Absolute Configuration and Biosynthesis of Tiliacorine and Tiliacorinine [1]

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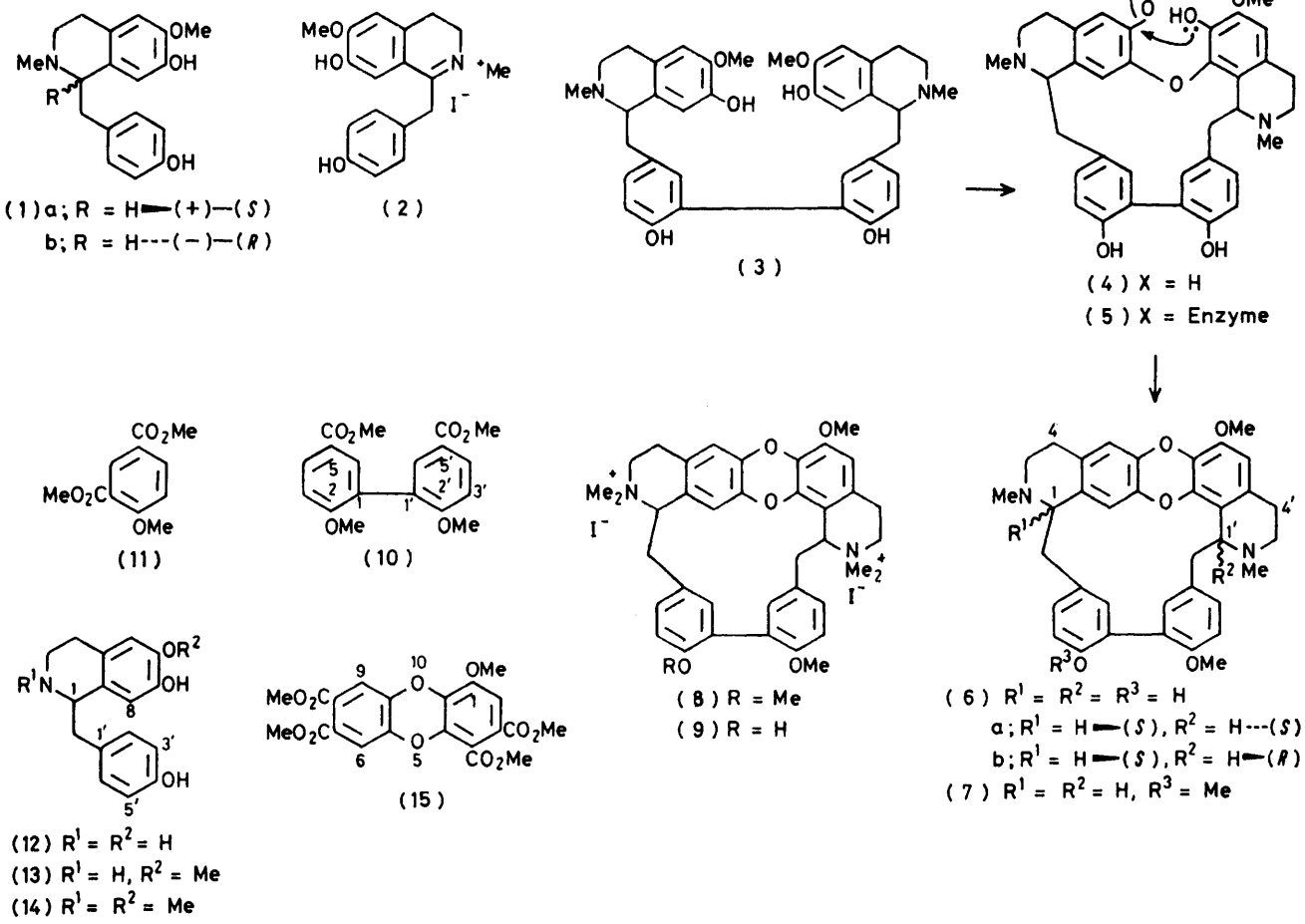
The incorporation of (±)-coclaurine, (±)-norcoclaurine, (±)-*N*-methylcoclaurine, and didehydro-*N*-methylcoclaurinium iodide into tiliacorinine and tiliacorine in *Tiliacora racemosa* Colebr. has been studied, and specific utilization of the (±)-*N*-methylcoclaurine demonstrated. The evidence shows that both 'halves' of these bases are derived from *N*-methylcoclaurine. Double-labelling experiments with (±)-*N*-methyl[1-^{3}H,6-*O*-methyl-^{14}C]coclaurine showed that the *O*-methyl function from one of the *N*-methylcoclaurine units is lost in the biotransformation into tiliacorine and tilacorinine. Experiments with (±)-*N*-[^{14}C]methyl[1-^{3}H]coclaurine demonstrated that the hydrogen atom at the asymmetric centre in the 1-benzyltetrahydroisoquinoline precursor is retained in the bioconversion into tiliacorine and tiliacorinine. Biosynthetic experiments with (*R*)- and (*S*)-*N*-methylcoclaurines established that tiliacorine has the *S*- and *R*-configuration at the asymmetric centres C-1 and C-1', respectively, and tiliacorinine has the *SS*-configuration at both centres.

