Phytochemistry, 1971, Vol. 10, pp. 2155 to 2159. Pergamon Press. Printed in England.

TWO NEW β-CARBOLINE ALKALOIDS FROM *AESCHRION CRENATA**

E. SÁNCHEZ and J. COMIN

Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Perú 222, Buenos Aires, Argentina

(*Received* 22 *October* 1970)

Abstract—From the bark of *Aeschrion crenata* Vell. (sin. *Picrasma crenata* (Vell.) Engl.) 1-carbomethoxy-β-carboline (I) and two new β-carboline bases, named *crenatine* (II) and *crenatidine* (III), have been isolated. The structures of II and III are 1-ethyl-4-methoxy-β-carboline and 1-ethyl-4,8-dimethoxy-β-carboline, respectively.

Aeschrion crenata Vell. (*Picrasma crenata* (Vell.) Engl. or *Picraena palo amargo* (Speg.) Speg., Simaroubaceae) is a tree that grows in the northeast tropical forest of Argentina. It was known to the guaraní indians as *paraih*, and used by them as insectifuge.[1] It was also employed in southern Brazil as folk medicine against diabetes.[2] Polonsky and Lederer[3] found 2,6-dimethoxybenzoquinone and quassine in the wood of this plant, and indicated the presence of other bitter principles. More recently, the isolation of canthin-6-one was reported.[4]

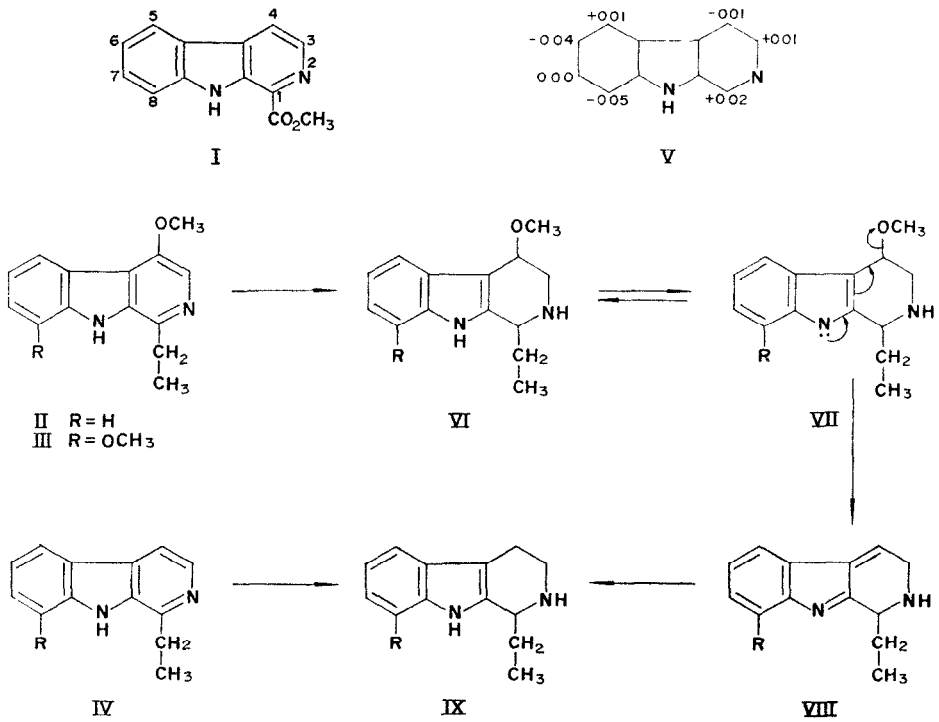
From the bark of *Aeschrion crenata* a basic fraction containing several alkaloids was obtained, from which three, all belonging to the β-carboline group, were isolated. One of them was identified as 1-carbomethoxy-β-carboline (I), first isolated from *Pleiocarpa mutica* (Apocynaceae).[5] Its identity was confirmed by direct comparison with a synthetic sample.

