Biosynthesis of the Bisbenzylisoquinoline Alkaloid Cocsulin

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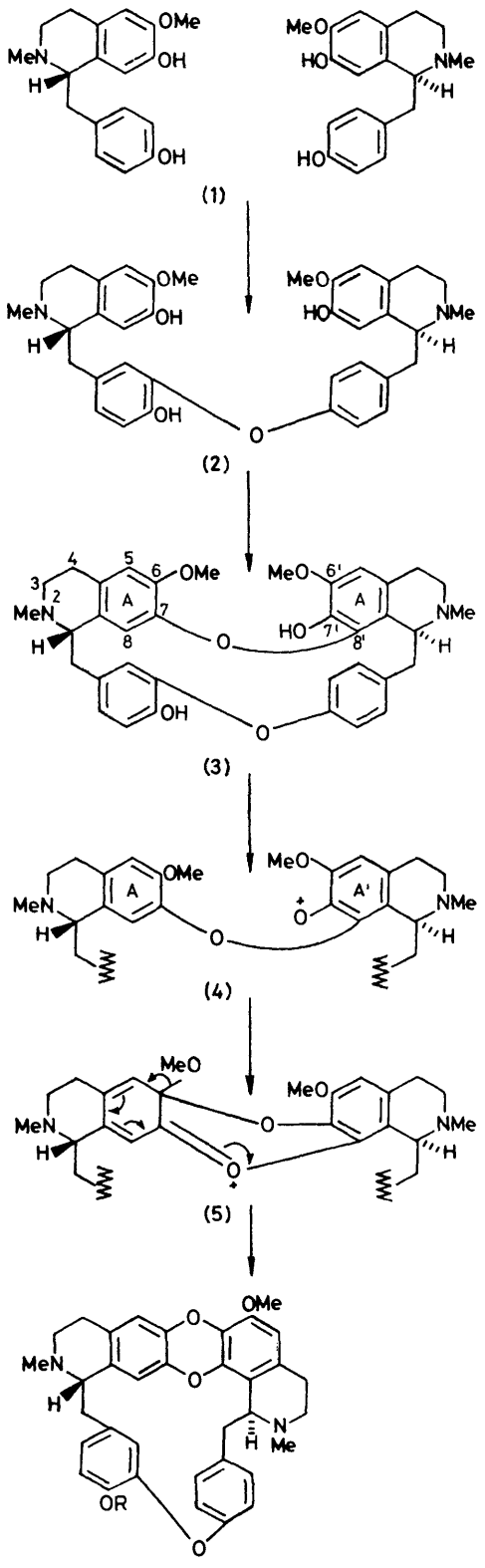
The incorporation of (±)-coclaurine, (±)-norcoclaurine, (±)-*N*-methylcoclaurine, and didehydro-*N*-methylcoclaurinium iodide into cocsulin in *Cocculus laurifolius* DC has been studied, and specific utilization of the (±)-*N*-methylcoclaurine demonstrated. The evidence supports the occurrence of oxidative dimerization of two *N*-methylcoclaurine units to give cocsulin. Double labelling experiments with (±)-*N*-methyl[1-^{3}H, *methoxy*-^{14}C]coclaurine showed that the *O*-methyl function from one of the *N*-methylcoclaurine units is lost in the bioconversion into cocsulin. Experiments with (±)-*N*-[^{14}C]methyl[1-^{3}H]coclaurine demonstrated that the hydrogen atom at the asymmetric centre in the 1-benzylisoquinoline precursor is retained in the bioconversion into cocsulin. Parallel feedings of (+)-(*S*)- and (−)-(*R*)-*N*-methylcoclaurines showed that the stereospecificity is maintained in the biosynthesis of cocsulin from the 1-benzylisoquinoline precursor.

