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GLUCOSINOLATES IN SEEDS OF *NESLIA PANICULATA*

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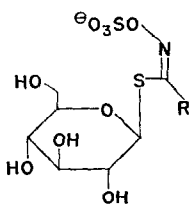
Key Word Index—*Neslia paniculata*; Cruciferae; glucosinolates; (R)$_S$-11-methylsulfinylundecylglucosinolate.

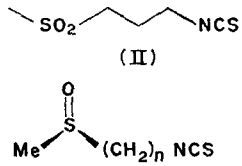
Abstract—The glucosinolate fraction in seeds of *Neslia paniculata* (L.) Desv., subsp. *thracica* (Velen.) Bornmüller, (Cruciferae), has been isolated and subjected to enzymic hydrolysis. The isothiocyanates thus produced have been separated and identified as cheirolin (II), (R)-9-methylsulfinylnonyl (III), (R)-10-methylsulfinyldecyl (IV), and (R)-11-methylsulfinylundecyl isothiocyanate (V), the last of which has not been previously encountered as a natural compound.

INTRODUCTION

THE CRUCIFER genus *Neslia* Desv. contains the single species *N. paniculata* (L.) Desv. (*Vogelia paniculata* (L.) Hornem.), occurring in Western Asia, Northern Africa, Central and Southeastern Europe. The taxon is frequently divided into the subspecies *paniculata* and *thracica* (Velen.) Bornm. (*N. apiculata* Fischer, Meyer et Avé-Lall.), with the former naturalized in the Northern, and subsp. *thracica* dominating in the Southern part of its range. We have had access to a large quantity of seeds of subsp. *thracica* and report the results of a study of its contents of glucosinolates, a class of natural anions possessing the general structure (I).



(I)



(II)

(III) n = 9
(IV) n = 10
(V) n = 11

No reference to glucosinolates in seed material of *Neslia* seems to be on record; Schultz and Wagner,[1] however, presented paper chromatographic evidence for the existence of an unidentified glucosinolate in fresh parts of *Vogelia paniculata* Hornem. (=*N. paniculata* (Desv.)).

RESULTS

The seed material employed in the present study was produced by large-scale cultivation of the annual herb during the spring of 1971 in the Botanic Garden of the University of Copenhagen.*

Paper chromatography of a methanolic seed extract revealed its contents of a hydrophilic,

* A herbarium voucher is deposited in the Botanical Museum of the University of Copenhagen.

[1] O.-E. SCHULTZ and W. WAGNER, *Z. Naturforsch.* **11B**, 73 (1956).

as well as one or more rather lipophilic glucosinolates. Various attempts to separate the glucosinolate fraction, produced by ion exchange technique, were unsuccessful. Hence, the glucoside mixture, from 300 g of seeds, was subjected to enzymic hydrolysis with a cell-free myrosinase preparation, and the resulting chloroform-soluble products, mainly isothiocyanates, were fractionated on silica gel columns, with chloroform as solvent.

The fastest moving material (225 mg) was identified as cheirolin, 3-methylsulfonylpropyl isothiocyanate (II), a long-known compound deriving by enzymic hydrolysis from 3-methylsulfonylpropylglucosinolate (I, $R = CH_3SO_2(CH_2)_3$), formerly encountered in several crucifer genera.[2]

Continued elution of the column with ethanol-containing chloroform yielded an additional isothiocyanate fraction (380 mg), consisting of several homologues according to mass spectrometry. Since attempts to separate these proved abortive, the total fraction was converted into the corresponding thioureas upon reaction with ammonia. Preparative paper chromatography in Bz–EtOH–H_2O (5:1:2),[3] followed by column chromatographic purification of the individual fractions, yielded three homogeneous thioureas.

