Biosynthesis of Reticuline[1]

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The incorporation of tyrosine, dopa, dopamine, and 4-hydroxy- and 3,4-dihydroxy-phenylpyruvic acids into reticuline in *Litsea glutinosa* has been studied, and it has been demonstrated that dopa and dopamine contribute only to the formation of the phenethylamine portion: the benzylic portion is biosynthesized from 3,4-dihydroxyphenylpyruvic acid not derived from dopa. Tyrosine and 4-hydroxy- and 3,4-dihydroxy-phenylpyruvic acids participate in the formation of both ' halves ' of reticuline. Tracer experiments have shown the intermediacy of norlaudanosoline-1-carboxylic acid, norlaudanosoline, and didehydronorlaudanosoline in the biosynthesis of reticuline. Incorporation studies with (±)-4'-*O*-methylnorlaudanosoline, (±)-laudanosoline, (±)-6-*O*-methylnorlaudanosoline, and (±)-nor-reticuline have demonstrated that *O*-methylation prededes *N*-methylation and that there is no rigid selectivity of *O*-methylation in the biosynthesis of reticuline.

RETICULINE[2] (1), an established precursor of a large number of 1-benzylisoquinoline-derived alkaloids, could be formed in nature from norlaudanosoline (12), which is considered to be derived from two fragments, (25) or (26) and (24), each derivable from dopa[3] (23).

Norlaudanosoline-1-carboxylic acid (14), which might be expected to be formed by condensation of dopamine (24) with 3,4-dihydroxyphenylpyruvic acid (25), has been shown to be a specific precursor of norlaudanosoline[4] (12) and morphine.[5] The *Papaver* species used in the latter tracer experiments also converts didehydronorlaudanosoline (16) into these alkaloids. Recently a considerable body of evidence has emerged concerning the duality of the role of tyrosine in 1-benzylisoquinoline biosynthesis. It has been suggested that tyrosine gives rise to two different constituent units by independent pathways.[6] Furthermore, dopa has been shown to be a precursor of only portions of the molecules of glaucine[7] and morphine.[8]

We have now shown in the biosynthesis of reticuline (1) that dopa contributes only to the formation of the phenethylamine portion in *Litsea glutinosa* (Lauraceae) plants, and that the benzylic portion is biosynthesised from 3,4-dihydroxyphenylpyruvic acid (25), not necessarily derived from dopa. Some other aspects of the biosynthesis of reticuline have also been studied.

Feeding of (±)-[2-^{14}C]dopa (experiment 1) and a mixture of (±)-[2-^{14}C]tyrosine and (±)-[3-^{14}C]dopa (specific activity ratio 45 : 55) (experiment 2) to young *Litsea glutinosa* plants showed that both these amino-acids were efficiently incorporated into reticuline (1) (Table). The location of the label at position 3 in reticuline derived from [2-^{14}C]dopa in the first experiment was established as follows. The reticuline (1) was converted into laudanosine (6) and then into the styrylphenethylamine (7) with no loss of activity. Catalytic hydrogenation of (7) gave the phenethylphenethylamine (8) with no loss of activity. A second Hofmann degradation yielded the olefin (9), which on ozonolysis gave formaldehyde, trapped as its dimedone derivative (97% of original activity). The labelled reticuline derived from the feeding of a mixture of [2-^{14}C]tyrosine and [3-^{15}C]dopa (experiment 2) (specific activity ratio 45 : 55) was similarly degraded[9] to give formaldehyde dimedone derivative (24% of original activity). Controlled oxidation of (7) with potassium permanganate gave veratric acid (20) (inactive). These results tentatively suggested that whereas tyrosine gives rise to both portions, dopa contributes only to the formation of the phenethylamine