COCSULIN [1] (6), a representative of the bisbenzylisoquinoline alkaloids having a dibenzo-*p*-dioxin system can be considered to be formed in nature by oxidative dimerization of coclaurine derivatives.[2,3] Intermolecular oxidative coupling of two *N*-methylcoclaurine (1) units would lead to a dauricine-type intermediate (2), which in turn can undergo intramolecular oxidative coupling to generate (3). Two possibilities have been considered for the formation of the dibenzo-*p*-dioxin system as is present in (6). In one [4] the intermediate (3) is oxidized to the corresponding phenoxy cation (4), which is then substituted into ring A to give the ion (5) which through loss of CH_3O^+ can generate (6). A similar mechanism involving radical substitution has also been considered.[4] In the second proposal [5] (Scheme) C–C bond formation is followed by a 1,2-*C* ⟶ *O* shift, leading to the dibenzo-*p*-dioxin system.

[1] D. S. Bhakuni and P. P. Joshi, *Tetrahedron*, 1975, **31**, 2575.
[2] F. Faltis and H. Frauendorfer, *Ber.*, 1930, **63**, 806.
[3] M. Shamma, ' The Isoquinoline Alkaloids,' Academic Press, New York, 1972, p. 138.
[4] 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.
[5] D. H. R. Barton and T. Cohen, *Festschr. A. Stoll*, 1957, 117.

It has been shown that (±)-coclaurine and (−)-*N*-methylcoclaurine are incorporated into the bisbenzylisoquinoline alkaloid epistephanine in *Stephania japonica* Miers.[6] We now present the first report on the biosynthesis of a bisbenzylisoquinoline alkaloid having a dibenzo-*p*-dioxin system.

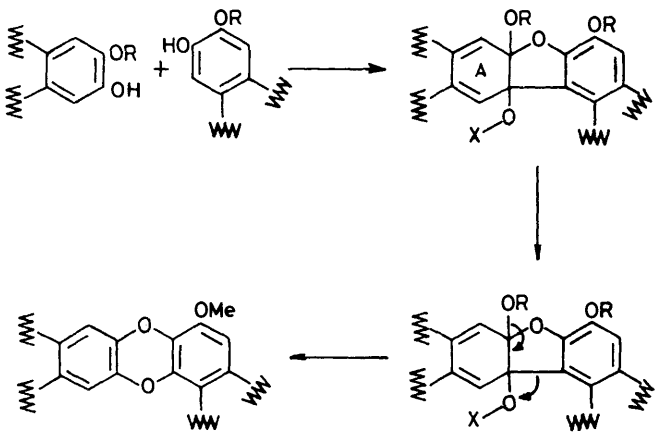


(±)-Tyrosine was initially fed to young cut branches of *Cocculus laurifolius* DC (Menispermaceae), and it was found that cocsulin (6) was being actively biosynthesised by the plants. In subsequent experiments labelled hypothetical precursors were fed to young cut branches of *C. laurifolius*. The results of several feedings are recorded in Table 1. Feeding of (±)-coclaurine (8) (experiment 2), (±)-*N*-methylcoclaurine (9) (experiment 3), and (±)-norcoclaurine (10) (experiment 9) established that (8)—(10) were efficient precursors of cocsulin (6).

TABLE 1

Tracer experiments on *C. laurifolius*

Expt.	Precursor fed	Incorporation (%) into cocsulin (6)
1	(±)-[2-^{14}C]Tyrosine	0.10
2	(±)-[3′,5′,8-$^{3}H_3$]Coclaurine (8)	0.14
3	(±)-*N*-Methyl[1-^{3}H]coclaurine (9)	0.17
4	(±)-*N*-[^{14}C]Methyl[1-^{3}H]coclaurine (9)	0.19
5	(±)-*N*-Methyl[1-^{3}H, *methoxy*-^{14}C]coclaurine (9)	0.22
6	Didehydro-*N*-[^{14}C]methylcoclaurine (14)	0.06
7	(±)-*N*-[^{14}C]Methylnorcoclaurine (11)	0.0002
8	(±)-*NOO*-Trimethyl[3′,5′,8-$^{3}H_3$]coclaurine (13)	0.002
9	(±)-[1-^{3}H]Norcoclaurine (10)	0.12
10	(+)-*N*-Methyl[3′,5′,8-$^{3}H_3$]coclaurine (1)	0.24
11	(−)-*N*-Methyl[3′,5′,8-$^{3}H_3$]coclaurine	0.004
12	(±)-*N*-[^{14}C]Methylcoclaurine (9)	0.19



