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ω-METHYLTHIOALKYLGLUCOSINOLATES AND SOME OXIDIZED CONGENERS IN SEEDS OF *ERYSIMUM RHAETICUM*

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Key Word Index—*Erysimum rhaeticum*; Cruciferae; ω-methylthio-3-hydroxyalkylglucosinolates; (−)-6-(3-methylthiopropyl)-tetrahydro-1,3-oxazine-2-thione.

Abstract—The glucosinolates in seeds of *Erysimum rhaeticum* Schleich. ex DC. have been identified by structure analysis of their sulfur-containing enzymic hydrolysis products, comprising 5-methylthiopentyl and 6-methylthiohexyl isothiocyanate, the corresponding 3-hydroxylated isothiocyanates, and the sulfoxides and sulfones of the latter. The phytochemical results are evaluated in terms of their possible taxonomic significance.

INTRODUCTION

AS PART of current studies on the distribution of glucosinolates—a class of naturally occurring anions possessing the general structure (I)—within the crucifer genus *Erysimum*, seed material of the species *E. rhaeticum* Schleich. ex DC.,* propagated from material collected in the wild near Meran in Tyrol, has been subjected to a detailed analysis. The present investigation supplements previous studies on the glucosinolate distribution within the systematically difficult genus *Erysimum* and was carried out in the hope of obtaining an additional set of characters of potential taxonomic interest.

RESULTS

Paper chromatographic analysis of a 70% MeOH extract of seeds of *E. rhaeticum* revealed its contents of several glucosinolates. These could be divided into four groups, A–D, arranged in the order of decreasing R_f-s in BuOH–EtOH–H_2O (4:1:3). Accordingly, group separation of the glucosinolates from 200 g of seeds into A–D was attempted and achieved by column chromatography on cellulose powder. Fractions A–C were individually subjected to enzymic hydrolysis with myrosinase, whereas fraction D was further divided into two subgroups, D_1 and D_2, by chromatography on cellulose, with BuOH–Py–H_2O (6:4:3) as the mobile phase, prior to hydrolysis.

An ethereal extract of the enzymically hydrolyzed glucoside fraction A contained, according to GLC analysis, two volatile compounds, with the same retention times as authentic specimens of 5-methylthiopentyl (II) and 6-methylthiohexyl (III) isothiocyanate.

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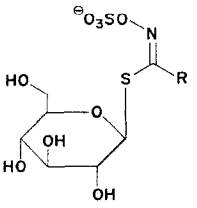
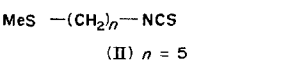
To verify the identity of the minute quantities of volatile isothiocyanates, an additional seed sample (150 g) was processed by extraction, enzymic hydrolysis, and steam distillation to give the same mixture. Combined GLC and MS served to firmly establish the identity of the volatile constituents as (II) and (III), most likely arising by enzymic hydrolysis from the corresponding glucosinolates, (Ia) and (Ib). Whereas the former of these has been repeatedly encountered in crucifer seeds,[1–3] including an *Erysimum* species,[3] (Ib) has thus far been recognized solely in seeds of the crucifer *Lesquerella lasiocarpa*.[4]

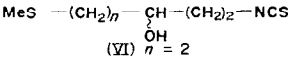


(I)

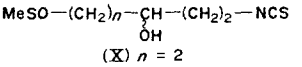
(Ia) R = $MeS(CH_2)_5$
(Ib) R = $MeS(CH_2)_6$
(Ic) R = $MeS(CH_2)_n\,CH(OH)(CH_2)_2$
(Id) R = $MeSO(CH_2)_n\,CH(OH)(CH_2)_2$
(Ie) R = $MeSO_2(CH_2)_n\,CH(OH)(CH_2)_2$



