ETHNOBOTANY AND PHYTOCHEMISTRY OF

TROPAEOLUM TUBEROSUM AND LEPIDIUM MEYENII

FROM ANDEAN SOUTH AMERICA

by

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Abstract

A systematic investigation of the ethnopharmacology of
Tropaeolum tuberosum Ruiz & Pavon and Lepidium meyenii Walp., in
the Tropaeolaceae and Brassicaceae respectively, was undertaken
to determine the physical basis for the medicinal and
nutritional uses of these species by natives of the Andes
mountains. The domestication of T. tuberosum in relation to
these uses was considered from the perspective of the
glucosinolate chemotaxonomy of the two subspecies, tuberosum and
silvestre .

High Performance Liquid Chromatography (HPLC) was used as
the primary tool for determining the isothiocyanates hydrolyzed
enzymatically from glucosinolates of the tubers, seeds, flowers
and leaves of both subspecies of T. tuberosum , and from the
roots of L. meyenii . On the basis of HPLC, paper chromatography
of thiourea derivatives, mass spectrometry and nuclear magnetic
resonance spectrometry it was concluded that the sole
isothiocyanate liberated from T. tuberosum subsp. tuberosum is
p-methoxybenzyl isothiocyanate. T. tuberosum subsp. silvestre is
characterized by benzyl, 2-propyl and 2-butyl isothiocyanates.
This difference in glucosinolates supports the existence of two
distinct subspecies. Hydrolysates of L. meyenii contain benzyl
isothiocyanate as the primary constituent, and p-methoxybenzyl
isothiocyanate as a minor constituent. N,N,Di(methoxy, 4-
benzyl)thiourea was detected in the isothiocyanate extracts of
T. tuberosum subsp. tuberosum .

A statistical survey of the ethnobotanical uses of
glucosinolate-containing plants from around the world was

carried out. The significant medicinal uses for glucosinolate-containing plants in general correlates positively with the uses from the Andes of _I. tuberosum_ and _L. meyenii_ . Pharmacological studies on crude plant material and extracts of _I. tuberosum_ , and on pure compounds were carried out in relation to the reputed uses of these species.

Tropaeolum tuberosum and _Lepidium meyenii_ are believed to affect human fertility. Feeding studies of female guinea pigs and _in vitro_ studies to test the 17β-estradiol binding inhibition of extracts and of pure isothiocyanates failed to substantiate any estrogenic activity of these taxa. However, preliminary results for N,N,Di(methoxy, 4-benzyl)thiourea suggest that this compound competitively inhibits estradiol binding and may have estrogenic activity. The antiaphrodisiac beliefs associated with _I. tuberosum_ subsp. _tuberosum_ were examined in male rats fed a diet containing tubers of this taxon. Experimental animals and controls showed equal capability in impregnating females, although animals fed _I. tuberosum_ showed a 45% drop in their blood levels of testosterone/dihyrotestosterone. This drop appears to be related to the antimetabolic effects of isothiocyanates in the tubers.

Tubers of _Tropaeolum tuberosum_ and purified isothiocyanates were shown to be antibiotic but not phototoxic against yeast and bacteria. Benzyl isothiccyanate was shown to be nematocidal. Tests of a tuber extract against Herpes Type I virus failed to substantiate possible antiviral activity for this species.

Analysis of free amino acids in tubers of _I. tuberosum_ failed to detect any non-protein or unusual amino acids.

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Introduction

Tropaeolum _tuberosum_ Ruiz & Pavon and _Lepidium_ _meyenii_
Walp., in the Tropaeolaceae and Brassicaceae respectively, are
two species of plants from Andean South America cultivated for
their edible and medicinal uses. To understand why they are so
used requires insight firstly into their origin and their
domestication, and secondly into their physical properties and
how these properties have contributed to the welfare of the
people who use them. The latter necessitates investigation into
the chemistry and pharmacology of the plants and will comprise
the bulk of this thesis.
The model of ethnobotanical investigation outlined by
Schultes (1960,1972) provides the basis, whether stated or
implied, for most studies into the pharmacology of folk
medicine. Schultes stresses the importance of ethnobotanical
work in the search for new drugs and suggests the following
methodology:

1. Bibliographic search of ethnobotanical and
herbalistic literature of the past.

2. Search of herbaria for ethnobotanical references.

3. Ethnobotanical field work among primitive
societies.

4. Phytochemical screening and investigation.

The contribution of ethnobotany to modern botanical,
pharmacological and medical sciences has generally been

negligible or neglected because of the often subjective nature of both the way in which plants are used by groups of human beings, and the way in which acquired information is compiled and evaluated. Considering the variety of cultural contexts in which the empirical knowledge of medicinal and edible plants is formulated and then in turn compiled, and considering the variation in concepts of health and disease, and of curing, it is often difficult to translate ethnobotanical data cross-culturally into information meaningful to the natural scientist. In this context the insights of the anthropologist are invaluable in interpreting the mythological and sociological aspects of folk medicine, and in preventing the pitfalls of a strictly ethnocentric approach in evaluating a particular folk remedy. In general, the means of effectively evaluating the extensive published information on the medicinal use of plants are lacking.

Phytochemical and pharmacological screening of plants in relation to their uses provides the only real test of the efficacy of a reputed native remedy. In many cases the path from native remedy to known constituent and even pharmacological preparation is straightforward. Such cases, however, become increasingly less frequent as the more dramatic folk remedies are investigated. Considering the diverse and unsystematic nature of ethnobotanical literature a non-critical selection of plants for study is little more than random phytochemical study in itself. If ethnobotany is to make a contribution to pharmacology and phytochemistry , it must do so by increasing the odds of success when looking for active principles, and by predicting beyond the level of chance the distribution of

particular chemicals.

One method of doing this is through more systematic evaluation of ethnobotanical data. In this thesis ethnobotanical information is considered from a plant taxonomic perspective with attention to both classical taxonomy and chemotaxonomy. Within this framework, data compiled and analyzed by modern computing methods is used to determine patterns of use in plant taxa and to relate this to known phytochemistry. Plants are then tested in relation to this analysis for chemical constituents and for biological activity.

The principle focus of this study, Tropaeolum tuberosum , is an edible tuber-producing plant of the high Andes cultivated for its food and medicinal uses. Pre-Columbian beliefs in the plant's properties as a male antiaphrodisiac and female fertility agent have continued to the present, and may be associated with the original domestication of the species. Its contribution to the adaptation of Andean Indians to their harsh hypoxic environment has interested the author since his sojourn in Peru in 1977. Material was collected then for study. Chemical and pharmacological investigation of the plant has been undertaken in this context.

The principle secondary constituents of the species are glucosinolates. Glucosinolates characterize several families, including the Tropaeolaceae, whose affinity in the order Capparales has been underlined by the presence of this class of compound (Dahlgren, 1975). In the Capparales, most obvious biological activity is attributable to the enzymatic breakdown products of glucosinolates, the isothiocyanates (Virtanen, 1965). Variation in chemical properties within the class of compounds is due to side chain differences in the aglycones.

Chemical investigation within glucosinolate-containing plants has been principally of a chemotaxonomic nature. Members of the order are widely distributed and are represented in most floras and ethnobotanical literature. Review of this literature has sought patterns of consistency of medicinal and nutritional use in relation to T. tuberosum . One species, Lepidium meyenii , in the Brassicaceae, stands out. It is sympatric with T. tuberosum , is cultivated for its edible root and is reportedly used medicinally to affect human fertility in a similar way to T. tuberosum . It was analyzed phytochemically for this reason.

Literature review

Ethnobotany of glucosinolate containing plants: data tabulation

References used in the compilation of a data base on the
ethnobotanical uses of the order Capparales in Central and South
America are listed in Supplementary Bibliography I. This
provides a fairly comprehensive bibliography of the ethnobotany
of Latin America and has been listed separately for this reason.
Most books or articles are regional in perspective, often
referring to a specific cultural group or geographical area.
References which compile information from diverse sources are
particularly useful. Altschul (1973) lists information gleaned
from the Gray Herbarium and covers a wide geographical area.
Ford (1975) compiles extensive ethnobotanical data from the arid
regions of northern Mexico and southwestern USA, as used by
Spanish speaking peoples.
     References on a global scale that were reviewed in relation
to the Capparales are listed in Supplementary Bibliography II.
Moerman's (1977) survey of American Indian medical ethnobotany
provides a basis for any study in Canada and the USA.
Particularly useful for tropical ethnobotany were Burkill (1966)
and Watt and Breyer-Brandwijk (1962).

Ethnobotany and ethnopharmacology of *Tropaeolum tuberosum* and *Lepidium meyenii*

The exploitation of <u>*Tropaeolum tuberosum*</u> as an edible tuber (Figure 1.) has received minimal attention other than to record its use in its native habitat. Survey volumes of useful plants invariably record the fact of its cultivation (Brucker, 1977;
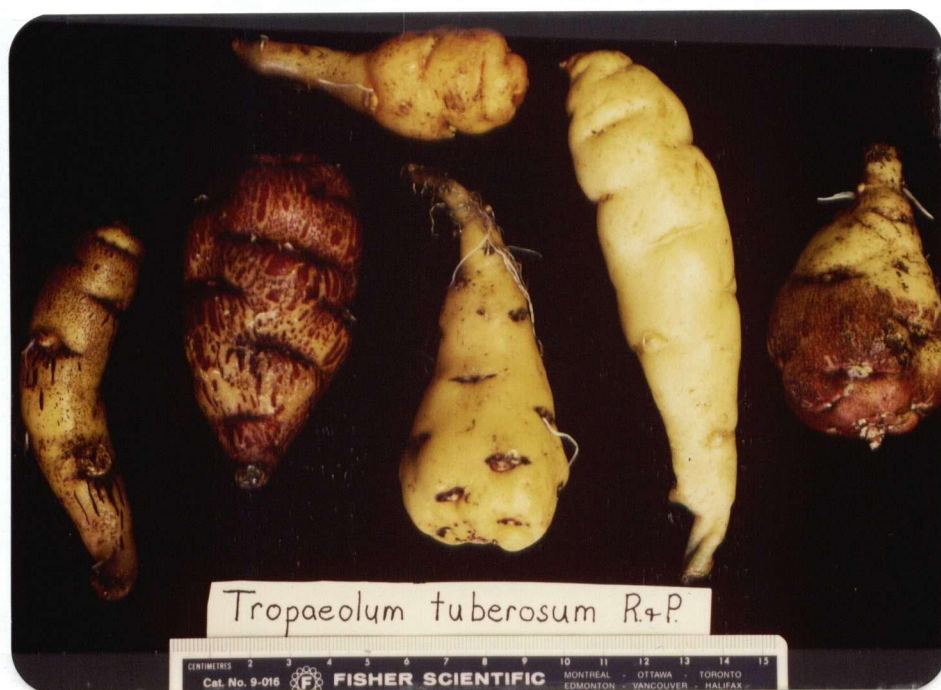
Figure 1. Tubers of <u>*Tropaeolum tuberosum*</u> subsp. <u>*tuberosum*</u>

Montaldo, 1977; Simmonds, 1976; Tanaka, 1976; Uphof, 1968). Various ethnographic reports (Baker and Little, 1976; Gade, 1975; Mazess and Baker, 1964; Stein, 1961; Vasquez Varela, 1952) mention the use of the plant in specific regions in the Andes. More detailed description of its cultivation and use is given by Hodge (1946, 1951) and Leon (1964). Fernandez (1973) provides

useful information into the history of its exploitation particularly in Argentina, and Williams (1978) gives evidence (although perhaps questionable) for the post-conquest introduction of the plant into, and current use in, Mexico. Beckett(1979) documents current interest in the plant in Britain, although primarily as an ornamental.

The nutritional contribution of tubers of _Tropaeolum_ _tuberosum_ is primarily due to their high carbohydrate content. Leung and Flores (1961) record 11 grams carbohydrate per 100 grams wet weight . Although no report is known defining the starch, leaves of _Tropaeolum_ _majus_ are known to contain mannitols (Gibbs, 1974) and it is possible that this sugar alcohol comprises the main low molecular weight carbohydrate in the tubers. The high ascorbic acid content (67.0 mg/100g wet weight) (Leung and Flores, 1961) contributes to the nutritional value of this plant. The protein content of the tubers of 1.6 g/100g wet weight (Leung and Flores, 1961), corresponds to the low values of most plant tubers. Potatoes contain 2g/100g wet weight by comparison.

Reports of medicinal uses of _Tropaeolum_ _tuberosum_ coincide with reports of its use as a food crop. In areas of the Southern Andes where cultivation of the plant is 'ornamental and pseudo-ornamental', Fernandez (1973) explains its presence as a relict of a primordial agricultural complex. Its propagation for non-economic reasons would underline a significant place in the past for this plant in the cultures of these peoples. The current medicinal uses and beliefs surrounding _T_. _tuberosum_ support this theory.

Beliefs concerning the medicinal uses of 'isaño', 'añu' or 'mashua' are fairly diverse but a few patterns do emerge. Garcia

Barriga (1975) reports that in Colombia the tubers are considered diuretic; they serve to break bladder and kidney stones and are used to treat kidney pain and other kidney diseases. Soukup (1970) and Oblitas Poblete (1969) report similar uses in Peru and Bolivia respectively. Hodge (1946,1951) reports that in Quito an Indian woman assured him that cooked tubers are especially good for the kidneys and liver. Strong diuretic effects in rats have been reported for allylisothiocyanate (Muztar et al., 1979b). Possible similar effects for other isothiocyanates provides the most immediate physiological explanation for any of these fairly consistent uses. Urine excreted by man and experimental animals after eating leaves of Tropaeolum majus , the garden nasturtium, has been reported to be antibiotic (Watt and Breyer-Brandwijk, 1962) and it is possible that such activity may favourably affect the urinary tract in some cases.

Garcia Barriga(1975) also reports that the tubers are effective in treating skin diseases such as eczemas and herpes. Valdizan and Maldonado, (1922) report that in Peru the flowers are rubbed on spots ('empeines') on the face and Oblitas Poblete(1969) lists the tubers as treatment for the skin ulcers caused by tropical insects. These conditions appear to be generally biotic or viral in etiology. Extracts and essential oils of Tropaeolum majus have been shown to be antibiotic against Bacillus subtilis, Escherichia coli, Staphylococcus aureus (Winter and Willeke, 1952); Candida albicans, Microsporum lanosum, Trichophyton gyrseum (Vickkanov et al., 1969); Saccharomyces spp., Lactobacterium buchneri and Acetobacter aceti (Shcherbanovski and Nilov, 1969). Dannenberg et al. (1956)

showed that the active antimicrobial principle of nasturtium is
identical with benzylisothiocyanate, one of the isothiocyanates
present in the seeds of wild T. tuberosum (Kjaer et al., 1978).
Glucosinolate containing plants, including Tropaeolum majus are
also known to be insecticidal (Sehgal and Ujagir, 1977; Blau et
al., 1978). Such activity supports the reported use from Bolivia
of T. tuberosum for killing lice (Oblitas Poblete, 1969).

A. Camino (pers. comm., 1977), an anthropologist working on
traditional agricultural practices in Cuyo-cuyo, Peru says that
the local farmers plant 'isaño' interspersed among other tuber
crops because they consider it resistant to pathogen attack and
capable of protecting other tubers from destruction. Certainly
antifungal and insecticidal properties are significant in this
case. Nematodes pose a particular problem in this area. Gommers
(1973) in a review of plant nematicidal principles quotes works
that show an inhibitory effect of isothiocyanate containing
plants on the emergence of larvae of Heterodera rostochiensis
Wollenweber.

Other folk uses of Tropaeolum tuberosum lack the possible
rational basis of the former cases. Oblitas Poblete(1969)
reports its being used to treat nervous diseases; as an
expectorant and as an anti-cough remedy; and to combat
polycethemia, a common ailment of high altitudes (Fuchs, 1978).
Cortes Bravo (1977), in a personal communication, stated that in
Cuzco the tubers are taken in soup to treat stomach ulcers.

