Roots of *Liatris pycnostachya* Mich. were extracted with a 1:1 mixture of petroleum–ether and the extract was concentrated and purified by chromatography over alumina with elution by pentane–ether (5:1) to give a major component, m.p. 92°, $C_9H_8O_2S$. Varusio spectra (see Experimental) were all consistent with the compound being 2-acetyl-3-hydroxy-5(prop-1-ynyl) thiophen (II), previously isolated[6] from *Artemisia arborescens* L., (sub-tribe Anthemideae).

I II

Qualitative examination by TLC of extracts obtained in the same way from *L. spicata* (L). Willd. and *L. scariosa* (L). Willd. indicated that the same compound was also present in these species.

EXPERIMENTAL

The NMR spectrum was obtained with a Varian HA 100 spectrometer (Imperial Chemical Industries Limited, Dyestuffs Division). The mass spectrum was determined on an A.E.I. MS9 mass spectrometer at 70 e.V., source temp. 220°.

Extraction of Plant Material

Air dried roots of *Liatris pycnostachya* Michx. (600 g) were quickly chopped by hand and allowed to stand in ether at room temp. for 2 weeks. The extract was evaporated under reduced pressure to give a brown oil (3·4 g) which was purified by chromatography over deactivated alumina (300 g). Elution with pentane–ether (5:1) gave 2-acetyl-3-hydroxy-5(prop-1-ynyl)thiophen (II), (87 mg) as colourless needles from pentane, m.p. 92–94° (uncorr.) (lit.[6] 100·5°). (Found: C, 59·8; H, 4·9; S, 17·5. Calc: $C_9H_8O_2S$: C, 60·0; H, 4·5; S, 17·8%); λ_{max} (ether) 301, 327 nm (log ϵ, 4·24, 4·11); ν_{max} (KBr) 2225 (R—C≡C—R), 1626 (conjugated OH-bonded >CO) and 838 cm^{-1} (2,5-disubstituted thiophen);[7] τ(CS_2), 7·96 (3, singlet, CH_3—CO—) 7·75 (3, singlet, CH_3—C≡C—), 3·53 (1, singlet, H in 4-position of thiophen ring), —1·24 (1, broad singlet, H-bonded—OH); *m/e* 180·0237 ± 0·0004, calc. $C_9H_8O_2S$, 180·0245.

Qualitative examination of the other *Liatris* sp. was carried out using TLC on silica-gel G (Merck) developed with benzene–$CHCl_3$ (1:1) (by D. M. Jones).

Acknowledgements—We are grateful to British Petroleum Limited, for a studentship (R.E.A.) and Professor C. H. Hassall for laboratory facilities.

[6] F. BOHLMANN, K-M. KLEINE and H. BORNOWSKI, *Chem. Ber.* **95**, 2934 (1962).
[7] R. F. CURTIS and G. T. PHILLIPS, *Tetrahedron* 4419 (1967).

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CRUCIFERAE

Dedicated to Prof. K. Mothes on the occasion of his seventieth birthday

GLUCOSINOLATES IN *LEPIDIUM* SPECIES FROM QUEENSLAND

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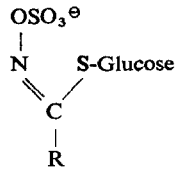
Abstract—The sole glucosinolate in the seed-bearing portion of *Lepidium bonariense* L., collected in Queensland, is shown to be *p*-methoxybenzylglucosinolate (IV). Seed material of *L. hyssopifolium* Desv., collected in Queensland, contains solely 3,4,5-trimethoxybenzylglucosinolate (V). A previous report on the presence of benzylglucosinolate in *L. hyssopifolium* is discussed.

INTRODUCTION

IN 1967, ONE of the authors (R.J.P.) reported the presence of benzylglucosinolate (I), along with a minor amount of an additional glucosinolate tentatively identified as the allyl derivative (II), in seed-bearing portions of plants collected in the wild in south-east Queensland and identified as "*Lepidium bonariense* (L.)."[1] This botanical diagnosis was questioned when it was established that *p*-hydroxybenzyl-(III) is the major, and *p*-methoxybenzyl-glucosinolate (IV) the minor constituent of seeds of authentic *Lepidium bonariense* L. of South American provenance.[2]



I, R: $C_6H_5CH_2$
II, R: $CH_2{=}CH{-}CH_2$
III, R: (*p*)-$OHC_6H_4CH_2$
IV, R: (*p*)-$CH_3OC_6H_4CH_2$
V, R: 3, 4, 5-$(CH_3O)_3C_6H_2CH_2$

Again, it was concluded that the seed-bearing portions of the species *Lepidium hyssopifolium* Desv., collected in the same area, contain solely benzylglucosinolate (I).[1] A reinvestigation has led to a different result which we now present.

