316. *Alkaloid Biosynthesis. Part III.* Amaryllidaceae Alkaloids: The Biosynthesis of Lycorine and its Relatives.*

By A. R. Battersby, R. Binks, S. W. Breuer, H. M. Fales, W. C. Wildman, and (in part) R. J. Highet.

Tracer experiments on Amaryllidaceae plants show that lycorine is built from an aromatic C_6–C_2 unit (Ar–C–C) and a C_6–C_1 unit (Ar–C) derivable in the plant from tyrosine and phenylalanine, respectively. The radioactive alkaloids have been degraded unambiguously to establish the sites of labelling. Evidence is presented that tyramine can serve as the C_6–C_2 unit and that phenylalanine is degraded to form the C_6–C_1 unit by way of *trans*-cinnamic acid.

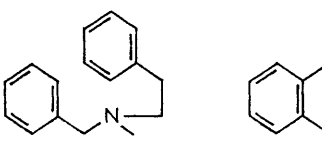
The biosynthetic intermediates have been studied by experiments with singly and doubly labelled derivatives of norbelladine (VI; R = R′ = H), and the intact incorporation of this substance into lycorine has been established. Supporting work aimed at the isolation of an intermediate is reported.

It is proved that the hydroxyl group at position 2 of lycorine is introduced late in the biosynthesis, and the conversion of norpluviine (VII; R = H) into lycorine (XII) is demonstrated; this finding uncovers an allylic oxidation system in Amaryllidaceae plants. Further, this conversion and experiments with [*methyl*-^{14}C]methionine are in keeping with the generation of the methylenedioxy-group of lycorine from an *o*-methoxyphenol.

The combined results allow a fairly complete biosynthetic pathway for lycorine to be considered.

Preliminary accounts of the main results have already been published.[1–3]

Plants of the Amaryllidaceae family produce many different alkaloids,[4] all of which can be considered in biogenetic theory to be based upon one or other of the four skeletons (I)—(IV). The present Paper is concerned with the biosynthesis of alkaloids with the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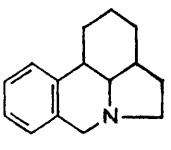
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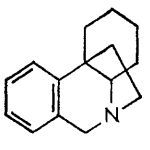
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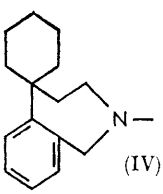
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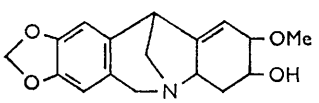
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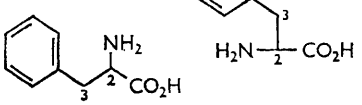
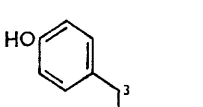
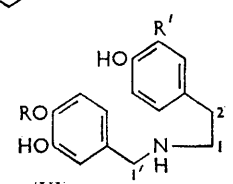





(VI)

pyrrolophenanthridine skeleton (II), of which lycorine (XII) is the commonest example. It is clear from inspection of the structures of the Amaryllidaceae alkaloids that the skeletons initially formed must, in many cases, be further modified by oxidative changes;

* Part II, *J.*, 1962, 3534.

[1] Battersby, Binks, and Wildman, *Proc. Chem. Soc.*, 1960, 410.

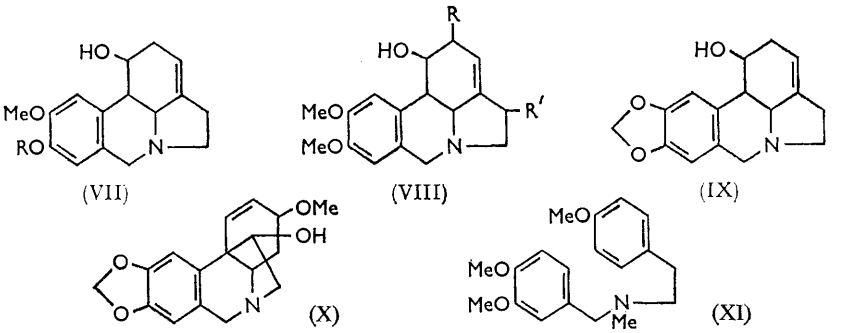
[2] Battersby, Binks, Breuer, Fales, and Wildman, *Proc. Chem. Soc.*, 1961, 243; Wildman, Fales, Highet, Breuer, and Battersby, *ibid.*, 1962, 180.

