Phenol Oxidation and Biosynthesis. Part XV.† The Biosynthesis of Roemerine, Anonaine, and Mecambrine

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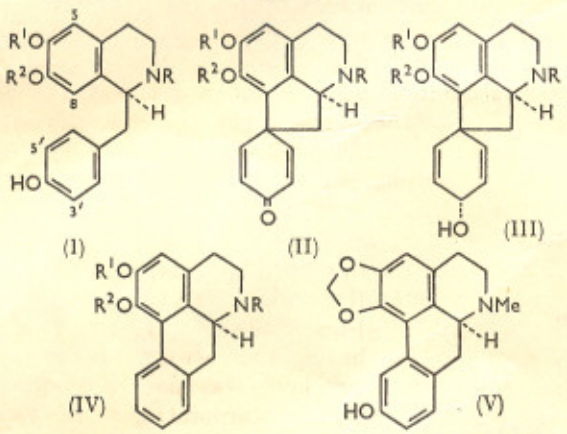
The relationship between the benzylisoquinoline alkaloid coclaurine and the aporphine alkaloid roemerine in *Papaver dubium* L. has been studied. Various congeners of coclaurine have also been utilised in the investigation of this biosynthesis. The corresponding biosynthesis of anonaine in *Anona reticula* L. has also been studied. The biosynthesis of mecambrine has been more briefly investigated. The relationship between mecambrine and roemerine has been demonstrated. The results as a whole provide good support for the previously enunciated hypothesis that aporphine alkaloids of the anonaine–roemerine type are derived from coclaurine-type precursors through phenol oxidation. Dienones of the crotonosine–mecambrine type are intermediates.

BIOGENETIC theory [1] suggests that aporphine alkaloids lacking an oxygen substituent in ring D are derived from a benzylisoquinoline (as I) by oxidation to a dienone



(as II), reduction to dienol (as III), and dehydration with rearrangement to the fully aromatic compound (as IV).

The first example to be found in Nature of a dienone of type (II) was crotonosine [2] (II; enantiomer, R = R^7 = H, R^2 = Me) followed closely by pronuciferine.[3] The biosynthesis of crotonosine does, in fact, follow the predicted route.[4,5] Another proaporphine dienone of common occurrence is mecambrine (fugapavine([6,7] (II; R = Me, $R^1 + R^2 = CH_2$).

The main objective of the present work was to study the biosynthesis of alkaloids of type (IV). Recently Slavik [8] showed that the poppy *Papaver dubium* contains both mecambrine and (+)-roemerine (IV; R = Me, $R^1 + R^2 = CH_2$). *Papaver dubium* is a plant well suited for biosynthetic studies and we have, therefore, used it in most of the work described in the sequel. We have, in addition, used *Meconopsis cambrica* which is a good source of mecambrine. At the same time we have also given some attention to anonaine (IV; enantiomer, R = H, $R^1 + R^2 = CH_2$), which is found in *Anona squamosa* [9] and in *A. reticulata*.[10,11] Supplies of the

† Part XIV, 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.

[1] D. H. R. Barton and T. Cohen, 'Festschrift A. Stoll,' Birkhauser A.G., Basel, 1957, p. 117.

[2] L. J. Haynes and K. L. Stuart, *J. Chem. Soc.*, 1963, 1784, 1789; L. J. Haynes, K. L. Stuart, D. H. R. Barton, and G. W. Kirby, *J. Chem. Soc.* (*C*), 1966, 1676; G. Snatzke and (Miss) G. Wollenberg, *ibid.*, 1966, 1681.

[3] K. Bernauer, *Helv. Chim. Acta*, 1963, **46**, 1783; 1964, **47**, 2119, 2122; see also M. P. Cava, K. Nomura, R. H. Schlessinger, K. T. Buck, B. Douglas, R. F. Raffauf, and J. A. Weisbach, *Chem. and Ind.*, 1964, 282.

[4] L. J. Haynes, D. H. R. Barton, D. S. Bhakuni, and G. W. Kirby, *Chem. Comm.*, 1965, 141.

[5] 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.

[6] J. Slavik, *Coll. Czech. Chem. Comm.*, 1960, **25**, 1663; 1965, **30**, 914.

[7] J. Slavik and J. Appelt, *Coll. Czech. Chem. Comm.*, 1965, **30**, 3687; S. Yu. Yunusov, V. A. Mnatsakanyan, and S. T. Akramov, *Doklady Akad. Nauk S.S.S.R.*, 1961, 43; L. Kuhn, S. Pfeifer, J. Slavik, and J. Appelt, *Naturwiss.*, 1964, **51**, 556; L. Kuhn and S. Pfeifer, *Pharmazie*, 1965, **20**, 520, 659.

[8] J. Slavik, *Coll. Czech. Chem. Comm.*, 1963, **28**, 1738.

[9] N. Trimurti, *J. Indian Inst. Sci.*, 1924, **7**, 232.

[10] A. C. Santos, *Phillipine J. Sci.*, 1930, **43**, 561.

[11] K. W. Gopinath, T. R. Govindachari, B. R. Pai, and N. Viswanathan, *Chem. Ber.*, 1959, **92**, 776.

latter plant were available. A preliminary account of our more important findings has already been published.[12] Related investigations on the biosynthesis of orientalinone and its transformation product isothebaine have recently been reported by Battersby and his colleagues,[13] and support, in an elegant manner, previously postulated [14] biosynthetic pathways.

