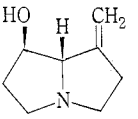
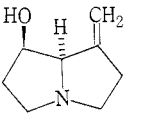
THE ALKALOIDS OF *CROTALARIA MAYPURENSIS* H. B. & K.*

By C. C. J. Culvenor,† R. S. Sawhney,†‡ and L. W. Smith†

Crotalaria maypurensis H. B. & K., a species native to Venezuela, has been suspected of causing chronic liver cirrhosis in cattle in Guyana.[1] An investigation of its alkaloids was undertaken to assist in deciding whether consumption of the plant was an aetiological factor in the disease. Dried vegetative material has been found to contain 0·18% alkaloid, of which the principal constituent is 7β-hydroxy-1-methylene-8β-pyrrolidizine (I) and most of the remainder is probably the isomeric 7β-hydroxy-1-methylene-8α-pyrrolizidine (II). These two bases occur in similar relative proportions



(I)



(II)

in *C. goreensis* Guill. & Perr.[2] which, like *C. maypurensis*, is classified in the series Digitatae, subseries Polyspermae of the genus. The mixed alkaloids from *C. goreensis*, largely (I), were found to be non-hepatotoxic on chronic administration to rats.[3] Although gas chromatography revealed the presence in *C. maypurensis* of trace amounts of an alkaloid with the properties of a macrocyclic diester, it is unlikely that the species would be hepatotoxic to grazing animals.

Experimental

Analyses were made by the Australian Microanalytical Service, Melbourne. Melting points are corrected. The solvent used in paper chromatography was the upper phase resulting from shaking n-butanol with an equal volume of 5% acetic acid. Thin-layer plates were prepared from a slurry of Merck silica gel G in 0·1N NaOH and developed in methanol. Gas chromatographic retention times were measured with a Packard 871 instrument using a 6-ft glass column of 4 mm i.d. packed with Gas Chrom P bearing 4% SE30, and operated at 120° with a flow rate of 60 ml N_2/min.

Alkaloid Assay

Dried vegetative material (288 g), assayed by the method of Culvenor *et al.*,[4] was found to contain 0·14% tertiary base and 0·04% *N*-oxide. The chromatographic behaviour of different

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† Division of Applied Chemistry, CSIRO Chemical Research Laboratories, P.O. Box 4331, Melbourne, Vic. 3001.

‡ Permanent address: Regional Research Laboratory, C.S.I.R., Jammu, India.

[1] Ford, E. J. H., personal communication.

[2] Culvenor, C. C. J., and Smith, L. W., *Aust. J. Chem.*, 1961, **14**, 284.

[3] Bull, L. B., Culvenor, C. C. J., and Dick, A. T., "The Pyrrolizidine Alkaloids. Their Chemistry, Pathogenesis and Other Biological Properties." (North Holland Publishing Co.: Amsterdam 1968.)

[4] Culvenor, C. C. J., O'Donovan, G. M., and Smith, L. W., *Aust. J. Chem.*, 1967, **20**, 757.

fractions is compared with that of authentic samples of known alkaloids in Table 1. The two main constituents have retention times identical with those of 7β-hydroxy-1-methylene-8β-pyrrolizidine (I) (5·0 min) and its 8α-isomer (II) (3·6 min). The identity of the base, R_T 5·0 min, being fully established as (I) (below), it is reasonably certain that the base, R_T 3·6 min, is (II), although isolation was not possible. The similarity in behaviour of the constituent, R_T 17 min, in the light petroleum extract is not close enough to that of usaramine, R_T 18 min, to indicate identity, but merely suggests that the two bases are of similar type and substitution.

TABLE 1

CHROMATOGRAPHIC DATA

Spots present in major amount indicated in *italics*

Fraction	R_F (t.l.c.)	R_F (paper)	R_T (min)
Light petroleum, ex. NH_3	0·42	0·34	17
Chloroform, ex. NH_3	0·14	*0·24*, 0·03	1·75, 3·6, *5·0*
Chloroform, ex. NaOH	0·07, *0·14*	0·24, 0·03	1·75, 3·6, *5·0*
7β-Hydroxy-1-methylene-8β-pyrrolizidine	0·14	0·24	5·0
7β-Hydroxy-1-methylene-8α-pyrrolizidine	0·07	0·24	3·6
Usaramine	0·36	0·33	18

Isolation of 7β-Hydroxy-1-methylene-8β-pyrrolizidine

Chloroform fractions were taken up in 0·5N sulphuric acid and the solution was basified with sodium bicarbonate and extracted with chloroform. The residual aqueous solution was further basified with NaOH and extracted with chloroform to give the major base, R_F (paper) 0·24. The picrate had $[\alpha]_D^{18}$ +21·87° (*c*, 1·6 in acetone), m.p. 172–173°, undepressed on admixture with picrate of 7β-hydroxy-1-methylene-8β-pyrrolizidine[2] (Found: C, 45·1; H, 4·4; N, 15·3. Calc. for $C_8H_{13}ON(C_6H_3O_7N_3)$: C, 45·6; H, 4·4; N, 15·2%). Base recovered from picrate by passage of a solution in aqueous acetone through a column of CG400 resin had $[\alpha]_D^{18}$ +42·8° (*c*, 1·82 in ethanol). 7β-Hydroxy-1-methylene-8β-pyrrolizidine is reported to have $[\alpha]_D^{18}$ +36·1° (*c*, 1·39 in ethanol).[2] The i.r. and n.m.r. spectra of the base and the i.r. spectrum of its picrate were identical with the corresponding spectra of authentic base (I) and its picrate.

Acknowledgment

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