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NEW NATURAL PRODUCTS FROM *PEGANUM HARMALA*

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Key Word Index—*Peganum harmala*; Zygophyllaccae; harmol; 5-hydroxytryptamine; 6-hydroxytryptamine; 8-hydroxyglucosylharmaline; gentisate-2,5-di-β-D-glucoside.

Abstract—Dihydroruine (8-hydroxyglucosylharmaline), a partially characterized phenolic dihydro-β-carboline, called YC2, and gentisate-2,5-di-β-D-glucoside have been isolated as minor components of *Peganum harmala*. Harmol, 5- and 6-hydroxytryptamine are reported for the first time from *P. harmala*.

INTRODUCTION

Previously we reported the isolation and characterization of ruine [1] (8-hydroxyglucosylharmine) from both seedlings and callus derived from roots and hypocotyls of *Peganum harmala*. The present paper deals with a detailed investigation of other secondary metabolites found in *P. harmala* seeds, seedlings and callus.

RESULTS AND DISCUSSION

The major β-carboline alkaloids which are known to occur [2] in *Peganum harmala*, harmine, harmaline and harmalol, are also the major alkaloids in the seeds, seedlings and callus grown on auxin-deficient medium [3]. Careful examination by TLC of methanolic extracts of callus [3] and the other plant material showed the presence of several minor components (Table 1).

Callus was grown on two media [3] which differed by the presence or absence of the auxin 2,4-D. Callus grown on auxin-deficient medium produced all the secondary metabolites reported here but callus grown on medium containing auxin produced only gentisate glucoside.

Harmol has not previously been reported in *P. harmala*. It is possible that harmol could have been produced artefactually by aerial oxidation of harmalol during extraction but this was excluded by the presence of harmol in the roots of differentiated callus i.e. from callus grown on auxin-deficient media crushed directly onto a TLC plate, and in extracts prepared rapidly at 0–4°. Dihydroruine (8-hydroxyglucosylharmaline) and the partially characterized alkaloid "YC2" are both new alkaloids. Dihydroruine is found in callus grown under conditions which favour the accumulation of harmaline, i.e. in callus grown in the light. YC2 had a UV spectrum typical of a phenolic dihydro-β-carboline, but could not be dehydrogenated, in marked contrast to other dihydro-β-carbolines. Its

Table 1. New natural products from *Peganum harmala*

	Seeds	Seedlings	Callus grown on medium with Auxin	Callus grown on medium with No auxin
Harmol	−	+	−	+
Dihydroruine	−	+	−	+
"YC2"	−	+	−	+
5-Hydroxy-tryptamine	−	−	−	trace
6-Hydroxy-tryptamine	+	+	−	+
Gentisate 2,5-β-diglucoside	−	+	+	+

All plant material was derived from seeds supplied by the Jardin Botanique de la Ville, Dijon.

Table 2. Properties of 5- and 6-hydroxytryptamine from *Peganum harmala*

	R_f/X100*	Colour on TLC with sprays: *p*-DMAB†	BB salt‡	λ_{max} (nm) (MeOH)	R_t
5-Hydroxy-tryptamine	36	Purple	Purple	275 (300/sh)	6 min
6-Hydroxy-tryptamine	39	Blue green	Bright red	295 (270/sh)	6 min 54 sec

* $CHCl_3$–MeOH–NH_3 (10:4:1) developed under N_2 in the dark.
† 1 g *p*-dimethylaminobenzaldehyde in 25 ml 10 M HCl, 75 ml MeOH.
‡ 1 g Fast Blue BB salt (diazotized 4-benzoylamino-2,5-diethoxy aniline) in 10 ml MeOH.

polarity indicated a glycoside, but it was resistant to acid hydrolysis, in contrast to ruine and dihydroruine. Neither 5- nor 6-hydroxytryptamine have been found in *P. harmala* before. The major isomer is 6-hydroxytryptamine. Traces of 5-hydroxytryptamine could be detected only on callus grown on medium containing no auxin. 5- and 6-hydroxytryptamine were distinguished from one another by comparison with standards and on the basis of the properties listed in Table 2.

Additional reagents which will distinguish between 5- and 6-hydroxyskatoles [4] give correspondingly the same colour reactions with 5- and 6-hydroxytryptamines. The occurrence of 6-hydroxytryptamine in *P. harmala* is of interest as it is a possible intermediate in the synthesis of the β-carboline alkaloids. It was found only in plant material derived from seed supplied from Dijon. This strain may be exceptional as seed contains 6-hydroxytryptamine and ruine (8-hydroxyglucosylharmine), neither of which are present in seed from Pakistan or from naturalized *P. harmala* from South Australia.

While callus grown on medium containing auxin does not produce alkaloids *de novo*, it can efficiently convert exogenous tryptamine to 5-hydroxytryptamine [5], harmaline to dihydroruine [5] and harmine to ruine [1]. The structural assignments of 5-hydroxytryptamine and dihydroruine reported here are based on material thus prepared.

Gentisate glucoside is produced in variable amounts by callus grown on both media. In the absence of a parent peak its identification as gentisate-2,5-di-β-D-glucoside is tentative. The possibility that it is a β-D-glucosyl derivative of this cannot be excluded.

EXPERIMENTAL

General. TLC was carried out on Merck silica gel plates (Cat. No. 5724 and 5714). A Facts and Method 400 Gas Chromatograph was used for GLC. The column was acid washed Diatoport S, 60–80 mesh, coated with 5% QF-1, with oven temp. at 205°, detector and inlet at 265°. MS measured on an Atlas MS-902 mass spectrometer, using a direct inlet system.