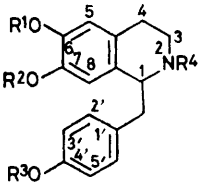
SCHEME

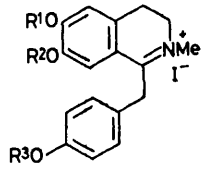
(±)-*N*-Methylnorcoclaurine (11) (experiment 7) was not incorporated. This result showed that *N*-methylation of norcoclaurine does not precede *O*-methylation in the biosynthesis of *N*-methylcoclaurine. The intermediate level of incorporation of the didehydro-*N*-methylcoclaurinium iodide (14) (experiment 6) is probably due to prior reduction *in vivo* to *N*-methylcoclaurine (9).

[6] D. H. R. Barton, G. W. Kirby, and A. Wiechers, *J. Chem. Soc.* (*C*), 1966, 2313.

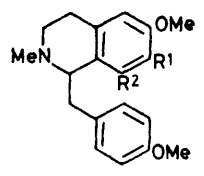
(±)-*NOO*-Trimethylcoclaurine (13) (experiment 8), as expected, was not incorporated into (6).

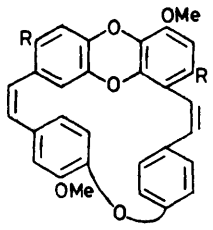
Labelled cocsulin (6) derived from (±)-*N*-methyl-[1-^{3}H]coclaurine (experiment 3) was treated with diazomethane to give *O*-methylcocsulin [1] (7) with no loss of





(8) R^1 = Me, R^2 = R^3 = R^4 = H (14) R^1 = Me, R^2 = R^3 = H
(9) R^1 = R^4 = Me, R^2 = R^3 = H (15) R^1 = R^2 = R^3 = $PhCH_2$
(10) R^1 = R^2 = R^3 = R^4 = H
(11) R^1 = R^2 = R^3 = H, R^4 = Me
(12) R^1 = R^2 = R^3 = $PhCH_2$, R^4 = Me
(13) R^1 = R^2 = R^3 = R^4 = Me





(16) R^1 = R^2 = H
(17) R^1 = H, R^2 = OMe
(18) R^1 = OMe, R^2 = H

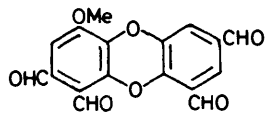
(19) R = $[CH_2]_2NMe_2$
(20) R = $CH{:}CH_2$

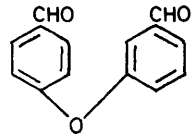
radioactivity. Reductive fission of (7) with sodium–liquid ammonia using an improved procedure [7] gave a phenolic product, which was purified and then treated with diazomethane to give the methyl ether. Second-stage fission of this compound with sodium–liquid ammonia afforded a mixture of phenolic and non-phenolic bases. The mixture of non-phenolic bases when separated by preparative t.l.c. afforded (16). The mixture of phenolic bases was treated with diazomethane. Separation of the methyl ethers by preparative t.l.c. chromatography gave (17) and (18). The 1-benzylisoquinolines (16)—(18) each had essentially half the molar activity of the parent base.

Feeding of (±)-*N*-[^{14}C]methyl[1-^{3}H]coclaurine (experiment 4) gave cocsulin (6) labelled both with ^{14}C and ^{3}H. The ^{14}C : ^{3}H ratios in the precursor and biosynthetic base were almost the same. This demonstrated that the hydrogen atom at the asymmetric centre in the benzylisoquinoline precursor is retained in the bioconversion into cocsulin.

Feeding of (±)-*N*-[^{14}C]methylcoclaurine (experiment 12) gave labelled cocsulin. The regiospecificity of the labelling was shown as follows. The labelled cocsulin (6) was treated with diazomethane to give (7), which was converted into its dimethiodide and then into its hydroxide form with no loss of activity. Hofmann degradation of this compound yielded the methine (19) which had essentially the same radioactivity as the parent base. Treatment of (19) with dimethyl sulphate followed by potassium hydroxide gave trimethylamine, trapped as its hydrochloride which had essentially half the molar activity of the parent base. The methine (20) was essentially radio inactive.