Tracer experiments on *L. glutinosa*

Expt.	Precursor(s) fed	% Incorporation into reticuline (1)
1	(±)-[2-^{14}C]Dopa (23)	0.35,† 0.20
2	(±)-[2-^{14}C]Tyrosine (21) and (±)-[3-^{14}C]-Dopa (23) (specific activity ratio 45 : 55)	0.34,† 0.18
3	(±)-[3-^{14}C]Tyrosine (21) and (±)-[2-^{14}C]-Dopa (23) (specific activity ratio 55 : 45)	0.31,† 0.12
4	[3,5-^{3}H$_2$]4-Hydroxyphenylpyruvic acid (22)	0.18
5	3,4-Dihydroxy[2,5,6-^{3}H$_3$]phenylpyruvic acid (25)	0.21
6	[1-^{14}C]Dopamine (24)	0.24
7	(±)-(3-^{14}C)Norlaudanosoline-1-carboxylic acid (14)	0.16
8	[3-^{14}C]Didehydronorlaudanosoline (16)	0.22
9	(±)-[1-^{3}H]Norlaudanosoline (12)	0.26
10	(±)-6-*O*-Methyl[1-^{3}H]norlaudanosoline (4)	0.29
11	(±)-4'-*O*-Methyl[1-^{3}H]norlaudanosoline (5)	0.35
12	(±)-[1-^{3}H]Laudanosoline (3)	0.002
13	(±)-[2',6',8-^{3}H$_3$]Nor-reticuline (2)	0.60
14	(±)-[*aryl*-^{3}H]Norcoclaurine (11)	0.002
15	(±)-[3-^{14}C]Norcoclaurine-1-carboxylic acid (13)	0.001

† Feeding results of 1975 season.

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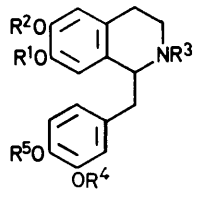
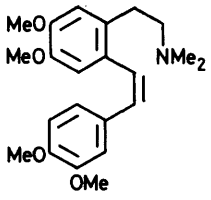
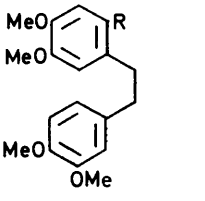


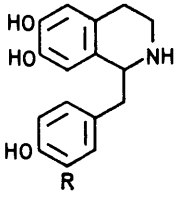
(1) $R^1 = R^4 = H, R^2 = R^3 = R^5 = Me$
(2) $R^1 = R^3 = R^4 = H, R^2 = R^5 = Me$
(3) $R^1 = R^2 = R^4 = R^5 = H, R^3 = Me$
(4) $R^1 = R^3 = R^4 = R^5 = H, R^2 = Me$
(5) $R^1 = R^2 = R^3 = R^4 = H, R^5 = Me$
(6) $R^1 = R^2 = R^3 = R^4 = R^5 = Me$



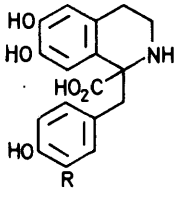
(7)



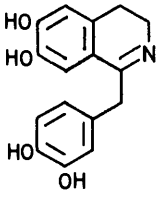
(8) $R = [CH_2]_2 \cdot NMe_2$
(9) $R = CH_2:CH$
(10) R = CHO



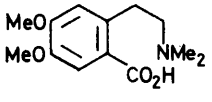
(11) R = H
(12) R = OH



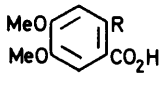
(13) R = H
(14) R = OH
(15) R = OMe



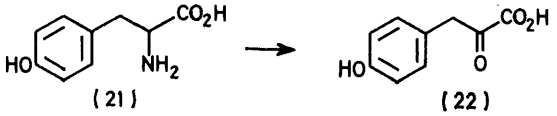
(16)



(17)



(18) $R = CH_2:CH$
(19) R = CHO
(20) R = H



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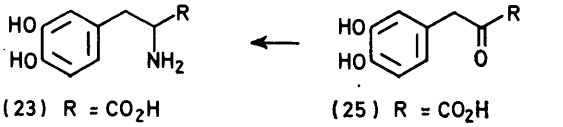
(22)



(23) $R = CO_2H$
(24) R = H

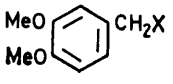
(25) $R = CO_2H$
(26) R = H

SCHEME

portion. Confirmation was obtained as follows. A mixture of (±)-[3-^{14}C]tyrosine and (±)-[2-^{14}C]dopa (specific activity ratio 55 : 45) (experiment 3) was fed to young *L. glutinosa* plants. The resulting labelled reticuline was degraded as above to yield veratric acid (20) (47% of the activity of the original base) and the amino-acid (17), which was treated with dimethyl sulphate followed by potassium hydroxide to afford vinylveratric acid (18) (58% of the activity of the original base) and trimethylamine (inactive). Treatment of (18) with osmium tetraoxide followed by oxidation with periodate gave the aldehyde (19) (42.4% of the activity of the original) and formaldehyde dimedone derivative (4.7% of the activity of the original).