THE diastereoisomeric alkaloids tiliacorine [2] and tiliacorinine,[2] representative of the biphenylbisbenzylisoquinoline alkaloids, have been assigned structure (6).[3] The absolute configuration at the asymmetric centres C-1 and C-1' in both bases cannot be determined by the usual sodium–ammonia cleavage method [4] because the two lower rings of these bases are linked through a direct carbon-to-carbon bond, rather than through the much more common diaryl ether bridge [5]

Tracer experiments have demonstrated that the bisbenzylisoquinoline alkaloids epistephanine,[6] cocsulin,[7] and cocsulinin [8] are formed in nature by oxidative dimerization of coclaurine. Biphenylbisbenzylisoquinoline bases of the tiliacorine and tiliacorinine types can similarly be formed in nature from coclaurine derivatives. Oxidative coupling [9,10] of *N*-methylcoclaurine (14) can give the biphenylbisbenzylisoquinoline intermediate (3). Intramolecular oxidative coupling



can form the oxygen ' bridge ' in the isoquinoline part of (3) to give (4). The methoxy-group from the isoquinoline portion of the molecule can then eliminate by the mechanism as shown in (5), probably as formaldehyde or its equivalent, to form the 1,4-dioxin bridge. Selective *O*-methylation can finally yield tiliacorine (6b) and tiliacorinine (6a).

(L)-[U-^{14}C]Tyrosine (experiment 1) was initially fed to young *Tiliacora racemosa* Colebr. (Menispermaceae) plants, and it was found that tiliacorine and tiliacorinine were being actively biosynthesized. In subsequent experiments labelled hypothetical precursors were fed to young (1—2 years old) *T. racemosa*. The results of several feedings are recorded in Table 1. Feeding of

TABLE 1

Tracer experiments on *Tiliacora racemosa* Colebr.

Expt.	Precursor	% Incorporation into Tiliacorine (6b)	% Incorporation into Tiliacorinine (6a)
1	(L)-[U-^{13}C]Tyrosine	0.09	0.06
2	(±)-[1-^{3}H]Norcoclaurine (12)	0.10	0.09
3	(±)-[3′,5′,8-$^{3}H_3$]Coclaurine (13)	0.14	0.12
4	(±)-*N*-Methyl[3′,5′,8-$^{3}H_3$]-coclaurine (14)	0.18	0.15
5	(±)-*NOO*-Trimethyl[3′,5′,8-$^{3}H_3$]-coclaurine	0.000 42	0.000 45
6	Didehydro-*N*-[^{14}C]methyl-coclaurine (2)	0.12	0.10
7	(±)-*N*-[^{14}C]Methyl[1-^{3}H]-coclaurine (14)	0.15	0.12
8	(±)-*N*-Methyl-[1-^{3}H,6-*O*-*methyl*]-coclaurine (14)	0.19	0.16
9	(*S*)-(+)-*N*-Methyl-[3′,5′,8-$^{3}H_3$]coclaurine (1a)	0.15	0.28
10	(*R*)-(−)-*N*-Methyl-[3′,5′,8-$^{3}H_3$]coclaurine (1b)	0.16	0.004

(±)-norcoclaurine (12) (experiment 2), (±)-coclaurine (13) (experiment 3), (±)-*N*-methylcoclaurine (14) (experiment 4), (±)-*NOO*-trimethylcoclaurine (experiment 5), and didehydro-*N*-methylcoclaurinium iodide (2) (experiment 6) demonstrated that (12), (13) and (14) were being metabolized by young *T. racemosa* plants to form tiliacorine and tiliacorinine. The incorporation of (2) is probably due to prior reduction *in vivo* to (14). As expected *NOO*-trimethylcoclaurine was not incorporated into tiliacorine and tiliacorinine.

Labelled tiliacorine and tiliacorinine derived from (±)-*N*-methyl[3′,5′,8-$^{3}H_3$]coclaurine (14) feedings were separately converted into *O*-methyltiliacorine dimethiodide [2] (8) and *O*-methyltiliacorinine dimethiodide [2] [as (8)] by treatment with methyl iodide–sodium methoxide. Alkaline permanganate oxidation of the dimethiodides,[2] followed by methylation of the acids so formed with diazomethane yielded, in each case, 2,2′-dimethoxy-5,5′-bismethoxycarbonylbiphenyl (10) and 1-methoxy-3,4,7,8-tetrakismethoxycarbonyldibenzo-*p*-dioxin (15). Oxidation of labelled *O*-methyltiliacorine dimethiodide (8) gave radioactive (10) and radioactive (15) having respectively 2/3 and 1/3 of the radioactivity of the parent base. Similar results were obtained for the degradation of *O*-methyltiliacorinine dimethiodide [as (8)]. Taking into account the loss of tritium in oxidative coupling, the distribution of the radioactivity in (10) and (15) formed by oxidative degradation of the biosynthetic bases derived from specifically and essentially equally labelled (14) demonstrated that (±)-*N*-methylcoclaurine (14) is specifically incorporated into both units of tiliacorine and tiliacorinine.

