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ALKALOIDS OF *ABRUS PRECATORIUS*

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Abstract—Two new alkaloids, viz., methyl ester of *N,N*-dimethyltryptophan metho cation (I) and precatorine (II), were isolated from the seeds of *Abrus precatorius* L. (Leguminosae Lotoideae). In addition, abrine, hypaphorine, and two previously uncharacterized bases, now identified as choline and trigonelline (III), were isolated from the seeds. Other parts such as the leaves, stems and roots also furnished the above bases in varying proportions. This is the first report of the occurrence in Nature of trigonelline as the gallic acid ester (II).

INTRODUCTION

Abrus precatorius L. (Leguminosae—Lotoideae) is distributed throughout the greater part of India ascending the outer Himalayas to 3500 ft. Almost every part of the plant has been used in indigenous medicine.[1,2] The leaves are said to remove biliousness, cure leucoderma, itching and skin diseases; the roots are emetic, alexiteric and the seeds are used as tonic in nervous disorders and antidote to cattle poisoning. Poultice of seeds is used as an abortifocient.

Previous investigations of the alkaloids showed the presence of abrine[3] and hypaphorine[4] in the scarlet seed kernels. Also the occurrence of two other bases, a hygroscopic solid, m.p. 143° and another isolated as the picrate, m.p. 242°, were reported.[4,5] The limited phytochemical evaluation of the species and its reported medicinal uses prompted the present authors to reinvestigate the alkaloidal constituents in all parts of the plant.

RESULTS AND DISCUSSION

An alcoholic extract of the defatted scarlet seeds on graded concentration afforded, in the following order, abrine, methyl ester of *N,N*-dimethyltryptophan metho cation, precatorine and hypaphorine. The minor bases, choline and hypaphorine, remaining in the alcoholic mother liquor were isolated through their reineckates formed at different pH.[6–8]

Attempts to prepare the hydroxide from the methyl ester of *N,N*-dimethyltryptophan metho cation (the anionic moiety could not be identified) resulted in hypaphorine. Acid hydrolysis of the ester also gave hypaphorine. Its identity as (I) was finally established by converting it to the corresponding iodide.[7]

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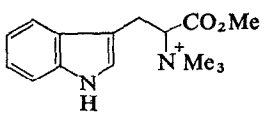
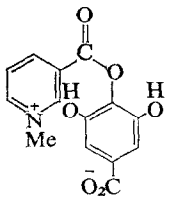
Precatorine, $C_{14}H_{11}NO_6$, fragmented without showing any molecular ion peak in its mass spectrum. Functional group analysis showed one NMe, two H^+, and no CMe or OMe function. It showed u.v. λ_{max} 210–16, 266–68, and 288–92 nm and λ_{min} 238 and 274–76 nm, typical of *N*-methylpyridinium-3-carboxylates.[9] The i.r. bands at λ 2·85, 3·10 (free and bonded OH), 5·95 (α,β-unsaturated CO_2R), 6·05 (CN), 6·30 (CO_2^-) and 13·05 μ (three adjacent aromatic protons) are consistent with the above assignment. The hydrochloride, $C_7H_8NO_2Cl$, of the alkaloid acetate showed u.v. λ_{max} 210–12, 268, and 288 nm; i.r. λ_{max} 3·0 (OH), 5·85 (CO_2H) and 13·05 μ; and the following pmr signals in D_2O. A sharp three proton singlet at 4·45 δ (N^+Me) and four aromatic protons, a one proton triplet at 8·3 δ (J = 6·5 c/s), a two proton multiplet at 8·8–9·1 δ, and a one proton singlet at 9·3 δ. These results revealed its identity as trigonelline hydrochloride.

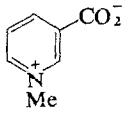
Mild hydrolysis of precatorine gave another product, $C_{14}H_{13}NO_7$, which exhibited similar u.v. spectrum but had i.r. spectrum different from precatorine. The α,β-unsaturated ester band of the parent alkaloid was replaced in the product by a new band at 5·4–5·45 μ due to a methylpyridinium carboxylic acid moiety.[10] Acid hydrolysis of precatorine gave trigonelline salt and gallic acid. Equimolecular mixture of gallic acid and trigonelline furnished trigonelline gallate salt identical in all respects to the mild hydrolysis product of precatorine. The above facts indicate that precatorine is the gallic ester of trigonelline. The hydroxyl group of gallic acid involved in the ester linkage was determined through methylation of precatorine with diazomethane and hydrolysis of the product with hydrochloric acid when trigonelline hydrochloride and 3,5-*O*-dimethyl gallic acid were obtained. Structure (II) was therefore assigned to precatorine. Although trigonelline (III) itself is known to occur in a number of plant families including the Leguminosae, this is for the first time that an ester derivative has been found in nature.