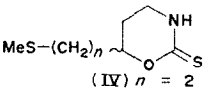
(II) n = 5
(III) n = 6



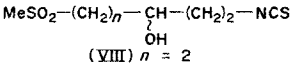
(VI) n = 2
(VII) n = 3



(X) n = 2
(XI) n = 3



(IV) n = 2
(V) n = 3



(VIII) n = 2
(IX) n = 3

Silica gel chromatography of the chloroform-soluble hydrolysis products from fraction B afforded a crystalline, and an oily product. The former, $C_8H_{15}NOS_2$, m.p. 44°, $[\alpha]_D^{22}$ −101°, possessed chemical and spectroscopic properties so similar to those of (IV)[3] that its identity as (−)-6-(3-methylthiopropyl)-tetrahydro-1,3-oxazine-2-thione (V), the higher homologue of (IV), was immediately obvious. Comparable rotation data suggest identity with regard to the unknown, absolute configurations of (IV) and (V). The small, oily fraction was slowly converted into (V) on chromatography, or, more rapidly, on treatment with triethylamine in chloroform, a behaviour analogous to that of (VI), undergoing cyclization to (IV).[3] Hence, (VII) is considered to represent the initial enzymic hydrolysis product, most likely deriving from the parent glucosinolate (Ic, n = 3), not previously encountered in Nature.

Similar treatment of fraction C resulted in the isolation of (IV), indistinguishable from a specimen formerly isolated from a hydrolyzed seed extract of *E. virgatum* Roth.[3,*] This finding strongly suggests the occurrence of the glucosinolate (Ic, n = 2), also in *E. rhaeticum*.

The most lipophilic entity D_1 of fraction D yielded, after enzymic hydrolysis and chromatography, a crystalline, dextrorotatory mustard oil, identified as (+)-3-hydroxy-5-methylsulfonylpentyl isothiocyanate (VIII) upon comparison with a specimen previously obtained from *E. virgatum*.[3] In addition, the cyclized counterpart (the sulfone corresponding to (IV)) was obtained in trace amounts, again identified on comparison with material isolated from *E. virgatum*.[3] It is of interest to note that the MS of both the isothiocyanate and the cyclized

* Referred to previously as *E. hieracifolium* L.[3] Expert studies have revealed, however, that the material employed should rightly be designated as *E. virgatum* Roth, a member of the *E. hieracifolium* group (Prof. C. FAVARGER, Neuchatel, and Dr. A. POLATSCHEK, Vienna; private communications).

[1] KJÆR, A., LARSEN, I. and GMELIN, R. (1955) *Acta Chem. Scand.* **9**, 1311.
[2] KJÆR, A. (1960) *Fortschr. Chem. Org. Naturstoffe* **18**, 122.
[3] KJÆR, A. and SCHUSTER, A. (1970) *Acta Chem. Scand.* **24**, 1631.
[4] DAXENBICHLER, M. E., VAN ETTEN, C. H. and WOLFF, I. A. (1961) *J. Org. Chem.* **26**, 4168.

product, contained minor peaks 14 m.u. above the molecular ions and several prominent fragment ions, signifying admixture with minor amounts of the higher homologues (IX), and the corresponding cyclized product (the sulfone of (V)). The amount of material available did not permit isolation and further characterization of the higher homologues, which, incidentally, were not detectable isolates from *E. virgatum*.[3] Assuming the usual progenitor type, the glucosinolates (Ie, $n = 2$ and 3) therefore appear to be present in *E. rhaeticum* seeds.

The least mobile fraction D_2, after enzymic hydrolysis and chromatographic separation of the $CHCl_3$-soluble hydrolysis products, afforded two major products, both in quantities insufficient for rigorous purification. According to chromatographic behaviour and MS data, however, one product consisted of the sulfoxide mustard oil (X), contaminated with small amounts of the higher homologue (XI). The other product, exhibiting virtually the same MS, was identified as a mixture of the cyclized sulfoxide (SO of (IV)), previously obtained from *E. virgatum*,[3] again accompanied by a minute amount of the higher homologue (SO of (V)). Again assuming an ordinary glucosidic derivation, the glucosinolates (Id, $n = 2$ and 3) are established as constituents of *E. rhaeticum* seeds.

DISCUSSION

In contrast to several other *Erysimum* species studied,[5] *E. virgatum* and *E. rhaeticum* appear to be limited in their glucosinolate-synthesizing capacity to the production of unbranched ω-methylthioalkyl chains containing 5 (or 6) methylene groups, with or without oxidation of the MeS-grouping and the 3-position of the carbon chain. The available evidence, including knowledge of the biosynthesis of 3-methylthiopropylglucosinolate and the corresponding sulfoxide,[6] suggests that methionine is operating as the principal amino acid progenitor in the biosynthesis of the above glucosinolates. Even when, unbranched ω-methylthiopropyl and ω-methylthiobutyl side chains, including their *S*-oxidized counterparts, are not unknown in other *Erysimum* species,[2,5] the biogenetic operation of methionine, though probably very common, can hardly be regarded as a characteristic of the genus as such, inasmuch as glucosinolates, derived from acid[7] or aromatic[5] amino acids, have been encountered within the genus.

