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PEPTIDE ALKALOIDS OF *DISCARIA LONGISPINA*

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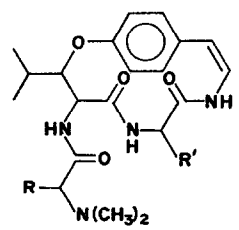
Abstract—Three peptide alkaloids have been isolated from *Discaria longispina*. They include frangulanine and the new substances discarine A (II) and B (III).

INTRODUCTION

OUR STUDY of the rhamnaceous Argentinian plant *Discaria longispina* (Hook and Arn.) Miers.[1] has been continued and the basic constituents investigated. The structure analysis of the root alkaloids forms the basis of this report.

RESULTS AND DISCUSSION

Extraction of the root of *D. longispina* and separation of the basic constituents by preparative TLC have yielded five alkaloids, three of which have been identified as frangulanine (I)[2] and the new substances discarine A (II) and discarine B (III).



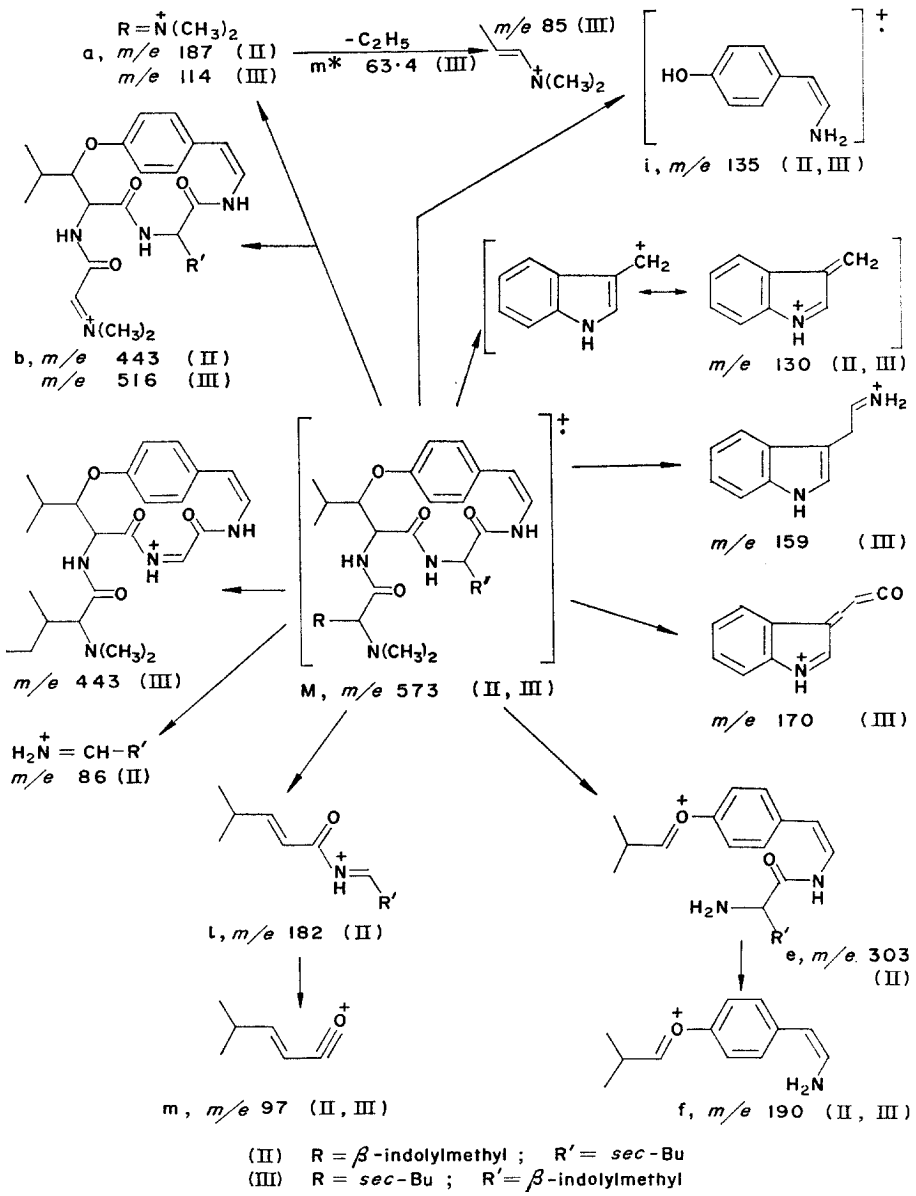
(I) R = *sec*-Bu, R′ = *i*-Bu
(II) R = β-indolylmethyl, R′ = *sec*-Bu
(III) R = *sec*-Bu, R′ = β-indolylmethyl