The least lipophilic of these (2 mg) proved identical, on comparison, with an authentic specimen of (*R*)-1-(9-methylsulfinylnonyl)-thiourea, derived from a previously characterized glucosinolate in *Arabis alpina* L.[4]

A homogeneous, more lipophilic, and predominant thiourea (101 mg) was identified as (*R*)-1-(10-methylsulfinyldecyl)-thiourea by means of its analytical composition and spectroscopic properties. Data for optical rotation and m.p. were somewhat at variance with values previously reported for a specimen of the same thiourea derived from (*R*)-10-methylsulfinyldecyl isothiocyanate (IV), in its turn resulting from enzymic hydrolysis of a glucosinolate, first discovered in seeds of *Camelina sativa* (L.) Crantz.[5] Closer inspection of the original thiourea specimen by improved GLC and MS methods revealed a notable, previously overlooked contamination with the lower homologue. Hence, *Camelina* seeds contain glucosinolates with both $CH_3SO(CH_2)_9$- and $CH_3SO(CH_2)_{10}$-side chains, the latter though in great preponderance.

The most lipophilic thiourea (4 mg) was also levorotatory, and possessed chemical and spectroscopic properties strikingly similar to those of the two thioureas described above. Thus, its IR spectrum virtually coincided with the spectra of the thioureas derived from (III) and (IV), whereas its MS, though very similar in type, differed from the spectra of the thioureas derived from (III) and (IV) by exhibiting numerous fragments displaced 28 or 14 m.u., respectively, towards the higher mass region. On this basis, the product is formulated as (*R*)-1-(11-methylsulfinylundecyl)-thiourea, deriving from the corresponding isothiocyanate (V). The absolute configuration is assigned on the basis of the sign and magnitude of its optical rotation, similar to those established for the configurationally known lower homologues. The new isothiocyanate most likely derives from a usual glucosidic progenitor, $(R)_S$-11-methylsulfinylundecylglucosinolate

(I, $R = -(CH_2)_{11}$ ▶ S(Me) ◀ O), a new natural product.

[2] A. KJÆR, *Fortschr. Chem. Org. Naturstoffe* **18,** 122 (1960).
[3] M. G. ETTLINGER and C. P. THOMPSON, *Studies of Mustard Oil Glucosides* (II), Final Rep. Contract DA-19-129-QM-1689 (1962), p. 12; report available from Office of Technical Services, U.S. Dept. of Commerce as AD-290 747.
[4] A. KJÆR and R. GMELIN, *Acta Chem. Scand.* **10,** 1358 (1956).
[5] A. KJÆR, R. GMELIN and R. BOE JENSEN, *Acta Chem. Scand.* **10,** 1614 (1956).

DISCUSSION

Unbranched $(R)_S$-ω-methylsulfinylalkylglucosinolates (I, $R = CH_3SO(CH_2)_n$–) are frequently encountered constituents of crucifer species, known today with every *n*-value from 3 to 10.[6,7] The present finding of the analogous glucoside with 11 methylene-groups, therefore, occasions little surprise. The simultaneous occurrence of sulfoxide isothisoyanates with 9, 10 and 11, and a sulfone with 3 methylene groups in enzymatically hydrolysed seed extracts of *Neslia* strongly suggests a common biosynthetic origin of the parent glucosinolates. Chisholm[8] has recently presented experimental evidence for the biosynthesis of 3-methylthiopropylglucosinolate (I, $R = CH_3S(CH_2)_3$, and the corresponding sulfoxide, from methionine, via homomethionine, in *Cheiranthus kewensis* (wallflower). The amino acid homologization involved in this conversion is likely to be operating repeatedly in *Neslia*, leading all the way to the observed C_9-, C_{10}-, and C_{11}-side chains. The failure to detect the sulfoxide-glucosinolates with 4–8 methylene groups, known from other plant sources, in *Neslia* by no means excludes their presence in trace amounts. On the other hand, it cannot be excluded that the enzymically operated chain lengthening process is accompanied by genetically rather than environmentally controlled competitive glucosinolate-producing reactions at the various homologue levels. Further insight into this problem could be of considerable value in connection with speculations on the biological affinity between various taxa, based on glucosinolate distribution patterns. More data are needed, however, to make such a discussion meaningful.