Incorporation of tyrosine into both halves of reticuline implies that 4-hydroxy- (22) and 3,4-dihydroxy-phenylpyruvic acid (25) are both intermediates (Scheme). Feeding of 4-hydroxy[3,5-$^3H_2$]phenylpyruvic acid (22) (experiment 4) to young *L. glutinosa* plants showed that (22) is efficiently utilised by the plants to form reticuline. This biosynthetic reticuline was degraded to [5-^{3}H]-veratric acid (20) (42% of the activity of the original base) and vinyl[2-^{3}H]veratric acid (18) (48.6% of the activity of the original). These results demonstrated that (22) gives rise to both the constituent units of reticuline.

3,4-Dihydroxy[2,5,6-$^3H_3$]phenylpyruvic acid (25) (experiment 5) when fed to young *L. glutinosa* plants was



(27) X = CN
(28) $X = CH_2NHAc$

also incorporated into reticuline (1). The labelled reticuline was degraded to vinyl[2,5-$^3H_2$]veratric acid (18) (32% of the activity of the original base) and [2,5,6-$^3H_3$]veratric acid (20) (60% of the activity of the original). These results showed that (25) is incorporated into both ' halves ' of reticuline (1). The incorporation of (25) into the phenethyl portion suggests that (25) is aminated in the plants to give dopa (23).

[1-^{14}C]Dopamine (24) (experiment 6) was fed to young *L. glutinosa* plants and was efficiently incorporated into reticuline (1). The labelled (1) was converted into laudanosine (6) with no loss of activity. Hofmann elimination of (6) gave (7) (no loss of activity). Catalytic hydrogenation of (7) yielded (8) (no loss of activity). A second Hofmann degradation furnished (9) (no loss of activity). Treatment of (9) with osmium tetraoxide–periodate gave formaldehyde dimedone derivative (37% of the activity of the original). Ozonolysis of the radioactive methine (9) furnished formaldehyde dimedone derivative having 98% of the activity of the original. The lower activity of the formaldehyde is conceivably due to dilution with unlabelled formaldehyde arising from oxidation elsewhere in the molecule, for example of *O*-methyl groups. The formation of formaldehyde in

such a way under these conditions has precedent.[10] The results thus established that dopamine (24) enters specifically only the phenethylamine portion of reticuline (1).

Norlaudanosoline-1-carboxylic acid (14) is the product of condensation of dopamine (24) with 3,4-dihydroxyphenylpyruvic acid (25). The intermediacy of (14) in the biosynthesis of reticuline (1) was demonstrated as follows. (±)-[3-^{14}C]Norlaudanosoline-1-carboxylic acid (14) (experiment 7) was fed to young *L. glutinosa* plants. The resulting labelled reticuline (1) was degraded to (9) as described earlier with no loss of activity. Ozonolysis of (9) gave formaldehyde (98% of the original activity).

Incorporation of [3-^{14}C]didehydronorlaudanosoline (16) (experiment 8) and of (±)-[1-^{3}H]norlaudanosoline (12) (experiment 9) suggested that (14) is decarboxylated to (16), which is then reduced to (12).

In many plant species tri- and tetra-hydroxylated 1-benzylisoquinoline derivatives co-occur. It is conceivable that in nature norlaudanosoline-type compounds could be formed by direct hydroxylation of norcoclaurine (11) derivatives. Feeding of norcoclaurine (11) (experiment 14) and norcoclaurine-1-carboxylic acid (13) (experiment 15) demonstrated that this biosynthetic route is not followed in the biosynthesis of reticuline in *L. glutinosa*.