Synthesis of Precursors.—The method of preparation of many of the precursors used has been described.[5] (±)-*N*-[^{14}C]Methylcoclaurine [(±)-I; R = Me-^{14}C, R^1 = Me, R^2 = H] was prepared by Eschweiler–Clarke methylation of (±)-coclaurine [(±)-I; R = R^2 = H, R^1 = Me) using radioactive paraformaldehyde. (±)-*N*-[^{14}C]-Methylnorcoclaurine [(±)-I; R = Me-^{14}C, R^1 = R^2 = H] was obtained by similar *N*-methylation of *OOO*-tribenzylnorcoclaurine, with subsequent catalytic debenzylation. (±)-[*N*-*Me*-^{14}C]Armepavine [(±)-I; R = Me-^{14}C, R^1 = R^2 = Me) was obtained by the same process on *O*-benzyl-*N*-norarmepavine followed by removal of the benzyl group.

N-Methyl-[3-^{14}C]coclaurine was prepared by treating 3-methoxy-4-benzyloxybenzyl chloride with radioactive cyanide, reduction to the amine,[15] and the usual series of reactions [5] to form the benzylisoquinoline.

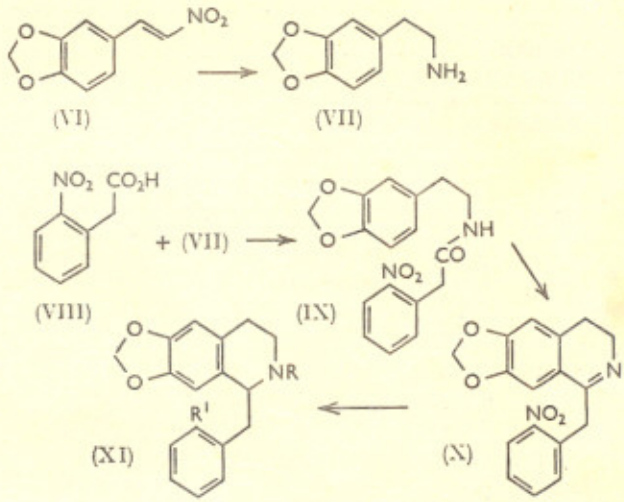
Labelled mecambrine (II; R = Me, R^1 + R^2 = CH_2) was prepared by exchange with base in tritiated water at room temperature.[16] Its radiochemical purity was checked by conversion into mecambroline (V), the molar activity remaining the same. In this labelling procedure the tritium must be introduced into the α-positions in the dienone ring by a base-catalysed hydration–(α)-tritiation–dehydration sequence.

Sources of Alkaloids.—(+)-Roemerine was obtained by isolation from *P. dubium* plants by essentially established procedures. (−)-Mecambrine and (+)-mecambroline were similarly isolated from *M. cambrica* roots.

(±)-Roemerine was synthesised by an essentially standard aporphine synthesis. Piperonaldehyde was converted into its nitrostyrene (VI) by an improved procedure (see Experimental section). Reduction of the latter gave the amine (VII). The acid (VIII) was prepared from *o*-nitrotoluene *via* *o*-nitrophenylpyruvic acid.[17] The amide (IX), formed from the acid (VIII) and the amine (VII), was converted into the dihydroisoquinoline (X) in the usual way. Reduction with sodium borohydride gave the tetrahydroisoquinoline (XI; R = H, R^1 = NO_2). The *N*-acetyl derivative of this compound (XI; R = Ac, R^1 = NO_2) showed in its n.m.r. spectrum two resonances for the acetyl methyl group as well as other double signals and apparently exists (on the n.m.r. time scale) in two isomeric forms.[18] *N*-Methylation of the tetrahydroisoquinoline and further reduction gave the amine (XI; R = Me, R^1 = NH_2) required for cyclisation. Diazotisation of this compound and decomposition of the diazonium salt in the presence of cuprous iodide gave (±)-roemerine in 35% yield.

(±)-Anonaine [(±)-IV; R = H, R^1 + R^2 = CH_2] was prepared from the dihydroisoquinoline (X) by the method of Barger and Weitnauer.[19]

Feeding Procedures and Results Obtained.—The poppies (*P. dubium* and *M. cambrica*) were fed by injecting the



precursors (except for tyrosine) as their hydrochlorides in water, into the seed-pods after the petals had dropped. The plants were left for ten days to metabolise the precursors and were then harvested. The precursors were administered to the stems of young *Anona reticulata* plants by wick-feeding.

The incorporations obtained for the first seasons (1964) feedings to *P. dubium* are shown in the Table. Tyrosine was incorporated (0·17%), showing that the plant was active in synthesising roemerine. As with other feedings where (+)-roemerine of the plant was diluted with (±)-roemerine, constant activity could only be obtained by recrystallisation of the optically inactive Hofmann degradation product, the methine base (XII).

As expected, (±)-isococlaurine [(±)-I; R = H, R^1 = H, R^2 = Me), having the 'wrong' methylation pattern for phenol coupling, was not incorporated. Since (±)-norcoclaurine was incorporated, no demethylation of isococlaurine had occurred in the plant.

