1185. *Phenol Oxidation and Biosynthesis. Part VIII.* Investigations on the Biosynthesis of Berberine and Protopine*

By D. H. R. Barton, R. H. Hesse, and G. W. Kirby

The " berberine bridge " carbon in both berberine and protopine has been shown to be derived in Nature by oxidative cyclisation of an *N*-methyl group. This was demonstrated using as precursor reticuline, a typical benzylisoquinoline. Alternative benzylisoquinoline-type precursors have been investigated. Some improvements in the synthesis of the benzylisoquinolines needed for this work have been noted. Comments have been made on the n.m.r. spectra of benzylisoquinoline derivatives.

The main substance of this work has already been reported in preliminary form.[1]

A biogenetic connection between the benzylisoquinoline and berberine (III) groups of alkaloids has been recognised for many years.[2] The one-carbon unit necessary to form the

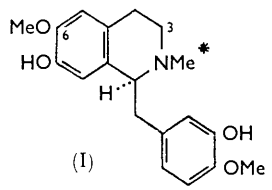
* Part VII, *J.*, 1965, 2423.

[1] D. H. R. Barton, R. H. Hesse, and G. W. Kirby, *Proc. Chem. Soc.*, 1963, 267.
[2] See Sir Robert Robinson, " The Structural Relations of Natural Products," Oxford University Press, London, 1955.

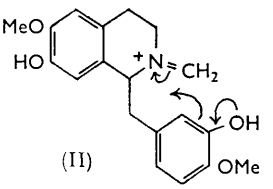
so-called " berberine bridge " [asterisk in (III)] has usually been referred to as " formaldehyde or its biological equivalent." The precise biological origin of the unit, however, was not defined prior to our own work.

We now present experimental evidence that the " berberine bridge " is a cyclised *N*-methyl group. This concept was first suggested at a Conference on " The Biosynthesis of Natural Substances " in Milan, Italy, in September 1962.[3,4] At that time Professor A. R. Battersby kindly informed us that he had independently conceived the same view.[5]

Earlier studies in the field of *Amaryllidaceae* alkaloids [6,7] had established that the methylenedioxy-group in hæmanthamine was derived biosynthetically by cyclisation of an *o*-methoxyphenol grouping. It was suggested that methylenedioxy-groups in general might be formed in Nature by this cyclisation mechanism. At present, in all five cases where the concept has been tested, it has been shown to be correct.[8] Having regard to the possible mechanisms of oxidative cyclisation of the *o*-methoxyphenol grouping,[7] it was easy to visualise a comparable process for oxidative cyclisation of the *N*-methyl group in a benzylisoquinoline derivative in the sense [(I) ⟶ (II) ⟶ (III)] to give the berberine ring system.



(I)



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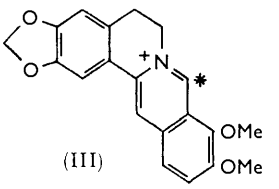
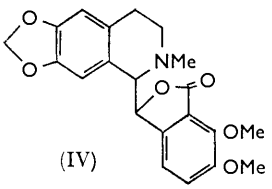


(III)



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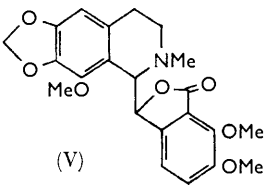

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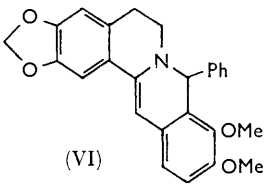


(VI)

Reticuline (I) was chosen as a suitable benzylisoquinoline with which to test the concept of oxidative cyclisation on the basis of the following argument. Reticuline is the precursor [9] of the morphine alkaloids and occurs in opium.[10] Since opium also contains narcotine (V) and protopine (VII), both of which possess the characteristic berberine carbon atom (see below), a biosynthetic connection between reticuline and the berberine group seemed probable. Moreover reticuline has, besides the obligatory *N*-methyl group, a phenolic hydroxyl suitably placed to effect cyclisation of the likely intermediate ion (II). The biosynthesis of berberine (III) and hydrastine (IV) from tyrosine and 3,4-dihydroxyphenethylamine has been thoroughly investigated in *Hydrastis canadensis*.[11] The carbon skeleton of both alkaloids was shown to be derived from two aromatic C_6–C_2 units. The same plant was chosen for our investigations with more sophisticated precursors.

Berberine.—[N-*Methyl*-^{14}C](±)-Reticuline was fed in spring into the stems of young *H. canadensis* plants through cotton wicks. After one week radioactive berberine (0·7% incorporation) was isolated. Conversion into phenyldihydroberberine (VI) and oxidation with chromic acid gave benzoic acid containing all (106%) of the radioactivity. Similarly

[3] D. H. R. Barton and G. W. Kirby, *Acad. Naz. dei Lincei*, 1964, 17.
[4] D. H. R. Barton, *Proc. Chem. Soc.*, 1963, 293.
[5] A. R. Battersby, *Proc. Chem. Soc.*, 1963, 189.
[6] D. H. R. Barton, G. W. Kirby, and J. B. Taylor, *Proc. Chem. Soc.*, 1962, 340.
[7] D. H. R. Barton, G. W. Kirby, J. B. Taylor, and G. M. Thomas, *J.*, 1963, 4545.
[8] See A. R. Battersby, R. J. Francis, E. A. Ruveda, and J. Staunton, *Chem. Comm.*, 1965, 89.
[9] D. H. R. Barton, G. W. Kirby, W. Steglich, G. M. Thomas, A. R. Battersby, T. A. Dobson, and H. Ramuz, *J.*, 1965, 2423, and references there cited.
[10] E. Brockmann-Hanssen and T. Furaya, *J. Pharm. Sci.*, 1964, **53**, 575.
[11] J. R. Gear and I. D. Spenser, *Canad. J. Chem.*, 1963, **41**, 783.

the precursor (I), labelled both in the 6-methoxyl group (5·9% of the total activity) and in its *N*-methyl group, (94%) gave doubly labelled berberine (0·9% incorporation). Hydrolysis with sulphuric acid gave radioactive formaldehyde (5·6%) and degradation, as above, gave benzoic acid (91%). Clearly reticuline is converted into berberine without fragmentation or demethylation and the berberine bridge carbon is derived solely from the *N*-methyl group. This experiment also provides another example of the biological cyclisation of an *o*-methoxyphenol group to a methylenedioxy-group (see above).

