Biosynthesis of Aromatic Isoprenoids. Part I. The Role of 3-Prenylquinolines and of Platydesmine in the Biosynthesis of the Furoquinoline Alkaloid, Dictamnine

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Tracer feeding experiments show that quinoline-2,4-diol and 4-methoxy-2-quinolone are involved in the biosynthesis of the furoquinoline alkaloid, dictamnine (4) and of the hydroxyisopropyldihydrofuroquinoline alkaloid, platydesmine metho-salt (5) in *Skimmia japonica* Thunb., and that 4-hydroxy- and 4-methoxy-3-(3-methyl-[1-^{14}C]but-2-enyl)-2-quinolone are incorporated specifically (3·6—4·8%) into the two alkaloids. Platydesmine (3) also is an efficient specific precursor of dictamnine (18·8% incorporation) in *S. japonica*, and trapping experiments indicate that it is a biosynthetic intermediate. There was no significant incorporation of dihydrodictamnine or of platydesmine metho-salt into dictamnine. The precursors were utilised to a small extent in the formation of 7,8-dimethoxydictamnine (skimmianine) in *S. japonica*, suggesting that aromatic hydroxylation can occur at a late stage of biosynthesis. A convenient preparation of 1-bromo-3-methyl[1-^{14}C]but-2-ene is described.

As discussed in the preliminary accounts of our work,[1] the proposal that the biosynthesis of the two-carbon fragment of the furan ring present in furocoumarins and in furoquinolines occurs by loss of an isopropyl group from an isoprenoid substituent was based on chemotaxonomic evidence and on the results of *in vitro* reactions [2] until [4-^{14}C]mevalonate was shown to be a precursor of the furocoumarins of *Pimmella magna*.[3] On the other hand, neither acetate nor mevalonate was incorporated into the furan carbon atoms of the furoquinoline alkaloid, dictamnine (4),[4,5] although [^{14}C]-acetate provided carbon atoms 2 and 3 of the quinoline ring.[4] In the light of these results, we decided to study the biosynthesis of furo- and isopropylfuro-quinolines by feeding ^{14}C-labelled compounds likely to feature later in the pathway, particularly quinoline-2,4-diol and its 3-(3,3-dimethylallyl) derivatives. *Skimmia japonica* Thunb., which contains dictamine (4) and platydesmine metho-salt (5) as principal alkaloids,[6] appeared to be suitable for our purpose.

[2,4-^{14}C$_2$]Quinoline-2,4-diol was prepared from sodium [^{14}C]cyanide *via* diethyl [1,3-^{14}C$_2$]malonate essentially

[1] J. F. Collins and M. F. Grundon, *Chem. Comm.*, 1969, 622; M. F. Grundon and K. J. James, *ibid.*, 1971, 1311.

[2] R. Robinson, 'The Structural Relations of Natural Products,' Clarendon Press, Oxford, 1955, p. 94; A. J. Birch and H. Smith, *Chem. Soc. Special Publ.*, 1958, No. 12, p. 4; R. Aneja, S. R. Mukerjee, and T. R. Seshadri, *Tetrahedron*, 1958, **4**, 256; G. A. Diment, E. Ritchie, and W. C. Taylor, *Austral. J. Chem.*, 1967, **20**, 565; A. J. Birch, M. Muang, and A. Pelter, *ibid.*, 1969, **22**, 1923; T. R. Seshadri, M. S. Hood, K. L. Handa, and Vishwapaul, *Tetrahedron*, 1967, **23**, 1883; A. P. Prokepeuko, *Khim. prirod. Soedinenii*, 1965, **3**, 215.

