866. *Phenol Oxidation and Biosynthesis. Part VI.* The Biogenesis of Amaryllidaceae Alkaloids.*

By D. H. R. Barton, G. W. Kirby, J. B. Taylor, and G. M. Thomas.

The incorporation of the four diphenols (Ia), (Ib), (Ic), and (Id) into the alkaloids galanthamine, galanthine, and hæmanthamine produced by the King Alfred daffodil has been investigated. The precursors have been labelled on the *N*-Me, on the *O*-Me, and at position 1 as appropriate. Degradation of the alkaloids demonstrated that there was no scrambling of the labels and that the precursors are incorporated intact. The application of multiply labelled precursors has been specially illustrated. Intermediate trapping experiments have also been made.

By a decisive experiment with the precursor (Ic), doubly labelled on *O*-Me and at position 1, it has been proved that the methylenedioxy-group of hæmanthamine is formed in Nature by cyclisation of the *O*-methoxyphenol group of (Ic). The generalisation and mechanism of this phenomenon are debated.

The results as a whole confirm the hypothesis that Amaryllidaceae alkaloids are produced in Nature by the oxidative coupling of diphenols. They also demonstrate that the de-*O*- and de-*N*-methylation are not rapid processes, at least in the King Alfred daffodil.

Preliminary communications summarising our main results have already appeared.[1-3]

The Amaryllidaceae alkaloids constitute a large group of naturally occurring compounds of widely diverse functionality and structural type.[4] Nevertheless the formation of all these compounds can be accommodated by a simple biogenetic hypothesis, *viz.*, that the carbon skeletons are produced by oxidative phenolic coupling of precursors of the type (I).[5] Since we have already elaborated this theory in detail in earlier Parts of this series, we shall only mention such specific points as are required for discussion of the results in the present paper. We shall deal with the alkaloids galanthamine (II), hæmanthamine (III), and galanthine (IV), which are theoretically produced by oxidative coupling of diphenols of type (I), the new carbon bonds being formed in principle between the *p*-*o*′, *p*-*p*′, and *o*-*p*′ positions.

The chemical synthesis [6] of galanthamine from the diphenol (Ia) supported the biogenetic hypothesis, as did the isolation [7] of the simplest Amaryllidaceae alkaloid, belladine, the *OO*-dimethyl ether of (Ia). However, a biogenetic hypothesis can only be tested properly by experiments with suitable isotopically labelled precursors. The present paper describes experiments of this type. Other workers have also been giving informative attention to the biogenesis of Amaryllidaceae alkaloids. Their findings are, in general, in agreement with our own. We shall refer specifically to this parallel work in so far as it is pertinent to the results which we now describe.

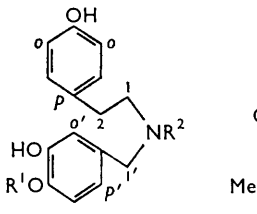
Synthesis of Precursors. Dimethylnorbelladine (Ia), labelled with carbon-14 in its *N*-methyl group, was prepared as described earlier.[6] [*N*-methyl-^{14}C]-*N*-Methylnorbelladine (Ib) was similarly obtained from [*N*-methyl-^{14}C]-3,4-dibenzyloxy-*N*-methylbenzylamine and *p*-benzyloxyphenylacetyl chloride. Norbelladine itself (Id) labelled at position-1

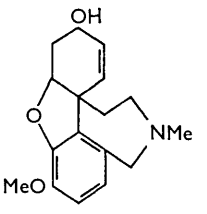
* Part V, *J.*, 1962, 806.

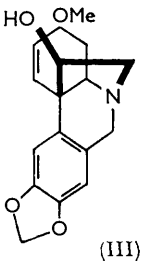
[1] Barton, Kirby, Taylor, and Thomas, *Proc. Chem. Soc.*, 1961, 254.
[2] Barton, Kirby, Taylor, and Thomas, *Proc. Chem. Soc.*, 1962, 179.
[3] Barton, Kirby, and Taylor, *Proc. Chem. Soc.*, 1962, 340.
[4] Wildman in "The Alkaloids," ed. Manske and Holmes, Academic Press, New York, 1960, Vol. VI, p. 290.
[5] Barton and Cohen, "Festschrift Arthur Stoll," Birkhauser, Basle, 1957, p. 117.
[6] Barton and Kirby, *J.*, 1962, 806.
[7] Warnhoff, *Chem. and Ind.*, 1957, 1385.

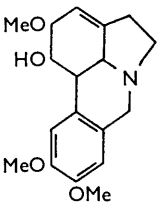
was synthesised as follows. 4-Benzyloxybenzyl chloride was treated with sodium [^{14}C]cyanide in dimethyl sulphoxide [8] and the derived nitrile reduced with lithium aluminium hydride to labelled *O*-benzyltyramine. Condensation of the latter with 3,4-dibenzyloxybenzaldehyde, and reduction of the imine thus formed with sodium borohydride, gave *OOO*-tribenzylnorbelladine. Hydrogenolysis over palladised charcoal then afforded [1-^{14}C]norbelladine (Id). *O*-Methyl[1-^{14}C]norbelladine (Ic) was obtained similarly from the same labelled *O*-benzyltyramine and *O*-benzylisovanillin.

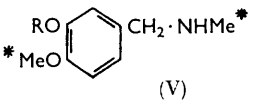
For the synthesis of [*O*-methyl-^{14}C]-precursors 3-benzyloxy-4-hydroxybenzaldehyde was required. Reimer–Tiemann formylation of *O*-benzylcatechol proved to be a convenient synthetic method and was preferable to direct benzylation of protocatechualdehyde.[9] Methylation of 3-benzyloxy-4-hydroxybenzaldehyde with [^{14}C]methyl iodide gave the desired *O*-methyl-labelled *O*-benzylisovanillin, condensation of which with [^{14}C]methylamine, and reduction of the derived imine with sodium borohydride, gave the doubly labelled † benzylamine (V; R = Ph·CH$_2$). This amine was used in the synthesis

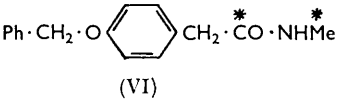


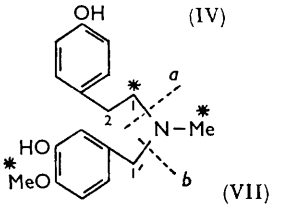












of the triply labelled phenol (Ia) with labels on *O*-methyl, *N*-methyl, and position 1. *p*-Benzyloxyphenyl[1-^{14}C]acetic acid, prepared from the corresponding nitrile (see above) was converted into its acid chloride and then condensed with the doubly labelled amine (V; R = Ph·CH$_2$). Reduction with lithium aluminium hydride followed by hydrogenolysis gave the desired triply labelled phenolic amine (Ia).

Doubly labelled *N*-methyltyramine, with the labels in the *N*-methyl group and at position 1, was prepared as follows. Condensation of *p*-benzyloxyphenyl[1-^{14}C]acetyl chloride (see above) with labelled methylamine gave the amide (VI). Reduction with lithium aluminium hydride followed by hydrogenolysis afforded the desired doubly labelled *N*-methyltyramine. [1-^{14}C]Tyramine itself was most conveniently prepared by decarboxylation of [2-^{14}C]tyrosine.[11]

The Experimental section contains further details of the preparation and radio-assay of these precursors.