(±)-Coclaurine was incorporated less efficiently than either (±)-norcoclaurine or (±)-*N*-methylcoclaurine, suggesting that *N*-methylation occurs at the norcoclaurine stage and precedes *O*-methylation. Since *N*-methylcoclaurine was incorporated more efficiently than coclaurine, it seemed likely that the *N*-methyl compound is the one that undergoes the actual phenol coupling.

[12] D. H. R. Barton, D. S. Bhakuni, G. M. Chapman, and G. W. Kirby, *Chem. Comm.*, 1966, 250.
[13] A. R. Battersby and T. H. Brown, *Chem. Comm.*, 1966, 170; A. R. Battersby, R. T. Brown, J. H. Clements, and G. Iverach, *ibid.*, 1965, 230; A. R. Battersby, T. H. Brown, and J. H. Clements, *J. Chem. Soc.*, 1965, 4550.
[14] A. R. Battersby, *Proc. Chem. Soc.*, 1963, 189.
[15] See A. R. Battersby, R. Binks, R. J. Francis, D. J. McCaldin, and H. Ramuz, *J. Chem. Soc.*, 1964, 3600.
[16] See G. W. Kirby and L. Ogunkoya, *J. Chem. Soc.*, 1965, 6914.
[17] W. B. Wright and K. H. Collins, *J. Amer. Chem. Soc.*, 1956, **78**, 221.
[18] See D. R. Dalton, M. P. Cava, and K. T. Buck, *Tetrahedron Letters*, 1965, 2687.
[19] G. Barger and G. Weitenauer, *Helv. Chim. Acta*, 1939, **22**, 1036.

The 1965 feedings with *P. dubium* were designed to confirm the biosynthesis of roemerine from *N*-methycoclaurine by use of a doubly-labelled precursor. The methylation pattern at the benzylisoquinoline stage and the stereospecificity of the biosynthesis were investigated. The results are shown in the Table.

Incorporation * of coclaurine derivatives into roemerine in *Papaver dubium*

1964 Season

Precursor	(±)-Coclaurine	(±)-Isococlaurine	(±)-Norcoclaurine	(±)-*N*-methylcoclaurine
Incorporation (%)	0·0062	0·00	0·34	0·48

(±)-Roemerine was used throughout for dilution. Precursors were labelled with tritium *ortho* to phenolic hydroxyl groups.

1965 Season

Precursor	(±)-Coclaurine	(±)-*N*-methylcoclaurine	(+)-*N*-methylcoclaurine	(−)-*N*-methylcoclaurine	(±)-*N*-methylnorcoclaurine
Labelling pattern	[8,3′,5′-$^3H_3$]	[*N*,*O*-*Me*-^{14}C]	[8,3′,5′-$^3H_3$]	[8,3′,5′-$^3H_3$]	[*N*-*Me*-^{14}C]
Diluting	(+)	(+)	(±)	(±)	(+)
Incorporation	0·15	0·19	0·11	0·000	0·10

1966 Season

Precursor	(±)-Armepavine	(±)-*N*-methylcoclaurine
Labelling pattern	[*N*-*Me*-^{14}C]	[*N*,*O*-*Me*-3-^{14}C]
Incorporation (%)	<0·001	0·19

(+)-Roemerine was used for dilution.

* Incorporations are corrected where appropriate for tritium loss inevitable in the cyclisation step.

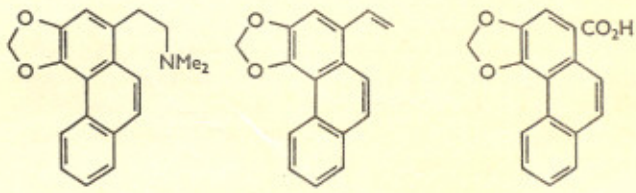
(±)-*N*-[^{14}C]Methylnorcoclaurine was incorporated less efficiently than (±)-coclaurine. It seems probable that biogenetic methylation of norcoclaurine in *P. dubium* can give either the *N*-methyl derivative or coclaurine, which are subsequently methylated. Herzig–Meyer demethylation of the radioactive roemerine showed that all the activity was, as expected, in the *N*-methyl group.

The incorporations of (+)-*N*-methylcoclaurine and (−)-*N*-methylcoclaurine (0·11 and 0·00%, respectively) showed the stereospecificity of the biosynthesis. With these feedings (±)-roemerine was used for dilution, since, if the plant was able to produce (−)-roemerine from (−)-*N*-methylcoclaurine, activity would have been retained in the purified (±)-roemerine.

The doubly-labelled (±)-*N*-methylcoclaurine contained 81% of the activity in the *N*-methyl group and 19% in the *O*-methyl group, the ratio being checked by a selective Herzig–Meyer determination. It was expected that the ratio would be the same in the derived roemerine, since conversion of an *o*-methoxyphenol into a methylenedioxy-group is well known [20] and generally not accompanied by demethylation. However, in the biosynthetic roemerine the *N*-methyl group contained 87% of the activity and the methylenedioxy-group 11%, the activity of the former being obtained by a Herzig–Meyer determination and of the latter by obtaining the dimedone derivative from the formaldehyde liberated by acid hydrolysis.

