PHENOLIC CONSTITUENTS

of

WESTERN HEMLOCK WOOD

(*Tsuga Heterophylla* (Raf.) Sarg.)

by

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ABSTRACT

The phenolic extractives from western hemlock wood (Tsuga heterophylla (Raf.) Sar.) have been examined The total extractive content of the wood amounted to 1.5% of the dry weight. A leucoanthocyanidin and two lignans, conidendrin and hydroxymatairesinol, were isolated from the phenolic fraction by precipitation of a methanol solution into peroxide-free ether followed by separation on silicic acid-calcium sulphate chromatobars.

The pigment produced by acid treatment of the isolated leucoanthocyanidin was shown by spectral studies and alkaline degradation to be a mixture of cyanidin and an unidentified anthocyanidin. The two anthocyanidins had identical R_f values in different solvents and similar ultra-violet spectra in ethanol-hydrochloric acid solution but the shift of the absorption maxima caused by addition of aluminium chloride was negligible in the case of the unknown compound and amounted to 30 mμ for cyanidin. Similar separations of the absorption maxima after complexing with aluminium ion were observed with the 3-methyl and 3-isopropyl ethers of the two anthocyanidins. The alkaline degradation products from the leucoanthocyanidin contained protocatechuic acid but no phloroglucinol. Degradation products of phloroglucinol, however, were present in the reaction mixture. These results suggested that the leucoanthocyanidin occurred in the wood in dimeric form and that alkaline degradation of this structure produced a symmetrical hexahydroxybenzophenone derivative which split up directly into fragments identical to those obtained from phloroglucinol under the same conditions.

New information on the structure of hydroxymatairesinol was obtained by comparison of the infrared spectra of the fully acetylated hydroxymatairesinol with that of the reduced compound and by neutral potassium permanganate oxidation of trimethylhydroxymatairesinol. The results obtained were in good

agreement with only one of the two structures previously proposed for hydroxymatairesinol by other workers.

The NMR spectra of hydroxymatairesinol and structurally related compounds were compared, but the interpretation of the spectrum of hydroxymatairesinol proved to be difficult because broad, incompletely resolved lines were obtained due to the complexity and asymmetry of the molecule.

ACHNOWLEDGEMENTS

I would like to express my sincere thanks and appreciation to Dr. L. D. Hayward for his help and encouragement during the course of the research and the preparation of this thesis.

I wish to thank the following for gifts of chemicals: Professor T. R. Seshadri, University of Delhi; Dr. J. A. F. Gardner and Mr. G. Barton, Forest Products Laboratory, Vancouver, B. C.; Dr. H. L. Hergert, Rayonier Corporation Shelton, Washington; Dr. R. D. Bennett, Du Pont Company of Canada, Montreal; Dr. J. L. Keays, Powell River Company, B. C.; Dr. K. G. Booth, Crown-Zellerbach Corporation, Camas, Washington; Dr. A. S. Gregory, Weyerhauser Timber Company, Longview, Washington; Dr. E. L. Lovell, Alaska Pine and Cellulose Company, Vancouver, B.C.

I wish also to express my thanks to Dr. D. McGreer, of this Department for his help in the analysis of NMR spectra, to Mr. B. I. Nist, Department of Chemistry, University of Washington, Seattle, for taking the NMR measurements, and to Drs. O. Goldschmid and H. L. Hergert for the opportunity to read the manuscript of their paper on hemlock lignin precursors in advance of publication.

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THE CHEMICAL CONSTITUTION OF WOOD

All wood contains cellulose, hemicellulose and lignin. The cellulose is the fibrous material of the cell wall of woody plants and consists of chains of D-glucopyranose molecules linked together as β-glucosides, through positions 1 and 4 of adjacent units. Physical methods indicate a degree of polymerisation (DP) for native wood cellulose of greater than 3,000 anhydroglucose units per molecule.

Hemicelluloses are the non-glucan carbohydrate polymers of wood and are interlaced with cellulose in the primary and secondary cell walls. Hemicellulose building units are D-xylose, D-mannose, D-glucose, L-arabinose, D-galactose and certain derivatives of these monosaccharides. The DP of hemicelluloses is considerably lower than that of cellulose, and they are usually soluble in 17.5% sodium hydroxide solution.

Lignin never occurs alone in nature but is always associated with cellulose or other carbohydrates. When wood is carefully treated with mineral acids, the carbohydrates are dissolved, leaving a lignin skeleton with hollow spaces, forming a sponge like system.

> "In the light of our present knowledge of lignin chemistry, lignin may now be defined as that incrusting material of the plant which is built up mainly, if not entirely, of phenyl propane building stones; it carries the major part of the methoxyl content of the wood; it is unhydrolysable by acids, readily oxidisable, soluble in hot alkali and bisulfite, and readily condenses with phenols and thio compounds". (1)

The purpose of lignification in the plant is a double one. It cements and anchors the cellulose fibers together and, at the same time stiffens them and protects them from chemical and physical attack (1).

With the exception of a small part of the lignin, the wood components mentioned above are insoluble in organic solvents, mainly because of their macro-molecular nature. In addition to these main structural constituents there are present in wood low molecular weight compounds which may be

removed by extraction with neutral solvents such as petroleum ether, benzene, ether, chloroform, acetone, alcohol, and water. These are the so-called "extraneous constituents" which are responsible for the pronounced differences in color, odor and physiological properties of different woods. The percentage composition of some typical plant materials is summarized in Table I.

Table I

Percentage Composition of Typical Plants (2)

Component	White Spruce	Aspen	Wheat Straw
Cellulose	50.2	49.43	50.7
Hemicellulose	16.39	21.18	-
Pentosans	-	-	29.4
Lignin	27.96	18.12	14.2
Extractives	3.75	4.64	-
Acetyl	1.08	3.41	8.3
Ash	0.29	0.38	7.4

The most important extraneous components of wood are the volatile oils, resins, and phenolic compounds. The last group may be further subdivided into (i) anthocyanins, leucoanthocyan(id)ins, and related pigments (flavonoids), and (ii) lignans.

The volatile oils include the odoriferous constituents of wood and are usually liquid at ordinary temperatures. Commercially, the most important are the oils produced by turpentining operations and steam distillation of wood. They are chiefly composed of the terpenes: α- and β-pinene, limonene, and phellandrene.

The resinous fraction of wood, which is extractable with organic solvents such as ether, acetone, or alcohol, is composed of resin acids and fatty acids, esters of these with terpenes and other alcohols, and unsaponifiable material consisting of sterols, alcohols, waxes and resenes.

Some species of wood contain rather large amounts of colored compounds and at one time a number of these were used as sources of natural dye stuffs (e.g. woad). The colored substances may be classified as unsaturated hydrocarbons, ketones, quinones and flavonoids.

Flavonoids constitute a class of phenolic compounds whose structure is based on the $C_6C_3C_6$ carbon skeleton (I) and (II). The range of structural variation found in the flavonoid type is associated primarily with variation in the oxidation level of the C_3 portion of the molecule (Figure 1). The range of oxidation level extends from the highly reduced catechin type (III) to the highly oxidized flavonol (X).

Anthocyanins are the coloring materials in red and blue flowers, in the red autumn leaves, and in red cabbage. Vegetable tannins which are capable of converting animal skins and hides into leather include flavonoid structures.

Earlier investigations of the chemical constituents of the wood of western hemlock (Tsuga heterophylla (Raf) Sarg.) have been concerned with the hemicellulose and lignin fractions and with the isolation of conidenrin in substantial quantity from the sulfite waste liquors obtained in the pulping process. In the most recent work the inner bark, cambium, and sapwood were examined for possible lignin precursors.

This thesis describes research on the isolation and molecular structure of phenolic extractives of western hemlock wood. Some of the compounds isolated have been previously reported but the postulated molecular structures required further investigation.

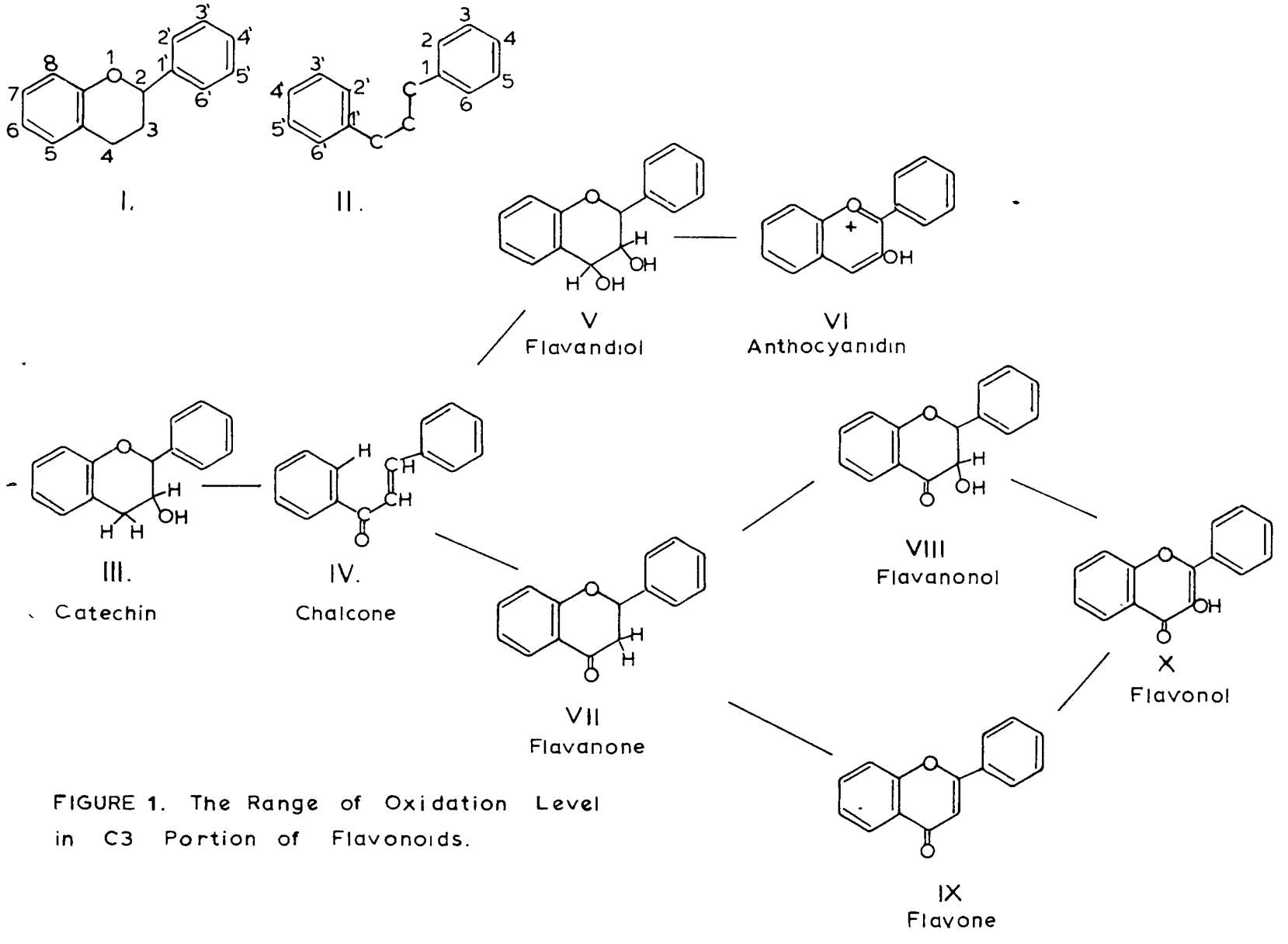


FIGURE 1. The Range of Oxidation Level in C3 Portion of Flavonoids.

HISTORICAL INTRODUCTION

LEUCOANTHOCYAN(ID)INS

Willstätter and Nolan (3) discovered the existence of colorless modifications of anthocyanidin pigments in rose petals and Rosenheim found similar compounds in grape vines and the skin of white grapes (4). These colorless substances changed into the red anthocyanidins on acidification and the term leucoanthocyanin was proposed for this group of flavonoids (4).

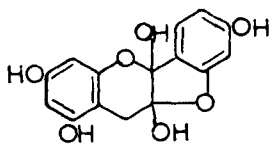
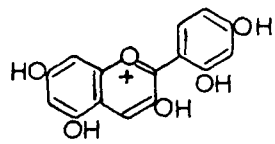
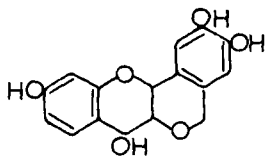
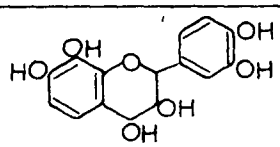
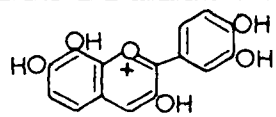
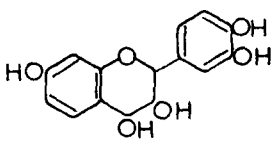
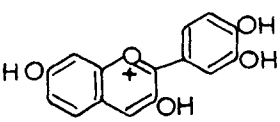
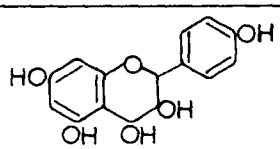
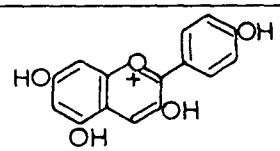
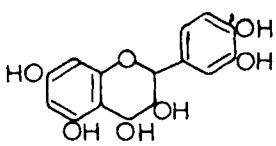
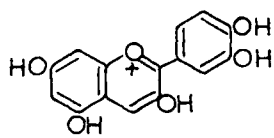
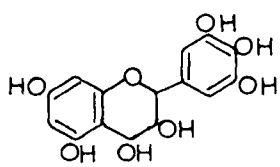
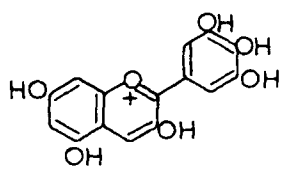
Robinson (5) suggested and Bate-Smith (6) confirmed that leucoanthocyanidins generally have the flavan 3,4-diol structure (V).

The first pure leucoanthocyanidins to be isolated were cyanomaclurin (XI) (7,8,9) and peltogynol (XII) (10,11). These two compounds had special features of structural complexity and yielded anthocyanidins on acidification which have not been found in nature (Table II). Later King and Bottomley (12) isolated melacacidin (XIII) and established its constitution and Keppler carried out a similar study of mollisacacadin (XIV), (13,14,15, 16).

The most widely-occurring groups of leucoanthocyanidins resisted isolation in pure condition until 1954. This was due to the experimental difficulties arising from their high instability toward trace amounts of moisture, light, oxygen, and other oxidising agents such as the peroxide content of diethyl ether. In addition, leucoanthocyanidins seemed to be easily polymerized and to react with aldehydic substances to form plastics (24). With modern techniques (-)-leucopelargonidin (XV), (+) - leucocyanidin (XVI), and (+) and (-) - leucodelphinidin (XVII) were recently isolated by Ganguly and Seshadri (17,18,19,20,21).

All of these colourless compounds belonged to the flavan-3,4-diol structure type (V) and could occur in more than one form because of asymmetric centers at carbon atoms 2,3 and 4 . As a consequence, eight optically active forms were possible (four pairs of enantiomers). The first reported example of the natural occurrence of an enantiomorphous pair of flavonoids

TABLE II. Leucoanthocyanidins

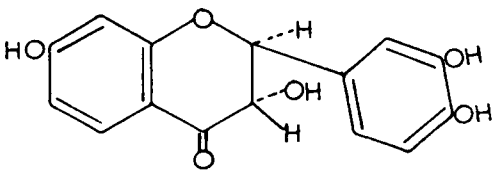
Leucoanthocyanidin	Source	Corresponding Anthocyanidin pigment
 XI. Cyanomaclurin (7, 8, 9)	Crataegus oxyacantha	 XVIII. Morinidin (26)
 XII. Peltogynol (10, 11)	Peltogyne porphyrocardia	—
 XIII. (-)-Melacacidin (12)	Acacia melanoxylon	 XIX —
 XIV. (+)-Mollisacacidin (13,14,15) (-)-Leucofisetinidin (16)	Acacia mollissima Gleditsia Schinopsis quebracho-colorado	 XX Fisetinidin
 XV (-)-Leucopelargonidin (17)	Eucalyptus Cabaphylla	 XXI Pelargonidin
 XVI (+)-Leucocyanidin. (18,19)	Tamarind-seed testa Butea frondosa gum	 XXII. Cyanidin
 XVII. (+)-Leucodelphinidin (20, 21)	Eucalyptus pilularis Karada bark Myrica nagi	 XXIII Delphinidin

was that of (+) - mollisaccacidin (XIV) (13) and (-) - leucofisetinidin isolated from two different woods (22). The enantiomorphism of the dextrarotatory and levorotatory forms of the 7, 3', 4' -trihydroxyflavan - 3,4-diol was unequivocally established by their identical melting points, and infrared absorption spectra and by formation of racemates from equal quantities of the (+) and (-) compounds. Moreover the melting point of the racemate was not depressed by admixture with the optically inactive compound prepared from natural fustin (XXIV) (23).

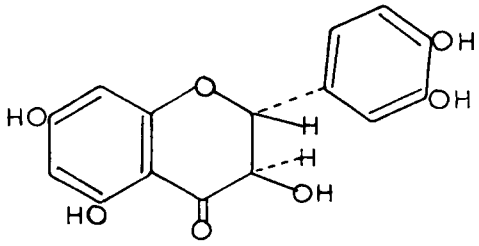
Fustin was extracted from wood and hydrogenated to yield (-) -leucofisetinidin (XXV) and further reduction yielded (+)-7, 3', 4' - trihydroxy flavan-3-ol (XXVI) (24). Hence, with regard to carbon atoms 2 and 3 the configuration of these three compounds was identical. Since the trimethylether of the leucofisetinidin (XXV) formed a cyclic acetone derivative (22) and an acid borate complex (13) the hydroxyls on carbon atoms 3 and 4 were in a cis relationship. The comparison of the rotation of fustin (XXIV) and fisetinid-3-ol (XXVI) on the one hand and of taxifolin (XXVII) and catechin (XXIX) on the other (26) led to the conclusion that (+) - fisetinidol corresponded configurationally to (-) - catechin (24), the mirror image of XXX. The absolute configuration of catechin had been established (27, 28,29,30) and therefore the configurations of the fisetin series were also determined on an "absolute" basis.

(+) - Mollisacacidin and (-) - leucofisetinidin were assigned the 2,3-trans-3,4-cis-configuration for which two half-chair conformations XXXII and XXXIII were possible (also their mirror images). They constituted an exception among optically active flavonoids which usually have been found to occur with 2,3-cis-configuration (27,31,32).

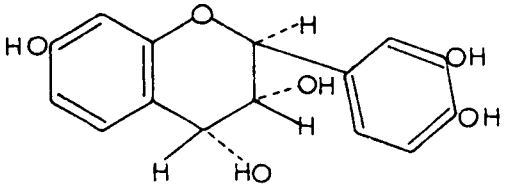
On examination of the nuclear magnetic resonance spectra (33) of a number of flavan-3-ols, flavan-3,4-diols, and their derivatives it was shown



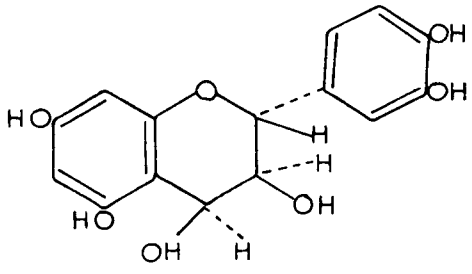
XXIV. (-)-Fustin



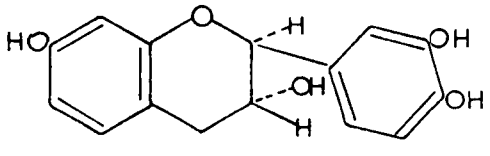
XXVII. (+)-Taxifolin



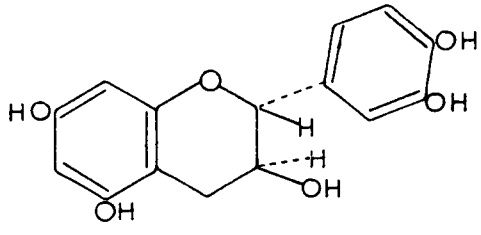
XXV. (-)-Leucofisetinidin



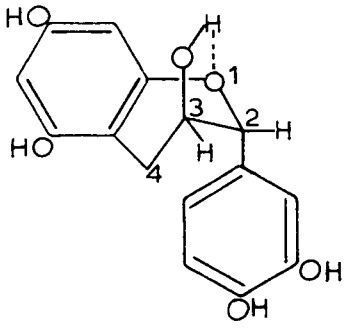
XXVIII (+)-Leucocyanidin



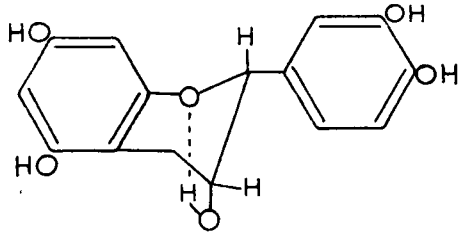
XXVI. (-)-Fisetinid-3-ol



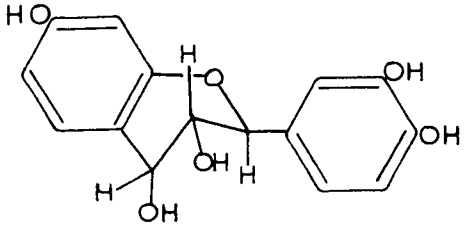
XXIX. (+)-Catechin



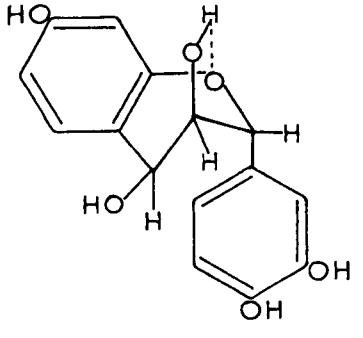
XXX. (+)-Catechin
2(a): 3(a)-trans



XXXI. (-)-Epicatechin
2(e): 3(a)-cis



XXXII 2(e): 3(e)-trans
3(e). 4(a)-cis



XXXIII. 2(a): 3(a)-trans
3(a): 4(e)-cis

that spin-spin coupling constants between the 2- and 3-, and 3- and 4-protons defined the stereochemistry and conformation of the heterocyclic ring. The low 2,3-cis coupling constants suggested that in the 2,3-cis derivatives one conformation was predominantly populated. In 2,3-trans flavan derivatives the conformation in which the 2- and 3- substituents are quasiequatorial was favoured at room temperature.

The leucoanthocyanidins were obtained in colorless crystalline or cream-colored amorphous forms; they darkened and shrank at elevated temperatures but did not melt. They were insoluble in light petroleum, ether, chloroform and benzene, but were freely soluble in water, ethanol, and acetone and were rapidly converted (especially leucocyanidin, XXVIII) to brown, insoluble polymers on standing in contact with moisture and oxygen. With alcohol and hydrochloric acid they developed an immediate purple color even in the cold, and this deepened on standing or on heating. A red, insoluble phlobaphene was obtained as a by-product, which was probably a polymer formed by condensation (23,34).

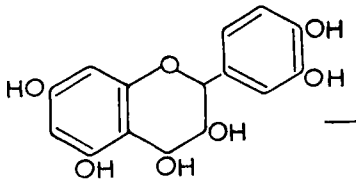
An important feature of the chemistry of the leucoanthocyanidins was that the anthocyanidins produced from them had lost the 4-hydroxyl and retained the 3-hydroxyl group (XXXIV). It was suggested (35) that the mechanism involved was an initial acid-catalyzed dehydration to a flavan-3-one (XXXV) and Roux and Bill (36) obtained evidence for the initial dehydration step. The mechanism for the required direction of dehydration was discussed by Birch for the corresponding tetrahydronaphthalenes (25). The flavan-3-ones, like flavan-3-4-diols, were in the same oxidation state as dihydroanthocyanidins so that the flavylium salt must have been produced by oxidation. Over-all mechanistic possibilities were direct oxidation and disproportionation (35,12). The reduced substances from disproportionation of a flavan-3-one would be at the catechin (XXXVI) level of

oxidation (39). Production of anthocyanidins from leucoanthocyanins seemed to be independent of the presence of oxygen in some cases, but not in others (37, 38).

The low yield of anthocyanidins from leuco-compounds was generally considered to be due to a competing condensation reaction leading to tannins (41). If favan-3-ones were intermediates, they would be very unstable, polymerization would occur by aldol-type condensation of the ketone group with the phloroglucinol nucleus (35). The polymerization of favan-3,4-diols would also be expected through the very reactive 4-hydroxyls groups, similar to the acid-catalyzed polymerization of catechins (34,41) (XXXVIII and XXXIX). High yields of anthocyanidins were obtained by Laumas and Seshadri (40) who first converted the flavan-3,4-diols into flav-3-en-3-ol acetates (XXXVII), so that self-condensations were inhibited by the lowered reactivity of the acetylated phenolic rings.

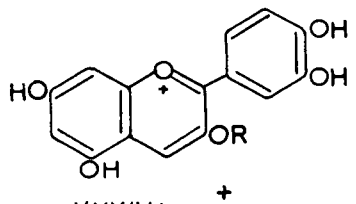
Under anhydrous conditions two anthocyanidins of similar coloration but with different rates of migration on paper chromatograms were generated from each flavan-3,4-diol (36). The anthocyanidin with the higher R_f value was found to be the 3-ol ether derivative (XXXIVb). Ether formation occurred prior to conversion into the corresponding anthocyanidin, and was promoted by anhydrous conditions. In the presence of moisture ether formation was almost completely inhibited.

The anthocyanidins obtained from the leuco-anthocyanidins were identified by their color reactions, paperchromatography, absorption spectra and by alkaline degradation. The production of distinctive colors with ferric chloride solution is a general property of all classes of polyhydroxy flavonoid compounds. The use of paperchromatography in the study of plant pigments has become wide-spread because of their characteristic solubilities, R_f values, and colors in visible and ultra-violet light. (42,43).



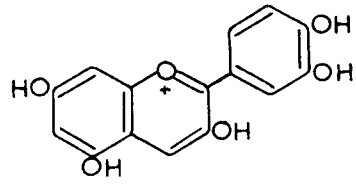
XXVIII.

ROH
H^+

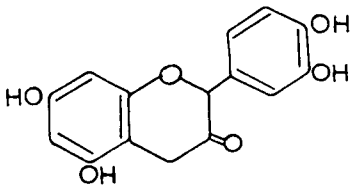


XXXIV b.

+



XXXIV a



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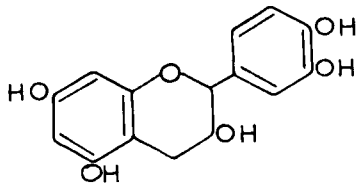

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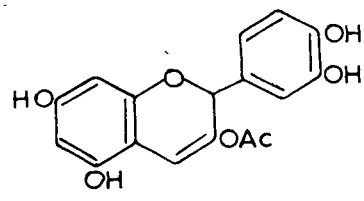

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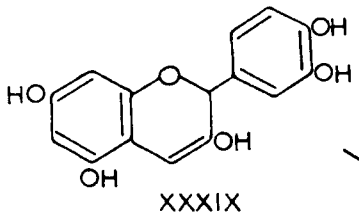

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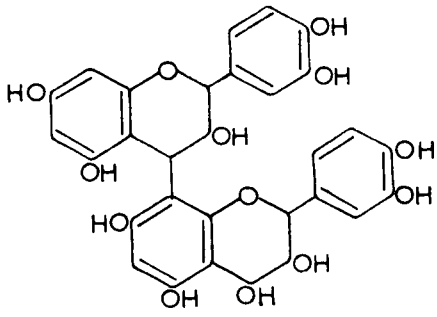

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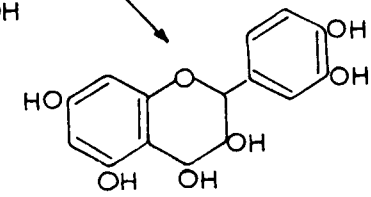
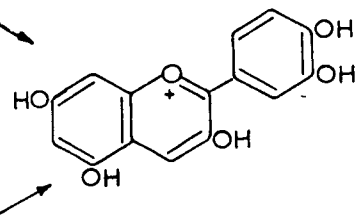


XXVIII

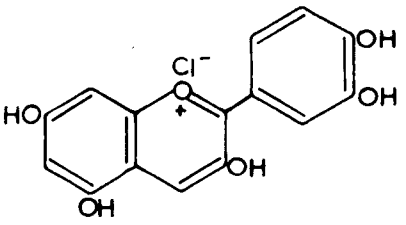


XXXIV

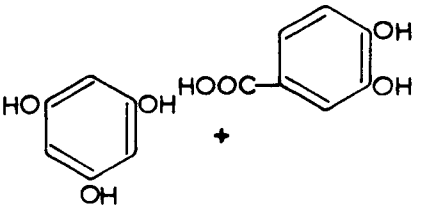
Anthocyanidin electronic absorption spectra were of limited value for identification. Only small differences occurred in the absorption maxima of the oxonium forms, but the shift in λ_{max} caused by complexing with aluminium chloride in the case of anthocyanidins with o-phenolic hydroxyl groups wasmore characteristic (126). The alkaline degradation products of anthocyanidins were identified by paperchromatography (XXXIV, XL, XLI).

Robinson (44) considered the flavonoid skeleton to be built up from two parts, C_6 and C_9, in biogenesis (XL, XLII, XLIII). The biosyntheses of quercetin (XLIV) and cyanidin (XXXIV) have been investigated. Birch and Donovan (45) carried out biogenetic experiments with the unicellular green algae which appeared to form quercetin through a pathway involving condensation of caffeic acid and phloroglucinol. Neish and co-workers (46) used buckwheat plants as experimental material since they were readily grown and contained rutin, a 3-rhamno-glucoside of the favonol quercetin, in the leaves. By the use of specifically labelled cinnamic acid and generally labelled phenylalanine it was shown that the phenyl propanoid skeleton gave rise to the B-ring, plus carbons 2,3 and 4 of quercetin as a unit. The formation of quercetin was studied in another experiment by feeding labelled acetate to buckwheat and the quercetin (XLIV) was degraded by fusion with potassium hydroxide into phloroglucinol (XL) and protocatechuic acid (XLVIII). Methyl- and carbonyl-labelled acetates were found to be equally good precursors of quercetin. This meant that acetate must have been used as a 2-carbon unit, and was not degraded to 1-carbon fragments in the biosynthetic process.

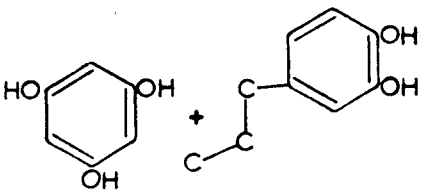
Birch and Donavan (45) noted that the structure of a large number of naturally occurring orcinol and phloroglucinol derivatives could be explained by assuming that they originated by cyclization of polyketo acids



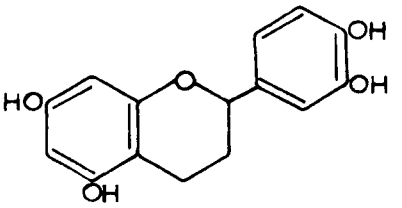
XXXIV



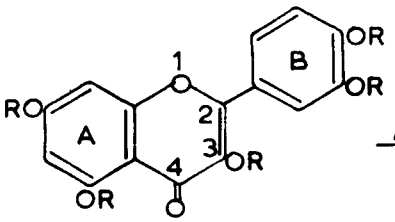
XL XLI



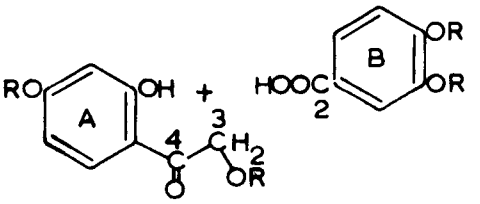
XL XLII



XLIII

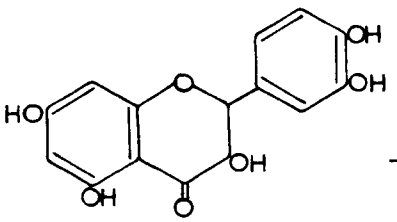


XLIV. R=H, Quercetin
XLV. R=CH_3, Quercetin pentamethyl ether

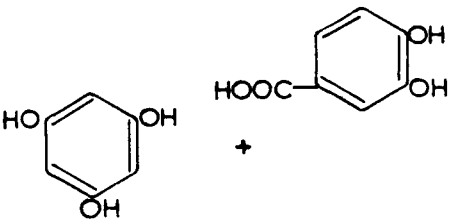


XLVI. R=CH_3
2'-Hydroxy-2,4,6'-trimethoxy acetophenone

XLVII. R=CH_3
Veratric acid



XLIV



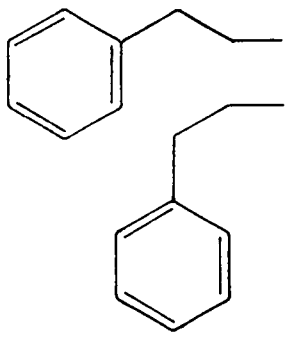
XL XLVIII

formed by head to tail condensation of acetate units. The finding that ring A came from acetate could have meant either that phloroglucinol was first formed from three acetate molecules and then condensed with the phenylpropanoid unit, or that the acetates condensed directly with the phenylpropanoid compound in a stepwise manner. The former alternative was eliminated by the finding that buckwheat did not indorporate radioactive phloroglucinal directly into quercetin (47).

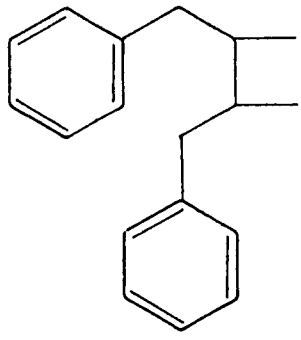
Grisebach (48) investigated the biogenetic relationship between quercetin and cyanidin in buckwheat with the help of C^{14} labelled compounds. These studies indicated that flavonol was not a precursor to anthocyanin in buckwheat. Grisebach also investigated the biogenesis of cyanidin in red cabbage (49,50) and the results obtained were in good agreement with those reported by Neish and co-workers.

LIGNANS

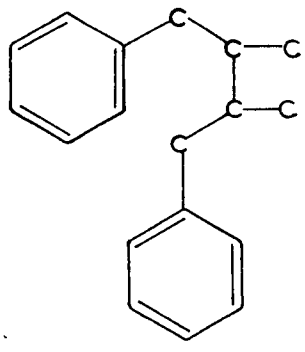
A number of phenolic compounds, called lignans, have been found among the alcohol-soluble components of various woods. The term was originated by Haworth (51) to describe a family of plant products which seemed to be formed by joining certain derivatives of n-propyl benzene (XLIX) at the β-carbon atoms of the side chain (L). This structure was found in a variety of modifications, including the 1,4-diarylbutane (LI), 1-phenyltetralin (LII), diphenylfuran (LIII), and furanofuran (LIV) types. The aromatic rings of the lignans were all oxygenated and carried hydroxyl, methoxyl, or methylenedioxy groups. The side chains existed in varying degrees of oxidation and in some cases were further modified by cyclization to tetrahydrofuran or tetrahydronaphthalene derivatives. In relationship to other non-carbohydrate plant products lignan appeared to be the dimer stage intermediate between monomeric propylphenol units and lignin (52). Erdtman (53) suggested that



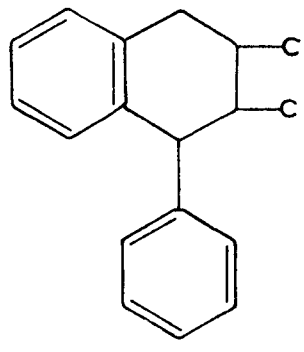
XLIX



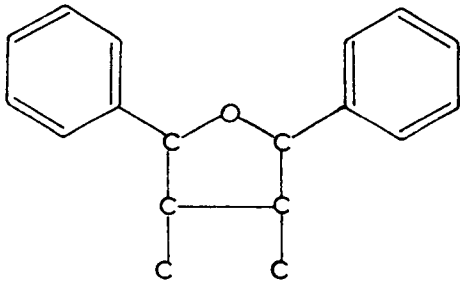
L



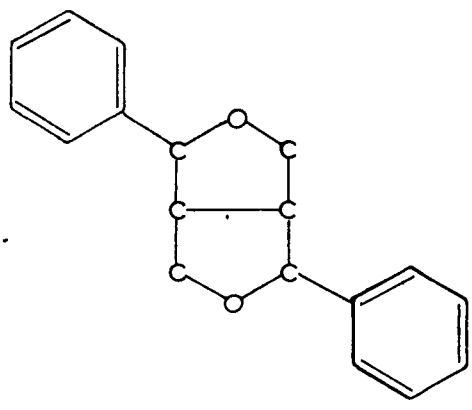
LI



LII



LIII



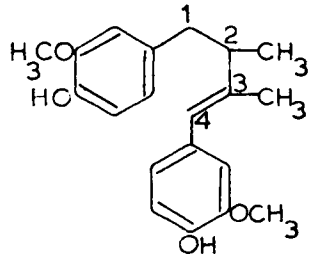
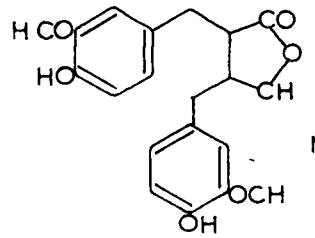
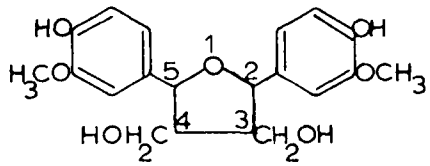
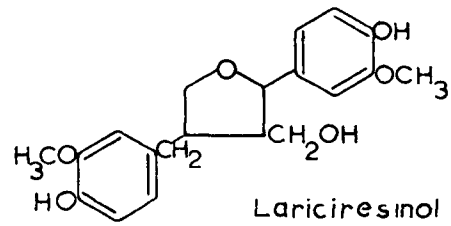
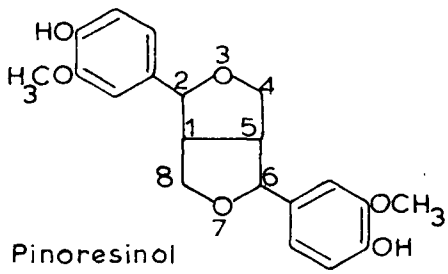
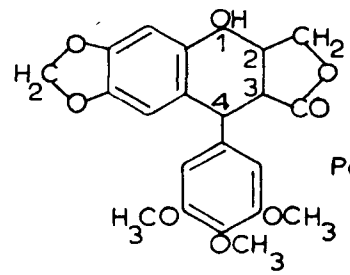
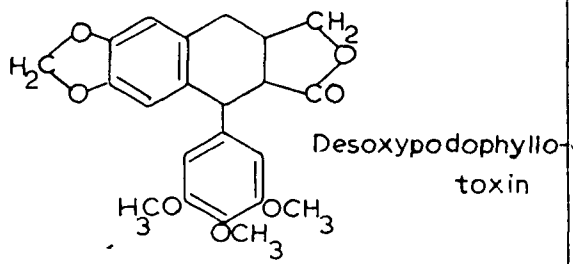
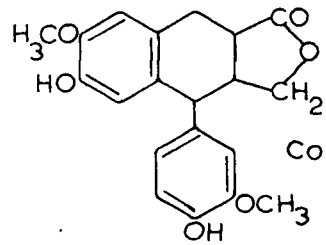
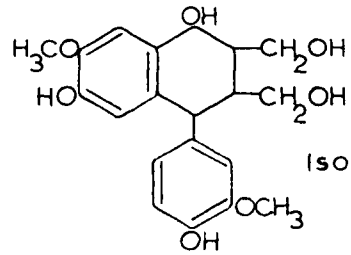
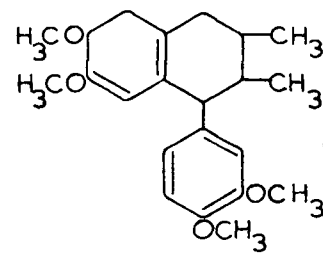
LIV

lignans as well as lignins were formed by dehydrogenation of simple primary C_6C_3 precursors. Naturally occurring trimers and tetramers have not been reported.

Lignans have been found widely distributed in the vegetable kingdom but the role they play in the plant has not been established. The lignans have been classified into five principal groups (A-E, Table III) on the basis of the structure formed by the side chains of the two combining C_6C_3 units. They are almost always designated by trivial names. The classification used in Table III is essentially that of Haworth (54).

Lignans generally have been obtained from wood extracts, but in some cases, especially when the starting material was ground wood, extraction with ether did not remove, or only incompletely removed, ether-soluble lignans. This has been attributed to the presence of an ether-insoluble "membrane" substance in the wood and not to adsorption (55). The lignans were sparingly soluble in light petroleum, soluble in ether, acetone, and lower alcohols and several yielded sodium and potassium salts. Chromatography and countercurrent extraction have been employed successfully in the separation of mixtures of related lignans. Many lignans were easily isomerized, especially in the presence of acids or alkali and some were sensitive to light (56). Recently it was found in this laboratory that some lignans were also sensitive to the oxygen of the air. Since separation of lignans was often found to be difficult they have been purified in some cases via ester or ether derivatives. The ultraviolet absroption spectra were not characteristic and, in the absence of double bonds conjugated with the aromatic nuclei, the spectra were very similar to the absorption of the aromatic portions of the molecules (55). The infrared absorption was, of course, more suitable for characterization. Dimorphism occurred frequently in the lignan field. Almost all isolated lignans were optically active, but the specific

TABLE III. LIGNANS.

DERIVATIVES OF	
A. 1,4-Diarylbutane	B. 2,3-Dibenzylbutyrolactone
 Guaiaretic acid	 Matairesinol
C. 2,5-Diaryltetrahydrofuran	C 2-Aryl-4-benzyltetrahydrofuran
 Olivil	 Lariciresinol
D. 2,6-Diaryl-3,7-dioxabicyclooctane	E. 4-Aryltetrahydronaphthalene
 Pinoresinol	 Podophyllotoxin
E. 4-Aryltetrahydronaphthalene	E. 4-Aryltetrahydronaphthalene
 Desoxypodophyllotoxin	 Conidendrin
E. 4-Aryltetrahydronaphthalene	E. 4-Aryltetrahydronaphthalene
 Isoolivil	 Galbulin

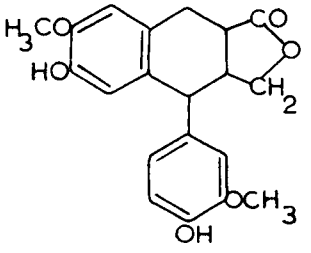
rotation was strongly dependent upon the solvent used. The structures of many lignans have been verified by comparison of the optical rotation, melting points and degradation products of the synthetic and natural compounds and their derivatives. The aromatic rings of lignans were substituted readily giving bromo and nitroderivatives (52). The hydroxyl groups were readily methylated and acetylated. The lactone ring was opened by heating with aqueous alkali to form the salt of the hydroxy acid and upon acidification the ring was again closed.

A. The Chemistry of Conidendrin

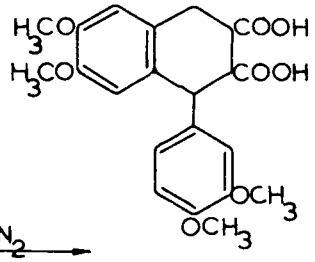
The earliest discovered and most thoroughly studied lignan was conidendrin (LV). It was found to occur widely in nature and was first isolated by Lindsey and Tollens in 1892 (57) and characterized in 1921 by Holmberg (58). The hypobromite oxidation of methylated conidendrin gave a neutral substance and two acids. The acids were shown to be dimethylconidendreic acid (LVI) and 2-veratroylveratric acid (LVII). The neutral substance was only recently identified as 6,7-dimethoxy-(3,4-dimethoxyphenyl)-2-naphthaldehyde (LVIII) (59).

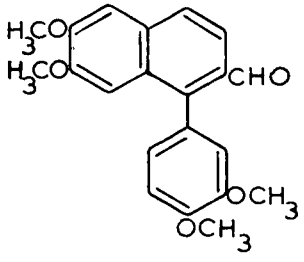
Holmberg (61) also established the presence of two methoxyl groups by analysis and two free hydroxyl groups by acetylation and methylation. The rapid ring closure of the opened lactone and the ready conversion of the dibasic acid to an anhydride showed that the lactone ring was five-membered. Oxidation to the dibasic acid showed the presence of a hydroxymethyl group, which must be on a carbon atom adjacent to the carbon atom carrying the carboxyl group. These facts, and the observation that conidendrin empirically was a dimer of coniferaldehyde led Erdtman to suggest one of two formulae (LIX and LX) for conidendrin (61).

As a further test of the proposed structures, Erdtman dehydrogenated the dibasic acid (LVI) with lead tetra-acetate to the phenylnaphthalene

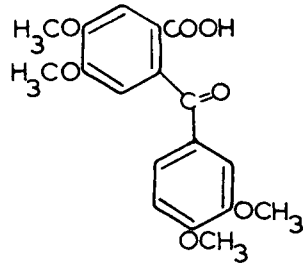


LV

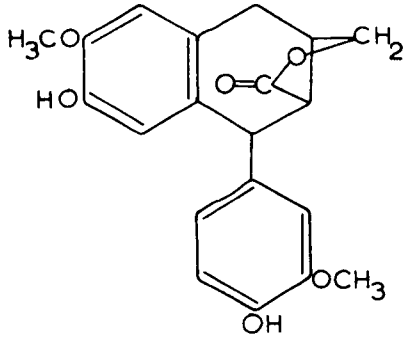




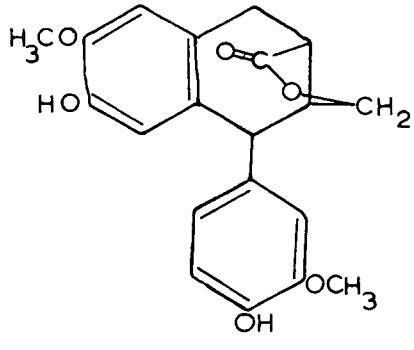
LVI + LVIII



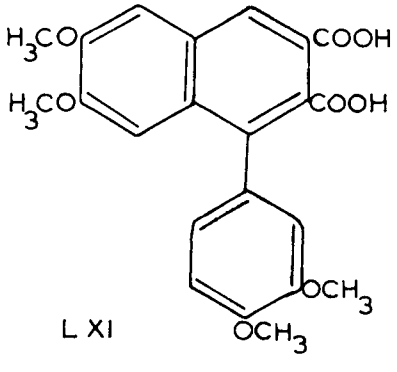
LVII



LIX



LX



LXI

dibasic acid (LXI) but he was unable to determine whether the lactone ring contained the carboxyl group on carbon 2 or on carbon 3.

The correct position of the lactone ring was established by Haworth (62,63) and the correct structure was confirmed by synthesis of the two possible naphthalene derivatives (Figures 2 and 3) which were compared with the corresponding naphthalene derivative prepared from dimethylconidendrin by dehydrogenation. A mixed melting point then demonstrated that conidendrin corresponded to structure LX.

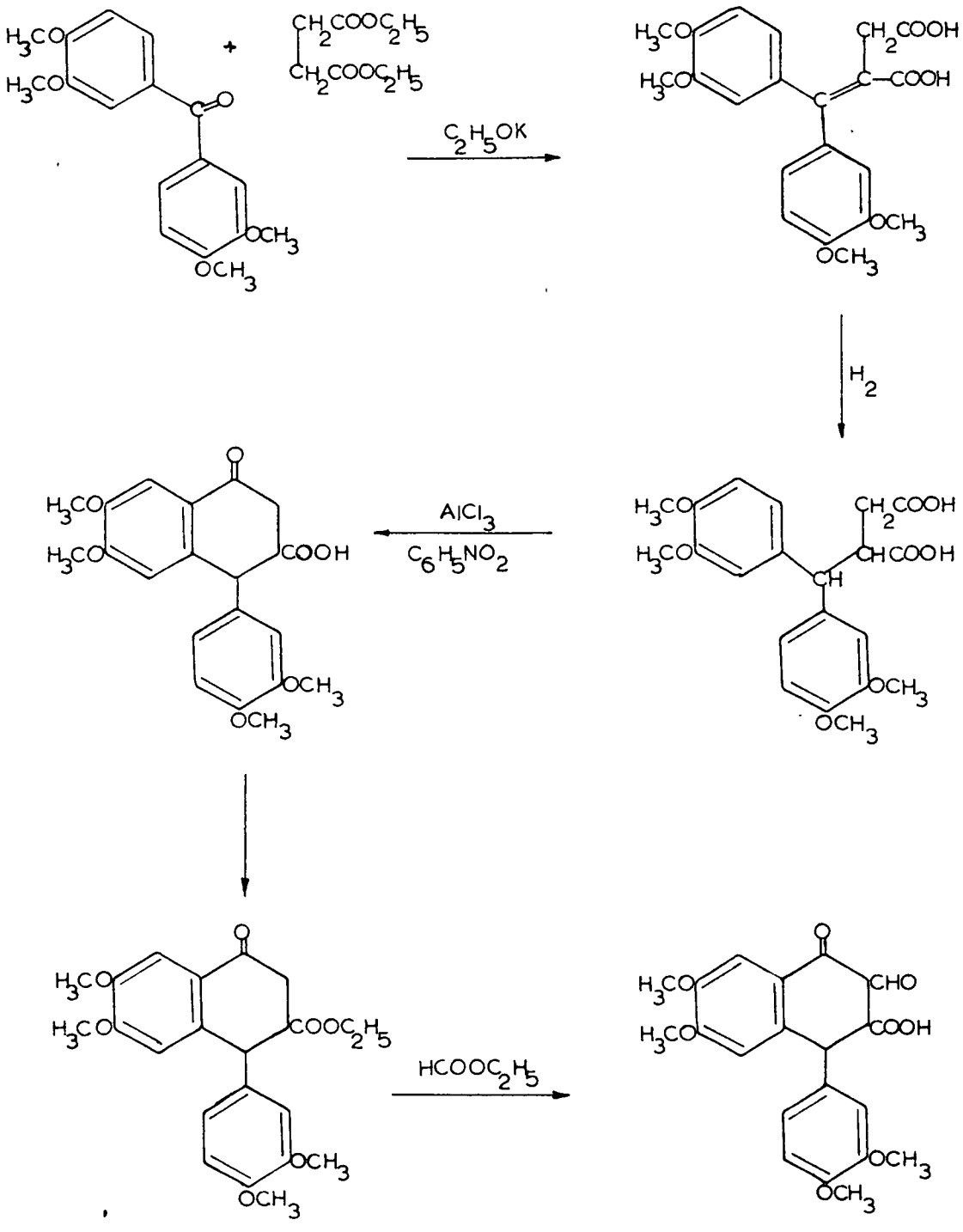
Demethylation of conidendrin was effected with pyridine hydrochloride. The products, α- and β- nor-conidendrin, possessed antioxidant properties for oils and fats (64,65,66). Holmberg (67) noted that conidenrin, when heated dry or treated with alcoholates, was transformed to another compound with different melting point and optical rotation. Also methylated conidendrin could be changed to an isomer. These isomeric forms have been designated β in the later literature (52). The hydroxy acids form the two methylated conidendrins had different melting points and optical rotations. Emde and Schartner (68) explained the isomerization of conidendrin by inversion of configuration at the carbon atom carrying the carboxyl group. In conidendrin the configuration of the lactone ring junction is trans, and the guaiacyl group has been suggested to be trans to the lactone methylene group, that is 2,3-trans- 3,4-trans (LXII), and in β-conidendrin the lactone ring junction is cis, that is 2,3-cis, that is 2,3-cis-3,4-trans (LXIII) (69,70).

B. The Chemistry of Matairesinol

Matairesinol, a butyrolactone lignan (LXIV), was isolated from coniferous woods along with conidendrin (63,71,72,73).

The matairesinol structure was verified by the synthesis of (-), (+), and (±)-matairesinol dimethylethers (73). Oxidation of the dimethyl ethers with alkaline permanganate gave a 55% yield of veratic acid (72,63)

FIGURE 2. Synthesis of Dimethyl Aromatized LIX.



continued

Figure 2. continued

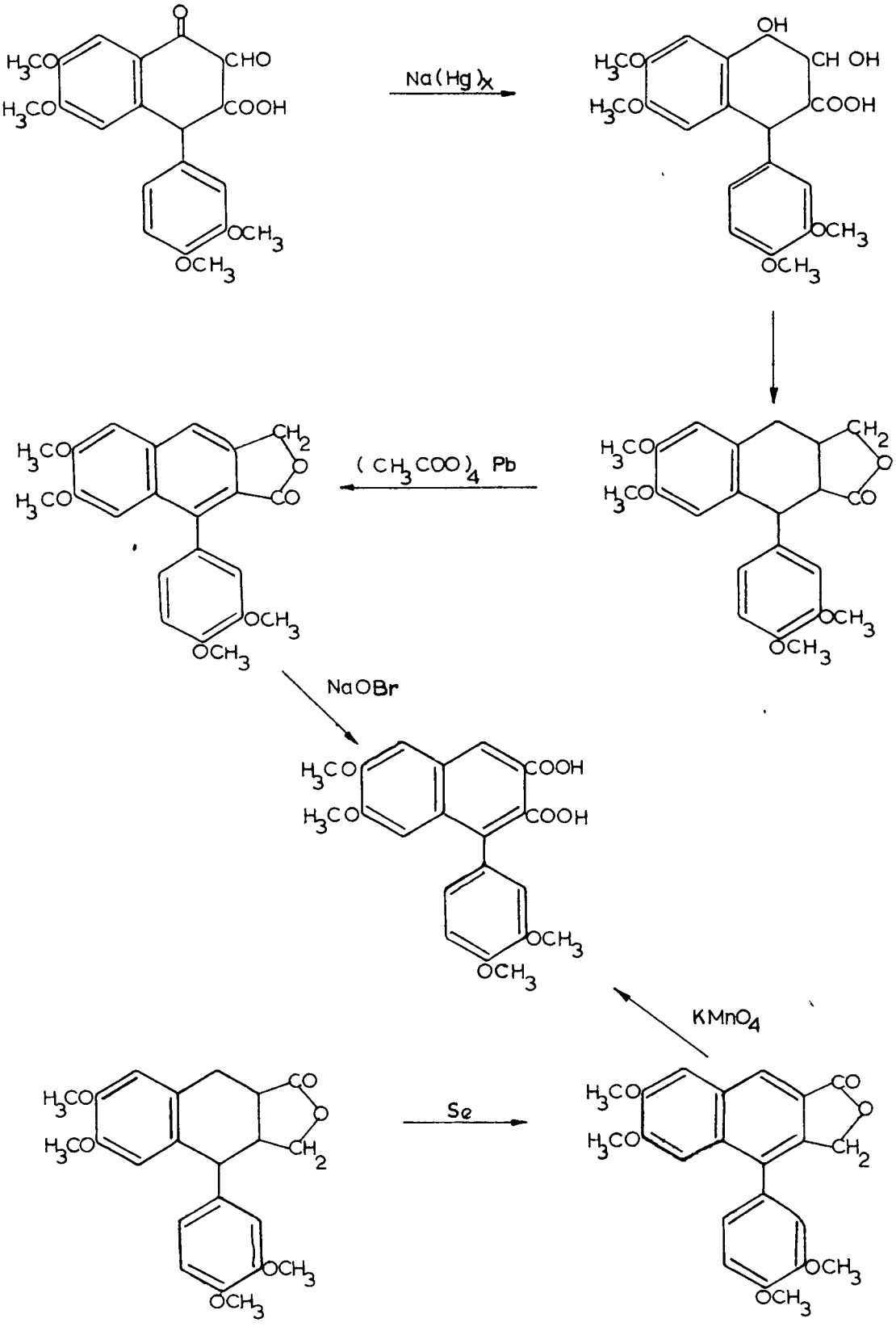
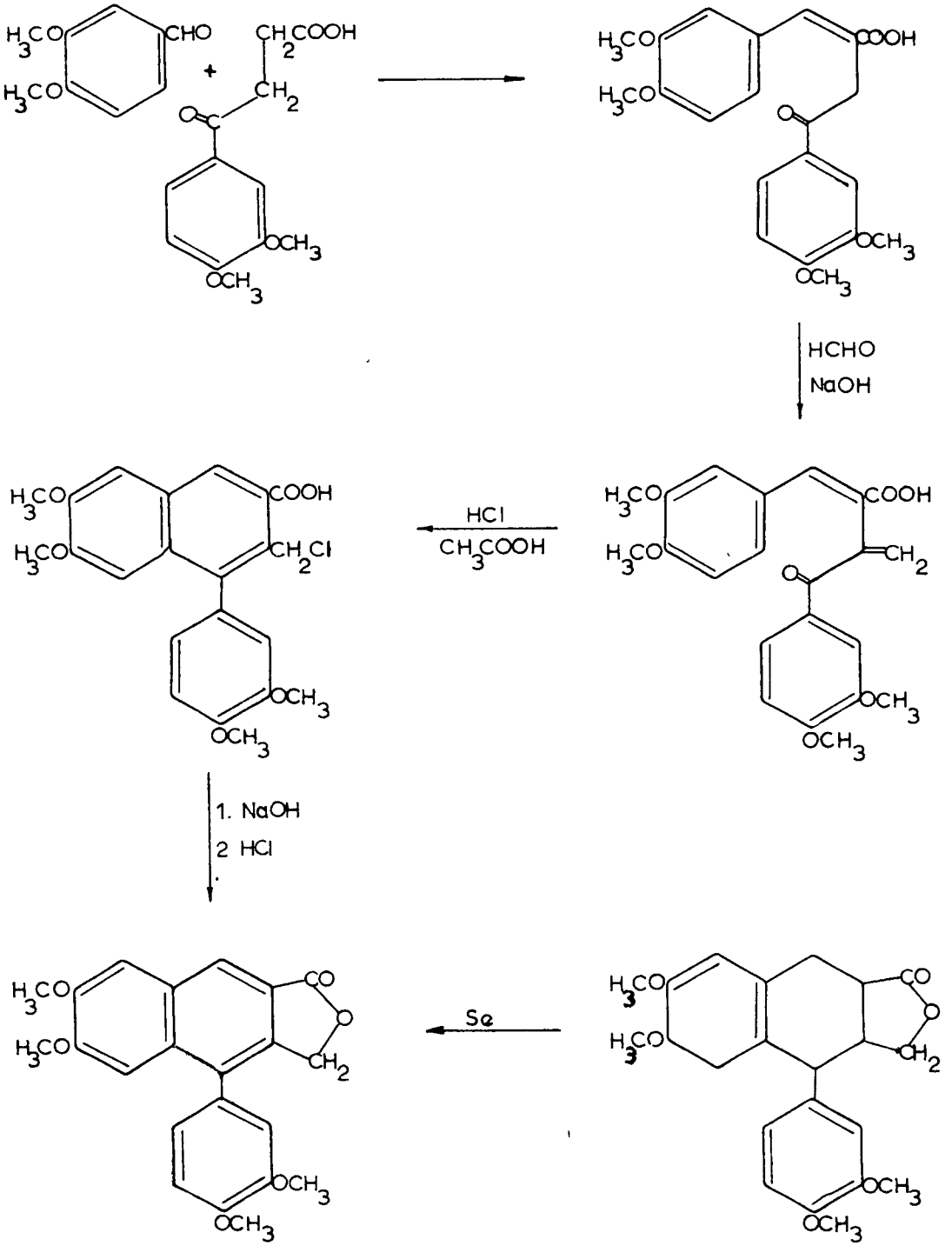


FIGURE 3. Synthesis of Dimethyl Aromatized LX.



which served to demonstrate the presence of two veratryl nuclei. Oxidation of dimethylmatairesinol with lead tetra-acetate in acetic acid caused cyclo-dehydrogenation and yielded a mixture of two naphthalene lactone (LXVIII and LXIX). (63). The mixture occurred because either of the two aromatic nuclei could be involved in the ring closure. The structures of the lactones were confirmed by syntheses (62,63).

