Biosynthesis of the Ipecac β-Carboline Alkaloid Tubulosine

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The incorporation of tryptamine, dopamine, strictosidine and vincoside, deacetylisoipecoside, deacetylipecoside, didemethyldeoxytubulosine, and deoxytubulosine into tubulosine in young *Alangium lamarckii* Thw. plants has been studied and a specific utilization of deacetylisoipecoside demonstrated. Results of parallel experiments with didemethyldeoxytubulosine and deoxytubulosine provide some evidence for the late stages of biosynthesis.

Tubulosine (8), an amoebicidal agent, was first isolated by Brauchli *et al.*[1] from the Argentinian plant *Pogonopus tubulosus* (DC) Schumann (Rubiaceae). Subsequently the base was isolated from the South African plant *Cassinopsis ilicifolia* Kuntze (Icacinaceae)[2] and from the root bark of *Alangium lamarckii* Thw. (Alangiaceae).[3]

The ipecac alkaloid cephaeline (10) which, like tubulosine, is produced by *A. lamarckii*, and has a structure closely related to that of (8), has been shown to derive from deacetylisoipecoside (12) in experiments using this plant;[4] similar results were obtained for emetine (11) and cephaeline (10) using *Cephaelis ipecacuanha*.[5] Thus the biosynthesis of tubulosine (8) would be expected to be from deacetylisoipecoside (12).[6] Enzymic hydrolysis of compound (12) and opening of ring c′ could yield the dialdehyde (13) in which at least the stereochemistry at C-1 and C-4′ † is retained. Condensation of the appropriate aldehyde function with the secondary amino group, followed by reduction of the corresponding iminium intermediate, could give rise to the protoemetine skeleton (16). Compound (16) would then react with tryptamine (derived from tryptophan) to furnish the key intermediate (5). Hydroxylation at C-6′ of (5), to give didemethyltubulosine (7), followed by selective *O*-methylation of the *ortho*-phenolic hydroxy groups, could finally give tubulosine (8). On the other hand, the presence of a partial structure corresponding to that of the terpenoid indole alkaloids suggests an alternative pathway beginning with strictosidine (1) which is the universal precursor for terpenoid indole alkaloids.[4,5] However, this pathway is argued against by the close relationship of cephaeline (10) and tubulosine (8) in terms of structure, including stereochemical detail, their co-occurrence in *A. lamarckii*, and the fact that any biosynthetic route from strictosidine (1) to tubulosine (8) must involve inversion of stereochemistry at C-4′ (biosynthetic label: C-15) of strictosidine (1). Thus hydrolysis of (1) followed by opening of the pyran ring and decarboxylation, could afford the dialdehyde (3). Condensation of compound (3) with dopamine (4) would generate the *trans*-quinolizidine moiety. The intermediate (5) thus formed could undergo hydroxylation and *O*-methylation to give tubulosine (8).

Initial feeding of (L)-[U-^{14}C]tyrosine (Table; experiment 1) to young *Alangium lamarckii* Thw. plants demonstrated that tubulosine (8) was biosynthesized by the young plants. Feeding of tryptamine (experiment 2) and dopamine (experiment 3) revealed that both amines were being used by the young plants to form tubulosine (8). Feeding a mixture of [3-^{14}C]strictosidine (1) ‡ and [3-^{14}C]vincoside (2) ‡ (experiment 4) demonstrated that neither (1) nor (2) was utilized by the young plants to form tubulosine (8). [3-^{14}C]Deacetylisoipecoside (12) (experiment 5) when fed to the young plants was, however, efficiently incorporated into tubulosine (8).

Biosynthetic tubulosine (8) derived from [3-^{14}C]deacetylisoipecoside (experiment 5) was heated with acetic anhydride–pyridine to give *N*,*O*-diacetyltubulosine (9) with essentially the same molar radioactivity as the parent base. Treatment of compound (9) with methyl iodide in the presence of sodium methoxide yielded *N*-acetyl-*O*-methyltubulosine methiodide (14) which was converted into its methohydroxide (15) by IR-400 anion-exchange resin. Hofmann degradation of the salt (15) yielded the methine (17) with essentially no loss of radioactivity. Ozonolysis of compound (17) gave radioactive formaldehyde (dimedone derivative, 98% of the original activity).

