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A REINVESTIGATION OF THE ALKALOIDAL CONSTITUENTS OF PEGANUM HARMALA

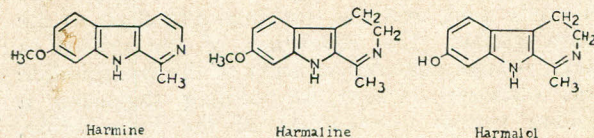
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As part of a general programme of reinvestigation of some of the well-known alkaloidal plants of economic importance to the country, following the mild methods of working evolved in the course of the present author's studies in this field, an examination of the seeds of *Peganum harmala* which grows in abundance in Pakistan and some of the neighbouring countries has been undertaken. Mature seeds from the West Punjab region in three separate lots were employed in this study.

Peganum harmala is known to contain four alkaloids, namely, harmaline ($C_{13}H_{14}ON_2$) reported by Goebel¹ as far back as 1841, harmine ($C_{13}H_{12}ON_2$) isolated by Fritsche,² harmalol ($C_{12}H_{12}ON_2$) noted by Goebel and prepared by O. Fischer³ from harmaline, and peganine ($C_{11}H_{12}ON_2$) obtained by Merk and later identified with vasicine isolated earlier by Hooper⁴ from *Adhatoda vasica* Nees. The constitution of the harmine group of bases and their interrelationship were established through a series of degradative and synthetic studies by O. Fischer, Perkin, Robinson and Manske,^{5,6,7} the following structures being eventually assigned to them:



As a result of the present studies, apart from harmine (m.p. 266°C.) and peganine/vasicine (m.p. 198°C.) in yields of 0.8 and 0.1% respectively on the weight of the seeds, a hitherto unrecorded

base has been isolated in a yield of 1.7% and provisionally named as harmidine. This base which analysed for $C_{13}H_{14}ON_2$ melts at 257-58°C., 18° higher than the melting point recorded for harmaline (239-40°C.), and shows a depression of 25° on admixture with harmine. It gives crystalline salts, all of which, except for the hydrohalides, have higher melting points than those noted for the corresponding salts of harmaline.

The isolation of harmine and harmidine, detailed account of which is given in the experimental, was mainly based on precipitating out the two bases in the form of their sparingly soluble hydroiodides from the water soluble portion of the alcoholic extractive of the crushed seeds through the addition of potassium iodide, purifying the iodide through repeated crystallizations from dilute alcohol, liberating the bases with dilute alkali, and fractionally precipitating them from their dilute acetic acid solution at controlled pH by the careful addition of dilute ammonia. In this process of separation the acetic acid salt of the weaker base, harmine, is hydrolysed on heating the clear solution in the water bath after bringing it to about 7.4 pH, harmidine being obtained from the filtrates of harmine on treatment with an excess of ammonia. Peganine and a base which appears to be deoxyvasicine, were obtained from the filtrate of the total hydroiodide of harmine and harmidine according to the procedure described in the experimental, but no phenolic bases could be obtained in these workings.

On the addition of 2 atoms of bromine in chloroform solution at zero degree, harmidine yielded an orange-yellow bromo product which

analysed for $C_{13}H_{14}ON_2Br_2 \cdot H_2O$, indicating an olefinic linkage, melted at 178° , and on liberation of the base in aqueous medium with NH_4OH gave a monobromo derivative melting at $220^\circ C$. (monobromoharmaline, m.p. $195^\circ C$.). Addition of bromine to the base in acetic acid and sulphuric acid solutions gave a yellowish dibromo product melting at $250-52^\circ C$. It was, however, noted that the dibromo product obtained on bringing the components together in chloroform medium also showed m.p. $250-52^\circ C$. when treated with alcohol, the original orange colour being thereby converted to yellow. In whichever way prepared, the bromo product analysed for $C_{13}H_{14}ON_2Br_2 \cdot H_2O$ and yielded an identical monobromo base.