As mentioned above, feeding experiments were also conducted on *Meconopsis cambrica* plants. (±)-[8,3′,5′-$^3H_3$]Coclaurine was incorporated into mecambrine in 0·66% yield. Acid treatment of the derived mecambrine gave mecambroline (V), which on treatment with aqueous alkali lost all its activity, indicating that the tritium was located at C(9) and C(11). (±)-[*N*,*O*-*Me*-^{14}C]*N*-Methylcoclaurine was also incorporated into mecambrine (0·03%).

For the 1966 season [*N*,*O*-*Me*-3-^{14}C]methylcoclaurine was fed to both *P. dubium* and *M. cambrica*, the ratio of the labels being: *N*-methyl, 61·6%, *O*-methyl, 13·0%, and C(3), 25·4%. The activities of the *N*-methyl and methylenedioxy-positions in roemerine were determined as before. The activity at C(5) of roemerine [corresponding to C(3) of the benzylisoquinoline] was found by a further Hofmann degradation on the methine base (XII) to give the vinylphenanthrene (XIII), which was oxidised to the phenanthrenecarboxylic acid (XIV). The difference between the latter activities represents that at C(5). For mecambrine the activities of the corresponding positions were obtained as above, after



(XII) (XIII) (XIV)

conversion of the alkaloid into roemerine. The roemerine from *P. dubium* had activities of: *N*-methyl, 72·0%, methylenedioxy, 1·2%, C(5), 29·4%. For mecambrine the corresponding activities were: 72·1, 1·8, and 32·0%. Thus although *O*-methyl activity was lost, the ratio between the *N*-methyl and C(3) activity remained essentially constant (initially 2·42 : 1, finally 2·44 : 1, and 2·22 : 1, respectively).

The discrepancies between the ' expected ' and found methylenedioxy-group activities both here, and in the experiment carried out earlier (see above), appear to require a rapid de-*O*-methylation followed by either (*a*) remethylation from the (unlabelled) methylating pool of the plant and subsequent oxidative cyclisation to the methylenedioxy-ring or (*b*) reaction of the catechol with a ' reagent ' at the formaldehyde oxidation level which constructs the methylenedioxy-ring in one step. The procedure (*a*) would reconcile our present results with the earlier work.[20]

(±)-[*N*-*Me*-^{14}C]Armepavine was not incorporated into roemerine showing that demethylation of the benzylisoquinoline did not occur.

Labelled mecambrine was well incorporated into roemerine (2·34%) in *P. dubium* and into mecambroline

[20] D. H. R. Barton, G. W. Kirby, J. B. Taylor, and G. M. Thomas, *J. Chem. Soc.*, 1963, 4545.

(2·76%) in *M. cambrica*, indicating that formation of the methylenedioxy-group occurs at the dienone stage.

The biosynthesis of anonaine (IV; enantiomer, R = H, $R^1 + R^2 = CH_2$) was investigated with *Anona reticulata* plants. (±)-[8,3′,5′-$^3H_3$]Coclaurine and (±)-[5,8,3′,5′-$^3H_4$]norcoclaurine were both incorporated into anonaine (0·44 and 0·49%, respectively). The activity was determined by conversion of the anonaine, diluted with (±)-anonaine, into the methine base (XII).

It is clear that these results are in good agreement with the correctness of the biosynthetic scheme that we suggested earlier [1] for roemerine, anonaine, and related alkaloids.

EXPERIMENTAL

Melting points were determined on the Kofler hot-stage apparatus. Unless otherwise stated ultraviolet absorption spectra refer to ethanol, infrared absorption spectra to chloroform, and n.m.r. spectra to deuteriochloroform solutions. The n.m.r. spectra were recorded on a Varian A-60 spectrometer on permanent loan from the Wellcome Trust. Mass spectra were recorded on an A.E.I. mass spectrometer, the samples being run with direct probe insertion with an electron beam of 70 ev. Chromatography was carried out, unless specified to the contrary, on neutral alumina (grade III). Light petroleum had b. p. 40—60°.

All ^{14}C- and tritium-labelled compounds were counted in a scintillation counter (Isotope Development Ltd., type 6012A), the samples being dissolved in dimethylformamide (0·2 ml.) and liquid scintillator (1·2 ml., Nuclear Enterprises Ltd., type NE 213) and are uncorrected for self-absorption except where stated. The relative efficiencies were obtained by counting [1,2-^3H]- and [2-^{14}C]-hexadecane standards.

Isolation of Alkaloids.—(a) *Anonaine.* This alkaloid was extracted from the bark of *Anona reticulata* according to the procedure of Govindachari and his co-workers.[11] Its properties are in accordance with the assigned structure as were those of its hydrochloride and *N*-acetyl derivative.

(b) *Roemerine. Papaver dubium* plants (2·0 kg.), harvested in June, were blended with ethanol (4 l.) and left to soak for 2 days. After removal of the ethanol the resulting gum was dissolved in N-hydrochloric acid (100 ml.); the solution was filtered, extracted with ether, and basified with aqueous 4N-sodium hydroxide; the basic solution was extracted with ether (3 × 50 ml.). After drying (K_2CO_3), the ether was removed to give the crude bases (430 mg.). These were chromatographed over alumina (30 g.) and the roemerine was eluted with carbon tetrachloride-benzene (1 : 1) as shown by t.l.c. (+)-Roemerine, crystallised as its hydrochloride (152 mg.) from ethanolic hydrogen chloride, had m. p. 265—270° (decomp.). This had R_F, infrared, and other properties identical with those of an authentic specimen of (−)-roemerine hydrochloride.