The most pervasive and perhaps the most puzzling of all
beliefs associated with T. tuberosum is that of the plant's
efficacy as an antiaphrodisiac of males. The sixteenth century
Spanish chroniquilists document that the Incas fed 'añu' to
their troops so that they would forget their women while on

military operations, and that the tuber suppresses sexual appetite and decreases the reproductive potential:

> "tiene virtud esta raíz de reprimir el apetito venéreo, según dicen los indios; y así afirman que mandaban los reyes Incas del Perú llevar la copia de este mantenimiento en sus ejércitos, para que comiendo de él los soldados, se olvidasen de sus mujeres." Father Cobo (1956)

> "dicen los indios que comida es contraria a la potencia generativa." Inca Garcilaso da la Vega (1960)

Although magical beliefs accompany these accounts, the use of _T. tuberosum_ in affecting human reproductive potential has continued to the present. Modern writers invariably cite Cobo and Garcilaso de la Vega (Soukup, 1970; Fernandez, 1973; Yacovleff and Herrera,1935). Hodge (1949, 1951), Gade (1975), Montaldo (1977) and Oblitas Poblete (1969) give evidence supporting the continuation of this belief into modern times in specific regions. In the Department of Ancash in Central Peru, Vasquez Varela (1952) records that 'mashuas' are considered as food for women. Men refuse to eat these tubers because they believe that to do so produces impotence and an incapacity to have children. A report in the Journal of the Horticultural Society of London from 1855 stated, in reference to the use and preparation of _T. tuberosum_ , that "the ladies of La Paz are all very fond of the Ysaño, and in the season of the taiachas large quantities are sopped in molasses, and taken as refreshments

during the heat of the day." In modern Bolivia T. tuberosum is believed to induce menstruation (Oblitas Poblete, 1969) and in Cuzco, Peru Tropaeolum majus and T. semanni Buch. are employed in popular medicine as emmenagogues (Herrera, 1940). In folk medicine generally, menstruation is seen as a sign of femininity and fertility (Conway and Slocumb, 1979) and efforts are made using herbs and other means to induce late menstrual periods.

The only report on the glucosinolates of Tropaeolum tuberosum is from seeds of wild plants collected in Peru. Kjaer et al., (1978) report benzylisothiocyanate and 2-propylisothiocyanate (Figure 2.) as the major components upon enzyme hydrolysis, with 2-butylisothiocyanate occurring in lesser amounts. Tropaeolum majus is reported to contain large proportions of erucic and eicosenoic acids in the seeds and flower petals (Radwan, 1976). Although erucic acid in Cruciferous seed oils has been implicated in producing lesions in cardiac tissue (McCutcheon et al., 1976), there is nothing to suggest that the presence of either of these fatty acids in T. tuberosum would contribute to the recorded medicinal uses.

Folk beliefs concerning Lepidium meyenii , the maca, have a striking correspondence to those of Tropaeolum tuberosum , although references are scarce. Leon (1964) provides the most accessible and recent overview of its biology and ethnobotany. Besides being cultivated for its edible root (Figure 3.), the most common use for maca is as a fertility medicine. It is

$$R-N=C=S$$

2-propyl $R=(CH_3)_2-CH-$

2-butyl $R=C_2H_5-\overset{\displaystyle |}{\underset{\displaystyle CH_3}{CH}}-$

ethyl $R=C_2H_5-$

2-methylpropyl $R=CH_3-\overset{\displaystyle |}{\underset{\displaystyle CH_3}{CH}}-CH_2-$

benzyl $R=$ ⬡$-CH_2-$

p-methoxybenzyl $R=$ $_3HCO-$⬡$-CH_2-$
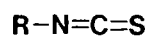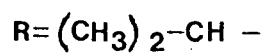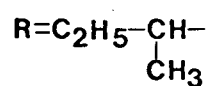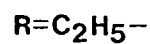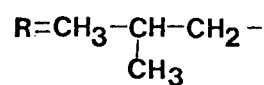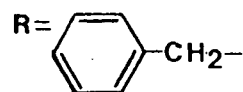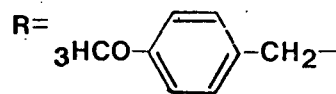
Figure 2. Isothiocyantes of the genus <u>Tropaeolum</u>.

reported by the chroniquilists that in the time of the Spanish conquest the Indians recommended feeding maca to domestic animals to combat low reproductive rates at high altitudes, and
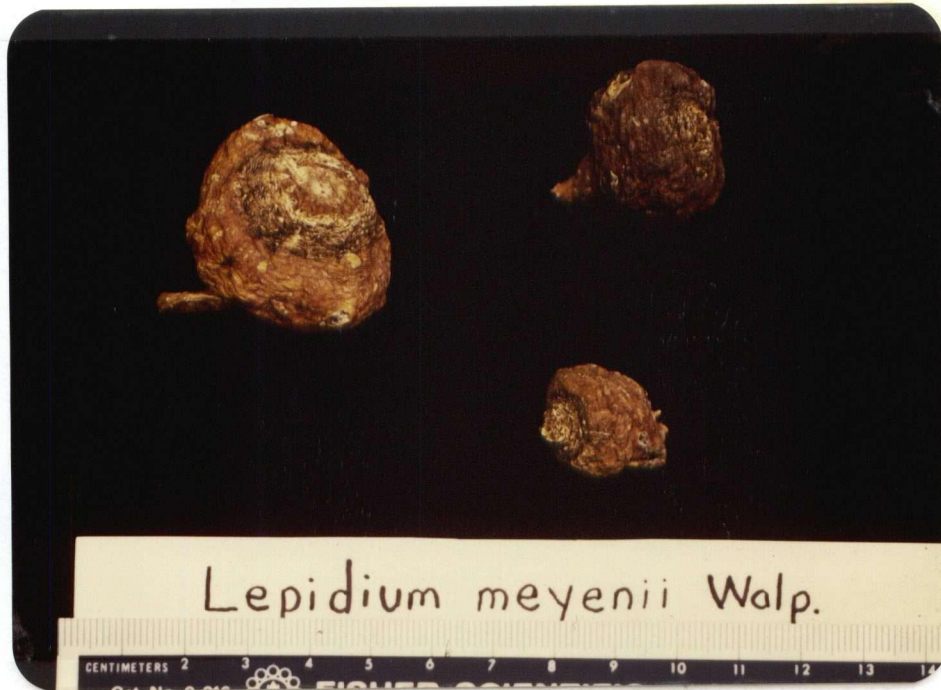
Figure 3. Edible roots of Lepidium meyenii

that the Spanish noticed the positive effects. Today maca is cultivated only in a few areas of central Peru. Leon reports that maca is now eaten by Indian and white women who want to have children. It is sold in the markets for this purpose. Michael F. Brown (1979), doctoral candidate in anthropology at the University of Michigan, reports that in 1973 when he worked in Junin, belief in the fertility effects of maca was widespread. However, the fact that the belief applied particularly to male fertility seems in contradiction to the other beliefs mentioned above.

Lepidium meyenii has not been studied phytochemically.

Lepidium species, as members of the Brassicaceae, are known to contain glucosinolates. Species from other areas of the world have been studied and found to contain a variety of glucosinolates including benzylisothiocyanate reported above from I. tuberosum . Variation comprising alkyl and alkenyl derivatives occur within the genus although aromatic glucosinolates, with or without hydroxy and methoxy substitutions in m- and p- positions, prevail (Kjaer and Wagniere, 1971). 3,4,5-Trimethoxybenzylglucosinolate occurs in L. sordida A. Gray (Kjaer and Wagniere, 1971) and L. hyssopifolium Desv. (Kjaer et al., 1971). The only species studied from South America, L. bonariense L. from Argentina, is reported to contain p-hydroxy and p-methoxybenzylisothiocyanate (Kjaer and Schuster, 1968). L. bonariense collected in Queensland, Australia has been shown to contain solely the p-methoxy compound (Kjaer et al., 1971).

The parallels between Tropaeolum tuberosum and Lepidium meyenii as agents affecting fertility may be coincidental or relate to cultural and environmental factors unconnected with the phytochemistry and physiological effects of the plants. Reproductive rates are indeed lower and a concern at high altitudes (Sobrevilla et al., 1968; Buck et al., 1968) and folk beliefs associated with this are to be expected. The association of two glucosinolate-containing 'root' crops with this concern may simply be a result of their strong and distinct flavour which tends to draw attention to the plants as medicinal agents.

Systematics and possible origin of Tropaeolum tuberosum

The best botanical description of the species Tropaeolum tuberosum is given by Sparre (1973). Hodge (1951) and Leon

Figure 4. Vegetative portions of Tropaeolum tuberosum

(1964) provide details of cultivation and tuber characteristics , while Herrera (1941) and Cardenas (1948) give reports on specific cultivated forms from around Cuzco, Peru and Bolivia respectively. Sparre differentiates two subspecies; Tropaeolum tuberosum subsp. tuberosum , the tuber producing cultivar, and subsp. silvestre Sparre, a wild type known to occur sympatrically . Sparre considers that subsp. silvestre can be distinguished by its gracile life form and lack of tubers. He states, however, that some specimens must contain tubers,

otherwise the species would not have been selected for cultivation.

Gibbs et al. (1978) have established the chromosome number of subsp. tuberosum definitively at 2n=52. The chromosome number of subsp. silvestre has not been established, although Gibbs et al., (1978) speculate that it is likely a diploid and that through autopolyploidy it has given rise to the tetraploid cultivar, subsp. tuberosum . Alternatively they suggested that the cultivar may have arisen through a hybridization of subsp. silvestre and another related species, perhaps Tropaeolum cochabambe Buch., followed by allopolyploidy. Huynh (1967) has reported a chromosome count of 2n=26 for T. cochabambe . Both T. cochabambe and T. tuberosum have been placed in the section Mucronata (Sparre, 1973). A base number of x=13 has been suggested for this section (Gibbs et al., 1978) in addition to x=7 previously established for Tropaeolum .

The report cited above on glucosinolates present in the species, refers presumably to seeds of T. tuberosum subsp. silvestre . Glucosinolates of subsp. tuberosum have not been identified. Kjaer et al. (1978) report the glucosinolates of several species in the genus including Tropaeolum cochabambe . Seeds of T. cochabambe produce, upon enzymatic hydrolysis, 2-propyl, 2-butyl, 2-methylpropyl, and benzylisothiocyanates. Because 4-methoxybenzyl alcohol and the corresponding aldehyde were detected, 4-methoxybenzylisothiocyanate is likely present as well. In addition, ethylisothiocyanate was detected in another species, T. peregrinum L.. Isothiocyanates known from the genus Tropaeolum are shown in Figure 2.

Androgens and plant estrogens

Glucosinolates as a class have not been studied directly in relation to animal fertility, but specific glucosinolate-containing plants have been included in surveys of estrogenic activity (Chaudher, 1966; Farnsworth et al., 1975ab). No consistent pattern of activity appears. Agents directly affecting reproduction are likely to be steroids or to approximate steroid hormones in activity in binding to the receptor sites responsible for mediating sexual and related physiological processes. Although such hormonal actions, particularly in females, are a result of complex interactions of more than one compound, it can be said generally that androgenic and estrogenic activities are opposite, that is a compound having stimulatory effect in a female might be antagonistic to androgen controlled processes in a male. Thus the folk uses of Tropaeolum tuberosum and Lepidium meyenii as female fertility agents and male antagonists have a certain logic to them. Agents that exhibit effects on estrogen receptors in the female may be estrogens themselves. Estrogenic activity is known from many plants (Liener, 1969), and estrone itself is a known plant constituent (Bennett et al., 1966) The Tropaeolaceae has not been studied for the occurence of estrogen. Isoflavones from plant sources have been shown to have estrogenic activity in cattle and sheep (Pope and Wright, 1954) and extensive studies have been done on the mechanism of action of this group of compounds (Shemish et al., 1972; Shutt and Cox, 1972). Isoflavanoids are known primarily from the Fabaceae and have

never been reported from the Tropaeolaceae. <u>Tropaeolum</u> <u>majus</u> , the only representative of this family to be examined for flavanoids, does not contain isoflavanoids (Delaveau, 1967). The one study on screening for the hemolytic activity characteristic of saponins reports negative results for the several species tested (Ricardi <u>et</u> <u>al</u>., 1958).

Anti-androgens are not known from plant sources. Mainwaring's (1977) review of the mechanism of action of androgens mentions the anti-androgenic effect of estrogen, but the bulk of work in this field is directed at various synthetic compounds such as cyproterone acetate and BCMT (Figure 5.) which have steroid-related structures. Other compounds, particularly flutamide (4'-nitro-3'trifluromethylisobutylanilide) (Figure 5.), exhibit similar competitiveness for binding sites on the androgen receptor protein in rat prostrate and seminal vesical (Peets <u>et</u> <u>al</u>., 1974), but show no structural relation to the steroids. The papers comprising <u>Acta</u> <u>Endocrinologia</u> 92(1979) <u>Supplementum</u> 229 concentrate on the steroidal anti-androgen TSAA-291 and illustrate the methodology available to study compounds with such activity. The desire for a safe and effective male oral contraceptive provides the current impetus into studying anti-androgens.

Muztar <u>et</u> <u>al</u>. (1978b) have shown that allylisothiocyanate lowers the amount of 17-keto steroids excreted in rat urine. 17-Keto steroids are breakdown metabolites of androgens and adrenocorticosteroids (Goodman and Gilman, 1975). Their lower levels may indicate a decrease in testosterone production and

cyproterone acetate

BOMT

flutamide
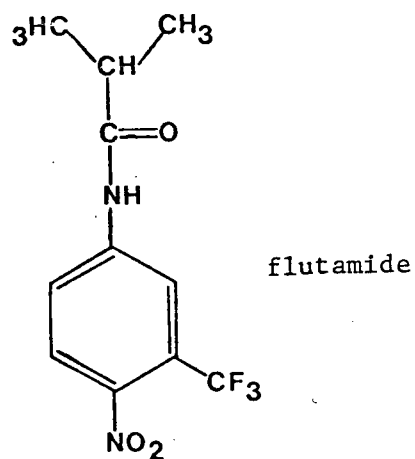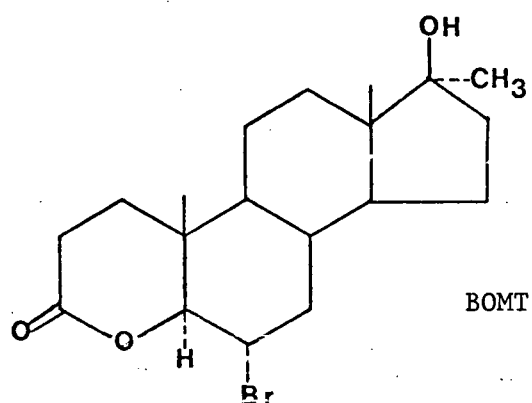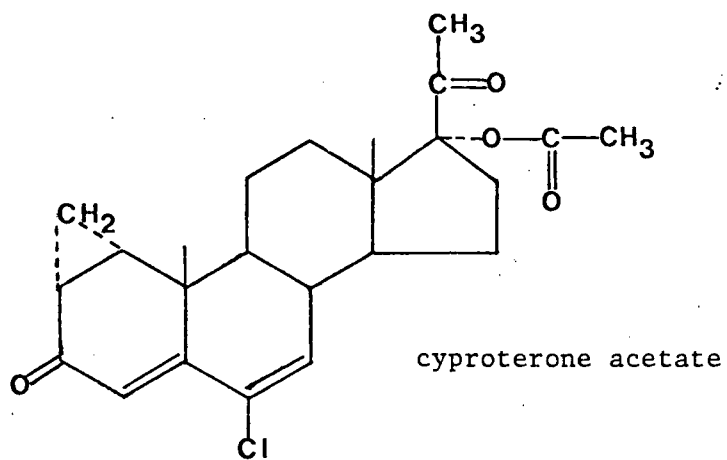
Figure 5. Structures of some known antiandrogens.

blood levels, although this has not been established in the case of allylisothiocyanate.

In studies of both estrogenic and anti-androgenic activity it has been shown that small changes in structure alter activity. Metabolism *in vivo* has been shown to enhance the activity of certain compounds. Katchen and Buxbaum, (1975) have demonstrated that flutamide is converted to trifluoro-2-methyl-4'nitro-m-lactoluidide, which may be the active principle. Nilsson (1961) demonstrated the demethylation of the isoflavanoid, Biochanin A (4'methylgenistein) to genistein, a slightly more potent phytoestrogen, in sheep and cattle ruminal fluid.

If in fact *I. tuberosum* and *L. meyenii* do affect reproductive processes, a mechanism of action is not immediately obvious. Isothiocyanates,because of their presence in the plant and their effect on urinary 17-keto steroids, are obvious candidates for study. All estrogenic and antiandrogenic compounds known, steroidal or otherwise, are aromatic. Significanty, *I. tuberosum* contains aromatic glucosinolates and *L. meyenii* is likely to contain them also . Isothiocyanates are known to be reactive compounds . Their biological activities will be discussed in relation to their chemistry .

Glucosinolates and their metabolites as biologically active compounds

The structure of the glucosinolate anion was correctly elucidated by Ettlinger and Lundeen, (1956). These secondary products are derived from amino acids and occur as potassium salts in plant tissues. Grob and Matile (1979) present evidence to suggest that glucosinolates and their associated enzymes, the myrosinases, are both compartmented in vacuoles. VanEtten et al. (1979) showed that in *Brassica oleracea* the greatest glucosinolate concentration is in the region of the vascular bundles. When the tissue is crushed or damaged the glucosinolate is hydrolyzed, usually forming the corresponding isothiocyanate (Figure 6.). Myrosinases mediate this process (Bjorkman, 1976) and are believed to function as thioglucoside glucohydrolases (E.C. 3.2.3.1.). Myrosinases are generally non-specific with respect to the side chain of the aglycone and their activity is often enhanced by the addition of ascorbic acid.