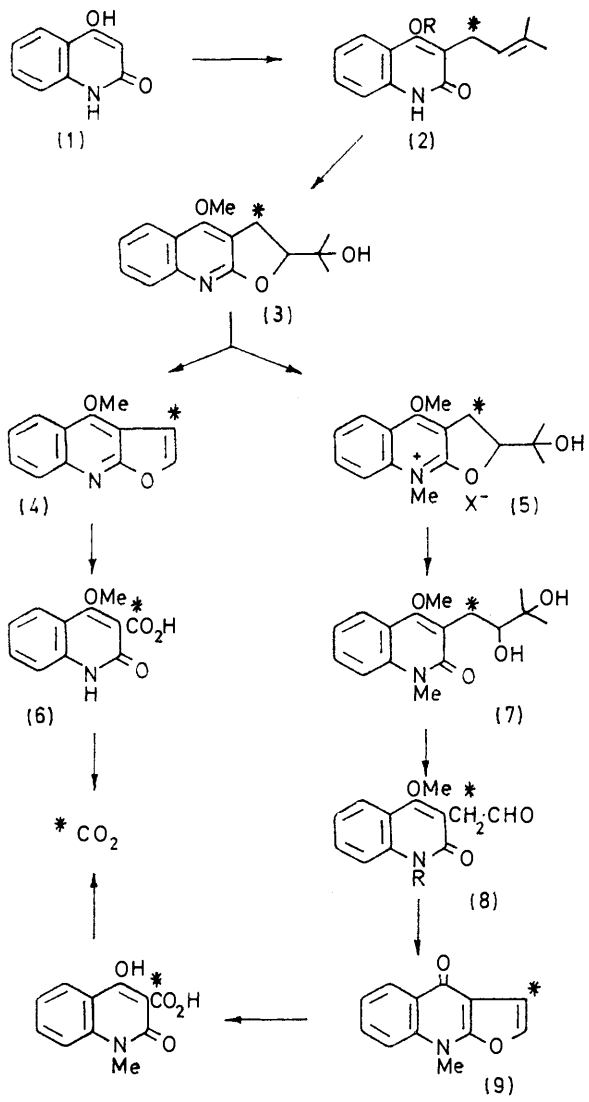
[3] H. G. Floss and U. Mothes, *Phytochemistry*, 1966, **5**, 169; H. G. Floss and H. Paikert, *ibid.*, 1969, **8**, 589.

[4] I. Monkovic, I. D. Spencer, and A. O. Plunkett, *Canad. J. Chem.*, 1967, **45**, 1935.

[5] E. Atkinson, D. R. Boyd, and M. F. Grundon, unpublished work.

[6] D. R. Boyd and M. F. Grundon, *J. Chem. Soc.* (*C*), 1970, 556.

by the method described previously [7,8] and was converted with diazomethane into the 4-methoxy-derivative. 3,3-Dimethylallylquinolones (2) specifically labelled with ^{14}C at the 1- or at the 2-position of the



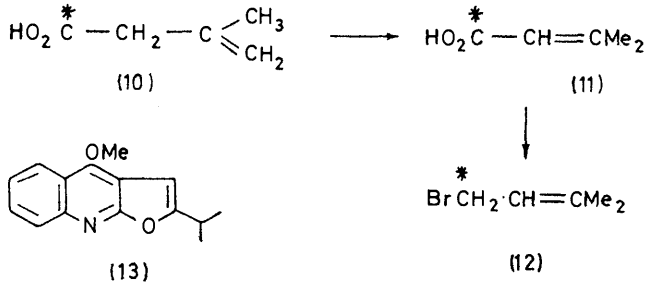
SCHEME

* Site of ^{14}C label.

side chain were also required, and for this purpose 1-bromo-3-methyl[1-^{14}C]but-2-ene (12) was prepared. Treatment of the Grignard reagent derived from 1-chloro-2-methylpropene with [^{14}C]carbon dioxide gave 3-methyl-[1-^{14}C]but-3-enoic acid [7,9] (10), isomerized by alkali to the 2-ene (11). Reduction of the ethyl ester with lithium aluminium hydride and treatment of the resultant alcohol with bromine and triphenyl phosphite or with phosphorus tribromide [10] furnished the required bromide (12) in 35% overall yield from 1-chloro-2-methylpropene. Two methods were available for the synthesis of 3-prenylquinolines (2). The reaction of aniline with diethyl (3-methyl[1-^{14}C]but-2-enyl)-malonate furnished the 4-hydroxy-2-quinolone (2; R = H),[11] and this was converted into the 4-methoxy-2-quinolone (2; R = Me). A more convenient procedure [12] for the preparation of the latter compound involved ether cleavage of 2,4-dimethoxy-3-(3-methylbut-2-enyl)quinoline obtained from 2,4-dimethoxyquinoline and 1-bromo-3-methyl[1-^{14}C]but-2-ene.