(c) *Mecambrine. Meconopsis cambrica* roots (1·37 kg.) were blended in ethanol (3·1) and left to soak for 3 days. After removal of the ethanol the crude gum (52·4 g.) was dissolved in 0·1N-hydrochloric acid (200 ml.) and filtered. The acid solution was extracted with ether (50 ml.), basified with ammonia, and extracted with ether (3 × 50 ml.). After drying (K_2CO_3), the ether was removed to give the crude bases (280 mg.) which were chromatographed over alumina (30 g.) and the mecambrine was eluted with benzene–chloroform (9 : 1). The free base (600 mg.), crystallised from ether, had m. p. 178° (lit.,[6] m. p. 178°). The t.l.c., m. p., mixed m. p., and the spectroscopic data were identical to those of an authentic specimen.

Synthesis of Anonaine and Roemerine.—*3,4-Methylenedioxy-ω-nitrostyrene.* Piperonaldehyde (25 g.) in redistilled nitromethane (60 ml.) was shaken at room temperature for 20 hr. with methylamine hydrochloride (5 g.) and anhydrous sodium acetate (5 g.). The crystalline precipitate was filtered off and recrystallised from glacial acetic acid to give *3,4-methylenedioxy-ω-nitrostyrene* [21] (VI) (30·5 g.) as yellow needles, m. p. 161°.

3,4-Methylenedioxy-β-phenethylamine. This nitrostyrene (15 g.) in dry tetrahydrofuran (400 ml.) was added dropwise [22] to lithium aluminium hydride (15 g.) in the same solvent (100 ml.) under reflux. Conversion of the product into the hydrochlorides and recrystallisation from ethanol gave the amine hydrochloride (VII) (10·2 g.), m. p. 210°.

N-(3,4-Methylenedioxyphenethyl)-o-nitrophenylacetamide and derivatives. *o*-Nitrophenylacetylchloride (from 5 g. of *o*-nitrophenylacetic acid by use of purified thionyl chloride) in benzene (100 ml.) was added dropwise with vigorous stirring to a mixture of 3,4-methylenedioxy-β-phenylethylamine (10 g.) suspended in aqueous sodium hydroxide (1N, 250 ml.) and benzene (50 ml.). After stirring for a further 30 min. the amide was filtered off. Further amide was obtained by evaporation of the benzene layer *in vacuo*. The amide,[19,23] crystallised from methanol (11·6 g.), had m. p. 122°. This amide was converted *via* the dihydroisoquinoline (X) into (±)-anonaine by the method of Barger and Weitnauer.[19]

1,2,3,4-Tetrahydro-6,7-methylenedioxy-1-(o-nitrobenzyl)-isoquinoline (XI; R = H, $R^1 = NO_2$). The dihydroisoquinoline (X) [the intermediate in the anonaine synthesis (see above)] (1·0 g.) in methanol (200 ml.) was treated at room temperature with sodium borohydride (370 mg.) during 30 min. and then stirred for a further hour. The methanol was removed *in vacuo*, sodium hydroxide solution (1N, 40 ml.) added, and the base extracted into ether. Removal of the ether and treatment with an excess of ethanolic hydrogen chloride gave the *hydrochloride*. Recrystallised from ethanol this formed plates (960 mg.), m. p. 234—235° (Found: C, 58·95; H, 5·1; Cl, 10·2; N, 7·8. $C_{17}H_{17}ClN_2O_4$ requires C, 58·6; H, 4·9; Cl, 10·2; N, 8·05%). The free base, crystallised from ether, had m. p. 98—99°. This compound (1·0 g.) was treated overnight at room temperature with acetic anhydride (5 ml.) and pyridine (5 ml.). Removal of the excess of reagents *in vacuo* and crystallisation from ethanol gave the *N-acetyl derivative* (840 mg.), m. p. 156—157° (Found: C, 64·5; H, 5·35; N, 8·3. $C_{19}H_{18}N_2O_5$ requires C, 64·4; H, 5·1; N, 8·0%).

1,2,3,4-Tetrahydro-2-methyl-6,7-methylenedioxy-1-(o-nitrobenzyl)isoquinoline (XI; R = Me, $R^1 = NO_2$). The tetrahydroisoquinoline (XI; R = H, $R^1 = NO_2$) hydrochloride (see above) (600 mg.) in formic acid (97%, 10 ml.) and formalin (40%, 10 ml.) was heated on the steam-bath for 45 min. The excess of reagents was removed *in vacuo*

[21] Y. Tanaka and T. Midzuno, *J. Pharm. Soc. Japan*, 1929, **49**, 255.

[22] M. Tomita and I. Kikkaua, *J. Pharm Soc. Japan*, 1957, **77**, 1011.

[23] L. Marion and V. Grassie, *J. Amer. Chem. Soc.*, 1944, **66**, 1290.

and the residue basified with 1N-sodium hydroxide. Extraction into ether, removal of the ether *in vacuo*, and treatment with excess of ethanolic hydrogen chloride gave the 2-methyl derivative *hydrochloride* (570 mg.), m. p. 204—205° (Found: C, 59·55; H, 5·05; Cl, 10·1; N, 7·75. $C_{18}H_{18}ClNO_4$ requires C, 59·6; H, 5·25; Cl, 9·75; N, 7·7%).