The second alkaloid, which was named *crenatine* (II), $C_{14}H_{14}H_2O$, M^+ 226, m.p. 177–179°, has an UV spectrum very similar to that of harman, suggesting the presence of the same chromophoric system, i.e. a β-carboline nucleus. Its substitution pattern was established by comparison of its NMR spectrum with that of 1-ethyl-β-carboline (IV: R = H) (Table 1), which shows a broad singlet at δ 9·97 (1H) due to the N-H proton, two doublets (J = 5 Hz, 1H each) at δ 8·45 and 7·80 due to H-3 and H-4 respectively, carbon 3 being the more electropositive (α-pyridine carbon). A slightly broadened doublet at δ 8·10 (J = 7 Hz, 1H) must be attributed to either H-5 or H-8. A Hückel MO calculation† of the β-carboline nucleus shows that C-5 has a net positive charge, whereas C-8 bears a strong negative one (V), providing good evidence that this doublet at low field is due to H-5. The three other

* Part XXX in the series "Studies on Argentine Plants". For Part XXIX see J. C. VITAGLIANO and J. COMIN, *Anales Asoc. Quìm. Argentina* **58**, 273 (1970). Preliminary communication: E. SÁNCHEZ and J. COMIN, *Anales Asoc. Quìm. Argentina* **57**, 57 (1969).

† Hückel MO calculations were carried out in the well established manner,[6] using as parameters for the heteroatom: N-2: $h_N = 0{\cdot}4$, $k_{C-N} = 1{\cdot}0$; N-9: $h_N = 1{\cdot}0$, $k_{C-N} = 0{\cdot}9$.

[1] R. PARDAL, *Medicina Aborigen Americana*, p. 100, Anesi, Buenos Aires (1937).
[2] J. R. PEREYRA, *Ann. Fac. Med. Univ. Sao Paulo* **14**, 269 (1938).
[3] J. POLONSKY and E. LEDERER, *Bull. Soc. Chim.* 1157 (1959).
[4] H. B. MACPHILLAMY, personal communication cited by W. I. TAYLOR, in *The Alkaloids* (edited by R. H. F. MANSKE), Vol. VIII, p. 250, Academic Press, New York (1965).
[5] H. ACHENBACH and K. BIEMANN, *J. Am. Chem. Soc.* **87**, 4177 (1965).
[6] See: A. STREITWIESER, *Molecular Orbital Theory for Organic Chemists*, Wiley, New York (1967).



protons in the benzene ring give rise to a complex pattern between δ 7·57 and 7·10. The ethyl group at C-1 is visible as the typical quartet-triplet pattern at δ 3·19 and 1·45 (J = 7 Hz). The proton at C-1 gives rise, when present, e.g. in nor-harman, to a singlet at about δ 9·00 in accordance with the positive charge a HMO calculation locates on that carbon atom.

The NMR spectrum of crenatine (Table 1) shows the N-H proton signal at δ 9·90 and, upfield of it, instead of the AX pair of doublets of the previous spectrum, a sharp singlet at δ 8·00 (1H). Either H-3 or H-4 has been substituted by the methoxy group that gives a signal at δ 4·10. As a methoxy group is expected to shift the signal of an *ortho* proton *ca.*

TABLE 1. NMR SPECTRAL DATA*

Compound	N-H	H-3	H-4	H-5	H-6,7,(8)	CH_3O-4	CH_3O-8	CH_2-CH_3	CH_2-CH_3
IV	9·97s	8·45d	7·80d	8·10d	7·57–7 10m	—	—	3·19q	1·45t
R = H	(broad)	J = 5	J = 5	J = 7				J = 7	J = 7
II	9·90s	8·00s	—	8·37d	7·50–7 10m	4·10s	—	3·13q	1 40t
	(broad)			J = 7				J = 7	J = 7
III	8 90s	8·00s	—	7 90d	7·33–6·80m	4·07s	3·90s	3·07q	1·40t
	(broad)			J = 7				J = 7	J = 7
IV	8·83s	8·36s	7 90d	7·77d	7 60–6·90m	—	4 00s	3·13q	1·45t
R = OCH_3	(broad)		J = 5	J = 7				J = 7	J = 7

* Chemical shifts in δ units; s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet Coupling constants in Hz.

0·4 ppm to higher field, the singlet at δ 8·00 must be due to H-3 (δ 8·45→8·00), and, in consequence the methoxy group can be located at C-4. The signals due to the protons in the benzene ring and the ethyl group remain practically unchanged, and, as the highly deshielded singlet characteristic of H-1 at δ 9·0 is absent, the latter must be attached to C-1.