Since *Skimmia japonica* is a bush growing up to 4 ft high, feeding experiments were carried out most conveniently by using excised green shoots immersed in a nutrient solution. The ^{14}C-compounds listed above were applied as emulsions containing light petroleum or chloroform or as solutions in aqueous dimethyl sulphoxide. The results are summarised in the Table. Specific incorporation of [^{14}C]quinolones (2) into the alkaloids was demonstrated by degradation (Scheme). Oxidation of dictamnine by the method previously described [4] furnished dictamnic acid (6), which yielded inactive quinoline-2,4-diol and carbon dioxide with 91% of the radioactivity present in the alkaloid. Platydesmine metho-salt (5) was counted as its base-cleavage product edulinine (7).[6] Oxidation of the diol with periodate furnished the aldehyde (8; R = Me), which after cyclisation to isodictamine (9) and oxidation gave [^{14}C]carbon dioxide showing 82% retention of activity.

The incorporation of 4-hydroxy-[2,4-$^{14}C_2$]-2-quinolone and of 4-methoxy-[2,4-$^{14}C_2$]-2-quinolone into dictamnine (4) and into platydesmine metho-salt (5) (Table) shows



that the biosynthetic pathway to these alkaloids in *S. japonica* involves formation of the quinoline system

[7] A. Murray and D. L. Williams, 'Organic Synthesis with Isotopes,' Interscience, New York and London, 1958.
[8] G. H. Patel and C. M. Mehta, *J. Sci. Ind. Res., India*, 1960, **19B**, 436.
[9] A. G. Geldberg and R. P. Linstead, *J. Chem. Soc.*, 1928, 2343.
[10] A. Schinz and G. Schnappi, *Helv. Chim. Acta*, 1947, **30**, 1483.
[11] R. M. Bowman and M. F. Grundon, *J. Chem. Soc.* (*C*), 1966, 1504.
[12] J. F. Collins, G. A. Gray, M. F. Grundon, D. M. Harrison, and C. G. Spyropoulos, *J.C.S. Perkin I*, 1973, 94.

before introduction of the side chains. 2,4-Dimethoxy-[2,4-$^{14}C_2$]quinoline was also fed, but was not incorporated. The 3-isoprenylquinolines (2; R = H or Me) serve as efficient precursors of the two alkaloids, thus indicating the isoprenoid origin of the furan ring of dictamine as well as of the isopropylfuro-group present in the quaternary alkaloid (5). The results, however, do not reveal the sequence of biological methylation, and the enzymic introduction and loss of methyl groups may be possible at each stage of the pathway.

Although the metho-salt (5) co-occurs with dictamnine (4), the corresponding tertiary base appeared to be a more probable biosynthetic intermediate in the transformation of the prenylquinolines (2) into dictamnine and we decided to feed specifically labelled platydesmine to *S. japonica*.

(±)-[^{14}C]Platydesmine (3) was obtained by oxidative cyclisation of 4-methoxy-3-(3-methyl[1-^{14}C]but-2-enyl)-2-quinolone (2; R = Me) with *m*-chloroperbenzoic acid, and a solution in aqueous dimethyl sulphoxide was fed to shoots of *S. japonica*. Radioactive dictamnine and platydesmine metho-salt were isolated, and each was degraded by the methods described earlier to show that significant randomisation of the labels had not occurred. Incorporations into dictamnine and platydesmine metho-salt were 18·8 and 4·3%, respectively; these figures are regarded as minimal since it is probable that only one enantiomer present in the racemic platydesmine is utilised in the biosynthesis.