It can always be argued that the conversion of a synthetic precursor into a natural product does not rigorously prove that the transformations involved form part of the normal biosynthetic processes of the organism. This objection can be overcome by demonstrating the presence of the alleged precursor in the organism (see, for example, refs. 7 and 9), especially when the demonstration be combined with appropriate enzyme studies.[12] We used a different technique.

Reticuline has one asymmetric centre but the derived alkaloid, berberine, is optically inactive. If the biological conversion is not stereospecific or proceeds non-enzymically then both enantiomers of reticuline would be incorporated with similar efficiency. The same result would also be observed if the precursor were racemised before incorporation. In contrast, efficient incorporation of only one enantiomer would show that at least one step in the biosynthetic pathway is stereospecific and therefore enzymically controlled. The (+)- and (−)-forms of reticuline [13] were each heated with acidified, tritiated water. A similar experiment with deuterium oxide showed (n.m.r. control) that all the aryl protons, and no others, are exchanged with tritium under these conditions.[14] The labelled enantiomers were fed separately, in parallel experiments, to *H. canadensis* in the usual way. (+)-Reticuline [absolute configuration (I)] [13] was converted fifteen times more efficiently than (−)-reticuline into berberine (Table 1). In this, and in all subsequent experiments

TABLE 1

Conversion of reticuline* into berberine in *Hydrastis canadensis*

No.	Year	1963	1963 †	1964
1	Chirality and labelling pattern	(±)-[N-*Methyl*-^{14}C]	(±)-[N-*Methyl*-^{14}C] (94%) (±)-[6-*Methoxy*-^{14}C] (5·9%)	(+)-[*Aryl*-^{3}H]
2	Incorporation (%)	0·7	0·9	9·9
3	Degradation products (% total activity)	$PhCO_2H$ (106)	CH_2O (5·6) $PhCO_2H$ (91)	—

No.	Year	1964	1964
1	Chirality and labelling pattern	(−)-[*Aryl*-^{3}H]	(±)-[3-^{14}C] (−)-[*Aryl*-^{3}H]
2	Incorporation (%)	0·66	1·5(^{14}C); 0·25(^{3}H)
3	Degradation products (% total activity)	—	—

* Control feeding (1963) of (±)-[2-^{14}C]dopa gave labelled berberine (0·90%) and hydrastine (0·62%). † Incorporation into canadine (0·035%) was also observed.

with aryl-tritiated phenols (see Tables 1 and 2 and below), incorporations were corrected for the loss of one tritium atom during cyclisation. The incorporation (9·9%) was unusually high for experiments with higher plants and supported the conclusion that we were observing the normal biosynthetic route to berberine. The significant incorporation of (−)-reticuline probably arose from incomplete resolution of the original racemate. However, reticuline is known to be racemised rapidly in *Papaver somniferum* during morphine biosynthesis [15] and partial racemisation might conceivably have occurred in *H. canadensis*.

[12] See, for example, H. M. Fales, J. Mann, and S. H. Mudd, *J. Amer. Chem. Soc.*, 1963, **85**, 2025.
[13] A. R. Battersby, R. Binks, D. M. Foulkes, R. J. Francis, D. J. McCaldin, and H. Ramuz, *Proc. Chem. Soc.*, 1963, 203.
[14] D. H. R. Barton, D. S. Bhakuni, and G. W. Kirby, unpublished observations.
[15] A. R. Battersby, D. M. Foulkes, and R. Binks, *J.*, 1965, 3323.

TABLE 2

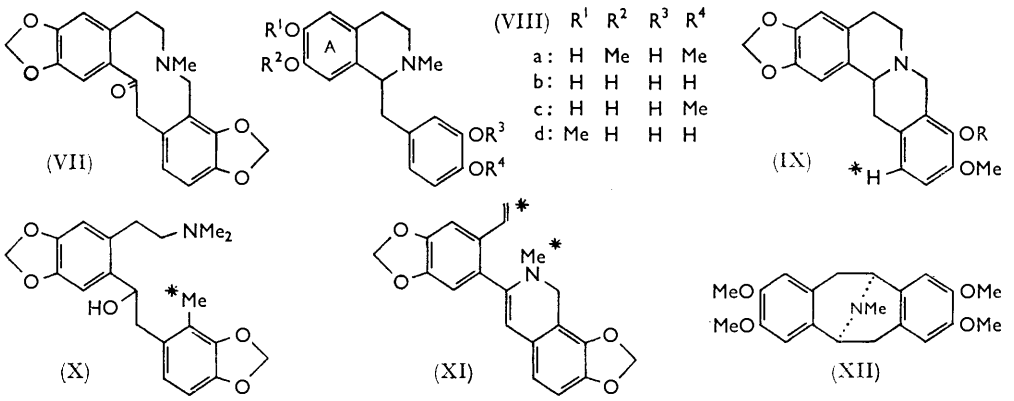
Conversion of reticuline* into protopine in *Dicentra spectabilis* (*D.*) and in *Argemone hispida* and *mexicana* (A.)