Feeding Experiments.—The King Alfred daffodil was chosen for the majority of our

† Multiply labelled compounds are, of course, mixtures of inactive and singly labelled species containing only a small number of molecules having more than one isotopic atom. If desired such multiply labelled materials can be conveniently obtained by mixing singly labelled species. (For example see Battersby, Breuer, Fales, Highet, and Wildman.[10])

[8] Smiley and Arnold, *J. Org. Chem.*, 1960, **25**, 257.
[9] Schering in Friedlaender's "Fortschritte der Theerfarbenfabrikation," Julius Springer, Berlin, Vol. IV, p. 1282.
[10] Batterby, Breuer, Fales, Highet, and Wildman, *Proc. Chem. Soc.*, 1962, 180.
[11] Schmidt and Nasse, *Annalen*, 1865, **133**, 214.

experiments. This plant is easy to grow and produces [12,13] useful amounts of galanthamine (II), hæmanthamine (III), and galanthine (IV), representing the three main groups of Amaryllidaceae alkaloids. Compounds were normally injected (in aqueous solution at pH 6) directly into the hollow flower stalks of the vigorously growing, mature plants. Incorporation of tyrosine into the alkaloids was also observed when the amino-acid was injected into the bulbs (corms) of freshly sprouted daffodils, but no incorporation occurred when dormant bulbs were used. Oblique hypodermic injection into the leaves of daffodils is also possible, and surprisingly large volumes of solution (*ca.* 0·5 ml. per leaf) may be introduced in this way.

A series of experiments was made to compare the efficiency of incorporation of the various dihydric phenols (I) and (±)-[2-^{14}C]tyrosine.[1] The phenols were singly labelled either at position 1 or, where applicable, in the *N*-methyl group. Similar chemical quantities of these precursors were fed to mature King Alfred daffodils growing in the same conditions, and the whole plants worked up after eight days. Injections appeared to have no ill effect on the plants' growth or alkaloid production. The alkaloid yields and precursor incorporations are given in Table 1. (±)-[2-^{14}C]Tyrosine was incorporated

TABLE 1.

Incorporation of precursors in galanthamine and hæmanthamine.

Precursors:	(Ia)		(Ib)		(Ic)		(Id)		Tyrosine	
	Yield* (%)	In-corpn. (%)	Yield* (%)	In-corpn. (%)	Yield* (%)	In-corpn. (%)	Yield* (%)	In-corpn. (%)	Yield* (%)	In-corpn. (%)
Galanthamine (II) ...	0·024	0·014	0·025	0·018	0·018	0·000	0·027	0·014	0·025	0·012
Hæmanthamine (III)	0·026	0·000	0·029	0·000	0·024	0·036	0·029	0·25	0·032	0·20

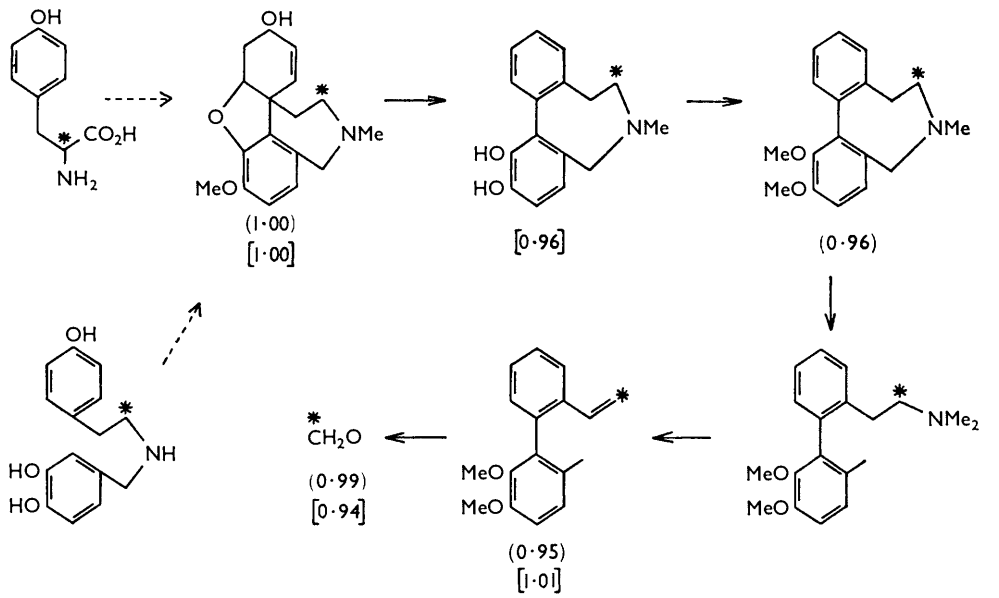
* Yield of alkaloid calculated on weight of fresh (wet) plant material.

much more efficiently into hæmanthamine than into galanthamine and this difference was also apparent in the corresponding incorporations of [1-^{14}C]norbelladine. However, these differences may merely reflect the relative rates of alkaloid production at the time of injection; in general, it is unwise to attach any great biosynthetic significance to the magnitude of precursor incorporation in intact plants. As expected, experiments with *N*-methyl-labelled phenols gave inactive hæmanthamine, showing that transfer of the *N*-methyl group to the " $C_{(1)}$ pool " did not occur to any measurable extent. Detailed accounts of the biosynthesis of galanthamine and hæmanthamine can now be given separately.

Galanthamine.—In early experiments, incorporation (0·14%) of (±)-[2-^{14}C]tyrosine into galanthamine in the snowdrop *Galanthus elwesii*,[14] was observed. Radioactive lycorine was also isolated but the incorporation (0·04%) into this alkaloid was much lower than that (0·44%) obtained [15] when using the " Texas " daffodil. A detailed study of the biosynthesis of lycorine has been made by Battersby, Wildman, and their co-workers [16,17] and, consequently, our radioactive material was not investigated further. A degradation of radioactive galanthamine, derived from (±)-[2-^{14}C]tyrosine, was carried out to show that no " scrambling " of the radioactivity had taken place. The reactions employed were based on the work of Kobayashi and Uyeo [18] and gave the results outlined in Scheme 1. In the same way it was shown that galanthamine, obtained from King Alfred daffodils injected with [1-^{14}C]norbelladine, also had its radioactivity confined to the expected carbon atom (Scheme 1).

[12] Fales, Giuffrida, and Wildman, *J. Amer. Chem. Soc.*, 1956, **78**, 4145.
[13] Boit and Ehmke, *Chem. Ber.*, 1956, **89**, 163.
[14] Boit and Ehmke, *Chem. Ber.*, 1955, **88**, 1590.
[15] Barton and Kirby, *Proc. Chem. Soc.*, 1960, 392.
[16] Battersby, Binks, and Wildman, *Proc. Chem. Soc.*, 1960, 410.
[17] Battersby, Binks, Breuer, Fales, and Wildman, *Proc. Chem. Soc.*, 1961, 243.
[18] Kobayashi and Uyeo, *Chem. and Pharm. Bull.* (*Japan*), 1953, **1**, 139.

Scheme 1.



( ) refers to relative molar activities of products derived from tyrosine and [ ] to products from norbelladine.

It was noted that, of the four biogenetically likely precursor diphenols (Ia—d), three [(Ia), (Ib), and (Id)] were converted into galanthamine with an efficiency similar to that observed with tyrosine. However the fourth, *O*-methylnorbelladine (Ic), did not serve as a precursor for this alkaloid and no incorporation was observed in two later experiments. Although negative experiments in biosynthetic work must be treated with great caution, this difference in behaviour of the closely related phenols of type (I) possibly suggests a definite order of methylation in the biosynthesis of galanthamine, thus: norbelladine ⟶ *N*-methylnorbelladine ⟶ *NO*-dimethylnorbelladine ⟶ galanthamine. In these experiments, galanthamine, derived from the *N*-methyl-labelled precursors [(Ia) and (Ib)], was shown to have all its radioactivity located in the *N*-methyl group.

