SOME ASPECTS OF NATURAL PRODUCT CHEMISTRY

I. CIRCULAR DICHROISM STUDY OF SOME 3- AND 20-KETO STEROIDS

II. THE STRUCTURAL DETERMINATION OF HIRSUTIC ACID C

III. BIOGENETIC-TYPE SYNTHESES OF ACETATE-DERIVED AROMATIC COMPOUNDS

by

FREDERICK WILLIAM COMER

B.Sc. Honours, University of British Columbia, 1963

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Chemistry

We accept this thesis as conforming to the

required standard

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August, 1966

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FACULTY OF GRADUATE STUDIES

PROGRAMME OF THE

FINAL ORAL EXAMINATION

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

of

FREDERICK W. COMER

B.Sc. (Hons.) University of British Columbia, 1963

TUESDAY, AUGUST 2nd, 1966 at 3:30 P.M.

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STUDIES IN NATURAL PRODUCT CHEMISTRY

ABSTRACT

In Part I, the C.D. spectra of a number of 3-keto and 20-keto steroids are reported. The 3-keto steroids were comprised of two series of compounds. For one series, the conformationally transmitted effects of olefinic centers located in, or exocyclic to, rings B and C were investigated. It was found that these effects were reflected in the C.D. spectra when the double bond was located in ring B, but not when the double bond was located in ring C. Further, no correlation could be made between the relative rates of alkali-catalyzed benzaldehyde condensation and the C.D. data. For the other series, the conformations of ring A in a number of steroids substituted at the 2- and 4- positions are discussed. The C.D. results are in agreement with other evidence on this subject, and suggest that a 1,3-diaxial methyl interaction leads to flattening of ring A. For the Δ^5 - 4,4-dimethyl-3-keto steroids, a non-chair conformation is indicated, but a distinction between a boat and a flat chair conformation cannot be made. For 2α -bromo-4,4-dimethylcholest-5-en-3-one, a conformational equilibrium is suggested.

The 20-keto steroids were comprised of a large number of 16-substituted pregnanone and 17α -pregnanone derivatives. The C.D. spectra of the 16,17 trans compounds and the 16α , 17α -cis-compounds were similar to those of the 16-unsubstituted parent compounds. Modifications in rings A and B had little effect upon the dichroism of the 20-keto group. The C.D. spectra of the 16β , 17β -compounds were very sensitive to the nature of the 16β -substituent. These results are interpreted in terms of the preferred conformation of the 17β -acetyl group. Finally, the C.D. spectra of a number of 16, 17-epoxy-20-keto steroids are reported and discussed with reference to the "reversed octant rule".

In Part II, the structural determination of the mould metabolite, hirsutic acid C, is reported. The functional groups were established from chemical information; however the X-ray analysis of the p-bromophenacyl ester was required to reveal the novel ring system. During the X-ray irradiation an unusual solid-phase rearrangement occurred, transforming the

α-epoxy hydroxyl system of p-bromophenacyl hirsutate to a β-hydroxy ketone system without disrupting the crystal structure. The X-ray analysis revealed a 50:50 mixture of starting material and rearrangement product. A combination of the X-ray and the chemical data was required to complete the structural determinations of both products. The generality of the rearrangement process was investigated. It occurred with methyl hirsutate and dihydromethylhirsutate, but not with hirsutic acid. It could not be induced thermally. It did not occur with the steroids 3α- and 3β-hydroxy-4β, 5-epoxy-5β-cholestane. The rearrangement process is probably controlled by the nature of the molecular packing and hydrogen-bond formation.

In Part III, the biogenetic-type syntheses of a number of acetate-derived aromatic compounds are reported. A discussion of the preparation of the condensed polypyrone intermediates is given, and in particular the synthesis of the tetrapyrone, 4-hydroxy-9-methyl-2,5,7,12-tetraketo 1,6,8,11-tetraoxachrysene from the condensation of bis (2,4-dichlorophenyl) malonate and the tripyrone, 7-methyl-1-hydroxy-3,5,10-triketo 4,6,9-trioxaphenanthrene is reported. Treatment of the tripyrone with methanolic potassium hydroxide solution resulted in ring opening to form a poly-β-keto chain, and subsequent aldol-type condensation to give aromatic compounds representative of the naturally-occurring 6,8 dihydroxy-isocoumarins and C-acetylorsellinic acid. Treatment with methanolic magnesium methoxide solution gave aromatic compounds derivable from different cyclization modes of the poly-β-keto chain. Among the compounds isolated were two representative of the naturally-occurring curvulinic acid and the 5,7-dihydroxychromones respectively. Structural assignments were made largely on the basis of the characteristic spectral properties of the compounds.

GRADUATE STUDIES

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PUBLICATIONS

A. I. Scott, F. McCapra, F. Comer, S. A. Sutherland, D. W. Young, G. A. Sim, and G. Ferguson, "Structure and Stereochemistry of Some Polycyclic Diterpenoids", Tetrahedron, 20, 1339 (1964).

P. Crabbe, F. McCapra, F. Comer and A. I. Scott, "Study of the Cotton effect of 16-substituted 20-keto Pregnane and 17 ∝ -pregnane derivatives", Tetrahedron, 20, 2455 (1964).

F. W. Comer, F. McCapra, I. H. Qureshi, A. I. Scott and J. Trotter, "The Structure and Stereochemistry of Hirsutic Acid C, a Novel Fungal Sesquiterpenoid", Chem. Comm. 310 (1965).

F. W. Comer and J. Trotter, "The Structure of Hirsutic Acid. X-ray Analysis of the p-Bromophenacyl Ester", J. Chem. Soc. (B), 11 (1966).

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T. Money, F. W. Comer, B. Webster, I. H. Qureshi, I. G. Wright and A. I. Scott, "Biogenetic-Type Synthesis of Phenolic Compounds", J. Chem. Soc. (C), 1966, in press.

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PART I

CIRCULAR DICHROISM STUDY OF SOME 3- AND 20-KETO STEROIDS

INTRODUCTION

The elucidation of the structure and stereochemistry of optically active compounds has been greatly facilitated by recent advances in instrumentation for measuring the two phenomena associated with the Cotton effect: optical rotatory dispersion (O.R.D.) and circular dichroism (C.D.).

An optically active medium has different indices of refraction for the left and the right circularly polarized components of plane-polarized light, a property defined as circular birefringence. As a result of circular birefringence, the two circularly polarized components traverse an optically active medium with unequal velocities, which in turn results in the rotation of the plane of polarization by the angle α. A plot of the molecular rotation $[\phi]$, which is proportional to α, path length and molarity, against the wavelength λ of the incident light gives a rotatory dispersion (O.R.D.) curve.[1] The molecular amplitude, $\underline{a}$, is defined [2] as the difference between the molecular rotation at the extremum of longer wavelength $[\phi]_1$, and at the extremum of shorter wavelength $[\phi]_2$, divided by 100.

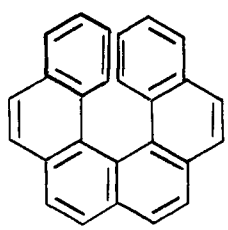
In addition to being circularly birefringent, an optically active medium absorbs the two circularly polarized components unequally, a property defined as circular dichroism. A plot of $\Delta\epsilon$, which is the difference in the molecular extinction coefficients of the left, ϵ_L, and the right, ϵ_R, circularly polarized components, i.e. $\epsilon_L - \epsilon_R$, against the wavelength λ of the incident light gives a C.D. curve.[3] As a result of circular

dichroism, when circularly polarized light traverses an optically active medium it emerges as elliptically polarized light, with the angle of ellipticity designated as Ψ . The molecular ellipticity, $[\theta]$, which is proportional to Ψ , path length and molarity, is defined by the equation: $[\theta] = 3300\Delta\epsilon$; and is often[4] used in place of $\Delta\epsilon$ for plotting C.D. curves. However it should be emphasized that present devices[5] for measuring circular dichroism are designed to detect absorption differences rather than changes in ellipticity.

It is in the regions in which optically active absorption bands are observed that the O.R.D. curves acquire anomalous character and the C.D. curves exhibit maxima. The two types of curves are closely related and an expression has been derived[3,6] to correlate them: $\underline{a} = 40.28\Delta\epsilon$. Although both O.R.D. and C.D. can often be used interchangeably for stereochemical problems,[7] the latter method is often preferred. Superimposed upon the anomalous O.R.D. curve is a plain curve due to rotational contributions from the various asymmetric centers of the molecule, and the tailing of other optically active bands of either the same chromophore or additional chromophores in the molecule. Although these background effects can be useful for characterizing a specific compound, they are more often a hindrance; and in the extreme they can obscure a weak Cotton effect or prevent the resolution of the anomalous effects due to two or more optically active absorption bands. On the other hand, the C.D. effect of a chromophore is only observed in the region of absorption maxima, and is largely influenced by substituents and asymmetric

centers in the immediate vicinity of the chromophore. These two properties render C.D. the method of choice for the resolution of several optically active absorption bands, and in fact for most configurational and conformational problems. Recent advances in C.D. instrumentation[8] now make both methods comparable in range (700 mμ to 190 mμ) and sensitivity.

Two types of optically active chromophores can be distinguished: the inherently dissymmetric chromophore, and the asymmetrically perturbed chromophore. Optical activity in the first type results from the intrinsic geometry of the chromophore, classical examples are hexahelicene (1)[6] and twisted biphenyl derivatives,[9] eg. (R)-9,10-dihydro-4,5-dimethylphenanthrene (2, $\Delta\epsilon$ 262 = +16.4). Compounds in this class exhibit



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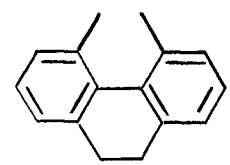


(2)

relatively strong Cotton effects, and do not necessarily require asymmetric carbon atoms for their optical activity. However it should be noted that the presence of asymmetric centers in a molecule could affect the chirality of an inherently dissymmetric chromophore.[10]

The most important example of the second type is the saturated carbonyl function. The carbonyl group has two

orthogonal reflection planes of symmetry and to the lowest order of approximation[6] the n $\rightarrow \pi^*$ transition should be optically inactive. Optical activity arises, however, through an asymmetric pertubation of the chromophoric electrons by a dissymmetric molecular environment. This type of chromophore is of particular value in stereochemical studies since the asymmetry of the rest of the molecule in relation to the symmetry elements of the chromophore is revealed in the Cotton effect. In the case of the carbonyl chromophore, a large body of empirical results has given rise to the octant rule.[11,12] The three mutually orthogonal planes of symmetry of the orbitals involved in the n $\rightarrow \pi^*$ transition define eight octants in space. The influence of the atoms of the molecular structure on the Cotton effect of the carbonyl group is characterized by a sign according to the octant in which they are located, and is considered negligible if they lie in any of the nodal planes. (For full details of the octant rule see reference 11). On the basis of the octant rule, assignments of the configuration of a ketone (absolute configuration if a suitable reference compound of known absolute configuration is available) can be made if its conformation is known, and similarly if the configuration of the substance is known information about its conformation can be obtained. In applying the octant rule, the final sign will be obtained from the resultant of the individual contributions made by the various substituents asymmetrically disposed about the carbonyl group. The influence of a given substituent will be largely determined by its steric and electronic nature,[13] and by its

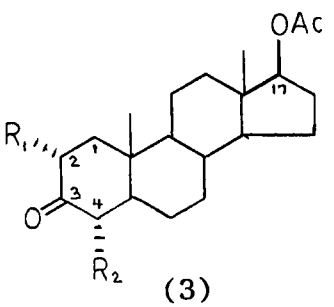
true position in the octant. At present very little is known about the quantitative contributions of various substituents, although Djerassi has studied the effects of several alkyl groups in some detail, eg. methyl,[14] isopropyl[15] and t-butyl.[16] For the most part a number of semiquantitative empirical rules are applied, eg. the nearer a substituent is to the carbonyl group the greater its effect, and the nearer it is to a nodal plane of the chromophore the less its effect. Despite the rather rigorous restrictions which a lack of quantitative data imposes upon the octant rule, it has found wide applicability as an important tool in stereochemical studies.

Historically the empirical basis of the octant rule developed from the Cotton effect studies of numerous steroidal ketones. The rigid fused-ring structure of this class of compounds makes it readily amenable to stereochemical studies. Consequently the steroid framework has been used extensively for studying the influences that various substituents and unsaturated centers have on the Cotton effect of the carbonyl chromophore.

The spectroscopic properties of α-halocyclohexanones have been studied in some detail; and subsequently have been used to assign the axial or equatorial configuration to the α-halogen atom. Thus it has been observed[17] that equatorial α-halogen substituents shift the infrared carbonyl band to higher frequency (ca. + $20cm^{-1}$), whereas the corresponding axial substituent has little effect upon this band. The electrostatic repulsion between the C-halogen and C=O dipoles, which will be

greatest when the halogen atom is equatorial, adequately explains this observation. The ultraviolet maxima associated with the saturated carbonyl chromophore undergoes a hypsochromic shift[18] of about 5 mμ when the α-bromine atom is equatorial, but is moved to longer wavelengths by about 28 mμ when the bromine atom possesses the axial orientation.

The Cotton effect of α-halocyclohexanones is similarly very sensitive to the orientation of the halogen atom. An equatorial halogen substituent has little effect upon the position and amplitude of the Cotton effect, whereas an axial halogen substituent shifts the anomalous effect to higher wavelengths (ca. + 20 mμ) and profoundly influences the amplitude. These observations were first stated in the "axial haloketone rule",[19] a forerunner of the octant rule, which is best illustrated by an example.[1] The parent compound 17β-acetoxyandrostan-3-one (3, R_1=R_2=H) as well as the equatorial 2α-bromo (3,R_1=Br,R_2=H) and 4α-bromo (3,R_1=H,R_2=Br) derivatives exhibit positive Cotton effects of similar amplitude. The axial 2β-bromo derivative (4,R_1=Br,R_2=H), however, exhibits a much enhanced positive amplitude, and the 4β-bromo derivative exhibits a strong negative Cotton effect. With the exception of fluorine, halogen



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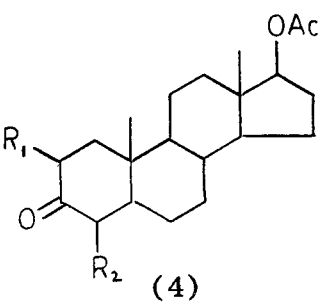

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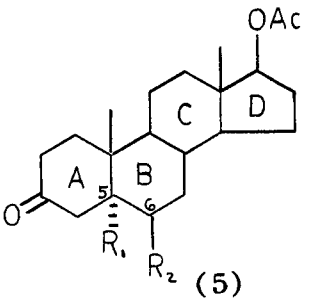

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substituents obey the more general octant rule. The fluorine[20] atom exhibits an effect opposite in sign to that of the other halogen atoms. The contribution of axial α-halogen substituents is generally so pronounced as to determine the sign of the observed Cotton effect.

Halogen substituents in β and γ-positions also have a significant influence upon the Cotton effect of 3-keto steroids. The negative contribution of 6β-halogen substituents (5,R_1=H, R_2=X) is attributed[21] to the location of the halogen atom in a rear negative octant. The negative contribution of 5γ-halogen substituents (5, R_1=X, R_2=H) is more difficult to rationalize. The two explanations offered are: (a) ring A adopts a boat-like conformation[21], and (b) ring A maintains a chair conformation but the halogen atom actually lies in a front[22] negative octant. Experimental evidence[22] favors the latter explanation. This example illustrates one weakness of the octant rule, i.e. the position of the plane perpendicular to the C=O bond has not been well defined either by theoretical or empirical studies.

Spectral evidence indicates that cyclopropane and oxiran groups have somewhat delocalized electron systems. The delocalized orbitals containing these electrons are believed to lie in the plane of the three-membered ring. The overlap of these delocalized orbitals with the vacant p-orbital of cyclopropyl carbonium ions[23] has been used to explain the considerable charge delocalization into the cyclopropyl ring of these ions; this charge delocalization is indicated by the

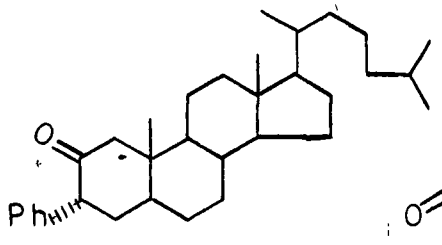
large (ca. 3 ppm.) downfield shift of the proton resonances of the ring hydrogen atoms. The presence of delocalized orbitals is also indicated by the ultraviolet spectra of α-epoxy[24] and α-cyclopropyl[25,26] ketones. In general the absorption band of the carbonyl chromophore is enhanced and shifted to slightly higher wavelengths.

The Cotton effect of α-epoxy and α-cyclopropylketones also indicates the presence of delocalized orbitals. Recent studies[27,28] of a large number of compounds have shown the α-epoxy and α-cyclopropyl rings make significant contributions to the Cotton effect of the carbonyl group which are opposite in sign to those made by alkyl and halogen groups. Also it has been shown[27] that a nonconjugated cyclopropyl ring makes no special contribution to the Cotton effect.

When a double bond is placed in conjugation with a keto group the resulting α,β-unsaturated ketone is characterized by two maxima in the ultraviolet region. The long wavelength transition is analogous to the $n \rightarrow \pi^*$ transition of saturated ketones but is noticeably enhanced ($\epsilon \simeq 100$) and shifted to longer wavelengths ($\lambda max \simeq 330 m\mu$). The other transition, described as $\pi \rightarrow \pi^*$, appears in the region 260 to 220$m\mu$ and shows very intense absorption ($\epsilon \simeq 10,000$). The Cotton effect of the $n \rightarrow \pi^*$ transition is very sensitive to conformational changes[3,29] and recently a modified octant rule has been proposed[30] to account for the empirical data. For cyclohexenones with planar chromophores the atoms lying off the plane determine

the Cotton effect, whereas the Cotton effect of nonplanar chromophores is dominated by the orientation of the double bond. The $\pi \rightarrow \pi^*$ transition is also optically active and is analogous to a similar transition in conjugated dienes. Hence a nonplanar α,β-unsaturated keto group may be regarded as an inherently dissymmetric chromophore.[10] The helicity rule[31,32] for nonplanar 1,3-dienes has been applied to determine the absolute conformation of α,β-unsaturated keto groups. Generally the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions of nonplanar α,β-unsaturated keto groups have Cotton effects of opposite sign; however recent[33] circular dichroism measurements in the region 400 to 200 mμ have indicated several exceptions to this rule, and have also revealed that there are two optically active bands in the $\pi \rightarrow \pi^*$ region (280 to 200 mμ).

The ultraviolet and circular dichroism spectra of β,γ-unsaturated ketones are critically dependent upon the molecular geometry of the system. Thus it has been observed that phenylcholestanones[34] exhibit an enhanced absorption band at 300 mμ and an enhanced Cotton effect only when the phenyl group is in an axial position, eg. 3α-phenylcholestan-2-one (6). The compound 4,4-dimethylcholest-5-en-3-one (7)[35] shows an unenhanced absorption band (λmax 293 mμ , ϵ =38) whereas cholest-5-en-3-one (8)[36] exhibits a slightly enhanced $n \rightarrow \pi^*$ transition (λmax 289 mμ , ϵ =59, $\Delta\epsilon$ 290 = 2.95). Partial orbital overlap between the two unsaturated groups of compound (8) is also indicated by the appearance of a new optically active



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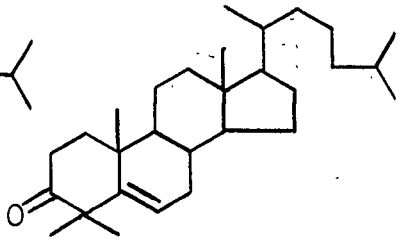

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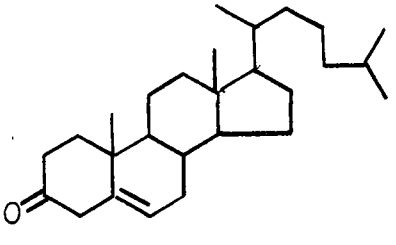

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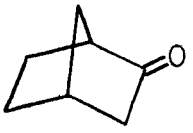
absorption band (λmax 217 mμ, ϵ=1300, $\Delta\epsilon$ 218 = -6). A number of postulates [37,38,39] have been put forward as to the nature of the orbital overlap. It is generally accepted that a π-orbital of the C=C group overlaps with both a p-orbital and a π^*-orbital of the C=O group. This model explains the observed enhanced $n \longrightarrow \pi^*$ transition and also rationalizes the appearance of a charge transfer band, $\pi_{c=c} \longrightarrow \pi^*_{c=o}$. Cholest-5-en-3-one (8) is an example of weak orbital overlap. In such cases[36] the orientation of the double bond influences but does not necessarily dominate the rotatory power of the $n \longrightarrow \pi^*$ transition. There are, however, numerous examples[40] of β,γ-unsaturated ketones where strong orbital overlap is indicated. In these cases the Cotton effect is dominated by the absolute conformation of the double bond, eg. norcamphor (9) exhibits a very weak Cotton effect ($\Delta\epsilon$ 310=-0.30) whereas dehydronorcamphor (10) shows a strong positive Cotton effect[40] ($\Delta\epsilon$ 305 = +4.7). When strong orbital overlap is indicated it is convenient to treat the β,γ-unsaturated ketone system as an inherently dissymmetric chromophore.[41] This viewpoint has led to useful applications in the determination of absolute configuration and conformat-

ion.[41,42]



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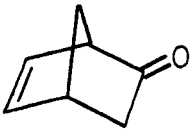

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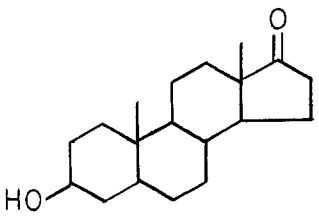
When the double bond is moved still further away from the carbonyl group in a system such as the steroid nucleus, the possibility of orbital overlap between the two unsaturated groups becomes virtually nil, eg. the ultraviolet and circular dichroism spectra[36] of the carbonyl chromophore of 3β -hydroxy-androstan-17-one (11, λmax 294 mμ, ϵ =43, $\Delta\epsilon$ 295 = +3.55) and the γ,δ -unsaturated ketone, 3β-hydroxyandrost-9(11)-en-17-one (12, λmax 295 mμ, ϵ =43, $\Delta\epsilon$ 300=+3.70) are nearly identical; in addition the latter compound shows no evidence of a charge



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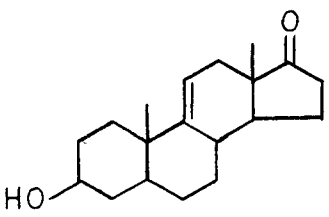
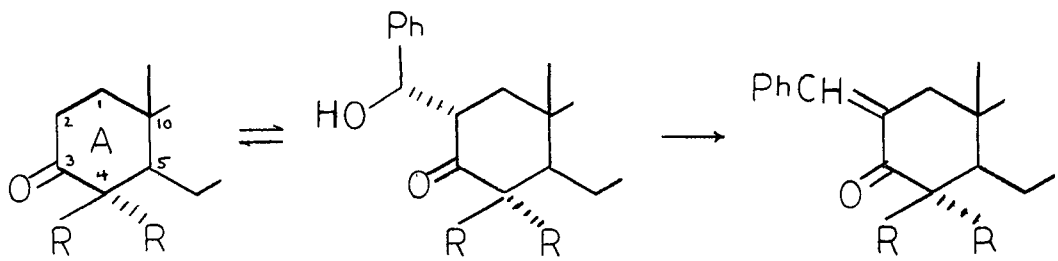


(12)

transfer band.

In the case of nonconjugated unsaturated ketones, even though there may be no direct electronic interaction between the olefinic and carbonyl groups, the steric arrangement of the atoms about the carbonyl group may vary according to the location of the olefinic center. This fact was first illustrated by Barton[43] in the study of the rates of alkali-catalyzed condensation of benzaldehyde with various 3-keto triterpenoids of partial structure (13, R=CH_3) to give benzylidene derivatives 15,R=CH_3). The mechanism has been shown[44,45] to consist of a series of reversible steps up to the formation of the anion



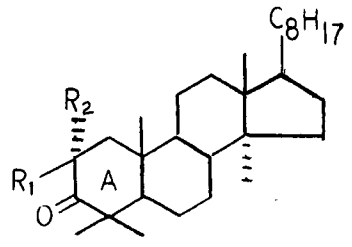
(13) (14) (15)

from (14) followed by an irreversible elimination to yield (15). It was observed that as the double bond was shifted to various positions in rings B,C,D the rate of reaction also changed. The study was extended[46,47] to include a series of 3-keto steroids (partial structure 13, R=H) and derivatives of β-decalone with similar results being obtained. By expressing the rate in terms of the rate of a saturated reference compound multiplied by a series of group rate factors, each of which is characteristic of the position of an unsaturated center, it was shown that the group rate factors for structurally analogous steroidal

and triterpenoid ketones were quantitatively related. This shows that in the triterpene series the influence of "axial buttressing" of the β-methyl groups (at C_4 and C_{10}) on the group rate factors is minor. The explanation advanced for the variation in rate with the position of the double bond is that the conformational distortion introduced at the original site of unsaturation is transmitted through the molecule and ultimately to ring A via flexing of valency angles and slight alterations in atomic coordinates. Such an effect is described as "conformational transmission".

Conformational transmission would also be expected to be reflected in the Cotton effect of unsaturated, nonconjugated steroidal and triterpenoid ketones. Djerassi[48,49] has shown that the O.R.D. curves of such compounds are sensitive to the location of the double bond. An attempted correlation with Barton's rate studies, however, was unsatisfactory.[46] Since the background contribution to the O.R.D. curves of these compounds, due largely to the tailing of the optically active $\pi \rightarrow \pi^*$ transition of the olefin, is large and critically dependent[49] upon the location of the double bond, it was felt that C.D. data might be more useful in relating the manifestation of conformational transmission in the Cotton effect and the rate of benzaldehyde condensation. Establishing the relationship between the C.D. spectra and the rate of benzaldehyde condensation for a number of steroidal ketones was thus one of the objects of this thesis.

Another illustration of conformational transmission is provided by a comparison of bromination experiments[50] with lanostan-3-one (16a) and lanost-8-en-3-one (17a). Monobromination of (16a) afforded only the 2α-isomer (16b). In addition



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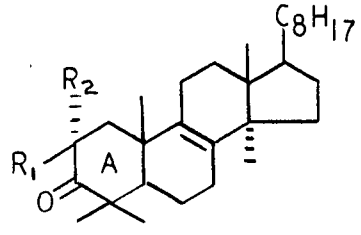
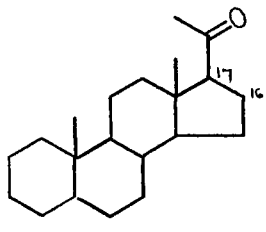
(a) $R_1 = R_2 = H$
(b) $R_1 = H, R_2 = Br$
(c) $R_1 = Br, R_2 = H$



(17)

acid-catalyzed (HBr/HOAc/$CHCl_3$) equilibration of either isomer (16b or 16c) resulted in a quantitative yield of 2α-bromolanostan-3-one (16b). Monobromination of (17a) however, gave a mixture of 2α- (95%) and 2β-bromolanost-8-en-3-one (5%). Acid-catalyzed equilibration of either (17b) or (17c) afforded a similar mixture. In addition to demonstrating the concept of conformational transmission, the above example has an important bearing on present concepts of ring A conformation. I.R. and U.V. spectra of (16b) and (16c), and similarly of (17b) and (17c), indicate the bromine atom is equatorial in all cases. This can best be explained[50] by postulating a boat conformation for ring A in the 2β-bromo isomers (16c and 17c). As part of the work reported in this thesis the C.D. spectra of several compounds of controversial ring A conformation have been obtained, and thus this subject will be discussed in some detail in the next section.

Aliphatic ketones generally exhibit weak Cotton effects[3] because conformational mobility destroys much of the asymmetry of the atomic environment of the carbonyl group. When conformational mobility is hindered, or more precisely, when one of the conformations predominates the Cotton effect will generally be enhanced, and correlations between the observed optical activity and structure can more easily be made. An example of a system where rotation is hindered is provided by the 17-acetyl steroids. It has been observed[51] that when the acetyl group has the β -configuration as in 17-acetylpregnanone (18) the Cotton effect is positive, whereas 17α -pregnanone (19), which has the acetyl group in the α -configuration, exhibits a negative Cotton effect. As part of the work reported in this thesis the C.D.



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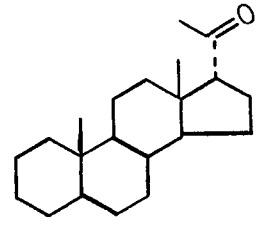

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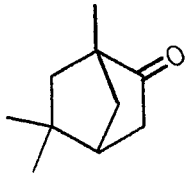
spectra of a large number of 16-substituted, 17-acetyl steroids were measured. A discussion of these results and how they relate to the problem of determining the configuration and the preferred conformation of the 17-acetyl sidechain will be given.

One of the problems associated with circular dichroism has been the interpretation of C.D. spectra exhibiting two maxima

of opposite sign for a single optically active transition. Examples of such curves are provided by the C.D. spectra in the 300 mμ region of a number of steroidal and triterpenoid ketones, particularly 3-ketotriterpenes;[52] and even by more conformationally rigid structures, eg. isofenchone (20)[53], see figure 1. Three proposals have been made to account for these irregular curves. For the case of rigid molecules, eg. (20), a solvational equilibrium has been postulated. Fundamental to this hypothesis is a considerable solvent dependence of the C.D. curve. In addition an increase in the population of the solvated species would be expected on lowering the temperature; hence if the view that the higher energy transition is due to the solvated species is accepted, then the short wavelength band of the C.D. curve is expected to increase at the expense of the long wavelength band.[54] The expected solvent[55] and temperature[56] dependence of the Cotton effect of isofenchone (20) is observed.



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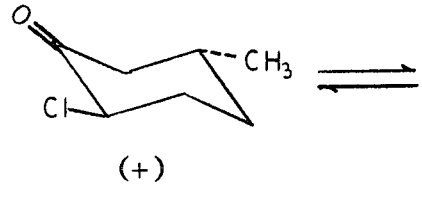
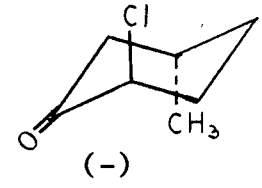




(21a) (21b)

A second phenomenon which can account for a curve exhibiting two maxima of opposite sign is a conformational equilibrium. Such an equilibrium would also be expected to be considerably solvent and temperature dependent, eg. (+)-trans-2-chloro-5-methylcyclohexanone [56,57,58] (see figure 1), which is present

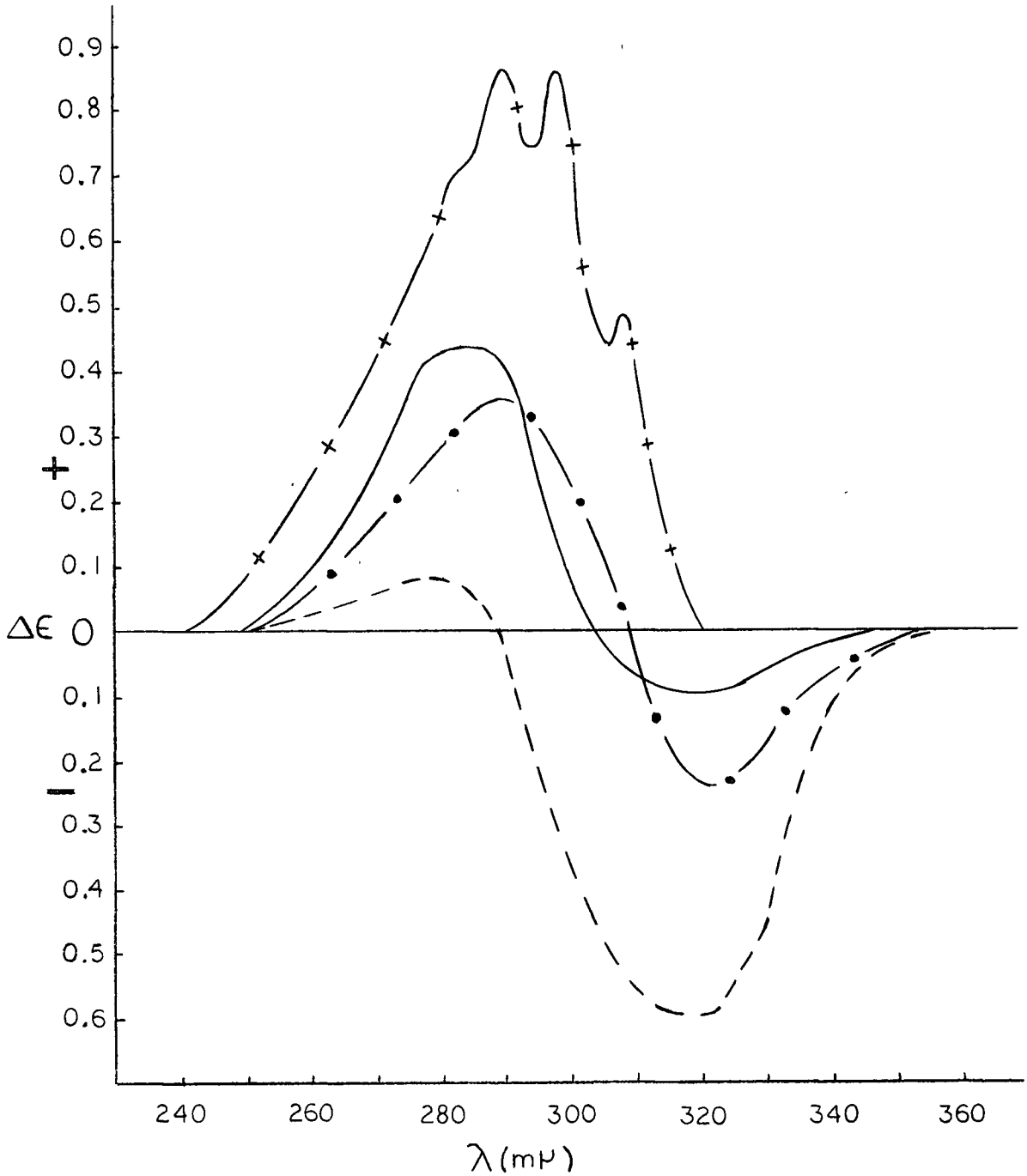


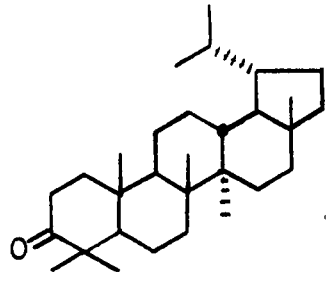
Figure 1

Circular Dichroism curves of:

isofenchone (20): ethanol, 25°
(+)trans-2-chloro-5-methylcyclohexanone (21)
ether:isopentane:ethanol, 5:5:2 (EPA) 25°
ether:isopentane:ethanol, 5:5:2 (EPA) -192°
carbon tetrachloride 25°

in solution as a mixture of the diequatorial (21a) and diaxial (21b) forms. The octant rule predicts a positive Cotton effect for (21a) and a negative Cotton effect for (21b).

In both of the explanations above the simplifying assumption has been made that the equilibrium involves only two species. On the basis of this assumption, calculations[54] have shown that the superposition of two Cotton effects of similar amplitude but opposite sign with a separation of their maxima of 1 to 20 mμ results in a relatively weak C.D. curve with apparent maxima separated by about 30 mμ. Empirically it is observed that usually one and often both of the maxima of an alternating-sign curve have very low intensity. It is obvious from the above arguments based on the solvent and temperature dependence of the C.D. curve that it will often be difficult to distinguish between a solvational or conformational equilibrium, eg. lupan-3-one[52,56] (22), λ ($\Delta\epsilon$), EPA: 25°, 330(-0.06), 295(+0.7); -192°, 330(-0.21), 290(+0.18). When the irregular curve persists in the vapor state then only the latter explanation is justified, eg. (-)-carvone[59] (23), $\lambda(\Delta\epsilon)$: 370(-0.5), 330(+2.6). A further



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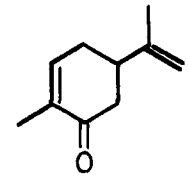


(23)

weakness of the solvational hypothesis is that it is difficult to rationalize the solvating power of hydrocarbon solvents.

Low temperature C.D. measurements often produce considerable enhancement of the vibrational fine structure[60] associated with an optically active band. A consideration of "vibrational structuring" has recently led to the proposal[61] that it may account for the alteration in sign of a C.D. band. Theoretical arguments[61] show that if it is assumed that both electric and magnetic transition dipole moments have significant components arising from molecular vibrations, then the sign of the dichroism band need not be invariant and in fact may alternate when the rotary power is weak, i.e. $|\Delta\epsilon| < 1.0$. Most of the features of the carbonyl dichroism band can be rationalized in terms of a vibronic scheme consisting of a totally symmetric, negative, "allowed" progression of the 1200 cm^{-1} stretch frequency originating at the 0-0 band, complemented by a positive, "forbidden" band system; the latter being the same 1200 cm^{-1} progression in combination with a single 900 cm^{-1}, "non-totally symmetric" mode. On this basis, hypothetical curves have been composed which are nearly identical to the C.D. curves of lupan-3-one (22, at -192°) and (-) carvone (23, at 25°). The temperature and solvent dependence to be expected on the basis of this proposal have not been defined, however vibrational structuring is expected to be important even at very low temperatures.

From the examples chosen it can be seen that a C.D. curve exhibiting two maxima of alternating sign for a single chromo-

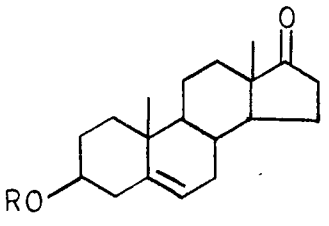
phore may be rationalized by one, two or all three of the postulated phenomena. During the course of the work reported in this thesis, several C.D. spectra of alternating sign were obtained. A brief discussion of these will be made with respect to the three phenomena: solvational equilibrium, conformational equilibrium, vibrational structuring.

DISCUSSION

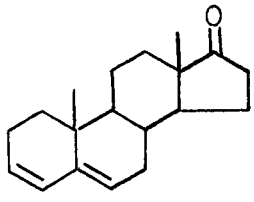
The intense ultraviolet absorption of conjugated olefins and ketones has proved useful for the detection and characterization of these chromophores. The two traditional and most fundamental criteria for purity of crystalline organic compounds are elemental analysis and melting point determination. A satisfactory elemental analysis is a necessary requirement for purity, but a sharp melting point, though desirable, can not be considered necessary for all classes of compounds. With respect to ultraviolet absorption, however, neither criterion is a sufficient requirement for purity. Thus trace amounts (ca. 1%) of a diene impurity present in a sample of a saturated steroidal ketone may not be reflected in either the melting point or the analysis, but will have a profound effect on the U.V. spectrum of the sample.

In a consideration of the effects of nonconjugated double bonds on the U.V. and C.D. spectra of the carbonyl group, Mason originally postulated[62,63] that a long range, charge transfer interaction between the two groups could occur via weak "conjugation" through the saturated carbon atoms. This postulate was based largely on the observation[62,63] of a weak absorption band at 239 mμ (ϵ =70, $\Delta\epsilon$ =0.2) in the electronic spectrum of 3β -hydroxyandrost-5-en-17-one (24a). An alternative explanation is that this weak absorption band is due to the presence of trace amounts of a diene impurity such as androst-3, 5-diene-17-one (25), λmax 290 mμ (ϵ 90), 236 mμ (ϵ 22,400). The U.V.

spectrum of a commercial sample of (24a) also showed a short wavelength band at 236 mμ (ϵ=143). However by a stepwise purification scheme of recrystallization, followed by chromatography of the corresponding acetate (24b), the peak at 236 mμ was eliminated. Subsequently Mason has reported[36] a similar result and



(24a, R=H)
(24b, R=OAc)



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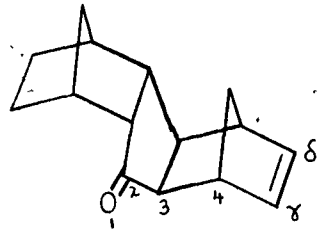


(26)

also has suggested that the impurity was the diene (25). The demonstration that (24a) does not exhibit a charge transfer band, plus additional evidence provided by Mason,[36] substantiates the view that the charge transfer interaction is of relatively short range and operates through space.

The question of whether σ-bonds can be involved in charge transfer interaction has very recently been reopened by the observation of a charge transfer band in a number of γ,δ-unsaturated ketones,[64] eg. (26), λmax: 307 mμ (ϵ 79), 224 mμ (ϵ 1330). The appearance of a $\pi_{cc} \rightarrow \pi^*_{co}$ transition in such systems is shown to be critically dependent on geometry. This has been interpreted in terms of a favorable geometry allowing the coupling of the two π-systems through overlap with the central C_3-C_4 σ-bond. In general γ,δ-unsaturated ketones do not possess the geometry defined in (26) and hence do not

show this type of overlap.

The results of a study of the long range effects of olefinic centers on the C.D. spectra of 3-keto steroids are summarized in Table 1. Some recently published results of Velluz[5] and Ourisson [52,65] are also included in the table, and all structural formula are given in figure 2. Most of the samples used in this study exhibited weak U.V. absorption maxima in the region 260 to 230 mμ in addition to the 290 mμ band of the carbonyl chromophore. It is felt the only satisfactory explanation is that the additional absorption bands are, in every case, indicative of trace amounts of highly absorbing impurities. These impurities would not be expected to have any measurable influence upon the C.D. spectra.

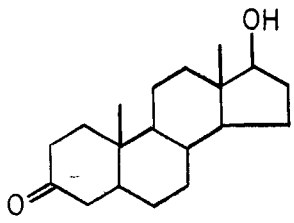
An examination of the C.D. results for the 4-demethylated series (27 to 36) reveals that the compounds fall into two groups. When the double bond is located in ring B (29,32) or exocyclic at C_7 (30,31) the intensity of the maxima varies from that of the saturated analogues (27,28). On the other hand, when the double bond is located in ring C (33,34,35) or exocyclic at C_{12} (36) the unsaturated center appears to have little effect upon the circular dichroism of the carbonyl group.

These results can be compared with the relative rates of the alkali-catalyzed benzaldehyde condensation[46] which are also listed in Table 1. It can be seen that in contrast to the C.D. results, the rate of condensation is quite sensitive to the location of the double bond for all of the compounds. In this

TABLE 1

Compound	λ max ($\Delta\epsilon$)	ref.	Relative rate[46]
17β -hydroxyandrostan-3-one (27)	294 (+0.96) 295 (+0.90)	5	188
cholestan-3-one (28)	295 (+1.13)	5	182
17β -hydroxy-7-methyleneandrostan-3-one (30)	295 (+1.56)	65	
cholest-6-en-3-one (29)	325(-0.04),294(+0.39)	65	645
7-methylenecholestan-3-one (31)	295 (+1.54)		365
ergost-7-en-3-one (32)	295 (+0.67)		47
ergost-8 (14)-en-3-one (33)	295 (+1.15)		94
$\Delta^{9(11)}$-dehydrotigogenone (34)	295 (+0.88)		221
Δ^{11}-dehydrotigogenone (35)	294 (+0.93)		380
12-methylenetigogenone (36)	295 (+0.90)		218
lanostan-3-one (16a)	ca. 300 (-0.34)*	49	55
lanost-8-en-3-one (17a)	318(-0.11),290(+0.13) 320(-0.13),290(+0.14)	52	100

* calculated from O.R.D. amplitude,[3,6] cf. (46), Table 2.



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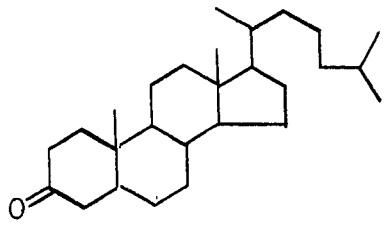

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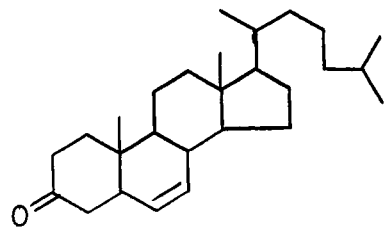

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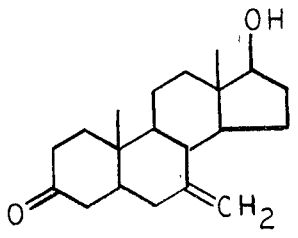

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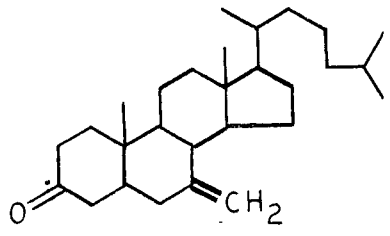

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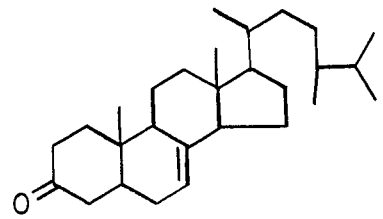

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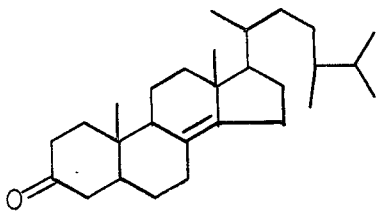

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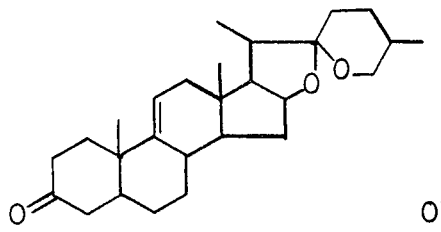

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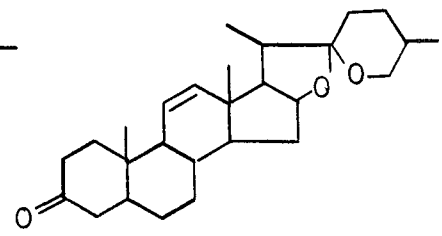

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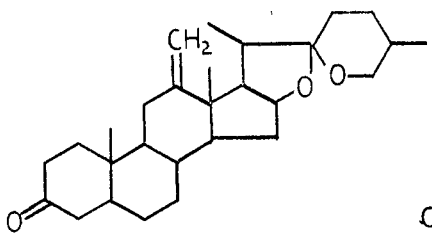

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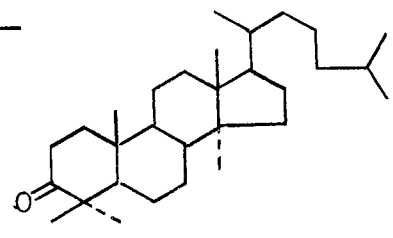

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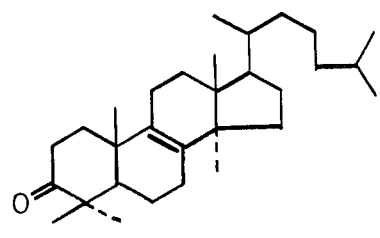
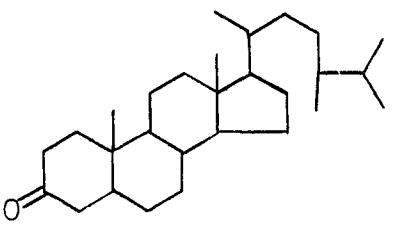


(17a)

Figure 2

regard it should be noted that the relative rates for ergostan-3-one (37, 188) and tigogenone (38, 174)[46] are similar to the rates for the other saturated analogues (27, 28). In addition even for the ring B unsaturated compounds a correlation between re-



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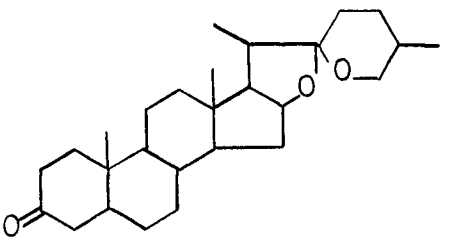
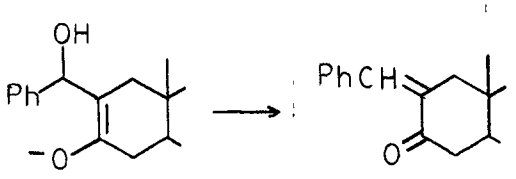


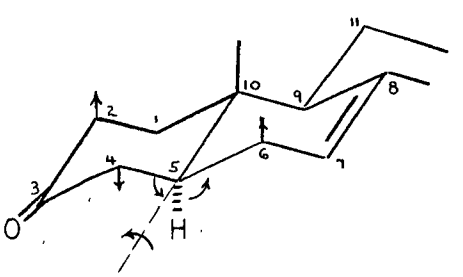
(38)

lative rates and circular dichroism can not be made, i.e. both cholest-6-en-3-one (29) and ergost-7-en-3-one show a diminished C.D. maxima, but the former compound exhibits a much enhanced and the latter a much depressed rate of condensation.

Despite this lack of correlation, it is felt that both sets of measurements reflect the phenomenon of conformational transmission. The relative rates have recently been rationalized[66] by considering the Δ^2-enolate anion (partial structure 14a) as a suitable model for the transition state in the rate determin-



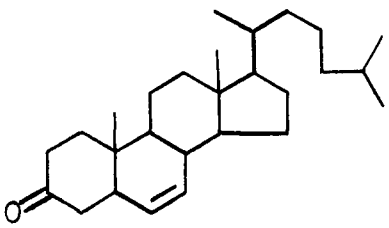
(14a) (15)



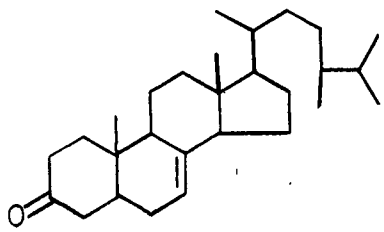
(32a)

ing step to form the benzylidene derivative (15). By the use of Dreiding models[67] it can be demonstrated that introduction of a $C_{7,8}$ double bond causes an upward displacement of C_6 in the β-direction. This distortion is relayed to C_5 causing angle deformation and resulting in an anticlockwise rotation about the C_5-C_{10} axis of the groups attached at these centers (32a). This in turn produces a downward displacement (α-direction) of C_4 which simultaneously causes an upward displacement of C_2. The strain may effectively be reduced by conversion of C_4 into a trigonal atom. Thus the formation of the Δ^4-enol is facilitated and simultaneously the tendency for Δ^2-enol formation is diminished, resulting in a reduced rate as observed (32). When the double bond is located at $C_{6,7}$ or exocyclic at C_7 the effect upon ring A is reversed to favor Δ^2-enol formation, and an enhanced rate is observed (29,30,31).

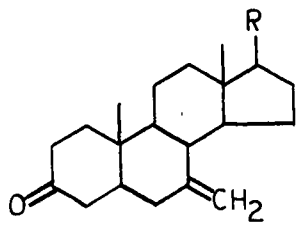
The C.D. results can similarly be rationalized by comparing the Drieding models of the saturated and unsaturated 3-keto steroids. For cholest-6-en-3-one (29) the major change in the octant diagram is that C_7 is displaced from its position in a positive octant towards a nodal plane. Thus a diminished C.D. curve would be expected as is observed. In ergost-7-en-3-one (32), C_6 is displaced towards a nodal plane, again diminishing the Cotton effect. For the 7-methylene-3-keto steroids (30,31). the methylene group is located in a positive octant and the expected enhancement of the C.D. maxima is observed. These explanations for the C.D. spectra of compounds (29) to (32) are grossly oversimplified and neglect the subtle changes in ring A conformation which a Dreiding model is unable to show; in addit-



(29)



(32)

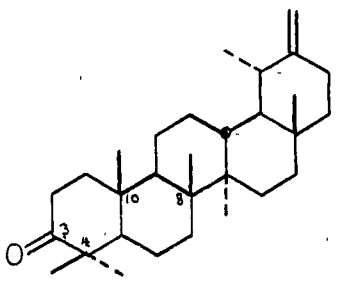


(30, R=OH)
(31, R=C_8H_{17})

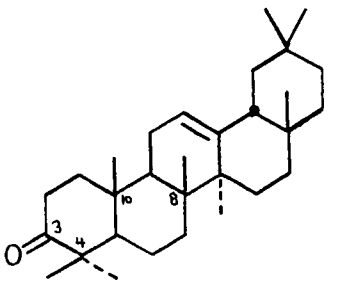
ion the influence of the π-bond as compared to the two C-H bonds that it replaces is not known. With respect to ring A conformation it is worth noting that any flattening of the ring would lead to diminished positive contributions from C_6 and C_7.

When the double bond is located in ring C it becomes more difficult to rationalize the results. By an extension of the above arguments, however, the relative rates of compounds (33) to (36) can be interpreted in terms of the strain induced in ring A. Similarly the C.D. results for these compounds suggest either the atomic coordinates of rings A and B vary negligibly or they vary in such a way as to give a negligible resultant effect. If the arguments used to explain the two sets of measurements are valid, then it follows that a correlation between the rates of benzaldehyde condensation and circular dichroism is not expected.

It is pertinent to note that the C.D. spectra of 3-keto triterpenes are more sensitive[52] to unsaturation in ring C, eg. taraxasterone (39), $\lambda(\Delta\epsilon)$: 293 (+0.75), whereas β-amyrone (40), $\lambda(\Delta\epsilon)$: 293(+0.30). This is not unexpected as the



(39)



(40)

positions of the 4∝, 4β and 8β-methyl groups would be influenced by conformational transmission.

