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A REINVESTIGATION OF THE CONSTITUENTS OF PEGANUM HARMALA

SALIMUZZAMAN SIDDIQUI AND RASHID KEMAL

Central Laboratories, Pakistan Council of Scientific and Industrial Research, Karachi

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In the preceding communication of this series,¹ the isolation of a new alkaloid, harmidine, $C_{13}H_{14}ON_2$ (m.p. 257–58°C.), was reported as the major alkaloidal constituent of the seeds of *Peganum harmala*, with an yield of 1.5%. On the basis of comprehensive studies on this base and its demethylation product, it was further shown to be structurally related to harmine as its dihydro derivative. In view of these facts and the eventual separation of basic fractions obtained in the process of isolation with m.p. 235° to 240°C. into harmidine and harmine, it was suggested that harmaline and its demethylation product, harmalol, the recorded melting points of which are 18°C. and 47°C. lower than those of harmidine and harmidol, respectively, should have little justification for being treated as uniform chemical entities. For a further clarification of this point, which was all the more necessary because of the reported synthesis of harmaline by Perkin, Robinson and Manske² and later by Spencer³ (m.p. 239–40°C.), examination of the seeds of *Peganum harmala* from other sources was carried out, as a check-up on possible variations in the nature of their alkaloidal constituents, due to differences in the soil and climatic conditions.

In the course of the present work, seeds obtained from India, Iraq, Morocco, and from a commercial source in Germany (through the courtesy of Messrs. Paul Muggenburg) were examined, but in each case the basic fractions melting around 235° to 240°C. could be separated into harmidine and harmine through careful fractional precipitation from their acetic acid solutions with ammonia. The present paper records the results of this study, giving comparative yields of harmidine and harmine in the seeds obtained from various sources, and the isolation of a new crystalline constituent, melting at 295–96°C. This product which was isolated from two bulk workings of seeds from West Pakistan, has been provisionally named as

pegaline. Studies in this constituent which are being further extended, indicate that it is a new amino acid corresponding to a homologue of hydroxy-proline.

Experimental

250 g. seeds of *Peganum harmala* obtained from Messrs. Paul Muggenburg, Germany, were ground to 30 mesh and extracted six times with ethanol at room temperature, and the combined extracts freed of the solvent under reduced pressure below 40°C. The dark red viscous residue was divided between water and petroleum ether whereby hardly any insoluble residue was left over. The ethereal layer containing the fatty material was not pursued in these studies. The aqueous layer was freed of the residual petroleum ether under reduced pressure, cooled and cottoned. The dark reddish solution thus obtained had pH 4.5. On bringing its pH to 6.5 with dilute ammonia and addition of ammonium sulphate, a dark brown spongy precipitate was thrown out which was cottoned off. The precipitate was repeatedly extracted with 2% acetic acid and the small quantity of a blackish, sticky, insoluble matter was neglected. The acetic acid extract was combined with the main solution which was heavily charcoaled and treated with excess of potassium iodide, whereby the sparingly soluble hydroiodide of the alkaloids was obtained as a reddish brown crystallisate, which was sucked and repeatedly washed with a dilute solution of potassium iodide (18 g.). On recrystallisations from dilute methanol in the hot with charcoaling, the iodide was obtained in the form of golden yellow glistening crystals. It was found necessary in the course of this work, to initially purify the alkaloids of coloured impurities, in the form of their iodides rather than as free bases, which have a tendency to get coloured in the process of repeated crystallisation through organic solvents. The iodide was then

treated with 10% ammonia and the liberated base sucked, washed and dried (9.6 g.). The base was dissolved in a minimum quantity of dilute acetic acid, the solution was charcoaled and filtered, and its pH brought up to 7.4 with dilute ammonia. On heating the clear reddish yellow solution in the water-bath it deposited a nearly colourless crystallisate which was sucked and washed with water. The pH of the solution which had gone down to 7.2 was again brought up to 7.4 and the process repeated. Subsequently, this operation was carried out after adjusting the pH to 7.5, 7.6 and finally above 8. Table 1 gives the comparative melting points of the crystallisates obtained at various pH in the course of this operation.

TABLE 1.

No. of fraction	pH	Melting point	Yields from 9.6 g. base
1	7.2	264°C.	1.7 g.
2	7.4	260°C.	1.2 g.
3	7.5	242°C.	1.4 g.
4	7.6	250°C.	1.6 g.
5	Above 8	252°C.	2.4 g.

