Phytochemistry, 1968, Vol. 7, pp. 307 to 311. Pergamon Press. Printed in England

ALKALOIDAL CONSTITUENTS OF *PEGANUM HARMALA* AND SYNTHESIS OF THE MINOR ALKALOID DEOXYVASCINONE

A. CHATTERJEE (Mrs.) and M. GANGULY (Miss)

Department of Chemistry, University College of Science, Calcutta, 9, India

(*Received* 5 *July* 1967)

Abstract—Of the four alkaloids isolated from the seeds of *P. harmala*, harmine constitutes the major component, the other three occurring as minor constituents. One of these is shown to be harmaline, the second minor base being deoxyvascinone, the third one being under characterization though its spectral analyses suggest it to be a 2 alkoxyquinolone derivative. A new synthesis of deoxyvascinone has been achieved from anthranilic acid and γ-aminobutyric acid. During this synthesis another product is also obtained for which a plausible structure has been postulated from spectral evidences.

IN CONTINUATION of our studies on the alkaloidal constituents of Indian medicinal plants and in view of the recent report on the isolation of a new alkaloid, harmidine,[1] $C_{13}H_{14}N_2O$ from *Peganum harmala* Linn. (Fam. *Zygophyllaceae*) to which no definite structure has yet been assigned we took up the systematic chemical investigation of this plant of Indian origin.

Extraction of the crushed seeds of *P. harmala* with different solvents and rigorous pH-gradient fractionation of the basic components obtained from the individual solvent extracts have so far resulted in the isolation of four crystalline alkaloids along with an amorphous red pigment of alkaloidal nature.

The major alkaloid of the seeds, $C_{12}H_9N_2(OCH_3)$, $M^+=212$, m.p. 258°, λ_{max}^{EtOH} 239, 299, 324 and 326 nm is a monoacidic tertiary base (pKa = 6·34) and was obtained in 0·3 per cent yield. It formed a monomethiodide, $C_{13}H_{12}N_2O.CH_3I$, m.p. 306° (dec.) which on catalytic reduction furnished a 6-methoxyindole derivative,[2] $C_{14}H_{18}N_2O$, m.p. 174°, λ_{max}^{EtOH} 226, 265 and 290 nm (log ε, 4·2, 3·66 and 3·75). The NMR spectrum of the alkaloid showed the presence of an aromatic C-methyl (2·78 δ, 3H singlet), an aromatic methoxyl (3·9 δ, 3H singlet) and 5 aromatic protons (6·9–8·5 δ) (Fig. 1). The data are consistent with the formulation of harmine (I). The alternative, N_a-methyl norharmine structure (II) considered on the basis of the absence of —NH band in the i.r. spectra of the alkaloid measured in nujol mull or KBr disc, was excluded on the grounds viz. (i) the NMR spectrum of the tetrahydro-N_b-methyl derivative of the alkaloid, obtained by the $NaBH_4$ reduction of the corresponding methiodide, showed the presence of a —CH—CH_3 grouping (methyl doublet at 1·44 δ, methine quartet at 3·2 δ, $J=6·5$ c/s) (Fig. 1a) and (ii) the base on oxidation with SeO_2, furnished an aminoaldehyde (III) though in poor yield, m.p. 183°, λ_{max}^{KBr} 6·1 μ, a transformation which can only be explained with the formulation (I). It is pertinent to mention in this connection that the chemical shift 3·2 δ of the C_1 hydrogen in the NMR spectrum of the tetrahydro-N_b-methyl derivative of harmine shows its axial configuration.[3]

[1] S. SIDDIQUI, *Chem. Ind.* 356 (1962).
[2] E. SCHLITTLER and R. SCHWYZER, *Helv. Chim. Acta* **37**, 59 (1954).
[3] W. E. ROSEN and J. N. SCHOOLERY, *J. Am. Chem. Soc.* **83**, 4816 (1961).