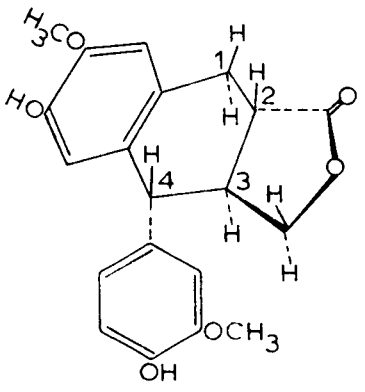
C. The Stereochemistry of Conidendrin and Matairesinol

Dimethylmatairesinol was isomerized by prolonged heating with strong alkali to an equilibrium mixture containing the salts of the hydroxy acids corresponding to dimethylmatairesinol and to an isomeric structure, dimethyl-isomatairesinol (78,79).

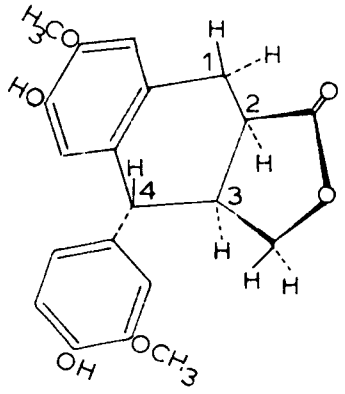
Haworth and Atkinson suggested that the isomerisation consisted in inversion about the asymmetric carbon atom, carbon 2, adjacent to the carbonyl group and that the inversion occurred without opening of the lactone ring under mild conditions. The two isomers had closely similar properties and both were dehydrogenated similarly with lead tetra-acetate (78). The tertiary hydrogen atoms (on carbons 2 and 3) on the lactone ring were trans in matairesinol (LXXIX) and cis in isomatairesinol (LXXVII), since reduction with lithium aluminium hydride gave the levorotatory- and meso-diols, respectively (80).

The behaviour of α-conidendrin (LX and LXII) and matairesinol (LXIV and LXXIX) were compared in the reactions concerned in the epimeriza-tion. In both compounds the tertiary hydrogen atoms on the lactone ring are in trans position while they are cis in β-conidendrin (LXIII) and isomatai-resinol(LXXVII).

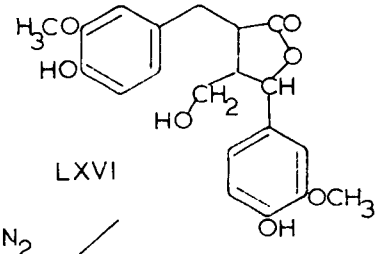
It was found (67) that the conidendrin hydroxy acid corresponding to the β-conidendrin (LXX) lactonised more rapidly than the one corre-sponding to α-conidendrin (LXXII) on acidification. In the case of the



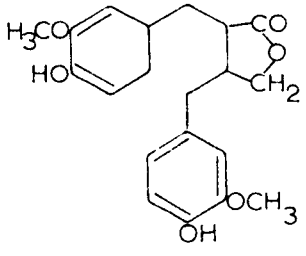
LXII



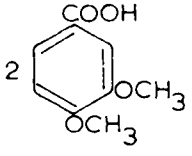
LXIII

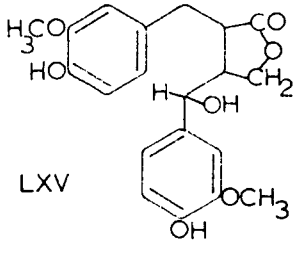


LXVI

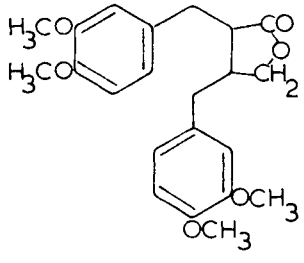


LXIV

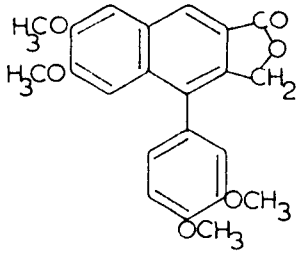




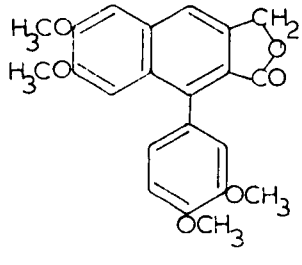
LXV



LXVII



LXVIII



LXIX

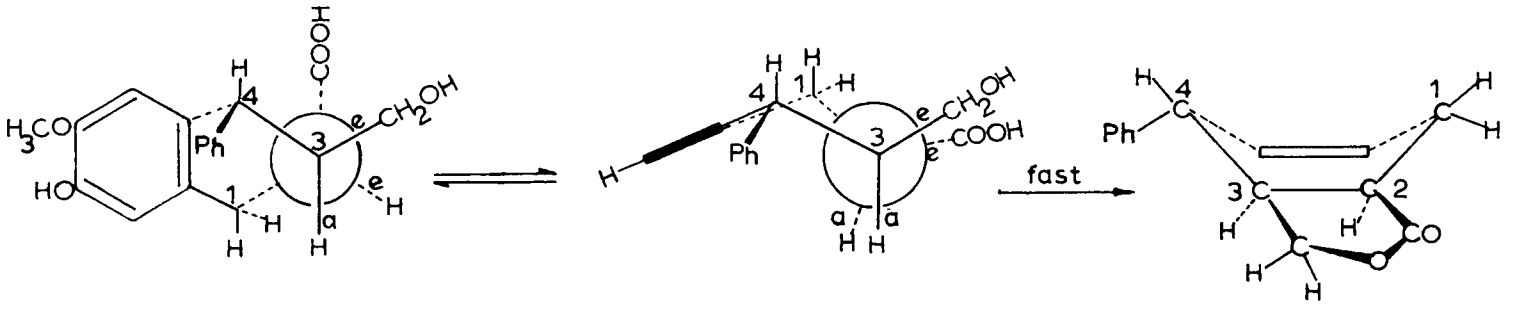
matairesinol isomers (78), the isomatairesinolic acid (LXXVI) lactonised more slowly than the matairesinolic acid (LXXVIII). The difference was consistent with the ease of rotation around the n-propylbenzene junction. In conidendrin hydroxyacids this rotation was prevented because of the phenyltetralin structure, while in the matairesinolic acids the diphenyl butane skeleton allowed the free rotation around carbon 2 - carbon 3 single bond. Since the rate of lactonization of hydroxyacids was more rapid in a configuration which allowed a closer approach of the $-CH_2OH$ and -COOH group, in the case of the conidendrin hydroxyacids (phenyltetralin skeleton) the cis-configuration (LXX and LXXI), and in the case of the matairesinol hydroxyacids (α,ω-diphenyl butane skeleton), the threo-configuration (LXXVIII), favored lactonization.

Although no discussion of the conformation features of the two conidendrin isomers (α and β), has so far appeared in the literature it seemed worthwhile to mention here that examination of molecular models indicated that the "cyclohexene ring" in naturally occurring α-conidendrin (LXII) was in a half-chair conformation (LXXIV) while the isomeric β-conidendrin (LXIII) was in a boat conformation (LXXV). These suggestions originated from the fact that it was impossible to construct the boat form of α-conidendrin or the half-chair conformation of the isomeric β-conidendrin from atomic models (Drieding Stereomedels, W. Buechi, Switzerland).

Since the half-chair conformation in the tetralin series was energetically the more favoured conformation, this analysis afforded an explanation (at least from the thermodynamic point of view) of the fact that the α-isomer is the more stable form of conidendrin.

D. The Chemistry of Hydroxymatairesinol

Freudenberg (74,75,76) claimed that the soluble native lignin or Braun's lignin (ethanol soluble part of lignin) was not true lignin but contained 30 to 40% of a lignan which crystallized with difficulty and for which

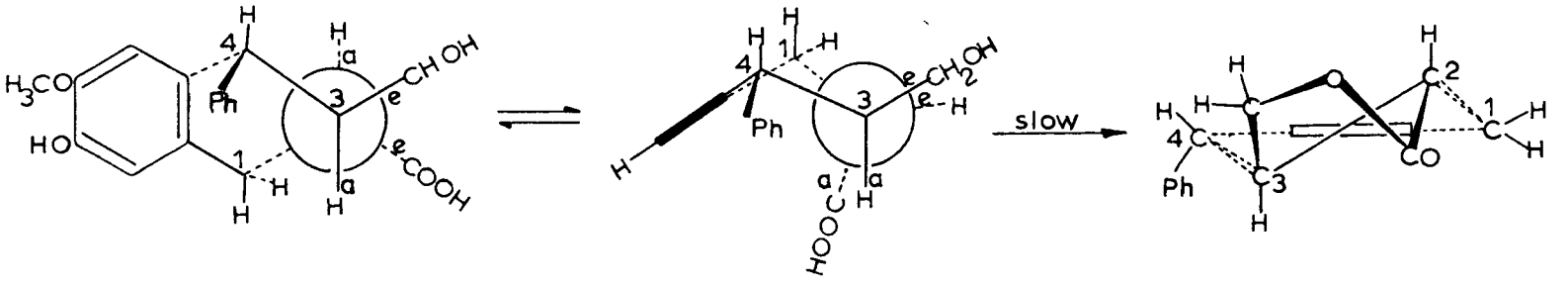


LXX. 2(e): 3(a): 4(a)
(half chair)

LXXI 2(a): 3(a) 4(a)
(boat)

LXXV. β - Conidendrin
(boat)

2.3-cis-3: 4-trans-Conidendrin hydroxyacid

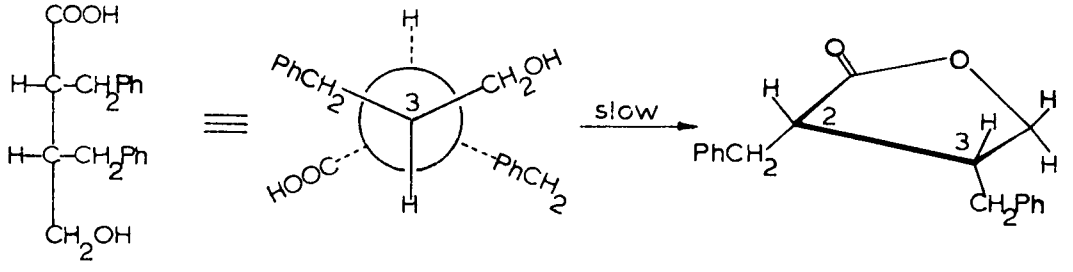


LXXII 2(a) 3(a): 4(a)
(half chair)

LXXIII 2(e): 3(a): 4(a)
(boat)

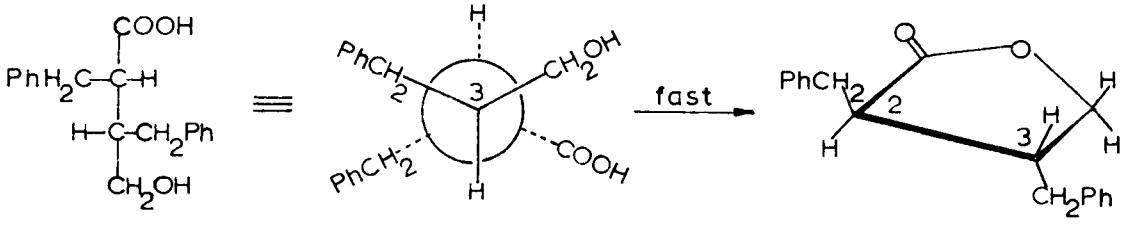
LXXIV α - Conidendrin
(half chair)

2·3-trans-3·4-trans- Conidendrin hydroxyacid



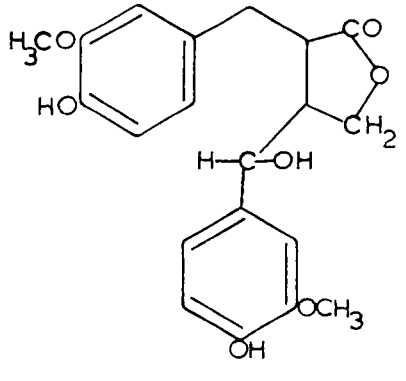
LXXVI erythro-iso-Mataıresinolic acid

LXXVII ısо-Mataıresinol

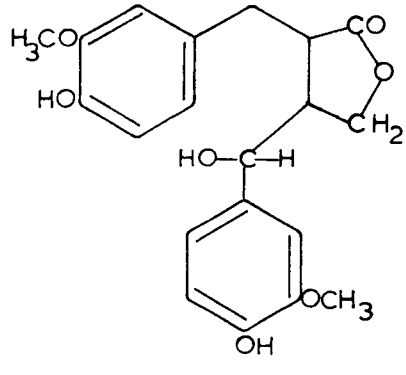


LXXVIII threo-Mataıresinolic acid

LXXIX Mataıresinol



LXXX



LXXXI

he proposed the structure of a hydroxymatairesinol (LXV or LXVI). This lignan was optically active, did not crystallise but formed a crystalline potassium-acetate adduct, and was readily transformed into conidendrin in the presence of acid. On hydrogenation with hydrogen and palladium in ethylacetate matairesinol (LXIV) was obtained (56). A strong peak at 1745 cm^{-1} in the infrared spectrum indicated the presence of a lactone ring. Freudenberg and Knopf also isolated from spruce wood allo-hydroxymatairesinol, an isomer of hydroxymatairesinol which was a crystalline compound also gave conidendrin in the presence of acid, and matairesinol on hydrogenation. The infrared spectra of the two hydroxymatairesinols were similar but the specific rotation of the allo-compound was somewhat more positive than that of the hydroxymatairesinol (56). Assuming structure LXV for hydroxymatairesinol the allo-hydroxymatairesinol could have been an optical isomer (epimer) with configuration LXXX or LXXXI. On the other hand if hydroxymatairesinol had structure LXVI, the allo-compound could also have been a geometrical isomer of the hydroxymatairesinol (LXVI).

E. The Lignan-Lignin Interrelationship in Biosynthesis

Haworth (51) drew attention to the fact that the presence of the C_6C_3 group in lignans and the current view that lignin was built up of such phenyl propane buildingstones indicated a close relationship between lignans and lignin. The low content of penolic hydroxyl groups in lignin, however, indicated a different type of condensation of the phenylpropane units in lignin and lignans. It has been suggested (81) that under normal conditions lignin was formed and under pathological conditions, lignans were formed. In 1949 Freudenberg began experiments on lignification by studying the formation of lignin-like products in vitro from coniferyl alcohol by enzymatic dehydrogenation. The first step of the action of the enzyme consisted in the removal of the hydrogen atom of the phenolic hydroxyl group of the

coniferyl alcohol (LXXXII) with the formation of radicals (LXXXIII to LIIIVI) (82). The dimerization of these radicals occurred readily and the intermediates that were first identified were the dimers dehydrodiconiferyl alcohol (LXXXVII), pinoresinol (LXXXVIII), and quaiacylglycerol- β-coniferylether (LXXXIX).

Freudenberg called these dimers secondary lignin building units because in a second step, which consisted in further dehydration and condensation they form lignin-like products. Recently there was reported (83, 84) the presence of small amounts of coniferyl alcohol and the three dimers in fresh woody cambial sap. The question now arises whether the basic structures of the above-mentioned secondary building stones are incorporated as such into the lignin molecule or are rearranged to a still unknown combinations in the molecule. In this connection, Freudenberg's experiment with pinoresinol, which is a tetrahydrofurofuran-type lignan is of interest. The dehydrogenated polymer of the pinoresinol did not show the presence of the pinoresinol structure and the polymer was optically inactive. It is not possible that the optically active lignans are combined as such into the lignin, because optically active lignin has never been found (75).

Purves and co-workers (85,86,87) and Chudokov and co-workers (88) have carried out oxidation experiments on lignin from which they obtained a mixture of benzenepolycarboxylic acids. Using radioactive formaldehyde-treated lignin they have obtained benzene hexa-(XCI), penta-, and tetracarboxylic acids. From these results they drew the conclusion that a minor part of the lignin macromolecule consisted of lignan elements in which the β-carbon atoms of the phenylpropane units were directly joined. (62).

An hydroxymethyl substitution in α-position of the lignan permits easy cyclization to a cyclohexane ring which, on oxidation, yields benzene hexa- and pentacarboxylic acids due to the five or six fold substitution of the ring (XC-XCI).

Zaburin and Tishchenko (89) claimed that the experiments (85,86,88) showed systematic errors so that conclusions as to the yield of benzene-pentacarboxylic acid in the oxidation of lignin could be held in doubt. These authors found that the oxidation of lignin yielded a mixture of only a small amount of benzenetri- and tetracaboxylic acids but no penta-analog was detected.

RESULTS AND DISCUSSION

I. Isolation of Phenolic Constituents from Western Hemlock Wood.

In preliminary experiments several attempts were made to apply previously reported procedures to the isolation and separation of the phenolic constituents of the defatted wood. The leucoanthocyanidin isolation methods used by Laumas and Seshadri on the gum of Butea frondosa (18) and by Manson on black spruce (Picea mariana BSP) (145) proved to be unsuccessful because the leucoanthocyanidin of the hemlock wood was insoluble in ethylacetate. The supposed "leucoanthocyanidin fraction" isolated by these methods (0.06% and 0.3% yield respectively) was actually found to be a mixture of lignans, and the ethylacetate-insoluble residue gave the characteristic leucoanthocyanidin reactions. The method of Pigman and co-workers (146) was found to be useful but the product (0.4%) was red-brown in color and appeared to be partially polymerized and oxidized. The absolute methanol-ether method described by Kranen, Finlayson and Hayward (147) proved to be the most useful for the isolation of undegraded leucocyanidin material, which was obtained as a cream-colored powder (0.4% yield).

On the basis of these preliminary experiments the fractionation procedure outlined in Figure 24 was devised for the isolation of both lignan and leucoanthocyanidin compounds.

II. Hemlock Leucoanthocyanidin

Fractions F and G obtained as shown in Figure 24 differed in methoxyl content (14.30% and 5.48% respectively) and the more pronounced color from fraction G obtained on acid treatment. These results indicated that fraction G was probably more closely related to the known leucoanthocyanidins and therefore this fraction was used in subsequent investigations. This material gave on paperchromatograms an elongated spot extending from R_f 0.00 to about 0.50 in solvent systems S-2, S-3, and S-4 using spray reagents R-1, R-2, R-4 and R-7*. The weak red color (strongest at R_f 0.0) obtained with vanillin-hydrochloric acid (R-4) indicated that some of the material present possessed a phloroglucinol nucleus with a vacant postion para to a free hydroxyl group (90). The slight pink color obtained with p-toluenesulfonic acid (R-1) and glycerol-hydrochloric acid (R-7) indicated a compound having the flavandiol structure (91).

The structure of the isolated leucoanthocyanidin was further investigated in two principally different ways: (i) by conversion into the corresponding anthocyanidin and (ii) by direct alkaline degradation.

When the leucoanthocyanidin was treated with isopropyl alcohol 0.03 normal in hydrochloric acid according to the procedure of Roux (36), two anthocyanidins with similar pink colors but with different rates of migration on paperchromatograms were obtained. The substance with lower R_f value corresponded to cyanidin (XXII) and the other was suspected to be the 3-isopropyl ether of cyanidin. To confirm this supposition the acid treatment of the

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*For convenience the numerous solvent systems (S), spray reagents (R), and absorbents and supporting media (M) used in chromatography are described in detail in Tables X, XI, and XII and are referred to elsewhere in this thesis by letter-number symbols.

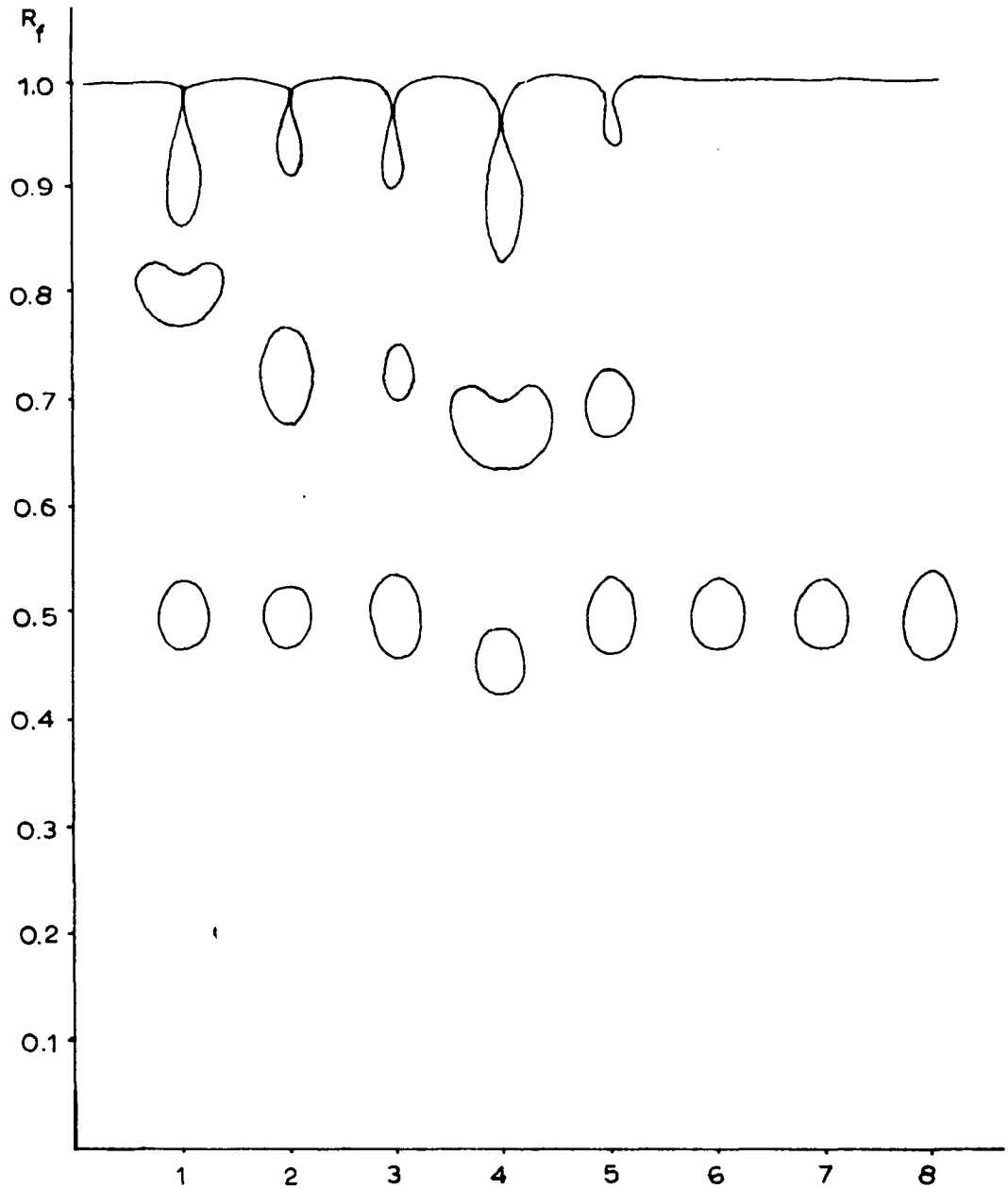


FIGURE 4. R_f Values of Cyanidin and Its Derivatives Obtained by Acid Treatment of the Leucoanthocyanidin of Hemlock Wood in Various Solvents.
The paperchromatogram was run in S-1. See Table IV for sample identification.

leucoanthocyanidin was carried out in isopropyl alcohol, methyl alcohol (both dry, and in the presence of water), acetone, dioxane, ethylacetate, and acetic acid. The results of the chromatographic examination of the products are shown in Table IV and Figure 4. When the reaction was repeated with authentic cyanidin only the spot with R_f 0.50 showed up on the paper-charomatogram. The "tailed" spots which appeared on the solvent front (Figure 4, position 1 to 5) were characteristic of the polymers formed from leucoanthocyanidins by condensation (23,24).

Table IV

R_f Values of Anthocyanidins from Hemlock Wood Prepared in Various Solvents

Sample No.	Solvents (0.03 N in HCl)	R_f Values[a] Lower Spot	Higher Spot
1	Isopropyl alcohol	0.50, 0.49	0.81
2	Dry Methanol	0.50, 0.49	0.72
3	Aqueous Methanol	0.50, 0.49	0.72
4	Acetone	0.46	0.67
5	Ethylacetate	0.50	0.70
6	Dioxane	0.50[b]	-
7	Acetic Acid	0.50[b]	-
8	Cyanidin	0.50	-

a Developing Solvent S-1.

b These spots were invisible in daylight after the paperchromatogram was air-dried, but they appeared in dark color under the UV lamp. After long standing they became visible in daylight with a greenish-grey color.

These results agreed with the theory (36) that the initial step in the conversion of flavan-3,4-diols into anthocyanidins (XXXIV) induced by hydrochloric acid was a dehydration of the 3,4-diol group in the heterocyclic ring, that is, a loss of the hydroxyl group at carbon 4 and a hydrogen atom at carbon 3. Ether formation, probably at the 3 position of the flavan-3,4-diol (36), occurred prior to the conversion into anthocyanidin and was

promoted by anhydrous conditions, since in aqueous methanol ether formation was decreased.

Absorption spectra were recorded according to the procedure of Harborne (92) for two reference samples of cyanidin, anthocyanidin from the wood, and the methyl - and isopropyl ethers of the wood anthocyanidin after chromatographic separation. In Table V are listed the wavelengths of maximum absorption of the anthocyanidins and the shifted maximum absorptions of their aluminium complexes. The spectra obtained are reproduced in Figures 5, 6 and 7.

Table V

Absorption Maxima of Anthocyanidins.

Compounds	R_f Values in S-1	λ_{max} (mμ) [a]	$\lambda_{max}(AlCl_3)$ (mμ) [b]	$\Delta\lambda_{max}$ (mμ)
Dahlia cyanidin	0.50	550.0	580.0	30
Synthetic cyanidin	0.50	550.0	580.0	30
Wood anthocyanidin	0.50	550.0	(562.4	12.5
			(580.4	30.4
Wood anthocyanidin methyl ether	0.72	542.0	(568.8	26.8
			(c	c
Wood anthocyanidin isopropyl ether	0.81	543.0	(567.2	24.2
			(593	50

a Concentration was 2.46×10^{-4} M in 95% ethanol containing 0.01 ml of concentrated hydrochloric acid per 100 ml.

b After addition of 0.1 ml of 5% aluminium chloride in ethanol per cell.

c A shoulder occurred at about 600 mμ but was not clearly defined.

It was observed that on addition of aluminium chloride to the ethanol-hydrochloric acid solution of the wood anthocyanidin a violet-blue color was obtained instead of the bright blue which was characteristic of pure

cyanidin. The absorption spectrum of the wood anthocyanidin showed a strong band with λ_{max} at 550.0 mμ (band W, Figure 6) while the corresponding aluminium complex gave a broader band with λ_{max} at 562.5 mμ and a shoulder at 580.4 mμ (band C, Figure 6). A similar result was obtained with the isopropyl ether of the wood anthocyanidin (Figure 7). The uncomplexed ether had λ_{max} at 543.0 mμ (band PW) and addition of aluminium chloride caused this to shift to 567.2 mμ while a shoulder appeared at about 593 mμ (band CPW). In the case of the methyl ether of the wood anthocyanidin, the addition of aluminium chloride caused a shift of λ_{max} from 542.0 mμ to 568.8 mμ, and the appearance of a shoulder at about 600 mμ; in this case, however, the shoulder was not clearly defined.

A reasonable interpretation of these results was provided if one assumed that the wood anthocyanidin was a mixture of cyanidin and another, unidentified, anthocyanidin which had an absorption maximum similar to that of cyanidin (550.0 mμ) and the same R_f values in various solvents, but lacked an adjacent pair of phenolic hydroxyl groups. In this basis the absorption spectrum of the wood anthocyanidin-aluminium complex (band C, Figure 6) resulted from superposition of two band indicated approximately by A and B in Figure 6. Band B represented the aluminium complex of cyanidin and band A represented the unknown and uncomplexed anthocyanidin. Similarly the isopropyl and methyl ethers of the unknown anthocyanidin and those of cyanidin gave combination absorption bands (e.g. E and F, Figure 7) on treatment with aluminium chloride due to the fact that only the cyanidin ethers formed aluminium complexes. A quantitative "difference spectrum" was therefore calculated by subtraction of the optical density of the pure cyanidin aluminium complex (Figure 5) from the optical density of the wood anthocyanidin aluminium complex (Figure 6) and the result (curve C, Figure 8) indicated clearly the presence of a second anthocyanidin compound (probably uncomplexed)

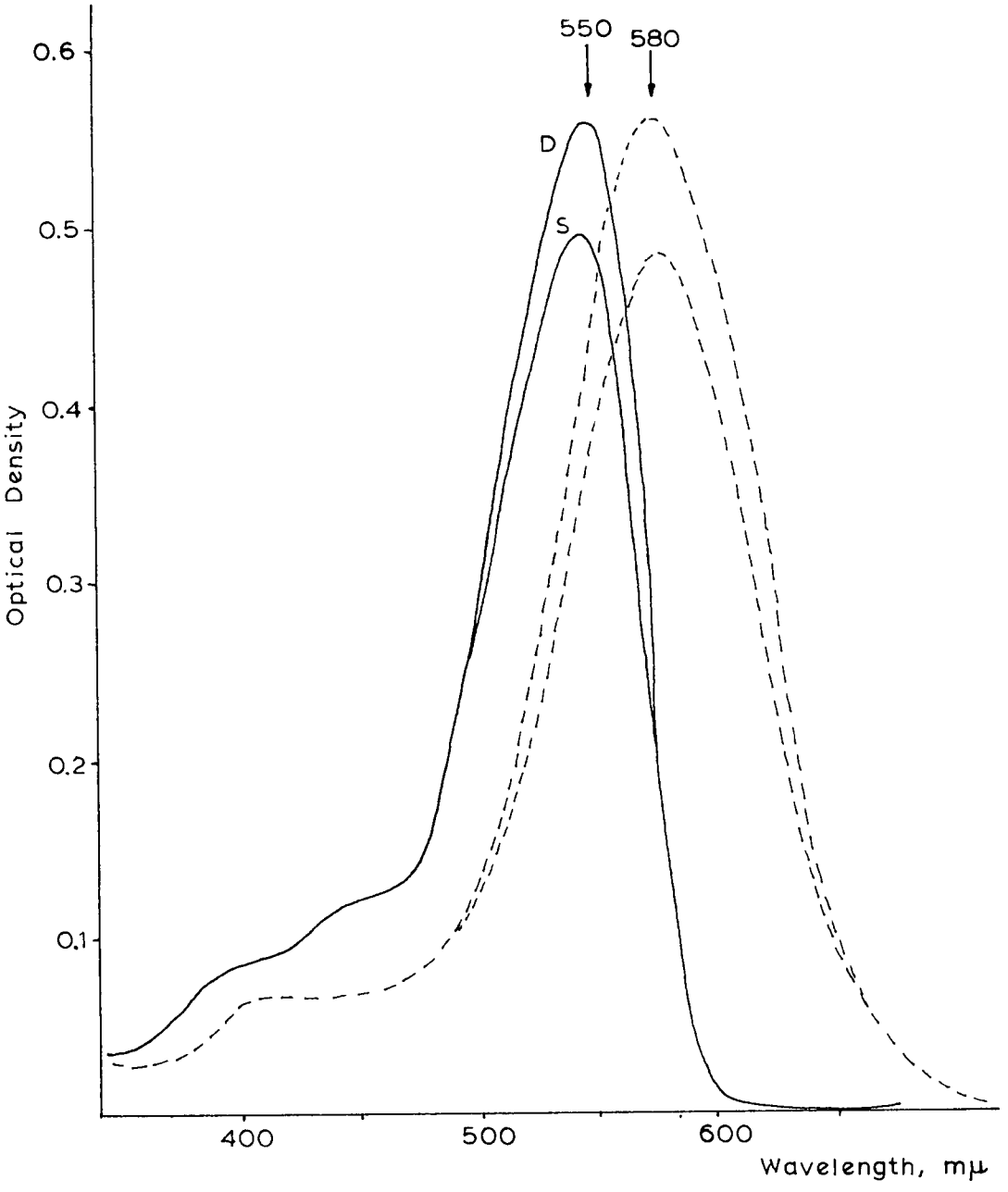


FIGURE 5 Absorption Spectra of Dahlia (D) and Synthetic (S) Cyanidin and Their Aluminium Complexes.

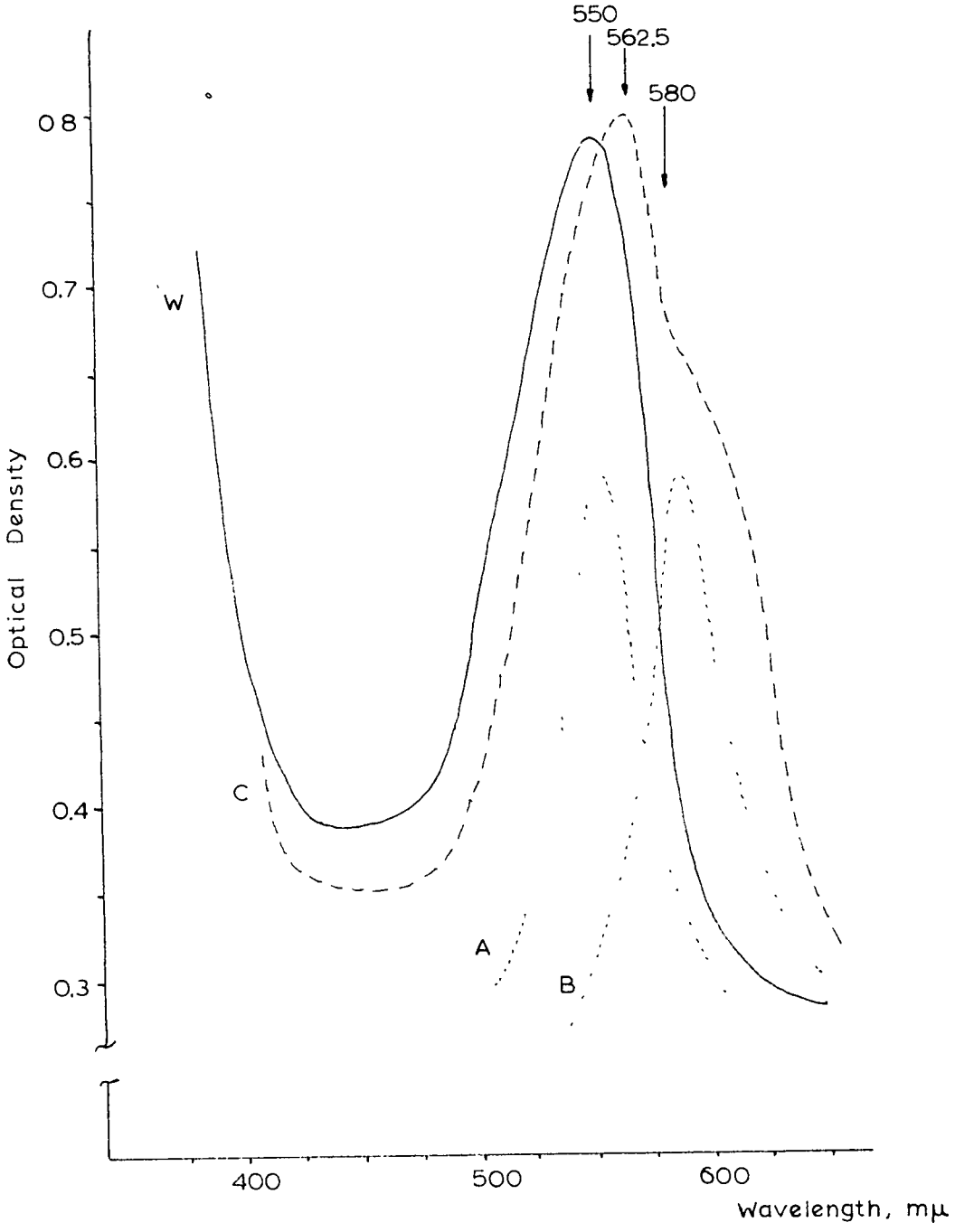


FIGURE 6. Absorption Spectra of Wood Anthocyanidin (W) and Aluminium Complexed Wood Anthocyanidin (C). A and B are assumed.

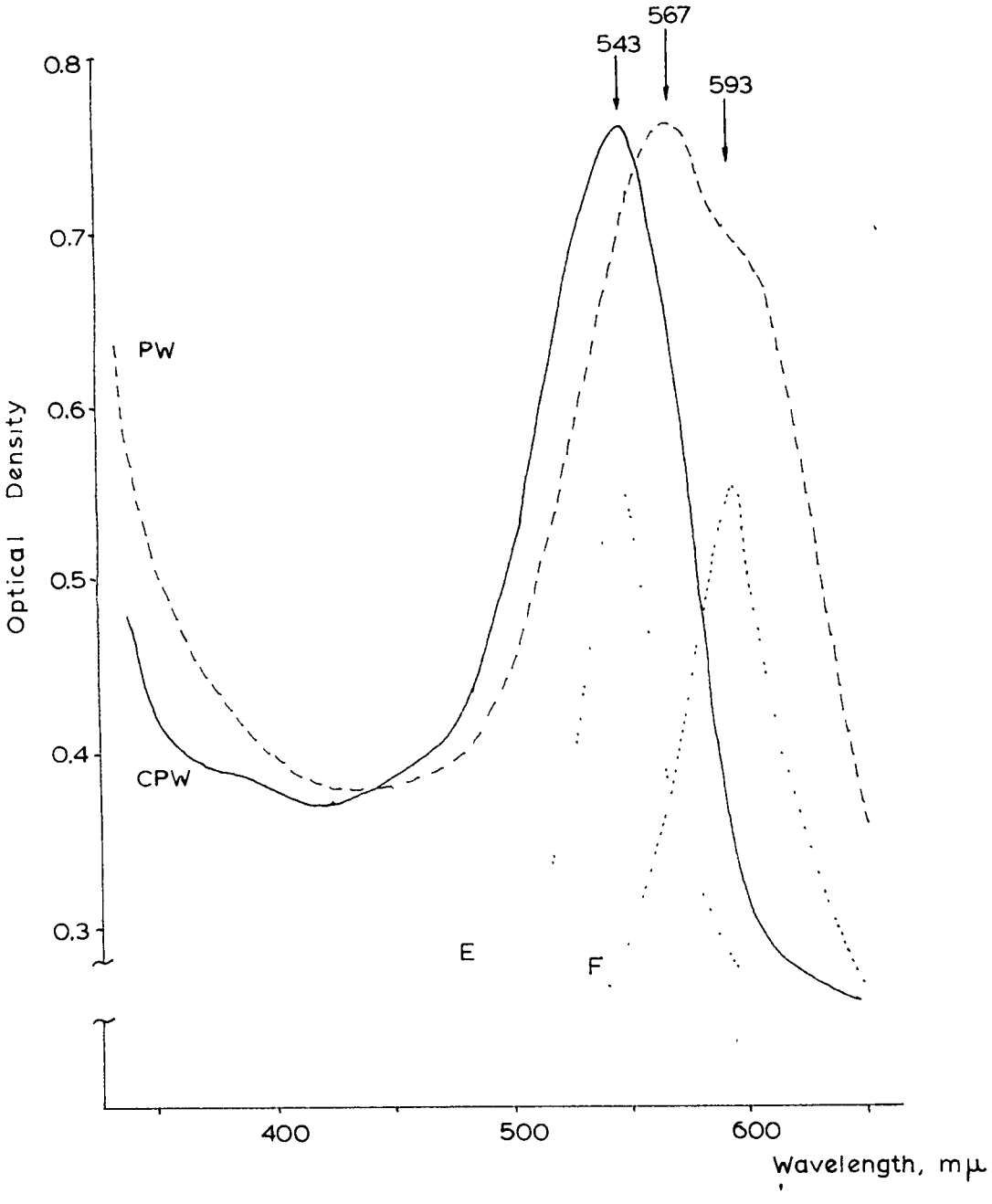


FIGURE 7. Absorption Spectra of the Isopropyl Ether of the Wood Anthocyanidin (PW) and the Corresponding Aluminium Complex (CPW). E and F are assumed.

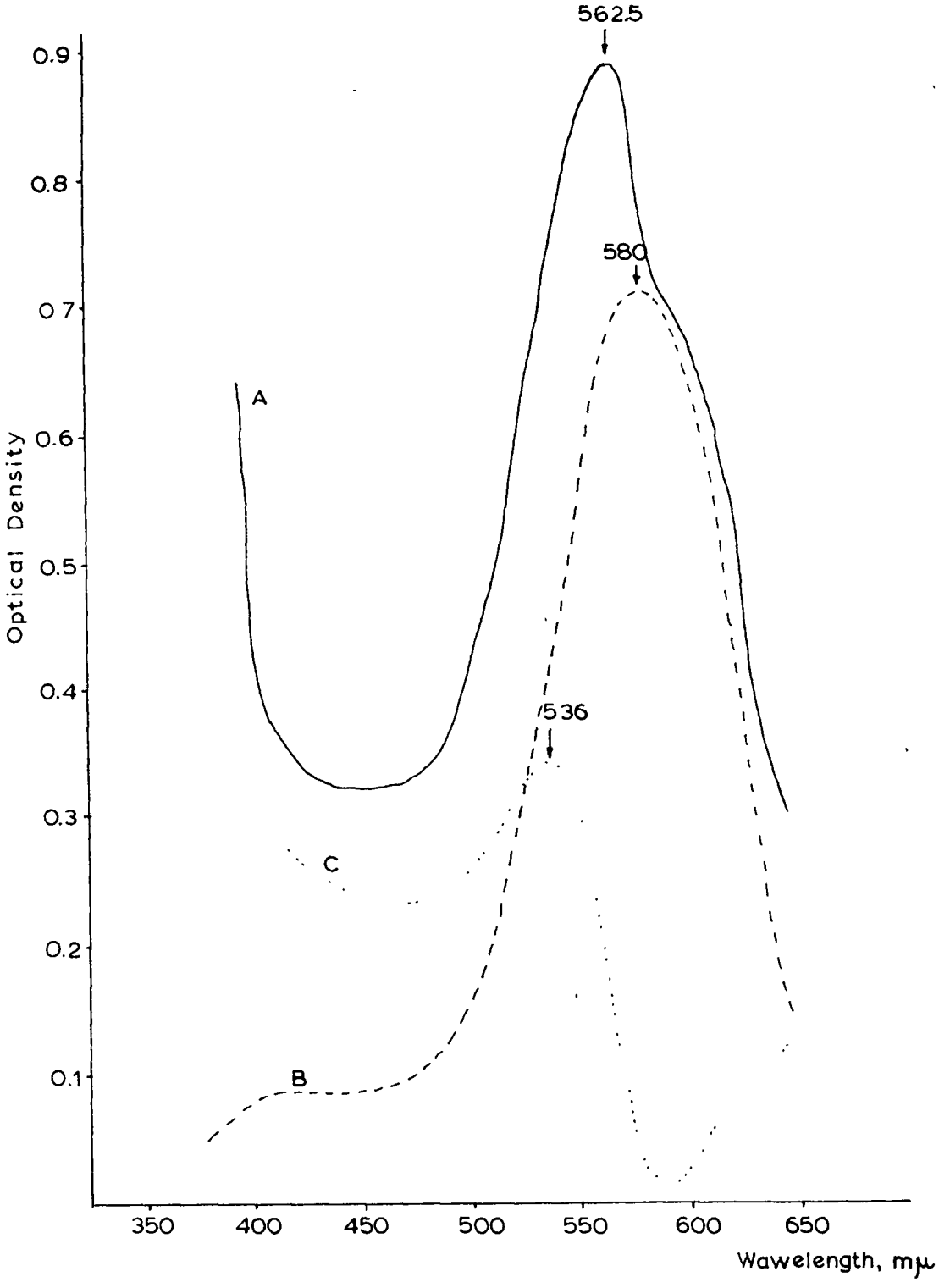


FIGURE 8 Difference Spectrum of Hemlock Anthocyanidin. (A) Aluminium chloride complexed wood anthocyanidin. (B) Aluminium chloride complexed dahlia cyanidin. (C) From curves A and B by subtraction.

with an absorption maximum at 536 mμ.

Further evidence concerning the structure of the hemlock leucoanthocyanidin was provided by alkaline degradation (93,94,95). The degradation products and reference compounds were chromatographed on paper in four different solvent systems (S-3, S-6, S-8, S-9), and R-3 was used as spray reagent. The degradation products from cyanidin and the leucoanthocyanidin contained protocatechuic acid but phloroglucinol and resorcinol were detected only in the case of cyanidin. The paperchromatogram is shown in Figure 9.

A control alkaline degradation of phloroglucinol indicated that the two pink spots with R_f values of 0.09 and 0.17 and the yellow spot with R_f 0.90 (identified as resorcinol) were degradation products of phloroglucinol itself (Table VI).

From these results it was concluded that the phloroglucinol nucleus must be present in the hemlock leucoanthocyanidin in a form different from that in which it occurs in leucocyanidins from other sources.

This could be explained if one assumed that the hemlock leucoanthocyanidin existed in a dimeric form similar to XXXVIII as was suggested recently by Freudenberg (41). The alkaline degradation of such a structure, e.g. XCII, would theoretically produce a symmetrical hexahydroxybenzophenone (XCIII) which would be a very labile substance (it has not been described in the literature). The ring system of this hypothetical intermediate would split up directly to fragments similar to those obtained from phloroglucinol under the same conditions and not via the liberation of free phloroglucinol (Figure 10). This would also exaplain the absence of resorcinol in the reaction mixture. The pink-orange spot detected at R_f = 0.92 in the alkaline degradation product of the leucoanthocyandin (Table VI) was clearly not due to the presence of resorcinol and will be discussed later.

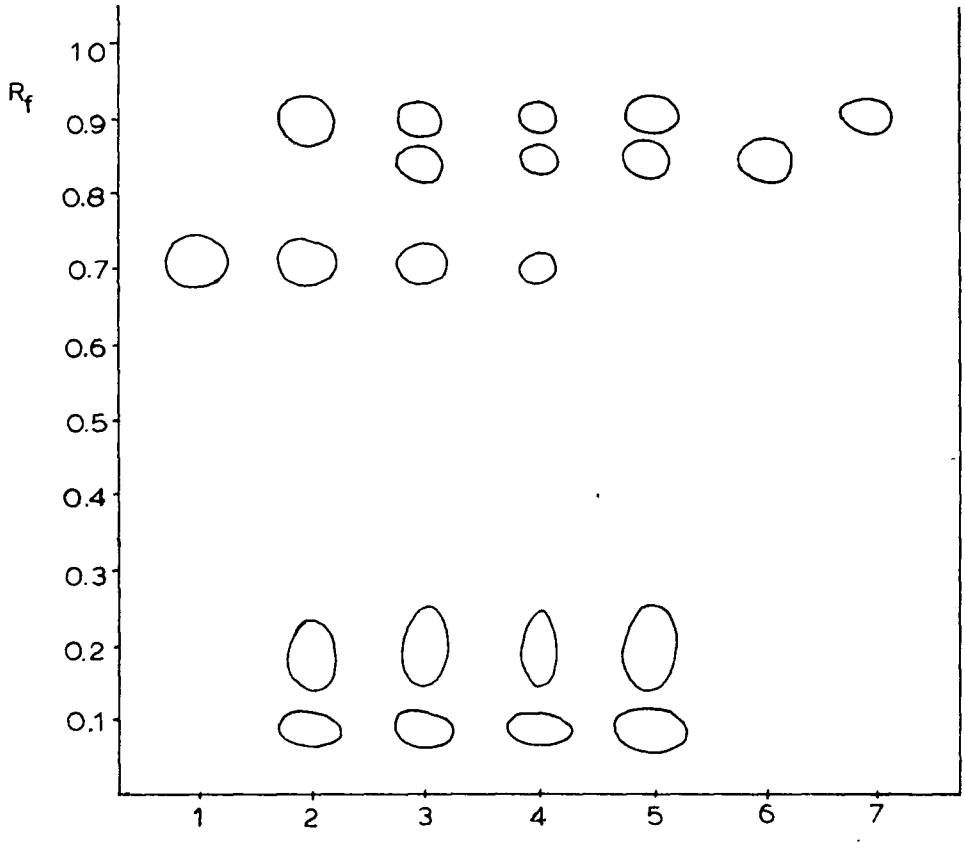
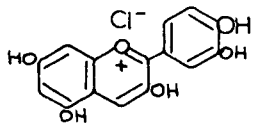
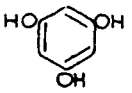
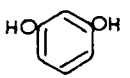
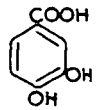
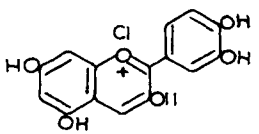
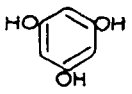
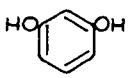
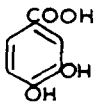
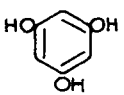
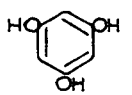
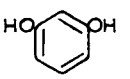
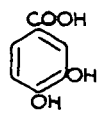


FIGURE 9 Paperchromatography of the Alkaline Degradation Products.

1 Phloroglucinol.
2 Alkali degraded phloroglucinol.
3 Degradation products of dahlia cyanidin.
4 Degradation products of synthetic cyanidin.
5. Degradation products of wood leucoanthocyanidin
6. Protocatechuic acid
7. Resorcinol

The chromatogram was run in S-3 and sprayed with R-3

TABLE VI. ALKALINE DEGRADATION PRODUCTS.

	NAME of Original Compounds	FORMULA	PRODUCTS OBTAINED ON ALKALINE DEGRADATION from RING A					from RING B	
1	DAHLIA CYANIDIN		 R_f = 0.72	 R_f = 0.90	Unknown "a" R_f = 0.17	Unknown "b" R_f = 0.09	—	 R_f = 0.84	—
2	SYNTHETIC CYANIDIN		 R_f = 0.72	 R_f = 0.90	Unknown "a" R_f = 0.17	Unknown "b" R_f = 0.09	—	 R_f = 0.84	—
3	PHLORO-GLUCINOL		 R_f = 0.72	 R_f = 0.90	Unknown "a" R_f = 0.17	Unknown "b" R_f = 0.09	—	—	—
4	WOOD LEUCOANTHO-CYANIDIN	"LAC"	—	—	Unknown "a" R_f = 0.17	Unknown "b" R_f = 0.09	Unknown "c" ? R_f = 0.92	 R_f = 0.84	Unknown "c" R_f = 0.92
Color of Spots with Diazonium Salt (R-3)			yellow	yellow	pink	pink	pink-orange	white	pink-orange

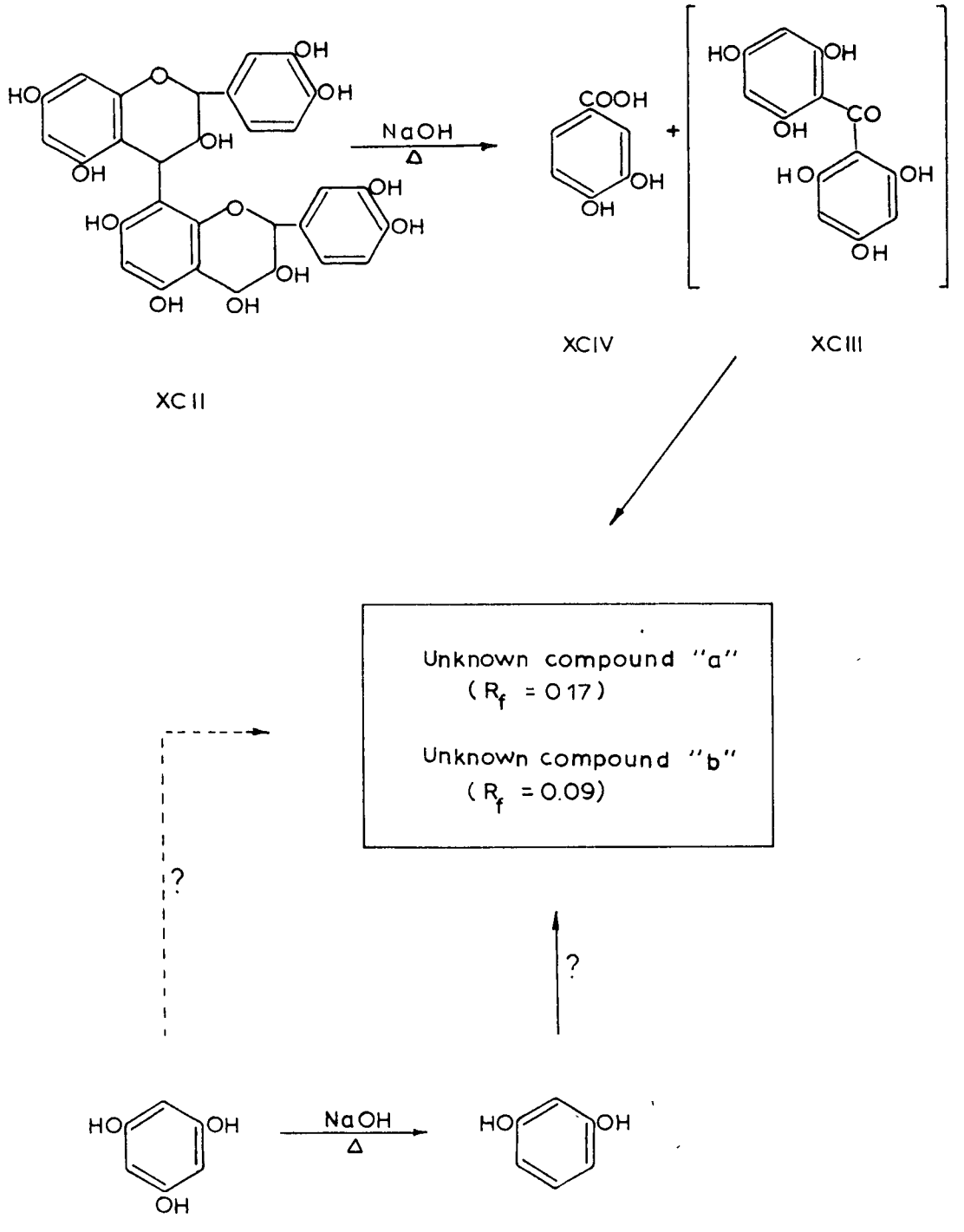


Figure 10 Proposed Alkaline Degradation of a Dimeric Leucoanthocyan(id)in.

The evidence obtained here concerning the molecular structure of the western hemlock leucoanthocyanidin may be summarized in the following points:

(1) From the study of the ultraviolet spectra, it was clear, that the leucoanthocyanidin on acid treatment produced cyanidin and a second pigment with the same R_f value but with a different absorption maximum when complexed with aluminium. One might have assumed that the second pigment was not an anthocyanidin but a humic acid similar to that reported by Raudnitz (96). However, this did not appear to be the case since the visible spectrum of Raudnitz's humic acid showed two characteristic maxima at 548 mμ and 459 mμ, while both cyanidin and the wood anthocyanidin had minimum at 459 mμ.

The second pigment might also have been a decomposition product of cyanidin itself which still retained the red color but did not form an aluminium complex. In this connection it was observed that the cyanidin obtained from the wood decomposed much more readily in handling than did cyanidin isolated from Dahlia. No explantion of this phenomenon was found.

(2) It was established by Roux that introduction of increasing numbers of hydroxyl groups into the flavan structure decreased the R_f values, and the stepwise masking of the hydroxyl groups in anthcoyanidins by methylation correspondingly increased the R_f values in aqueous solvents (97). The hypsochromic shift caused by introducing O-methyl groups was reported to be between 8-9 mμ (98). This agreed with the results reported here (Table V). The shift was 8 mμ on introduction of an O-methyl group in the C_3 position of cyanidin, that is, the absorption maximum of cyanidin was 550 mμ while that of cyanidin-3-methyl ether was 542 mμ.

The unknown anthocyanidin pigment, therefore, probably had five hydroxyl groups as does cyanidin (same R_f values). The positions of the two hydroxyl groups in ring B, however, were different from those in

cyanidin. Either they were disposed in meta positions as, for example, in morinidin (XVIII, Table II), or, more likely, the 3-hydroxyl group of the normally occurring 3′, 4′-vicinal pair was methylated and the extra hydroxyl groups was present at the 2′-position.

(3) The presence of two different anthocyanidins in the mixture from the acid-treated leucoanthocyanidin suggested that the dimeric leucoanthocyanidin was constructed from two different flavan monomers. If the two B rings in the dimer differed in substitution upon sodium hydroxide fusion two different carboxylic acids would show up in the degradation mixture. As shown in Table VI and Figure 9, in addition to protocatechuic acid the presence of another compound (orange-pink spot, R_f = 0.92) was detected by paper chromatography. This unidentified compound probably represented the second carboxylic acid.

The suggested structure of the dimeric hemlock wood leucoanthocyanidin and the postulated degradation pathway are summarized in Figure 11.

Although there was no conclusive evidence for the nature and location of the R group in the second carboxylic acid, the methoxyl content (5.48%) of the leucoanthocyanidin indicated that R probably was a methoxyl group. The calculated methoxyl content of a monomethyl dimer was 4.97%.