The aglycones of glucosinolates decompose non-enzymatically usually to the isothiocyanates. However, under particular conditions nitriles and thiocyanates (Miller, 1965) , and occasionally oxazolidine-2-thiones and cyano-epithioalkanes are known to appear, either spontaneously or by an enzymatically controlled reaction. Benn (1977) reviews the catabolism of glucosinolates and the biological effects associated with this catabolism. Isothiocyanates, or mustard oils, are the compounds responsible for the sharp characteristic mustard taste in glucosinolate containing plants. MacLeod (1976) reviews the role

of isothiocyanates, thiocyanates and nitriles in the flavour
palatability of Cruciferous vegetables. In the Andes the sharp
taste associated with _T. tuberosum_ is removed from tubers by

$$R-C \overset{N-OSO_3^-}{\underset{S-Glu}{}} \xrightarrow{H_2O} R-C \overset{N-OSO_3^-}{\underset{S^-}{}} \xrightarrow{} \begin{cases} R-NCS + SO_4^{2-} \quad \text{isothiocyanate} \\ R-CN + S + SO_4^{2-} \quad \text{nitrile} \\ [R^+] + SCN^- + SO_4^{2-} \quad \text{thiocyanate} \end{cases}$$

$$+ \\ C_6H_{12}O_6$$

Figure 6. Breakdown of glucosinolates

boiling before they are eaten. Heating destroys myrosinase
activity thus preventing isothiocyanate release.

The well known goitrogenic effects of cabbage and other
cruciferous vegetables is due to the oxazolidine-2-thiones
derived from the cyclization of 2-hydroxyalkylisothiocyanates,
and also to thiocyanate ions. These antithyroid agents have been
shown to inhibit enzymatic halogenation reactions (Morris and
Hager, 1966; Silverstein and Hager, 1974) and in this way are
thought to interfere with the iodination of tyrosine to produce
thyroxin. Isothiocyanates as well may be goitrogenic. Benn
(1977) suggests that soluble thiourea resulting from the

reaction of isothiocyanate with basic amino acids may be responsible for this activity. Bjorkman (1973) demonstrated the reaction of isothiocyanates with sulphydryl and amino groups in proteins, thus providing a mechanism to account for their toxicity towards microorganisms and insects.

'El bocio', or goitre has been known from the Andes since the conquest (Lastres, 1951). Although 2.7-4.8% of the population have enlarged thyroids in areas where T. tuberosum is consumed (Buck et al., 1968), the incidence is not higher than from other regions in Peru and there is no evidence to associate this condition specifically with the use of T. tuberosum .

Glucosinolate containing plants are known to have an antimetabolic effect on domestic animals (VanEtten and Tookey, 1978). Animals fed more than 10% of rapeseed meal, Brassica napus, suffered weight loss while continuing to consume normal amounts of feed. Muztar et al. (1979a) showed that allylisothiocyanate increased activity of both succinate dehydrogenase and lactate dehydrogenase in rat liver. They suggest that the catalytic effect on oxidative reactions may be similar to that of thyroxine which increases the metabolic rate in sub-cellular mitochondria and microsomes. The significance of this information in the overall homeostasis of the organism is not known.

The fate of isothiocyanates, nitriles and thiocyanates in the test tube or in living systems is not well studied. Benn (1977) suggests that amines and aldehydes, carboxylic acids and thiols respectively are the logical final products. Detailed gas chromatography (GLC) of the seed volatiles of Tropaeolum perigrinum and T. cochatambe by Kjaer et al. (1978) supports the breakdown of isothiocyanates to aldehydes and ketones.

El Migirab et al. (1977) have isolated and described isothiocyanates, thioureas and thiocarbamates derived from Pentadiplandra brazzeana Baillon., an African plant in the family Pentadiplandraceae. Although the unusualness of this
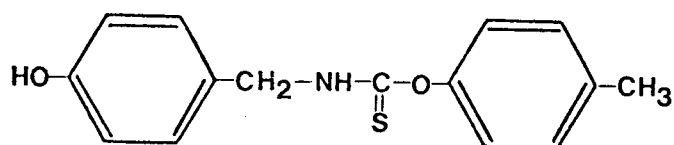
N-(4-hydroxy,benzyl) methylthiocarbamate
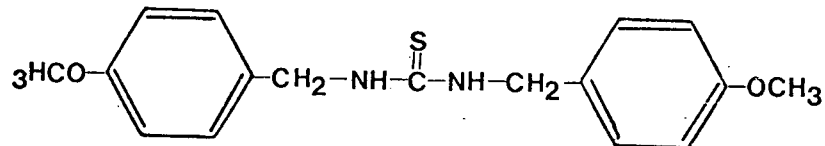
N,N,Di(methoxy,4-benzyl) thiourea

Figure 7. Examples of thioureas and thiocarbamates from glucosinolate containing plants

report would suggest all but the isothiocyanates to be artifacts

of the extraction procedure, the aromatic structures of the particular compounds and their possible relation to estrogenic or anti-androgenic compounds makes them potentially interesting in this investigation. Thioureas specifically will be considered as fertility affecting compounds.

Scheline (1978) reviews the mammalian metabolism of glucosinolates in humans and animals. In vivo metabolism of glucosinolates is complicated by the necessity for thioglucoside hydrolysis before absorption takes place. Thioglucosidase activity appears to be present in some human intestinal bacteria (Oginsky et al., 1965) and leads to the production of isothiocyanates and related compounds and their absorption in the intestinal lumen. Tani et al. (1974) isolated Enterobacter cloacae as a bacterium with particularly high myrosinase activity.

When sinalbin was administered to rats by stomach tube (Griffiths, 1969) it underwent ester hydrolysis. Sinapic acid, dihyrosinapic acid and p-hydroxybenzoic acid were excreted in the 24 hour urine. 3-Hydroxy-5-methoxyphenylpropionic acid was released on the second day. Work by Brusewitz et al. (1977) suggests that isothiocyanates may be conjugated with glutathione and excreted in the urine as mercapturic acids. A series of intermediate reactions were detected in vitro when benzylisothiocyanate was incubated with rat liver and kidney homogenates. Benzyl isothiocyanate and the corresponding mercapturic acid (Figure 8.) were detected in the urine of rats administered 10mg/kg orally. According to Brusewitz et al. (1977) the mercapturic acid of benzylisothiocyanate has been detected in the urines of rats, hamsters, dogs, pigs and humans,

but not in the urines of rabbits and guinea pigs, suggesting different routes of metabolic breakdown in these two groups of animals.
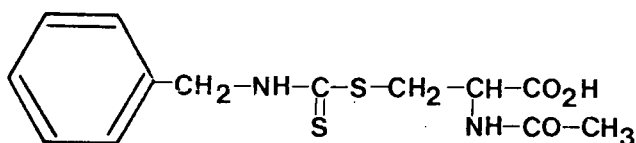
Figure 8. Mercapturic acid of benzyl isothiocyanate

Seventy-three different glucosinolates were known as of 1977. Reviews by Ettlinger and Kjaer (1968), and Kjaer and Olsen Larsen (1973, 1976, 1977) provide a cummulative summary of ongoing investigations and new reports. The distribution of these compounds throughout the plant kingdom is dealt with by Ettlinger and Kjaer (1968) and Kjaer (1973). The current preferred method for glucosinolate detection and determination is GLC-Mass Spectroscopy of isothiocyanates released by enzymatic hydrolysis. Coles's (1976) work on the Brassicaceae perhaps best illustrates this method in the screening of large numbers of plants when standards are available. Kjaer *et al*. (1978) combined the classic method of paper chromatography (PC)

of thiourea derivatives of isothiocyanates with GLC-MS in the
only comprehensive study on the family Tropaeolaceae. Paper
chromatraphy was used to determine the isothiocyanates of
Tropaeolum tuberosum as described below.

Materials and Methods

Experiments which follow were carried out to determine why _Tropaeolum tuberosum_ and _Lepidium meyenii_ are used for food and medicine in relation to the specific points raised in the above discussion.

Outline of Research

1. Ethnobotanical literature on glucosinolate containing plants was surveyed and studied statistically for patterns which support the specific uses of _T. tuberosum_ and _L. meyenii_ .

2. Glucosinolates of _T. tuberosum_ subsp. _tuberosum_ and _L. meyenii_ were determined and related to the similar medicinal uses of these plants.

3. Crude extracts, glucosinolates and their breakdown metabolites were assayed for estrogenic and anti-androgenic activity to determine the basis for the prevalant beliefs concerning such properties for these plants.

4. Isothiocyanates and crude extracts were assayed biologically in relation to the antibiotic, antiviral and nematocidal uses of the plants.

5. The glucosinolates of _T. tuberosum_ subsp. _silvestre_ and _T. tuberosum_ subsp. _tuberosum_ were compared and are discussed in relation to the derivation of the cultivated

plant. The chromosome number of I. tuberosum subsp. silvestre was determined.

6. Free amino acids were analyzed to screen for any non-protein biologically active members of this class of compound.

Statistical Analysis and Data Collection

Information obtained from the literature sources listed in Supplementary Bibliographies I and II was compiled onto cards filed by genus, and subsequently coded and stored in a 2-D raw data matrix. Data were recorded by case, each case referring to a specific mention of a use of a particular species, either by an author or in a specific geographical or cultural region. Care was taken to eliminate repetition of cases where information had been compiled from or interchanged between literature sources. Data were stored under various category headings (variables) and coded to record the possible conditions (values) in the specific case. Medicinal uses which compose the bulk of the data were categorized broadly by functional system, generally as outlined by Lewis and Elvin-Lewis(1977). New categories were created to cover cases that did not fit those originally defined and the values recorded increased and evolved to meet the increasing scope of the study. The way data was coded does reflect the interpretation of folk medicinal taxonomies by the author. Although this interpretation is a constant factor in the

investigation, its effect on subsequent results is difficult to ascertain, but it is believed to be minimal.

The raw data matrix was structured to meet the requirements of the Statistical Package for the Social Sciences(SPSS) (Nie et al., 1975). All analyses were carried out on the Amdahl 470V/6 Model II computer run under the Michigan Terminal System (MTS) at the UBC Computing Centre. Data retrieval was carried out on the SPSS systems files as required. The transformation powers of the package were used to redefine the data matrix, and the XFrequency(Kita, 1978) and Crosstabs programs were used to generate frequency data. For the bulk of the analysis on medicinal and nutritional uses, all information recorded as values and variables was transformed into 41 new yes or no categories reflecting the following concepts:

1 Insecticide	22 Respiratory:infection
2 Disinfectant	23 Respiratory:anticough
3 Vermifuge	24 Respiratory:other
4 Emetic	25 Skin:dermatitis
5 Digestive	26 Skin:ulcer
6 Purgative	27 Snakebite
7 Stomach ache	28 Wound
8 Antidysentry	29 Inflamation
9 Rheumatism	30 Skin:other
10 Febrifuge	31 Tonic
11 Headache	32 Urinary system
12 Anesthetic	33 Liver
13 Nervous:other	34 Circulatory system
14 Eye	35 Disease(misc.)
15 Ear	36 Condiment
16 Metabolic	37 Dye
17 Oral problem	38 Edible and Beverage
18 Toothache	39 Internal bleeding
19 Poison:dermatitis	40 Poison
20 Reproductive:hormonal	41 Miscellaneous
21 Reproductive:other	

Frequencies of these variables were obtained and compared statistically to a similar array of ethnobotanical uses compiled by tabulating the frequencies of use of all the plants contained in Altschul(1978) . The data in this volume is taken as representative of a normal distribution of medicinal and

nutritional uses of plants. Positive differences from this distribution were considered to indicate a significant selectivity for particular uses.

Data from both sources were tabulated as a three-way contingency table by fitting a hierarchial log-linear model to the cell frequencies using the program P3F in the Biomedical Computer Programs P-series(BMDP) (Dixon and Brown, 1979) and UBC Act, both available at the UBC Computing Centre. The model AC,AB,BC was tested and a likelihood ratio chi-square calculated to test for independence. To determine the specific medicinal use responsible for any statistical significance, use variables were collapsed and the modelling procedure repeated. Variables were collapsed into the following super-categories;

        A) Antiorganismal

        B) Internal organs

        C) Nervous system

        D) Reproductive system

        E) Respiratory system

        F) Skin

Positively differing categories of the original 41 were tested in turn in a table containing the six super-categories. Once significant categories were identified the frequency of the original uses from the raw data matrix was determined using the Crosstabs program.

Table I. Analysis of Contingency Tables

Uses (A)

Sample (C)	Presence (B)	1	2	3	.....	39	40	41
Lit. Sample	Yes							
	No							
Altschul	Yes							
	No							

Plant Material

Tubers and seeds of _Tropaeolum tuberosum_ were obtained from
Peru. The source and geographical origin of the collections used
in this investigation are listed in Table II. Plants of all
collections were propagated on the campus of the University of
British Columbia during May through November in 1978 and 1979.
Vouchers of original collections and propagated specimens of
both _T. tuberosum_ subsp. _tuberosum_ and subsp. _silvestre_ are
deposited in the UBC Herbarium. Vegetative, floral and seed
material was collected at various times throughout the growing
season, and was used fresh, or frozen at -70° C. Tubers were
harvested immediately after frost-kill on October 25, 1978 and
November 6, 1979, and were either stored at 4° C or freeze dried
and stored at -70° C.

Roots of _Lepidium meyenii_ were collected in Wayri,
Department of Junin, Peru on July 15, 1973 by Michael F. Brown.
They were subsequently preserved in p-dichlorobenzene and
deposited (Catalogue No. 26323) at room temperature in the
Museum of Anthropology, University of Michigan. They were
obtained on request from that institution on December 8, 1979.

Sinapis alba , a source of sinalbin and the enzyme
myrosinase, was donated by the Canadian Grain Commission,
Vancouver, from samples taken from Canadian export shipments.

Table II. Collections of _Tropaeolum tuberosum_

No.	Name	Proveniance	Collector	Date
1	_T. tuberosum_ ssp. _silvestre_ 'kipa isano' *	Cuyo-cuyo, Puno,Peru	Johns(505)	Dec3 77
2	_T. tuberosum_ ssp. _tuberosum_ 'chaucha isano'	Cuyo-cuyo	Johns(466)	Nov30 77
3	ssp. _tuberosum_ (seeds)	Cuzco,Peru	Cortes Bravo,	Nov25 77
4	ssp. _tuberosum_ 'oke isano'	Cuyo-cuyo	Camino	May 78
5	ssp. _tuberosum_ 'isala isano'	Cuyo-cuyo	Camino	May 78
6	ssp. _tuberosum_ 'kello isano'	Cuyo-cuyo	Camino	May 78
7	ssp. _tuberosum_ 'yani nawi isano'**	Cuyo-cuyo	Camino	Apr14 78
8	ssp. _tuberosum_	Huancayo, Peru	Camino	Jun11 78
9	ssp. _tuberosum_	Huancayo	Camino	Jun11 78

Source-)

* All collections are tubers unless otherwise stated;
** 'yani nawi isano' is equivalent to 'isala isano'

Chemicals

Glucosinolates, isothiocyanates and their thiourea derivatives were obtained from various sources and used as standards or in biological testing.

Glucosinolates:

Benzylglucosinolate and sinalbin as tetramethyl ammonium salts, and sinigrin were obtained from Drs. E.W. Underhill and L.R. Wetter of the National Research Council Canada, Prairie Regional Laboratory.

Isothiocyanates:

Specimens of benzylisothiocyanate were obtained from Dr. E.W. Underhill, and from Fluka AG. Allylisothiocyanate, phenethylisothiocyanate and phenylisothiocyanate were purchased from Eastman Kodak Co.. Dr. Anders Kjaer provided samples of 2-butyl and 2-propyl isothiocyanates upon request but these compounds unfortunately decomposed too quickly to be useful. p-Methoxybenzylisothiocyanate was obtained from _T. tuberosum_ subsp. _tuberosum_ using the HPLC methods described below.

Thioureas:

The thiourea derivative of 2-butyl isothiocyanate was obtained from Dr. E. W. Underhill and phenylthiourea was purchased from Eastman Kodak. Thiourea derivatives were prepared from benzyl and p-methoxybenzyl isothiocyanates by the methods described below.

Materials used in assays for estrogenic and anti-androgenic activities:

$17\beta-{}^{3}H$-estradiol was purchased from the Amersham Corporation. Genistein was purchased from Eastman Kodak Co.. Androgen levels in rat blood were tested using a Testosterone/Dihydrotestosterone RIA kit prepared by Amersham Corporation.