EXPERIMENTAL

Chromatography, extraction, and enzymic hydrolysis of glucosinolates. On PC, with (a) BuOH–EtOH–H_2O (4:1:3) or (b) BuOH–Py–H_2O (6:4:3) as the mobile phases,[9] a methanolic extract of ground seeds of *Neslia paniculata*, subsp. *thracia* exhibited glucosinolate spots with R_B-values (i.e. R_f relative to that of benzylglucosinolate (I, $R = C_6H_5CH_2$–)) of 0·23 and about 1·40 in (a), 0·5 and 1·0–1·1 in (b). Dry seeds (300 g) were finely ground and extracted with two 750-ml portions of boiling 70% MeOH. The combined extracts were freed of MeOH *in vacuo*, the residue was diluted with H_2O to a total vol. of 1 l. and passed through a column of Dowex 1 × 1 ion exchange resin, preloaded with Cl^-. The column was thoroughly rinsed with H_2O, and the glucosinolates were eluted with a 5% K_2SO_4-solution, 250 ml fractions being collected. With TLC analysis, the non-separated, total glucosinolate fraction was present in fractions 2–15. These were combined and evaporated to dryness. Repeated extractions of the residue with 85% EtOH served to remove considerable quantities of inorganic salt. After concentration to dryness of the EtOH extracts, an amorphous glucosinolate fraction (6 g) resulted, still containing some salt. Further purification was achieved by passing the total fraction, dissolved in H_2O (150 ml), through a column of basic Al_2O_3 (Woelm) (300 g). The column was rinsed with H_2O (100 ml), and eluted with a 2% KOH solution (100 ml). The total glucosinolate fraction was present in the rinsing H_2O and the first 50 ml of the eluate. Concentration to dryness of the combined solutions afforded the purified glucosinolates (3 g). They were dissolved in a citrate buffer (pH 6·5) (100 ml), a trace of ascorbic acid and a cell-free myrosinase preparation (15 ml) were added, and the mixture was set aside at room temperature for 4 hr. The hydrolysis mixture was repeatedly extracted with $CHCl_3$, the extract was dried (over Na_2SO_4), and the solvent removed, leaving a crude, oily isothiocyanate mixture (*c.* 1 g).

Separation and identification of hydrolysis products. The hydrolysis products, dissolved in a small volume of $CHCl_3$, were placed on a column of silica gel (50 g, deactivated with 15% of H_2O), and the column was eluted with 200 ml-portions of $CHCl_3$, $CHCl_3$ + 1% EtOH, $CHCl_3$ + 2% EtOH, etc. up to $CHCl_3$ + 6% EtOH. 15-ml fractions were collected and controlled by TLC analysis on silica gel, with $CHCl_3$ + 5% EtOH as the solvent. Fractions 9–15 were combined and evaporated to dryness (480 mg); likewise the combined fractions 15–23 gave a residue (142 mg), which was divided up into a major (131 mg) and a minor fraction (5 mg) by preparative TLC (in $CHCl_3$ + 5% MeOH). The contents from fractions 15–23 and the major fraction from the TLC-plate were united and subjected to column chromatography on silica gel (25 g). Elution with EtOAc removed a chromatographically homogeneous, crystalline compound (480 mg);

[6] M. G. ETTLINGER and A. KJÆR, in *Recent Advances in Phytochemistry* (edited by T. J. MABRY, R. E. ALSTON and V. C. RUNECKLES), Vol. 1, p. 58, Appleton–Century–Crofts, New York (1968).
[7] R. GMELIN, A. KJÆR and A. SCHUSTER, *Acta Chem. Scand.* **24**, 3031 (1970).
[8] M. D. CHISHOLM, *Phytochem.* **11**, 197 (1972).
[9] R. GMELIN and A. KJÆR, *Phytochem.* **9**, 591 (1970).