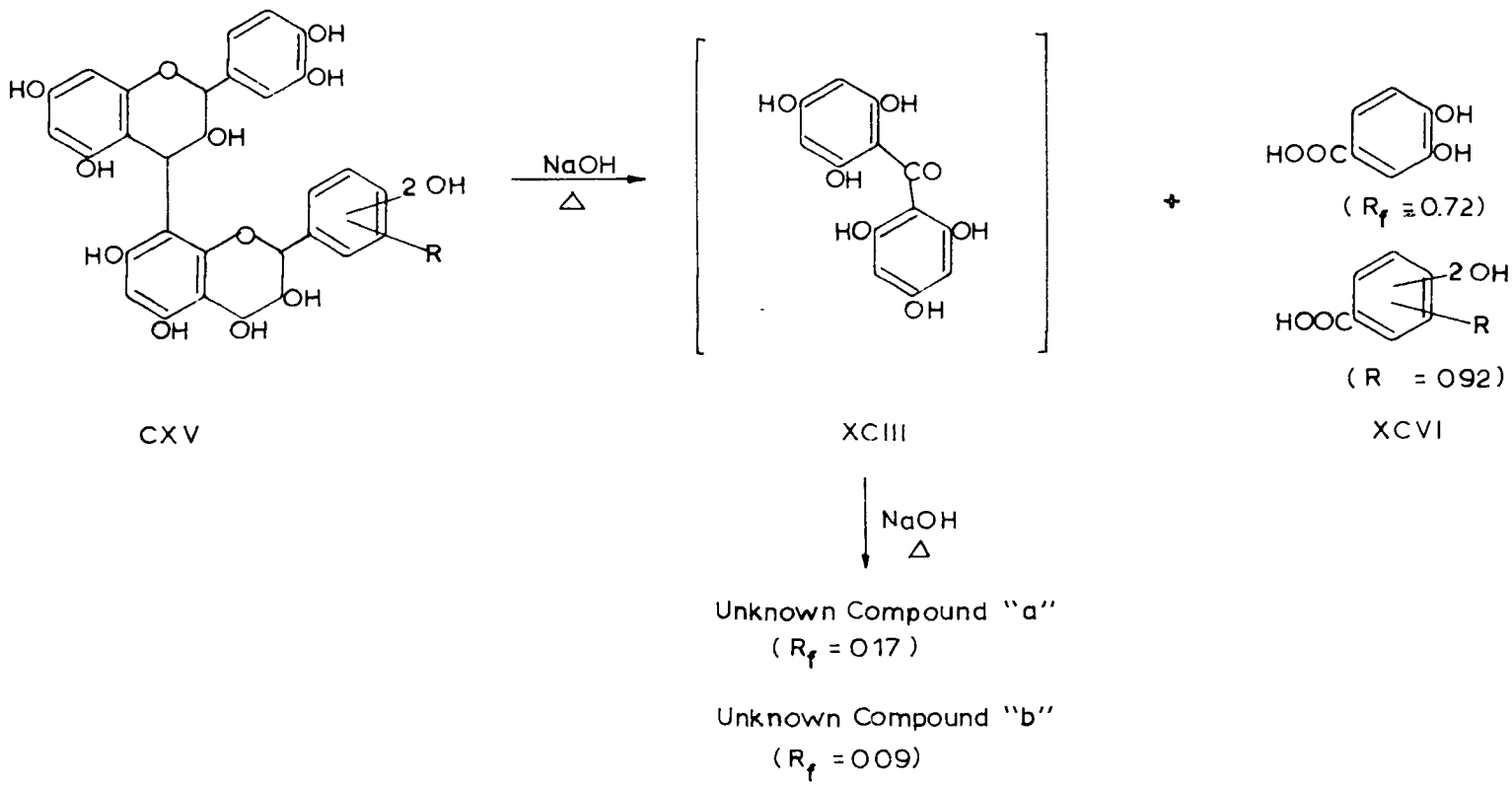


FIGURE 11. Suggested Structure and Degradation Products of Hemlock Leucoanthocyanidin.

III. Hemlock Lignans

A. Separation of Lignans

Since fractions B, C, D and E (Figure 24) appeared to be similar on chromatographic examination they were combined and evaporated to a solid cream-colored foam. It proved to be extremely difficult to separate the lignans due to the complexity of the mixture of compounds present. This was illustrated by the fact that the lignan mixture was examined by paper-chromatography in eighteen different solvent systems (S-2 to S-19) but only benzene-acetic acid-water (S-15) was found to give suitable separation of lignans detected by diazotized sulfanilic acid (R-3) as spray reagent. The R_f values obtained are listed in Table VII.

Table VII

R_f Values of Lignans in the Methanol Extract of Hemlock Wood

Spot	R_f values[a]	Color with R-3
a	0.660	brownish-pink
b	0.399	brownish-pink
c	0.271	pink
d	0.169	orange
e	0.116	orange
f	0.064	orange
g	0.030	orange-red
(h,i,j,k)[b]	0.000	yellow

[a]Irrigation time 7 hrs., solvent S-15

[b]These spots appeared after 13 hrs. irrigation time.

When the chromatograms were developed for 12 and 18 hours (the solvent was allowed to drain off the paper) three more yellow spots appeared in the lower R_f region (h,i, and j). The travelled distances of spots on chromatograms developed for 7, 13 and 18 hours are plotted in Figure 12 for comparison.

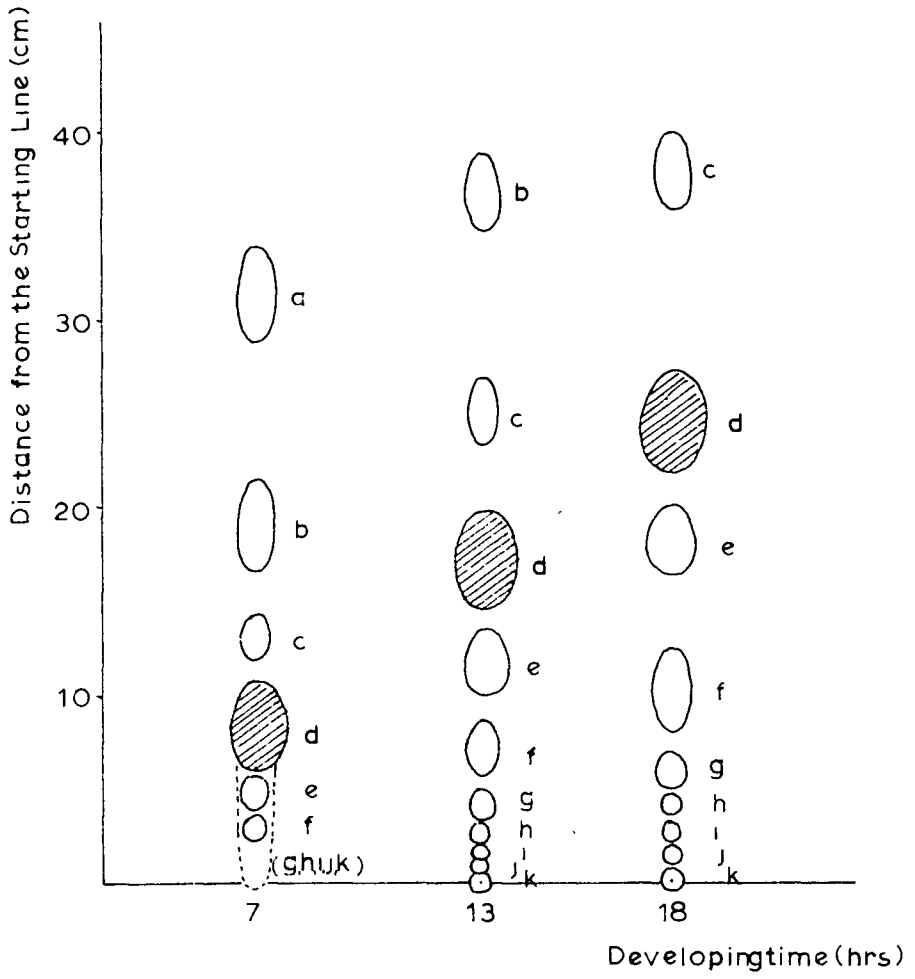


FIGURE 12. Separation of Lignans with Time of Development.

The chromatograms were run in S-15 and sprayed with R-3

d = Hydroxymataıresinol

A two-dimensional chromatogram was run in solvent S-15 for 24 and 38 hours and spots a, b and c (Table VII) were allowed to drain off the paper (Figure 13).

The colors produced on spraying with diazotized sulfanilic acid (R-3) were characteristic of the phenols present (Table VII) (56,77). A lemon yellow color indicated p-hydroxyphenyl groups in compounds giving spots with very low R_f values (h, i, j, and k). The orange color indicated that compounds d, e, f with R_f values 0.169, 0.116, and 0.064 respectively possessed an α-hydroxyguaiacyl nucleus, and the pink color obtained with compounds a, b, and c with R_f values 0.660, 0.399, and 0.271 showed them to be guaiacyl derivatives with a methylene or methine group in the alpha position. The Gierer test (99) for the α-hydroxyguaiacyl nucleus also was positive.

Several attempts were made to separate the lignans on a preparative scale. Cellulose columns (M-5) irrigated with S-15 gave some separation but required several days for development and the evaporation of the acetic acid from hundreds of fractions at low temperature proved to be extremely tedious, moreover, the acidic developing solvent system was undesirable because of the easy conversion of hydroxymatairesinol into conidendrin in the presence of acid. The silicic acid-plaster-of-Paris-nylon powder column (M-9) used with solvent system S-22 gave a very good separation of conidendrin from the lignan mixture but here also an acidic solvent was required and the fractions from the column contained some foreign material, possible dissolved from the nylon powder, and therefore this separation method was not further used. No separation occurred on Celite, alumina, or silica gel columns.

Counter-current distribution (12 units) using the organic and aqueous layers of benzene-acetic acid-water solvent mixture (S-15) as distribution system was also applied. The organic phase contained only

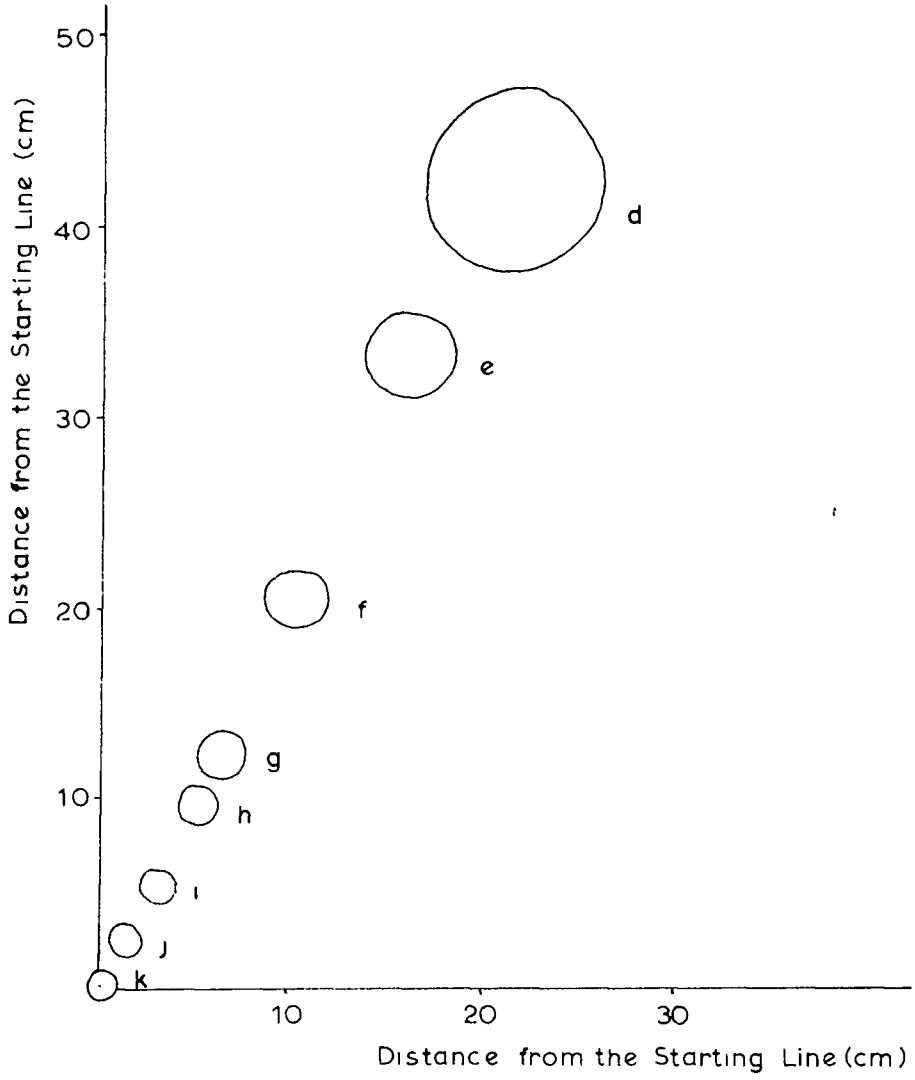


FIGURE 13 Separation of Lignans by Two-dimensional Paper Chromatography.
S-15 was used as irrigating solvent in both directions and R-3 as spray reagent.

non-phenolic material, the aqueous fraction I contained phenolic materials, which gave yellow and orange colors with diazonium salts, and fractions VII to XII contained pure conidendrin. The chromatographic analysis is illustrated in Figure 14.

A similar result was obtained from counter-current distribution between ether and an aqueous solution of sodium carbonate.

Since only partial separation of the lignans was obtained with the conventional methods a new technique was devised, namely the use of thin-layer chromatography (TLC) and chromatobars for separation and identification of the lignans. The advantages of the thin layer chromatoplate (using S-24 and R-9) over a paperchromatogram were that the developing time was decreased from 18 hours to 3 hours, good separation of the lignan mixture was obtained in neutral solvent systems, and the spots could be made visible in characteristic colors (with R-9) as indicated in Figure 15 and Table VIII.

By means of the chromatobar technique followed by repeated purification on thick layer chromatoplates using solvent system S-24 a second lignan was isolated in addition to conidendrin. This was shown to be hydroxymatairesinol by comparison with an authentic sample kindly supplied by Dr. H.L. Hergert and Mr. G.M. Barton. The separation of conidendrin and hydroxymatairesinol on chromatobars is illustrated in Figure 16.

Several efforts were made to isolate other lignans but only partially separated fractions were obtained.

The lignan first isolated, conidendrin (LX) previously reported in western hemlock by Brauns (100), was obtained in 0.1% yield which is lower than that reported (0.15 - 0.2%) by Goldschmid and Hergert (77). The conidendrin was identified by its melting point, chromatograms, infrared spectrum and methoxyl content.

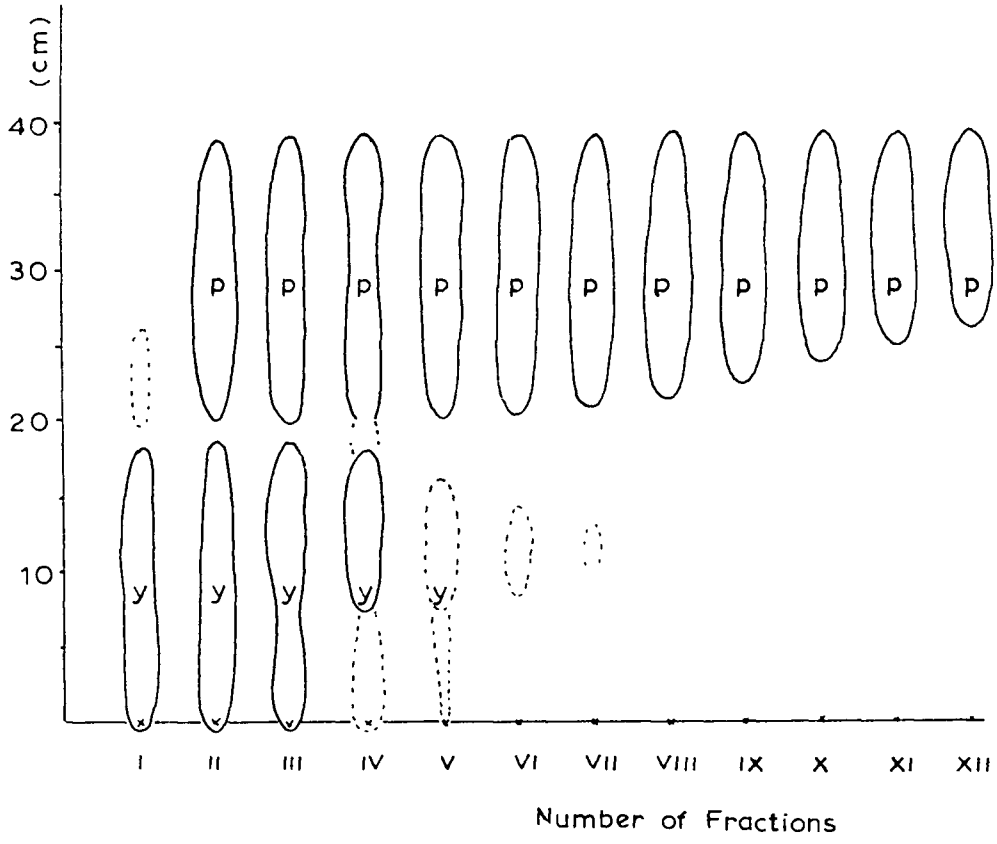


FIGURE 14. Separation of Lignans by Counter-current Distribution

Fraction I Hydroxymatairesinol and unidentified lignans

Fractions VII-XII Conidendrin

p = pink, y = yellow

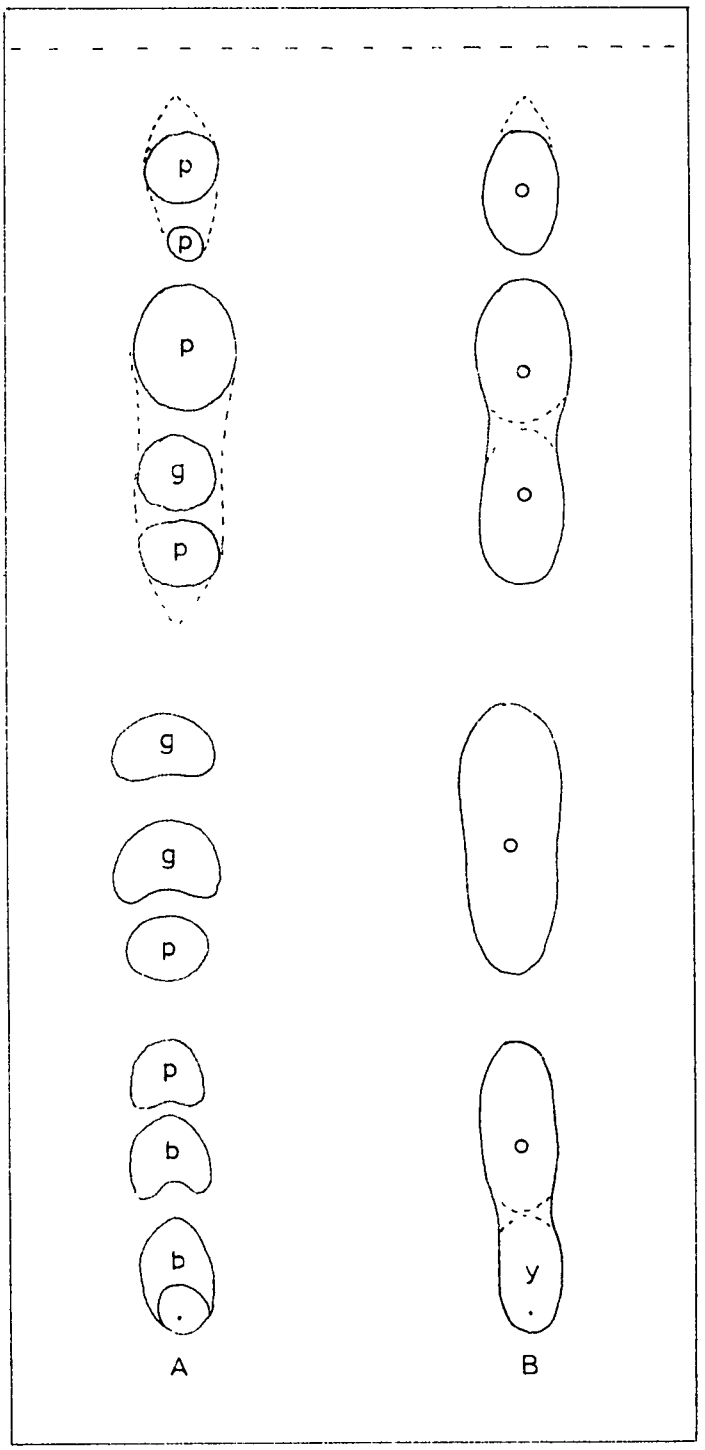


FIGURE 15. Thin Layer Chromatography of Lignans in Solvent S-24. (A) With spray reagent R-9. (B) With spray reagent R-3 or R-8. p = pink, g = green, o = orange, b = brown, y = yellow.

Table VIII

Colors and R_f Values of Lignans in TLC with Three Different Spray Reagents*

Spray reagent R - 9		Spray reagent R - 9		Spray reagent R - 3	
R_f	Color	R_f	Color	R_f	Color
0.928	pink	0.904	green-yellow	0.904	orange
0.866	pink				
0.715	pink	0.715		0.715	orange
0.679	green	0.649	green-yellow	0.649	orange
0.619	pink				
0.461	green				
0.379	green	0.388	green-yellow	0.388	orange
0.296	pink				
0.194	pink	0.139	green-yellow	0.139	orange
0.136	brownish				
0.042	brownish	0.033	green-yellow	0.033	yellow

* The TLC-plate was run in S-24

It was reported by Wise and Jahn (101) that conidendrin crystallized in two forms, one melting at 238°C and another (the stable form) melting at 255-256°C. The conidendrin isolated during this research was found to melt at 239-240°C after recrystallization from aqueous acetone.

The next lignan isolated was characterized as hydroxymatairesinol. This compound was first isolated by Freudenberg and Knopf (56) from spruce wood (Picea excelsa) and more recently Goldschmid and Hergert (77) reported the occurrence of this compound in western hemlock wood.

The hydroxymatairesinol (HMR) was obtained as a colorless amorphous powder from ethylacetate-petroleum ether; efforts to obtain it in crystalline form were unsuccessful. The product softened at 95°C but did not melt. It was levorotatry $[\alpha]_D^{25}$ - 11.5 ± 2° (c, 4.0 in tetrahydrofuran).

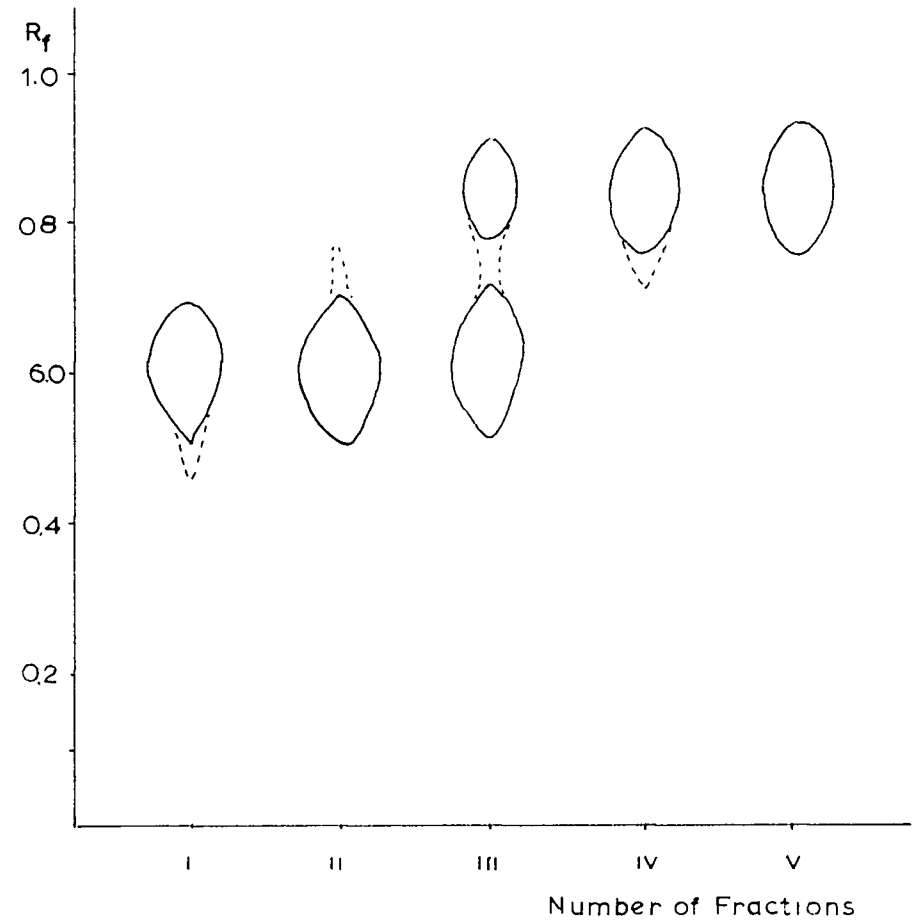


FIGURE 16. Separation of Conidendrin and Hydroxymatairesinol on Chromatobars.

Fractions I and II : Hydroxymatairesinol(HMR)
Fraction III Conidendrin and HMR
Fractions IV and V Conidendrin

The reported optical rotations were -10.4° and 11.0° (c, 4.0 in tetrahydrofuran) (56,77). Calcd. for $C_{20}H_{22}O_7$: OCH_3, 16.58%. Found OCH_3, 16.57%. The product was examined on paper- and thin layer chromatograms in various solvent systems and the results obtained are listed in Table IX.

Table IX

Chromatographic Tests of Hydroxymatairesinol (HMR)

	Solvents	Rf	Spray Reagent	Color
Paper-Chromatogram	S-3	0.90	R-3	Orange
	S-15	0.17		
	S-25	0.45		
	S-26	0.59		
Thin layer chromatogram	S-23	0.52 ± 0.02	R-9	Pink
	S-24	0.17 ± 0.02		

B. Structural Analysis of Hydroxymatairesinol (HMR)

Hydroxymatairesinol in the presence of acid was converted into conidendrin nearly quantitatively. This reaction indicated that the structure of these lignans were very closely related. The positive α-hydroxyl quaiacyl test indicated that HMR possessed an hydroxyl group alpha to a phenyl ring which was very likely the point of ring closure in the formation of conidendrin. (LX). The infrared spectrum showed the presence of a five-membered lactone ring in HMR similar to that in conidendrin (Figure 29).

These results agreed with those of Freudenberg and Knopf who proposed two possible structural formulae, (LXV and LXVI) for HMR (56). Goldschmid and Hergert (77) recently pointed out, that on the basis of the positive Gierer test structure LXV should be preferred.

Further information on the structure of HMR was obtained in three different ways:

(i) Comparison of the infrared spectra of the fully acetylated HMR with that of the reduced and acetylated HMR.

(ii) Nuclear magnetic resonance spectroscopic study of HMR and structurally related known compounds (conidendrin and vanillyl alcohol)

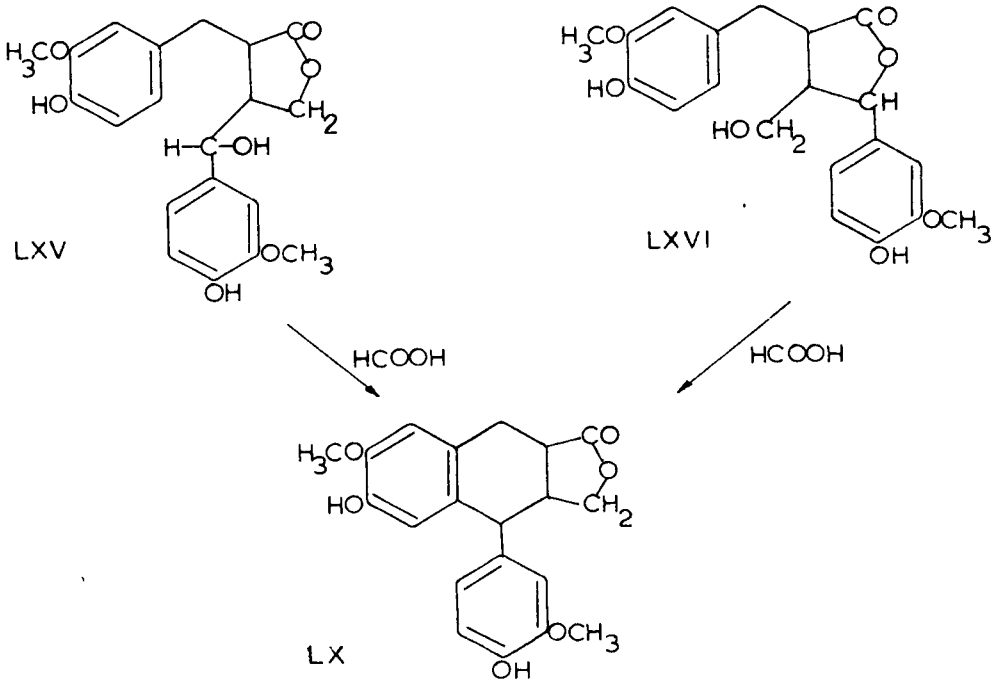
(iii) Neutral potassium permanganate oxidation of trimethyl HMR.

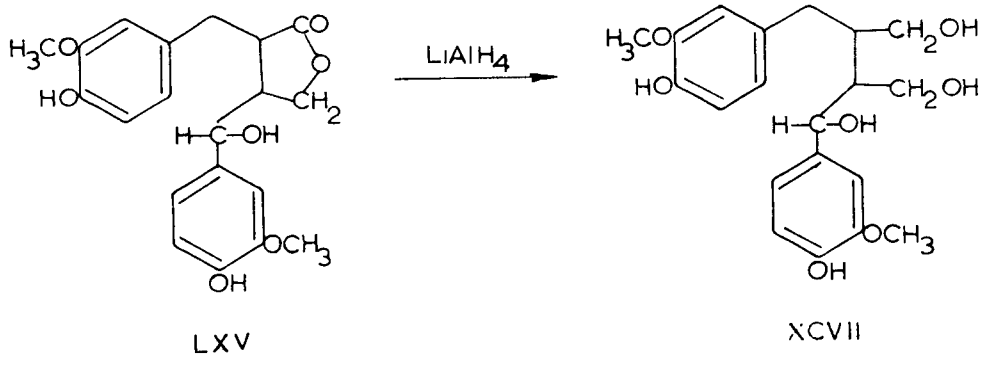
The five-membered lactone ring of HMR was reduced by lithium aluminium hydride to the corresponding diol as indicated by the complete disappearance of the lactone carbonyl stretching frequency at 1750 cm^{-1} in the infrared spectrum and the appearance of a broad peak in the 3100-3600 cm^{-1} region due to the strong hydrogen-bonding effect (Figure 17). This reduction product was therefore a triol (XCVII). The dimethyl ether of this triol (XCVIII) was previously obtained by Haworth and Woodcock from catalytic hydrogenation of olivil dimethyl ether (XCIX) (107) but the triol itself (XCVII) has not been previously reported.

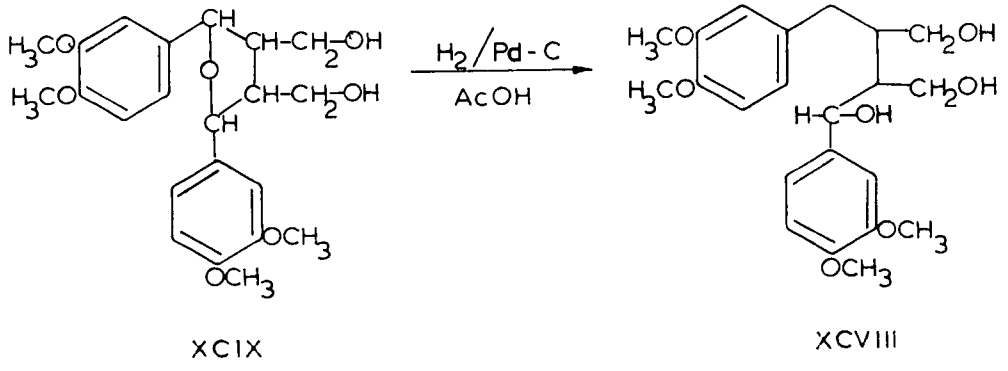
Both the original and reduced hydroxymatairesinol were acetylated and the infrared spectra of both acetate derivatives showed bands in the regions 1760-1770 cm^{-1} and 1730-1740 cm^{-1} indicating the presence of phenolic acetoxy carbonyl (vinyl ester type, C=C-O-C=O) and aliphatic acetoxy carbonyl groups respectively (Figure 17).

According to the proposed formulae LXV and XCVII aliphatic acetoxy and aromatic acetoxy groups would be present in the fully acetylated compounds in the ratios of 1:2 for HMR acetate and 3:2 for reduced HMR acetate. As expected the aliphatic carbonyl band of the HMR-acetate was the weaker (Figure 18, B) while in the reduced HMR-acetate the two bands had about equal intensity (D in Figure 18).

The situation was complicated however in the case of the HMR-acetate


LXV
LXVI
HCOOH
HCOOH
LX


LiAlH4
LXV
XCVII


H2/Pd-C
AcOH
XCIX
XCVIII

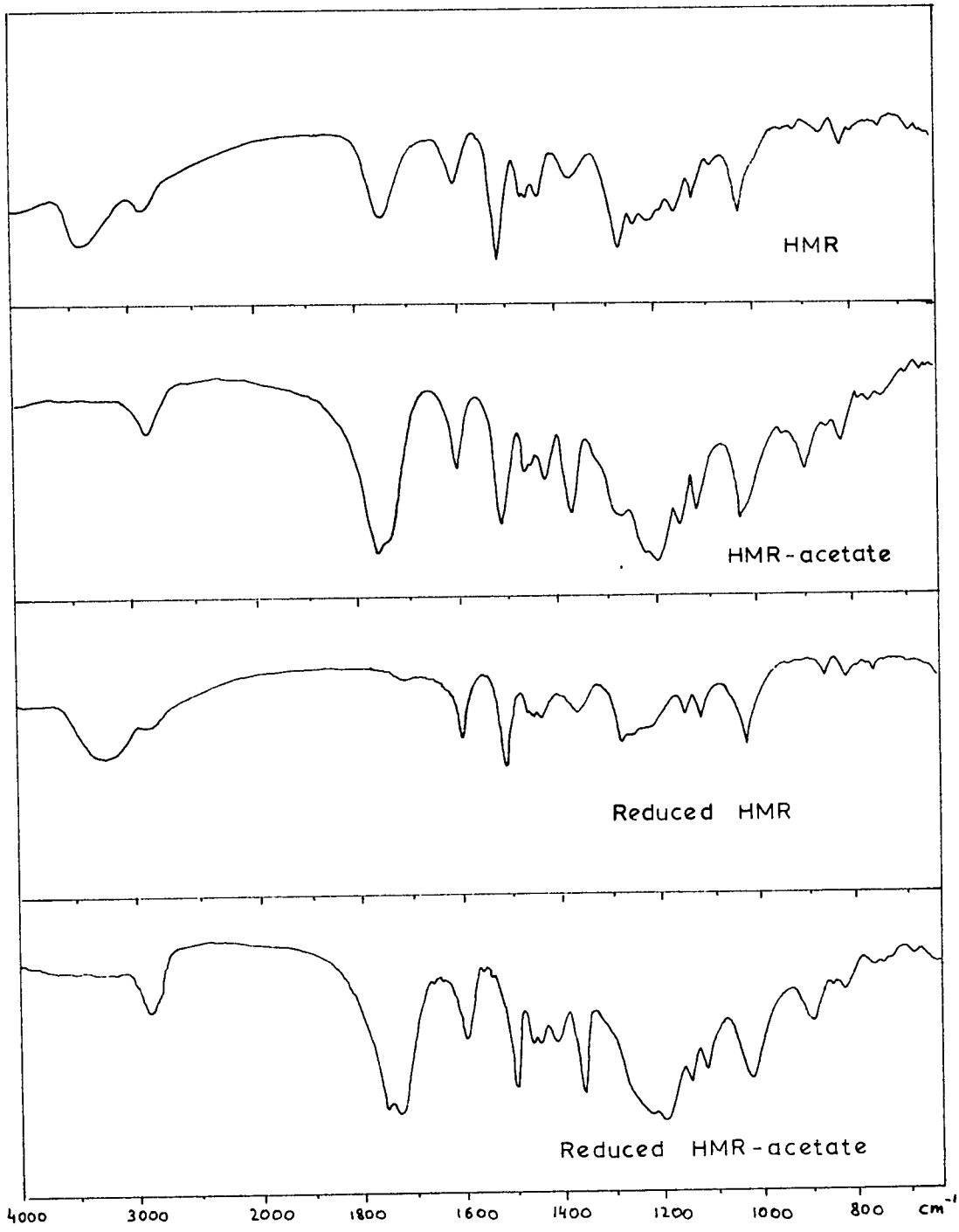


FIGURE 17. Infrared Spectra of Hydroxymatairesinol (HMR) and Derivatives.

because the carbonyl absorption of the five-membered lactone (1750 cm^{-1}) contributed also to the acetyl carbonyl band intensity (Figure 18, A and B). The "difference spectrum" (Figure 18, C) was obtained by subtraction of the lactone absorption (A) from the HMR-acetate curve (B). The different intensities of the two peaks (1737 and 1770 cm^{-1}) in the "difference spectrum" (C) was then in good agreement with the predicted 1:2 ratio proving the existence of only one aliphatic hydroxyl group in HMR.

The 60 Mc/sec. NMR spectra of vanillyl alcohol, conidendrin and hydroxymatairesinol are compared in Figure 19. The interpretation of the spectrum of HMR proved to be very difficult because broad, not fully resolved lines were obtained due to the complexity and assymetry of the HMR molecule. Only a few of the many lines could be identified. Aromatic ring protons appeared at τ = 3.22 and 3.32 as a doublet, and the sharp line at τ =6.23 represented the two methoxyl groups on the two benzene rings. (The very weak splitting was caused by coupling with one vicinal proton (103)).

One of the structures of HMR suggested by Freudenberg (LXV) was further confirmed by neutral potassium permanganate oxidation of the trimethyl HMR. If HMR had structure LXVI, the only product to be expected from oxidation of the trimethyl ether (CVI) would be veratric acid (CIV). If, however, HMR possessed structure LXV then the two products veratric acid and veratric acid methyl ester (CV) would be expected in the oxidation mixture from trimethyl HMR (CIII).

Since the oxidation required very careful control because of the easy hydrolysis of the expected methyl ester product neutral oxidation media (acetone plus magnesium sulphate or aqueous magnesium sulphate) were employed. Benzoin methyl ether (CVII) was used as a model compound in the study of the oxidation and since both benzoic acid (CVIII) and

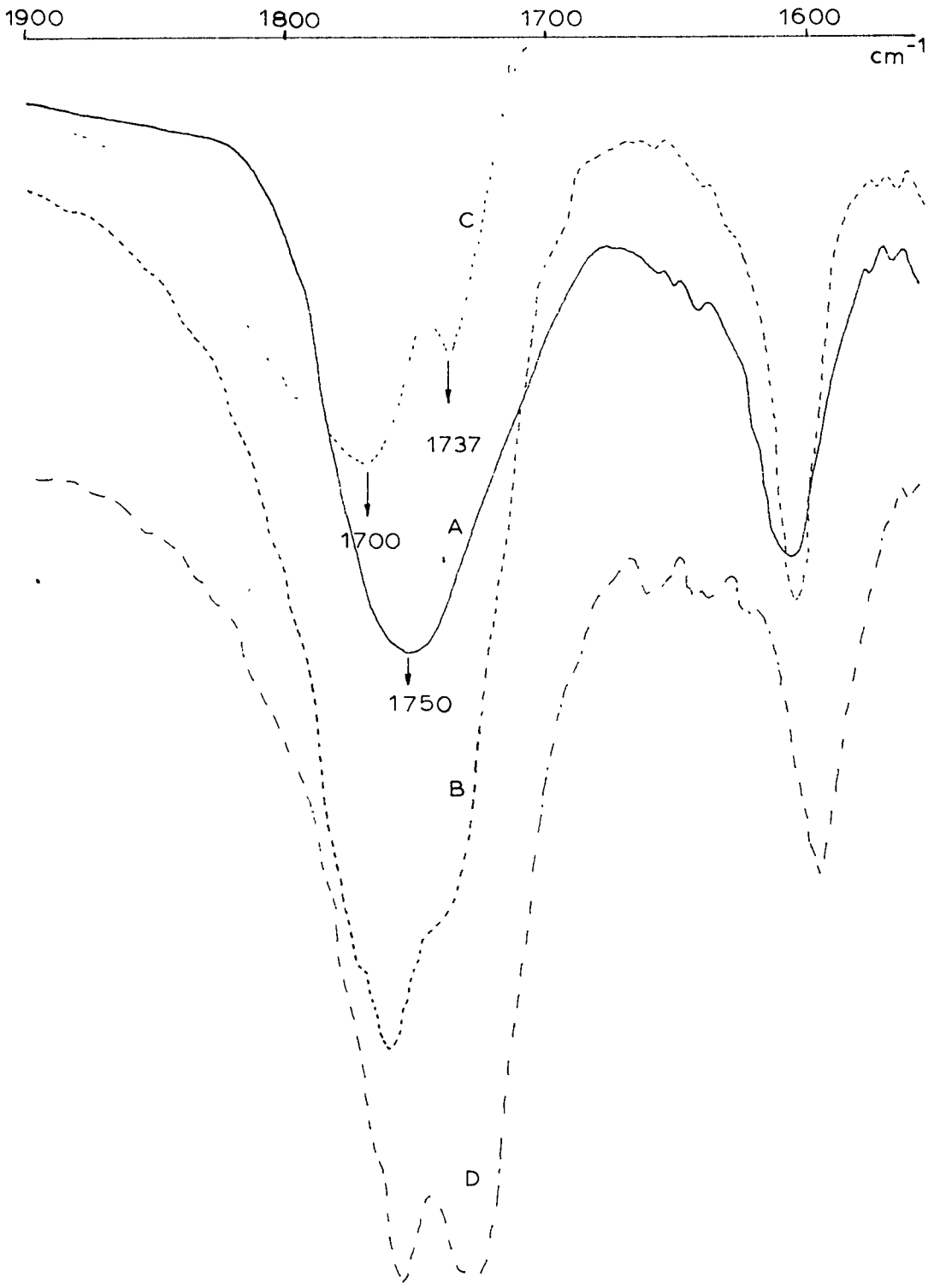


FIGURE 18. Infrared Spectra of HMR, Reduced HMR and Their Acetate Derivatives (A) HMR, (B) HMR-acetate, (C) B-A, (D) Reduced HMR-acetate

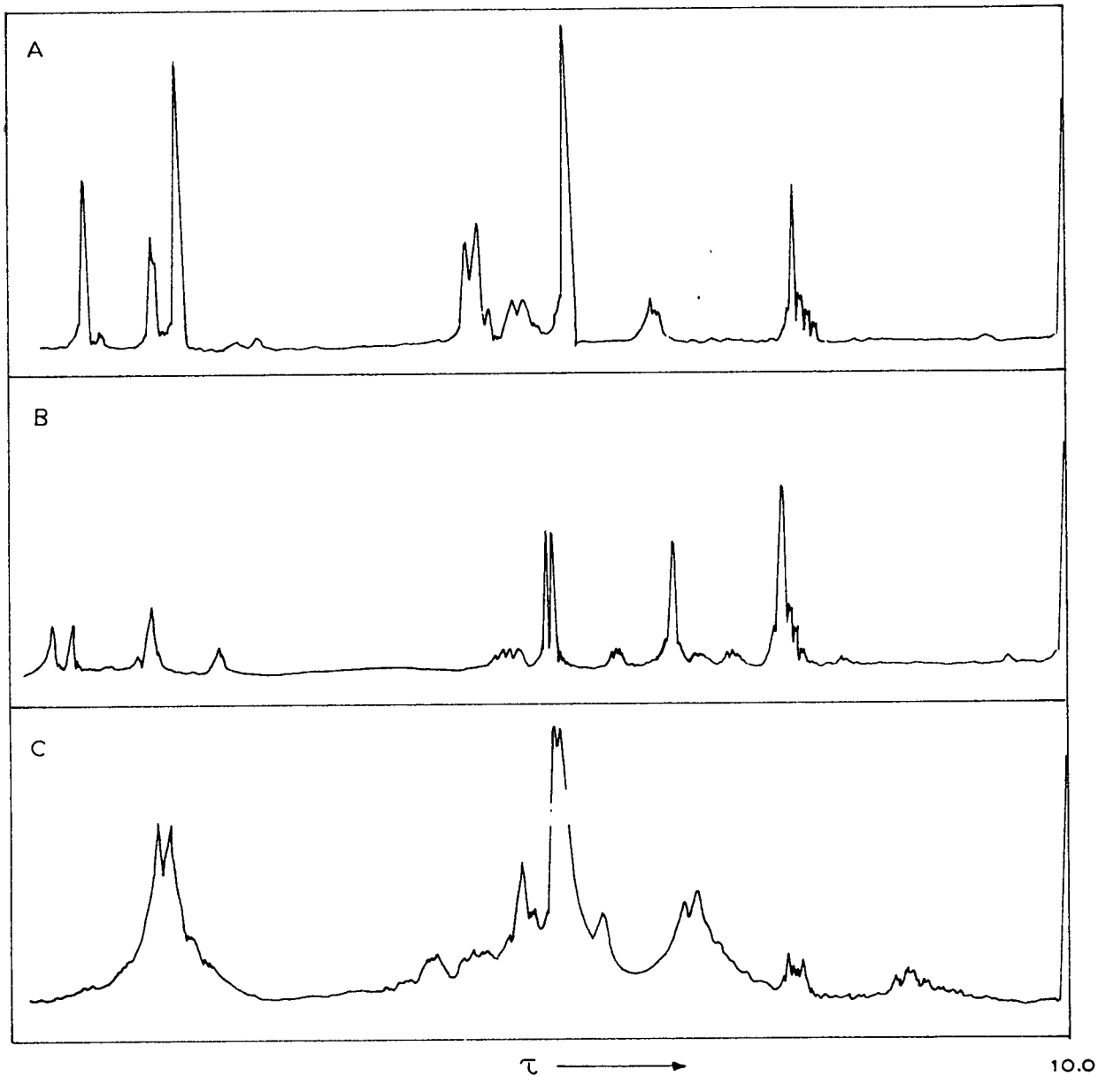
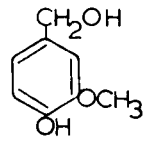


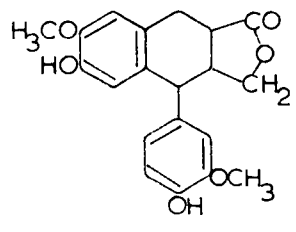
FIGURE 19. Nuclear Magnetic Resonance Spectra of Hydroxymatairesinol and Structurally Related Known Compounds.

A



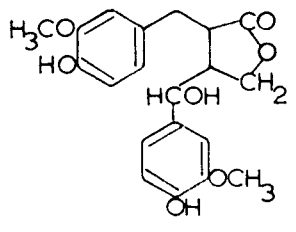
Vanillyl alcohol
C

B

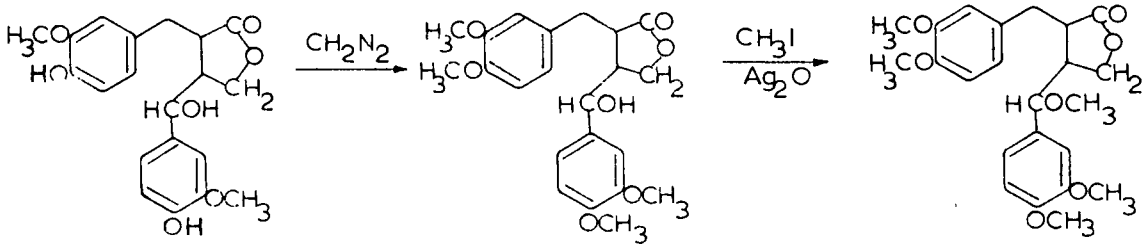


Conidendrin
LX

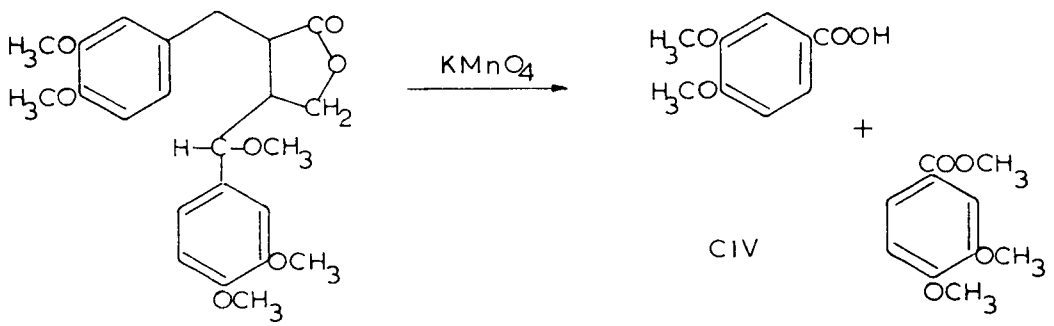
C



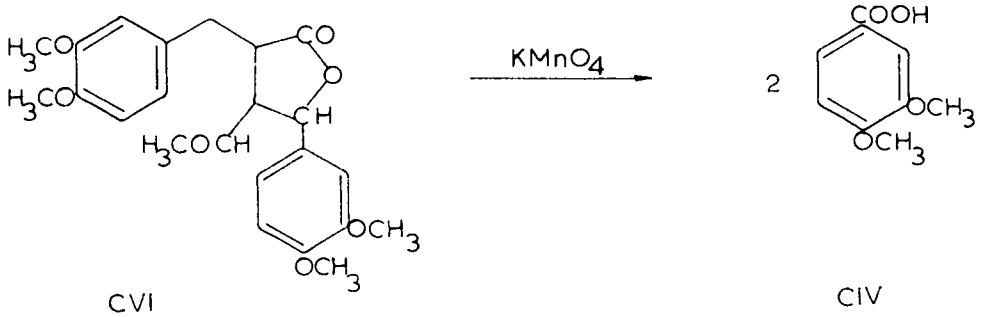
HMR
LXV



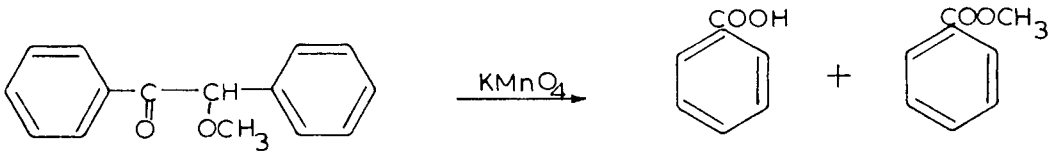
LXV HMR CII DMHMR CIII TMHMR



CIII CIV CV



CVI CIV



CVII CVIII CIX

benzoic acid methyl ester (CIX) were isolated and identified by TLC, the oxidation technique was considered to be definitive. Control oxidations were also carried out on the reference compound veratric acid methyl ester (CV) in both media and a very small amount of decomposition product was observed by TLC.

Both veratric acid and its methyl ester were detected by TLC among the oxidation products from hydroxymatairesinol trimethyl ether. This result clearly pointed to structure CIII for trimethylhydroxymatairesinol. Since the conditions employed in the methylation did not alter the lactone ring of hydroxymatairesinol as judged from a comparison of the infrared spectra of HMR and of CII and CIII (Figure 31) it followed that hydroxymatairesinol had structure LXV and structure LXVI was ruled out.

The structure of allo-hydroxymatairesinol remains uncertain. Since it readily yielded the same matairesinol (LXIV) as HMR on hydrogenation (56) it seemed most probable that the structure and configuration of the lactone rings were the same in all three compounds and that HMR and allohydroxymatairesinol therefore constituted a pair of epimers represented by formulae LXXX and LXXXI. The assignment of configuration of the benzyl alcohol group in HMR will require further investigation.

SUGGESTIONS FOR FURTHER WORK

A. Leucoanthocyanidins.

1. Chromatography of the wood anthocyanidin mixture and reference compounds on both paper- and chromatoplates containing aluminium chloride to separate and identify the anthocyanidin compounds detected in the present work.

2. Identification of the new hemlock anthocyanidin by means of chromatographic investigation of the unidentified alkaline degradation product and by comparison with the available hydroxylated and methylated benzoic acids in at least four different solvent systems.

3. NMR investigation of hemlock leucoanthocyanidin and related compounds.

B. Lignans

1. Investigation of the colors obtained in the formaldehyde-sulphuric acid reaction with a series of phenolic compounds of known structure in order to find out what structural features are responsible for the formation of the different colors. This might provide sufficient knowledge to predict the structural elements of the unidentified lignans detected in the present work.

2. Methylation of HMR with diazomethane and reduction of dimethyl-HMR with lithium aluminium hydride to obtain the triol derivative for comparison of its physical and chemical properties to that of the known triol obtained from dimethyl olivil by catalytic hydrogenation.

3. Further nuclear magnetic resonance spectroscopic study of hydroxymatairesinol and its acetylated, methylated, reduced, reduced-acetylated and other derivatives.

4. Although the structure of HMR is now well established by Freudenberg's color and transformation reactions and by the spectroscopic evidence and oxidative degradation in this thesis, the complete synthesis of HMR would be required as final verification of the structure. Two different

routes (A and B) are suggested for the total synthesis. The basic ideas of the syntheses are summarized as follows:

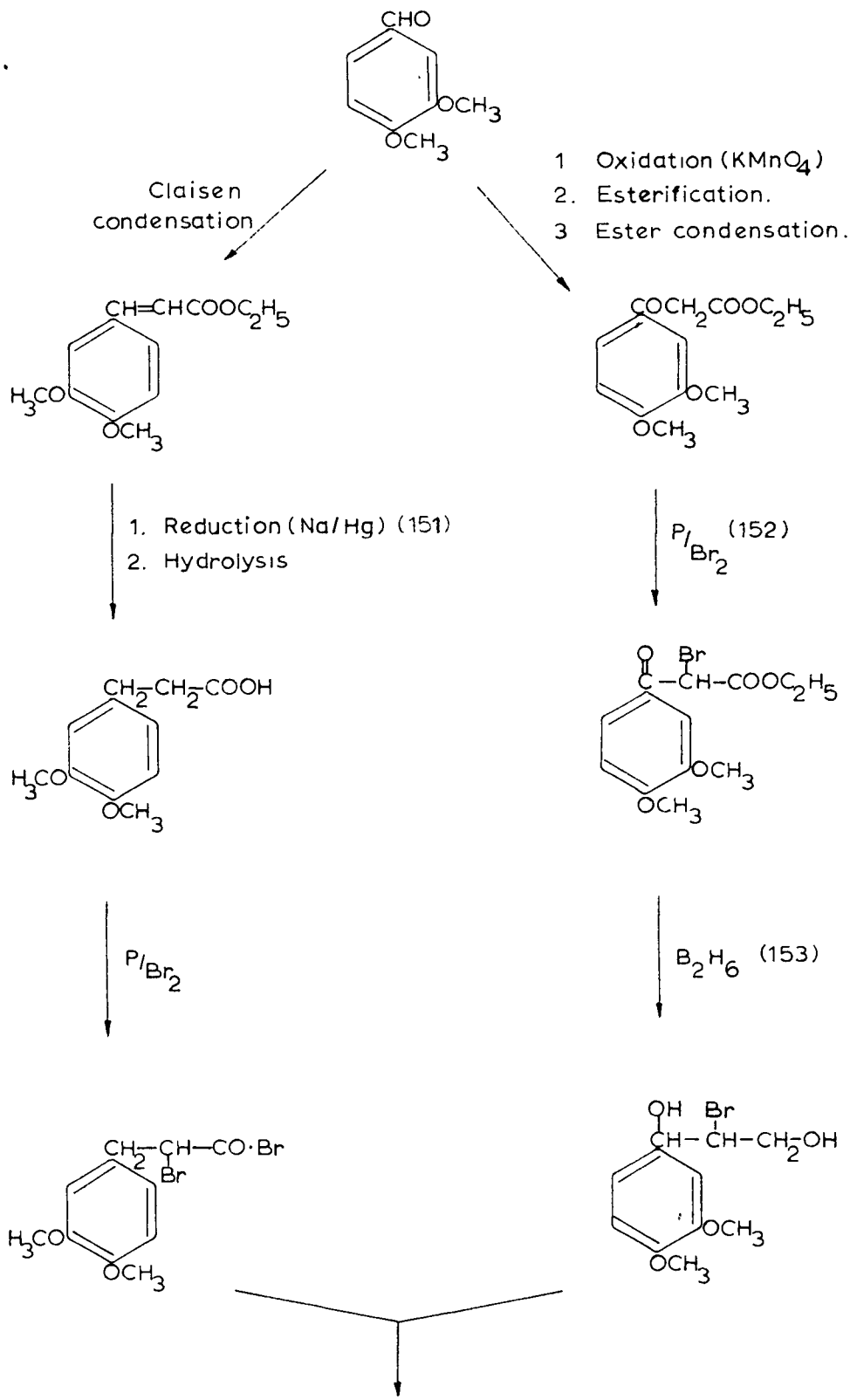
(A) $C_6 - C + C_2 \longrightarrow C_6 - C_3$

$2C_6 - C_3 \longrightarrow C_6 - C_3 - C_3 - C_6$

(B) $C_3 + C_3 \longrightarrow C_3 - C_3$

$C_6 + C_3 - C_3 + C_6 \longrightarrow C_6 - C_3 - C_3 - C_6$

The details of the suggested syntheses are shown in Figure 20 and 21.