Bioconversion of norlaudanosoline (12) into reticuline (1) involves *N*- and *O*-methylation. It is generally believed and has been demonstrated experimentally in several instances that biosynthetic methylations do not occur at random but according to a definite sequence. The results of parallel feeding of (±)-6-*O*-methylnorlaudanosoline (4) (experiment 10), (±)-4′-*O*-methylnorlaudanosoline (5) (experiment 11), and (±)-laudanosoline (3) (experiment 12) suggests that *O*-methylation precedes *N*-methylation in the biosynthesis of reticuline from norlaudanosoline (12). Since 6-*O*-methylnorlaudanosoline (4) (experiment 10) and 4′-*O*-methylnorlaudanosoline (5) (experiment 11) are incorporated into reticuline with almost equal efficiency, these results suggest that there is no rigid selectivity of *O*-methylation in the biosynthesis of reticuline. Efficient incorporation of (±)-nor-reticuline (2) (experiment 13) into reticuline suggests that *N*-methylation is the terminal step in the bioconversion of norlaudanosoline (12) into reticuline. *N*-Methylation of nor-reticuline has been reported to be a specific process.[11]

Reticuline occurs in plants in (+)-,[12,13] (−)-,[14] and (±)-forms. It is suggested that this asymmetry is generated at the norlaudanosoline stage.[10] Racemization of reticuline, which is a necessary step for the biosynthesis of the morphine alkaloids,[14] appears to be very substrate-specific.

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EXPERIMENTAL

Unless otherwise stated u.v. spectra refer to solutions in ethanol, i.r. spectra to KBr discs and n.m.r. spectra to solutions in deuteriochloroform. T.l.c. was carried out, unless specified to the contrary, on silica GF-254.

Counting Methods.—Liquid scintillation counting was used for the measurement of ^{3}H and ^{14}C activities (Packard 314 Ex instrument). Radioactive samples were dissolved in methanol or dimethylformamide (0.2 ml) and liquid scintillator (7 ml); quoted activities are not corrected for self absorption except where stated. The relative efficiencies were obtained by counting [1,2-$^{3}H_2$]- and [2-^{14}C]-hexadecane standards.

Synthesis of Precursors.—The racemates of norlaudanosoline (12), nor-reticuline (2), norcoclaurine (11), 6-*O*-methylnorlaudanosoline (4), 4′-*O*-methylnorlaudanosoline (5), and laudanosoline (3) were prepared by standard methods.[15,16]

3,4-Dimethoxy[1-^{14}C]*benzyl cyanide* (27). To a stirred suspension of potassium cyanide (2.0 mCi + 67 mg of inactive KCN) in dry dimethyl sulphoxide (4 ml) was added dropwise a solution of 3,4-dimethoxybenzyl chloride in dry dimethyl sulphoxide (5 ml). Stirring was continued for 3 h and the mixture was worked up in the usual manner. The crude product was chromatographed on a column of neutral alumina (8 g). Elution with light petroleum–benzene (1 : 1) yielded the nitrile (27) (40 mg), m.p. 64—65° (lit.,[17] 64—65°), specific activity 0.0043 mCi mg^{-1}.

N-*Acetyl*-2-(3,4-*dimethoxyphenyl*)[1-^{14}C]*ethylamine* (28). A mixture of the nitrile (27) (176 mg), acetic anhydride (5 ml), Raney nickel (100 mg), and anhydrous sodium acetate (100 mg) was hydrogenated at 50 lb in^{-2}. After 24 h the mixture was filtered and the catalyst was washed with ethyl acetate (5 × 50 ml). The combined filtrate and washings were concentrated to an oily residue (195 mg), which was chromatographed on a column of silica (8 g). Elution with chloroform afforded the acetate (28) (158 mg), m.p. 94—95° (lit.,[18] 94—95°).

2-(3,4-*Dihydroxyphenyl*[1-^{14}C]*ethylamine* (*dopamine*). A solution of the amide (28) (158 mg) in hydrobromic acid (48%; 6 ml) was heated at 140 °C for 4 h. The excess of hydrobromic acid was removed *in vacuo* and the residue was crystallised from methanol–ether to give dopamine hydrobromide (150 mg), m.p. 210° (lit.,[19] 212°), specific activity 0.00398 mCi mg^{-1}.