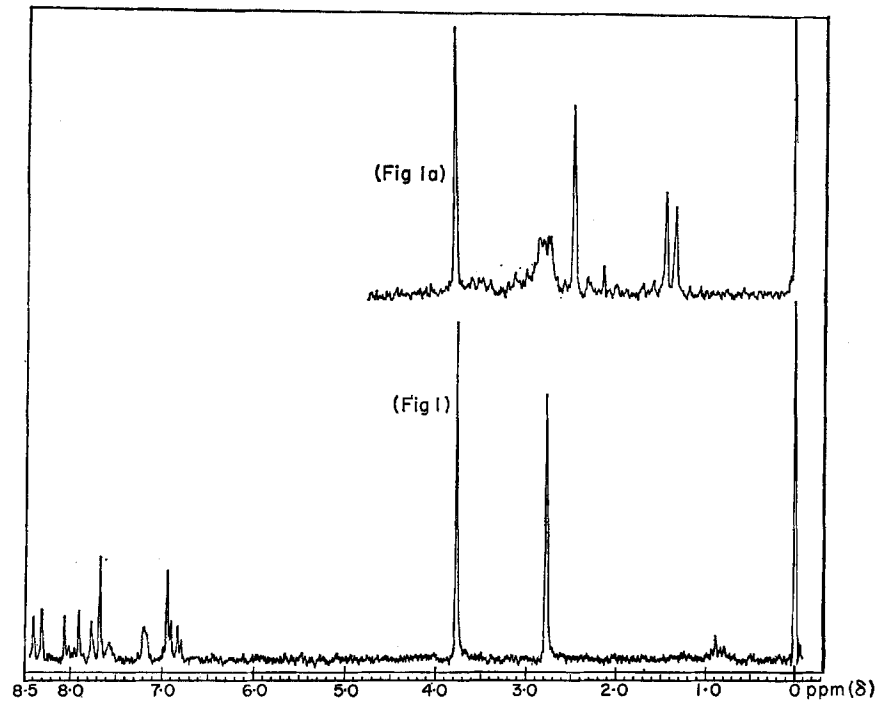
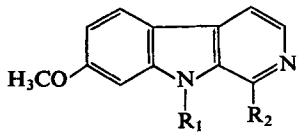


FIG. 1. NMR SPECTRA OF HARMINE (FIG. 1) AND N_b-METHYL 1,2,3,4-TETRALYDROHARMINE (FIG. 1a).



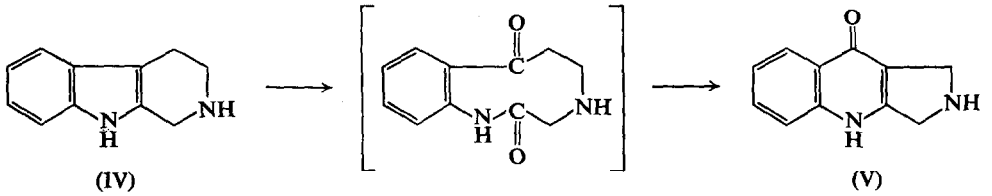
I, $R_1=H$; $R_2=CH_3$
II, $R_1=CH_3$; $R_2=H$
III, $R_1=H$, $R_2=CHO$

The second alkaloid, $C_{13}H_{14}N_2O$, $M^+=214$, m.p. 220–22°, λ_{max}^{EtOH} 231, 259 and 333 nm, obtained in a yield of 0·0001 per cent, was found to have the molecular formula same as that reported for harmidine.[1] It formed a crystalline hydroiodide, $C_{13}H_{14}N_2O.HI$, m.p. 232–35° and a methiodide, $C_{13}H_{14}N_2O.CH_3I$. Sodium borohydride reduction of the methiodide furnished a dihydro derivative, $C_{14}H_{18}N_2O$, m.p. 174°, identical in all respects with the tetrahydro-N_b-methyl derivative of harmine. The data are thus consistent with the properties of dihydroharmine, i.e. harmaline with which it is found to be identical by the usual procedure.

The third alkaloid, m.p. 192°, $M^+=264$, obtained as a minor base exhibits characteristic u.v. spectrum, λ_{max}^{EtOH} 242, 266, 302, 322 and 334 nm (log ϵ, 2·03, 4·58, 1·89, 2·12 and 2·42) comparable to that of 2-alkoxy 4-quinolone[4] chromophoric system. Isolation of this alkaloid for further work is in progress.

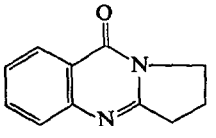
[4] A. W. SANGSTER and K. L. STUART, *Chem. Rev.* **65**, 69 (1965).

The fourth minor constituent (yield, 0·0008 per cent), $C_{11}H_{10}N_2O$, m.p. 106–7°, M^+= 186, λ_{max}^{EtOH} 224, 267, 272, 302 and 314 nm (log ϵ, 4·2, 3·63, 3·59, 3·4 and 3·33) appears to be interesting. From an examination of its composition, its non-indole u.v. spectrum and its co-occurrence with harmine it would be rather tempting to assume it to be pyrrolidino-quinolone compound (V), an oxidation product of tetrahydronorharman[5] (IV), though such presumption is not unlikely from biogenetic considerations.