On reduction with zinc and hydrochloric acid, harmidine gave a base which analysed for $C_{13}H_{16}ON_2$, melted at $199^\circ C$. and showed no depression in m.p. on admixture with tetrahydroharmine prepared directly from harmine. On refluxing with hydroiodic acid, harmidine furnished a crystalline golden yellow base which analysed for $C_{12}H_{12}ON_2$ and has been named as harmidol. Crystallized from methanol, it showed m.p. $259-60^\circ C$. as against $212^\circ C$., the melting point recorded for harmalol, and gave well-defined crystalline salts. On crystallisation from dilute methanol or moist acetone, the base was obtained in the form of a golden yellow coloured hydrate which frothed up at $135^\circ C$., losing its water of crystallization, and melted down at $259-60^\circ C$.

The findings recorded above would suggest that harmidine and harmidol are isomers of harmaline and harmalol, respectively. In so far, however, as from none of the three lots of harmala seeds worked up in the course of this study any base corresponding in its melting point to harmaline could be obtained as a pure product, caution is indicated against such a conclusion. In fact, the eventual separation of crystalline fractions melting in the range $235-40^\circ C$. into harmine and harmidine by the process employed in the isolation of these bases, would raise doubts as to the justification of harmaline and harmalol, with melting points 18° and 47° lower than those of harmidine and harmidol respectively, being treated as uniform chemical entities. In view of the fact, however, that harmaline was actually synthesised by Perkin, Robinson and Manske,⁷ more recently also by Spencer,⁸ and the synthetic base was found by them to show the same melting point ($239-40^\circ C$.; $235-37^\circ C$.) as harmaline from the natural source, a definite conclusion in this regard can not be arrived at on the basis of the present data. To clarify this position with

particular reference to the fact that harmaline which is reported as the major alkaloidal constituent (1.5% of the weight of the seeds) could not be obtained in the course of this investigation, examination of the seeds of *Peganum harmala* from other sources, and further studies in harmidine are in progress.

Experimental

8 kg. of *Peganum harmala* seeds were powdered to 30 mesh and percolated 8 times with alcohol at room temperature. On removal of the solvent from the combined percolates at reduced pressure below $40^\circ C$., a reddish green, viscous residue was obtained—2.9 kg., which was divided up into petroleum ether and water-soluble fractions by a process of repeated partitioning in the two solvents. In the course of this operation it was noted that hardly any material insoluble in the two solvents is left over, if there has been no over-heating in the removal of the solvent from the alcoholic percolates. The water-soluble layer was freed of the residual organic solvent *in vacuo*, treated with ammonia to bring the pH from the original 4.5 to 6.5 and then with ammonium sulphate. The resulting precipitate of dark brown resinous impurities was cottoned off, and the slightly turbid reddish solution was heavily charcoaled and filtered, whereby the colour of the solution became much lighter. On the addition of a concentrated solution of KI to the filtrate, a light brown crystalline precipitate of the hydroiodide of the bases was obtained, which was sucked, washed with water and dried—the yield being 600 g. On concentration and treatment with KI the washings gave a further quantity of hydroiodide—40 g., bringing the total yield of the crude iodide to 640 g.

ISOLATION OF HARMINE AND HARMIDINE

The total crude hydroiodide (640 g.) was dissolved in dilute alcohol in the hot, charcoaled and filtered through hot funnel. The charcoal mud was repeatedly extracted in the hot with dilute alcohol with the addition of small quantities of acetic acid, and the washings added on to the main filtrate. On repetition of the process a clear, orange coloured solution of the hydroiodide was obtained, which on cooling deposited a golden yellow crystallizate of the hydroiodide, the mother liquors yielding further quantities of it on concentration (total yield 490 g.).

For the liberation of the base the hydroiodide was treated with 10% NH_4OH with slight warming; the buff coloured base was sucked and washed first with 2% NH_4OH , then repeatedly with

water, and dried on porous plates (270 g.). The total base was dissolved in 10% acetic acid with slight warming, keeping the pH of the solution between 6 and 6.5. After cooling to room temperature the slightly reddish solution was charcoaled and filtered, and its pH was brought up to about 7.2 with the addition of dilute NH_4OH , taking care that no precipitation of the base occurs at room temperature. On heating the orange coloured solution in a boiling water bath a sizable crystallize was formed which showed a tendency to redissolve on cooling; it was, therefore, sucked hot, washed with water and dried (m.p. 260-62°C.). The pH of the filtrate which fell from 7.2 to 6.8 was again brought up to 7.4 with the careful addition of dilute NH_4OH and a second crop of crystals was obtained (m.p. 260°C.). A third crop of crystals was obtained on bringing the pH to 7.4-7.6 and heating in a water bath till the pH came down to 7.4; this crop of crystals had a pinkish colour and m.p. 236-40°C. The filtrate was now treated with an excess of fairly concentrated NH_4OH bringing the pH above 8, and the crystallize thereby obtained had m.p. 252°-54°C.