1-(o-*Aminobenzyl*)-1,2,3,4-*tetrahydro*-2-*methyl*-6,7-*methylenedioxyisoquinoline* (XI; R = Me, $R^1 = NH_2$). The above-mentioned nitro-compound (2·0 g.) in aqueous hydrochloric acid (1 part conc. HCl to 2 parts H_2O, 86·5 ml.) was stirred for 30 min. during the addition of zinc dust (860 mg.) and the stirring continued for a further 30 min. After the excess of zinc had been filtered off, the free base was liberated by concentrated aqueous ammonia (*d* 0·880) and extracted into ether. Removal of the ether *in vacuo* and addition of methanolic hydrogen chloride afforded the base dihydrochloride. Recrystallised from methanol this (1·7 g.) formed needles, m. p. 282—283°, in agreement with the literature.[23] This dihydrochloride (1·0 g.) in aqueous sulphuric acid (2N, 50 ml.) was treated at 0° during 30 mins. with sodium nitrite (250 mg.) and then kept at 0° for 5 hr. The solution was then heated at 100°; until nitrogen evolution ceased (3 min.), ammonia (*d* 0·880) was added and the precipitated bases extracted into ether. After drying (potassium hydroxide pellets) and removal of the solvent the product was chromatographed over alumina. Elution with carbon tetrachloride–benzene (1 : 1) (t.l.c. control) gave (±)-roemerine (IV; R = Me, $R^1 + R^2 = CH_2$) (246 mg.). Recrystallised from ethanolic hydrogen chloride the hydrochloride has [24] m. p. 262—267°. When cuprous iodide (1·0 g.) was added before decomposition of the diazonium salt the yield of (±)-roemerine rose to 35%.

(±)-*N*-[^{14}C]*Methylcoclaurine*.—(±)-Coclaurine hydrochloride (40 mg.) in formic acid (0·5 ml.) and 4N-sodium hydroxide (0·3 ml.) was treated with radioactive paraformaldehyde (0·71 mg., 0·1 mc). After heating at 100° under nitrogen for 10 min., inactive paraformaldehyde (30 mg.) was added and the heating continued for 15 min. Finally, formalin (40%, 0·3 ml.) was added and the solution heated for a further 7 min. The solvent was removed under reduced pressure and the residual acid neutralised with saturated aqueous sodium hydrogen carbonate solution. Extraction with chloroform (3 × 3 ml.), which was in turn washed with water (2 ml.) and dried (Na_2SO_4), followed by removal of the solvent *in vacuo* gave the labelled base. This was crystallised from methanolic hydrogen chloride–ether as needles, m. p. 250—253° (decomp.) (lit.,[25] m. p. 252—254°) (radiochemical yield, 73%).

4-*Benzyloxy*-3-*methoxy*-[1-^{14}C]*ethylamine Hydrochloride*.—4-Benzyloxy-3-methoxy-benzyl chloride [15] (100 mg.) in freshly distilled dimethyl sulphoxide (2 ml.) was heated on the steam-bath for 15 min. with radioactive potassium cyanide (1·77 mg., 1 mc). Further potassium cyanide (17·2 mg.) was added and the heating continued for 3 hr. Dilution with water, addition of carrier 4-benzyloxy-3-methoxybenzyl cyanide (18 mg.), and extraction into ether gave the radioactive product. The ether was dried (Na_2SO_4) and removed *in vacuo*. The residue, in anhydrous ether (5 ml.), was added slowly to lithium aluminium hydride (400 mg.) in the same solvent (5 ml.) under reflux and the refluxing continued for 1 hr. The excess of reductant was destroyed by cautious addition of water and ether, the ether layer decanted, and the residue washed (2 × 5 ml.) with ether. Removal of the ether and crystallisation from ethanolic hydrogen chloride gave the required amine hydrochloride (18 mg.), m. p. 176—177° (lit.,[26] m. p. 173—175°).

(±)-*N*-[^{14}C]*Methylnorcoclaurine*.— *OOO*-Tribenzylnorcoclaurine [12] (66 mg.) in formic acid (1·3 ml.) and 4N-sodium hydroxide (0·8 ml.) was treated with radioactive paraformaldehyde (0·71 mg., 0·1 mc) on the steam-bath for 10 min. Paraformaldehyde (2·75 mg.) was added and the mixture heated for a further 15 min. Finally, formalin (40%, 1 ml.) was added and the reaction completed by a further 10 minutes' heating. After removal of the solvent under reduced pressure, the solution was made alkaline with aqueous sodium hydrogen carbonate and ether-extracted. This gave the *N*-methyl compound (54·3 mg.) which, crystallised from ether, had m. p. 96°.

This *OOO*-tribenzyl compound (250 mg.) in ethanol (12 ml.), methanol (3 ml.), and concentrated hydrochloric acid (0·24 ml.) was hydrogenolysed over 10% palladised charcoal for 3 hr. (3 mol. uptake). Removal of the solvent and catalyst gave, from methanol–ether, the desired radioactive *N*-methylnorcoclaurine hydrochloride (120 mg.).

