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SHORT COMMUNICATION

3,4,5-TRIMETHOXYBENZYLGLUCOSINOLATE: A CONSTITUENT OF *LEPIDIUM SORDIDUM*

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Abstract—The predominant glucosinolate in the crucifer *Lepidium sordidum* A. Gray is shown to be 3,4,5-trimethoxybenzylglucosinolate (III), not previously encountered in Nature, but present also in *L. hyssopifolium* Desv., and characterized as a crystalline potassium tetraacetate. The identification is based on chemical and enzymic hydrolysis, the latter producing 3,4,5-trimethoxybenzyl isothiocyanate (II), indistinguishable from a synthetic specimen. The natural occurrence of ring-oxidized benzylglucosinolates is briefly discussed.

INTRODUCTION

THE GENUS *Lepidium* (Cruciferae), traditionally comprising well above a hundred distinct species of nearly cosmopolitan distribution,[1] was first established as a source of isothiocyanates in 1899, when Gadamer[2] isolated and identified benzyl isothiocyanate as a product of enzymic hydrolysis of a glucosinolate present in seeds of ordinary garden cress (*L. sativum* L.). Within the last twenty years, several other isothiocyanates have been recognized as enzymic hydrolysis products of glucosinolates in the nearly twenty additional *Lepidium* species studied.[3–8] Notwithstanding interesting variations in the chemical type of isothiocyanates encountered, comprising, e.g. alkyl and alkenyl derivatives,[3,5,8] glucosinolates affording benzyl isothiocyanate, without or with hydroxy or methoxy groups in *m*- or *p*-positions (I), seem to prevail, at least in seed material, within the genus. Ring-oxidized benzylglucosinolates, observed in *Lepidium* species, include *p*-hydroxy,[3,6,8] *p*-methoxy,[6–8] *m*-hydroxy,[4,8] and *m*-methoxy[4,8] substituents (Ib–e), not infrequently occurring pairwise together.

Recently, 3,4,5-trimethoxybenzyl isothiocyanate (II) was reported as a product of enzymic hydrolysis of a seed extract of *L. hyssopifolium* Desv.[7] Its identity was established upon comparison with a specimen obtained by enzymic hydrolysis of a glucosinolate isolated from *L. sordidum* A. Gray; the latter isothiocyanate, in its turn, was reported identical with a synthetic specimen of (II).[7] The natural source for the work quoted was whole plants

[1] O. E. SCHULZ in A. ENGLER and K. PRANTL, *Die Natürlichen Pflanzenfamilien*, 2nd edn, Vol. 17b, p. 407, (edited by H. HARMS), Duncker & Humblot, Berlin (1936).
[2] J. GADAMER, *Arch. Pharm.* **237**, 507 (1899).
[3] A. KJÆR, *Fortschr. Chem. Org. Naturstoffe* **18**, 122 (1960).
[4] P. FRIIS and A. KJÆR, *Acta Chem. Scand.* **17**, 1515 (1963).
[5] M. E. DAXENBICHLER, C. H. VANETTEN, F. S. BROWN and Q. JONES, *Agricult. Food Chem.* **12**, 127 (1964).
[6] A. KJÆR and A. SCHUSTER, *Phytochem.* **7**, 1663 (1968).
[7] A. KJÆR, A. SCHUSTER and R. J. PARK, *Phytochem.* **10**, 455 (1971).
[8] M. G. ETTLINGER, personal communication.

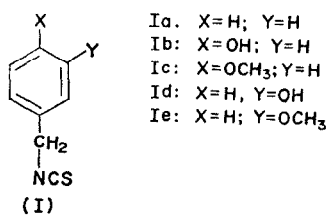
of *L. sordidum*, a biennial indigeneous to Mexico and West Texas, collected in the wild* and generously placed at our disposal as dried material by Dr. M. Ettlinger who also kindly confirmed the botanical authenticity.

We describe the isolation, identification, and characterization of the new glucosinolate.

RESULTS AND DISCUSSION

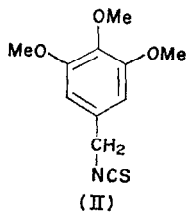
According to paper chromatography in two solvent systems, a 70% methanol extract of dried pods and seeds of *L. sordidum* contained one major glucosinolate, migrating significantly slower than benzylglucosinolate (cf. Experimental); trace amounts of a second, more hydrophilic glucosinolate were barely noticeable. A qualitatively similar picture was obtained when extracts of the remaining parts of the plants, mainly stems, were analysed.