On repeated crystallization from a mixture of methanol and benzene in which both the bases are appreciably more soluble than in either of the two solvents individually, the fractions melting above 260°C. gave harmine m.p. 266°C. (65 g.). Its hydrochloride showed m.p. 285°C., and the tetrahydroharmine prepared from it melted at 199°C. The fractions melting between 250-54°C. yielded harmidine (140 g.) which melted at 257-58°C. and gave a depression of 25° in its mixed melting point with harmine.

The fraction melting in the range 236-40°, when treated in the manner described for the isolation of harmine and harmidine from the total base, was ultimately separated into the two bases, giving once again low melting intermediate fractions, m.p. 236-40°, which appeared to be eutectic mixtures of the two.

ISOLATION OF VASICINE

The filtrate from the total hydroiodide was concentrated to one fourth of its bulk and repeatedly extracted with amyl alcohol. The alcoholic layer was extracted with water after the addition of petroleum ether (1:1). The aqueous extract was freed of the solvent *in vacuo* and the brownish semi-solid residue (40 g.) washed with water to remove inorganic matter, when 13.4 g. of a nearly white crystalline product was obtained. This was taken up in hot alcohol, charcoaled and filtered, when on cooling it deposited a white

crystalline product (0.8 g.), which on repeated crystallization from methanol finally melted at 285°C. and analysed for $\text{C}_{11}\text{H}_{12}\text{N}_2\cdot\text{HI}$ (deoxy vasicine hydroiodide?). Found after drying to constant weight over P_2O_5 at 100°C. *in vacuo*: C, 43.86; H, 4.29; N, 9.2; I, 42.6. Required: C, 44.00; H, 4.33; N, 9.33; I, 42.33.

The alcoholic mother liquor yielded 9.1 g. of a white crystalline hydroiodide, m.p. 206°C., which on liberation from alcohol furnished vasicine (peganine), m.p. 198°C. Found after drying to constant weight over P_2O_5 at 100°C. *in vacuo*: C, 70.13; H, 6.9; N, 14.71; O, 8.57. $\text{C}_{11}\text{H}_{12}\text{ON}_2$ requires: C, 70.18; H, 6.43; N, 14.88; O, 8.50.

CHARACTERISATION OF HARMIDINE

Harmidine is fairly soluble in mixtures of benzene and methanol or ethanol, sparingly soluble in these solvents individually, and nearly insoluble in ether and petroleum ether. It crystallizes from these solvents in light amber coloured prismatic plates, which, when crushed, form a white powder melting at 257-58°C. On columning the base on neutral or basic alumina in benzene and alcohol medium the melting point is slightly lowered, but is restored on crystallization from the mixed solvents.

Harmidine Hydrochloride.—On treating the base with an excess of alcoholic HCl a yellow coloured paste was formed, which was taken up in alcohol. On diluting the solution with ether harmidine hydrochloride was precipitated out, and on crystallization from alcohol formed long yellow needles, m.p. 236°C. Found on drying to constant weight over P_2O_5 at 100°C. *in vacuo*: Cl, 14.95. $\text{C}_{13}\text{H}_{14}\text{ON}_2\cdot\text{HCl}$ requires: Cl, 14.15.

Harmidine Hydroiodide.—On treating a solution of harmidine in dilute acetic acid with a concentrated solution of KI a yellow crystalline was obtained, which when sucked and washed with water, and crystallized from alcohol, formed bright yellow rods and needles melting at 242°C. Found on drying to constant weight over P_2O_5 at 100°C. *in vacuo*: I, 37.56. $\text{C}_{13}\text{H}_{14}\text{ON}_2\cdot\text{HI}$ requires: I, 37.43.

