The Biosynthesis of the β-Carboline Alkaloids, Harman and Eleagnine †

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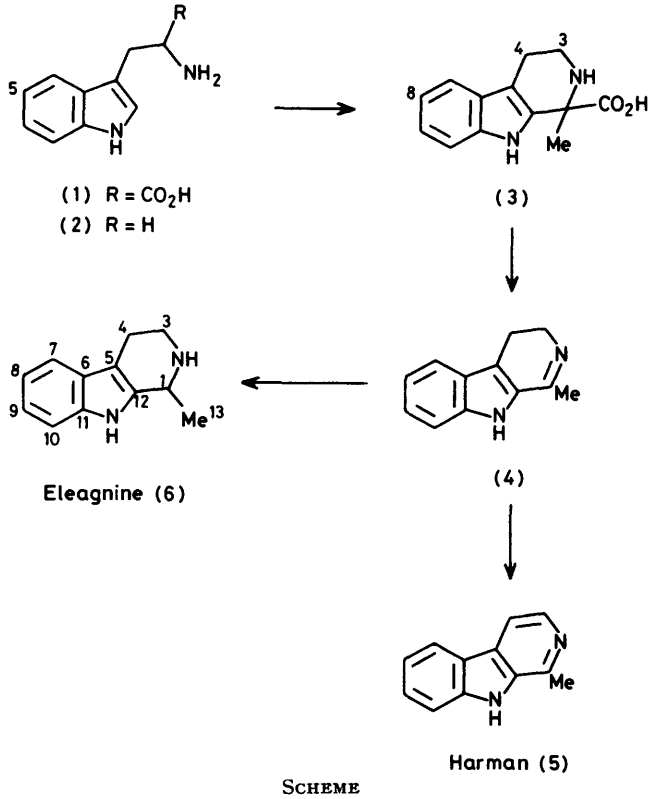
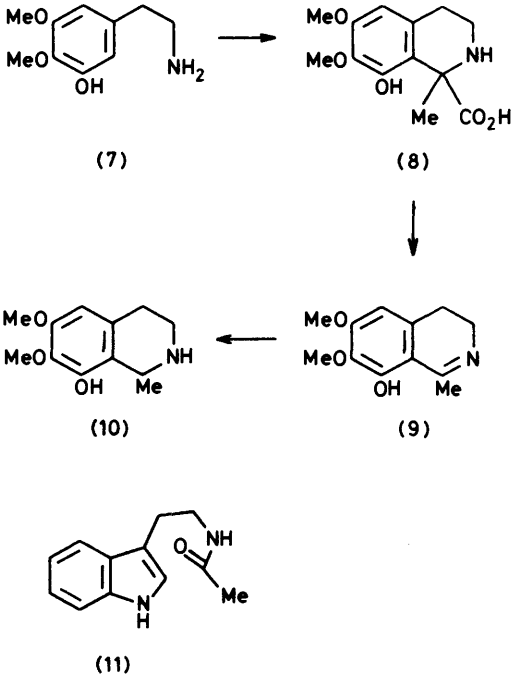
1-Methyl-1,2,3,4-tetrahydro-β-carboline-1-carboxylic acid (3) is shown to be an intact precursor for harman (5) in *Passiflora edulis* and for eleagnine (6) in *Eleagnus angustifolia*; it is a natural constituent of both these plants; (3) is at least an 8-fold better precursor for (5) than is *N*-acetyltryptamine (11).