No.	Plant (year)	*D.* (1963)	*D.* (1964)	*D.* (1964)
1	Chirality and labelling pattern	(±)-[N-*Methyl*-^{14}C]	(±)-[N-*Methyl*-^{14}C] (75%) (±)-[3-^{14}C] (25%)	(+)-[*Aryl*-^{3}H]
2	Incorporation (%)	1·0	3·4	7·4
3	Degradation products (% total activity)	CH_3NH_2 (96) MeI (<2)	CH_2O (23)	—

No.	Plant (year)	*D.* (1964)	*A.* (1964)	
1	Chirality and labelling pattern	(−)-[*Aryl*-^{3}H]	(±)-[N-*Methyl*-^{14}C] (+)-[*Aryl*-^{3}H]	
2	Incorporation (%)	0·56	0·050(^{14}C); 0·12(^{3}H)	—
3	Degradation products (% total activity)	—	—	

* Control feeding (1963) of (±)-[2-^{14}C]tyrosine gave labelled protopine (2·3%).

A further incisive experiment to establish the stereospecificity of the overall biosynthesis was carried out as follows. A mixture of (−)-[^{3}H]reticuline and (±)-[3-^{14}C]reticuline was administered to a single plant. The ^{3}H : ^{14}C ratio in the derived berberine then afforded



a direct comparison of the incorporations of the enantiomeric forms. The observed values (Table 1) were in good agreement with those from the separate feeding experiments.

Further evidence for the special status of (+)-reticuline as a berberine precursor was secured in the following way. "Protosinomenine" (VIIIa)[16] differs from reticuline only in the arrangement of methoxyl and hydroxyl groups in ring A, and possesses all the structural features necessary, in principle, to permit cyclisation to the berberine ring system. (±)-Protosinomenine was labelled with tritium in positions *ortho* and *para* to phenolic hydroxyl groups by exchange with alkaline tritium oxide.[17] However, this phenol was not significantly converted into berberine in *H. candensis*, the incorporation being $<2 \times 10^{-3}$ that observed for (±)-reticuline.

Independent studies by Battersby and his colleagues[18] have established the conversion of (±)-laudanosoline (VIIIb) into berberine (0·07% incorporation) in *Berberis japonica*. In this work also it was clearly shown that the berberine bridge carbon was derived exclusively from the *N*-methyl group of the precursor.

[16] R. Robinson and S. Sugasawa, *J.*, 1931, 3163; 1932, 789.
[17] D. H. R. Barton, (Mrs.) A. J. Kirby, and G. W. Kirby, unpublished observations.
[18] A. R. Battersby, R. J. Francis, M. Hirst, and J. Staunton, *Proc. Chem. Soc.*, 1963, 268.

We have, in addition, made a preliminary search for intermediates lying on the biosynthetic pathway between laudanosoline and reticuline and between reticuline and berberine. The isomeric (±)-nor-reticulines (VIIIc) and (VIIId) were chosen for the first experiment. A mixture of isomer (VIIIc), labelled with ^{14}C in the *N*-methyl group, and isomer (VIIId) labelled with ^{3}H by exchange in alkali (as above), was fed to *H. canadensis*. Measurement of the $^{14}C : {}^{3}H$ ratio in the derived berberine gave incorporations of 2·5 and 1·0% for (VIIIc) and (VIIId) respectively. Thus both phenols are efficiently methylated to give reticuline and two routes to the latter precursor are possible. The berberine derivative (IX; R = H) [19] is a possible intermediate in the later stages of biosynthesis. However, the racemic material, labelled with tritium as shown (IX), was only poorly incorporated (0·064%) into berberine. In view of the ready methylation of *both* nor-reticulines it cannot be assumed that the phenol (IX; R = H) is an obligatory intermediate in berberine biosynthesis. The corresponding methyl ether, (±)-canadine (IX; R = Me), was very efficiently incorporated (8·9%). This result must, however, be interpreted with caution since autoxidation of canadine to berberine occurs readily. Nevertheless (−)-canadine, having the same absolute configuration as (+)-reticuline, does occur as a minor alkaloid in *H. canadensis*.[20] Examination of the akaloid residues from an early reticuline feeding (1963 double labelling experiment, Table 1) showed the presence of canadine and, after dilution with the racemate, radiochemically pure (±)-canadine (0·035% incorporation) was obtained.

The incorporation of one-carbon units from formate and from methionine into berberine has recently been studied by Gupta and Spenser.[21] The methylenedioxy-group and the berberine bridge have been shown to come from one-carbon units in agreement with our results with large precursors.

Protopine.—Dicentra spectabilis, which grows easily in temperate climates, was the main plant for our investigation of protopine biosynthesis. Protopine is the major alkaloid and can be extracted in quantity from the roots; precursors were, however, administered in the usual way through the stem. Again, (±)-reticuline was found to be an efficient precursor of the alkaloid (Table 2). Chromic acid oxidation of the Emde reduction product (X) gave acetic acid which was converted (Schmidt degradation) into methylamine containing all the activity derived from the ^{14}C labelled *N*-methyl group of reticuline. In this investigation radioactive samples were counted as thin films. For this purpose the methylamine was converted into the convenient, crystalline derivative, *N*-methyl-*O*-benzylisovanillylamine (see Experimental). Protopine from the double labelling experiment (Table 2) was degraded to the methine (XI). Ozonolysis then afforded formaldehyde representing the labelled C-3 of reticuline. Protopine, like berberine, contains therefore a one-carbon bridge derived exclusively from the *N*-methyl group of the versatile precursor reticuline. Following the method described for berberine (see above) it was shown (Table 2) that only (+)-reticuline is efficiently incorporated into protopine in *D. spectabilis*. There can be little doubt that here also we are studying a highly specific biosynthetic process. It was of interest to see if protopine biosynthesis in other plants had the same stereochemical requirements. *Argemone hispida* and *Argemone mexicana* were selected for this purpose since it seemed that the alkaloid argemonine (XII) [22,23] might also be derived from reticuline. In practice only small amounts of argemonine were detected in our plants and the incorporation of reticuline was not significant. However, useful incorporation into protopine was observed. When a mixture of (±)-reticuline, labelled with ^{14}C in the *N*-methyl group, and (+)-reticuline labelled with tritium in the aromatic rings (as above), was fed to one plant of *A. hispida* and *A. mexicana* the derived protopine

[19] E. Spath and G. Burger, *Ber.*, 1926, **59**, 1486.
[20] T. A. Henry, " The Plant Alkaloids," J. and A. Churchill Ltd., London, 1949, p. 336.
[21] R. N. Gupta and I. D. Spenser, *Biochem. and Biophys. Res. Comm.*, 1963, **13**, 115; *Canad. J. Chem.*, 1965, **43**, 133.
[22] M. J. Mastell, T. O. Soine, and L. B. Kies, *J. Amer. Chem. Soc.*, 1963, **85**, 1022.
[23] F. R. Stermitz, S.-Y. Luo, and G. Kallos, *J. Amer. Chem. Soc.*, 1963, **85**, 1551.