The significance of the strikingly similar glucosinolate patterns encountered in *E. rhaeticum* and *E. virgatum* is difficult to assess. On the basis of current taxonomic classification,[8] the former belongs to the *E. sylvestre*, the latter to the *E. hieracifolium* group, two complexes of no established close affinity. Preliminary analyses of seeds of *E. sylvestre* (Crantz) Scop. indicated a glucosinolate pattern virtually indistinguishable from that of *E. rhaeticum*, in accord with expectations. Further comments on the value of glucosinolate patterns in taxonomic studies within the genus *Erysimum* will have to await additional data compiled from a broader selection of well-authenticated species. It appears possible, however, that such data, supplemented with other chemical studies, e.g. of cardenolides,[9] may prove useful as an auxiliary tool in the task of bringing clarity to the systematics of this extremely difficult genus.

[5] To be published.
[6] CHISHOLM, M. D. (1972) *Phytochem.* **11,** 197.
[7] CHISHOLM, M. D. (1973) *Phytochem.* **12,** 605.
[8] BALL, P. W. JR. (1964), *Flora Europaea* Vol. 1, p. 270, Cambridge University Press.
[9] GMELIN, R. and BREDENBERG, J. B. (1966) *Arzneimittelf.* **16,** 123.

EXPERIMENTAL

Extraction, chromatography, and enzymic hydrolysis of glucosinolates. PC was performed as recently described.[3] Multicomponent spots were observed in BuOH–EtOH–H_2O (4:1:3), with R_B values of A *ca.* 1·2; B *ca.* 1·0; C *ca.* 0·8; and D *ca.* 0·2. A large seed portion (200 g) was crushed and defatted with light petrol.; the dry residue (170 g) was extracted 2× with 1 l. portions of hot 70% MeOH. After filtration, evaporation *in vacuo* of MeOH, and filtration through Hyflo-Supercel, the aqueous solution (1 l.) was passed through a column of Dowex 1 × 1 ion exchange resin, preloaded with Cl^-. The column was thoroughly rinsed with H_2O, before the glucosinolates were eluted with a 5% K_2SO_4 solution, 300 ml fractions being collected. Fractions 1–15, containing the total glucosinolate fraction, were combined and concentrated to dryness. Repeated extractions with hot 85% EtOH served to remove considerable amounts of inorganic salt. 1 g portions of the residue (12·5 g) were subjected to group separation by chromatography on a column of 100 g of microcrystalline cellulose powder (Merck), preloaded with the lower phase (70 ml) of the BuOH–EtOH–H_2O (4:1:4) system, and packed by suspension in the upper phase. 10 ml fractions were collected, analyzed by PC, and combined into the four groups; a typical separation was: A, fractions 16–21 (22 mg); B, 22–30 (36 mg), C, 39–49 (152 mg); and D, 59–98 (217 mg). The appropriate fractions from four such separations were combined, evaporated to dryness and subjected to enzymic hydrolysis by the addition of citrate buffer (20 ml, pH 6·7), a few drops of a myrosinase solution, and a trace of ascorbic acid. After 2 hr at room temp. the solutions were saturated with $(NH_4)_2SO_4$ and extracted with $CHCl_3$ (Et_2O, in the case of fraction A). The dried solutions were concentrated to dryness and treated individually as described below.

Fraction A. The ethereal solution from fraction A was gas-chromatographed at 100° on a DEGA-packed column, with 5-methylthiopentyl[1] and 6-methylthiohexyl isothiocyanate[10] as reference compounds. Small peaks with retention times identical with those of the reference compounds were observed. In order to substantiate the finding, a new seed portion (150 g) was defatted with light petrol., and the glucosinolates extracted with 70% MeOH as described above. The resulting aqueous solution was extracted with $CHCl_3$ and, after removal of organic solvents by evaporation, subjected to enzymic hydrolysis by the addition of a myrosinase solution. After 4 hr at room temp., volatile products were removed by steam distillation and collected in Et_2O. Combined GC–MS permitted the unambiguous identification of 5-methylthiopentyl (II) as the major, and 6-methylthiohexyl isothiocyanate (III) as the minor constituent.