[3] Wildman, Battersby, and Breuer, *J. Amer. Chem. Soc.*, 1962, **84**, 4599; Archer, Breuer, Binks, Battersby, and Wildman, *Proc. Chem. Soc.*, 1963, 168.

[4] Wildman, "The Alkaloids," ed. Manske, Academic Press, New York, 1960, Vol. VI, p. 289.

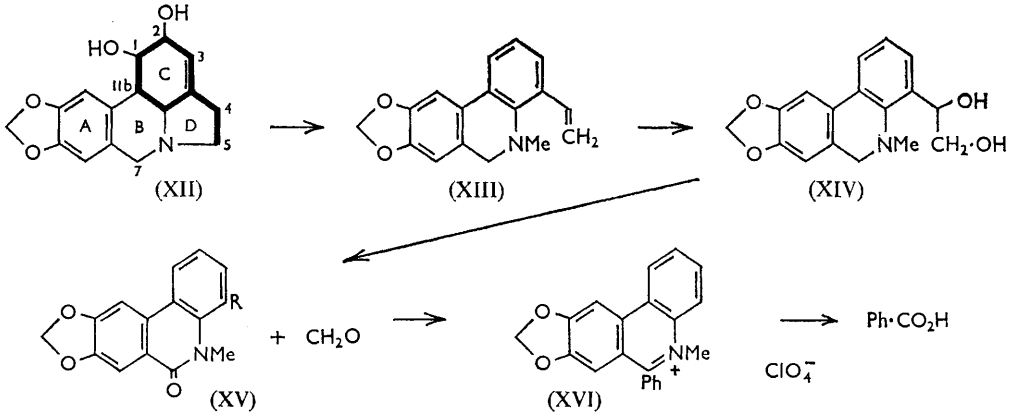
for montanine (V) and its relatives, rearrangement of the skeleton must also be postulated.[5] Future Papers will report our experiments on these modifications of the skeletons; full biosynthetic studies on the systems (I), (III), and (V) will also be described.

Several theories have been proposed [6,7] to account for the formation of these alkaloids in Nature, the most satisfying being that of Barton and Cohen.[7] Their proposal which, was elaborated in detail in the original survey [7] and also more recently,[8] makes use of phenol oxidation [7,9] and the coupling of phenoxide radicals to generate a new carbon–carbon bond between the two phenolic rings of the postulated precursor (VI; R′ = H or OH) or of its *N*-methyl derivative; *ortho–para*, *para–para*, and *para–ortho* coupling are required to generate the systems (II), (III), and (IV), respectively.



The present experimental study falls into three parts: (a) origin of the carbon skeletons; (b) aromatic intermediates; (c) modification of the skeleton initially produced. Other investigators have carried out much parallel or complementary work in this field, and those contributions which are directly related to the matter of this Paper are considered at the appropriate points.

Our experiments have been carried out mainly on " Twink " or " Deanna Durbin " narcissus plants, which were chosen because they allow one to study not only the formation of the major alkaloids but also the biosynthetic relations of several similar bases. The



original isolation work [10] showed the presence of lycorine (XII) as the major alkaloid, together with a small quantity of hæmanthamine (X); traces of pluviine (VII; R = Me)

[5] Inubushi, Fales, Warnhoff, and Wildman, *J. Org. Chem.*, 1960, **25**, 2153.
[6] Wenkert, *Experientia*, 1959, **15**, 165; Steglich, *Tetrahedron*, 1957, **1**, 195.
[7] Barton and Cohen in " Festschrift Arthur Stoll," Birkhäuser, Basle, 1957, p. 117.
[8] Barton, Hugo Muller Lecture, *Proc. Chem. Soc.*, 1963, 293.
[9] Erdtman and Wachtmeister in " Festschrift Arthur Stoll," Birkhäuser, Basle, 1957, p. 144.
[10] Boit and Ehmke, *Chem. Ber.*, 1956, **89**, 163.

and galanthine (VIII; R = OMe, R′ = H) were also isolated. Both vapour-phase chromatography and preparative chromatography on alumina [11] show that norpluviine (VII; R = H) is the major base present and that, in addition to the alkaloids listed above, caranine (IX) can be isolated in useful amounts. *Nerine bowdenii* plants, a rich source of belladine [12] (XI), have also been used for tracer experiments. The various labelled materials, usually as the hydrochlorides, were administered in aqueous solution at pH 5·5—7·0 to mature plants, normally by injection into the flower stems or into the bulbs of blooming plants. Some precursors, however, were fed by uptake through a leaf. After 2—3 weeks, the alkaloids were extracted from the plants, and separated by established methods.[10,11]

Origin of the Carbon Skeletons.—Earlier experience with the isoquinoline alkaloids of *Papaver somniferum* [13,14] led us to expect that the C_6–C_2 unit of lycorine (XII), drawn with

TABLE 1.

