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SHORT COMMUNICATION

PYRROLIZIDINE ALKALOIDS. THE CO-OCCURRENCE OF MONOCROTALINE AND TRICHODESMINE IN *CROTALARIA RECTA* STEUD. EX A. RICH.

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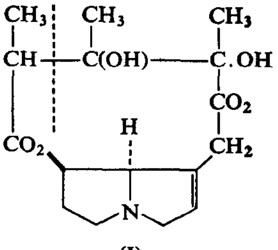
Abstract—*Crotalaria recta* (Leguminosae) has been shown to produce two alkaloids, monocrotaline (I) and trichodesmine (II). This finding lends support to current theories of the biogenesis of the pyrrolizidine alkaloids.

Pyrrolizidine alkaloids, which have long been known to cause liver disease in animals, may also have been implicated in human liver disease in at least one instance.[1] A number of plant species from genera known to produce pyrrolizidine alkaloids have been screened for toxicity as part of an investigation into possible causes of human liver disease in Ethiopia. One of the most toxic species examined[2] was *Crotalaria recta* Steud. ex A. Rich., the alkaloid content of which has now been investigated.

The dried aerial portions of the plant* gave on extraction 0·36 per cent of a crystalline alkaloid fraction which was shown to be a mixture of two components by thin-layer chromatography. These were designated alkaloids A and B, the minor, faster-running component being alkaloid A. After two recrystallizations from chloroform–acetone, pure crystalline alkaloid B was obtained and identified as monocrotaline (I) by direct comparison with authentic material.



(I)

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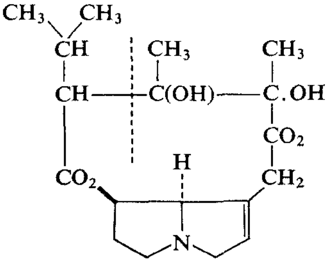
* The plant material was collected by Dr. A. Coady, at Mulu Farm, 50 miles north of Addis Ababa, by kind permission of Brigadier and Mrs. D. Sandford. We are indebted to the Keeper of the Herbarium, Royal Botanic Gardens, Kew, and his staff, for identification of the species.

[1] (*a*) R. Schoental, *Bull. World Health Organ.* **29**, 823 (1963); (*b*) F. L. Warren, *Fortschr. Chem. Org. Naturstoffe* **24**, 329 (1966).

[2] R. Schoental, private communication.

The combined mother liquors were chromatographed on acid-washed, activated alumina, whereupon partial separation of the two alkaloids was achieved and a homogeneous sample of alkaloid A obtained. This alkaloid crystallized only with difficulty but formed a nicely crystalline picrate which was used for subsequent purification.

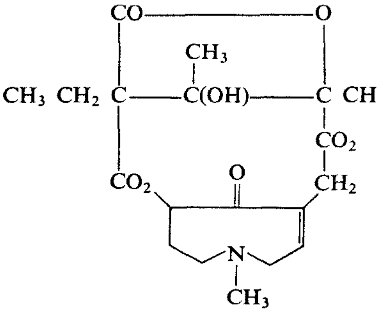
The nuclear magnetic resonance spectrum of the alkaloid, reisolated from the picrate, showed signals due to four C-methyl groups with values 8·57, 8·61 (singlets) and 8·96, 9·04 (doublets, $J = 6{\cdot}0$ c/sec). The chemical shifts and coupling constants of these peaks were almost identical to those recorded for the alkaloid trichodesmine (II).[3] The identity of alkaloid A and trichodesmine was subsequently confirmed by direct comparison with authentic material.



(II)

Further proof of the identity of alkaloid A was obtained from the mass spectrum which showed peaks due to the molecular ion at m/e 353 and a very intense peak at m/e 264, as described for trichodesmine.[3]

The co-occurrence of monocrotaline and trichodesmine is noteworthy from a biogenetic point of view since alkaloids of these two structural types have not before been reported from a single *Crotalaria* species. Previously, monocrotaline has only been found in association with alkaloids of the same structural type (crispatine, fulvine[4] and retusine[5]) or with the unique alkaloid retusamine (III).[6] The present finding lends further support to the view[7]



(III)

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[3] C. K. ATAL, R. K. SHARMA, C. C. J. CULVENOR and L. W. SMITH, *Australian J. Chem.* **19**, 2189 (1966).

[4] (*a*) R. SCHOENTAL, *Australian J. Chem.* **16** (2), 233 (1963); (*b*) C. C. J. CULVENOR and L. W. SMITH, *Australian J. Chem.* **16** (2), 239 (1963).