A comparison of either the condensation rates or the C.D. spectra of the compounds lanostan-3-one (16a) and lanost-8-en-3-one (17a, see Table 1) suggests conformationally transmitted effects in the latter compound. As for the triterpenes, it is quite likely that the detection of these effects by circular dichroism is facilitated by the presence of the gem-dimethyl groups at C_4.

The explanation of the C.D. spectrum of lanost-8-en-3-one (17a) is complicated by the fact that there are two maxima of opposite sign. The C.D. curve of cholest-6-en-3-one (29) is also of this type. Of the three hypotheses to explain this type of curve, the solvational equilibrium hypothesis is the least tenable since it is difficult to see why these two compounds should solvate so differently from the others listed in Table 1. A conformational equilibrium is more plausible since it is known that the exocyclic keto group lowers the barrier to chair-boat conversion in cyclohexane ring systems, and the

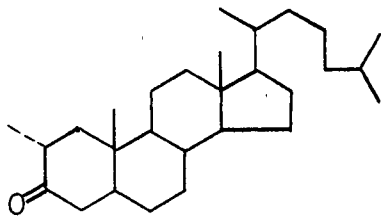
conformationally transmitted effects of the double bond could possibly lower this barrier still further. However it is again difficult to see why these two compounds should differ from the others with respect to ring A flexibility. The vibrational structuring hypothesis offers an attractive alternative. Both (17a) and (29) exhibit weak Cotton effects in accord with this hypothesis. Low temperature measurements might help distinguish the latter two hypotheses since a large temperature variation would support a conformational equilibrium.

In recent years the subject of the conformation of ring A in substituted 3-keto steroids has received considerable attention. Table 2 lists the C.D. maxima of a number of such compounds and it will be shown that these results are consistent with present views on the subject. To substantiate our own data, Table 2 includes a number of results recently published by Ourisson, et.al.[52] The structural formulae for the compounds listed in the table are given in figure 3.

A number of studies have indicated that the introduction of an axial methyl group at the 2β- or 4β- position of a saturated 3-keto steroid leads to flattening of ring A. It is believed the flattening occurs concurrently with the lateral displacement of the 10β and 4β (or 2β) methyl groups in relieving the steric interaction between these groups. X-ray determinations of the structures of 3β-iodoacetoxylanost-8-ene (55)[68] and 3β-acetoxy-7α, 11α-dibromolanostane-8α, 9α-epoxide (56)[69] showed that the bonds to the 4β and 10β methyl groups

TABLE 2

Compound	λmax ($\Delta\epsilon$)	ref.
17β -hydroxyandrostan-3-one (27)	294 (+0.96)	
cholestan-3-one (28)	295 (+1.17)	52
2α -methylcholestan-3-one (41)	295 (+0.75)	
2,2-dimethyl-17β -hydroxyandrostan-3-one (42)	303-290 (+1.52)	
2,2-dimethylcholestan-3-one (43)	302-295 (+1.93)	52
2,2,17α -trimethyl-19-norandrostan-3-one (44)	303-290 (+2.78)	
17β -hydroxy-19-norandrostan-3-one (45)	294 (+1.37)	52
4,4-dimethylcholestan-3-one (46)	305 (-0.30)	52
4,4-dimethylcholest-5-en-3-one (47)	295 (+0.96)	
4,4-dimethyl-17β -hydroxyandrost-5-en-3-one (48)	294 (+1.28)	
4,4-dimethyl-17β -hydroxy-19-norandrost-5-en-3-one (50)	303-295 (+2.11)	
4,4-dimethyl-17β -acetoxy-19-norandrost-5-en-3-one (51)	307-297 (+1.84)	52
4,4-dimethyl-17β -acetoxy-19-norandrostan-3-one (52)	301 (-0.47)	52
2α -bromo-4,4-dimethylcholest-5-en-3-one (53)	309 (+0.70)	
2α -bromo-4,4,6-trimethylcholest-5-en-3-one (54)	312 (+1.07)	



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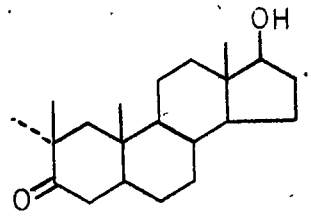
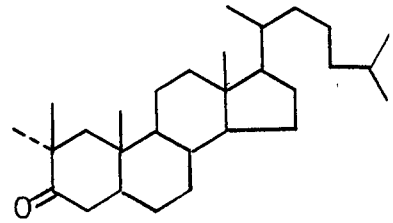
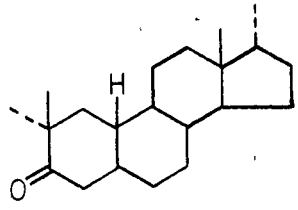


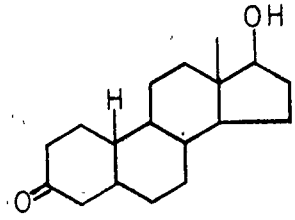
(42)



(43)



(44)



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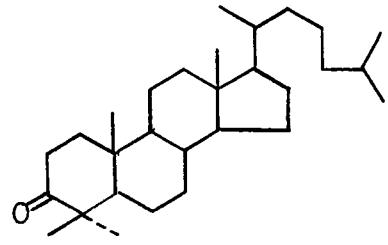

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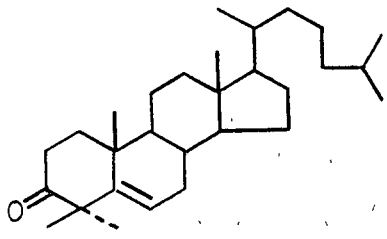

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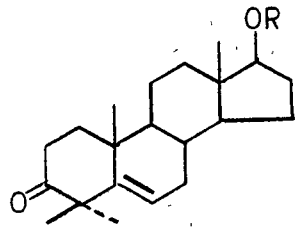
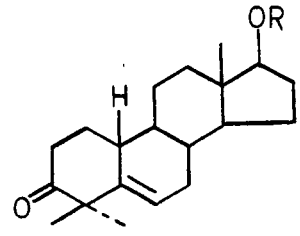
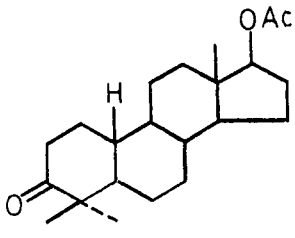


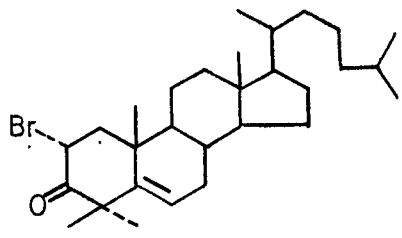
(48, R=H)
(49, R=Ac)



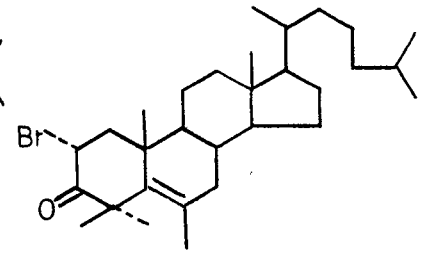
(50, R=H)
(51, R=Ac)



(52)

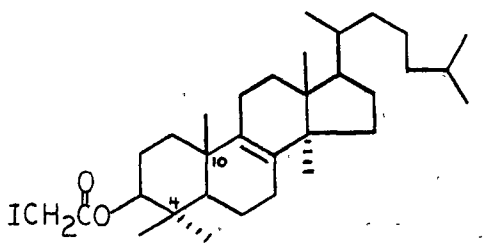


(53)

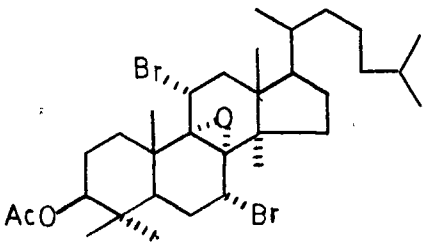


(54)

Figure 3



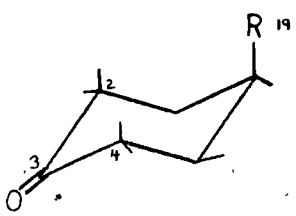
(55)



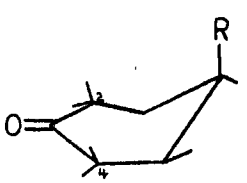
(56)

were not parallel, and the difference between the carbon atom centers of these methyl groups was respectively 3.2 ± 0.1 Å and 3.28 ± 0.03 Å; these values correspond to a lateral displacement of about 15°, and are in agreement with an interference radius[70] of 1.7 Å which has been found for the methyl group from a consideration of the effects of steric interactions upon U.V. spectra.

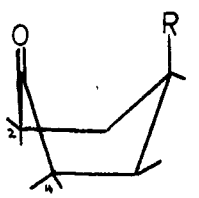
Our understanding of ring A conformation in saturated 3-keto steroids has been greatly facilitated by the dipole moment studies [71,72] and energy calculations [73,74] of Allinger. In these studies the possible conformations of ring A considered were the chair (57a), the flat chair (57b) and the flexible boat forms of which the two extremes are (57c) and (57d). The results suggest that conformation (57b) is favored when a



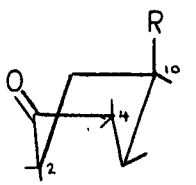
(57a)



(57b)



(57c)



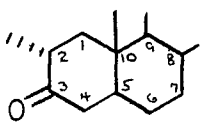
(57d)

1,3-dimethyl diaxial methyl interaction is present in ring A, eg. 2β -methyl, 4β -methyl, 2,2-dimethyl and 4,4-dimethylcholestan-3-one ($R=CH_3$). In the corresponding 19-nor compounds (R=H) this interaction is lacking and the chair conformation (57a) is favored. Similarly conformation (57a) is favored when the substituents are equatorial, eg. 2α -and 4α -cholestan-3-one.

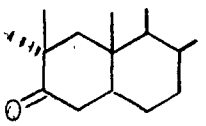
Infrared and N.M.R. spectral data also support these assignments. In going from conformation (57a) to (57b) the C_2-C_3-C_4 angle is expanded and hence a decrease in the carbonyl stretch frequency is expected.[75] This decrease (ca.6cm^{-1}) is observed[72] for cholestanone derivatives with an axial methyl group at C_2 or C_4, but is not observed for the corresponding 19-nor compounds. The proton resonance of the 2β -methyl group in several 19-nor-3-keto steroids[76] suffers an upfield shift of about 0.2 ppm. on going from deuteriochloroform to benzene solvent. This is in agreement with the reported[77] shift for an axial methyl group adjacent to a carbonyl group.

The most convincing spectral evidence for these assignments is provided by O.R.D.[72] and C.D. data. The results given in Table 2 overlap considerably with the O.R.D. results previously reported[72], and in this case a rationale based on either set of measurements is equally valid. The presence of a 2α -methyl group (41, partial structure 41a) produces a C.D. maximum of slightly diminished amplitude (cf. 27,28) due to the weak negative contribution of the equatorial methyl group. The 2,2-dimethyl compounds (42,43, partial structure 42a) show

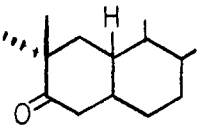
enhanced C.D. curves but not nearly as enhanced as that shown by the corresponding 19-nor compound (44, partial structure 44a).



(41a)

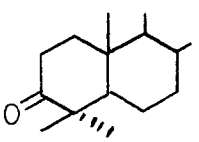


(42a)

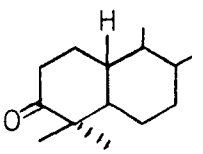


(44a)

This is compatible with ring flattening for the former compounds since this would diminish the C.D. amplitude in three ways: the positive contribution of the 2β -methyl group decreases as it becomes less axial; the negative contribution of the 2∝-methyl group increases as it becomes more axial; the positive contributions of the C_6 and C_7 methylene groups become less as the ring flattens. For the 4,4-dimethyl compounds (16a, 17a, 46, partial structure 46a) the situation is complicated by the fact that ring flattening decreases the negative contribution of the



(46a)



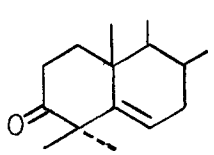
(52a)

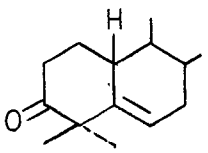
gem-dimethyl groups which is opposite to the effect upon the contributions of the ring B methylene groups, which become less positive. Nevertheless the weak Cotton effects of these compounds are compatible with a flat ring A conformation; and the more negative curve of the 19-nor compound (52, partial structure 52a) is probably indicative of a more axial 4β -methyl

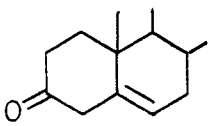
group as in the chair conformation (57a).

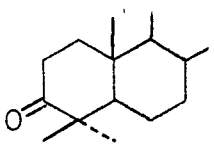
The C.D. results provide strong support for the assignment of a flat chair conformation to the 2,2-dimethyl compounds (42,43) since, from the octant rule, it is highly unlikely that any of the boat conformations would exhibit an enhanced positive Cotton effect. Conversely, one or several of the flexible forms of the 4,4-dimethyl compounds (16a, 17a, 46) would be expected to give weak Cotton effects; thus in the latter case a choice between the flat chair and the boat conformations can not be made on the basis of the C.D. results alone.

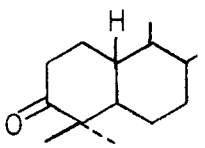
The evidence for the conformation of ring A in Δ^5-4,4-dimethyl-3-keto steroids is less conclusive. A comparison of the U.V. spectra[78,79,80] of 17β -acetoxy-4,4-dimethylandrost-5-en-3-one (49, partial structure 47a) and the corresponding 19-nor compound (51, partial structure 50a) shows the former compound has an unenhanced carbonyl band (ϵ = 30) whereas the latter (51) exhibits not only an enhanced n $\rightarrow \pi^*$ transition (ϵ = 75) but also strong end absorption indicative of a new π cc $\rightarrow \pi^*$ co band. Our results for the corresponding 17β -hydroxy compounds (48,50) are similar except that for the 19-nor sample (50) a trace amount of impurity is probably dominating the spectrum. Since cholest-5-en-3-one (8, partial structure 8a) also shows enhanced U.V. absorption,[36] the results suggest that ring A is in a chair conformation for the 19-nor compounds (50,51, partial structure 50a), but in a nonchair conformation for the compounds (47,48,49, partial structure 47a).

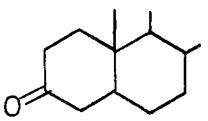

(47a)


(50a)


(8a)


(46a)


(52a)


(28a)

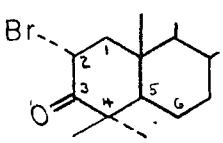
The decreased infrared carbonyl stretch frequency of the latter compounds[81] is also compatible with a nonchair conformation. The proton resonance of the C_{19} methyl group in compounds (47, 48,49) is at relatively high field[78-81] again suggesting a nonchair conformation for ring A. Finally a slight temperature dependence of this resonance may or may not be indicative[81] of a conformational equilibrium. None of these spectral data can adequately distinguish between a flat chair or a boat conformation.

The C.D. results (Table 2) are equally inconclusive. A comparison of the C.D. spectra of the 19-nor saturated ketone (52, partial structure 52a) and the 19-nor Δ^5 ketones (50,51, partial structure 50a) shows that the introduction of the β,γ-double bond results in a large positive contribution to the Cotton effect and also produces a broadened maxima. This same effect has been observed[5] for the compounds cholestan-3-one (28, partial structure 28a), $\lambda(\Delta\epsilon)$: 295 (+1.13) and cholest-5-en-3-one (8, partial structure 8a), $\lambda(\Delta\epsilon)$: 304-295 (+2.52).

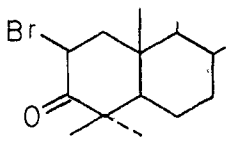
Hence the results are consistent with a chair conformation for the 19-nor compounds (50,51) with the double bond in a positive octant dominating the effect (cf. the saturated analogue (52) where the 4β -methyl group in a negative octant dominates the circular dichroism). The pronounced effect of the β,γ -double bond is not unexpected since the previously mentioned U.V. results suggested orbital overlap between the two unsaturated groups. A similar comparison of the C.D. spectra of the saturated 4, 4-dimethyl-3-keto steroid (46, partial structure 46a) and the corresponding Δ^5 compounds (47,48, partial structure 47a) shows that again the introduction of the β,γ -double bond results in a change in sign of the Cotton effect. However (47,48) exhibit much weaker Cotton effects than the 19-nor compounds (50,51) and do not show broadened maxima. These results support the U.V. evidence which suggested a nonchair conformation for (47,48). A distinction between the various nonchair forms can not be made however, since the flat chair conformation or any one of several boat forms would be expected to give a positive Cotton effect.

The conformation of ring A in 2-bromo-4, 4 dimethyl-3-keto steroids has been shown[82-85] to be dependent not only on the α-or β-orientation of the bromine atom, but also on the presence or absence of a Δ^5 double bond. For the saturated series, I.R. and U.V. spectral data[82] show the bromine atom is equatorial for both the 2α - and 2β -isomers. This indicates a chair (or flattened chair) conformation for the α-isomer (partial structure 58), and a boat conformation for the β -isomer (partial

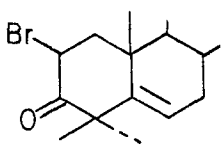
structure 59). These conclusions have been substantiated by energy calculations,[74] and a consideration of the coupling constants[83,84] between the protons on C_1 and C_2. For the Δ^5-series, I.R. and U.V. spectral data[82,85] show the bromine atom is equatorial for the 2β-isomer (partial structure 60), but axial for the 2α-isomer (partial structure 53a). This suggests



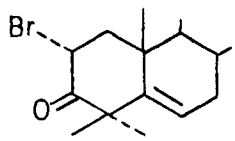
(58)



(59)



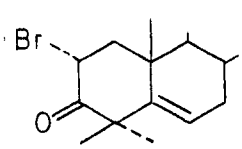
(60)



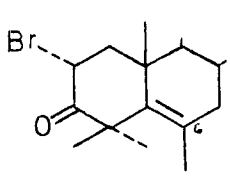
(53a)

both isomers have ring A in a boat conformation.

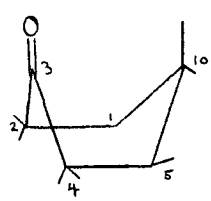
The chemical shifts and coupling constants of the C_1 and C_2 protons for the 2α-bromo compounds (53,54, partial structures 53a, 54a)[83] are almost identical, suggesting the same ring A conformation. Furthermore, from the coupling constants it was concluded that the preferred conformation was quite close to a classical boat with C_3 and C_{10} at the stem-stern positions (57c).



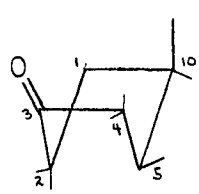
(53a)



(54a)



(57c)



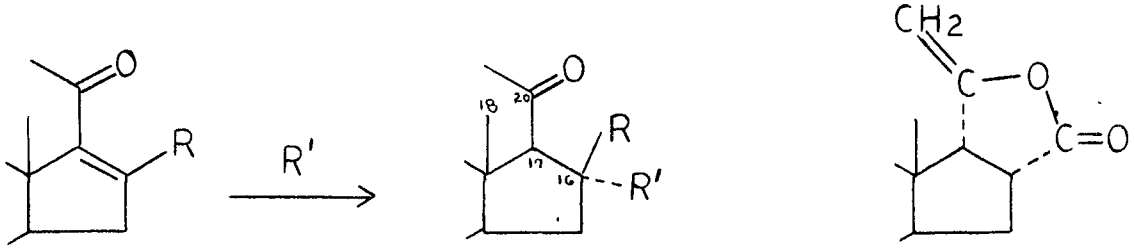
(57d)

From an examination of the C.D. spectra (see Table 2) it can be seen that the 4,4,6-trimethyl compound (54) exhibits an enhanced positive Cotton effect relative to the dimethyl compound (53).

If it is assumed that (53) and (54) have the same ring A conformation, then the C.D. results are incompatible with the classical boat conformation (57c). However a relatively minor distortion from this conformation would place the C_6 methyl group of (54) in a positive octant to account for the enhancement. That either compound exhibits a positive Cotton effect is surprising because the axial bromine atom is in a negative octant for all boat conformations. In addition, for the conformation (57c) or slight deviations from it, the Δ^5 double bond and C_7 also lie in a negative octant. Conformation (57d) with C_2 and C_5 at the stem-stern positions might be expected to give a positive Cotton effect; but the N.M.R. evidence and also the strong steric interaction between the 4α and 6-methyl groups (54) makes this conformation highly unlikely. It can only be concluded that either the double bond has an unusual effect upon the dichroism or else a conformational equilibrium exists. In support of the latter explanation, unpublished dipole moment studies[86] suggest that ring A of (53) exists as a mixture of chair and boat forms. In this regard it is worth noting that both the N.M.R. and C.D. data are consistent with a chair conformation.

The stereochemical problems associated with 16-substituted, 17-acetyl steroids are three-fold: (i) the assignment of the configurations at C_{16} and C_{17}; (ii) the determination of the conformation of ring D; (iii) the determination of the preferred conformation of the 17-acetyl sidechain.

Assignments of the configurations at C_{16} and C_{17} have largely been made on the basis of chemical arguments [87,88] involving the preparation and subsequent transformations of these compounds. These arguments include: preferential attack[89] of the Δ^{16} precursor (partial structure 61a) from the least



(61a) (61b) (61c)

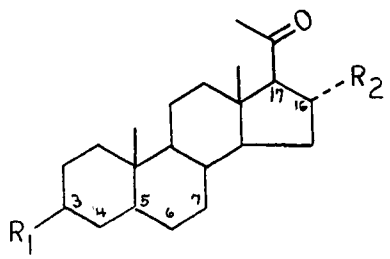
hindered, α-face of the molecule to give the 16-substituted compound (partial structure 61b, R=H, R^1= functional group or vice-versa), acid or base-catalyzed epimerization at C_{17} and at C_{16} when the substituent contains a carbonyl group adjacent to this position, greater thermodynamic stability of the C_{16},17 trans isomers, attainment of the cis-configuration by ring formation, eg. γ-lactone[87] formation (61c).

Spectroscopic methods have also been useful in making configurational assignments at C_{16} and C_{17}. Correlations between configuration and substituent effects upon the proton resonance of the C_{18} methyl group have been made;[88,90,91] the additivity principle[92] has been shown to hold except for the 16β, 17β - isomers, where steric crowding probably results in conformational changes in ring D and 17β-acetyl sidechain. O.R.D. and C.D. measurements have also proved useful, a positive Cotton effect[51]

being associated with a 17β-acetyl group and a negative effect with a 17α-acetyl group. In general, substituents at C_{16} do not alter the sign of the Cotton effect of the C_{20} carbonyl group, but the amplitude does vary with both the nature and configuration of the C_{16} substituent. Several O.R.D.[93,94] and C.D.[95,96] studies of 16-substituted 20-keto steroids have appeared recently, the most comprehensive[96] forming part of this thesis.

The circular dichroism results are collected in Tables 3 to 7. A general feature is that substituents in rings A and B have little effect upon the dichroism of the C-20 carbonyl group. Table 3 lists the results for the 16α-substituted, 17β-acetyl steroids. It can be seen that certain substituents, eg. hydroxyl, methoxyl and methyl result in a slightly diminished amplitude, whereas others, eg. isopropyl, dicarboxymethyl, dicarbethoxymethyl, cyano, carboxamido, carboxy, carbomethoxy and acetyl result in an enhanced Cotton effect. Table 3 includes a number of compounds containing two optically active carbonyl chromophores. For these compounds the observed C.D. curve is the sum of the two Cotton effects, eg. the large amplitude of 16α-cyanopregn-5-en-3, 20-dione (62q, +6.82) includes the contribution from the Δ^5-3-keto chromophore (+2.52).[5] For those compounds containing the Δ^4-3-keto chromophore, the two Cotton effects are well resolved (see figure 4, 62o, 62p), and the effects upon the C_{20} carbonyl C.D. maxima are slight as the C.D. curve of the Δ^4-3-keto chromophore is only weakly

TABLE 3



Compound	Position of substituents			C.D. max. of 20-keto-group	
	$3R_1$	4,5,6,7	$16R_2$	λmax (mμ)	$\Delta\epsilon$
62a	β-OH	5αH	H	293	+3.50*
b	ketone	$\Delta^{1,4}$	H	292	+3.74*
c	βOAc	5αH	OH	293	+3.48
d	βOAc	Δ^5	OCH_3	293	+3.50
e	βOAc	5αH, Δ^7	OCH_3	292	+3.00
f	βOH	5αH	CH_3	292	+3.05
g	βOAc	5αH	CH_3	292	+3.10
h	βOH	Δ^5	$CH(CH_3)_2$	293	+4.44
i	βOH	Δ^5, $6CH_3$	$CH(CH_3)_2$	294	+5.00
j	ketone	Δ^4, 6$\alpha$$CH_3$	$CH(CH_3)_2$	293	+4.23*
k	βOH	Δ^5	$CH(CO_2H)_2$	290	+4.90
l	βOAc	Δ^5	$CH(CO_2Et)_2$	292	+4.47*
m	βOAc	5αH	C$\equiv$N	289	+4.40*
n	βOAc	Δ^5	C$\equiv$N	286	+4.57*
o	βOH	Δ^5	C$\equiv$N	287	+4.52
p	ketone	Δ^4	C$\equiv$N	287	+3.80
q	ketone	Δ^5	C$\equiv$N	290	(+6.82)
r	βOH	Δ^5	$CONH_2$	288	+5.00
s	ketone	Δ^4	CO_2H	288	+3.70
t	ketone	Δ^4	CO_2CH_3	288	+4.12
u	ketone	Δ^4	$COCH_3$	288	(+4.35)

*These compounds were provided by Dr. P. Crabbe, but the C.D. spectra were run in the laboratories of G. Ourisson, University of Strasburg.

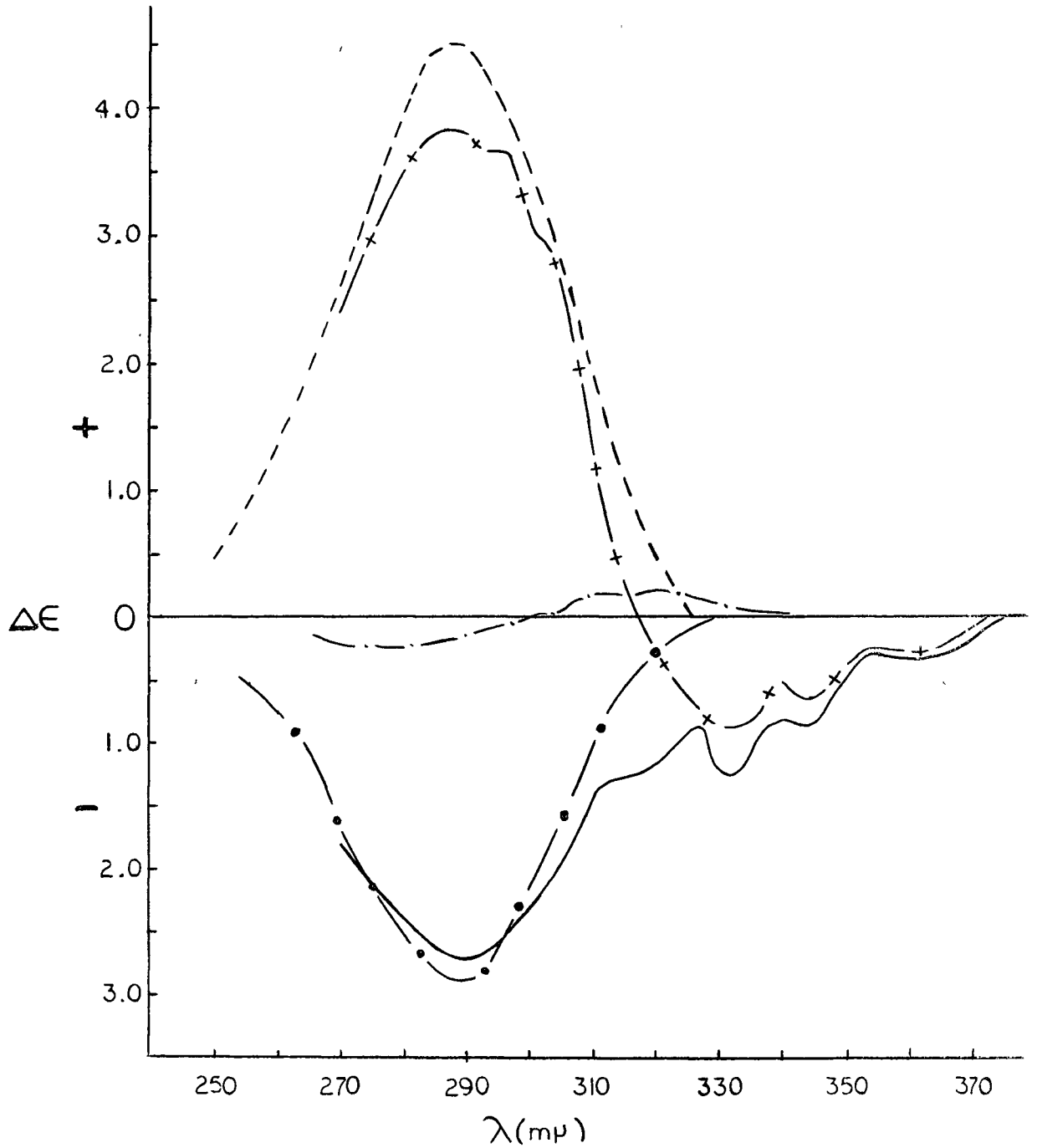


Figure 4

Circular Dichroism curves of:

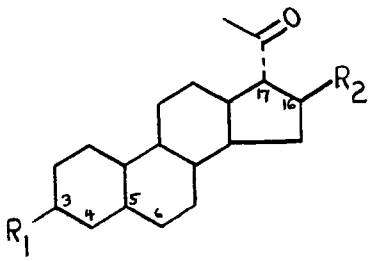
3β-hydroxy-16∝-cyanopregn-5-en-20-one (62o)
16∝-cyanoprogesterone (62p)
3β-acetoxy-16β-carbomethoxy-17∝-pregn-5-en-20-one (63q)
16β-carbomethoxy-17∝-progesterone (63t)
3β-acetoxy-16β-methyl-pregn-5-en-20-one (65i)

negative[97] in this region. The excellent resolution of these two chromophores by circular dichroism can be contrasted with the O.R.D. curves[93] which are dominated by the Cotton effect of the C_{20} carbonyl group. For the 16α, 17β-diacetyl compound (62u) the observed C.D. curve again appears to be a sum of the two individual Cotton effects, as the 16α-acetyl group is known to exhibit a weak positive effect.[93] However this explanation is oversimplified as it ignores the possible steric and electronic interactions between the two chromophores.

Table 4 lists the results for the 16β-substituted, 17α-acetyl steroids. It can be seen that most substituents, eg. methyl, hydroxymethyl, acetoxymethyl, cyano, carboxy and carbomethoxy have little effect upon the C.D. amplitude. However the carboxamido group produces a definite enhancement of the negative dichroism. The Cotton effects of the Δ^4-3-keto and C_{20} carbonyl chromophores are well-resolved (figure 4, 63q, 63t) and again this may be contrasted with the O.R.D. curves[93] which are dominated by the Cotton effect of the unsaturated chromophore. The Δ^5-3-keto (63d) and the 3,5-cyclo-6-keto (63s)[27] compounds exhibit C.D. curves which are the sums of the Cotton effects due to the individual chromophores; the latter is an example of an α-cyclopropylketone which obeys a "reversed" octant rule.[27] This simple summation also applies to the 16β, 17α-diacetyl compound (63u),[93] although as mentioned previously this may be an oversimplification in this case.

Table 5 lists the results for the 16α-substituted, 17α-acetyl

TABLE 4

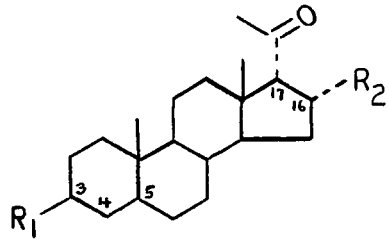


Compound	Position of substituents			C.D. max. of 20-keto-group	
	$3R_1$	4,5,6	$16R_2$	λmax (mμ)	$\Delta\epsilon$
63a	βOAc	Δ^5	H	289	-2.70
b	ketone	Δ^4	H	289	-2.30
c	ketone	$\Delta^{1,4}$	H	292	-2.36*
d	ketone	Δ^5	H	294	(+0.30)
e	ketone	Δ^4	CH_3	294	-2. 58*
f	βOH	Δ^5	CH_2OH	285	-2.66
g	ketone	Δ^4	CH_2OH	292	-2.31*
h	ketone	Δ^4	CH_2OAc	292	-2.52*
i	βOAc	Δ^5	$C\equiv N$	286	-2.86
j	βOAc	5αH	$CONH_2$	290	-3.15
k	βOAc	Δ^5	$CONH_2$	290	-3.18*
l	ketone	Δ^4	$CONH_2$	290	-3.13
m	βOAc	Δ^5	$CONEt_2$	287	-3.33*
n	βOAc	5αH	CO_2H	289	-2.80
o	βOH	Δ^5	CO_2H	289	-2.64
p	βOAc	Δ^5	CO_2H	290	-2.74
q	βOAc	Δ^5	CO_2CH_3	289	-2.89
r	3,5 α-cyclo	6β OH	CO_2CH_3	290	-2.94
s	3,5 α-cyclo	6-ketone	CO_2CH_3	291	(-4.40)
t	ketone	Δ^4	CO_2CH_3	290	-2.75
u	ketone	Δ^4	$COCH_3$	288	(-4.30)

*These compounds were provided by Dr. P. Crabbe but the C.D. spectra were run in the laboratories of G. Ourisson, University of Strasburg.

steroids. Several of the substituents, eg. methyl, dicarboxymethyl and dicarbethoxymethyl have little effect upon the Cotton effect of the 17∝-acetyl group. Hence circular dichroism can not distinguish between the 16∝(64c) and 16β-methyl-17∝-progesterones (63e). The 16∝-carbomethoxy group, however, definitely enhances the negative dichroism; and thus C.D. can distinguish between the 16∝ (64g, 64h, 64i) and 16β-carbomethoxy-17∝-acetyl steroids (63q, 63r, 63s, 63t).

TABLE 5

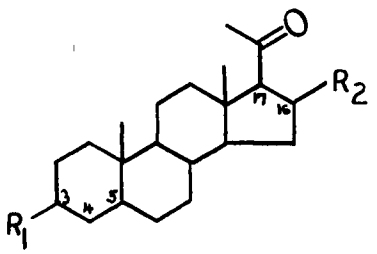


Compound	Position of substituents			C.D. max. of 20-keto group	
	$3R_1$	4,5	$16R_2$	λmax (mμ)	Δε
63a	βOAc	Δ^5	H	289	-2.70
b	ketone	Δ^4	H	289	-2.30
64c	ketone	Δ^4	CH_3	295	-2.70
d	βOH	Δ^5	$CH(CO_2H)_2$	285	-3.06
e	βOAc	5∝Cl	$CH(CO_2Et)_2$	286	-3.00
f	βOAc	Δ^5	$CH(CO_2Et)_2$	290	-2.30
g	βOAc	5∝H	CO_2CH_3	290	-3.85
h	βOAc	Δ^5	CO_2CH_3	292	-4.06
i	ketone	Δ^4	CO_2CH_3	290	-3.54

Table 6 lists the results for the 16β -substituted, 17β -acetyl steroids. In each case the 16β substituted compound has a C.D. curve different from the corresponding 16α compound (Table 3). This is not unexpected as steric and electronic interaction between the C_{18} methyl group and the 16β and 17β substituents can lead to conformational changes in either ring D or the 17β -acetyl group. Thus electrostatic interaction between the cyano and carbonyl dipoles in compound (65c) probably results in a reorientation of the C_{20} carbonyl axis to account for the diminished dichroism. For the 16β -carboxy compound (65d), hydrogen bonding between the carboxyl and carbonyl groups may account for the weak Cotton effect; the corresponding carbomethoxy compounds (65e, 65f) exhibit undiminished C.D. curves supporting this hypothesis. The steric interaction induced by the introduction of a 16β -methyl group results in a dramatic change in the Cotton effect of the C_{20} carbonyl group (65i, see figure 4).

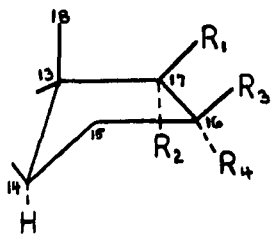
To interpret the results in Table 6, both the conformation of ring D and the preferred orientation of the 17β -acetyl group must be considered. Three favorable ring D conformations have been considered: the envelope form (67a) with C_{14} below the plane of the other four carbon atoms, the half-chair form (67b) with C_{13} and C_{14} equidistant above and below the $C_{15}C_{16}C_{17}$ plane, and the envelope form (67c) with C_{13} above the plane of the other four carbon atoms. Energy calculations[98] suggest that for 17β -substituted steroids (R_1=CH_3 or OH, R_2=R_3=R_4 = H) conformations

TABLE 6

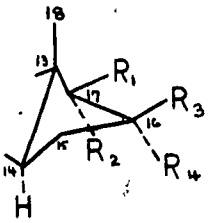


Compound	Position of substituents			C.D. max. of 20-keto group	
	$3R_1$	4,5	$16R_2$	λmax (mμ)	Δε
62a	βOH	5α-H	H	293	+3.50*
b	ketone	Δ^{4}	H	292	+3.74*
65c	βOAc	Δ^{5}	C≡N	290	+2.23*
d	βOH	Δ^{5}	CO_2H	290	+1.40
e	βOAc	Δ^{5}	CO_2CH_3	290	+3.68
f	ketone	Δ^{4}	CO_2CH_3	290	+3.40
g	βOAc	5α-H	CH_3	320-307	+0.21
				280-271	-0.26
h	βOH	Δ^{5}	CH_3	344	-0.034*
				326	+0.103
				314	+0.062
				285	-0.207
i	βOAc	Δ^{5}	CH_3	321	+0.22
				280-271	-0.25
j	ketone	Δ^{4}	CH_3	307	-0.72*
				296	-0.49

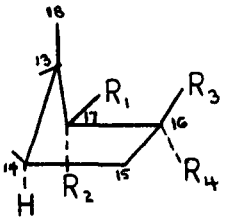
* These compounds were provided by Dr. P. Crabbe but the C.D. spectra were run in the laboratories of G. Ourisson, University of Strasburg.



(67a)



(67b)

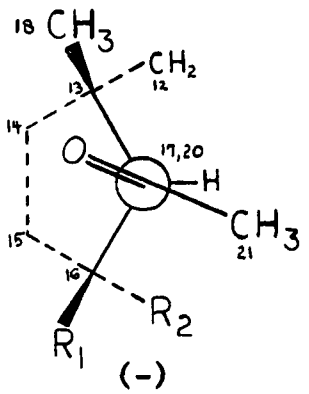


(67c)

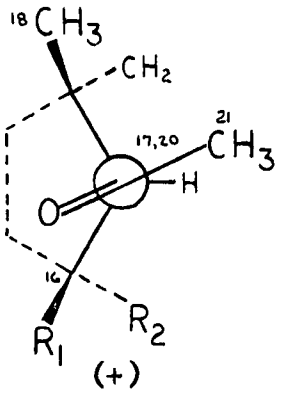
(67b) and (67c) are favored over (67a) by about 2 k.cal./mole, whereas for 17α-substituted steroids (R_2 = CH_3 or OH, R_1=R_3=R_4=H) conformations (67a) and (67b) are favored over (67c) by a similar amount. An N.M.R. study[90] of the four possible isomers of 16-carbomethoxy-17-acetyl androst-4-en-3-one (62t, 63t, 64i, 65f) has been carried out in which the coupling constants between the protons at C_{16} and C_{17} have been compared with those calculated[99] for the three probable ring D conformations (67a, 67b, 67c). For the 16,17-<u>trans</u> isomers, the results support the energy calculations mentioned above with conformations (67b) and (67c) being favored for the 16α-carbomethoxy-17β- acetyl steroid (62t, partial structure 67, R_1 = CH_3CO, R_4 = CO_2CH_3, R_2 = R_3 = H), and conformations (67a) and (67b) being favored for the corresponding 17α-acetyl <u>trans</u> isomer (63t). Unfortunately, for the <u>cis</u> isomers (64i, 65f) no distinction could be made. Nevertheless, from a consideration of the octant rule, it is highly unlikely that the dramatic effect of a 16β-methyl group on the dichroism of 17β-acetyl steroids (Table 6) is caused simply by variations in ring D conformation.

In considering the orientation of the 17-acetyl group,

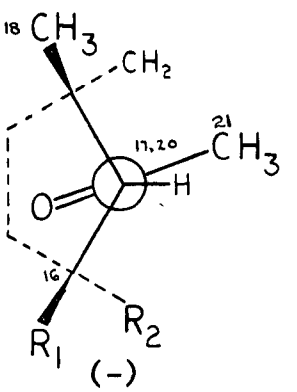
Dreiding models are useful since they provide a half-chair (67b) conformation for ring D, which is probably quite close to the preferred conformation (see above). A consideration of the steric interactions suggested by Dreiding models[100] has indicated



(68a)

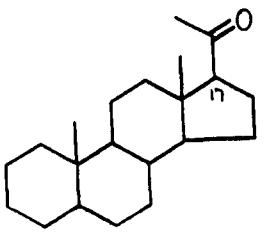


(68b)

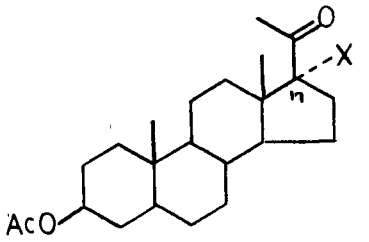


(68c)

that the preferred conformation for the 17α-acetyl group is (68c), whereas for the 17β-acetyl group two low energy conformations (68a) and (68b) are probable. The C.D. results in Tables 3,4,5 are consistent with the preferred conformations (68b) and (68c) for the 17β and 17α-acetyl groups respectively. In addition, dipole moment studies[101] and hydride reduction experiments[102] on the unsubstituted 17β-acetyl compound, pregnan-20-one (69), and the O.R.D. curves of 17α-halopregnan-20-ones (70)[103] support conformation (68b) for the 17β-acetyl group. With the 16β-substituted-17β-acetyl compounds however, steric (eg. R_1=CH_3) or electronic (eg. R_1=CN) interaction would destabilize conformation (68b) with respect to (68a). Since a negative Cotton effect is predicted for the latter a diminished dichroism would be expected as has been observed (Table 6). Low



(69)



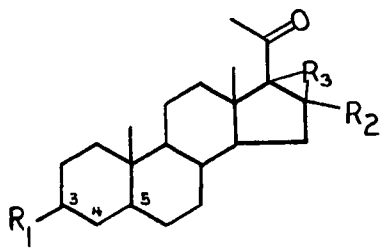
(70)

temperature C.D. studies[100] support these conclusions; thus while the C.D. curves of the unsubstituted 17β (62a) and 17α-acetyl (63a) steroids[104] remain essentially unchanged on cooling to -192°C, the curves[100] of both the 16β-cyano (65c) and 16β-methyl (65i) 17β-acetyl compounds change dramatically on cooling ($\Delta\epsilon$ -192° = 0.8 and -1.6 respectively). Consequently the weak, alternating sign C.D. curve of (65i, figure 4) is conveniently explained on the conformational equilibrium hypothesis.

Table 7 lists the C.D. results for various 16, 17-epoxy and cyclopropyl 20-keto steroids. These compounds are characterized by weak Cotton effects. If it is assumed that the 17β-acetyl group has the preferred conformation (68b) then the epoxy (or cyclopropyl) group lies in a negative octant (cf. 17α-halopregnan-20-one[103]); thus the observed Cotton effects would appear to be in contradiction to the "reversed" octant rule[27] postulated for α-epoxy and α-cyclopropyl ketones. However it is doubtful that the results in Table 7 can be used either to establish a preferred conformation for the 17-acetyl group, or to verify the "reversed" octant rule because the electronic interaction between the carbonyl and epoxy (or cyclopropyl) groups would be expected to be unique for each possible orientat-

ion of the sidechain, i.e. a conformation of low population might have a profound influence upon the resultant Cotton effect through strong electronic interaction of these two groups.

TABLE 7



Compound	Position of substituents				C.D. max. of 20-keto group	
	$3R_1$	4,5	R_2	R_3	λmax (mμ)	$\Delta\epsilon$
66a	βOH	Δ^5	16βH	α-cyclopropyl	278	+0.92
b	βOH	5αH	16βH	α-epoxy	300	-0.38
c	βOAc	Δ^5	16βH	α-epoxy	301	-0.33
d	βTHP	Δ^5	16βH	α-epoxy	302	-0.38
e	βOH	Δ^5	16βCH	α-epoxy	304	+2.13*
f	βOH	Δ^5	16αH	β-epoxy	310	-0.16

* This compound was provided by Dr. P. Crabbe but the C.D. spectrum was run in the laboratories of G. Ourisson, University of Strasburg.

EXPERIMENTAL

Melting points were determined on a Kofler hot stage microscope. Ultraviolet spectra (U.V.) were determined in ethanolic solutions using a Cary 14 spectrophotometer, and maxima are reported in millimicrons (mμ). The maxima in the region 260 to 230 mμ are believed indicative of trace amounts of unsaturated impurities. Circular dichroism measurements were made in dioxan solutions at room temperature with apparatus[105] assembled according to Mitchell.[106,107] According to convention,[4] C.D. values are reported for the following wavelengths: starting point of the curve, positions of maxima and inflections (inflections are denoted by "s"), final point of the curve. C.D. values are reported as $\lambda(\Delta\epsilon)$ with the wavelengths in millimicrons; Γ is the bandwidth (in millimicrons) at half-maximum and $\Gamma/2$ is reported when the band is not well resolved.

I would like to thank Dr. F. McCapra of this department for supplying samples of most of the 3-keto steroids, and Dr. P. Crabbe of Syntex S.A. Mexico for supplying the 20-keto steroids used in the circular dichroism study.

Androstenolone, (3β-hydroxyandrost-5-en-17-one), (24a):

Commercial (Upjohn) androstenolone, m.p. 145-150°, λ max: 293 (ϵ 45), 236 (ϵ 143) was recrystallized once from ethanol: water and then three times from benzene: n-hexane, m.p. 151-153°, reported m.p. 153°.[108,109] The intensity of the peak at 236 mμ

decreased in a stepwise manner from ϵ=115 to ϵ =76, whereas the peak at 293 mμ remained constant at ϵ=33.

Androstenolone acetate, (3β -acetoxyandrost-5-en-3-one), (24b):

1.5 g. recrystallized androstenolone was acetylated (16 hrs. 25°) with a solution of dry pyridine (10 ml.) and acetic anhydride (10 ml.). The reaction mixture was acidified with dilute sulphuric, extracted with ether and the organic layer subsequently extracted with sodium bicarbonate solution, washed, dried and concentrated in vacuo. Crystallization from ether followed by recrystallization from ethanol: water gave 1.2 g. of acetate, m.p. 166-168°, λmax: 293 (ϵ 36), 236sh (ϵ 54). (When the reaction was repeated using commercial androstenolone the crystallized acetate had λmax: 293 (ϵ 43), 236(ϵ 150).)

Further purification was effected by chromatography on a grade III alumina column (120g, 38 x 2 cm.dia.) Petroleum ether (b.p. 60-80°) was used for elution with 50 ml fractions being collected. Fraction 5 proved to be the largest (583 mg.) and also the most pure. Recrystallization from ethanol:water gave the final product, m.p. 168-169°, reported 170-171°.[110] The U.V. spectrum showed a single peak at 293 mμ (ϵ 36); the previously observed peak at 236 mμ had completely disappeared (ϵ = 13 at this wavelength).

Lanost-8-en-3-one (17a).

5g. commercial (K & K) lanosterol, m.p. 135-138°, λmax:

251 (ε 1630), 243 (ε 2080), 235 (ε 1850), in 200 ml. ethyl acetate was hydrogenated for 4 hrs. over 0.5g. Adams catalyst. Recrystallization from ethyl acetate gave 4.5g. lanost-8-enol, m.p. 142-145°, λmax: 252 (ε 854), 243 (ε 1070), 235 (ε 925); reported m.p. 145-146°.[111]

Chromic acid oxidation (Jones' reagent[112,113]) of lanost-8-enol (3g.) in acetone (150 ml.) gave, in the usual way,[112] lanost-8-en-3-one (2.1g) as plates from acetone:methanol, m.p. 118-120°, $[\alpha]_D$ + 81° (chloroform, C=2.6), λmax: 286 (ε 35), 252 (ε 693), 243 (ε 1010), 236 (ε 926); reported m.p. 119-120°, $[\alpha]_D$ + 78°.[111]

Further purification was attempted by chromatography on silica gel. However elution with ether:petroleum ether 1:19 gave lanost-8-en-3-one which still showed the U.V. maxima believed due to the diene impurity lanost-7,9(11)-dien-3-one.

17β-hydroxyandrostan-3-one (27): U.V.: 367sh (9), 330sh (20), 283sh (52), 252sh (68), 237sh (74); C.D.: 330 (0), 294(+0.96), 270(+0.32), Γ=37 (c=1.24 g./100 ml.).

7-methylenecholestan-3-one (31): U.V.: 285(26); C.D.: 330(0), 311s(+0.82), 308s(+0.99), 301s(+1.40), 295(+1.54), 256(+0.14), Γ= 38 (c=1.74).

ergost-7-en-3-one (32): U.V.: 360sh (24), 320sh (49), 287 (65), 252 (109), 241sh (117); C.D.: 324(0), 297-293(+0.67), 280s(0.48), 262(+0.13), Γ=37 (c=1.25).

ergost-8(14)-en-3-one (33): U.V.: 288sh (36), 251(172); C.D.: 330(0), 298-292(+1.15), 268(+0.40), Γ=37 (c=2.21).

$\Delta^{9(11)}$dehydrotigogenone (34): U.V.: 365sh (6), 325sh (15), 282(37), 252(41), 242(42); C.D.: 330(0), 311s(+0.48), 304s(+0.75), 295(+0.88), 256(+0.09), Γ=39 (c=2.24).

Δ^{11} dehydrotigogenone (35): U.V.: 365sh(4), 325sh(13),285(31), 256(27), 246(27), 239(26); C.D.: 328(0), 310s(+0.51), 303s(+0.73), 294(+0.93), 258(+0.11), Γ=37 (c=1.68).

12-methylenetigogenone (36): U.V.: 365sh(40), 345sh(57), 330sh(68), 285sh(114), 254(216), 240sh(205); C.D.: 328(0), 303s(+0.80), 297-294(+0.90), 270(+0.31), Γ=38 (c=0.78).

lanost-8-en-3-one (17a): U.V.: 286(35), 252(693), 243(1010), 236(926); C.D.: 336(0), 318(-0.11), 308(0), 296-280(+0.13), 268(+0.04), (c=2.77).

2α-methylcholestan-3-one (41): U.V.: 365sh(12), 340sh (15), 330sh(16), 282sh(43), 265sh(54), 255(65); C.D.:328(0), 310sh(+0.49), 298-292(+0.75), 256(+0.05), Γ=36 (c=1.57).

2,2-dimethyl-17β-hydroxyandrostan-3-one (42): U.V.:288(31); C.D.: 336(0), 312s(+1.02), 309s(+1.18), 306s(+1.31), 303-290(+1.52), 260(+0.18), Γ=40 (c=2.64).

2,2,17α-trimethyl-19 nor-androstan-3-one (44): U.V.: 286(35); C.D.: 334(0), 315s(+1.28), 312s(+1.65), 309s(+1.84), 306s(+2.24), 303-290(+2.78), 287s(2.40), 285s(+2.30), 266(+0.22), Γ=37(c=0.83).

4,4-dimethylcholest-5-en-3-one (47): U.V.: 325sh(46), 283sh(98), 242sh(242), reported: 293(38)[35]; C.D.: 324(0), 310s(+0.52), 297-292(+0.96), 262(+0.05), Γ =39 (c=0.61).

4,4-dimethyl-17β-hydroxyandrost-5-en-3-one (48): U.V.:287(44); C.D.: 328(0), 309s(+0.77), 303s(+1.14), 294(+1.28), 260(+0.16), Γ=37(c=1.46).

4,4-dimethyl-17β-hydroxy-19-nor-androst-5-en-3-one (50): U.V.: 285sh(443), 230(2160); C.D.:330(0), 315s(+1.18), 303-295(+2.11), 264(+0.08), Γ=39 (c=0.25).

2α-bromo-4,4-dimethylcholest-5-en-3-one (53): U.V.: 365sh(26), 314(167); C.D.: 344(0), 335s(+0.14), 324s(+0.47), 309(+0.70), 300s(+0.60), 284s(+0.37), 272(+0.05), Γ =44 (c=0.76).

2α-bromo-4,4,6-trimethylcholest-5-en-3-one (54): U.V.: 321(169); C.D.: 352(0), 337s(+0.38), 325s(+0.69), 312(+1.07), 299s(+0.77), 286s(+0.37), 278(+0.05), Γ=40 (c=0.93).

3β-acetoxy-16α-hydroxy-5α-pregnan-20-one (62c):
C.D.: 328(0), 293(+3.48), 255(+0.36), Γ =35 (c=0.34).

3β-acetoxy-16α-methoxypregn-5-en-20-one (62d):
C.D.: 330(0), 292(+3.50), 269(+0.30), Γ =39 (c=0.29).