Preparation of plant extracts

Glucosinolates and Isothiocyanates:

Two procedures were followed to obtain and to identify the isothiocyanate breakdown products of the glucosinolates. The first uses endogenous enzyme, the second a myrosinase preparation obtained from the seeds of Sinapis alba .

Use of endogenous enzyme (Cole, 1976):

The plant material, either fresh or dried, was mashed or ground to a fine consistency. If necessary, water was added to give a paste-like consistency. The mixture was incubated for 2 hours at 37^0 C, after which it was shaken and mixed with several portions of $MeCl_2$ to extract isothiocyanates.

Use of myrosinase preparation:

The plant material was ground in a Waring blender with 70% hot methanol. Solvent was added to cover and the procedure repeated until pigment in the extract was negligible. The blended mixture was filtered, and the extract filtered hot through Celite and concentrated in vaccuo to a volume of less than 5ml. To the flask were added 50ml of citrate-phosphate buffer (Dawson et al., 1972) pH 6.6, 0.1ml of ascorbic acid solution (Rodman, 1978) and 2ml of the myrosinase preparation, prepared as outlined by Rodman(1978). Incubation took place at room temperature overnight. Released isothiocyanates were extracted into either diethyl ether or $MeCl_2$.

Free amino acids:

One to two gram portions of tubers of $\underline{T}$. $\underline{tuberosum}$ were extracted twice with 15ml of 85% hot ethanol, filtered, evaporated to dryness under vacuum and taken up in 1ml of lithium citrate buffer pH 2.2. Buffer was prepared as specified for use on the Beckman Model 120C Amino Acid Analyzer. Samples were frozen and stored until analyzed.

Detection and Determination of Constituents

Glucosinolates:

Matsuo(1970) has provided several solvent systems for separation of glucosinolates on silica gel thin layer chromatography (TLC). The following systems were used with success: acetone-chloroform-ethanol-water (6:3:3.4:3); butanol-benzene-ethanol-ammonia (4:1:2:3); butanol-propanol-acetic acid-water (3:1:1:2). Paper chromatography (PC) of glucosinolates was carried out on Whatman No.1 paper using a solvent system of butanol-ethanol-water (4:1:4)(Gmelin and Kjaer,1970). Spots were detected on Polygram SilG/uv254 TLC plates using a short wave ultraviolet lamp, and by the exposure of chromatograms to iodine vapor. TLC plates and paper chromatograms were also sprayed with silver nitrate-NaOH reagent(Gmelin and Kjaer, 1970). Phenols were detected using a 0.1% p-nitrobenzenediazonium

tetrafluoroborate spray reagent oversprayed with 5% NaOH (Phillips, pers. comm.). Chromatograms can be sprayed for phenols, left to dry and subsequently sprayed with the $AgNO_3$-NaOH reagent.

Isothiocyanates:

The determination of isothiocyanates from the enzymatic hydrolysis of glucosinolates provides a superior route to the identity of glucosinolates. For a rough determination of constituents, ether extracts were divided into 3 portions and the method of Rodman(1978) was used to test for SCN^-, cyclic oxozolidinethenes and isothiocyanates. Thiocyanate(SCN^-) is produced in alkaline medium from p-hydroxybenzylglucosinolate (sinalbin) and indolic glucosinolates and was assayed with a ferric nitrate reagent. The cyclic derivatives of β-hydroxy and γ-hydroxy substituted glucosinolates can be detected by spotting on paper and spraying with Grote's reagent(Grote, 1931) and promptly steaming. A blue colour indicates a positive result. Isothiocyanates were detected as thiourea derivatives. The ether extract was reacted with an excess of ethanolic ammonia (one part concentrated aqueous ammonium hydroxide and five parts of ethanol). After several hours the sample was evaporated and spotted on paper. Grote's reagent was used as above to determine presence. If thioureas were present they were separated by paper chromatography or on Avicel TLC plates. The classic method using paper(Kjaer and Rubinstein, 1953) is superior to TLC in providing better resolution. Standard thioureas where available

were used to identify spots either through comparison of Rf values or co-chromatography. The most successful solvent systems (Rodman,1978; Kjaer et al., 1978) for the present investigation were found to be: benzene-ethanol-water (5:1:2); chloroform-water (5:1); toluene-acetic acid-water (3.75:1.5:3). Rph's were calculated as the ratio of the distance of the spot/distance of phenylthiourea.

A more convenient approach to the determination of isothiocyanates was worked out using High Performance Liquid Chromatography (HPLC) and ultraviolet detection at 245nm. Considerable effort was applied to finding an HPLC system that would separate the compounds detected by PC of thioureas. Although isothiocyanates show no strong absorption maxima they do absorb in the range of 245 nm, the extinction coefficient of ethylisothiocyanate being 1,200(Scott, 1964). Aromatic isothiocyanates have increased absorption in this region.

MeCl$_2$ extracts were concentrated and passed through a small (10-20ml) pre-column of Silica gel. The MeCl$_2$ elutant was again concentrated to a few ml. Samples applied to a normal phase column were injected in MeCl$_2$. For reverse phase chromatography the MeCl$_2$ elutant was dried, taken up in acetonitrile and before injection was filtered through a fritted disc funnel to remove precipitated lipids. .

A Varian Model 500 Liquid Chromatograph with a Varian Series 634 variable wavelength detector was used and chromatography was carried out at ambient temperature. For reverse phase chromatography a Micropak m-CH-10 analytical column with acetonitrile and water as the mobile phase was used. Samples were injected at 35% CH$_3$CN and run at a flow rate of 1ml/minute over a gradient of 10%/minute to 70% CH$_3$CN.

Preparative chromatography was carried out by a similar method using a m-CH-10 preparative column and a flow rate of 2ml/minute.

For normal phase chromatography a Micropak NH_2-10 column with $MeCl_2$:isooctane(30:70) as the mobile phase provided the best results. Standards were co-chromatographed with all samples where possible. For preparative work fractions from repeated runs were pooled, extracted into $MeCl_2$ and concentrated under vacuum. Identity of samples prepared in this way were confirmed by the thiourea-PC method described above, and by mass spectral analysis and/or NMR.

Mass Spectral Analysis:

Mass spectrometry was carried out on an Atlas MAT (Bremen) CH4-B Mass Spectrometer.

Nuclear Magnetic Resonance Spectrometry:

The ^{1}H-NMR spectrum of p-methoxybenzylisothiocyanate (20mg) was recorded on a Bruker WP-80 Fourier transform spectrometer in $CDCl_3$ (0.5ml) with TMS (10%) as an internal standard.

Amino Acid Analysis:

Free amino acids were determined using a Beckman Model 120C Automatic Amino Acid Analyzer. Basic amino acids were analyzed according to manufacturers recommendations (Beckman, 1966) on a 14 by 1 cm column. Acidic and neutral amino acids and amides were separated using lithium salts as directed by the manufacturer(Beckman, 1966) on a 56 by 1 cm column. Amino acids present in tuber samples were identified by comparison of retention times to those of the standard calibration mixture of protein amino acids and of other known common amino acids.

Determination of Chromosome Number

Tubers of the collection, 'kipa isaño' (Table II), confirmed in identification as Tropaeolum tuberosum subsp. silvestre on the basis of the glucosinolates it contains (see below), were sprouted in vermiculite. Root tips were fixed in Carnoy's solution and stored at 4^0 C. Tips were stained in aceto-carmine and the chromosome numbers were determined by Dr. Gerald B. Straley, VanDusen Botanical Garden, Vancouver.

Figure 9. Tubers of _Tropaeolum tuberosum_ subsp. _silvestre_

Tests for Biological Activity

Antibiotic activity:

Tests for antibiotic and phototoxic activity were carried out using the method of Daniels (1965). Antibiotic tests of tuber material of both subspecies of T. tuberosum , and of pure benzyl isothiocyanate and p-methoxybenzyl isothiocyanate, were carried out against: Candida albicans , Escherichia coli , Pseudomonas fluorescens , and Staphylococcus albus . Candida albicans was grown on plates containing Sabouraud Dextrose Agar while, E. coli , P. fluorescens and S. albus were cultured on Difco Bacto-Agar. . Plates were inoculated with a lawn of bacteria or yeast. Paper disks (7mm diameter) were inoculated with 10ul of ether solutions of pure compound in concentrations of 10 and 1mg/ml (100 and 10 ug respectively), allowed to air dry and placed on the inoculated plates. Plant material was surface sterilized in ethanol. Dissected pieces were placed on the plates. All plates were incubated at 37^0 C. After 24 hours plates were observed for the clear zones around the disk or plant material which indicates antibiosis.

Phototoxicity of tuber and leaf material of the two subspecies of T. tuberosum were tested against Candida albicans . Plates were prepared as above, in duplicate. One set of plates were exposed to longwave ultraviolet light for 5 hours at 30^0 C (four Sylvannia black-light blue fluorescent lamps, F20T12-BLB. Light intensity 0.6mWatt/cm^2). Controls were kept wrapped in aluminium foil. Incubation and observations were as above.

Antiviral activity:

To test the possible antiviral effect of _Tropaeolum_ _tuberosum_ subsp. _tuberosum_ , an ether extract of the tubers, prepared as above, was tested in duplicate against a sample of Herpes type I virus using the method of Van Den Berge _et_ _al._ (1978). Monolayers of confluent VERO cells maintained by Mr. Brian Judd, Department of Medical Microbiology, U.B.C., were grown in Linbro multiwell plates and incubated with serial 10-fold dilutions of the virus strain M2989, with or without the addition of the maximum nontoxic dilution (MNTD) of the plant extract. Strain M2989 was isolated by Dr. D.M. McLean, Department of Medical Microbiology, U.B.C., and grown in VERO cells maintained on Dulbecco's Modified Eagle Medium (MEM) containing 3.7g/l sodium bicarbonate with 10% of Gibco Fetal Bovin Calf Serum (FCS) obtained from the American Type Culture.

The MNTD, a measure of the cytotoxicity of the plant extract, was determined prior to the virus inhibitory assay. Serial 10-fold dilutions of the extract in MEM and 10% FCS were added to VERO monolayers which were examined under 100X magnification after 2 days of incubation at 37^0 C. Cytopathic effect (CPE) was indicated by a rounding of cells and a subsequent destruction of the monolayer.

Virus control, tissue culture control, and solvent controls were included in the test. To test the possible protective effect of the extract upon the cells, an aliquot (0.5ml) of the concentrated Herpes sample was incubated for 1 hour at 37^0 C with 0.5ml of the MNTD and then serially diluted and added to VERO cells as above.

Virus titres in the presence and absence of the plant

extract were determined after 2 days of incubuation at 37^0 C. CPE was determined as above. Antiviral activity was expressed as a reduction factor of the viral titre.

Nematocidal activity:

Nematodes (Caenorhabditis elegans) were obtained from Dr. D.L. Baillie, Department of Biological Sciences, Simon Fraser University, in 35 by 10mm petri plates supporting Escherichia coli on the Nematocidal Growing Medium (NGM) used in that laboratory. 1ul amounts of ethanolic solutions of benzyl isothiocyanate in concentrations from 0-2% were dropped onto the centre of the plates. The plates were examined after one half an hour to determine the LD_{100} of the compound.

Estrogenic activity:

Tests for estrogenic acitivity were carried out using the methods of, and under the direction of, Frances Newsome, Department of Animal Science, UBC.
Both in vivo and in vitro experiments were done to test the estrogenic effects of extracts of T. tuberosum and pure compounds.

Effects of <u>Tropaeolum</u> <u>tuberosum</u> on the estrus cycle of the guinea pig:

A simple test using female guinea pigs was undertaken to test the fertility related emmenagogic beliefs surrounding T. <u>tuberosum</u> . Guinea pigs exhibit a regular 14-18(16.5 on the average) day estrus cycle (Cooper and Schiller, 1975). The onset of estrus can be readily detected by the disintigration of the vaginal closure membrane, a unique structure in the guinea pig, and the opening of the vaginal crifice. These characteristics make the guinea pig an ideal animal for this type of study. Early onset of estrus would be indicative of an emmenagogic effect.

Twelve recently weaned virgin albino guinea pigs were obtained from the Animal Care Centre at UBC and were maintained under controlled light and temperature conditions in individual 10.5" by 19" by 6.2" cages over the course of the experiment . A diet of Purina Guinea Pig Chow supplemented with lettuce, apple and sweet potato was provided through 3 normal estrus cycles to test the regular periodicity of the animals . Six control animals were maintained on the basal diet. Six experimental animals were fed a diet of ground pellets mixed with approximately 20% by dry weight of freeze dried tubers of T. <u>tuberosum</u> subsp. <u>tuberosum</u> . Control animals during this stage of the experiment consumed on the average 70g of dried pellets per day. Experimental animals were given comparable amounts. Body weights were determined before and during the experiment . The animals were observed and their estrus cycles recorded for 40 days afer the experimental feeding began.

Radioactive competitive binding assay:

Extracts of tubers of <u>Tropaeolum</u> <u>tuberosum</u> , seeds of <u>Sinapis</u> <u>alba</u> , standard glucosinolates and isothiocyanates, and fractions obtained from HPLC were assayed for estrogenic activity. Samples and $17\beta-^3H$-estradiol were incubated with a preparation of estrogen receptor from calf uterus. Competitive inhibition was measured as a factor of the drop in the label bound to the receptor. Results were compared to those obtained using known concentrations of the isoflavone, genistein(Pope and Wright, 1953), a well-known phyto-estrogen.

Plant extracts made using peroxide-free and recently distilled diethyl ether were divided and placed in 5ml test tubes. The aliquots were dried and stored in the freezer in 1.0ml of recently distilled ethanol. Standard compounds and HPLC fractions were dissolved in 1.0ml ethanol, and serially divided and made up to 1.0ml. Prior to the assay all tubes were dried under a stream of nitrogen. Genistein standards of 10, 50 and 200 ul of a 200ng/ml solution were placed in tubes and dried. The receptor containing cytisol fraction was prepared from calf uterus and tested for binding capacity by Frances Newsome.

100ul of tris buffer (pH 8) was added to all tubes, vortexed and allowed to stand for 30 minutes at 25^0 C. 50ul(1.25 x 10-2 uCi) of ^3H-estradiol and 50-100 ul of the cytisol fraction(depending on the test for binding capacity) were added and after 30 minutes at room temperature 1ml of dextrose coated charcoal (0.5g Norit activated charcoal and 5 mg dextrose T40 in 200 ul of tris buffer (pH 8)) was added to precipitate unbound ^3H-estradiol. Tubes were centrifuged at 3000g for 10 minutes and

the supernatant counted for radioactivity in 10ml of Amersham's PSC . Activity of test samples was compared to a plot of the genistein standard.

Dixon plot:

To determine whether inhibition of estrogen binding was competitive or non-competitive, the procedure of Dixon (1953) for enzyme inhibition was adapted. Concentration of benzylisothiocyanate inhibitor was varied over two different substrate (S) (^{3}H-estradiol) concentrations. Inhibitor concentrations were used in the range of 0.07 to 2.94 X 10^{-5} moles/reaction tube. Substrate concentrations of 25 and 50ul/reaction tube were used. The reaction rate (v) was measured as DPM of estradiol bound. To determine the nature of inhibition 1/v was plotted versus [I] for both substrate concentrations. The intercept position in relation to the x axis determines the nature of the inhibition.

Anti-androgenic activity:

To test the anti-aphrodisiac beliefs associated with Tropaeolum tuberosum , tubers of subsp. tuberosum were fed to male rats. Wistar white rats were obtained from the Zoology Research Small Animal Unit, UBC. Male rats weighing 280 to 330 grams at 12 weeks of age were housed under controlled light and

temperature conditions in pairs in 10.5" by 19" by 6.2" cages. Control animals were fed a diet of ground Purina Rodent Laboratory Chow over the course of the experiment. Experimental animals were fed a mixture of ground chow and pulverized freeze dried tubers. Initially the animals were offered a mixture of 10% by weight of the tubers. Animals were weighed regularly and the composition of the diet was varied up to a maximum of 25% tuber material so as to maintain a constant body weight.

After two weeks five experimental animals and five controls were separated and each caged with a virgin female rat for a period of one week (estrus cycle is 5 days). Female rats weighed 225-275 grams at 12 weeks of age. Control and experimental males were maintained on their respective diets. Females were removed from the cage for 6 hours each day and allowed to feed on normal pellets. After the 'breeding' period females were maintained for 22 days, their normal maximum gestation period, to determine if they were pregnant.

A third group of male rats was maintained for 2 weeks on a diet that limited their weight gain to as close to zero as possible.