O-*Benzylarmepavine*.—(with Dr. A. Wiechers). 4-Benzyloxyphenyl-*N*-(3,4-dimethoxyphenethyl)-acetamide[27] (3·2 g.) in dry toluene (40 ml.) containing freshly redistilled phosphorus oxychloride (10 ml.) was refluxed for 20 min. (infrared control) whilst a stream of dry oxygen-free nitrogen was passed. On cooling, the imine hydrochloride crystallised out. Recrystallisation from ethanol–ether afforded the hydrochloride (2·95 g.) as plates, m. p. 210° (lit.,[27] m. p. 210—211°) (Found: C, 70·6; H, 6·35; Cl, 8·35; N, 3·35. Calc. for $C_{25}H_{26}ClNO_3$: C, 70·8; H, 6·2; Cl, 8·35; N, 3·3%). This dihydroisoquinoline [recovered from the hydrochloride (1·0 g.)] in methanol (20 ml.) at 0° was treated with sodium borohydride (800 mg.) added in small portions and the resultant solution stirred overnight at room temperature. Working up and crystallisation of the product, after conversion into the hydrochloride, from ethanol–ether gave 1-(4-*benzyloxyphenyl*)-1,2,3,4-*tetrahydro*-6,7-*dimethoxy-isoquinoline hydrochloride*, m. p. 198—200° (Found: C, 70·85; H, 6·5; Cl, 8·6; N, 3·3. $C_{25}H_{28}ClNO_3$ requires C, 70·5; H, 6·6; Cl, 8·3; N, 3·3%). The corresponding amine, which did not crystallise, in formic acid (1·5 ml.) was treated with aqueous 4N-sodium hydroxide until the pH was 5. Formalin (40%; 1·5 ml.) was added and the mixture heated under nitrogen for 90 min. (t.l.c. control) on the steam-bath. The solvent was removed *in vacuo* and the residue treated with 4N-sodium hydroxide. Extraction into chloroform gave an oily amine (353 mg.) which afforded crystalline O-*benzylarmepavine hydrochloride*. Recrystallised from ethanol–ether this had m. p. 195—197° (Found: C, 71·3; H, 6·95; Cl, 8·65; N, 3·4. $C_{26}H_{30}ClNO_3$ requires C, 71·0; H, 6·85; Cl, 8·05; N, 3·2%). This *O*-benzyl ether hydrochloride (101 mg.) in ethanol (10 ml.) containing concentrated HCl (1 drop) was hydrogenolysed over pre-reduced 10% palladised charcoal (25 mg.) for 8 hr. (1 mol. uptake). The basic product crystallised from acetone–ether to give (±)-armepavine [27,28] (80 mg.), m. p.

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164—166°. The hydrochloride crystallised from ethanol–ether, m. p. 209—211° (decomp.).

(±)[*N*-*Me*-^{14}C]*Armepavine*.—*O*-Benzyl-*N*-norarmepavine (34 mg.) in formic acid (1·0 ml.) and 4N-sodium hydroxide (0·3 ml.) were treated on the steam-bath with radioactive paraformaldehyde (0·77 mg., 9·1 mc) under nitrogen for 15 min. Further inactive paraformaldehyde (2·0 mg.) was added and the heating continued for a further 10 min. Formalin (40%, 0·5 ml.) was introduced and the heating continued for 10 min. After removal of the solvent *in vacuo* aqueous N-sodium hydroxide was added and the amine extracted into ether (3 × 4 ml.). After being washed with water (5 ml.) and dried (K_2CO_3), the *O*-benzylamine (28 mg.) was crystallised as its hydrochloride, m. p. 221—224°, from ethanol–ether (radiochemical yield 35%).

This radioactive *O*-benzylarmepavine hydrochloride (28 mg.) in ethanol (2 ml.) and concentrated hydrochloric acid (2 drops) was hydrogenolysed over 10% palladised charcoal (1 mol. uptake). After removal of the catalyst and solvent the residual foam was taken up in 5% aqueous sodium hydrogen carbonate (5 ml.) and extracted with chloroform (3 × 3 ml.). The chloroform layer was washed with water (2 ml.) and the solvent removed. Crystallisation from ethanol–ether gave (±)[*N*-*Me*-^{14}C]armepavine hydrochloride (17 mg.), m. p. 210—212°.

Feeding and Work-up Procedure with Anona reticulata *Plants*.—The *Anona reticulata* plants were wick-fed (in three separate places on the stem) with the hydrochlorides of the precursors in water and left for ten days. The plant was washed and then blended with ethanol (4 l.) and allowed to stand for three days. After removal of the ethanol 0·1N-hydrochloric acid was added. The acid solution was filtered, extracted with ether (25 ml.), and basified with sodium hydroxide. The basic solution was extracted with ether (3 × 25 ml.) and removal of the solvent gave the crude non-phenolic alkaloids. Chromatography over alumina, and elution with benzene–chloroform (1 : 1), gave anonaine. Anonaine hydrochloride was prepared by precipitation from ethanolic hydrogen chloride with ether. In one case (±)-anonaine hydrochloride was added.

The anonaine hydrochloride was converted into its free base and treated with formic acid and formalin (0·3 ml.) for 15 min. After removal of the solvent under reduced pressure and addition of 2N-sodium hydroxide (until basic), roemerine was extracted into ether (2 × 5 ml.). The ether solution was washed with water, dried (Na_2CO_3), and evaporated. The resulting gum was dissolved in methanol and methyl iodide (0·1 ml.) was added. The resulting methiodide was treated as described under the feedings to *Papaver dubium* plants.