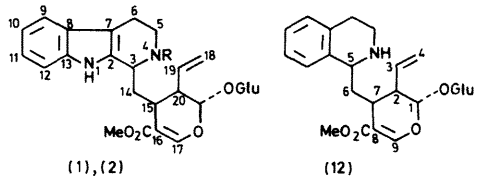
Table. Tracer experiments on *A. lamarckii* Thw.

Experiment	Precursor	% Incorporation into tubulosine (8)
1	(L)-[U-^{14}C]Tyrosine	0.114
2	(±)-[1-^{14}C]Tryptamine	0.11
3	(±)-[1-^{14}C]Dopamine	0.59
4	[3-^{14}C]Strictosidine (1) and [3-^{14}C]Vincoside (2)	0.003
5	[3-^{14}C]Deacetylisoipecoside (12)	3.0
6	[3-^{14}C]Deacetylipecoside	0.21
7	[*aryl*-^{3}H]Didemethyldeoxytubulosine (5)	0.020
8	[*aryl*-^{3}H]Deoxytubulosine (6)	0.09

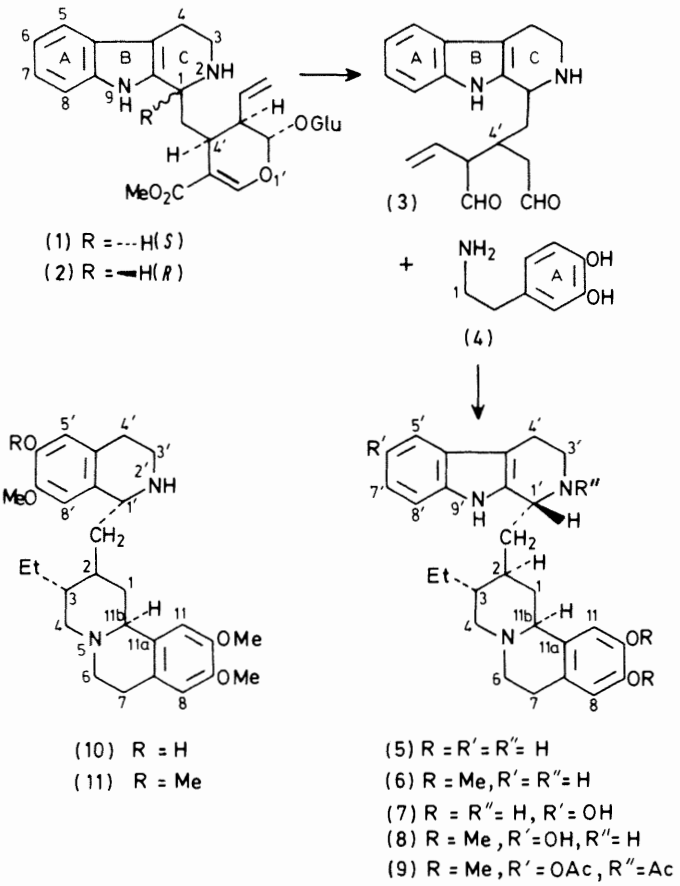
Low incorporations into tubulosine (8) of [*aryl*-^{3}H]didemethyldeoxytubulosine (5) (experiment 7) and of [*aryl*-^{3}H]-deoxytubulosine (6) (experiment 8) were recorded. These results must be regarded as tentative but they do point to the overall biosynthetic sequence to tubulosine as shown below.