[3-^{14}C]*Norcoclaurine-1-carboxylic acid* (13). A mixture of 4-hydroxyphenylpyruvic acid (46 mg) and [1-^{14}C]dopamine hydrobromide (75 mg) in water (0.4 ml) was adjusted to pH 4.0—4.5 with ammonium hydroxide, then left at room temperature for 24 h. The crystalline product was filtered off and dissolved in aqueous 1% sodium hydroxide (5 ml), and the alkaline solution was acidified with concentrated hydrochloric acid. During 24 h the acidic solution (pH 2.0) deposited light yellow needles, which were filtered off and washed with water (2 ml) and methanol (0.5 ml) to give the *acid* (13)*hydrochloride* (36 mg), m.p. 263—264° (decomp.), $\lambda_{max.}$ (0.1N-HCl) 284 nm; $\nu_{max.}$ (KBr) 1 730

[14] A. R. Battersby, D. M. Foulkes, and R. Binks, *J. Chem. Soc.*, 1965, 3323.
[15] S. Teitel, J. O'Brien, and A. Brossi, *J. Medicin. Chem.*, 1972, **15**, 845.
[16] S. Kubota, T. Masui, E. Fujita, and S. M. Kupchan, *J. Org. Chem.*, 1966, **31**, 516.
[17] A. Kaufmann and H. Muller, *Ber.*, 1918, **51**, 123.
[18] E. Spath and N. Polgar, *Monatsh.*, 1929, **51**, 190.
[19] G. Barger and J. Ewins, *J. Chem. Soc.*, 1910, **97**, 2258.

cm^{-1} (Found: C, 57.65; H, 5.45; N, 3.8. $C_{17}H_{18}ClNO_5$ requires C, 58.0; H, 5.1; N, 4.0%); base *m/e* 315 (M^+).

3′-O-*Methylnorlaudanosoline*-1-*carboxylic acid* (15). A mixture of 3-methoxy-4-hydroxyphenylpyruvic acid (100 mg) and dopamine hydrobromide (150 mg) in water (0.5 ml) was adjusted to pH 4.0—4.5 with ammonium hydroxide, then left at room temperature under nitrogen for 24 h. The crystalline product was filtered off and dissolved in aqueous 1% sodium hydroxide (5 ml), and the alkaline solution was acidified with concentrated hydrochloric acid. During 24 h the acidic solution (pH 2.0) deposited light yellow needles, which were recrystallised from concentrated hydrochloric acid–methanol (1 : 2) to give the *acid* (15) *hydrochloride* (210 m.p. 240° (decomp.); $\lambda_{max.}$ (0.1N-HCl) 283 nm; $\nu_{max.}$ (KBr) 1 725 cm^{-1}; τ (NaOD) 6.18 (3 H, s, OCH_3), 6.35—7.5 (6 H, m, 3 CH_2), and 3.0 (1 H, s, ArH) (Found: C, 56.05; H, 5.65; N, 4.05. $C_{18}H_{20}ClNO_5$ requires C, 56.6; H, 5.25; N, 3.65%).

Norlaudanosoline-1-*carboxylic acid* (14). A mixture of the methyl ether (15) (100 mg), hydrobromic acid (48%; 1 ml), and glacial acetic acid (2 ml) was refluxed in an oil-bath for 2 h. Excess of hydrobromic acid and acetic acid were removed *in vacuo*. The residue was basified with ammonium hydroxide and then treated with concentrated hydrochloric acid to give the acid (14) hydrochloride, which was recrystallised from concentrated hydrochloric acid–methanol (1 : 2); yield 70 mg; m.p. 285—290° (decomp.) [lit.,[4] 287—295° (decomp.)]; $\lambda_{max.}$ (0.1N-HCl) 282 nm; τ (CD_3OD) 2.41—3.34 (5 H, m, ArH); base *m/e* 331 (M^+), 284, 208, 164, and 124.