Feeding and Work-up Procedure with Papaver dubium *Feedings*.—The precursors, except tyrosine, as their hydrochlorides in water were injected into the seed-pods of *Papaver dubium* plants after the petals had dropped. Ten days later the plants were harvested. The plants were washed and blended with ethanol (2·1.) and left in ethanol for three days. They were then worked up as in the large-scale extraction.

When the alkaloid of the plant was diluted with (±)-roemerine the roemerine was converted into its methine base, which was crystallised to constant activity. The activity of this was checked by making its methiodide.

Methine Base from Roemerine.—Roemerine methiodide was prepared by taking up roemerine in methanol (minimum volume) and treating with a few drops of methyl iodide at room temperature for 24 hr. The precipitated methiodide [24] was filtered off, m. p. 222—225°. Roemerine methiodide (15 mg.) in methanolic potassium hydroxide (20%, 4 ml.) was refluxed for 3½ hr. The methanol was removed *in vacuo*, water (5 ml.) added, and the methine base (80%) extracted into ether. Crystallisation from ethanolic hydrogen chloride gave the hydrochloride,[24] m. p. 220—225°. The methine base hydrochloride was recrystallised from ethanol to constant activity.

The methine base methiodide was prepared as in the procedure detailed above and had [24] m. p. 283—284°.

In the case of the triply-labelled precursor feeding, the activity at C(3) was determined by converting the methine base methiodide into 3,4-methylenedioxy-1-vinylphenanthrene. This indicated the activity of the C(3) and methylenedioxy-positions combined. The activity at C(3) was then found by oxidation of the vinylphenanthrene to 3,4-methylenedioxyphenanthrene-1-carboxylic acid.

3,4-*Methylenedioxy-1-vinylphenanthrene*.—The methine base methiodide (15 mg.) was refluxed in methanolic potassium hydroxide (20%, 2 ml.) for 3 hr. The methanol was removed *in vacuo*, water (5 ml.) added, and the vinyl derivative extracted into chloroform (3 × 3 ml.). Crystallisation from methanol gave 3,4-methylenedioxy-1-vinylphenanthrene (5·75 mg.), m. p. 87° (lit.,[24] m. p. 86—87°).

3,4-*Methylenedioxyphenanthrene-1-carboxylic acid*.— The vinylphenanthrene (above) (8 mg.) in acetone (2 ml.) was treated with potassium permanganate (21 mg.) at room temperature for 20 min. 0·1N-Sodium hydroxide (5 ml.) was added and the solution ether-extracted (3 ml.). Concentrated hydrochloric acid (excess) was added and the precipitate extracted into benzene (3 × 4 ml.). After being washed with water (4 ml.) and dried (Na_2SO_4) the benzene was removed *in vacuo*. Crystallisation from benzene gave the carboxylic acid [19,24] (5·8 mg.), m. p. 245—247°.

For the determination of the activity of the methylenedioxy-group in roemerine the alkaloid (15 mg.) in aqueous sulphuric acid (35%, 40 ml.) was distilled at *constant volume* into aqueous dimedone solution (0·6%, 25 ml.) until 100 ml. had been collected. After being left overnight the dimedone derivative (m. p. and mixed m. p.) was collected and recrystallised from ethanol to constant activity.

The *N*-methyl group activity of roemerine was determined by conversion into triethylmethylammonium iodide by the standard Herzig–Meyer method.

Feeding and Work-up Procedure with Meconopsis cambrica. —The precursors were fed as with the feedings to *Papaver dubium*, and the plants were worked-up as with the large-scale extraction of mecambrine.

Mecambrine was crystallised to constant activity as its free base from ether. The *N*-methyl group and methylenedioxy-group were determined as with roemerine. The position of the tritium in the mecambrine, derived from (±)-[8,3′,5′-^{3}H$_3$]coclaurine, was determined by converting mecambrine into mecambroline and exchanging with aqueous base. A trial experiment, exchanging mecambroline with base in deuterium oxide, showed that two protons exchanged.

The activity at C(3) of mecambrine, derived from triply-labelled *N*-methylcoclaurine, was determined by conversion into roemerine and the same series of reactions as with the aporphine.

Mecambroline was isolated from the other phenols by precipitation from an aqueous solution with concentrated

hydrochloric acid and recrystallisation from water. Its activity was checked by crystallisation of its free base.

In order to convert mecambrine into mecambroline the mecambrine in aqueous hydrochloric acid (conc. HCl–water, 1 : 4) was heated in the steam-bath for 20 min. After cooling, the mecambroline hydrochloride was filtered and recrystallised from water.

Mecambrine (64·5 mg.) in ether (21 ml.) was treated at room temperature with lithium aluminium hydride (50 mg.) added during 20 min. The solution was stirred for a further ½ hr. at room temperature. The excess of reducing agent was destroyed with water and the ether layer decanted off. The aqueous layer was extracted with ether. The combined ether extracts were dried (K_2CO_3), the solvent removed, and the residue crystallised from ethanolic hydrogen chloride to give (+)-roemerine hydrochloride (57·5 mg.).

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