INSPECTION of the structures of the simple β-carboline alkaloids, harman (5) and eleagnine (6), suggests that they are biosynthesized in part from tryptophan. This has been substantiated by the specific incorporation of DL-[3′-^{14}C]tryptophan [as (1)] into C-4 of eleagnine (6) in *Eleagnus angustifolia*.[1] Tryptophan has also been found to be incorporated into harman (5) in *Passiflora edulis*, as has tryptamine (2).[2] The origin of the remaining two carbon atoms in (5) and (6), and also the course of biosynthesis, is suggested by knowledge of the biosynthesis of isoquinoline alkaloids, most notably anhalonidine (10).[3,4] This alkaloid is formed by condensation of the phenethylamine (7) with pyruvic acid to give the amino-acid (8). Oxidative decarboxylation affords compound (9), reduction of which gives anhalonidine (10). In a parallel sense, the β-carboline alkaloids could arise by condensation of tryptamine with pyruvic acid to give 1-methyl-1,2,3,4-tetrahydro-β-carboline-1-carboxylic acid (3) as a key intermediate. This hypothesis is argued against, however, by the report that *N*-acetyltryptamine (11), labelled in the acetyl group, is a specific precursor for harman (5) in *P. edulis* and that it is a natural constituent of this plant.[2] On the other hand, *N*-acetyltryptamine (11) was found neither to be a precursor for eleagnine (6) in *E. angustifolia* nor a natural constituent of this plant.[5] We set out to resolve uncertainties about β-carboline biosynthesis by examining the possible biosynthetic role of the amino-acid (3) in *P. edulis* and *E. angustifolia*.



SCHEME

1-Methyl-1,2,3,4-tetrahydro-β-carboline-1-carboxylic acid (3) was synthesized as described earlier from tryptamine and pyruvic acid.[6] Material for feeding experiments was prepared with a tritium label in the benzene ring and a ^{14}C label on the methyl group to provide a sensitive monitor for intact incorporation.



The amino-acid (3) was found to be a significantly better precursor than tryptophan for harman (5) and for eleagnine (6) (Tables 1 and 2). Maintenance of isotope ratio in the alkaloids formed established that the precursor was incorporated intact. This was true for

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eleagnine only with the highest of three incorporations recorded (Table 2, Experiment 4). The isotope ratio showed a change from precursor to alkaloids in the other two results obtained (Experiments 2 and 3), the larger change being associated with the lower incorporation (Experiment 2). This correlation of isotope ratio with efficiency of incorporation emphasises the sensitivity of the double labelling experiment. In both cases the compound (3) and it has been found to be a precursor for both harman (5) and eleagnine (6).[2,5] It may be concluded that the biosynthesis of these alkaloids parallels that of isoquinolines and is as shown in the Scheme.

The ^{13}C n.m.r. spectra of harman (5) and eleagnine (6) were obtained and the signals for the latter were assigned from literature values[7] and off-resonance decoupled spectra (see Experimental section).

TABLE 1

Incorporation of precursors into harman (5) in *P. edulis*

Experiment	Substance fed	Activity (μCi)	$^3H/^{14}C$	Metabolite isolated	Incorporation (%)	$^3H/^{14}C$
1	L-[5-^{3}H]Tryptophan	100	—	(5)	0.002	—
2	(3) [a] 17 μM	20.8	4.7	(5)	0.50 [b]	4.3
3	(3) [a] 14 μM	14.3	6.8	(5)	1.56 [b]	6.6
4	L-[5-^{3}H]Tryptophan	158	—	(5)	0.002	—
				(3)	0.014	—
5	L-*N*-Acetyl[5-^{3}H]tryptamine 7.4 μM	9.0	—	(5)	0.065	—
	(3) [c] 20 μM	1.3		(5)	0.51	—

[a] 1-[^{14}C]Methyl-1,2,3,4-tetrahydro[8-^{3}H]-β-carboline-1-carboxylic acid. [b] Value for ^{14}C. [c] As [a] but ^{14}C label only.