CHO
OCH3
OCH3
Claisen condensation
1 Oxidation (KMnO4)
2. Esterification.
3 Ester condensation.
CH=CHCOOC2H5
H3CO
OCH3
COCH2COOC2H5
OCH3
1. Reduction (Na/Hg) (151)
2. Hydrolysis
P/Br2 (152)
CH2-CH2-COOH
Br
C-CH-COOC2H5
P/Br2
B2H6 (153)
CH2-CH-CO·Br
Br
OH Br
CH-CH-CH2-OH
continued

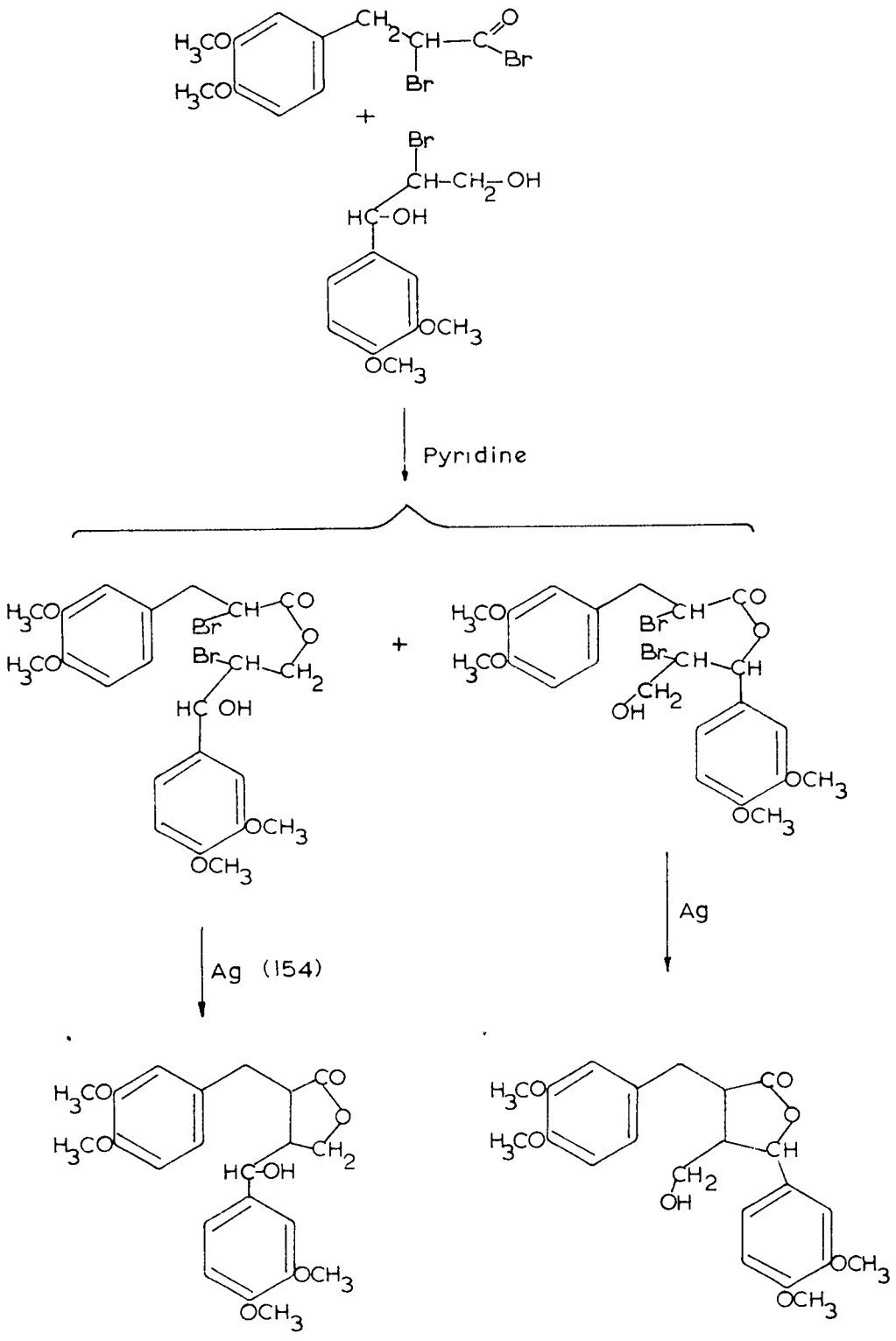
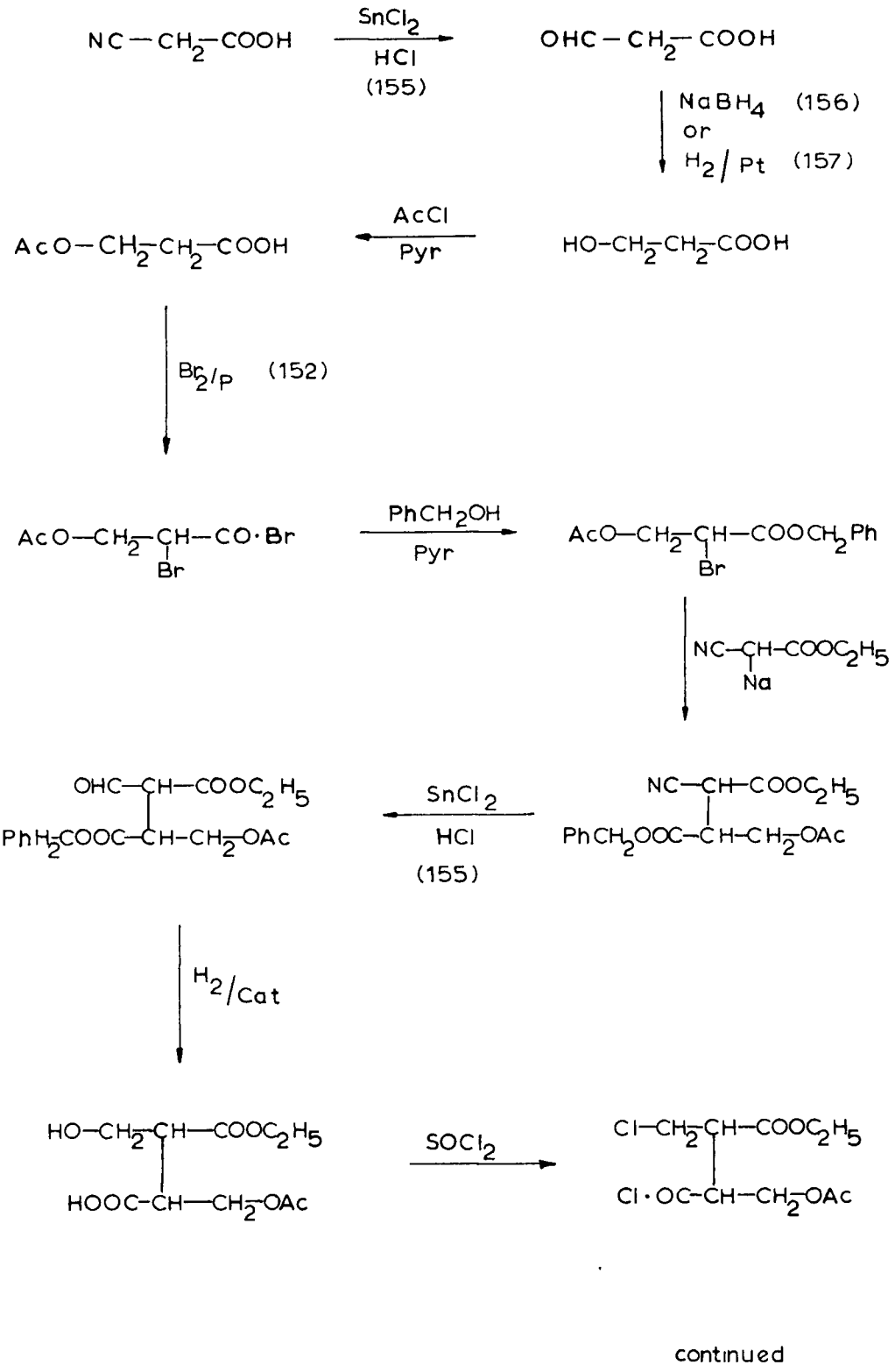


FIGURE 20. Suggested Synthetic Route A to Dimethyl Hydroxymatairesinol.


NC—CH2—COOH
SnCl2
HCl
(155)
OHC—CH2—COOH
NaBH4 (156)
or
H2/Pt (157)
HO—CH2—CH2—COOH
AcCl
Pyr
AcO—CH2—CH2—COOH
Br2/P (152)
AcO—CH2—CH—CO·Br
Br
PhCH2OH
Pyr
AcO—CH2—CH—COOCH2Ph
Br
NC—CH—COOC2H5
Na
NC—CH—COOC2H5
PhCH2OOC—CH—CH2—OAc
SnCl2
HCl
(155)
OHC—CH—COOC2H5
PhH2COOC—CH—CH2—OAc
H2/Cat
HO—CH2—CH—COOC2H5
HOOC—CH—CH2—OAc
SOCl2
Cl—CH2—CH—COOC2H5
Cl·OC—CH—CH2—OAc

continued

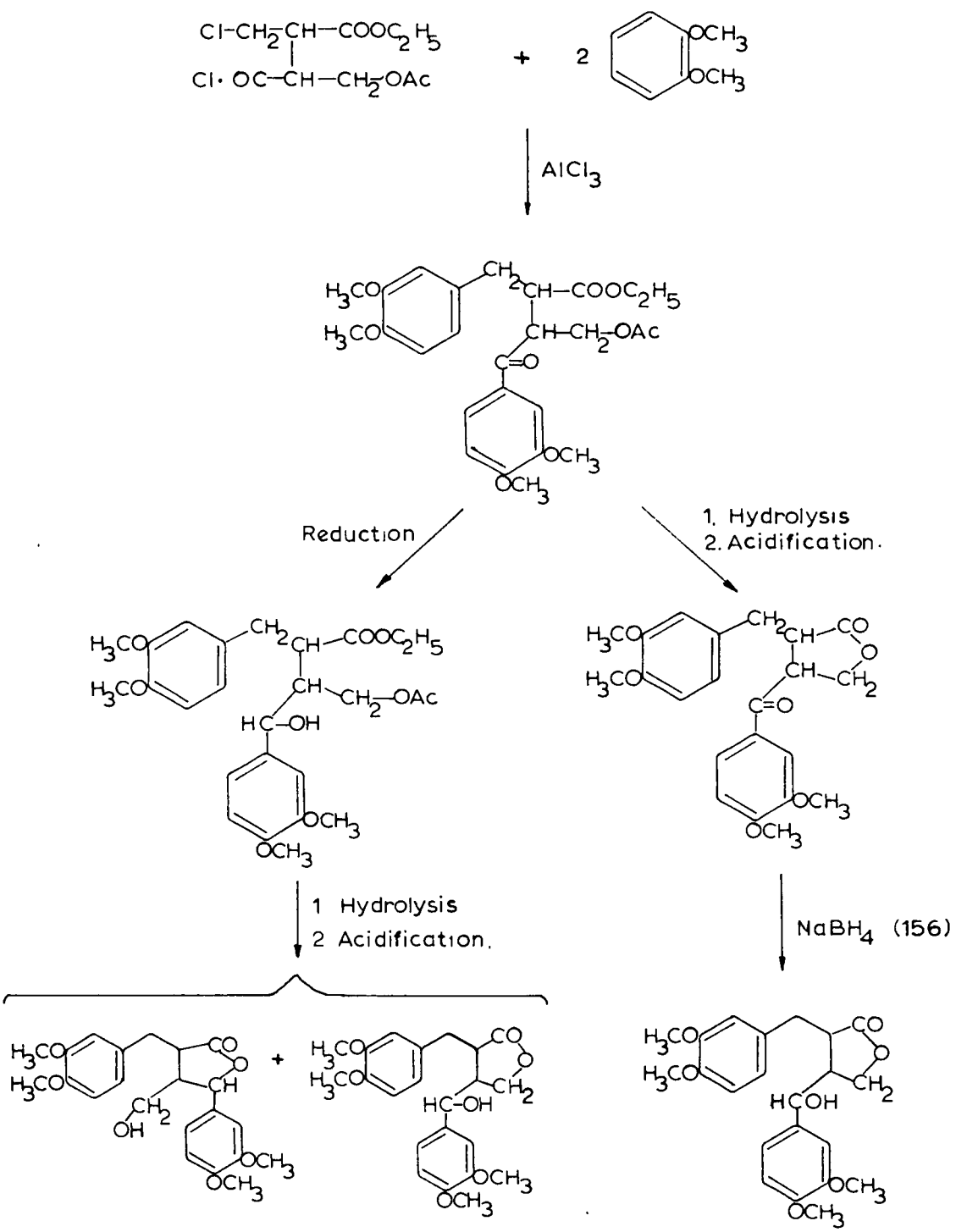


FIGURE 21. Suggested Synthetic Route B to Dimethyl Hydroxymatairesinol.

EXPERIMENTAL

I. Materials.

A. Solvents.

All solvents were reagent grade and were distilled through a 22 cm Vigreaux column before use unless otherwise stated. Peroxide-free ether was prepared by distilling anhydrous ether from lithium aluminium hydride and storing in the dark in a refrigerator.

B. Reagents

Diazomethane

Diazomethane was generated from N-methyl-N-nitroso-p-toluenesulfonamide (Diazald, Aldrich Chemical Co.) and the ether solution obtained (104, 105) was used immediately.

Hydrochloric Acid in Isopropyl Alcohol

Dry hydrogen chloride gas was bubbled into 50 ml of anhydrous isopropyl alcohol (b.p. 81.5°C) and the solution was titrated with standardized sodium hydroxide solution. An aliquot of the alcohol solution was then diluted to 0.03 N with more isopropyl alcohol

Diazotized Sulfanilic Acid

Diazotized sulfanilic acid was prepared according to Cramer (106) and the air-dried solid product was stored in the refrigerator. A solution of 0.1 g of the diazonium salt in 20 ml of 10% sodium carbonate solution was prepared immediately before use.

Quinone Monochlorimide

p-Amino-phenol hydrochloride was prepared from technical grade p-amino-phenol by treatment with concentrated hydrochloric acid and repeated recrystallizations with alternate charcoal treatments. The pure product was used for the preparation of quinone monochlorimide as described

by Willstätter and Meyer (107). The yellow product was stored in the refrigerator.

Silver Oxide

Dry silver oxide was prepared according to Young's procedure (108,109) and used within a week of preparation.

Hydroxamic Acid-Ferric Chloride Reagent

This test reagent for organic esters was adapted from that used in a spot test described by Feigl (110, 150).

Reagent A: A saturated alcoholic solution of hydroxylamine hydrochloride (50 ml) was mixed with 50 ml of a saturated alcoholic solution of potassium hydroxide, and the resulting solution was filtered. Water, 10 ml, was added to the filtrate and the reagent was stored in the refrigerator.

Reagent B: Reagent grade ferric chloride, 1 g, was dissolved in 50 ml of a 50 per cent solution of hydrochloric acid in ethanol.

C. Reference Compounds

Isolation of Cyanidin Chloride (Cyanidin) from Dahlia (Dahlia variabilis var. Smokey).

The air-dried, powdered, defatted, dark-red dahlia petals were treated with dry hydrogen chloride gas and then extracted with methanol (107). The red solution was evaporated to dryness below 40°C bath temperature. The residue, a dark red powder, was a mixture of about eleven different plant pigments which were separated on a cellulose column, and the cyanidin-glucoside (cyanin) was obtained in a pure state. The glucoside was hydrolyzed with boiling 2 N hydrochloric acid and the cyanidin chloride separated as a dark red powder from the cooled reaction mixture in 60% yield; λ_{max}, 550 mμ; λ_{max}, $AlCl_3$, 580 mμ; R_f values:

0.50 in acetic acid-water-hydrochloric acid (30:10:3) (S-1), and 0.22 in formic acid-water-hydrochloric acid (5:3:2) on papergrams. The reported values were 0.49 and 0.22 respectively (92). The infrared spectrum (potassium bromide pellet) is shown in Figure 32.

Synthesis of Cyanidin Chloride (Cyanidin)

(a) Cyanidin chloride was synthesized from reductively acetylated quercetin pentaacetate according to the procedure of King and White (111) in 3% yield. (b) Procedure (a) was modified and acetic acid-water-hydrochloric acid (30:10:3) was used instead of 3N hydrochloric acid-isopropyl alcohol solution and the reaction mixture was heated in an open flask instead of in sealed tubes. The yield of pure cyanidin was thereby increased to 10% of the theoretical value.

The ultraviolet absorption maxima and R_f values of these products were identical with those of natural cyanidin chloride isolated from dahlia petals.

Methyl Veratrate

Purified veratric acid (m.p. 181°C) was methylated with freshly prepared ethereal diazomethane solution. The product was obtained after recrystallization in the form of fine, colourless, needle-like crystals, in 96.6% yield, and melted at 59-59.5°C. The reported melting point was 59.0°C (112).

Conidendrin Dimethyl Ether

α-Conidendrin (a gift of the Crown-Zellerbach Corporation, Camas, Washington) with m.p. 254-256°C was methylated with diazomethane (100, 113, 114) and the dimethyl conidendrin was obtained as a white powder, which melted at 178-179°C. Calculated for $C_{22}H_{24}O_6$:OCH_3, 32.25%. Found: OCH_3 32.48%. No hydroxyl band appeared in the infrared spectrum of the methylated product.

Benzoin Methyl Ether

Benzoin (Eastman-Kodak) (m.p. 136-137°C) was methylated by Purdie's method (115) with the modifications that freshly ignited "Drierite" was added to the reaction mixture and refluxing was continued for 44 hours. A yellow oil was initially obtained as methylation product. The infra-red spectrum was taken as a film on a sodium chloride plate and no hydroxyl band was apparent.

Calculated for $C_{15}H_{14}O_2$: OCH_3, 13.70%; Found: OCH_3 12.56%.

The oil crystallized after standing at room temperature for three weeks, and the colourless crystals melted at 48°C. The reported melting point was 49°C (112).

II. Analyses

A. Melting Points

The corrected melting points were observed (± 0.5°C) with a hot stage polarizing microscope (Wetzlar, No. 48114, Ernst Leitz, Germany).

B. Methoxyl Determination

Analyses for methoxyl groups were carried out by Mrs. A.E. Aldridge, Microanalytical Laboratory, Department of Chemistry, University of British Columbia.

C. Specific Rotation

Optical rotation was measured at the wavelength of the sodium D line on a Lippich polarimeter (Model 219, O.C. Rudolph and Sons, Calwell, N.J.).

D. Colour Tests

Test for α,β-Unsaturated Lactones

Tests were carried out according to Haynes (116, 117) using Tollen's reagent, ammonical silver nitrate, and the Legal reaction.

Test for the α-Hydroxyguaiacyl Nucleus

α-Hydroxyguaiacyl groups were detected by their reaction with quinone monochlorimide in weakly alkaline solution (99).

Test for the Methylenedioxyphenyl Group

The methylenedioxyphenyl group was detected with gallic acid in strongly acidic solution (118).

Test for Esters

The original test (110) was adapted for use as a chromatographic spray reagent. The ester to be tested was spotted on a thin-layer chromatoplate and was developed in a suitable solvent. The dried plate was sprayed

with reagent A and heated in an oven for 15 minutes at 120°C. After cooling the plate was sprayed with reagent B. A violet colour on a pale yellow background was developed by organic esters such as methylbenzoate and methyl-veratrate.

III. Spectra

A. Ultraviolet Spectra

All ultraviolet spectroscopic measurements were made on a Cary recording spectrophotometer (Model 14) in 95% ethanol containing 0.01 ml of concentrated hydrochloric acid per 100 ml. Five per cent aqueous aluminium chloride solution (0.1 ml) was added per cell when measuring the absorption maxima shifts caused by complex formation at vicinal hydroxyl groups (92).

B. Infrared Spectra

Infrared spectra were recorded on a Perkin-Elmer Model 21 Infrared Spectrometer in potassium bromide windows, paraffin oil (Nujol) mulls, or as film deposited on sodium chloride plates from an appropriate solvent.

C. Nuclear Magnetic Resonance Spectra

The nuclear magnetic resonance spectra were run on a 60 Mc high resolution spectrometer (Varian Associates, Palo Alto, California) in deuteroacetone with tetramethylsilane as internal reference.

IV. Chromatography

Several types of chromatographic methods were used in the isolation and identification experiments. Paperchromatography was conducted by the descending and ascending techniques, one- and two-dimensionally, in glass tanks or in a Reco A-125 Chromatocab at room temperature. For preparative purposes, chromatobars (119), conventional column chromatography, thin-(TLC)and thick-(TkLC) layer chromatography were applied.

The supporting materials and adsorbents, solvents, and spray reagents used are summarized in Tables, X, XI and XII respectively. Since separations on rectangular chromatobars (119) were not efficient because of unsymmetrical zone distribution, cylindrical chromatobars were prepared in dialysis tubing. The supporting rod was omitted from the center of the bar and an external supporting devise was applied (Figure 22). The composition and dimensions of various chromatobars and chromatoplates are summarized in Table XIII.

Table X

Supporting Materials and Adsorbents for Chromatography

No.	Supporting Materials and Adsorbents	Used as	Ref.
M-1	Paper (Whatman No. 1)	Sheet	-
M-2	Formamide paper (Whatman No. 1)	Sheet	56
M-3	Borate-acetate paper (Whatman No. 1)	Sheet	120
M-4	Silicic acid-plaster-of-Paris (For composition see Table XIII) or Silica Gel G (Merck and Co. Darmstadt)	TLC[a] TkLC[b] CCB[c]	121,122,123 this work 119
M-5	Cellulose powder (Whatman, Standard)	Column	-
M-6	Celite No. 535 (Johns-Manwille)	Column	-
M-7	Alumina (Merck, "Acid washed, chromatographic grade", 100 mesh)	Column	-
M-8	Silica gel. (B.D.H. "for chromatography")	Column	-
M-9	Silicic acid-plaster-of-Paris-nylon powder[d] (60:15:20)	Column	this work

[a]Thin-layer chromatoplates

[b]Thick-layer chromatoplates

[c]Cylindrical chromatobars

[d]A gift of the DuPont Company of Canada

Table XI

Solvents for Chromatography

No	Solvents[a]	Ratio of Volumes	Ref.
S-1	Acetic acid-water-hydrochloric acid	30:10:3	6
S-2	1-Butanol-water (50 ml of organic phase plus 1 ml of ethylene glycol)	8:2	124
S-3	1-Butanol-acetic acid-water	4:1:5	125
S-4	1-Butanol-27% acetic acid	1:1	126
S-5	0.3 Sulfurous acid	-	127
S-6	Isopropyl alcohol-NH_3-water	8:1:1	128
S-7	1-Butanol-acetic acid-water	4:1:1	129
S-8	Benzene-propionic acid-water	2:2:1	128
S-9	m-Cresol-acetic- acid-water	50:2:48	129
S-10	Ethyl acetate-acetic acid-formic acid water	18:3:1:4	130
S-11	Benzene-ligroin-methanol-water	50:50:1:50	131
S-12	Ethanol-ammonia-water	80:4:16	132
S-13	2% Acetic acid	-	133
S-14	n-Amyl alcohol saturated with water	-	135
S-15	Benzene-acetic-acid-water	8:2:1	135
S-16	1-Butanol-acetone-water	4:5:1	136
S-17	α-Bromo-naphthalene-90% acetic acid	1:1	137
S-18	Methylethyl ketone saturated with water	-	138
S-19	Distilled water	-	139
S-20	Acetal saturated with formamide	-	56
S-21	1-Butanol saturated with water	-	120
S-22	Chloroform-acetic acid	9:1	140
S-23	Benzene-ethylacetate	3:7	b
S-24	Benzene-ethylacetate	7:3	b
S-25	Benzene-ethylacetate	2:8	b
S-26	Benzene-ethanol-water	150:35:15	b
S-27	5% Acetic acid	-	b

[a]S-1 to S-19 and S-26, S-27, were used on papergrams (M-1), S-20 on formamide-treated paper (M-2), S-21 on borate-acetate paper (M-3) and S-22 to S-25 for chromatoplates and chromatobars (M-4).

[b]This work

Table XII

Spray Reagents for Chromatography

No.	Spray Reagents*	Ref.
R-1	3% p-Toluene sulfonic acid in ethanol	91
R-2	2% Ferric chloride in methanol	125
R-3	Diazotized sulfanilic acid (DSA)	128
R-4	Vanillin-hydrochloric acid	129
R-5	Aniline-phthalic acid	141
R-6	Phloroglucinol-hydrochloric acid	142
R-7	Glycerol-hydrochloric acid-methanol	143
R-8	1% Potassium permanganate in 2%	144
R-9	Sulfuric acid-formaldehyde (9:1)	140
R-10	Hydroxylamine-ferric chloride	110

* R-1 to R-7 were used on papergrams (M-1). R-3 was also used on treated papers (M-2, M-3), and chromatoplates (M-4). R-8, R-9, R-10 were used on chromatoplates and chromatobars only (M-4).

Table XIII

Composition and Dimensions of Chromatoplates and Chromatobars

Use	Dimensions (mm)			Composition of Slurry		
	Length	Width	Thick-ness	Silicic acid[a](g)	Plaster-of-Paris[b](g)	Water ml
TLC[c]	300	150	0.25	30	7.5	60
TkLC	300	150	10.0	120	30	240
CCB large	300	47[d]	-	210	90	450
CCB small	300	27[d]	-	105	45	225

[a]Mallinckrodt, 100 mesh

[b]Gypsum, Lime and Alabastine Company Limited, Vancouver, B.C.

[c]Silica Gel G was also used as supplied by Brinkman Instrument Inc., Great Neck, L.I., N.Y.

[d]Diameter

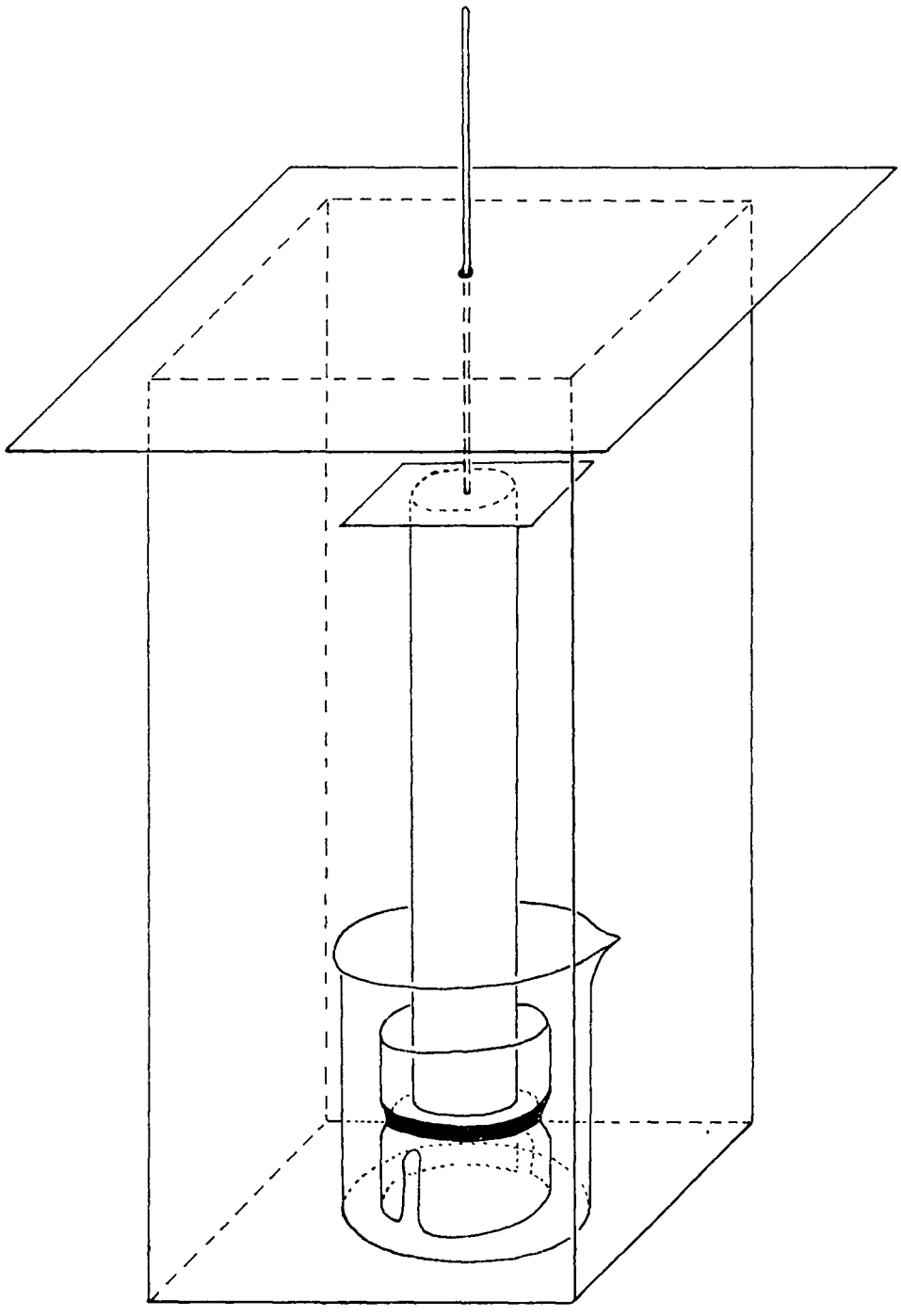


FIGURE 22 . Distributor for Chromatobars .

V. Extraction of Western Hemlock Wood.

A. Collection and Preparation of Wood Samples.

1. A western hemlock tree, grown on the University of British Columbia Endowmentland, 8.1 cm. in diameter, having 36 annual rings, was cut at 51 cm. above the ground, and a 91 cm piece was immediately cleared of bark and cambium and cut into four lengths. These samples were placed in polyethylene bags and stored in the dark at room temperature until required. All samples were examined within two months of collection.

Immediately after felling (not more than 2 hours) one of the samples was shaved to very thin strips with a draw-knife and these were cut into short pieces with scissors. The finely divided sample thus obtained was stored under nitrogen until required.

The other three samples were reduced to matchsticks, dried in air at 32°C, then ground in a Wiley mill to pass a 40-mesh screen, and stored in air-tight jars.

2. Four western hemlock trees, a gift of the Powell River Co. Limited were selected and felled in the Gordon Pasha Lake watershed, near Powell River, B.C. The four trees measured as follows:

	Ring count	Diameter	Bark Thickness
1	203	16"	3/4"
2	125	15"	1/2"
3	101	13 1/2"	1/2"
4	111	12 1/2"	1/2"

Wood samples from these trees were reduced to sawdust with a table saw and the air-dried sawdust was stored in polyethylene bags. No attempt was made to separate heartwood and sapwood in the sample.

Methanol extraction of the hemlock wood samples, prepared under

the various conditions described above and chromatographic examination of the extracts revealed a considerable variation in the proportion of leucoanthocyanidin present and the rate at which the anthocyanidins were generated on acid treatment. No firm correlation could be established, however, between these tests and the age or history of the sawdust sample. The wood sample prepared by method 2, therefore, was used in all subsequent experiments.

B. Extraction of Fats, Waxes and Related Compounds.

The air-dried sawdust was extracted in 2 kg batches in a Soxhlet-type apparatus shown in Figure 23, which was designed so that the distillate was cooled to 18 - 20°C before returning to the extraction chamber. The extractor was constructed of glass except for two short Tygon tubing connections to the siphon tube which permitted slight flexibility for assembly.

Extraction with petroleum ether (b.p. 30-60°C) was continued for a period of 2 weeks. The extract became dark yellow in color within a few days, and was then drained off and replaced with a fresh portion of petroleum ether. The combined extracts were concentrated by distillation under reduced pressure and below 50°C bath-temperature to dryness. The yield of amorphous, yellow residue was 0.142% on the basis of the air-dried wood.

The petroleum ether extracted sawdust was dried in a stream of purified nitrogen and the extraction was continued in the same apparatus with chloroform. The coloured extract was drained off and replaced with fresh chloroform from time to time and this operation was repeated until no more coloration of the chloroform took place (10 to 15 days). The residue from the chloroform extracts was recovered as described above and amounted to 0.407 % of the weight of the air-dried wood.

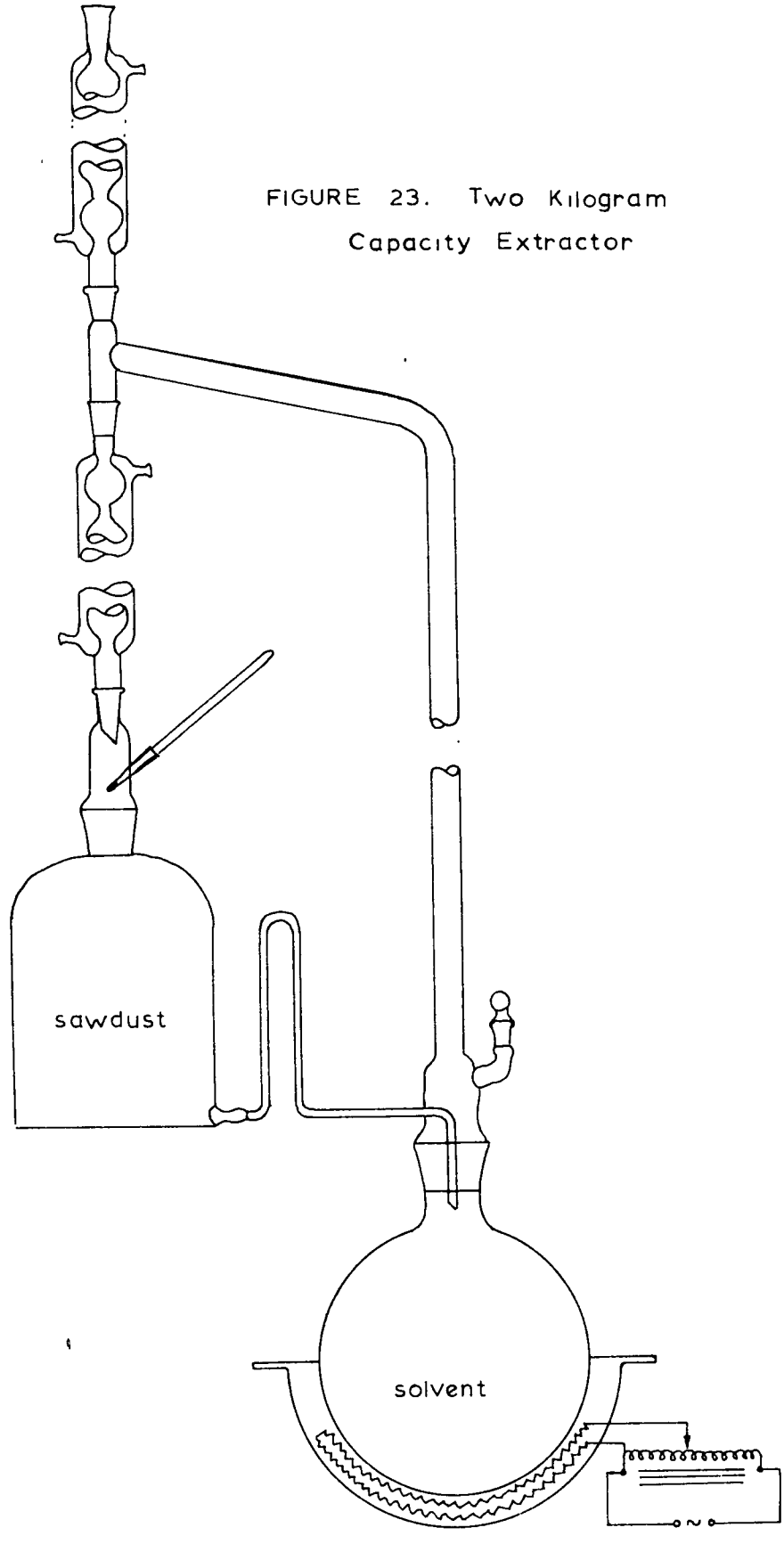


FIGURE 23. Two Kilogram Capacity Extractor

The nitrogen-dried sawdust was then extracted with peroxide-free ether for two weeks with several solvent changes. The yield of residue from the collected extracts amounted to 0.053% of the air-dried wood. The completely defatted sawdust was finally nitrogen-dried until the odor of ether was no longer detectable and was stored in the dark in polyethylene bags.

C. Extraction of Phenolic Constituents.

Anhydrous solvents were used throughout and all evaporations to dryness were conducted at temperatures from -5° to +5°C in an atmosphere of purified nitrogen.

In a typical experiment sawdust (530 g) which had been pre-extracted as described above in section B, was shaken mechanically with anhydrous methanol at room temperature with frequent solvent changes. The methyl alcohol extract was evaporated to dryness, and the residue was extracted successively with petroleum ether (b.p. 30-60°C) (A), benzene (B), chloroform (C), ether (D), ethylacetate (E), acetone (F), and methyl-alcohol (G). (Figure 24). A small amount of insoluble dark brown material (H) was obtained as a final residue. The extracts were evaporated to dryness and the residues were re-extracted successively with the solvents named above and dried again by evaporation. The yields of the various fractions of the methanol extract are given in Table XIV. Fractions B, C, D, and E showed the chromatographic behavior and gave qualitative tests typical of a mixture of lignans while fractions F and G responded to the typical tests for leucoanthocyanidins.

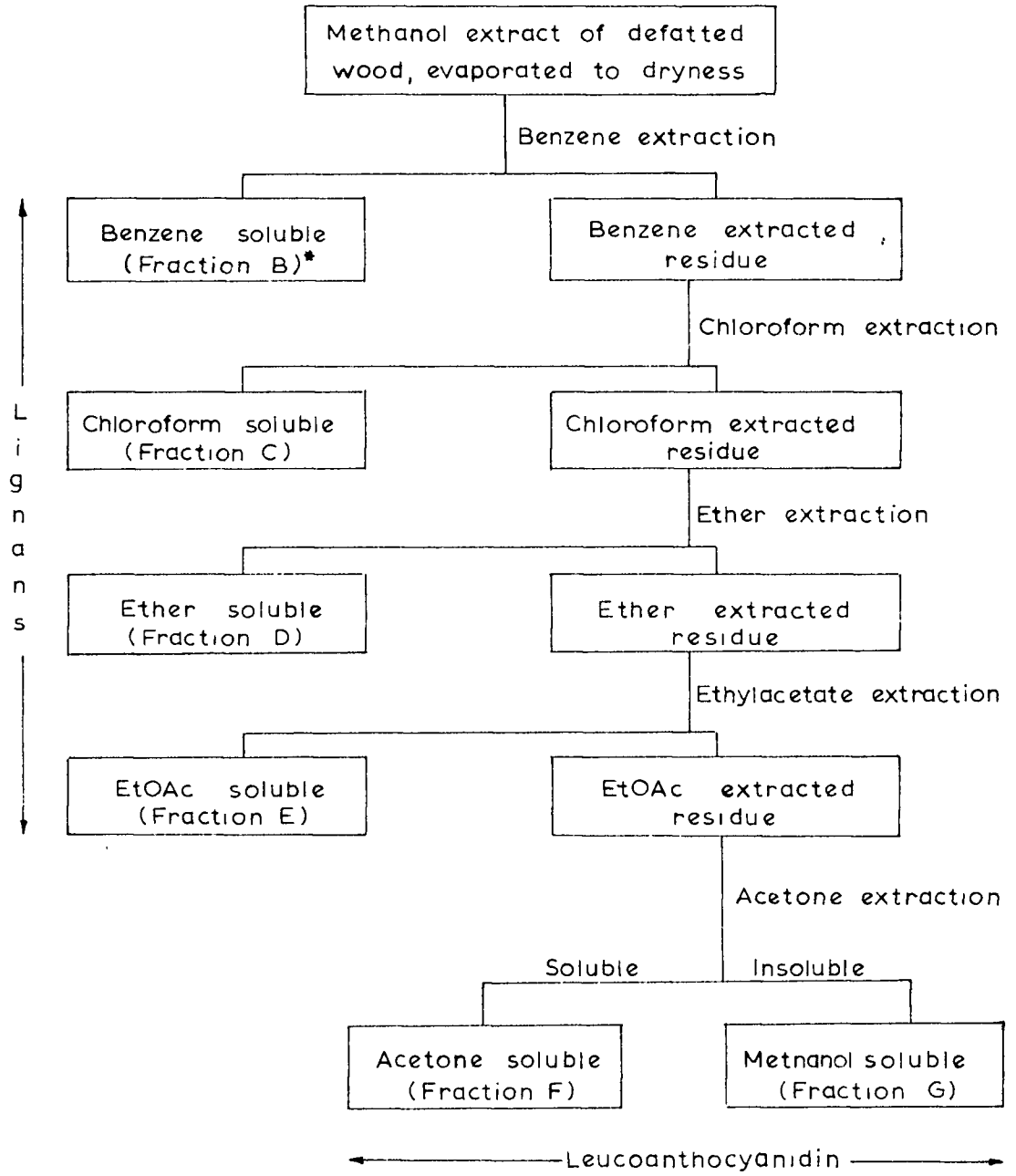


FIGURE 24. Fractionation of the Methanol Extract of Hemlock Wood.

*Fraction A is the petroleum ether extract which did not contain phenolic subtances

Table XIV

Yields of the Fractions from the Methanol Extract of Wood.

	Fraction from	Yield based on Wood %	Yield based on total MeOH extract %	OCH_3%
A	Petroleum ether	0.035	2.40	-
B	Benzene	0.042	2.80	15.92
C	Chloroform	0.850	55.10	12.17
D	Ether	0.021	1.30	15.35
E	Ethylacetate	0.220	14.61	16.13
F	Acetone	0.136	8.90	14.3
G	Methylaclohol	0.212	14.05	5.48
	Total	1.516	99.25	

Larger quantities of the lignan mixture corresponding to fractions C, D, and E were obtained from the methanol extract as follows:

The extract was evaporated to dryness at -5 to +5°C and the dark brown sticky residue was extracted with peroxide-free ether. The yellow-green ether solution was filtered from a light brown powder. The powder was washed with ether and extracted with 100 ml of dry acetone. After filtration the acetone solution was concentrated to about 25 ml and precipitated into 2 liters of peroxide-free ether. The filtered ether solution was evaporated to give a pale yellow solidified foam which failed to crystallize from several solvents.

Further amounts of leucoanthocyanidin corresponding to fractions F and G were obtained directly from the methanol extract as follows:

The extract was concentrated to about 25 ml under reduced pressure and 75 ml of dry acetone was added. The dark yellow solution was purged with nitrogen and allowed to stand in the refrigerator overnight. The small amount of precipitate which separated was filtered off and the filtrate

was concentrated to about 25 ml and added drop by drop to 2 liters of peroxide-free ether, stirred with a rapid stream of nitrogen. A light brown, flocculent precipitate settled out after standing in the refrigerator for a few hours. The precipitate was separated and dried under nitrogen to give a cream-colored powder, 0.4 % of the weight of dry wood.

The infrared spectrum of the hemlock leucoanthocyanidin (potassium bromide pellet) is shown in Figure 32 together with that of a synthetic leucocyanidin prepared by Mr. G. Barton, Forest Products Laboratory, Vancouver, B.C., by sodium borohydride reduction of dihydroquercetin. The two spectra show some differences, particualrly in the region 900-1200 cm^{-1}, where the synthetic product has a broad, not clearly defined band, however in the region 1300-4000 cm^{-1} the principal absorption band are in reasonable good agreement. The spectrum of the tetramethyl ether of leucocyandin prepared by Ganguly and Seshadri (19) also shown in Figure 32 contained bonds at 1230 and 1030 cm^{-1} which indicated that the hemlock leuco-compound probably contained methoxyl groups.

The chromatographic separation and ultraviolet spectra of the anthocyanidin solutions prepared by acid treatment of the hemlock leucoanthocyanidin in several different solvents are shown in Table IV and V and Figure 4, 6, 7 and 8.

VI. Separation of Lignans on Cylindrical Chromatobars

Fraction C (6.7 g) was dissolved in 30 ml of ethylacetate in a 150 ml beaker, and this solution was loaded on three freshly prepared cylindrical chromatobars (CCB large, Table XIII) by dipping the bars into the solution to a depth of 0.5 cm. Without drying, the loaded bars were placed in distributors set up in closed glass tanks (Figure 22) and irrigated with S-25. When developed, the wet bars were dried in a stream of nitrogen, then were covered with aluminium foil except for a 2 cm wide strip along the 30 cm axis and sprayed with a very thin film of reagent R-9. After location of zone the sprayed layer (about 0.5 mm deep) was immediately scraped off, and the zones were separated with a knife. Since the separation was not sharp (zones overlapped each other) the columns were cut up into subfractions smaller than the visible zone. The zones were powdered, placed in glass tubes, and eluted with ethylacetate followed by methanol and acetone.

Samples for thin layer chromatographic analysis were taken from the first few concentrated drops of the eluates. The result of the first chromatobar separation is shown in Figure 25. The corresponding fractions of the three bars were combined and four new fractions were obtained (I, II, III and IV). These solutions were concentrated (Figure 26) to about 10 ml and were rechromatographed on four bars as before. Thin layer chromatographic tests were carried out on the fractions from the second set of four chromatobars (I to IV) and the results are shown in Figure 27 and 28.

The two first fractions from bar IV were evaporated to dryness and the pale cream-colored residue was dissolved in 5 ml of ethylacetate and precipitated into 500 ml petroleum ether (b.p. 30-60°C). The resulting

precipitate was filtered off and dried, to give 100 mg of a colorless powder which failed to crystallize from any solvents. It was identified as hydroxymatairesinol on the basis of comparisons of its physical and chemical properties with those of an authentic sample. The infrared spectrum (potassium bromide pellet) is shown in Figure 29, 30 and 31 for comparison with other flavonoids.

The fourth and fifth fractions from bar IV were concentrated to small volume and allowed to stand in refrigerator. The resulting white powder was filtered off and dried. It was identified as conidendrin. The infrared spectrum of the conidendrin isolated is shown in Figure 29, it was identical with that of an authentic sample.

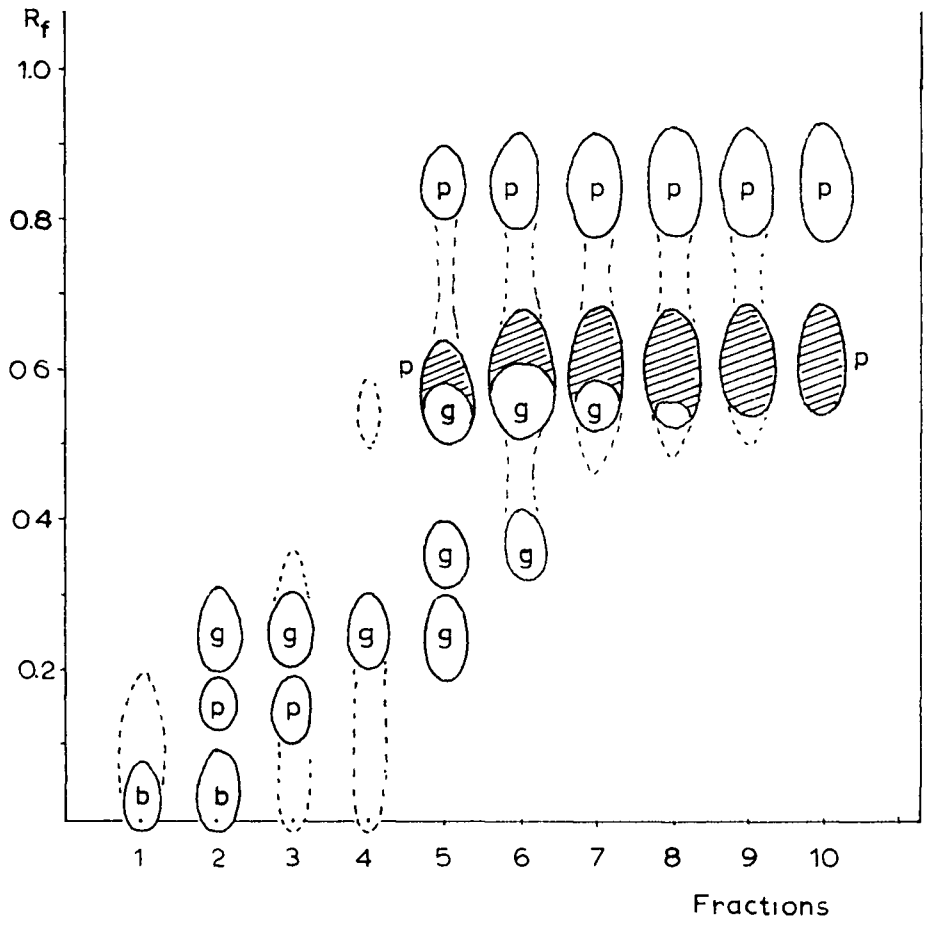


FIGURE 25. First Separation of Fraction C on Chromatobars.

Chromatoplate M-4, developed with S-24, sprayed with R-9. b = brown, g = green, p = pink.

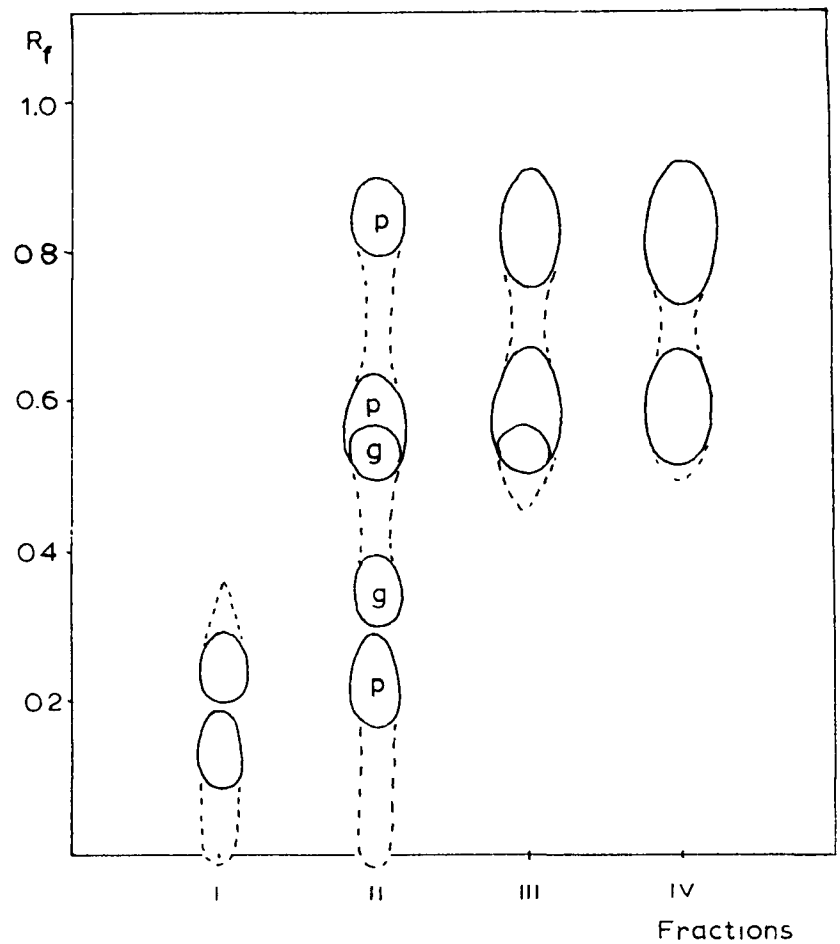


FIGURE 26. Combined Fractions I to IV of Lignans from First Chromatobar Separation. The TLC-plate was run in S-24 and was sprayed with R-9.

Fractions III and IV: Conidendrin and hydroxy-matairesinol.

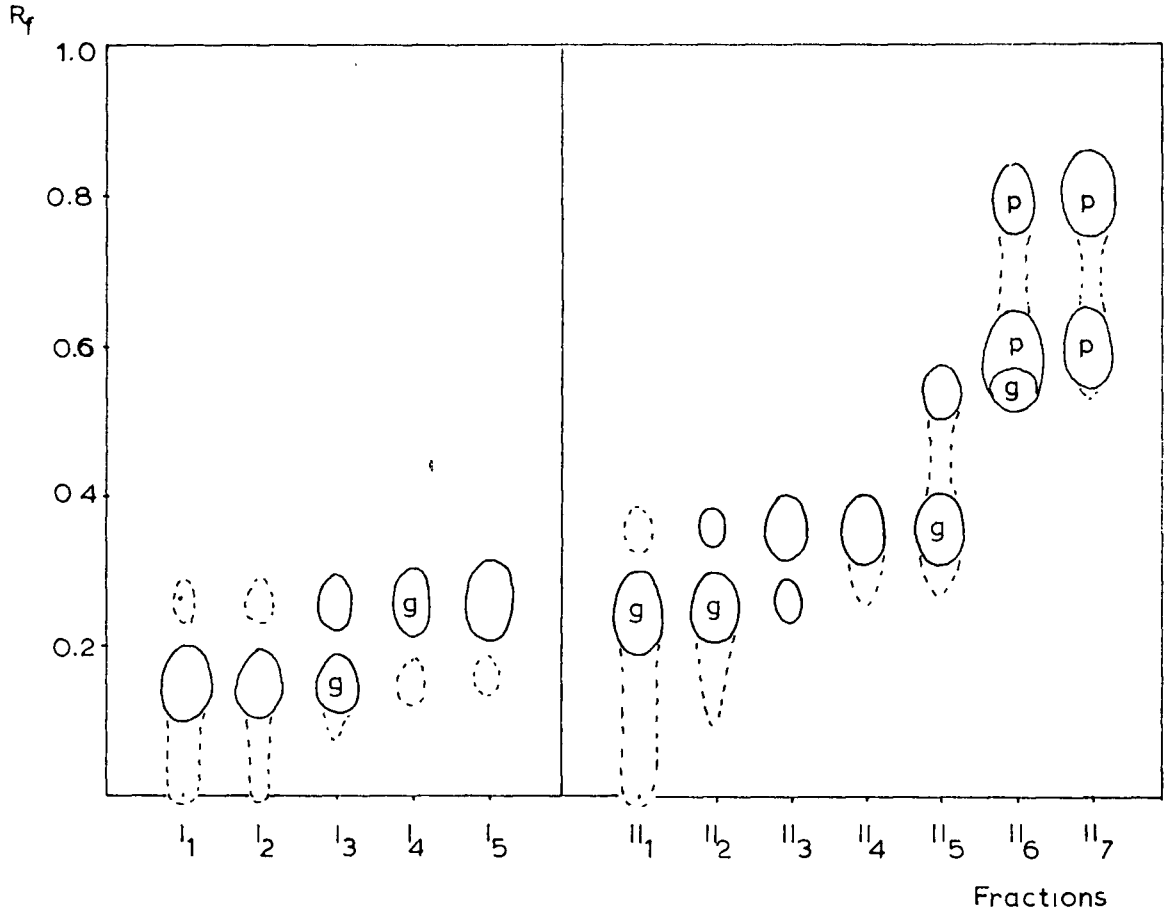


FIGURE 27. Thin Layer Chromatography of Fractions from Chromatobars I and II. The plates were irrigated with S-24, sprayed with R-9, and heated in an oven at 110°C for 15 minutes p = pink, g = green

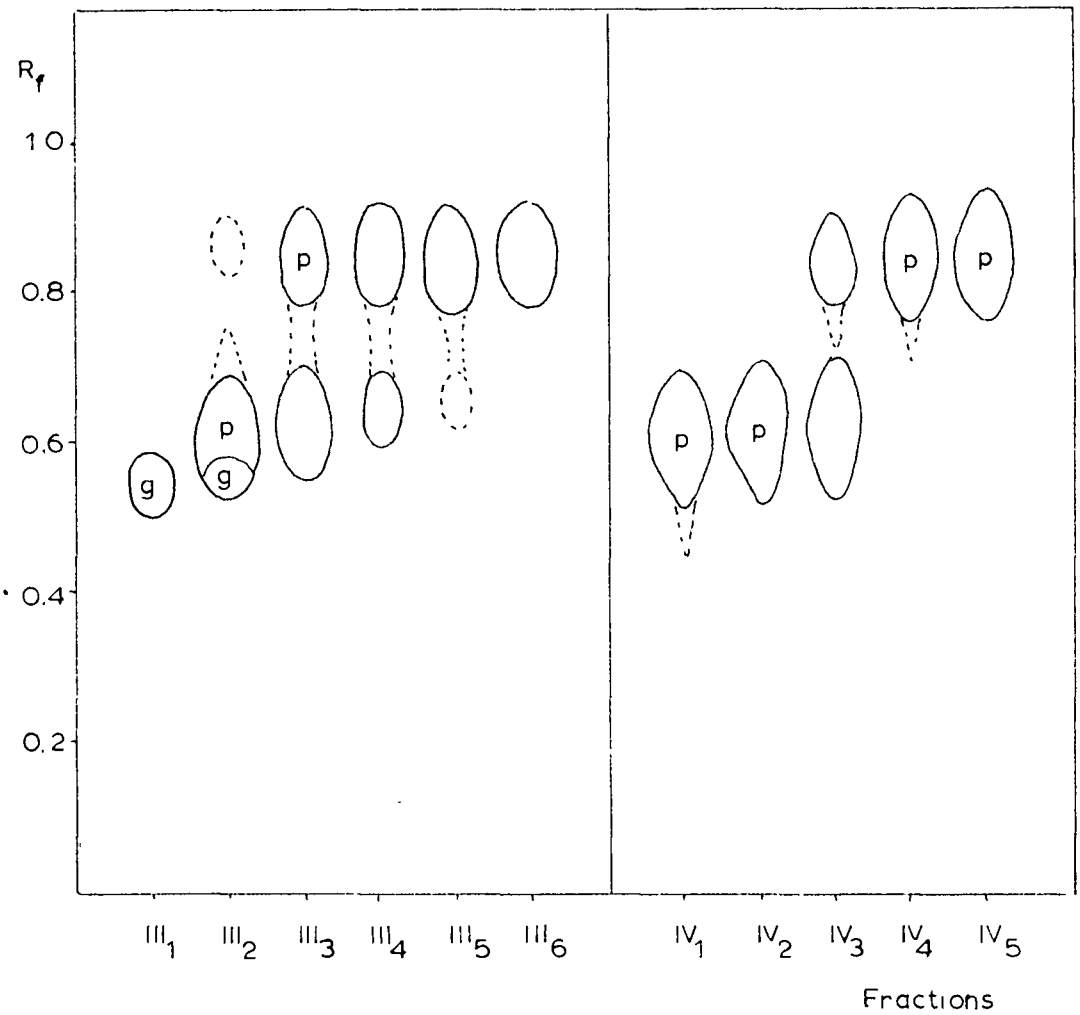


FIGURE 28 Thin Layer Chromatography of the Fractions from Chromatobars III and IV The plates were irrigated with S-24 sprayed with R-9 and heated in an oven at 110°C for 15 minutes p = pink, g = green

VII. Reactions and Derivatives

A. Western Hemlock Leucoanthocyanidin

Attempted Isolation of Anthocyanidins

Leucoanthocyanidin, 1.25 g, was dissolved in 750 ml of isopropyl alcohol and 7.57 ml of 3.2 N hydrochloric acid in isopropyl alcohol was added and the mixture was relfuxed on a steam bath at 80°C for 50 minutes. The carmine-red solution was concentrated to about 10 ml under reduced pressure in a nitrogen stream at room temperature and was adsorbed on cellulose powder which was then dried in a vacuum desiccator over solid sodium hydroxide. The dried powder was loaded on the top of a powdered cellulose column which was developed with solvent system S-1. After separation the pink band was removed, dried, and eluted with methanol and examined by paperchromatography in solvent S-1. In addition to a bright pink spot at R_f 0.51 a brown-red phlobaphene spot appeared at the solvent front. After a second chromatographic separation no phlobaphene showed up on the papergram but only the pure pink spot. The bright red solution was concentrated under reduced pressure at room temperature and a nearly colorless flocculent precipitate appeared. A papergram of the solution again indicated the phlobaphene spot at the solvent front. The precipitate was filtered off and the solution was concentrated further. The white precipitate appeared again and after standing for 30 minutes the solution became colorless indicating that the anthocyanidin chlorides had decomposed.

Alkaline Degradation of Hemlock Leucoanthocyanidin

Leucoanthocyanidin isolated from wood, cyanidin from dahlia petals, and synthetic cyanidin were degraded by the three different methods described by Tayean and Masquilier (93), Harris and Rickets (94) and Masquilier and Point (95). The degradation products and reference compounds were

examined by paperchromatography in solvent systems S-4, S-6, S-8 and S-9 using spray reagent R-3. The results are shown in Figure 9.

B. Lignans

Tri-O-acetyl-hydroxymatairesinol

Acetylation of curde hydroxymatairesinol with acetic anhydride and pyridine for 48 hours at room temperature, and isolation of the product by pouring onto crushed ice gave a sticky gum which dried to a powder in a desicator over potassium hydroxide. On recrystallization from ethyl-acetate-petroleum ether a white powder was obtained in 94% yield which softened at about 80-82°C but did not melt. The infrared spectrum showed no hydroxyl absorption.

Tri-O-benzoyl-hydroxymatairesinol

Treatment of crude hydroxymatairesinol with benzoyl chloride and pyridine at room temperature for 48 hours and isolation of the product in the usual manner gave an oil having a pleasant odor. The oil crystallized from petroleum ether as a colorless powder, and melted at 155-157°C. The infrared spectrum (KBr pellet) is shown in Figure 30.

Reduction of Hydroxymatairesinol

Crude hydroxymatairesinol was treated with lithium aluminium hydride under nitrogen at 40°C for 24 hours and isolation of the product by ethyl-acetate extraction and petroleum ether precipitation gave a white powder in 20.2% yield which softened at 88-90°C. The infrared spectrum showed no lactone ring carbonyl band, but a broad, hydrogen bonded hydroxyl band at 3380 cm^{-1}.