Males from all three groups were anesthetized under diethyl ether. Heparin (120 units in 0.1 ml of physiological saline) was injected intravenously. The testicles were exposed surgically and the testicular vein was clamped and cut. Approximately 0.2ml of testicular venous blood was collected dropwise into a test tube (Sudo et al., 1979). The aorta was severed and an arterial blood sample of at least 1ml was collected. Samples were immediately frozen at -70^0 C and stored until analysis. Androgen levels were determined using Amersham's Testosterone/Dihyrotestosterone RIA kit following the

manufacturer's instructions for Total(testosterone + DHT) levels.

Results

Statistical Analysis of Data Bank of Glucosinolate-containing

Plants

The total data bank of glucosinolate-containing plants was comprised of 774 cases, 15.2% of which referred either to edible or beverage uses. This frequency was considerably less than the 28.5% of edible and medicinal usage tabulated from the work of Altschul.

To test the significance of the various medicinal categories the table was reduced to 595 cases referring specifically to medicinal uses. Table III contains categories having a positive frequency difference from the distribution of Altschul that was considered great enough to be worth testing. The probabilities of the likelihood ratio chi-squares performed in relation to the collapsed data base showed that only categories 3,16,20,24 and 32 were used significantly more than expected. Within each of these categories one specific use from the raw data matrix contributed the greatest number of cases. Within the category vermifuge, all cases were concerned with the treatment of intestinal worms. Within the category metabolic, 38 cases or 92.7% were antiscorbutics. Within the category reproductive:hormonal, 13 cases or 44.8% were emmenagogues; 78.5% were associated specifically with female conditions, and only 14.3% with male concerns. Within the category respiratory:other, 13 cases or 54.2% were expectorants, while

Table III Use categories analyzed for significant

medicinal effects

Category	Cases	%Frequency (Sample)	%Frequency (Altschul)	Probability P**
3 Vermifuge	13	2.2	1.0	0.0416*
4 Emetic	8	1.3	0.6	0.3807
13 Nervous:other	28	4.7	2.5	0.0644
16 Metabolic	41	6.9	0.4	<0.0001*
20 Reproductive:hormomal	29	4.9	2.4	0.0326*
24 Respiratory:other	24	4.0	1.5	0.0058*
30 Skin:other	46	7.7	5.7	0.1316
31 Tonic	20	3.4	1.7	0.1491
32 Urinary system	49	8.2	3.4	<0.0001*
33 Liver	16	2.7	1.7	0.4461
34 Circulatory system	25	4.2	2.3	0.2534

** Probability P of the collapsed data base was 0.7031.

* P <0.05

20.8% were concerned with treating asthma. Within the category urinary system, 39 cases or 79.5% were diuretics.

Detection and Determination of Constituents

TLC of glucosinolates of _Tropaeolum tuberosum_ subsp. _tuberosum_ and _Lepidium meyenii_ :

Glucosinolates occur naturally as potassium salts. Standards as tetramethyl ammonium salts were not useful for chromatography. In the absence of standards, TLC was carried out to determine the number of constituents and to compare the glucosinolate profiles of _Tropaeolum tuberosum_ subsp. _tuberosum_ and _Lepidium meyenii_ .

Extracts from both species gave negative results when sprayed for phenolic glucosinolates. Phenols were present in extracts from both species but did not give spots that corresponded to those revealed by the $AgNO_3$-NaOH reagent. Rf's of spots detected in both species are recorded in Table IV. In both solvent systems the major constituents correspond in Rf. _Lepidium meyenii_ has at least one unique glucosinolate, while both species show a minor constituent in common.

Paper chromatography of glucosinolates:

Extracts of _Tropaeolum tuberosum_ subsp. _tuberosum_ and _Lepidium meyenii_ were compared as with TLC. Rf's are recorded in Table IV. The major compound (Rf=0.21) produced a single spot when samples of both species were co-chromatographed. _Lepidium meyenii_ also produced a minor spot with an Rf of 0.38. No

phenolic conpounds were detected.

Table IV   Thin layer and paper chromatography of glucosinolates

(Rfs)

Chromatography	Solvent system	Samples	
		T.tuberosum subsp.tuberosum	L.meyenii
Thin layer	butanol	0.18(major)	0.18(major)
	benzene		0.25
	ethanol	(0.40)	(0.40)
	ammonia		
	butanol	0.43(major)	0.43(major)
	propanol	0.21	0.21
	acetic acid		
	water		
Paper	butanol	0.21(major)	0.21(major)
	ethanol		0.38

Analysis of isothiocyanates:

Chemical tests and paper chromatography of thioureas

Extracts of isothiocyanates liberated by endogenous and exogenous (from seeds of _Sinapis_ _alba_) myrosinases of both subspecies of _T. tuberosum_ , and of _L. meyenii_ were negative for thiocyanates and cyclic oxozolidinethenes. All samples of _T. tuberosum_ tested did react with ethanolic ammonium to produce thioureas. The incubation of ground root of _L. meyenii_ with water failed to release detectable isothiocyanates. Isothiocyanates were liberated by an exogenous enzyme preparation and were detected as thiourea derivatives. Results of the paper chromatography of thioureas of all three taxa are tabulated in Table V. _T. tuberosum_ subsp. _tuberosum_ appears to contain one isothiocyanate which was identified as p-methoxybenzyl isothiocyanate. Tubers of the subspecies _silvestre_ ('kipa isaño') appear to contain benzyl isothiocyanate as the major constituent and a minor constituent similar in Rph to published results for 2-propyl thiourea. This secondary constituent was detected only in the benzene-ethanol-water solvent system, and then not consistently. Seeds of _T. tuberosum_ subsp. _silvestre_ contain 2-butyl and 2-propyl isothiocyanates as major constituents and only a trace of benzyl isothiocyanate. _Lepidium meyenii_ , in the one solvent system tested, produced one spot corresponding to benzyl isothiocyanate.

Table V   Paper chromatography of thiourea derivatives (Rph's)

Solvent system	Standard thioureas			Samples		
	Benzyl	p-Met*	s-Butyl	T.t. s.tub.	T.t. s.sil.	L.m.
Benzene ethanol water	1.05	1.10	0.80	1.07	tuber 1.0 0.32 seed (1.0) 0.77 0.36	1.03
Toluene acetic acid water	0.8	0.8	0.7	0.77	0.8	
Chloroform water	0.92	1.0	0.85	1.0	tuber 0.92 seed 0.86 0.54	

*p-Met = p-Methoxybenzyl isothiocyanate

HPLC analysis of isothiocyanates

Isothiocyanates resolved by HPLC methods were identified by comparison of retention values and co-chromatography with standard compounds, and by paper chromatography (of thiourea derivatives), mass spectrometry and NMR spectrometry of collected fractions. Data for the HPLC analysis is recorded in Table VI. Samples of 2 grams of fresh material were sufficient

Table VI   HPLC analysis of isothiocyanates

Mode	Compound	tr(min.)	α	k'
Reverse phase	p-methoxybenzyl isothiocyanate(A)	11.3	0	4.7
	benzyl isothiocyanate(A)	11.3	1.15	4.7
	2-propyl isothiocyanate(B)	10.1		4.1
Normal phase	p-methoxybenzyl isothiocyanate(D)	3.8	1.65	0.52
	benzyl isothiocyanate(C)	3.4	1.80	0.34
	2-propyl isothiocyanate(B)	3.1		0.19

for analysis when standards were available. Retention times(tr) given are mean values. Variability in retention times necessitated the regular use of standards, and the identity of compounds in sample extracts was based primarily on comparison with standard compounds. Column selectivity (α) (Johnson and Stevenson, 1978) is measured by the relative separation of peaks and is defined by the equation

$$\alpha = \frac{tr2 - trm}{tr1 - tm}$$

where: $tr1$ and $tr2$ = the retention times of components

1 and 2 respectively.

tm = retention time of unretained compounds

(solvent front)

α indicates the relative separation of components. The greater the value the greater the separation of two successive peaks. The column capacity factor, k', (Johnson and Stevenson, 1978) is given by the expression

$$k' = \frac{tr1 - tm}{tm}$$

and is a measure of the retention of compounds relative to the solvent front.

The reverse phase system provided the best resolution for isothiocyanates in general (Figure 10) but failed to separate p-methoxybenzyl and benzyl isothiocyanates. The peak having the same retention time as either of these compounds is labelled A. Normal phase chromatography provided a convenient method for distinguishing these two similar compounds (Figure 11). Benzyl isothiocyanate is referred to as C; p-methoxybenzyl isothiocyanate is referred to as D. The small k' values in the normal phase system indicate the low resolution of this system. The possibility of other compounds having identical retention times and being mistakenly identified is high under these conditions and the usefulness of the system for the analysis of unknown samples is limited without comparison with results from other methods of analysis.

Reverse phase chromatography of extracts of tubers of 'kipa isaño' (T. tuberosum subsp. silvestre) revealed 4 peaks (Figure 10) . Preparative chromatography of extracts from approximately 40 grams of fresh tubers provided sufficient pure compounds for application to other methods of analysis. Two of the collected peaks (A and B) reacted with ethanolic ammonia to produce thioureas. Paper chromatography indicated that compound A is benzyl isothiocyanate and B is 2-propyl isothiocyanate. Mass spectrometry (Table VII) confirmed the identity of A but

Figure 10.
Reverse phase analysis of
Tropaeolum tuberosum subsp. silvestre

0.02 AU

A

B

E

0 1   5   10   15

MINUTES
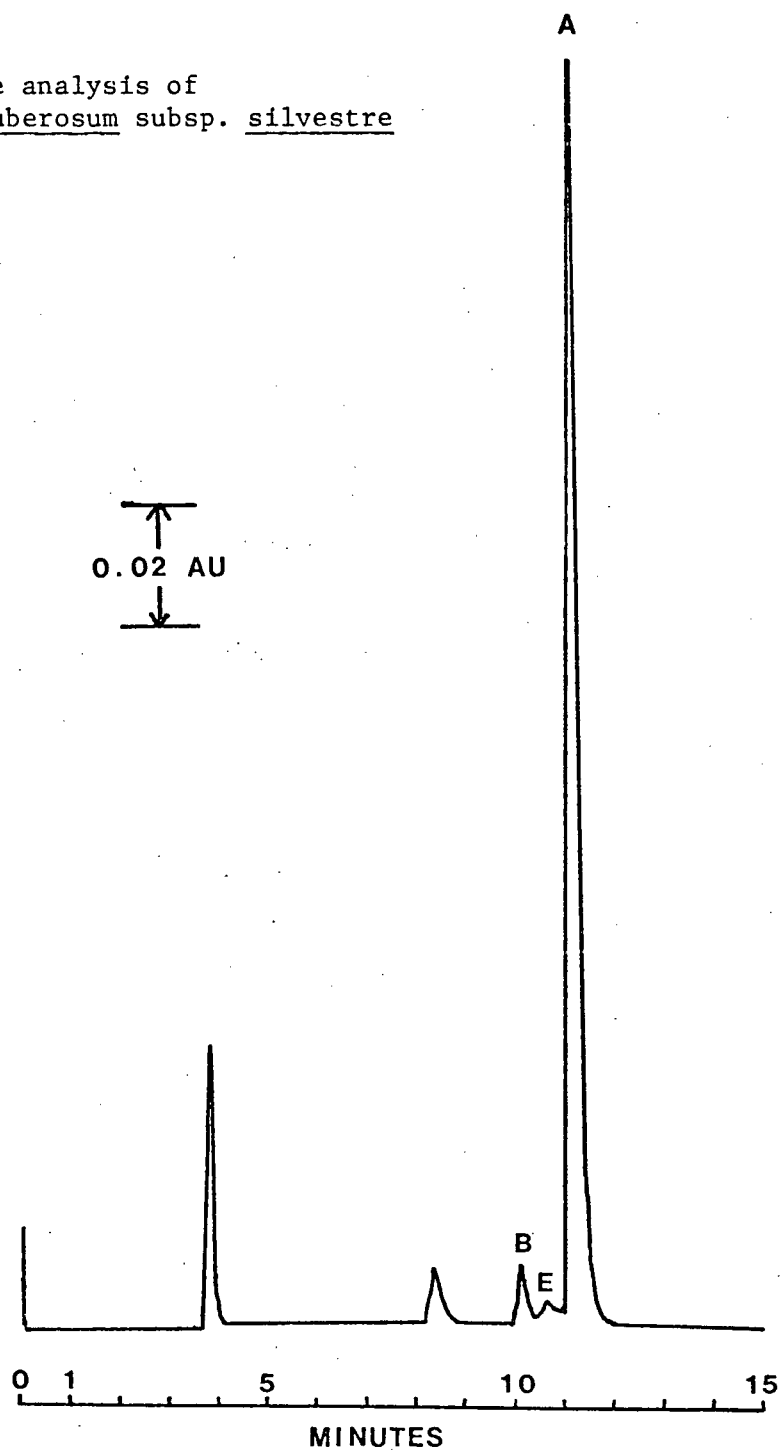
Figure 11. Resolution of benzyl and p-methoxybenzyl isothiocyanates by normal phase HPLC.
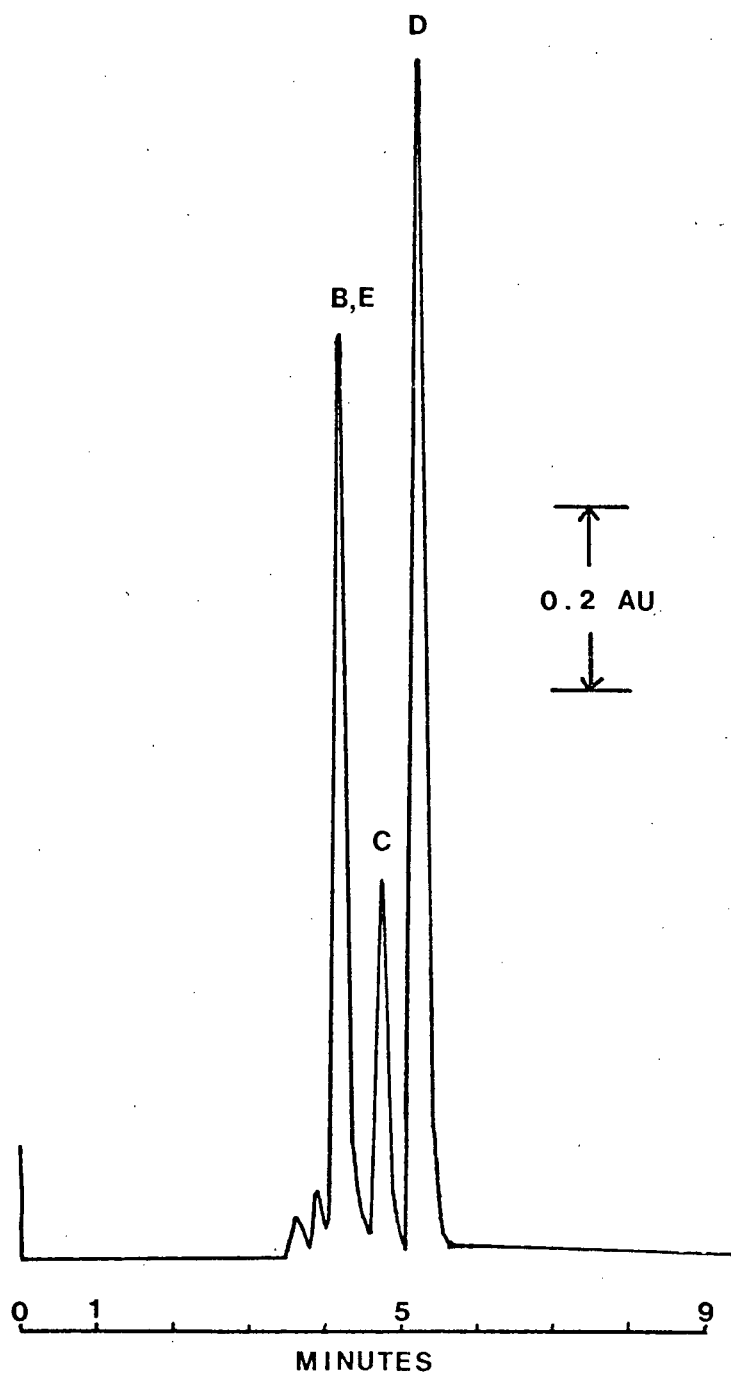
failed to give a meaningful result for B because of the low
boiling point of the sample (B.P. 2-propyl isothiocyanate is
138⁰ C).