Crenatidine (III), the other isolated alkaloid, $C_{15}H_{16}N_2O_2$, M^+ 256, m.p. 157–158°, has an UV spectrum that strongly resembles the one of 1-methyl-8-methoxy-β-carboline, suggesting it has the same chromophoric system. The NMR spectrum (Table 1) is very similar to those previously discussed. The N-H signal is shifted upfield *ca.* 1 ppm, to δ 8·90, and the H-5 doublet 0·4 ppm to δ 7·90, whereas the H-3 singlet remains unchanged at δ 8·00. At δ 3·90 there appears the signal of a second methoxy group that can only be attached to the benzene ring. It is neither joined to C-5, as the doublet due to its proton is still present, nor to C-6, because in this case H-5 would have appeared as a singlet. On the other hand, C-7 can be discarded on account that harmine, the C-7 methoxy substituted harman, has a different NMR spectrum in the aromatic region, and, besides, an entirely different UV spectrum.[7] This leaves C-8 as the only possible point of attachment for the second methoxy group, a position that explains the diamagnetic shift of H-5 and of the N-H proton signal. The latter effect is probably enhanced by the diamagnetic anisotropy of the neighboring C-0 bonds or steric inhibition of hydrogen bonding. The NMR spectrum of 1-ethyl-8-methoxy-β-carboline (IV; R = OCH_3), synthesized by known procedures[8] (Table 1), confirms the above conclusions. The N-H signal is again located at δ 8·83, and the H-5 broad doublet at δ 7·77. The H-7 signal appears as a pair of doublets centered at δ 6·94, while H-6 remains as a complex signal between δ 7·30 and 7·00.

The structures proposed for both alkaloids were confirmed by a simple reaction. Reduction of crenatine and crenatidine with sodium in absolute ethanol gives 1-ethyl-1,2,3,4-tetrahydro-β-carboline (IX; R = H) and 1-ethyl-8-methoxy-1,2,3,4-tetrahydro-β-carboline (IX; R = OCH_3), respectively. These were identified with synthetic samples obtained by sodium–ethanol reduction of the corresponding β-carbolines. This reduction of β-carbolines to their 1,2,3,4-tetrahydro derivatives is a well known reaction.[9,10] In the case of crenatine and crenatidine, however, the conjugate bases (VII) of the hypothetical tetrahydro intermediates (VI), lose the methoxy group attached to C-4, and are then further reduced to the final compounds. This reductive elimination of a methoxy group has been described for similar compounds, e.g. 3-methoxymethylindoles, in reaction with a strong reducing and basic reagent such as $LiAlH_4$.[11]

The mass spectra of both alkaloids are consistent with the proposed structures.

In addition to crenatine and crenatidine, two other β-carboline alkaloids with a 4-methoxy substituent have been recently isolated from Simaroubaceae species.[12,13] As the only other two bases previously isolated from this family belong to the canthin-6-ones,[4,14] it appears that the β-carboline nucleus is a rather constant characteristic of the Simaroubaceae alkaloids.

[7] G. G. Doig, J. D. Loudon and P. McCloskey, *J. Chem. Soc.* 3912 (1952).
[8] H. R. Snyder, S. M. Parmenter and L. Katz, *J. Am. Chem. Soc.* **70**, 222 (1948).
[9] O. Fischer, *Ber. Chem.* **22**, 637 (1889).
[10] M. M. Janot, J. Keufer and J. Le Men, *Bull. Soc. Chim. Fr.* 230 (1952).
[11] E. Leete and L. Marion, *Can. J. Chem.* **31**, 775 (1953).
[12] S. R. Johns, J. A. Lamberton and A. A. Sioumis, *Australian J. Chem.* **23**, 629 (1970).
[13] N. Bourguignon-Zylber and J. Polonsky, *Bull. Chim. Therap.* 396 (1970).
[14] N. Inamato, Sh. Masuda, O. Sinamura and T. Tsuyukis, *Bull. Chem. Soc. Japan* **34**, 888 (1961).

