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GLUCOSINOLATES IN *LEPIDIUM BONARIENSE* L.*

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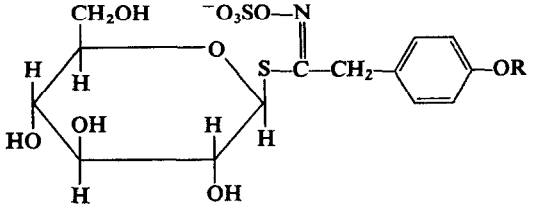
Abstract—The major glucosinolate in seeds of the South American crucifer *Lepidium bonariense* L. is shown to be the *p*-hydroxybenzylglucosinolate (glucosinalbate) ion (Ia), isolated as the tetramethylammonium salt and characterized as a potassium salt of the corresponding pentaacetate. The second, and minor, glucosinolate undergoes enzymatic hydrolysis with the production of *p*-methoxybenzyl isothiocyanate, typically formed from *p*-methoxybenzylglucosinolate (glucoaubrietin) (Ib). The seeds also contain sinapine, isolated as the thiocyanate. These results are at variance with a recent literature report on the glucosinolates in *L. bonariense*.

INTRODUCTION

A RECENT communication by Park,[1] dealing with the occurrence of mustard oil glucosides (glucosinolates) in the seed-bearing portions of three Queensland crucifers, including "*Lepidium bonariense* (L.)",† contains conclusions which are in obvious conflict with results recently obtained in our laboratory from studies of seeds of *L. bonariense* L. We present our data here.

RESULTS

Paper chromatographic analysis of the glucosinolates in a methanolic extract of seeds of *Lepidium bonariense* L.‡ disclosed the presence of a major and a minor component, possessing



(Ia) R=H
(Ib) R=CH_3

* Part LVIII of a series of papers on isothiocyanates and their natural progenitors. Part LVII: *Phytochem.* **7**, 131 (1968).

† This (L.)-nomenclature leaves open the question as to the identity of the plant material as *L. bonariense* L., indigenous to the south-eastern states of South America (cf. Hitchcock[2]).

‡ The seed employed in the present investigation was propagated during the summer of 1967 in the Botanic Garden of the University of Copenhagen from seed collected in the wild by Hawkes, Hjerting, and Rahn, in February 1966, near Quebrada de las Pavas (2600 m), in the province Tucumán of Argentina. A herbarium sheet is deposited in the Botanic Museum of the University of Copenhagen under the file No. 3577 (Hawkes, Hjerting, Rahn).

[1] R. J. PARK, *Australian J. Chem.* **20**, 2799 (1967).
[2] L. C. HITCHCOCK, *Lilloa* **11**, 75 (1945).

the same R_f values as the known *p*-hydroxybenzyl- (Ia) and *p*-methoxybenzylglucosinolate (Ib) ions, respectively. On spraying with diazotized sulphanilic acid, only the major constituent exhibited, like authentic (Ia), a brick-red colour.

Isolation of the glucosinolate fraction (*ca.* 5 g) from a 100 g seed sample was achieved by defatting, extraction with 70 per cent methanol, absorption on an anion exchange resin, and elution with potassium sulphate. Part of the crude fraction was subjected to acetylation yielding, after repeated recrystallizations, a homogeneous specimen of *potassium penta-acetylglucosinolate sesquihydrate* (the fully acetylated derivative of (Ia)). Its structure appeared from the analytical data, the u.v., i.r., and NMR spectra, as well as from the recognition of the expected products, *p*-hydroxyphenylacetic acid, hydroxylamine, and glucose, formed upon acid hydrolysis of the non-acetylated glucoside fraction whence (Ia) derives.[3] Again, the latter typically affords thiocyanate ion subsequent to enzymic hydrolysis and base treatment of the initially expected *p*-hydroxybenzyl isothiocyanate.

A second seed portion was utilized for isolating the previously described tetramethylammonium *p*-hydroxybenzylglucosinolate (glucosinalbate) (Ia)[3,4] by absorption on anionotropic alumina followed by elution with tetramethylammonium hydroxide. The purified salt proved identical, by i.r. spectra, with an authentic specimen. From the aqueous solution, passing the alumina column, sinapine was isolated as the sparingly soluble thiocyanate in amounts of about 0·2 g per 100 g of seed.