Platydesmine is a constituent of at least two rutaceous plants [13] but it has not been detected in *S. japonica*. In order to assist in the interpretation of our biosynthetic results, we carried out an *in vivo* isotope-trapping experiment by feeding the [^{14}C]-3-dimethylallyl-2-quinolone (2; R = Me) in the presence of unlabelled (±)-platydesmine. After 24 h, isolation of platydesmine and dictamnine showed that incorporations of the quinolone (2; R = Me) into the alkaloids were 0·86 and 0·82%, respectively. The radioactive purity of platydesmine was checked by conversion into anhydroplatydesmine (13). Thus, platydesmine is not only utilised efficiently *in vivo* for the synthesis of dictamnine but is also an intermediate in the biosynthetic pathway.

There was the possibility that platydesmine metho-salt could also serve as a precursor of dictamine. However, when [^{14}C]platydesmine methiodide was fed, only a very small incorporation (0·03%) into dictamnine was observed, thus showing that this is not a major pathway. During our work, it was reported that the dihydrofurocoumarin (14) was a precursor of the corresponding furocoumarin, psoralen (0·02% incorporation; general tritium label) in *Ficus carica*.[14] We find, however, that this is not an important route in *S. japonica*, since [^{14}C]dihydrodictamnine, prepared by hydrogenation of radioactive dictamnine obtained from platydesmine feeding experiments, was not incorporated significantly into dictamnine.

Our work indicates that the furan ring of dictamnine and the hydroxyisopropyldihydrofuro-ring of platydesmine metho-salt in *S. japonica* are isoprenoid-derived, and shows that the major route to the alkaloids involves the sequence (1) ⟶ (2) ⟶ (3) ⟶ (4) or (5) (Scheme); later studies [15] suggest that this is a general pathway to furoquinoline alkaloids and to furocoumarins.

Tracer experiments with *Skimmia japonica* Thunb.

Precursor	Method of feeding	Incorporation [a] (%) into Dictamnine	Incorporation [a] (%) into Quaternary salt (5)
[2,4-$^{14}C_2$]quinoline-2,4-diol	In N-Na_2CO_3	1·3	2·0
4-Methoxy-[2,4-$^{14}C_2$]-2-quinolone (2; R = H)	Dispersion in $CHCl_3$–H_2O	1·5	2·1
(2; R = Me)	Dispersion in $CHCl_3$–H_2O	3·8	4·7
	Dispersion in light petroleum–H_2O	3·6	4·8
(±)-[^{14}C]Playdesmine (3)	In H_2O–Me_2SO	18·8	4·3
(±)-[^{14}C]Platydesmine methiodide (5)	In H_2O	0·03	
[^{14}C]Dihydrodictamnine	In H_2O–Me_2SO	<0·1	

[a] Mean values of two or more experiments.

Skimmianine (15) is a minor constituent of *S. japonica* [6] and the radioactive alkaloid (0·6% incorporation) was obtained from our feeding experiments with labelled quinoline-2,4-diol. The latter compound stimulated the production of skimmianine, as observed by Steck with tissue cultures of *Ruta graveolens*.[16] We showed also that [^{14}C]platydesmine (3) was incorporated (0·1%) into skimmianine, suggesting that hydroxylation of the homocyclic ring of the quinoline nucleus occurs late in the biosynthetic pathway to skimmianine; using rutaceous plants in which skimmianine is a major alkaloid we established subsequently that dictamnine is a specific precursor of skimmianine.[17]

EXPERIMENTAL

Measurements of radioactivity were made with an Isotope Developments Limited 1DL 6012 scintillation counter

[13] F. Werny and P. J. Scheuer, *Tetrahedron*, 1963, **19**, 1293; S. R. Johns and J. A. Lamberton, *Austral. J. Chem.*, 1966, **19**, 1991.