Acetylation of Reduced Hydroxymatairesinol

The reduced hydroxymatairesinol was acetylated in the usual manner and a white powder was obtained with softened at 69-72°C. The infrared

spectrum showed no hydroxyl band but two strong acetate carbonyl bands at 1760 and 1735 cm^{-1}.

Conversion of Hydroxymatairesinol to Conidendrin

Treatment of crude hydroxymatairesinol with formic acid according to the procedure of Freudenberg and Knopf (56) yielded conidendrin melting at 238-240°C. The infrared spectrum was identical to that of authentic conidendrin (Figure 29).

Dimethylhydroxymatairesinol

Purified hydroxymatairesinol dissolved in ether-methanol was methylated with diazomethane in the usual manner. The product failed to crystallize in various solvents and was precipitated into petroleum ether. The white powder softened at 85-87°C. Calculated for $C_{22}H_{26}O_7$: OCH_3, 30.8%. Found: OCH_3, 27.42%. The infrared spectrum showed hydroxyl absorption at 3470 cm^{-1} (Figure 31).

Trimethylhydroxymatairesinol

Dimethylhydroxymatairesinol was further methylated according to Purdie's method with the modification that Drierite was added to the reaction mixture and refluxing was continued for 44 hours. The product was an oily residue which could not be induced to crystallize. On precipitation into petroleum ether a yellow sticky powder was obtained which softened at ca. 80°C, calculated for $C_{23}H_{28}O_7$: OCH_3, 37.26%, Found: OCH_3, 30.36%. The infrared spectrum showed no hydroxyl band (Figure 31).

Potassium Permanganate Oxidation of Trimethylhydroxymatairesinol and Related Compounds (148).

Preliminary oxidation studies were carried out on methylbenzoin, conidendrin dimethylether, and veratric acid methyl ester as follows:

(a) Methylbenzoin (100 mg, 0.44 mM) was dissolved in 5 ml of acetone, 300 mg of magnesium sulphate in 5 ml of water was added and the mixture was

stirred magnetically while 140 mg (2mM) of potassium permanganate in 4 ml of water was added drop by drop. The reaction was allowed to proceed at 50°C for 5 hours then the mixture was cooled and sulphur dioxide gas was introduced until a clear, colorless solution was obtained. The acidic solution was extracted 5 times with ether and the ether extract was washed with saturated sodium bicarbonate solution, then with water, dried over anhydrous sodium sulphate and evaporated to dryness. A mixture of colorless crystalline and yellow oily products was obtained in 72.2% yield. This mixture contained unreacted methylbenzoin and methylbenzoate as shown by TLC using authentic samples for comparison, S-23 as developing solvent and R-10 as spray reagent. The aqueous sodium bicarbonate solution was acidified with hydrochloric acid, extracted with ether, washed with water, dried and evaporated. A colorless crystalline product was obtained in 72.2% yield, which was identified as benzoic acid by thin layer chromatography and by mixed melting point.

(b) The oxidation was repeated on 67.8 mg of dimethylconidendrin, 500 mg of potassium permanganate and 900 mg of magnesium sulphate. Four oxidation products were detected but not identified because of the lack of reference samples.

(c) Potassium permanganate oxidation was carried out on veratric acid methylester in dry acetone, and in the aqueous medium and the oxidized samples were examined by thin layer chromatography as described in (a).

Trimethylhydroxymatairesinol (66.5 mg) was dissolved in 20 ml of acetone and 900 mg of magnesium sulphate was added in 10 ml of water, followed by 410 mg of potassium permanganate in 20 ml of water. The reaction mixture was stirred and kept at 50°C for 4 hours and then was worked up as in (a) except that the sodium bicarbonate extraction was omitted. The ether extract was concentrated and examined by thin layer chromatography as before.

Standard samples of veratric acid and veratric acid methyl ester were used as reference compounds.

The chromatographic examination showed that the oxidation product contained unreacted starting material and about 10 to 12 unidentified intermediate oxidation products. It was taken up in 25 ml of dry acetone and 400 mg of solid potassium permanganate and 900 mg of solid magnesium sulphate were added and the oxidation procedure was carried out as before. The final oxidation product was examined on a thin layer chromatoplate.

APPENDICES

Appendix I

Calculation of the Proton Nuclear Resonance Shielding Values (τ).

The τ-Values were calculated according to the conventional method (149) and as follows:

$$\tau \text{ (in ppm)} = 10.000 - 10^6 \quad \frac{\nu_{obs.} - \nu_{Me_4Si}}{\nu_{Me_4Si}}$$

where $\nu_{Me_4Si} = 60 \times 10^6$ cps, and $\nu_{obs.} - \nu_{Me_4Si}$ were measured as a distance in mm between the observed and standard peaks and were converted to cps units upon multiplication with a conversion factor (f) obtained from side band measurements.

Table XV

Constants for τ Value Calculation

Compound	Side band (cps)	Average distance (mm)	f (cps/mm)
Vanillyl alcohol	300.2	243.43	1.23
Conidendrin	300.2	246.80	1.22
HMR	300.2	246.51	1.22

Table XVI

τ-Values from NMR Spectrum of Vanillyl Alcohols

Number of peaks	$\nu_{obs} - \nu_{Me_4Si}$ mm	cps.	cps/60	τ (ppm)
1	94.6	116.36	1.939	8.061
2	96.3	118.45	1.974	8.026
3	97.9	120.42	2.007	7.993
4	99.9	122.88	2.048	7.952
5	153.4	188.68	3.145	6.238
6	184.5	225.71	3.762	6.238
7	199.1	244.89	4.082	5.918
8	203.2	249.94	4.166	5.834
9	212.1	260.88	4.348	5.652
10	215.9	265.56	4.426	5.574
11	219.9	270.48	4.508	5.492
12	326.8	401.96	6.699	3.301
13	335.8	413.03	6.884	3.116
14	355.7	437	7.292	2.708

Table XVII

τ-Values from NMR Spectrum of Conidendrin

Number of peaks	$\nu_{obs} - \nu_{Me_4Si}$ mm	cpc	cps/60	τ (ppm)
1	97.6	119.07	1.985	8.015
2	99.6	121.51	2.025	7.975
3	101.3	123.59	2.060	7.940
4.	103.2	125.90	2.098	7.902
5	122.1	148.96	2.483	7.517
6	123.6	150.79	2.513	7.487
7	143.1	174.58	2.910	7.090
8	187.0	228.14	3.802	6.159
9	188.9	230.46	3.841	5.927
10	200.3	244.37	4.073	5.927
11	205.9	251.20	4.187	5.813
12	299.9	365.88	6.098	3.902
13	335.1	433.22	7.220	2.780
14	364.2	444.32	7.405	2.595
15	377.3	454.21	7.570	2.430

Table XVIII

τ-Values from NMR Spectrum of Hydroxymatairesinol (HMR)

Number of peaks	$\nu_{obs} - \nu_{Me_4Si}$ mm	cps	cps/60	τ (ppm)
1	52.4	63.93	1.066	8.934
2	56.3	68.69	1.145	8.855
3	57.8	70.52	1.175	8.826
4	63.2	77.10	1.285	8.715
5	97.8	119.32	1.989	8.011
6	98.9	120.66	2.011	7.989
7	100.5	122.61	2.044	7.956
8	102.5	125.05	2.084	7.916
9	135.5	165.31	2.755	7.245
10	141.4	172.51	2.875	7.125
11	170.8	208.37	3.473	6.527
12	185.5	226.31	3.772	6.228
13	200.6	244.73	4.079	5.921
14	213.0	259.86	4.331	5.669
15	217.7	265.59	4.427	5.573
16	222.1	270.96	4.516	5.484
17	231.6	282.55	4.709	5.291
18	314.7	383.93	6.399	3.601
19	321.8	392.60	6.543	3.457
20	328.5	400.77	6.680	3.320
21	333.3	406.63	6.777	3.223

Appendix II

Infrared Spectra

Table XIX

Index of Infrared Spectra

Compound	Symbol	Origin	Figure
Hydroxymatairesinol	HMR	Isolated	29,30,31
Hydroxymatairesinol-benzoate	HMRB	Synthesized	30
Dimethylhydroxymatairesinol	DMHMR	Synthesized	31
Trimethylhydroxy-matairesinol	TMHMR	Synthesized	31
Conidendrin		Isolated	29
Conidendrin		a	
Hemlock leucoantho-cyanidin	LAC	Isolated	32
Synthetic leucoyanidin	LAC	b	32
Tetramethyl ether of leucocyanidin		c	32
Dahlia cyanidin		Isolated	32

[a] A gift from the Crown-Zellerbach Corp., Camas, Washinton.

[b] A gift from Dr.J.A.F. Gardner, Forest Products Laboratories, B.C.

[c] From gum of Butea frondosa; a gift from Dr. T.R. Seshadri, University of Delhi.

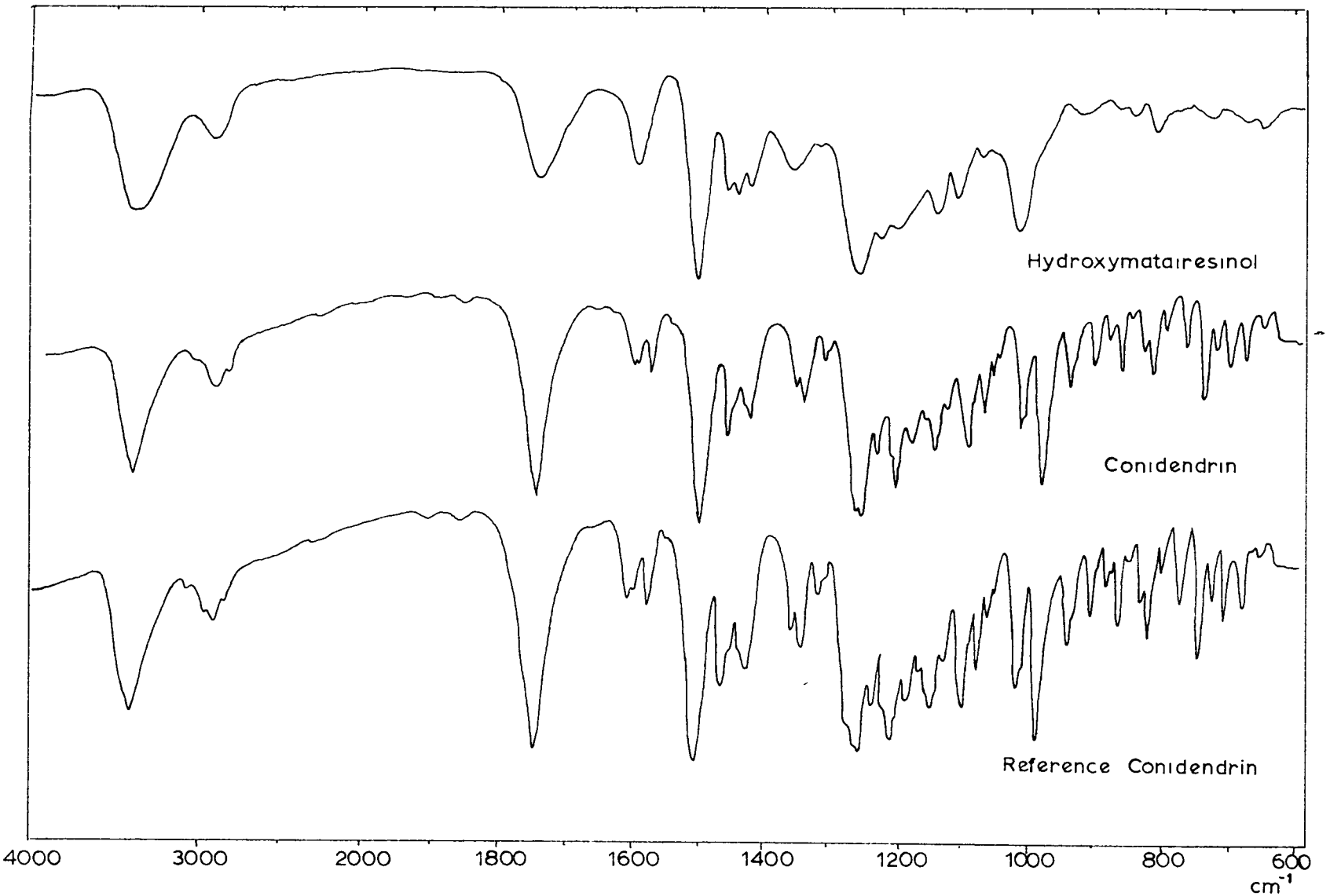


FIGURE 29 Infrared Spectra of Lignans from Hemlock Wood

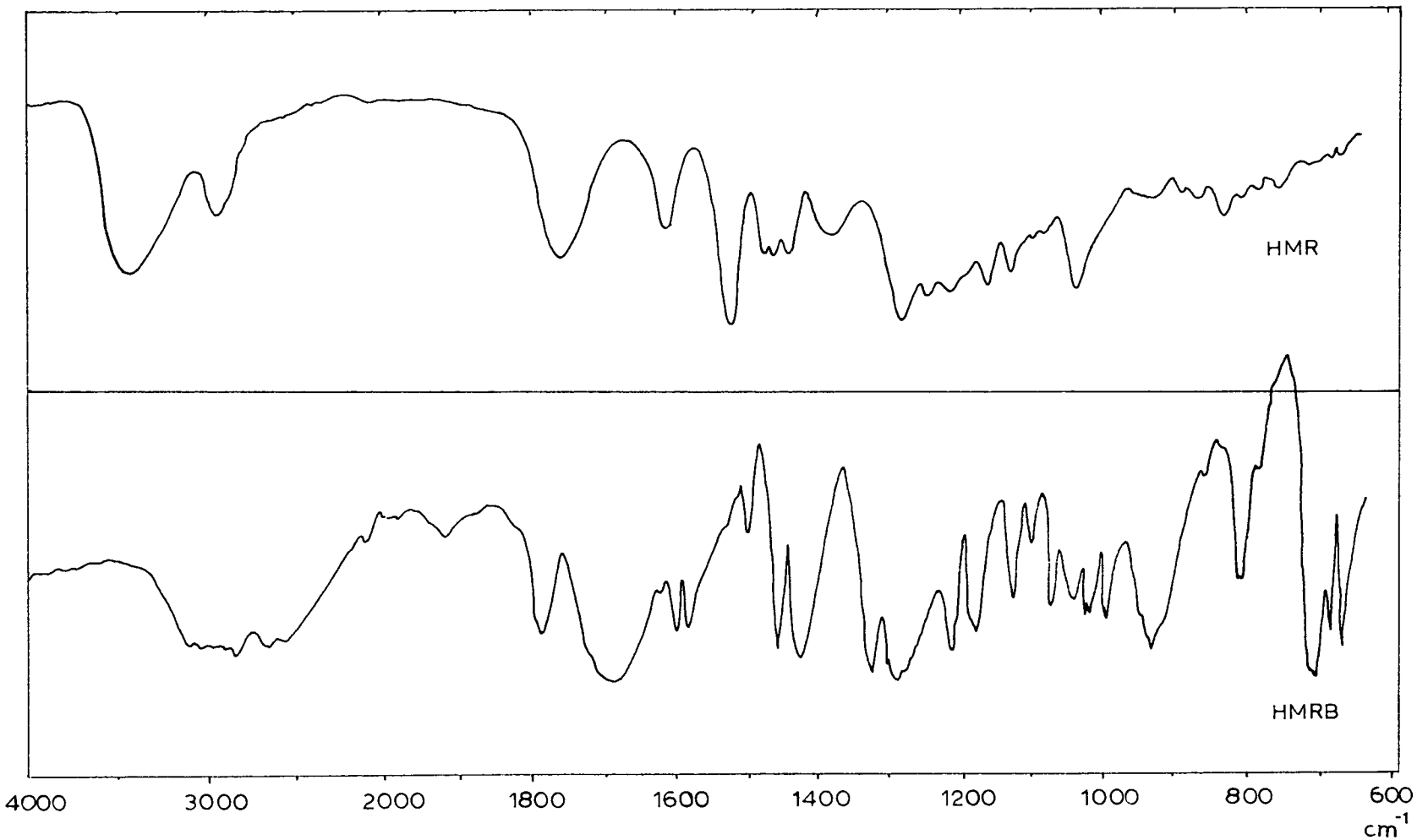


FIGURE 30 Infrared Spectra of Hydroxymatairesinol (HMR) and HMR-benzoate.

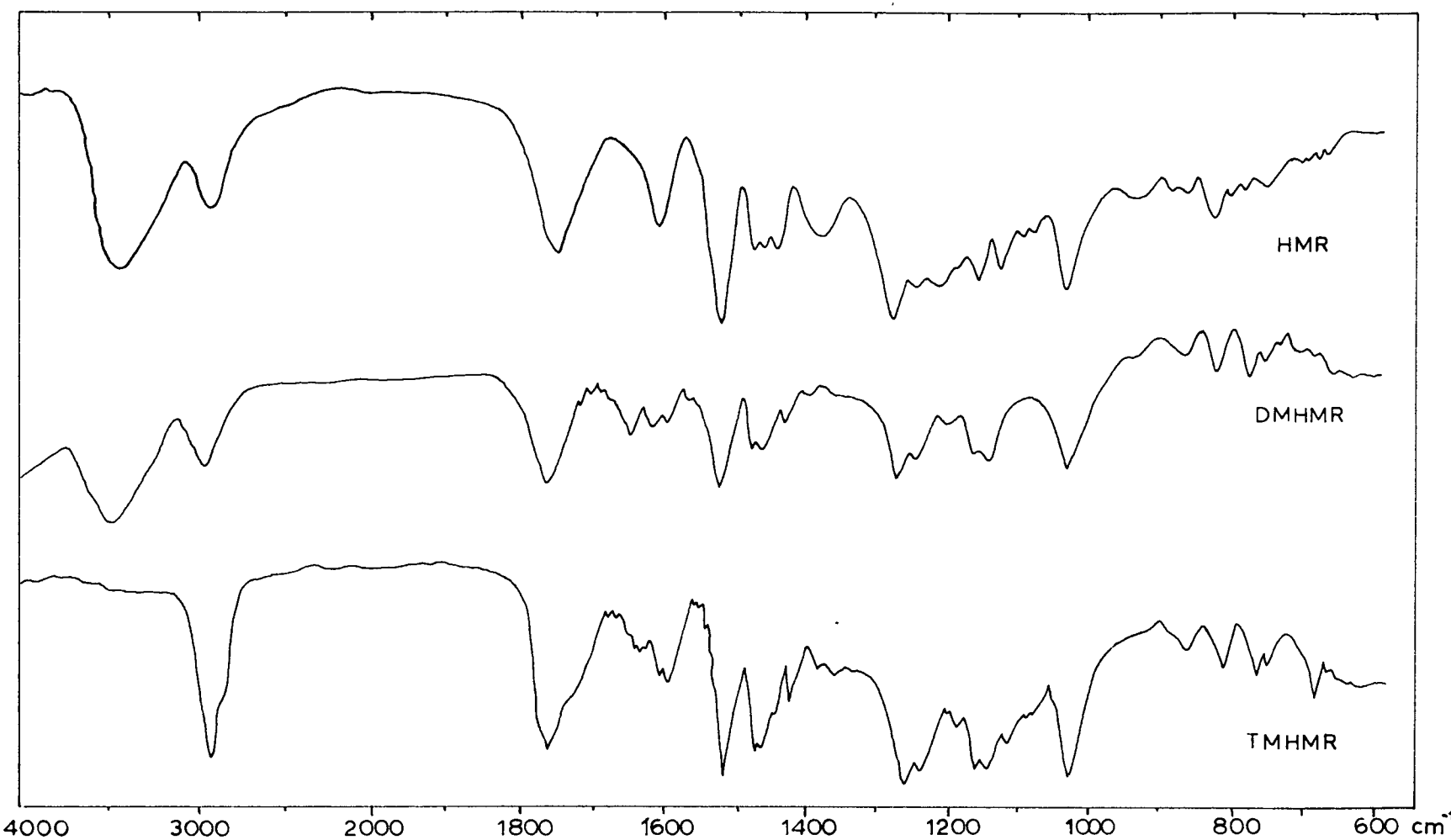


FIGURE 31 Infrared Spectra of Hydroxymatairesinol and Derivatives.
(DM = dimethyl; TM = trimethyl)

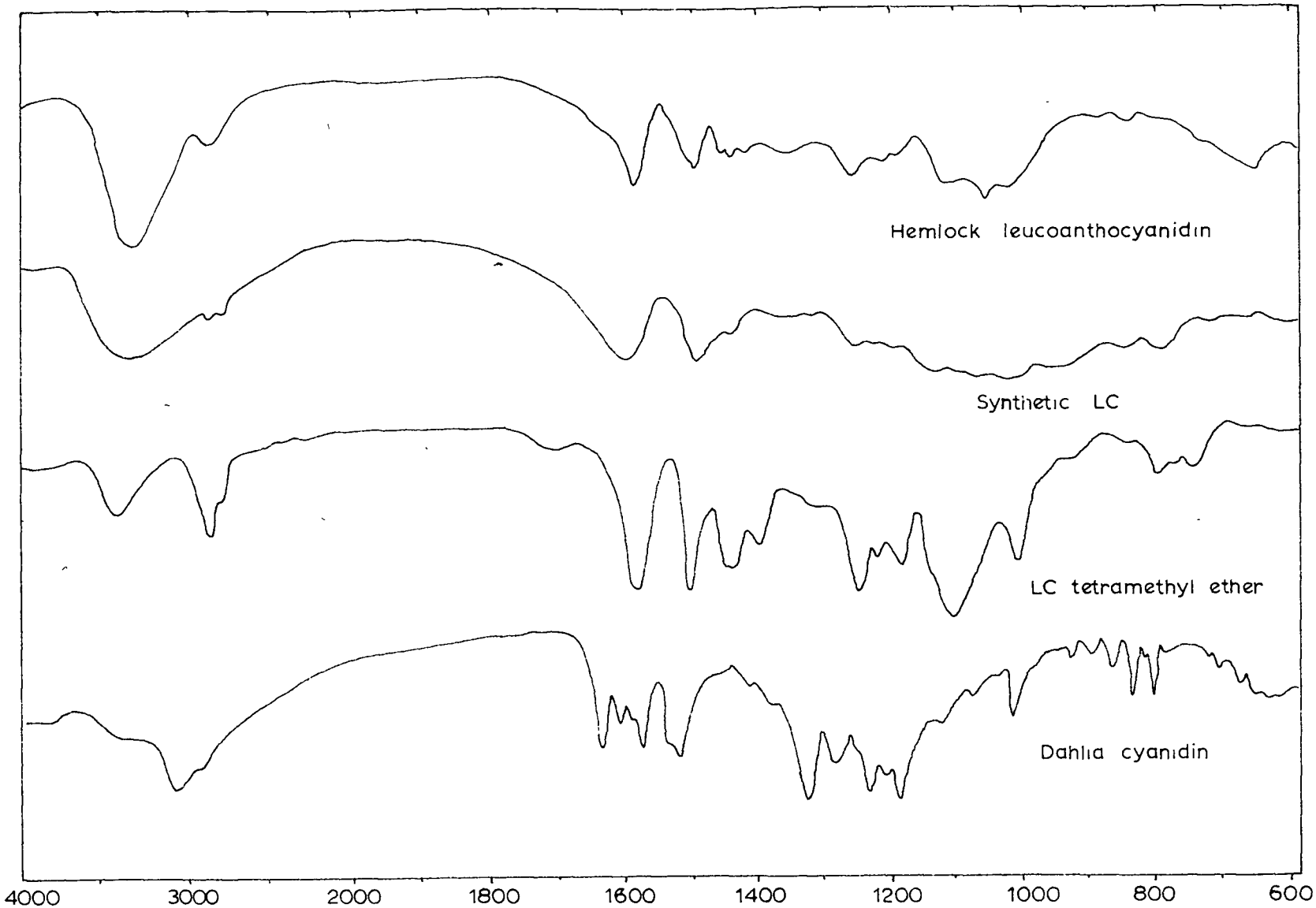


FIGURE 32. Infrared Spectra of Hemlock Leucoanthocyanidin and Related Compounds.

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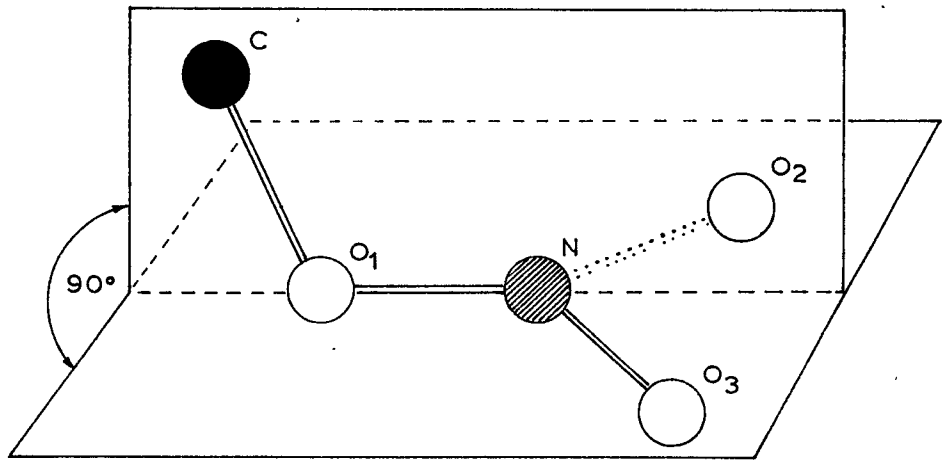


FIGURE 2. The Structure of the Organic Nitrate Group.

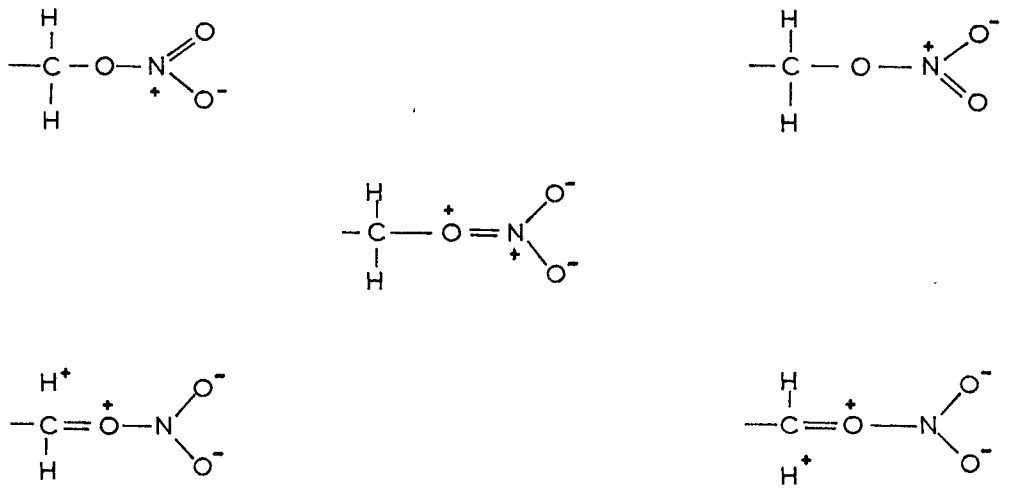
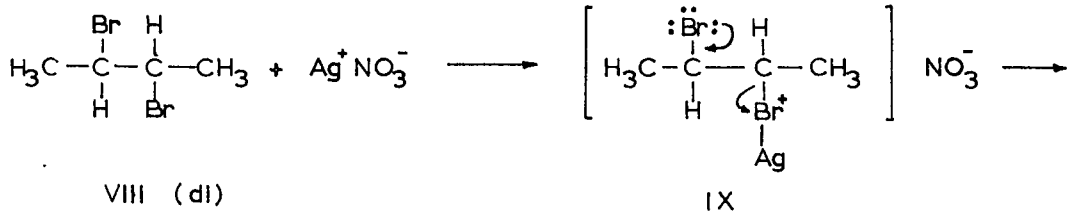
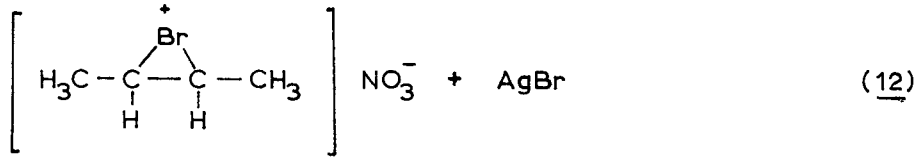


FIGURE 3. Resonance Structures of the Nitroxy Group.



VIII (dl) IX



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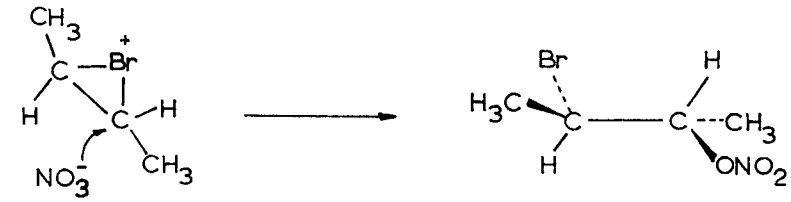
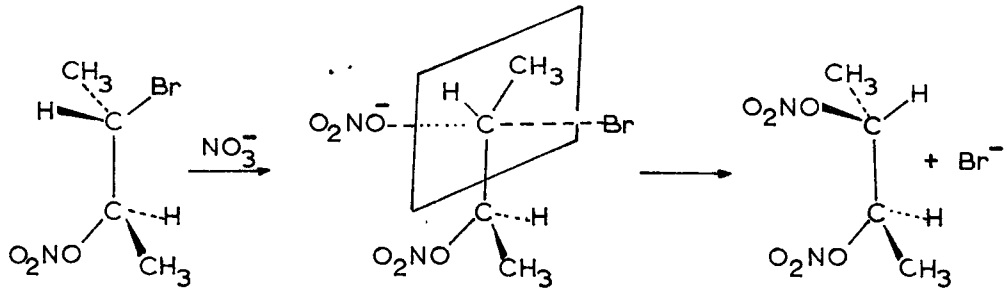
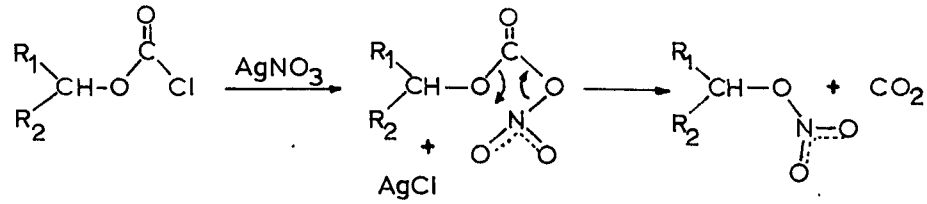


X XI (13)



XI XII XIII (14)



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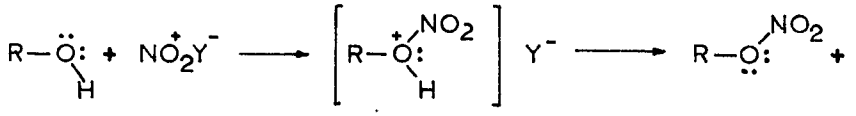

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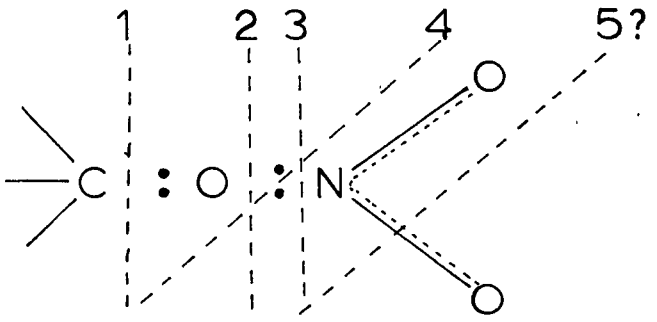
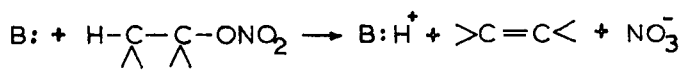
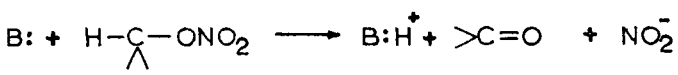
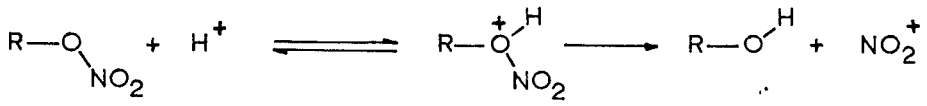


FIGURE 4. Modes of Scission of the Nitroxy Group.

TABLE IV. Reaction Mechanisms.

	Reaction Mechanisms	Mode of Scission
1. Electrophilic substitution on oxygen : 	S_{EO}	3
2. Nucleophilic substitution on carbon: 	S_{NC}	1
3. Nucleophilic substitution on nitrogen: 	S_{NN}	3
4. β-Hydrogen elimination: 	$E_{C=C}$	1
5. α-Hydrogen elimination: 	$E_{C=O}$	2



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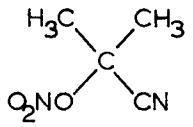

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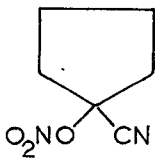

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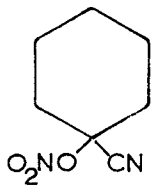

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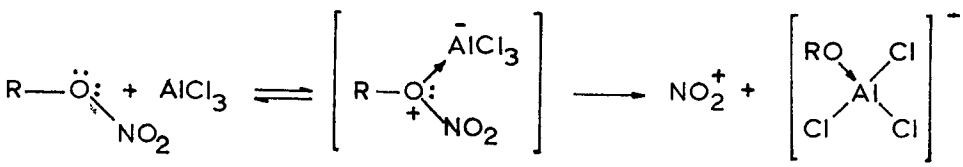

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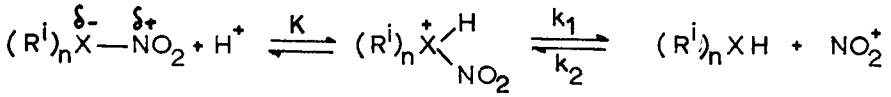

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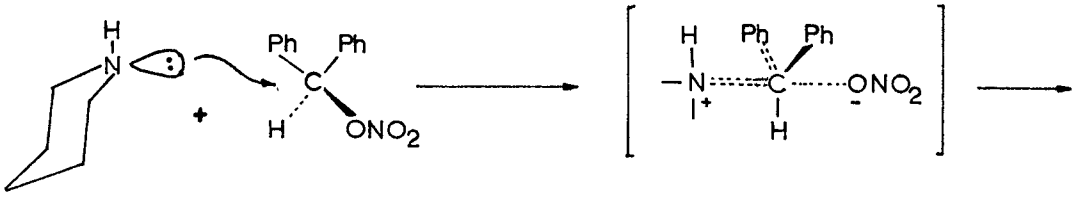

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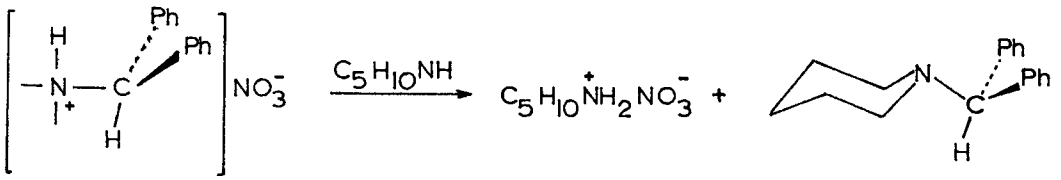
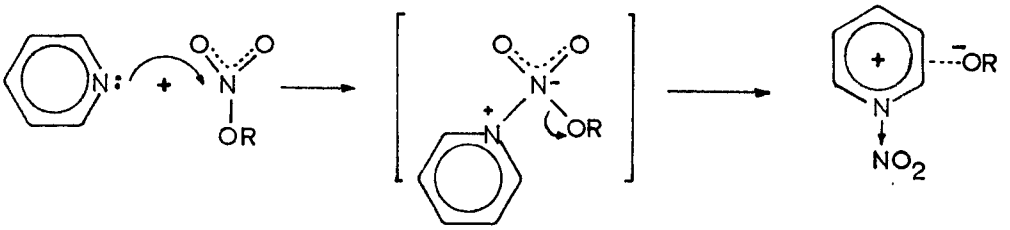


$$PhCH_2ONO_2 + OH^- \longrightarrow PhCH_2OH + NO_3^- \qquad (23)$$

$$t\text{-}BuONO_2 + 2\ H_2O \longrightarrow t\text{-}BuOH + H_3O^+ + NO_3^- \qquad (24)$$

$$^{18}OH + \text{-C-O-NO}_2 \longrightarrow H^{18}O\text{-C-} + NO_3^- \qquad (S_{NC}) \qquad (25)$$

$$\text{-C-O-NO}_2 + {}^{18}OH^- \longrightarrow \text{-C-O}^- + H^{18}ONO_2 \qquad (S_{NN}) \qquad (26)$$



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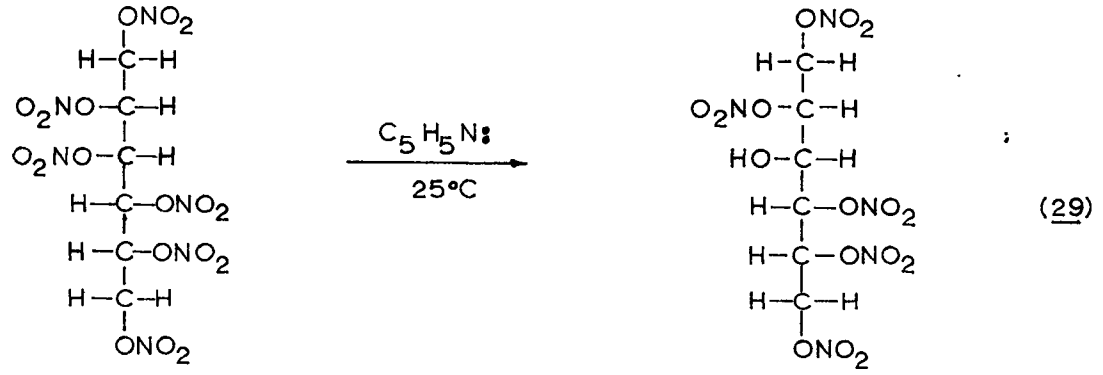
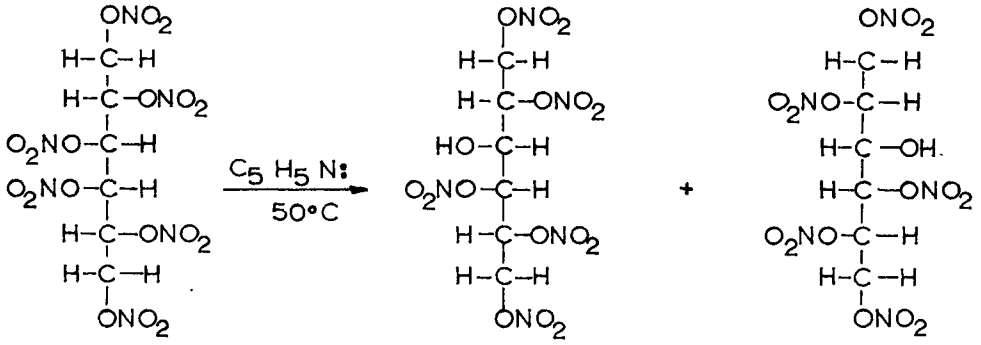

ONO2
H–C–H
O2NO–C–H
O2NO–C–H
H–C–ONO2
H–C–ONO2
H–C–H
ONO2
C5 H5 N:
25°C
ONO2
H–C–H
O2NO–C–H
HO–C–H
H–C–ONO2
H–C–ONO2
H–C–H
ONO2

(29)


ONO2
H–C–H
H–C–ONO2
O2NO–C–H
O2NO–C–H
H–C–ONO2
H–C–H
ONO2
C5 H5 N:
50°C
ONO2
H–C–H
H–C–ONO2
HO–C–H
O2NO–C–H
H–C–ONO2
H–C–H
ONO2
+
ONO2
H–C–H
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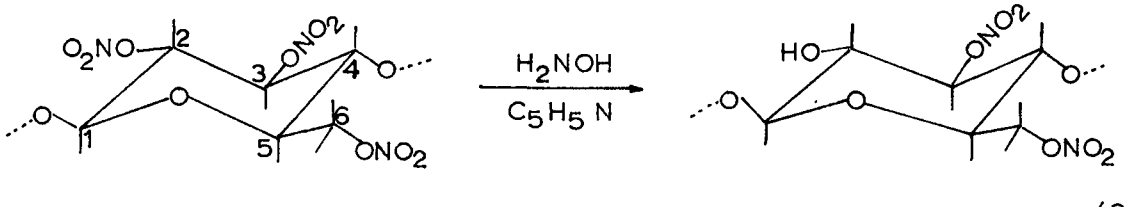
H–C–OH
H–C–ONO2
O2NO–C–H
H–C–H
ONO2

(30)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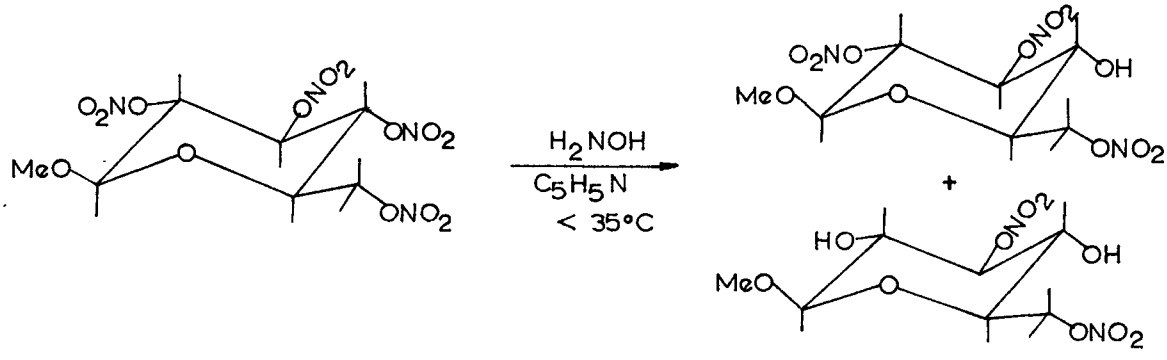
2
ONO2
3
4
O
O
1
O
6
5
ONO2
H2NOH
C5H5 N
HO
ONO2
O
O
O
ONO2

(31)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MeO
O
ONO2
ONO2
H2NOH
C5H5 N
< 35°C
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O
ONO2
+
HO
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OH
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O
ONO2

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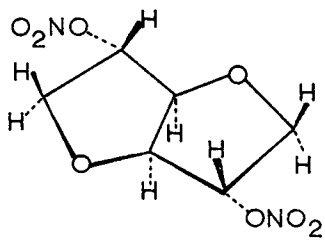

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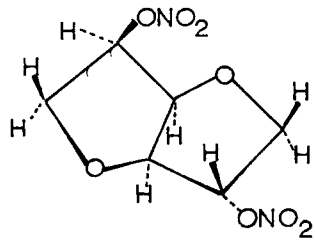

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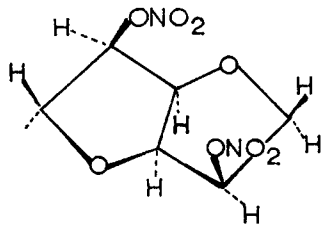

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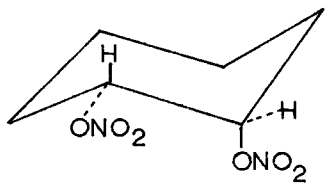

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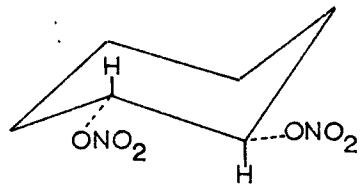

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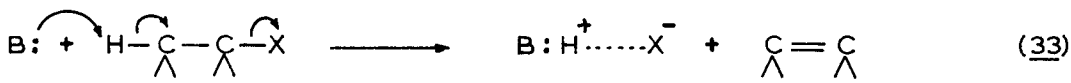
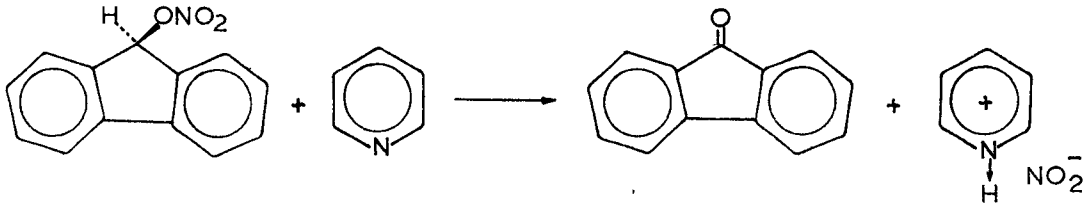


(33)

(34)



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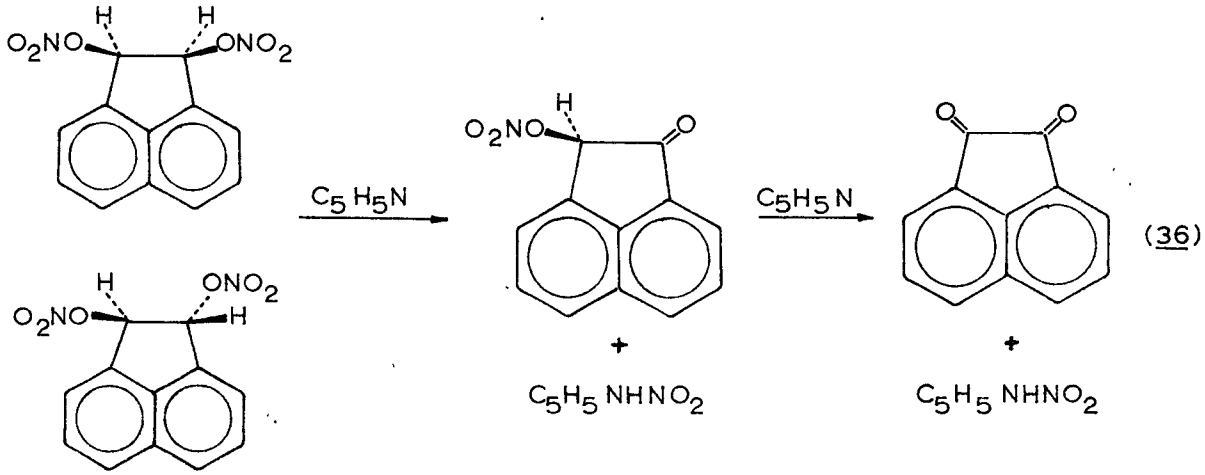

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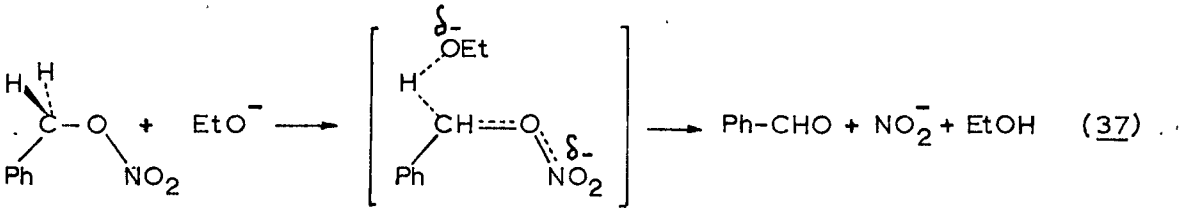

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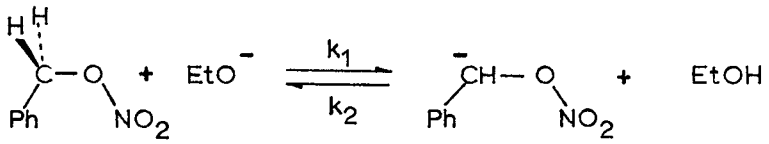

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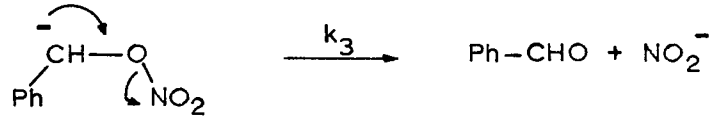

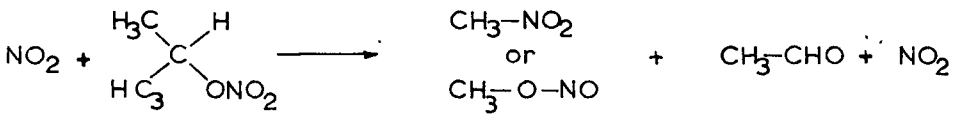

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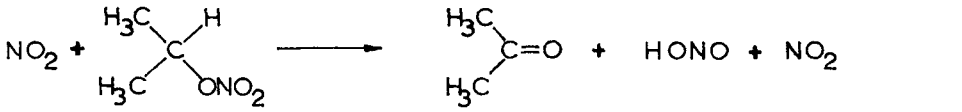

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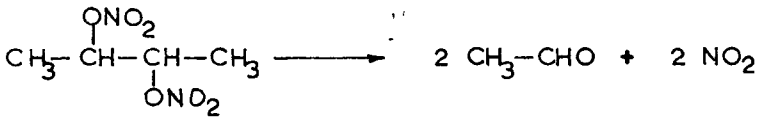
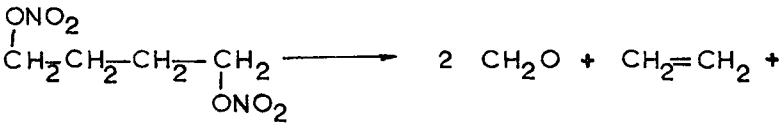


(47)



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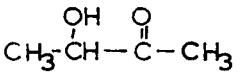
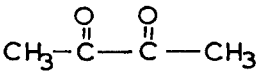


XXIII



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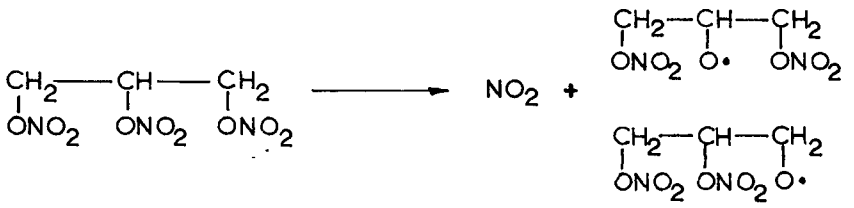

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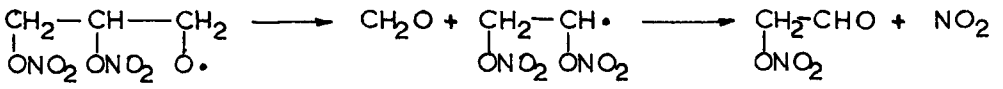

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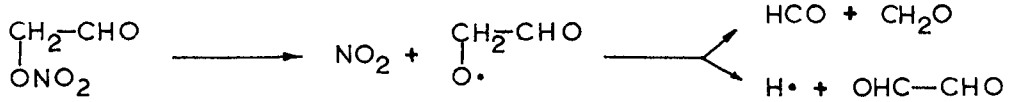

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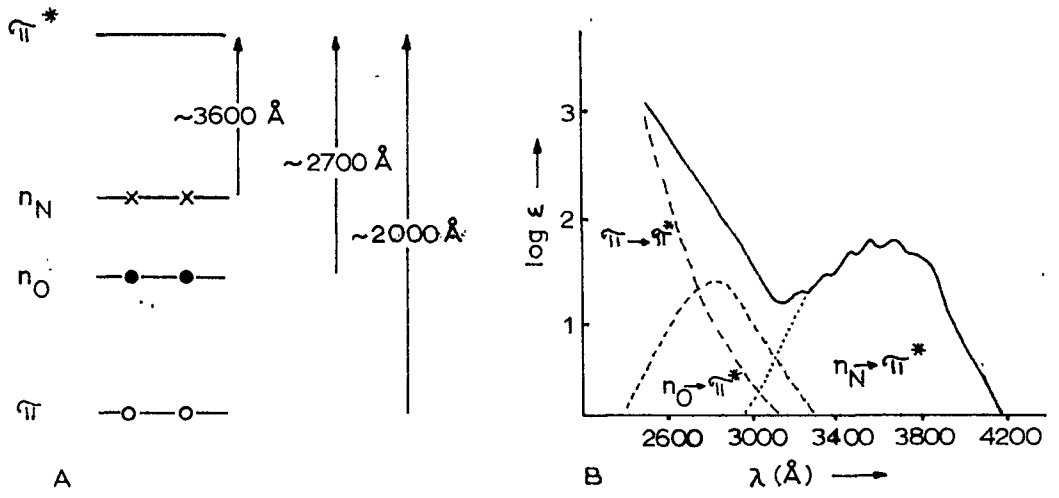


Figure 5.

A: Energy Levels of Molecular Orbitals and Possible Electronic Transitions for Nitrite Esters.

B: Typical Electronic Spectrum of a Nitrite Ester (2-butyl nitrite in ether (95)). The dotted lines represent the estimated separation of the various transitions.

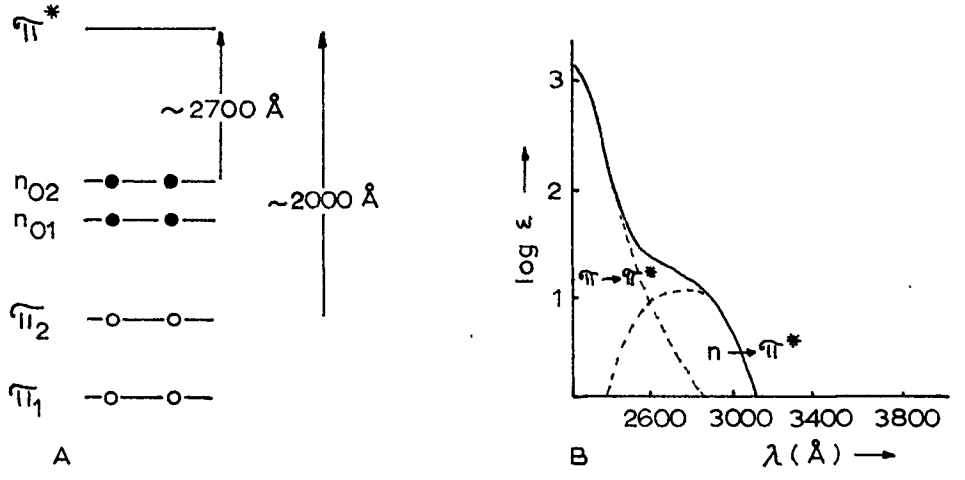
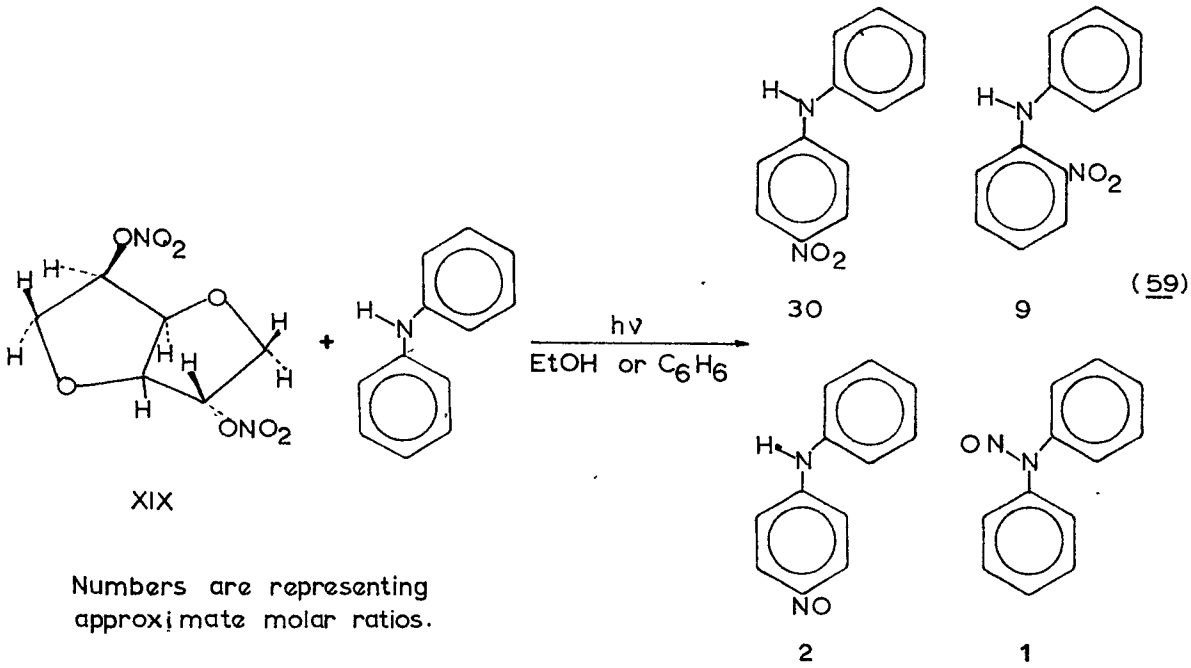


Figure 6.

A: Energy Levels of Molecular Orbitals and Possible Electronic Transitions for Nitrate Esters.

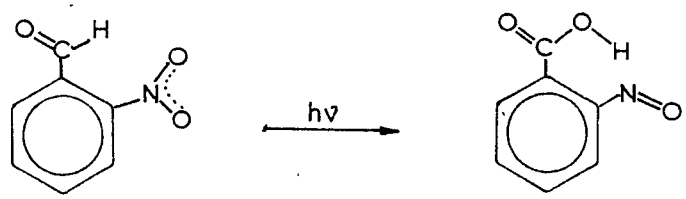
B: Typical Electronic spectrum of a Nitrate Ester (2-butyl nitrate in ethanol (95)). The dotted lines represent the estimated separation of the various transitions.



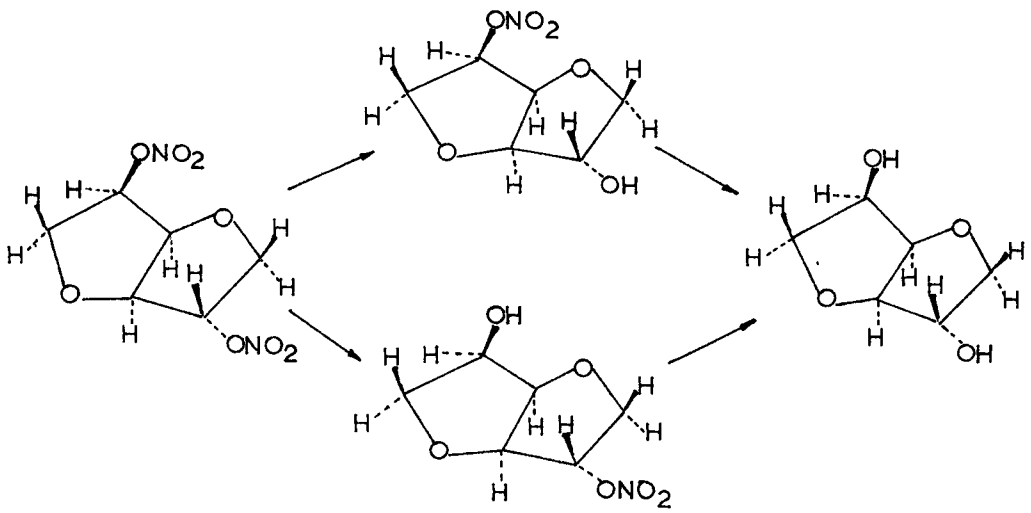
XIX

(59)

Numbers are representing approximate molar ratios.