Feeding of (±)-*N*-[^{14}C]methyl[1-^{3}H]coclaurine (14) (experiment 7) gave tiliacorine and tiliacorinine labelled both with ^{14}C and ^{3}H. The ratios of these radioatoms in the precursor and biosynthetic bases were essentially unchanged. Since there is no loss of hydrogen from C-1 in the precursor in the biotransformation, and stereospecificity is maintained in the biosynthesis of other bisbenzylisoquinoline alkaloids from the 1-benzyltetrahydroisoquinoline precursors,[6-8] this demonstrates that the stereochemistry of these asymmetric centres remains unchanged during biosynthesis. Feeding of (±)-*N*-methyl[1-^{3}H,6-*O*-*methyl*-^{14}C]coclaurine (14) (experiment 8) yielded tiliacorine and tiliacorinine labelled with ^{14}C and ^{3}H, but the ^{14}C and ^{3}H ratios in the precursor was 1 : 30 and in the biosynthetic tiliacorine and tiliacorinine was 1 : 61 and 1 : 59.5 respectively. The results conformed with the biogenetic proposal [5,10] that the methoxy-group from one of the *N*-methylcoclaurine units may be lost in the formation of the 1,4-dioxin bridge.

Parallel-feeding experiments with (−)-(*R*)-*N*-methyl-[3′,5′,8-$^{3}H_3$]coclaurine (1b) (experiment 10) and (+)-(*S*)-*N*-[3′,5′,8-$^{3}H_3$]coclaurine (1a) (experiment 9) gave in each case, radioactive tiliacorine. Labelled tiliacorine (6b) derived from the (+)-(*S*)-form (1a) was converted into tiliacorine dimethiodide (9) by treatment with methyl iodide with essentially no loss of radioactivity. Alkaline permanganate oxidation which destroys the phenolic ring of the tiliacorine dimethiodide (9) gave dimethyl 4-methoxyisophthalate (11) (radioactive) and radioactive (15). Tiliacorine derived from the (−)-(*R*)-form (1b) was converted into radioactive tiliacorine dimethiodide (9) and similarly degraded to give the radioactive (11) and radioinactive (15). These results thus established the (*S*)-and (*R*)-configurations at the asymmetric centres C-1 and C-1′ respectively in tiliacorine.

Parallel feeding of the (+)-(*S*)-, and (−)-(*R*)-isomers (1a) and (1b), demonstrated that the former was incorporated into tiliacorinine 70 times more efficiently than the latter. Labelled tiliacorinine (6a) derived from the (+)-(*S*)-form (1a) was converted into the radioactive *O*-methyltiliacorinine dimethiodide [as (8)] by treatment with methyl iodide–sodium methoxide. Alkaline permanganate oxidation of the radioactive [as (8)] followed by methylation with diazomethane of the acids, so formed, gave the radioactive (10) and the radioactive (15) having essentially 2/3 and 1/3 radioactivity respectively. The results thus established the (*SS*) configuration at the two asymmetric centres C-1 and C-1′ respectively in tiliacorinine (6a).

EXPERIMENTAL

For general directions (spectroscopy details and counting method) see ref. 7.

Synthesis of Precursors.—The racemates of norcoclaurine [11] (12), coclaurine [12] (13), and *N*-methylcoclaurine [13] (14) were prepared by the known procedures.

Resolution of OO′-Dibenzylcoclaurine.—The salt from (±)-*OO′*-dibenzylcoclaurine (1.76 g) and (+)-dibenzoyltartaric acid (1.37 g) was fractionally crystallized successively from benzene–ether, benzene, ethanol–ether, ethanol, and methanol to give the salt as needles (1.0 g); $[\alpha]_D$ +73° (*c*, 0.90 in methanol) (lit.,[14] 72°). The salt was decomposed with sodium hydroxide and the liberated *OO′*-dibenzylcoclaurine was chromatographed on alumina (grade III) and crystallized from ethanol to give plates m.p. 87—88 °C; $[\alpha]_D$ +14° (*c*, 0.7 in methanol) and −26° (*c*, 0.9 in chloroform) (lit.,[14] m.p. 86—87 °C, $[\alpha]_D$ +15°, in methanol). The corresponding hydrochloride crystallised from ethanol as needles, m.p. 169—170 °C; $[\alpha]_D$ +46° (*c*, 0.2 in methanol) (lit.,[14] m.p. 170—171 °C; $[\alpha]_D$ +47° in methanol).

(−)-*Coclaurine Hydrochloride.*—(+)-*OO′*-Dibenzylcoclaurine hydrochloride (240 mg) was hydrogenolysed in ethanol (2 ml) using 10% palladium–carbon catalyst (120 mg). The resulting (−)-coclaurine hydrochloride crystallized from ethanol as needles, m.p. 166—167 °C, $[\alpha]_D$ −14° (*c*, 1.2 in methanol). The free base had $[\alpha]_D$ −15° (*c*, 1.2 in methanol) (lit.,[14] −16° in methanol).