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[15] A. O. Colonna and E. G. Gros, *Chem. Comm.*, 1970, 674; M. Cobet and M. Luckner, *Phytochemistry*, 1971, **10**, 1031; S. A. Brown, M. El Dakhakpay, and W. Steck, *Canad. J. Biochem.*, 1970, **48**, 863.

[16] W. Steck, personal communication.

[17] J. F. Collins, W. J. Donnelly, M. F. Grundon, D. M. Harrison, and C. G. Spyropoulos, *J.C.S. Chem. Comm.*, 1972, 1029.

or with a Packard Tri-Carb 3320 instrument. The scintillation solution used was a PPO–POPOP toluene-based mixture, and correction for quenching was carried out by the channels-ratio method. Percentage incorporations were calculated as (total number of counts in isolated product $\times 100$)/(total number of counts fed). Barium carbonate was counted as a suspension in Instagel–water (8 : 3).

[2,4-$^{14}C_2$]*Quinoline-2,4-diol and 4-Methoxy-*[2,4-$^{14}C_2$]*-2-quinolone.*—Diethyl [1,3-$^{14}C_2$]malonate, prepared from sodium [^{14}C]cyanide,[7] reacted with aniline to give a mixture of mono- and di-anilides, which was converted with polyphosphoric acid into [2,4-^{14}C]quinoline-2,4-diol (41%). Treatment of this with diazomethane afforded the 4-methoxy-derivative.

1-*Bromo-3-methyl*[1-^{14}C]*but-2-ene.*—Methallyl chloride (38 g) in ether (80 ml) was added to magnesium (10·08 g) and a crystal of iodine in ether (300 ml) at 0°. After 2 h, the mixture, attached to a vacuum manifold, was treated with [^{14}C]carbon dioxide generated by dropping degassed sulphuric acid (40 ml) onto barium [^{14}C]carbonate (15·5 g). Water was added, and the mixture was acidified with 2N-hydrochloric acid, and extracted with ether. 3-Methyl-[1-^{14}C]but-3-enoic acid was obtained from the ethereal solution with aqueous sodium hydroxide and was refluxed in aqueous potassium hydroxide (100 ml; 40%) for 4 h. Acidification, extraction with ether, and careful removal of the solvent to avoid sublimation furnished 3-methyl-[1-^{14}C]but-2-enoic acid as a solid (3·2—4·6 g, 39—56%), m.p. 66—68° (lit.,[18] 66—69°).

A solution of the 2-enoic acid (3·2 g) in ethanol (10 ml) and benzene (20 ml) containing sulphuric acid (2 drops) was refluxed in a Soxhlet extractor containing anhydrous magnesium sulphate (20 g). Water (20 ml) was added, the solution was extracted with ether (3 × 25 ml), and the extract was washed with aqueous 10% sodium hydrogen carbonate. Evaporation gave ethyl 3-methyl[1-^{14}C]-but-2-enoate (3·8 g, 94%), b.p. 49° at 12 mmHg, identical (i.r.) with an authentic sample.

A suspension of lithium aluminium hydride (1·1 g) in ether (25 ml) was added to a solution of the ester (3·8 g) in ether (20 ml), and the mixture was refluxed for 1 h. Water was added cautiously, followed by 2N-sulphuric acid (50 ml). Extraction with ether gave 3-methyl-[1-^{14}C]but-2-enol as an oil (2·4 g, 98%), identical with an authentic sample.

Bromine (1·0 g) was added dropwise to triphenyl phosphite (5·4 g) in ether (5 ml) at 0° under nitrogen to give a precipitate of triphenyl phosphite dibromide. The 2-enol (1·7 g) in ether (5 ml) was then added dropwise, followed by N-sodium thiosulphate until the solution was colourless. Ether was removed, and the residue was distilled under reduced pressure through a short fractionating column to give 1-bromo-3-methyl[1-^{14}C]but-2-ene (2·3 g, 75%), b.p. 75° at 12 mmHg. The following preparation of the bromide was also used. Phosphorus tribromide (2·2 g) in light petroleum (b.p. 60—80°) (10 ml) was added slowly to a stirred solution of the 2-enol (2 g) in light petroleum at 0°. After 24 h, water was added, the layers were separated, and the aqueous layer was extracted with dichloromethane. Evaporation of the combined dichloromethane–light petroleum solution afforded the bromide (1·5 g, 45%).