The results described so far do not rigorously establish that precursors (as VII) are not cleaved at bonds *a* and/or *b* before conversion into the " true " precursors of the alkaloids. To clarify this point, experiments with multiply labelled precursors were made.[2] The diphenol (VII) with both *N*- and *O*-methyl labelling was fed to King Alfred daffodils in the usual way. When the derived galanthamine was demethylated to give the separate activities for the *N*- and *O*-methyl groups, it was found that the ratio of activities was, within the limits of the experimental method, the same as in the precursor. For confirmation, the galanthamine was oxidised with manganese dioxide to narwedine (the corresponding ketone) which was also demethylated (Table 2). Clearly cleavage of bond *b*

TABLE 2.

Distribution of carbon-14 in alkaloids and their precursors.

	Fraction of ^{14}C in			Fraction of ^{14}C in		
Doubly labelled	*O*-Me	*N*-Me	Triply labelled	*O*-Me	*N*-Me	Remainder
Precursor (VII)	0·48	0·51	Precursor (VII)	0·19	0·21	0·60
Galanthamine (II)	0·48	0·48	Galanthamine (II)...	0·18	0·19	0·63
Narwedine	0·45	0·49	Narwedine	0·18	0·18	0·64

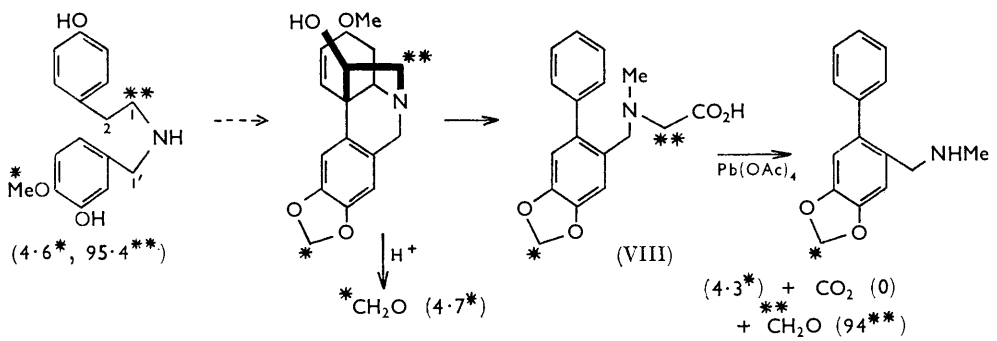
does not take place. In a similar way the triply labelled precursor (VII), with labels on the *O*-methyl, on the *N*-methyl, and at position 1, was used to exclude cleavage of bonds *a* and *b* and to show that partial *O*- and *N*-demethylation did not take place.

In independent studies, Battersby, Wildman, and their co-workers [10] have shown that norbelladine, labelled at positions 1 and 1', is incorporated intact into belladine, lycorine, and crinamine. These results are in accord with our own on galanthamine and with the original biogenetic hypothesis.

Hæmanthamine.—Battersby, Fales, and Wildman [19] have reported experiments on hæmanthamine biosynthesis in " Twink " double narcissus plants. Degradation of the radioactive alkaloid showed that both [2-^{14}C]tyrosine and [1-^{14}C]norbelladine were incorporated without " scrambling " of the label. The incorporation of norbelladine (0·15%) observed in their work is in good agreement with our own value (0·25%) obtained with the King Alfred daffodil. Our special interest in hæmanthamine arose from the observation (Table 1) that radioactive alkaloid was produced in feeding experiments with *O*-methyl[1-^{14}C]norbelladine (Ic). This result could be explained in one of two ways. First, the precursor could be demethylated and the methylenedioxy-group of hæmanthamine formed from a formaldehyde equivalent at a later stage. Secondly, the methylenedioxy-group could be produced directly by oxidative cyclisation of the methoxyl group. Since demethylation of precursors before alkaloid formation did not occur in the work on galanthamine (see above), we were led to favour the second possibility. The following critical experiment proved the correctness of the hypothesis in the case of hæmanthamine.[3]

O-Methylnorbelladine (Ic) was labelled in the *O*-methyl group (19%) and at position 1 (81%). The biosynthetically derived hæmanthamine had 20% of the activity in the methylenedioxy-group, the methylene carbon being isolated as formaldehyde after hydrolysis with 20% sulphuric acid. In another experiment, hæmanthamine, derived from the doubly labelled precursor (Ic) containing 4·6% of the activity in the methoxyl group and the remainder at position 1, was degraded as shown in Scheme 2. The inter-

Scheme 2.

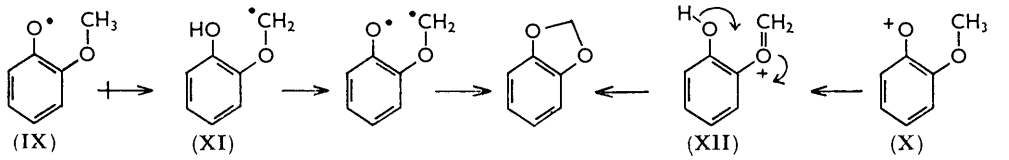


mediate amino-acid (VIII) [19,20] was most conveniently cleaved by lead tetra-acetate. Both the formaldehyde and the *N*-methyl-6-phenylpiperonylamine [21] fragment had the expected activities. In this experiment the incorporation (1·0%) of *O*-methylnorbelladine (Ic) into hæmanthamine was much higher than had been obtained in the two previous experiments. This variation again makes plain the need for caution in interpreting the magnitude of incorporation values.

The hypothesis that methylenedioxy-groups could be derived biogenetically by cyclisation of *o*-methoxyphenols was first mentioned by Sribney and Kirkwood [22] who found that methionine was a much more effective precursor than formate for the methylenedioxy-groups of protopine. However, the present results appear to constitute the first experimental proof of this hypothesis.

[19] Battersby, Fales, and Wildman, *J. Amer. Chem. Soc.*, 1961, **83**, 4098.
[20] Fales and Wildman, *J. Amer. Chem. Soc.*, 1960, **82**, 197.
[21] Warren and Wright, *J.*, 1958, 4696.
[22] Sribney and Kirkwood, *Nature*, 1953, **171**, 931.

Since *o*-dimethoxybenzenes, *o*-methoxyphenols, and methylenedioxybenzenes frequently occur together in closely related natural products, it seems reasonable that methylenedioxy-groups in general are formed in Nature by a cyclisation mechanism. Clearly the intermediates involved can be either radical or cationic in character. It is tempting to speculate that an oxidation process applied to the phenolic hydroxyl of an *o*-methoxyphenol can furnish either the radical (as IX) or the cation (as X). In so far as the radical (as IX) is stabilised to the alternative radical (as XI) and the cation (as X) is



destabilised relative to the oxonium ion (as XII), the latter process could appear, at least thermodynamically, to be more probable. However, hydroxylation of methyl groups attached to saturated carbon is commonly observed in Nature (*e.g.*, in the terpenoids); consequently, hydroxylation of an *o*-methoxyphenol might take place without involving the phenolic hydroxyl group, and the resulting hemiformal could then lose water to give the ion (XII). The biological de-*O*-methylation of codeine to give morphine [23,24] might also involve hydrolysis of an intermediate ion (as XII). We have experiments in hand on the biogenetic origin of methylenedioxy-groups in other alkaloids.