The glucosinolate fraction from 50 g of seeds and pods was isolated as the crude potassium salt by ion exchange technique. A portion of the salt was converted into the nicely crystalline *potassium glucosinolate tetraacetate monohydrate*, $C_{25}H_{32}O_{16}NS_2K$, H_2O, characterized by analysis, m.p., IR spectrum, and optical rotation. Hydrolysis at 60° with conc. HCl of a small aliquot of the salt resulted in the production, *inter alia*, of glucose and hydroxylamine, both identified by appropriate chromatographic methods. These fragments, combined with the observed production of sulphate ions on enzymic hydrolysis, are considered valid proof for the *L. sordidum* glucoside being of the general glucosinolate type (see Ref. 9).

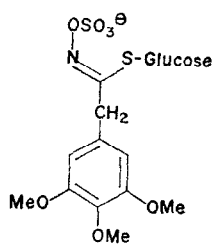


(I)

Ia. X=H; Y=H
Ib: X=OH; Y=H
Ic: X=OCH_3; Y=H
Id: X=H, Y=OH
Ie: X=H; Y=OCH_3



(II)



(III)

About half of the non-purified potassium glucosinolate was subjected to enzymic hydrolysis in a citrate buffer (pH 6·5) with a cell-free, crude myrosinase preparation[10] and a trace of ascorbic acid. Solvent extraction of the mixture, followed by short-path distillation of the residue, afforded a low-melting, crystalline product identified as 3,4,5-trimethoxybenzyl isothiocyanate (II) by critical comparison (GLC, mixed m.p., IR and mass spectra) with an authentic specimen of (II), synthesized by standard methods as described in a forthcoming publication.[11] Again, the thiourea, produced from the naturally derived isothiocyanate on reaction with ammonia, proved indistinguishable from a synthetic specimen of 1-(3,4,5-trimethoxybenzyl)-thiourea.[11] Hence, in conclusion, *L. sordidum* contains *3,4,5-trimethoxybenzylglucosinolate* (III), not previously encountered in Nature.

* The collection (*Powell 1552*, 19 August 1967) was made in Madera Canyon, Jeff Davis County, Texas, by Dr. A. Michael Powell, Biological Department, Sul Ross State College, Alpine, Texas. A voucher is deposited in the College herbarium (SRSC).

[9] M. G. ETTLINGER and A. J. LUNDEEN, *J. Am. Chem. Soc.* **78**, 4172 (1956).
[10] C. NEUBERG and J. WAGNER. *Biochem. Z.* **174**, 457 (1926).
[11] E. BACH and A. KJÆR, *Acta Chem. Scand.* **25** (1971) (in press)

The finding of the aromatic 3,4,5-trimethoxy pattern, so common in natural products, within the class of natural glucosinolates, already encompassing representatives with aromatic oxygen-substitution in 3-, 4-, and 3,4[12]-positions can hardly be surprising, neither from analogy nor from a biosynthetic viewpoint. It may be worth remembering here that sinapine, containing sinapic acid with the syringyl pattern (3,5-dimethoxy-4-hydroxy) is a common, but far from general or family-specific constituent of crucifers. The natural distribution of 3,4,5-trimethoxybenzylglucosinolate, thus far observed with certainty only in *L. hyssopifolium*[7] and *L. sordidum*, is unknown; it seems unlikely, however, that restriction in natural occurrence should parallel other obvious characters.

EXPERIMENTAL

Paper Chromatographic Analysis

A 70% methanol extract of disintegrated, dry seeds and pods of *L. sordidum* was chromatographed descendingly, on Schleicher & Schüll paper No. 2043b, in (i) butanol–ethanol–water (4:1:4) and (ii) butanol–pyridine–water (6:4:3), with benzylglucosinolate serving as a reference compound (R_B 1·0). Spray reagent: ammoniacal $AgNO_3$. A strong glucosinolate spot, possessing R_B-values of 0·75 and *ca.* 0·9 in solvents (i) and (ii), respectively, was observed. A faint trace of an additional glucosinolate, not observable on chromatograms run in solvent (ii), possessed the R_B-value 0·15 in solvent (i). A qualitatively similar picture was obtained when extracts of the remaining parts of the dried, whole plants were chromatographed.