EXPERIMENTAL

M.ps are uncorrected. Microanalyses were performed by Dr. B. B. de Deferrari and by Dr. A. Bernhardt, Germany. UV spectra were recorded in EtOH soln., IR spectra in Nujol mulls, NMR spectra in $CDCl_3$ soln. with TMS as internal standard, and mass spectra were measured in a Varian-MAT CH7 instrument. TLC on alumina G (Merck) and $CHCl_3$ (A) and EtOAc (B) as solvents; preparative TLC on alumina PF_{254}, type E (Merck) and cyclohexane–$CHCl_3$–MeOH 10:1:1 as solvent.

Extraction and Separation

The bark of *Aeschrion crenata*, collected near Puerto Iguazú, Misiones (Argentina), was dried and ground. A 2·5 kg sample was extracted first with light petroleum and then with MeOH. The MeOH extract was con. centrated to 1·5 l., cooled and filtered The filtrate was concentrated to 200 ml, poured on 1·5 l. of 1% HCl, and the resulting suspension cooled and filtered. The filtrate was made alkaline with conc. NH_3; 2 ppt. formed that was filtered and dried (16·0 g). A portion of it (7·0 g) was dissolved in $CHCl_3$ and chromatographed on 250 g of silica gel, eluting with $CHCl_3$ (3·0 l.; fraction 1, discarded), and then with $CHCl_3$–MeOH 99:1 (1·5 l.; fraction 2, discarded), 98:2 (3·2 l.; fraction 3, 1·0 g and fraction 4, 1·7 g), and 97·3 (2·2 l.; fraction 5, 1·3 g)

1-*Carbomethoxy-β-carboline* (I)

Fraction 3 was dissolved in 50 ml of 1% HCl, filtered, and the filtrate made alkaline with $NaHCO_3$ and extracted with Et_2O. The extract was dried and evaporated to dryness, yielding a residue (0·3 g) which shows one spot on TLC. A sample was sublimed at 140° (0·001 mm), needles, m.p. 160–161° (lit.[5] m.p. 166°) undepressed by admixture with a synthetic sample;[15] IR and NMR spectra, identical to those of a synthetic sample. R_f 0·86 (A).

Crenatine (*II*)

Fraction 5 was purified in the same way, yielding a crystalline residue (1·0 g), which after sublimation at 140° (0·001 mm) melted at 177–179°; UV spectrum: λ_{max} 243 nm (ϵ 28,200), 265 (5800), 274 (6500), 284 (11,700), 331 (5500) and 348 (6200); NMR spectrum (Table 1); mass spectrum: *m/e* 227 (M + 1), 226 (M), 225, 211, 210, 198, 183, 169, and 168; R_f 0·60 (A). (Found: C, 74·47; H, 6·45; N, 12·68. $C_{14}H_{14}N_2O$ required: C, 74·31; H, 6·24; N, 12·38%)

Crenatidine (*III*)

Fraction 4 was also purified by the described procedure, yielding a residue (0·9 g) which was further purified by preparative TLC. A crystalline alkaloid was obtained (0·4 g), which after sublimation at 140° (0·001 mm) melted at 157–158°; UV spectrum: λ_{max} 243 nm (ϵ 53·600), 267 (7·900), 283 (6·600), 334 (6·500) and 346 (6·600); NMR spectrum (Table 1); mass spectrum: *m/e* 257 (M + 1), 256 (M), 255, 241, 227, 226, 225 and 198; R_f 0·75 (A). (Found· C, 70·15; H, 6·23; N, 11·12. $C_{15}H_{16}N_2O_2$ required: C, 70·29; H, 6·29; N, 10·93%.)

1-*Ethyl-β-carboline* (*IV*. R = H)

It was prepared from tryptophan by known procedures,[8] m.p. 195–197° (lit.[16] 193–194°); NMR spectrum (Table 1); mass spectrum· *m/e* 197 (M + 1), 196 (M), 195, 181, 168, 167, and 140.