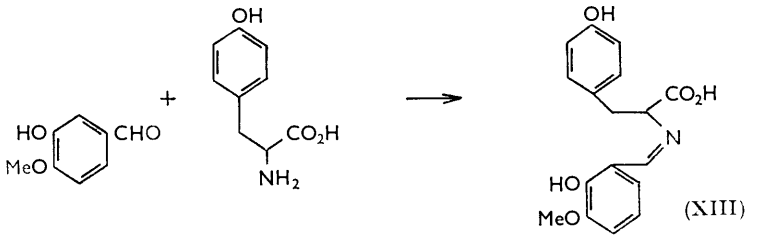
That tyrosine provides the C_6–C_2 unit of the major Amaryllidaceae alkaloids has been established by several groups of investigators.[15,16,19,25-28] However, less is known about the origin of the remaining C_6–C_1 unit. To gain insight into this problem the doubly labelled *N*-methylbenzylamine (V; R = H) was fed to King Alfred daffodils. Radioactive galanthamine was isolated (0·019% incorporation), all the radioactivity being located in the *O*-methyl group. This suggests that degradation of the amine to either 3-hydroxy-4-methoxybenzylamine or, more probably, isovanillin had preceded incorporation. Feeding experiments with *O*-methyl-labelled isovanillin were unfortunately frustrated by lack of absorption of this substance by the plant. However, in preliminary experiments, Suhadolnik, Fischer, and Zulalian [25] have observed incorporation of tritiated protocatechualdehyde into lycorine, although the distribution of radioactivity in the alkaloid remains to be determined. The benzylamine (V) was also incorporated (0·018%) into hæmanthamine. Hydrolysis gave formaldehyde containing all the radioactivity. This provides further confirmation of the methoxyl–methylenedioxy transformation.

Jeffs [26] has shown that [3-^{14}C]tyrosine does *not* provide the C_6–C_1 unit of hæmanthamine, and Wildman, Fales, and Battersby [27] have independently obtained similar results for hæmanthamine, hæmanthidine, and tazettine. Suhadolnik, Fischer, and Zulalian,[25] and Wildman, Battersby, and Breuer [28] have made the important observation that [3-^{14}C]phenylalanine is incorporated into the C_6–C_1 unit, but not into the C_6–C_2 unit of lycorine, and the same holds true for belladine.[28] This is consistent with the view that hydroxylation of the aromatic ring occurs only after further transformation of phenylalanine. Phenylserine [25] or cinnamic acid [28,29] may be an intermediate in this process.

Our work on galanthamine and hæmanthamine has established two separate routes leading from aromatic amino-acids to the alkaloids. The first, involving step-wise methylation of norbelladine, has already been discussed. The second proceeds from an

[23] Battersby and Harper, *Tetrahedron Letters*, 1960, No. 27, 21.
[24] Stermitz and Rapoport, *J. Amer. Chem. Soc.*, 1961, **83**, 4045; *Nature*, 1961, **189**, 310.
[25] Suhadolnik, Fischer, and Zulalian, *J. Amer. Chem. Soc.*, 1962, **84**, 4348.
[26] Jeffs, *Proc. Chem. Soc.*, 1962, 80.
[27] Wildman, Fales, and Battersby, *J. Amer. Chem. Soc.*, 1962, **84**, 681.
[28] Wildman, Battersby, and Breuer, *J. Amer. Chem. Soc.*, 1962, **84**, 4599.
[29] McCalla and Neish, *Canad. J. Biochem. Physiol.*, 1959, **37**, 537.

aromatic unit bearing a methoxyl group (*e.g.*, isovanillin) and, for galanthamine biosynthesis, must not involve the intermediate formation of *O*-methylnorbelladine (Ic).



(XIII)

One possible intermediate might be the aldimine (XIII), analogous to that formed by condensation of pyridoxal phosphate and tyrosine during the enzymic decarboxylation of the amino-acid. Methylation, decarboxylation, and reduction of this aldimine (XIII) would then give *NO*-dimethylnorbelladine and hence galanthamine, while decarboxylation and reduction alone would give *O*-methylnorbelladine, the precursor of hæmanthamine. An equally acceptable scheme could be written using tyramine but we have been unable to observe any incorporation of tyramine or *N*-methyltyramine into galanthamine. However, these negative results must, as always, be regarded with caution, especially since tyramine [25] is known to be an efficient precursor for lycorine.

The tracer studies described so far establish unambiguously that the plant is capable of effecting certain well-defined chemical operations. However, they do not prove conclusively that these processes are part of the organism's normal metabolism. This is especially true when we are feeding compounds which have not been shown to exist in the plant. A clearer view of biosynthetic sequences may, however, be obtained by intermediate trapping experiments. For example, if labelled tyrosine is injected together with a large amount of an inactive compound, *X*, which is a true biosynthetic intermediate, then two effects should be observed. First, the incorporation of tyrosine into the alkaloids should be considerably diminished since the label will become diluted as it passes through the intermediate, *X*. This will hold true providing that the plant cannot convert all of *X* into alkaloid during the course of the experiment. Secondly, if *X* can be re-isolated, it should be radioactive.

With these principles in mind, we fed mixtures of (±)-[2-^{14}C]tyrosine and the inactive phenols (Ia), (Ic), and (Id) to King Alfred daffodils and compared the activities of alkaloids, isolated after about one week, with those from plants fed with (±)-[2-^{14}C]tyrosine alone. The results are shown in Table 3.

TABLE 3.

Incorporations (%) of (±)-[2-^{14}C]tyrosine.

		Tyrosine + Precursors		
	Tyrosine	(Ia)	(Ic)	(Id)
Galanthamine	0·013	0·000	0·000	0·000
Hæmanthamine	0·13	0·049	0·021	0·020

Inhibition of tyrosine incorporation into the alkaloids was observed with all three phenols, although the plants appeared to flourish during the experiment and gave normal yields of alkaloids. However, inhibition cannot be caused simply by intermediate dilution since the phenol (Ic) was earlier found *not* to be a precursor for galanthamine and the *N*-methyl phenol (Ia) clearly cannot be on a direct path to hæmanthamine, which lacks an *N*-methyl group. It seems likely therefore that all the phenols (I) can compete with one another for the enzymes controlling the oxidative coupling step.

Isolation of the precursors (I) from plant extracts is generally difficult and attention was confined to the most tractable member (Ia). This substance was recovered in 29%

yield from the trapping experiment described above. The phenol was radioactive and retained its activity after dilution with pure material and repeated crystallisation. However, the activity was very low (0·0016% incorporation based on tyrosine). Methylation with diazomethane gave belladine which was converted into its methiodide and this was cleaved with sodium amalgam. O-Methylhordenine, isolated from the reaction mixture, contained essentially all (99%) of the original activity. Further degradation was not possible with the small amounts of substance available. The results of these investigations are consistent with the ideas presented earlier in this paper but are not decisive. Further progress in elucidating the finer points of Amaryllidaceae alkaloid biosynthesis may come from experiments with plant homogenates and work towards this end is in hand. However, the results as a whole provide good support for the suggested mode of biosynthesis.

Experimental

M. p.s were taken on the Kofler block. Light petroleum refers to the fraction of b. p. 60—80°.

Counting Methods.—All activities were measured by using thin films (*ca.* 0·5 mg. per cm.^{2}) and were not corrected for self-absorption or back-scattering. A gas-flow (methane) proportional counter was used, giving a back-ground of *ca.* 15 counts per min.

Determination of Incorporation.—The specific activities of alkaloids and their degradation products are expressed as counts per min. per mmole. These values were used to determine the relative molar activities given earlier. Radioactive alkaloids were crystallised to constant activity and then converted into one or two derivatives which were further purified. Agreement between the molar activities of an alkaloid and its derivatives was taken as proof of radiochemical purity. Measurements were made in duplicate or, for very low activities, in triplicate. A typical set of values for galanthamine hydrochloride and the derived narwedine (six independent determinations) gave a standard deviation from the mean of 4%, and a similar set for hæmanthamine and its picrate (eight determinations) a standard deviation of 2%. The main source of error lay in non-uniform films, the measured activities of duplicate samples commonly differing by 10%. Incorporations were calculated from the total activity of injected precursors (see below), the specific activity of purified alkaloid, and the yield of crude alkaloid after chromatography. The last-named quantity was taken as the best estimate of the plant's true alkaloid content.