(combined from both plants)* had an isotope ratio showing, again, preferential incorporation of (+)-reticuline (Table 2). If argemonine is also formed in the plant from (+)-reticuline then its absolute configuration (as yet unknown) should be represented by (XII). Experiments with the tritium-labelled phenol (IX; R = H) established incorporation (0·3%) into protopine in *D. spectabilis*.

In preliminary studies (1963) with *Hydrastis candensis* (±)-3-(3,4-dihydroxyphenyl)-[2-^{14}C]alanine (dopa) was converted efficiently into both berberine (0·90%) and hydrastine (IV) (0·62%). However, in all subsequent experiments (1963 and 1964) with large precursors no significant incorporation into hydrastine was observed. Nevertheless it seems likely that lactone alkaloids of the hydrastine group are derived from reticuline. Battersby and Hirst [24] have observed conversion of reticuline into the related alkaloid, narcotine (V), in *Papaver somniferum*. They showed, in agreement with biosynthetic theory, that the *N*-methyl group of reticuline provided the lactonic carbonyl of the alkaloid. Also, Spenser and his colleagues [11,21] have shown that the lactonic carbonyl of hydrastine is derived from the one-carbon precursors, formate and methionine. It is possible therefore that our failure to observe incorporation of reticuline into hydrastine may have a trivial explanation. Alternately, it is possible that an isomer of reticuline is involved in the biosynthesis of hydrastine.

A large body of evidence has now accumulated implicating reticuline as an intermediate in the biosynthesis of many groups of phenolic alkaloids.[25] Recent investigations by Battersby and his colleagues with *Chelidonium majus* [8] have made two further additions to the list of reticuline-derived alkaloids. (+)-Reticuline was shown to be a specific precursor for protopine, chelidonine † (a benzophenanthridine), and stylopine (a protoberberine). So far, experimental evidence exists for the biological conversion of one or other of the enantiomers of reticuline into about a dozen phenolic alkaloids.

In the discussion above we have represented the formation of the berberine bridge as involving the cyclisation of a methyleneiminium salt. Such a salt could be obtained by direct dehydrogenation of an *N*-methyl group [1] or *via* the corresponding *N*-oxide.[18] It is also possible [25] to visualise the two-electron oxidation of a phenol to a phenoxonium ion (XIII) from which (II) could be derived by intramolecular hydride transfer. A further possibility would be oxidation of the phenolic hydroxyl of (I) to a phenolate radical followed by hydrogen-atom transfer from the *N*-methyl group. Further phenolate oxidation to the biradical (XIV) would then be followed by ring closure as in normal C–C bond formation in phenolate oxidation reactions. The mechanistic paths correspond to those already discussed for the cyclisation of *o*-methoxyphenols to methylenedioxy-groups [7] with added complications. It is not possible to select the correct pathway on the basis of present knowledge.

In the course of the present investigations a number of benzylisoquinolines was prepared by essentially routine procedures (see Experimental). We draw attention here only to a few selected improvements in procedure which may be useful to others.

For the methylation of phenolic hydroxyl the anion was formed using sodium hydride in dimethylformamide before addition of the labelled (or unlabelled) methyl iodide. We have shown that this procedure is of general applicability.

For the conversion of a protocatechuic aldehyde into the corresponding phenethylamine we proceeded *via* the corresponding nitrostyrene. In collaboration with Dr. D. S.

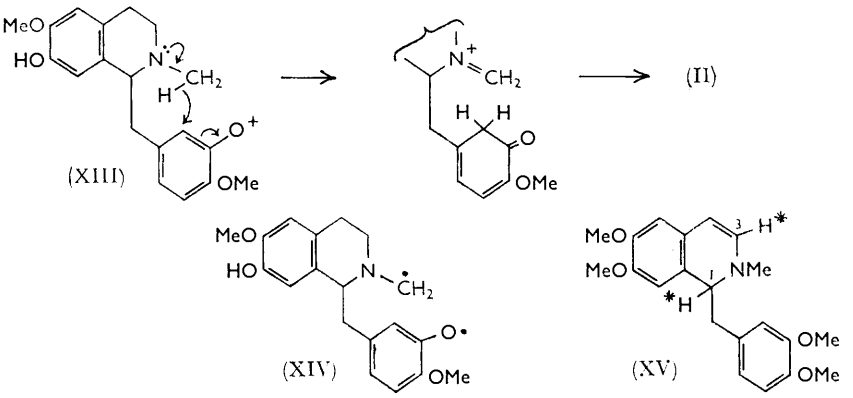
* Seeds of *A. hispida* and of *A. mexicana* were obtained from reputable botanical sources. The derived plants were, however, identified at Kew as *A. mexicana* and *A. hispida* respectively. In order to avoid becoming involved in a botanical controversy, we decided to feed one of each kind of plant in the same way at the same time and to work both up together.

† We had also observed incorporation of (±)-reticuline into chelidonine (R. H. Hesse, Ph.D. Thesis, London, 1965) but, by friendly arrangement with Professor A. R. Battersby, our studies were prosecuted no further.

[24] A. R. Battersby and M. Hirst, *Tetrahedron Letters*, 1965, 669.
[25] D. H. R. Barton, *Pure and Applied Chemistry*, 1964, **9**, 35.

Bhakuni [14] we showed that improved yields of nitrostyrenes could be obtained by treatment of the aldehyde in pure nitromethane with sodium (or potassium) acetate and methylamine hydrochloride.