Discarine A, m.p. 229–231°, was shown to be a $C_{33}H_{43}N_5O_4$ compound whose IR NH and CO bands and UV maxima revealed it to possess peptide linkages and an indole chromophore, respectively. Hydrolytic degradation by acid yielded isoleucine and by base, N_b,N_b-dimethyltryptophan.[3] For comparison this compound was prepared by methylation

[1] V. M. MERKUZA, O. A. MASCARETTI, R. CROHARE and E. A. RUVEDA, *Phytochem.* **10**, 908 (1971).
[2] R. TSCHESCHE, H. LAST and H.-W. FEHLHABER, *Chem. Ber.* **100**, 3937 (1967).
[3] Since the preparation of this compound by the procedure of S. CORSANO and E. PERROTTI, *Ann. Chim.* **48**, 1037 (1958) could not be duplicated, the synthesis reported by T. SUYAMA and S. KANAO [*Yakugaku Zasshi* **85**, 284 (1965); *Chem. Abs.* **63**, 7096 c (1965)] was repeated. However, neither this method of preparation nor the procedure kindly furnished by Dr. S. KANAO (heating of tryptophan, formalin and zinc dust in 40% AcOH at 55–60° for several hours) led to N_b,N_b-dimethyltryptophan. Instead, 2-methyl-3-carboxy-1,2,3,4-tetrahydroharman was obtained and shown to be the previously claimed tryptophan derivative.

of tryptophan with methyl iodide,[4] iodide exchange by chloride and demethylation[5] of the resultant methyl N_b,N_b-dimethyltryptophanate N_b-methochloride.

Hydrogenation of the alkaloid and acid hydrolysis of the resultant dihydro product led to *p*-tyramine and β-hydroxyleucine. These facts reflected a frangulanine-like structure and, when combined with a complete analysis of the high resolution mass spectrum (Scheme 1),[6]



SCHEME 1.

[4] P. VAN ROMBURGH and G. BARGER, *J. Chem. Soc.* **99**, 2068 (1911).
[5] M. SHAMMA, N. C. DENO and J. F. REMAR, *Tetrahedron Letters* 1375 (1966).
[6] The assignment of all the fragment ions, shown in Scheme 1, was confirmed by extensive high resolution mass measurements.

were consonant only with structure II for discarine A. The 220 Mc PMR spectrum supported this structure. The singlet of the *N,N*-dimethylamino group was situated at δ 2·26, the isopropyl residue of the β-hydroxyleucine moiety produced, as in other cases,[7] a pair of doublets (J = 7 c/s) at δ 0·86 and 1·08, and the signals corresponding to the C-methyl groups of the isoleucine residue appeared at δ 0·61 as an asymmetric doublet. This may be due to the overlap of a triplet and a doublet.

A similar analysis was applied to discarine B, m.p. 235–236°. It was shown spectrally to be an isomer of discarine A and also to possess peptide bonds and a tryptophan moiety. Its alkaline hydrolysis yielded tryptophan, while acid hydrolysis of its dihydro product gave *N,N*-dimethylisoleucine, β-hydroxyleucine and *p*-tyramine. These results as well as interpretation of the high resolution mass spectrum (Scheme 1)[6] showed discarine B to possess structure III. The 220 Mc PMR spectrum of discarine B showed, as in discarine A, the pair of doublets (J = 7 c/s) at δ 0·89 and 1·07 corresponding to the isopropyl residue of the β-hydroxyleucine moiety. The singlet at δ 2·13, the triplet (J = 7 c/s) at δ 0·70, and the doublet (J = 7 c/s) at δ 0·44, corresponding to the *N,N*-dimethylamino and *C*-methyl groups of the *N,N*-dimethylaminoisoleucine moiety respectively, were detected also. The structure elucidation of the remaining alkaloids of this plant is under investigation.