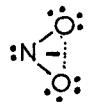
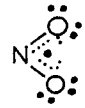
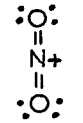
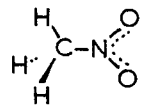
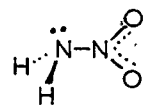
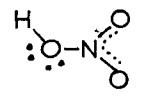
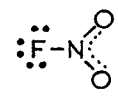
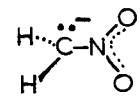
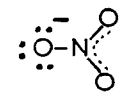
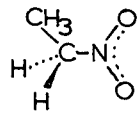
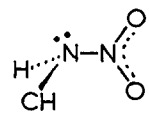
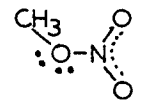


(60)



(61)

TABLE IX. Structural Properties* of Nitrogen-Oxygen Compounds.

Valence electrons N—O			N≡O 11 1.150 Å			
Valence electrons ∠ ONO N—O	 18 115.4° 1.236 Å		 17 134.1° 1.188 Å			 16 180.0° 1.154 Å
Valence electrons ∠ ONO N—O X—N		 24 127° 1.22 Å 1.48 Å	 24	 24 130° 1.206 Å 1.405 Å	 24 125° 1.23 Å 1.35 Å	
Valence electrons ∠ ONO N—O X—N		 24 ---- ---- ----	—	 24 120.5° 1.24 Å 1.25 Å	—	
Valence electrons ∠ ONO N—O X—N		 30 --- --- ---	 30 --- --- ---	 30 125.3° 1.26 Å 1.36 Å	—	

* Values taken from (133)

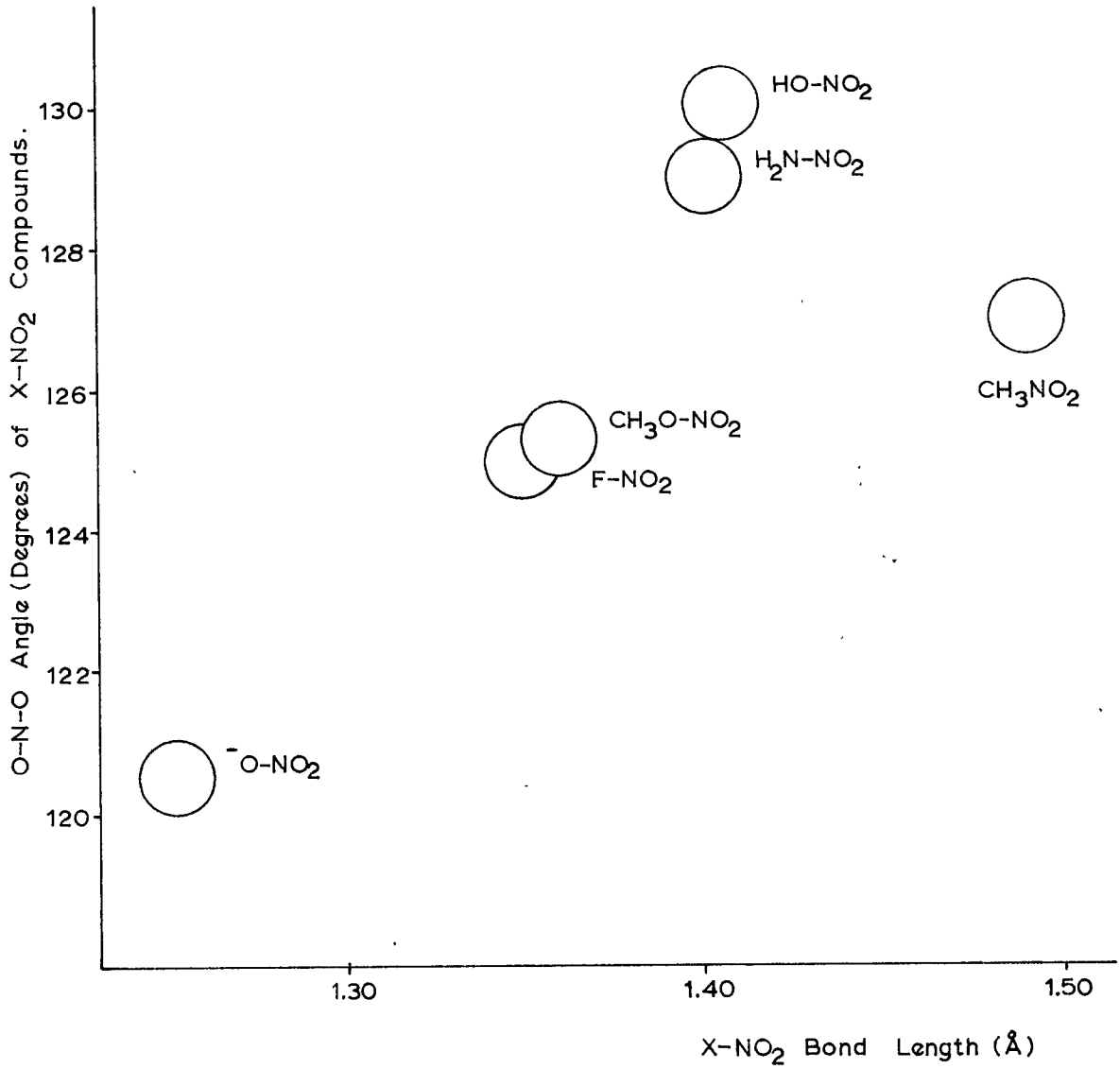
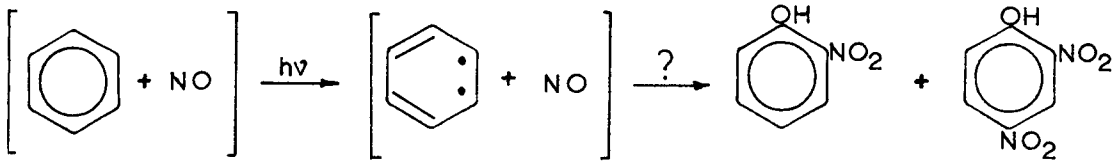
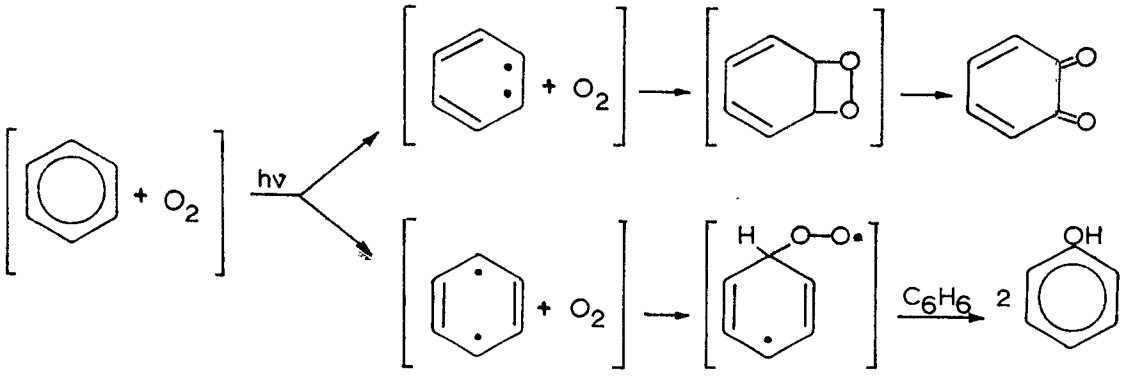


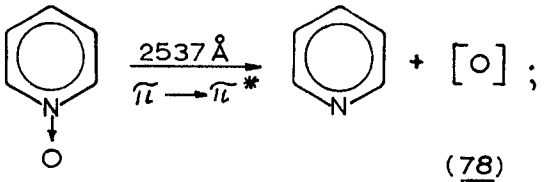
FIGURE 7. Correlation of ∠ ONO and $X-NO_2$ Bond Length in $(R^i)_n XNO_2$ Compounds.



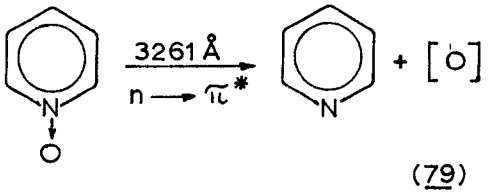
(67)



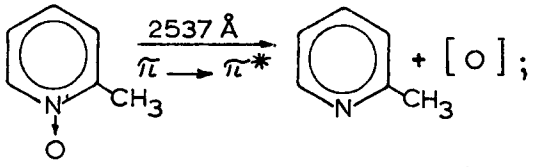
(68)



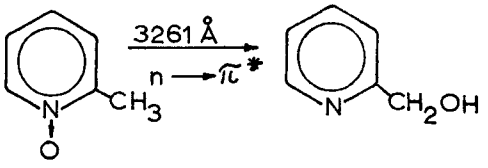
(78)



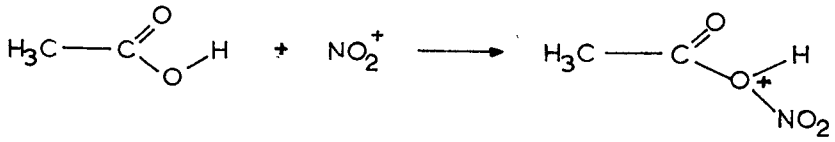
(79)



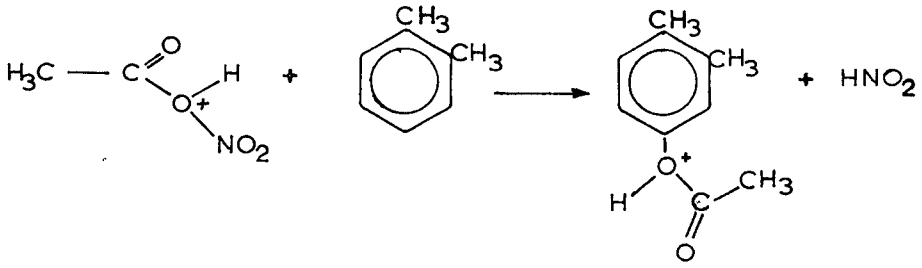
(80)



(81)



(86)



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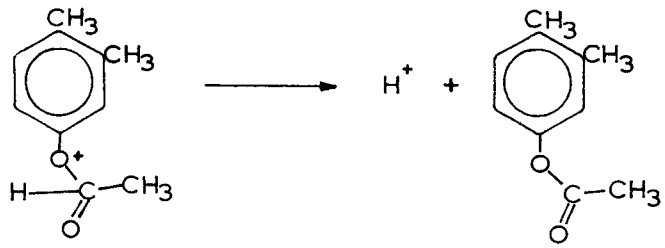

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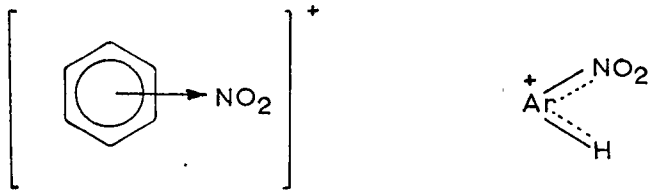
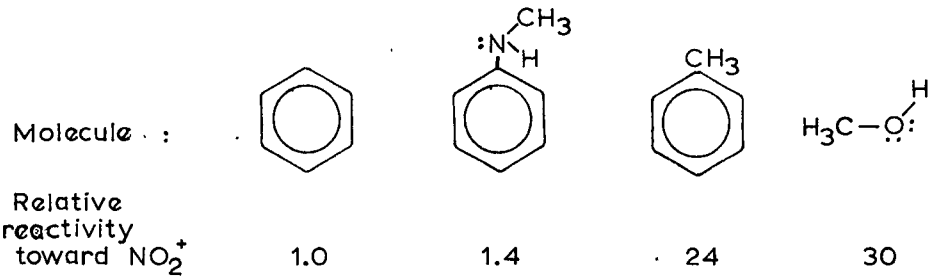


XXVII XXXVIII



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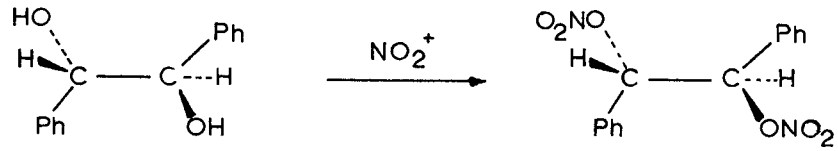
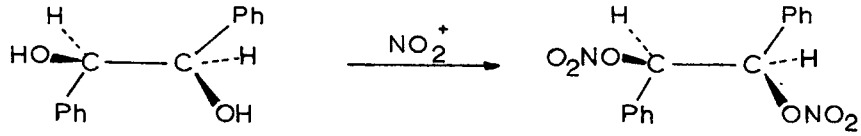
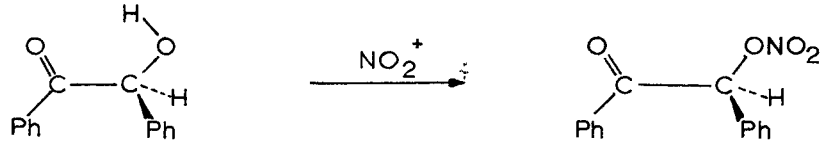


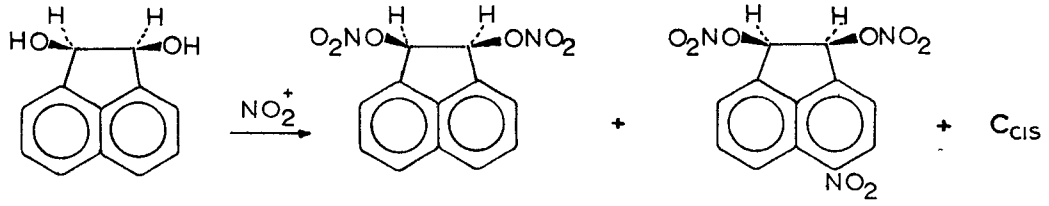
XXIX XXX (93)



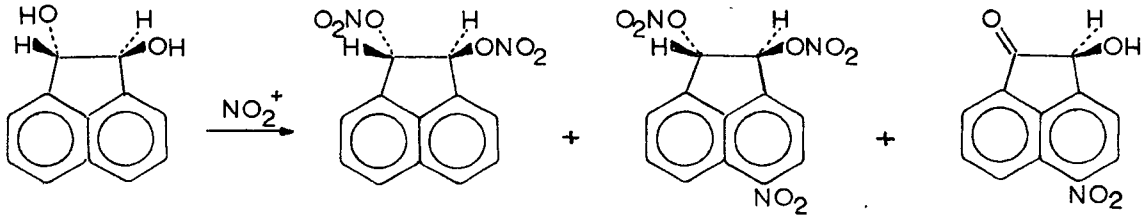
XXXI XXXII (94)



XXXIII XXXIV (95)



XXXV XXXVI (A_{cis}) XXXVII (B_{cis}) XXXVIII (96)



XXXIX XL (A_{trans}) XLI (B_{trans}) XLII (C_{trans}) (97)

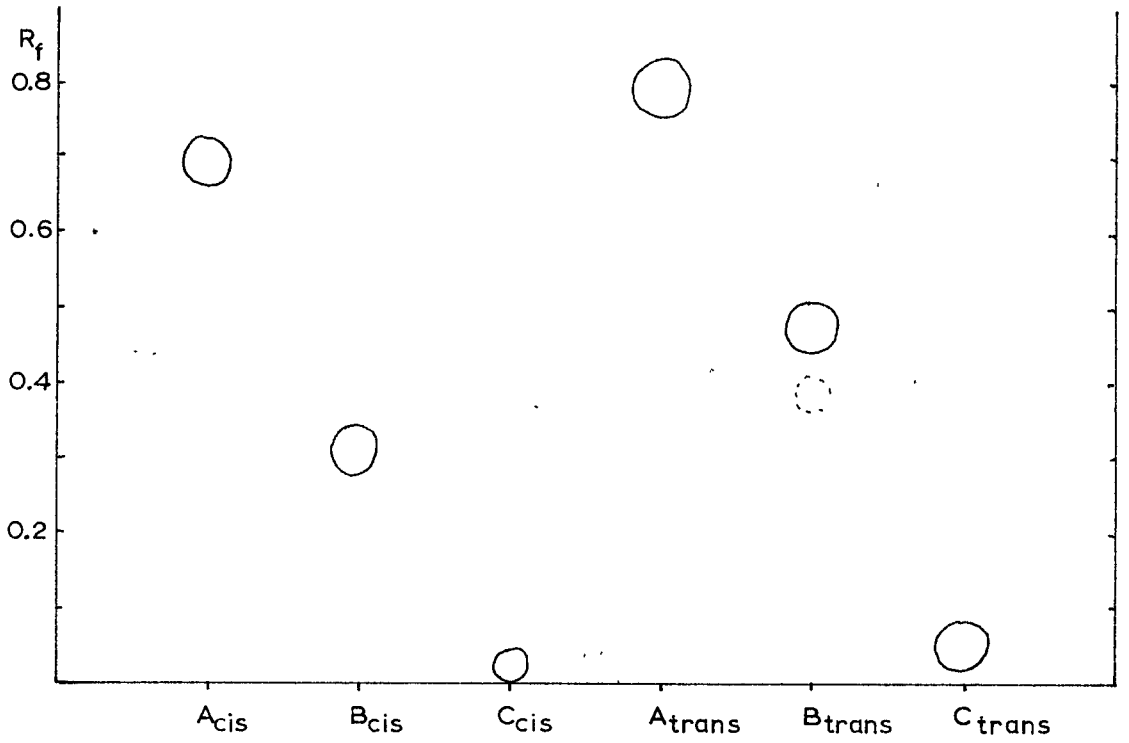


FIGURE 8. Thin-layer Chromatography of Nitration Products from cis- and trans-1,2-Acenaphthenediols (M-3, S-1, R-1)

TABLE XII Nitration Products from cis-and trans-1,2-Acenaphthenediols.

Product		Formula		R_f (M-3 S-1 R-1)	M.P. (°C)	N %	
Symbol	Isomer	Number	Empirical			Obt.	Calc.
A	cis-	XXXVI	$C_{12}H_8O_6N_2$	0.69	129.5–132.5	10.03	10.15
	trans-	XL		0.79	98–100	10.07	
B	cis-	XXXVII	$C_{12}H_7O_8N_3$	0.32	95 - 98	12.50	13.10
	trans-	XLI		0.47	oil	11.81	
C	cis-	XXXVIII	$C_{12}H_7O_4N$	0.02	----	8.89	6.10
	trans-	XLII		0.05	210 - 215	6.36	

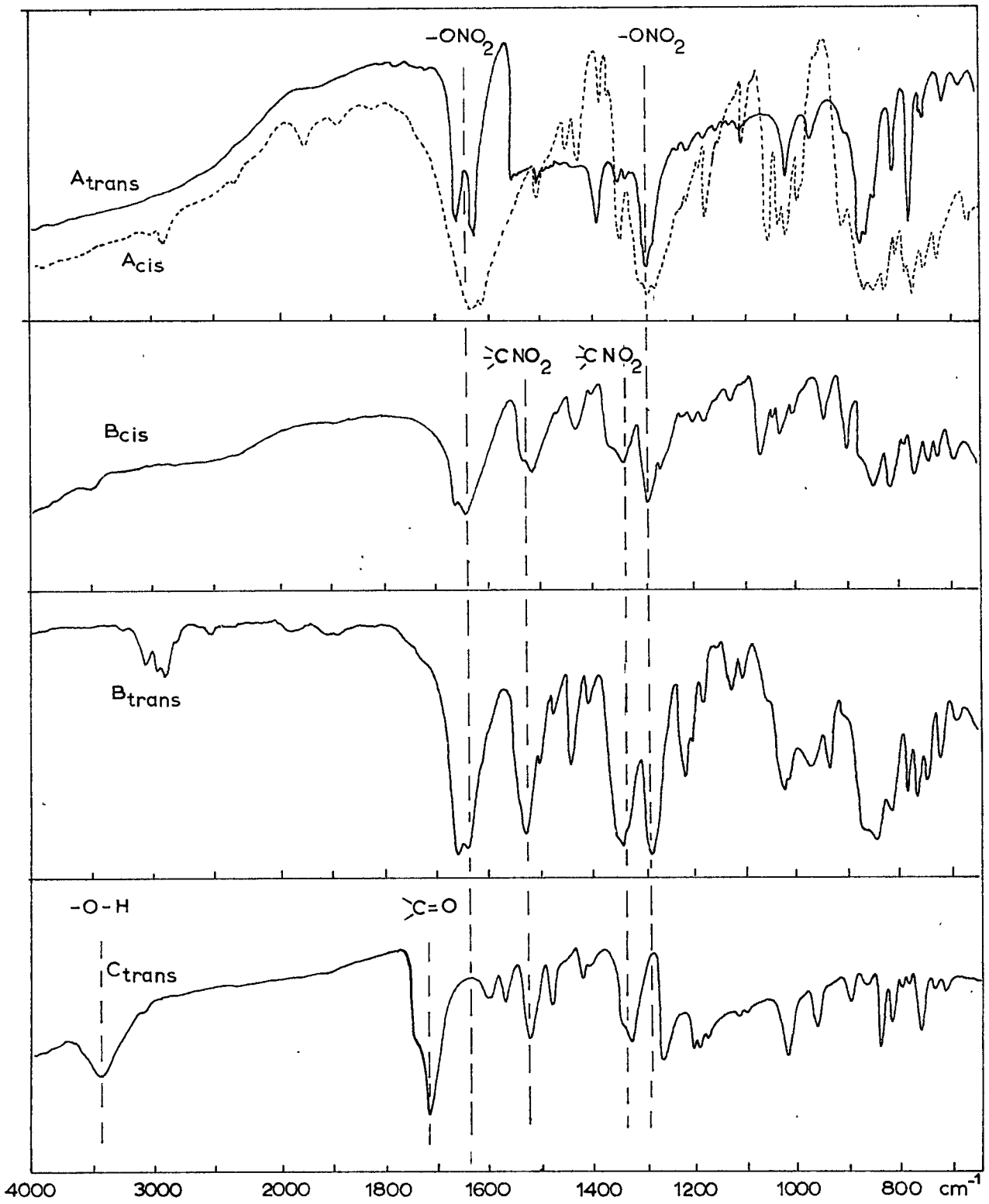


FIGURE 9. Infrared Spectra of Nitration Products of cis- and trans-1,2-Acenaphthenediol.

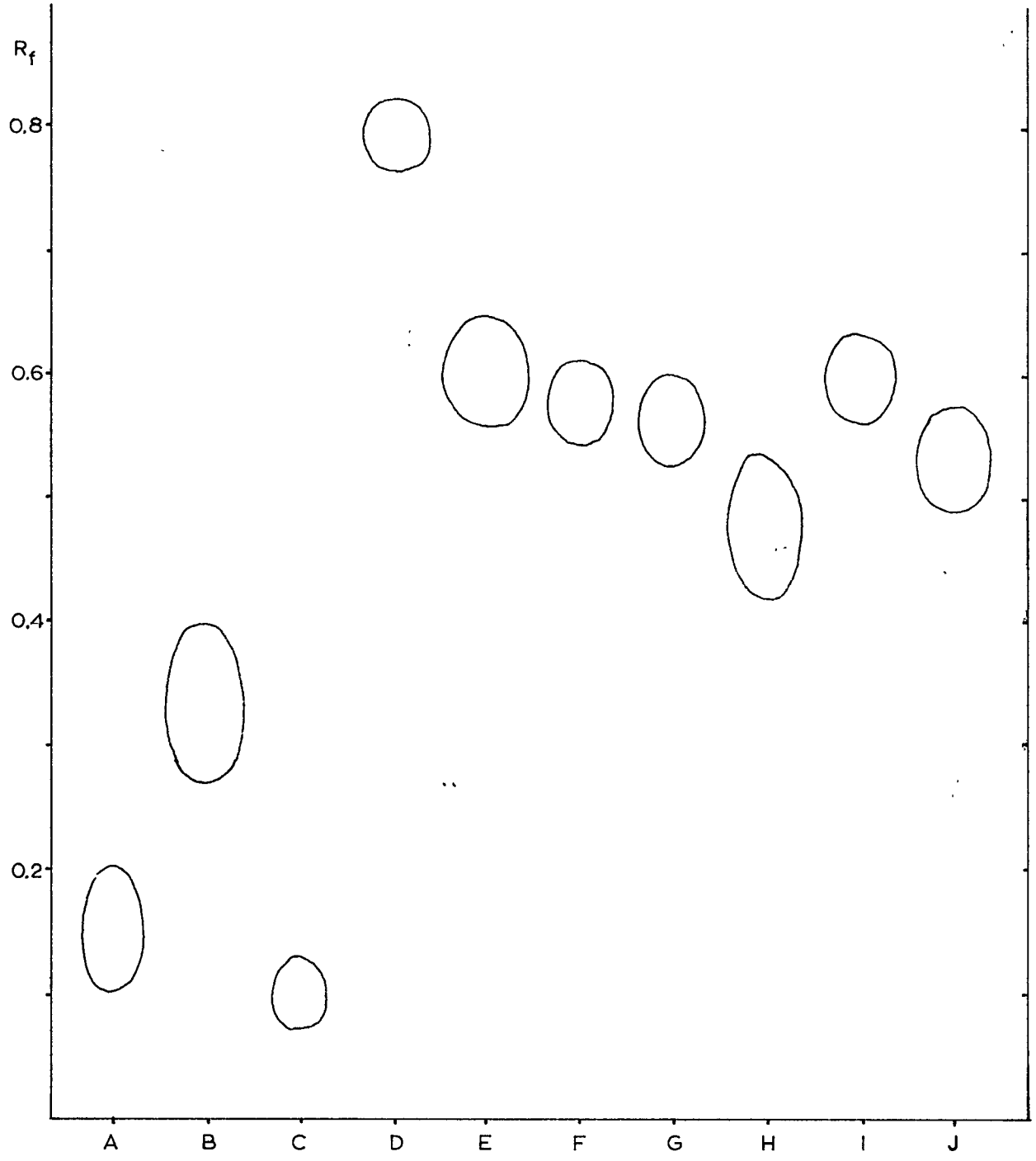


FIGURE 10. Chromatographic Pattern of Representative Nitrate Esters. TLC; M-3, S-1, R-2. The compounds and calculated R_M, $\Delta F°$, and K values are listed in Table XIV.

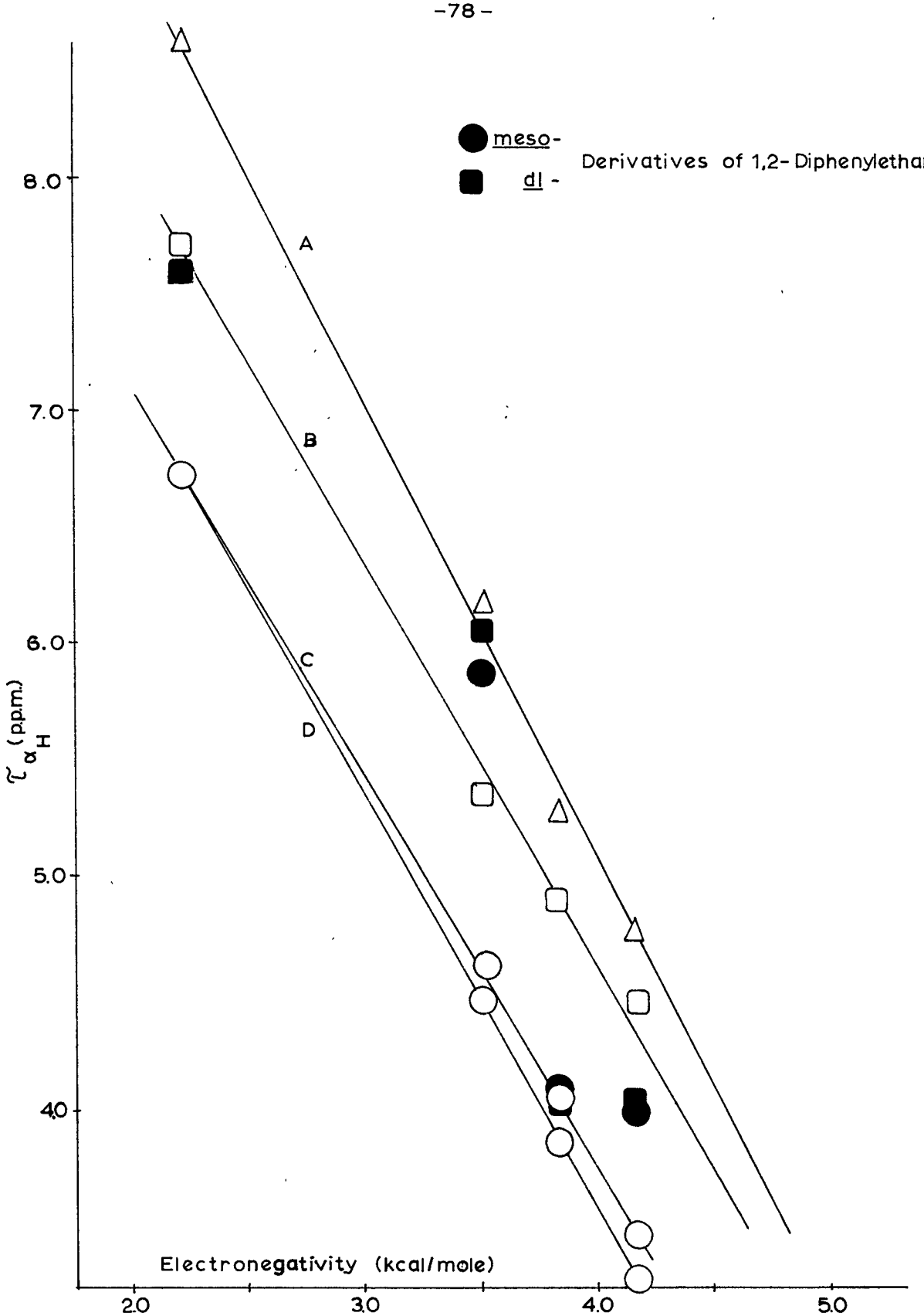


FIGURE 11. Correlation of τ_{α_H} with Group Electronegativities in (A) trans-1,2-Cyclohexane-, (B) Benzyl-, (C) trans- and (D) cis-1,2-Acenaphthenyl-Derivatives.

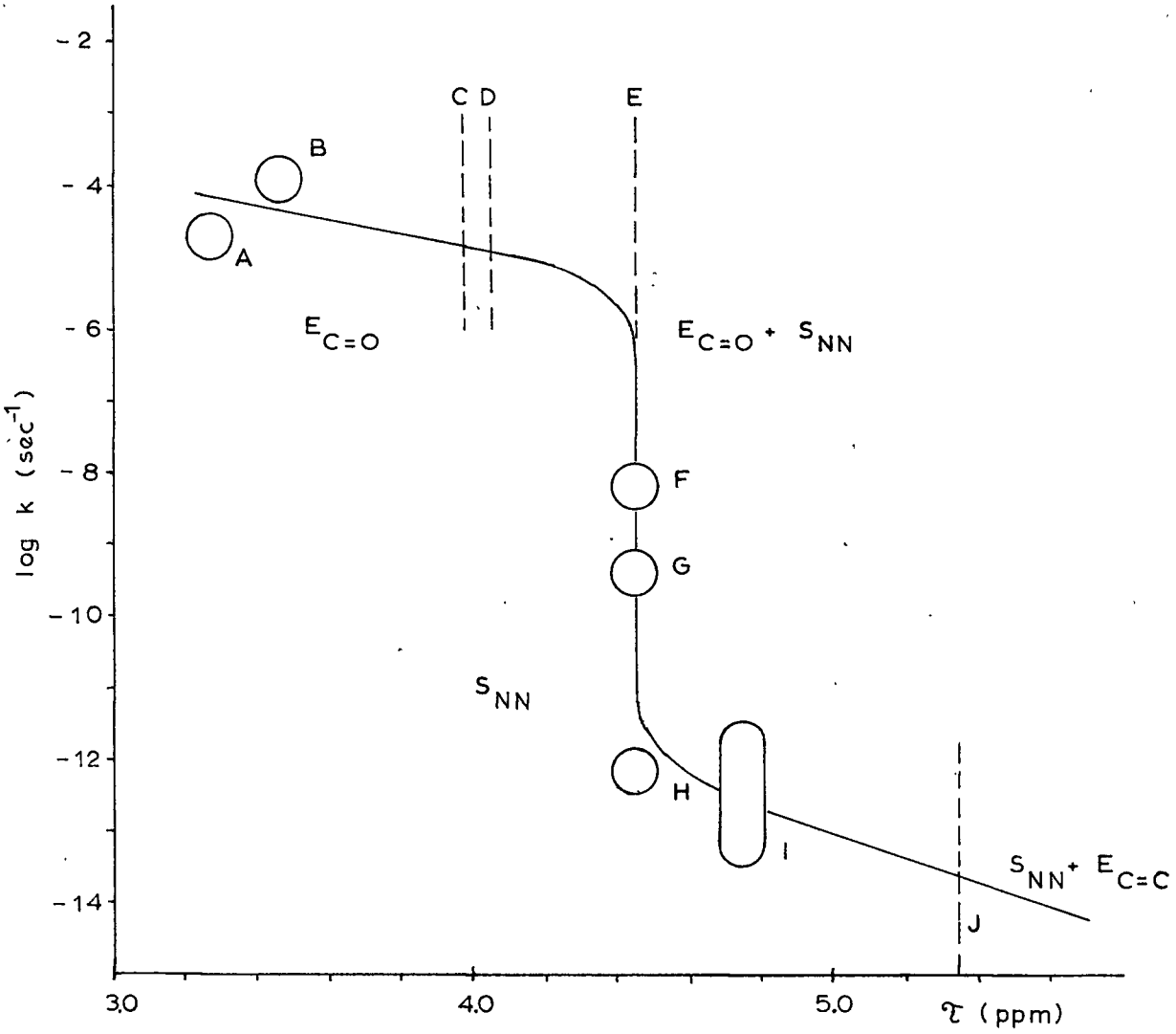


FIGURE 13. Tentative Correlation of τ_{α_H} and Mechanism in the Reaction of Nitrate Esters with Pyridine at 25°.

A: cis-1,2-Acenaphthenediol Dinitrate (XXXVI)
B: trans-1,2-Acenaphthenediol Dinitrate (XL)
C: meso-Hydrobenzoin Dinitrate (XXIX)
D: dl-Hydrobenzoin Dinitrate (XXXI)
E: Benzyl Nitrate
F: Isoidide Dinitrate (XVIII) (165)
G: Isosorbide Dinitrate (XIX) (165)
H: Isomannide Dinitrate (XX) (165)
I: trans-1,2-Cyclohexanediol Dinitrate (XXII)
J: Ethyl Nitrate.

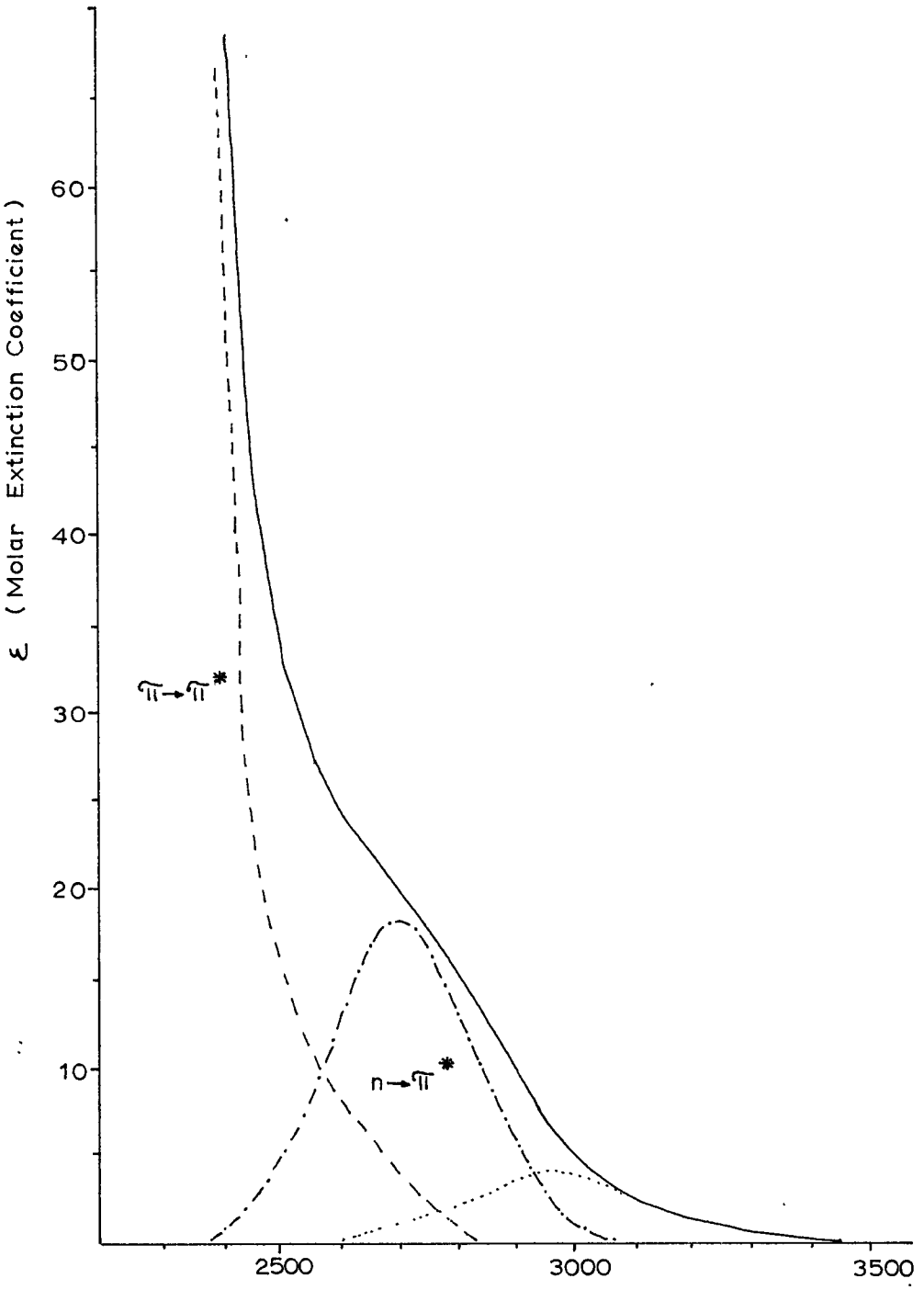


FIGURE 13. Ultraviolet Spectrum of iso-Amylnitrate in Methanol.

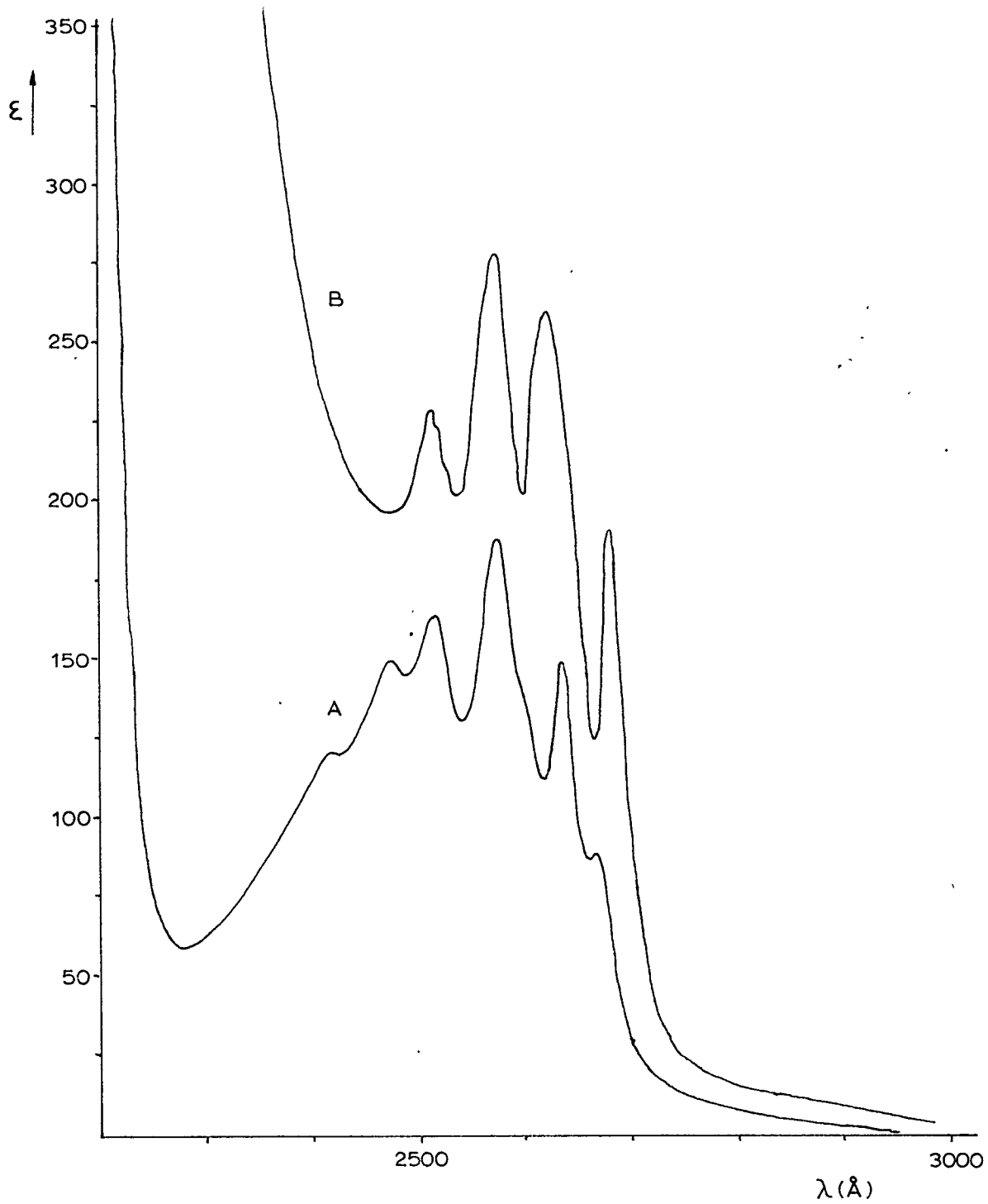


FIGURE 14. Benzenoid Absorption of Benzylalcohol (A), Benzylnitrate (B) in Hexane Solution.

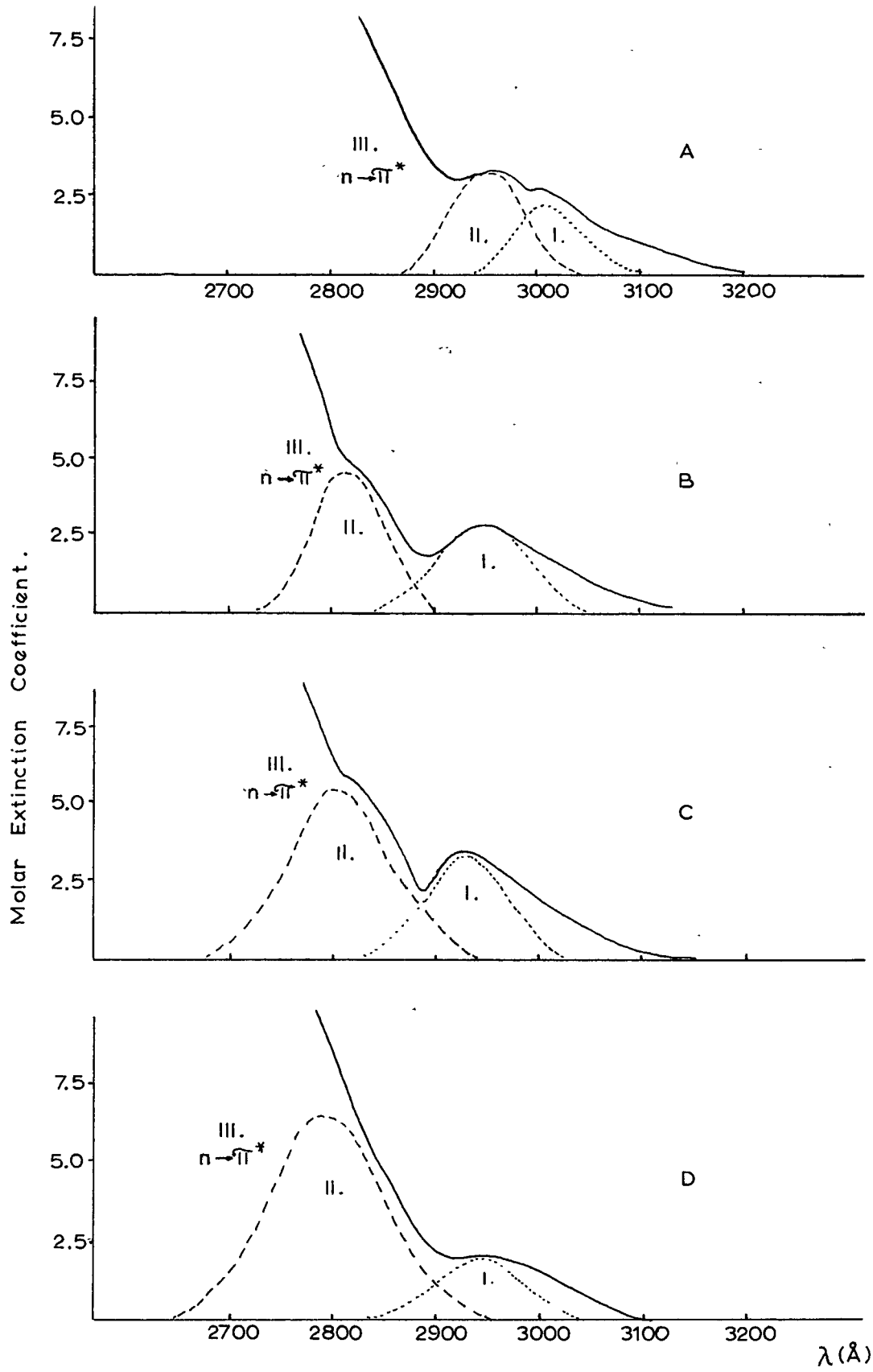


FIGURE 15. Difference Spectrum of Benzyl Nitrate and Benzyl Alcohol in Various Solvents. A: benzene, B: ether, C: hexane, D: alcohol.

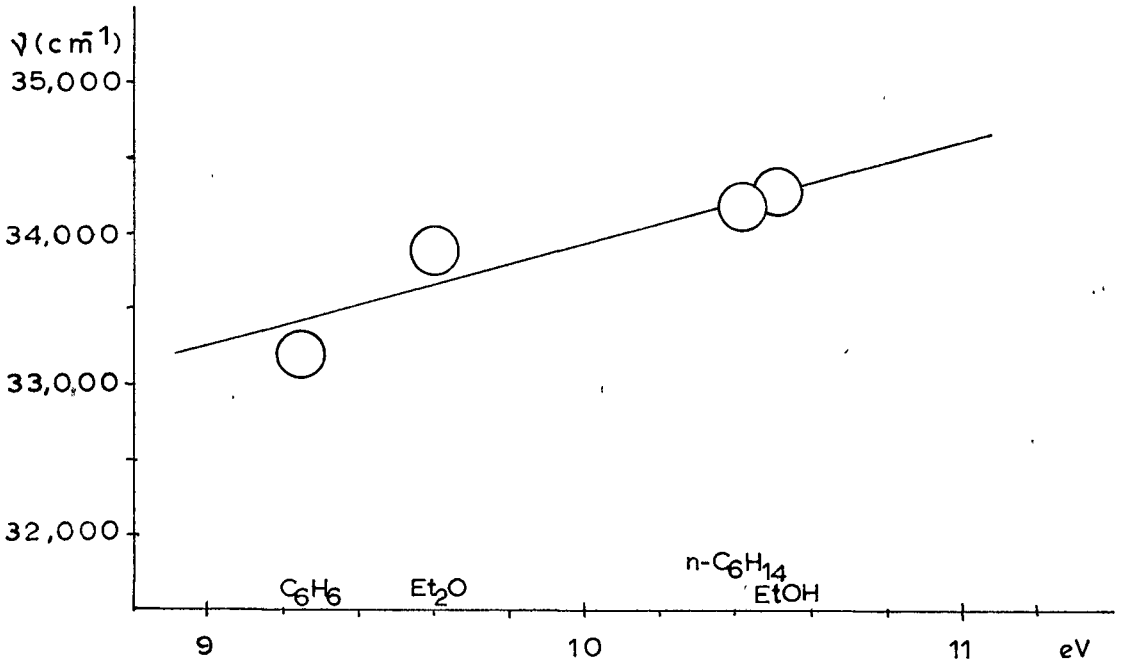


FIGURE 16. Correlation of the Frequency of Band I with the Solvent Ionization Potential

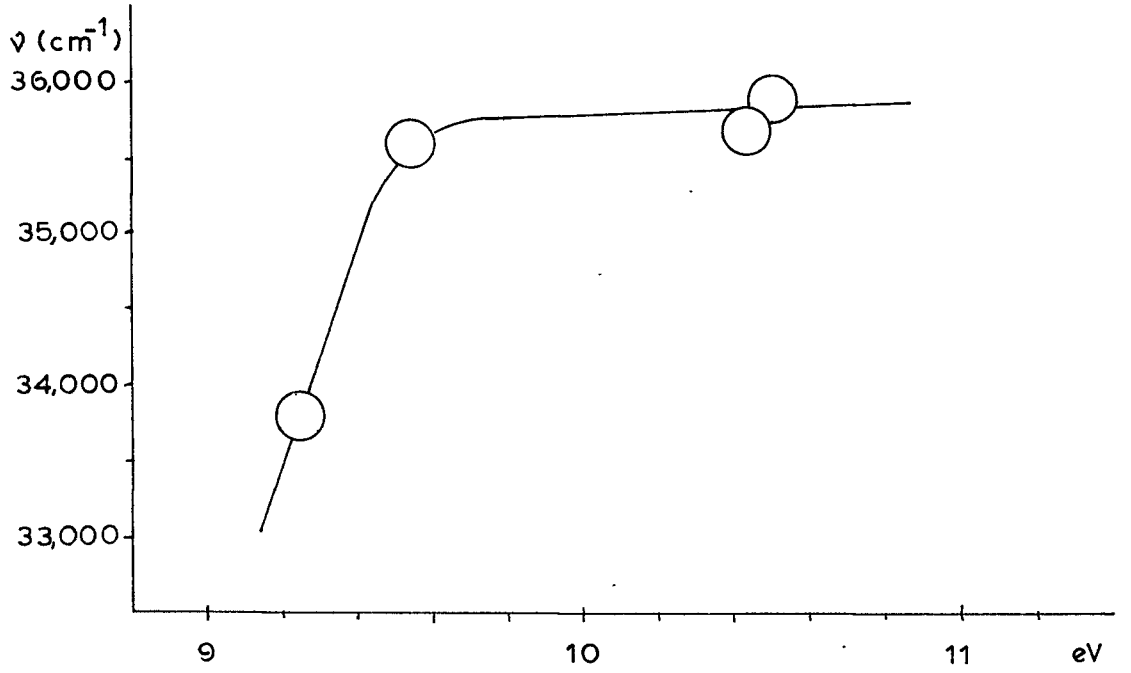


FIGURE 17. Correlation of the Frequency of Band II with the Solvent Ionization Potential.

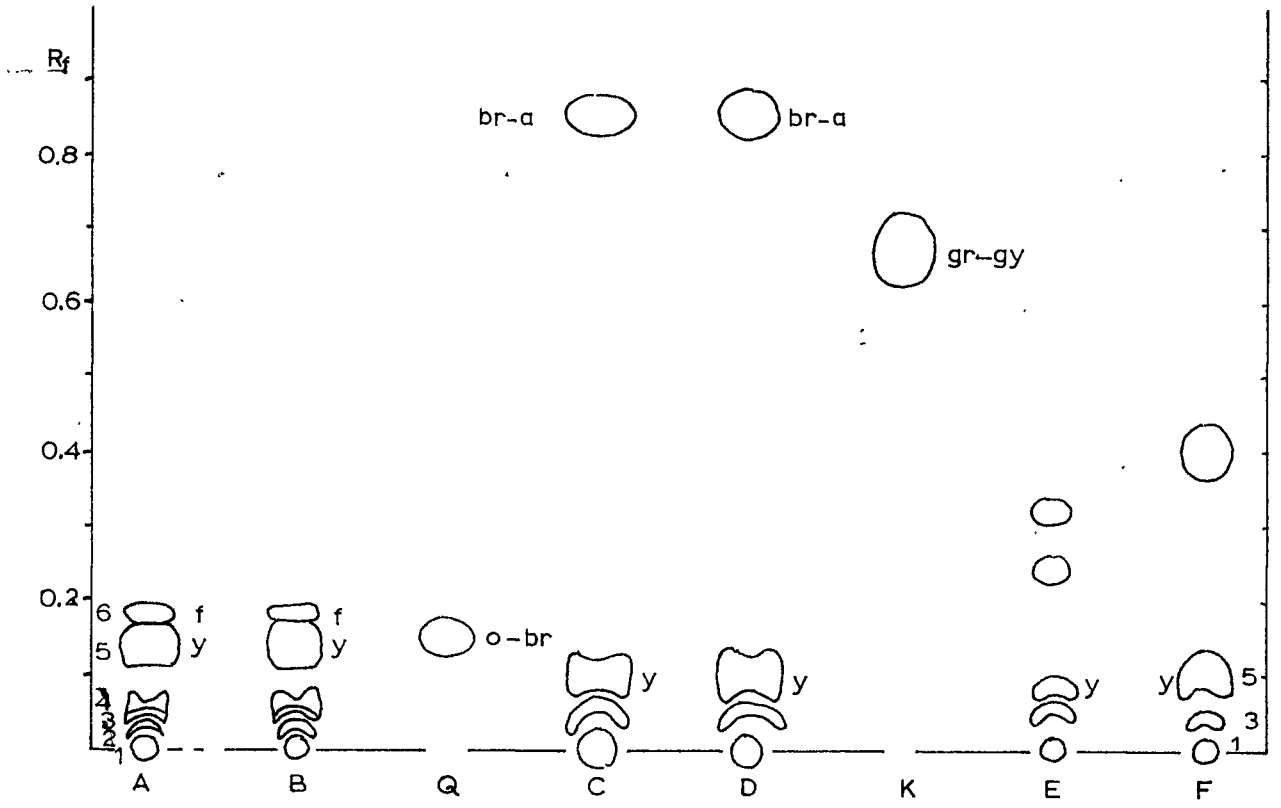


Figure 18. Chromatographic Separation of Photolysis Products of Nitrate Esters (TLC; M-3, S-6).

A.	cis-1,2-Acenaphthenediol Dinitrate	f:	fluorescence (UV)
B.	trans-1,2-Acenaphthenediol Dinitrate	y:	yellow
Q.	Acenaphthenequinone	o:	orange
C.	meso-Hydrobenzoin Dinitrate	r:	red
D.	dl-Hydrobenzoin Dinitrate	br:	brown
K.	Benzil (1,2-Diketo-1,2-diphenylethane)	br-a:	brown absorption (UV)
E.	Benzoin nitrate	gr-gy:	green-gray
F.	Isosorbide Dinitrate		

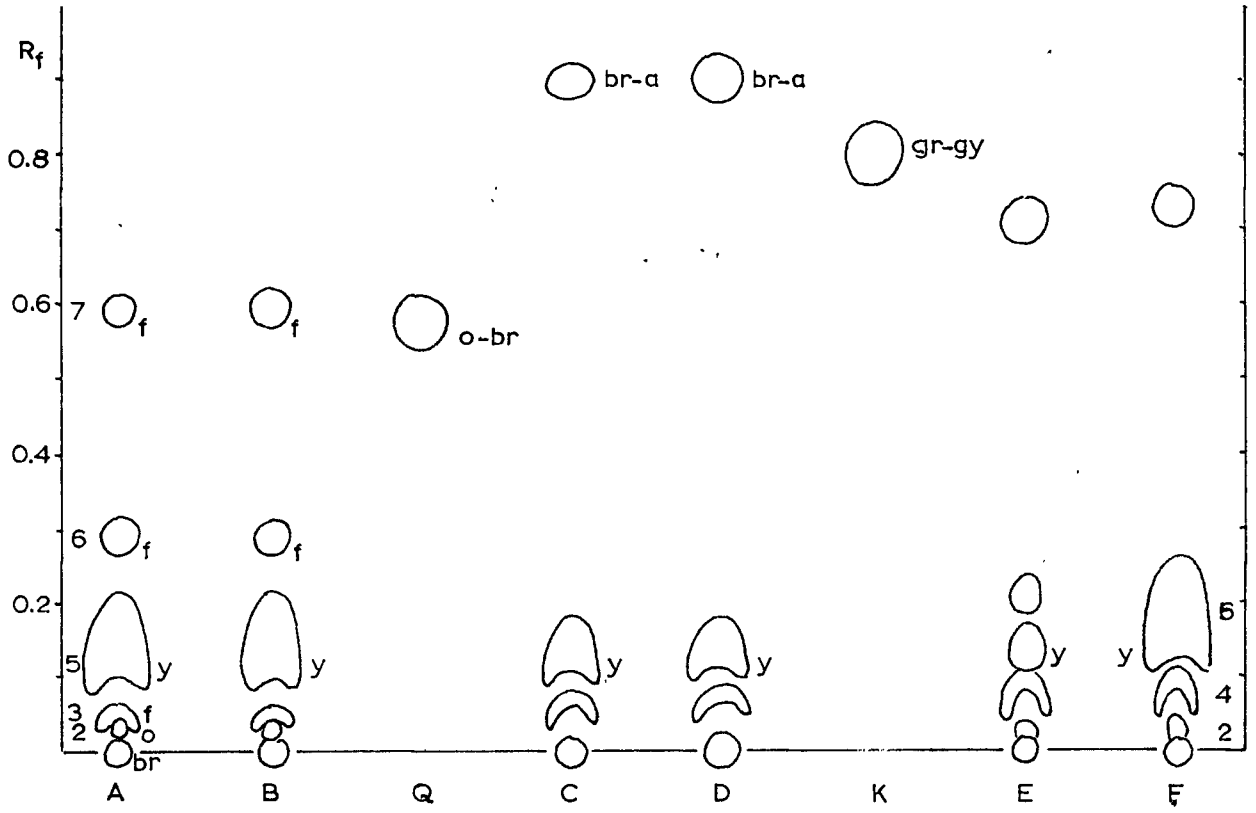


Figure 19. Chromatographic Separation of Photolysis Products of Nitrate Esters (TLC; M-3, S-10).