(IV) (V)

But the fact that the alkaloid exhibits a 3H-quinazoline-4-one[6] rather than a quinolone[5] u.v. spectrum and that its i.r. spectrum exhibits no —NH absorption and instead shows strong bands at 5·95 and 6·15 μ due to amide carbonyl and C=N-groupings, emphasized that the alkaloid is, in all probability, deoxyvascinone (VI). The NMR spectrum of the compound which showed 3 sets of methylene signals around 1·15, 2·3 and 3·3 δ lent additional support to this view.



(VI)

Its identity with deoxyvascinone was finally confirmed by direct comparison m.m.p., TLC and superimposable i.r. with the synthetic sample obtained by the condensation reaction of γ-amino butyric acid and anthranilic acid.

Appropriate condensation of anthranilic acid and γ-amino butyric acid in boiling xylene with P_2O_5, furnished in addition to deoxyvascinone (yield 50 per cent) a second product (designated as DVQ), $C_{22}H_{17}N_3O$, M^+=339, m.p. 285°, λ_{max}^{EtOH} 280 and 306 nm (log ϵ, 4·53 and 4·3) in about 30 per cent yield. The i.r. spectrum of the compound λ_{max}^{KBr} 5·9, 6·05 and 6·3 μ was comparable to that of deoxyvascinone and suggested the presence of same functionalities in the DVQ (*vide supra*) as that in deoxyvascinone. Structural information of DVQ was secured by the application of differential u.v. spectral technique as the subtraction spectrum λ_{max}^{EtOH} 238, 285, 308 and 320 nm (log ϵ, 4·09, 3·87, 3·52 and 3·63) of the compound (DVQ) and deoxyvascinone was a typical quinoline spectrum,[7] (Fig. 2). DVQ thus bears in its molecule two isolated chromophoric systems viz., a 3H-quinazoline-4-one and a quinoline. On the basis of these accumulated evidences the structure of this compound is tentatively formulated as (VII), presumably formed through enamine reactions of deoxyvascinone with anthranilic acid and γ-aminobutyric acid. Further evidence in support of the postulate (VII) and its mode of formation would be presented in our forthcoming publication.

[5] B. Witkop and S. Goodwin, *J. Am. Chem. Soc.* **75**, 3371 (1953).
[6] S. C. Pakrashi, J. Bhattacharya, L. F. Johnson and H. Budzikiewicz, *Tetrahedron* **19**, 1011 (1963).
[7] J. M. Hearn and J. C. Simpson, *J. Chem. Soc.* 3318 (1951).

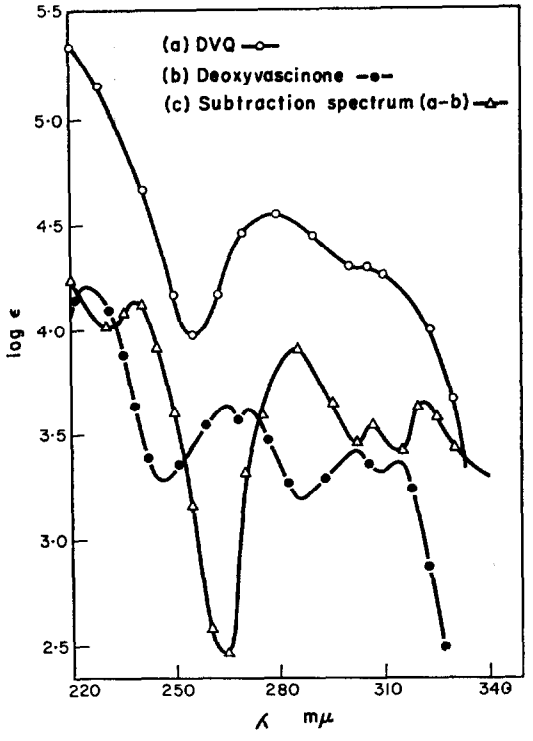
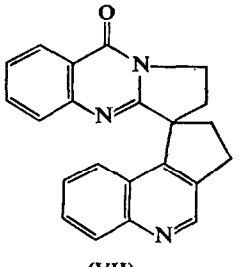


FIG. 2. U.V. SPECTRA OF DVQ(*a*) AND DEOXYVASCINONE (*b*), AND THEIR SUBTRACTION SPECTRUM (*c*).