The regiospecificity of the labelling in the tritium labelled cocsulin (6) derived from (±)-*N*-methyl[1-^{3}H]-coclaurine was established as follows. Labelled cocsulin (6) was converted into the methine (20) according to the degradation procedure described above; the product had essentially the same radioactivity as the parent base. Treatment of (20) with osmium tetraoxide–sodium periodate gave the aldehyde (21), which had the same molar activity as the methine (20), and the aldehyde (22), which was essentially radioinactive.





(21) (22)

The foregoing feeding experiments thus established that cocsulin (6) is specifically biosynthesised from *N*-methylcoclaurine in *C. laurifolius*. According to biogenetic theory,[4] however, the methoxy-function from one of the *N*-methylcoclaurine units should be lost during the biotransformation. Feeding of (±)-*N*-methyl[1-^{3}H, *methoxy*-^{14}C]coclaurine (experiment 5) yielded cocsulin (6) labelled with ^{14}C and ^{3}H. The ^{14}C : ^{3}H ratio in the precursor was 1 : 23 whereas in the biosynthetic base the ratio was 1 : 42. These results were thus in conformity with the biogenetic proposal.

In the majority of the bisbenzylisoquinoline alkaloids there are two asymmetric centres. Although the foregoing experiments established that *N*-methylcoclaurine is a specific precursor of cocsulin in *C. laurifolius*, the precursors used were racemic. It would be expected that the enzyme system involved in the relevant biotransformation would be stereospecific, and that only one of the two optical isomers would normally act as a direct substrate. Parallel feedings with (+)-(*S*)- and (−)-(*R*)-*N*-methylcoclaurines showed that the stereospecificity is maintained in the oxidative dimerization of *N*-methylcoclaurine into cocsulin. The *S*-form (experiment 10) was incorporated into cocsulin about 60 times more efficiently than the *R*-form (experiment (11).

(+)-(*S*)-*N*-Methylcoclaurine (1) has been found to be specifically incorporated into cocsulin (6), and it has also been isolated from *C. laurifolius* [8] DC. Thus (1) is a true precursor of (6). The foregoing results strongly support the following sequence for the biosynthesis of

[7] Y. Inubushi, K. Momura, and M. Miyawaki, *J. Pharm. Soc. Japan*, 1963, **83**, 282.
[8] J. Kunitomo, *J. Pharm. Soc. Japan*, 1961, **81**, 1253.

cocsulin in *C. laurifolius* DC: tyrosine ⟶ norcoclaurine (10) ⟶ coclaurine (8) ⟶ (+)-(*S*)-*N*-methylcoclaurine ⟶ (dimerisation) ⟶ cocsulin (6).

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 (recorded with a Varian A-60D spectrometer) to solutions in deuteriochloroform. T.l.c. was carried out, unless specified to the contrary, on silica GF254.

Counting Methods.—Liquid scintillation counting was used for the measurement of ^3H and ^{14}C activities (Packard 3320 automatic Tricarb instrument). Samples were counted in 7 ml of scintillator, after dissolution in methanol or dimethyl sulphoxide (0.2 ml) and values are not corrected for self-absorption. Relative efficiencies were obtained by counting [1,2-$^3H_2$]- and [2-^{14}C]-hexadecane standards.

Synthesis of 1-Benzylisoquinoline Precursors.—The racemates of coclaurine,[9] norcoclaurine,[10] and *N*-methylcoclaurine [11] were prepared by standard procedures.

6,7-*Bisbenzyloxy*-1-(p-*benzyloxybenzyl*)-3,4-*dihydroisoquinoline Methiodide* (15).—6,7-Bisbenzyloxy-1-(*p*-benzyloxybenzyl)-3,4-dihydroisoquinoline [10] (250 mg) in dry benzene (10 ml) was treated with methyl iodide (2 ml). The resulting mixture when kept at room temperature for 20 h gave the *methiodide* (15) as yellow needles (220 mg), m.p. 200—202° (Found: C, 66.45; H, 5.7; N, 2.5. $C_{38}H_{36}INO_3$ requires C, 66.95; H, 5.3; N, 2.05%); $\nu_{max.}$ 1 600, 1 560, 1 430, 1 270, 1 220, and 730 cm^{-1}.