(I)



(II) Precatorine



(III) Trigonelline

The relative per cent yield of the individual bases isolated from the different parts of *A. precatorius* is given in Table 1.

EXPERIMENTAL

All m.ps were determined on a Köfler block, in open capillary, and are uncorrected. Microanalyses were performed by the CDRI, Lucknow and by Dr. A. Bernhardt, Mulheim (Ruhr), Germany.

All parts of *A. precatorius* were extracted and worked up as described below for the seeds. Descending paper chromatography was performed on Whatman No. 1 paper using *n*-BuOAc–*n*-BuOH–HOAc–H_2O (85:15:20:40), the alkaloids being detected with the Dragendorff and Ehrlich reagents.

[9] P. BLADON, in *Physical Methods in Organic Chemistry* (edited by J. C. P. SCHWARZ), p. 126, Oliver & Boyd, Edinburgh (1964).

[10] K. NAKANISHI, *Infrared Absorption Spectroscopy*, p. 194 Holden-Day, San Francisco (1962).

TABLE 1. RELATIVE % YIELD OF THE ALKALOIDS IN *Abrus precatorius*

Components	Scarlet seeds	Leaves and Stems	Roots	Pods
Abrine	70	55	78	Nil
Hypaphorine	7	10	7	Nil
Precatorine	11	12	11	Nil
Trigonelline	traces	5	Nil	100
Methyl ester of *N,N*-dimethyltryptophan metho cation	2	Nil	Nil	Nil
Choline	10	18	4	Nil

Isolation of alkaloids from the scarlet seeds. Dried and finely ground seeds (2 kg) were defatted with benzene and then extracted with alcohol under reflux in a Soxhlet for 16 hr. The alcoholic solution was concentrated (500 ml) under reduced pressure to give a cream coloured solid (18 g).

Abrine. The solid material crystallized from aqueous alcohol as colourless needles, m.p. 295° (dec.) (lit.[3] m.p. 295°); R_f, 0·45; Dragendorff, negative; Ehrlich, purple; u.v. max 220–24, 277, 290–92 nm; i.r. bands at 2·95 (NH), 3·58 (NMe), 5·88 μ (CO_2H). The picrate crystallized from alcohol as orange needles, m.p. 194° (lit.[3] m.p. 195°). (Found: N, 15·55. Calc. for $C_{12}H_{14}N_2O_2$, $C_6H_3N_3O_7$: N, 15·65%.)

Methyl ester of N,N-*dimethyltryptophan metho cation.* Further concentration of the alcoholic solution, after the separation of abrine, to *ca.* 250 ml afforded methyl ester of *N,N*-dimethyltryptophan metho cation (0·52 g) as straw coloured amorphous solid. It crystallized from MeOH as cream coloured needles, m.p. 272° (dec.); R_f, 0·52; Dragendorff, orange; Ehrlich, purple. (Found: C, 61·72; H, 5·39; OMe, 4·21; NMe, 11·30; H^+, 0·8.)

Methyl ester of N,N-*dimethyltryptophan methiodide.* The above compound was dissolved in EtOH (48 mg in 50 ml) and was passed through a column of De-Acidite E (I^-). Elution with EtOH furnished three fractions (20 ml). The solvent was removed and the residue crystallized from aqueous alcohol (1:1) as straw coloured needles, m.p. 200–202°. The compound was found to be identical in all respects (mixed m.p. superimposable i.r. spectra and R_f) with the methyl ester of *N,N*-dimethyltryptophan methiodide, m.p. 200–202°, prepared by refluxing hypaphorine with MeI in MeOH for 8 hr.

Hydrolysis of the ester. The above ester (220 mg) was hydrolyzed with 6NHCl (15 ml) for 30 min on a steam bath. The product was cooled in ice and was treated with a saturated aqueous solution of ammonium reineckate. The precipitated reineckate complex was filtered, the residue taken in acetone, and the acetone solution was passed through a column of De-Acidite FF (pH, 8) which was eluted with EtOH. The solvent was removed and the residue crystallized from alcohol as colourless needles, m.p. 260–262°. It was identified as hypaphorine, m.p. 260–261°, from mixed m.p. which remained undepressed, co-chromatography, R_f, 0·47 and superimposable i.r. spectra, λ_{max} (Nujol) 2·95 (NH) and 6·28 (CO_2^-) μ.

Precatorine. The alcoholic mother liquor, after separation of methyl ester of *N,N*-dimethyltryptophan metho cation, was further concentrated (to *ca.* 100 ml) and kept at room temp. for about 2 weeks when a cream coloured amorphous material (2·3 g) separated. It crystallized from methyl alcohol as needles, m.p. 218–220°, R_f, 0·14, Dragendorff, orange; Ehrlich, negative. ($C_{14}H_{11}NO_6$ requires: C, 58·13; H, 3·80; N, 4·84. Found: C, 57·84; H, 4·01; N, 4·93%.) The acetate hydrochloride crystallized from MeOH as needles, m.p. 245–248°. A mixed m.p. with trigonelline hydrochloride, m.p. 250–251°, remained undepressed. Co-chromatography with authentic trigonelline hydrochloride showed a single spot. R_f, 0·14. (Found: C, 48·85; H, 4·72; N, 7·89; $COCH_3$, 1·45. Calc. for C_7H_8NOCl: C, 48·41; H, 4·61; N, 8·07%.)