Tracer experiments on Amaryllidaceae plants.

Precursor	Plants; year	Wt. of alkaloid	% incorpn.
0·1 mc (±)-[2-^{14}C]Tyrosine	8 Twink; 1960	Lycorine, 100 mg.	0·23
		Norpluviine, 181 mg.	0·15
		Caranine, 53 mg.	0·08
		Hæmanthamine, 99 mg.	0·08
0·1 mc (±)-[3-^{14}C]Tyrosine	8 *N. bowdenii*; 1961	Lycorine, 159 mg.	0·11
		Belladine,[a] 303 mg.	0·82
0·1 mc (±)-[1-^{14}C]Tyrosine	5 Deanna Durbin; 1961	Lycorine, 40 mg.	$4·7 \times 10^{-4}$
		Norpluviine, 75 mg.	$2·4 \times 10^{-3}$
0·2 mc [1-^{14}C]Tyramine	8 Deanna Durbin; 1962	Lycorine, 81 mg.	0·023
		Norpluviine, 77 mg.	0·14
0·3 mc (±)-[3-^{14}C]Phenylalanine	6 *N. bowdenii*; 1961	Lycorine, 104 mg.	0·09
		Belladine,[a] 134 mg.	0·42
0·135 mc Sodium *trans*-[3-^{14}C]cinnamate	4 *N. bowdenii*; 1962	Lycorine, 78 mg.	0·02
0·25 mc [*carboxyl*-^{14}C]Protocatechuic acid	6 Deanna Durbin; 1961	Lycorine, 56 mg.	$1·7 \times 10^{-3}$
		Norpluviine, 102 mg.	1×10^{-3}
2·5 mc Tritiated isovanillin	7 Deanna Durbin; 1962	Lycorine, 160 mg.	$6·5 \times 10^{-4}$
		Norpluviine, 267 mg.	$4·7 \times 10^{-3}$
2·5 mc Tritiated isovanillin	4 *N. bowdenii*; 1962	Lycorine, isolated by dilution	8×10^{-4}
0·09 mc [1-^{14}C]Norbelladine	8 Twink; 1961	Lycorine, 184 mg.	0·24
		Norpluviine, 300 mg.	0·74
0·354 mc [1- and 1′-^{14}C]Norbelladine	11 *N. bowdenii*; 1961	Lycorine, 313 mg.	0·07
		Belladine,[a] 680 mg.	2·64
0·15 mc [1-^{14}C]Hydroxynorbelladine	3 Deanna Durbin; 1961	Lycorine, 38 mg.	8×10^{-5}
		Norpluviine, 50 mg.	1×10^{-3}
0·2 mc [1-^{14}C]Hydroxynorbelladine	5 Twink; 1962	Lycorine, 208 mg.	$6·8 \times 10^{-4}$
		Norpluviine, 351 mg.	$1·7 \times 10^{-3}$
0·5 mc [*methyl*-^{14}C]methionine	5 Twink; 1962	Lycorine, 73 mg.	0·01
		Norpluviine, 182 mg.	0·01
3·5 mc Tritiated norpluviine	7 Deanna Durbin; 1962	Lycorine, 109 mg.	10·5
		Norpluviine, 333 mg.	57
69 μc Tritiated norpluviine	6 Twink; 1963	Lycorine, 181 mg.	1·7
		Norpluviine, 443 mg.	33
37 μc Tritiated lycorine	6 Twink; 1963	Lycorine, 134 mg.	85
		Norpluviine, 330 mg.	<0·01

[a] As hydrochloride.

thickened bonds, would be derivable in the plant from tyrosine. In fact, when (±)-[2-^{14}C]-tyrosine was fed to " Twink " daffodils, radioactive lycorine, norpluviine (VII; R = H), caranine (IX), and hæmanthamine (X) were isolated (Table 1). The active lycorine was degraded by Hofmann's method, without separation of the α- and β-methiodides,[15] to give