3β-acetoxy-16α-methoxy-5α-pregn-7-en-20-one (62e):
C.D.: 328(0), 292(+3.00), 287s(+2.90), 267(+0.20), Γ =34 (c=0.20).

3β-hydroxy-16α-methyl-5α-pregnan-20-one (62f):
C.D.: 330(0), 292(+3.05), 252(+0.20), Γ=40 (c=1.40).

3β-acetoxy-16α-methyl-5α-pregnan-20-one (62g):

C.D.: 326(0), 292(+3.10), 269(+2.10), Γ=39 (c=0.71).

3β-hydroxy-16α-isopropylpregn-5-en-20-one (62h):

C.D.: 328(0), 293(+4.44), 266(+1.34), Γ=37 (c=0.78).

3β-hydroxy-6-methyl-16α-isopropylpregn-5-en-20-one (62i):

C.D.: 330(0), 294(+5.00), 291s(+4.93), 267(+1.03), Γ=37(c=0.56).

3β-hydroxy-16α-dicarboxymethylpregn-5-en-3-one (62k):

C.D.: 326(0), 305s(+2.45), 290(+4.90), 264(+1.05), Γ=34 (c=0.49).

3β-hydroxy-16α-cyanopregn-5-en-20-one (62o):

C.D.: 326(0), 287(+4.52), 250(+0.40), Γ=39 (c=1.51).

16α-cyanoprogesterone (62p): C.D.: 372(0), 362(-0.28), 345(-0.66), 332(-0.88), 318(0), 303s(+2.90), 295s(+3.66), 287(+3.80), 270(+2.40), (c=1.47).

16α-cyanopregn-5-en-3,20-dione (62q):

C.D.: 326(0), 290(+6.82), 252(+0.60), Γ=40 (c=0.24).

3β-hydroxy-16α-carboxamidopregn-5-en-20-one (62r):

C.D.: 328(0), 288(+5.00), 253(+0.60), Γ=38 (c=0.31).

16α-carboxyprogesterone (62s): C.D.: 372(0), 362s(-0.30), 344s(-0.74), 330(-1.14), 316(0), 301s(+2.74), 288(+3.70), 274(+2.60), (c=1.50).

16α-carbomethoxyprogesterone (62t): C.D.: 376(0), 360s(-0.36), 343s(-1.10), 330(-1.50), 323s(-1.00), 316(0), 301s(+3.00), 288(+4.12), 270(+2.36), (c=1.50).

16∝-acetylprogesterone (62u): C.D.: 372(0), 361s(-0.30), 344(-1.10), 330(-1.31), 316(0), 303s(+2.80), 288(+4.35), 270(+2.50), (c=1.50).

3β-acetoxy-17∝-pregn-5-en-20-one (63a):
C.D.: 336(0), 303s(-2.10), 289(-2.70), 256(-0.40), Γ=40 (c=1.50).

17∝-progesterone (63b): C.D. 376(0), 360s(-0.30), 346(-0.90), 331(-1.16), 303s(-2.04), 295s(-2.26), 289(-2.30), 270(-1.05), (c=1.50).

17∝-pregn-5-en-3,20-dione (63d):
C.D.: 320(0), 294(+0.30), 266(+0.05), Γ=32 (c=0.30).

3β-hydroxy-16β-hydroxymethyl-17∝-pregn-5-en-20-one (63f):
C.D.: 326(0), 286(-2.66), 251(-0.49), Γ=36 (c=0.37).

3β-acetoxy-16β-cyano-17∝-pregn-5-en-20-one (63i):
C.D.: 326(0), 286(-2.86), 271(-2.00), $\Gamma/2$ = 17 (c=0.46).

3β-acetoxy-16β-carboxamido-5∝, 17∝-pregnan-20-one (63j):
C.D.: 330(0), 290(-3.15), 250(-0.26), Γ=37 (c=1.51).

16β-carboxamido-17∝-progesterone (63l): C.D.: 374(0), 360s(-0.26), 345s(-1.02), 331(-1.33), 314s(-1.80), 288(-3.13), 261(-1.23), (c = 0.18).

3β-acetoxy-16β-carboxy-5∝, 17∝-pregnan-20-one (63n):
C.D.: 330(0), 317s(-0.31), 289(-2.80), 250(-0.26), Γ=39 (c=1.52).

3β-hydroxy-16β-carboxy-17∝-pregn-5-en-20-one (63o):

C.D.: 330(0), 319s(-0.33), 309s(-1.80), 295s(-2.20), 289(-2.64), 280s(-2.15), 250(-0.33), Γ =37 (c=1.51).

3β-acetoxy-16β-carboxy-17∝-pregn-5-en-20-one (63p):
C.D.: 328(0), 307s(-1.25), 294s(-2.56), 290(-2.74), 285s(-2.59), 250(-0.24), Γ =38 (c=1.49).

3β-acetoxy-16β-carbomethoxy-17∝-pregn-5-en-20-one (63q):
C.D.: 328(0), 289(-2.89), 254(-0.50), Γ =39 (c=1.51).

3,5∝-cyclo-6β-hydroxy-16β-carbomethoxy-17∝-pregnan-20-one (63r):
C.D.: 330(0), 290(-2.94), 250(-0.33), Γ=39 (c=1.51).

3,5∝-cyclo-16β-carbomethoxy-17∝-pregnan-6,20-dione (63s):
C.D.: 330(0), 291(-4.40), 260(-0.90), Γ=37 (c=1.50).

16β-carbomethoxy-17∝-progesterone (63t): C.D.: 376(0), 361(-0.33), 344(-0.86), 332(-1.24), 317s(-1.26), 297s(-2.46), 290(-2.75), 270(-1.80), (c=1.50).

16β-acetyl-17∝-isoprogesterone (63u): C.D.: 374(0), 360(-0.40), 346(-1.10), 331(-1.40), 303s(-2.80), 288(-4.30), 279s(-3.90), 272(-2.80), (c=1.50).

16∝-methyl-17∝-progesterone (64c): C.D.: 372(0), 340s(-1.28), 325s(-1.78), 314s(-2.42), 295(-2.70), 269(-1.14), Γ=52 (c=0.11).

3β-hydroxy-16∝-dicarboxymethyl-17∝-pregn-5-en-20-one (64d):
C.D.: 330(0), 285(-3.06), 266(-1.79), $\Gamma/2$ =18 (c=0.88).

3β-acetoxy-5∝-chloro-16∝-dicarbethoxymethyl-17∝-pregnan-20-one (64e):
C.D.: 328(0), 286(-3.00), 267(-1.76), $\Gamma/2$ = 19 (c =0.88).

3β-acetoxy-16∝-dicarbethoxy-17∝-pregn-5-en-20-one (64f):

C.D.: 328(0), 290(-2.30), 254(-0.53), ⌐=39 (c=1.51).

3β-acetoxy-16∝-carbomethoxy-5∝,17∝-pregnan-20-one (64g):

C.D.: 332(0), 301s(-2.93), 290(-3.85), 250(-0.33), ⌐=37 (c=1.50).

3β-acetoxy-16∝-carbomethoxy-17∝-pregn-5-en-20-one (64h):

C.D.: 328(0), 292(-4.06), 251(-0.62), ⌐=39 (c=0.23).

16β-carbomethoxy-17∝-progesterone (64i): C.D.: 374(0), 362s(-0.40), 345(-1.00), 330(-1.40), 309s(-2.20), 303s(-2.83), 290(-3.54), 263(-1.00), (c=1.50).

3β-hydroxy-16β-carboxy-pregn-5-en-20-one (65d):

C.D.: 328(0), 290(+1.40), 260(+0.10), ⌐=39 (c=0.21).

3β-acetoxy-16β-carbomethoxy-pregn-5-en-20-one (65e):

C.D.: 328(0), 298s(+3.52), 290(+3.68), 251(+0.30), ⌐=43 (c=0.36).

16β-carbomethoxyprogesterone (65f): C.D.: 378(0), 360(-0.44), 346(-0.96), 332(-1.31), 323s(-0.73), 317(0), 297s(+3.13), 290(+3.40), 270(+1.80), (c=1.51).

3β-acetoxy-16β-methyl-5∝-pregnan-20-one (65g):

C.D.: 332(0), 320-307(+0.21), 297(0), 280-271(-0.26), (c=0.67).

3β-acetoxy-16β-methyl-pregn-5-en-20-one (65i):

C.D.: 344(0), 321(+0.22), 313s(+0.18), 301(0), 280-271(-0.25), (c=1.30).

3β-hydroxy-16, 17α-cyclopropylpregn-5-en-20-one (66a):

C.D.: 300(0), 278(+0.92), 265(+0.48), $\Gamma/2$ =13 (c=0.41).

3β-hydroxy-16,17α-epoxy-5α-pregnan-20-one (66b):

C.D.: 336(0), 300(-0.38), 276(-0.13), Γ =33 (c=1.50).

3β-acetoxy-16,17α-epoxypregn-5-en-20-one (66c):

C.D.: 338(0), 301(-0.33), 266(-0.14), Γ =55 (c=1.03).

3β-hydroxy-16,17α-epoxypregn-5-en-20-one 3-tetrahydropyranyl ether (66d):

C.D.: 338(0), 302(-0.38), 268(-0.14), Γ=47 (c=0.58).

3β-hydroxy-16,17β-epoxy-17α-pregn-5-en-20-one (66f):

C.D.: 332(0), 310(-0.16), 292(-0.01), Γ =22 (c=1.08).

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PART II

THE STRUCTURAL DETERMINATION OF HIRSUTIC ACID C

INTRODUCTION

In Part I some applications of circular dichroism to specific stereochemical problems were given. In the discussion many limitations of this method were revealed. In general, each of the physical methods has a limited or specific application to organic chemistry. Collectively, however, the physical tools provide a powerful means of solving structural and stereochemical problems. Although the application of physical methods has modernized structural organic chemistry, chemical methods still form an integral part of this modern approach. Many structures are proved by synthesis, or by degradation or transformation to compounds of known structure or stereochemistry, and the nature and relative orientations of the various functional groups of a molecule are generally established by a combination of both physical and chemical methods.

The integration of chemical and physical methods is one aspect of modern structural organic chemistry; the application of X-ray diffraction methods[1-4] is another. In contrast to other physical and chemical methods, which provide a collection of fragmentary evidence, X-ray diffraction presents an all-or-nothing approach to molecular structural determination. Another unique feature of this method is that it provides a three-dimensional picture of the molecule, including, in many cases, the correct absolute configuration. The only chemical information required to carry out an X-ray analysis is the empirical formula; a previous knowledge of structural data can often

greatly facilitate the X-ray analysis; however the incorporation of incorrect structural information into the analysis will lead to time-consuming delays. Thus it is usually best to proceed with the analysis, at least in the early stages, without the aid of any supporting evidence. It should be emphasized that it is virtually impossible to derive an incorrect molecular structure from a satisfactory X-ray analysis; at the same time, it is very difficult to derive any partial structural information from an unsatisfactory X-ray analysis.

As for the other physical methods, there are certain limitations to the use of X-ray diffraction in structural organic chemistry; the major restriction is that a satisfactory crystalline form must be available. Another restriction, which is generally met, but has particular relevance to the structural determination described in this thesis, is that the crystal structure must be stable to X-ray irradiation. With regards to natural products chemistry, the use of X-ray diffraction is largely limited to application of the "heavy-atom" method,[5] i.e. the X-ray analysis is carried out on a derivative containing a heavy atom, eg. I, Br, Cl, S, Fe, Zn. Before describing some of the salient features of this method it is important to note that the structural correlation between the heavy atom derivative and the parent compound must be made by other physical and chemical methods.

The Heavy-Atom Method of X-ray Analysis:

Experimentally the X-ray diffraction pattern, which consists of a series of diffracted beams of varying intensities, is recorded photographically or by counter techniques. Each beam is diffracted by a set of parallel planes in the crystal referred to by Miller indices hkl. The pattern defines the space group of the crystal structure; and more important, the intensities are related to the structure amplitudes $|F(hkl)|$. The structure amplitudes are related to the electron density distribution in the unit cell, $\rho(xyz)$, by the triple Fourier series:

$$\rho(xyz) = \frac{1}{V} \sum_h \sum_k \sum_l F(hkl) \exp[-2\pi i (hx + ky + lz)]$$

where V is the volume and x, y, z the fractional coordinates of the unit cell. The function F(hkl) is known as the structure factor and is the resultant of the waves scattered by each of the N atoms with fractional coordinates x_j, y_j, z_j. It is expressed by the summation:

$$F(hkl) = \sum_j f_j \exp[2\pi i (hx_j + ky_j + lz_j)]$$

where f_j is the scattering factor of atom j. The complex quantity F(hkl) is characterized by the structure amplitude $|F(hkl)|$ and a phase angle $\alpha(hkl)$ according to the two expressions:

$$|F(hkl)| = \sqrt{A^2 + B^2}$$

$$\alpha(hkl) = \arctan (B/A)$$

where $A = \sum_j f_j \cos 2\pi (hx_j + ky_j + lz_j)$

$B = \sum_j f_j \sin 2\pi (hx_j + ky_j + lz_j)$

The electron density can be put in a more convenient form by replacing F(hkl) by its amplitude and phase:

$$\rho(xyz) = \frac{1}{V}\sum_{h}\sum_{k}\sum_{l}|F(hkl)|\cos\left[2\pi(hx + ky + lz) - \alpha(hkl)\right]$$

This last equation illustrates the phase problem[6] in X-ray crystallography, i.e. in the recording of the X-ray data the structure amplitudes $|F(hkl)|$ are obtained, but unfortunately the phase angles $\alpha(hkl)$ are lost. Before the electron density distribution can be revealed the phase angles must be recovered by some indirect method. The heavy-atom method is the one most commonly used.

The first step of this method is the location of the heavy atom. Generally this is accomplished by using the structure amplitudes to evaluate the Patterson function:[7,8]

$$P(uvw) = \frac{1}{V^2}\sum_{h}\sum_{k}\sum_{l}|F(hkl)|^2\cos 2\pi(hu + kv + lw)$$

where u,v,w are fractional coordinates. The peaks of a Patterson map occur at the ends of vectors starting from the origin of the unit cell, which correspond to the vectors between every pair of atoms in the cell. If all of these vectors could be found it would be theoretically possible to derive the atomic positions from this vector distribution. However for n atoms in the unit cell there are $(n^2-n)/2$ independent peaks, and for most organic structures the interpretation of the Patterson map is extremely difficult due to overlap of the numerous peaks. The relative intensity of a Patterson peak is determined by the product of the atomic numbers of the two atoms involved. Thus it is usually possible to locate the heavy atom in a Patterson distribution.

It is possible to carry out the heavy-atom method with a derivative containing more than one heavy atom; but the presence of too many heavy atoms could result in a Patterson map which is complicated even for the location of the heavy atoms. This is particularly true when these atoms are of different atomic species, since in addition to the strong peaks characteristic of vectors between heavy atoms there are strong peaks formed from the overlap of many peaks characteristic of vectors between light atoms.

After the coordinates of the heavy atom are established, they are used to evaluate the phase angles $\alpha(hkl)$; then using the observed structure amplitudes $|F(hkl)|$ and the calculated phases based on the heavy atom a Fourier series is summed. The location of many of the light atoms, i.e. C,N,O is usually possible from the resulting electron density map. The process is repeated, incorporating the coordinates of the light atoms into the phase angle calculations, until all of the non-hydrogen atoms are located.

The success of the heavy-atom method depends on the ability of an atom of relatively high scattering power to determine a sufficient number of phases[6] to initiate the Fourier analysis. The choice of a heavy atom is often restricted by chemical considerations, but some numerical estimate can serve as a useful guide. For this purpose a "heaviness index" has been defined:[5] $\sum Z_H^2/\sum Z_L^2$ where Z_H and Z_L refer to the atomic numbers of the heavy and light atoms respectively. For com-

pounds of moderate complexity a value of about 1.0 is usually used, although values from 0.2 to above 2.0 have been used. If the index is too low there is the danger that either the heavy atom may not be located in the Patterson distribution, or the electron-density distribution may give inconclusive results because of the determination of an insufficient number of accurate phase angles.

On the other hand, if the heaviness index is too large then the heavy atom may so dominate the structure amplitudes that the light atoms are poorly resolved on the electron-density map. In general the hydrogen atoms are not resolved in X-ray analysis. In the few cases that they have been located,[5] a large amount of quite accurate data was available, and the method used was a "difference synthesis" in which the function:

$$D = \frac{1}{V}\sum_{h}\sum_{k}\sum_{\ell}(|Fo| - |Fc|)\cos\left[2\pi(hx + ky + lz) - \alpha(hkl)\right]$$

is plotted where |Fo| and |Fc| are the observed and calculated structure amplitudes respectively. Although the hydrogen atoms are not generally located in an X-ray analysis, they can usually be placed on the basis of chemical considerations, since a satisfactory analysis will give bond lengths and angles sufficiently accurate to define atom type, bond multiplicity and type of hybridization.

After all of the non-hydrogen atoms have been found, further refinement of the structure generally proceeds by the method of least squares.[9] The object of the refinement procedure is to find the atomic parameters which give the best agreement bet-

ween the observed and calculated structure amplitudes. In the least squares method the function minimized is: $\sum w\ (|Fo| - |Fc|)^2$ where w is a weighting factor inversely proportional to the probable error in $|Fo|$, and the summation extends over all the crystallographically independent planes. One of the advantages that the least squares method has over the Fourier method is that it is free from series termination errors. The parameters included in the refinement are the positional parameters, isotropic or anisotropic thermal parameters for each atom, and a scale factor relating the observed and calculated structure amplitudes.

As part of the least squares process the standard deviations of the parameters are calculated, and these can subsequently be used to calculate the standard deviations of the bond lengths and angles. The standard deviation $\sigma(AB)$ of the bond length between the two atoms A and B is given by:[4]

$$\sigma^2(AB) = \sigma^2(A) + \sigma^2(B)$$

where $\sigma(A)$ and $\sigma(B)$ are the standard deviations of the coordinates of A and B along the direction of the bond. The standard deviation (in radians) of the angle between the bonds AB and BC is given by:[4]

$$\sigma^2(\Theta) = \frac{\sigma^2(A)}{AB^2} + \sigma^2(B)\left(\frac{1}{AB^2} - \frac{2\cos\Theta}{AB \cdot BC} + \frac{1}{BC^2}\right) + \frac{\sigma^2(C)}{BC^2} \quad .$$

The structure revealed by the electron-density distribution is generally confirmed by a suitable refinement of the positional and thermal parameters. A popular means of expressing the correctness of a structure is by use of the discrepancy factor,

R, which is defined by:

$$R = \sum(|Fo| - |Fc|)/\sum|Fo|$$

Not too much significance should be attached to the R value since it indicates the accuracy of the intensity measurements as well as the correctness of the structure; and the latter is determined by the extent as well as the accuracy of the empirical data. After a suitable refinement procedure, a necessary but insufficient condition for a correct structure is that $R < 0.25$.

As a final step in a structure analysis by the heavy atom method, it is often possible to determine the absolute configuration of the molecule.[10] Normal scattering of X-rays by a non-centrosymmetric crystal results in the equality: $|F(hkl)| = |F(\bar{h}\bar{k}\bar{l})|$, i.e. the intensity of reflections (hkl) and $(\bar{h}\bar{k}\bar{l})$ are the same (Friedel's Law). The consequence of this law is that normally it is not possible to distinguish between two enantiomers by X-ray diffraction. However it has been observed[11,12] that some atoms scatter X-rays anomalously; this anomalous scattering is greatest when the frequency of the incident radiation is near but still greater than an absorption edge frequency of the atom. The scattering factors for atoms which scatter X-rays anomalously are defined by:

$$f = fo + \Delta f^{1} + i\Delta f^{11}$$

where fo is the normal scattering factor, and Δf^{1} and $i\Delta f^{11}$ are real and imaginary corrections. The imaginary part is scattered $\pi/2$ ahead of the phase of the real part $(fo + \Delta f^{1})$ resulting in

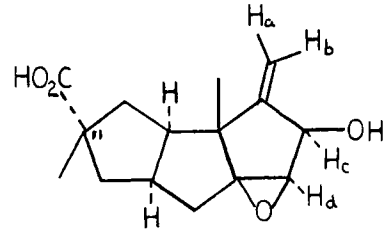
the breakdown of Friedel's Law. Thus if a molecule contains two or more atomic species, one of which is an anomalous scatterer, it should be possible to distinguish between the two enantiomers. Empirically it is observed that the commonly available radiations, eg. Cu-K_α, Mo-K_α, are scattered anomalously by most heavy atoms.

In order to distinguish between two enantiomers, structure amplitudes based on the coordinates of one enantiomer and using the anomalous scattering factor for the heavy atom are calculated for the reflection pairs (hkl) and $(\bar{h}\bar{k}\bar{l})$. These amplitudes are compared with the observed intensities I(hkl) and $I(\bar{h}\bar{k}\bar{l})$; and if it is found that when $|F(hkl)| > |F(\bar{h}\bar{k}\bar{l})|$, $I(hkl) > I(\bar{h}\bar{k}\bar{l})$, and similarly when $|F(hkl)| < |F(\bar{h}\bar{k}\bar{l})|$, $I(hkl) < I(\bar{h}\bar{k}\bar{l})$, then the correct enantiomer has been chosen. If the inequalities do not agree then the mirror image of the assumed structure has the correct absolute configuration. Care must be taken to choose a right-handed system of coordinates while indexing the reflections since the formula for the structure amplitudes[4] are given for a right-handed system of coordinates. Usually only a few pairs of reflections exhibit a significant intensity difference, and even for these very accurate intensity measurements may be required; however only one pair of reflections is necessary to establish the absolute configuration, and accurate intensity measurements can be made by counter techniques.[13]

DISCUSSION

Hirsutic acid C is one of the metabolites isolated from Stereum hirsutum[14] and has the molecular formula $C_{15}H_{20}O_4$. The elucidation of its structure and absolute stereochemistry (1) has been accomplished by a necessary combination of chemical[15] and X-ray[16] methods of analysis.

The functional groups were characterized via the transformations outlined in figure 1. The spectral properties of hirsutic acid (1) and its methyl ester (3) suggested the nature of most of the substituents. The acid (1) showed only end-absorption in the ultraviolet spectrum, and exhibited peaks in



(1)

the infrared region at 3520 (hydroxyl), 3200-2400 (carboxyl), 1700 (carbonyl), 1655 and 890 cm^{-1} (exocyclic methylene); the methyl ester (3) showed similar I.R. absorption bands for the exocyclic methylene and hydroxyl functions, as well as the characteristic ester peaks at 1720 and 1160 cm^{-1}. The N.M.R. spectra of (1) and (3) were identical with the exception of the ester methyl resonance (6.34τ); methyl hirsutate (3) also had singlet peaks at 8.66 and 8.97 τ (tertiary methyls) and doublets at 4.72, 5.00 (Ha,Hb) and 6.55 τ (Hd) as well as a multiplet at

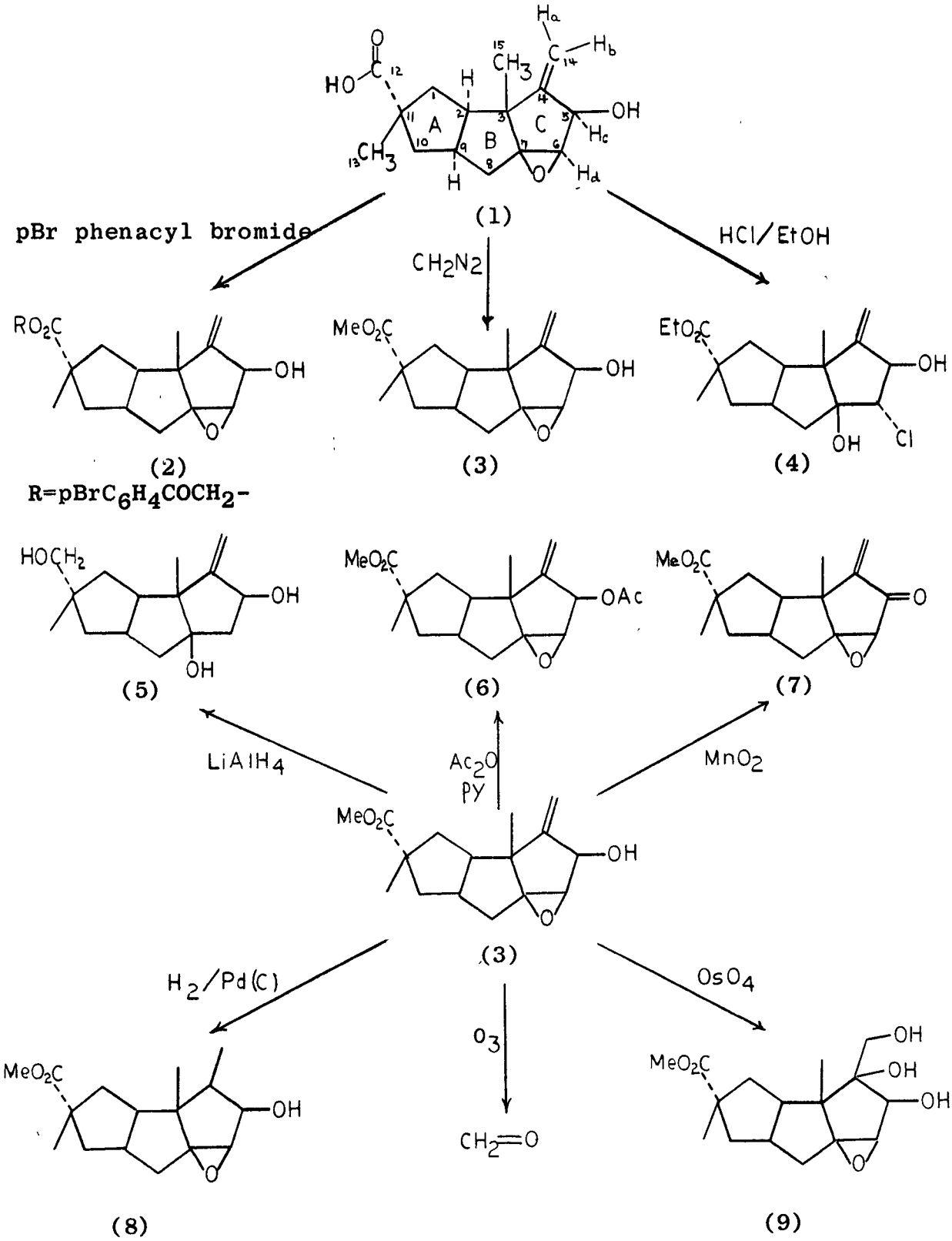
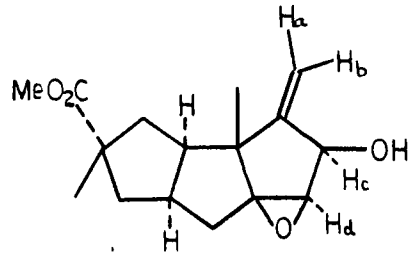
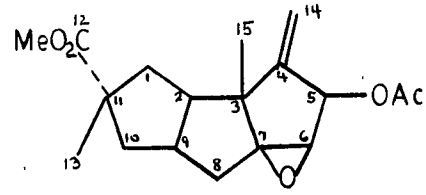

(1)
pBr phenacyl bromide
CH2N2
HCl/EtOH
(2)
R=pBrC6H4COCH2-
(3)
(4)
(5)
(6)
(7)
LiAlH4
Ac2O
py
MnO2
H2/Pd(C)
O3
OsO4
CH2=O
(8)
(9)

Figure 1



(3)

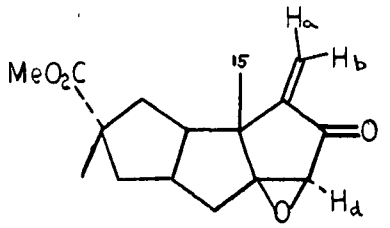


(6)

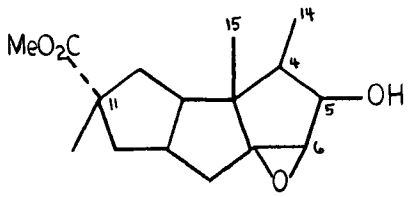
5.40 τ (Hc). The long range coupling constants between the exocyclic methylene protons Ha,Hb and the C_5 proton Hc were 2.1 and 2.7 c.p.s., whereas JHaHb = 0 and JHcHd = 1.9 c.p.s. Finally the mass spectrum of methyl hirsutate showed a strong parent peak (m/e = 278) as well as peaks due to the loss of methyl, hydroxyl and carbomethoxy groups (m/e = 263,260,219 and 201).

The presence of a secondary hydroxyl group was confirmed by the formation of the monoacetate (6). The infrared spectrum of (6) showed no absorption above 3000 cm^{-1}, but exhibited peaks at 1735, 1375 and 1210 cm^{-1} characteristic of the acetoxy group. The N.M.R. spectrum showed a singlet at 7.85 τ due to the acetate protons; in addition the chemical shifts of the protons on carbon atoms C_5,C_6,C_{14} and C_{15} were different from the parent compound (3), but the coupling constants were the same.

The allylic relationship between the double bond and the hydroxyl group was established after manganese dioxide oxidation of methyl hirsutate gave an α,β-unsaturated ketone (7, λmax: 231 mμ (ε 5350); λcalc:[17] 230 mμ). The infrared spectrum of (7) showed no absorption above 3000 cm^{-1} but exhibited peaks at 1727 and 1645 cm^{-1} characteristic of the α,β-unsaturated ketone



(7)



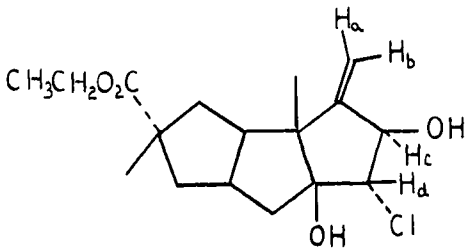
(8)

system. The value for the carbonyl stretch frequency (1727 cm^{-1}) suggested a five-membered ring. The N.M.R. spectrum showed that the keto group shifted the protons on C_{15} downfield by 0.15τ, and more important Ha,Hb and Hd appeared as singlets, thus confirming the assignment of long range coupling in methyl hirsutate (3) and also confirming the presence of oxygen functions on adjacent carbon atoms.

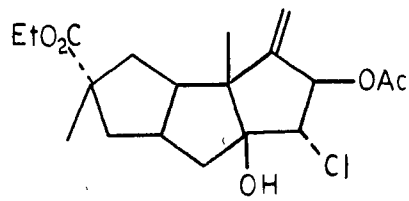
The presence of the exocyclic double bond was confirmed by several experiments. Formaldehyde, as the 2,4-dinitrophenylhydrazone, was isolated by the ozonolysis of methyl hirsutate (3). Catalytic reduction of (3) resulted in the uptake of one mole of hydrogen and the isolation of the dihydromethyl hirsutate (8). The N.M.R. spectrum of (8) was consistent with the replacement of an exocyclic methylene group by a secondary methyl group (9.05τ, doublet, J = 6.0 c.p.s.); and again the chemical shifts of the protons at C_5,C_6 and C_{15} had altered. Finally oxidation of (3) with osmium tetroxide gave a triol (9).

The epoxide function was the most difficult to establish. By elimination, the remaining oxygen function had to be in an ether linkage, and as suggested above, probably was attached to a carbon atom adjacent to the secondary hydroxyl group. The

presence of an epoxide was confirmed by the formation of the chlorohydrin (4). The infrared spectrum of (4) showed enhanced absorption at 3500 cm^{-1} as well as peaks at 1720 and 1190 cm^{-1}



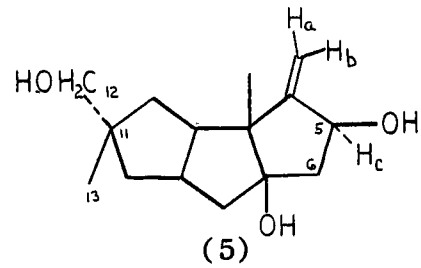
(4)



(10)

characteristic of the ethyl ester. The N.M.R. spectrum of (4) showed peaks due to the ethyl ester (τ 5.86q, 8.76t, J=7 c.p.s.); in addition Hd appeared at lower field (6.04τ) with the coupling between Hc and Hd considerably enhanced (J=9 c.p.s.). The structure of the chlorohydrin (4) was supported by the failure to form an isopropylidene derivative, a negative periodate test and the formation of a monoacetate (10). The acetylation product (10) exhibited strong infrared absorption at 3480 cm^{-1} in addition to the acetate peaks at 1735 and 1370 cm^{-1}. The N.M.R. spectrum clearly showed the presence of a single acetoxy group (7.87τ, 3H).

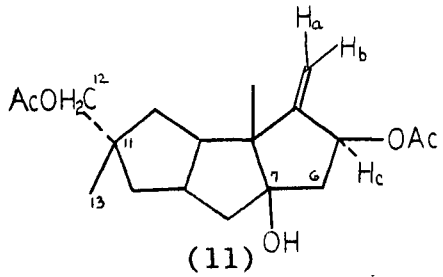
Further evidence for the epoxide was provided by reduction of methyl hirsutate (3) with lithium aluminum hydride. The reduction product (5) showed strong absorption in the infrared spectrum at 3500 cm^{-1}, but no absorption in the carbonyl region. The mass spectrum showed a strong parent peak (m/e = 252) as well as peaks due to the loss of one hydroxyl, two hydroxyl



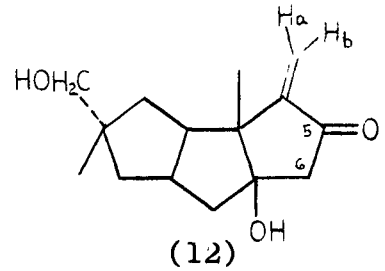
(5)

and hydroxymethyl groups (m/e = 234, 216, 221). The presence of a hydroxymethyl group was also revealed in the N.M.R. spectrum by a two proton singlet at 6.70 τ . Both tertiary methyl groups appeared at 8.90 τ (resolved on 100 Mc spectrum); this suggested the gem-substitution pattern at C_{11} since the C_{13} methyl group appeared at 8.66 τ in methyl hirsutate. The exocyclic methylene protons Ha, Hb appeared as quartets (4.82, 5.03 τ) with long range coupling constants with Hc of 1.8,1.9 c.p.s. and a geminal splitting of 0.5 c.p.s. The C_5 proton Hc appeared as a broad peak but addition of a drop of acid to a deuterioacetone solution resulted in a triplet of triplets pattern (121,242,121) consistent with a splitting of 1.8 c.p.s. with Ha, Hb and a splitting of 6 c.p.s. with the protons on C_6. Acid treatment of the N.M.R. sample also resulted in the disappearance of peaks at 6.19, 6.53 and 7.15 τ assigned to hydroxyl protons.

Acetylation of the reduction product (5) gave a diacetate (11). The infrared spectrum showed strong absorption at 3500 cm^{-1} in addition to the acetate peaks at 1725 and 1365 cm^{-1}. The N.M.R. spectrum established the presence of two acetoxy groups (τ 7.94s, 6H). The C_{12} protons appeared as a singlet at 6.18 τ , and peaks due to the tertiary methyl groups and exocyclic methylene appeared as in the parent (5). The proton Hc appeared as ten



(11)

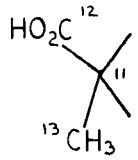


(12)

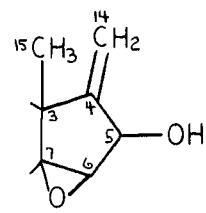
peaks in a triplet quartet triplet pattern (121 1331 121) consistent with splittings of 5.8 and 7.7 c.p.s. with the protons on C_6 as well as a long range splitting of 1.9 c.p.s. with Ha,Hb.

Other transformations of the reduction product (5) included catalytic hydrogenation of the double bond, and chromic acid/pyridine oxidation of the secondary hydroxyl group at C_5. The α,β-unsaturated ketone system of the latter (12) was characterized by its U.V. (λmax 234 mμ), I.R. (1720, 1640 cm^{-1}) and N.M.R. (τ4.8s, 4.0s = Ha,Hb) spectra. The N.M.R. spectrum of the oxidation product (12) also showed a sharp peak at 7.48 τ which could be ascribed to the two protons at C_6.

The structural information provided by the chemical evidence given above can be summarized by the two partial structures (13a) and (13b). Although all of the functional groups are



(13a)

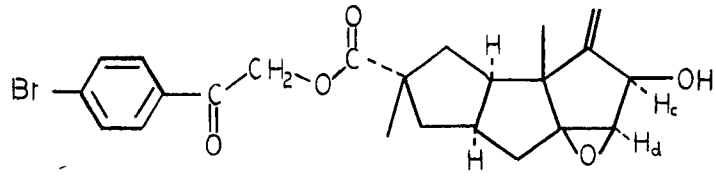


(13b)

contained in these partial structures, very little of the

hirsutic acid ring system is revealed. In addition, acid, alkali or selenium treatment of methyl hirsutate (3) resulted in complex mixtures lacking in any distinctive chromophore. Since our original supply of hirsutic acid only consisted of a few grams,* and since the strain of Stereum hirsutum which produces this metabolite has not been rediscovered, it was apparent that a complete chemical degradation of hirsutic acid was not possible. Thus an X-ray analysis[16] of the p-bromophenacyl ester (2) was carried out.

The p-bromophenacyl ester of hirsutic acid (2) was characterized by spectral and analytical data. The ester group was



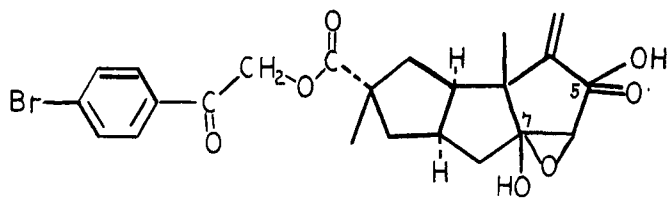
(2)

well-defined by the U.V. (λmax: 255 mμ (ϵ 15,500), I.R. (1735, 1705, 1590 cm^{-1}), N.M.R. (4 aromatic protons, phenacyl methylene at 4.71τ) and mass spectra (parent m/e = 460,462). Crystallization from benzene-ethanol gave colorless prisms belonging to the space group $P2_12_12_1$.

Initially a set of intensities was collected on film and estimated visually. On the first electron-density distribution,

* I would like to thank Dr. N.G. Heatley for providing us with his total supply of hirsutic acid C.

which was calculated using the coordinates of the bromine atom, 28 of the 29 non-hydrogen atoms were resolved, and it appeared that X-ray analysis would give the structure without the aid of any chemical data. In the subsequent analysis the missing atom (epoxide oxygen) was located; however, in spite of a new set of more accurate intensities measured on a scintillation counter, the positional and isotropic thermal parameters did not refine very well. The completed analysis defined the nature and positions of 27 full atoms and 4 "half-atoms" as shown by the

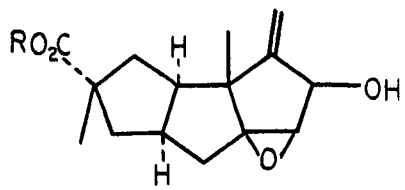


(14)

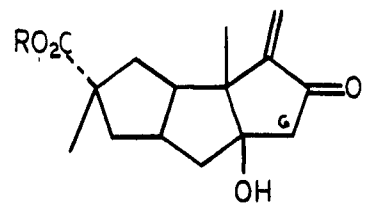
structural formula (14) where the four oxygen atoms attached to C_5 and C_7 are "half-atoms". The X-ray result suggested that the irradiated crystals contained an almost equal mixture of two randomly distributed and chemically distinct molecules. Thus it appeared that during the irradiation an unusual solid-phase rearrangement had occurred, but the crystal structure remained essentially unchanged with only the positional parameters of two non-hydrogen atoms altering significantly.

A combination of the X-ray evidence (structure 14) with the chemical evidence (partial structure 13b) established the structure of the p -bromophenacyl ester of hirsutic acid (2), and

suggested the rearrangement product had the structure (15). To confirm the latter assignment, a sample of p-bromophenacyl hirsutate (2) was irradiated, and the rearrangement product (15) separated from the unchanged ester (2) by preparative thin-layer chromatography. Characterization of (15) followed from the spectral data. The tertiary hydroxyl group was indicated by



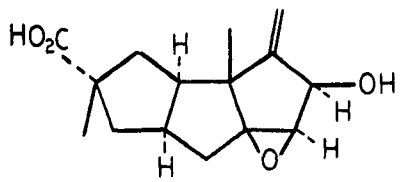
(2) R = $BrC_6H_4COCH_2$-



(15)

strong absorption in the infrared spectrum at 3480 cm^{-1}. The α,β-unsaturated ketone system was characterized by I.R. (1720, 1625 cm^{-1}) and N.M.R. (τ4.72s, 3.96s Ha,Hb) spectra. In addition the rearrangement product exhibited enhanced absorption in the ultraviolet region, λmax:255 mμ (ϵ 18,500). The C_6 methylene group appeared in the N.M.R. spectrum as a two proton singlet at 7.48τ.

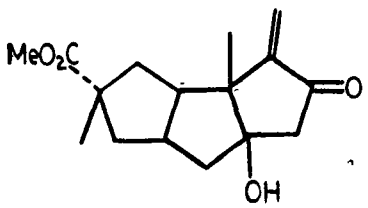
As a final step in the X-ray analysis the absolute configurations of both p-bromophenacyl hirsutate (2) and the rearrangement product (15) were determined by the anomalous dispersion method.[11] The structure and absolute stereochemistry of hirsutic acid C (1) follows from the chemical correlation between the acid and the ester (2).



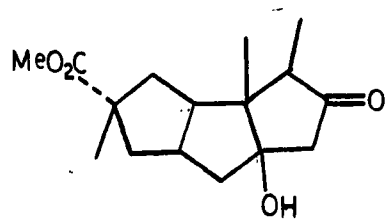
(1)

From an examination of the X-ray results it was apparent that the rearrangement process was strongly influenced by the nature of the molecular packing and the hydrogen bonding scheme. A discussion of the X-ray results and the mechanism of the rearrangement are given in the Experimental section following the X-ray data.

The generality of the X-ray-induced rearrangement was then investigated. Irradiation of methyl hirsutate (3) and dihydromethyl hirsutate (8) gave respectively about 30% and 70% rearrangement. Analytically pure samples of the rearrangement products were not obtained; however spectral data obtained in each case from samples containing about 70% rearrangement product were consistent with a rearrangement process analogous to the one observed with p-bromophenyl hirsutate (2). Thus the rearrangement product from ethyl hirsutate is believed to have structure (16) and the one from dihydromethyl hirsutate, structure (17). Irradiation of hirsutic acid (1) itself resulted in no rearrangement. In the free acid (1), hydrogen bonds involving the carboxyl group probably dominate the molecular packing. Attempts to induce the rearrangement thermally in the esters (2) and (3) also were unsuccessful.



(16)



(17)

Further to the investigation of the generality of the rearrangement process, the model compounds 4β, 5-epoxy-5β-cholestan-3β-ol (21) and 4β, 5-epoxy-5β-cholestan-3∝-ol (23), and the expected rearrangement product, 5β-hydroxy-cholestan-3-one (25) were synthesized (see figure 2). The ring system of (21) and (23) bears little resemblance to the hirsutic acid skeleton; however the ∝-hydroxy-epoxy functional groups were expected to dominate the molecular packing. Irradiation of the 3β-hydroxy isomer (21) for 2 days resulted in no degradation, and a similar bombardment of the 3∝-isomer (23) produced only slight decomposition. A sample of (23) which had been irradiated for 7 days showed four components on T.L.C. in addition to ca.90-95% starting material. Of the new products, the major one (ca.5%) could not be identified. Of the minor components, T.L.C. and U.V. (λmax 236 mμ) evidence suggested that one was cholest-4-en-3-one (19) and T.L.C. evidence suggested that another could possibly be the expected rearrangement product (25).

The results of the irradiation of the two model compounds (21,23) suggests that the unusual solid phase rearrangement observed with p-bromophenacyl hirsutate (2) does not generally apply to other ring systems containing the ∝-hydroxy-epoxy

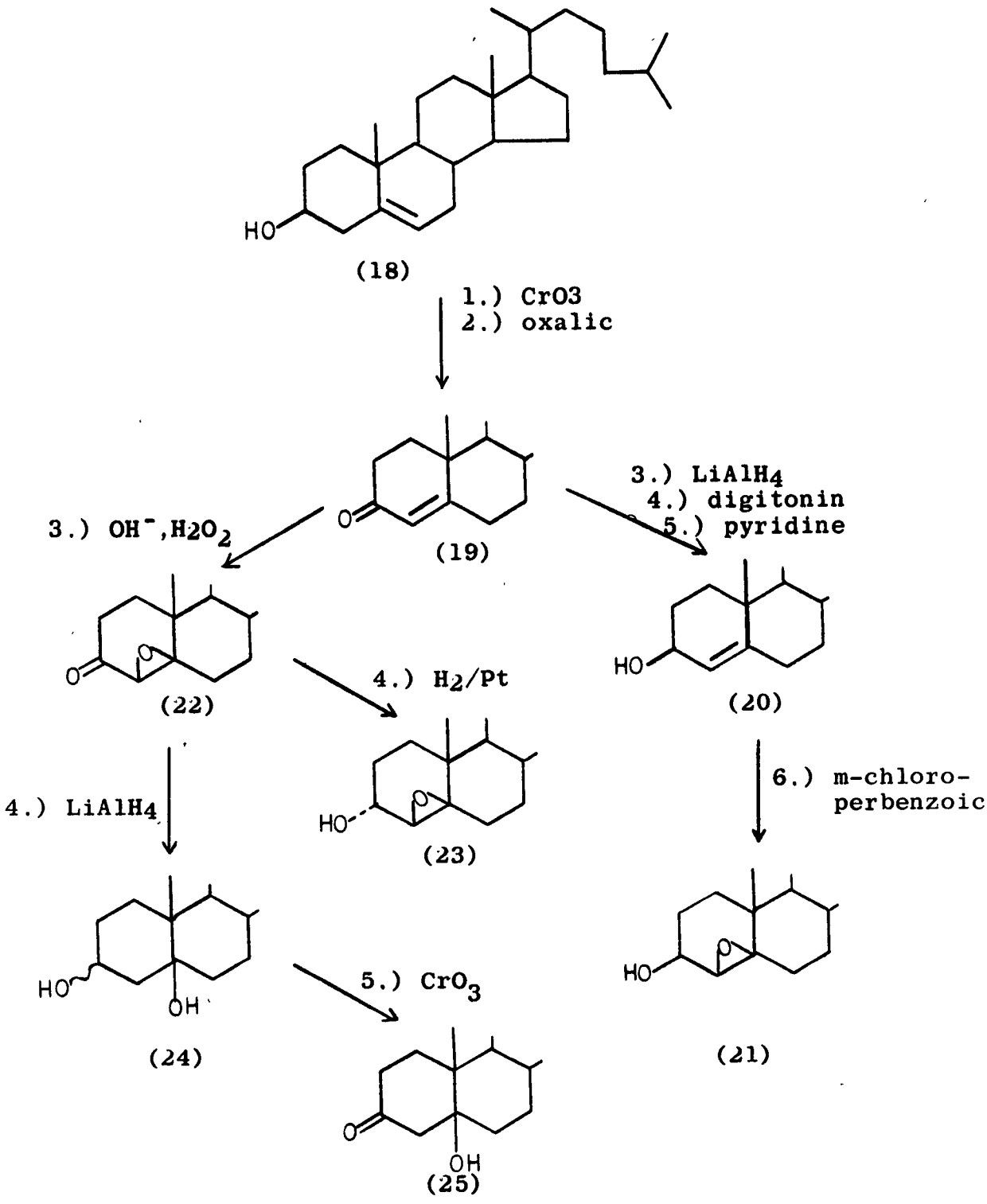
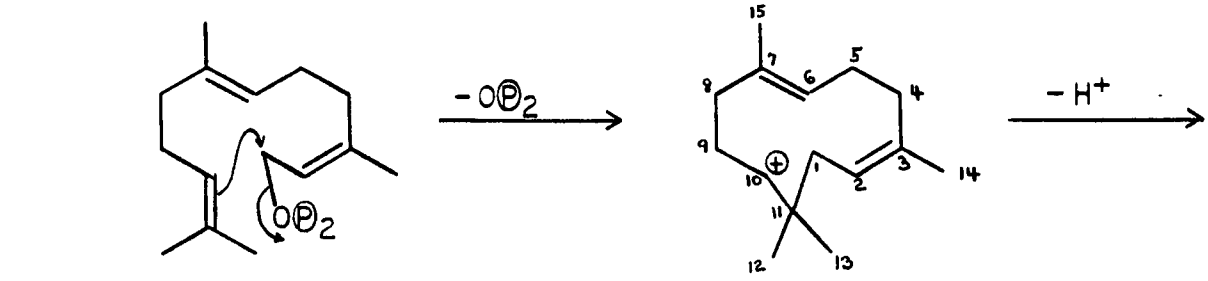

HO
(18)
1.) CrO3
2.) oxalic
3.) LiAlH4
4.) digitonin
5.) pyridine
3.) OH⁻,H2O2
(19)
(22)
(20)
4.) H2/Pt
4.) LiAlH4
6.) m-chloro-
perbenzoic
(23)
(24)
(21)
5.) CrO3
(25)

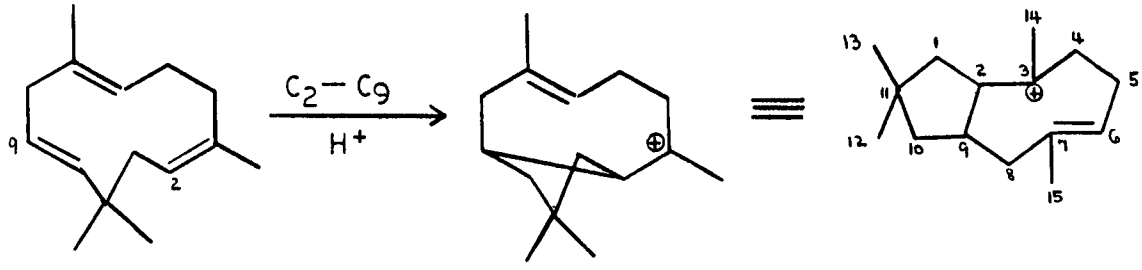
Figure 2

functional groups. Thus the nature of the molecular packing in p-bromophenacyl hirsutate (2) and the related methyl esters (3,8) might be a unique feature of the peculiar ring system.

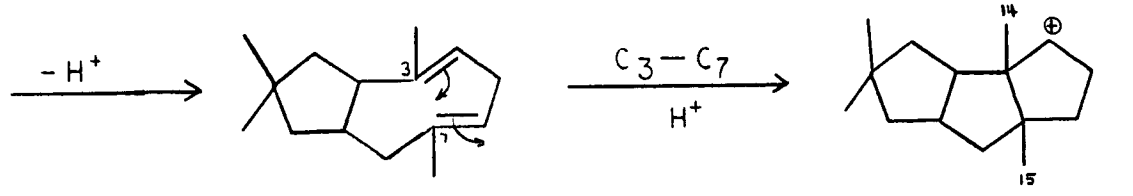
Since hirsutic acid C possesses a novel ring system it is interesting to speculate on its biosynthetic origin. The molecular formula, $C_{15}H_{20}O_4$, suggests that it is a sesquiterpene[18,19] and hence derivable from farnesyl pyrophosphate (26). Hirsutic acid also bears a structural resemblance to humulene (28)[20]; this suggests a possible biosynthetic scheme as outlined in figure 3.



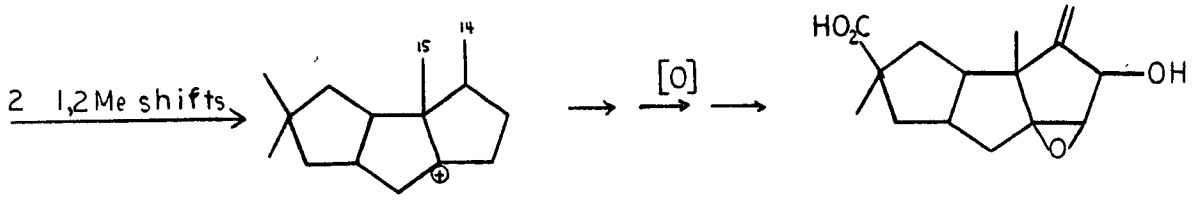
(26) (27)



(28) (29)



(30) (31)



(32) (1)

Figure 3

EXPERIMENTAL

Chemical Data:

Melting points were determined on a Kofler hot stage microscope. Ultraviolet spectra (U.V.) were determined in ethanolic solutions using a Cary 14 spectrophotometer; and infrared spectra (I.R.) were taken on a Perkin Elmer Model 137B spectrophotometer in chloroform solutions using matched 0.1 mm. cells unless stated otherwise. Optical rotation measurements were taken on a Rudolph instrument in chloroform solutions with one decimeter tubes. Nuclear magnetic resonance spectra (N.M.R.) were taken on a Varian A60 Mc instrument in deuteriochloroform solutions unless indicated otherwise. The mass spectra were determined on an A.E.I. MS 9 Double Focusing Mass Spectrometer. Elemental microanalyses were carried out by Dr. A. Bernhardt and his associates at the Max Planck Institute, Mulheim, Ruhr, West-Germany.

The U.V. maxima are reported in millimicrons; pertinent I.R. peaks are reported in cm^{-1}; and the N.M.R. peaks are reported in τ units with the coupling constants, J, reported in cycles/sec; the assignments of the N.M.R. peaks and the coupling constants are enclosed in brackets. The mass spectra data are reported as m/e; a rationale of the complete spectra has not been attempted; however, in addition to the parent peak several strong peaks which are probably indicative of the loss of functional groups without rearrangement of the skeleton are reported; the suggested fragment that is eliminated is enclosed

in brackets. Standard abbreviations for peak intensity (I.R.) and shape (N.M.R.) are used.