The identity of the minor glucosinolate in seed of *L. bonariense* was established by enzymatic hydrolysis of the glucosinolates, followed by conversion of the non-phenolic isothiocyanate into a benzylthiourea derivative, which, upon thin-layer chromatography in two solvent systems, proved indistinguishable from authentic 1-benzyl-3-(*p*-methoxybenzyl)-thiourea,[5a] almost certainly deriving from (Ib) (glucoaubrietin).[5a,b]

DISCUSSION

The glucosinolate (Ia), probably best known as the classical salt sinalbin containing the quaternary base sinapine as a cation, is of relatively limited known distribution (cf. Refs. 6, 7). However, its appearance, as here, in a typical *Lepidium* species is not entirely surprising in view of the previously recorded occurrence of (Ia) within the genus, e.g. in *L. campestre* R. Br.[6]

Park[1] recently concluded, on the basis of paper chromatographic analysis of seed-bearing plants of "*L. bonariense* (L.)", collected in Queensland, that the two observed glucosinolates were benzyl- (glucotropaeolin) and, supposedly, allyl-glucosinolate (sinigrin). The R_B values reported (1·0 for the major, and 0·30 for the minor component) in the solvent system butanol:ethanol:water (4:1:4), do not seem to be reconcilable with our R_B values in the same system (0·7 for the major, 1·03 for the minor glucosinolate). Hence, we can offer no other explanation for the discrepancy than an erroneous botanical diagnosis of the Australian

[3] M. G. ETTLINGER and A. J. LUNDEEN, *J. Am. Chem. Soc.* **78**, 4172 (1956).
[4] A. J. LUNDEEN, *The Structure of the Mustard Oil Glucosides and Synthesis of the Glucotropaeolate Ion.* Thesis, p. 29, Rice University, Houston, Texas (1957).
[5] (*a*) A. KJÆR, R. GMELIN and R. BOE JENSEN, *Acta Chem. Scand.* **10**, 26 (1956); (*b*) R. GMELIN, A. KJÆR and A. SCHUSTER, *Acta Chem. Scand.* **22**, 713 (1968).
[6] A. KJÆR, *Fortschr. Chem. Org. Naturstoffe* **18**, 122 (1960).
[7] M. ETTLINGER and A. KJÆR, in *Recent Advances in Phytochemistry* (edited by T. J. MABRY, R. E. ALSTON and V. C. RUNECKLES), p. 59, Appleton-Century-Crofts, New York (1968).

material studied by Park. It may be significant here that the Queensland flora lists a number of *Lepidium* species, possibly with affinity to *L. ruderale* L., a reported source of benzyl isothiocyanate and hence presumably of benzylglucosinolate ion.[6]

We thus consider it established that seeds of *L. bonariense* L. contain the ions (Ia) and (Ib).

EXPERIMENTAL

Melting points are uncorrected and determined in capillary tubes in an Anschütz-Hershberg apparatus. Analytical samples are dried in a vacuum over $CaCl_2$ at room temperature. Rotations are measured photoelectrically in a 1 dm tube.

Paper Chromatographic Analysis

A 70 per cent methanol extract of seeds of *Lepidium bonariense* L. was chromatographed, without further purification, in the two solvent systems: (i) butanol:ethanol:water (4:1:4) and (ii) butanol:pyridine:water (6:4:3), with benzylglucosinolate (glucotropaeolin) serving as a reference compound (R_B=1·0). Spray reagent: ammoniacal silver nitrate.

The major glucosinolate appeared as a spot with R_B 0·69 in the solvent system (i); R_B 0·93 in solvent system (ii), indistinguishable from authentic *p*-hydroxybenzylglucosinolate (glucosinalbate) (Ia). Like the latter, the major *L. bonariense* spot produced a brick-red colour on spraying with a solution of diazotized sulphanilic acid.

The minor glucosinolate possessed the R_B values: 1·02 in system (i), and 0·98 in system (ii), and gave no colour with the diazo reagent.

Potassium p-*Hydroxybenzylglucosinolate Pentaacetate Sesquihydrate*

A 100 g seed portion of *L. bonariense* L. was finely ground and defatted by extraction with four 250-ml portions of light petroleum. The dry seed powder (85 g) was extracted with three 200-ml portions of 70 per cent methanol. The solvent was removed *in vacuo* and the residue dissolved in 1·5 l. of water. After filtration through Hyflo Supercel the solution was slowly passed through an Amberlite i.r. 4B column (75 ml resin on the Cl^- form). The absorbed glucosinolates were eluted from the column by means of a 5 per cent K_2SO_4 solution, and 100-ml fractions were collected. Fractions 2–26 were pooled, evaporated to dryness *in vacuo*, and the residue treated with hot 85 per cent ethanol, leaving inorganic salt undissolved. After cooling, and filtration from additional inorganic matter, a solution was obtained which, after evaporation of the solvents, gave a partly crystalline residue (5·0 g) mainly consisting of the potassium glucosinolates.