Harmidine Picrate.—To a methanol benzene solution of harmidine a solution of picric acid in methanol was added, when a yellow microcrystalline precipitate was obtained which soon changed its colour to orange red and on crystallization through moist acetone yielded orange red prismatic rods, m.p. 246°C.

Harmidine Chloroplatinate.—Harmidine hydrochloride was dissolved in water and a 3% solution of H_2PtCl_6 was added to it with good ice

cooling. The resulting precipitate was sucked, washed with water, dried and crystallized through moist acetone, when the chloroplatinate was obtained in orange yellow prismatic rods and needles m.p. 259-60°C., which after drying to constant weight over P_2O_5 at 100°C. *in vacuo* gave: Pt, 23.44. $(C_{13}H_{14}ON_2)_2.H_2PtCl_6$ requires: Pt, 23.33.

Reduction of Harmidine

2 g. of harmidine was suspended in 50 ml. of 2:1 water and concentrated HCl, and 5 g. of zinc dust was gradually added with constant heating on the water bath and occasional shaking till the yellow colour of the reaction mixture disappeared. Unreacted zinc was filtered off, and the clear solution strongly ammoniated. The resulting white precipitate which consisted of the reduced base and $Zn(OH)_2$ was sucked and washed. The reduced base was extracted out by ethyl acetate in the hot; the ethyl acetate solution was washed with water, dried over anhydrous sodium sulphate, filtered and freed of the solvent on the water bath. On crystallization of the residue from alcohol, dihydroharmidine was obtained in colourless needles (1 g.) which melted at 199°C., and gave no depression in melting point on admixture with an authentic sample of tetrahydroharmine. Found after drying to constant weight over P_2O_5 , at 100°C. *in vacuo*: C, 72.06; H, 7.47; N, 13.16; O, 7.51. $C_{13}H_{16}ON_2$ requires: C, 72.19; H, 7.46; N, 12.95; O, 7.40.

Bromination of Harmidine

Bromination in Chloroform Medium.—0.5 g. of harmidine was dissolved in 30 ml. dry chloroform with the help of 2-3 drops of glacial acetic acid, and 23.4 ml. of 1.6% bromine in chloroform was slowly run into the ice-cooled solution with constant shaking. The reaction mixture was kept in the cold overnight, and the orange crystalline deposit (needles and rods) of the bromo product was collected and washed with chloroform, m.p. 178°C. A further crop of the bromo product was obtained on diluting the mother liquor with ether (total yield, 0.42 g.). Found on drying to constant weight over P_2O_5 at 60°C. *in vacuo*: C, 40.11; H, 3.99; N, 6.98; Br, 40.65. $C_{13}H_{14}ON_2.Br_2.H_2O$ requires: C, 39.95; H, 4.08; N, 7.12; Br, 40.82.

When refluxed with alcohol for 5 to 10 minutes, it was converted into yellow coloured needles which melted at 252°C. and also analysed for $C_{13}H_{14}ON_2.Br_2.H_2O$, after drying to constant weight over P_2O_5 , at 60°C. *in vacuo*. Found: C, 41.28; H, 4.19; N, 6.71; Br, 40.27. Required: C, 39.95; H, 4.08; N, 7.12; Br, 40.28.

Bromination in Acetic Acid Medium.—To a solution of 0.5 g. of harmidine in 10% acetic acid was added with ice cooling a solution of 1.548% bromine prepared by dissolving 1.548 g. bromine in 5 g. glacial acetic acid and then diluting it with water. After the drop-by-drop addition of 24.2 ml. (corresponding to 2 atoms of bromine) with constant shaking, the reaction mixture was allowed to stand in the cold. The greenish yellow glistening needles of the bromo product which soon started to form, were collected, washed with cold water and dried on porous plate, m.p. 252°C. (yield nearly theoretical). Found after drying to constant weight over P_2O_5 , at 60°C. *in vacuo*: C, 40.06; H, 4.19; N, 7.41; Br, 40.58. $C_{13}H_{14}ON_2.Br_2.H_2O$ requires: C, 39.95; H, 4.08; N, 7.12; Br, 40.82.