Radiochemical Purity of Precursors.—For convenience, the total activities of synthetic precursors are given in millicuries (mc), a counter efficiency of 50% (*i.e.*, 1 mc $\equiv 0{\cdot}5 \times 2{\cdot}2 \times 10^9$ counts per min.) being assumed. An accurate value for this efficiency was, however, not needed in the present investigations. Samples of synthetic precursors were diluted with pure inactive material and the mixtures recrystallised several times. In no case did the specific activity fall significantly during crystallisation, *i.e.*, the radiochemical purity was >95%. The precursors were also examined by chromatography on Whatman No. 1 paper, and autoradiographs were taken (Kodak " Kodirex " *X*-ray film); no radioactive impurities were detected. This procedure was particularly useful for showing the absence of [^{14}C]tyramine in [1-^{14}C]phenols (I). Chromatographs were developed with Pauly's spray (diazotised sulphanilic acid) and typical R_F values in the butan-1-ol–acetic acid–water (4 : 1 : 5) system are recorded.

Compound	(Ia)	(Ic)	(Id)	Hordenine	*N*-Methyltyramine	Tyramine	Tyrosine
R_F	0·85	0·83	0·77	0·65	0·60	0·56	0·40

Zeisel Determinations.—The usual microanalytical procedure [30] for *O*- and *N*-methyl groups was followed on samples (*ca.* 10 mg.) of alkaloid or diluted precursor. The evolved methyl iodide was collected in ethanolic triethylamine [31] which was then evaporated; the residual quaternary salt was plated in the usual way. Control experiments showed that up to 5% of the *N*-methyl group was liberated along with the *O*-methyl group but no correction has been made for this source of error.

[30] Grant, " Quantitative Organic Microanalysis," Churchill, London, 1951, p. 194.
[31] Brown and Bjerrum, *J. Amer. Chem. Soc.*, 1952, **74**, 1523.

Isolation of Alkaloids.—King Alfred daffodils (whole plants; typically, 350 g. wet wt.) were extracted with ethanolic tartaric acid by the procedure of Fales, Giuffrida, and Wildman.[12] The alkaloid mixture (0·5—1·0 g.) in chloroform (1 ml.) was placed on a column of grade III alumina (50 g.) in benzene. Elution was carried out with a linear gradient of ethyl acetate in benzene, the ethyl acetate concentration increasing by 2% per 20 ml. fraction collected. The course of elution was followed by ultraviolet absorption at 280 mμ. After removal of galanthamine (25—35% of EtOAc) and galanthine (35—55% of EtOAc), gradient elution was stopped and hæmanthamine was eluted with pure ethyl acetate and ethyl acetate–ethanol (95 : 5). Galanthamine was purified through its hydrochloride, and radiochemical purity checked by conversion into narwedine.[6] Hæmanthamine was crystallised several times from ethyl acetate and converted into its picrate.

3,4-*Dibenzyloxy*-N-*methylbenzylamine Hydrochloride.*—To 3,4-dibenzyloxybenzaldehyde [32] (320 mg.) in methanol (50 ml.) was added methylamine hydrochloride (80 mg.) in methanol (10 ml.) containing 4N-sodium hydroxide (0·8 ml.). After 1 hr. at room temperature, potassium borohydride (200 mg.) was added and the suspension set aside for 2 hr. The solvent was evaporated and the residue shaken with water and ether. Evaporation of the ether layer gave the oily amine which was dissolved in methanol (1 ml.). Concentrated hydrochloric acid (0·5 ml.) was added, followed by ether (5 ml.), and the mixture kept at 0° for 1 hr. The *hydrochloride* which separated crystallised from ethanol–ether as needles (310 mg., 83%), m. p. 169—170° (Found: C, 71·4; H, 6·5; N, 3·5. $C_{22}H_{24}ClNO_2$ requires C, 71·4; H, 6·5; N, 3·8%). Decomposition of the hydrochloride with sodium carbonate and extraction with ether gave the *amine* (272 mg.) which did not crystallise.

4-*Benzyloxy*-N-(3,4-*dibenzyloxybenzyl*)-N-*methylphenethylamine.*—To a suspension of *p*-benzyloxyphenylacetic acid (100 mg.) in benzene (3 ml.) was added oxalyl chloride (1 ml.) and the mixture warmed until all the acid had dissolved. After 1 hr. at room temperature the solvent and excess of oxalyl chloride were evaporated and the remaining acid chloride was redissolved in benzene (2 ml.). This solution was added dropwise with stirring to 3,4-dibenzyloxy-*N*-methylbenzylamine (272 mg.) in benzene (2 ml.). After 2 hr. the precipitated amine hydrochloride was removed (160 mg.) and the filtrate washed successively with N-hydrochloric acid, water, N-sodium hydrogen carbonate, and water, dried, and evaporated to give the expected amide (176 mg.) which did not crystallise. This amide in benzene (3 ml.) was added slowly to a refluxing suspension of lithium aluminium hydride (500 mg.) in ether (10 ml.). The excess of reagent was decomposed with ethyl acetate, water was added, and the ether layer separated from the pasty aqueous layer which was extracted with further quantities of ether. Evaporation of the dried ($MgSO_4$) ethereal solutions gave the *amine* which crystallised under ether as prisms (149 mg., 85%), m. p. 65—66° (Found: C, 81·6; H, 6·7; N, 2·5. $C_{37}H_{37}NO_3$ requires C, 81·75; H, 6·8; N, 2·6%).

4-*Hydroxy*-N-(3,4-*dihydroxybenzyl*)-N-*methylphenethylamine Hydrochloride.*—The corresponding tribenzyl ether (149 mg.) in ethanol (5 ml.) containing concentrated hydrochloric acid (0·1 ml.) was hydrogenated over 10% palladised charcoal (20 mg.). After 3 hr. the solution was filtered and evaporated to dryness. The *hydrochloride* crystallised from ethanol–ether as needles (76 mg., 95%), m. p. 207—208° (Found: C, 60·5; H, 6·4; N, 4·3. $C_{16}H_{20}ClNO_3$ requires C, 61·0; H, 6·5; N, 4·5%).

4-*Benzyloxybenzyl Chloride.*—4-Benzyloxybenzyl alcohol [33] (7 g.), prepared from the corresponding aldehyde by reduction with potassium borohydride, in benzene (100 ml.) was added slowly to thionyl chloride (7 ml.) in benzene (20 ml.) containing pyridine (0·1 ml.) under reflux. After 1 hr. the reaction mixture was cooled and treated with ice–water. The benzene layer was washed with N-sodium hydrogen carbonate and water, and dried (Na_2SO_4). Evaporation of the solvent gave 4-benzyloxybenzyl chloride, which crystallised from light petroleum as plates (5·4 g., 70%), m. p. 77·5—78·5° (lit.,[32] 79—80°) (Found: C, 72·2; H, 5·9. Calc. for $C_{14}H_{13}ClO$: C, 72·3; H, 5·7%). This substance is a powerful skin-irritant and can cause dermatitis when handled.

4-*Benzyloxybenzyl Bromide.*—4-Benzyloxybenzyl alcohol (640 mg.) in benzene (20 ml.) containing pyridine (0·1 ml.) was treated with phosphorus tribromide (300 mg.) in benzene (5 ml.) and the mixture heated under reflux for 2 hr. The product was cooled and treated

[32] Burton and Praill, *J.*, 1951, 522.
[33] Shelton, van Campen, Meisner, Parmerter, Andrews, Allen, and Wyckoff, *J. Amer. Chem. Soc.*, 1953, **75**, 5491.

with water and the benzene layer was washed with water, dried (Na_2SO_4), and evaporated. The crude *4-benzyloxybenzyl bromide* (75%) had m. p. 86—87° (from light petroleum–benzene) (Found: C, 60·7; H, 4·9. $C_{14}H_{13}BrO$ requires C, 60·3; H, 4·7%).