Part of this material (1·24 g) was acetylated with acetic anhydride (7 ml) in pyridine (7 ml) by standing at room temperature for 1½ hr, and overnight in the ice box. After removal of volatile products *in vacuo*, the residue was recrystallized from methanol. Colourless needles separated (760 mg). An analytical specimen of potassium *p*-hydroxybenzylglucosinolate pentaacetate sesquihydrate was produced by two additional recrystallizations from methanol; m.p. 166–167°; $[\alpha]_D^{28}$ −11·2° (*c* 0·6, H_2O); $\lambda_{max}^{H_2O}$ 220 nm (ε 14,000); the i.r.-spectrum (in KBr) exhibited strong bands at 3470, 1750, 1500, 1430, 1360, 1240 (br.), 1060, 920, and 790 cm^{-1}. The NMR spectrum (in D_2O) showed the expected signals corresponding to five CH_3COO groups (δ2·0–2·1), two benzylic protons (singlet, δ2·33), seven sugar protons (δ3·6–5·2), and four aromatic protons (δ7·1–7·5). (Found: C, 41·20; H, 4·60; N, 2·08; S, 8·85; H_2O (Karl Fischer), 3·93. $C_{24}H_{28}O_{15}NS_2K$, 1·5 H_2O required: C, 41·13; H, 4·46; N, 2·00; S, 9·15; H_2O 3·86 per cent.) The corresponding tetramethylammonium salt (anhydrous) has previously been described.[4] An analysed specimen had m.p. 165–169°. For a less rigorously dried sample the values λ_{max}^{MeOH} 221 nm (ε 14,900) and $[\alpha]_D^{30}$ −8° (*c* 1·4, H_2O) were reported.[4]

Acid Hydrolysis of the Glucosinolate Fraction

Part of the glucosinolate fraction (1 g) obtained from the ion exchange resin and containing, according to paper chromatography, virtually homogeneous *p*-hydroxybenzylglucosinolate, was heated for 4 hr at 60° with 20 per cent HCl (cf. Ref. 3). After cooling and ether extraction, a crystalline organic acid was obtained (147 mg), which, after recrystallization from benzene (78 mg), had the m.p. 147–148° and proved identical (by i.r. and u.v. spectra as well as mixed m.p. determination) with authentic *p*-hydroxyphenylacetic acid.

A similar hydrolysis experiment (2 hr hydrolysis at 60° with conc. HCl) was conducted in order to prove the presence, by paper and thin-layer chromatography, of glucose in the hydrolysis mixture.

Formation of Thiocyanate on Enzymic Hydrolysis

A small portion (50 mg) of the crude glucosinolate was subjected to enzymic hydrolysis with myrosinase in the usual way. The combined chloroform and ether extracts from the reaction mixture were treated with 2 N NaOH whereby thiocyanate ions were liberated as demonstrated by the red colour, produced upon addition of ferric ions.

Tetramethylammonium Glucosinolate

An aqueous solution of glucosinolates, prepared from seeds of *L. bonariense* (50 g) by defatting, methanol extraction, and evaporation, as described above, was passed through a column containing anionotropic alumina (Woelm) (85 g). The column was washed with water and then eluted with a 5 per cent solution of tetramethylammonium hydroxide. The eluate (200 ml) was evaporated to dryness, and the residue dissolved in 96 per cent ethanol. On cooling, and addition of absolute ethanol, the crystalline glucoside salt separated (1·32 g). After five recrystallizations, performed in a similar fashion, a specimen of tetramethylammonium glucosinalbate was obtained (343 mg) which proved homogeneous on paper chromatography. M.p. 183–185° (dec., bath preheated to 175°); $[\alpha]_D^{28}$ −20·0° (*c* 0·79, H_2O); λ_{max}^{MeOH} 228 nm (17,000), 278·5 nm (2,700). Previously described specimen[4]: m.p. 187–188° (dec., preheated to 180°); $[\alpha]_D^{35}$ −18·86° (*c* 3·3, H_2O); λ_{max}^{MeOH} 228 (ε 14,850), 278 nm (ε 1600). The i.r. spectrum proved identical with that of an authentic sample of tetramethylammonium glucosinalbate isolated from *Sinapis alba* L. seed.

The aqueous column washings, before elution began, were concentrated to a small volume. A solution of ammonium thiocyanate was added resulting in the separation of sinapine thiocyanate which was recrystallized twice from water (85 mg) and identified by comparison with an authentic specimen.

Identity of Minor Glucosinolate

A 25-g seed portion of *L. bonariense* was ground, defatted with light petroleum, and extracted with 70 per cent methanol as described above. The residue was extracted with ether and dissolved in 25 ml of citrate buffer (pH 6·5) to which a trace of ascorbic acid and 1 ml of a myrosinase preparation were added. After standing for 12 hr at room temperature the mixture was extracted with three 25-ml portions of ether. The extract was washed with a small volume of 1 N NaOH, then with water, and dried over Na_2SO_4. After addition of benzylamine (0·3 ml), the reaction mixture was left overnight at room temperature. Excess benzylamine was removed by washing with 2 per cent HCl, and an alcoholic solution of the benzylthiourea was subjected to thin-layer chromatography on silica gel in the solvent systems $CHCl_3$:MeOH (95:5) and EtOAc:EtOH (95:5); the spot, appearing with R_f values of 0·63 and 0·77, respectively, was observed in u.v. light, and was indistinguishable from that obtained from 1-benzyl-3-(*p*-methoxybenzyl)-thiourea [5a] in the two solvent systems.

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