The usual work-up included washing three times with water, drying with sodium sulfate and removal of the solvent on a rotary evaporator at 45°, the latter being designated by the phrase in vacuo.

The X-ray irradiation experiments were carried out using unfiltered molybdenum radiation. The sample (up to 50mg) was placed in a thin-walled, soft glass capsule which was then placed in the cavity of a stainless steel block. The block was attached to the X-ray tube outlet.

Thin-layer chromatography (T.L.C.) was carried out with silica gel G (acc. to Stahl) plates unless indicated otherwise. Detection was carried out with: U.V. irradiation, iodine vapor and charring with a 5% ceric sulfate/10% sulfuric acid spray.

Hirsutic acid (1)

The crude sample provided by Dr. N.G. Heatley[14] was recrystallized from ethanol to give colorless prisms; m.p. 179-180°; $[\alpha]_D^{23}$ + 116° (c=1.05); I.R.($CHCl_3$): 3520 w, 3200-2400 b, 2950 s, 1700 vs, 1660 w, 920 s, 890 m; U.V.: end absorption; N.M.R.: 4.73 d, 5.00 d (Ha,Hb: JHa,Hb:Hc = 1.9, 2.6, JHaHb = 0), 5.39m (Hc), 6.54 d (Hd: JHcHd = 2.0), 8.62 s (C_{13} methyl), 8.97s (C_{15} methyl), 7.4 - 8.2 - 9.0 (8 skeleton protons); Anal. Found: C 68.30, H 7.47, $C_{15}H_{20}O_4$ requires: C 68.16, H 7.63.

X-ray irradiation: 30mg. was bombarded for 42 hours, but an undepressed m.p., an infrared spectrum identical with starting material and only end absorption in the ultraviolet region indicated that no rearrangement had occurred.

p-Bromophenacyl hirsutate (2)

150 mg. of hirsutic acid (0.57 mmoles) and 160 mg. of p-bromophenacyl bromide (0.62 mmoles) were dissolved in 15 ml. of potassium carbonate-dried acetone containing 1 g. of potassium carbonate. The mixture was refluxed for 50 min. under anhydrous conditions, then filtered and the solvent removed in vacuo. T.L.C. indicated greater than 95% reaction had occurred. Crystallization from ether-petroleum ether, followed by two recrystallizations from benzene-ethanol gave a 50% yield of colorless prisms; m.p. 129-130°; $[\alpha]_D^{22}$ + 97° (c = 1.55); T.L.C. (benzene:ether, 1:1): R_f = 0.5; I.R. ($CHCl_3$): 3600 m, 3500 w, 2920 m, 1735 vs, 1705 vs, 1660 w, 1590 vs, 920 s, 890 m; U.V.: 255 mμ (15,500); N.M.R.: 2.18 nq, 2.37 nq (4 aromatic protons, J=9), 4.71 s (phenacyl methylene), 4.70 d, 498 d (Ha,Hb, JHaHb = 0, JHa,Hb : Hc = 2.1), 5.36 b (Hc), 6.52 d (Hd, JHcHd = 2.1), 8.57 s (C_{13} methyl), 8.95 s (C_{15} methyl), 7.3 - 8.2 - 9.0 (8 skeleton protons); mass spec.: 460, 462 (parent), 445, 447 ($-CH_3$), 442, 444 ($-H_2O$), 263 ($-CH_2COC6H4Br$),245 ($263^+ - H_2O$), 219 ($-CO_2CH_2COC_6H_4Br$), 201 ($219^+ - H_2O$); Anal. Found: C 60.08, H 5.78, Br 17.44, $C_{23}H_{25}O_5Br$ requires: C 59.88, H 5.46, Br 17.30.

X-ray irradiation product (15)

(i) 3 mg. of analytically pure p-bromophenacyl ester was bombarded and the rate of reaction followed by T.L.C. After about 10 hours the reaction was complete with about 60% of the ester being converted. The irradiated sample showed a depressed m.p. 70-100°, an enhanced U.V. maximum 255 (ϵ=17,900) and enhanced absorption in the infrared region at 3500 cm^{-1}.

(ii) Single crystals were mounted on both the Weissenberg camera and the Spectrogoniometer (X-ray structure determination conditions). After 15 hours irradiation (Cu-Kα) they were crushed separately. Pieces from both crystals had depressed m.p. and exhibited a spot on T.L.C. plates corresponding to the rearrangement product (15).

(iii) On a preparative scale, 200 mg. of ca. 95-98% pure ester was irradiated for 60 hours. For this impure ester only 30-40% conversion was obtained. Separation of the starting material and rearranged products was carried out by preparative T.L.C. using two silica gel plates (20 x 60 x 0.05 cm.) containing G.E. phosphor and developed along the long axis with benzene:ether, 1:4 for 24 hours. The compounds were eluted from the silica with ether; the recovered ester crystallized instantly; however the rearrangement product did not crystallize. It appears that the latter decomposes partially on elution from silica. Therefore the spectral data were acquired using the oil (ca. 95% pure). $[\alpha]_D^{19}$+ 30° (c = 1.10); T.L.C. (benzene: ether, 1:1): R_f = 0.45; I.R. (oil): 3480 s, 2920 s, 1735 s (sh), 1720 vs, 1705 vs, 1625 m, 1585 s, 910 m; U.V.: 255 mμ(ϵ 18,500);

N.M.R.: 2.20 nq, 2.40 nq (4 aromatic protons, J = 9), 4.72 s (phenacyl methylene), 3.96s, 4.72 s (Ha,Hb, JHaHb = 0), 7.48 s (C_6 methylene), 8.58 s (C_{13} methyl), 8.85 s (C_{15} methyl), 7.3-8.3-9.0 (8 skeleton protons).

Methyl hirsutate (3)

1 g. of hirsutic acid was dissolved in 30 ml of ether and cooled to 0°. Freshly prepared diazomethane/ether solution[21] was added dropwise at 0° until an excess was clearly indicated. The excess reagent was destroyed with dilute acid and the ether layer washed, dried, filtered and removed in vacuo. Recrystallization from ethanol gave a quantitative yield of the methyl ester as colorless prisms; m.p. 161-162°; $[\alpha]_D^{20}$+ 119° (c = 2.25); T.L.C. (benzene:ether, 1:1): R_f = 0.4; I.R. ($CHCl_3$): 3660 m, 3500 w, 3000 s, 1720 vs, 1655 w, 1160 vs, 915 s, 885 m; N.M.R. (100 Mc): 4.72 d, 5.00 d (Ha,Hb, JHaHb = 0, JHa,Hb:Hc = 2.1, 2.7), 5.40 b(Hc), 6.55 d (Hd, JHcHd = 1.9), 6.34 s (ester methyl), 8.66 s (C_{13} methyl), 8.97 s (C_{15} methyl), 7.4-8.2-9.0 (8 skeleton protons); mass spec.: 278 (parent), 263 ($-CH_3$), 260 ($-H_2O$), 219 ($-CO_2CH_3$), 201 ($219^+ - H_2O$); Anal. Found: C 69.09, H 7.98, $C_{16}H_{22}O_4$ requires: C 69.04, H 7.97.

X-ray irradiation: 100 mg. of methyl hirsutate was bombarded for 150 hours. The irradiated sample had a depressed m.p. 105-140° and T.L.C. indicated ca. 30% conversion to a new product had occurred. The ultraviolet spectrum of the irradiated sample exhibited a maximum at 238 mμ; but the new product was shown to be different from the manganese dioxide oxidation

product (7) by comparative T.L.C. The new product (16) was separated from starting material by preparative T.L.C., however considerable decomposition of the former resulted during its elution and subsequent handling. The spectral data were acquired with ca. 70% pure product. The U.V. spectrum appeared to be dominated by the impurities (λmax 246 mμ, ϵ 11,000, eg. Δ^6 - 5 - keto chromophore); however the infrared and N.M.R. data were consistent with structure (16); I.R. (oil): 3400 m, 1725 vs, 1620 s, 1160 vs, 880 m; N.M.R.: 4.12 s, 4.82 s (Ha,Hb, JHaHb = 0), 6.35 s (ester methyl), 8.61 s (C_{13} methyl), 8.82 s (C_{15} methyl), 7.2 - 8.4 - 9.0 (8 skeleton protons); T.L.C. (benzene:ether, 1:1): R_f = 0.6.

Ethyl hirsutate chlorohydrin (4)

100 mg. of hirsutic acid was dissolved in 20 ml. ethanol and 2 ml. conc. HCl added. The solution was refluxed on a steam bath for 5 hours; the ethanol was removed in vacuo, ether added and the ether layer washed, dried and evaporated. Crystallization from ether-petroleum ether followed by two recrystallizations from benzene-petroleum ether gave a 50% yield of colorless prisms; m.p.: 95-96; $[\alpha]_D^{23}$ + 67° (c = 2.42); T.L.C. (benzene:ether, 1:1): R_f = 0.4; U.V.: end absorption; I.R.: 3610 m, 3480 m, 2980 s, 1720 vs, 1665 w, 1190 vs, 910 s; N.M.R.: 4.68 d, 4.81 d (Ha,Hb, JHaHb = 0, JHa,Hb:Hc = 2.0, 2.0), 5.7b (Hc), 6.04 d (Hd, JHcHd = 9), 5.86 q, 8.76 t (ester ethyl, J = 7), 8.67 s (C_{13} methyl), 8.87 s (C_{15} methyl), 7.4 - 8.4 - 9.0 (8 skeleton protons); Anal. Found: C 62.32, H 7.48, Cl 10.34,

$C_{17}H_{25}O_4Cl$ requires: C 62.09, H 7.67, Cl 10.80.

Three reactions supported the structural assignment (4):

(i) Attempted isopropylidene formation:

30 mg. of the chlorohydrin was dissolved in 15 ml. dry acetone containing 1.5 g. anhydrous copper sulphate, and the mixture shaken for 2 days. T.L.C. showed that no reaction had occurred, and starting material was recovered.

(ii) Negative periodate test[22]

(iii) Monoacetate formation (10):

30 mg. of chlorohydrin was acetylated (2 ml. pyridine + 1 ml. acetic anhydride) for 3 days. The solution was added to cold water and then extracted with chloroform. The organic layer was washed, dried and removed in vacuo; the pyridine was azeotroped off with toluene in vacuo. T.L.C. of the residue indicated a single product which was characterized as the monoacetate of (4) from its spectral properties; T.L.C. (benzene: ether, 1:1): R_f = 0.7; I.R. (oil): 3480s, 2940 s, 1735 vs, 1725 vs. 1655 w, 1370 s, 915 s, 870 m; N.M.R.: 4.81 d (Ha,Hb, JHaHb = 0, JHa,Hb = Hc = 2.1), 5.8 b (Hc), 5.81 d (Hd, JHcHd = 8.5), 5.85 q, 8.75 t (ester ethyl, J = 7), 7.87 s (acetate methyl), 8.67 s (C_{13} methyl), 8.86 s (C_{15} methyl), 7.3 - 8.4 - 9.0 (8 skeleton protons).

Lithium aluminum hydride reduction product (5)

The experiments concerning the preparation and subsequent transformations of (5) were carried out with Dr. I.H. Qureshi, whose assistance is gratefully acknowledged.

500 mg. of methyl hirsutate in 25 ml. tetrahydrofuran was added dropwise to a refluxing solution of 2g. $LiAlH_4$ in 100 ml. tetrahydrofuran, and the mixture refluxed for 5 hours. Excess $LiAlH_4$ was decomposed cautiously with ethyl acetate followed by a saturated solution of ammonium chloride. The product was extracted with ether and the organic layer washed, dried and removed <u>in vacuo</u>. Recrystallization from ethanol gave a 30% yield of colorless prisms; m.p. 117-118°; $[\alpha]_D^{19}$ + 54° (c = 1.13); I.R. ($CHCl_3$): 3500 s, 2900 s, 1655 w, 910 s; N.M.R. (100 Mc), $CDCl_3$: 4.82 q. 5.03 q (Ha,Hb, JHaHb = 0.5, JHa,Hb:Hc = 1.8, 1.9), 5.50 b (Hc), 6.70 s (C_{12} methylene), 8.90 d (6H, C_{13} and C_{15} methyls, δ = 1 cps), 7.4 - 9.0 (8 skeleton protons), $(CD_3)_2CO$: 4.91 q, 5.10 q, 5.6 b, 6.76 s, 8.92 d (6H), 7.4 - 9.0 (8H), and broad peaks at 6.19, 6.53, 7.15 believed due to three hydroxyl protons, $(CD_3)_2CO/HCl$: hydroxyl peaks disappear and Hc appears as a triplet of triplets (121 242 121) pattern, (J = 1.8, 6); mass spec.: 252 (parent), 234 ($-H_2O$), 221 ($-CH_2OH$), 216 ($-2H_2O$), 31 (CH_2OH^+); Anal. Found: C 70.92, H 9.09, $C_{15}H_{24}O_3$ requires: C 71.39, H 9.59.

The reduction product (5) was characterized further by several transformations:

(i) Diacetate formation (11):

Acetic anhydride/pyridine acetylation of (5) gave an oil which was shown to be the diacetate (11) from its spectral properties; I.R. ($CHCl_3$): 3560 m, 3460 m, 2910 s, 1725 vs, 1655 w, 1365 s, 910 s, 865 w; N.M.R. (100 Mc): 4.80 d, 4.91 d (Ha,Hb, JHaHb = 0, JHa,Hb:Hc = 1.9,1.9), 4.47 m (Hc, 121 1331 121

pattern, J = 7.7, 5.8, 1.9 (triplet)), 6.18 s (C_{12} methylene), 7.94 s (6H, two acetate methyls), 8.89 d (6H, C_{13} and C_{15} methyls, δ= 1.5 cps); 7.4 - 9.0 (8 skeleton protons).

(ii Chromic acid oxidation product (12):

33 mg. of (5; 0.13 m moles) in 1 ml. pyridine was added to a solution of 13 mg. chromic acid (0.13 mmoles) in 0.5 ml. pyridine. After 16 hours the mixture was pourred into ice and dilute HCl, then extracted with ether. The organic layer was washed, dried and removed <u>in vacuo</u> to yield 18 mg. of a gum. The spectral properties of the residue suggest oxidation of the secondary hydroxyl (C_5) has occurred; I.R. ($CHCl_3$): 3400 s, 2900 s, 1720 vs, 1640 m; U.V.: 234 mμ; N.M.R.: 4.0 s, 4.8 s (Ha,Hb, JHaHb = 0), 6.65 s (C_{12} methylene), 7.48 s (C_6 methylene), 8.85 (C_{15} methyl), 8.90 (C_{13} methyl), 7.2 - 8.5 (8 skeleton protons).

(iii) Catalytic hydrogenation

28 mg. of (5) in 30 ml. ethanol was hydrogenated using platinum oxide. The spectral data of the residue was consistent with reduction of the exocyclic methylene group; I.R. (oil): 3400 vs; N.M.R. $(CD_3)_2CO$: 6.0 b (Hc), 6.55 b (2 or 3 hydroxyl protons, removed with D_2O), 6.75 s (C_{12} methylene), 8.95 s (C_{13} methyl), 9.06 s (C_{15} methyl), 9.05 d (C_{14} methyl, J = 6), 7.4 - 9.0 (8 skeleton protons).

Acetylation of the hydrogenation product above gave a

residue which by its spectral properties was a diacetate, I.R. (oil): 3450 m, 2900 s, 1720 vs, 1370 vs; N.M.R. $(CD_3)_2CO$: 5.0 b (Hc), 6.19 s (C_{12} methylene), 7.92 s, 7.99 s (6H, two acetate methyls), 8.95 s (C_{13} methyl), 9.05 s (C_{15} methyl), 9.05 d (C_{14} methyl, J = 4), 7.4 - 9.0 (8 skeleton protons).

Methyl hirsutate acetate (6)

145 mg. of methyl hirsutate was dissolved in 2 ml. dry pyridine and 2 ml. acetic anhydride added. The solution was left standing for 4 days with occasional swirling, and then the organic reagents were removed under high vacuum; ethanol was added to remove traces of acetic anhydride. The residue appeared to be at least 95% homogeneous by T.L.C., however crystallization could not be induced. The residue was sublimed at 85° (0.02 mm Hg) to give a homogeneous, colorless oil. This oil was characterized as the monoacetate; $[\alpha]_D^{23}$ + 106° (c = 1.78, 11.1); T.L.C. (benzene:ether, 1:1): R_f = 0.75); I.R. ($CHCl_3$): 2990 s, 1735 s, 1725 vs, 1675 w, 1375 s, 1210 vs, 1170 s, 915 s, 875 w; N.M.R.: 4.29 d, 4.92 d (Ha,Hb, JHaHb = 0, JHa,Hb:Hc = 2.2, 2.2), 4.98 m (Hc), 6.44 d (Hd, JHcHd = 2.0), 6.33 s (ester methyl), 7.85 s (acetate methyl), 8.65 s (C_{13} methyl), 8.91 s (C_{15} methyl), 7.4 - 8.2 - 9.0 (8 skeleton protons); Anal. Found: C 67.87, H 7.59, $C_{18}H_{24}O_5$ requires: C 67.48, H 7.55.

Manganese dioxide oxidation product (7)

1 g. of active manganese dioxide[23] was added to a solution of 155 mg. methyl Hirsutate in 15 ml. chloroform and the mixture

was shaken for 1 day. The solution was filtered and the solvent removed in vacuo. Crystallization from ether-petroleum ether, followed by two recrystallizations from benzene-petroleum ether gave a 50% yield of colorless prisms; m.p. 103-104°; $[\alpha]_D^{22}$ - 96° (c = 2.23); T.L.C. (benzene:ether, 1:1): R_f = 0.8; U.V.: 231 mμ (ϵ = 5350); I.R. ($CHCl_3$): 2980 s, 1727 vs, 1720 vs, 1645 m, 1170 s, 915 m, 885 m; N.M.R.: 3.92 s, 4.69 s (Ha, Hb, JHaHb = 0), 6.58 s (Hd), 6.34 s (ester methyl), 8.62 s (C_{13} methyl), 8.82 s (C_{15} methyl), 7.3 - 8.1 - 9.0 (8 skeleton protons); Anal. Found: C 69.61, H 7.34, $C_{16}H_{20}O_4$ requires: C 69.54, H 7.30.

Dihydromethyl hirsutate (8)

70 mg. of 5% Pd/charcoal catalyst was added to 6 ml. ethyl acetate and hydrogenated; after 45 min. 50 mg. of methyl hirsutate was introduced and shaking continued for 35 min.; the reaction was over in 3 min. with 1 mole equivalent of hydrogen being absorbed. The solution was filtered through a celite pad and the solvent removed in vacuo. Two recrystallizations from ethyl acetate gave a 25% yield of colorless prisms; m.p.: 119 - 120°; $[\alpha]_D^{23}$ + 25° (c = 1.70); T.L.C. (benzene:ether, 1:1): R_f = 0.4; I.R. ($CHCl_3$): 3600 w, 3460 w, 2980 s, 1720 vs, 1165 vs, 920 m, 885 w; N.M.R.: 5.9 b (Hc), 6.77 d (Hd, JHcHd = 2.0), 6.40 s (ester methyl), 8.70 s (C_{13} methyl), 9.10 s (C_{15} methyl), 9.05 d (C_{14} methyl, J = 6.0); Anal. Found: C 67.99, H 8.48, $C_{16}H_{24}O_4$ requires: C 68.54, H 8.64.

X-ray irradiation: 45 mg. of dihydromethyl hirsutate was

bombarded for 50 hours. After irradiation the sample was a liquid and T.L.C. (alumina plate, $CHCl_3$) indicated that about 70% rearrangement had occurred. The spectral data can be rationalized by assuming the irradiated sample contains 30% starting material and 70% rearrangement product (17). The latter is believed to be the dihydro compound analogous to (16). U.V.: 288 sh (ϵ =25); I.R. (oil): 3450 s, 2960 s, 1730 vs, 1720 vs, 1165 s, 915 w; N.M.R. ($CHCl_3$): 5.9 b (1/3 H, Hc), 6.77 d (1/3 H, Hd), 6.34 s (1H), 6.36 s (2H, ester methyl), 7.62 s (4/3 H, C_6 methylene), 8.69 s (3H, C_{13} methyl), 8.96, 9.09 s (6 H, C_{15} methyl and C_{14} methyl, J = 8), C_6H_6: 6.94 d (1/3H), 6.54 s (1H), 6.59 s (2H), 7.74 (4/3 H), 8.74 s (3H), 8.95,9.04,9.06,9.15 s (6H, J=7).

Osmium tetroxide oxidation product (9)

A solution of 200 mg. methyl hirsutate (0.72 mmoles) and 267 mg. of osmium tetroxide (1.05 mmoles) in 10 ml. dry dioxan was allowed to stand for 14 days protected from light. The black solution was saturated with hydrogen sulfide for 30 min. and then the osmium sulfide precipitate removed by filtration through a celite pad. After removal of the solvent in vacuo, recrystallization from ethanol gave a 10% yield of triol as a colorless powder; m.p. 165-173°; $[\alpha]_D^{22}$ 0° (c = 1.27), I.R. ($CHCl_3$): 3500 s, 2970 s, 1725 vs, 1180 s, 915 m, 875 w; N.M.R. (DMF): 6.35 s (ester methyl), 8.61 s (C_{13} methyl), 9.08 s (C_{15} methyl), 5.7 - 8.4 - 9.0 (other protons poorly resolved); Anal. Found: C 60.94, H 7.44, $C_{16}H_{24}O_6$ requires: C 61.52, H 7.75.

Ozonolysis

This experiment was carried out by Dr. I.H. Qureshi.

To a solution of 53 mg.methyl hirsutate in 20 ml. dichloromethane at -78° was bubbled a stream of ozonized oxygen for 1 hour. The ozonide was decomposed by warming with water and the reaction mixture steam distilled. The distillate was treated with 2,4-dinitrophenylhydrazine. Recrystallization from ethanol gave yellow crystals; m.p. 162-163°, undepressed mixed m.p. with authentic formaldehyde 2, 4-dinitrophenylhydrazone derivative.

4β, 5-epoxy-5β-cholestan-3β-ol (21)

Cholest-4-en-3-one (19) was prepared from cholesterol (18) by oxidation with Jones' Reagent [24,25] followed by isomerization with oxalic acid according to Fieser.[26]

Cholest-4-en-3β-ol (20) was prepared by lithium aluminum hydride reduction of cholest-4-en-3-one (19) followed by separation from the α-isomer via the digitonide according to McKennis and Gaffney[27]. Oxidation of (20) with m-chloroperbenzoic acid[28] in chloroform at 0° for 36 hours gave a homogeneous oil which eventually crystallized from methanol. Two recrystallizations from methanol gave 4β, 5-epoxy-5β-cholestan-3β-ol (21) as colorless prisms; m.p. 95-97°, $[\alpha]_D^{20}$ + 5.7° (c = 3.14), I.R. (nujol): 3450 s; (cf. Plattner[28]: m.p. 95-96°, $[\alpha]_D$ + 5.4°).

X-ray irradiation: a few mg. of 4β, 5-epoxy-5β-cholestan-3β-ol (21) was bombarded for 2 days. However, T.L.C. evidence,

an undepressed m.p. and negligible absorption in the U.V. spectrum confirmed that no rearrangement had occurred.

4β, 5-epoxy-5β-cholestan-3∝-ol (23)

4β, 5-epoxy-5β-cholestan-3-one (22) was prepared from cholest-4-en-3-one (19) by alkaline hydrogen peroxide epoxidation according to Plattner.[29] Catalytic hydrogenation of (22)[29] gave a crude mixture which failed to yield a sharp melting compound by successive recrystallizations from methanol. However column chromatography with grade III Woelm neutral alumina[30] and elution with benzene, followed by recrystallization from methanol gave 4β, 5-epoxy-5β-cholestan-3∝-ol (23) as colorless prisms; m.p. 163-164°, $[\alpha]_D^{20}$ + 25° (c = 0.41), I.R. (nujol): 3450 s (sharp); (cf. Plattner[29] m.p. 158-159°, $[\alpha]_D$ + 31.4°; Collins[30] m.p. 161-162°, $[\alpha]_D$ + 25°).

X-ray irradiation: a few mg. of 4β, 5-epoxy-5β-cholestan-3∝-ol (23) was bombarded for 2 days yielding material of depressed m.p. 154-161°. 30 mg. was irradiated for 1 week yielding material of m.p. 140-157°. Comparative infrared spectra (KBr) of starting material and the irradiated sample showed broad absorption in the carbonyl region of the latter with a weak maximum at 1715 cm^{-1}. The U.V. spectrum of the irradiated sample also possessed a maximum at 240 mμ (ε = 236). T.L.C. (alumina, benzene:ether 4:1) showed the major component was starting material, ca. 90-95%, R_f = 0.52, but at least four new products also were indicated: R_f = 0.03, ca. 5%; R_f = 0.82, ca. 1%;

R_f = 0.35, ca. 0.1%; R_f = 0.10, ca. 0.1%. Of the new products, the major component (ca. 5%) as well as one of the minor ones (R_f = 0.10) remain unidentified. The 1% component (R_f = 0.82) is believed to be cholest-4-en-3-one (19) by comparative T.L.C. and U.V. evidence. (From the U.V. data the amount of cholest-4-en-3-one (λmax 240 mμ, ϵ = 16,000)[31] could not be more than 1.5%.) The other minor component has the same R_f value (0.35) as the expected rearrangement product, 5β-hydroxycholestan-3-one (25).

5β-hydroxycholestan-3-one (25)

Lithium aluminum hydride reduction of 4β, 5-epoxy-5β-cholestan-3-one (22) according to Plattner[28] gave a mixture of the 3, 5β-dihydroxycholestane isomers. Oxidation of the mixture with Jones' reagent[32] and successive recrystallizations from methanol[33] and n-hexane gave 5β-hydroxycholestan-3-one (25) as colorless plates; m.p. 159-161°, $[\alpha]_D^{24}$ + 45°, I.R. (nujol): 3400 s (sharp), 1720 s; (cf. Plattner[33] m.p. 152-153°, $[\alpha]_D$ + 62.3°; Burgess[32] m.p. 158-159°).

X-RAY DATA:

Crystals of the p-bromophenacyl ester of hirsutic acid C are colorless prisms elongated along a, with (001) and (010) developed. The density was measured by flotation in aqueous potassium iodide; and the unit-cell dimensions and space group were determined from various rotation, Weissenberg and precession photographs and on the G.E. spectrogoniometer. During the

analysis it became apparent that irradiation was initiating a molecular rearrangement, which, however, produced only very minor changes in crystal structure. One manifestation of this rearrangement was a small change in unit-cell parameters; unit-cell dimensions corresponding as closely as possible to those of the unirradiated crystals were determined from films with relatively short exposures, and the final cell dimensions were measured on the Spectrogoniometer after the completion of the intensity measurements.

Crystal Data (λ, Cu-K_α = 1.5418 Å; λ, Mo-K_α =0.7107 Å) p-bromophenacyl ester of hirsutic acid, $C_{23}H_{25}O_5Br$; M = 461.3. Orthorhombic, initial: a = 6.49 + 0.03, b = 9.14 + 0.03, c = 35.64 + 0.08 Å, final: a = 6.56 + 0.03, b = 9.38 + 0.03, c = 35.14 + 0.08 Å. U = 2114 $Å^3$ (initial), 2162 $Å^3$ (final); D_m = 1.46 + 0.01 (unirradiated crystal), Z = 4, D_c = 1.45 (initial), 1.42 (final). Absorption coefficient for X-rays, μ(Cu-K_α) = 31 cm^{-1}. F (000) = 952. Space group $P2_12_12_1$ (D_2^4); absent spectra: hoo when h is odd, oko when k is odd, ool when l is odd.

Three crystals were used to obtain two complete sets of three-dimensional intensity data. The two main crystals had dimensions 1.1, 0.02 and 0.1 mm. parallel to a,b and c respectively. One crystal was used to record the intensities on film, and the other for direct intensity measurement by counter techniques. In the film measurements, the intensities of the 0kl, 1kl ... 4kl layers were recorded on equi-inclination

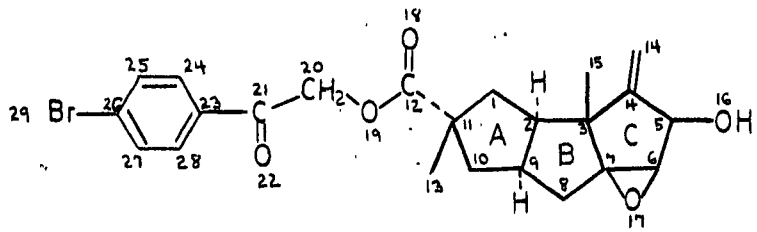
Weissenberg films with Cu-K_α radiation, and were estimated visually. The layers were scaled initially by timing the exposures. A third, larger crystal was used for recording the weakest reflexions. A total of 800 reflexions was observed, 150 of these being very weak and visible only on the films of the larger crystal.

For the counter data, the crystal was similarly mounted about the a axis. The intensities of all reflexions with 2Θ (Cu-K_α) $\leqslant 102^\circ$ (corresponding to a minimum interplanar spacing d = 0.99 Å) were measured on a General Electric XRD-5 Spectrogoniometer, with Single-crystal Orienter, scintillation counter, approximately monochromatic Cu-K_α radiation (nickel filter and pulse-height analyser) and using the moving crystal-moving counter technique.[13] Of the 1379 reflexions in the range $0 < 2\Theta \leqslant 102^\circ$, 798 had intensities greater than twice the background, and these were used in the structure determination and refinement. Final structure factors were calculated also for the 186 reflexions with intensity between one and two times the background, and for the 395 unobserved reflexions. For both the visual and counter data, the appropriate Lorentz and polarization factors were applied and the structure amplitudes derived. No absorption corrections were considered necessary.

Structure Analysis:

The visual data, obtained first, was used in the preliminary stages of the structural analysis. The position of the bromine atom was determined from the three Harker sections of

the three-dimensional Patterson function as (0.101, 0.216, 0.146), and a three-dimensional Fourier series was summed with phases based on the bromine atom. On the resulting electron-density distribution 28 of the 29 non-hydrogen atoms in the molecule were resolved, the epoxide oxygen atom (atom 17) being the missing atom. Structure factors were calculated for the 28 atoms



using the scattering factors for Br, C, O of the International Tables for X-ray Crystallography,[4] and R, the usual discrepancy factor, was 0.44. A second Fourier series was summed with phases based on all 28 atoms, but the electron density map did not reveal the final atom, and in fact suggested some errors in the location of the atoms and substituents of ring C; atom 16 was rather poorly defined, and there was considerable electron-density near atoms 16 and 6. The electron-density map was recalculated, with all the atoms of ring C omitted from the phasing, but this only confirmed the positions already deduced. The positional and isotropic thermal parameters were then refined by (block-diagonal) least-squares using a program for the IBM 1620 computer.[34] The function minimized was $\sum w\,(|F_o| - |Fc|)^2$, with $\sqrt{w} = |Fo|/25$ when $|Fo| < 25$ and $\sqrt{w} = 25/Fo$ when $|Fo| \geqslant 25$. Four cycles reduced R to 0.34. The scale factor of each layer

was then adjusted separately by application of the relation: $k|Fo| = |Fc| \exp(\Delta B \sin^2\theta/\lambda^2)$, and a further three least-squares cycles reduced R to 0.26.

At this stage the intensities were measured with the counter equipment, and the analysis proceeded using the 798 reflexions with intensities greater than twice background. Since the measurements were reliable for all but the very weak reflexions, the weighting scheme used was $\sqrt{w} = 1$ when $|Fo| \geqslant 25$ and $\sqrt{w} = |Fo|/25$ when $Fo < 25$. At this stage also, the IBM 7040 computer was available and the computations were carried out using Fortran iv programmes devised by Professor Trotter. Two least-squares cycles using the coordinates of the 28 atoms reduced R from 0.196 to 0.170. In an effort to locate the remaining atom three separate Fourier series were summed, with phases based on: (i) all 28 atoms, (ii) all atoms except 5,6,7,16, (iii) atoms of the p-bromophenacyl group only. In addition, a difference synthesis based on the phases of all atoms except 5,6,7,16 was carried out. The three electron-density distributions obtained from the Fourier series summations were all very similar; they confirmed the positions of the atoms already found, and could best be interpreted by placing "half-atoms" at positions 16, 16^1 and 17, 17^1 (see Figure 4). The difference synthesis provided further support for this interpretation. Four cycles of least-squares reduced R to 0.124. Attempts to refine structures with full atoms in any of the positions 16, 16^1, 17, 17^1 gave anomalously high thermal parameters for these atoms, and gave larger R values. The chemical

nature of these "half atoms" and the bond distances involving them (discussed later) indicate that they are oxygen atoms. A final set of structure factors was calculated for all planes with 2θ (Cu-K_α) $\leqslant 102^\circ$; the measured and calculated values for the 1379 planes are given in Table 1, R being 0.136 for the 984 observed reflexions. A final three-dimensional Fourier series was summed, and superimposed sections of the electron-density distribution taken through the atomic centers are shown in Figure 5; a perspective drawing of the molecule is shown in Figure 4.

Refinement with the less accurate visual data gave similar results. However the electron-density distribution favored the structure with atoms at positions 16, 17. This preference was also revealed in the discrepancy value: four least-squares cycles using "half-atoms" at positions 16, 16^1, 17, 17^1 reduced R to 0.214; when "full atoms" were used at positions 16,17, R was correspondingly reduced to 0.216, insignificantly different. The visual structure factor data for the latter case are given in Table 1A.

At this point, the X-ray structure analysis was complete, and some chemical information was required to clarify the anomalies in the X-ray results. The original crystals were pure p-bromophenacyl ester, but the results of the X-ray analysis suggested that after irradiation there was a mixture of at least two compounds in the crystal, distributed randomly. Thus it appeared that the molecular structure, but not the crystal

Table 1. Measured and calculated structure amplitudes (Counter data).

h	k	l	F_o	F_c
0	0	2	33.8	52.9
0	0	4	22.6	22.5
0	0	6	83.9	95.0
0	0	8	10.0	26.6
0	0	10	108.4	121.4
0	0	12	68.2	66.5
0	0	14	61.8	71.1
0	0	16	25.6	24.8
0	0	18	49.7	54.8
0	0	20	32.4	35.2
0	0	22	6.0	3.9
0	0	24	26.6	28.4
0	0	26	6.7	4.2
0	0	28	20.8	22.9
0	0	30	6.3	2.2
0	0	32	0.	0.1
0	0	34	8.2	6.2
0	2	0	231.7	240.4
0	2	2	123.7	126.3
0	2	4	104.8	109.6
0	2	6	66.0	68.7
0	2	8	18.2	26.4
0	2	10	132.6	138.8
0	2	12	15.8	16.6
0	2	14	12.5	22.2
0	2	16	35.0	41.5
0	2	18	48.4	54.1
0	2	20	21.8	28.2
0	2	22	9.2	9.1
0	2	24	12.2	18.3
0	2	26	17.1	15.2
0	2	28	12.3	11.0
0	2	30	8.9	7.8
0	2	32	0.	8.5
0	2	34	0.	6.3
0	4	0	60.8	57.0
0	4	2	48.1	47.8
0	4	4	21.1	23.3
0	4	6	36.7	35.9
0	4	8	30.1	25.0
0	4	10	58.2	58.2
0	4	12	35.1	31.4
0	4	14	40.4	44.9
0	4	16	9.5	11.6
0	4	18	12.1	15.0
0	4	20	10.8	9.4
0	4	22	0.	2.3
0	4	24	11.7	14.7
0	4	26	12.0	14.3
0	4	28	9.7	5.8
0	4	30	0.	3.1
0	4	32	0.	4.9
0	6	0	0.	4.8
0	6	2	5.3	7.8
0	6	4	10.7	6.7
0	6	6	7.0	5.8
0	6	8	14.3	13.5
0	6	10	8.0	6.5
0	6	12	4.7	3.4
0	6	14	9.1	6.6
0	6	16	21.4	19.4
0	6	18	6.2	3.0
0	6	20	0.	0.1
0	6	22	0.	3.1
0	6	24	0.	0.7
0	6	26	0.	3.5
0	8	0	5.1	0.7
0	8	2	0.	1.4
0	8	4	5.2	1.5
0	8	6	0.	3.1
0	8	8	0.	0.9
0	8	10	0.	0.1
0	8	12	0.	3.9
0	8	14	0.	3.0
0	8	16	0.	2.8
0	8	18	0.	3.3
1	1	1	54.9	48.0
1	1	3	91.1	89.7
1	1	5	144.8	139.3
1	1	7	41.9	39.6
1	1	9	51.8	49.9
1	1	11	23.8	17.8
1	1	13	23.5	29.5
1	1	15	32.2	32.7
1	1	17	8.9	2.5
1	1	19	29.7	34.1
1	1	21	18.7	16.9
1	1	23	18.6	21.3
1	1	25	8.1	7.3
1	1	27	13.4	16.2
1	1	29	10.1	11.7
1	1	31	0.	1.0
1	1	33	0.	0.5
1	3	1	16.0	12.5
1	3	3	52.9	49.4
1	3	5	37.8	42.1
1	3	7	81.0	78.9
1	3	9	23.8	24.9
1	3	11	23.5	23.8
1	3	13	35.3	38.0
1	3	15	10.6	15.5
1	3	17	25.8	26.9
1	3	19	11.4	16.4
1	3	21	11.6	16.2
1	3	23	13.5	14.9
1	3	25	9.3	12.7
1	3	27	0.	5.3
1	3	29	10.9	15.2
1	3	31	5.1	7.3
1	3	33	0.	3.2
1	5	1	30.7	28.8
1	5	3	53.3	51.1
1	5	5	11.6	13.9
1	5	7	27.5	25.4
1	5	9	13.2	12.5
1	5	11	18.3	19.4
1	5	13	18.6	18.9
1	5	15	10.3	8.9
1	5	17	15.8	14.6
1	5	19	13.0	13.9
1	5	21	10.7	13.3
1	5	23	10.3	9.0
1	5	25	8.2	9.1
1	5	27	6.3	5.2
1	5	29	0.	4.9
1	7	1	6.9	7.9
1	7	3	20.7	17.0
1	7	5	10.5	8.7
1	7	7	23.1	21.6
1	7	9	6.2	7.2
1	7	11	7.2	9.1
1	7	13	0.	4.8
1	7	15	3.7	6.3
1	7	17	7.4	11.2
1	7	19	0.	3.5
1	7	21	8.2	7.5
1	9	1	0.	1.3
1	9	3	0.	2.6
1	9	5	5.1	4.8
1	9	7	0.	9.4
2	0	0	0.	6.9
2	0	2	21.5	31.8
2	0	4	24.5	18.8
2	0	6	21.7	27.1
2	0	8	9.0	1.8
2	0	10	20.5	17.7
2	0	12	27.2	29.5
2	0	14	19.7	16.9
2	0	16	3.8	7.7
2	0	18	7.1	18.1
2	0	20	0.	3.7
2	0	22	0.	0.7
2	0	24	7.6	6.6
2	0	26	13.2	14.3
2	0	28	0.	0.8
2	0	30	7.4	9.9
2	0	32	0.	5.8
2	2	0	27.6	26.9
2	2	2	30.1	27.8
2	2	4	10.9	6.8
2	2	6	51.3	47.3
2	2	8	22.4	22.0
2	2	10	24.9	27.4
2	2	12	29.1	28.4
2	2	14	0.	2.9
2	2	16	17.0	17.7
2	2	18	9.0	11.0
2	2	20	8.3	12.9
2	2	22	0.	8.1
2	2	24	0.	7.7
2	2	26	6.2	11.1
2	2	28	0.	4.9
2	2	30	0.	1.0
2	2	32	0.	1.6
2	4	0	9.9	1.5
2	4	2	42.7	41.3
2	4	4	24.8	24.4
2	4	6	45.3	40.0
2	4	8	25.2	22.6
2	4	10	18.4	16.2
2	4	12	30.1	28.6
2	4	14	20.9	20.9
2	4	16	15.2	19.7
2	4	18	16.3	14.9
2	4	20	10.4	12.1
2	4	22	14.2	16.6
2	4	24	5.1	8.4
2	4	26	8.2	12.1
2	4	28	0.	0.5
2	4	30	0.	2.5
2	6	0	0.	5.7
2	6	2	21.3	15.6
2	6	4	18.8	17.0
2	6	6	18.7	16.8
2	6	8	17.7	15.9
2	6	10	11.5	10.5
2	6	12	25.4	24.6
2	6	14	15.2	10.5
2	6	16	0.	6.2
2	6	18	9.7	7.4
2	6	20	5.2	6.4
2	6	22	9.8	10.2
2	6	24	0.	1.4
2	8	0	0.	1.2
2	8	2	7.4	5.5
2	8	4	6.4	7.5
2	8	6	0.	4.3
2	8	8	0.	5.8
2	8	10	0.	7.5
2	8	12	14.2	13.2
2	8	14	0.	3.7
3	1	1	37.0	28.3
3	1	3	39.5	37.4
3	1	5	62.6	53.0
3	1	7	26.3	21.7
3	1	9	23.1	20.4
3	1	11	35.8	37.4
3	1	13	24.3	24.9
3	1	15	29.2	33.8
3	1	17	0.	4.5
3	1	19	15.6	18.1
3	1	21	13.1	13.8
3	1	23	12.7	14.4
3	1	25	13.0	14.4
3	1	27	0.	3.1
3	1	29	8.1	9.7
3	1	31	0.	4.1
3	3	1	35.5	28.6
3	3	3	40.5	38.7
3	3	5	34.9	31.1
3	3	7	28.9	25.8
3	3	9	30.8	25.2
3	3	11	19.3	14.5
3	3	13	7.0	8.5
3	3	15	27.3	24.9
3	3	17	16.8	19.6
3	3	19	15.5	13.2
3	3	21	10.7	11.8
3	3	23	0.	6.4
3	3	25	0.	5.1
3	3	27	0.	3.0
3	3	29	0.	5.8
3	5	1	18.4	14.4
3	5	3	15.4	14.5
3	5	5	15.6	11.8
3	5	7	13.0	11.8
3	5	9	16.7	11.5
3	5	11	6.0	11.2
3	5	13	10.6	12.4
3	5	15	12.5	9.1
3	5	17	20.7	18.8
3	5	19	24.2	21.4
3	5	21	6.4	5.1
3	5	23	0.	2.9
3	5	25	0.	2.6
3	7	1	5.2	3.7
3	7	3	5.2	6.2
3	7	5	5.2	5.0
3	7	7	0.	12.6
3	7	9	6.4	6.8
3	7	11	0.	5.9
3	7	13	0.	7.6
3	7	15	0.	3.3
3	7	17	0.	7.7
4	0	0	42.6	36.4
4	0	2	7.8	10.9
4	0	4	29.6	25.9
4	0	6	11.3	3.9
4	0	8	24.3	20.5
4	0	10	16.1	17.9
4	0	12	7.3	3.3
4	0	14	31.7	33.7
4	0	16	19.4	23.0
4	0	18	10.6	10.3
4	0	20	12.5	15.4
4	0	22	0.	0.7
4	0	24	0.	5.5
4	0	26	0.	3.5
4	0	28	6.2	4.7
4	2	0	45.0	39.6
4	2	2	13.5	10.8
4	2	4	19.0	17.0
4	2	6	13.8	9.9
4	2	8	22.7	22.1
4	2	10	20.6	20.4
4	2	12	9.4	6.1
4	2	14	21.3	18.0
4	2	16	4.9	12.0
4	2	18	14.6	17.9
4	2	20	10.2	11.0
4	2	22	0.	2.0
4	2	24	9.6	7.6
4	2	26	0.	7.1
4	4	0	22.2	21.9
4	4	2	9.6	9.9
4	4	4	15.7	14.5
4	4	6	0.	5.0
4	4	8	17.1	16.0
4	4	10	10.0	13.5
4	4	12	0.	5.3
4	4	14	8.9	7.3
4	4	16	0.	4.9
4	4	18	12.2	8.9
4	4	20	0.	1.9
4	4	22	6.3	3.1
4	6	0	0.	3.5
4	6	2	6.4	7.9
4	6	4	0.	4.3
4	6	6	0.	4.3
4	6	8	6.4	7.9
4	6	10	0.	3.4
4	6	12	0.	2.5
4	6	14	0.	3.3
4	6	16	0.	2.7
5	1	1	4.9	6.4
5	1	3	14.0	11.8
5	1	5	6.0	9.2
5	1	7	0.	2.4
5	1	9	5.0	4.1
5	1	11	0.	2.9
5	1	13	0.	2.4
5	1	15	0.	4.9
5	1	17	5.2	3.5
5	1	19	0.	3.1
5	1	21	0.	4.2
5	3	1	7.2	5.2
5	3	3	15.8	11.9
5	3	5	6.3	6.8
5	3	7	5.2	3.4
5	3	9	5.2	5.7
5	3	11	0.	5.0
5	3	13	0.	4.5
5	3	15	0.	1.6
5	3	17	0.	3.7
5	3	19	0.	2.9
5	5	1	0.	4.0
5	5	3	5.2	5.2
5	5	5	0.	4.4
5	5	7	12.2	11.7
5	5	9	0.	2.8
5	5	11	5.2	6.0
5	5	13	6.2	2.5
6	0	0	3.7	10.2
6	0	2	10.4	12.3
6	0	4	5.2	1.6
6	0	6	0.	0.3
6	0	8	0.	5.7
6	0	10	0.	8.0
6	0	12	0.	1.5
6	0	14	0.	7.0
6	2	0	0.	1.4
6	2	2	6.4	4.3
6	2	4	0.	5.0
6	2	6	0.	2.9
6	2	8	0.	0.9
6	2	10	5.1	3.9
6	2	12	0.	1.9
0	2	1	79.0	81.5
0	2	3	17.5	12.5
0	2	5	15.8	22.3
0	2	7	8.2	12.5
0	2	9	17.5	14.2
0	2	11	19.5	20.3
0	2	13	38.8	39.7
0	2	15	29.8	35.8
0	2	17	12.9	18.4
0	2	19	18.4	22.9
0	2	21	16.4	16.6
0	2	23	10.0	11.9
0	2	25	9.4	15.5
0	2	27	0.	0.2
0	2	29	0.	8.7
0	2	31	5.2	7.8
0	2	33	0.	0.3
0	4	1	13.4	9.1
0	4	3	25.5	18.1
0	4	5	46.5	49.6
0	4	7	11.8	17.0
0	4	9	39.5	42.1
0	4	11	26.9	26.1
0	4	13	15.5	14.3
0	4	15	32.2	32.7
0	4	17	16.7	18.4
0	4	19	5.5	8.2
0	4	21	0.	2.1
0	4	23	11.0	15.7
0	4	25	6.2	15.0
0	4	27	8.9	9.6
0	4	29	12.7	13.0
0	4	31	0.	1.6
0	6	1	16.4	16.1
0	6	3	31.0	29.4
0	6	5	15.3	12.5
0	6	7	5.5	4.4
0	6	9	28.1	22.1
0	6	11	30.3	30.9
0	6	13	0.	5.0
0	6	15	16.7	16.1
0	6	17	0.	6.4
0	6	19	8.9	12.8
0	6	21	0.	0.9
0	6	23	0.	4.3
0	6	25	11.5	14.6
0	6	27	0.	0.9
0	8	1	25.5	23.4
0	8	3	0.	2.6
0	8	5	0.	3.0
0	8	7	0.	1.4
0	8	9	0.	7.6
0	8	11	6.4	7.3
0	8	13	0.	2.8
0	8	15	5.2	7.6
0	8	17	0.	1.1
1	1	0	118.8	112.3
1	1	2	64.3	60.7
1	1	4	44.9	44.0
1	1	6	86.4	92.4
1	1	8	115.7	115.4
1	1	10	48.3	51.6
1	1	12	36.0	43.4
1	1	14	28.3	23.4
1	1	16	42.7	50.4
1	1	18	39.5	44.5
1	1	20	10.3	9.9
1	1	22	9.2	13.2
1	1	24	10.2	10.1
1	1	26	14.4	18.8
1	1	28	8.7	9.5
1	1	30	0.	2.9
1	1	32	7.3	6.0
1	1	34	0.	4.5
1	3	0	35.6	37.9
1	3	2	37.5	40.3
1	3	4	46.1	46.2
1	3	6	31.2	27.4
1	3	8	17.7	24.5
1	3	10	55.0	51.7
1	3	12	26.3	26.8
1	3	14	19.5	20.5
1	3	16	17.0	21.9
1	3	18	31.1	30.8
1	3	20	11.7	13.7
1	3	22	15.8	21.8
1	3	24	0.	5.6
1	3	26	10.6	13.9
1	3	28	0.	5.4
1	3	30	0.	7.7
1	3	32	0.	6.0
1	5	0	36.9	43.3
1	5	2	11.7	14.2
1	5	4	27.4	23.7
1	5	6	38.2	38.6
1	5	8	13.7	13.5
1	5	10	25.6	21.9
1	5	12	0.	7.1
1	5	14	17.6	16.9
1	5	16	5.7	8.5
1	5	18	0.	2.4
1	5	20	10.0	11.9
1	5	22	14.8	12.4
1	5	24	0.	5.7
1	5	26	6.4	7.6
1	5	28	5.1	5.5
1	7	0	12.9	12.9
1	7	2	0.	0.5
1	7	4	16.7	16.6
1	7	6	18.6	16.9
1	7	8	0.	4.3
1	7	10	0.	4.1
1	7	12	0.	7.9
1	7	14	0.	2.1
1	7	16	13.3	13.8
1	7	18	5.2	3.9
1	7	20	0.	7.9
1	7	22	0.	1.0
1	9	0	0.	2.1
1	9	2	0.	1.0
1	9	4	7.3	7.3
1	9	6	0.	4.9
1	9	8	0.	2.5
2	0	1	25.6	26.2
2	0	3	137.2	125.5
2	0	5	25.4	20.1
2	0	7	80.8	75.2
2	0	9	21.6	24.3
2	0	11	86.9	86.9
2	0	13	83.8	85.5
2	0	15	25.4	21.3
2	0	17	19.0	25.5
2	0	19	11.1	11.5
2	0	21	22.2	24.2
2	0	23	23.4	26.3
2	0	25	6.0	6.0
2	0	27	0.	3.4
2	0	29	0.	0.4
2	0	31	0.	8.0
2	0	33	0.	4.0
2	2	1	42.1	34.4
2	2	3	91.0	83.9
2	2	5	16.3	21.9
2	2	7	47.1	48.0
2	2	9	20.3	17.3
2	2	11	57.5	60.8
2	2	13	50.0	57.3
2	2	15	6.2	3.3
2	2	17	33.3	35.0
2	2	19	6.9	5.7
2	2	21	12.1	27.3
2	2	23	21.9	24.8
2	2	25	7.0	10.9
2	2	27	5.1	7.8
2	2	29	0.	2.4
2	2	31	5.1	7.6
2	4	1	23.7	20.7
2	4	3	36.4	39.4
2	4	5	18.6	16.3
2	4	7	12.1	13.3
2	4	9	0.	6.3
2	4	11	22.9	19.1
2	4	13	22.7	25.2
2	4	15	22.3	25.0
2	4	17	13.1	14.9
2	4	19	4.8	11.1
2	4	21	12.6	18.7
2	4	23	11.4	13.2
2	4	25	0.	4.3
2	4	27	6.3	5.5
2	4	29	0.	2.3
2	6	1	5.7	4.2
2	6	3	13.6	13.3
2	6	5	17.0	14.2
2	6	7	0.	7.3
2	6	9	6.9	9.1
2	6	11	0.	4.0
2	6	13	19.5	19.0
2	6	15	17.7	15.8
2	6	17	0.	2.7
2	6	19	0.	2.1
2	6	21	0.	3.1
2	6	23	0.	3.2
2	8	1	0.	2.8
2	8	3	3.7	2.7
2	8	5	5.2	7.1
2	8	7	0.	1.2
2	8	9	0.	8.7
2	8	11	0.	4.0
2	8	13	0.	1.5
2	8	15	0.	4.0
3	1	0	0.	5.0
3	1	2	19.1	20.1
3	1	4	21.7	25.2
3	1	6	37.8	39.1
3	1	8	36.9	41.8
3	1	10	14.8	23.0
3	1	12	8.4	12.2
3	1	14	6.5	5.9
3	1	16	0.	4.4
3	1	18	8.5	11.0
3	1	20	7.4	8.5
3	1	22	0.	4.0
3	1	24	5.0	1.2
3	1	26	0.	3.0
3	1	28	0.	3.7
3	1	30	0.	4.0
3	3	0	45.9	44.1
3	3	2	17.4	14.5
3	3	4	17.5	14.7
3	3	6	9.0	12.9
3	3	8	8.3	10.6
3	3	10	32.3	31.4
3	3	12	6.9	5.2
3	3	14	11.9	12.9
3	3	16	10.9	15.4
3	3	18	21.8	27.1
3	3	20	15.7	19.8
3	3	22	0.	2.8
3	3	24	7.3	7.6
3	3	26	6.3	7.7
3	3	28	0.	6.1
3	5	0	31.9	28.2
3	5	2	17.3	12.6
3	5	4	20.9	18.9
3	5	6	14.2	13.7
3	5	8	16.9	16.3
3	5	10	20.4	17.4
3	5	12	0.	2.8
3	5	14	13.8	15.5
3	5	16	7.3	10.1
3	5	18	18.8	18.9
3	5	20	15.7	12.9
3	5	22	0.	0.4
3	5	24	0.	2.5
3	7	0	13.2	13.8
3	7	2	0.	2.5
3	7	4	14.8	14.1
3	7	6	0.	5.1
3	7	8	0.	4.1
3	7	10	11.7	10.6
3	7	12	0.	3.3
3	7	14	15.6	12.5
3	7	16	0.	1.2
4	0	1	0.	1.1
4	0	3	15.1	10.8
4	0	5	4.2	6.7
4	0	7	22.8	17.3
4	0	9	0.	7.5
4	0	11	0.	7.5
4	0	13	0.	9.1
4	0	15	0.	4.4
4	0	17	16.7	18.8
4	0	19	6.2	3.0
4	0	21	14.6	16.8
4	0	23	0.	0.8
4	0	25	0.	10.2
4	0	27	5.1	6.0
4	2	1	18.4	14.4
4	2	3	15.2	10.6
4	2	5	12.9	8.2
4	2	7	14.6	11.9
4	2	9	17.6	12.4
4	2	11	17.1	18.2
4	2	13	11.7	12.8
4	2	15	12.9	15.7
4	2	17	7.9	4.5
4	2	19	0.	3.9
4	2	21	0.	5.7
4	2	23	8.2	3.2
4	2	25	10.9	8.2
4	2	27	5.0	5.5
4	4	1	20.1	13.3
4	4	3	21.6	15.3
4	4	5	13.3	7.8
4	4	7	24.1	18.8
4	4	9	17.3	16.1
4	4	11	16.4	15.2
4	4	13	0.	7.1
4	4	15	10.4	7.0
4	4	17	9.7	6.2
4	4	19	0.	5.1
4	4	21	0.	4.6
4	4	23	8.0	7.8
4	6	1	18.0	13.4
4	6	3	6.4	3.0
4	6	5	12.3	7.4
4	6	7	0.	2.1
4	6	9	9.8	7.0
4	6	11	6.4	7.5
4	6	13	0.	2.5
4	6	15	0.	4.5
5	1	0	0.	5.2
5	1	2	37.2	27.9
5	1	4	27.1	22.3
5	1	6	11.6	7.2
5	1	8	27.8	20.8
5	1	10	0.	2.5
5	1	12	15.6	9.5
5	1	14	8.9	5.8
5	1	16	8.1	5.4
5	1	18	8.9	8.0
5	1	20	0.	7.2
5	1	22	10.1	10.0
5	3	0	0.	3.7
5	3	2	22.9	13.9
5	3	4	17.0	11.2
5	3	6	23.0	20.8
5	3	8	26.2	20.3
5	3	10	0.	5.0
5	3	12	16.5	11.7
5	3	14	5.2	2.9
5	3	16	0.	5.0
5	3	18	0.	2.7
5	5	0	0.	2.6
5	5	2	11.6	6.9
5	5	4	5.2	3.1
5	5	6	9.0	9.7
5	5	8	9.0	7.6
5	5	10	0.	3.4
5	5	12	0.	5.6
6	0	1	7.4	8.6
6	0	3	11.7	9.7
6	0	5	0.	7.0
6	0	7	0.	4.7
6	0	9	0.	0.0
6	0	11	0.	2.4
6	0	13	0.	4.8
6	2	1	5.2	5.1
6	2	3	12.2	12.9
6	2	5	7.4	7.0
6	2	7	0.	4.3
6	2	9	0.	4.7
6	2	11	8.0	6.0
0	1	3	23.6	33.7
0	1	5	49.0	48.8
0	1	7	22.7	26.7
0	1	9	29.5	32.8
0	1	11	51.1	59.6
0	1	13	27.8	30.8
0	1	15	35.4	42.6
0	1	17	26.9	37.1
0	1	19	0.	3.1
0	1	21	16.1	27.3
0	1	23	8.2	15.0
0	1	25	18.8	23.4
0	1	27	15.4	24.8