4-Benzyloxybenzyl Cyanide.—4-Benzyloxybenzyl chloride (2 g.) in freshly distilled dimethyl sulphoxide (10 ml.) was added to sodium cyanide (500 mg., excess) in the same solvent (20 ml.) and the mixture heated at 100° for 3 hr. with occasional stirring, cooled, and treated with water (100 ml.). The resulting suspension was extracted with ether. The ether extract was washed with water, dried (Na_2SO_4), and evaporated. The residue gave 4-benzyloxybenzyl cyanide as plates (1·4 g., 73%), m. p. 68—69° (from light petroleum) (lit.,[34] 69°) (Found: C, 80·6; H, 5·9; N, 6·3. Calc. for $C_{15}H_{13}NO$: C, 80·7; H, 5·9; N, 6·3%). Experiments with 4-benzyloxybenzyl bromide gave lower yields of the nitrile.

O-Benzyltyramine.—4-Benzyloxybenzyl cyanide (1 g.) in anhydrous ether (20 ml.) was added during 1 hr. to a boiling suspension of lithium aluminium hydride (5 g.) in ether (20 ml.). After a further 30 min. the excess of reagent was decomposed with ethyl acetate, and water added slowly with stirring. The ethereal layer was decanted from the residual paste of aluminium salts which was shaken with further quantities of ether. The combined ether extracts were washed, dried (Na_2SO_4), and evaporated. The residual amine, m. p. 50°, was converted into its hydrochloride (800 mg., 68%) with ethanolic hydrogen chloride. The *hydrochloride* of *O*-benzyltyramine formed plates, m. p. 202—204° (from ethanol–ether) (Found: C, 68·2; H, 6·9; N, 5·5. $C_{15}H_{18}ClNO$ requires C, 68·3; H, 6·8; N, 5·3%).

4-Benzyloxy-N-(3,4-dibenzyloxybenzyl)phenethylamine (OOO-*Tribenzylnorbelladine*).—To 3,4-dibenzyloxybenzaldehyde [33] (650 mg.) in methanol (15 ml.) was added *O*-benzyltyramine (450 mg.) in methanol (10 ml.), and the mixture was left at room temperature for 1 hr. In one experiment the expected imine crystallised (m. p. 101—102°), but in general the mixture was treated directly with an excess of potassium borohydride (500 mg.) at room temperature for 3 hr. After evaporation of solvent the residue was treated with water and the desired amine extracted into ether. Treatment of the ether solution with concentrated hydrochloride acid (2 ml.) caused OOO-*tribenzylnorbelladine hydrochloride* to separate at the interface. The hydrochloride formed plates (880 mg., 78%), m. p. 148—149° (from ethanol) (Found: C, 76·3; H, 6·7; N, 2·4. $C_{36}H_{36}ClNO_3$ requires C, 76·4; H, 6·4; N, 2·5%).

4-Hydroxy-N-(3,4-dihydroxybenzyl)phenethylamine (*Norbelladine*) (Id).—The corresponding tribenzyl ether (500 mg.) was hydrogenolysed in methanol (25 ml.) containing concentrated hydrochloric acid (0·1 ml.) and 10% palladised charcoal (50 mg.), hydrogen uptake being complete in 30 min. Filtration of the solution and evaporation of solvent gave *norbelladine hydrochloride*. This crystallised from ethanol–ether as plates (240 mg., 91%), m. p. 175—176° (Found: C, 61·3; H, 6·3; N, 4·6. $C_{15}H_{18}ClNO_3$ requires C, 60·9; H, 6·1; N, 4·7%).

[1-^{14}C]*Norbelladine* (Id).—Sodium [^{14}C]cyanide (5 mg.; 0·1 mc) in dimethyl sulphoxide (0·7 ml.) containing 4-benzyloxybenzyl chloride (30 mg.) was heated for 3 hr. at 100°, cooled, and diluted with saturated sodium chloride solution. The product was extracted with ether (4 × 2 ml.), carrier 4-benzyloxybenzyl cyanide (20 mg.) being added in the first portion of ether. The extract was washed with water (2 × 2 ml.), dried, and evaporated. The total crude product was reduced with lithium aluminium hydride as described above and *O*-benzyltyramine isolated as its hydrochloride which was converted into the free base (36 mg.). The latter was condensed with an excess of 3,4-dibenzyloxybenzaldehyde (150 mg.), and the remainder of the preparation was carried out as described earlier to give [1-^{14}C]norbelladine hydrochloride (29 mg., 0·039 mc), m. p. 175—176°.

4-Benzyloxy-N-(3-benzyloxy-4-methoxybenzyl)phenethylamine.—To *O*-benzylisovanillin [35] (500 mg.) in methanol (15 ml.) was added *O*-benzyltyramine (450 mg.) in methanol (10 ml.) and the mixture was left at room temperature for 1 hr. Potassium borohydride (500 mg.) was added and, after 3 hr., the solvent was evaporated and the residue treated with water and extracted with ether. Addition of conc. hydrochloric acid (2 ml.) to the ether solution caused *4-benzyloxy-N-(3-benzyloxy-4-methoxybenzyl)phenethylamine hydrochloride* to separate. It formed needles (0·80 g., 85%), m. p. 123—124° (from ethanol–ether) (Found: C, 73·3; H, 6·7; N, 2·5. $C_{30}H_{32}ClNO_3$ requires C, 73·6; H, 6·5; N, 2·9%).

4-Hydroxy-N-(3-hydroxy-4-methoxybenzyl)phenethylamine (O-*Methylnorbelladine*) (Ic).—The corresponding dibenzyl ether hydrochloride (300 mg.) in methanol (25 ml.) was hydrogenolysed

[34] Tomita, Nakaguchi, and Takagi, *J. Pharm. Soc. Japan*, 1951, **71**, 1046.
[35] Späth, Orechoff, and Kuffner, *Ber.*, 1934, **67**, 1214.

on 10% palladised charcoal (30 mg.). The product, isolated in the usual way, gave O-*methylnorbelladine hydrochloride* (160 mg., 81%), m. p. 205—207° (from ethanol–ether) (Found: C, 61·8; H, 6·5; N, 4·9. $C_{16}H_{20}ClNO_3$ requires C, 62·0; H, 6·5; N, 4·5%).

O-methyl[1-^{14}C]norbelladine was prepared from labelled *O*-benzyltyramine in the way described for norbelladine and with similar radiochemical yield. Doubly labelled material was made by using *O*-benzyl[*methyl*-^{14}C]isovanillin (see below).

3-Benzyloxy-4-hydroxybenzaldehyde [with Mr. H. P. Tiwari].—*O*-Benzylcatechol [36] (2·5 g.) in ethanol (20 ml.) and water (10 ml.) containing NaOH (9·5 g.) was treated with chloroform (7·5 g.) added dropwise with stirring at room temperature. After 2 hr. the mixture was heated under reflux for 30 min. Chloroform and ethanol were removed under reduced pressure and the aqueous solution was acidified with hydrochloric acid and extracted with ether. The extract was washed with water, dried ($MgSO_4$), treated with charcoal, and evaporated. The residual dark oil was chromatographed on neutral alumina (grade V) (200 g.) in benzene. Elution with benzene gave *O*-benzylcatechol (1·4 g.); elution with benzene–chloroform (1 : 1) gave 3-benzyloxy-4-hydroxybenzaldehyde which crystallised from benzene as plates (530 mg., 19%), m. p. 113—114° (lit.,[9] 113—114°) ($\lambda_{max.}$ 252 and 349 mμ; ε 11,400 and 23,600 in 0·1N-NaOH). Methylation with methyl iodide and potassium carbonate (see below) gave *O*-benzylisovanillin, m. p. and mixed m. p. 60°.