Isolation and Hydrolysis of Glucosinolate

50 g of ground, dry seeds and pods of *L. sordidum* were extracted with three 400-ml portions of hot 70% methanol. The combined extracts were freed of methanol by evaporation *in vacuo*, and the aqueous solution was filtered through Celite. The filtrate, diluted to 1 l. with water, was passed through a column of 75 ml of Amberlite IR-4B ion exchange resin on Cl^--form. The column was rinsed with water, and the glucosinolate was eluted with 5% K_2SO_4, 100 ml-fractions being collected. The fractions Nos. 1–16 were combined and evaporated to dryness. The residue, containing large amounts of inorganic salt, was extracted with 85% EtOH; the extract was concentrated to dryness. A new extraction with hot 85% EtOH gave (i) a hot filtrate and (ii) a crystalline residue.

(i) On cooling, the hot filtrate deposited a crystalline salt (260 mg); the mother liquor on concentration afforded an additional crop (123 mg) of a hygroscopic salt which was employed for acetylation (see below).

An aliquot of the salt was kept for 2 hr at 60° in conc. HCl (6 ml); after cooling, the solution was extracted three times with $CHCl_3$, and the aqueous phase was concentrated to dryness and redissolved in a minimum amount of water. The presence of glucose in the solution was established by TLC (CH_3COONa-buffered Kieselguhr G; upper phase of the system ethyl acetate: 2-propanol (65:35)-water 2–1; spray reagent anisaldehyde) along with authentic glucose. The presence of hydroxylamine in the solution was established by paper chromatographic comparison with authentic hydroxylamine (Whatman paper No. 4; 70% MeOH–6 N HCl (7–3); spray reagent: picryl chloride). Another aliquot of the salt (20 mg) was subjected to enzymic hydrolysis with a sulphate-free myrosinase preparation. Addition of barium ions to the chloroform-extracted aqueous phase served to establish the liberation of sulphate ions.

Preparation of Glucosinolate Tetraacetate

The above hygroscopic salt (115 mg) was treated with anhydrous pyridine (1 ml) and Ac_2O (1 ml) at room temp. Crystals started to deposit after 1 hr. Next morning, the mixture was concentrated to dryness, and the residue was recrystallized three times from methanol to give an analytical specimen of *potassium glucosinolate tetraacetate (monohydrate)* (20 mg), m.p. 190–195° (dec.) $[\alpha]_{D}^{28}$ −11° (*ca.* 0·6; H_2O). (Found: C, 41·29; H, 4·70; N, 1·95; S, 8·31; H_2O, 2·82 (Karl Fischer). $C_{25}H_{32}O_{16}NS_2K$, H_2O required: C, 41·49; H, 4·73; N, 1·94; S, 8·86; H_2O, 2·49%.

Enzymic Hydrolysis and Isothiocyanate Characterization

The crystalline residue ((ii) above) was dissolved in a citrate-buffer (pH 6·5); a cell-free myrosinase preparation[10] (0·5 ml) and a trace of ascorbic acid were added, and the solution was kept at ambient temp. for 2 hr. The solution was extracted with $CHCl_3$, and the dried extract was evaporated to dryness. The residue was distilled onto a 'cold finger' (bath 70–80°; 0·01 mm) where colourless needles deposited. After 'redistillation', the crystals melted at 34–35°, undepressed on admixture with synthetic

[12] M. G. ETTLINGER, A. KJÆR, C. P. THOMPSON and M. WAGNIÈRES, *Acta Chem. Scand.* **20**, 1778 (1966).

3,4,5-trimethoxybenzyl isothiocyanate[11] (m.p. 36°). Identical retention times on vapour phase chromatography (at 175°), and coinciding IR spectra (film between KBr discs) and mass spectra further served to establish the identity of the naturally derived and synthetic isothiocyanate.

On brief treatment with methanolic ammonia, the naturally derived isothiocyanate afforded a crystalline thiourea which, after recrystallization first from water and then from methanol, had m.p. 194° (uncorr.), alone or in admixture with a synthetic specimen of 1-(3,4,5-trimethoxybenzyl)-thiourea[11] (m.p. 193–194° (uncorr.)). Again, IR and UV spectra of the two samples were identical, and they were indistinguishable on paper chromatography in four solvents.

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