Table 1. continued:

0	1	29	0.	0.2
0	1	31	10.3	19.0
0	1	33	0.	7.1
0	1	35	9.4	12.9
0	3	1	83.6	78.6
0	3	3	60.5	58.7
0	3	5	7.0	14.5
0	3	7	32.3	38.9
0	3	9	7.1	0.8
0	3	11	13.2	3.0
0	3	13	7.9	6.9
0	3	15	17.4	17.2
0	3	17	18.9	25.8
0	3	19	5.2	2.4
0	3	21	19.2	25.1
0	3	23	4.7	2.6
0	3	25	15.9	19.2
0	3	27	10.7	14.3
0	3	29	0.	1.1
0	3	31	0.	0.9
0	3	33	0.	2.7
0	5	1	2.7	5.1
0	5	3	35.5	35.0
0	5	5	4.8	8.4
0	5	7	13.2	14.9
0	5	9	4.1	5.0
0	5	11	0.	6.1
0	5	13	0.	2.3
0	5	15	9.1	1.3
0	5	17	0.	2.2
0	5	19	10.9	8.5
0	5	21	7.9	12.4
0	5	23	0.	4.7
0	5	25	5.2	4.7
0	5	27	0.	2.0
0	5	29	0.	3.7
0	7	1	4.8	3.5
0	7	3	15.3	15.8
0	7	5	4.9	5.7
0	7	7	0.	2.9
0	7	9	12.2	12.5
0	7	11	0.	4.0
0	7	13	7.2	7.9
0	7	15	0.	5.7
0	7	17	5.2	0.9
0	7	19	0.	0.7
0	7	21	0.	3.2
0	7	23	0.	2.9
0	9	1	0.	4.8
0	9	3	10.3	10.0
0	9	5	6.3	4.3
0	9	7	0.	5.5
0	9	9	5.1	2.4
1	0	2	39.2	34.8
1	0	4	161.5	164.9
1	0	6	42.5	41.5
1	0	8	33.7	41.7
1	0	10	13.6	9.0
1	0	12	52.3	57.0
1	0	14	21.3	22.3
1	0	16	20.8	20.4
1	0	18	21.1	27.2
1	0	20	0.	1.1
1	0	22	19.3	24.7
1	0	24	0.	2.5
1	0	26	0.	9.4
1	0	28	16.3	22.1
1	0	30	11.6	11.3
1	0	32	12.2	19.3
1	0	34	0.	5.9
1	2	0	26.9	27.3
1	2	2	44.1	47.7
1	2	4	86.8	77.2
1	2	6	89.0	86.8
1	2	8	88.1	85.2
1	2	10	13.9	14.2
1	2	12	28.5	27.1
1	2	14	26.8	26.9
1	2	16	8.3	7.7
1	2	18	18.9	21.4
1	2	20	7.3	9.0
1	2	22	19.8	23.8
1	2	24	0.	11.8
1	2	26	12.9	14.1
1	2	28	6.2	6.5
1	2	30	0.	6.2
1	2	32	0.	9.8
1	2	34	0.	5.3
1	4	0	46.8	51.0
1	4	2	32.6	35.1
1	4	4	29.9	32.0
1	4	6	69.9	67.2
1	4	8	35.6	38.6
1	4	10	13.9	19.3
1	4	12	6.3	6.0
1	4	14	32.0	32.6
1	4	16	6.8	5.5
1	4	18	8.4	12.8
1	4	20	9.4	9.1
1	4	22	6.9	12.2
1	4	24	8.7	7.0
1	4	26	0.	3.8
1	4	28	0.	6.1
1	4	30	0.	4.9
1	6	0	24.1	23.6
1	6	2	8.8	9.6
1	6	4	24.0	21.8
1	6	6	11.5	8.5
1	6	8	10.3	12.9
1	6	10	23.0	23.7
1	6	12	0.	6.9
1	6	14	19.3	16.0
1	6	16	12.8	13.5
1	6	18	8.9	9.2
1	6	20	8.2	10.8
1	6	22	0.	2.5
1	6	24	0.	1.2
1	6	26	0.	1.4
1	8	0	16.4	14.5
1	8	2	0.	6.2
1	8	4	7.3	7.3
1	8	6	7.4	7.7
1	8	8	9.8	9.6
1	8	10	0.	7.4
1	8	12	0.	4.7
1	8	14	0.	6.6
1	8	16	0.	5.2
2	1	1	46.0	43.9
2	1	3	31.9	31.4
2	1	5	14.3	10.7
2	1	7	67.5	70.1
2	1	9	34.3	33.2
2	1	11	38.2	43.1
2	1	13	28.8	29.0
2	1	15	25.1	23.5
2	1	17	24.3	19.8
2	1	19	8.9	6.9
2	1	21	8.3	7.8
2	1	23	4.7	8.7
2	1	25	6.0	7.6
2	1	27	5.0	6.3
2	1	29	0.	8.6
2	1	31	0.	2.5
2	1	33	0.	1.5
2	3	1	37.5	29.6
2	3	3	20.0	22.3
2	3	5	29.5	24.5
2	3	7	22.7	16.3
2	3	9	48.6	48.0
2	3	11	61.8	61.4
2	3	13	6.9	9.7
2	3	15	21.0	21.6
2	3	17	6.9	11.7
2	3	19	17.9	15.7
2	3	21	11.2	6.5
2	3	23	4.9	3.4
2	3	25	13.0	16.4
2	3	27	8.1	6.8
2	3	29	5.2	5.7
2	3	31	0.	3.8
2	5	1	41.2	38.8
2	5	3	0.	12.8
2	5	5	22.6	21.9
2	5	7	0.	0.3
2	5	9	33.5	34.8
2	5	11	22.6	21.3
2	5	13	18.5	15.9
2	5	15	26.7	31.5
2	5	17	9.3	5.7
2	5	19	14.8	10.3
2	5	21	6.3	6.7
2	5	23	9.0	6.1
2	5	25	8.2	7.7
2	5	27	0.	2.5
2	7	1	18.5	17.7
2	7	3	15.9	15.5
2	7	5	5.1	13.0
2	7	7	5.1	5.3
2	7	9	12.1	11.7
2	7	11	7.3	12.1
2	7	13	11.7	7.6
2	7	15	13.9	14.7
2	7	17	0.	3.7
2	7	19	5.2	6.5
3	0	2	79.2	68.8
3	0	4	21.9	19.7
3	0	6	40.8	41.5
3	0	8	55.2	54.5
3	0	10	0.	16.2
3	0	12	61.0	57.6
3	0	14	12.7	6.7
3	0	16	16.8	17.6
3	0	18	13.2	13.4
3	0	20	9.4	10.9
3	0	22	17.0	19.9
3	0	24	0.	0.1
3	0	26	11.0	13.5
3	0	28	0.	4.0
3	0	30	0.	3.5
3	2	0	9.6	6.8
3	2	2	35.9	28.8
3	2	4	43.5	40.4
3	2	6	47.5	45.3
3	2	8	52.2	48.1
3	2	10	23.2	27.3
3	2	12	32.2	32.5
3	2	14	9.6	6.5
3	2	16	19.5	20.1
3	2	18	15.0	17.5
3	2	20	7.6	11.7
3	2	22	17.2	19.4
3	2	24	0.	5.3
3	2	26	0.	8.1
3	2	28	0.	2.5
3	2	30	0.	3.8
3	4	0	13.4	15.9
3	4	2	19.0	15.3
3	4	4	11.8	10.9
3	4	6	11.1	11.4
3	4	8	28.3	22.2
3	4	10	12.9	10.9
3	4	12	13.2	14.5
3	4	14	13.1	12.4
3	4	16	13.0	11.3
3	4	18	17.0	15.5
3	4	20	8.1	9.1
3	4	22	8.2	9.2
3	4	24	0.	3.4
3	4	26	8.1	8.1
3	6	0	6.1	4.5
3	6	2	5.0	4.1
3	6	4	3.5	4.0
3	6	6	0.	3.7
3	6	8	6.2	5.3
3	6	10	7.3	5.9
3	6	12	8.2	11.3
3	6	14	7.4	4.6
3	6	16	0.	3.4
3	6	18	11.1	10.9
3	6	20	0.	6.0
3	8	0	0.	2.0
3	8	2	0.	1.2
3	8	4	0.	2.1
3	8	6	0.	2.0
3	8	8	0.	2.8
4	1	1	29.9	23.9
4	1	3	44.6	36.9
4	1	5	15.1	11.3
4	1	7	33.2	26.8
4	1	9	7.6	5.7
4	1	11	18.9	19.3
4	1	13	16.4	18.6
4	1	15	7.5	11.6
4	1	17	19.6	22.5
4	1	19	0.	2.5
4	1	21	10.8	13.1
4	1	23	0.	7.8
4	1	25	0.	1.5
4	1	27	0.	6.4
4	3	1	24.1	25.2
4	3	3	21.5	20.7
4	3	5	4.6	9.2
4	3	7	18.8	15.0
4	3	9	8.3	7.8
4	3	11	18.2	17.4
4	3	13	19.0	19.5
4	3	15	0.	2.4
4	3	17	8.8	8.0
4	3	19	10.3	12.4
4	3	21	6.4	8.5
4	3	23	0.	5.7
4	3	25	0.	2.5
4	5	1	16.7	15.0
4	5	3	7.2	8.2
4	5	5	3.6	5.8
4	5	7	0.	4.2
4	5	9	0.	3.4
4	5	11	13.3	12.9
4	5	13	8.3	8.7
4	5	15	0.	4.4
4	5	17	0.	6.7
4	5	19	0.	3.8
4	7	1	5.2	2.8
4	7	3	0.	2.9
4	7	5	7.3	6.1
4	7	7	0.	3.0
4	7	9	0.	3.0
5	0	2	0.	0.7
5	0	4	0.	6.1
5	0	6	0.	1.3
5	0	8	8.6	12.9
5	0	10	0.	0.9
5	0	12	0.	1.4
5	0	14	0.	0.9
5	0	16	6.4	7.6
5	0	18	0.	3.6
5	0	20	0.	6.2
5	0	22	0.	1.9
5	2	0	5.0	4.6
5	2	2	5.0	4.1
5	2	4	9.4	9.8
5	2	6	7.2	7.2
5	2	8	8.8	11.2
5	2	10	9.5	12.7
5	2	12	0.	8.5
5	2	14	12.7	13.6
5	2	16	0.	1.9
5	2	18	0.	4.5
5	2	20	0.	2.7
5	4	0	14.2	13.9
5	4	2	0.	2.7
5	4	4	15.2	13.3
5	4	6	11.1	10.6
5	4	8	0.	5.8
5	4	10	0.	3.9
5	4	12	0.	3.2
5	4	14	12.7	10.8
5	4	16	0.	3.5
5	6	0	8.8	6.3
5	6	2	0.	3.4
5	6	4	6.2	8.5
6	1	1	13.9	7.7
6	1	3	13.9	7.1
6	1	5	0.	4.5
6	1	7	14.6	12.2
6	1	9	10.3	5.6
6	1	11	7.2	6.2
6	1	13	8.0	4.8
6	3	1	0.	2.7
6	3	3	7.3	2.7
6	3	5	5.2	4.1
6	3	7	10.9	7.7
0	1	2	44.7	47.2
0	1	4	87.6	91.8
0	1	6	77.9	77.7
0	1	8	5.0	11.4
0	1	10	13.0	23.1
0	1	12	28.6	33.8
0	1	14	40.5	41.6
0	1	16	9.9	13.2
0	1	18	11.6	17.5
0	1	20	3.9	4.8
0	1	22	9.4	15.1
0	1	24	4.5	0.2
0	1	26	11.1	7.7
0	1	28	12.6	5.0
0	1	30	10.8	8.8
0	1	32	0.	4.7
0	1	34	0.	2.5
0	3	2	66.1	68.9
0	3	4	9.6	8.9
0	3	6	52.8	55.1
0	3	8	26.9	29.5
0	3	10	20.5	17.7
0	3	12	57.0	62.0
0	3	14	54.6	54.7
0	3	16	8.6	14.3
0	3	18	11.6	16.0
0	3	20	0.	4.5
0	3	22	28.2	30.5
0	3	24	0.	4.0
0	3	26	9.9	14.0
0	3	28	0.	0.8
0	3	30	0.	5.1
0	3	32	6.3	5.5
0	5	2	44.0	47.9
0	5	4	44.1	41.0
0	5	6	53.6	54.3
0	5	8	36.0	36.1
0	5	10	22.7	23.8
0	5	12	22.5	24.3
0	5	14	13.0	10.4
0	5	16	19.9	20.7
0	5	18	19.4	17.3
0	5	20	17.5	22.1
0	5	22	19.5	21.8
0	5	24	6.3	7.4
0	5	26	9.7	12.6
0	5	28	0.	9.2
0	7	2	30.0	29.6
0	7	4	16.8	17.6
0	7	6	16.3	13.2
0	7	8	24.5	26.5
0	7	10	5.0	3.7
0	7	12	24.2	23.7
0	7	14	5.2	6.7
0	7	16	19.5	14.2
0	7	18	0.	3.6
0	7	20	0.	8.2
0	7	22	11.6	10.4
0	9	2	9.0	9.2
0	9	4	7.3	9.1
0	9	6	0.	2.2
0	9	8	7.3	8.0
0	9	10	0.	3.0
1	0	1	103.2	108.1
1	0	3	65.7	72.5
1	0	5	72.4	72.2
1	0	7	43.1	46.8
1	0	9	131.2	135.0
1	0	11	4.5	14.9
1	0	13	22.5	14.1
1	0	15	56.3	63.0
1	0	17	0.	3.5
1	0	19	58.2	63.6
1	0	21	23.4	27.9
1	0	23	12.6	18.1
1	0	25	14.1	17.5
1	0	27	0.	1.5
1	0	29	14.4	17.2
1	0	31	0.	5.6
1	0	33	0.	3.7
1	2	1	93.3	89.4
1	2	3	99.3	89.5
1	2	5	57.9	54.1
1	2	7	30.0	28.4
1	2	9	85.3	86.7
1	2	11	78.7	76.3
1	2	13	29.3	30.3
1	2	15	38.4	40.5
1	2	17	8.6	14.3
1	2	19	44.1	44.3
1	2	21	17.0	15.1
1	2	23	7.2	5.0
1	2	25	18.2	22.3
1	2	27	0.	6.1
1	2	29	8.1	9.8
1	2	31	0.	7.3
1	2	33	0.	2.4
1	4	1	51.6	46.8
1	4	3	28.7	25.9
1	4	5	41.0	40.3
1	4	7	28.6	30.6
1	4	9	60.9	59.1
1	4	11	25.7	19.3
1	4	13	10.7	6.1
1	4	15	32.5	32.6
1	4	17	16.1	16.7
1	4	19	13.8	9.3
1	4	21	17.9	21.9
1	4	23	7.0	6.5
1	4	25	9.5	11.7
1	4	27	5.2	3.2
1	4	29	0.	6.3
1	4	31	0.	2.1
1	6	1	6.2	4.4
1	6	3	28.8	27.6
1	6	5	19.0	18.5
1	6	7	27.2	26.1
1	6	9	6.6	9.6
1	6	11	16.1	14.8
1	6	13	5.9	8.0
1	6	15	13.6	18.6
1	6	17	5.1	6.1
1	6	19	6.3	8.5
1	6	21	12.3	12.5
1	6	23	0.	3.6
1	6	25	0.	6.1
1	8	1	0.	2.2
1	8	3	12.7	10.4
1	8	5	8.2	7.1
1	8	7	16.5	14.6
1	8	9	5.2	8.2
1	8	11	6.4	8.7
1	8	13	0.	2.6
1	8	15	0.	7.8
1	8	17	0.	3.3
2	1	0	83.0	81.5
2	1	2	27.0	24.9
2	1	4	107.1	105.2
2	1	6	16.0	12.0
2	1	8	19.4	17.2
2	1	10	45.6	48.1
2	1	12	25.9	27.9
2	1	14	59.3	60.8
2	1	16	29.2	36.3
2	1	18	33.9	37.6
2	1	20	22.0	25.1
2	1	22	6.4	11.5
2	1	24	24.6	27.8
2	1	26	9.3	11.8
2	1	28	11.4	12.7
2	1	30	0.	2.0
2	1	32	6.3	10.0
2	3	0	81.6	85.1
2	3	2	24.4	23.0
2	3	4	40.8	36.2
2	3	6	34.9	29.1
2	3	8	24.2	21.2
2	3	10	50.1	47.3
2	3	12	32.5	34.5
2	3	14	50.6	52.9
2	3	16	19.9	21.9
2	3	18	15.7	16.9
2	3	20	5.7	5.1
2	3	22	10.8	9.9
2	3	24	7.9	6.3
2	3	26	7.2	7.6
2	3	28	8.2	8.1
2	3	30	0.	3.0
2	5	0	33.2	30.5
2	5	2	10.3	9.2
2	5	4	15.6	11.8
2	5	6	0	5.7
2	5	8	7.0	5.8
2	5	10	25.1	25.3
2	5	12	8.0	6.8
2	5	14	11.7	11.8
2	5	16	9.2	6.9
2	5	18	13.8	10.3
2	5	20	0.	3.8
2	5	22	0.	5.6
2	5	24	0.	4.1
2	5	26	0.	1.9
2	7	0	0.	3.6
2	7	2	6.2	5.4
2	7	4	0.	4.1
2	7	6	0.	3.7
2	7	8	0.	4.6
2	7	10	6.3	5.2
2	7	12	6.4	9.6
2	7	14	0.	6.0
2	7	16	0.	3.9
2	7	18	8.2	6.1
2	7	20	0.	2.2
3	0	1	8.3	4.9
3	0	3	26.0	27.8
3	0	5	24.1	20.1
3	0	7	29.6	29.5
3	0	9	52.0	46.5
3	0	11	16.7	17.7
3	0	13	0.	8.3
3	0	15	22.2	24.2
3	0	17	12.9	17.0
3	0	19	18.2	21.5
3	0	21	4.8	6.7
3	0	23	10.6	9.3
3	0	25	0.	3.0
3	0	27	5.2	8.7
3	0	29	0.	6.7
3	0	31	0.	0.8
3	2	1	3.6	19.8
3	2	3	28.9	24.3
3	2	5	33.1	27.8
3	2	7	30.9	28.5
3	2	9	32.3	32.3
3	2	11	24.5	27.7
3	2	13	14.3	12.4
3	2	15	13.1	10.4
3	2	17	21.6	22.7
3	2	19	10.5	9.9
3	2	21	6.0	10.2
3	2	23	6.2	3.9
3	2	25	0.	3.8
3	2	27	5.2	7.7
3	2	29	0.	4.1
3	4	1	12.0	13.9
3	4	3	24.8	25.2
3	4	5	21.9	26.6
3	4	7	30.4	31.2
3	4	9	11.5	16.1
3	4	11	17.3	17.3
3	4	13	12.5	10.1
3	4	15	6.9	7.6
3	4	17	20.1	21.4
3	4	19	14.7	13.2
3	4	21	9.6	8.2
3	4	23	0.	3.4
3	4	25	0.	3.9
3	4	27	5.1	4.7
3	6	1	13.1	10.6
3	6	3	16.5	10.8
3	6	5	5.0	3.8
3	6	7	17.9	16.1
3	6	9	12.0	7.7
3	6	11	8.9	7.9
3	6	13	0.	7.4
3	6	15	0.	3.0
3	6	17	16.1	12.2
3	6	19	0.	7.0
3	6	21	0.	4.8
3	8	1	0.	4.9
3	8	3	8.9	7.2
3	8	5	0.	3.9
3	8	7	8.9	6.2
3	8	9	0.	2.8
4	1	0	25.9	19.9
4	1	2	28.1	22.5
4	1	4	28.5	25.0
4	1	6	28.2	24.4
4	1	8	14.2	14.7
4	1	10	14.8	14.0
4	1	12	0.	5.2
4	1	14	13.7	17.2
4	1	16	6.8	5.3
4	1	18	0.	1.6
4	1	20	5.1	1.9
4	1	22	5.1	5.5
4	1	24	0.	2.0
4	1	26	0.	5.0
4	3	0	19.8	10.3
4	3	2	29.7	27.0
4	3	4	20.9	16.0
4	3	6	17.4	12.8
4	3	8	18.6	13.3
4	3	10	12.7	11.3
4	3	12	17.4	17.4
4	3	14	5.0	4.6
4	3	16	7.1	4.2
4	3	18	5.1	5.0
4	3	20	7.3	8.7
4	3	22	8.2	8.3
4	3	24	5.1	6.7
4	5	0	0.	1.2
4	5	2	17.8	15.5
4	5	4	13.5	9.7
4	5	6	8.9	10.3
4	5	8	7.3	12.1
4	5	10	14.7	6.2
4	5	12	14.8	15.4
4	5	14	11.1	10.3
4	5	16	0.	0.9
4	5	18	0.	2.6
4	5	20	0.	4.5
4	7	0	7.3	6.9
4	7	2	6.3	5.7
4	7	4	5.2	5.4
4	7	6	0.	5.2
4	7	8	0.	7.8
5	0	1	22.3	19.2
5	0	3	10.9	7.3
5	0	5	16.3	15.6
5	0	7	6.1	4.3
5	0	9	20.1	14.8
5	0	11	13.4	14.1
5	0	13	9.6	8.7
5	0	15	9.0	8.9
5	0	17	0.	5.3
5	0	19	0.	0.2
5	0	21	0.	0.3
5	2	1	12.7	10.7
5	2	3	15.9	12.4
5	2	5	18.9	15.8
5	2	7	0.	4.9
5	2	9	8.0	6.6
5	2	11	0.	6.4
5	2	13	5.2	3.0
5	2	15	8.2	6.8
5	2	17	0.	4.0
5	2	19	8.1	7.3
5	2	21	0.	1.6
5	4	1	5.2	1.4
5	4	3	7.4	7.7
5	4	5	7.4	3.8
5	4	7	5.2	4.5
5	4	9	0.	5.0
5	4	11	0.	5.6
5	4	13	0.	1.5
5	4	15	0.	3.0
5	4	17	0.	2.4
5	6	1	0.	2.1
5	6	3	0.	1.8
6	1	0	23.3	14.7
6	1	2	12.3	8.3
6	1	4	6.4	4.1
6	1	6	5.2	2.2
6	1	8	0.	3.0
6	1	10	7.2	5.1
6	1	12	0.	2.1
6	3	0	7.3	6.1
6	3	2	0.	2.1
6	3	4	5.2	6.0
6	3	6	0.	1.5
6	3	8	0.	4.3

Table 1A. Measured and calculated structure amplitudes (Visual data).

k	l	F_o	F_c
h = 0			
0	2	0.	50.2
0	4	35.5	29.2
0	6	112.4	103.9
0	8	17.7	21.5
0	10	148.3	123.0
0	12	77.6	57.6
0	14	85.3	76.3
0	16	26.6	23.2
0	18	60.1	45.7
0	20	33.6	30.4
0	22	0.	6.3
0	24	35.5	27.9
0	26	0.	2.8
0	28	22.8	17.9
0	30	0.	0.1
0	30	9.1	0.1
0	34	11.9	3.9
2	0	269.6	250.5
2	2	131.5	120.4
2	4	129.2	103.1
2	6	80.9	74.7
2	8	19.5	24.7
2	10	159.9	125.9
2	12	17.5	19.4
2	14	21.8	23.4
2	16	37.0	35.7
2	18	71.9	45.6
2	20	23.2	21.0
2	22	10.1	9.4
2	24	15.0	16.6
2	26	7.1	10.0
2	28	11.6	10.2
2	32	12.0	6.1
4	0	50.2	53.3
4	2	34.0	32.0
4	4	24.4	25.6
4	6	29.6	33.7
4	8	20.0	11.9
4	10	56.4	51.7
4	12	30.7	27.0
4	14	41.5	32.6
4	16	0.	6.0
4	16	4.4	6.0
4	18	10.2	13.8
4	20	10.7	4.6
4	22	7.0	1.2
4	24	7.3	8.3
4	24	11.5	8.3
4	26	11.8	8.8
4	28	12.0	5.7
6	2	20.2	4.0
6	8	21.0	4.2
6	10	21.4	3.7
6	14	11.2	5.1
6	18	5.2	4.0
6	22	5.4	1.0
6	24	9.3	1.1
2	1	80.9	78.2
2	3	7.4	17.2
2	5	11.1	15.3
2	7	5.9	8.4
2	9	15.7	12.2
2	11	25.8	25.3
2	13	47.0	34.8
2	15	35.7	31.4
2	17	21.0	20.3
2	19	22.4	18.6
2	21	23.9	16.1
2	23	10.3	10.9
2	25	6.9	7.3
2	27	0.	1.5
4	1	3.4	6.3
4	3	15.3	11.0
4	5	39.8	36.2
4	7	0.	4.2
4	9	31.2	32.3
4	11	17.4	14.7
4	13	12.8	11.9
4	15	26.9	27.0
4	17	19.9	18.3
4	19	0.	5.1
4	19	4.7	5.1
4	21	6.9	4.2
4	23	7.2	10.3
4	23	12.4	10.3
4	25	0.	9.4
4	25	9.0	9.4
4	27	0.	3.3
4	27	9.2	3.3
4	29	12.0	6.3
6	1	7.8	4.5
6	3	14.3	19.2
6	7	8.0	4.8
6	9	21.2	14.0
6	13	11.1	0.3
6	15	16.0	10.6
6	17	5.2	1.9
6	23	5.4	2.8
8	1	11.9	10.0
1	1	35.6	46.7
1	3	17.6	25.4
1	5	55.2	51.0
1	7	32.3	31.4
1	9	25.5	24.7
1	11	65.6	48.7
1	13	41.5	25.1
1	15	64.8	47.6
1	17	30.5	23.5
1	19	6.8	0.6
1	21	18.8	15.6
1	23	10.0	7.1
1	25	26.1	21.3
1	27	15.8	13.2
1	29	0.	0.0
1	31	16.9	10.2
1	35	11.8	7.5
1	37	10.1	6.5
3	1	76.6	65.1
3	3	77.9	68.9
3	5	6.7	5.9
3	7	27.8	31.9
3	9	14.6	11.5
3	11	7.7	2.7
3	13	8.2	2.6
3	15	17.3	19.3
3	17	18.4	18.3
3	19	0.	1.5
3	21	25.2	17.5
3	23	0.	2.5
3	25	22.6	16.3
3	27	11.7	8.1
3	29	5.3	1.7
5	3	35.6	32.5
5	13	10.1	0.5
5	15	10.4	5.2
5	19	5.0	6.1
5	21	16.3	7.1
5	25	9.3	6.4
5	27	7.6	1.6
5	27	10.7	1.6
5	33	4.8	0.7
7	1	15.9	6.5
7	3	11.2	3.1
7	7	5.1	1.6
7	15	7.5	0.9
1	2	47.9	43.0
1	4	101.5	91.9
1	6	99.6	74.5
1	8	5.4	6.3
1	10	22.5	28.3
1	12	36.2	31.9
1	14	58.7	38.6
1	16	15.7	15.6
1	18	12.0	18.6
1	20	0.	5.9
1	20	4.1	5.9
1	22	0.	8.6
1	22	10.7	8.6
1	24	0.	5.0
1	26	6.9	2.5
1	26	9.8	2.5
1	28	0.	1.6
1	28	8.9	1.6
1	30	7.5	3.0
1	30	10.6	3.0
3	2	71.6	63.3
3	4	0.	10.8
3	4	2.9	10.8
3	6	45.0	44.0
3	8	28.4	30.0
3	10	28.1	27.4
3	12	48.9	55.1
3	14	51.9	51.2
3	16	8.9	12.6
3	18	0.	10.1
3	18	6.0	10.1
3	20	0.	1.5
3	22	29.8	23.5
3	24	0.	4.7
3	24	4.9	4.7
3	26	11.5	7.9
5	2	35.4	38.0
5	4	35.8	33.3
5	6	40.7	41.9
5	8	34.9	27.9
5	10	23.5	20.7
5	12	24.3	22.1
5	14	10.3	10.0
5	16	21.3	16.0
5	18	8.5	12.4
5	20	16.1	12.5
5	22	16.5	14.3
5	24	11.9	4.2
5	26	12.0	7.6
5	32	5.0	1.9
7	2	11.2	16.8
7	4	11.3	12.8
7	6	11.4	11.3
7	8	11.5	16.7
7	12	7.4	15.5
h = 1			
1	1	34.9	39.1
1	3	67.7	81.4
1	5	109.6	141.5
1	7	20.2	27.1
1	9	50.9	47.6
1	11	15.5	17.6
1	13	24.0	26.4
1	15	26.1	31.2
1	17	3.5	1.0
1	19	30.4	33.2
1	21	17.8	12.6
1	23	19.0	19.4
1	25	2.6	6.2
1	27	8.6	12.0
1	29	6.3	10.1
3	1	8.5	10.8
3	3	29.6	36.3
3	5	28.2	40.5
3	7	61.8	73.1
3	9	17.9	20.4
3	11	13.4	20.7
3	13	31.6	30.9
3	15	6.7	12.2
3	17	22.5	22.6
3	19	13.0	11.5
3	21	5.6	11.6
3	23	11.8	10.9
3	25	8.7	9.0
3	27	4.9	6.0
3	29	9.1	9.5
5	1	13.7	22.0
5	3	32.3	39.6
5	7	18.8	21.4
5	11	7.5	10.7
5	13	11.0	14.0
5	15	5.7	5.0
5	17	8.3	8.3
5	19	6.1	7.7
5	21	6.3	7.7
5	23	8.1	6.7
5	25	6.5	5.2
5	27	2.9	1.4
7	3	8.6	15.9
7	7	8.8	12.2
1	0	69.4	91.8
1	2	43.7	56.9
1	4	32.7	35.7
1	6	76.7	96.8
1	8	107.3	112.9
1	10	53.5	53.6
1	12	33.2	38.8
1	14	31.7	22.3
1	16	42.9	48.9
1	18	37.1	35.9
1	20	7.0	8.3
1	22	10.6	12.6
1	24	13.8	9.6
1	26	11.9	10.2
1	28	4.8	3.9
1	32	5.0	1.7
3	0	19.6	22.9
3	2	23.7	29.4
3	4	34.0	42.2
3	6	25.2	26.0
3	8	9.5	17.9
3	10	41.1	46.1
3	12	23.8	22.2
3	14	17.2	18.8
3	16	13.8	14.6
3	18	35.0	25.7
3	20	13.4	11.8
3	22	11.5	15.3
3	24	2.7	3.9
3	26	7.9	8.7
5	0	18.1	29.5
5	2	4.8	7.5
5	4	15.5	18.3
5	6	18.6	25.6
5	10	14.8	15.4
5	12	4.2	3.3
5	14	11.2	12.6
5	16	4.5	5.7
5	20	7.8	7.3
5	22	8.9	10.2
5	24	2.9	1.9
5	26	2.9	3.6
5	28	5.0	2.6
7	0	2.7	9.6
7	4	8.7	10.5
7	6	8.7	9.8
0	2	19.3	23.5
0	4	157.8	170.0
0	6	40.8	51.2
0	8	22.7	26.0
0	10	7.8	2.0
0	12	49.8	57.3
0	14	17.3	14.0
0	16	14.6	15.2
0	18	15.8	19.7
0	22	14.9	14.9
0	28	8.7	9.2
0	30	9.0	5.8
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2	6	80.6	85.8
2	8	82.6	69.6
2	10	10.3	12.5
2	12	31.4	29.9
2	14	23.9	22.8
2	16	6.4	4.3
2	18	16.8	14.6
2	20	5.2	7.9
2	22	19.0	18.1
2	26	8.6	6.1
2	28	6.3	5.0
2	32	5.0	4.9
4	0	29.9	35.4
4	2	18.6	33.4
4	4	15.8	32.0
4	6	55.9	58.5
4	8	20.0	29.6
4	10	5.9	11.2
4	14	25.9	25.7
4	18	5.6	10.8
4	20	5.8	6.0
4	22	6.0	11.1
4	24	7.9	5.3
6	0	11.0	15.3
6	4	11.1	18.5
6	10	11.7	11.6
6	14	7.7	11.9
6	16	6.2	5.4
6	20	2.9	4.6
6	22	4.1	2.4
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8	2	2.9	2.0
8	6	4.1	4.2
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0	9	120.8	121.1
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0	13	16.5	16.4
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0	21	12.5	18.2
0	23	10.9	16.5
0	25	8.1	14.7
0	29	8.9	12.8
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2	5	42.9	42.6
2	7	18.4	21.3
2	9	85.8	77.6
2	11	67.6	62.5
2	13	32.5	30.3
2	15	40.1	39.9
2	17	4.7	12.7
2	19	40.7	37.4
2	21	13.0	13.0
2	23	3.6	6.4
2	25	11.9	15.4
2	29	8.1	7.1
4	1	33.2	34.1
4	3	16.8	25.2
4	5	21.8	33.4
4	7	13.9	23.4
4	9	52.4	52.2
4	11	15.0	18.7
4	13	12.2	12.2
4	15	26.5	28.3
4	17	13.3	12.6
4	19	13.9	10.6
4	21	16.8	15.3
4	23	4.8	3.5
4	25	11.0	7.0
4	29	5.0	3.0
6	3	11.1	15.7
6	5	7.9	10.4
6	7	11.3	17.5
6	11	4.6	10.2
6	15	8.7	12.5
6	17	2.8	4.3
6	21	6.4	8.0
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0	6	11.4	8.3
0	8	2.3	11.1
0	10	15.0	12.3
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0	14	23.1	17.1
0	18	6.8	14.2
0	24	5.2	5.7
0	26	4.7	7.6
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2	2	19.7	21.9
2	4	7.6	2.3
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2	14	2.0	5.8
2	16	11.7	12.8
2	18	3.2	5.1
2	20	3.4	9.9
2	24	4.7	7.9
2	26	4.8	7.8
2	28	2.9	2.7
4	0	2.0	1.8
4	2	29.2	35.0
4	4	20.0	18.1
4	6	37.1	29.8
4	8	17.7	15.6
4	10	15.5	9.9
4	12	22.8	25.8
4	14	18.4	13.6
4	16	11.1	11.4
4	18	11.6	11.0
4	20	8.5	8.6
4	22	8.8	10.2
4	24	2.9	4.4
4	26	11.2	8.9
6	2	10.9	10.9
6	4	8.2	11.5
6	6	8.3	9.9
6	8	8.4	12.2
6	10	6.0	7.1
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6	14	6.3	7.9
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2	9	18.1	13.3
2	11	54.4	52.5
2	13	42.9	49.8
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2	17	25.1	26.3
2	19	4.1	4.3
2	21	15.7	19.0
2	23	16.6	17.4
2	25	4.7	9.6
4	1	12.4	14.5
4	3	25.0	29.2
4	5	11.1	16.2
4	7	9.3	8.3
4	11	17.3	11.9
4	13	18.0	20.3
4	15	18.8	16.8
4	17	13.9	11.3
4	21	11.6	11.1
4	23	8.9	9.1
4	25	2.9	2.9
6	3	7.3	9.6
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6	13	8.3	11.9
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3	15	19.9	14.3
3	19	15.7	8.3
3	21	7.8	5.4
3	25	6.3	8.8
3	27	4.1	4.3
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5	5	12.7	17.6
5	9	21.6	23.3
5	11	15.7	13.9
5	13	14.0	14.2
5	15	14.5	17.3
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1	24	16.6	20.7
1	26	4.8	8.5
1	28	6.4	9.5
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3	8	18.4	20.1
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3	12	23.3	27.4
3	14	48.4	47.2
3	16	16.2	13.1
3	18	18.7	16.2
3	20	7.2	6.9
3	22	8.0	6.7
3	24	6.2	3.9
3	26	4.0	3.8
3	28	4.1	5.9
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5	2	2.3	9.1
5	4	2.3	10.4
5	10	15.5	17.7
5	12	2.5	4.1
5	14	3.7	13.7
5	16	2.7	2.7
5	18	4.8	6.8
h = 3			
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1	9	16.3	14.4
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1	13	19.6	21.7
1	15	23.5	25.7
1	19	13.4	12.5
1	21	9.0	9.9
1	23	9.4	7.9
1	25	9.8	5.2
1	27	1.6	3.6
1	29	1.6	5.0
3	1	22.4	17.5
3	3	27.0	25.9
3	5	27.8	26.8
3	7	19.6	16.0
3	9	20.6	15.7
3	11	11.9	16.2
3	13	6.9	5.6
3	15	19.6	14.4
3	17	11.6	13.2
3	19	12.2	7.7
3	21	4.8	9.6
3	23	4.4	5.3
3	25	1.6	4.0
3	29	1.6	3.3
5	1	10.4	10.8
5	3	7.4	7.8
5	5	7.5	8.0
5	7	8.8	7.0
5	9	11.9	6.5
5	11	2.5	7.1
5	13	4.7	6.4
5	15	4.8	6.5
5	17	15.6	13.1
5	19	15.9	10.7
5	21	2.3	1.4
1	0	1.8	6.4
1	2	14.5	20.7
1	4	13.6	21.2
1	6	25.3	30.5
1	8	27.1	31.6
1	10	9.4	14.1
1	12	4.6	13.7
1	14	3.5	4.1
1	16	6.6	7.6
1	18	8.2	12.5
1	20	3.9	6.0
1	22	2.1	2.3
1	24	2.2	2.1
3	0	31.6	30.6
3	2	7.0	12.6
3	4	5.6	12.6
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3	10	21.1	16.8
3	12	3.9	1.6
3	14	7.1	13.6
3	16	7.4	10.0
3	18	16.2	17.0
3	20	12.4	11.0
3	24	2.7	3.5
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3	28	3.6	3.8
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5	2	9.5	7.7
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5	10	12.1	12.8
5	12	2.1	1.8
5	14	4.8	7.0
5	16	2.2	3.6
5	18	15.8	11.5
5	20	13.3	6.8
0	2	33.0	39.1
0	4	18.8	21.7
0	6	29.7	39.8
0	8	36.3	38.3
0	10	2.0	6.2
0	12	51.3	44.5
0	14	1.6	3.6
0	16	5.3	11.9
0	18	4.0	11.4
0	20	4.3	10.4
0	22	4.6	16.7
0	26	4.9	9.9
0	28	1.6	1.1
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2	2	25.7	25.1
2	4	30.7	32.8
2	6	39.6	34.7
2	8	42.5	37.2
2	10	19.4	21.8
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2	16	20.1	17.3
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2	22	17.0	13.9
2	24	3.5	5.1
2	26	5.0	6.1
2	28	1.6	1.3
2	30	1.6	2.3
4	0	5.2	11.2
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4	18	12.6	9.0
4	20	3.5	5.8
4	22	3.5	4.0
4	26	4.5	3.9
6	6	4.8	3.6
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0	11	5.2	9.7
0	13	2.3	4.6
0	15	13.0	17.9
0	17	5.5	18.6
0	19	13.2	17.0
0	23	4.7	5.8
0	27	4.2	5.5
2	1	9.1	16.2
2	3	16.8	18.7
2	5	27.7	24.2
2	7	19.0	24.7
2	9	31.9	32.7
2	11	19.2	14.1
2	13	11.5	6.2
2	15	12.3	11.1
2	17	19.4	12.9
2	19	8.8	8.8
2	21	9.2	7.3
2	23	4.3	4.1
2	25	3.5	2.6
2	27	3.6	4.3
2	29	1.6	1.9
2	31	1.6	0.3
4	1	5.2	12.3
4	3	11.8	20.9
4	5	10.1	20.5
4	7	17.5	23.0
4	9	7.0	17.2
4	11	9.4	10.2
4	13	3.1	6.3
4	17	14.8	14.4
4	19	11.8	8.7
4	21	5.0	4.4
4	27	2.2	2.7
6	1	3.3	7.6
6	3	3.4	6.2
6	7	1.5	10.9
6	9	1.5	5.0
6	11	1.6	5.5
6	17	3.6	5.6
h = 4			
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0	6	5.9	5.1
0	8	17.2	15.5
0	10	14.1	15.0
0	12	8.5	5.2
0	14	21.4	20.6
0	16	15.2	13.9
0	18	8.7	8.1
0	20	9.1	7.8
0	22	2.1	0.5
0	24	4.9	3.5
0	28	2.2	2.3
2	0	22.4	24.7
2	2	6.9	6.2
2	4	11.0	9.8
2	6	8.8	10.1
2	8	14.2	18.1
2	10	15.2	10.3
2	12	5.4	4.3
2	14	15.2	12.9
2	16	3.3	6.3
2	18	11.1	8.8
2	20	6.0	6.4
2	24	4.9	4.2
2	26	2.2	2.5
4	0	11.4	10.3
4	2	2.5	4.6
4	4	8.2	8.7
4	6	1.9	1.6
4	8	12.1	9.1
4	10	5.6	6.8
4	12	2.0	2.0
4	14	2.1	4.8
4	18	3.8	5.4
0	1	1.0	4.6
0	3	5.4	12.0
0	7	16.1	10.6
0	17	10.3	7.3
0	21	9.3	10.7
2	1	8.8	9.6
2	3	9.9	10.1
2	5	7.4	6.8
2	7	10.2	9.5
2	9	12.0	12.7
2	11	10.5	8.4
2	13	7.9	11.1
2	15	10.3	10.2
2	17	4.0	1.8
2	19	2.1	1.0
2	25	3.1	5.0
4	1	9.8	10.7
4	3	11.5	9.3
4	5	8.2	8.6
4	7	14.6	9.3
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4	15	3.7	4.5
4	17	2.2	2.8
4	21	2.2	2.3
6	1	9.7	8.7
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6	5	4.9	5.8
1	1	14.2	19.2
1	3	22.0	28.4
1	5	8.6	10.5
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1	13	14.9	14.6
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1	21	6.6	8.5
3	1	11.9	19.1
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5	9	6.0	2.4
5	11	6.8	7.6
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1	2	12.6	17.1
1	4	14.3	18.5
1	6	16.3	11.9
1	8	8.4	6.5
1	10	9.2	7.2
1	12	2.3	5.7
1	14	10.9	14.8
1	16	5.8	1.0
1	20	2.9	0.7
3	0	9.7	8.1
3	2	17.6	21.0
3	4	11.2	10.2
3	6	11.6	0.3
3	8	12.0	7.6
3	10	8.0	6.7
3	12	13.2	11.7
3	16	5.7	1.1
3	18	4.7	2.4
3	20	3.1	1.9
3	22	3.1	4.1
3	24	3.8	3.3
5	2	9.0	9.5
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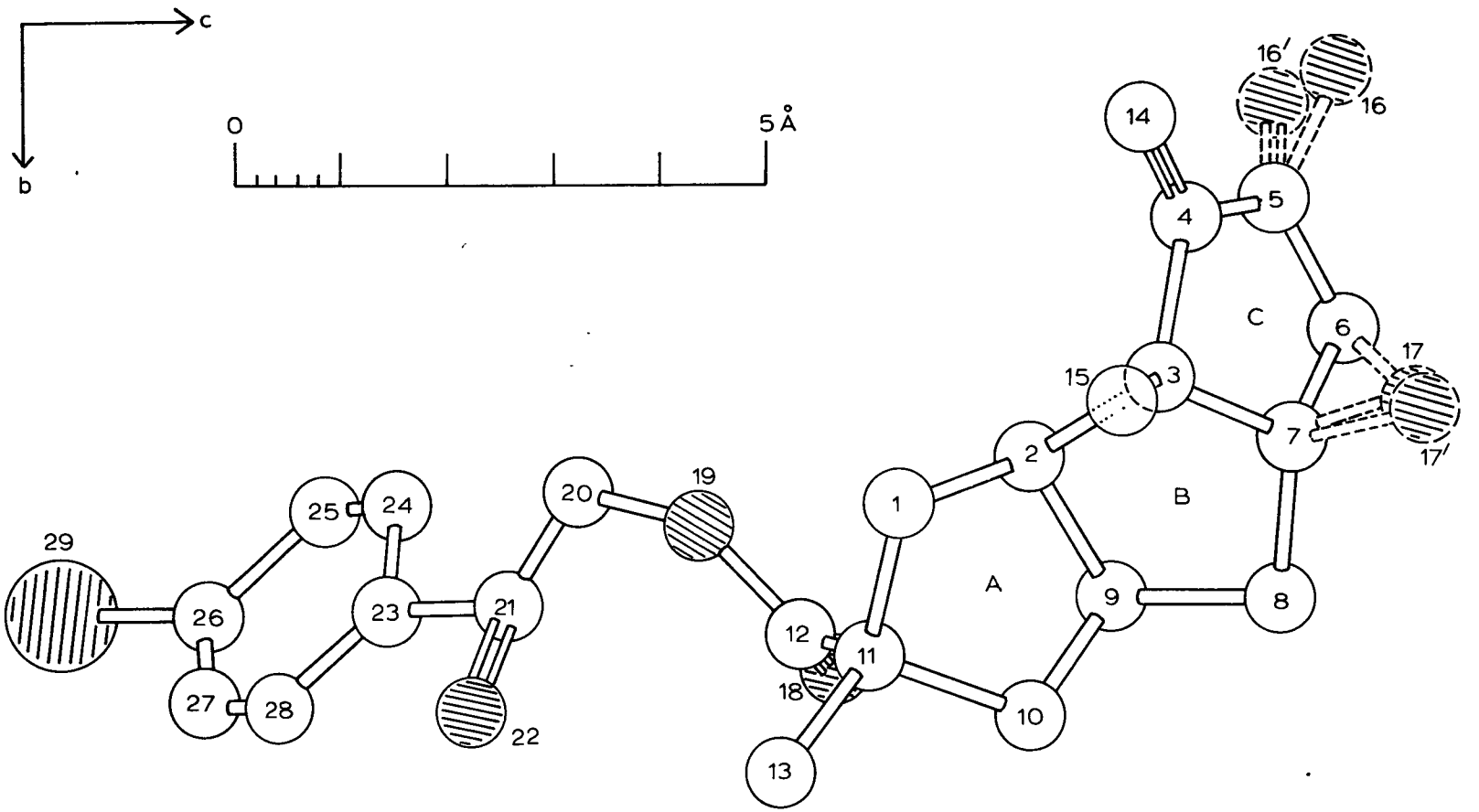


Figure 4. A perspective drawing of the molecule with the atom numbering used. The correct absolute configuration is shown, the positive direction of the a-axis being towards the viewer.

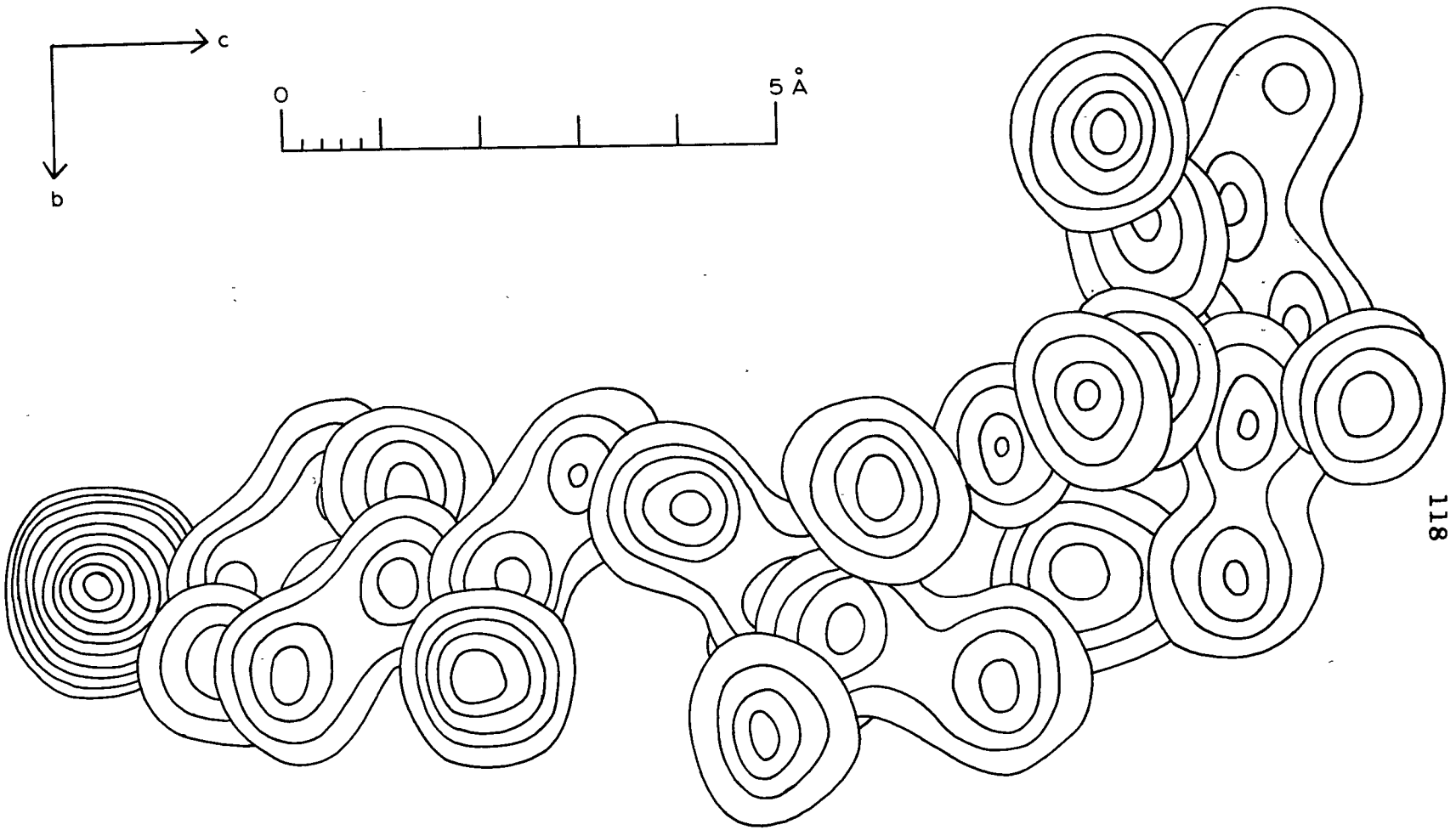
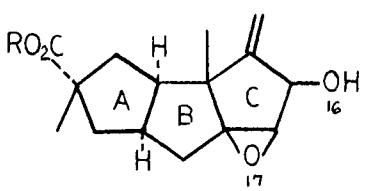


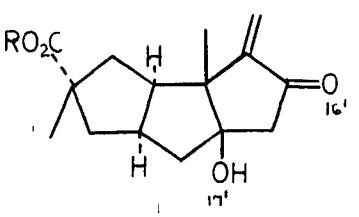
Figure 5. Superimposed sections of the three-dimensional electron-density distribution, through the atomic centres parallel to (100); contours for carbon and oxygen atoms are at intervals of $1e.Å^{-3}$ starting at $1e.Å^{-3}$, and for the Br atom at intervals of $3e.Å^{-3}$ starting at $3e.Å^{-3}$. The correct absolute configuration is shown, the positive direction of the a-axis being towards the viewer.

structure, was decomposing on exposure to X-rays. This suspicion was confirmed by irradiating samples of the derivative with unfiltered molybenum radiation. After 10 hr. the decomposition was essentially complete, yielding a mixture of two compounds which were separated by thin-layer chromatography (T.L.C.). One (ca. 40% yield) was the starting material, and the other (ca. 60% yield) was shown by I.R., U.V. and N.M.R. spectroscopy to be a rearranged product. As a final check a single crystal was irradiated with Cu-K_α radiation under the conditions of intensity measurement; when this crystal was crushed it showed depressed melting point and give two spots on T.L.C. plates which were consistent with the two spots obtained with the previously irradiated sample.