O-Benzyl[methyl-^{14}C]*isovanillin*.—[^{14}C]Methyl iodide (*ca.* 3 mg.; 0·1 mc) was distilled *in vacuo* with an acetone carrier into 3-benzyloxy-4-hydroxybenzaldehyde (9 mg.) in dry acetone (5 ml.) containing anhydrous potassium carbonate (50 mg.). The reaction vessel was sealed *in vacuo* and heated at 60° for 72 hr. with stirring. Inactive methyl iodide (1 ml.) was then added and the mixture refluxed for a further 6 hr. to complete methylation. Inactive *O*-benzylisovanillin [35] (16 mg.) was added, the reaction mixture centrifuged, and the supernatant solution evaporated. The product was chromatographed on alumina (grade III) (3 g.). Elution with benzene afforded *O*-benzylisovanillin (25 mg., 0·08 mc).

3-Hydroxy-4-methoxy-N-methylbenzylamine Hydrochloride.—The hydrochloride [6] of the *O*-benzyl ether (V; R = Ph·CH_2) (500 mg.) in methanol (50 ml.) was hydrogenolysed over 10% palladised charcoal (50 mg.). After 2 hr. the solution was filtered and evaporated to dryness to give *3-hydroxy-4-methoxy-N-methylbenzylamine hydrochloride*. This crystallised from ethanol–ether as plates (250 mg.), m. p. 171—172° (Found: C, 53·4; H, 6·7; N, 6·8. $C_9H_{14}ClNO_2$ requires C, 53·0; H, 6·9; N, 6·9%).

p-*Benzyloxy-N-methylphenylacetamide* (doubly labelled as in VI).—*p*-Benzyloxyphenylacetic acid (24 mg.), labelled in the carboxyl group, was obtained by hydrolysis of the corresponding [^{14}C]nitrile with alkaline hydrogen peroxide. This was converted, by treatment with oxalyl chloride, into the corresponding acid chloride which was stirred in benzene (3 ml.); and [^{14}C]methylamine, liberated from its hydrochloride (2 mg.) by addition of sodium hydroxide, was distilled in. After 15 min. the reaction was completed by addition of an excess of inactive methylamine (from 50 mg. of the hydrochloride) and, after a further 5 min., water was added and the benzene layer separated. Benzene-extraction of the aqueous phase and evaporation of the combined, dried benzene solutions gave p-*benzyloxy-N-methylphenylacetamide* (22 mg., 95%), m. p. 138—142°. In a separate experiment on inactive material the amide had m. p. 144·5—145·5° (from ethanol) (Found: C, 75·1; H, 6·2; N, 5·7. $C_{16}H_{17}NO_2$ requires C, 75·3; H, 6·7; N, 5·5%).

N-Methyltyramine.—The doubly labelled amide prepared above (22 mg.) was reduced with lithium aluminium hydride (500 mg.) in ether (10 ml.) for 36 hr. and the resulting amine isolated and debenzylated by hydrogenolysis in the usual way. *N*-Methyltyramine was obtained as its hydrochloride which crystallised from ethanol–ether as needles, m. p. 146—147° (lit.,[37] 148·5°). Radiochemical yields: *N*-methyl label 43; chain label 20%.

Degradation of Galanthamine.—The procedure follows the work of Kobayashi and Uyeo [18] with some modifications. Galanthamine (174 mg.) was refluxed with 45% aqueous hydrogen bromide (1·7 ml.) under nitrogen for 4 hr. Apogalanthamine hydrobromide, m. p. 228—230° [lit.,[18,38] 228—230°, 234° (decomp.)] crystallised from the cooled reaction mixture and was collected, dissolved in water, and treated with aqueous ammonia. The precipitated free base (110 mg.) in ethanol (5 ml.) was treated with diazomethane (large excess) in ether (40 ml.) for

[36] Druey, *Bull. Soc. chim. France*, 1955, **2**, 1737.
[37] Walpole, *J.*, 1910, **97**, 944.
[38] Proskurnina and Yakovleva, *Zhur. obshchei Khim.*, 1955, **25**, 1035.

2 days at room temperature. The product was chromatographed on alumina (grade III). Elution with benzene–ethyl acetate (9 : 1) gave *OO*-dimethylapogalanthamine as an oil (73 mg.), then benzene–ethyl acetate (1 : 1) removed a crystalline compound (12 mg.), m. p. 212—214° (decomp.), presumably a partially methylated product. This was not investigated further. The dimethyl ether was treated with methyl iodide (1 ml.) in methanol (3 ml.) under reflux for 30 min. to give the corresponding methiodide (104 mg.), m. p. 143—150° (lit.,[38] 144—150°). The methiodide was shaken with freshly precipitated silver chloride in water (5 ml.), the silver salts were filtered off, and the filtrate was heated with 4% sodium amalgam (10 g.) at 100° for 4 hr. The cooled reaction mixture was extracted with ether and, after evaporation of solvent, the extracted Emde base was converted into its methiodide (93 mg.), m. p. 220—223° (lit.,[18] 221—223°), which was shaken in water with freshly precipitated silver oxide; after filtration, 0·1N-sodium hydroxide (1·75 ml.) was added to the aqueous solution which was evaporated *in vacuo*. The resulting residue was sublimed (100—140°/$1·5 \times 10^{-6}$ mm.) and an ethereal solution of the sublimate was washed with hydrochloric acid, water, dried (Na_2SO_4), and evaporated to give the oily vinylbiphenyl (22 mg.). Ozonolysis of this material was carried out at −25° in ethyl chloride (10 ml.) and was complete in 40 min. (ultraviolet control). The solvent was evaporated at room temperature and the ozonide treated with zinc dust (1 g.) and then steam-distilled. The distillate was collected in aqueous dimedone, to give formaldehyde dimedone (14 mg.), m. p. 184—186° (from aqueous ethanol).

Lead Tetra-acetate Oxidation of N-*Benzyl*-N-*methylglycine.*—*N*-Benzyl-*N*-methylglycine [39] (215 mg.) in water (2 ml.) was added to lead tetra-acetate (443 mg.) in acetic acid (10 ml.), and the mixture heated at 100° for 3 hr. The system was flushed with nitrogen (CO_2-free), the evolved carbon dioxide (0·90 mol., by titration) being collected in saturated aqueous barium hydroxide, and the formaldehyde (83 mg. of dimedone derivative, 28%) in aqueous dimedone reagent. At the end of the reaction the solution was made alkaline with sodium hydroxide and extracted with ether (5 × 5 ml.). The ether solution was extracted with N-hydrochloric acid (5 × 5 ml.), the acidic solution made alkaline, and the liberated amine taken into ether. After evaporation of ether the crude *N*-methylbenzylamine [40] was converted into its hydrochloride (90 mg., 57%), m. p. and mixed m. p. 175—176°.

Degradation of Hæmanthamine.—Hæmanthamine (70 mg.) was converted into the hydrated sodium salt of *N*-methyl-*N*-(6-phenylpiperonyl)glycine (45 mg.) by the method of Fales and Wildman.[20] This salt (20 mg.) in water (1 ml.) was oxidised with lead tetra-acetate (27 mg.) in acetic acid (5 ml.), as above, to give carbon dioxide (0·88 mole), formaldehyde dimedone derivative (8 mg., 44%), and *N*-methyl-6-phenylpiperonylamine hydrochloride (8·2 mg., 59%), m. p. 159—160° (lit.,[21] 160°). In one experiment the formaldehyde dimedone derivative was contaminated with an oil; the semi-solid mixture was collected, and chromatographed in benzene over alumina (grade III); the pure derivative was eluted with benzene–ethyl acetate (1 : 1).

Hydrolysis of Hæmanthamine with Sulphuric acid.—Hæmanthamine was hydrolysed by a procedure, based on the work of Sarkar,[41] kindly communicated to us by Dr. S. Kirkwood. The alkaloid (21 mg.) in 20% sulphuric acid (40 ml.) was slowly distilled at constant volume. The distillate was collected in dimedone (150 mg.) in water (25 ml.). After 150 ml. had distilled the precipitated formaldehyde dimedone derivative (11 mg., 56%) was collected.