6,7-*Bisbenzyloxy*-1-(p-*benzyloxybenzyl*)-1,2,3,4-*tetrahydro*-2-*methylisoquinoline* (12).—To a suspension of the methiodide (15) (200 mg) in methanol (25 ml) at 0 °C was added sodium borohydride (500 mg). After 1 h the solvent was removed; the residue was treated with 4N-sodium hydroxide (5 ml) and extracted with ether (15 × 4 ml). The extract was washed with water, dried, and evaporated, and the residue was chromatographed on alumina (grade III; 50 g). Elution with benzene–chloroform (1 : 1) gave the product (12) (90 mg), m.p. 75—77°; *hydrochloride*, m.p. 178—180° (Found: C, 76.85; H, 6.0; N, 2.15. $C_{38}H_{38}ClNO_3$ requires C, 77.1; H, 6.4; N, 2.35%); $\nu_{max.}$ 1 600, 1 370, 1 235, and 725 cm^{-1}.

1,2,3,4-*Tetrahydro*-6,7-*dihydroxy*-1-(p-*hydroxybenzyl*)-2-*methylisoquinoline* (11).—Compound (12) (200 mg) in ethanol (10 ml) was refluxed with 8N-hydrochloric acid for 1 h. The resulting mixture was worked up to give the product (11); *hydrochloride* (110 mg), m.p. 165—170° (decomp.) (Found: C, 63.75; H, 6.7; N, 4.75. $C_{17}H_{20}ClNO_3$ requires C, 63.45; H, 6.2; N, 4.35%); $\nu_{max.}$ 3 230, 1 600, 1 360, 1 210, and 820 cm^{-1}.

Resolution.—(±)-Di-*O*-bisbenzyl-*N*-methylcoclaurine was resolved by treatment with (−)- and (+)-di-*p*-toluoyltartaric acid. Hydrolysis of the benzyl ethers with hydrochloric acid furnished (−)-(*R*)- and (+)-(*S*)-*N*-methylcoclaurines.[11]

Labelling of Precursors.—Tritiation. Tritium was introduced by the published [12] procedure earlier. (±)-Coclaurine hydrochloride (110 mg) in tritiated water (0.5 ml, 60 mCi) containing potassium t-butoxide (200 mg) was heated under nitrogen (sealed tube) for 100 h at 100 °C to give (±)-[3′,5′,8-$^3H_3$]coclaurine, which was purified as its hydrochloride (70 mg) to constant activity. The other 1-benzyltetrahydroisoquinoline precursors were tritiated in the same way.[12]

(±)-*N*-Methyl[1-^3H]coclaurine and (±)-[1-^3H]norcoclaurine were prepared by reduction of the corresponding dihydroisoquinolines with potassium [^3H]borohydride in dry dimethylformamide.

(±)-*N*-[^{14}C]Methylcoclaurine, (±)-*N*-[^{14}C]methylnorcoclaurine, and didehydro-*N*-[^{14}C]methylcoclaurinium iodide were prepared by treating the corresponding dihydroisoquinolines with [^{14}C]methyl iodide and subsequent reduction with sodium borohydride.

(±)-*N*-Methyl[*methoxy*-^{14}C]coclaurine was prepared by treatment of (±)-[*methoxy*-^{14}C]coclaurine with formic acid–formaldehyde. (±)-*NOO*-Trimethyl[3′,5′,8-$^3H_3$]coclaurine was prepared by treating (±)-*N*-methyl-[3′,5′,8-$^3H_3$]coclaurine with diazomethane.

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

Feeding Experiments.—For feeding purposes *N*-methyl- and *NOO*-trimethyl-coclaurine were dissolved in water (1 ml) containing tartaric acid (10 mg). Coclaurine hydrochloride, didehydro-*N*-methylcoclaurinium iodide, norcoclaurine hydrochloride, and *N*-methylnorcoclaurine hydrochloride were dissolved in aqueous dimethyl sulphoxide (1 ml). Freshly cut young branches of *C. laurifolius* were dipped into the solutions and allowed to take up the precursor. When uptake was complete water was added for washing. The twigs were then dipped in water, left for 5—6 days, and worked up for cocsulin.