(+)-(S)-N-*Methylcoclaurine* (1a).—(−)-Coclaurine hydrochloride (120 mg) in water (1 ml) under nitrogen was treated successively with 2M-sodium hydroxide (1 ml), formic acid (1.4 ml; 98%), and aqueous formaldehyde (1.5 ml; 38%), at pH *ca.* 5. The mixture was heated at 100 °C for 15 min and the basic products were isolated in the usual way. Chromatography on alumina (grade III; 30 g) and elution with chloroform–ethanol (94 : 6 v/v) gave (+)-(*S*)-*N*-methylcoclaurine (1a) (64 mg), $[\alpha]_D$ +123° (*c*, 0.8 in methanol) (lit.,[14] +123° in methanol).

(+)-*Coclaurine Hydrochloride.*—*OO′*-Dibenzylcoclaurine enriched with the (+)-enantiomer (from the resolution described above) was converted into the corresponding (−)-dibenzoyltartarate, $[\alpha]_D$ −73° (lit.,[14] −72°). The salt was treated with sodium hydroxide to give *OO′*-dibenzylcoclaurine, m.p. 85—86 °C, $[\alpha]_D$ −15° (*c*, 0.8 in methanol) (lit.,[14] −16° in methanol). The benzyl ether was hydrogenolysed with 10% palladium–carbon catalyst to give (+)-coclaurine hydrochloride (105 mg), $[\alpha]_D$ +13° (*c*, 0.8 in methanol) (lit.,[14] +13° in methanol).

(−)-(R)-N-*Methylcoclaurine* (1b).—(+)-Coclaurine hydrochloride (100 mg) in water (1 ml) (N_2 atmosphere) was treated with 2M-sodium hydroxide (1 ml), formic acid (1.5 ml; 98%), and aqueous formaldehyde (1.5 ml; 38%) at pH *ca.* 5. The mixture was heated at 100 °C for 15 min and worked up as above to give (−)-(*R*)-*N*-methylcoclaurine (1b) (60 mg), $[\alpha]_D$ −121° (*c*, 0.9 in methanol) (lit.,[14] −120° in methanol).

Labelling of Precursors. Tritiation.—Tritium was introduced in the precursors specifically into positions *ortho* to the phenolic hydroxy-groups by base-catalysed exchange [15] reaction. (±)-Coclaurine hydrochloride (120 mg) in tritiated H_2O (0.5 ml, 80 mCi) containing potassium t-butoxide (210 mg) was heated under N_2 (sealed tube) at 100 °C for 120 h to give (±)-[3′,5′,8-$^3H_3$]coclaurine which was purified as its hydrochloride (85 mg) and crystallized from MeOH to constant activity. (±)-*N*-Methyl[3′,5′,8-$^3H_3$]coclaurine (14) was tritiated in the similar manner. (±)-*N*-Methyl-[3′,5′,8-$^3H_3$]coclaurine (14) was diluted with radioinactive (±)-*N*-methylcoclaurine and the diluted material treated with CH_2N_2 to give (±)-*NOO*-trimethyl[3′,5′,8-$^3H_3$]-coclaurine with essentially no loss of radioactivity. Hofmann elimination of the radioactive *O*-methyl derivative gave the corresponding stilbene which was oxidized to anisic acid containing 66% of the total ^3H activity.

(*S*)-(+)-*N*-Methylcoclaurine (145 mg) in tritiated H_2O (0.5 ml, 80 mCi) containing potassium t-butoxide (200 mg) was heated under N_2 (sealed tube) at 100 °C for 125 h to give (*S*)-(+)-*N*-methyl[3′,5′,8-$^3H_3$]coclaurine. (*R*)-(−)-*N*-Methyl[3′,5′,8-$^3H_3$]coclaurine was prepared in the similar manner. (±)-[1-^3H]Norcoclaurine (12) and (±)-*N*-methyl[1-^3H]coclaurine (14) were prepared by reduction of the corresponding dihydroisoquinolines with potassium [^3H]borohydride in dry dimethylformamide. (±)-*N*-[^{14}C]-Methylcoclaurine was prepared by treating the corresponding dihydroisoquinolines with [^{14}C]methyl iodide and subsequent reduction of the methiodide with sodium borohydride. Dehydro-*N*-[^{14}C]methylcoclaurinium iodide (2) was prepared by treating the corresponding dihydroisoquinoline with [^{14}C]methyl iodide.

(±)-*N*-Methyl[6-O-*methyl*-^{14}C]coclaurine was prepared as follows: 4-benzyloxy-3-hydroxybenzaldehyde in DMF in the presence of NaH was treated with radioactive $^{14}CH_3I$ at ambient temperature to give radioactive 4-benzyloxy-3-methoxy[^{14}C]benzaldehyde which was then used to prepare (±)-*N*-methyl[6-*O*-*methyl*-^{14}C]coclaurine as described earlier.[13]

Doubly labelled (±)-*N*-methyl[1-^3H,6-O-*methyl*-^{14}C]coclaurine was prepared by mixing (±)-*N*-methyl[1-^3H]-coclaurine and (±)-*N*-methyl[6-O-*methyl*-^{14}C]coclaurine. (±)-*N*-[^{14}C]Methyl[1-^3H]coclaurine was prepared by mixing (±)-*N*-methyl[1-^3H]coclaurine and (±)-*N*-[^{14}C]methyl-coclaurine.