[5] C. C J. CULVENOR and L. W. SMITH, *Australian J. Chem.* **8**, 556 (1955).

[6] (*a*) C. C. J. CULVENOR and L. W. SMITH, *Australian J. Chem.* **10**, 464 (1957); (*b*) J. A WUNDERLICH, *Chem and Ind.* 2089 (1962).

[7] D. H. G. CROUT, M. H. BENN, H. IMASEKI and T. A. GEISSMAN, *Phytochem.* **5**, 1 (1966).

that the necic acid components of monocrotaline and trichodesmine have a common biogenesis for the five-carbon fragment to the right of the dotted line in (I) and (II) and that the remaining portions are derived from alanine and valine (or their α-keto analogues) respectively.

EXPERIMENTAL

All melting points are corrected. Kieselgel G adsorbent with the system $CHCl_3$:MeOH:NH_4OH (85:14:1) was used for TLC and alkaloids were visualized with the modified Dragendorff reagent.[8] Alkaloid mobilities are quoted as R_m values where R_m = distance travelled by alkaloid/distance travelled by monocrotaline. NMR spectra were determined at 60 MHz on a Perkin–Elmer R10 instrument. Mass spectra were run on an AEI MS9 mass spectrometer.

Isolation

The finely ground plant material (467 g) was extracted continuously with methanol in a Soxhlet apparatus for 24 hr. The extract was evaporated, the residue dissolved in $CHCl_3$ (300 ml) and the solution extracted with 2 N H_2SO_4 (150 ml). The acid solution was re-extracted with two 100-ml portions of $CHCl_3$ and stirred with Zn dust for 90 min. The solution was filtered (Keiselguhr) and the filtrate again extracted with two 100-ml portions of $CHCl_3$. The solution was then made alkaline with conc. NH_3 (80 ml) and re-extracted with four 100-ml portions of $CHCl_3$. The combined $CHCl_3$ extracts were dried (Na_2SO_4) and evaporated to give a slightly coloured, crystalline alkaloid mixture (1·70 g, 0·36 per cent).

Examination of the crude mixture by TLC showed two alkaloids to be present, a major compound with R_m 1·00 (alkaloid B) and a minor alkaloid with R_m 1·26 (alkaloid A).

Two recrystallizations from chloroform–acetone of the crude alkaloid mixture gave pure alkaloid B (785 mg), needles, m.p. 197–198·5°. There was no depression of the mixed m.p. with authentic monocrotaline (m.p. 196–198°) and the i.r. spectra (KBr disc) of the two alkaloids were identical.

The combined mother liquors were evaporated and the residue chromatographed on a column of acid-washed (50 per cent HNO_3), activated (110°, 16–24 hr) alumina (60 g). Benzene:$CHCl_3$ (1:1), benzene: $CHCl_3$ (1:4) and $CHCl_3$ failed to elute any alkaloidal material. The solvent was accordingly changed to $CHCl_3$ containing 2 per cent MeOH and five 50-ml fractions were collected. The first two fractions gave pure alkaloid A, the remaining three, a mixture of alkaloid A and monocrotaline. Subsequent elution with CHCl + 5 per cent MeOH (200 ml) and $CHCl_3$ + 10 per cent MeOH (100 ml) gave only monocrotaline.

The fractions containing alkaloid A were evaporated and the residue converted to the picrate; 73 mg yellow needles from ethanol, m.p. 228–230° (decomp.). Literature m.p. 228°.[9]

The free base, re-isolated from the picrate (40 mg), crystallized slowly from water to give prisms, m.p. 155–158° (decomp.). Literature m.p. 161°.[10] The R_f value of the alkaloid and the i.r. spectra of the alkaloid and its picrate were identical to those of authentic trichodesmine. The mass spectrum indicated M.W. 353, with a major fragmentation peak at m/e 264, as reported for trichodesmine. The NMR spectrum also gave similar peaks to those reported for trichodesmine.[3]

Acknowledgements—I am grateful to Dr. J. A. Ballantine for determining the mass spectra and to the Medical Research Council for financial support.

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[8] R. Munier and M. Macheboef, *Bull. Soc. Chim. Biol.* **33**, 846 (1951).

[9] G. Menshikow and W. Rubinstein, *Ber.* **68**, 2039 (1935).

[10] R. Adams and M. Gianturco, *J. Am. Chem. Soc.* **78**, 1922 (1956).