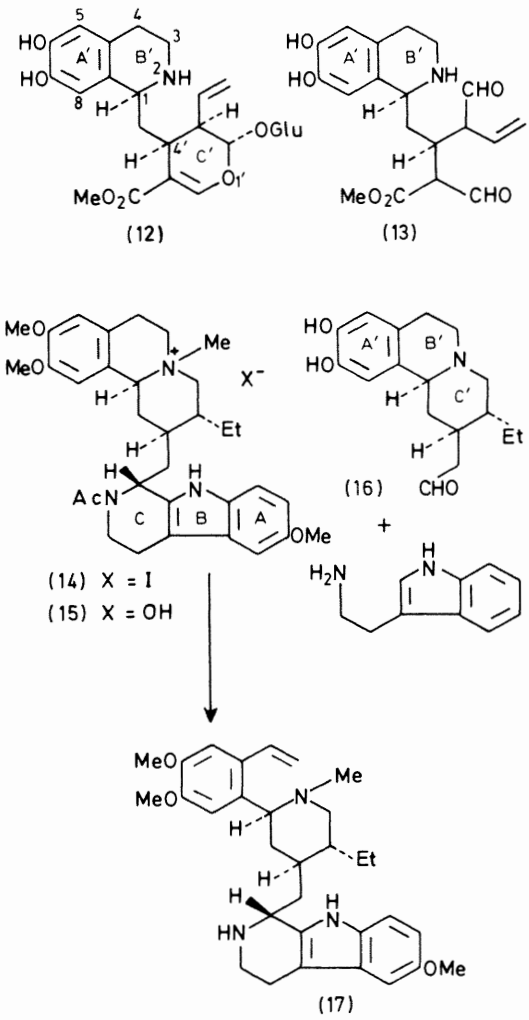
Deoxytubulosine (6)[7] and dihydroprotoemetine[8] have been isolated earlier from *A. lamarckii* Thw. The presence of deoxytubulosine in the plant was again confirmed by feeding (L)-[U-^{14}C]tyrosine (incorporation 0.20%). The foregoing

† Biosynthetic numbering: C-5 and C-7, respectively. Systematic numbering schemes are used in this paper. As a reference for readers, the biosynthetic numbering given in previous publications is reproduced below.



‡ [5-^{14}C]-Labelled compounds in the biosynthetic numbering scheme.





experiments thus support the following sequence for the biosynthesis of tubulosine (8) in young *A. lamarckii* Thw. plants: tyrosine ⟶ dopamine + secologanin ⟶ deacetylisoipecoside (12) ⟶ deoxytubulosine (6) ⟶ tubulosine (8).

Experimental

Unless otherwise stated, u.v. absorption spectra refer to solutions in ethanol, i.r. absorption spectra to KBr discs, and n.m.r. spectra to solutions in deuteriochloroform. The n.m.r. spectra were recorded with a Varian A-60 spectrometer. T.l.c. was carried out, unless specified to the contrary, on silica gel GF 254.

Counting Methods.—Liquid scintillation counting was used for the measurement of ^{3}H and ^{14}C activities (Packard 314 Ex instrument). Samples were counted in the scintillator (7 ml), after dissolution in methanol or dimethyl sulphoxide (DMSO) (0.2 ml), and values are not corrected for self-absorption except where stated. Relative efficiencies were obtained by counting [1,2-$^{3}H_2$]- and [2-^{14}C]-hexadecane standards.

The compounds under investigation were identified and analysed by t.l.c., co-chromatography, radioscanning, dilution with unlabelled material, crystallization to constant specific activity, and in some cases formation of derivatives.

Synthesis and Labelling of Precursors.—3,4-Dihydroxyphenyl[1-^{14}C]ethylamine ([1-^{14}C]dopamine) (4) was prepared by a known procedure.[9]

[1-^{14}C]*Tryptamine.* Gramine was heated with methyl iodide to give 3-dimethylaminomethylindole methiodide [10] which was then treated with K^{14}CN to furnish indol-3-yl[1-^{14}C]acetonitrile, catalytic reduction of which afforded [1-^{14}C]tryptamine.

[3-^{14}C]*Strictosidine* (1) *and* [3-^{14}C]*vincoside* (2). Secologanin was condensed with [1-^{14}C]tryptamine according to the literature procedure [11] to yield a mixture of [3-^{14}C]strictosidine (1) and [3-^{14}C]vincoside (2) which was used as such for feeding experiments.

[3-^{14}C]*Deacetylisoipecoside* (12) *and* [3-^{14}C]*deacetylipecoside.* Secologanin was condensed with [1-^{14}C]dopamine (pH 5) and the mixture was purified and resolved according to the method of Zenk and his co-workers [4] to give [3-^{14}C]-deacetylisoipecoside (12) and [3-^{14}C]deacetylipecoside.

[aryl-^{3}H]*Didemethyldeoxytubulosine* (5) *and* [aryl-^{3}H]*deoxytubulosine* (6). Deoxytubulosine (6) was demethylated by HBr in AcOH to give nordeoxytubulosine (5). Both compounds (5) and (6) were tritiated in an acid-catalysed exchange reaction [12,13] to furnish [*aryl*-^{3}H]didemethyldeoxytubulosine (5) and [*aryl*-^{3}H]deoxytubulosine (6).