Feeding Experiment.—The solution of the precursors was introduced into young *T. racemosa* plants by wick feeding. When uptake was complete the plants were left for 8 to 10 days to metabolise the precursor and then worked up for tiliacorine and tiliacorinine.

Isolation of Tiliacorine and Tiliacorinine.—Young plants (typically 135 g wet wt) of *T. racemosa* fed with the precursor were harvested and macerated in ethanol (250 ml, containing 1% AcOH) with radioinactive tiliacorine (110 mg) and tiliacorinine (103 mg) and left for 10 h. The ethanolic extract was decanted and the plant material was percolated with fresh ethanol (4 × 200 ml). The combined ethanolic extract was concentrated under reduced pressure to give a greenish viscous mass which was extracted with 5% acetic acid (4 × 10 ml). The aqueous acidic extract was defatted with ether (5 × 25 ml) and then basified with aqueous Na_2CO_3. The liberated bases were extracted with chloroform–methanol (85 : 15 v/v) (5 × 25 ml). The combined organic layer was washed with H_2O and dried (Na_2SO_4), and the solvent was removed under reduced pressure to yield the crude base (240 mg) which was chromatographed over a column of neutral alumina (30 g). Elution with benzene–chloroform (1 : 1 v/v) gave a mixture of tiliacorine and tiliacorinine. The mixture of bases was subjected to preparative t.l.c. on silica gel (solvent: chloroform–methanol, 99 : 1 v/v; double run) to give tiliacorine (6b) (74 mg), m.p. 262—263 °C [lit.,[2] 262—264 °C (decomp.)],

crystallized to constant activity from chloroform–acetone, and tiliacorinine (6a) (68 mg), m.p. 195—196 °C (decomp.), crystallized from acetone–ether to constant activity.

Feeding of (L)-[U-^{14}C]*Tyrosine*.—(L)-[U-^{14}C]Tyrosine (experiment 1) (0.1 mCi) in water (1 ml) containing tartaric acid (12 mg) was fed to young *T. racemosa* (5 plants). After 8 days the plants were harvested. The plant material (137 g wet wt) was macerated with radioinactive tiliacorine (87 mg) and tiliacorinine (100 mg). The plant material was extracted with ethanol (5 × 200 ml, containing 1% acetic acid). The ethanolic extract was worked up as above to give the radioactive tiliacorine (58 mg) (specific activity 2.285×10^3 disint. min^{-1} mg^{-1}; molar activity 1.316×10^6 disint. min^{-1} mmol^{-1}; incorporation 0.09%) and the radioactive tiliacorinine (70 mg) (specific activity 1.33×10^4 disint. min^{-1} mg^{-1}; molar activity 7.67×10^6 disint. min^{-1} mmol^{-1}; incorporation 0.06%).

Feeding of (±)-[1-^{3}H]*Norcoclaurine*.—(±)-[1-^{3}H]Norcoclaurine (12) (experiment 2) hydrochloride (0.355 mCi) in water (1 ml) containing dimethyl sulphoxide (0.2 ml) was fed to young *T. racemosa* (4 plants). After 9 days the plants were harvested and the plant material (121 g wet wt) was macerated with radioinactive tiliacorine (102 mg) and tiliacorinine (50 mg), and worked up as above gave the radioactive tiliacorine (72 mg) (specific activity 7.720×10^3 disint. min^{-1} mg^{-1}; molar activity 4.45×10^6 disint. min^{-1} mmol^{-1}; incorporation 0.1%) and the radioactive tiliacorinine (33 mg) (specific activity 1.42×10^4 disint. min^{-1} mg^{-1}; molar activity 8.18×10^6 disint. min^{-1} mmol^{-1}; incorporation 0.09%).

Feeding of (±)-[3′,5′,8-$^3H_3$]*Coclaurine*.—(±)-[3′,5′,8-$^3H_3$]-Coclaurine (13) (experiment 3) hydrochloride (0.3496 mCi) in water (1 ml) containing dimethyl sulphoxide (0.3 ml) was fed to young *T. racemosa* (5 plants). After 8 days the plants were harvested and worked up. Radioinactive tiliacorine (85 mg) and tiliacorinine (80 mg) were added to isolate the radioactive tiliacorine (57 mg) (specific activity 1.235×10^4 disint. min^{-1} mg^{-1}; molar activity 7.114×10^6 disint. min^{-1} mmol^{-1}; incorporation 0.14%) and the radioactive tiliacorinine (52 mg) (specific activity 1.16×10^4 disint. min^{-1} mg^{-1}; molar activity 6.68×10^6 disint. min^{-1} mmol^{-1}; incorporation 0.12%).

Feeding of (±)-N-*Methyl*[3′,5′,8-$^3H_3$]*coclaurine*.—(±)-*N*-Methyl[3′,5′,8-$^3H_3$]coclaurine (14) (experiment 4) (0.124 mCi) in water (1 ml) containing tartaric acid (8 mg) was fed to young *T. racemosa* (4 plants). After 10 days the plants were harvested. Radioinactive tiliacorine (60 mg) and tiliacorinine (67 mg) were used as carriers to isolate the radioactive tiliacorine (36 mg) (specific activity 5.048×10^3 disint. min^{-1} mg^{-1}; molar activity 2.907×10^6 disint. min^{-1} mmol^{-1}; incorporation 0.18%) and the radioactive tiliacorinine (40 mg) (specific activity 6.29×10^3 disint. min^{-1} mg^{-1}; molar activity 3.62×10^6 disint. min^{-1} mmol^{-1}; incorporation 0.15%).