Table VII   Mass spectrometry of HPLC fractions

Compound	Mass-to-Charge Ratio(m/e)
Benzyl isothiocyanate(C)	149(51),91(100),65(21),51(6),39(9), 32(12),28(50)
P-methoxybenzyl isothiocyanate(D)	179(20),121(100),91(4),77(4),65(2), 51(2),32(11),28(44)
N,N,Di(4-methoxy,benzyl) thiourea	316(18),282(2),195(15),179(2),153(3), 136(35),121(100),109(3),91(5),77(7)
N-(4-methoxy, benzyl) methylthiocarbamate	271(3),164(5),108(100),77(3),51(3)

Reverse phase and preparative chromatography of tubers of
T. tuberosum subsp. tuberosum produced one peak (A) that could
be identified as an isothiocyanate (Figure 12). Paper
chromatography of its thioureas gave its identity as
methoxybenzyl isothiocyanate. Mass spectral analysis (Table VII)
confirmed this determination. The parent ion (m/e 179) and the
fragmentation pattern giving a major fragment at m/e 121
($CH_3OC_6H_4CH_2-$) corresponds to that expected for methoxybenzyl
isothiocyanate (M.W. 179.25). Figure 13 shows the H^1-NMR
spectrum for 23mg of the compound. Chemical shifts ( ) of
7.05(multiplet), 4.60(singlet) and 3.80(singlet) correspond with
the shifts of aromatic, aliphatic and methoxy protons

Figure 12. Reverse phase HPLC of tubers of _Tropaeolum_ _tuberosum_
subsp. _tuberosum_.
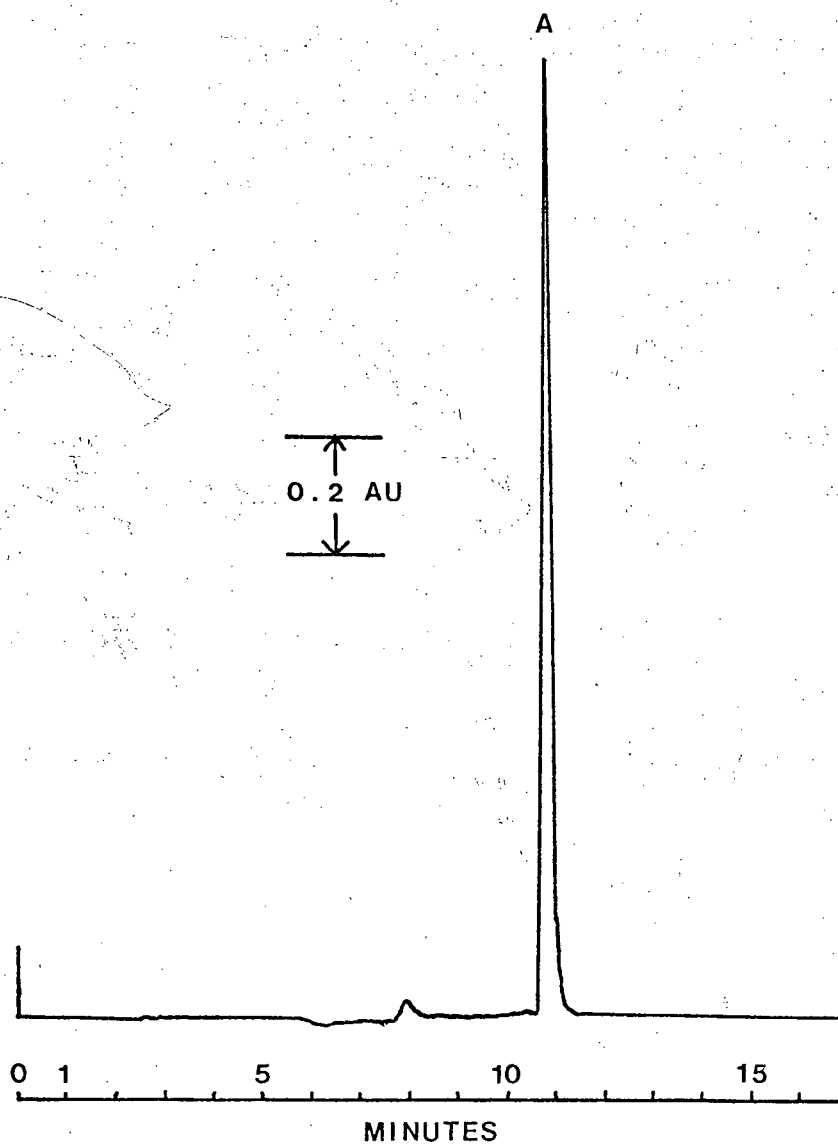
Figure 13. H^{1}-NMR spectrum of p-methoxybenzyl isothiocyanate from T. tuberosum subsp. tuberosum.

* contaminant
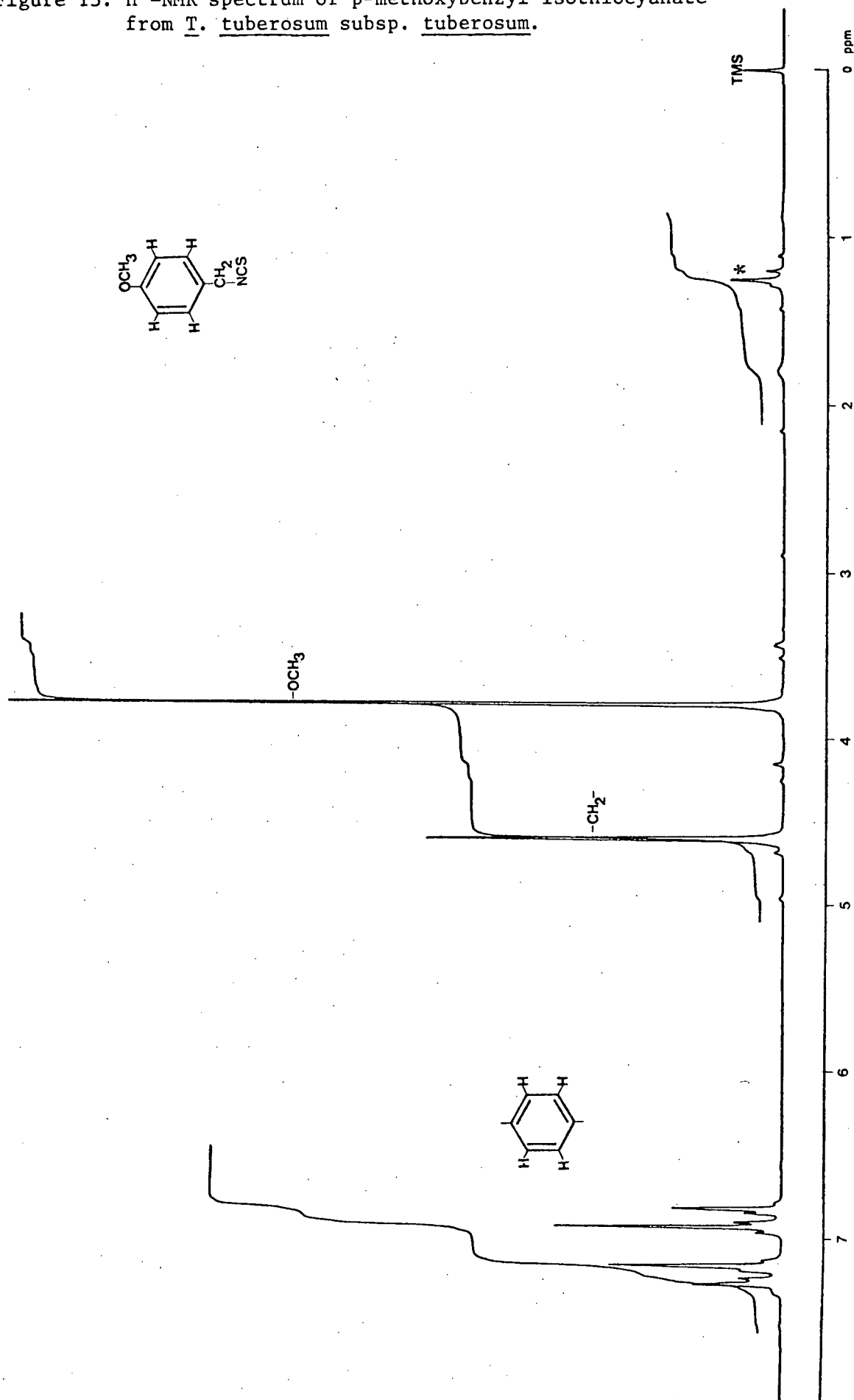
respectively (Silverstein et al., 1974). The integrals of the peaks have the ratio of 4:2:3 expected for methoxybenzyl isothiocyanate. The multiplet has the splitting pattern of an AB quartet, typical of an aromatic ring substituted in the para position. From the above evidence it is conclusive that the sole isothiocyanate liberated in extracts of I. tuberosum subsp. tuberosum is p-methoxybenzyl isothiocyanate.

Table VIII   NMR data for p-methoxybenzyl isothiocyanate

^{1}H-NMR (80MHz, CDCl$_3$) δ(ppm) 7.05(4H,m), 4.60(2H,s), 3.80(3H,s)

A peak having a large retention time on the normal phase system (17.0 minutes) was collected preparatively and analyzed by mass spectroscopy. The parent mass (m/e 316) (Table VII) coincides with a formula of $C_{17}H_{20}O_2N_2S$. The fragmentation pattern is consistent with the structure of N,N,Di(methoxy,4-benzyl)thiourea (Figure 7). The mass-to-charge ratios correspond with that recorded for this compound by El Migirab, et al. (1977).

Extracts of Sinapis alba produced a peak with a retention time of 7.9 minutes on the reverse phase column. The mass spectrum of this compound (Table VII) suggests that it is N-(4-hydroxy, benzyl)methylthiocarbamate (Figure 7). The largest fragment of this spectrum corresponds to $HOC_6H_4CH_2-$. This is the expected fragment in the breakdown of p-hydroxybenzyl isothiocyanate to thiocyanate.

Tubers of all the collections in Table II and various plant parts from each of the two subspecies of Tropaeolum tuberosum

were screened by normal phase HPLC. All tuber samples of

T. tuberosum subsp. tuberosum and samples of seeds, flowers and

leaves of collection no.6 showed similar HPLC profiles to that

recorded in Figure 14. An extract of tubers that had been cooked

by boiling produced p-methoxybenzyl isothiocyanate when

incubated with the exogenous enzyme preparation.

Tubers, leaves, seeds and flowers of 'kipa isaño' (

T. tuberosum subsp. silvestre) all contained benzyl

isothiocyanate and the peak identified as 2-propyl

isothiocyanate(Figure 15). However, the relative quantities

varied. Tubers contained the greatest relative amount of benzyl

isothiocyanate and only negligible amounts of other

constituents. Seeds contained relatively more of 2-propyl

isothiocyanate than any other plant part, as well as one unknown

peak. Peak E from reverse phase chromatography cochromatographed

with A in the normal phase system. E was suspected to be 2-butyl

isothiocyanates but its identity was not confirmed. If this were

2-butyl isothiocyanate then the 2-propyl peak (A) in the normal

phase system is a combination of 2-butyl and 2-propyl

isothiocyanates.

An extract of 7 g of ground roots of Lepidium meyenii

incubated with an enzyme preparation from Sinapis alba ,

produced isothiocyanates that were detectable by HPLC. Reverse

phase and normal phase profiles are recorded in Figure 16. The

largest peak in both systems corresponded to benzyl or p-

methoxybenzyl isothiocyanates. Reverse phase chromatography

produced one unidentified peak. By normal phase chromatography

the sample was resolved into 4 peaks. The largest of these

corresponded to benzyl isothiocyanate and a smaller one to

69

Figure 14. Normal phase HPLC of tubers of Tropaeolum tuberosum
subsp. tuberosum.
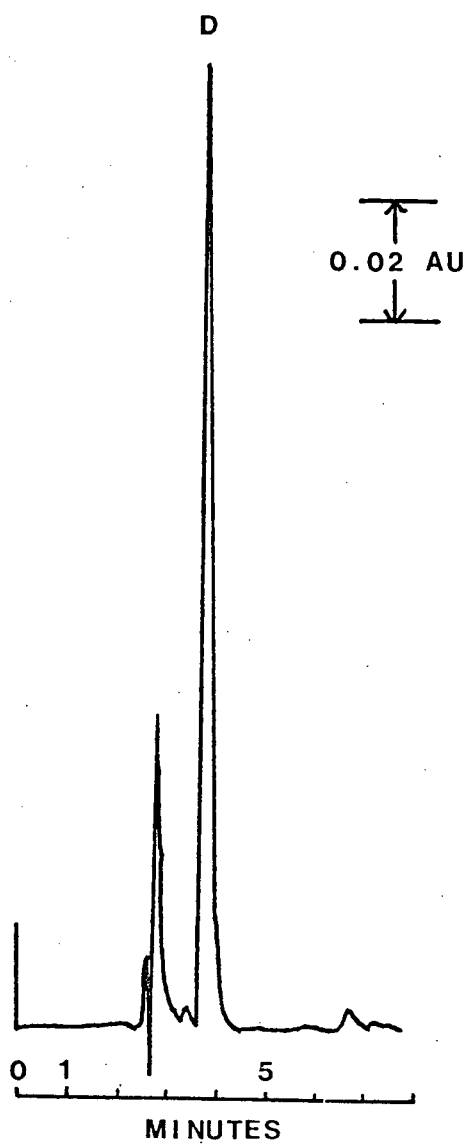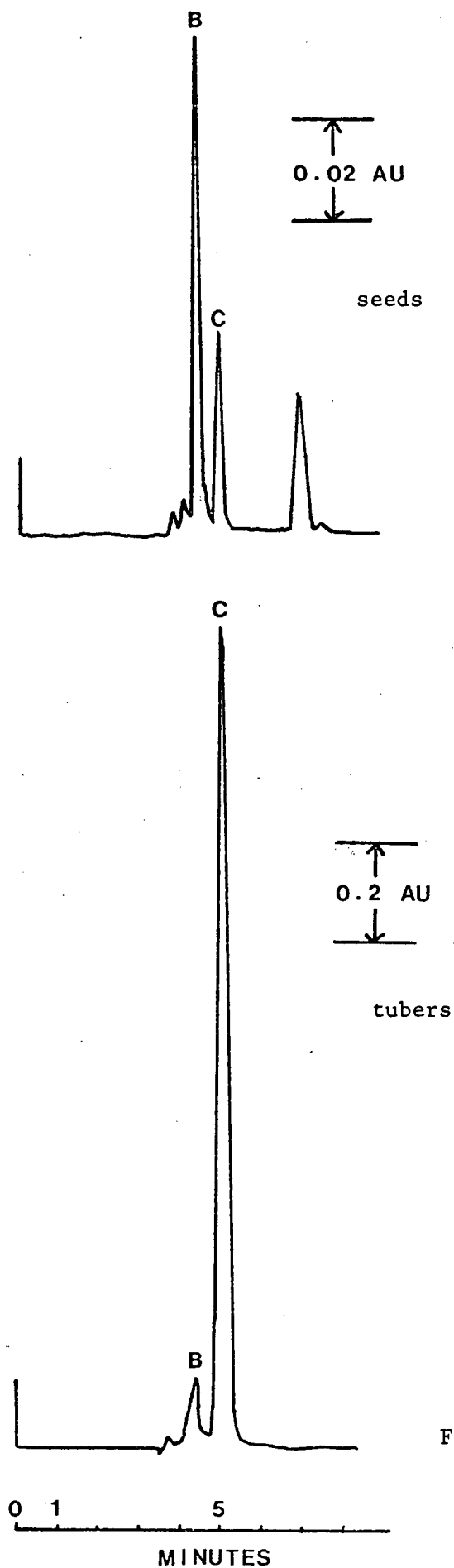
Figure 15. Normal phase HPLC of _Tropaeolum tuberosum_ subsp. _silvestre._
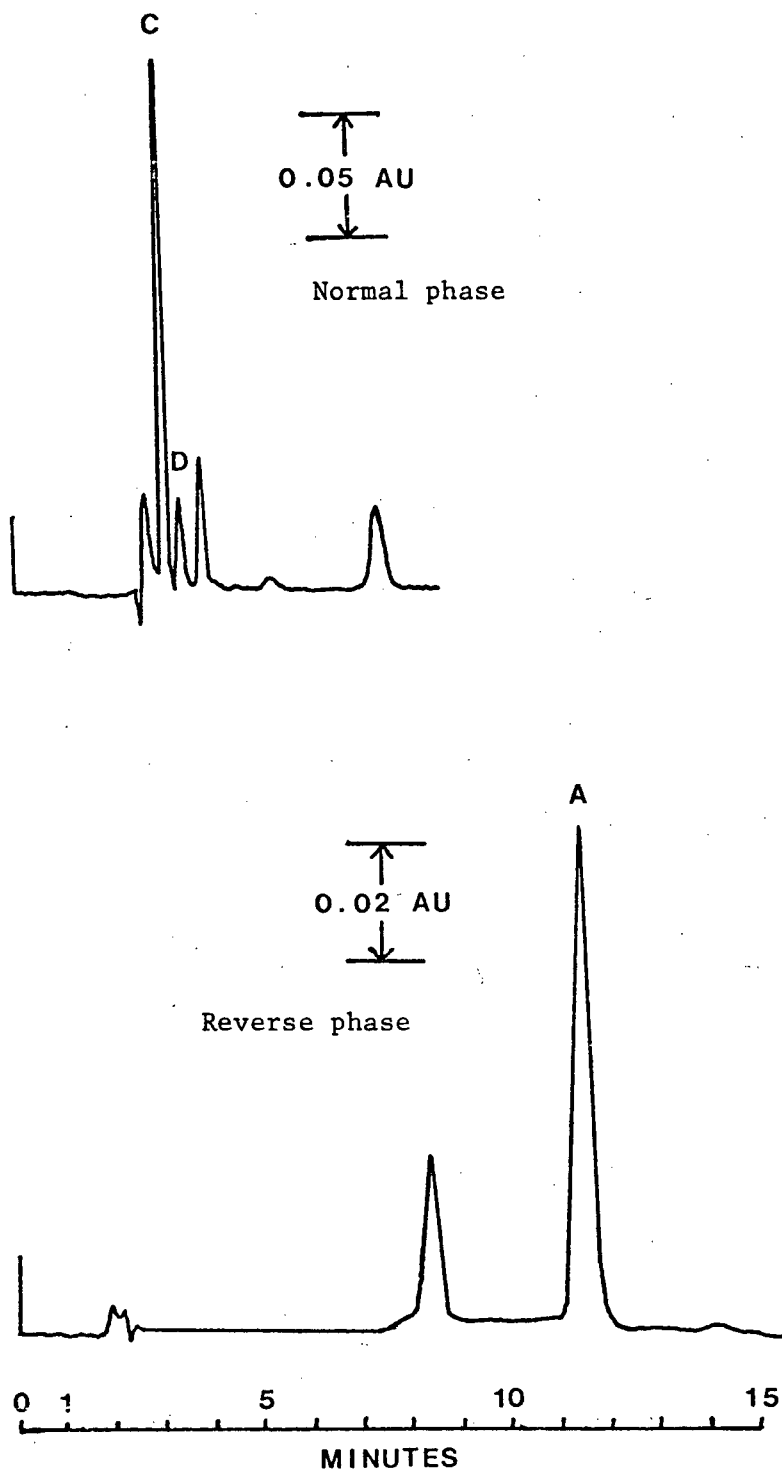
Figure 16. HPLC of Lepidium meyenii