Feeding Experiments.—For feeding purposes, dopamine, deacetylisoipecoside, deacetylipecoside, strictosidine, and vincoside were dissolved in water (1 ml). Tryptamine, didemethyldeoxytubulosine, and deoxytubulosine were dissolved in water (1 ml) containing DMSO (0.2 ml). The labelled precursors were fed to young *A. lamarckii* Thw. plants by the wick-feeding technique. When uptake was complete, water was added to wash out any remaining precursor. The plants were then kept alive for 7—8 d to metabolize the precursor

and were then worked up as described for the isolation of biosynthetic tubulosine.

Isolation of Tubulosine (8).—Young *A. lamarckii* Thw. plants (150 g wet weight) were macerated in EtOH (280 ml) with inactive tubulosine (100 mg) and left for 20 h. The EtOH was then decanted and the plant material was percolated with EtOH (5 × 250 ml). The combined ethanolic extracts were concentrated under reduced pressure to afford a greenish viscous mass which was extracted with 10% AcOH (5 × 10 ml). The combined acidic extracts were defatted with light petroleum (b.p. 40—60 °C) (4 × 20 ml), basified with Na_2CO_3 (pH 9), and the liberated bases were extracted with $CHCl_3$ (5 × 50 ml). The combined $CHCl_3$ extracts were washed with water, dried (anhydrous Na_2SO_4), and the solvent was removed under reduced pressure. The crude alkaloidal mixture thus obtained was subjected to preparative t.l.c. [SiO_2; $CHCl_3$–MeOH (93 : 7)] to give tubulosine (8) (60 mg), m.p. 258 °C (from MeOH) (lit.,[1] 258—261 °C). The radiochemical purity of the base was established by dilution techniques.

Isolation of Secologanin.—Fresh leaves of *Lonicera japonica* (800 g) were percolated with EtOH (5 × 800 ml). The percolate was concentrated at <40 °C under reduced pressure to give a thick syrup (40 g) which was then diluted with water (50 ml), defatted with benzene (6 × 50 ml), and extracted with Bu^nOH (5 × 100 ml). The combined n-butanol extracts were washed with H_2O, dried (anhydrous Na_2SO_4), and concentrated under reduced pressure. The residue (20 g) so obtained was chromatographed over a column of silica gel (250 g). Elution was effected with $CHCl_3$ and $CHCl_3$–MeOH (with increasing proportions of MeOH). Elution was monitored by t.l.c. [SiO_2 GF_{254}; $CHCl_3$–MeOH (8 : 2)]. Elution with $CHCl_3$–MeOH (9 : 1) gave secologanin (800 mg) as an amorphous powder, $[\alpha]_D$ −95.6° (*c* 1.12 in MeOH) [lit.,[14] $[\alpha]_D$ −96° (MeOH)].

Degradation of the Biosynthetic Tubulosine Derived from [3-^{14}C]*Deacetylisoipecoside.*—A mixture of radioactive tubulosine (8) (300 mg) (molar activity 7.196 × 10^5 disint. min^{-1} $mmol^{-1}$), pyridine (5 ml), and acetic anhydride (5 ml) was heated for 2 h on a water-bath and was then left at room temperature for 24 h. Excesses of pyridine and acetic anhydride from the resulting mixture were removed under reduced pressure. Water was added to the residue, the product was extracted with $CHCl_3$ (5 × 50 ml), and the combined extracts were washed with water, dried (anhydrous Na_2SO_4), and evaporated to afford a crude powder which was chromatographed over a column of silica gel. Elution with C_6H_6 gave *N,O*-diacetyltubulosine (9) (285 mg), m.p. 148 °C (lit.,[1] 149—150 °C) (molar activity 7.082 × 10^5 disint. min^{-1} $mmol^{-1}$).