An alternative route to phenethylamines is by reduction of the corresponding phenylacetonitrile. This reduction, which does not always give a satisfactory yield with lithium



aluminium hydride alone, was carried out with improved efficiency using the lithium aluminium hydride–aluminium trichloride complex.[26]

Quaternised dihydroisoquinolines are normally reduced to isoquinolines with sodium borohydride. We have shown that pyridine is an improved solvent for such reductions. We have also shown that this solvent and reducing agent provide a much improved method for the synthesis of 1,2-dihydro-*N*-methylpapaverine [27] (XV) starting with readily available papaverine methiodide. The further reduction of intermediate (XV) to the tetra-

TABLE 3

Shielding effects in the n.m.r. spectra* of benzylisoquinolines

Compound	(XVIa)	(XVIb)	(XVIc)	(XVId)	(XVIe)	(XVIf)	(XV)	(XVII)
R^2	Me	$PhCH_2$	Me	$PhCH_2$	Me	$PhCH_2$	Me	Me
Line position (τ) ...	6·40	5·15	6·40	5·22	6·45	5·20	6·41	6·68
Position (τ) of other like groups	6·14 †	4·90	6·15	4·92	6·19	4·95	6·20 †	6·20 †
Shielding $\Delta\tau$	0·26	0·25	0·25	0·30	0·26	0·25	0·21	0·48

* Spectra measured in $CDCl_3$ with tetramethylsilane (10τ) internal standard. † Mean values of three closely spaced ($\Delta\tau < 0{\cdot}1$) lines.

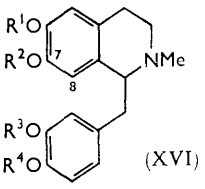
hydroisoquinoline stage, which occurs readily in protonic solvents, is avoided in the aprotic solvent pyridine. Berberine was also reduced in quantitative yield to 1,2-dihydroberberine under these conditions.

During the course of the present, and of related, work the n.m.r. spectra of numerous benzylisoquinolines have been measured. It had been noticed [17] that the n.m.r. spectra of reticuline (I) and of its isomer protosinomenine (VIIIa) differed markedly in one respect. The methoxyl groups in reticuline gave a single line at 6·18 τ whereas those in protosinomenine appeared clearly separated at 6·17 and 6·46 τ. This is in agreement with an earlier observation [28] on the spectra of laudanosine and *o*-methylarmepavine. Examination of a series of tetrahydrobenzylisoquinoline ethers (Table 3) established that in general the methyl protons of methoxyl groups, and the methylene protons of benzyloxyl groups, attached to C-7 [R^2 in (XVI)] are displaced upfield from their expected positions.

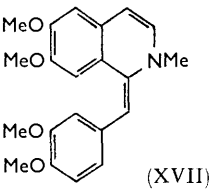
[26] R. F. Nystrom, *J. Amer. Chem. Soc.*, 1955, **77**, 2544.
[27] H. Schmid and P. Karrer, *Helv. Chim. Acta*, 1949, **32**, 960.
[28] I. R. C. Bick, J. Harley-Mason, N. Sheppard, and M. J. Vernengo, *J.*, 1961, 1896.

Other methoxyl and benzyloxyl groups in the same molecule provided internal references for measuring this shielding effect. A shielding value, $\Delta\tau$, of *ca.* 0·25 was observed for substituents at C-7 in six tetrahydrobenzylisoquinolines. This is similar in magnitude to that observed [28,29] for substituents at the analogous position in aporphines. A similar shift is observed in the dihydrobenzylisoquinoline (XV) while the corresponding shielding in *N*-methylisopapaverine (XVII) is twice as large. The uniformity of this shielding effect

(XVI)	a	b	c	d	e	
R^1	Me	Me	$PhCH_2$	Me	$PhCH_2$	Me
R^2	Me	$PhCH_2$	Me	$PhCH_2$	Me	$PhCH_2$
R^3	Me	$PhCH_2$	$PhCH_2$	Me	Me	$PhCH_2$
R^4	Me	Me	Me	$PhCH_2$	$PhCH_2$	$PhCH_2$



(XVI)



(XVII)

is valuable for the identification of an alkoxyl group at C-7. The aryl proton at C-8 in all these derivatives, except (XVII), also appears at abnormally high field (*ca.* 3·9 τ). In the dihydro-derivative (XV) the olefinic protons gave rise to a pair of doublets at 3·95 and 4·75 τ (J = 7 c./sec.). The components of the low-field doublet showed further fine splitting (J = *ca.* 1 c./sec.). A spin-decoupling experiment,[30] kindly performed by Dr. D. W. Turner, established long-range coupling between protons at C-1 and C-3 [asterisks in (XV)]. Similar coupling has been observed [31,32] in simple cyclohexenes and the known dependence of coupling constant on stereochemistry suggests a quasi-axial conformation for the benzyl group in (XV).

Experimental

Melting points were taken on a Kofler hot-stage apparatus. The n.m.r. spectra were measured with a Varian A60 Spectrometer on permanent loan from the Wellcome Trust using $CDCl_3$ as solvent.

Counting Methods.—The earlier procedures [9] were employed throughout. Tritium-labelled berberine was converted into the colourless derivative, (±)-canadine (see below), before counting by scintillation methods.

Feeding Methods.—Vigorously growing plants were fed in spring or summer with aqueous solutions (typically 3 ml.) of the precursors (*ca.* 10 mg.) adjusted to *ca.* pH 6 with acetic or phosphoric acid. The solutions were allowed to pass slowly into two or three plants through wicks of untreated cotton drawn through the stems in the usual way. A period of one week was generally allowed for metabolism of the precursor.