Feeding of (±)-NOO-*Trimethyl*[3′,5′,8-$^3H_3$]*coclaurine*.—(±)-*NOO*-Trimethyl[3′,5′,8-$^3H_3$]coclaurine (0.065 mCi) (experiment 5) in water (1 ml) containing tartaric acid (10 mg) was fed to young *T. racemosa* (4 plants). After 9 days the plants were harvested. The plant material (112 g wet wt) was macerated with radioinactive tiliacorine (86 mg) and tiliacorinine (72 mg) and worked up in the usual way to give the radioactive tiliacorine (58 mg) (specific activity 7.06 disint. min^{-1} mg^{-1}; molar activity 4.066×10^3 disint. min^{-1} mmol^{-1}; incorporation 0.000 42%) and the tiliacorinine (49 mg) (specific activity 9 disint. min^{-1} mg^{-1}; molar activity 5.184×10^3 disint. min^{-1} mmol^{-1}; incorporation 0.000 45%).

Feeding of Didehydro-N-[^{14}C]*methylcoclaurinium Iodide*.—Didehydro-*N*-[^{14}C]methylcoclaurinium iodide (2) (experiment 6) (0.005 mCi) in water (1 ml) containing dimethyl sulphoxide (0.3 ml) was fed to young *T. racemosa* (5 plants). After 8 days the plants were harvested and worked up. Radioinactive tiliacorine (58 mg) and tiliacorinine (52.2 mg) were added to isolate the radioactive tiliacorine (37 mg) (specific activity 2.30×10^2 disint. min^{-1} mg^{-1}; molar activity 1.325×10^5 disint. min^{-1} mmol^{-1}; incorporation 0.12%) and the radioactive tiliacorinine (29 mg) (specific activity 2.13×10^2 disint. min^{-1} mg^{-1}; molar activity 1.22×10^5 disint. min^{-1} mmol^{-1}; incorporation 0.10%).

Feeding of (±)-N-[^{14}C]*Methyl*[1-^{3}H]*coclaurine*.—(±)-*N*-[^{14}C]Methyl[1-^{3}H]coclaurine (experiment 7) (^{14}C activity 0.007 15 mCi and ^{3}H, 0.156 mCi; ^{14}C : ^{3}H, 1 : 22) in water (1 ml) containing tartaric acid (9 mg) was fed to young *T. racemosa* (5 plants). After 10 days the plants were harvested and worked up. Radioinactive tiliacorine (103 mg) and tiliacorinine (98 mg) were used to isolate the radioactive tiliacorine (70 mg) (^{14}C specific activity 2.343×10^2 disint. min^{-1} mg^{-1}; molar activity 1.35×10^5 disint. min^{-1} mmol^{-1}) (^{3}H specific activity 5.04×10^3 disint. min^{-1} mg^{-1}; molar activity 2.00×10^6 disint. min^{-1} mmol^{-1}) (^{14}C : ^{3}H, 1 : 21.5) and the radioactive tiliacorinine (61 mg) (^{14}C specific activity 2.00×10^2 disint. min^{-1} mg^{-1}; molar activity 1.15×10^5 disint. min^{-1} mmol^{-1}) (^{14}C : ^{3}H, 1 : 21).

Feeding of (±)-N-*Methyl*[1-^{3}H,6-O-methyl-^{14}C]*coclaurine*.—(±)-*N*-Methyl[1-^{3}H,6-O-*methyl*-^{14}C]coclaurine (experiment 8) (^{14}C activity 0.002 mCi and ^{3}H activity 0.06 mCi; ^{14}C : ^{3}H, 1 : 30) dissolved in water (1 ml) containing tartaric acid (11 mg) was fed to young *T. racemosa* (5 plants). After 10 days the plants were harvested and worked up. Inactive tiliacorine (113 mg) and tiliacorinine (95.2 mg) were used as a carrier to isolate the radioactive tiliacorine (83 mg) (^{14}C specific activity 36.718 disint. min^{-1} mg^{-1}; molar activity 2.12×10^4 disint. min^{-1} mmol^{-1} (^{3}H specific activity 2.24×10^3 disint. min^{-1} mg^{-1}; molar activity 1.29×10^6 disint. min^{-1} mmol^{-1}) (^{14}C : ^{3}H, 1 : 61) and the radioactive tiliacorinine (67 mg) (^{14}C specific activity 37.63 disint. min^{-1} mg^{-1}; molar activity 2.24×10^3 disint. min^{-1} mmol^{-1}) (^{3}H specific activity 2.24×10^3 disint. min^{-1} mg^{-1}; molar activity 1.29×10^6 disint. min^{-1} mmol^{-1}) (^{14}C : ^{3}H, 1 : 59.5).