Two pieces of chemical evidence were now sufficient to fix the structures of both the p-bromophenacyl ester of hirsutic acid and the rearrangement product: (i) the rearrangement product, but not the starting material, contains an α,β -unsaturated ketone system; (ii) each compound contains a hydroxyl group. The starting material is therefore (2, R=p-bromophenacyl) and



(2)



(15)

the rearrangement product is (15, R = p-bromophenacyl).

Coordinates and Molecular Dimensions:-

Only the parameters from the final least-squares cycle with the counter data are given, since these are the most accurate results. The final positional and isotropic thermal parameters are given in Table 2, together with their standard deviations calculated from the least-squares residuals.

The bond distances and valency angles are given in Table 3; and the shorter intermolecular distances in Table 4. The molecular packing, including possible hydrogen bonds, is illustrated in Figure 6.

The three five-membered rings were examined for planarity. The rings were all non-planar, and in each case it was found that one of the five possible four-atom planes was much better defined than the others; but only for the ring containing atoms 1,2,9,10,11 (ring A) was an envelope conformation sharply defined. Table 5 summarizes the equations of various planes (including the aromatic ring), deviations from the planes and several pertinent dihedral angles.

Absolute Configuration:-

As a final step in the analysis the absolute configurations of the molecules (both starting material and rearrangement product) were determined by the anomalous dispersion method.[11] Structure factors were calculated for all the hkl and $\bar{h}\bar{k}\bar{l}$ reflexions, using a scattering factor for Br of the form:

$$f = (f_{Br} + \Delta f'_{Br}) + i\Delta f''_{Br}$$

Table 2. Fractional positional parameters and standard deviations (each x 10^4), isotropic thermal parameters and standard deviations ($Å^2$).

Atom	x	y	z	σ(x)	σ(y)	σ(z)	B	σ(B)
C(1)	3075	1321	3699	42	29	7	5.22	0.78
C(2)	1897	0751	4030	51	32	9	7.31	0.97
C(3)	3156	-0038	4357	50	35	8	7.20	0.91
C(4)	2486	-1616	4378	51	34	9	8.34	1.02
C(5)	0743	-1605	4624	63	40	10	11.58	1.27
C(6)	0540	-0289	4865	60	39	10	10.15	1.18
C(7)	2318	0539	4705	56	36	10	9.24	1.15
C(8)	1706	2176	4651	54	41	9	10.16	1.06
C(9)	0903	2118	4229	47	33	7	6.50	0.73
C(10)	1549	3437	4001	46	30	8	6.10	0.83
C(11)	1931	2801	3627	41	32	7	6.39	0.79
C(12)	0134	2253	3394	47	37	8	8.44	1.01
C(13)	3106	3672	3332	54	35	9	7.71	0.98
C(14)	3588	-2745	4215	63	43	10	12.09	1.21
C(15)	5639	0191	4297	61	41	10	10.74	1.20
O(16)	0717	-2896	4873	97	67	14	14.75	1.89
O(16')	-0513	-2662	4651	73	55	13	11.39	1.51
O(17)	2428	0313	5019	80	53	13	9.88	1.52
O(17')	3806	0370	5083	85	45	11	8.39	1.28
O(18)	-1641	2865	3434	32	23	5	8.67	0.60
O(19)	0076	1321	3114	31	23	5	7.00	0.60
C(20)	-1570	1042	2858	52	33	9	7.46	0.91
C(21)	-1798	2207	2584	48	37	8	8.05	0.93
O(22)	-0652	3260	2574	33	21	5	8.03	0.62
C(23)	-3560	2224	2321	42	31	7	5.45	0.71
C(24)	-5183	1121	2377	52	35	8	7.67	1.00
C(25)	-6745	1161	2121	47	32	8	6.42	0.84
C(26)	-6821	2062	1832	41	33	7	5.79	0.77
C(27)	-5166	3189	1768	43	32	8	6.21	0.84
C(28)	-3594	3129	2028	46	31	7	6.11	0.77
Br(29)	-8947	2132	1463	7	5	1	9.58	0.09

Table 3. Bond distances (Å) and valency angles (degrees). Standard deviations are about 0.05 Å and 3°.

C(1)-C(2)	1.50	C(5)-C(4)-C(14)	131.6
C(1)-C(11)	1.59	C(4)-C(5)-O(16)	111.0
C(2)-C(3)	1.60	C(6)-C(5)-O(16)	109.2
C(2)-C(9)	1.59	C(4)-C(5)-C(6)	114.8
C(3)-C(4)	1.54	C(4)-C(5)-O(16')	123.1
C(3)-C(7)	1.45	C(6)-C(5)-O(16')	122.0
C(3)-C(15)	1.65	C(5)-C(6)-C(7)	98.1
C(4)-C(5)	1.43	C(5)-C(6)-O(17)	116.8
C(5)-C(6)	1.50	C(7)-C(6)-O(17)	44.7
C(6)-C(7)	1.51	C(6)-O(17)-C(7)	69.8
C(7)-C(8)	1.59	C(6)-C(7)-O(17)	65.5
C(8)-C(9)	1.58	C(3)-C(7)-C(6)	114.7
C(9)-C(10)	1.53	C(3)-C(7)-C(8)	110.7
C(10)-C(11)	1.47	C(3)-C(7)-O(17)	137.4
C(11)-C(12)	1.52	C(3)-C(7)-O(17')	115.0
C(11)-C(13)	1.58	C(6)-C(7)-C(8)	110.1
C(4)-C(14)	1.40	C(6)-C(7)-O(17')	95.8
C(5)-O(16)	1.49	C(8)-C(7)-O(17)	108.1
C(5)-O(16')	1.29	C(8)-C(7)-O(17')	109.5
C(6)-O(17)	1.46	C(7)-C(8)-C(9)	99.3
C(7)-O(17)	1.13	C(2)-C(9)-C(8)	107.7
C(7)-O(17')	1.66	C(2)-C(9)-C(10)	107.7
C(12)-O(18)	1.30	C(8)-C(9)-C(10)	112.0
C(12)-O(19)	1.32	C(9)-C(10)-C(11)	101.0
O(19)-C(20)	1.43	C(1)-C(11)-C(10)	106.7
C(20)-C(21)	1.46	C(1)-C(11)-C(12)	99.0
C(21)-O(22)	1.24	C(1)-C(11)-C(13)	111.8
C(21)-C(23)	1.48	C(10)-C(11)-C(12)	119.2
C(23)-C(24)	1.49	C(10)-C(11)-C(13)	116.3
C(23)-C(28)	1.33	C(12)-C(11)-C(13)	102.3
C(24)-C(25)	1.37	C(11)-C(12)-O(18)	118.9
C(25)-C(26)	1.32	C(11)-C(12)-O(19)	130.4
C(26)-C(27)	1.53	O(18)-C(12)-O(19)	110.2
C(27)-C(28)	1.38	C(12)-O(19)-C(20)	127.9
C(26)-Br(29)	1.91	O(19)-C(20)-C(21)	110.9
		C(20)-C(21)-O(22)	123.4
C(2)-C(1)-C(11)	101.1	C(20)-C(21)-C(23)	120.1
C(1)-C(2)-C(3)	117.4	O(22)-C(21)-C(23)	116.4
C(1)-C(2)-C(9)	105.4	C(21)-C(23)-C(24)	117.5
C(3)-C(2)-C(9)	105.4	C(21)-C(23)-C(28)	120.3
C(2)-C(3)-C(4)	109.3	C(24)-C(23)-C(28)	122.0
C(2)-C(3)-C(7)	104.0	C(23)-C(24)-C(25)	115.1
C(2)-C(3)-C(15)	110.8	C(24)-C(25)-C(26)	123.7
C(4)-C(3)-C(7)	102.0	C(25)-C(26)-C(27)	121.7
C(4)-C(3)-C(15)	114.2	C(25)-C(26)-Br(29)	125.1
C(7)-C(3)-C(15)	115.6	C(27)-C(26)-Br(29)	113.1
C(3)-C(4)-C(5)	104.4	C(26)-C(27)-C(28)	113.8
C(3)-C(4)-C(14)	123.7	C(23)-C(28)-C(27)	123.5

Table 4. Shorter intermolecular distances.

(All the crystallographically-independent contacts $\leqslant 3.70$ Å are listed).

Atom (Molecule 1)	to Atom	in Molecule	d(Å)
O(22)	C(25)	2	3.60
C(13)	O(18)	2	3.56
O(22)	C(20)	3	3.35
C(27)	C(1)	3	3.63
C(13)	C(24)	3	3.60
C(13)	C(25)	3	3.64
O(17)	O(16)	4	3.14
O(17)	O(16')	4	3.05
C(7)	O(16)	4	3.64
C(4)	O(16)	4	3.41
C(5)	O(16')	4	3.61
C(6)	O(16')	4	3.64
C(15)	O(16)	4	3.63
C(14)	O(16)	4	3.55
O(16)	C(6)	4	3.70
O(16)	O(16)	4	3.47
O(16)	O(16')	4	3.03
O(17')	O(16)	4	2.63
O(17')	O(16')	4	2.74
O(17')	C(9)	5	3.64
O(17')	C(8)	5	3.12

Molecule	Coordinates
1	$\underline{x}, \underline{y}, \underline{z}$
2	$1+\underline{x}, \underline{y}, \underline{z}$
3	$-\underline{x}, \frac{1}{2}+\underline{y}, \frac{1}{2}-\underline{z}$
4	$\frac{1}{2}+\underline{x}, -\frac{1}{2}-\underline{y}, 1-\underline{z}$
5	$\frac{1}{2}+\underline{x}, \frac{1}{2}-\underline{y}, 1-\underline{z}$

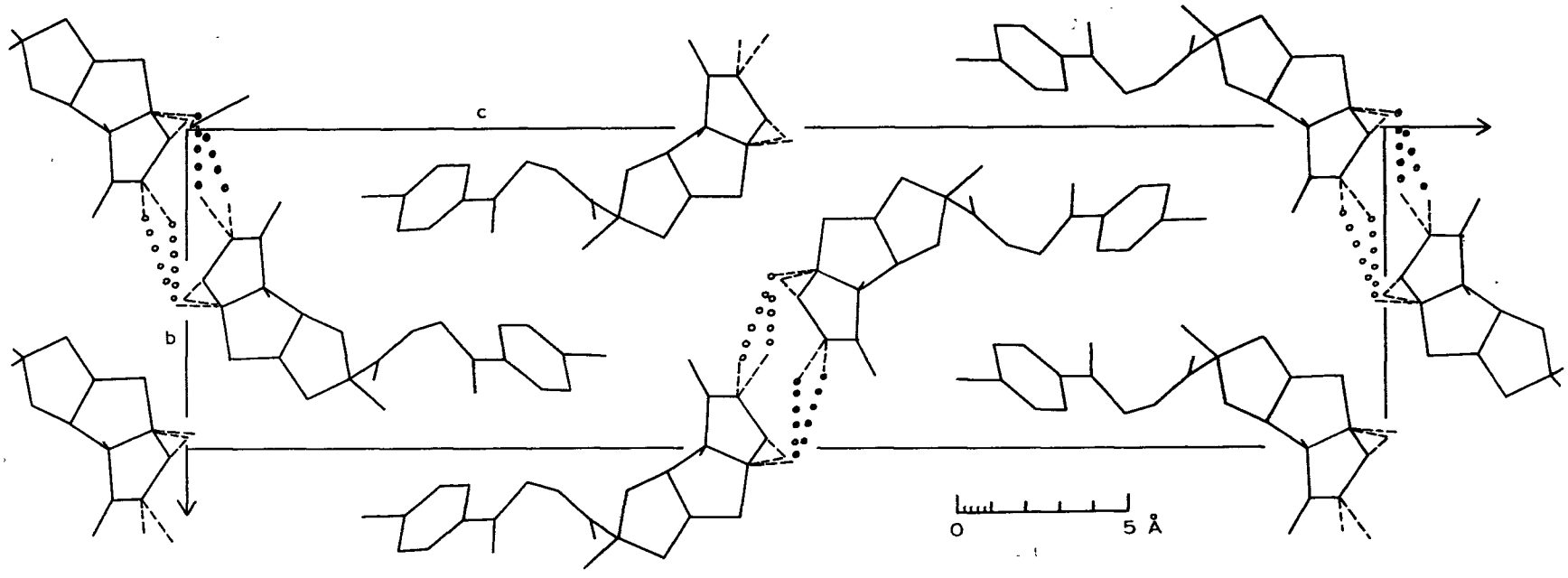


Figure 6. Projection of the structure along (100), illustrating the packing of the molecules and the hydrogen bonding between atom 17' and atoms 16 or 16'.

o o o hydrogen bonding between molecules:

x, 1+y, z and $-\frac{1}{2}+x, \frac{1}{2}-y, 1-z$
$1-x, \frac{1}{2}+y, \frac{3}{2}-z$ and $\frac{1}{2}-x, -y, \frac{1}{2}+z$
$1-x, \frac{1}{2}+y, \frac{1}{2}-z$ and $\frac{1}{2}-x, -y, -\frac{1}{2}+z$

● ● ● hydrogen bonding between molecules:

x, 1+y, z and $\frac{1}{2}+x, \frac{1}{2}-y, 1-z$
$1-x, \frac{1}{2}+y, \frac{3}{2}-z$ and $\frac{3}{2}-x, -y, \frac{1}{2}+z$
$1-x, \frac{1}{2}+y, \frac{1}{2}-z$ and $\frac{3}{2}-x, -y, -\frac{1}{2}+z$

Table 5. Equations of planes.

Atoms in plane	Equation	Symbol
C(1)C(2)C(9)C(10)	0.8096X+0.0972Y+0.5788Z = 9.2875	(A)
C(3)C(2)C(9)C(8)	0.8051X+0.5230Y-0.2798Z = -2.6324	(B)
C(4)C(5)C(6)C(7)	-0.5767X+0.3928Y-0.7163Z =-12.5574	(C)
C(21)C(23)C(24)C(25)-C(26)C(27)C(28)C(29)	0.4973X-0.6494Y-0.5753Z = -7.1831	(D)

Deviations from mean planes (Å).

Atom	A	B	Atom	C	Atom	D
1	-0.0005	+	3	0.3646	21	0.0236
2	0.0007	0.0280	4	-0.0162	23	-0.0251
3	+	-0.0168	5	0.0255	24	0.0018
7		-0.5183	6	-0.0240	25	-0.0109
8	+	0.0117	7	0.0147	26	0.0021
9	-0.0007	-0.0223	14	-0.4348	27	-0.0144
10	0.0005	+	16'	0.0445	28	0.0066
11	-0.6196		16	-0.5247	29	0.0164

Dihedral Angles (degrees).

Plane	Plane	ϕ
A	C(1)C(11)C(10)	42.8
A	B	122.7
B	C(3)C(7)C(8)	36.7
C	C(4)C(3)C(7)	22.8
C	C(6)O(17)C(7)	118.2
C(3)C(7) and C(2) or C(8)	C(3)C(7) and C(4) or C(6)	120 *

* An average of the four dihedral angles.

With Cu-K_α radiation the differences between $|Fc(hkl)|$ and $|Fc(\bar{h}\bar{k}\bar{l})|$ were quite small. The intensities for twelve sets of planes with relatively large differences were measured with the scintillation counter, and the results (Table 6) indicate unambiguously that the parameters used to calculate the structure factors (those of Table 2 referred to a conventional right-handed set of axes) represent the true absolute configuration. Figure 4 and (2) and (15) therefore depict the correct absolute configuration.

Discussion of X-ray results:-

The present X-ray analysis has established, with the assistance of some chemical evidence, the structure and absolute configuration of the p-bromophenacyl ester of hirsutic acid as (2, R=p-bromophenacyl). Hirsutic acid is therefore (2, R=H). The ester rearranges on irradiation with X-rays to form a product for which the X-ray analysis gives the structure and absolute configuration as (15, R=p-bromophenacyl). This rearrangement proceeds without disrupting the crystal structure, and produces only minor changes in lattice parameters and in the intensities of the reflexions. After irradiation the crystal contains the two different molecules distributed randomly at the lattice sites, since no superlattice reflexions or diffuseness were observed. Each atomic position determined by the X-ray analysis is therefore the mean of the positions in the separate molecules, which coincide very closely, except for the outer atoms in ring C. The relatively high thermal parameters (Table 2) are probably a

Table 6. Determination of the absolute configuration

(CuK_α radiation)

h	k	l	Io(hkl)	Io($\bar{h}\bar{k}\bar{l}$)	Fc(hkl)	Fc($\bar{h}\bar{k}\bar{l}$)	$\frac{Io(hkl)}{Io(\bar{h}\bar{k}\bar{l})}$	$\frac{\lvert Fc(hkl)\rvert^2}{\lvert Fc(\bar{h}\bar{k}\bar{l})\rvert^2}$
1	1	2	4102	6057	57.6	65.2	0.68	0.78
1	1	11	338	273	19.9	16.9	1.24	1.39
1	2	5	2328	2220	54.3	50.3	1.05	1.17
1	2	8	4601	5223	82.6	86.4	0.88	0.91
1	2	16	30	46	5.9	8.6	0.65	0.47
1	3	1	173	106	14.4	10.1	1.63	2.03
1	3	2	814	714	41.2	37.3	1.14	1.22
1	3	8	225	164	26.0	22.2	1.37	1.37
1	3	12	267	328	24.4	27.9	0.81	0.77
2	1	6	205	108	12.7	9.2	1.90	1.91
2	1	8	226	168	17.8	15.0	1.35	1.41
2	3	4	773	572	37.4	33.6	1.35	1.24

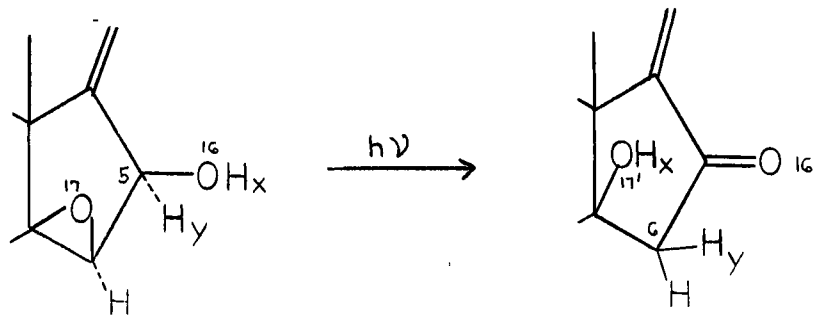
result of this disorder rather than large vibrations; these parameters are greatest in ring C where the changes in atomic position accompanying the rearrangement process are largest.

The general shapes of the molecules of the p-bromophenacyl ester of hirsutic acid, and of the rearrangement product, are clear from Figures 4 and 5. Table 5 defines the five-membered rings as envelopes or slightly distorted envelopes. Not too much significance should be attached to the detailed shape of ring C given in Table 5, as there are undoubtedly two centers at each atomic position, and only a mean position has been derived.

The measured bond distances and valency angles are not particularly accurate as a result of the disordered arrangement; however they are accurate enough to define unambiguously C_{14} as an exocyclic methylene group, atom 16' as a carbonyl oxygen and atom 16 as a hydroxyl oxygen, atom 17 as an epoxide oxygen and atom 17' as a hydroxyl oxygen. These features and the two tertiary methyl groups are in agreement with the chemical and spectroscopic properties of hirsutic acid and its derivates.

Nearly all of the intermolecular distances (Table 4) correspond to van der Waals interactions. The two exceptions are the O(17')···O(16) and O(17')···O(16') contacts of 2.63 and 2.74 Å, which represent O-H···O hydrogen bonds. The original crystal does not contain any hydrogen bonds since the intermolecular distance between O(16) and O(17) is 3.14 Å, and the intramolecular distance is 3.24 Å. However the nature of the hydrogen-bonding

scheme formed during the irradiation-induced rearrangement probably has a strong influence on the rearrangement process. The rearrangement involves oxidation of the secondary hydroxyl at C(5) to a ketone, together with reduction of the epoxide ring. During the rearrangement at least three bonds are broken and formed, and two hydrogen atoms are shifted. The hydrogen atom shifts probably involve a 1,2 shift from C(5) to C(6), and



the shift of the secondary hydroxyl proton O(16)-H_x to the tertiary hydroxyl group O(17')-H_x. The tertiary hydroxyl group of the rearrangement product can then form intermolecular hydrogen bonds either with the secondary hydroxyl group of the starting material (O-16) or with the keto group of the rearrangement product (O-16'). The nature of the hydrogen bonds formed and the molecular packing (Figure 6) can account for the observed mixture (ca. 60% rearrangement); however a distinction between an intra-and an intermolecular hydrogen atom transfer from O(16) to O(17') can not be made.

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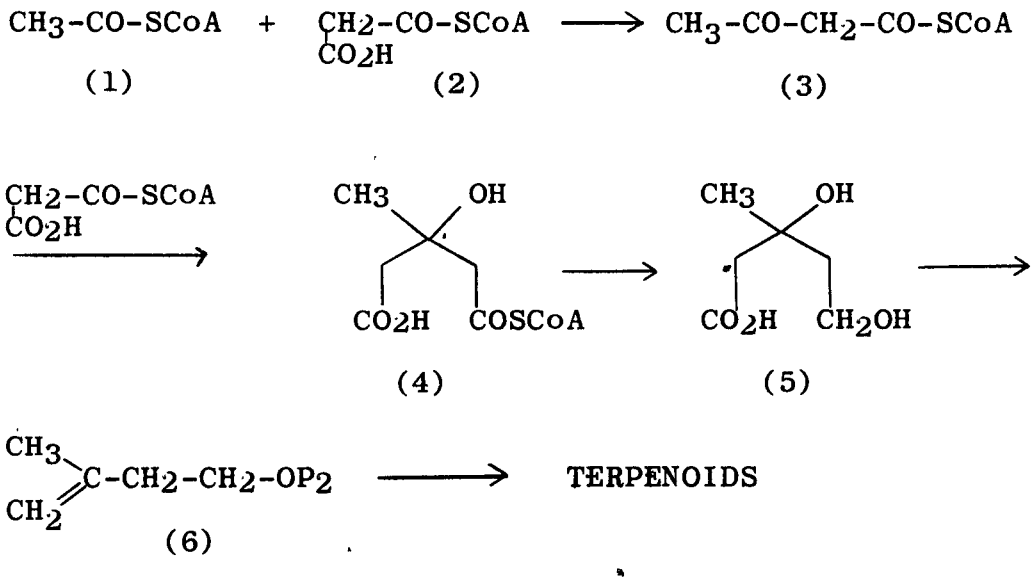
PART III

BIOGENETIC-TYPE SYNTHESES OF ACETATE-DERIVED AROMATIC COMPOUNDS

INTRODUCTION

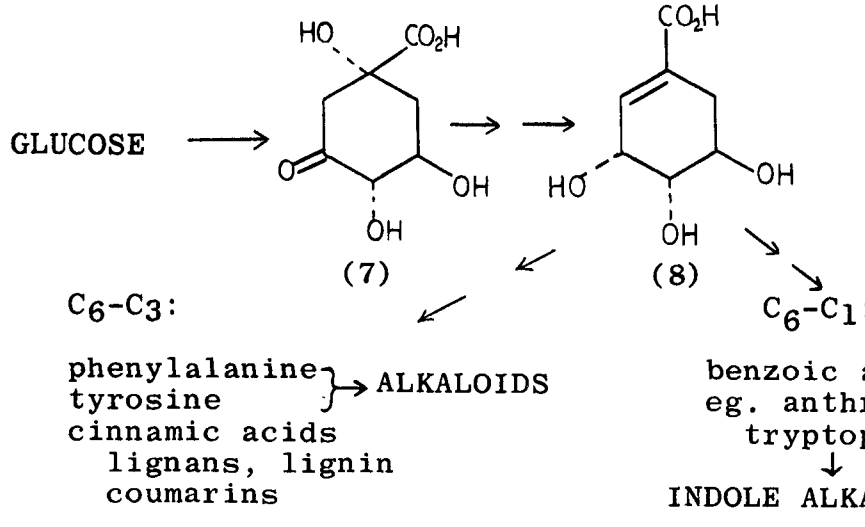
In Part II the structure elucidation of a sesquiterpenoid compound was carried out. The biosynthetic classification was made on the basis of the molecular formula, and on the fact that a logical rationale of its biogenesis could be made. Proof of the terpenoid nature of the compound could be provided by successful feeding experiments with labelled mevalonic acid or farnesyl pyrophosphate. The unique role of mevalonic acid (5) as a terpene precursor has been established,[1] and farnesyl pyrophosphate, which is formed by the head-to-tail condensation of three biological isoprene units (6),[2,3] is believed to be a precursor of the sesquiterpenes.[1] The precursors of other terpene families are similarly derivable from condensations of the basic isoprene unit (6).



The biogenetic theory of isoprenoid compounds[4] has proved invaluable in the elucidation and correlation of the great variety of structural types characteristic of this class of

natural products. Similarly, other biosynthetic schemes have been proposed to correlate many of the nonisoprenoid compounds.

An important and large group of natural products which have received considerable investigation are the aromatic compounds. The biogenesis of aromatic compounds[1] appears to be largely confined to two distinct pathways. One of these, the carbohydrate or shikimic acid (8) pathway, can lead to numerous classes of natural products. These classes arise from either a C_6-C_1 or a



C_6-C_3 aromatic precursor; included in the C_6-C_1 group are the benzoic acids which can be metabolized further, for example, anthranilic acid is the precursor of tryptophan which in turn is the precursor of the indole alkaloids; included in the C_6-C_3 group are the amino acids phenylalanine and tyrosine which are alkaloid precursors, and cinnamic acids which are precursors of coumarins (C_6-C_3 monomer), lignans (C_6-C_3 dimer) and lignin (C_6-C_3 polymer). In addition to the C_1 or C_3 sidechain, aromatic compounds derived from the shikimic acid (8) pathway are usually characterized by the following oxygenation patterns: none, 4-,

3,4- or 3,4,5- (numbering with respect to the sidechain).

The other well-established biosynthetic route to aromatic compounds is the acetate pathway.[1,5-9] This pathway (see figure 1) commences with the condensation of a malonate unit (2) with the chain starter, acetyl CoA (1,9 R=CH_3). The condensation of another malonate unit with acetoacetyl CoA (3, 10 R=CH_3) can occur at two sites. Condensation at the keto group produces a branched-chain intermediate (4) essential to the terpenoid pathway, whereas condensation at the ester group produces the poly-β-keto intermediate (11) in the acetate pathway. According to the acetate hypothesis the poly-β-keto chain is built-up by further head-to-tail condensations of acetate units. Reduction of the carbonyl groups while the chain is being constructed leads to the fatty acids and polyacetylenes. However if the carbonyl groups are retained several condensation modes are possible. These are illustrated for the poly-β-keto chain (12): O-acylation can lead to formation of pyrones (13); aldol condensation can lead to "orcinol-type" aromatic compounds (14); and finally Claisen-type condensation can lead to acyl phloroglucinols (15). The simplest of the α-pyrone structures, triacetic acid lactone (16), which is derived from three acetate units, has recently been isolated from Penicillium patulum.[10] Similarly the simplest aromatic compounds, which are derivable from four acetate units, are natural products;[1] orsellinic acid (17) and the decarboxylation product, orcinol (18) arise from aldol-type condensation, and acetylphloroglucinol (19) from Claisen-type condensation

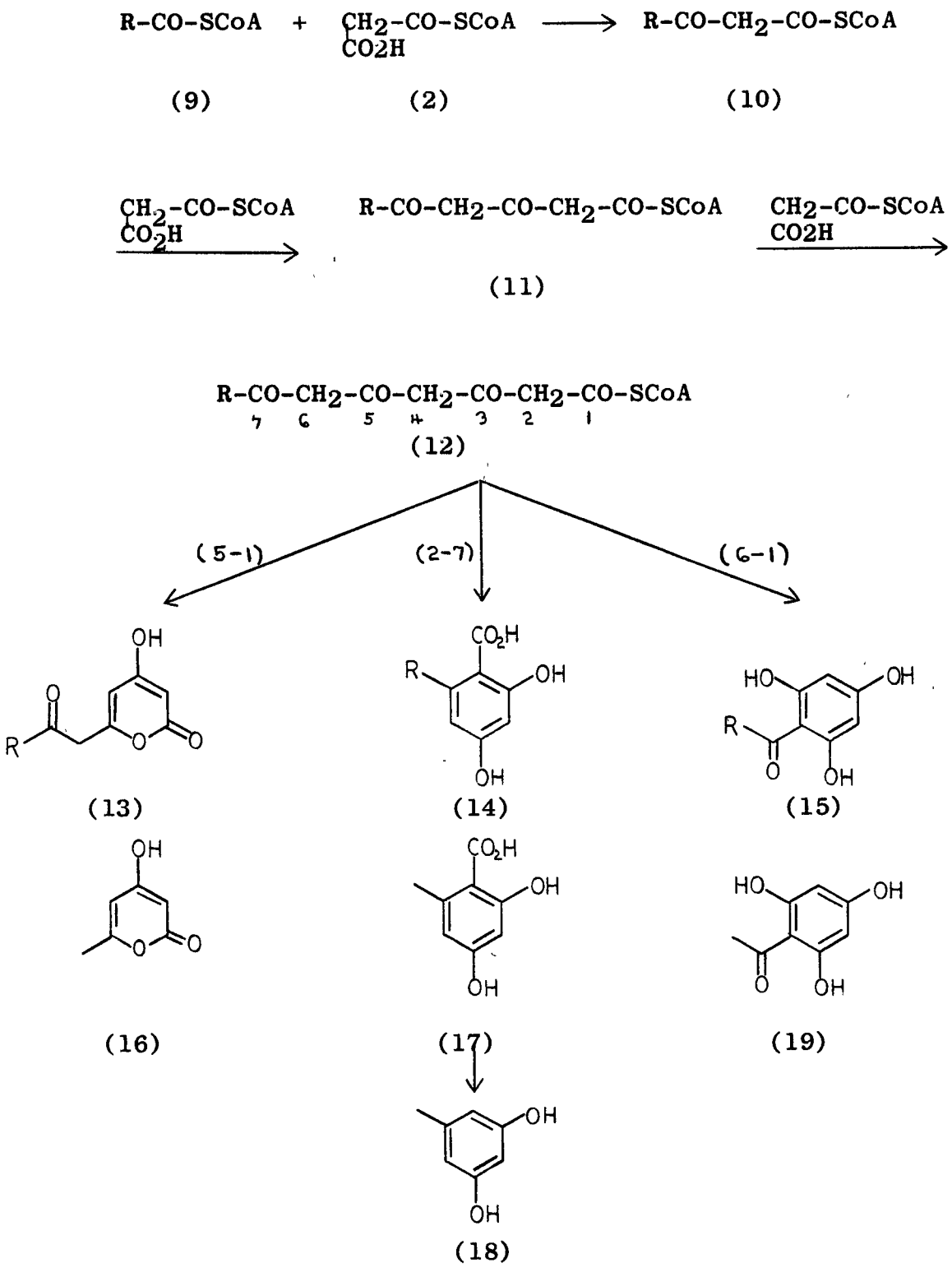
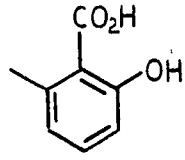

R-CO-SCoA + CH2-CO-SCoA (CO2H) → R-CO-CH2-CO-SCoA
(9)
(2)
(10)
CH2-CO-SCoA (CO2H)
R-CO-CH2-CO-CH2-CO-SCoA
CH2-CO-SCoA (CO2H)
(11)
R-CO-CH2-CO-CH2-CO-CH2-CO-SCoA
7 6 5 4 3 2 1
(12)
(5-1)
(2-7)
(6-1)
(13)
(14)
(15)
(16)
(17)
(19)
(18)

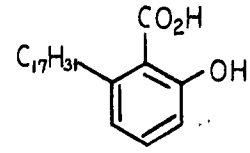
Figure 1

of the poly-β-keto precursor (12 R=CH_3).

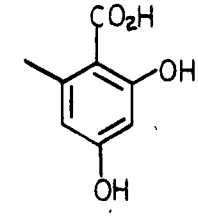
A large number of aromatic compounds can be accommodated by the acetate hypothesis if the following extensions are considered.[1] The poly-β-keto chain may be partially reduced prior to cyclization as illustrated by the products, 6-methylsalicylic acid (20) and pelanjuoic acid (21). Alternatively oxidation may



(20)

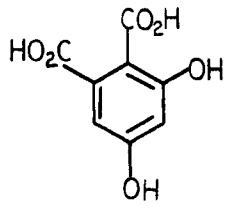


(21)



(17)

occur as in the metabolite (22). In addition to oxidation, C- and O-alkylation with a C-1 unit may occur as in cyclopaldic acid (23). Oxidation may also involve hydroxylation and subsequent quinone formation as in the product fumigatin (24). In



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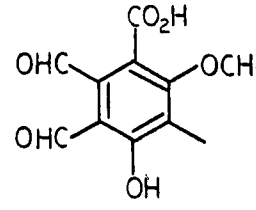
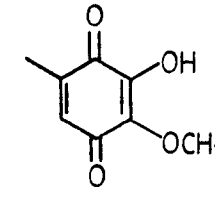
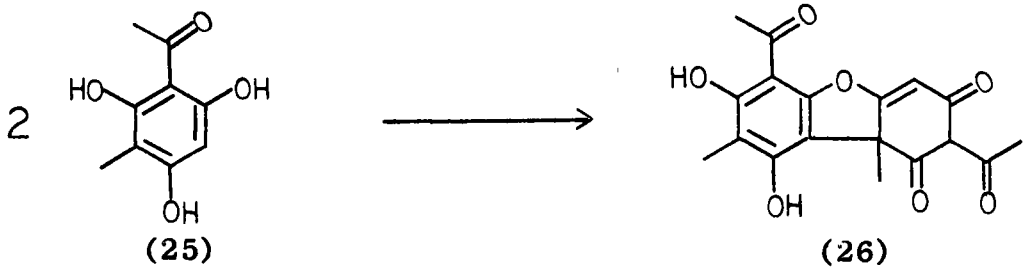


(23)



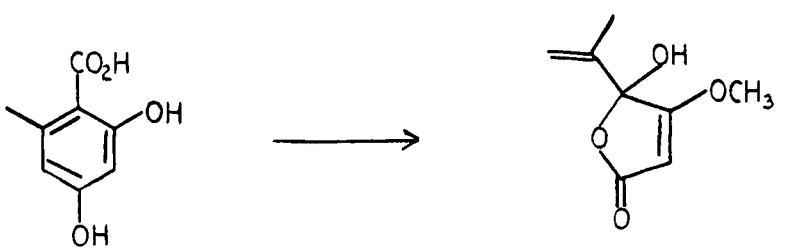
(24)

each of the above examples the characteristic alkylation and/or hydroxylation pattern of orsellinic acid (17) has been masked. Another mode of oxidation is the formation of dimeric species through oxidative coupling as shown by the coupling of two units



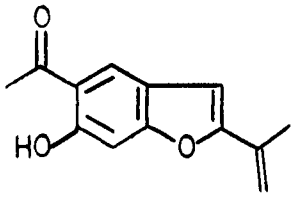
(25) (26)

of methylphloroacetophenone (25) to form the lichen product, usnic acid (26). This reaction has been carried out *in vitro* by Barton,[11] utilizing a one-electron oxidizing agent. Finally, oxidative cleavage of the aromatic ring may occur as illustrated by penicillic acid (27) which is produced from orsellinic acid (17) by *Penicillium cyclopium*.[12]



(17) (27)

There are two important variants of the acetate hypothesis which result in the overlap of the terpenoid and shikimic acid pathways[1] with the acetate route. First, alkylation by an isoprene unit can occur in a fashion analogous to the alkylation by a C-1 unit; for example, euparin (28) and evodionol (29) are acylphloroglucinols containing isoprenoid units. Second, the initial acid of the poly-β-keto chain, i.e. the chain starter, need not always be acetic acid. In fact a large number of natural products appear to be acetate-derived from cinnamic (C_6-C_3) or benzoic (C_6-C_1) acids as chain initiators. Important examples



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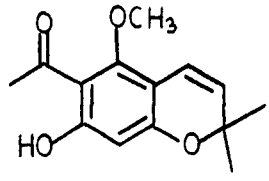
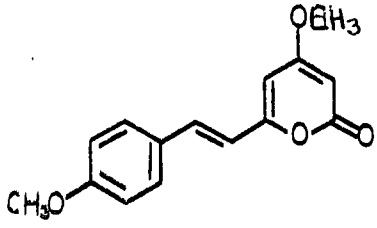


(29)

of the mixed shikimate-acetate pathway include pyrone structures such as yangonin (30), stilbenes, eg. pinosylvin (31), benzophenones, eg. maclurin (32) and the flavanoids, eg. naringenin (33).



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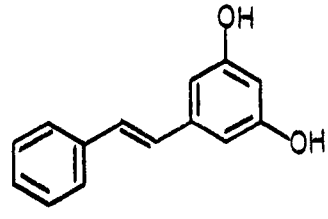
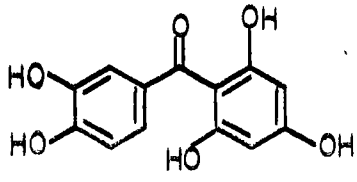
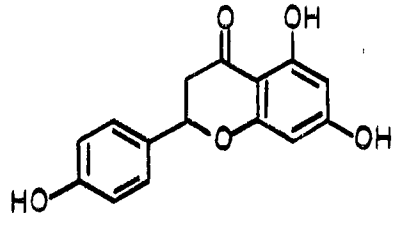


(31)



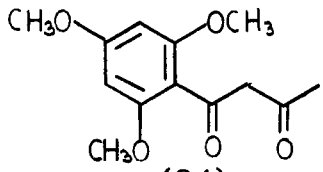
(32)



(33)

In each of the above examples the hydroxylation pattern not only distinguishes the shikimate and acetate-derived rings, but also illustrates the cyclization mode of the acetate-derived part.

With longer poly-β-keto chains the possibilities of cyclization become more varied and complex. For a chain of five acetate units, cyclizations entirely analogous to those outlined in figure 1 are possible, as illustrated by the acylphloroglucinols, eugenone (34) and eugenin (35) which co-occur in the species



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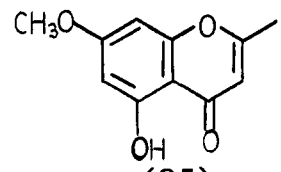
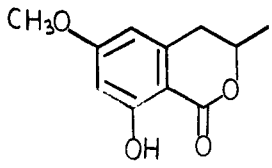
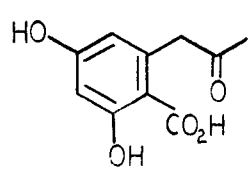


(35)

Eugenia caryophyllata.[13] The chromone structure (35) is formally derivable from the acylphloroglucinol structure (34) by further O-acylation. "Orcinol-type" compounds are also represented in nature, eg. the isocoumarin (36) and the C-acetylorsellinic acid (37). Pyrolysis or acid treatment of the latter[14] yields the



(36)



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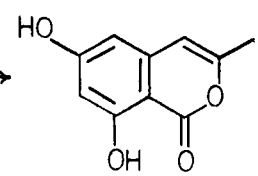
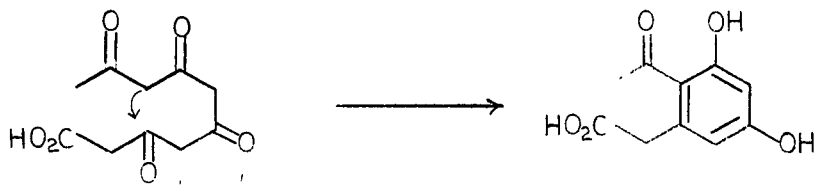


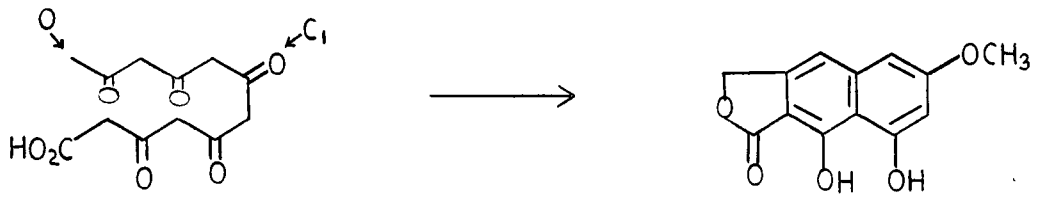
(38)

corresponding isocoumarin (38). In addition to the above cyclization modes, other aldol condensations are possible as illustrated by the product, curvulinic acid (39).[15]



(39)

Chains of six to ten acetate units are shown in figure 2. The most important group derivable from six acetate units[1] are



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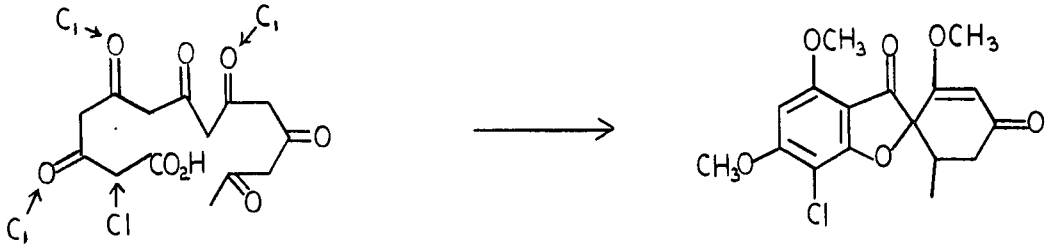
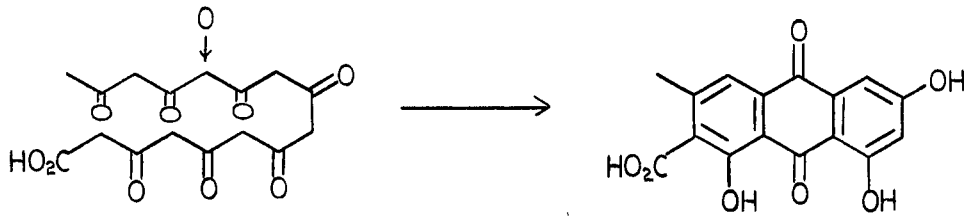
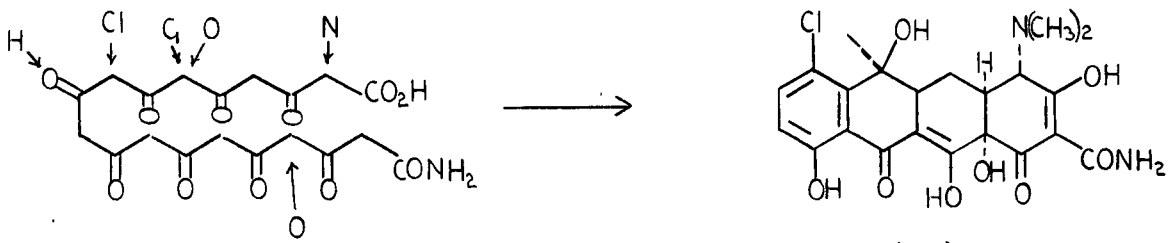


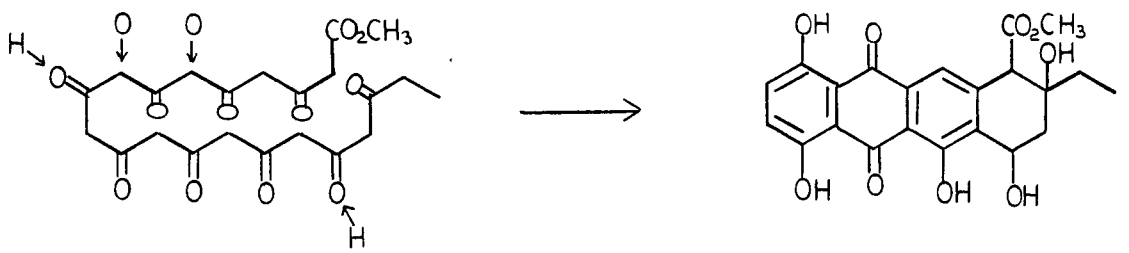
(41)



(42)



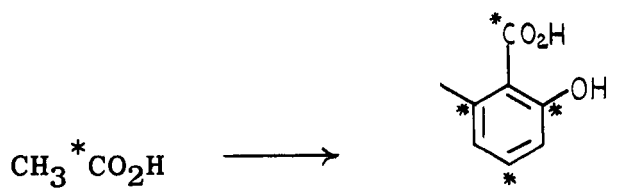
(43)



(44)

Figure 2

the naphthalenes, eg. ∝-sorigenin (40),[16] and the corresponding naphthoquinones. The cyclization of a chain of seven acetate units gives rise to a great variety of natural polycyclic compounds,[1] griseofulvin (41)[17] being one of the more unusual structures. The anthraquinones, eg. endocrocin (42), form the largest group derivable from eight acetate units. The limit to the length of the hypothetical poly-β-keto chain appears to be about ten units. Such long chains give rise to two important groups of antibiotics,[1] the tetracyclines, eg. the chlorotetracycline (43), and the mycinones, eg. ϵ-pyrromycinone (44). The tetracyclines can be considered to be derivable from a chain of nine units with the monoamide of malonic acid initiating the chain.[18] The mycinones are derivable from a chain of ten units with propionic acid initiating the chain.[19]



(20)

The first direct experimental verification of the acetate hypothesis was provided by Birch[20] who showed that 1-C^{14}-acetic acid was incorporated into 6-methylsalicylic acid (20) with the labelled atoms in the expected positions. Since then, similar labelling experiments have verified the acetate pathway[1,8] for many naturally occurring compounds. In addition, feeding experiments with labelled mevalonic[1,21] and shikimic[1,22] acids have verified several examples of the mixed acetate-terpenoid and

acetate-shikimate pathways respectively. Feeding experiments have also established methionine[1,23] as a source of C-1 fragments.

The biogenesis of 6-methylsalicylic acid (20) has been studied in considerable detail. Feeding experiments with 1-C^{14}-acetate,[24] 2-C^{14}-ethyl malonate, [25] O^{18}-acetate[26] and labelled acetyl coenzyme A[27] have shown that (20) is derived from one acetate and three malonate units. In addition, Lynen[28] has isolated an enzyme extract from a Penicillium species which catalyzes the reaction of one acetyl CoA and three malonyl CoA molecules to produce 6-methylsalicylic acid (20). These experiments and related experiments in connection with fatty acid biogenesis[1] have resulted in the only major modification to Birch's original[6] acetate hypothesis, i.e. malonyl CoA is the chain-building unit. It is quite likely that malonyl CoA is the major, if not sole chain-builder for the acetate-derived aromatic compounds, although methylmalonyl CoA is believed to be the building unit for some of the macrolides.[1,29]

In addition to defining the chain builder, studies in fatty acid biosynthesis have provided a detailed description of the carboxylation of acetyl CoA.[1] Further, Lynen[30] observed that the activity of the enzyme "fatty acid synthetase" was quenched by sulfhydryl-blocking agents; and thus he has postulated a detailed mechanism of fatty acid biosynthesis involving a sulfhydryl-containing enzyme. A modification of Lynen's model[1] (see figure 3) also serves as a useful working hypothesis for

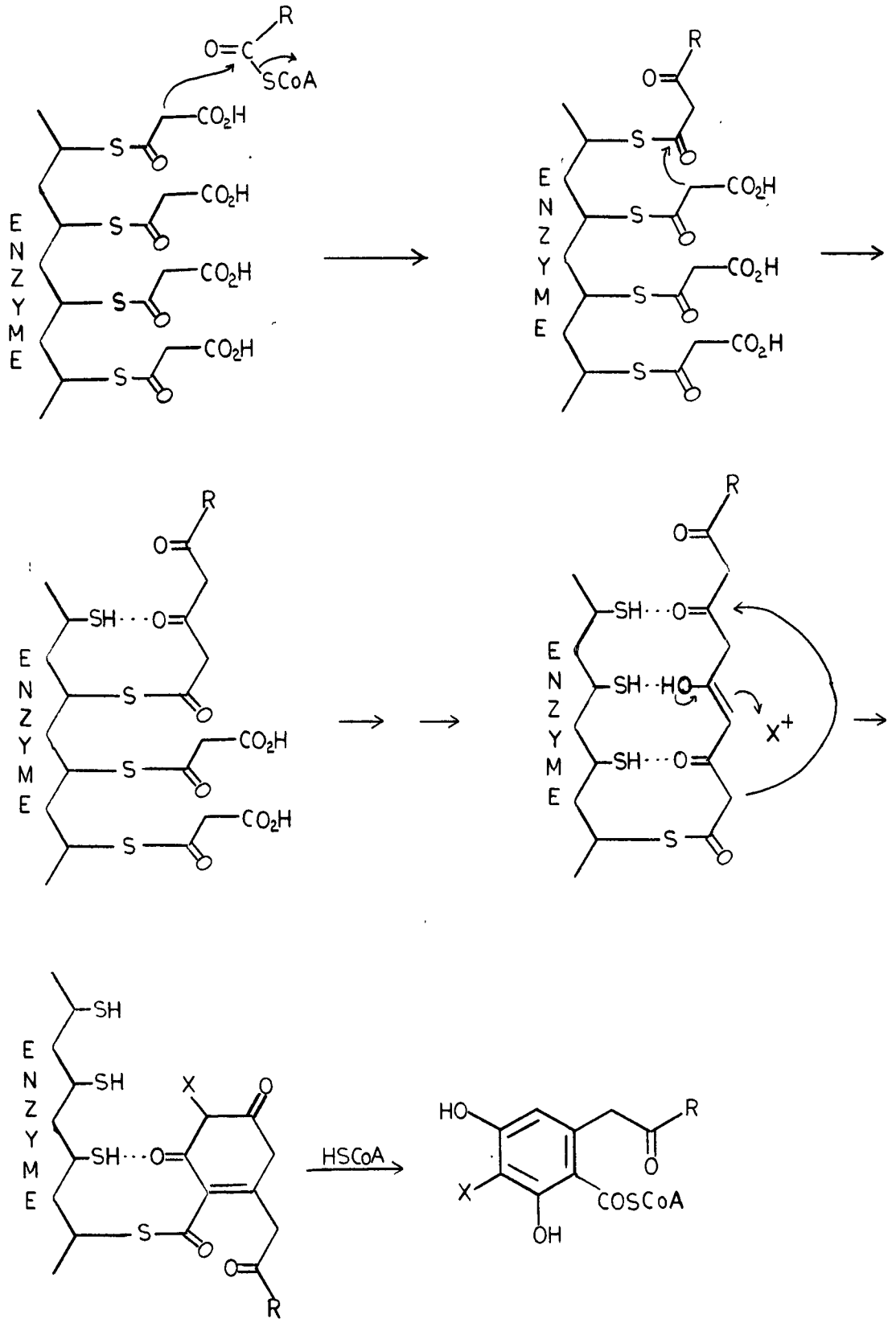
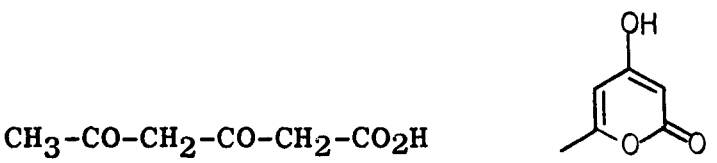


Figure 3

the biogenesis of acetate-derived aromatic compounds. It will be noted that in this model the carboxyl groups of the malonate units are assumed to be lost during the build-up of the poly-β-keto chain. There is direct support for this concept for the biogenesis of fatty acids;[1,31] and it is quite likely that the concept applies to acetate-derived aromatic compounds since there have been no verified examples of natural products which retain the residual carboxyl groups. However it is important to realize that the postulated poly-β-keto intermediates in the acetate pathway have never been isolated from nature with the exception of the C-6 unit, 3, 5-diketohexanoic acid (45)[1] which is the straight-chain form of triacetic acid lactone (16). Thus at present there is no direct evidence of the intermediate steps in the biogenesis of acetate-derived aromatic compounds; this



(45) (16)

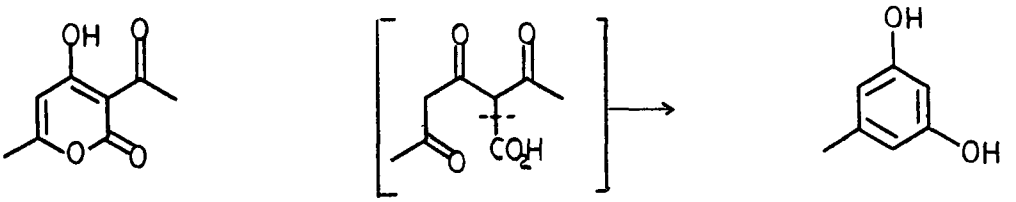
pertains not only to carboxyl loss but also to several of the variants of the acetate hypothesis, eg. reduction and alkylation, which probably occur at the poly-β-keto stage.