Labelling of Precursors.—Tritium in the precursors was introduced by the technique described [20] earlier. (±)-Norreticuline (100 mg) in tritiated water (0.5 ml; activity 60 m-Ci) containing potassium t-butoxide (200 mg) was heated under nitrogen (sealed tube) for 100 h at 100 °C. The resulting mixture was diluted with water, ammonium chloride was added (pH 7), and the liberated base was extracted with chloroform. The extract was washed with water and evaporated. The crude product was chromatographed on a column of neutral alumina. Elution with chloroform–methanol (95 : 5) afforded (±)-[2′,6′,8-$^3H_3$]norreticuline.

(±)-[aryl-^{3}H]*Norcoclaurine*. Thionyl chloride (0.4 ml) was added to tritiated water (0.6 ml) and to the solution was added (±)-norcoclaurine hydrochloride (100 mg). The resulting mixture was heated under nitrogen (sealed tube) for 90 h at 100 °C, diluted with water, and basified with sodium carbonate. The liberated base was extracted with chloroform–methanol (80 : 20) and solvent was removed. The residue was taken up in ethanolic hydrochloric acid to give (±)-[*aryl*-^{3}H]norcoclaurine hydrochloride.

(±)-[1-^{3}H]Laudanosoline (3) was prepared by reduction of 6,7-bisbenzyloxy-1-(3,4-bisbenzyloxybenzyl)-3,4-dihydroisoquinoline methiodide in dry dimethylformamide with potassium [^{3}H]borohydride followed by debenzylation. (±)-6-*O*-Methyl[1-^{3}H]norlaudanosoline (4), (±)-4′-*O*-methyl[1-^{3}H]norlaudanosoline (5), and (±)-[1-^{3}H]norlaudanosoline (12) were prepared similarly from the corresponding 3,4-dihydroisoquinoline derivatives. [3-^{14}C]Didehydronorlaudanosoline was prepared by standard procedures. (±)-[3-^{14}C]Norlaudanosoline-1-carboxylic acid, and (±)-[3-^{14}C]norcoclaurine-1-carboxylic acid were prepared by condensation of [1-^{14}C]dopamine with 4-hydroxy- (22) and 3,4-dihydroxy-phenylpyruvic acid (25), respectively.

Feeding Experiments.—For feeding purposes, dopa, the mixture of dopa and tyrosine, dopamine hydrochloride, 4′-*O*-methyl-laudanosoline (5), 6-*O*-methyl-laudanosoline (4), and laudanosoline (3) hydrochlorides were dissolved in aqueous dimethyl sulphoxide (1 ml). Nor-reticuline (2) was dissolved in water (1 ml) containing tartaric acid (10 mg). Freshly cut young *L. glutinosa* plants without roots were dipped into the precursor solutions and allowed to take up the precursor. When uptake was complete, water was added for washing. The young plants were then dipped in water, left for 5–6 days, and worked up for reticuline.

Isolation and Purification of Reticuline.—Stems and leaves (typically 100 g wet weight) of the young plants were macerated in ethanol (500 ml) with inactive reticuline (136 mg) and left for 24 h. The ethanol was then decanted and the plant material was percolated with fresh ethanol (6 × 250 ml). The solvent from the combined ethanolic extract was removed and the dark green viscous residue was extracted with N-hydrochloric acid (5 × 10 ml). The acidic solution was defatted with hexane (6 × 10 ml) and then basified with aqueous sodium hydrogen carbonate. The liberated basic material was extracted with chloroform (6 × 20 ml); the extract was washed with water, dried, and evaporated to afford crude reticuline (115 mg), which was chromatographed over a column of neutral alumina. Elution with chloroform–ethanol (98 : 2) gave reticuline (1); perchlorate, m.p. 143—144° (lit.,[2] 144—145°).

Degradations of Labelled Reticulines.—Labelled (±)-reticuline (1) (350 mg) in methanol (5 ml) was treated with an excess of ethereal diazomethane to give (±)-laudanosine (6), m.p. 113—114° (lit.,[9] 114—115°).