Feeding of (+)-(S)-*N-methyl*[3′,5′,8-$^3H_3$]*coclaurine*.—(+)-(*S*)-*N*-Methyl[3′,5′,8-$^3H_3$]coclaurine (1a) (experiment 9) (0.347 mCi) in water (1 ml) containing tartaric acid (10 mg) was fed to young *T. racemosa* (5 plants). After 8 days the plants were harvested and worked up. Radioinactive tiliacorine (69 mg) and tiliacorinine (62 mg) were used as carriers to isolate the radioactive tiliacorine (41 mg) (specific activity 1.02×10^4 disint. min^{-1} mg^{-1}; molar activity 5.87×10^6 disint. min^{-1} mmol^{-1}; incorporation 0.152%) and the radioactive tiliacorinine (37 mg) (specific activity 3.48×10^3 disint. min^{-1} mg^{-1}; molar activity 2.0×10^6 disint. min^{-1} mmol^{-1}; incorporation 0.28%).

Feeding of (−)-(R)-N-*Methyl*[3′,5′,8-$^3H_3$]*coclaurine*.—(−)-(*R*)-*N*-Methyl[3′,5′,8-$^3H_3$]coclaurine (1b) (experiment 10) (0.585 mCi) in water (1 ml) containing tartaric acid (12 mg) was fed to young *T. racemosa* (5 plants). After 8 days the plants were harvested and worked up. Radioinactive tiliacorine (117 mg) and tiliacorinine (100 mg) were used as carriers to isolate the radioactive tiliacorine (92 mg)

(specific activity 1.776×10^4 disint. min^{-1} mg^{-1}; molar activity 1.022×10^7 disint. min^{-1} $mmol^{-1}$; incorporation 0.16%) and the radioactive tiliacorinine (81 mg) (specific activity 5.19×10^2 disint. min^{-1} mg^{-1}; molar activity 2.99×10^5 disint. min^{-1} $mmol^{-1}$; incorporation 0.004%).

Degradation of the Biosynthetic Tiliacorine (6b) *derived from* (±)-N-*Methyl*[3′,5′,8-$^3H_3$]*coclaurine* (14).—Labelled tiliacorine (experiment 4) was diluted with radioinactive material. Diluted tiliacorine (220 mg) suspended in MeOH (2 ml) was heated with MeONa (prepared from 50 mg Na in 1 ml MeOH) and MeI (1 ml). MeONa (1 ml) and MeI (1 ml) were again added after 6 h. The resulting mixture was worked up as described earlier to give the radioactive *O*-methyltiliacorine dimethiodide (8) (235 mg), m.p. 360 °C (decomp.) (lit.,[2] 360 °C).

To a stirred solution of the preceding radioactive *O*-methyltiliacorine dimethiodide (8) (230 mg) in H_2O (10 ml) at 70—80 °C was added dropwise an aqueous solution of $KMnO_4$ (4%; 25 ml). The mixture was kept at 70—80 °C for 5 h. The precipitated MnO_2 from the resulting mixture was filtered off. The filtrate was concentrated under reduced pressure to 5 ml and acidified with concentrated HCl. The liberated acid A (65 mg) was filtered off. The filtrate was kept for the isolation of the acid B.

The crude radioactive acid A (60 mg) in MeOH (2 ml) was treated with an excess of ethereal CH_2N_2 to give the radioactive 2,2′-dimethoxy-5,5′-bismethoxycarbonylbiphenyl (10) (30 mg), m.p. 171—172 °C (lit.,[2] 172—173 °C).

The filtrate and water washings after removal of (10) were combined and evaporated to dryness *in vacuo*. The residue, so obtained, was suspended in MeOH (2 ml) and to it added an excess of ethereal CH_2N_2 to give the radioactive 1-methoxy-3,4,7,8-tetrakismethoxycarbonyldibenzo-*p*-dioxin (15) (9.2 mg), m.p. 180—181 °C (lit.,[2] 180—181 °C). The radioactivity of the products is shown in Table 2.

TABLE 2

Compound	Specific activity/ disint. min^{-1} mg^{-1}	Molar activity/ disint. min^{-1} $mmol^{-1}$
(6b)	4.60×10^3	2.65×10^5
(9)	3.01×10^2	2.63×10^5
(15)	1.874×10^2	8.36×10^4
(10)	5.4×10^2	1.78×10^5

Degradation of the Biosynthetic Tiliacorine (6b) *derived from* (+)-(S)-N-*Methyl*[3′,5′,8-$^3H_3$]*coclaurine* (1a).—Labelled tiliacorine (experiment 9) was diluted with inactive material. A mixture of diluted labelled tiliacorine (250 mg), $CHCl_3$ (4 ml), and MeI (1 ml) was left at room temperature for 20 h. The yellow crystalline solid that separated out was filtered off and crystallized from acetone–MeOH to afford the radioactive *tiliacorine dimethiodide* (9) (265 mg) as needles m.p. 294—295 °C (decomp.) [lit.,[2] 294—295 °C (decomp.)].

The preceding radioactive dimethiodide (9) (260 mg) in H_2O (20 ml) at 75—80 °C was oxidized with aqueous solution of $KMnO_4$ (4%; 60 ml) as above to give the radioinactive dimethyl 4-methoxyisophthalate (11) (55.4 mg), m.p. 94 °C (lit.,[2] 95 °C).