Mild hydrolysis of precatorine. Precatorine (0·4 g) in water (25 ml) was boiled for 10 min. The solvent was removed under reduced pressure and the residue crystallized from MeOH as cream coloured needles, m.p. 206–208°; u.v. λ_{max} (EtOH) 212–216, 263–265 and 288–292 nm; mixed m.p. with precatorine, m.p. 218–220°, was depressed (195–203°). ($C_{14}H_{13}NO_7$ requires: C, 54·72; H, 4·23; N, 4·56. Found: C, 54·40; H, 4·20; N, 4·30 per cent.)

Trigonelline gallate. Trigonelline (0·1 g), gallic acid (0·12 g) and MeOH (25 ml) were boiled together for about 15 min. The solution was then kept at room temperature when cream coloured needles, m.p. 208°, separated. The salt was identical in all respects with the mild hydrolysis product of precatorine, m.p. 206–208°.

Acid hydrolysis of precatorine. Precatorine (0·18 g) in 10 ml 2% HCl was heated on a steam bath for 30 min. The solution was cooled and extracted with ether (25 ml × 3). The organic layer was washed with water, dried, and the solvent was removed. The residue crystallized from water as colourless needles, m.p. 248°; M^+, *m/e* 170; significant peaks at *m/e* 153 and 125. Mmp with gallic acid, m.p. 248°, remained undepressed. The trimethyl ether crystallized from aqueous alcohol as needles, m.p. 166°. Mixed m.p. with an

authentic sample, m.p. 165–166°, remained undepressed. The aqueous acidic layer from the above hydrolysis was evaporated to dryness. The residue crystallized from MeOH as needles, m.p. 250–251°. Mixed m.p. with trigonelline hydrochloride remained undepressed.

Methylation and hydrolysis of precatorine. Precatorine (0·2 g) Et_2O (25 ml) was treated with CH_2N_2 in Et_2O till the mixture retained yellow colour after 24 hr. The mixture was kept at room temp. for 1 week. The solvent was removed and the gummy residue left was treated with a few drops of 1N-HCl. The product was diluted with water (20 ml) and then extracted with ether. The ether layer afforded 3, 5-*O*-dimethylgallic acid, m.p. 204–205° (lit.[11] 204–205°); M^+, *m/e* 198. The 4-acetyl derivative crystallized from alcohol as needles, m.p. 191°. (Found: C, 54·32; H, 4·98. Calc. for $C_9H_{10}O_5$: C, 54·54; H, 5·05%.)

The aqueous acidic mother layer, after the separation of 3,5-*O*-dimethylgallic acid, when processed in the usual way furnished trigonelline hydrochloride, m.p. 248–250°.

Choline. An aliquot (10 ml) of the alcoholic concentrate, after the separation of precatorine, was evaporated to dryness. The gummy residue was taken in water (50 ml), and treated with ammonia (pH 8). The suspended impurities were filtered and the clear filtrate was treated with a saturated aqueous solution of ammonium reineckate. The precipitated reineckate complex was filtered, the residue washed with water, and dried. Regeneration of the base from the reineckate complex (5·9 g) in the usual way[8] furnished a hygroscopic solid (2·4 g); R_f, 0·18; Dragendorff, pink; Ehrlich, negative. The pharmacological properties of the base were identical to that of choline.[12] A portion of the base was taken in alcohol and was treated with a saturated solution of picric acid in the same solvent. The picrate crystallized from alcohol as orange needles, m.p. 240–242° (lit.[8] m.p. 240–242°). (Found: N, 16·34. Calc. for $C_5H_{14}NO,C_6H_3N_3O_7$: N, 16·81%.)

Hypaphorine. The mother liquor, after the separation of choline reineckate, was cooled in ice, acidified with H_2SO_4, and was treated with an excess of ammonium reineckate solution at room temperature for 4 hr. The precipitated complex was filtered, washed with water and dried. The residue (4·3 g) was processed in the usual way[8] for liberating the alkaloid when hypaphorine was obtained as colourless needles, m.p. 260–261°.

The base hydrochloride crystallized from alcohol as needles, m.p. 232°. Mixed m.p. with an authentic sample, m.p. 232°, remained undepressed. (Found: N, 9·85. Calc. for $C_{14}H_{19}N_2O_2Cl$: N, 9·99%.)

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