subsequent elution with $CHCl_3$ yielded an oily fraction (380 mg), which, according to MS, was clearly a mixture of homologues. The crystalline material was recrystallized from $CHCl_3$, containing light petrol., to give colourless crystals (225 mg), m.p. 45°. The compound was easily identified as cheirolin (II) by comparison with a synthetic specimen (IR, m.m.p., MS).[10] The oily fraction, mainly consisting of isothiocyanates, was converted into the corresponding thioureas on reaction with methanolic ammonia at room temp. for 4 hr. The mixture could not be separated by column chromatography or counter-current distribution (emulsions!). Recourse was finally taken to separation of the available quantity (295 mg) on 23 sheets of Whatman 3MM paper, with Bz–EtOH–H_2O (5:1:2).[3] Three thiourea-zones were recognized in UV light, they were separately eluted with MeOH. The most hydrophilic thiourea was taken up in $CHCl_3$, and the solution was repeatedly washed (H_2O) to remove impurities from the paper. The residue was chromatographed on a very small column of silica gel, with $CHCl_3$, containing increasing amounts of EtOH. The thiourea-containing fractions were combined and the residue (4·3 mg) was recrystallized from EtOAc to give a homogeneous thiourea (2·0 mg), m.p. 104–105°, undepressed on admixture with an authentic specimen of (*R*)-1-(9-methylsulfinylnonyl)-thiourea.[4] Coinciding IR and MS further served to confirm the identity. Identical chirality of the two specimens was established by comparing their CD-curves. The more lipophilic, and major, thiourea was recrystallized from EtOAc to give colourless crystals (118 mg), m.p. 103–104°. An analytical specimen was produced on two additional recrystallizations from EtOAc to give colourless needles (101 mg), m.p. 108°, $[\alpha]_D^{22}$ −63° (*c*. 1·7, EtOH); $[\alpha]_D^{22}$ −56° (*c*. 1·7, $CHCl_3$). (Found: C, 51·5; H, 9·25; N, 9·8; S, 22·9. $C_{12}H_{26}ON_2S_2$. Required: C, 51·9; H, 9·42; N, 10·1; S, 23·0). The composition, UV, IR and MS suggested that the thiourea was (*R*)-1-(10-methylsulfinyldecyl)-thiourea, previously reported as a derivative of the corresponding isothiocyanate (IV), obtained from an enzymically hydrolyzed seed extract of *Camelina sativa*.[5] The thiourea was reported with a m.p. of 92–92·5° and a rotation of $[\alpha]_D^{24}$ −65° (*c*. 1·03, 96% EtOH).[5] Renewed investigation of the original thiourea specimen, notably by PC in the Bz–Et–H_2O system,[3] and by MS, revealed a marked contamination of the original specimen with the lower homologue, the above described thiourea derived from (III). The analytical figures, UV and IR spectra, rotational data, and GLC behaviour in standard solvents, will not reveal moderate contaminations with the nearest homologous thioureas. The most lipophilic thiourea was dissolved in $CHCl_3$, the solution was washed (H_2O), and dried. The residue was chromatographed on silica gel with $CHCl_3$, containing gradually increasing amounts of EtOH (up to 6%), as the solvent. The residue was recrystallized from EtOAc to give colourless crystals (10 mg), homogeneous on GLC. Two additional recrystallizations from EtOAc gave the pure compound (4 mg), m.p. 124–125°, $[\alpha]_D^{23}$ −54° (*c*. 0·6, EtOH). The amount of substance did not permit combustion analyses, but the sign and magnitude of rotation, the IR spectrum, R_F on PC and, particularly, the MS established the identity of the thiourea as (*R*)-1-(11-methylsulfinylundecyl)-thiourea, derived from the mustard oil (V).

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[10] A. KJÆR, F. MARCUS and J. CONTI, *Acta Chem. Scand.* **7**, 1370 (1953).