The aqueous filtrate and the washings after removal of (11) were mixed and the water was removed under reduced pressure. The residue so obtained was taken up in MeOH and treated with an excess of ethereal CH_2N_2 to give radioactive (15) (15 mg), m.p. 180—181 °C (lit.,[2] 180—181 °C). The radioactivity of the products is shown in Table 3.

TABLE 3

Compound	Specific activity/ disint. min^{-1} mg^{-1}	Molar activity/ disint. min^{-1} $mmol^{-1}$
(6b)	1.875×10^3	1.08×10^6
(9)	1.24×10^3	1.07×10^6
(15)	1.164×10^3	5.19×10^5
(11)	0	0

Degradation of the Biosynthetic Tiliacorine (6b) *derived from* (−)-(R)-N-*Methyl*[3′,5′,8-$^3H_3$]*coclaurine* (1b).—Labelled tiliacorine (210 mg) (experiment 10) was converted into radioactive tiliacorine dimethiodide (9) and degraded as above, to afford radioinactive (15) and radioactive (11). The radioactivity of the products is shown in Table 4.

TABLE 4

Compound	Specific activity/ disint. min^{-1} mg^{-1}	Molar activity/ disint. min^{-1} $mmol^{-1}$
(6b)	3.125×10^2	1.80×10^5
(9)	2.035×10^2	1.75×10^5
(15)	0	0
(11)	7.00×10^2	1.568×10^5

Degradation of the Biosynthetic Tiliacorinine (6a) *derived from* (±)-N-*Methyl*[3′,5′,8-$^3H_3$]*coclaurine*.—Labelled tiliacorinine (6a) (268 mg) (experiment 4) was converted into the radioactive *O*-methyltiliacorinine dimethiodide [as (8)] and degraded according to the scheme as described above for the degradation of the biosynthetic tiliacorine, to give radioactive (15) and radioactive (10). The radioactivity of the products is shown in Table 5.

TABLE 5

Compound	Specific activity/ disint. min^{-1} mg^{-1}	Molar activity/ disint. min^{-1} $mmol^{-1}$
(6a)	1.23×10^3	7.08×10^5
(8)	8.12×10^2	7.1×10^5
(15)	5.15×10^2	2.3×10^5
(10)	1.42×10^3	4.69×10^5

5. *Degradation of the Biosynthetic Tiliacorinine* (6a) *derived from* (+)-(S)-N-*Methyl*[3′,5′,8-$^3H_3$]*coclaurine* (1a).—Labelled tiliacorinine (297 mg) (experiment 9) was converted into *O*-methyltiliacorinine dimethiodide and then

TABLE 6

Compound	Specific activity/ disint. min^{-1} mg^{-1}	Molar activity/ disint. min^{-1} $mmol^{-1}$
(6a)	3.2×10^2	1.843×10^5
(8)	2.05×10	1.80×10^5
(15)	1.31×10^2	5.84×10^4
(10)	3.7×10^2	1.22×10^2

oxidized with $KMnO_4$ as above to give radioactive (15) and radioactive (10). The radioactivity of the products is shown in Table 6.

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REFERENCES

[1] Preliminary communication, D. S. Bhakuni, A. N. Singh, S. Jain, and R. S. Kapil, *J. Chem. Soc., Chem. Commun.*, 1978, 226.
[2] B. Anjaneyulu, T. R. Govindachari, S. S. Sathe, N. Viswanathan, K. W. Gopinath, and B. R. Pai, *Tetrahedron*, 1969, **25**, 3091.
[3] M. Shamma, J. E. Foy, T. R. Govindachari, and N. Viswanathan, *J. Org. Chem.*, 1976, 1293.
[4] Y. Inubushi, K. Momura, and M. Miyawaki, *J. Pharm. Soc., Jpn.*, 1963, **83**, 282.

[5] M. Shamma, ' The Isoquinoline Alkaloids,' Academic Press, New York, 1972, p. 138.
[6] D. H. R. Barton, G. W. Kirby, and A. Wiechers, *J. Chem. Soc. C*, 1966, 2313.
[7] D. S. Bhakuni, V. M. Labroo, A. N. Singh, and R. S. Kapil, *J. Chem. Soc., Perkin Trans. 1*, 1978, 121.
[8] D. S. Bhakuni, S. Jain, and A. N. Singh, *J. Chem. Soc., Perkin Trans. 1*, 1978, 380.
[9] D. H. R. Barton and T. Cohen, '*Festschr. A. Stoll*', Birkhauser, Basel, 1957, p. 117.
[10] A. R. Battersby in ' Oxidative Coupling of Phenols,' eds. A. R. Battersby and W. I. Taylor, Dekker, New York, 1967; C. W. Thornber, *Phytochemistry*, 1970, **9**, 157.
[11] H. Yamaguchi, *J. Pharm. Soc., Jpn.*, 1958, **78**, 692.
[12] K. Kratzl and G. Billek, *Monatsh. Chem.*, 1951, **82**, 568.
[13] H. Yamaguchi, *J. Pharm. Soc., Jpn.*, 1958, **78**, 678.
[14] 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.
[15] G. W. Kirby and L. Ogunkoya, *J. Chem. Soc.*, 1965, 6914.