Isolation and Purification of Cocsulin.—The young stems and leaves (typically 110 g wet wt) were macerated in ethanol (300 ml) containing inactive cocsulin (140 mg) and left for 12 h. The ethanol was then decanted and the plant material was percolated with fresh ethanol (6 × 200 ml). The combined ethanolic extract was concentrated *in vacuo*. The green viscous mass so obtained was treated with 5% hydrochloric acid (5 × 10 ml). The acidic solution was defatted with ether (3 × 15 ml) and then basified with aqueous sodium carbonate. The liberated bases were extracted with chloroform (5 × 20 ml), and the combined extracts were washed with water, dried (Na_2SO_4), and evaporated to afford crude cocsulin (130 mg), which was chromatographed over a column of alumina (15 g; grade III; deactivated with 1% water). Elution with benzene–chloroform (1 : 3) afforded cocsulin (6) (95 mg), m.p. 272—274° (from ethyl acetate) (lit.,[1] 272—274°). The radiochemical purity of the biosynthetic cocsulin was checked by the dilution method.

Reductive Fission of Tritium-labelled Cocsulin.—Cocsulin (6) (430 mg) (molar activity 2.4 μCi mmol^{-1}) derived from (±)-*N*-methyl[1-^3H]coclaurine feeding was treated with ethereal diazomethane and left at room temperature for 60 h to give *O*-methylcocsulin (7) (400 mg), m.p. 213—214° (lit.,[1] 212—214°) (molar activity 2.3 μCi mmol^{-1}).

Liquid ammonia (150 ml) was treated with sodium hydride (1 g), and to the product was gradually added sodium (500 mg). A solution of radioactive *O*-methylcocsulin (380 mg) in toluene (12 ml) was then added dropwise. The resulting mixture was stirred at −68 °C and

[9] K. Kratzl and G. Billek, *Monatsh.*, 1951, **82**, 568.
[10] H. Yamaguchi, *J. Pharm. Soc. Japan*, 1958, **78**, 692.
[11] H. Yamaguchi, *J. Pharm. Soc. Japan*, 1958, **78**, 678.
[12] D. S. Bhakuni, S. Satish, H. Uprety, and R. S. Kapil, *Phytochemistry*, 1974, **13**, 2767.

again sodium (200 mg) was added till a blue colour persisted. The mixture was left for 3 h. Ammonia was then allowed to evaporate off at room temperature. Water was added to the residue and the non-phenolic products were extracted with chloroform. The aqueous alkaline solution was saturated with ammonium chloride. The biphenyl derivative from the chloroform solution was extracted with citrate–phosphate buffer (pH 6.5). The remaining phenolic bases in methanol were treated with ethereal diazomethane to give *O*-methyl derivatives, which in dry toluene (15 ml) were added to liquid ammonia (100 ml) (dried over sodium) containing sodium (200 mg). More sodium was added until a blue colour persisted. After stirring at −60 °C for 3 h, the mixture was left for 16 h at room temperature, then worked up in the usual manner, and phenolic and non-phenolic bases were separated.

The mixture of non-phenolic bases was chromatographed on basic alumina. The material from elution with hexane–benzene (1 : 3) when further separated by preparative t.l.c. (solvent chloroform–methanol, 19 : 1) afforded compound (16) (10 mg), m.p. 62—64° (lit.,[7] 64—65°) (molar activity 1.12 μCi mmol^{-1}).

The mixture of phenolic bases was treated with diazomethane to give methyl ethers, which were subjected to preparative t.l.c. on alumina (solvent benzene–ethyl acetate, 96 : 4) to give compounds (17) (8 mg), m.p. 134—135° (lit.,[7] 135—136°) (molar activity 1.12 μCi mmol^{-1}), and (18) (9 mg), m.p. 60° (lit.,[13] 61—62°) (molar activity 1.13 μCi mmol^{-1}).

Degradation of Tritium-labelled Cocsulin.—Tritium-labelled cocsulin derived from (±)-*N*-methyl[1-^{3}H]coclaurine was degraded as follows.