Feeding Experiments with King Alfred Daffodils.—(±)-[2-^{14}C]Tyrosine, the phenols (Ia) and (Ib) (labelled in the *N*-methyl groups with ^{14}C), and (Ic) and (Id) (labelled in the carbon chain) were fed to flowering King Alfred daffodils. An aqueous solution (10 ml.; pH 6) of each precursor (*ca.* 25 mg.) was injected with a hypodermic syringe into the hollow flower stalks of three plants, injections being spread over 3 days. After 8 days from the first injection the whole plants (wet wt. *ca.* 350 g.) were worked up. The weights and specific activities (mg.; counts per min. per mmole) of isolated alkaloids are tabulated. The calculated incorporation values are given in the Discussion section (Table 1).

Precursor	Tyrosine (0·007 mc)	(Ia) (0·034 mc)	(Ib) (0·020 mc)	(Ic) (0·022 mc)	(Id) (0·039 mc)
Galanthamine ...	78; $3·36 \times 10^3$	72; $2·10 \times 10^4$	58; $1·98 \times 10^4$	63; 0·00	88; $2·01 \times 10^4$
Hæmanthamine	102; $4·63 \times 10^3$	78; 0·00	67; 0·00	120; $2·21 \times 10^4$	96; $4·00 \times 10^5$

[39] Mannich and Kuphal, *Ber.*, 1912, **45**, 314.
[40] Cromwell, Babson, and Harris, *J. Amer. Chem. Soc.*, 1943, **65**, 312.
[41] Sarkar, *J. Indian Chem. Soc.*, 1934, **11**, 691.

Zeisel demethylations performed on galanthamine derived from the phenols (Ia) and (Ib) gave ^{14}C values of *N*-methyl 95%, *O*-methyl 2% and *N*-methyl 95%, *O*-methyl 3%, respectively. The galanthamine derived from norbelladine (Id) was diluted with inactive material and degraded as described earlier (see Scheme 1).

Tyrosine Feeding of Immature Daffodils.—Dormant King Alfred daffodil bulbs (200 g.) were injected (*ca.* 30% mechanical loss of solution occurred) with (±)-[2-^{14}C]tyrosine (0·007 mc). After 7 days, extraction and purification gave inactive galanthamine and hæmanthamine. (±)-[2-^{14}C[Tyrosine (0·005 mc) was injected into the bulbs of young sprouting plants (95 g.). After 7 days, radioactive galanthamine (0·013% incorporation) was obtained.

Feeding Experiments with Snowdrops.—(±)-[2-^{14}C]Tyrosine hydrochloride (20 mg.; 0·01 mc) in water (6 ml.) was injected into the bulbs of 30 flowering snowdrops (85 g. wet wt.), *Galanthus elwesii*, during 7 days. Working up after a further 3 days gave radioactive galanthamine [29 mg., $1{\cdot}55 \times 10^4$ counts per min. per mmole, 0·13 (5%) incorporation] and lycorine, purified through its hydrochloride and perchlorate (40 mg., $3{\cdot}58 \times 10^4$ counts per min. per mmol., 0·04% incorporation). The galanthamine was diluted and degraded as outlined in Scheme 1. In a parallel experiment, [1-^{14}C]norbelladine (Id; 21 mg.; 0·026 mc) gave radioactive galanthamine (30 mg.; $1{\cdot}45 \times 10^5$ counts per min. per mmole, 0·05% incorporation). The corresponding incorporation into lycorine was *ca.* 0·03% but it was not possible to obtain the alkaloid rigorously pure in this experiment.

Multiple Labelling Experiments with King Alfred Daffodils.—The doubly and triply labelled phenols (Ia) were converted into galanthamine in the King Alfred daffodil with incorporations of 0·014% and 0·018%, respectively, by the methods described above for the singly labelled precursors. Demethylation gave the results in Table 2; hæmanthamine, isolated in both experiments, was inactive. On two separate occasions the phenol (Ic), labelled both at position 1 and in the methoxyl group, was fed to daffodils at similar stages of growth. Incorporations into hæmanthamine of 1·0% and 0·036% were observed. Degradation of the hæmanthamine gave the results presented (Scheme 2) in the Discussion section.

Experiments with the doubly labelled 3-hydroxy-4-methoxy-*N*-methyl benzylamine (V; R = H) gave radioactive galanthamine (0·019% incorporation) and hæmanthamine (0·018% incorporation), incorporations being calculated on the precursor's methoxyl-group activity. The results of Zeisel determinations on the benzylamine (V; R = H), galanthamine, and the derived narwedine are tabulated.

	Precursor (V; R = H)	Galanthamine	Narwedine
Fraction of ^{14}C in MeO	0·48	0·92	0·88
Fraction of ^{14}C in MeN	0·51	0·02	0·02

Hydrolysis of the hæmanthamine with 20% sulphuric acid gave formaldehyde having 101% of the alkaloid's radioactivity.

Neither [2-^{14}C]tyramine (0·04 mc) nor *N*-methyltyramine (0·064 mc), labelled in the *N*-methyl group (62%) and at position 2 (38%), was incorporated significantly into galanthamine or hæmanthamine in the King Alfred daffodil.

Intermediate Trapping Experiments.—Solutions of (±)-[2-^{14}C]tyrosine (*ca.* 1 mg.), and mixtures of (±)-[2-^{14}C]tyrosine with the inactive phenols (Ia), (Ic), and (Id), were fed to King Alfred daffodils, and the whole plants worked up after 1 week in the usual way. The quantities used are tabulated and the resulting incorporations of tyrosine into galanthamine and hæmanthamine are given in the Discussion section (Table 3).

Tyrosine (mc)	0·02	0·01	0·01	0·005
Precursor	(Ia)	(Ic)	(Id)	
wt. (mg.)	100	80	80	None
No. of plants	2	1	1	1

The total basic extract (537 mg.) from the experiment with tyrosine and the phenolic precursor (Ia) was chromatographed on neutral alumina (grade V) (50 g.). Elution with benzene containing increasing amounts of ethyl acetate gave galanthamine (99 mg.), galanthine (105 mg.), and hæmanthamine (112 mg.) in that order. Further elution with ethanol–chloroform (1 : 20) then gave *NO*-dimethylnorbelladine (Ia), isolated as its hydrochloride (29 mg.), m. p. 212—220°. This radioactive material was diluted with the pure inactive hydrochloride (30 mg.), and the mixture ($2{\cdot}91 \times 10^3$ counts per min. per mmole) recrystallised three times from ethanol–ether, the activities after each crystallisation being successively 1·99, 2·06, and

$1{\cdot}92 \times 10^3$ counts per min. per mmole, corresponding to an incorporation of 0·0016%. The *NO*-dimethylnorbelladine (Ia; 30 mg.) was methylated with diazomethane in the usual way and the product chromatographed on alumina (grade III) to give belladine which was converted into its hydrochloride (18 mg.; $2{\cdot}12 \times 10^3$ counts per min. per mmol.), m. p. 182—186° (lit.,[6,42] 181—182°). The free base was regenerated and converted into its methiodide and thence into its methochloride which was heated in aqueous solution with 4% sodium amalgam at 100° for 6 hr. The oil which separated in ether (10 ml.) was extracted with N-hydrochloric acid (4×5 ml.). The acidic solution was made alkaline and extracted with ether, and this extract dried (Na_2SO_4) and evaporated to give *O*-methylhordenine. This was converted into its hydrochloride (5 mg.; $1{\cdot}99 \times 10^3$ counts per min. per mmole), m. p. 172—174° (lit.,[43] 176°).

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