Isolation of Alkaloids.—(a) *Hydrastis canadensis.* Whole plants were disrupted in 50% aqueous methanol containing 5% acetic acid and a trace of hydrochloric acid. The resulting mass was heated on the steam-bath for several hours then filtered through Celite. The filtrate and aqueous methanolic washings were concentrated *in vacuo*, treated with excess of aqueous ammonia, and extracted with chloroform. The aqueous layer was acidified, treated with excess of sodium hydrogen carbonate, and again extracted with chloroform and ether. The combined chloroform and ether extracts were evaporated and the berberine-rich residue dissolved in aqueous acetic acid and treated with 6N-sulphuric acid. The berberine sulphate which separated was removed and inactive berberine sulphate added to the mother liquors, brought into solution by heating, and allowed to crystallise. The combined portions of berberine sulphate were recrystallised from hot water and treated with aqueous barium hydroxide. Barium sulphate was removed and the aqueous solution acidified with excess of 6N-hydrochloric acid, concentrated *in vacuo*, and set aside to yield crystalline berberine chloride. The barium sulphate was washed with ethanol and the washings added to the combined aqueous mother liquors from the berberine crystallisations. This aqueous ethanolic solution was made alkaline with 2N-sodium hydroxide and extracted with chloroform. Evaporation then gave a residue which was chromatographed on alumina to yield hydrastine and canadine. Berberine was best purified

[29] W. H. Baarschers, R. R. Arndt, K. Pachler, J. A. Weisbach, and B. Douglas, *J.*, 1964, 4778.
[30] D. W. Turner, *J.*, 1962, 847.
[31] G. W. Kirby and H. P. Tiwari, *J.*, 1964, 4655.
[32] E. W. Garbisch, *Chem. and Ind.*, 1964, 1715, and references cited therein.

by crystallisation of its sulphate and chloride from water containing the corresponding acid. As a further check on its radiochemical purity berberine was reduced with sodium borohydride in aqueous methanol [33] to give (±)-canadine. Hydrastine was recrystallised by treating a hot concentrated chloroform solution with hot methanol.

(b) *Dicentra spectabilis.* Whole plants were treated as described for *H. canadensis* (above). The aqueous methanolic extract was concentrated, washed with chloroform, basified with 2N-sodium hydroxide, and extracted with chloroform. Evaporation of the chloroform and treatment of the residue with methanol gave crystalline protopine. Further purification was achieved by dissolving the alkaloid in hot 5% aqueous acetic acid and treating the solution with concentrated aqueous potassium nitrate. The crystalline protopine nitrate which separated was then shaken with aqueous ammonia and chloroform. The chloroform layer was evaporated to small volume and hot methanol added. Pure crystalline protopine then separated.

(c) *Argemone hispida* and *Argemone mexicana.* The total bases were obtained by chloroform extraction as described for *D. spectabilis.* Only a small amount of argemonine could be detected in the extract. Inactive, synthetic (±)-argemonine [34] was added and protopine and argemonine separated by preparative think-layer chromatography on silica gel G (Merck) plates developed in methanol. The eluted alkaloids were crystallised in the usual way.

Synthesis of Precursors.—Benzylisoquinoline derivatives were synthesised, and labelled,[9] by the standard Bischler–Napieralski procedure.[35] Many of the compounds used in the present work have already been reported.[9,36–38] Modifications of some published methods were employed and are illustrated below.

4-Benzyloxy-3-[methyl-^{14}C]methoxybenzaldehyde.[7] Sodium hydride (55% suspension in mineral oil, 3 mg.) was added to a solution of 4-benzyloxy-3-hydroxybenzaldehyde [7] (15 mg.) in dry dimethylformamide (1 ml.) and the mixture warmed until evolution of hydrogen had ceased. [^{14}C]Methyl iodide (2·9 mg., 0·1 mc) was distilled (vacuum line) onto the frozen phenoxide solution and the reaction mixture sealed *in vacuo.* After 4 days at room temperature excess of inactive methyl iodide was added and after 1 hr. the mixture was diluted with excess of N-sodium hydroxide. The product was extracted with ether and crystallised in the usual way to give the desired methyl-labelled aldehyde (16 mg., 0·07 mc), m. p. 61—63°.

4-Benzyloxy-3-methoxyphenethylamine. Lithium aluminium hydride (1 g.) was added with swirling to a solution of aluminium chloride (4 g.) in dry ether (50 ml.) and the resulting suspension filtered. 4-Benzyloxy-3-methoxyphenylacetonitrile [9] (370 mg.) in ether (25 ml.) was added slowly to the filtrate under reflux. After 30 min. the mixture was decomposed with moist tetrahydrofuran (20 ml.) followed by water and was then adjusted to pH < 2 with 6N-sulphuric acid. The ether layer was removed and the aqueous phase made alkaline and extracted with ether in the usual way to give the desired amine (320 mg.). Reduction also occurs in the absence of aluminium chloride [9] but the yields are variable.

3,4-Dibenzyloxy-ω-nitrostyrene (with D. S. Bhakuni). 3,4-Dibenzyloxybenzaldehyde [39] (5 g.) in redistilled nitromethane (15 ml.) was treated with methylamine hydrochloride (600 mg.) and anhydrous sodium acetate (600 mg.) with shaking at room temperature. After 5 hr. the crystalline deposit was filtered off and washed with water to give substantially pure nitrostyrene (2·4 g.). The filtrate was evaporated *in vacuo* and the residue washed with ether and water then crystallised from acetic acid to yield more (3·3 g.) nitrostyrene. The combined products were crystallised from ethanol–acetic acid (10 : 1) to give the nitrostyrene as yellow rods (5·2 g.), m. p. 120° (lit.,[40] 118—119°). The literature procedure and many standard variants failed to give satisfactory yields of the products.