3-(3-*Methyl*[1-^{14}C]*but-2-enyl)-2-quinolones* (2).—The reaction of 1-bromo-3-methyl[1-^{14}C]but-2-ene (1·5 g) with diethyl malonate furnished diethyl 3-methyl[1-^{14}C]but-2-enyl)malonate (1·6 g, 74%).[19] The ester (2·7 g) was converted as described previously[11] into 4-hydroxy-3-(3-methyl[1-^{14}C]but-2-enyl)-2-quinolone (2; R = H) (1·2 g), m.p. 180—182° (lit.,[20] m.p. 180—182°), which with diazomethane gave 4-methoxy-3-(3-methyl[1-^{14}C]but-2-enyl)-2-quinolone (2; R = Me) (71%), m.p. 133° (lit.,[11] 132—134°). The 4-methoxy-compound was also prepared (40% yield) from 1-bromo-3-methyl[1-^{14}C]but-2-ene (1·5 g) and 2,4-dimethoxyquinoline.[12]

(±)-[^{14}C]*Platydesmine* (3) *and its Methiodide.*—In a modification of the previous method,[11] a solution of 4-methoxy-3-(3-methyl[1-^{14}C]but-2-enyl)-2-quinolone (0·16 g) and *m*-chloroperbenzoic acid (0·35 g) in chloroform (15 ml) was kept for 4 days, extracted with 2N-sodium hydroxide, and evaporated. Chromatography of the residue on alumina and elution with ether–chloroform (4 : 1) gave [^{14}C]-3,4-dihydro-3-hydroxy-5-methoxy-2,2-dimethyl-2*H*-pyrano[2,3-*b*]quinoline (0·035 g, 20%), m.p. and mixed m.p. 172—175° (lit.,[11] m.p. 175—176°). Elution with ether–chloroform (4 : 1) afforded (±)-[^{14}C]platydesmine (0·115 g, 67%), m.p. and mixed m.p. 135—137° (lit.,[11] 137—138°).

(±)-[^{14}C]Platydesmine (0·045 g) was refluxed in methyl iodide (10 ml) for 20 h. Evaporation, and extraction of the residue with water gave (±)-[^{14}C]platydesmine methiodide (0·041 g, 67%), m.p. and mixed m.p. 147—149° (lit.,[11] 156—158°).

[^{14}C]*Dihydrodictamnine.*—[^{14}C]Dictamnine (0·05 g), obtained from feeding experiments, in ethanol was hydrogenated over palladium–charcoal for 1·5 h. Preparative t.l.c. of the product on silica with chloroform–ethyl acetate (4 : 1) gave [^{14}C]dihydrodictamnine (0·31 g, 62%), m.p. 105° (lit.,[21] 105°).

Feeding Experiments with Skimmia japonica.—During April–May, young shoots of *S. japonica* were excised under water to avoid the formation of air-locks and placed in separate vials containing Hoagland's solution. The tracer mixture (Table) was added and the shoots were illuminated with 1000 W bulbs for 3 days. As absorption occurred the level was maintained by addition of Hoagland's solution.

The alkaloids were isolated as described previously.[6] Dictamnine and skimmianine were separated by preparative t.l.c. on silica with chloroform–ethyl acetate (4 : 1) and dictamnine was crystallised to constant radioactivity. 'Carrier' platydesmine metho-perchlorate was added to the aqueous solution obtained after removal of the ether-soluble constituents, and the quaternary alkaloid was isolated as its metho-perchlorate, m.p. 190—194° (from methanol–ether), which was counted as the diol, edulinine (7), m.p. 139—140° (lit.,[6] 141—142°). The residual radioactivity in the containers (always <2%) was estimated and deducted from the total activity fed. The results are summarised in the Table.