A. cis-1,2-Acenaphthenediol Dinitrate
B. trans-1,2-Acenaphthenediol Dinitrate
Q. Acenaphthenequinone
C. meso-Hydrobenzoin Dinitrate
D. dl-Hydrobenzoin Dinitrate
K. Benzil (1,2-Diketo-1,2-diphenylethane)
E. Benzoin nitrate
F. Isosorbide Dinitrate

f: fluorescence (UV)
y: yellow
o: orange
r: red
br: brown
br-a: brown absorption
gr-gy: green-gray

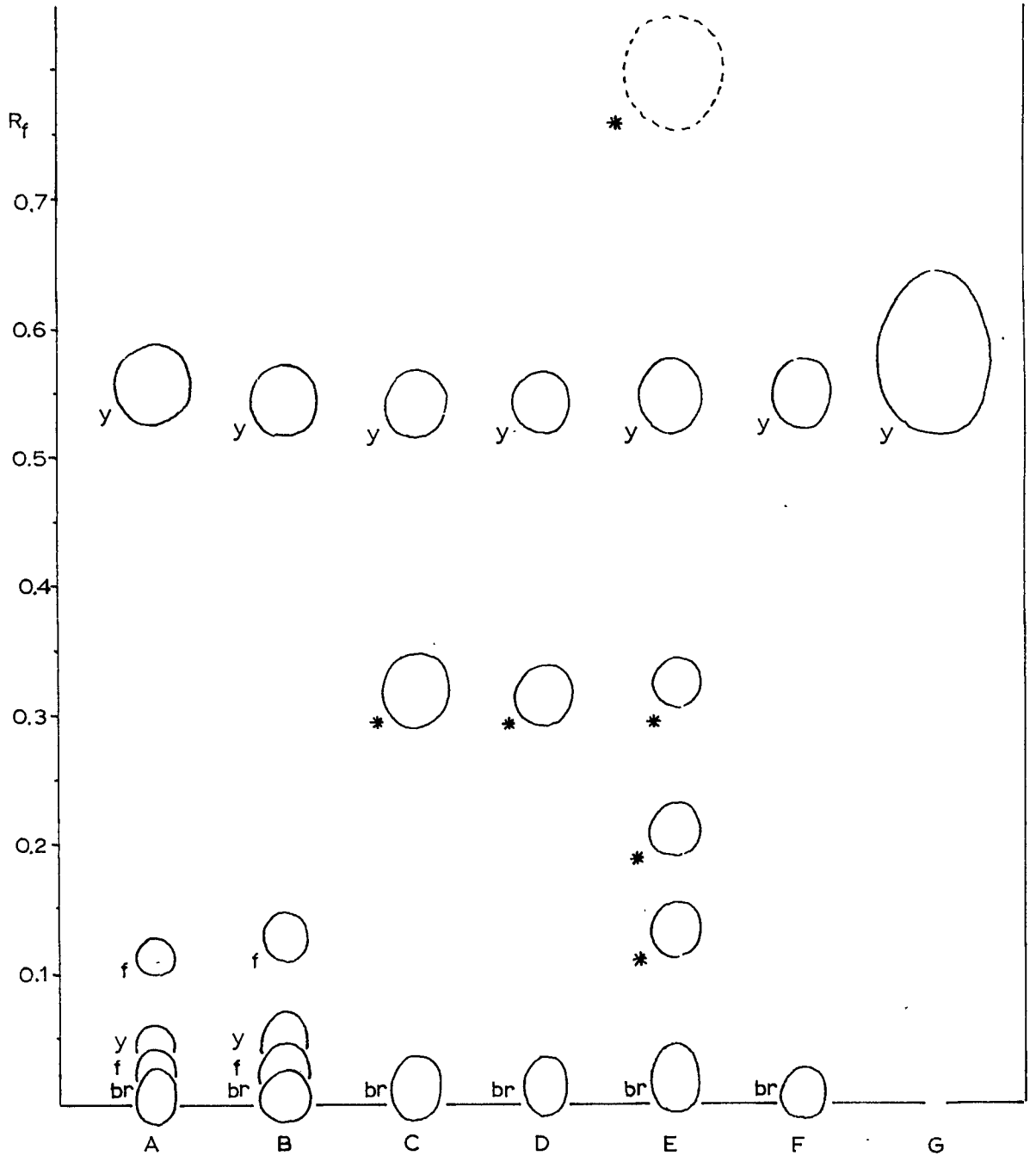


FIGURE 20. Chromatographic Separation (M–3, S–1, R–3) of Photolysis Products of Nitrate Esters.

A: cis-1,2-Acenaphthenediol Dinitrate; B: trans-1,2-Acenaphthenediol Dinitrate; C: meso– ; D: dl-Hydrobenzoin Dinitrate; E: Benzoin Nitrate; F: Isosorbidedinitrate; G: orto-Nitrophenol; f: fluorescence (UV); y: yellow; b: brown; *: colourless before spraying, white spot on pink background after spraying with R–3

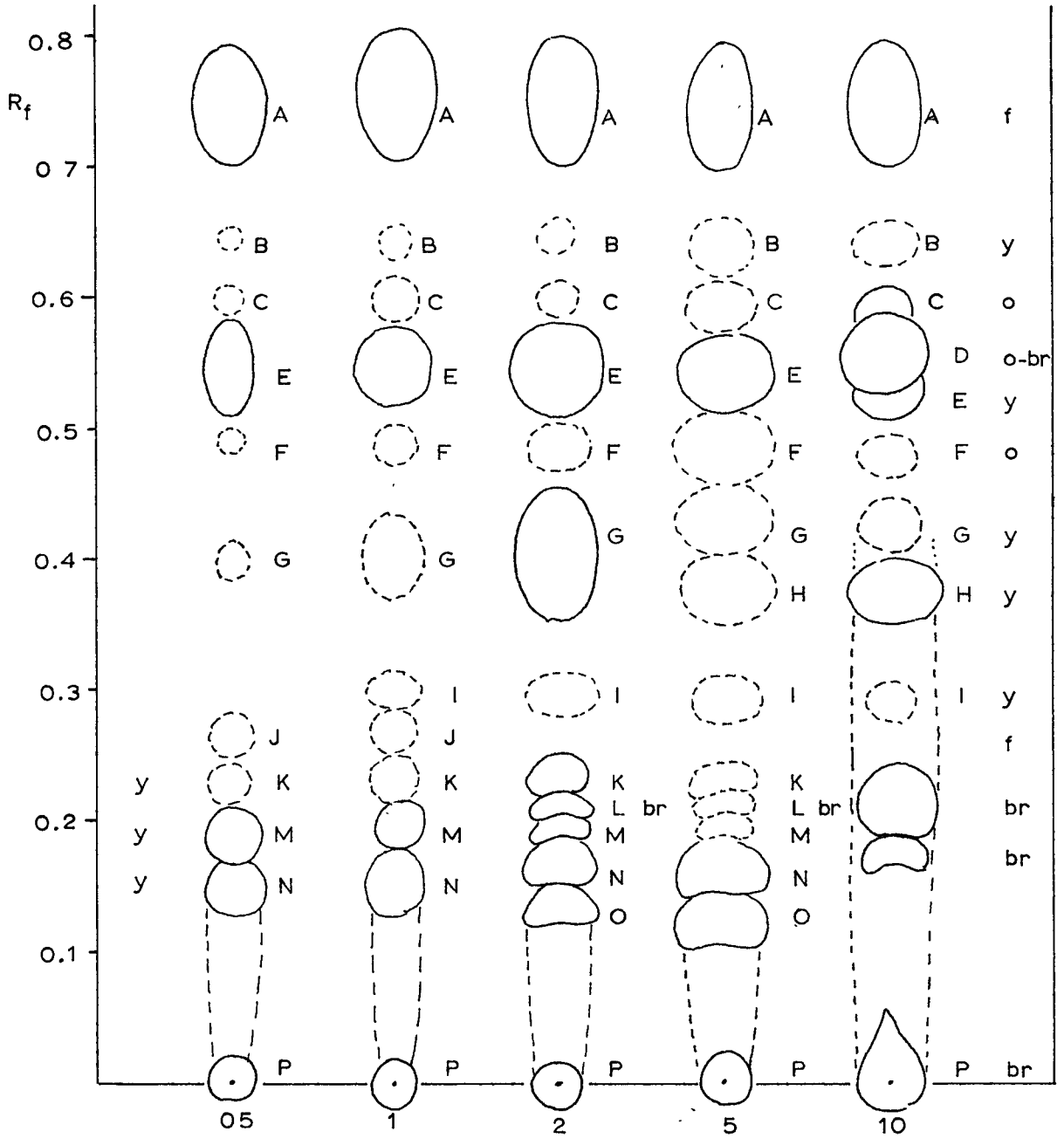


FIGURE 21 Chromatographic Separation of Products from Photolysis of meso-Hydrobenzoin Dinitrate in Benzene Solution. (M-3, S-5)

f: fluorescence (UV); y: yellow; o: orange; o-br: orange brown; brown = br.

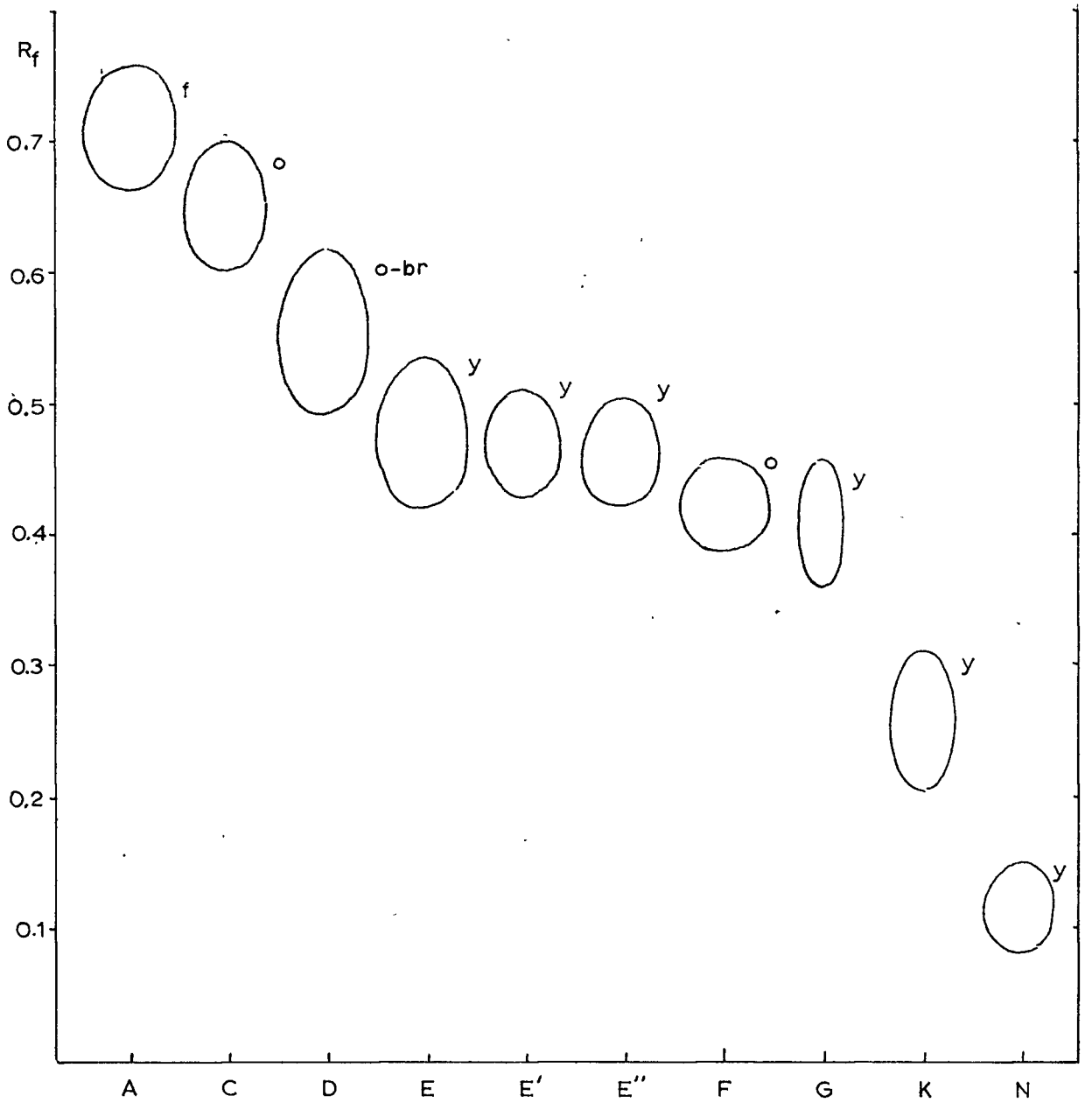


FIGURE 22. Phenolic Products Isolated from Photolyzed (in C_6H_6) meso – Hydrobenzoin Dinitrate.

f: fluorescence (UV); y: yellow; o: orange;
o-br: orange brown; b: brown.

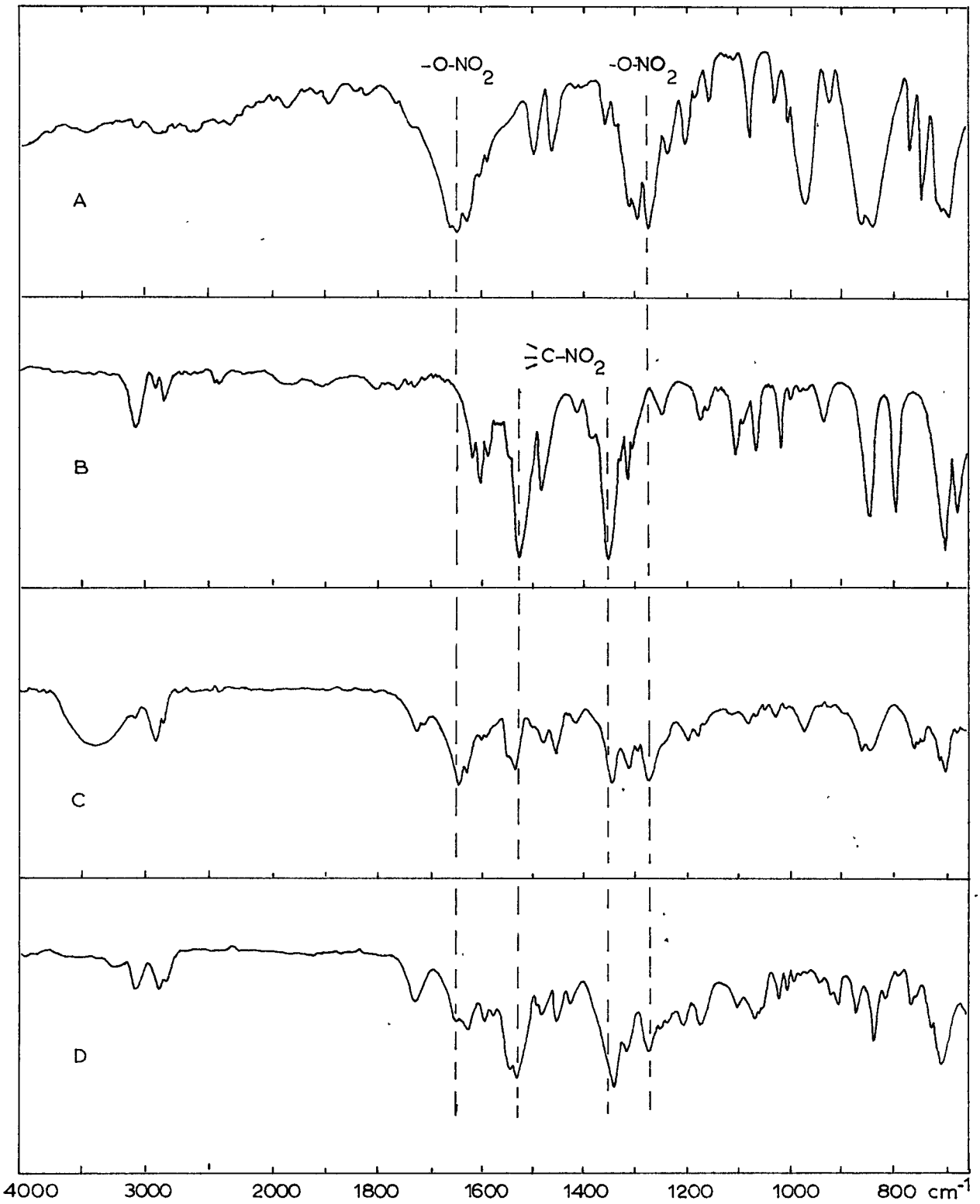


FIGURE 23. Infrared Spectra of meso-Hydrobenzoin Dinitrate (A), Nitrobenzene (B) and Photolysis Products of A (C and D).

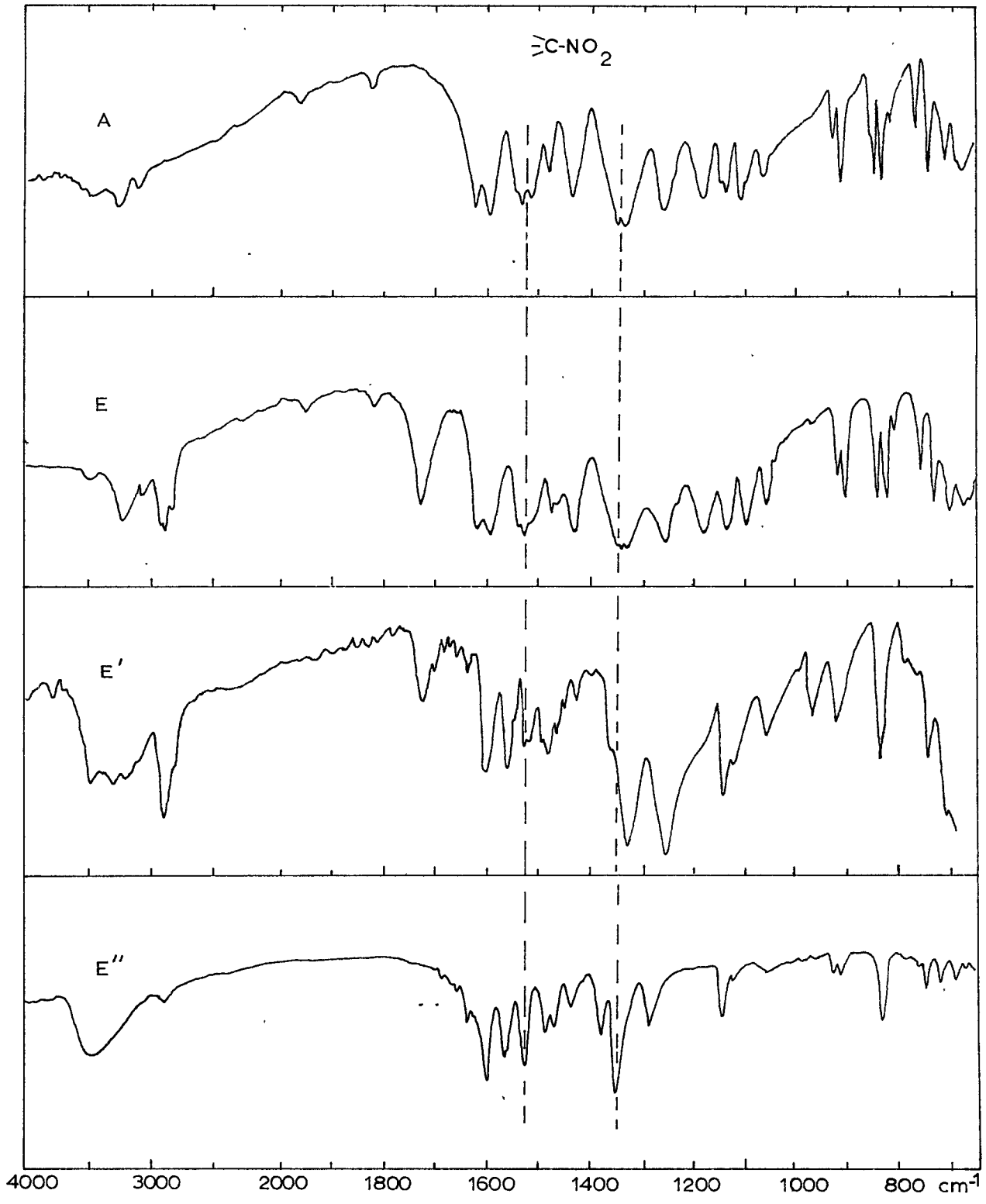


FIGURE 24. Spectra (IR) of 2,4-Dinitrophenol (A) and Photolysis Products from meso-Hydrobenzoin Dinitrate (E, E' and E'').

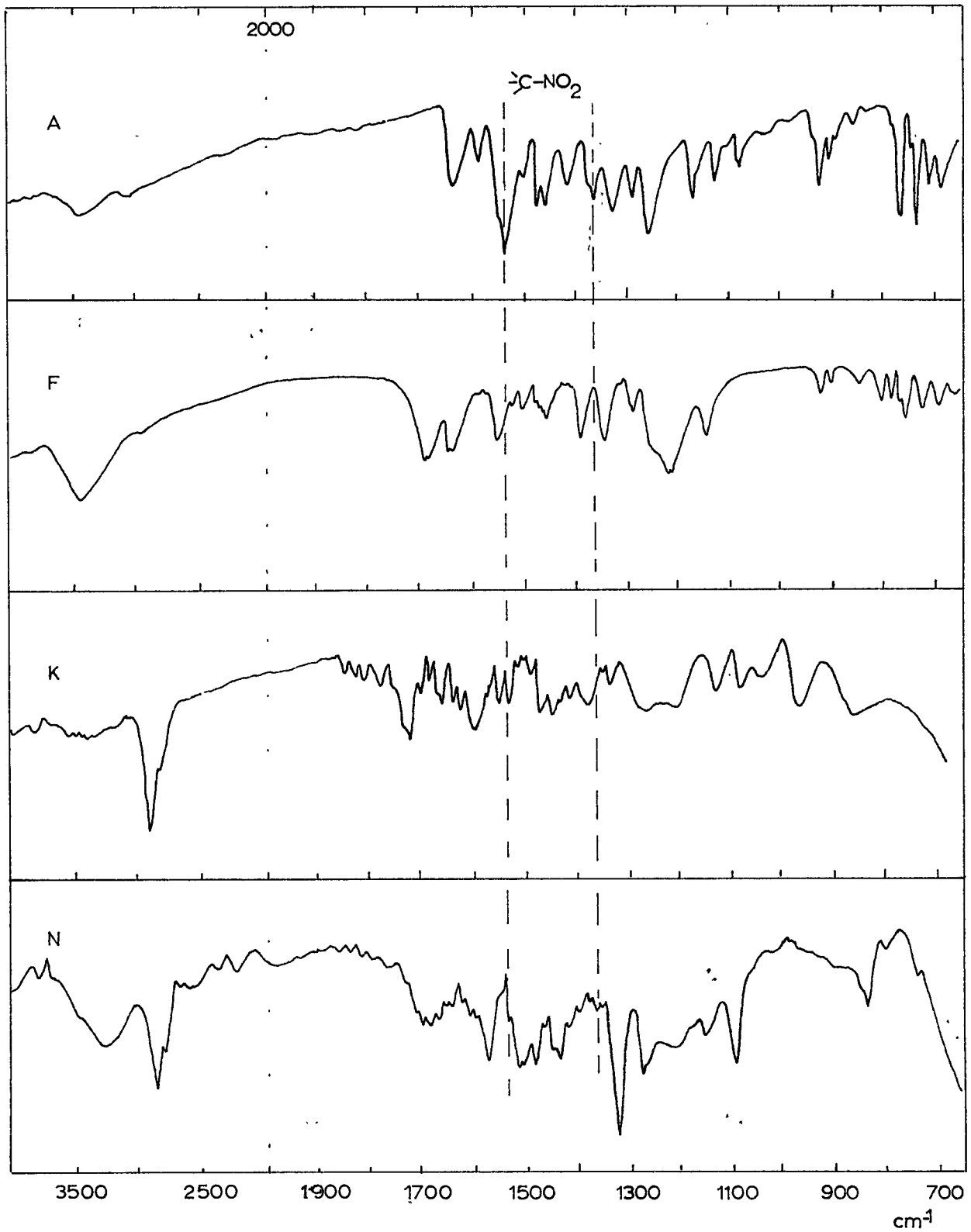


FIGURE 25. Infrared Spectra of 2,6-Dinitro-4-Phenylphenol (A) and Photolytic Products from meso-Hydrobenzoin Dinitrate (F, K and N).

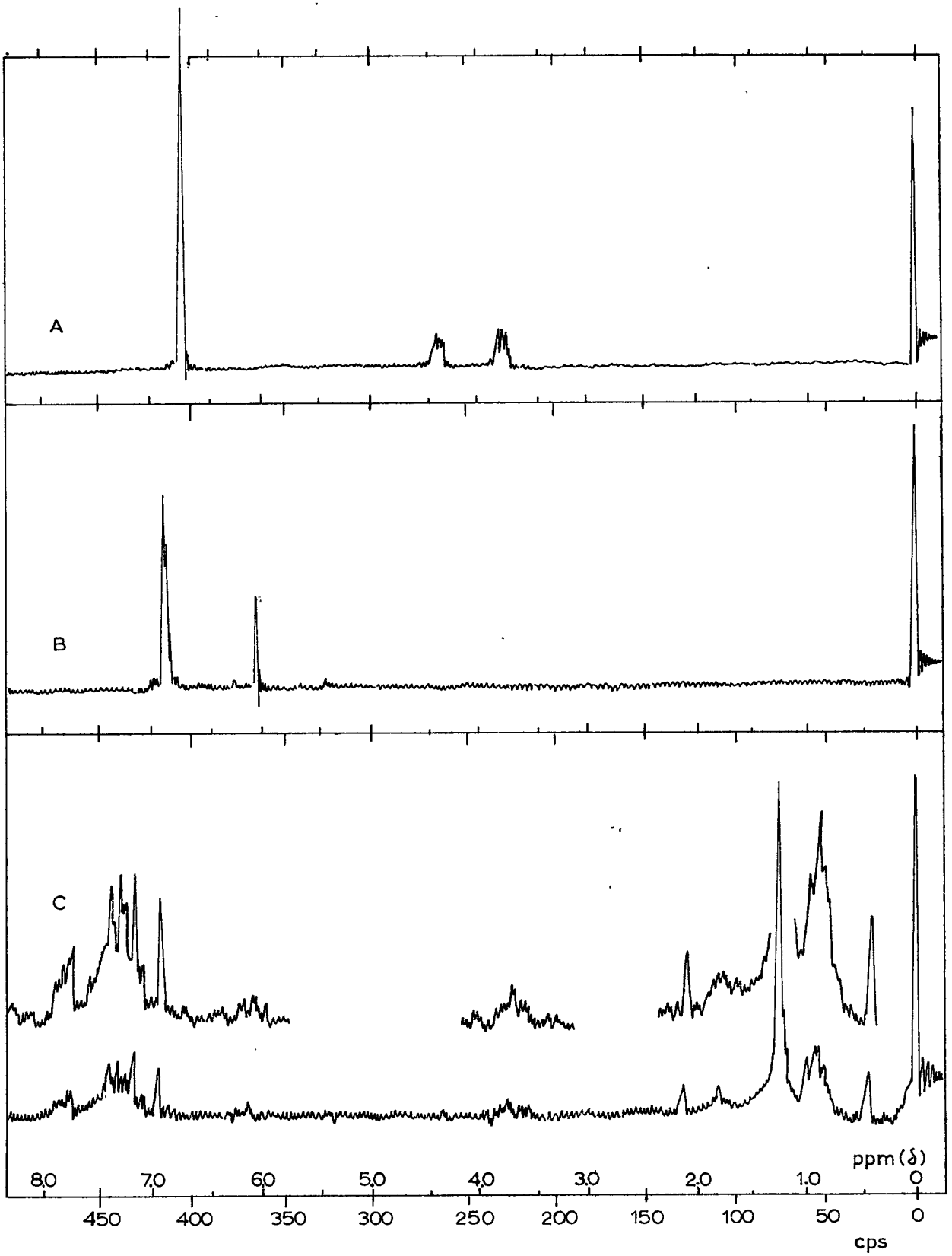
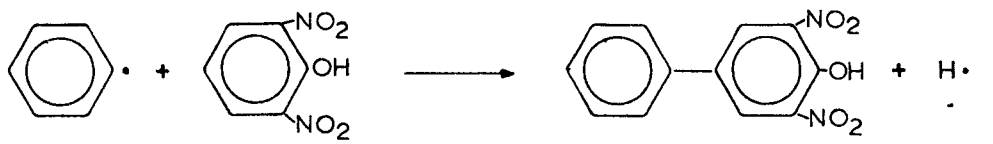
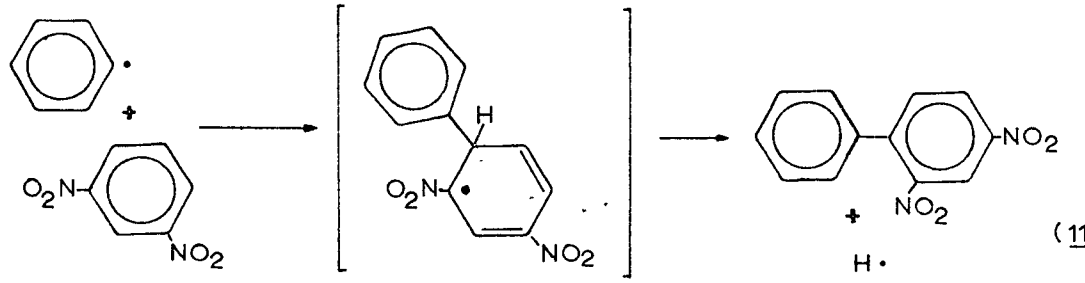


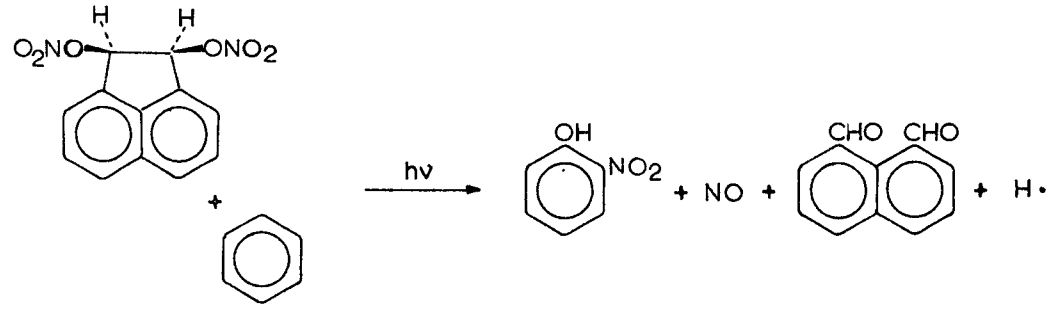
FIGURE 26. NMR Spectra of 1,2-Diphenylethane Derivatives.
A: meso-Hydrobenzoin, B: meso-Hydrobenzoin Dinitrate, C: Product from B Irradiated in Ether Solution.



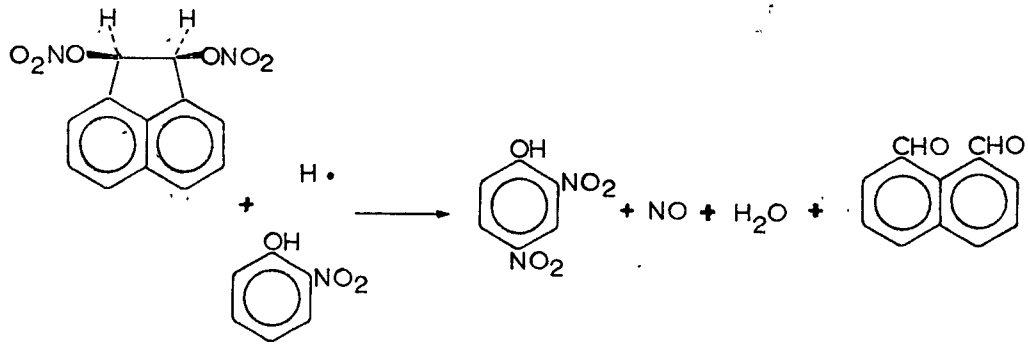
(110)



(111)

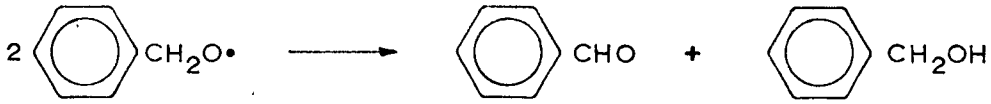


(112)

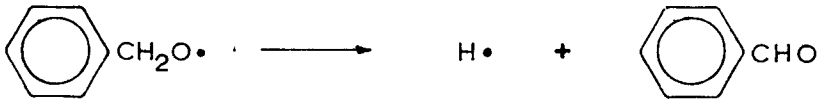


(113)

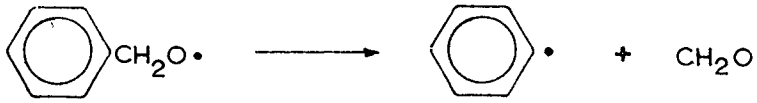
(114)



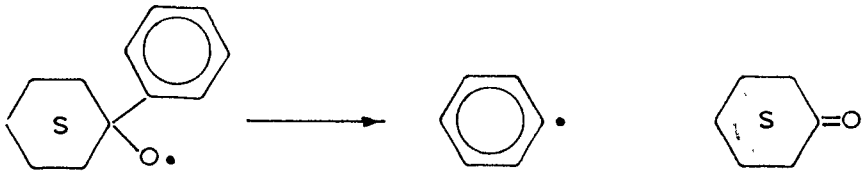
(115)



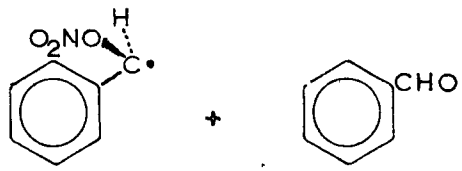
(116)



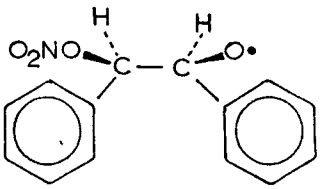
(117)

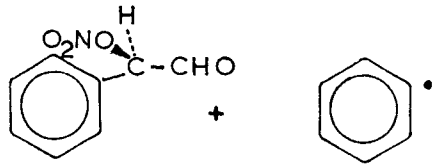


(118)



(119)





(120)

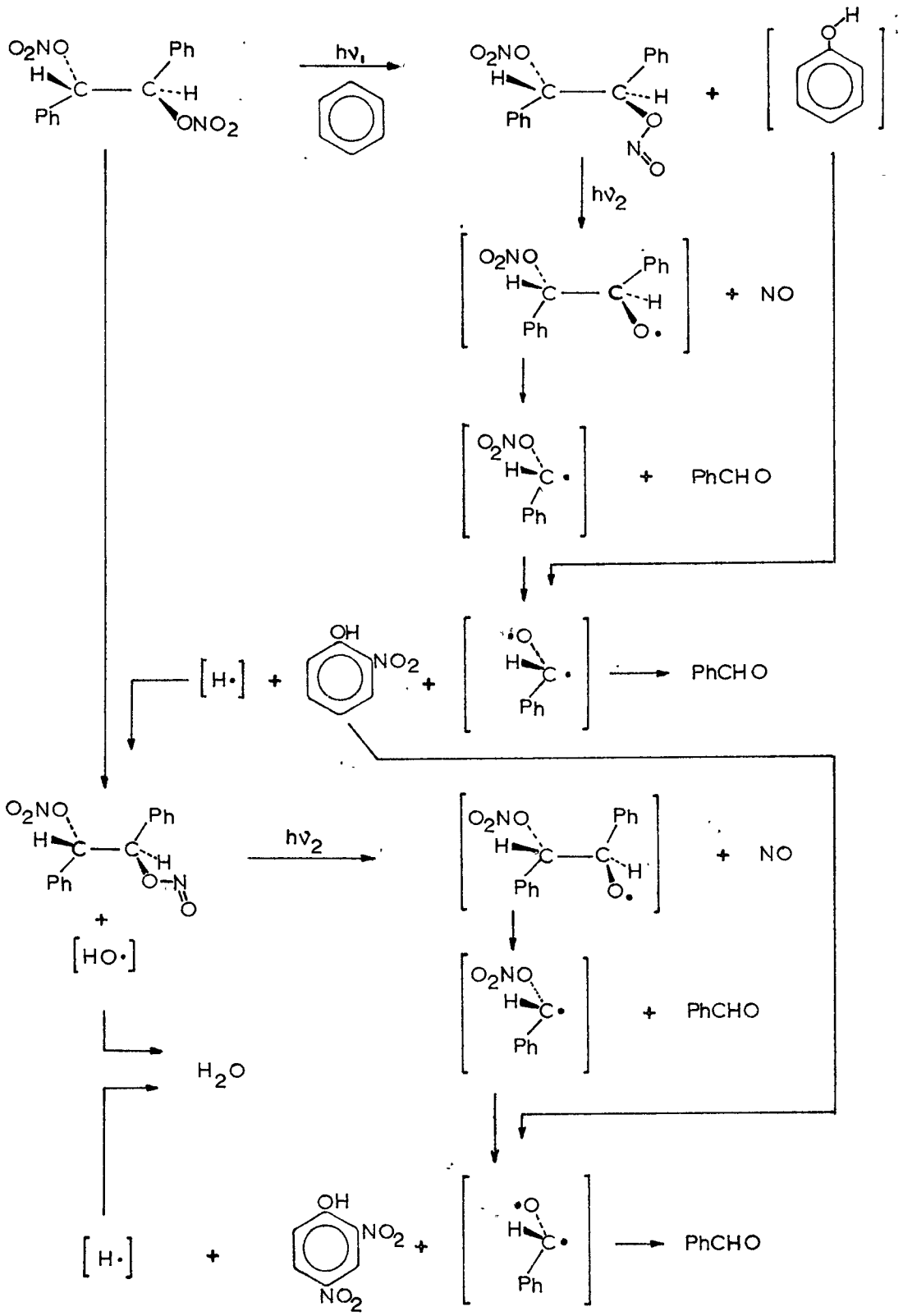
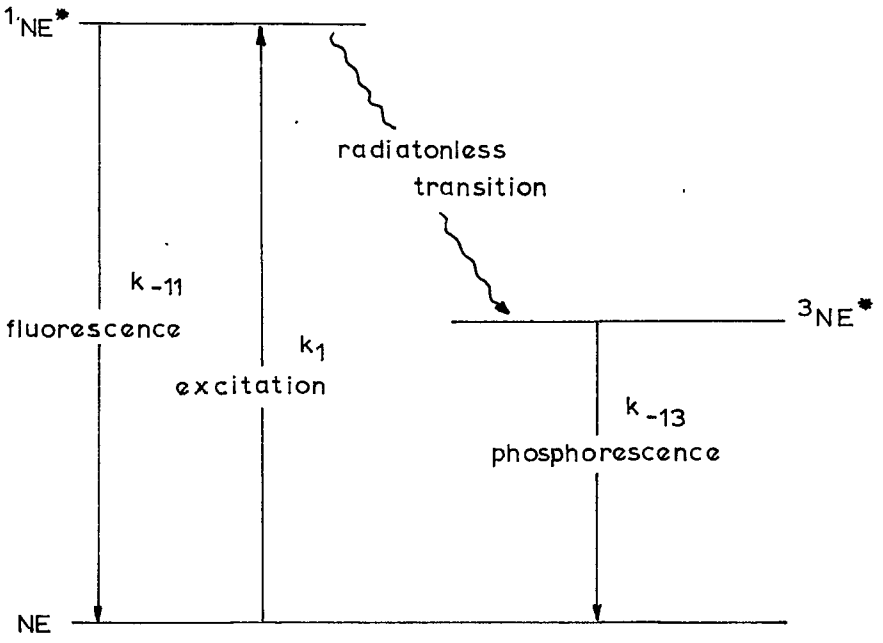


FIGURE 27. Possible Mechanism of Photolysis of meso-Hydrobenzoin Dinitrate.



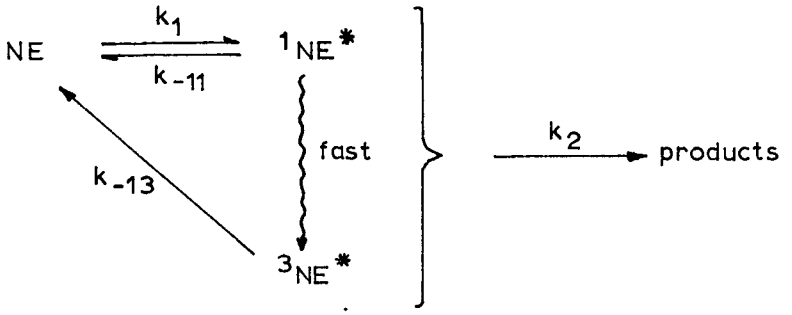


FIGURE 28. Primary Photochemical Reactions of Nitrate Esters (NE).

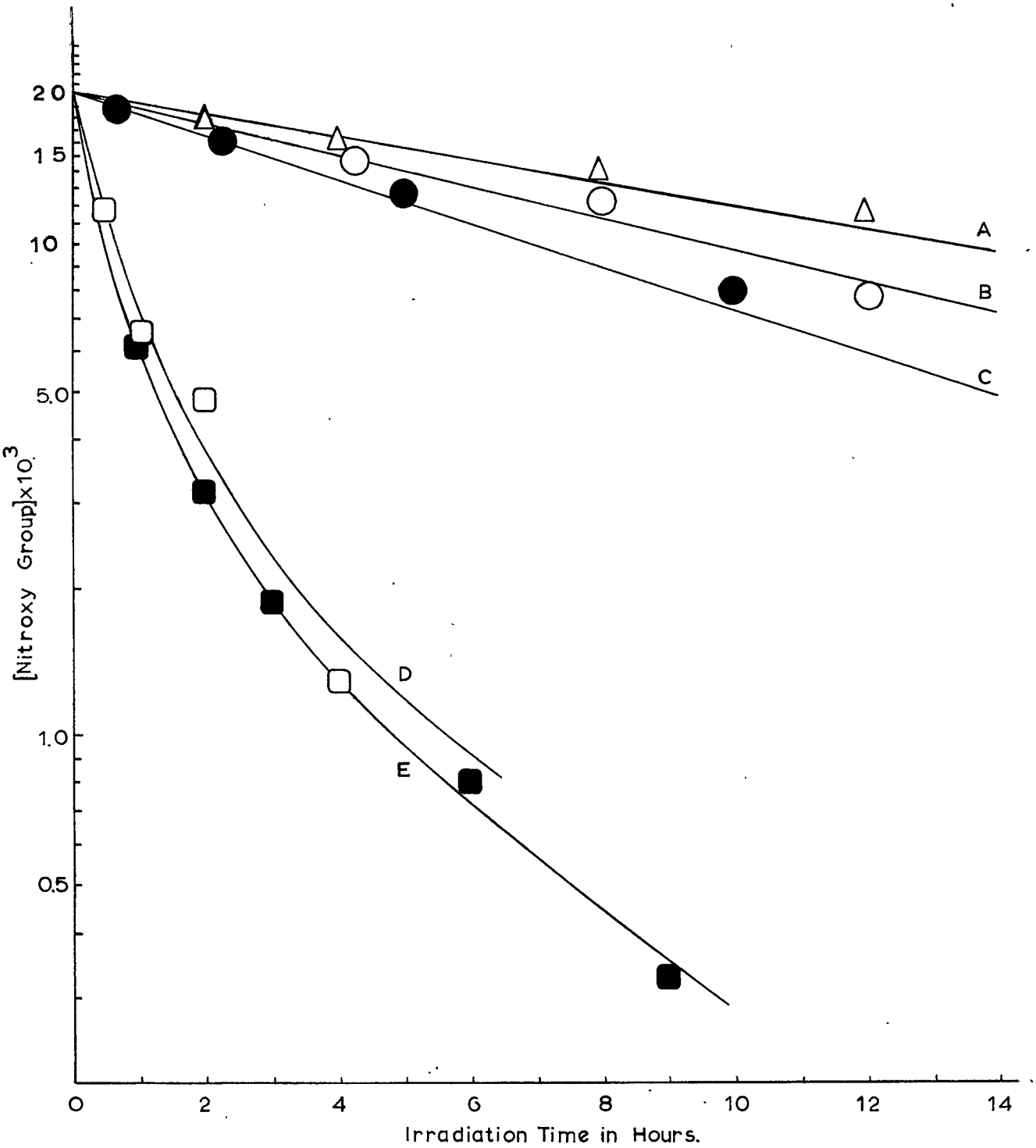


FIGURE 29. Rates of Photoreaction of (A) Benzyl Nitrate, (B) dl- and (C) meso-Hydrobenzoin Dinitrates, (D) trans- and (E) cis-1,2-Acenaphthenediol Dinitrates in Benzene Solution at 25°.

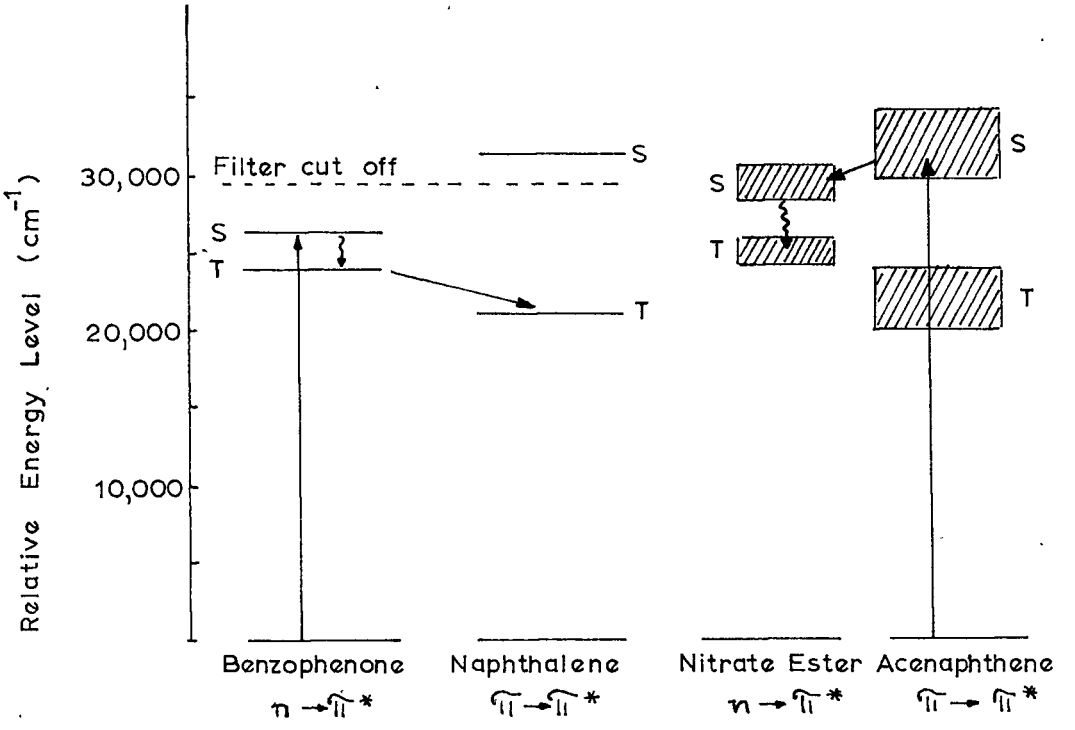
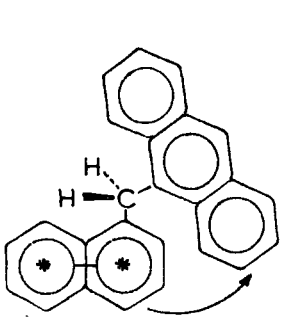
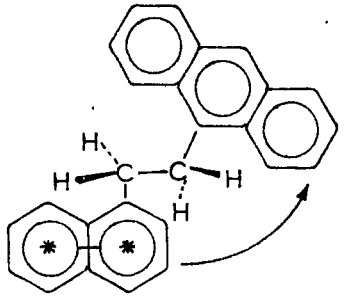


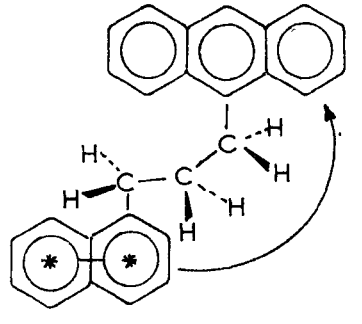
FIGURE 30. Triplet–Triplet Energy Transfer Between Benzophenone and Naphthalene (180) and Singlet–Singlet Energy Transfer within 1,2 Acenaphthenediol Dinitrates.



XLIII



XLIV



XLV

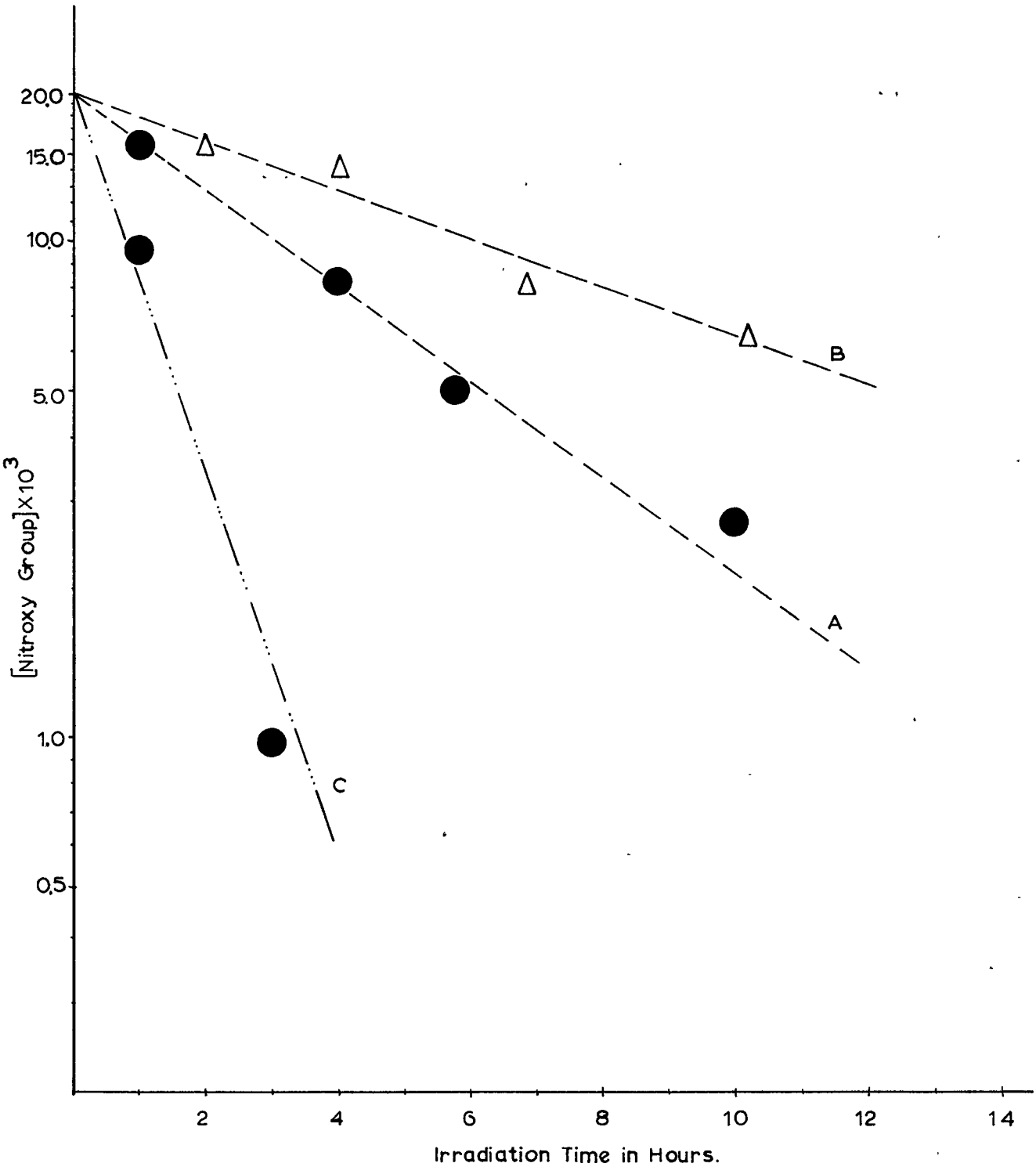


FIGURE 31. Rates of Photoreactions of meso-Hydrobenzoin Dinitrate (A) and Benzyl Nitrate (B) in Ethanol and of meso-Hydrobenzoin Dinitrate in Ether$_{\wedge}^{(C)}$ at 24.2°C.

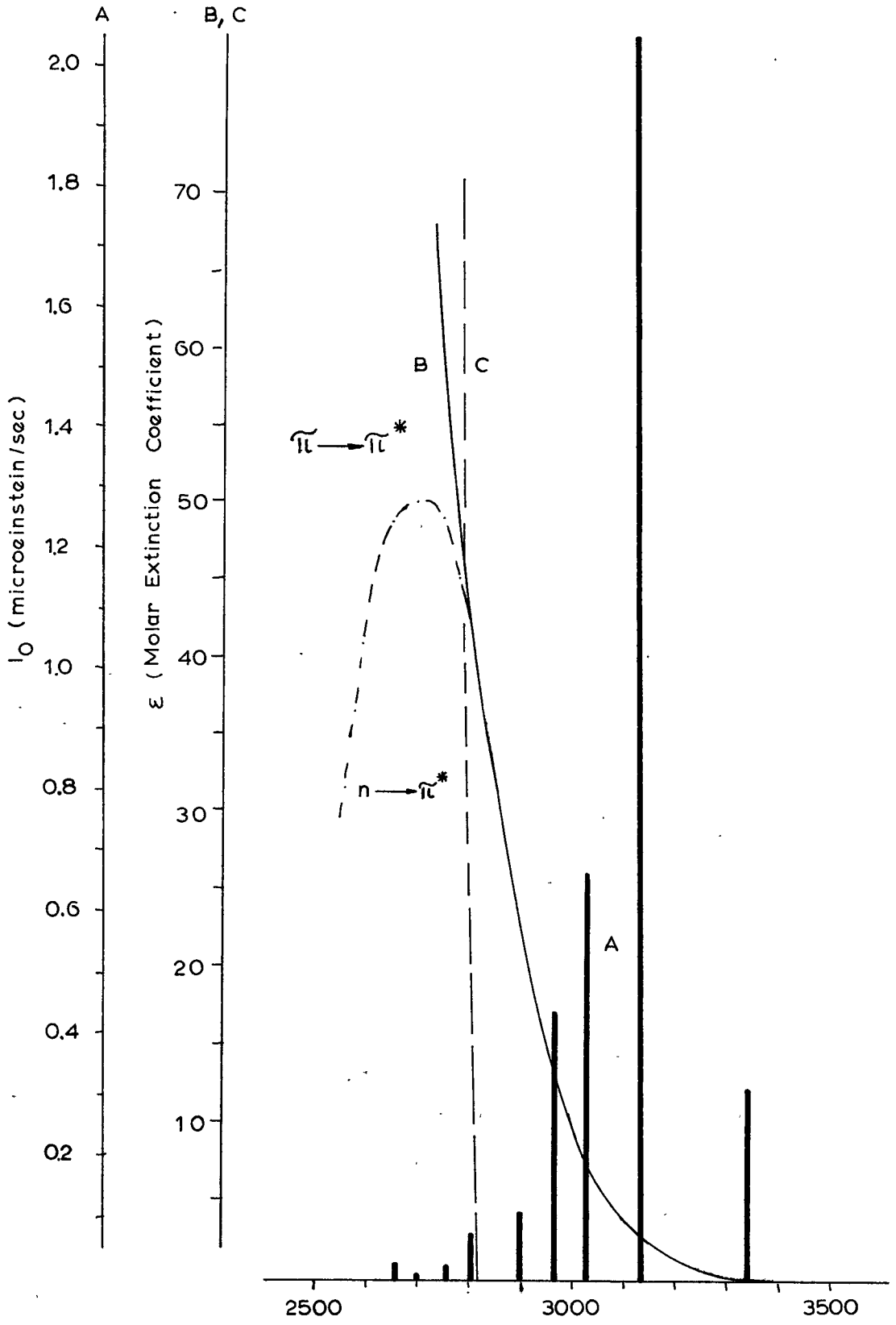


FIGURE 33. Light Energy Emitted by Source (A) and Absorbed by meso Hydrobenzoin Dinitrate (B) and Solvent Benzene (C) in the Photoreaction at 24.2°C.