ω-Diazo-3,4-dibenzyloxy-acetophenone. 3,4-Dibenzyloxybenzoyl chloride, prepared from the corresponding acid (800 mg.) with oxaloyl chloride in the usual way, in ether (50 ml.) and benzene (15 ml.) was added slowly to excess of ethereal diazomethane (from 5 g. of nitrosomethylurea). The solution was kept overnight at 5° in the dark then evaporated to give the *diazoketone*

[33] R. Mirza, *J.*, 1957, 4400.
[34] A. R. Battersby and R. J. Binks, *J.*, 1955, 2888.
[35] W. M. Whaley and T. R. Govindachari, "Organic Reactions," ed. R. Adams, John Wiley and Sons, New York, 1951, vol. 6, 83.
[36] C. Schopf, I. Jackh, and H. Perrey, *Annalen*, 1932, **497**, 59.
[37] K. Kratzl and G. Billek, *Monatsh.*, 1952, **83**, 1409.
[38] J.-I., Kunitomo, *J. Pharm. Soc. Japan*, 1961, **81**, 1253, 1257.
[39] H. Burton and P. F. G. Praill, *J.*, 1951, 522.
[40] K. E. Hamlin, *Chem. Abs.*, 1959, **53**, 7101.

(720 mg.), m. p. 100—102°. Crystallisation from ether gave pure material, m. p. 103° (Found: C, 73·5; H, 5·1; N, 8·0 $C_{22}H_{18}N_2O_3$ requires C, 73·7; H, 5·1; N, 7·8%).

4-Benzyloxy-N-(3-benzyloxy-4-methoxyphenethyl)-3-methoxyphenylacetamide. 3-Benzyloxy-4-methoxyphenethylamine (from the hydrochloride, 215 mg.) [16] and 3-methoxy-4-benzyloxy-ω-diazoacetophenone [41,42] (206 mg.) in benzene (50 ml.) were irradiated for 18 hr. under nitrogen in the usual way.[9] The resulting *amide* (315 mg.), after crystallisation from ethanol–ether, had m. p. 128° (Found: C, 74·9; H, 6·6; N, 2·9. $C_{32}H_{33}NO_5$ requires C, 75·1; H, 6·5; N, 2·7%).

3,4-Dibenzyloxy-N-(3-methoxy-4-benzyloxyphenethyl)phenylacetamide. 4-Benzyloxy-3-methoxyphenethylamine [43] (from the hydrochloride, 290 mg.) and 3,4-dibenzyloxy-ω-diazoacetophenone (360 mg.) in benzene (60 ml.) were irradiated in the usual way.[9] Work-up afforded two crops of the *amide*, m. p. 128—132° (405 mg.) and m. p. 125—129° (75 mg.). Crystallisation from ethyl acetate–ether gave material, m. p. 131° (Found: C, 77·5; H, 6·3; N, 2·45. $C_{38}H_{37}NO_5$ requires C, 77·7; H, 6·35; N, 2·4%).

7-Benzyloxy-1-(3,4-dibenzyloxybenzyl)-6-methoxy-3,4-dihydroisoquinoline hydrochloride. The appropriate phenylacetamide (see above) (0·52 g.), in dry refluxing toluene (20 ml.), was treated with freshly distilled phosphorus oxychloride (0·5 ml.). After 1 hr. solvent was removed *in vacuo* and the residual salt triturated with ether to give the *dihydroisoquinoline hydrochloride* (0·45 g.). Crystallisation from ethanol–ether gave material, m. p. 182—184° (Found: C, 75·4; H, 6·22. $C_{38}H_{36}ClO_4N$ requires C, 75·3; H, 6·0%).

7-Benzyloxy-1-(3,4-dibenzyloxybenzyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline. The appropriate hydrochloride (see above) (320 mg.) in methanol (10 ml.) was treated with potassium t-butoxide (61 mg.) and methyl iodide (0·4 ml.). The resulting suspension was filtered and the filtrate refluxed for 45 min. Concentration of the solution and addition of ether gave the expected methiodide (400 mg.), m. p. 180°. This was added to a suspension of sodium borohydride (60 mg.) in pyridine (5 ml.). After 15 min. the mixture was poured into the water and the product extracted with ether. Chromatography on alumina (grade III) gave two crystalline products; (*a*) (35 mg.), m. p. 155—160° and (*b*) (221 mg.), m. p. 62°. Reduction of the methiodide with sodium borohydride in cold methanol in the usual way gave only product (*b*). Crystallisation of this material from methanol yielded the required *tetrahydroisoquinoline*, m. p. 62° (Found: C, 79·1, 79·3; H, 6·9, 6·9; N, 1·8, 2·0; O, 12·1. $C_{39}H_{39}NO_4,0{\cdot}5CH_3OH$ requires C, 78·9; H, 6·7; N, 2·3; O, 11·9%). Product (*a*) like the amine (*b*), gave a positive Dragendorff reaction but showed strong infrared bands at *ca.* 2400 cm.$^{-1}$. It appeared to be a C–BH_2 compound and was not investigated further.

Tritiated 1-(3,4-dihydroxybenzyl)-7-hydroxy-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline. The corresponding tribenzyl ether (300 mg.) was hydrogenated in ethanol, containing hydrochloric acid, with 10% palladium–carbon catalyst (300 mg.) in the usual way. Filtration, evaporation, and addition of N-sodium hydrogen carbonate liberated the derived phenolic amine which was extracted into chloroform. Evaporation gave the product as a white solid (164 mg.). The n.m.r. spectrum confirmed the presence of one *N*-methyl group, one methoxyl group, five aryl protons, and the absence of *O*-benzyl groups. This catechol deteriorated on keeping in air and was tritiated directly as follows. The catechol (20 mg.) was heated in dimethylformamide (0·2 ml.) and tritiated water (0·5 ml., 0·1 c) containing triethylamine (0·2 ml.) under nitrogen at 100° for 5 days. The solvents were removed *in vacuo* and methanol added to the residue and evaporated to remove labile tritium. The residue was treated with N-sodium hydrogen carbonate and extracted with chloroform to give tritiated material (5 mg., 2×10^7 disintegrations per min. per mg.). A similar experiment in deuterium oxide showed (n.m.r. control) exchange at positions *ortho* and *para* to the phenolic hydroxyl groups.