Labelled laudanosine (6) was degraded to veratric acid (20) and vinylveratric acid (19) according to the procedures of Battersby and his co-workers.[9]

Laudanosine (300 mg) in methanol (10 ml) was refluxed with methyl iodide (2 ml) to give laudanosine methiodide (375 mg), which was taken up in methanol and passed through a column of freshly regenerated Amberlite IR-410 anion exchange resin (8 g) to afford laudanosine methohydroxide. The solution was concentrated to 5 ml and refluxed for 2 h with potassium hydroxide (1.2 g). It was then cooled, diluted with water, and extracted with ether–chloroform (3 : 1 v/v; 5 × 50 ml). The extracts were washed with water and evaporated to yield a mixture of *trans*- and *cis*-laudanosine methine.[9] This mixture in ethanol was hydrogenated over platinum oxide to give the dihydro-derivative (8), which was treated with methyl iodide to afford the methiodide. This was converted into the hydroxide form with IR-410 resin and then treated with potassium hydroxide to yield (9).

Ozonolysis. Ozonized oxygen was passed through a solution of (9) (150 mg) in ethyl acetate (8 ml) at −78 °C for 10 min. The solvent was removed under reduced pressure and to the residue water (35 ml), zinc dust (350 mg), and silver nitrate (18 mg) were added. The resulting mixture was refluxed for 20 min and then distilled. The distillate was collected in a solution of dimedone (320 mg) in aqueous ethanol (80 ml). After 1 h the solution was concentrated to 10 ml and left for 12 h. The precipitated solid in chloroform was chromatographed over silica. Elution with chloroform (t.l.c. control) afforded the formaldehyde dimedone derivative, m.p. 193—194°.

Cleavage of the methine (9) *with osmium tetraoxide–periodate*.

[20] D. H. R. Barton, D. S. Bhakuni, G. M. Chapman, G. W. Kirby, L. J. Haynes, and K. L. Stuart, *J. Chem. Soc.* (*C*), 1967, 1295 and references cited therein.

To a stirred solution of (9) (80 mg) in t-butyl alcohol (4 ml) and water (3 ml) was added aqueous osmium tetraoxide (4%; 0.75 ml). Immediately an orange complex separated. To this suspension, sodium periodate (60 mg) was added. More periodate (10 mg) was added after 2 h. Stirring was continued for another 3 h and the mixture was decomposed with saturated aqueous arsenious oxide (35 ml). It was then extracted with ether (3 × 25 ml); the extracts were washed with water, dried, and evaporated to afford a gum (40 mg). This was chromatographed on silica (2 g). Elution with benzene furnished (10).

The aqueous solution left after the extraction of (10) was adjusted to pH 10 with potassium carbonate. Dimedone (180 mg) was then added and after 10 min the pH was adjusted to 6. The resulting mixture was left for 20 h at room temperature, and the solid which had settled was filtered off, washed with water, dried, and dissolved in chloroform. This solution was chromatographed on basic alumina to give the labelled formaldehyde dimedone derivative, m.p. 193—194°.

Oxidation of laudanosine methines. The mixture of *cis*- and *trans*-laudanosine methines was oxidised according to the method of Battersby and his co-workers.[9] A stirred solution of (7) (100 mg) in 50% v/v aqueous pyridine (3 ml) was treated at room temperature during 1 h with a solution of potassium permanganate (120 mg) in 50% aqueous pyridine (20 ml). The precipitated manganese dioxide was removed. The alkaline filtrate was acidified with hydrochloric acid and extracted with ether–chloroform (3 : 1 v/v; 4 × 60 ml). Removal of the solvent and crystallisation of the residue from aqueous ethanol gave veratric acid (20) (30 mg), m.p. 181—182°.

The acidic solution containing the amino-acid (17) was concentrated to 8 ml, adjusted to pH 10 with potassium hydroxide, and then stirred at 0 °C with dimethyl sulphate (0.4 ml) and 10N-potassium hydroxide (0.2 ml) for 1 h. Three more portions of dimethyl sulphate and potassium hydroxide were added at hourly intervals. The mixture was then refluxed with potassium hydroxide (2.5 g) for 2 h. The resulting solution was acidified with hydrochloric acid and extracted with ether–chloroform (3 : 1 v/v). Removal of the solvent from the extract and crystallisation of the residue from benzene gave vinylveratric acid (18), m.p. 183—184° (lit.,[9] 184°).

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