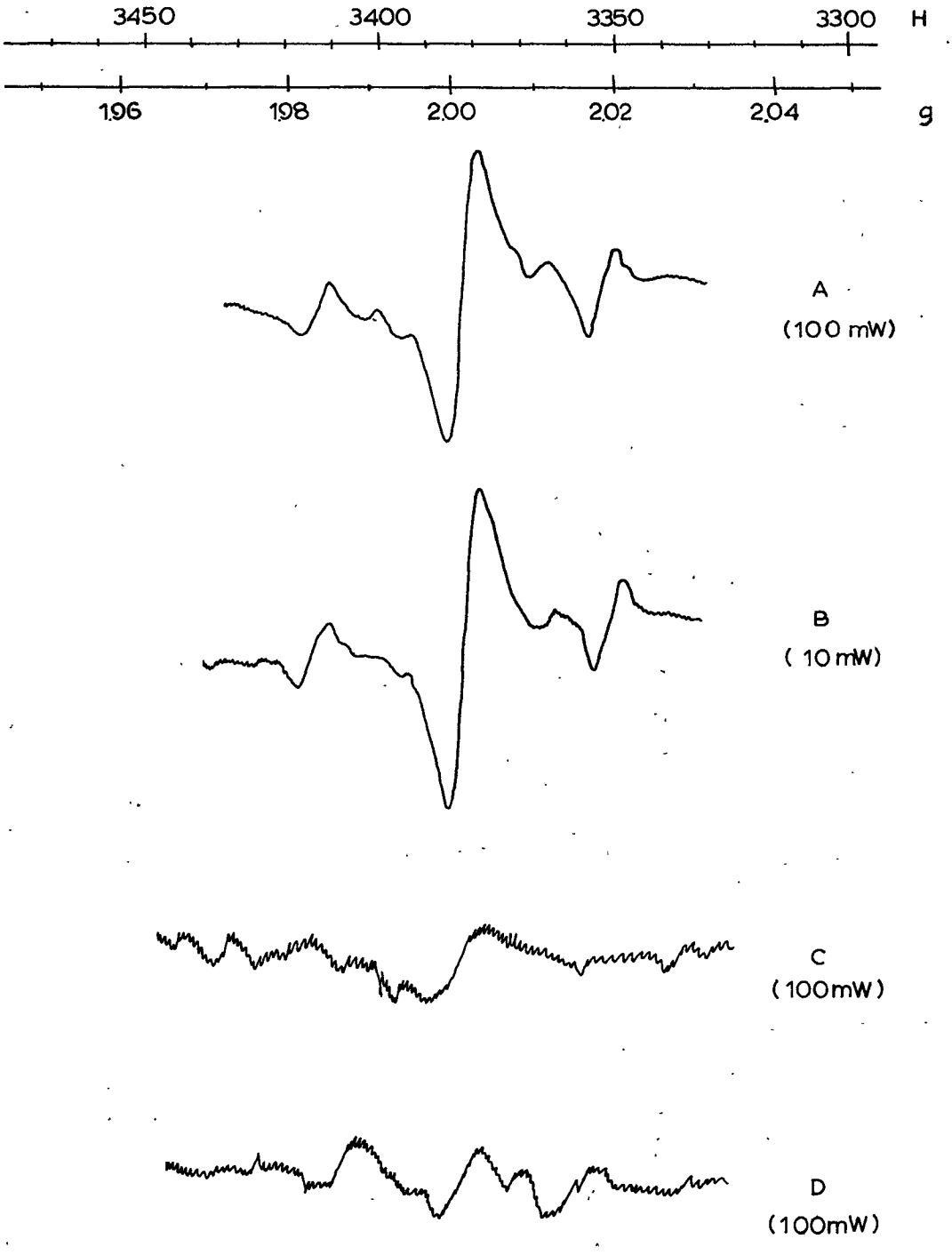


FIGURE 34. Steady State ESR Spectra of Irradiated cis-(A) and trans-(B) 1,2-Acenaphthenediol Dinitrates and meso-(C) and dl-(D) Hydrobenzoin Dinitrates in Benzene Solution at Room Temperature

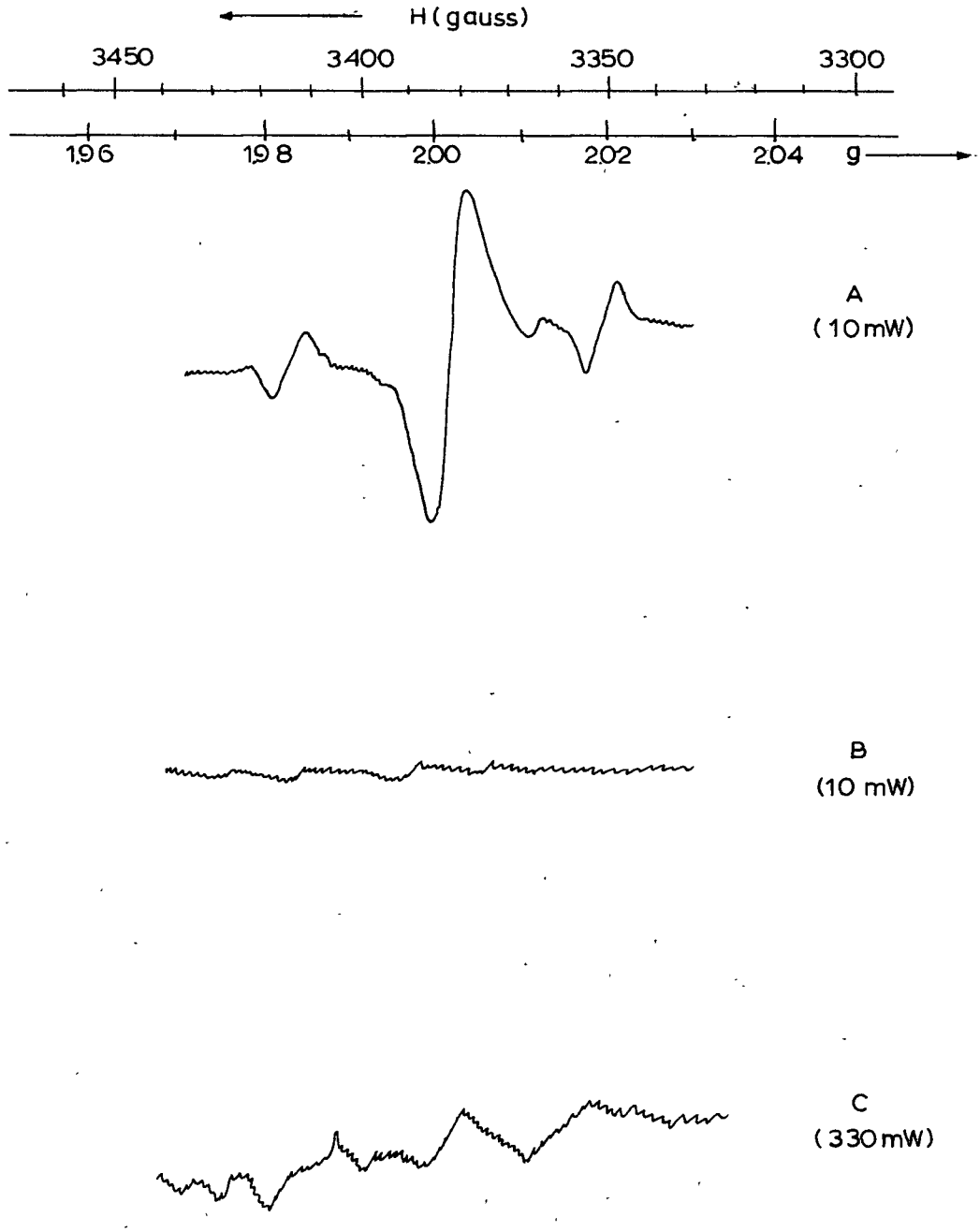


FIGURE 35. ESR Signals of Irradiated trans-1 2-Acenaphthene-diol Dinitrate. Obtained Initially (A) and After Ten Days in the Dark (B and C).

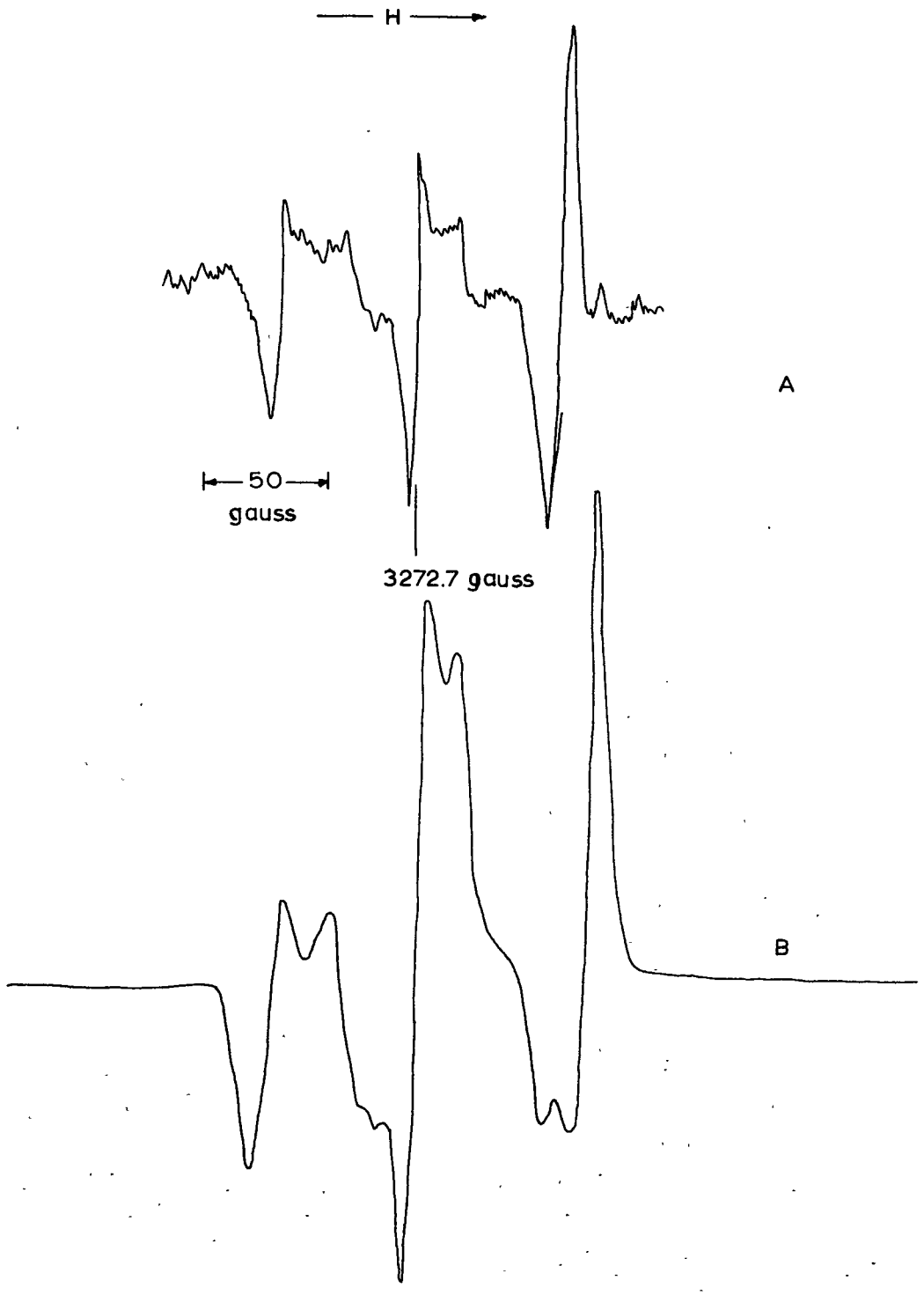


FIGURE 36. ESR Spectrum of NO_2 (A) in Solid Argon (214) and (B) Generated from trans-1,2-Acenaphthenediol Dinitrate in EPA at 77°K.

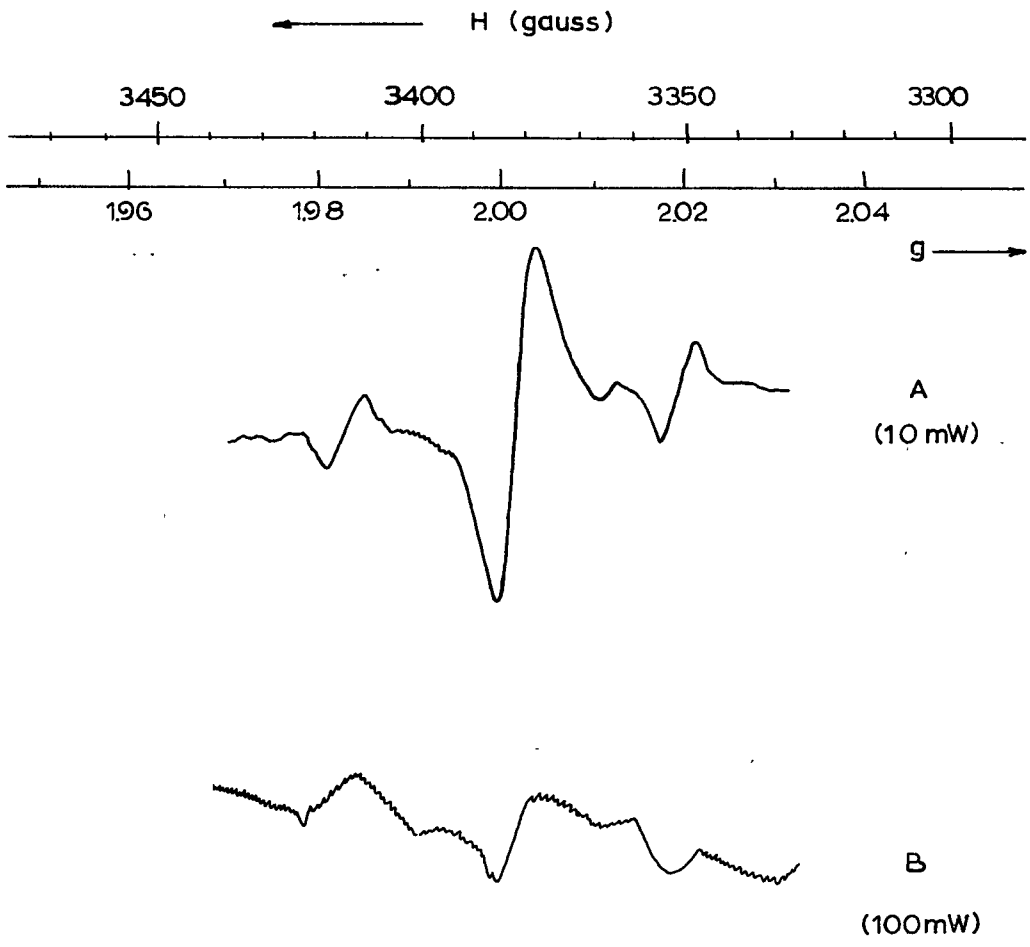


FIGURE 37. ESR Spectra of Irradiated trans-1,2-Acenaphthenediol Dinitrate (0.1 M)(A) and NO (0.2 M)(B) in Benzene Solution.

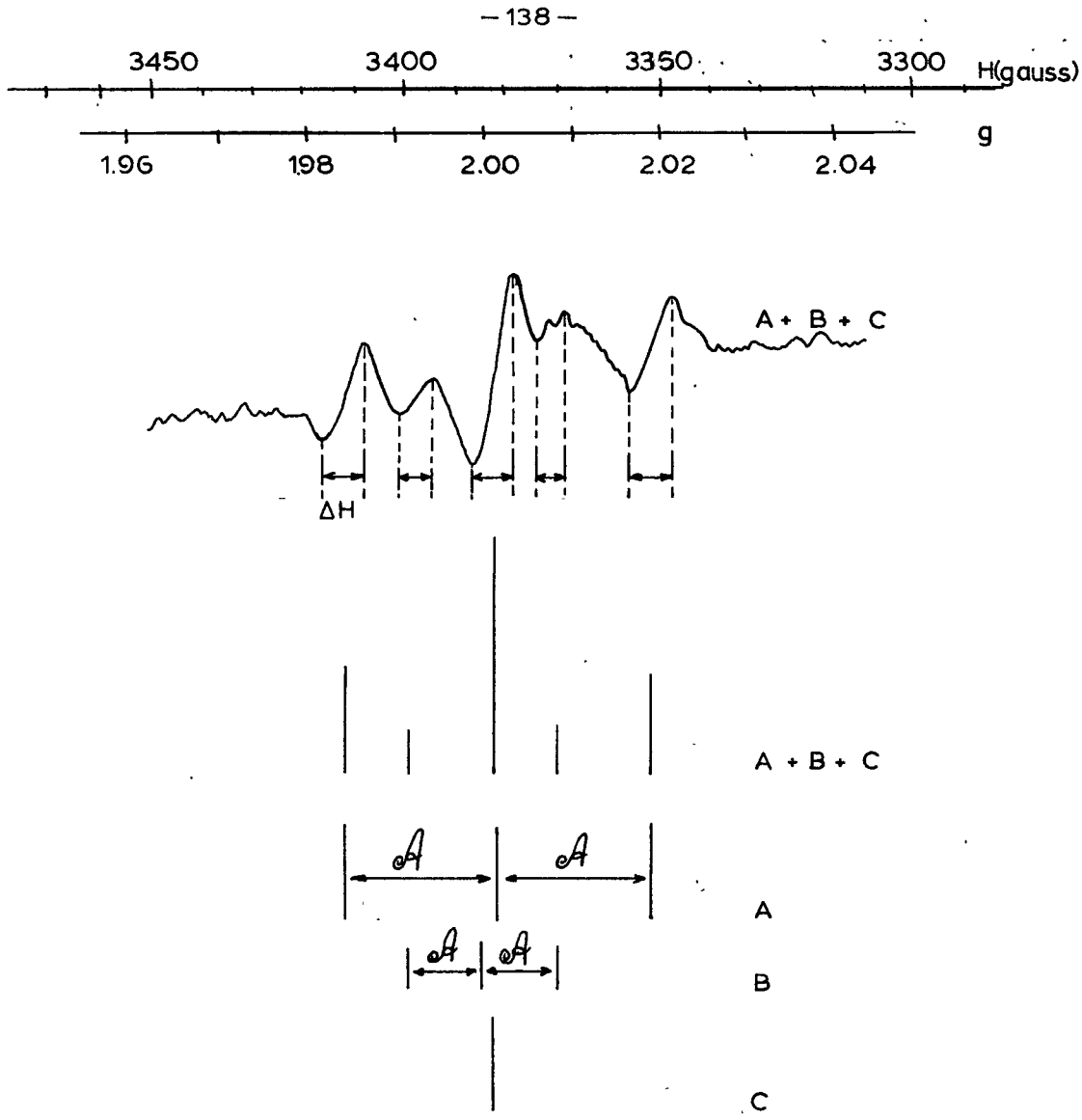


FIGURE 38. ESR Spectrum and Components of Irradiated trans-1,2-Acenaphthenediol Dinitrate.

TABLE XXVI. Observed Components of ESR Spectra of Irradiated Nitrate Esters.

Component	Multiplicity	g value	Splitting (A) Mc/s	Splitting (A) gauss	Line Width (ΔH) gauss
A	triplet	2.00121	~~0.1252~~ 82.44	29.41	7.95
B	triplet	2.00302	~~0.0552~~ 36.33	12.96	5.16
C	singlet	2.00162	—	—	8.36

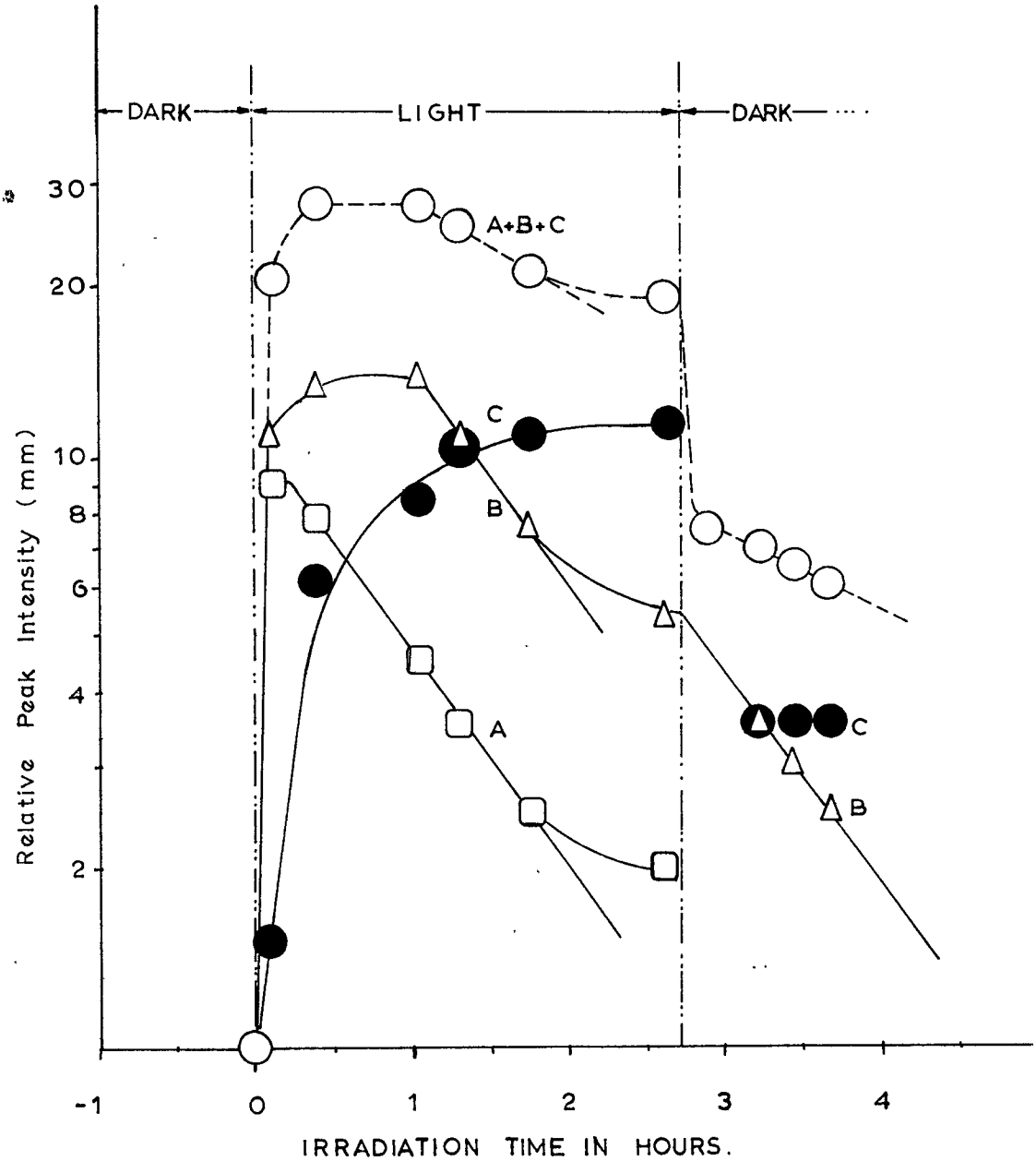


FIGURE 39. Rates of Generation of Components of ESR Spectrum of Irradiated Nitrate Ester.

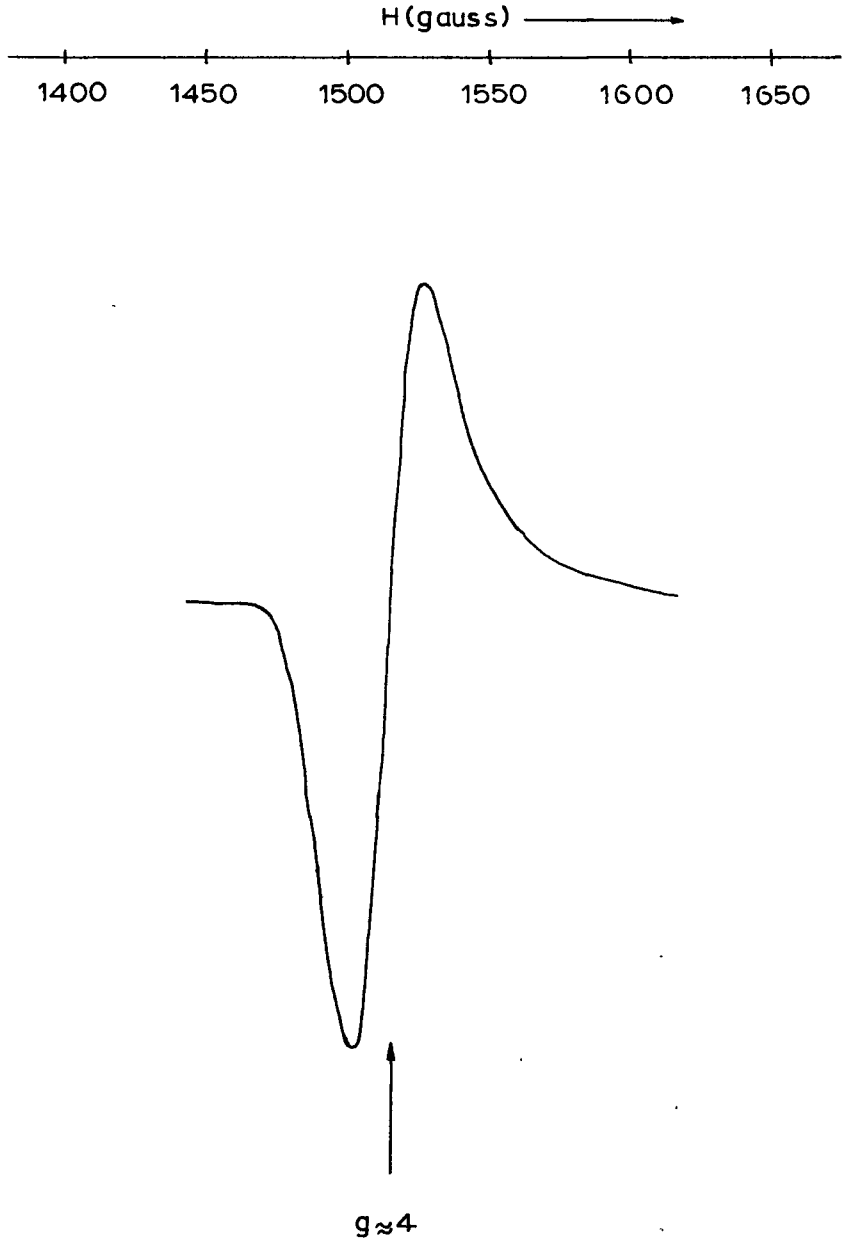
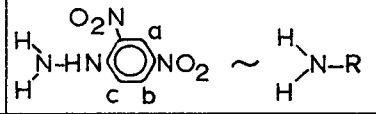
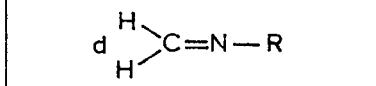
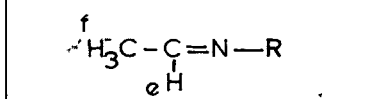
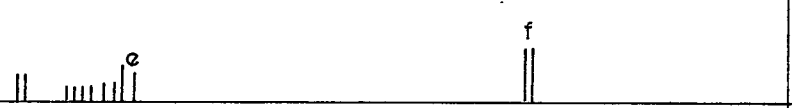
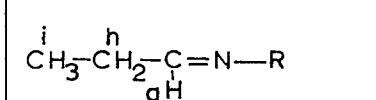
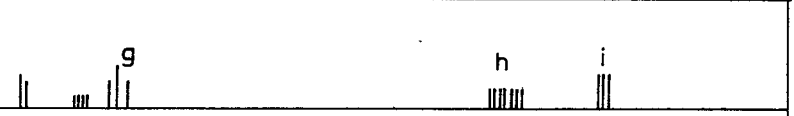
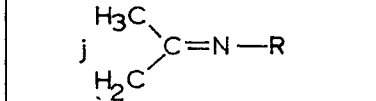
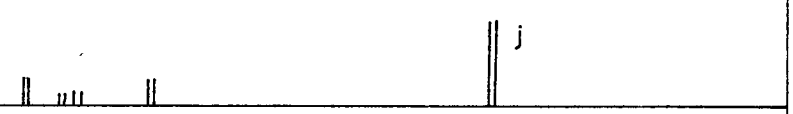
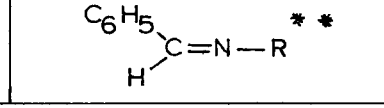


FIGURE 40. ESR Spectrum of Acenaphthene $\pi \rightarrow \pi^*$ Triplet State in EPA.

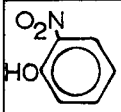
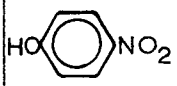
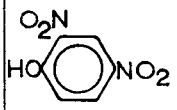
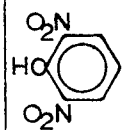
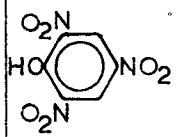
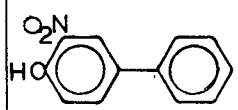
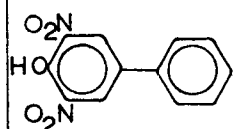
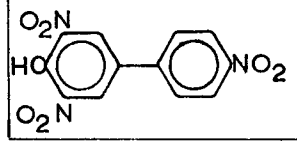
TABLE XXVII. Melting Points and NMR Spectra of 2,4-Dinitrophenylhydrazones.

Parent Compound	Formula	M.P. (°C) Reported*	M.P. (°C) Obtained	NMR Spectra in Trifluoroacetic Acid
—		192-193	195-197	
Formaldehyde		166	167-168	
Acetaldehyde		168	165.0-165.5	
Propion-aldehyde		155	154-155	
Acetone		128	126-127	
Benzaldehyde	 **	237	238-239	Insoluble in trifluoroacetic acid

* Reference (196)

** Calculated for $C_{13}H_{10}O_4N_4$: C, 54.55; H, 3.52; N, 19.57%. Found: C, 54.28; H, 3.69; N, 19.43%.

TABLE XXVIII. Melting Points and NMR Spectra of Nitrophenols.

COMPOUND		m.p. (°C) Obt.	Rep.	Ref.	NMR Spectra in Acetone
	XLVIII	44.5-45.0	44.9	197	
	XLIX	114.5-115.5	114	197	
	L	116.5	113	197	
	LI	63.5-64.0	63-64	197	
	LII	123	122.5	197	
	LIII	60-62	67	215	
	LIV	157-158	154-155	216	
	LV	208-209	201-202	216	

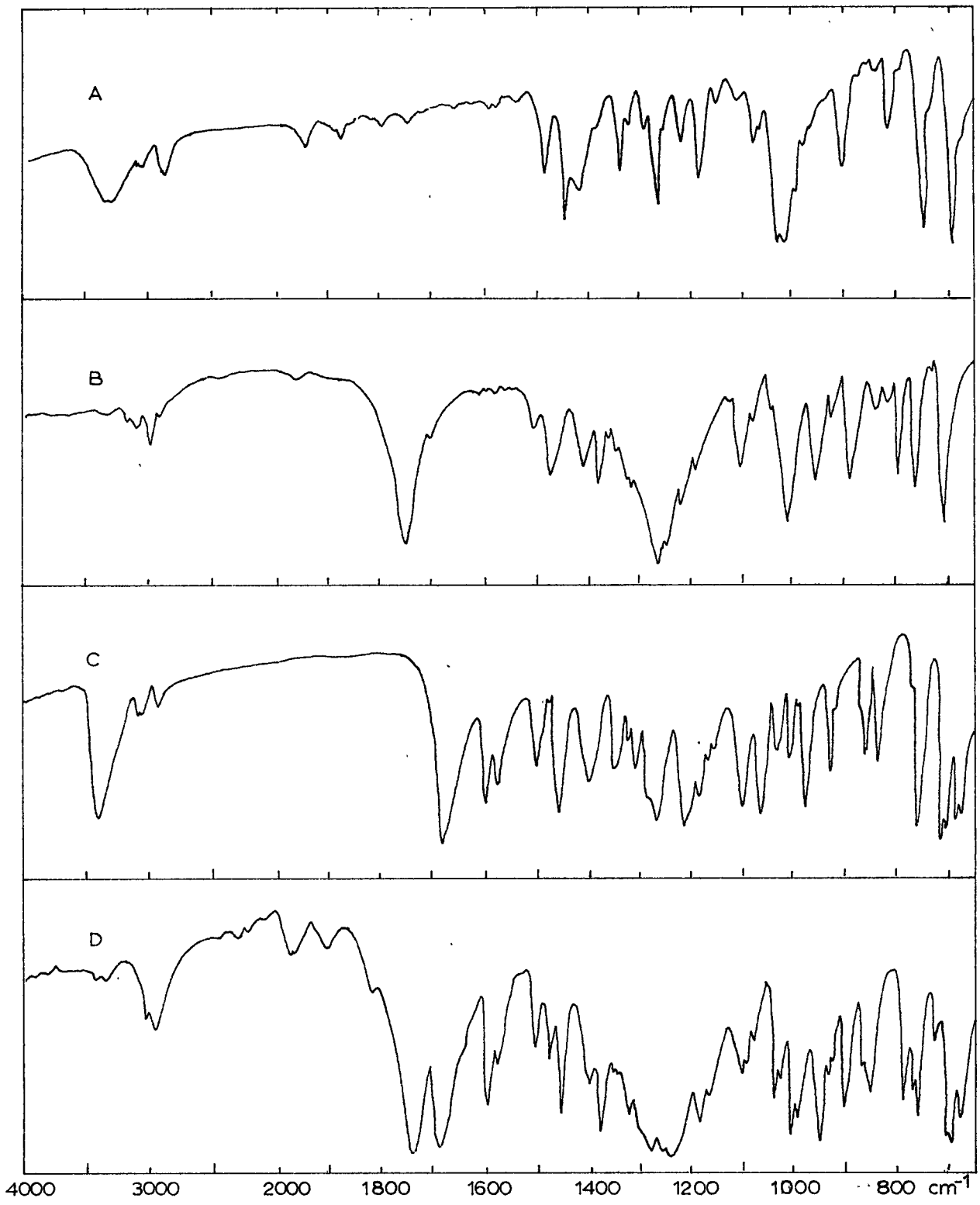


FIGURE 42. Infrared Spectra of Ethylcarbonates of 1,2-Diphenylethane Derivatives. A: meso-Hydrobenzoin; B: meso-Hydrobenzoin Di-(Ethylcarbonate); C: Benzoin; D: Benzoin Ethylcarbonate.

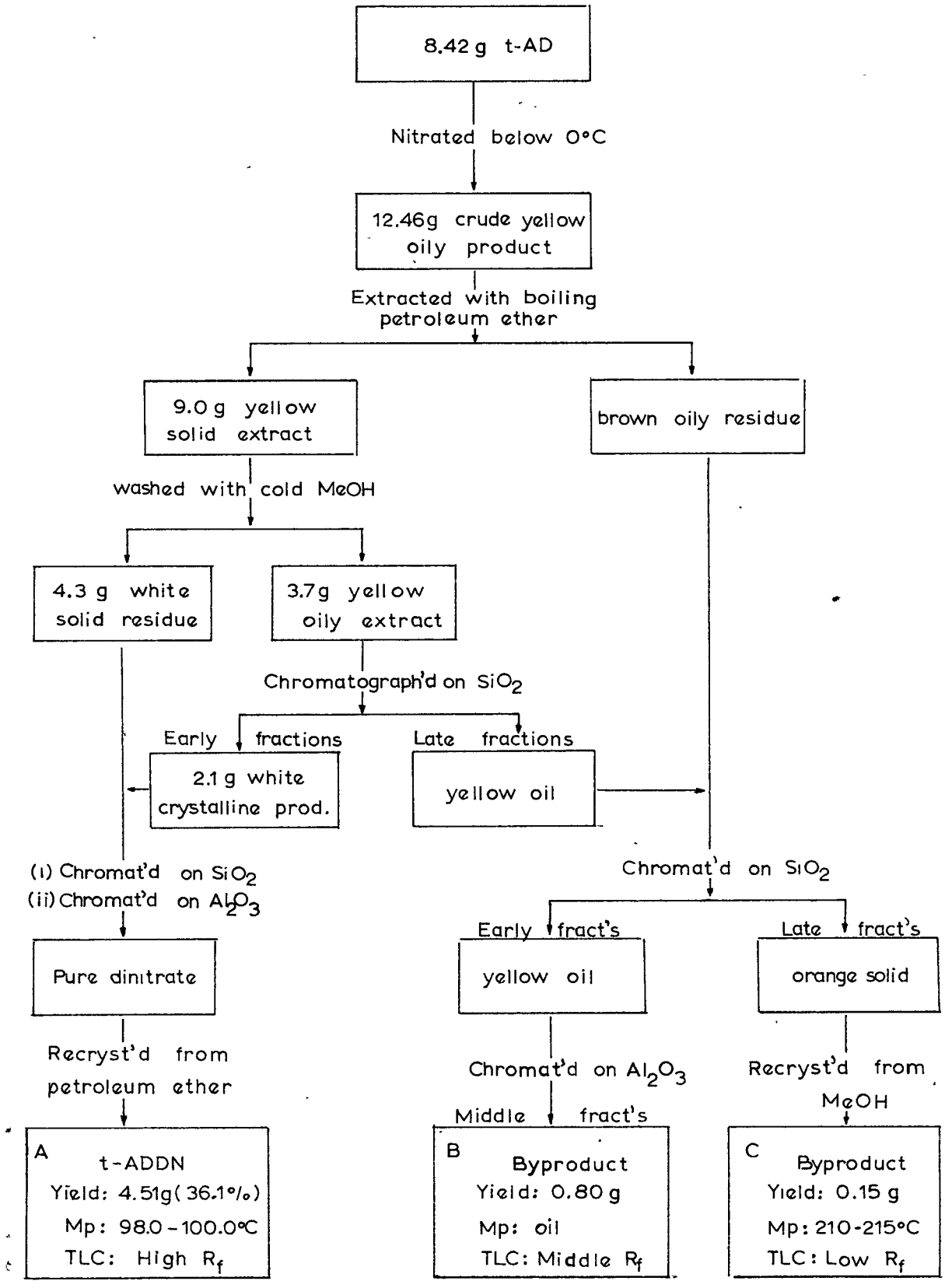
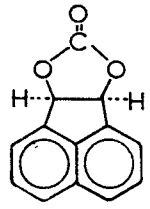
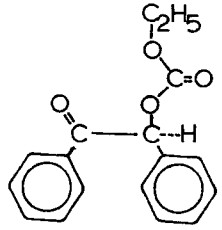
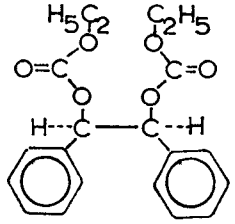


FIGURE 43. Flow Sheet of Separation of Nitration Products from trans-1,2-Acenaphthenediol. (t-AD)

TABLE XXX. Attempted Syntheses of Aromatic Nitrate Esters from Cyclic and Ethylcarbonates.

Carbonates	Sample (mg)	Solvent * (ml)	$AgNO_3$ (mg)	Reaction Condition**		M.P. (°C)		
				Temp. (°C)	Time (hrs)	Starting Material	Expected Nitrate Ester	Product Obtained
 cis-1,2-Acenaphthenediol Carbonate (LVI)	5.4	5.0	14.4	60	20	231	128-130	228-234
 Benzoin Ethylcarbonate (LVII)	43.0	5.0	179	Bp.	3	74-76	77-78	76-78
 meso-Hydrobenzoin Di (Ethylcarbonate) (LVIII)	50.7	5.0	142	Bp	3	113.5-115.5	148.5-149.5	116.5-118.5

* Acetonitrile

** Procedure according to Boschan (37)

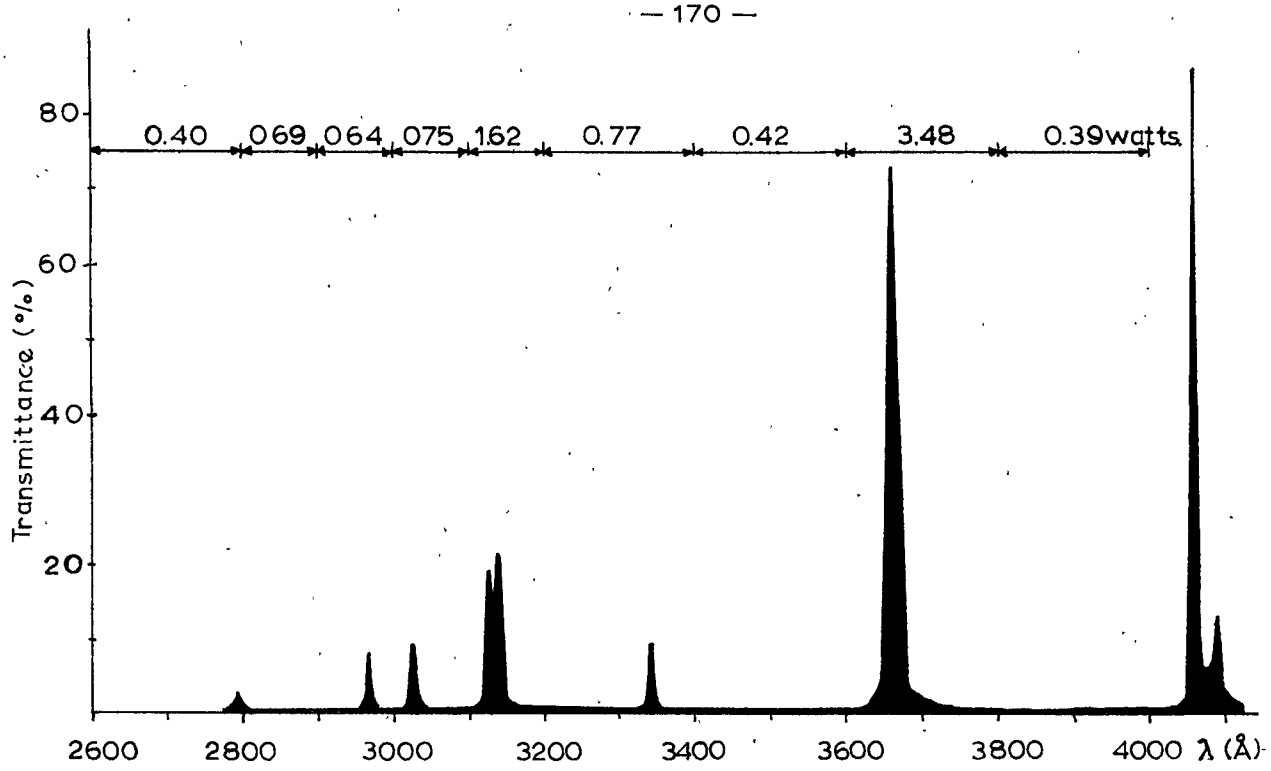


FIGURE 44. Reported Power Output and Observed Spectral Distribution for G.E.-H85-A3 Medium Pressure Mercury-Arc Lamp.

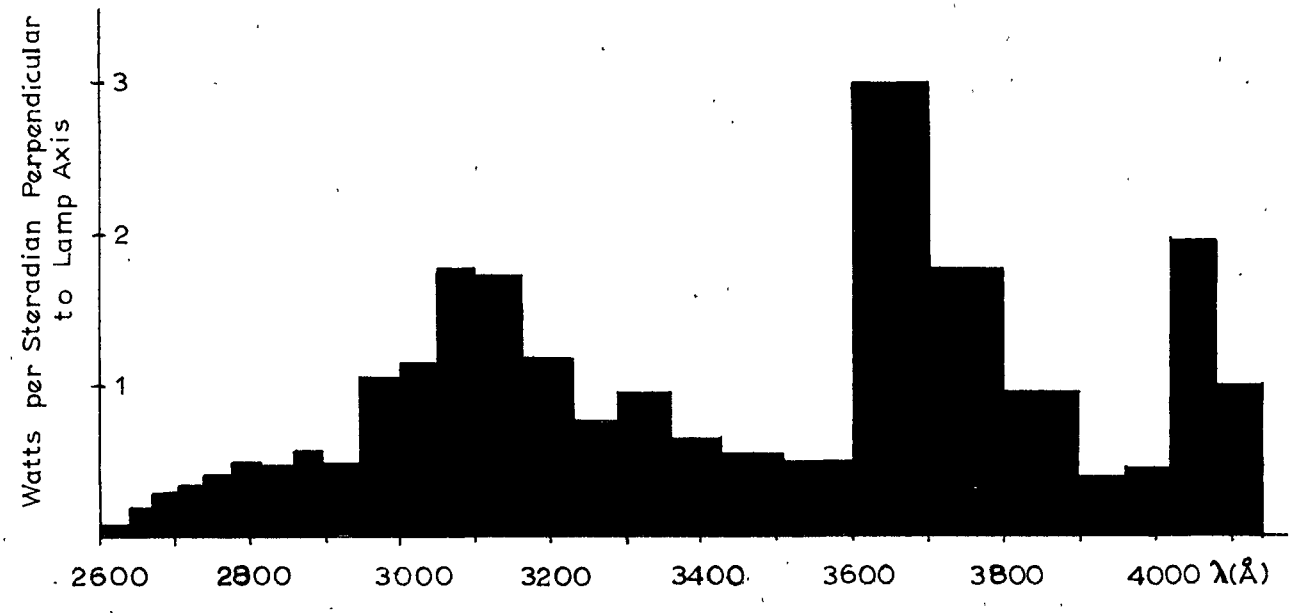


FIGURE 45. Reported Power Output for GE-A-H6 High Pressure Mercury-Arc Lamp.

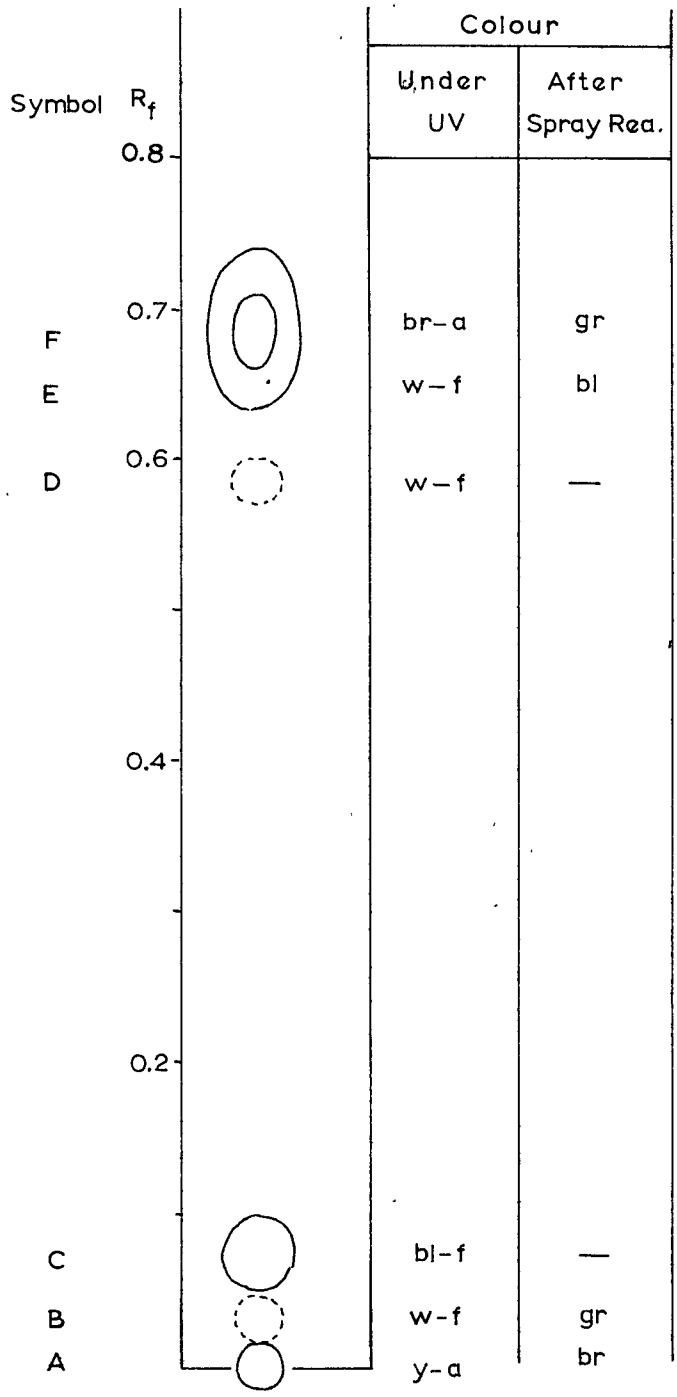


FIGURE 48. TLC (M-5, S-1, R-1) of trans-1, 2-Acenaphthenediol Dinitrate Rhotolysed in Benzene Solution.

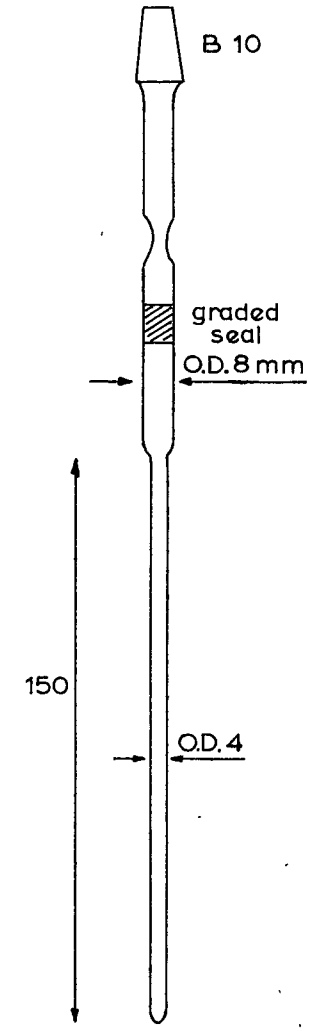


FIGURE 49. ESR Tube. Scale 2:1

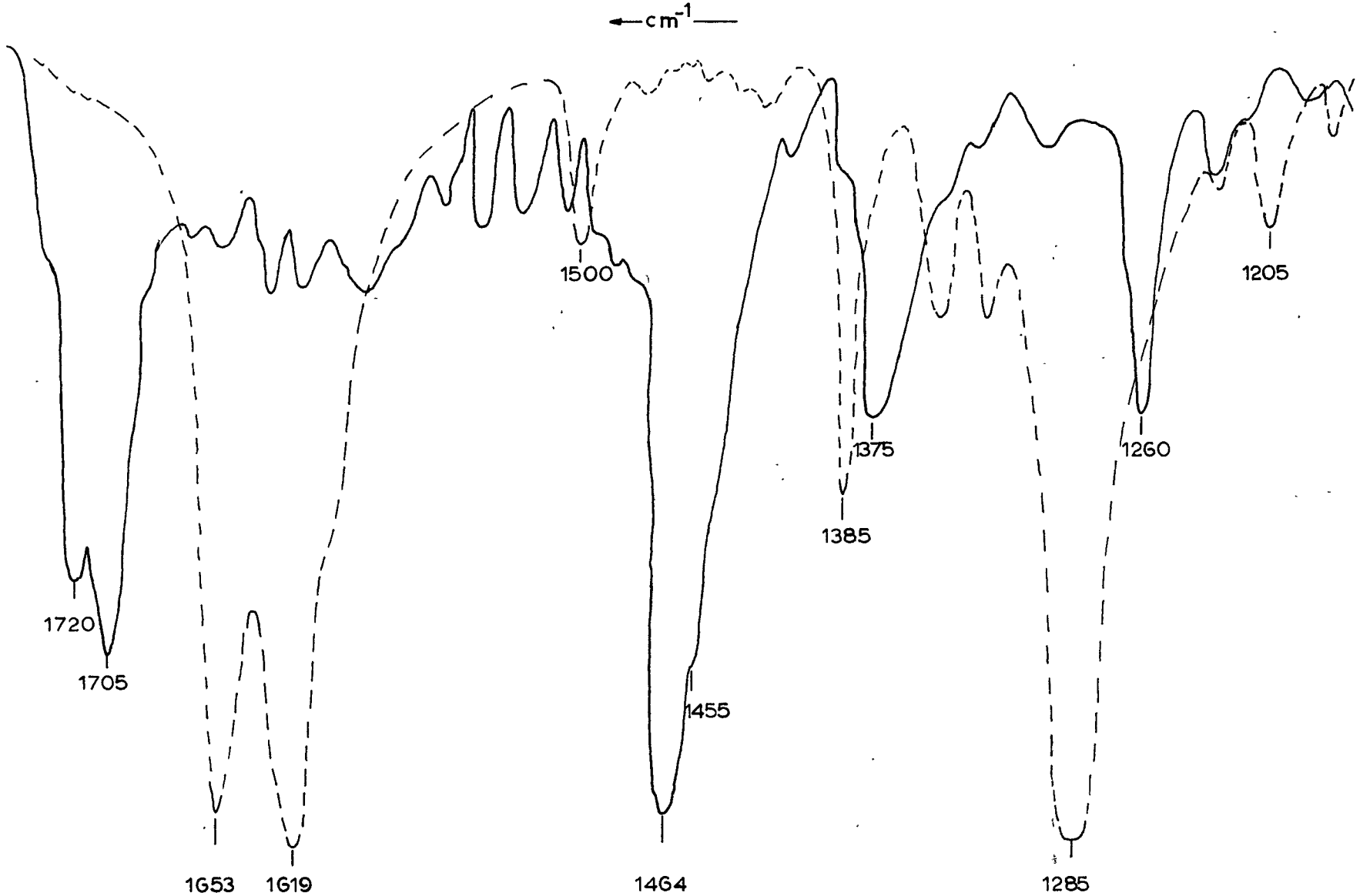


FIGURE 50. Solid State Infrared Spectra of <u>trans</u>-1,2-Acenaphthenediol Dinitrate and One of Its Photolysis Products. (- - - - - - - t-ADDN; ——— fluorescent photolysis Product (C)).

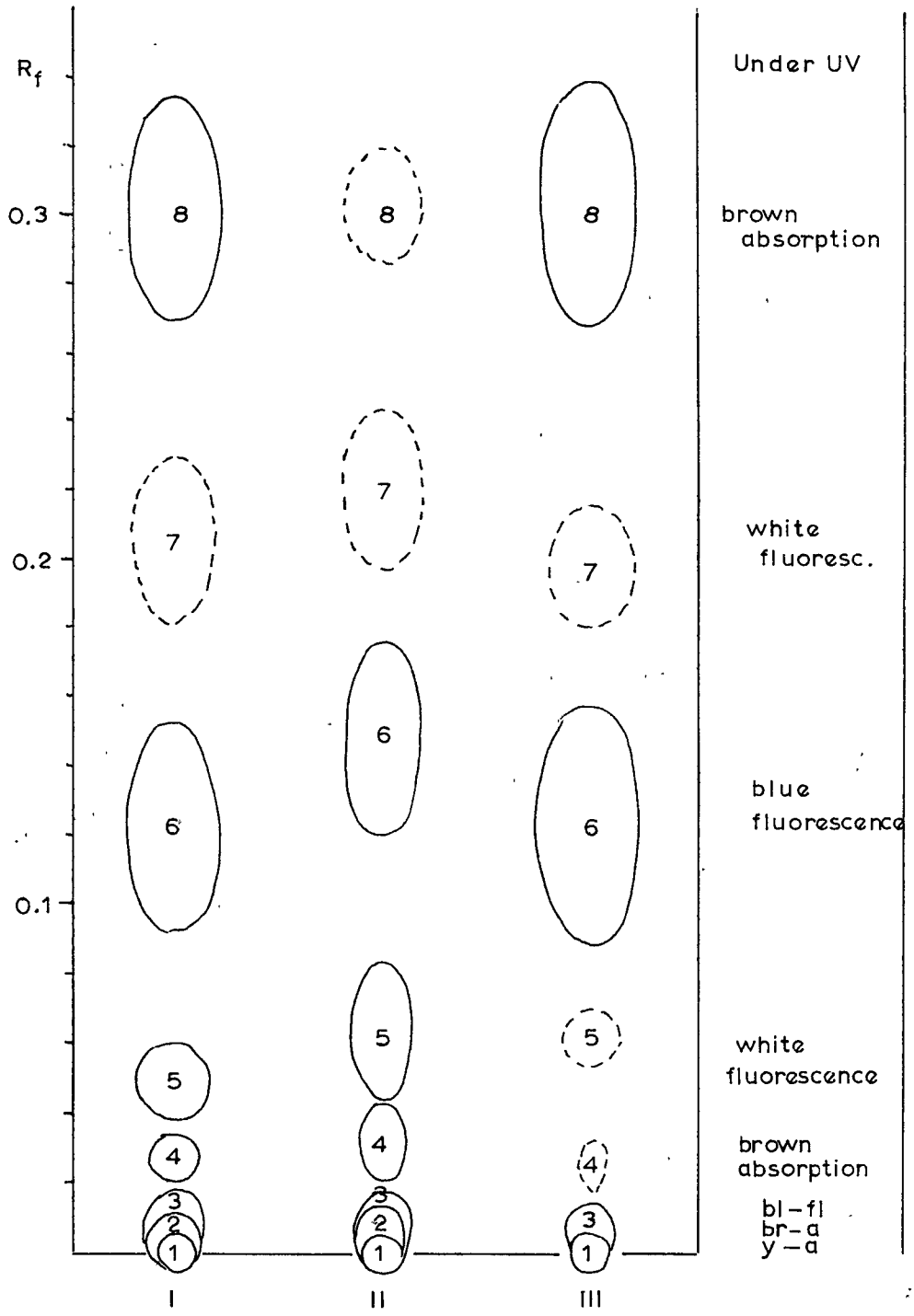


FIGURE 51. TLC (M-5, S-6) of trans 1 2 Acenaphthenediol Dinitrate after Photolysis in Benzene Solution.
I. in Quartz Cell (0.01 M) II. Quartz ESR Tube (0.1 M)
III. in Corex Cell (0.01 M)

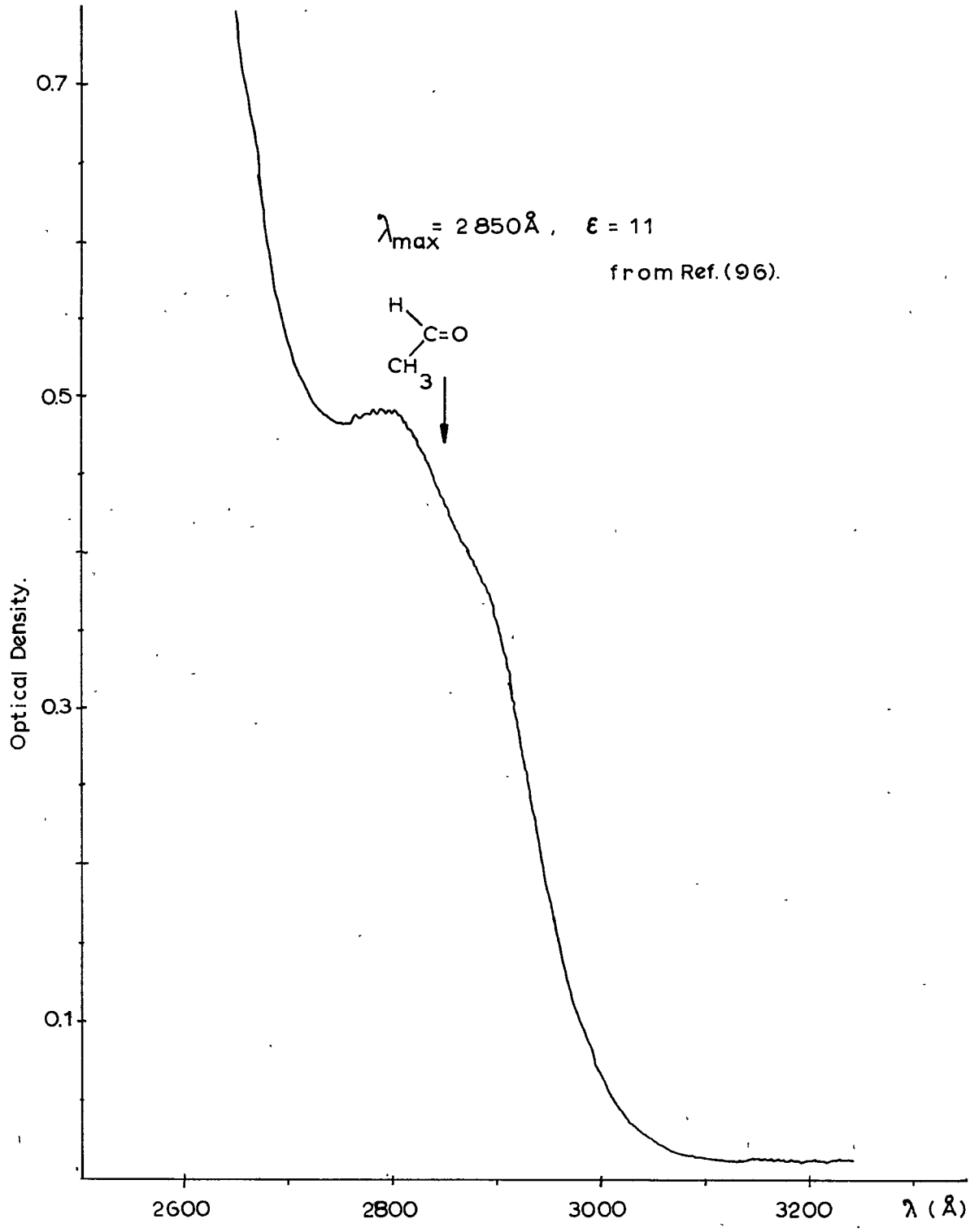


FIGURE 52. UV Spectrum of Acetaldehyde from the Photolysis of meso-Hydrobenzoin Dinitrate in Ethanol