(VII)

EXPERIMENTAL

General

The melting points were determined on the Kofler block and are uncorrected. The u.v. spectra were measured in 95 per cent ethanol (aldehyde-free), the i.r. spectra in nujol mull or KBr disc and electrometric titration in dimethyl formamide. The analytical samples were dried at 80° over P_2O_5 for 24 hr *in vacuo*. Anhydrous Na_2SO_4 was used for drying the organic solvents and Brockmann alumina was used for column chromatography throughout.

Isolation of the Alkaloids

1·5 kg of crushed dried seeds of *P. harmala* were defatted for 30 hr with light petroleum (60–80°) in a soxhlet apparatus. The petroleum extract was concentrated to a thick oil and then churned with 5 per cent aqueous citric acid (500 ml) for 5 hr. The aqueous acid layer was separated and washed with petroleum (60–80°) to remove entrained oil. The acid solution was adjusted to pH 4 by addition of Na_2HPO_4 and then exhaustively extracted with benzene (4 × 500 ml). The benzene extract was washed with aqueous NH_3 and

water, dried and concentrated. The oily concentrate (100 mg) was chromatographed using petroleum-benzene (3:1) as the eluent. Earlier fractions furnished deoxyvascinone (8 mg) which crystallized from benzene-petroleum (60–80°) as needles, m.p. 104° (lit. m.p. 110–1°).[8]

The latter fractions gave a base, crystallized from acetone as needles m.p. 192–4° (M^+=264).

The acid extract left after extraction with benzene was then basified with NH_3 (pH 11) and extracted with $CHCl_3$ (4 × 600 ml). The $CHCl_3$ extract was dried, concentrated and chromatographed. Elution with benzene gave harmine, m.p. 258° (lit. m.p. 262°).[9]

The defatted seeds were then extracted with alcohol for 15 days. The alcoholic extract was concentrated under reduced pressure, the concentrate was extracted with citric acid as before. Aqueous acid was basified (pH 11) with NH_3, extracted with $CHCl_3$, dried, concentrated and then chromatographed. Elution with benzene furnished harmine, crystallized from methanol as needles m.p. 258° (lit. m.p. 262°),[9] with chloroform harmaline, crystallized from benzene m.p. 222° (lit. m.p. 229–231°).[9] The column was then washed with $CHCl_3$–methanol (1:1) when an amorphous red pigment migrated out.

2. *N_b-methyl 1,2,3,4 Tetrahydro Harmine*

To a solution of harmine methiodide (500 mg) prepared from harmine by treatment with methyl iodide in 75 ml of methanol, $NaBH_4$ (600 mg) was added in portions and the reaction mixture was kept overnight. The methanolic solution was concentrated and then diluted with water when a crystalline solid separated. Recrystallization from benzene-acetone yielded N_b-methyl 1,2,3,4 tetrahydro harmine (300 mg), m.p. 174°, λ_{max}^{EtOH} 226, 265 and 290 nm (log ϵ, 4·2, 3·66, and 3·75).

3. *Selenium Dioxide Oxidation of Harmine*

To a solution of harmine (100 mg) in 50 ml of xylene, freshly sublimed SeO_2 (100 mg) was added with stirring and the mixture was refluxed in an oil bath at 170–80° for 6 hr. The solution was then cooled when a crystalline compound, m.p. 180–82° separated out. Recrystallization from benzene furnished needles, m.p. 182°, λ_{max}^{KBr} 6·1 μ. (Identical with 3-formyl-norharman).

SYNTHESIS OF DEOXYVASCINONE

A mixture of dry anthranilic acid (200 mg), γ-aminobutyric acid (200 mg) and P_2O_5 (600 mg) in dry xylene was refluxed at 170–80° for 3 hr. The reaction mixture was poured over crushed ice, the xylene layer was separated and treated with 5 N HCl (3 × 50 ml). The total acidic extract was then basified with NH_3 and extracted with $CHCl_3$ (3 × 100 ml). The $CHCl_3$ extract was washed, dried, concentrated and chromatographed.

Elution with benzene-petroleum (1:1) furnished a solid, m.p. and mixed m.p. with natural deoxyvascinone 104° (100 mg). Elution with chloroform gave a second compound, DVQ which crystallized from chloroform-acetone, m.p. 285°, $C_{22}H_{17}N_3O$ (M^+, 339).

Acknowledgement—The authors express their sincere thanks to the C.S.I.R. (India) for the award of fellowship to one of them (M.G.).

[8] H. G. Boit, "*Ergebnisse der Alkaloid-chemie Bis 1960*", p. 742 Akademie-Verlag, Berlin (1961).

[9] M. Hesse, *Indole alkaloide in Tabellen*, p. 95, Springer-Verlag, Berlin (1964).