Plant material. Seed of *P. harmala* was donated by the director of the Jardin Botanique de la Ville, Dijon, Dr. D. Liljgren of Adelaide and Dr. A. Kamahl from Pakistan.

Extraction and isolation of natural products. Seeds were ground with sand and EtOH–H_2O (7:3); the extract was filtered, concentrated, and chromatographed as for extracts of callus [3]. Seedlings were extracted in the same manner as callus [3]. Harmine, harmaline, harmolol, harmol and ruine were isolated and identified as described previously [3].

Dihydroruine. MeOH extracts from callus were chromatographed by TLC and developed with $CHCl_3$–MeOH (2:1). Dihydroruine (R_f 0·3) was eluted with MeOH–H_2O–HCO_2H (89:10:1) and re-chromatographed with $CHCl_3$–MeOH–NH_3 (20:8:1), then again with $CHCl_3$–MeOH (2:1). Dihydroruine could not be crystallized. UV spectrum: λ_{max} nm (MeOH ± OH^-) 255 (sh), (MeOH + H^+) 260, 335 (sh), 360, 400 (sh). Dihydroruine readily oxidized in air to ruine (coTLC, UV spectrum) [1]. Acetylation of dihydroruine (Ac_2O pyridine) gave a yellow, major product (R_f 0·6; $CHCl_3$–MeOH, 9:1) which could not be crystallized but could be converted to ruine tetra-acetate [1] (m.m.p.) by aerial oxidation or by dehydrogenation with 5% Pd on $CaCO_3$.

YC2. YC2 isolated (R_f 0·17; $CHCl_3$–MeOH–NH_3, 10:4:1) from auxin-deficient callus had a bright yellow fluorescence, and on TLC plates gave a red to purple colour with diazotized sulphanilic acid (excess HNO_2 removed with ammonium sulphamate). UV spectrum: λ_{max} nm (MeOH) 265, 340; (MeOH + H^+) 265, 380; (MeOH + OH^-) 360. Treatment with $NaBH_4$ gave a non-fluorescent phenolic product (red colour with Fast Blue BB salt and a red brown colour with acidic xanthydrol). UV spectrum: λ_{max} nm (MeOH) 270, 290 (sh). YC2 could be regenerated by heating this product with formaldehyde solution. Insufficient YC2 was obtained to attempt crystallization. It was resistant to acid hydrolysis, and to dehydrogenation either by aerial oxidation or using 5% Pd on $CaCO_3$. YC2 reacted with Ac_2O pyridine, but the main, aqua-fluorescing, yellow product was non-phenolic (no reaction with Fast Blue BB salt) and still quite polar (R_f 0·4 with $CHCl_3$–MeOH–NH_3 10:4:1).

5-Hydroxytryptamine. For identification, 5-hydroxytryptamine was isolated [3] from callus grown on auxin-containing

medium to which tryptamine had been added [5]. It was crystallized as the creatinine . SO_4.

6-*Hydroxytryptamine*. Extracts from seeds (0·1 g) or from callus (40 g) were chromatographed as described for the isolation of hydroxytryptamines from callus [3]. Attempts to crystallize 6-hydroxytryptamine as the creatinine . SO_4 were unsuccessful. The parent peak was mass matched (Found 176·0945; $C_{10}H_{12}N_2O$ requires 176·0949).

*Gentisate-2,5-di-β-*D*-glucoside*. Callus (37 g) grown on auxin-containing medium was freeze-dried and extracted exhaustively with MeOH. The concentrated extract, diluted to MeOH–H_2O (9:1) was exchanged on DEAF-Sephadex A-25 (OH^- form, equilibrated in EtOH–H_2O, 9:1). Fractions were eluted with EtOH–H_2O (9:1), EtOH–H_2O–HCO_2H (44:5:1) and EtOH–H_2O–HCO_2H (81:9:10). The last fraction was neutralized with $(NH_4)_2CO_3$. The yellow oil remaining after evaporation and sublimation of the NH_4CO_2H was chromatographed by TLC ($CHCl_3$–MeOH–NH_3, 9:9:1). A colourless oil (50 mg) was obtained from the blue fluorescent band (R_f 0·6). Fluorescence spectrum λ_{max} nm (MeOH) excitation 315, emission 440; UV spectrum: λ_{max} nm (MeOH ± OH^-) 226 (sh), 305; (MeOH + H^+) 229 (sh), 315. Prominent peaks in the MS show the fragmentation of a dihydroxybenzoic acid [6]: *m/e* 154 (M^+, 59%), 136 (100), 108 (25), 80 (22), 52 (19). Minor peaks attributable to a hexose moiety [7] appear at *m/e* 162, 149, 144, 73 and 60. The whole spectrum is consistent with a phenolic hexoside with a vanishingly small true molecular ion peak [8]. The aglycone, gentisic acid, was obtained by hydrolysis with β-D-glucosidase in 0·05 M citrate buffer, pH 4·9 overnight and identified by comparison with standard gentisic acid (m.p. and m.m.p.). The glucoside could also be hydrolysed by 2 M HCl at 80°. Using DEAE-Sephadex A-25 to neutralize the reaction and to absorb the aglycone, the presence of D-glucose in the unexchanged solution was demonstrated by the action of D-glucose oxidase shown by the peroxidase mediated conversion of tyramine to a fluorescent biphenyl [9].

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