(±)-[^{3}H]-Phenol (IX; R = H, *labelled as indicated*). Berberine chloride (500 mg.) was heated in a stream of carbon dioxide at 185—205° for 75 min. The residue was suspended in water and the red phenol betaine extracted into chloroform. The chloroform was evaporated and the residue dissolved in aqueous methanol (90%, 20 ml.) and treated at 4° with sodium borohydride (150 mg.). The resulting tetrahydro-base was isolated by chloroform extraction, as usual, and chromatographed on alumina (grade III). Crystallisation from methanol gave (±)-phenol (IX; R = H) (185 mg.), m. p. 170° (lit.,[19] 167°). (±)-Phenol (IX; R = H) (100 mg.) was treated under nitrogen in tritiated water (0·5 ml., 0·1 c) containing potassium

[41] T. Kametani and J. Serizawa, *J. Pharm. Soc. Japan*, 1952, **72**, 1084.
[42] D. H. Hey and L. C. Lobo, *J.*, 1954, 2246.
[43] J. Finkelstein, *J. Amer. Chem. Soc.*, 1951, **73**, 550.

t-butoxide (72 mg.) at 100° for 6 days. The reaction vessel was opened, dimethylformamide (1 ml.) added, and heating continued for a further 4 days. The labelled product ($7{\cdot}1 \times 10^6$ disintegrations per min. per mg.) was isolated in the usual way. A similar experiment with deuterium oxide showed (n.m.r. control) exchange only at the position *para* to the phenolic hydroxyl group. Methylation with diazomethane in methanol–ether, in the usual way, gave the correspondingly labelled (±)-canadine.

1,2-*Dihydro*-N-*methylpapaverine*. Papaverine methiodide (from papaverine, 500 mg.) was added to a suspension of sodium borohydride (60 mg.) in pyridine (20 ml.). After 10 min., more sodium borohydride (40 mg.) was added. The mixture was allowed to stand, with occasional shaking, for 10 min. Excess of ether and water were added and the ether layer washed with more water. The ethereal extract was dried ($MgSO_4$) and evaporated, the remaining traces of pyridine being removed by addition and evaporation of toluene. The residue crystallised from ether–light petroleum to give the product (370 mg.), m. p. 134—136° (lit.,[27] 135°).

Degradation of Labelled Alkaloids.—The cleavage of *N*-methyl groups to give methyl iodide (Herzig–Meyer method) and of methylenedioxy groups to give formaldehyde (isolated as the dimedone derivative) has been described earlier.[7,9] Other degradations used in the present investigation are given below. The results have already been recorded in Tables 1 and 2.

1-*Phenyl*-1,2-*dihydroberberine*. To a suspension of anhydrous berberine chloride (63 mg.) in ether (0·5 ml.) was added 1-M-ethereal phenyl magnesium bromide (1 ml.). The suspension was stirred under reflux for 2·5 hr. and then decomposed with water and 6N-hydrochloric acid. A white solid separated when the walls of the vessel were scratched. This was collected by centrifugation, dissolved in hot water, and treated with aqueous ammonia. The free base which separated was collected and dissolved in hot acetic acid. Addition of ethanol, then aqueous ammonia, gave the product as crystals (52 mg.), m. p. 194—198° (lit.,[44] 195°). Oxidation with chromic acid in the usual way (Kuhn–Roth degradation) gave benzoic acid.

Emde Base (*X*) *from Protopine*.—4% Sodium amalgam (20 g.) was slowly added to a solution of protopine methosulphate (134 mg.) in 5% aqueous sulphuric acid (5 ml.). The mixture was heated on a steam-bath and kept acidic by repeated additions of sulphuric acid. After the addition of amalgam was complete the reaction mixture was poured into excess of 4N-sodium hydroxide. The resulting suspension was extracted with ether, chloroform, and ethyl acetate. The combined extracts were dried and evaporated and the residue crystallised from ether to give the *Emde base* (66 mg.), m. p. 110—112° (Found: C, 68·0; H, 6·8. $C_{21}H_{25}NO_5$ requires C, 67·9; H, 6·8%).

Ozonolysis of Anhydromethylprotopine (XI).—Isoprotopine chloride and anhydromethylprotopine were obtained by Perkin's method.[45] Phosphorus oxychloride free from hydrogen chloride was essential for good yields in the preparation of the former derivative. A slow stream of ozone was passed through a solution of anhydromethylprotopine (40 mg.) in ethyl chloride (20 ml.) at −78°. After 45 min. ozone appeared in the exit gases. The solvent was allowed to evaporate at atmospheric pressure and the residue was treated with water (15 ml.) and zinc dust (800 mg.). The thoroughly mixed suspension was kept for 1 hr. then steam-distilled. Formaldehyde was collected in saturated aqueous dimedone to give, on standing overnight, the usual derivative, m. p. 190—191°.

Degradation of Acetic Acid.—Sodium acetate (5 mg., from the Kuhn–Roth degradation of protopine Emde base) was subjected to the Schmidt degradation.[46] The resulting methylamine was distilled into aqueous hydrochloric acid in the usual way. The solution was evaporated and the residue dissolved in methanol. *O*-Benylisovanillin (25 mg.) was added followed by one drop (excess) of 2N-sodium hydroxide. After 1 hr. sodium borohydride (30 mg.) was added and the solution left for a further hour. *O*-Benzyl-*N*-methylisovanillylamine was isolated as the hydrochloride (12 mg.), m. p. 212° (lit.,[7] 212°).

We thank Mr. R. H. Young for expert horticultural assistance. Financial support from the Government Grants Committee of the Royal Society and the D.S.I.R. is gratefully acknowledged. One of us (R. H. H.) thanks the National Science Foundation (of the U.S.A.) for a Fellowship.

IMPERIAL COLLEGE, LONDON S.W.7. [*Received, May 13th*, 1965.]

[44] M. Freund and H. Beck, *Ber.*, 1904, **37**, 4673.
[45] W. H. Perkin, *J.*, 1919, **115**, 713.
[46] E. F. Phares, *Arch. Biochem. Biophys.*, 1951, **33**, 173.