EXPERIMENTAL

All m.ps were determined on a Kofler block or in open capillaries and are uncorrected. UV spectra were determined in 95% EtOH. IR spectra were obtained in KBr discs and PMR were determined with Varian Associates 220 Mc and T 60 spectrometers.

Hydrogenation. The following conditions were used for all the peptide alkaloids. Hydrogenation of the alkaloid (15 mg) in MeOH (10 ml) was carried out for 6 hr at room temp. in the presence of 10% Pd–C (10 mg) in a H_2 atmosphere. The dihydro product was isolated upon filtration through a celite pad and vacuum removal of the solvent.

Hydrolysis. Total hydrolysis of the alkaloids or their dihydro derivatives were performed by heating them in sealed tubes with 6 N HCl for 24 hr at 110° or with $Ba(OH)_2 . 8H_2O$ for 24 hr at 125–130°. After the usual work-up the mixture was analyzed by the following techniques and the products identified by comparison with authentic samples.

(i) *Paper chromatography*: one part of the hydrolysis mixture was spotted on Whatman No. 1 chromatographic paper, previously treated with a buffer at pH 4 according to the McFarren procedure[8] and developed with the upper phase of a *n*-BuOH–pH 4 buffer. The components were detected with *p*-diazobenzenesulfonic acid.[9] In this way *p*-tyramine was differentiated unambiguously from *o*-tyramine and *m*-tyramine.

(ii) *TLC*: in order to distinguish β-hydroxyleucine from β-hydroxyisoleucine, the hydrolysis mixture was chromatographed on cellulose MN 300 TLC plates using a 4:1:5 *n*-BuOH–HOAc–H_2O mixture and developed continuously during 6 hr. The spots were detected with ninhydrin. The differentiation between *N,N*-dimethylleucine and *N,N*-dimethylisoleucine followed the same technique but by the use of plates coated with 'microcrystalline' cellulose which were developed continuously with 70:15:20:2 butanone–pyridine–HOAc–H_2O during 8 hr.[7] The spots were detected with iodine. Tryptophan and N_b,N_b-dimethyltryptophan were identified on silica gel plates developed with 4:1:5 *n*-BuOH–AcOH–H_2O and detected with ninhydrin and Erlich's reagent.

(iii) An automatic amino acid analysis differentiated leucine from isoleucine.

Extraction of Discaria longispina. The plant material was collected at Salliquelo (Provincia de Buenos Aires) in September 1969. The powdered roots (9·4 kg) were extracted with EtOH for 24 hr × 5, after which time the solvent was drained off. Concentration of the combined extracts yielded an oily residue which was suspended in water, acidified to pH 1·5 with 2 N HCl and extracted throughly with Et_2O; the ether extracts were examined as described before.[1] The remaining aqueous phase was made alkaline with NH_3 to pH 9 and again extracted throughly with Et_2O. The combined ether extracts were washed with H_2O, dried (Na_2SO_4) and evaporated to dryness to yield a solid residue (9·3 g).

[7] R. E. Servis, A. I. Kosac, R. Tschesche, E. Frohberg and H.-W. Fehlhaber, *J. Am. Chem. Soc.* **91**, 5619 (1969).
[8] E. F. McFarren, *Analyt. Chem.* **23**, 168 (1951).
[9] E. Stahl, *Thin-Layer Chromatography*, p. 899, Springer-Verlag, Berlin (1969).