RESULTS AND DISCUSSION

Two years ago a small amount of seed material was collected by one of us (R.J.P.) within 100 yards of the site of the original "*L. bonariense* (L.)". Chromatographic analysis failed to confirm the presence of benzylglucosinolate (I) in this material, but served to establish its content of another, unidentified glucosinolate. When a larger amount of seed-bearing plants, identified by Mr. S. Everist, the Queensland Government Botanist, as *L. bonariense* L.* were collected in the original area, it was possible to establish its content of *p*-methoxybenzylglucosinolate (IV) as the sole glucosinolate. The original finding[1] of benzylglucosinolate (I) in "*L. bonariense* (L.)" was solely based on paper chromatographic evidence and may have been incorrect due to the fact that the glucosinolates (I) and (IV) are virtually indistinguishable in most solvent systems. The *p*-methoxybenzyl-derivative (IV) has formerly been encountered within the genus *Lepidium* as a minor constituent of *L. bonariense* L. of South American origin,[2] but was first recorded as a typical glucosinolate in the crucifer genus *Aubrietia*.[3] *L. bonariense* L., indigeneous to the south-eastern states of South America,[2] was accidentally introduced to Queensland about two hundred years ago,[4] and may have been isolated long enough for a mutation to appear, representing an infra-specific variant or "chemical race" of the parent, native species.

Recollection of the material, originally described as *L. hyssopifolium* Desv. and reported to contain benzylglucosinolate (I),[1] was difficult and uncertain due to housing development in the original area. A collection, made within 300 m of the original site, and identified by Mr. Everist as *L. hyssopifolium*,*,[4] has now been reinvestigated. Seed extracts, containing

* Herbarium voucher specimen deposited in the Queensland Herbarium.

[1] R. J. PARK, *Australian J. Chem.* **20**, 2799 (1967).
[2] A. KJÆR and A. SCHUSTER, *Phytochem.* **7**, 1663 (1968).
[3] A. KJÆR, R. GMELIN and R. BOE JENSEN, *Acta Chem. Scand.* **10**, 26 (1956).
[4] S. EVERIST, private communication to R. J. PARK.

only one glucosinolate according to chromatographic analysis, were subjected to enzymic hydrolysis. The resulting isothiocyanate was identified by mass spectrometry as well as by comparison with a synthetic specimen[5] as 3,4,5-trimethoxybenzyl isothiocyanate, almost certainly deriving from the glucosinolate (V). Conversion, by treatment with ammonia, into the corresponding 1-(3,4,5-trimethoxybenzyl)-thiourea and comparison with an authentic specimen[5] served to confirm the identity. The glucosinolate (V) has previously been identified as a constituent of seeds of *L. sordidum* A. Gray.[6] The absence of observable amounts of 1-benzylthiourea in the ammonia-treated, enzymically hydrolyzed seed extract renders the botanical identity of the original, benzylglucosinolate-bearing species obscure. Attempts are now being made to ascertain whether another *Lepidium* species, containing benzylglucosinolate and growing on the same locality, might have been misidentified as *L. hyssopifolium*.

EXPERIMENTAL

Paper Chromatographic Analysis

70% methanol extracts of ground seed material were chromatographed, without further purification, in the two solvent systems: (i) butanol–water (4:1:4) and (ii) butanol–pyridine–water (6:4:3), with benzylglucosinolate (I) serving as a reference compound (R_B 1·0). Spray reagent: ammoniacal $AgNO_3$.

A seed extract, (A), prepared from plants identified as *L. bonariense* L.[4] and collected on the same locality as the plants previously described as "*L. bonariense* (L.)",[1] gave a single spot, possessing R_B values of 1·02 and 0·98 in the solvents (i) and (ii), respectively.

A seed extract, (B), of plants, identified as *L. hyssopifolium* Desv.[4] and collected about 300 m from the locality of the plants previously collected under the same name,[1] gave a single spot with R_B-values of 0·79 and 0·93 in solvents (i) and (ii), respectively.

Enzymatic Hydrolysis

Extract (A) was freed of methanol and subjected to enzymic hydrolysis with a myrosinase preparation at pH 6·9. The resulting isothiocyanate was extracted with $CHCl_3$; on TLC chromatography in $CHCl_3$–5% MeOH ($AgNO_3$ spray), only one spot was observed. When the $CHCl_3$ solution, obtained from 15 g of seeds, was concentrated and treated with methanolic ammonia, a crystalline thiourea was produced. After recrystallization from water, the thiourea had m.p. 134°, alone or in admixture with an authentic specimen of 1-(4-methoxybenzyl)-thiourea.[3] Coinciding i.r. spectra (in KBr) further served to secure the identity.

Extract (B), prepared from 2 g of seeds, was similarly subjected to enzymic hydrolysis, and the resulting isothiocyanate was extracted with $CHCl_3$. Mass spectrometry (70 eV, solid inlet, 90°) disclosed a strong molecular ion at *m/e* 239, and a fragment at *m/e* 181 representing the base peak. The MS was strongly indicative of a trimethoxybenzyl isothiocyanate and was, in fact, identical with that obtained from a synthetic specimen of 3,4,5-trimethoxybenzyl isothiocyanate.[5] The i.r. spectra (film between KBr plates) of the synthetic[5] and naturally derived isothiocyanate were coinciding. A thiourea-solution, produced on treatment of the isothiocyanate solution with methanolic ammonia, was co-chromatographed with a solution of authentic 1-(3,4,5-trimethoxybenzyl)-thiourea[5] in various solvent systems, and identity thus further confirmed.

Acknowledgements—The authors are much indebted to Mr. S. Everist, the Queensland Herbarium, Brisbane, Australia, for the botanical identifications and to Dr. M. G. Ettlinger for seed material *L. sordidum*.

[5] A. KJÆR and M. WAGNIÈRES, unpublished results.
[6] E. BACH and A. KJÆR, unpublished results.