TABLE 2

Incorporation of precursors into eleagnine (6) in *E. angustifolia*

Experiment	Substance fed	Activity (μCi)	$^3H/^{14}C$	Metabolite isolated	Incorporation (%)	$^3H/^{14}C$
1	L-[5-^{3}H]Tryptophan	129	—	(6)	0.06	—
				(3)	0.19	—
2	(3) [a] 12 μM	6.7	2.9	(6)	0.04 [b]	3.8
3	(3) [a,c] 44 μM	25.2	13.2	(6)	0.15 [b]	14.5
4	(3) [a] 40 μM	24.2	15.1	(6)	0.27 [b]	15.8

[a] 1-[^{14}C]Methyl-1,2,3-tetrahydro[8-^{3}H]-β-carboline-1-carboxylic acid. [b] Value for ^{14}C. [c] Experiment with cut branches.

change in isotope ratio was associated with an increase of tritium relative to ^{14}C. This may be attributed to catabolism of compound (3) to [^{3}H]tryptamine which was then re-used for alkaloid synthesis with consequent increase in the measured tritium to ^{14}C ratio.

Two further sets of results indicate strongly that compound (3) is a normal intermediate in the biosynthesis of eleagnine (6) and harman (5). Firstly, [5-^{3}H]tryptophan was fed to the two plants and, during work-up, inactive amino-acid (3) was added. This material was then re-isolated and, in both plants, was found to be substantially radioactive showing that compound (3) is a normal plant constituent (Table 1, Experiment 4; Table 2, Experiment 1). Secondly, the β-carboline-acid (3) was found to be an eight-fold better precursor than *N*-acetyltryptamine (11) for harman (5) (Table 1, Experiment 5). The very low incorporation of the latter indicates that it may have been utilized only by prior conversion into tryptamine. The original positive results with *N*-acetyltryptamine (11) are difficult to understand, but we noted that the preparation, and handling, of *N*-acetyltryptamine can produce small amounts of harmalan, a β-carboline precursor.

Harmalan (4) is the logical decarboxylation product of

EXPERIMENTAL

L-[5-^{3}H]Tryptophan and [3-^{14}C]pyruvic acid were purchased from The Radiochemical Centre, Amersham. Radioactivity was measured using a Packard 300 CD scintillation counter. Plant metabolites were recrystallized to constant radioactivity. Eleagnine (6) was synthesized as described.[8] Dehydrogenation (*cf.* ref. 9) gave harman (5). Unless otherwise stated whole plants were fed with aqueous solutions of the precursors, take-up being through wicks in the base of the stems; isolation of metabolites was after 10 d. In the single experiment with cut branches of *E. angustifolia* the branches were allowed to stand in an aqueous solution of the precursor; isolation of eleagnine (6) was after 4 d.

[5-^{3}H]*Tryptamine*.—An excellent published procedure for the preparation of unlabelled tryptamine was followed.[10] To DL-tryptophan (25 mg) and L-[5-^{3}H]tryptophan was added diphenyl ether (1.25 ml) and the mixture was refluxed for 15 min. After the mixture had been cooled, diethyl ether (1.25 ml) was added to it and it was extracted with 2M hydrochloric acid (3 × 2 ml). The extract was basified with 6M aqueous sodium hydroxide and extracted with diethyl ether (4 × 3 ml). The ether solution was dried ($MgSO_4$) and filtered, and hydrogen chloride was passed into it. The precipitate was collected and recrystallized from methanol–ether to give [5-^{3}H]tryptamine

hydrochloride (13 mg, 57%), m.p. 247 °C; $\nu_{max.}$ (Nujol) 3 300 cm^{-1}.

N-Acetyl[5-^{3}H]*tryptamine*.—[5-^{3}H]Tryptamine, obtained from [5-^{3}H]tryptophan (50 mg; 300 μCi), and imidazole (5 mg) in acetic anhydride (2 ml) were stirred overnight at room temperature. The mixture was evaporated to dryness and the *N*-acetyl[5-^{3}H]tryptamine was purified by preparative t.l.c. (10% methanol in chloroform plus 5 drops of triethylamine in 100 ml) and h.p.l.c. (Varian 5000, Micropak Si 10 column; ethyl acetate containing 0.2% propan-2-ol). *N*-Acetyltryptamine decomposed slowly when heated or with time when dissolved in an organic solvent. One of the products was harmalan (identified on t.l.c. by its R_F and characteristic u.v. fluorescence).