Bromination in Dilute Sulphuric Acid Medium.—The addition of 2 atoms of bromine in the acetic acid medium used in the preceding experiment to a solution of 0.5 g. harmidine in 5% sulphuric acid gave a bright yellow crystalline bromo product (needles) which melted at 252°C. Found on drying to constant weight over P_2O_5 at 60°C. *in vacuo*: C, 40.74; H, 4.09; N, 7.03; Br, 40.74. $C_{13}H_{14}ON_2.Br_2.H_2O$ requires: C, 39.95; H, 4.08; N, 7.12; Br, 40.82.

The bromo product as prepared in the various mediums was fairly soluble in water, methanol and ethanol, less soluble in chloroform, and practically insoluble in ethyl acetate, ether and petroleum ether.

Monobromoharmidine.—On treatment of the bromo product ($C_{13}H_{14}ON_2.Br_2.H_2O$) with 10% NH_4OH , a white crystalline base was obtained which on recrystallization through methanol formed prismatic rods of monobromoharmidine, m.p. 220°C., readily soluble in methanol and ethanol, less so in benzene, sparingly soluble in ethyl acetate and nearly insoluble in ether and petroleum ether. Found on drying to constant weight over P_2O_5 , at 60°C. *in vacuo*: C, 53.37; H, 4.59; N, 9.73; Br, 26.99. $C_{13}H_{14}ON_2.Br$ requires: C, 53.06; H, 4.76; N, 9.52; Br, 27.21.

DEMETHYLATION OF HARMIDINE—HARMIDOL

0.5 g. finely powdered harmidine was refluxed on a sand bath for about 15 minutes, after which the heating was continued on a boiling water bath till crystallization started, when it was allowed to stand in the cold. The crystallize was sucked, washed with a dilute solution of KI, and recrystallized through water after charcoaling. The hydroiodide thus obtained gave on treatment with NH_4OH an orange-coloured phenolic base which crystallized in golden yellow prismatic rods from

methanol, and was readily soluble in dilute alkali. The (demetho) base, which has been named as 'harmidol', melted after a few crystallizations from the same solvent at 259-60°C. as against m.p. 212°C. recorded for harmalol. It is fairly soluble in acetone and alcohol, less so in ethyl acetate, sparingly soluble in benzene and ether, and nearly insoluble in petroleum ether. After drying to constant weight over P_2O_5 , at 100°C. *in vacuo* it analysed for $C_{12}H_{12}ON_2$. Found: C, 71.56; H, 6.19; N, 14.09; O, 7.99. Required: C, 71.98; H, 6.04; N, 13.99; O, 7.99.

When crystallized through dilute methanol, harmidol formed golden yellow prismatic rods of the hydrate, which frothed up at 135°C., melted down at 259-60°C., and analysed after drying in air for $C_{12}H_{12}ON_2 \cdot 3H_2O$. Found: C, 57.20; H, 7.02; N, 10.59. Required: C, 56.96; H, 7.09; N, 11.02.

Salts of Harmidol

Harmidol Hydrochloride.—The hydrochloride of the base was obtained on bringing the reactants together in alcoholic medium and precipitating the salt with the addition of ether. It is readily soluble in water and methanol, less so in alcohol, and crystallizes through these solvents in yellow coloured prismatic rods and plates m.p. 265-267°C. Found after drying to constant weight over P_2O_5 , at 100°C. *in vacuo*: Cl, 14.95. $C_{12}H_{12}ON_2 \cdot HCl$ requires: Cl, 15.10.

Harmidol Picrate.—It was obtained on adding a dilute aqueous solution of picric acid to 1% acetic acid solution of the base. It is fairly soluble in moist acetone and methanol, sparingly in water in the hot, and crystallized from these solvents

in aggregates of orange coloured prismatic rods and plates, melting at 249-50°C.

Harmidol Chloroplatinate.—The chloroplatinate was prepared by dissolving the hydrochloride of the base in water and treating it with an excess of 3% solution of H_2PtCl_6 in water. It crystallized from moist acetone in orange coloured prismatic rods and melted at 294-95°C. Found on drying to constant weight over P_2O_5 , at 100°C. *in vacuo*: Pt, 23.56. $(C_{12}H_{12}ON_2)_2 \cdot H_2PtCl_6$ requires: Pt, 24.1.

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