p-methoxybenzyl isothiocyanate. The area of the 'benzyl' peak in reverse phase was 63%, while the area of the combined benzyl and p-methoxybenzyl peaks in normal phase was 65%. This rough measure supports the supposition that the two peaks were resolved from the major peak in the reverse phase system. The identity of the two other peaks remains unknown.

Amino acid analysis:

Six clones of _J. tuberosum_ subsp. _tuberosum_ were analyzed. The following free amino acids were detected in all samples: aspartic acid, threonine, serine, asparagine, glutamic acid, proline, glycine, alanine, valine, cystine, methionine (very low), isoleucine, leucine, tyrosine, phenylalanine, arginine, histidine, lysine, ornothine, tryptophan . Small amounts of another neutral amino acid were detected in some samples. This compound had a retention time slightly faster than that of valine and is probably γ-amino butyric acid. Another unknown appeared in the analysis of basic amino acids. This compound was readily detectable in all samples and had a retention time slightly faster than ammonia. Its identity remains undetermined.

Quantitatively, the results show considerable variation. Relative amounts of amino acids changed from sample to sample. No patterns that might relate to glucosinolate biosynthesis were discernible.

Determination of Chromosome Number

Root squashes of tubers of 'kipa isaño' (T. tuberosum subsp. silvestre) gave a chromosome count of 2n>42. The small size of the chromosomes made it difficult to determine the number more precisely.

Tests for Biological Activity

Antibiotic activity:

Tubers of both subspecies of T. tuberosum showed antibiotic activity (Table IX) against Candida albicans , while only subsp. tuberosum was antibiotic against Escherichia coli and Staphylococcus albus . Neither taxa was antiobiotic against Pseudomonas fluorescens . Pure p-methoxybenzyl and benzyl isothiocyanates, detected in subsp. tuberosum and subsp. silvestre respectively, showed a similar pattern to the plant material, except that benzyl isothiocyanate inhibited the growth of E. coli and was antibiotic towards S. albus . Antibiosis was dose dependent against some organisms in the concentration difference tested. The yeast, C. albicans , was the most sensitive to both compounds, while E. coli and S. albus were sensitive only to 100ug of either p-methoxybenzyl or benzyl isothiocyanate.

Phototoxicity tests of crude plant material of both

subspecies against C. albicans showed an identical result to that recorded above. The organisms were killed in either light or dark indicating a lack of phototoxic affect.

Table IX   Antibiotic activity

Test organisms	Tropaeolum tuberosum subspecies		Isothiocyanates			
			p-Methoxybenzyl		Benzyl	
	tuberosum	silvestre	100ug	10ug	100ug	10ug
C.albicans	++	+	++	++	++	++
E.coli	+	-	+	-	i	-
P.fluorescens	-	-	-	-	-	-
S.albus	++	-	++	-	+	-

+ = antibiotic

i = inhibitive

- = negative

Antiviral activity:

An ether extract of tubers of Tropaeolum tuberosum subsp. tuberosum showed no antiviral activity against Herpes type I virus. The results of the assay for the antiviral activity are recorded in Table X. The experimental titre showed no reduction in CPE (cytopathic effect) in relation to the viral

Table X   Antiviral activity

Experiment and controls	Cytopathic effect (CPE)						
	Herpes dilution						
	10^{-1}	10^{-2}	10^{-2}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
1. Experiment	4+	4+	4+	4+	3+	2+	?+
MNTD + Herpes + VERC							
2. Virus control	4+	4+	4+	4+	3+	2+	?+
Herpes + VERC							
3. Protective control	4+	4+	4+	2+	+	?+	0

0.5ml of 10^{-1} dilution of Herpes and 0.5ml MNTD incubated

1 hour and then titred.

4. Solvent control(viral)	–	–	–	3+	3+	+	0

0.5ml of 10^{-4} dilution of ether + VERC

	Solvent dilution			
	10^{-1}	10^{-2}	10^{-3}	10^{-4}
5. Solvent control(VERC)	0	0	0	0
6. Tissue culture control	CPE = 0			

The maximum nontoxic dilution (MNTD) of the plant extract was

10^{-4}.

titre (2. Virus control). Similarly when the MNTD (maximum non-toxic dilution) was incubated first with Herpes (3. Protective control) no significant drop in CPE was observed. Solvent controls 4 and 5 indicate that the solvent did not affect either viral activity in the range where CPE would be observable, or VERO cells in the concentrations used in the incubation.

Nematocidal activity:

Benzyl isothiocyanate had a LD??? against nematodes (Caenorhabditis elegans) of approximately 0.4% after half an hour. The amount of compound applied to the plate was 4.5ug. A concentration of 0.1%(1.1ug) killed some nematodes at the centre of the plate but those near the periphery were able to avoid the toxic effects. After 6 and 24 hours the results were the same as after half an hour.

Estrogenic activity:

Effect of Tropaeolum tuberosum on the estrus cycle of the guinea pig

A diet containing 20% by weight of T. tuberosum subsp. tuberosum failed to affect the regularity of the estrus

cycle of female guinea pigs over a period of 40 days (2 cycles) (Table XI). Experimental animals showed a drop in weight over the first 2 weeks of the feeding, but as they became accustomed to the diet they gained weight at a rate comparable to control animals. This initial weight drop was not reflected in any change in the estrus cycle of the guinea pigs.

Table XI   Effect of _Tropaeolum_ _tuberosum_ on estrus cycle

Group	Number of animals	Mean length of cycle (days)	Probability P
Experimental	6	15.67 ± 0.82	
			0.9000*
Control	6	15.60 ± 0.89	

* $P > 0.05$

Radioactive competitive binding assay

Crude ether extracts of _T._ _tuberosum_ subsp. _tuberosum_ and of _Sinapis_ _alba_ produced a qualitative inhibition of estradiol binding when compared to solvent controls. Pure isothiocyanates also produced inhibition. An unquantified p-methoxybenzyl isothiocyanate fraction obtained from HPLC showed inhibition proportional to several dilutions. Inhibition by standard benzyl and phenethyl isothiocyanates is tabulated in Table XII in relation to nanograms of genistein that would produce an equal

Table XII   Estrogenic activity

Compound	ng/tube	Equivalent genistein(ng)
Phenethyl isothiocyanate	3.3×10^6	43
	1.1×10^6	16
	1.0×10^5	13
	1.0×10^4	3
Benzyl isothiocyanate	3.4×10^6	42
	1.1×10^6	19
	1.0×10^6	7
	1.0×10^5	3
	1.0×10^4	2
N,N,Di(methoxy,4-benzyl) thiourea	10	38
	2	17
	0.2	7
	0.01	—

inhibition. Benzyl glucosinolate in amounts up to 0.25mg produced no measurable inhibition in estradiol activity. The difference between the amount of isothiocyanate added and the equivalent weight of genistein that would produce the same inhibition is in the order of 10^5. The magnitude of the difference suggests that the inhibition observed is not

competitive. The Dixon plot (Figure 17) confirms that the inhibition is non-competitive i.e. that the isothiocyanates act by affecting the estrogen receptor other than at its active site.

One portion of N,N,Di(4-methoxy, benzyl)thiourea, obtained from extracts of _Tropaeolum_ _tuberosum_ subsp. _tuberosum_ by HPLC and identified by mass spectrometry was tested quantitatively for estrogenic activity. These results suggest that this compound inhibits estradiol binding in the same order of magnitude as genistein.

Anti-androgenic activity:

Rat breeding

Male rats fed a diet containing tubers of _T._ _tuberosum_ subsp. _tuberosum_ did not show any decline in their success at impregnating female rats. Experimental and control groups of male rats each were 60% successful at impregnating females (Table XIII).
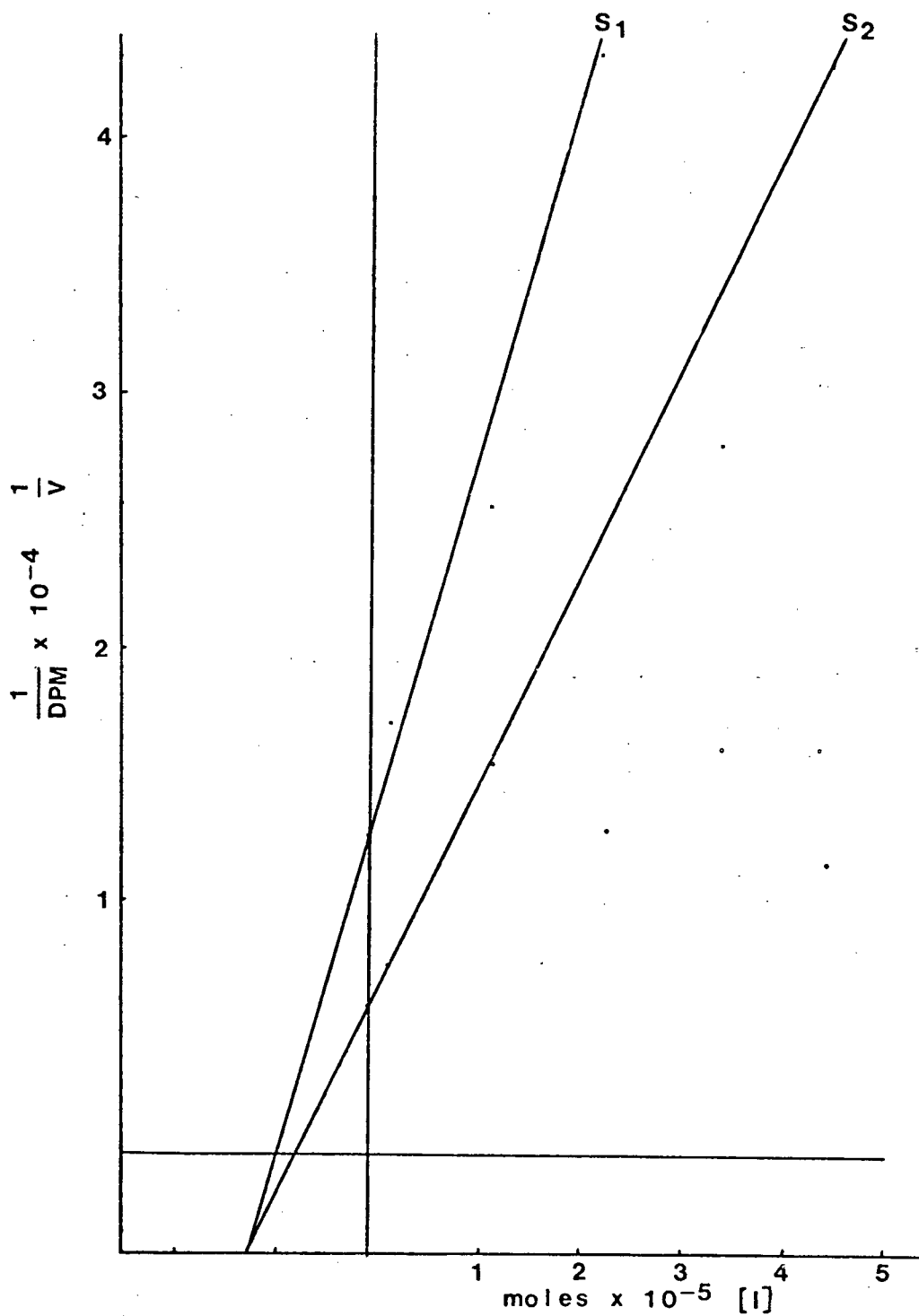
Figure 17. Dixon plot of inhibition of estradiol binding
by benzyl isothiocyanate.

Table XIII   Rat breeding experiment

Group	Number of animals	Number (and %) of pregnant females	Probability P
Experimental	5	3 (60%)	
			1.0*
Control	5	3 (60%)	

* P>0.05

Androgen levels

Male rats fed a diet containing tubers of _I_. _tuberosum_ subsp. _tuberosum_ showed a 45% drop in total levels of testosterone plus dihydrotestosterone. The arterial blood of experimental animals and control animals had mean androgen levels of 145 and 264 ng/ml of blood serum (Table XIV), respectively. A Student's t-test performed on the two samples showed the result to be significant (p<0.05). However, in comparison to animals starved to the point where they gained only 2.5%/week of their body weight for 2 weeks, the drop in androgen levels was insignificant. Starved rats had mean androgen levels of 155ng/ml, a 41% drop compared to controls(P=0.0127). The drop in testosterone/dihyrotestosterone is related to the diet and not to body weight. Control animals

weighing from 309 to 396 grams had comparable testosterone levels. Control rats gained 6.6%/week of their body weight. Experimental rats gained no weight over the course of the study. Testosterone levels in blood collected from testicular veins showed too much variation unrelated to the particular group of animals, to be meaningful in this investigation.

Table XIV   Levels of testosterone + dihydrotestosterone in rat

blood serum

Group	Number of animals	Testosterone + dihydrotestosterone (ng/ml)	Probability P
Control	13***	264 ± 99	
			0.0036*
Experimental	9	145 ± 54	
			0.6773**
Starved	7	155 ± 42	

* P<0.05

** P>0.05

*** Control and experimental animals fed for 2 or 3 weeks showed no observable difference in levels and are grouped together.

Discussion

Glucosinolates of Tropaeolum tuberosum

The results of paper chromatography of thiourea derivatives, HPLC on reverse phase and normal phase systems, mass spectroscopy and NMR all confirm that the sole isothiocyanate obtained by hydrolysis from extracts of tubers of Tropaeolum tuberosum subsp. tuberosum is p-methoxybenzyl isothiocyanate. Paper chromatography of thioureas and HPLC analysis of seeds, flowers and leaves of I. tuberosum subsp. tuberosum gave identical results to those obtained from tubers. Paper chromatography of glucosinolates revealed a single compound although TLC revealed a second spot in two solvent systems when sprayed with $AgNO_3$-NaOH. This spot may indicate a minor glucosinolate constituent in the tubers but, considering the consistency of the evidence from other methods of analysis, it is probably a glycoside other than a glucosinolate. The spot is considered of minor importance. The sole detectable glucosinolate in Tropaeolum tuberosum subsp. tuberosum is therefore p-methoxybenzyl glucosinolate.