Fraction B. Four combined B fractions, totaling 150 mg of glucosinolate, yielded 42 mg of enzymic hydrolysis products, soluble in $CHCl_3$. Chromatography on silica gel (18 g), with EtOAc as the mobile phase, gave both an oily (18 mg), and, subsequently, a crystalline fraction (18 mg). The latter could be recrystallized from $CHCl_3$–hexane to give colourless needles of (V) (12 mg), m.p. 61°, $[\alpha]_D^{22}$ −101° (*c* 0·6, abs. EtOH). (Found: C, 46·0; H, 7·09; N, 6·5. $C_8H_{15}NOS_2$ required: C, 46·80; H, 7·37; N, 6·82.) UV absorption (in EtOH): λ_{max} 252 nm (ϵ 14 700); IR (KBr): strong bands at 1560, 1430, 1338, 1303, 1250, 1243, 1220, 1170, 1152 and 1043 cm^{-1}. The MS showed a parent ion at *m/e* 205 and fragments corresponding to losses of 33 (SH), 47 (MeS), and 89 ($MeS(CH_2)_3$); a strong peak at 61 was indicative of the stable ion $MeSCH_2^+$. The above oily fraction was rechromatographed in EtOH on silica gel, resulting in the production of further quantities of (V). The latter could be more easily produced by letting the oily material, representing the mustard oil (VII), stand in EtOAc solution containing a few drops of NEt_3; the UV absorption at 252 nm was employed for following the cyclization reaction.

Fraction C. A total of *ca.* 500 mg of the C-fraction was subjected to enzymic hydrolysis in the usual way, giving a $CHCl_3$-soluble mixture (93 mg) which was chromatographed on silica gel with EtOAc as an eluent. A crystalline fraction (66 mg) was obtained; after recrystallization from EtOAc-hexane, the product, m.p. 57°, $[\alpha]_D^{22}$ −138° (*c* 0·9, EtOH), was identified as (IV) by comparison with an authentic specimen[3] (m.m.p., IR and MS). An oily fraction consisting of (VI), was identified on the basis of its NEt_3-induced cyclization to (IV).

Fraction D. The combined D-fractions (1·26 g) were chromatographed, in 200 mg portions, on a cellulose column (50 g), with BuOH–Py–H_2O (6:4:3) as the mobile phase. The fastest-moving fractions D_1 were combined to give 357 mg of a homogeneous product, according to paper chromatography. Similarly, the slow-moving fractions D_2 were pooled to give 529 mg of another apparently homogeneous glucosinolate. D_1. After enzymic hydrolysis in the usual way, a $CHCl_3$-soluble product mixture (44 mg) was obtained. Chromatography on silica gel, with EtOAc and a gradient of EtOH as the eluants, yielded two crystalline fractions, one with $[\alpha]_D^{22}$ + 20·4° (*c* 0·6, EtOAc), and m.p. 98–99°, undepressed on a mixture with authentic (VIII),[3] and exhibiting virtually the same IR spectrum as the latter, and another, levorotatory compound, possessing properties (IR and MS), indistinguishable from those of the sulfone corresponding to (IV).[3] The MS of both crystalline products exhibited, in addition to the peaks of the authentic specimen, minor molecular ion signals at *m/e* 237, in addition to several fragment ions displaced 14 m.u. towards the higher mass region when compared with the ions observed in the MS of (VIII) and its cyclized counterpart. D_2. Similarly, myrosinase-catalyzed hydrolysis yielded a $CHCl_3$-soluble product mixture (31 mg) which was

[10] KJÆR, A. and CHRISTENSEN, B. (1957) *Acta. Chem. Scand.* **11**, 1298.

fractionated by silica gel chromatography with $CHCl_3$, containing increasing amounts of EtOH, as the eluant. Again, two fractions were obtained, one (11 mg) possessing the properties (IR, MS) expected for (X), and another (4 mg), identified as essentially pure cyclized product (a sulfoxide corresponding to (IV)) on comparison with an authentic specimen[3] (IR and MS). Again, MS of the isothiocyanate as well as the cyclized product indicated the presence of minute quantities of the higher homologues: (XI, and a sulfoxide of (V)).

Acknowledgements—The authors are grateful to Dr. R. Gmelin for the original seed sample, and to Dr. J. P. Hjerting and the Botanic Garden of the University of Copenhagen for generous assistance in its propagation. MS were recorded by Dr. J. Øgaard Madsen of this Institute. Microanalyses were performed by Mr. G. Cornali and his staff.