In addition to the direct experimental verification provided by feeding experiments, the acetate hypothesis is supported by two other criteria. One of these is the cooccurrence in a single species of several compounds of similar structure, of

which there are numerous examples.[1] Another criterion is that the reactions postulated should be chemically sound. This latter criterion is particularly important to the organic chemist since it forms the basis of the "biogenetic-type synthesis" of natural products. In such a synthesis, the chemist need not attempt to carry out the reaction under physiological conditions, but can choose any desirable set of laboratory conditions to overcome the lack of an enzyme system.

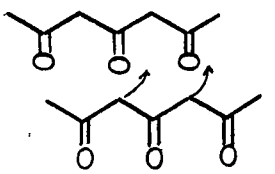
The historical development of the acetate hypothesis is remarkable in that it was first stated in 1893 and again in more detail in 1907 by Collie.[5] Unfortunately Collie's ideas remained dormant for over 50 years until the hypothesis was independently rediscovered by Birch.[6] Birch subsequently elaborated on the hypothesis[7,8] and is responsible for much of the experimental verification. What is also remarkable is that Collie developed his hypothesis from a consideration of several biogenetic-type syntheses, whereas Birch derived the same hypothesis from a correlation of structures.

Collie first observed[31] that treatment of dehydroacetic acid (46) under weakly alkaline conditions (aqueous barium hydroxide) yielded the natural product, orcinol (18). As expected, similar treatment [5,31] of heptane-2,4,6-trione (47) also yielded orcinol (18), but in addition three other aromatic compounds were obtained. The structures of these compounds have been established[5,32,33] as (48), (49), (50), and it is obvious that they arise from an intermolecular condensation of two

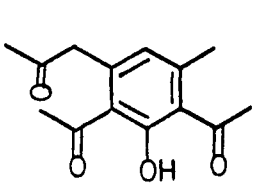


(46) (18)

molecules of heptane-2,4,6-trione (48) with subsequent cyclization (49) and dehydration (50).



(47)



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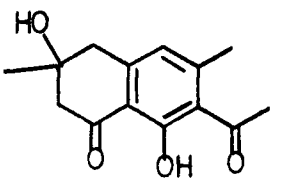
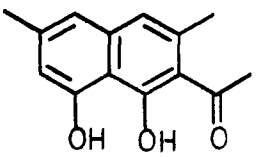


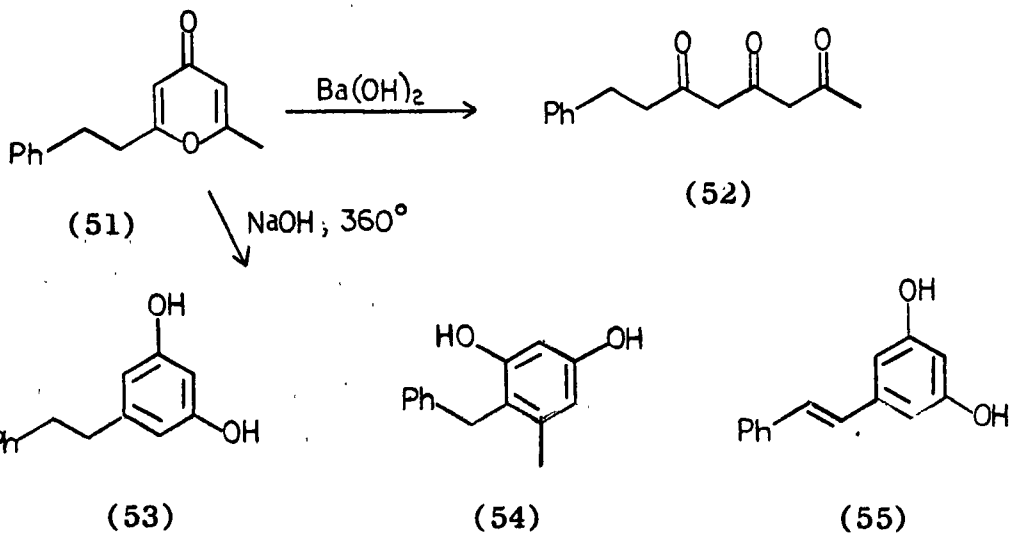
(49)



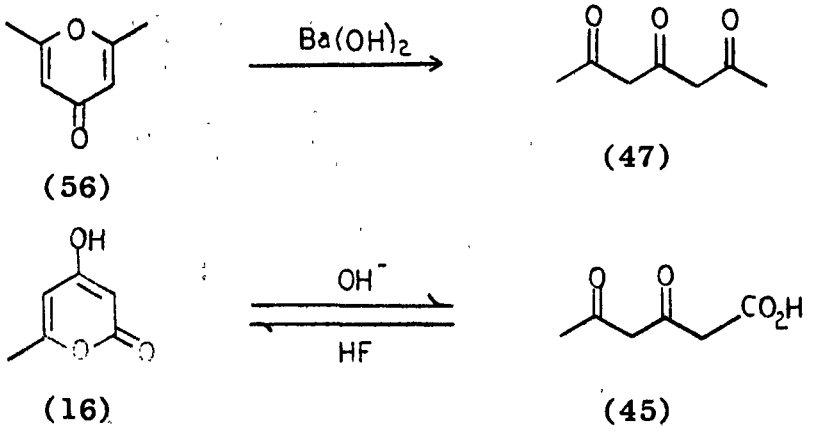
(50)

Recently, Birch[33] has carried out a biogenetic-type synthesis of dihydropinosylvin (53) by alkaline treatment of the γ-pyrone (51) or the corresponding trione (52). In addition the compound (54) was obtained which would result from the alternative intramolecular aldol condensation. Attempts to form pinosylvin (55) by an analogous procedure failed however.

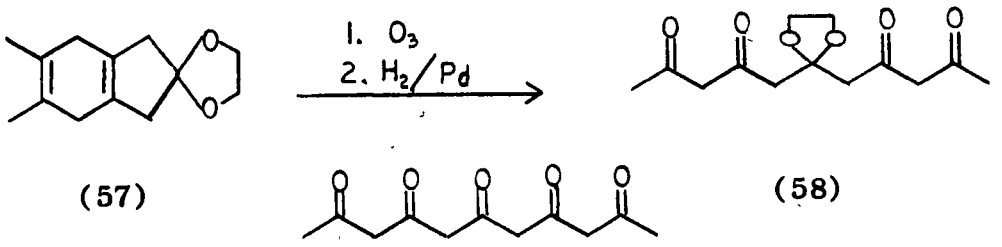
The biogenetic-type syntheses of acetate-derived aromatic compounds has been hindered by the difficulty in preparing poly-β-keto compounds. Prior to work in these laboratories the



largest members in this series were heptane-2,4,6-trione (47) which is formed by alkaline hydrolysis[31] of the dimethyl-γ-pyrone (56), and 3,5-diketohexanoic acid (45) which is formed in



a similar manner[34] from triacetic acid lactone (16). The latter reaction has been reversed by treatment of (45) with hydrofluoric acid.[10] Birch[35] has attempted to prepare longer poly-β-keto chains by ozonolysis of dihydroaromatic compounds. He succeeded in isolating the tetraketone (59) from the dihydroindanone ketal (57). However attempts to isolate the pentaketone (59) were unsuccessful; and attempts to cyclize (58) or (59) by treatment

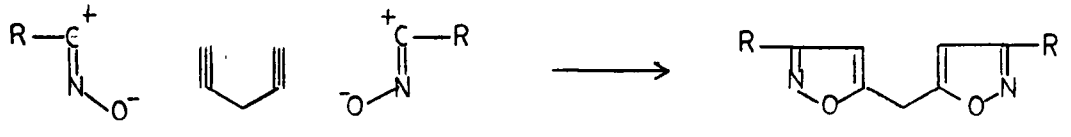


(57) (58)

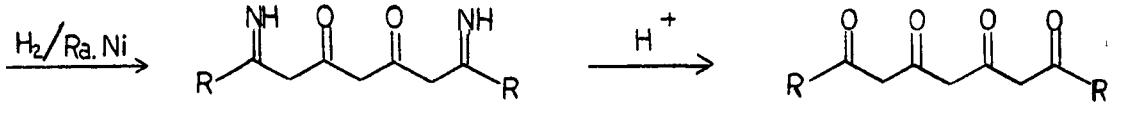
(59)

with base gave complex mixtures which were not investigated further.

Recently a convenient synthesis of tetra-β-ketones has been reported.[36] The reaction sequence involves the formation of a diisoxazolylmethane (61) from the addition of two molecules of nitrile oxide, RCNO, to diethynylmethane (60), followed by catalytic reduction to form the imino derivative (62), and subsequent acid hydrolysis to the tetraketone (63). In this way dibenzoylacetylacetone (63, R = phenyl) has been synthesized.

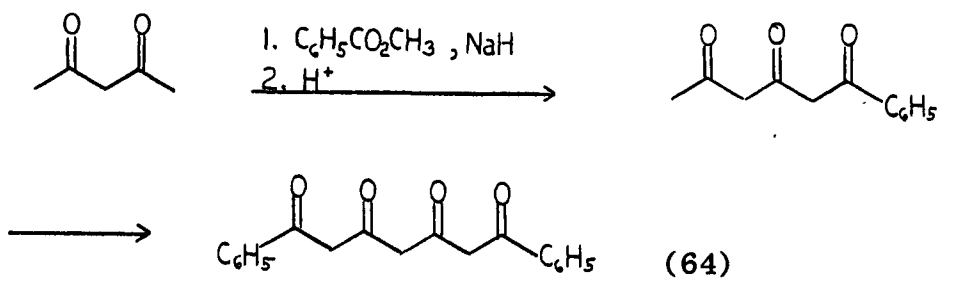


(60) (61)



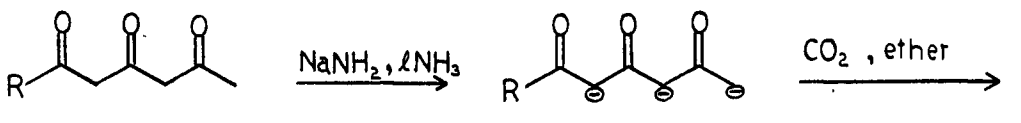
(62) (63)

Dibenzoylacetylacetone (64) has also been synthesized from acetylacetone by successive aroylation with methyl benzoate in a sodium hydride: 1,2-dimethoxyethane system.[37] Alternatively, the system sodium amide-liquid ammonia[38] has been used for

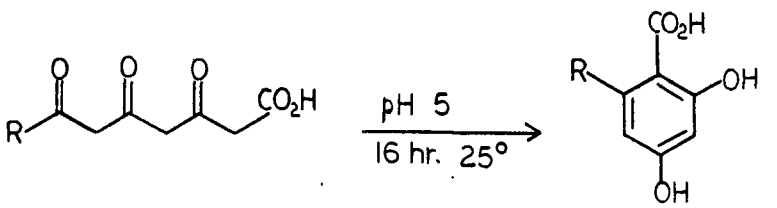


(64)

similar acylations and alkylations. This latter system has proved very useful for synthesizing diketo and triketo acids by carboxylation[10,38,39] of the corresponding ketones (65). The syntheses of the triketo acids (67) were carried out by first generating the anions (66), followed by rapid removal of the liquid ammonia with simultaneous replacement by ether; and finally the addition of dry ice effected the carboxylation. By



(65) (66)



(67) (68)

this method the acids (67) with R = $C_6H_5CH=CH$, nC_7H_{15}, nC_5H_{11}, nC_3H_7 and CH_3 have been formed in yields of 52%,30%,14%,2% and trace respectively. Treatment of these acids[39] under very mild conditions (aqueous buffer, pH 5, 25°, 16 hr.) resulted in the conversion (ca. 90% yield) to the respective aromatic acids (68): pinosylvic, spheropherolcarboxylic, olivetolcarboxylic, divaric and orsellinic. All of these acids except pinosylvic (68,

R = $C_6H_5CH=CH$) are natural products, and the latter was easily decarboxylated to the naturally-occurring pinosylvin (55), thus completing a remarkable biogenetic-type synthesis. The ease with which the poly-β-keto acid (67) cyclizes under essentially physiological conditions provides good support for its intermediacy in the biogenesis of acetate-derived aromatic compounds.

In these laboratories it was felt that a convenient way of overcoming the difficulties in the preparation and isolation of poly-β-keto chains would be to hold the chains in the form of condensed polypyrone structures (see figure 4). These polypyrone structures could be constructed by successive condensations with malonate units in a manner analogous to the biogenesis of poly-β-keto chains, with the exception that the retention of the carboxyl groups is required for pyrone formation. The generation, in basic media, of the poly-β-keto acid analogue of the monocyclic α-pyrone, triacetic acid lactone (16) has already been demonstrated,[34,40,41] and in fact the biogenetic-type syntheses of both Collie[31], and Birch[33] were based on the alkaline treatment of monocyclic pyrone structures. Thus, in basic media, the dipyrone (69) would be expected to generate a poly-β-keto chain of four acetate units; the tripyrone (70), a chain of five acetate units; and the tetrapyrone (71)*, a chain of six acetate units. Unfortunately these chains do retain the residual carboxyl groups (see figure 4); however, it is quite likely that they could be lost at some later stage in the biogenetic-type synthesis.

*for nomenclature based on condensed aromatic systems see experimental section.

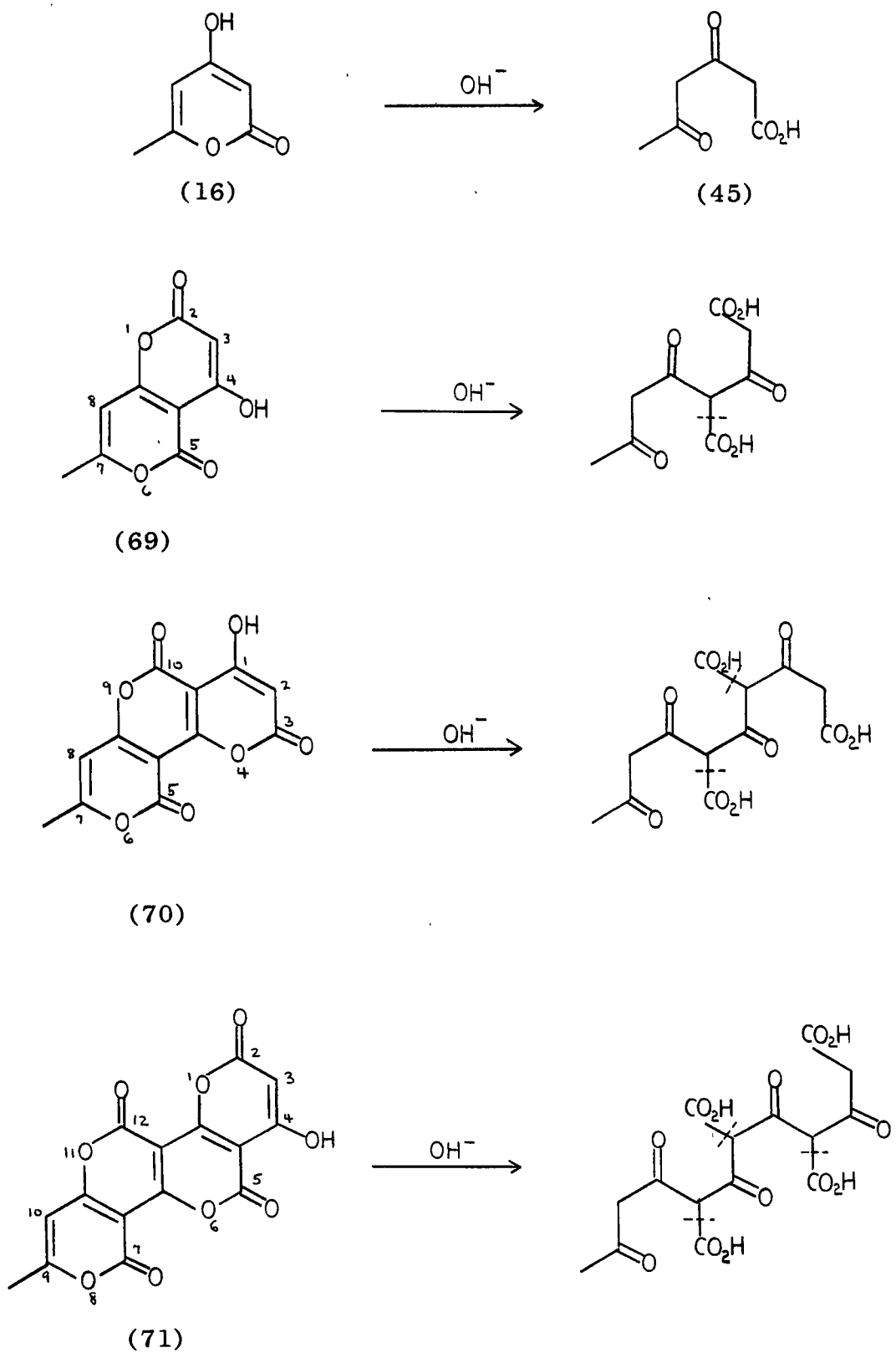
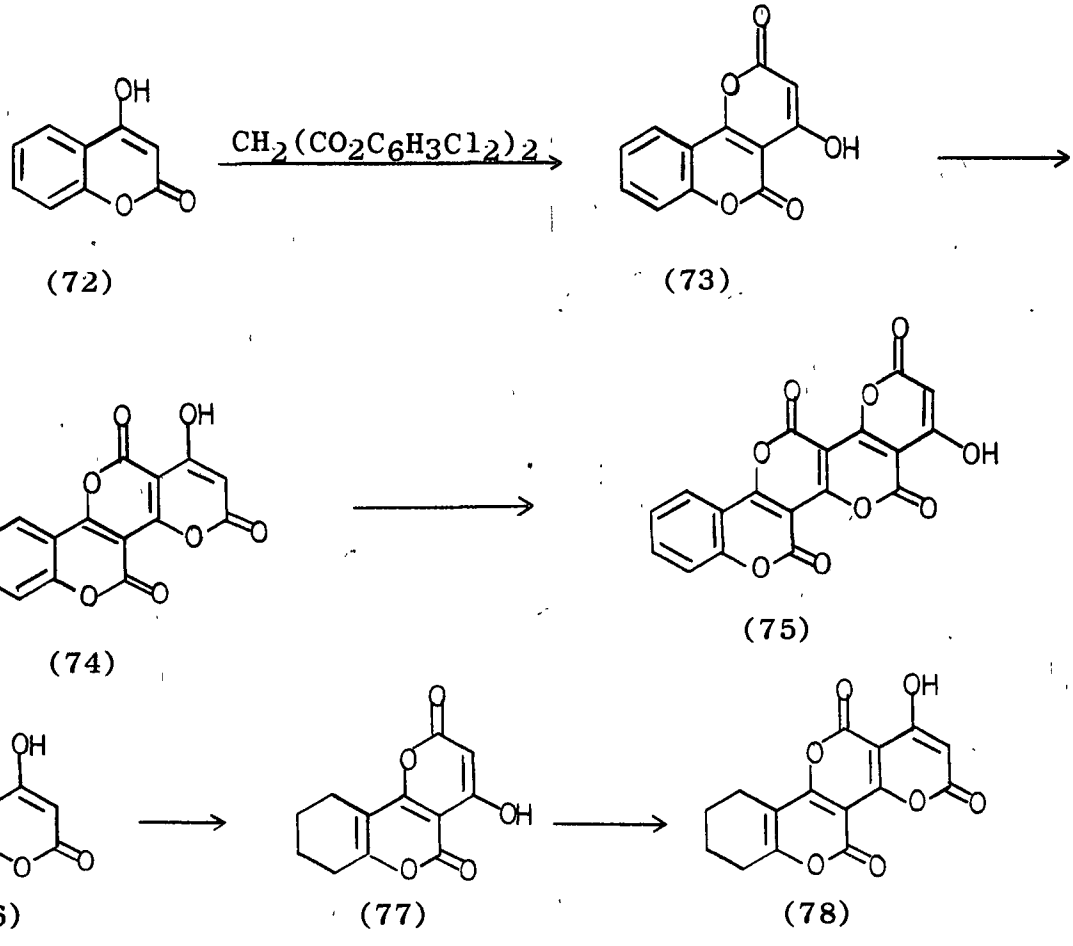
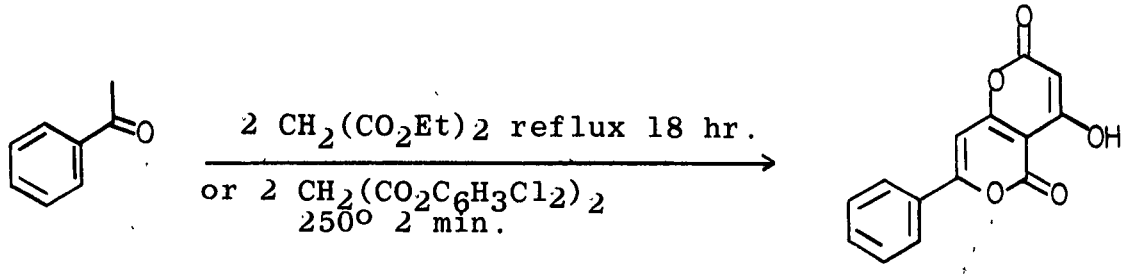

OH
OH⁻
CO₂H
(16)
(45)
OH⁻
CO₂H
CO₂H
(69)
OH
OH⁻
CO₂H
CO₂H
CO₂H
(70)
CO₂H
OH⁻
CO₂H
CO₂H
CO₂H
(71)

Figure 4

A search of the literature revealed that condensed polypyrone structures have been synthesized, notably by Ziegler[42] and Mentzer.[43] In his investigations on the structure of "red-carbon", the polymerization product of carbon suboxide (C_3O_2), Ziegler has synthesized a large number of condensed polypyrone structures. The closest analogies to the pyrones shown in figure 4 are the two series (73),(74),(75) and (77),(78) which were synthesized in a stepwise manner from the 4-hydroxycoumarin (72)



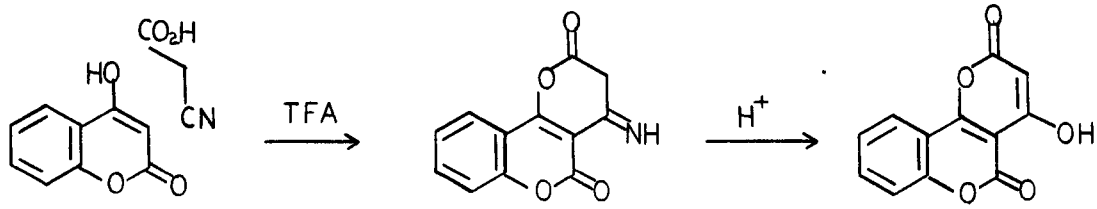
and the 4-hydroxytetrahydrocoumarin (76)[44] respectively. The syntheses were best carried out by fusing equimolar quantities of pyrone and the condensing unit, bis (2, 4-dichlorophenyl)



(79)

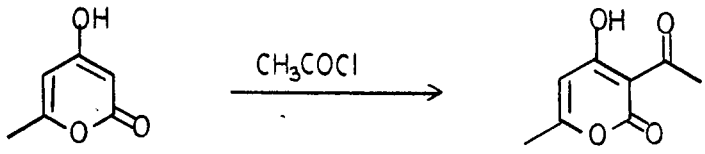
malonate at 250°. Mentzer[43] has synthesized the phenyldipyrone (79) by refluxing acetophenone in diethylmalonate; this pyrone (79) has also been synthesized independently[44] by Ziegler using bis (2,4-dichlorophenyl) malonate as the condensing reagent.

The aromatic dipyrone (73) has also been synthesized by Woods[45] by condensing cyanoacetic acid with 4-hydroxycoumarin (72) in refluxing trifluoroacetic acid, followed by acid hydrolysis of the imine (80).



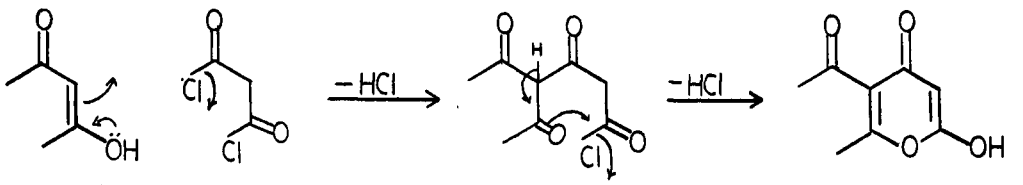
(72) (80) (73)

Woods also had observed that triacetic acid lactone (16) was readily acylated with acetyl chloride at the 3-position to give back dehydroacetic acid (46). In addition Elvidge[47] has



(16) (46)

carried out a number of heterocyclic syntheses with malonyl dichloride; for example, malonyl dichloride condenses readily with the β-diketo compound, acetylacetone, to form 3-acetyl-6-hydroxy-2-methyl-4-pyrone (81), an isomer of dehydroacetic acid



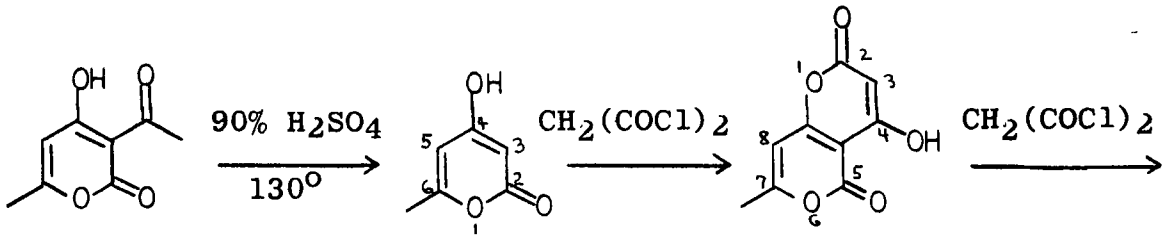
(81)

(46). It is pertinent to note that the synthesis of (81) involves both C- and O- acylation of acetylacetone by malonyl dichloride.

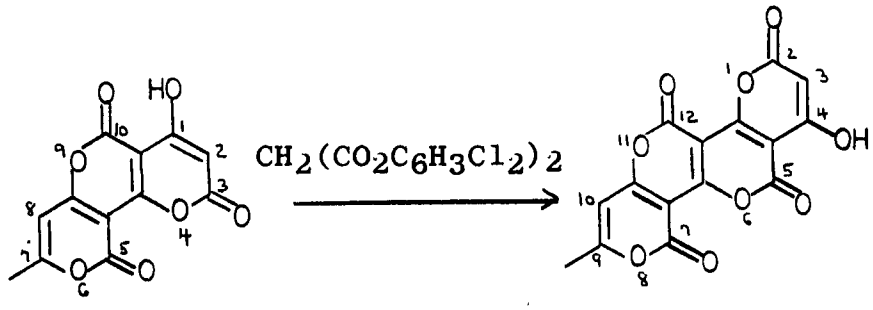
Utilizing the ideas outlined in the papers mentioned above, workers in these laboratories[48,49] have synthesized the poly-pyrones listed in figure 4; and in addition have treated the dipyrone (69) and the tripyrone (70) under a variety of basic conditions, and have isolated and characterized the aromatic compounds which were formed.

DISCUSSION

The syntheses of the polypyrones listed in figure 4 are outlined below:



(46) (16) (69)

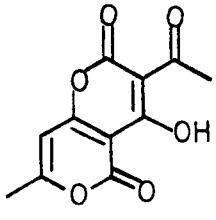


(70) (71)

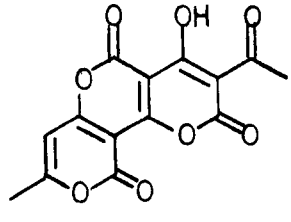
Triacetic acid lactone (16) was prepared by deacetylation of the commercially available dehydroacetic acid (46) by the method of Collie.[50] The dipyrone (69) had previously been synthesized[51] in these laboratories by refluxing a trifluoroacetic acid solution of triacetic acid lactone (16) and a two-fold excess of malonyl dichloride. The tripyrone (70) had been synthesized from the dipyrone (69) in a similar fashion except that an eight-fold excess of acid chloride was required. The synthesis of dipyrone (69) had also been carried out using the condensing reagents cyanoacetic acid and ethylchloroformylacetate, but neither of these reagents could effect the synthesis of tripyrone (70). In addition previous attempts[51] to synthesize the tetrapyrone (71) from tripyrone by treatment with malonyl dichloride

had failed. As part of the work reported in this thesis, the methods of synthesis, and notably the methods of isolation and purification of the pyrones (36), (69) and (70) were improved; and the synthesis of the tetrapyrone (71) was achieved.

As part of the synthesis of tetrapyrone, the method of preparation of tripyrone was investigated. From the steadily increasing difficulty in the formation of the polypyrones (69), (70),(71), it was obvious that as the condensed polypyrone structure was built-up the reactivity at the acylating positions decreased. This was also revealed by the acylation of dipyrone and tripyrone with acetyl chloride[51] to give respectively the acetyldipyrone (82) and the acetyltripyrone (83); the synthesis of the latter requiring more vigorous conditions. Acetyltripyrone (83) has also been isolated[51] in low yield from the attempts to prepare tetrapyrone from the condensation of malonyl dichloride with tripyrone, and was an impurity in the analogous preparation of tripyrone.



(82)



(83)

In the investigation of the reagent, malonyl dichloride, it was observed that its use was limited by the heat-catalyzed formation of a red-brown polymer. The diester reagents[42] were

more stable and could be used for synthesizing both tripyrone and tetrapyrone. The formation of a melt (230°, 10 min.) of dipyrone and bis (2,4-dichlorophenyl)malonate yielded a mixture of dipyrone, tripyrone and tetrapyrone in the ratio of about 2:1:0.4. This procedure provides a useful synthesis of both of the higher pyrones and has the advantages over the malonyl dichloride method of increased yields and the absence of the acetylpolypyrone impurities. Tetrapyrone was also synthesized in a similar fashion from tripyrone and isolated in about 30% yield. The other diester reagents, diphenylmalonate and bis(2,4,6-trichlorophenyl)malonate were less effective in the preparation of higher pyrones than bis(2,4-dichlorophenyl)malonate; and diethylmalonate was ineffective.

Tetrapyrone was characterized by analytical and spectral data. The N.M.R. spectrum was nearly identical with the spectra of the other two polypyrones in this series exhibiting, in trifluoroacetic acid, singlet peaks at 3.43 ($C_{10}H$), 3.99 (C_3H) and 7.45 τ (C_9CH_3). The I.R. spectrum in the carbonyl and olefinic stretch regions was also similar with peaks at 1755, 1705, 1645, 1590 and 1555 cm^{-1}. As expected the U.V. absorption was displaced to longer wavelengths than for tripyrone with maxima at 398,385 and 272 mμ; and finally the mass spectrum showed a strong parent peak at m/e = 330.

Treatment of these condensed polypyrone structures with base results in ring opening and subsequent condensation to form aromatic compounds. The results which had previously been ob-

tained[51,52] from the ring opening experiments with dipyrone (69) and acetyldipyrone (82) are summarized in figure 5. The open form of dipyrone (69a) can cyclize via either a Claisen or aldol-type condensation to give respectively compounds of the acetylphloroglucinol (84) or orcinol-type (85). Compounds of the latter type[51] were obtained when dipyrone was treated with either 1N aqueous potassium hydroxide or 1N methanolic potassium hydroxide solutions. Under aqueous conditions, the natural product orsellinic acid (17) was obtained, while from methanolic potassium hydroxide solutions methyl orsellinate (86), 2,4-dicarbomethoxyorcinol (87) and p-O-methylorsellinic acid (88) were isolated. The formation of methyl esters and ethers under the methanolic conditions is not surprising since the predominant basic species is probably methoxide anion.[53,54] The Claisen condensation mode was effected by the system magnesium methoxide-methanol[52] to give carbomethoxyacetylphloroglucinol (89). Thus both of the cyclization modes observed in nature for a poly -β- keto chain of four acetate units have been carried out in vitro.

The ring-open form of acetyldipyrone (82a) can similarly cyclize via two condensation modes to give compounds of the diacetylphloroglucinol (90) and the acetylorcinol type (91). Treatment of acetyldipyrone with methanolic potassium hydroxide solution[51] gave the expected aldol condensation product, orcacetophenone (92); however from the methanol-magnesium methoxide system[52] only the degradation product, dicarbomethoxy-orcinol (87) was isolated.

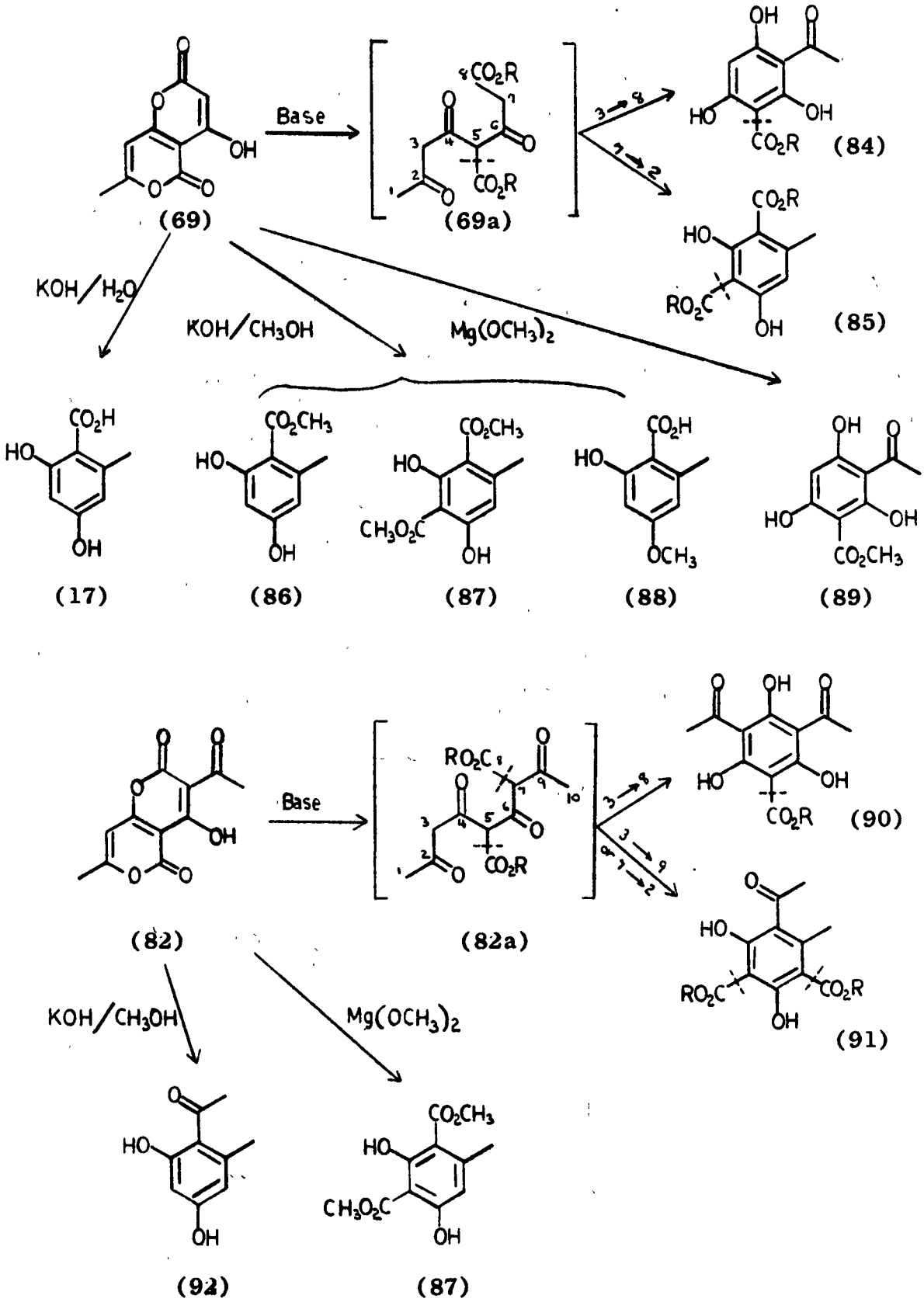
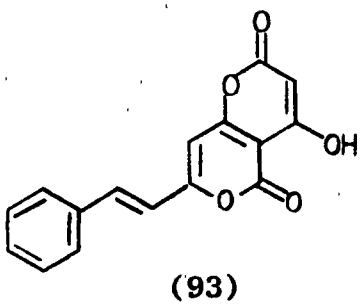
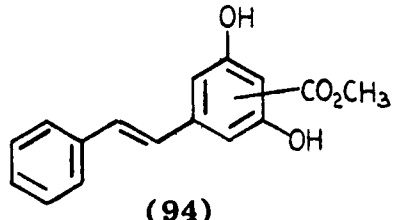

Base
(69)
(69a)
(84)
(85)
KOH/H2O
KOH/CH3OH
Mg(OCH3)2
(17)
(86)
(87)
(88)
(89)
Base
(82)
(82a)
(90)
(91)
KOH/CH3OH
Mg(OCH3)2
(92)
(87)

Figure 5

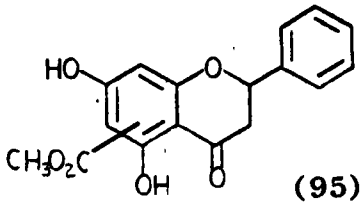
The styryldipyrone (93) has also been prepared by workers in these laboratories[55] and base treatment has given structures representative of the stilbenes (94) and the flavanones (95). The selectivity in the condensation modes was achieved as for the dipyrone (69). The dihydrostyryldipyrone (96), when



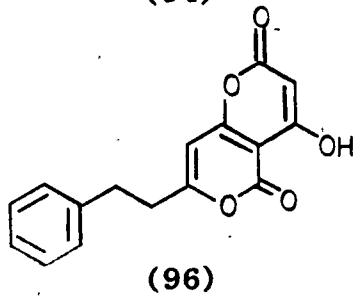
(93)



(94)



(95)



(96)

similarly treated, gave structures representative of dihydrostilbenes and dihydrochalcones.[55]

As part of the work reported in this thesis, tripyrone (70) was treated under the basic conditions described above, and the aromatic products formed were isolated and characterized. The ring-open form of tripyrone (70a) can cyclize in many ways, the three aldol condensation modes (3 → 9, 7 → 2, 10→4) and the three Claisen-type condensation modes (3 → 8, 5 → 1, 10 → 12) are illustrated in figure 6. From subsequent O-acylation and dehydration various oxygen-containing heterocyclic structures are possible as shown in figure 6. Empirically, a large number of aromatic compounds were formed, and those which were isolated

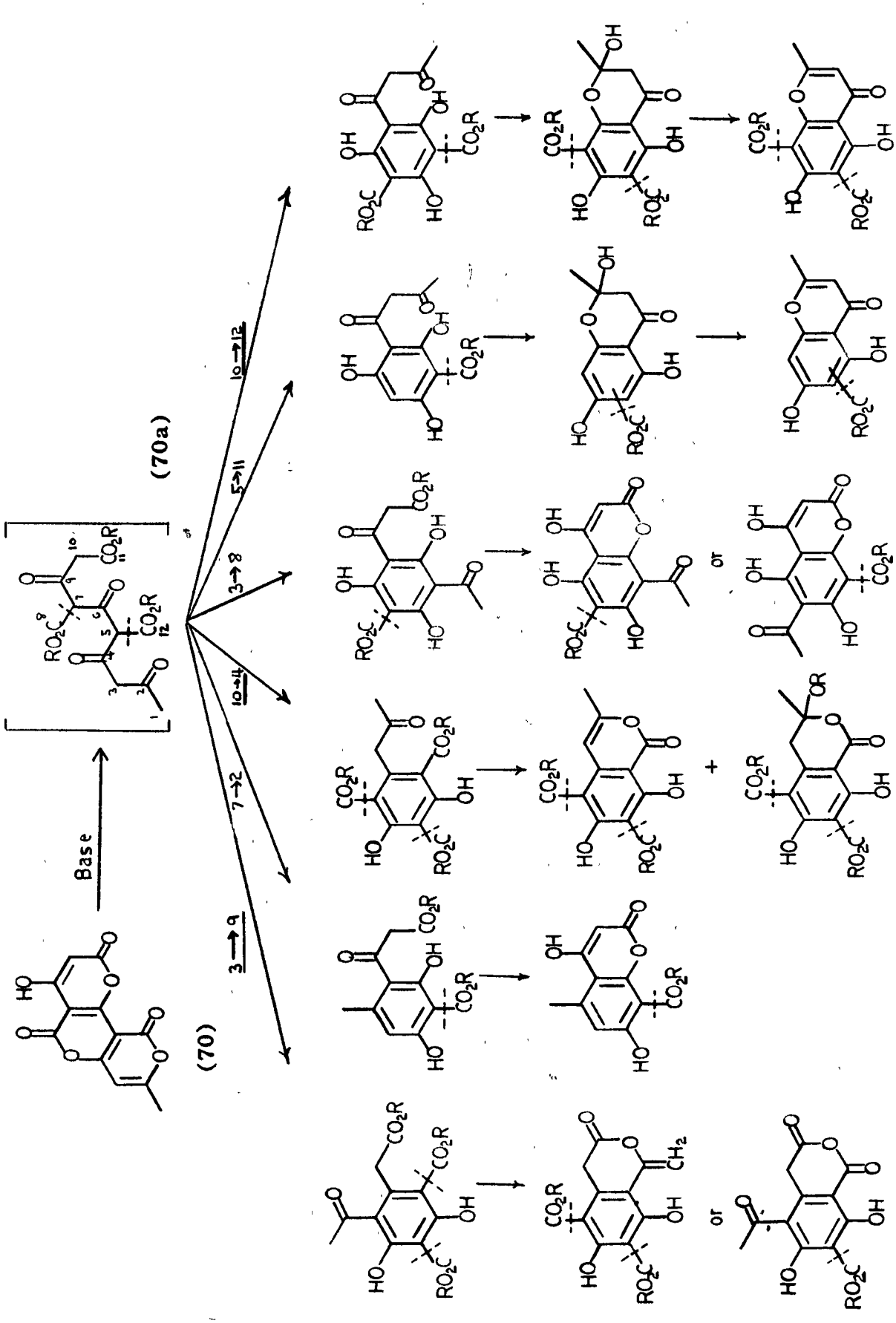


Figure 6

in sufficient quantity and purity to be characterized are given in figure 7.

From the treatment of tripyrone with 1N aqueous potassium hydroxide at 25° only one aromatic compound was isolated, and this was shown to be the degradation product, orcacetophenone (92). Proof of structure was provided by comparison of spectral properties, m.p. and undepressed mixed m.p. with authentic material previously prepared[51] by the method of Hoesch.[56] This compound has been isolated[51] from base treatment of acetyldipyrone (82); it is quite likely that in the base treatment of tripyrone it formed via acetyldipyrone (82) since this degraded pyrone was also isolated from the reaction mixture.

From the treatment of tripyrone with 1N methanolic potassium hydroxide at 25° several aromatic compounds were isolated and characterized (see figure 7). The total yield of aromatic products was ca. 15-20%, however about one-third of this was degraded material. Orcacetophenone (92) was again isolated, and another degradation product, 2,4-dicarbomethoxyorcinol (87) was also isolated. This latter compound (87) had previously been isolated[51] from base treatment of dipyrone (69); the structural assignment was made on the basis of spectral and analytical data, and on the similarity of the U.V. spectrum (λmax:316,262sh, 247sh, 232 mμ) with that of the known compound,[57,58] 3,5-dihydroxy-2, 4-dicarboxyphenylacetic acid trimethyl ester (97) λmax: 314, 260 infl, 247sh, 228 mμ.

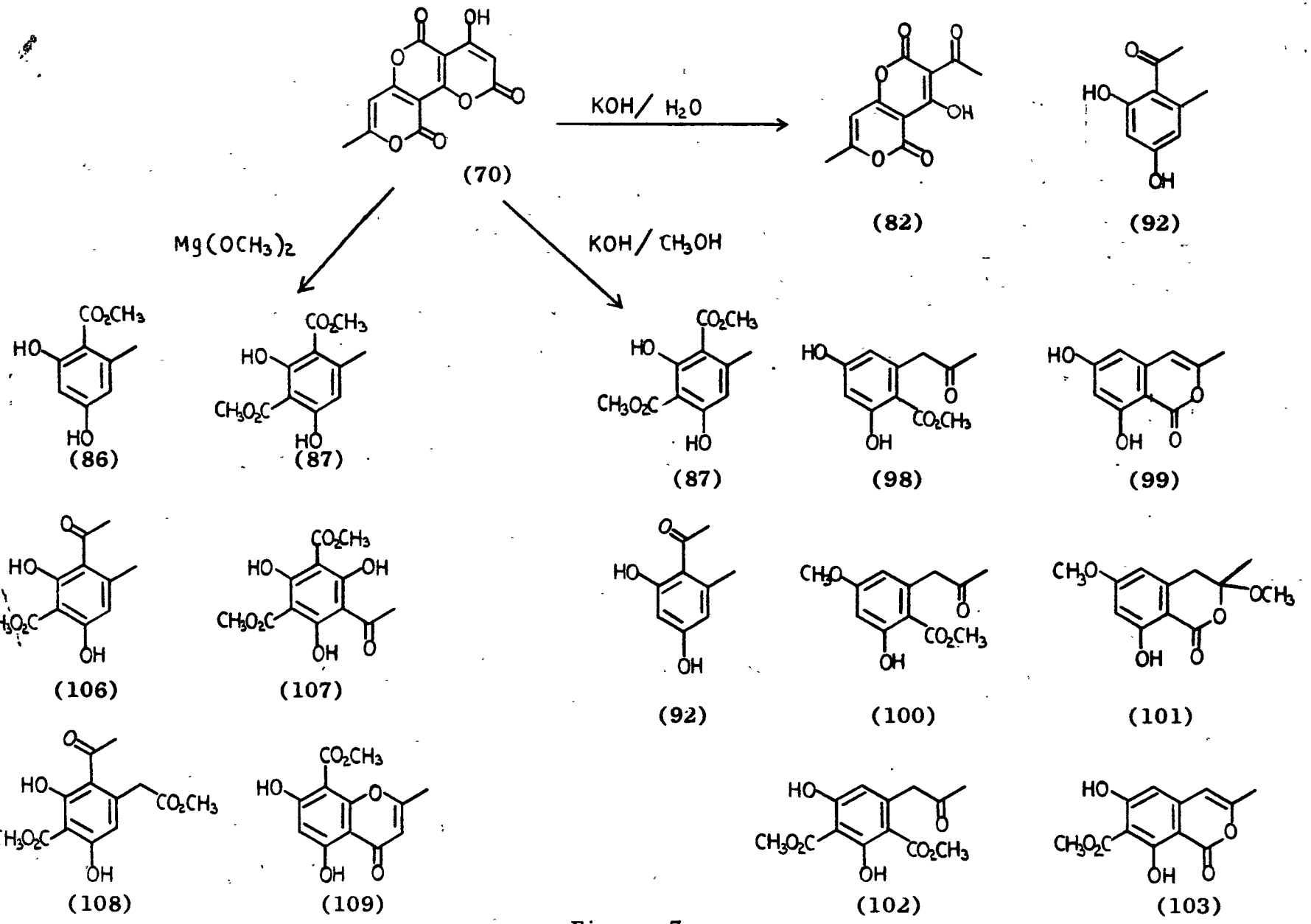
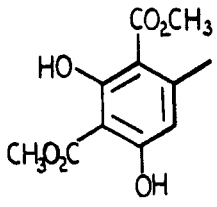
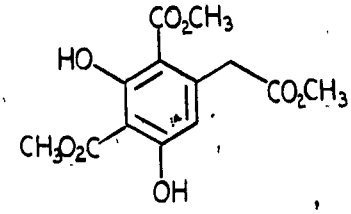

KOH/ H2O
Mg(OCH3)2
KOH/ CH3OH
(70)
(82)
(92)
(86)
(87)
(87)
(98)
(99)
(106)
(107)
(92)
(100)
(101)
(108)
(109)
(102)
(103)

Figure 7



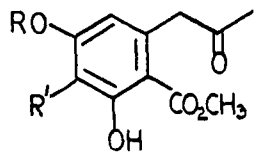
(87)



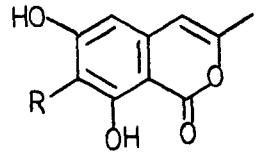
(97)

In addition to these degradation products, six new aromatic compounds were obtained (see figure 7). All of these compounds appear to arise via the aldol cyclization mode 10 → 4, of the open form of tripyrone (70a, figure 6). Further, the compounds can be grouped into three pairs with the isocoumarin structures (99), (101), (103) probably arising via the corresponding phenylpropanone structures (98), (100) and (102) during the acid work-up. Compounds of both type (98) and (99) occur in nature,[1] probably arising via the same cyclization modes.

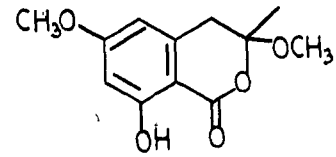
The characteristic properties of each of these compounds are in excellent agreement with the structural assignments. All compounds exhibited a positive ferric chloride test, and for each the molecular weight was indicated by a strong parent peak in the mass spectrum. The saturated carbonyl groups of compounds (98), (100), (102) appeared in the infrared spectrum at 1700-1710 cm^{-1} whereas the conjugated ester carbonyls appeared at 1660-1670 cm^{-1}, and the hydrogen-bonded isocoumarin carbonyls of (99), (101), (103) at 1675-1685 cm^{-1}. The N.M.R. spectra defined all of the protons for each of the compounds. The hydrogen-bonded hydroxyl groups were indicated by sharp signals at very low field (-1.2 to -2.8 τ) whereas the free



(98) R=R'=H
(100) R=CH_3, R'=H
(102) R=H, R'=CO_2CH_3



(99) R=H
(103) R=CO_2CH_3

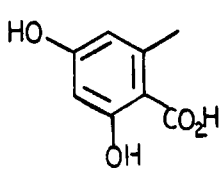


(101)

hydroxyls (98,99) appeared as broad signals at ca. + 0.5 τ. The methyl ethers and esters appeared at 5.97-6.17 τ with the exception of the ketal methyl ether of (101, 6.68 τ). The protons on the aromatic rings as well as the olefinic protons of (99), (103) exhibited signals in the region 3.51-3.72 τ. The CH_3-CO-CH_2-Ar system of (98), (100), (102) was characterized by a methyl peak at 7.82-7.84 τ and a methylene peak at 6.00-6.37 τ. The methyl group of the system $CH_3-\overset{O}{C}=C-Ar$ of the isocoumarins (99) and (103) appeared respectively at 7.78 and 7.75 τ, whereas the system $CH_3-\overset{O}{\underset{O}{C}}-CH_2-Ar$ of the methoxydihydroisocoumarin (101) was characterized by a methyl peak at 8.37 τ and a methylene peak at 6.84 τ.

The ultraviolet spectra of these compounds was also diagnostic. The phenylpropanones (98) and (100) and the methoxydihydroisocoumarin (101) all exhibit U.V. spectra characteristic of the natural products orsellinic acid[59,60] (17), λmax: 300(ε 4,000), 260 (ε 12,600) and C-acetylorsellinic acid[14,61] (37), λmax: 305 (ε 6200), 270 (ε 12,900). For compounds (100), (101) this provides further proof of the location of the methyl ethers since it has recently been shown[62] that compounds of structure (104,

R=R'=H) and (104, R=Me, R'=H) have orsellinic acid U.V.'s whereas



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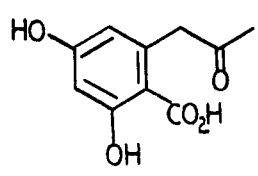

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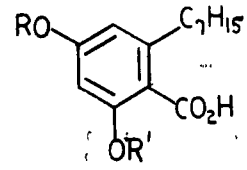
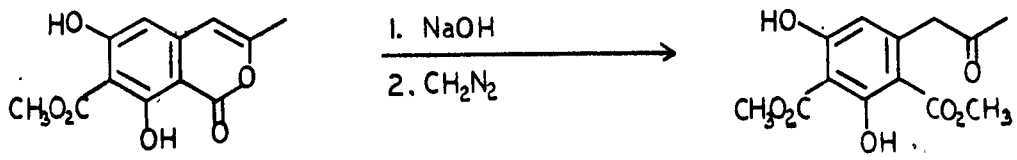


(104)

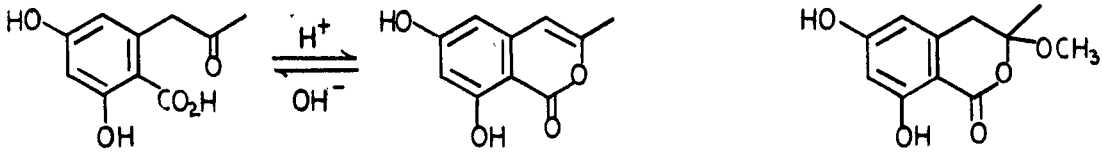
compound (104, R=H, R'=Me) has a different U.V. spectra (λmax: 282, 245sh). The presence of a second carbomethoxy group attached to the ring alters the U.V. spectra; however the spectra of (102, λmax: 316, 265sh, 248, 231 mμ) was very similar to that of 2,4-dicarbomethoxyorcinol (87), thus suggesting the second carbomethoxy group was located ortho to both hydroxyl groups. A positive Gibbs test[63,64,65] for (102) confirmed this assignment. The isocoumarin (99) is a known compound and the U.V. spectrum (λmax: 324, 289, 278, 257sh, 245, 237 mμ) and m.p. 250-253° are in agreement with that reported.[14,61] The U.V. spectrum of compound (103), λmax: 340, 325sh, 303, 291, 274sh, 257, 250sh, 214sh mμ, was typical of an isocoumarin structure despite the effect of the carbomethoxy group. The location of the carbomethoxy group was again established by a positive Gibbs test.