Isolation of the alkaloids. The mixture of alkaloids was submitted to preparative TLC (0·75 mm) using silica gel GF 254 and 19:1 $CHCl_3$–MEOH as solvent. After developing the plates showed three major bands of R_f 0·75; 0·65 and 0·56 in addition to two smaller ones of R_f 0·88 and 0·83. Each band was scraped off the plate and eluted with 97:3 CH_2Cl_2–MeOH.

Frangulanine. The solid residue (1·3 g) obtained from the R_f 0·75 band was crystallized from $CHCl_3$–Et_2O; m.p. 272–274°; homogeneous on TLC (silica gel, 11 solvents); $[\alpha]_D$ −296° (*ca.* 0·1, $CHCl_3$). IR cm^{-1} 3360, 1630. Acid hydrolysis of the dihydro derivative [m.p. 312°; UV max. 231 nm (log ε 3·92), 287 (2·95)] yielded *p*-tyramine, β-hydroxyleucine, *N,N*-dimethylisoleucine and leucine. It was identical (mass spectrum; IR; mixed m.p.; TLC) with an authentic sample.

Discarine A. From the R_f 0·65 band a solid residue was obtained (0·7 g) which crystallized from CH_2Cl_2–Et_2O; m.p. 229–231°; homogeneous on TLC (silica gel, 11 solvents); $[\alpha]_D$ −282° (*ca.* 0·05, $CHCl_3$); $C_{33}H_{43}N_5O_4$ (M^+ 573·3427, required 573·3315).[10] IR cm^{-1} 3350, 1645; UV max. 223 nm (log ε 4·56); 273 (3·82); 284 (3·83); 294 (3·73). PMR (d_6-DMSO): δ 0·61 (6H) asymmetric doublet; 0·86 (3H) doublet, J = 7 c/s; 1·08 (3H) doublet, J = 7 c/s; 2·26 (6H) singlet. Dihydrodiscarine A, crystallized from MeOH; m.p. 286–288°; homogeneous on TLC (silica gel, 3 solvents).

Discarine B. The solid residue (0·3 g) obtained from the R_f 0·56 band was crystallized from $CHCl_3$–Et_2O; m.p. 235–236°; homogeneous on TLC (silica gel, 11 solvents); $[\alpha]_D$ −172° (*ca.* 0·1, $CHCl_3$); $C_{33}H_{43}N_5O_4$ (M^+ 573·3269, required 573·3315). IR cm^{-1} 3350, 1650; UV max. 226 nm (log ε 4·68); 2·73 (3·95); 284 (3·95); 294 (3·82). PMR (d_6-DMSO): δ 0·44 (3H) doublet, J = 7 c/s; 0·70 (3H) triplet, J = 7 c/s; 0·89 (3H) doublet, J = 7 c/s; 2·13 (6H) singlet. Dihydrodiscarine B, crystallized from MeOH–H_2O; m.p. 263–263·5°; homogeneous on TLC (silica gel, 3 solvents).

Reference compounds. Isoleucine, tryptophan and *p*-tyramine were commercial samples. *m*-Tyramine and *o*-tyramine were synthesized according to the procedure of Coulson *et al.*[11] *N,N*-Dimethylleucine and *N,N*-dimethylisoleucine were prepared by reductive methylation of the corresponding amino acids.[12] β-Hydroxyleucine was prepared by the procedure of Wieland *et al.*[13] and β-hydroxyisoleucine according to the procedure of Dobson and Vining.[14]