1-*Ethyl-1,2,3,4-tetrahydro-β-carboline* (*IX*: R = H)

(*A*) *From* 1-*ethyl-β-carboline* (*IV*: R = H). To a solution of 1-ethyl-β-carboline (375 mg) in absolute EtOH (7·5 ml), Na (1·5 g) was added in small pieces and the suspension kept at 100–105° for 2 hr. The same amounts of EtOH and Na were added again and the whole heated for an additional 2 hr. EtOH (25 ml) was added, and the warm solution poured into 200 ml of water The resulting suspension was extracted with Et_2O, and the Et_2O then extracted with 1% HCl. The acid solution was basified with 10% NaOH, extracted with Et_2O and the extract dried and concentrated to dryness. A crystalline residue (273 mg) was obtained, which was sublimed at 190° (0·001 mm), m.p. 229–230° (sint. 114–115°); hydrochloride m.p. 257–258° (lit.[17] 261–262°); UV spectrum: λ_{max} 223 nm (ϵ 32,400), 274 (7100 sh), 281 (7200) and 290 (6000); R_f 0·35 (B).

(*B*) *From crenatine* (*II*). A sample of crenatine (306 mg) was reduced as in (A), yielding 183 mg of a crystalline product which after sublimation at 190° (0·001 mm), melts at 228–229°, hydrochloride m.p. 256–257°, undepressed by admixture with the precedent hydrochloride; IR spectra of both the free base and the hydrochloride identical with those of the precedent products; R_f 0·35 (B)

[15] H. R. SNYDER, H. G. WALKER and F. X. WERBER, *J. Am. Chem. Soc.* **71**, 527, (1949).
[16] N. J. LEONARD and R. C. ELDERFIELD, *J. Org. Chem.* **7**, 556 (1942).
[17] B. T. HO, W. M. ISAACS, K. E. WALKER and V. ESTEVEZ, *J. Pharm. Sci.* **57**, 269, (1968).

1-Ethyl-8-methoxy-β-carboline (IV: R = OCH_3)

A suspension of 7-methoxytryptophan[18] (1·0 g) in 100 ml of water was acidified with 1·0 ml of 2 N HCl, freshly distilled propionaldehyde (4·0 ml) added and the mixture left overnight at room temp. with stirring. The resulting suspension was diluted with 300 ml of water and boiled 15 min. Acetic acid (10 ml) and 10% aq. $K_2Cr_2O_7$ (50 ml) were added and the solution kept boiling for 2–3 min, after which it was cooled and enough solid Na_2SO_3 added to eliminate the excess oxidizing agent. It was then made alkaline with 2 N NaOH and extracted with Et_2O. The extract was washed with H_2O, dried and concentrated to dryness, yielding a crystalline residue (528 mg), which after sublimation at 140° (0·001 mm) melted at 167–169°; UV spectrum: λ_{max} 242 nm (ε 57,500), 276 (10,200), 286 (11,500) and 339 (7100); NMR spectrum (Table 1); mass spectrum: *m/e* 227 (M + 1), 226 (M), 225, 211, 210, 198, and 197; R_f 0·40 (A), (Found: C, 74·11; H, 6·13; N, 12·28; $C_{14}H_{14}N_2O$ required: C, 74·31; H, 6·24; N, 12·38%.)

1-Ethyl-8-methoxy-1,2,3,4-tetrahydro-β-carboline (IX: R = OCH_3)

(*a*) *From 1-ethyl-8-methoxy-β-carboline (IV: R = OCH_3)*. Reduction of this compound (122 mg) with Na and EtOH by the procedure described above yielded a crystalline residue (53 mg), which was sublimed at 120° (0·001 mm), m.p. 129–131°; UV spectrum: λ_{max} 224 nm (ε 22·400), 269 (7·800), and 290 (4·100); R_f 0·10 (A). (Found: C, 72·87; H, 7·76; N, 12·02; $C_{14}H_{18}N_2O$ required: C, 73·01; H, 7·88; N, 12·17%.)

(*b*) *From crenatidine (III)*. Reduction of crenatidine (100 mg) by the same procedure gave a residue that was purified by preparative TLC and sublimation at 120° (0·001 mm); m.p. 126–128°, undepressed my admixture with the precedent compound; IR spectrum: identical to that of the above product; R_f 0·10 (A).

Acknowledgements—This work was supported by grant 930 from the Consejo Nacional de Investigaciones Científicas y Técnicas of Argentina and by grant GM-11994-02 from the National Institutes of Health, U.S. Public Health Services. We are indebted to the Dirección General de Parques Nacionales of Argentina for the collection of plant material.

[18] E. D. Bergmann and E. Hoffmann, *J. Chem. Soc.* 2827 (1962).