Only isothiocyanates were analyzed in 'kipa isaño', the wild collection identified tentatively as I. tuberosum subsp. silvestre . Kjaer et al., (1978) detected benzyl and 2-propyl thioureas as the major isothiocyanate derivatives and 2-

butyl thiourea as a minor constituent from seeds of wild *T. tuberosum* . The HPLC and paper chromatography methods for thioureas used in this study confirmed the presence of benzyl and 2-propyl isothiocyanates in tubers, seeds, flowers and leaves of the one collection available of wild material. 2-Butyl isothiocyanate was detected in seeds but not in tubers. Benzyl isothiocyanate was the major constituent in tubers but a minor constituent in seeds. Mass spectral analysis of the benzyl isothiocyanate fraction from reverse phase chromatography confirmed the presence of this compound. p-Methoxybenzyl isothiocyanate was not detected in this material. From these results it can be concluded that the material analyzed by Kjaer et al. (1978) and 'kipa isaño' are distinct from subsp. *tuberosum* . It is concluded that they are both examples of *T. tuberosum* subsp. *silvestre* , and that benzyl, 2-propyl and 2-butyl isothiocyanates characterize this subspecies.

Glucosinolates of Lepidium meyenii

Because of the limited amount of material available determinations of the glucosinolates of *Lepidium meyenii* are tentative. Analysis of isothiocyanates liberated by an exogenous myrosinase preparation indicate that the major constituent is benzyl isothiocyanate, while p-methoxybenzl isothiocyanate occurs in comparatively smaller amounts. Both HPLC and paper chromatography of thioureas support this conclusion. Analysis of

glucosinolates by paper chromatography and TLC consistently detected 2 constituents. The major component co-chromatographed with the major glucosinclate in T. tuberosum subsp. tuberosum (p-methoxybenzyl). This spot is likely to be benzyl and p-methoxybenzyl glucosinolates combined. The identity of the other spot is undetermined and may be a non-glucosinolate glycoside. Although no other thiourea derivatives appear, other peaks on the HPLC could possibly correspond to a third glucosinolate. However, until further work can be carried out the summation of these results is that benzyl and p-methoxybenzyl glucosinolates have been detected in roots of L. meyenii .

Thiocarbamates and thioureas in glucosinolate-containing plants

Results obtained from mass spectral analysis of HPLC fractions indicate the presence of thiocarbamates and thioureas in isothiocyanate extracts from glucosinolate-containing plants. In both cases the structure of the detected constituents can easily be seen as a reaction product of two molecules of the isothiocyanate known from the plant. N-(4-hydroxy, benzyl) methylthiocarbamate is formed from p-hydroxybenzyl isothiocyanate in Sinaris alba . One molecule of the $OH-C_6H_4CH_2-$ fragment formed by the liberation of thiocyanate from the isothiocyanate reacts with a molecule of the parent isothiocyanate to form the thiocarbamate. Two molecules of p-methoxybenzyl isothiocyanate from T. tuberosum subsp. tuberosum

react to form N,N,Di(methoxy, 4-benzyl)thiourea. These two
products have not been widely reported from glucosinolate-
containing plants, but their detection in extracts from
T. tuberosum subsp. tuberosum and S. alba confirms the report of
El Migirab et al. (1977) that they exist. Conventional methods
of analysis of isothiocyanates using paper chromatography of
thioureas and GC-MS may not have detected these compounds even
if they were present. Their presence may have been overlooked
because they were not known to occur. Whether these products are
naturally formed in biological systems, or whether they are
artifacts of the extraction procedure cannot be determined from
this study. Chemotaxonomically their presence is not relevant as
they give no new information on the glucosinolates present.
However, their biological activity is largely unstudied. El
Migirab et al. (1977) report thiocarbamates to be antibiotic. If
these thiocarbamates and thioureas can be shown to be present in
living systems, their biological activity in relation to the
reported effects of glucosinolate-containing plants is a
relevant concern.

Evaluation of HPLC methods of analysis of isothiocyanates

The method of HPLC analysis developed in this study is more
convenient and more sensitive than the classic approach of paper
chromatography of thiourea derivatives. This method was more
successful in consistently detecting 2-propyl isothiocyanate and

was decidedly superior in resolving p-methoxybenzyl and benzyl isothiocyanates, the two constituents most important in this study.

It is difficult to evaluate the method in relation to the GC-Mass Spectral method of analysis as no direct comparisons were attempted. When standards are available HPLC is probably comparable in resolution with GC. The relatively low extinction coefficient of isothiocyanates suggests that HPLC is less sensitive in detecting these compounds. The mass spectrometry facility of a GC-MS unit would generally make this system more useful. A liablity of HPLC, is that for further analysis by mass spectrometry, fractions must be laboriously collected and then the solvent evaporated. With low boiling point compounds such as 2-propyl isothiocyanate this method would be unsuccessful. With stable compounds, however, preparative HPLC provides a convenient method for isolation of compounds of high purity. The possibility of using further analytical tools such as NMR, and of having material available for biological work is therefore enhanced.

Systematics of Tropaeolum tuberosum

Analysis of the glucosinolates of _Tropaeolum tuberosum_ supports the assessment of two distinguishable subspecies within the species (Sparre, 1973). This study has determined that p-methoxybenzyl glucosinolate is the characteristic constituent of

subsp. *tuberosum* and confirmed that benzyl, 2-propyl and 2-butyl glucosinolates characterize subsp. *silvestre* as determined by Kjaer *et al*. (1978). The chromosome number of 'kipa isaño' is 2n>42. If x=13, as is typical for the section Mucronata of this genus (Gibbs *et al*., 1978), then this clone of *T. tuberosum* subsp. *silvestre* is possibly a tetraploid with 2n equal to 52 as for subsp. *tuberosum* . However, more thorough investigation of the chromosome number including the examination of meiotic tissue is necessary to rule out the possibility that 'kipa isaño' is a triploid (2n=39). If *T. tuberosum* subsp. *silvestre* is a tetraploid or a triploid, the ploidy level and the difference in chemistry rule out an autotetraploid origin of subsp. *tuberosum* with subsp. *silvestre* as the progenitor. p-Methoxybenzyl glucosinolate is a constituent of several members of the genus *Tropaeolum* including *Tropaeolum cochabambe* (Kjaer *et al*., 1978), a species that Sparre (1973) suggests as having taxonomic affinity with *T. tuberosum* . The suggestion that subsp. *tuberosum* arose as a hybrid from *T. cochabambe* (or another species) and *T. tuberosum* subsp. *silvestre* (Gibbs *et al*., 1978) can be supported by these results. However *T. cochabambe* has been reported to have a chromosome count of 2n=26 (Huynh, 1967), and therefore would not hybridize with a tetraploid subspecies of *Tropaeolum tuberosum* to form tetraploid progeny. If the collection studied is not typical and diploid strains of subsp. *silvestre* do exist, an allotetraploid origin of the cultivar with subsp. *silvestre* and/or *T. cochabambe* as progenitors is still a possibility. The limited sample size of subsp. *silvestre* makes it impossible to rule out the existence

of populations with chemical differences. Strains which produce
p-methoxybenzyl glucosinolate could have given rise to
subsp. tuberosum through selection. Further systematic studies
of the chromosome numbers and the crossing capabilities within
the genus Tropaeolum would be necessary to make more conclusive
statements about the exact origin of the cultivated subspecies
of T. tuberosum .

An obvious assumption of the above discussion is that
subsp. tuberosum arose from subsp. silvestre rather than vice
versa. Generally it is assumed that cultivated forms arise from
wild forms, but the possibility that the wild subspecies arose
from the hybridization of subsp. tuberosum with another taxon
cannot be ruled out. The so-called wild subspecies may in fact
be part of a crop-weed complex (Harland, 1975). 'Kipa isaño'
does exist in Cuyo-cuyo in relatively close proximity to fields
of the cultivar and hybridization between it and cultivated
plants may have occurred in the past or be ongoing.

A sterile triploid would be the likely product of a
hybridization between T. cochabambe and T. tuberosum . 'Kipa
isaño' sets seeds from the majority of its flowers when grown in
Vancouver, although the seed was not tested for viability.

Differences in the tubers of T. tuberosum subsp. silvestre
('kipa isaño') and T. tuberosum subsp. tuberosum support the
separation of a wild and a cultivated subspecies. Tubers of
'kipa isaño' (Figure 9) are more elongated in shape, and were
observed in the field and in cultivation to have longer stolons.
Tubers of subsp. silvestre sprout readily even when kept in cold
storage. Sprouting occurs from the apex of the tuber, and many
of the tubers appear to have an apex that is capable of

continued growth. The wild subspecies is better adapted for vegetative propagation and dispersal than is the obligate cultivar.

The role of human intervention into the origin and selectivity of the cultivated subspecies is a factor that is difficult to assess. Selection of short stolon length and enlarged tubers with greater dormancy is expected. Selection for the chemical content of the cultivar in relation to cultural concepts of consumers is a possibility. However, p-methoxybenzyl glucosinolate in subsp. tuberosum could not have been selected for if plants with genetic capabiltiy to produce it were unknown. If subsp. tuberosum did arise from subsp. silvestre , it must have done so through hybridization or from varieties which produce p-methoxybenzyl glucosinolate.

Ethnopharmacology of Tropaeolum tuberosum and Lepidium meyenii

The ethnobotanical uses of Tropaeolum tuberosum and Lepidium meyenii are consistent with the patterns shown by the survey of all glucosinolate-containing plants. The use of both of these species to affect human reproduction corresponds to a significant use for glucosinolate-containing plants in ways that reflect some effect on reproductive hormonal processes. T. tuberosum , is used as well to affect kidney conditions and as an expectorant, both medicinal uses that are significantly greater in the survey sample. The use of tubers of T. tuberosum

to treat skin conditions parallels a positive (although not significant) selection for glucosinolate-containing plants to treat such conditions.

This statistical support for the medicinal uses of T. tuberosum and L. meyenii has a pharmacological basis in some cases. The similarity in the chemistry of T. tuberosum subsp. tuberosum and L. meyenii indicates that, at least in the conception of Andean peoples, there is a relationship between the presence of aromatic glucosinolates and human reproductive processes. The decrease in testosterone/dihydrotestosterone levels in rats fed tubers of T. tuberosum subsp. tuberosum supports the beliefs attached to T. tuberosum as an anti-reproductive agent. The fact that experimental rats were as capable and apparently as inclined towards impregnating females as were controls contradicts the use of the plant as an anti-aphrodisiac for males. Varying testosterone levels do not have an effect on sexual behaviour in mammals below a threshold level considerably less than that seen in this study (Gorzalka and Mogenson, 1977). Testosterone levels do affect sperm count (Purvis et al., 1975), although no obvious effect on spermatogenisis is evident from this investigation.

The lack of a significant difference in the androgen levels of experimental and starved animals indicates that T. tuberosum exerts its effect on basic metabolism and not by directly affecting androgen production. The fact that the experimental rats did not gain weight and the drop in testosterone associated with this is likely a result of the known antimetabolic and counter-nutritional activity of isothiocyanates. The rate of food consumption by experimental animals, although not

noticeably less than that of controls, was not determined and lack of weight gain may to some extent be related to the animals' aversion to the isothiocyanates contained in the food. Cooking the tubers does not destroy the glucosinolates. While cooking improves the palatability of the food (particularly if large quantities are consumed) by preventing enzymatic production of isothiocyanates in the mouth, spontaneous and bacterial hydrolysis can still liberate isothiocyanates and other breakdown products. The drop in 17-keto steroid levels in urine of rats fed allyl isothiocyanate (Muztar et al., 1979b) may be a result of an effect on androgens similar to that seen in this experiment. Muztar et al. (1979b) make no mention of the effect of allyl isothiocyanate on weight gain.

The use of a starvation inducing factor as an antiaphrodisiac seems rather drastic and counterproductive in maintaining an effective army. Reports of the use of 'isaño' by the Incas in such a way are unsubstantiated by this study. However, these experimental results do support the empirical basis for Tropaeolum tuberosum producing the decrease in reproductive potential reported by the chroniquilists and modern observers. The ideal male contraceptive would decrease spermatogenesis while maintaining libido. The current concern in finding a useable male contraceptive would warrant further investigation into the behavioural and biochemical actions of Tropaeolum tuberosum and isothiocyanates. The general antimetabolic and antinutritional activities of isothiocyanates are not well understood, and further study in this area might lead to greater understanding of specific biochemical changes, such as the effect on testosterone levels.

The antimetabolic effects of isothiocyanates are not sex specific and would affect females adversely as well. It is not known whether they affect female hormonal levels. Female fertility is often associated with menstruation in folk beliefs (Conway and Slocumb, 1979) and, as shown in the statistical survey, beliefs of emmenagogic effects are the largest contributers to the use of glucosinolate-containing plants in affecting human reproduction. This study found no conclusive experimental evidence to support these beliefs. A diet containing tubers of I. tuberosum subsp. tuberosum did not affect the regular periodicity of the estrus cycle in the guinea pig. Although in vitro studies on extracts of I. tuberosum and Sinapis alba showed a qualititative inhibition of estradiol binding, quantitatively isothiocyanates did not competitively inhibit estradiol binding. N,N,Di(methoxy, 4-benzyl) thiourea extracted from tubers of I. tuberosum subsp. tuberosum does significantly inhibit estradiol binding. However, the lack of information on the importance, or the existence, of this compound in biological systems (either plant or animal tissue), and the one time nature of the experiment make it impossible to draw meaningful conclusions as to the likelihood of this compound having estrogenic activity in glucosinolate-containing plants.

Guinea pigs are known to metabolize isothiocyanates differently than rats or man and it is possible that the feeding experiment repeated with another test organism would produce a positive result. This uncertainty, the ambiguity surrounding N,N,Di(methoxy, 4-benzyl)thiourea, and the specific nature of the experiments do not rule out the possibility that other

experiments might show results that would support the folk beliefs relating to female reproductive processes.

Folk uses of *T. tuberosum* that could be produced by toxicity towards lower organisms are well supported pharmacologically. The antibiotic activity of p-methoxybenzyl isothiocyanate is as high or higher than that of benzyl isothiocyanate against several species of yeast and bacteria. The treatment of some skin ailments and positive effects on the urinary system could be explained by antibiotic activity (see Literature Review). The nematocidal activity of benzyl isothiocyanate shown here supports the reports (Gommers, 1973) that glucosinolate-containing plants are toxic to nematodes. Antibiotic and nematocidal activities could both contribute to the reputed crop protective benefits of interplanting with *T. tuberosum* subsp. *tuberosum* . Insecticidal uses of *T. tuberosum* are supported by studies on other glucosinolate-containing plants (Blau *et al.*, 1978).

The folk beliefs on the effects of *T. tuberosum* on the kidneys corresponds to the high use of glucosinolate-containing plants as diuretics. The diuretic effect of allyl isothiocyanate shown by Muztar *et al.* (1979b) supports these folk uses.

Oblitas Poblete (1969) reports the use of *T. tuberosum* as an expectorant and glucosinolate-containing plants in general are used for this purpose. The pharmacological basis for such use is undetermined. The use of mustard in plasters to treat colds and chest conditions relates to the irritant effect of isothiocyanates on the skin. This counter-irritant effect may contribute to the reputed expectorant properties of isothiocyanates.

Although antiscorbutic uses of *T. tuberosum* are not known,

the important use of glucosinolate-containing plants for such a purpose is substantiated in T. tuberosum by the high levels of ascorbic acid reported from the tubers. The use of T. tuberosum for this purpose would be warranted. The nutritional benefits of the high ascorbic acid levels of tubers of T. tuberosum have been discussed.

From the above discussion it can been seen that the nutritional and medicinal uses of Tropaeolum tuberosum and Lepidium meyenii by human beings living in the Andes mountains have in most cases some physical basis. The contribution of these plants to the well being of these groups of people is real and their utilization by these peoples generally rational, particularly if taken in the context of the native etiology of disease (Crtiz de Montellano, 1975). The domestication of T. tuberosum subsp. tuberosum in the Andes is explainable by the Indians' longstanding concern for both the nutritional and medicinal properties of its tubers.

The strong correlation between the statistically significant uses of glucosinolate-containing plants, and the pharmacological basis for these uses as borne out in the study of Tropaeolum tuberosum subsp. tuberosum and Lepidium meyenii , demonstrates the validity and potential of a systematic approach to the evaluation of ethnobotanical reports in general. The tabulation and statistical analysis of ethnobotanical data is greatly simplified using modern computing methods. If subsequent phytochemical and pharmacological investigations concentrate on significantly positive correlations, the chances of detecting active constituents can be increased. The association of folk uses, statistical patterns and phytochemistry in this study

indicates that pharmacological activity of known phytochemical constituents could be predicted by analysis of the relationship between ethnobotanical use and phytochemistry. A data bank comprising ethnobotanical reports as well as phytochemical , chemotaxonomic and pharmacological information would be a valuable tool in interpreting research results and in increasing the predicability of results whether one approaches ethnopharmacological problems from the direction of ethnobotany, phytochemistry or pharmacology.

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