N_b,N_b-*Dimethyltryptophan.*[15] The method of van Romburgh and Barger[4] gave (3·6 g; 37% yield from tryptophan) methyl N_b,N_b-dimethyltryptophanate N_b-methiodide, m.p. 200–202°. By using an anion exchange resin the corresponding methochloride was obtained (2·5 g), m.p. 200° from EtOH–EtOAc. (Found: C, 57·20; H, 7·06; N, 8·56. $C_{15}H_{21}N_2O_2Cl.H_2O$ required: C, 57·20; H, 7·32; N, 8·90%.) The latter product (2·25 g) was dissolved in absolute EtOH (60 ml) and treated with freshly prepared sodium thiophenoxide[16] (4·35 g) dissolved in absolute EtOH (60 ml). After being stirred for 20 min the mixture was filtered and the filtrate evaporated to dryness. Freshly distilled butanone (300 ml) was added to the residue and the mixture heated to reflux for 26 hr. The solvent was evaporated *in vacuo* to give an oily residue which was dissolved in H_2O (50 ml) and acidified with 10% HCl. The solution was extracted with $CHCl_3$ and the remaining aqueous phase neutralized (pH 6·5) and extracted with *n*-BuOH. The combined butanolic extracts were evaporated to dryness. The residue gave a crystalline product by addition of absolute EtOH (0·9 g); m.p. 240–242°; recrystallized from EtOH–H_2O, m.p. 243°. PMR (D_2O): δ 2·98 (6H) singlet; 3·42 (2H) asymmetric doublet; 4·0 (1H) multiplet; 7·16–7·8 (5H) multiplet. (Found: C, 67·40; H, 7·27; N, 11·92. Calc. for $C_{13}H_{16}N_2O_2$: C, 67·22; H, 6·94; N, 12·06%.)

2-*Methyl*-3-*carboxy*-1,2,3,4-*tetrahydroharman.*[3] Recrystallized from HOAc-H_2O; m.p. 245°. Mass: (M^+) 230, 185, 143 (base ion peak), etc. *m/e.* (Found: C, 67·70; H, 6·33; N, 11·83. Calc. for $C_{13}H_{14}N_2O_2$: C, 67·81; H, 6·13; N, 12·17%). Methyl ester, m.p. 193–195°; UV max 225 nm (log 4·66); 274 (3·91); 280 (3·90); 285 (3·89); 290 (3·80). PMR ($DCCl_3$): δ 2·6 (3H) singlet; 3·18 (2H) asymmetric doublet; 3·6–4·2 (6H) multiplet and singlet; 7·0–7·6 (4H) multiplet; 8·0 (1H) broad singlet. (Found: C, 68·50; H, 6·85; N, 11·60. $C_{14}H_{16}N_2O_2$ required C, 68·83; H, 6·60; N, 11·47%.)

[10] The difference of +11·2 m.m.u. between the found mass and the calculated value for the elemental composition is probably due to the low intensity of the parent peak. The ions $C_{33}H_{42}N_5O_4$ (M–H) [(572·3141, required 572·3237)] and $C_{33}H_{41}N_5O_4$ (M–2H) [(571·3099, required 571·3159)], which confirm the elemental composition of discarine A, were also detected.

[11] W. F. COULSON, A. D. SMITH and J. B. JEPSON, *Analyt. Biochem.* **10,** 101 (1965).

[12] R. E. BOWMAN and H. H. STROUD, *J. Chem. Soc.* 1342 (1950).

[13] TH. WIELAND, H. CORDS and E. KECK, *Chem. Ber.* **87,** 1312 (1954).

[14] T. A. DOBSON and L. C. VINING, *Can. J. Chem.* **46,** 3007 (1968).

[15] This preparation was carried out by Mr. MANUEL GONZALEZ SIERRA under the tenure of a fellowship from the Instituto Nacional de Farmacologia y Bromatologia.

[16] S. I. MILLER, C. E. ORZECH, C. A. WELCH, G. R. ZIEGLER and J. I. DICKSTEIN, *J. Am. Chem. Soc.* **84,** 2020 (1962).

Acknowledgements—We thank Professor A. R. Battersby (Cambridge) for help in obtaining some low resolution mass spectra and the Battelle High Resolution Mass Spectrometry Center for high resolution mass spectra, Dr. M. J. Escalante for the plant collection and identification, Professor J. A. Santome for the automatic amino acids analysis and Professor R. Tschesche (Bonn) for a generous gift of frangulanine. Financial support from Consejo Nacional de Investigaciones Cientificas y Tecnicas de Argentina is acknowledged gratefully. One of us (O.A.M.) thanks the Universidad de Buenos Aires for a fellowship.

Key Word Index—Discaria longispina; Rhamnaceae; peptide alkaloids; frangulanine.