A suspension of the foregoing compound (9) in MeOH (1 ml) was heated with MeONa [prepared from Na (50 mg) in MeOH (1 ml)] and MeI (4 ml). MeONa (1 ml) and MeI (2 ml) were again added at an interval of 6 h. After 9 h the solvent from the resulting mixture was removed under reduced pressure and water was added to the residue which was then filtered. The filtrate was cooled and the precipitated solid was filtered off. The solid thus obtained was then redissolved in hot water and the solution was refluxed for 10 min with freshly precipitated copper powder (100 mg) and filtered while hot. The filtrate was cooled and concentrated to give the radioactive N-*acetyl*-O,O-*dimethyltubulosine methiodide* (14) (250 mg), m.p. 232 °C (MeOH–water) (molar activity 7.120 × 10^5 disint. min^{-1} $mmol^{-1}$); $\nu_{max.}$ KBr 3 450 (indole NH), 1 630 (NAc), and 1 230 cm^{-1} (OMe); τ($CDCl_3$ + [$^2H_6$]DMSO) 9.29 (3 H, m, CH_2CH_3), 9.08 (2 H, m, CH_2-CH_3), 7.8 (3 H, m, NAc), 6.82 (3 H, s, $\overset{+}{N}$-CH_3), 6.26, 6.23 and 6.21 (each 3 H, s, OCH_3), 4.35 (1 H, m, 1′-*H*), and 2.8—3.5 (5 H, m, ArH); *m/z* 546 (M^+), 531 (M^+ − 15), 489 (M^+ − 57), 288 (M^+ − 258), 272—275 (M^+ − 274 to 271), 246 (M^+ − 300), 244 (M^+ − 302), 215 (M^+ − 331), 206 (M^+ − 340), 205 (M^+ − 341), 201 (M^+ − 345) (base peak), 199 (M^+ − 347), 192 (M^+ − 354), and 191 (M^+ − 355) (Found: C, 58.8; H, 6.55; N, 6.25. $C_{33}H_{44}N_3O_4$ requires C, 58.84; H, 6.53; N, 6.24%).

A solution of the preceding radioactive methiodide (14) (220 mg) in MeOH (100 ml) was passed through a column of freshly generated Amberlite IR-400 anion-exchange resin (OH^- form) (5.0 g) and the eluate was recycled five times. The resin was finally washed with MeOH (100 ml). The combined eluates were evaporated under reduced pressure to afford the radioactive methohydroxide (15). A solution of the labelled salt (15) in MeOH (10 ml) was refluxed with KOH (5.0 g) in water (2 ml) for 5 h. The solvent from the resulting mixture was removed, water (5 ml) was added, and the mixture was extracted with $CHCl_3$ (5 × 50 ml). The combined $CHCl_3$ extracts were washed with water, dried (anhydrous Na_2SO_4), and the solvent was removed under reduced pressure to give (17), the radioactive methyl methine of O,O-*dimethyltubulosine* (150 mg), m.p. 197—200 °C (MeOH–hexane) (molar activity 7.035 × 10^5 disint. min^{-1} $mmol^{-1}$); $\nu_{max.}$ KBr 3 350 (NH), 2 900, and 1 460 cm^{-1} (CH=CH_2); τ 9.18 (3 H, m, CH_2CH_3), 8.72 (2 H, m, CH_2CH_3), 8.02 (3 H, s, NCH_3), 6.28, 6.21, and 6.16 (each 3 H, s, OCH_3), 5.51 (1 H, m, 1′-H), 4.4—5.0 (3 H, m, CH=CH_2), and 2.7—3.4 (5 H, m, ArH); *m/z* 503 (M^+), 302 (M^+ − 201), 287 (M^+ − 216), 272 (M^+ − 231), 247 (M^+ − 256), 245 (M^+ − 258), 215 (M^+ − 288), 204 (M^+ − 299), 201 (base peak) (M^+ − 302), 199 (M^+ − 304), 191 (M^+ − 312), and 190 (M^+ − 313) (Found: C, 73.8; H, 8.1; N, 8.3. $C_{31}H_{41}N_3O_3$ requires C, 73.95; H, 8.15; N, 8.35%).

Ozonised O_2 was passed through a solution of the radioactive compound (17) (130 mg) in EtOAc (10 ml) at −78 °C for 30 min. The solvent from the resulting mixture was removed under reduced pressure. To the residue were added water (25 ml), Zn dust (250 mg), and $AgNO_3$ (20 mg). The mixture was refluxed for 20 min and then subjected to distillation. The distillate was collected in a solution of dimedone (300 mg) in aqueous EtOH (3 : 1 v/v; 80 ml) which was purified by column chromatography over silica gel and the product was crystallized from MeOH–Et_2O to give formaldehyde dimedone (15 mg), m.p. 188—189 °C (lit.,[15] 188 °C) (molar activity 7.016 × 10^5 disint. min^{-1} $mmol^{-1}$).

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