The weakly basic fractions melting at 260°C. and 264°C. were taken together and crystallised through a methanol benzene (1:1) mixture with charcoaling, when they gave colourless prismatic rods and needles of harmine melting at 266°C., 2.8 g.—1.11% on the weight of seeds.

The fractions melting in the range 250–52°C. on similar treatment gave harmidine m.p. 257–58°C., 4.6 g.—1.84%.

When fraction 3 noted in Table 1 with m.p. 242°C. was subjected to fractional precipitation with ammonia, following the procedure described above, it yielded a small quantity of harmine along with harmidine, and again a small quantity of low melting product m.p. 238–40°C., when worked according to the described procedure. Table 2 below gives the percentage of the two bases in seeds from the noted sources.

TABLE 2.

Source of seeds	Percentage of harmine	Percentage of harmidine
India	0.9	1.7
Iraq	1.0	1.8
Morocco	0.7	1.5
Commercial source in Germany	1.1	1.8

Isolation of a New Amino Acid C₆H₁₁O₃N (Pegaline).—In the course of this series of investigations with bulk quantities of *Peganum harmala* seeds (loc. cit.), it was observed that when the alcoholic extracts of the seeds were allowed to stand over a period of 1 to 2 weeks after concentration, they deposited a sizeable quantity of a reddish brown crystallisate. In one of these workings, 50 g. of the crude crystallisate were obtained from the first and the second alcoholic extract of 20 kg. of seeds. The crystallisate was sucked and washed with petroleum ether to remove the adhering fatty matter. It was then dissolved in hot water and heavily charcoaled to remove coloured impurities. The resulting greenish yellow fluorescent solution, when concentrated and cooled, gave from the first crop of crystals a product which could be identified with harmine hydrochloride. The tail fractions from this process of fractional crystallisation finally yielded a product which melted at 295–96°C., and has been provisionally named as pegaline.

In another working, the defatted crystalline deposit was taken up in hot water, charcoaled and treated with dilute ammonia to precipitate out harmine. The filtrate was freed of the solvent *in vacuo*, and the residue was taken up in dilute alcohol and kept in the cold. The resulting crystallisate, when recrystallised twice from the same solvent, gave a product melting at 295–96°C., the melting point remaining unchanged on further crystallisation from dilute methanol or ethanol, and showing no depression in m.p. on admixture with pegaline as obtained according to the preceding operation.

Pegaline is readily soluble in water, very sparingly in methanol and ethanol even in the hot, and insoluble in other bench solvents. It gave a yellow spot with ninhydrin, indicative of its amino acid character corresponding to the behaviour of prolines. When thoroughly rubbed with zinc dust and heated over a free flame the vapours gave a red stain to a pine splinter moistened with dilute HCl, indicating a pyrrole nucleus in it. It shows m.p. 295–304°C. depending on the rate of heating.

Its uniformity was established through paper chromatography, and its R_f value found to be 0.23 in butanol-acetic acid-water mixture (4:1:5) as against 0.25 and 0.203, the corresponding values for proline and hydroxy proline, respectively. On slight warming with barium hydroxide it gave off ammonia. On treating with nitrous acid it formed a nitroso compound as verified through Liebermann's reaction, but the product could not be studied further due to lack of sufficient material. On drying at 60°C. over P₂O₅ *in vacuo* it analysed

for $C_6H_{11}O_3N$, found C, 49.52; H, 7.53; N, 9.83; O, 33.27. $C_6H_{11}O_3N$ requires C, 49.65; H, 7.58; N, 9.65; O, 33.10.

Copper Salt of Pegaline.—When pegaline (50 mg.) was heated on the water-bath with excess of cupric carbonate suspended in water, the initial greenish solution turned deep blue. After continuing the heating for about 15 minutes, the suspended matter was filtered off and the bluish filtrate freed of the solvent *in vacuo*, finally after addition of a little alcohol to remove the last traces of moisture. The deep blue amorphous residue was extracted out with absolute ethanol, and filtered off from the unreacted material. The filtrate was again freed of solvent and taken up in slightly diluted alcohol, which on standing over-night in the cold deposited transparent blue prismatic plates melting at 235°C. (dec.).

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