Isotope Trapping of Platydesmine.—A mixture of 4-methoxy-3-(3-methyl[1-^{14}C]but-2-enyl)-2-quinolone (0·01 g) (total activity, $1{\cdot}28 \times 10^6$ disint. min^{-1}) and inactive (±)-platydesmine (0·05 g) was fed in aqueous dimethyl

[18] R. E. Buckles and G. V. Mock, *J. Org. Chem.*, 1950, **15**, 680.
[19] E. A. Clarke and M. F. Grundon, *J. Chem. Soc.*, 1964, 438.
[20] R. M. Bowman and M. F. Grundon, *J. Chem. Soc.* (*C*), 1966, 1084.
[21] T. Ohta, T. Miyazaki, and Y. Mori, *J. Pharm. Soc. Japan*, 1954, **74**, 708.

sulphoxide to shoots of *S. japonica*. After 24 h, the tertiary bases were extracted as usual, separated by preparative t.l.c., and crystallised to constant radioactivity, giving dictamnine (total activity $1\cdot0 \times 10^4$ disint. min^{-1}, 0·78% incorporation) and platydesmine (total activity $1\cdot1 \times 10^4$ disint. min^{-1}, 0·86% incorporation). Treatment of [^{14}C]platydesmine with sulphuric acid gave anhydroplatydesmine (13) quantitatively, which, after crystallisation from ether–light petroleum (b.p. 40—60°) to give needles, m.p. 106—108° (lit.,[22] 107—108°), showed no significant loss of radioactivity.

Degradation of Dictamnine.[4]—A solution of dictamnine (0·081 g; specific activity 0·64 μCi $mmol^{-1}$) and potassium permanganate (0·20 g) in acetone (30 ml) was refluxed for 1·5 h, and evaporated. The residue in water (25 ml) was saturated with sulphur dioxide, giving a precipitate of dictamnic acid (0·049 g, 65%) [specific activity 0·60 μCi $mmol^{-1}$ (94% retention)]. Dictamnic acid (0·40 g) in conc. hydrochloric acid was refluxed under nitrogen for 30 min, the evolved gas being passed into a suspension of barium hydroxide in water. Separation of the precipitate by centrifugation gave barium carbonate [specific activity 0·58 μCi $mmol^{-1}$ (97% retention)]. Evaporation of the filtrate and sublimation of the residue gave inactive quinoline-2,4-diol (0·008 g), m.p. >300°.

Degradation of Edulinine (7).—A solution of edulinine (0·025 g; specific activity $5\cdot30 \times 10^{-2}$ μCi $mmol^{-1}$), obtained from [^{14}C]platydesmine perchlorate, in methanol (1 ml) and water (1 ml) containing periodic acid (0·5 ml), was stirred at 20° for 24 h. Addition of water (10 ml), extraction with dichloromethane, and evaporation gave the aldehyde (8) (0·010 g, 50%), m.p. 113°, identical (i.r. and t.l.c.) with an authentic sample, m.p. 113—115°;[12] the specific activity was $5\cdot05 \times 10^{-2}$ μCi $mmol^{-1}$ (96% retention).

The aldehyde (specific activity $6\cdot39 \times 10^{-3}$ μCi $mmol^{-1}$) was cyclised[12] to isodictamnine (9), m.p. 178—181° (lit.,[12] m.p. 185—188°). [^{14}C]Isodictamnine (0·014 g) was diluted with an equal quantity of cold isodictamnine and oxidised with potassium permanganate (50 mg) in refluxing acetone (5 ml) for 1·5 h. Work-up as usual gave *N*-methyldictamnic acid (0·013 g, 84%), m.p. 220—232°, which was decarboxylated as described for dictamnic acid to give barium carbonate (0·011 g, 95%) [specific activity $5\cdot76 \times 10^{-3}$ μCi $mmol^{-1}$ (90% retention)].

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[22] S. R. Johns, J. A. Lamberton, and A. A. Sioumis, *Austral. J. Chem.*, 1967, **20**, 1975.