As final proof of the isocoumarin structure (103), it was converted to (102) by aqueous sodium hydroxide treatment according to Hassall,[61] followed by methylation with diazomethane. Hassall[61] has effected a similar conversion of the isocoumarin (99) to C-acetylorsellinic acid (37).



(103) (102)

It is not surprising that isocoumarin and methoxydihydroisocoumarin formation has occurred during the acid work-up. Raistrick[14] has converted the natural product, C-acetylorsellinic acid (37) to the isocoumarin (99), by acid treatment; and on acid work-up of a methanolic solution containing this acid (37) he obtained the methoxydihydroisocoumarin (105).



(37) (99) (105)

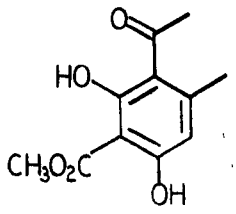
Treatment of tripyrone with 1M methanolic magnesium methoxide solution at 25° also resulted in the formation of many aromatic compounds (total yield 10-15%); however different cyclization modes were preferred and degradation was more severe. The compounds which were isolated in sufficient quantity and purity to be characterized are shown in figure 7. In addition characteristic properties of two other compounds (110) and (111) are given; however structural assignments for these two compounds could not be made and it is quite likely that the properties reported are not those of homogeneous compounds. Once again, 2,4-dicarbo-

methoxyorcinol (87) was isolated and identified by comparison with an authentic sample.[51] Another "dipyrone product", methyl orsellinate, (86) was also isolated and identified by comparison of its spectral properties, m.p. and undepressed mixed m.p. with an authentic sample.[51]

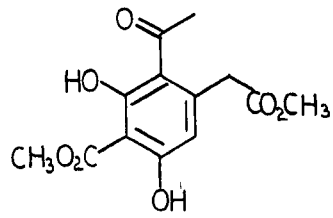
Four new compounds were characterized. Unfortunately each of these contains carbomethoxy groups attached to the ring, thus obscuring the characteristic U.V. spectra of orcacetophenone, acetylphoroglucinol or 6,8-dihydroxychromones. Nevertheless the characteristic properties of each of the compounds (106,107, 108,109) provide good support for the structural assignments made.

All of the compounds exhibited a positive ferric chloride test, and for each the molecular weight was indicated by a strong parent peak in the mass spectrum. Compound (106) is believed to be the "acetyldipyrone product", 3-acetyl-4-carbomethoxyorcinol. The acetyl function was defined by the I.R. (1645 cm^{-1}) and N.M.R. spectra (7.47 τ, 3H), as was the carbomethoxy group (1675 cm^{-1}, 5.95 τ). The N.M.R. spectra also characterized the hydrogen-bonded hydroxyl groups (-1.35, -0.10τ), the ring methyl (7.70τ) and the aromatic proton (3.68τ). Finally, a positive Gibbs test suggested the location of the carbomethoxy group.

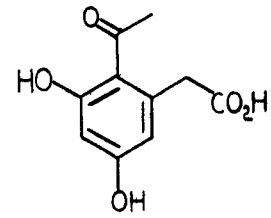
The U.V. spectrum of compound (108), λmax: 327, 265sh, 250, 236sh, was very similar to that of compound (106). In addition



(106)



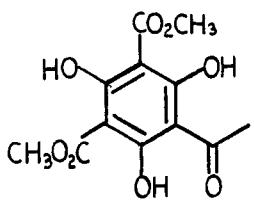
(108)



(39)

the Ar-CH_2CO_2Me group was defined by the I.R. (1725 cm^{-1}) and N.M.R. spectra (6.29, 6.31τ). The other carbonyl groups and protons were defined as for (106), and a positive Gibbs test suggested the position of the carbomethoxy group. This compound (108) arises from the aldol cyclization mode 3 → 9 (figure 6) and is in fact the 4-carbomethoxy derivative of the natural product, curvulinic acid (39).[15] Thus another of the "naturally-occurring" cyclization modes for a poly-β-keto chain of five acetate units has been demonstrated in vitro.

The major product isolated from the magnesium methoxide treatment of tripyrone was the dicarbomethoxyacetylphloroglucinol (107). All of the functional groups were established from the I.R. and N.M.R. spectra. Compound (107) probably arises via cleavage of the structure (112, R=CO_2Me) which in turn is formed from the open form of tripyrone (70a) via the Claisen cyclization



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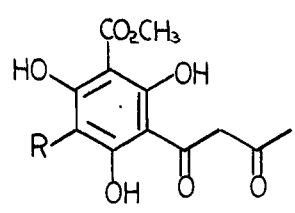
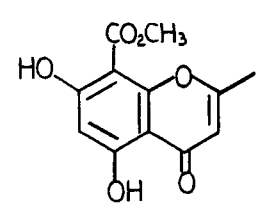


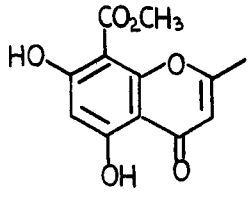
(112)



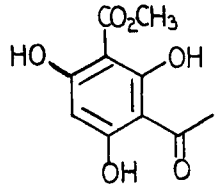
(109)

mode 10 → 12 (figure 6). The structure (112) is capable of further O-acylation and dehydration to form carbomethoxy derivatives of the naturally-occurring dihydroxymethylchromones. The chromone analogue of (112, R=CO_2Me) was not isolated; however compound (109) is believed to be the chromone analogue of structure (112, R=H).

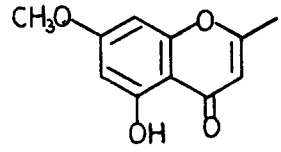
The N.M.R. spectrum of (109) was very similar to that of the isocoumarin structural isomer (103); in addition a distinction between these two compounds could not be made on the basis of their I.R. spectra. However the U.V. absorption was diagnostic; whereas (103) has a U.V. spectrum not unlike that of the parent isocoumarin (99), (109) has a spectrum (λmax: 315sh, 280sh, 257, 225 mμ) very similar to that of the carbomethoxyphloroglucinol (89), λmax: 317sh, 280sh, 256 mμ,[66] and not unlike that of the



(109)



(89)



(35)

naturally-occurring chromone, eugenin (35), λmax: 318,288,257, 248,228 mμ.[67] A negative Gibbs test suggested the position of the carbomethoxy group to complete the structural assignment.

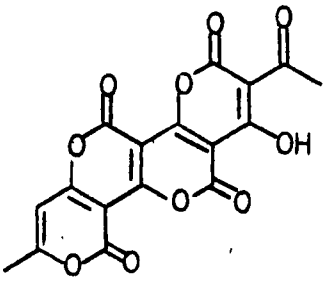
Thus another structural type formed from the intramolecular condensation of a chain of five acetate units has been carried out in vitro. The naturally-occurring chromones, eg. eugenin (35),

probably arise via the condensation mode 5 → 11 (figure 6) followed by subsequent O-acylation and dehydration. The carbomethoxychromone (109) could arise via the same cyclization mode; however since the degradation product (103) was the major compound isolated, it is more probable that (109) arises via cyclization on to one of the residual carboxyl groups, i.e. 10 → 12; in nature, presumably, the carboxyl groups C_8 and C_{12} are not present in the poly-β-keto chain and the only Claisen mode possible is 5 → 11.

The results reported in this thesis show that the tripyrone (70) is a convenient means of holding a chain of five acetate units together. From the base treatment of tripyrone four structural types: C-acetylorsellinic acid, isocoumarin, curvulinic acid and chromone, which are believed to arise in nature from a poly-β-keto chain of five acetate units, have been isolated. In addition, previously reported work[48,51] showed that base treatment of the dipyrone (69) resulted in the two structural types derivable from a poly-β-keto chain of four acetate units, i.e. orsellinic acid and acetylphloroglucinol. Future work in this field will involve: the opening of the tetrapyrone (71), which should generate a chain of six acetate units; the construction of larger polypyrone structures; and the search for new basic systems to effect more efficient cyclizations.

The construction of larger polypyrone structures will require considerable effort since these compounds are quite insoluble and thus difficult to handle. An attempt to prepare

the pentapyrone (113) by condensation of bis(2,4-dichlorophenyl) malonate with tetrapyrone failed, although traces of the pentapyrone were indicated by T.L.C. evidence. Acetyltetrapyrone (114) was prepared by refluxing a trifluoroacetic acid solution of tetrapyrone and a large excess of acetylchloride. Perhaps



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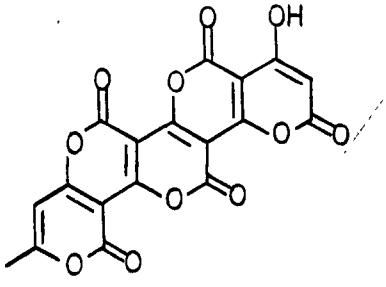

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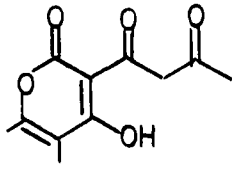
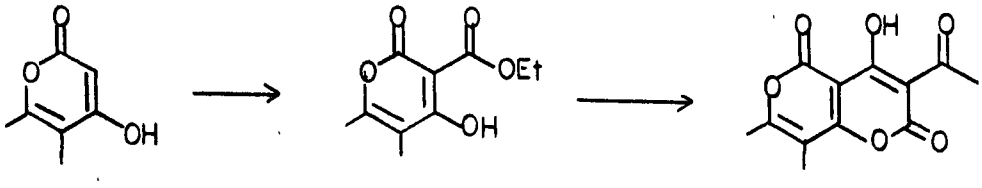


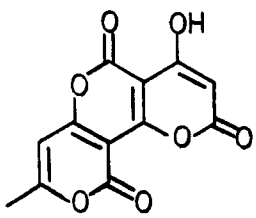
(115)

the synthesis of the pentapyrone (113) might be accomplished by acylation of (114) with ethylchloroformate. Alternatively, acylation of acetyltetrapyrone (114) with acetic anhydride[67] might extend the poly-β-keto chain by one unit (partial structure 115). The synthesis of acetylpentapyrone (partial structure 116) from tetrapyrone might also be accomplished in two steps, eg. acylation with ethylchloroformate followed by condensation with ethylacetylacetate.



(116)

A more profitable approach to the problem of constructing larger polypyrone structures might be to couple two condensed



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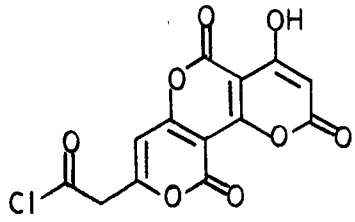

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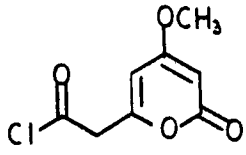

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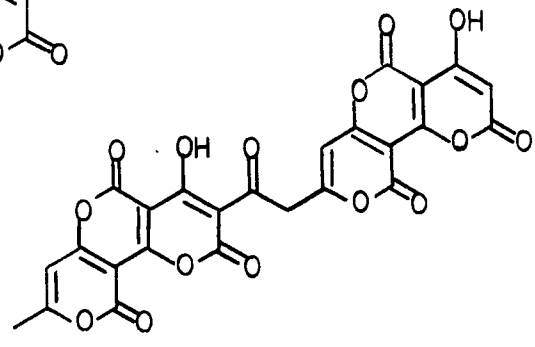

(118)

pyrone structures together. For example, acylation of tripyrone (70) with the tripyrone acid chloride (117) could give the bis-tripyrone (118). In this regard, the monocyclic acid chloride (119) has been prepared,[72] and its acylating properties are under investigation. Base opening of (118) would give rise to a poly-β-keto chain of ten acetate units, and thus the biogenetic-type synthesis of tetracyclic structures representative of the tetracyclines (43) and mycinones (44) would be possible. Work towards this goal is proceeding in these laboratories.

Before attempting the syntheses of compounds such as (118) a more critical examination of systems for opening condensed polypyrones should be made. The real weakness in the biogenetic-type synthetic scheme based on condensed polypyrones is that the conditions for generating the poly-β-keto chains might preferably degrade these chains rather than promote intramolecular condensations. Harris[39] has shown that poly-β-keto chains of four acetate units cyclize to aromatic compounds under essentially

physiological conditions; and he has postulated that such cyclizations may require no enzyme catalysis. However the formation and the retention of poly-β-keto chains do require enzyme catalysis; thus it is doubtful that chains much longer than four acetate units will actually be isolated. If this proves to be the case, then the polypyrone structures still provide an attractive alternative for the biogenetic-type syntheses of acetate-derived aromatic compounds.

EXPERIMENTAL

Melting points were determined on a Kofler hot stage microscope. Ultraviolet spectra (U.V.) were determined in ethanolic solutions using a Cary 14 spectrophotometer; and infrared spectra (I.R.) were taken in nujol with a Perkin Elmer 137B spectrophotometer. Nuclear magnetic resonance spectra (N.M.R.) were taken on a Varian A60 Mc instrument in deuteriochloroform solutions unless indicated otherwise. Tetramethyl silane was used as the internal standard. The mass spectra were determined on an Atlas spectrometer. Elemental microanalyses were carried out by Dr. A. Bernhardt and his associates at the Max Planck Institute, Mulheim, Ruhr, West Germany, and by Mr. P Borda of this department.

The U.V. maxima are reported in millimicrons; λ(base) refers to the spectra obtained after adding 1 drop of aqueous potassium hydroxide solution to the ethanolic U.V. sample. I.R. peaks are reported in cm^{-1}; and the N.M.R. peaks are reported in τ units with the coupling constants, J, reported in cycles/sec; the assignments of the N.M.R. peaks and the coupling constants are enclosed in brackets. The mass spectrum data are reported as m/e. Standard abbreviations for peak intensity (I.R.) and shape (N.M.R., U.V.) are used.

Organic solvents were routinely removed on a rotary evaporator at 45° (<u>in vacuo</u>). Thin-layer chromatography (T.L.C.) was carried out with silica gel G (acc. to Stahl) plates. For preparative purposes 20 x 60 x 0.10 cm plates were run along

the 60 cm axis while inclined at a 15° angle.

The ferric chloride tests were run using a 1% ethanolic ferric chloride solution. The Gibbs tests were carried out by dissolving a small quantity (<1 mg.) of compound in ethanol,[63] adding a small quantity (<1 mg.) of the reagent, 2,6-dichloro-p-benzoquinone-4-chlorimine (B.D.H.), and then 2 ml. of sodium borate buffer, pH 9.24.[62]

Triacetic acid lactone (16),[50] (4-hydroxy-6-methyl∝-pyrone):

100 g. of dehydroacetic acid (46, Eastman Organic) was dissolved in 165 ml. 90% sulfuric acid by warming on a steam bath. The reaction flask was partially immersed in a 150° oil bath; the solution was stirred magnetically, and nitrogen was blown through the flask. The temperature of the reaction mixture was brought up to 130° and then kept at 130-136° for 5 min. The resulting deep reddish-brown solution was cooled rapidly in an ice-bath and then added to 700 ml. of ice-water. Crystallization was essentially complete within 5 min. The white precipitate was collected in a Buchner funnel and washed with 500 ml. ice-water. Recrystallization (3 crops) from ethyl acetate gave a 60% yield (45g.) of slightly discolored (orange tinge) but sharp melting triacetic acid lactone (16); m.p. 190-191°; T.L.C. (chloroform:acetic acid 4:1): R_f=0.30; ferric chloride: negative; I.R. (nujol): 3100-2600 m, 1720s, 1660s, 1630s, 1590m, 1540m, 1510 m, 1490m, 1340m, 1300s, 1250s, 1190m, 1150m, 1040w, 990s, 880m, 843s, 815m, 730w, 690w; U.V.:[47]

284(ε 6,500); N.M.R. (trifluoroacetic acid): 3.48m (C_5H, long range coupling with methyl group and C_3H), 3.79d (C_3H, J=2 c.p.s.), 7.53s (methyl); Anal. Found: C57.28, H 4.90, $C_6H_6O_3$ requires: C 57.14, H 4.76.

Dipyrone (69), (4-hydroxy-7-methyl-2,5-diketo 1,6-dioxanaphthalene):

To 100 g. of triacetic acid lactone (0.8 moles) in 300 ml. trifluoroacetic acid (Matheson Coleman & Bell) was added 222 g. (1.6 moles) of malonyl dichloride (Aldrich). The deep red-brown solution was refluxed gently under anhydrous conditions for 5 hours. The reaction mixture was cooled in an ice-bath and then 130 ml. of cold ethyl acetate added. After 10 min. chilling the fine brown precipitate was collected and washed with ethyl acetate, crude yield 100 g.

The crude dipyrone was purified further by dissolving it in 13 liters of chloroform and filtering the solution through a 400 g. silica gel column (35 x 5 cm. dia.) at the rate of 2 liters per hour. The column was washed with an additional 10 liters of chloroform. The dipyrone was recrystallized from chloroform (2 crops) to give 82 g. dipyrone (53% yield). The major impurity, acetyl dipyrone (69)[51] (characteristic fluorescence under U.V. irradiation) was present in the later fractions and the second crops. The earlier fractions were colorless and sharp melting; m.p. 230-232°; T.L.C. (chloroform-acetic acid 4:1): R_f=0.65; ferric chloride:positive, dark red; I.R. (nujol): 3200m,3050m,1755s,1690s,1625s,1560s,1340m,1285w,1200s,1170s, 1125m,1100m,1050s,990m,860m,793m,770m,740m,720m; U.V.:329(ε 7,300),

271 (ε 11,800); N.M.R. (trifluoroacetic acid): 3.30m (C_8H), 4.02s (C_3H), 7.44s (methyl); mass spec.:194 (parent); Anal. Found: C 55.90, H 3.19, $C_9H_6O_5$ requires: C 55.65, H 3.12.

Tripyrone (70), (1-hydroxy-7-methyl-3,5,10-triketo 4,6,9-trioxaphenanthrene):

To 10 g. of dipyrone (0.05 mole) in 20 ml. trifluoroacetic acid was added 58g. malonyl dichloride (0.4 mole). The reaction mixture was refluxed (100° oil bath) for 1.5 hr. under anhydrous conditions. The reaction flask was cooled and the black sludge triturated with 50 ml. ether. The light brown powder was collected and washed with ether.

The crude tripyrone was purified further by chromatography on a 100 g. silica gel column. The sample was initially put on the column with 12 liters of hot benzene. Elution with chloroform (7 liters) yielded dipyrone in the first fractions (2g.) followed by tripyrone. Recrystallization from acetone (2 crops) gave 2.7 g. (20% yield) of tripyrone. The major impurity was acetyltripyrone[51] which appeared in the second crop. The first crop contained tiny yellow needles; m.p. 260° dec.; T.L.C. (chloroform-acetic acid 4:1): R_f = 0.30; ferric chloride: positive (weak); I.R. (nujol): 3200w, 1755s, 1720s, 1635 m, 1570(sh)m, 1540s, 1325m, 1290w, 1220m, 1185m, 1170m, 1110m, 1045m, 1028m, 1010w, 975w, 880w, 860w, 830w, 802m, 770w, 740m; U.V.: 392 infl. (ε 5100), 370 (ε 8,900), 360 (ε 8,900), 281(ε 8,700), 255sh (ε 5,600); N.M.R. (trifluoroacetic acid): 3.30m (C_8H), 3.96s (C_2H), 7.43s (methyl); mass spec.: 262 (parent); Anal. Found:

C 54.95, H 2.59, $C_{12}H_6O_7$ requires: C 54.97, H 2.31.

For an alternate synthesis of tripyrone from dipyrone which gives better yields, see under the tetrapyrone section.

Tetrapyrone (71), (4-hydroxy-9-methyl-2,5,7,12-tetraketo 1,6,8,11 tetraoxachrysene):

Attempts to synthesize tetrapyrone from the condensation of tripyrone with malonyl dichloride yielded only trace amounts of the desired product. The major reaction which accompanied these attempts was the heat-catalyzed polymerization of malonyl dichloride to form a red-brown polymer; m.p. 330° dec.; I.R. (nujol): 1755vs, 1710(sh)s, 1625s, 1540s, 800m, 1300-850w. Trifluoroacetic acid did not appear to catalyze this polymerization since a similar product was obtained when tetrahydrofuran, dioxan or chloroform was used as solvent. Pyridine catalyzed the polymerization rather than the condensation reaction; and aluminum chloride was ineffective as a catalyst.

The use of malonic acid diesters as C_3O_2 condensing units[42] was more successful. A series of preliminary experiments were carried out using dipyrone and a number of diesters. Treatment of dipyrone with refluxing diethylmalonate (215° oil bath), or with refluxing 10% sulfuric acid/diethylmalonate solution resulted in no condensation. The fusion of dipyrone with various aryl diesters resulted in the formation of substantial amounts of tripyrone and tetrapyrone. The order of reactivity for the diesters was: bis(2,4,6-trichlorophenyl)malonate <diphenylmalonate <bis(2,4-dichlorophenyl)malonate. The reactions were carried out

by fusing a mixture of dipyrone and diester at 230°. The reactions were over within 10 to 30 min. when the melt solidified due to the polymerization of the diester. The use of the respective phenols to maintain the melt repressed the condensation; and the use of diethylcarbitol for the same purpose did not increase the yield. The use of an aluminum chloride-sodium chloride 5:1 melt[69] was also unsuccessful. The most successful experiment was as follows: a mixture of 100 mg. dipyrone (0.5 mmoles) and 508 mg. bis(2,4-dichlorophenyl)malonate (1.3mmoles) was heated (230° oil bath) for 10 min.; T.L.C. indicated the ratio of dipyrone:tripyrone:tetrapyrone to be about 2:1:0.4. On a large scale this reaction could be used to prepare both tripyrone and tetrapyrone. For our purposes, tetrapyrone was synthesized from tripyrone and purified in the following manner.

A mixture of 300 mg. tripyrone (1.1 mmoles) and 550 mg. bis(2,4-dichlorophenyl)malonate (1.4 mmoles) was heated (250° oil bath) for 2 min. T.L.C. of the reaction mixture indicated the ratio of tripyrone:tetrapyrone was about 2:1. The mixture was then chromatographed on a 30g. silica gel column. The sample was put on the column with 4 liters of hot benzene and the pyrone materials were eluted with 8 liters of chloroform; bis(2,4-dichlorophenyl)malonate (175 mg.) was eluted with the benzene. Most of the tripyrone (160 mg.) was eluted with the first two liters of chloroform; a mixture 90-95% pure in tetrapyrone was collected from the last 6 liters of chloroform (125 mg.). The tetrapyrone was purified further by subliming off the tripyrone impurity (0.01 mm. Hg, 195°, 4 hr.). Charcoal-

ing an acetone solution of the residue, followed by recrystallization from acetone gave tetrapyrone as tiny yellow prisms; m.p. 280° dec.; T.L.C. (chloroform-acetic acid): R_f = 0.15; ferric chloride:very weak; I.R. (nujol): 1755s, 1705s, 1645m, 1590m, 1555s, 1320w, 1290w, 1240m, 1210w, 1165w, 1135w, 1120m, 1055w, 1030w, 1005w, 965vw, 860w, 805m, 700vw, 678w; U.V.: 420 infl. (ε 6,000), 398 (ε 9,800), 385 (ε 9,800), 332sh (ε 4,600), 272 (ε 8,600); N.M.R. (trifluoroacetic acid): 3.43s ($C_{10}H$), 3.99s (C_3H), 7.45s (methyl); mass spec.: 330 (parent; Anal. Found: C 54.67, H 2.02, $C_{15}H_{16}O_9$ requires: C 54.56, H 1.83.

Attempts to prepare pentapyrone (113) from tetrapyrone by an analogous procedure were unsuccessful, as T.L.C. of the reaction mixture indicated only trace amounts of a compound believed to be pentapyrone.

Acetyltetrapyrone (114):

1 ml. acetylchloride was added to a solution of 20mg. tetrapyrone (71) in 1 ml. trifluoroacetic acid, and the solution was refluxed (100° oil bath) under anhydrous conditions for 24 hrs. Hydrogen chloride gas was given off and a yellow compound precipitated. The reaction mixture was cooled; the precipitate was collected and washed with water and then acetone; m.p. 330° dec.; I.R. (nujol): 1750vs, 1640s, 1600s, 1540vs, 1130s, 1000s, 920m, 860m, 810s, 687m; U.V.: λmax: 397, 265 mμ; mass spec.: 372 (parent). The acetyltetrapyrone was very insoluble in all the common solvents; it sublimed at 230° (0.1 mm Hg) but only very slowly.

Bis(2,4-dichlorophenyl)malonate:

A mixture of 10.4 g. (0.1 mole) malonic acid (Eastman), 32.6 g. (0.2 mole) 2,4-dichlorophenol (Eastman) and 30.6 g. (0.2 mole) phosphorus oxychloride (B.&A.) was refluxed (120° oil bath) for 3 hr. The reaction mixture was pourred into ice-water and the crystalline material collected. The product was taken up in 600 ml. ether and the organic layer extracted with sodium bicarbonate, washed, dried and the solvent removed in vacuo. Recrystallization from benzene:n-hexane gave a 70% yield of colorless needles; m.p. 99-100°; T.L.C. (chloroform-acetic acid 4:1): R_f = 0.9; I.R. (nujol): 1775vs, 1750m, 1585m, 1245m, 1215s, 1150s, 1135vs, 1095vs, 1060m, 965w, 940w, 872m, 845m, 825m, 783s, 698w, 685w; N.M.R. (deuterioacetone): 2.40q (2H, J ≈ 2,1), 2.60d (4H, J=1.8), 5.82s (2H); Anal. Found: C 46.08, H 2.19, Cl 35.99, $C_{15}H_8O_4Cl_4$ requires: C 45.72, H 2.05, Cl 35.99.

Diphenylmalonate:

A solution of 9.4 g. (0.1 mole) phenol (B.D.H.) in 30 ml. of chloroform was heated to a gentle reflux (75° oil bath), and a solution of 14.0 g. (0.1 mole) malonyl dichloride in 15 ml. chloroform slowly added over 2 hr. Refluxing was continued for a further 30 min. and then excess malonyl dichloride destroyed with water. The chloroform layer was concentrated in vacuo, 100 ml. water added and the aqueous solution extracted with ether. The organic layer was extracted with sodium bicarbonate, washed, dried and the solvent removed in vacuo. Crystallization

from ethanol (3 crops) gave 10.3 g. (80% yield) of diphenylmalonate. Recrystallization from ethanol gave colorless needles; m.p. 46-47° (cf. reported[70] m.p. 50°); I.R. (nujol): 1780vs, 1750vs, 1595m, 1490s, 1360s, 1340s, 1255m, 1200vs, 1150s, 1125vs, 1060m, 1025m, 1005w, 965m, 955m, 940m, 910m, 845m, 820m, 760s, 750s, 705s, 695s; N.M.R. (deuterioacetone): 2.68, 2.74m (10H), 6.05s (2H); Anal. Found: C 70.33, H 4.91, $C_{15}H_{12}O_4$ requires: C 70.30, H 4.72.

Bis (2,4,6-trichlorophenyl)malonate:

A solution of 5.0g (0.025 mole) 2,4,6-trichlorophenol (Eastman) in 20 ml. of chloroform was heated to a gentle reflux, and a solution of 3.0 g. (0.021 mole) malonyl dichloride in 5 ml chloroform added slowly over 15 min. Refluxing was continued for a further 15 min. and then the excess malonyl dichloride destroyed with water. The chloroform layer was taken to dryness *in vacuo* and the residue taken up in 150 ml. benzene. The benzene layer was extracted with sodium bicarbonate, washed, dried and concentrated *in vacuo*. Crystallization from benzene (3 crops) gave a 65% yield (3.8 g.) of diester. Recrystallization from benzene gave colorless prisms; m.p. 154-156°; I.R. (nujol): 3100w, 1795s, 1780s, 1570m, 1420w, 1400w, 1385m, 1330s, 1255m, 1230s, 1195w, 1155m, 1135w, 1105vs, 1075w, 960w, 938m, 920w, 875w, 862s, 822s, 805m, 790m; N.M.R. (deuteriochloroform): 2.59s (4H), 5.95s (2H); Anal. Found: C 39.12, H 1.21, Cl 45.92, $C_{15}H_6O_4Cl_6$ requires: C 38.92, H 1.31, Cl 45.95.

It is interesting to note that *bis* (2,4,6-trichlorophenyl)

malonate is unstable in several common organic solvents. When ether was used in the work-up very little of the diester was isolated; attempted recrystallization from ethanol failed with degradation of the diester; and the N.M.R. spectrum of a sample of the diester in deuterioacetone indicated considerable degradation had occurred in this solvent also.

Base Treatment of Tripyrone (70):

1. 1N KOH (aqueous):

A solution of 200 mg. tripyrone in 120 ml. 1N aqueous potassium hydroxide was magnetically stirred under nitrogen at room temperature for 2 hr. The solution was initially a dark green color which gradually changed to orange over a few minutes. After 2 hr. the solution was cooled (ice-bath), acidified (pH2) with dropwise addition of hydrochloric acid (11 ml.) and then extracted with chloroform. Evaporation of the organic layer yielded 15 mg. of residue, which was shown by T.L.C. (chloroform: acetic acid 4:1) to contain a number of minor components including tripyrone.

The aqueous layer was freeze-dried and the residue extracted with refluxing acetone to yield 140 mg. of crude material, which was shown by T.L.C. to contain two major components. The two components were separated by preparative T.L.C. (chloroform-acetic acid 9:1). One of these components was tripyrone; the other (R_f=0.7, crude yield 30 mg.) was recrystallized from benzene to give colorless needles; m.p. 157-158°. Comparative

T.L.C., I.R., U.V. and N.M.R. spectra, and undepressed mixed m.p. with authentic material[51] proved the aromatic component was orcacetophenone (92).

The above reaction was repeated with a solution of 1 g. tripyrone in 1 liter of 1N aqueous potassium hydroxide. After 3 days the solution was worked up as before to yield over 500 mg. of starting material along with about 50 mg. of acetyldipyrone (82).

2. 1N KOH/MeOH:

A mixture of 1 g. tripyrone in 1 liter of 1N methanolic potassium hydroxide was magnetically stirred under nitrogen at room temperature for 2 days. The solution was initially a bright green color, gradually changing to bright orange during the first hour. After 1 day all of the solid had gone into solution. After 2 days the solution was cooled (ice-bath), acidified (pH2) by dropwise addition of hydrochloric acid (80 ml.) and then concentrated *in vacuo*. The solution was diluted with 200 ml. water and then extracted with chloroform.

The above reaction was repeated twice. The combined aqueous layers were freeze-dried and the residue extracted with refluxing acetone to yield *ca*. 1 g. of crude material, which was shown by T.L.C. and I.R. spectra to consist largely of tripyrone. Another major component was isolated (95 mg.) by preparative T.L.C. (benzene-ether 4:1); recrystallization from ethyl acetate: n-hexane gave colorless needles; m.p. 157-158°; T.L.C. (benzene-

ether 4:1):R_f = 0.55; ferric chloride: positive (dark red); Gibbs test:positive (purple); I.R. (nujol): 3200s, 1620s, 1575s, 852m, 827m; U.V.: 315sh (ε 3,200), 282 (ε 6,700), 231sh (ε 5,000), 221 (ε 7,400); N.M.R. (deuterioacetone): -3.00b (1H, H-bonded OH), 0.93b (1H,OH), 3.76m (2H, aromatic), 7.40s (methyl), 7.48s (methyl). Comparative T.L.C., spectral data and undepressed mixed m.p. with an authentic sample[51] proved the aromatic component was orcacetophenone (92).

The combined chloroform layers were washed, dried and the solvent removed in vacuo to yield ca. 2 g. of crude material. The material was initially fractionated by elution from a silica gel column with benzene, chloroform and acetone solvents. Further fractionation was carried out by preparative T.L.C. using the solvent systems: benzene-ether 4:1, ether, and carbon tetrachloride:chloroform: acetic acid 10:9:1. In addition to the fractionated material there was 120 mg. of relatively insoluble material, m.p. 255-260 dec. Comparative T.L.C., spectral data and undepressed mixed m.p. identified this compound as tripyrone. This data combined with the T.L.C. evidence of the residue from the aqueous layers indicates that ca. 30% starting material was present in the reaction mixture.

From the fractionation procedure a large number (ca. 20) of compounds were indicated, and of these 7 were isolated in sufficient purity and quantity to be characterized by spectral data. The spectral data and probable structures for these compounds are presented below. The yields given are the weights

of the material collected from the last chromatographic step and probably represent minimum reaction yields in each case.

2,4-dicarbomethoxyorcinol (87):

Yield: 12 mg.; T.L.C. (benzene:ether 4:1): R_f=0.60; recrystallization from benzene: n-hexane gave colorless needles; m.p. 108-110°; ferric chloride: positive; Gibbs test: positive (purple); I.R. (nujol): 1670s, 1650s, 1620m, 1575m, 1260s, 1240s, 1200m, 1095m, 970m, 852w, 830m, 815m, 715w; U.V.: 316 (ε 6,300), 262sh (ε 11,400), 247sh (ε 15,600), 232 (ε 21,600), λ(base): 310sh (ε 8,000), 286 (ε 10,000), 250sh (ε 13,000); N.M.R. (deuterioacetone): -1.94b (1H, H-bonded hydroxyl), -1.05b (1H, H-bonded hydroxyl), 3.62s (1H, aromatic), 5.98s (ester methyl), 6.09s (ester methyl), 7.60s (methyl), (deuteriochloroform): -2.65s, -1.62s, +3.68s, 6.01s, 6.09s, 7.56s, (trifluoroacetic acid): 3.48s, 5.85s, 5.91s, 7.53s; mass spec.: 240 (parent); Anal. Found: C 55.27, H 5.24, $C_{11}H_{12}O_6$ requires: C 55.00, H 5.05.

By comparative T.L.C., spectral data and undepressed mixed m.p., the above diester (87) was shown to be identical with one of the products previously isolated[51] from the treatment of dipyrone (69) with 1N methanolic potassium hydroxide solution. The structural assignment was made on the basis of the above data, and on the similarity of the spectral properties (particularly U.V.) with those of the known compound[57,58] 3,5-dihydroxy-2,4-dicarboxyphenyl acetic acid trimethyl ester (97): U.V.: 314 (ε 7,600), 260 infl., 247sh (ε 15,000), 228 (ε 21,700);

I.R. (nujol): 1730s, 1675s, 1600m, 1580m, 1310m, 1270vs, 1260vs, 1200vs, 1175s, 1100m, 1135vw, 1060vw, 990vw, 980w, 950vw, 905vw, 870vw, 828s, 755vw; N.M.R. (deuteriochloroform): -2.92s (1H, H-bonded hydroxyl), -2.00s (1H, H-bonded hydroxyl), 3.60s (1H, aromatic), 5.98s, 6.12s, 6.30s (9H, ester methyls), 6.12s (2H, methylene).

2-carbomethoxy-3,5-dihydroxyphenyl propan-2-one (98):

Yield: 30mg.; T.L.C. (benzene-ether 4:1): R_f = 0.20; recrystallization from ethyl acetate: n-hexane gave colorless prisms; m.p. 126-131°; ferric chloride: positive (dark red); Gibbs test: positive (blue-purple); I.R. (nujol): 3410s, 3290s, 1700s, 1670vs, 1625s, 1610s, 1320s, 1260s, 1218s, 1190s, 1160s, 1100s, 1055m, 945m, 853m, 835w, 797m, 730m; U.V.: 303 (ε 5,520), 265 (ε 11,900), 225sh (ε 14,400), 215 (20,700), λ(base): 330sh (ε 6,000), 305 (ε 9,000), 254sh (ε 25,000), 246 (ε 28,000); N.M.R. (deuterioacetone): -1.51s (1H, H-bonded hydroxyl), +0.5b (hydroxyl), 3.66d, 3.73d (2H, aromatic, J=2.4 cps), 6.00s (2H, methylene), 6.05s (ester methyl), 7.83s (methyl); mass spec.: 224 (parent); Anal. Found: C 58.97, H 5.48, $C_{11}H_{12}O_5$ requires C 58.92, H 5.40.

The structural assignment was made on the basis of the above data and by the similarity of the U.V. spectra with that of the known acids, 2-carboxy-3, 5-dihydroxyphenyl propan-2-one (37):[61] 305 (ε 6,200), 270 (ε 12,900), and orsellinic acid (17): 300 (ε 4,000), 260 (ε 12,600).[60]

6,8-dihydroxy-3-methyl isocoumarin (99):

Yield 40 mg.; T.L.C. (benzene:ether 4:1): R_f = 0.40; recrystallization from ethyl acetate gave discolored prisms, m.p. 216-243° dec.; recrystallization from acetone:water gave colorless needles; m.p. 250-253°; ferric chloride:positive (dark red); Gibbs test: positive (blue); I.R. (nujol): 3250m, 1685s, 1625s, 1580m, 1240s, 1180s, 1070m, 963m, 867m, 838m, 795m; U.V.: 324 (ε 6,200), 289 (5,200), 278 (7,000), 257sh (12,000), 245 (49,000), 237 (42,000), λ(base): 344 (ε 8,600), 300(11,400), 280sh (13,000), 263sh (28,000), 253sh (32,000), 245 (37,000); N.M.R. (deuterioacetone): -1.17b (1H, H-bonded hydroxyl), +0.47b (hydroxyl), 3.62s (3H, aromatic, olefinic), 7.78s (methyl); mass spec.: 192 (parent); Anal. Found: C 61.90, H 4.41, $C_{10}H_8O_4$ requires: C 62.50, H 4.20.

6,8-dihydroxy-3-methyl isocoumarin (99) is a known compound and the above data are in agreement with that reported: m.p. 244-248°[14], 245-248°[61]; U.V.: 317 (6,300), 276(29,000), 260 (31,000), 244 (56,000), 237 (45,000).[61]

2-carbomethoxy-3-hydroxy-5-methoxyphenyl propan-2-one (100):

Yield: 8 mg; T.L.C. (benzene:ether 4:1): R_f = 0.45; sublimation at 85° (0.01 mm. Hg) followed by recrystallization from benzene:n-hexane gave colorless needles; m.p. 101-103°; ferric chloride:positive (red-brown); Gibbs test:positive (purple); I.R. (nujol): 3400w, 1710s, 1660s, 1620m, 1585m, 1310s, 1270m, 1230s, 1165m, 1110s, 960w, 880w, 815s, 780m, 720m; U.V.: 315

315 (ε 2,800), 258 (ε 11,300), λ(base): 350sh (ε 1,400), 303 (ε 2,800); N.M.R. (deuteriochloroform): -1.51s (1H, H-bonded hydroxyl), 3.51s (1H, aromatic), 3.72s (1H, aromatic), 6.06s (methyl), 6.15s (methyl), 6.37s (2H, methylene), 7.82s (methyl); mass spec.: 238 (parent, $C_{12}H_{14}O_5$).

3,6-dimethoxy-8-hydroxy-3-methyl-3,4-dihydroisocoumarin (101):

Yield: 23 mg.; T.L.C. (benzene:ether 4:1): R_f=0.55; recrystallization from n-hexane gave colorless cubes; m.p. 99-101°; ferric chloride: positive (dark red); Gibbs test: positive (purple); I.R. (nujol): 1675s, 1640m, 1590w, 1510m, 1315s, 1285m, 1245w, 1220m, 1190m, 1165s, 1110vw, 1070s, 1040s, 970vw, 950vw, 935w, 915w, 855m, 815w, 785s, 755w, 725vw, 690w, 680w; U.V.: 303 (ε 5,800), 268 (13,300), 228sh (12,800), 216 (21,400), λ(base): 337 (8,000), 268 (10,000), 237sh (28,000); N.M.R. (deuterioacetone): -1.28s (1H, H-bonded hydroxyl), 3.65s (2H, aromatic), 6.17s (methyl), 6.68s (methyl), 6.84s (2H, methylene), 8.37s (methyl); mass spec.: 238 (parent); Anal. Found: C 60.49, H 6.12, $C_{12}H_{14}O_5$ requires: C 60.50, H 5.92.

2,4-dicarbomethoxy-3,5-dihydroxyphenyl propan-2-one (102):

Yield: 20 mg; T.L.C. (benzene:ether 4:1): R_f = 0.40; recrystallization from benzene:n-hexane gave colorless needles; m.p. 149-151°; ferric chloride:positive (dark red); Gibbs test:positive (blue-purple); I.R. (nujol): 1710m, 1665s, 1610m, 1570m, 1330s, 1255s, 1235s, 1215s, 1165m, 1095s, 975w, 950w, 840w, 820w, 720w; U.V.: 316 (ε 3,100), 265sh (9,800), 248 (11,800),

231 (14,600), λ(base): 348 (5,400), 272 (16,300), 249 (17,200); N.M.R.: -2.62s (1H), -1.78s (1H, H-bonded hydroxyls), 3.64s (1H, aromatic), 5.97s (ester methyl), 6.11s (5H, ester methyl, methylene), 7.84 (methyl); mass spec.: 284 (parent, $C_{13}H_{16}O_7$).

The similarity of the U.V. spectra of (102) and 2,4-dicarbomethoxyorcinol (87) also suggests that the second carbomethoxy group is located at the 4-position.

7-carbomethoxy-6,8-dihydroxy-3-methyl isocoumarin (103):

Yield: 95 mg.; T.L.C. (benzene:ether 4:1): R_f = 0.50; recrystallization from ethyl acetate gave colorless needles; m.p. 196-198°; ferric chloride:positive (dark red); Gibbs test: positive (blue); I.R. (nujol): 1685s, 1650s, 1625(sh)m, 1570s, 1280m, 1245m, 1225s, 1150m, 1080s, 980w, 945vw, 865m, 830m, 815m, 785w; U.V.: 340 (ε 7,700), 325sh (6,200), 303(8,300), 291 (8,400), 274sh (12,000), 257 (39,000), 250sh (34,000), 214sh (10,100), λ(base): 352 (8,700), 272 (25,000), 246 (27,000); N.M.R. (deuteriochloroform): -2.78s (1H), -2.42s (1H, H-bonded hydroxyls), 3.72s (1H, aromatic), 3.86s (1H, olefinic), 6.00s (ester methyl), 7.75s (methyl); mass spec.: 250 (parent); Anal. Found: C 57.73, H 4.51, $C_{12}H_{10}O_6$ requires: C 57.60, H 4.03.

The structural assignment was made on the basis of the above data and by the conversion of (103) to the corresponding phenyl propanone (102). The ring opening was carried out (see below) by a method used by Hassall[61] to effect a similar conversion of 6,8-dihydroxy-3-methyl isocoumarin (99) to 2-carboxy-3,5-dihydro-

xyphenyl propan-2-one (37). The conversion not only establishes the isocoumarin ring system but also proves that the location of the ester groups of (102) and (103) are the same. Thus the carbomethoxy group of (103) is probably located at position 7 of the isocoumarin ring system (see above).

The conversion of (103) to (102):

A solution of 5 mg. compound (103) in 2 ml. aqueous 0.02 M sodium hydroxide was refluxed (130° oil bath) for 20 min. The solution was cooled, acidified and extracted with 20 ml. ether; and the ether layer washed, dried and the solvent removed in vacuo. The U.V. spectrum of the residue (λmax: 323, 265 infl., 246, 232) was very similar to that of compound (102).

Three-quarters of the residue was taken up in 2 ml. ether, cooled (ice-bath) and treated with diazomethane at 0°. The excess reagent was immediately destroyed with dilute hydrochloric acid and the ether layer was washed, dried and the solvent removed in vacuo. Crystallization from benzene:n-hexane gave needles, m.p. 145-150°. The reaction product was shown to be compound (102) by T.L.C., undepressed mixed m.p. and superimposable I.R. spectra.

3. 1M $Mg(OCH_3)_2$/MeOH:

A magnesium methoxide solution was prepared by adding 6.0 g. of magnesium powder (B.D.H. reagent) to 225 ml. anhydrous methanol[71] and refluxing the mixture for 1 hr. The solution was

cooled to room temperature and a slurry of 1.0 g. tripyrone in 25 ml. methanol added. The solution was stirred at room temperature under nitrogen for 2 days, and then the solvent was concentrated *in vacuo*; 100 ml. water was added, the solution was cooled in an ice bath and then acidified by dropwise addition of *ca*. 40 ml. hydrochloric acid. The aqueous solution was extracted with chloroform.

The above reaction was repeated; and the combined aqueous layers were freeze-dried and the residue extracted with refluxing acetone to yield *ca*. 0.2 g. of crude material. Two aromatic compounds were isolated by preparative T.L.C. with benzene:ether 4:1 solvent system. By comparison with authentic samples these compounds were shown to be methyl orsellinate (86, yield 7 mg.) and 2,4-dicarbomethoxyorcinol (87, yield 8 mg.), both of which had been previously obtained[51] by base treatment of dipyrone (69). Both of these compounds were also isolated from the chloroform extract (see below) and the total yields for (86) and (87) were 20 mg. and 9 mg. respectively. Since 2,4-dicarbomethoxyorcinol (87) was also isolated from the methanolic potassium hydroxide treatment of tripyrone its characteristic properties are given above.

Recrystallization of *methyl orsellinate* (86) from benzene gave colorless prisms; m.p. 141-143°; T.L.C. (benzene:ether 4:1): R_f=0.60; Gibbs test:positive (purple); I.R. (nujol): 3340m, 1645s, 1620m, 1585m, 1495w, 1320s, 1305s, 1260s, 1200m, 1170s, 1160s, 1110w, 1060w, 955w, 855w, 840w, 803m; U.V.: 300 (ϵ 4,400),

264 (13,000), 217 (18,000), λ(base): 306 (21,000), 240 (8,200); N.M.R. (deuteriochloroform): -1.71s (1H, H-bonded hydroxyl), 0.28bd (1H, hydroxyl), 3.75s (2H, aromatic), 6.10s (ester methyl), 7.53 (methyl), deuterioacetone: -1.58s, +1.03s, 3.73s, 6.11s, 7.54s; mass spec.: 182 (parent).

The combined chloroform layers were washed, dried and the solvent removed in vacuo to give ca. 1.2 g. crude material. Fractionation was carried out by preparative T.L.C. using the solvent systems: benzene:ether 4:1, carbon tetrachloride:chloroform:acetic acid 10:9:1. In addition to the fractionated material there was 50 mg. of relatively insoluble material. T.L.C. and N.M.R. evidence indicated this material was a 1:3 mixture of tripyrone and an unidentified product. Unfortunately the unidentified product decomposed upon attempted purification by T.L.C.; however its solubility and T.L.C. characteristics, and its N.M.R. spectrum: 3.26s (1H), 5.91s (3H), 7.29s (3H), 7.43s (3H) suggest that it is a new pyrone product, perhaps the methyl ether of the acetyldipyrone (82).

From the fractionation procedure a large number (ca. 20) of compounds were indicated, of these 8 were isolated in sufficient purity and quantity to be characterized by spectral data. As mentioned above, two of these, methyl orsellinate (86) and 2,4-dicarbomethoxyorcinol (87) were isolated from the aqueous layer as well. For the others, the spectral properties, and in most cases, probable structures, are given below. The yields given are the weights of the material collected from the last

chromatographic step, and thus probably represent minimum reaction yields.

2-acetyl-4-carbomethoxyorcinol (106):

Yield: 12 mg.; T.L.C. (benzene:ether 4:1): R_f = 0.55; recrystallization from n-hexane gave colorless prisms; m.p. 96-98°; ferric chloride:positive, dark red; Gibbs test:positive (purple); I.R. (nujol): 3420m (sharp), 1675s, 1645m, 1570m, 1245m, 1165s, 802m; U.V.: 325 (ϵ 3,900), 265sh (8,100), 249 (14,200), λ(base): 353 (9,900), 300 (8,600), 266 (11,100); N.M.R. (deuteriochloroform): -1.35s (1H, H-bonded hydroxyl), -0.10s (1H, H-bonded hydroxyl), 3.68s (1H, aromatic), 5.95s (ester methyl), 7.47s (methyl), 7.70s (methyl), deuterioacetone: 3.66s, 5.90s, 7.54s, 7.77s; mass spec.: 224 (parent).

3,5-dicarbomethoxyacetylphloroglucinol (107):

Yield: 104 mg.; T.L.C. (ether): R_f = 0, (carbon tetrachloride: chloroform:acetic acid 10:9:1): R_f = 0.60; recrystallization from benzene:n-hexane gave colorless needles; mp. 172-173°; ferric chloride: positive, dark red; Gibbs test:negative (brown); I.R. (nujol): 1640s, 1570s, 1320s, 1240s, 980m, 970m, 955m, 910m, 870m, 815s, 690s; U.V.: 270sh (20,100), 256 (41,700), λ(base): 317 (22,200), 277 (26,600); N.M.R. (deuteriochloroform): -5.38bd (2H, H-bonded hydroxyls), -4.08bd (1H, H-bonded hydroxyl), 6.02s (6H, 2 ester methyls), 7.29s (methyl); mass spec.: 284 (parent); Anal. Found: C 50.49, H 4.59, $C_{12}H_{12}O_8$ requires C 50.71, H 4.26.

4-carbomethoxymethyl curvulinate (108):

Yield: 14 mg.; T.L.C. (benzene:ether 4:1): R_f = 0.45; recrystallization from benzene: n-hexane gave colorless needles; m.p. 110-112°; ferric chloride: positive, purple; Gibbs test: positive (blue-purple); I.R. (nujol): 3400w (sharp), 1725vs, 1680s, 1645s, 1565m, 1330s, 1255s, 1220s, 1170vs, 820m; U.V.: 327 (ε 5,650), 265sh (11,400), 250 (19,200), 236sh (16,000), λ(base): 353 (14,100), 305 (11,700), 263 (14,600); N.M.R. (deuteriochloroform): -0.66s (1H, H-bonded hydroxyl), +0.34s (1H, H-bonded hydroxyl), 3.61s (1H, aromatic), 5.90s (ester methyl), 6.29s (2H, methylene) 6.31s (ester methyl), 7.44s (methyl); mass spec. 282 (parent).

2-methyl-5,7-dihydroxy-8-carbomethoxychromone (109):

Yield: 8 mg.; T.L.C. (benzene:ether 4:1): R_f = 0.45); recrystallization from benzene: n-hexane gave colorless needles; m.p. 178° dec.; ferric chloride: positive, dark red; Gibbs test: negative (brown); I.R. (nujol): 3200w, 1675s, 1620m, 1585m, 1330s, 1180m, 1130w, 970w, 860w, 805w; U.V.: 280sh (ε 8,000), 257 (26,000), 225 (17,200), λ(base): 324 (10,500), 279 (16,200); N.M.R. (deuteriochloroform): -3.71s (1H, H-bonded hydroxyl), -2.42s (1H, H-bonded hydroxyl), 3.68s (1H, aromatic), 3.88 (1H, aromatic), 6.01s (ester methyl), 7.60s (methyl); mass spec.: 250 (parent).

The characteristic properties of compounds (110) and (111) are given below. Both of these compounds were difficult to

purify and appear to be unstable; for these reasons there may be some inconsistencies in the reported properties, and also no structural assignments have been made.

Compound (110):

Yield: 12 mg.; T.L.C. (ether): R_f=0, (carbon tetrachloride: chloroform: acetic acid 10:9:1): R_f=0.30; sublimation at 60° (0.01 mm. Hg) gave colorless prisms; m.p. 92-93°; ferric chloride: negative; Gibbs test:negative; I.R. (nujol): 1725s, 1640s, 1620m, 1570m, 1330w, 1290m, 1240m, 1155w, 995m, 985w, 940w, 778w, 710w, 700w; U.V.: 311 (ϵ 8,700), 228 (6,700), λ(base): 283 (9,500); N.M.R. (deuteriochloroform): -5.76s (1H), +4.01s (1H), 5.97s (2H), 6.25s (3H), 7.71s (3H); mass spec.: 250 (parent).

Compound (111):

Yield: 70 mg; T.L.C. (ether): R_f=0, (carbon tetrachloride: chloroform:acetic): R_f=0.55; recrystallization from either dimethylsulfoxide or dimethylformamide gave colorless prisms; m.p. 154-155°, 161-162° (at 154° crystal structure breaks down and needles form); ferric chloride:positive, dark red; Gibbs test: negative (brown); I.R. (nujol): 1730s, 1680s, 1640-1610s, 1550m, 1310s, 1240-1225s, 1105m, 1090m, 982m, 952m, 850m, 820m; U.V.: 307 (ϵ 7,000), 270sh (16,500), 260 (26,200), λ(base): 350 (7,000), 272 (23,400), 258 (22,800); N.M.R. (trifluoroacetic acid): 2.65s (1H), 5.87s (8 or 9 H), 7.60s (3H), (dioxan): -2.80bd (1H), 3.15s (1H), (dimethylformamide): 2.82bd (1H), (6.07); mass spec.: 340 (very weak), 308 (perhaps parent).

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