Radioactive cocsulin (350 mg) (molar activity 0.61 μCi mmol^{-1}) in methanol (5 ml) was treated with diazomethane to give the *methyl ether* (7) (220 mg), m.p. 213—214° (lit.,[1] 212—214°) (molar activity 0.56 μCi mmol^{-1}).

The radioactive (7) (300 mg) in methanol (8 ml) was heated gently under reflux with methyl iodide (1.5 ml) for 4 h. The resulting mixture was worked up to give the corresponding *dimethiodide* (302 mg), m.p. 264—266° (decomp.) (lit.,[1] 263—264°) (molar activity 0.58 μCi mmol^{-1}).

The radioactive dimethiodide (300 mg) in methanol was passed through a column of Amberlite IR 410 anion-exchange resin (10 g) to afford the corresponding methohydroxide, which was heated with potassium hydroxide (1.5 g in 10 ml of water) at 100 °C for 4 h to give the methine (19) (230 mg), m.p. 111—113° (lit.,[1] 112—115°) (molar activity 0.57 μCi mmol^{-1}).

The radioactive methine (19) (200 mg) in aqueous ethanol (10 ml) at 0 °C was stirred with dimethyl sulphate (0.5 ml) and 10N-potassium hydroxide (0.25 ml) for 1 h. At hourly intervals three more portions of dimethyl sulphate (0.25 ml) and 10N-potassium hydroxide (0.13 ml) were added. After 5 h stirring potassium hydroxide (6 g) was added and the resulting mixture was heated under reflux for 2 h. The trimethylamine thus formed was collected in 20% hydrochloric acid. The remaining alkaline solution was extracted with ether–chloroform (3 : 1 v/v; 4 × 50 ml). The combined organic solutions were washed with water, dried (Na_2SO_4), and evaporated to give the methine (20) (160 mg), m.p. 120—121° (lit.,[1] 121—122°) (molar activity 0.558 μCi mmol^{-1}).

To a stirred solution of the radioactive methine (20) (160 mg) in t-butyl alcohol (5 ml) and water (4 ml) was added aqueous osmium tetraoxide (4%; 1.5 ml). The orange complex formed was treated with sodium periodate (140 mg). More sodium periodate (60 mg) was added after 2 h. Stirring was continued for another 3 h and the resulting mixture was decomposed with saturated aqueous arsenious oxide solution (50 ml). The resulting solution was extracted with ether–chloroform (3 : 1 v/v; 4 × 20 ml) and the extract was washed with water and dried (Na_2SO_4). Removal of the solvent left a mixture which was separated by preparative t.l.c. (solvent chloroform–methanol, 98.5 : 1.5) to give *4-methoxydibenzo*-p-*dioxin*-1,2,7,9-*tetracarbaldehyde* (21) (12 mg), m.p. 225—227° (molar activity 0.545 μCi mmol^{-1}) and the aldehyde (22),[14] which was essentially inactive. A parallel experiment with radioinactive material gave the aldehyde (21), m.p. 227° (decomp.) (Found: M^+, 326.1558. $C_{17}H_{10}O_7$ requires M, 326.1576).

Degradation of [^{14}C]*Cocsulin.*—Labelled cocsulin derived from (±)-*N*-[^{14}C]methylcoclaurine was converted into *O*-methylcocsulin (7) as above. Hofmann degradation of (7) as earlier gave the methine (19). Treatment of (19) with dimethyl sulphate and potassium hydroxide yielded trimethylamine. The radioactivities of the degradation products are given in Table 2.

TABLE 2

Activities of cocsulin degradation products

Compound	Molar activity (disint. min^{-1} mmol^{-1})
Cocsulin (6)	3.52 × 10^4
O-Methylcocsulin (7)	3.40 × 10^4
O-Methylcocsulin dimethiodide	3.51 × 10^4
Methine (19)	3.44 × 10^4
Trimethylamine hydrochloride	1.73 × 10^4

[7/1040 *Received*, 17*th June*, 1977]

[13] M. Tomita and J. Kunitomo, *J. Pharm. Soc. Japan*, 1962, **82**, 734.

[14] F. V. Bruchhausen, H. Oberembt, and A. Feldhans, *Annalen*, 1933, **507**, 144.