1,2,3,4-*Tetrahydro*-1-*methyl*-β-*carboline*-1-*carboxylic Acid* (3).—This material was prepared as described,[6] and a similar procedure, employing [5-^{3}H]tryptamine and [3-^{14}C]pyruvic acid, was used to obtain the labelled compound (93%), m.p. 223 °C (lit.,[6] 220 °C); single spot by t.l.c. [methanol–chloroform (1 : 4) + 0.2% acetic acid]; $\nu_{max.}$ (Nujol) 3 550, 3 470, 3 400, 2 900—2 200, and 1 620 cm^{-1}; *m/z*, 230 (M^+, 1%), 186 ($M^+ - CO_2$, 100), 185 (62), 171 (82), and 117 (47) (Found: C, 67.8; H, 6.25; N, 12.2. Calc. for $C_{13}H_{14}N_2O_2$: C, 67.80; H, 6.10; N, 12.20%).

The acid gave a methyl ester hydrochloride,[6] m.p. 133—134 °C; $\nu_{max.}$ ($CHCl_3$), 3 480, 1 740; δ($CDCl_3$) 1.7 (3 H, s), 2.3 (1 H, s, NH), 2.75 (2 H, t, *J* 6 Hz), 3.21 (2 H, t, *J* 6 Hz), 3.77 (3 H, s), 7.0—7.6 (4 H, m), and 8.25 (1 H, broad s, NH).

Isolation of Metabolites.—(*a*) *From* P. edulis. The plants were macerated in methanol containing 2% acetic acid and set aside for 2 d. The mixture was then filtered, and the filtrate was evaporated to dryness. The residue was taken up in 2M sulphuric acid, and the acidic solution was washed with ether and basified with conc. aqueous ammonia. This solution was then extracted continuously with chloroform for 12 h; t.l.c. showed the presence of harman in the extract. The chloroform extract was evaporated to dryness and unlabelled harman (50 mg) was added. The residue was taken up in methanol, then filtered and evaporated; this sequence was repeated in turn with chloroform and benzene. The solid obtained was almost pure harman, and was recrystallized from benzene.

To isolate the amino-acid (3) from *P. edulis* the following procedure was used. The residue obtained as above, after evaporation of the methanol containing 2% acetic acid, was extracted with water. Unlabelled compound (3) was dissolved in this solution and the pH was adjusted to 7.0. The solution was filtered and applied to a column of Amberlite IRC-50 (H^+ form). Compound (3) was eluted from the column with water (500 ml), and the harman was eluted with 2M sulphuric acid. The solution of compound (3) was applied to a column of Amberlite IR-120 (H^+ form), and compound (3) was eluted with 5% aqueous ammonia. Evaporation gave compound (3) as a solid which was recrystallized from methanol.

(*b*) *From* E. angustifolia. The metabolites were isolated as described above under (a); in all cases, the eleagnine was purified by chromatography on Amberlite IRC-50 (H^+ form) and was then recrystallized from benzene–acetone (1 : 1).

^{13}C *N.m.r. Spectra*.—Spectra were obtained using a JEOL FX 90 Q Spectrometer. Harman (5): δ[$(CD_3)_2SO$]: 20.58 (Me), 121.2, 127.0, 134.7, 140.5, 142.4 (quaternary carbons), 112.0, 112.6, 119.2, 121.7, 127.8, and 137.6. Eleagnine (6): δ($CDCl_3$): 20.8 (C-13), 22.7 (C-4), 42.7 (C-3), 48.2 (C-1), 108.4 (C-5), 110.7 (C-10), 118.1 (C-7), 119.3 (C-8), 121.4 (C-9), 127.5 (C-6), 135.7 (C-12), and 137.1 (C-11).

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