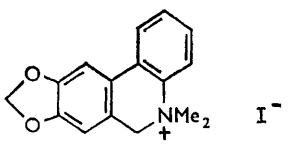
[11] Fales, Giuffrida, and Wildman, *J. Amer. Chem. Soc.*, 1956, **78**, 4145.
[12] Lyle, Kielar, Crowder, and Wildman, *J. Amer. Chem. Soc.*, 1960, **82**, 2620.
[13] Battersby and Harper, *J.*, 1962, 3526.
[14] Battersby, Binks, and Harper, *J.*, 1962, 3534.
[15] Kondo, Katsura, and Uyeo, *Ber.*, 1938, **71**, 1529.

the anhydromethine [16] (XIII). This underwent ready glycol formation with osmium tetroxide, and the product (XIV) was cleaved with periodate. Formaldehyde was isolated as its dimethone and the basic aldehyde was oxidised (permanganate), without purification, to the known [17] acidic lactam (XV; R = CO_2H). The results (Table 2) prove the original lycorine (XII) to be specifically labelled at position 5.

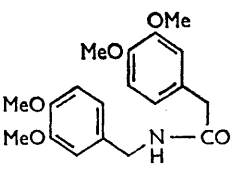
The incorporation of (±)-[2-^{14}C]tyrosine into lycorine has been reported by Barton and Kirby [18] but, by friendly arrangement, their study was carried no further than this.

(±)-[3-^{14}C]Tyrosine was also used by the plants to yield radioactive lycorine (Table 1) which was degraded as above, to afford inactive formaldehyde and the acidic lactam (XV; R = CO_2H). This was decarboxylated over copper chromite in quinoline to give the non-radioactive lactam (XV; R = H). Thus, the original lycorine (XII) is proved to be labelled only at position 4 (see Table 2). Since the conversion of prephenic acid into tyrosine is accepted to be irreversible,[19,20] it follows [14] from these two feeding experiments that ring c and carbon atoms 4 and 5 of lycorine arise in the plant from an aromatic C_6–C_2 unit (Ar–C–C) which is derivable from tyrosine in the living system. Any possibility of degradation of the precursor followed by re-incorporation of fragments is eliminated by the specific labelling of lycorine. Decarboxylation of the tyrosine-derived unit must occur at some stage in the biosynthesis, and this was confirmed by feeding (±)-[1-^{14}C]tyrosine to plants at the stage of development when good incorporations of activity occur from (±)-[2-^{14}C]tyrosine; the isolated lycorine did not contain a significant amount of activity. Moreover, [1-^{14}C]tyramine was incorporated by " Deanna Durbin " plants into lycorine and norpluviine (Table 1), and the former alkaloid was degraded as above to establish specific labelling at position 5 (Table 2). [2-^{14}C]Tyramine and (±)-[3-^{14}C]tyrosine have also been shown to act as precursors of lycorine by Suhadolnik, Fischer, and Zulalian,[21] but the site of labelling of the alkaloid has yet to be determined; ring A and its attached two carbon atoms (7 and 11b) were, however, shown to carry no activity.

The experiment with [3-^{14}C]tyrosine also shows that ring A and carbon atom 7 of lycorine cannot be derived from tyrosine and its close relatives. However, the source of this unit was discovered in the present work, and independently by Suhadolnik and his co-workers,[21] when radioactive lycorine was obtained using (±)-[3-^{14}C]phenylalanine as the precursor. In our work, the lycorine was degraded as above to the lactam (XV; R = H). No loss of radioactivity occurred up to this point in the degradation (Table 2). Treatment of this lactam with phenyl-lithium gave the phenanthridinium derivative (XVI) which was oxidised by permanganate to benzoic acid. The carboxyl carbon atom of benzoic acid corresponds to carbon 7 of the original alkaloid, which is thus proved (Table 2) to be specifically and solely labelled at this position. Suhadolnik and his co-workers [21] proved that hydrastic acid obtained by oxidation of their lycorine carried all the original activity, a result which is in agreement with ours.



(XVII)



(XVIII)

During exploratory degradative work on non-radioactive materials, the phenanthridone (XV; R = H) was reduced by lithium aluminium hydride to the corresponding dihydrophenanthridine, which was isolated as its methiodide (XVII). Emde degradation of this

[16] Humber, Kondo, Kotera, Takagi, Takeda, Taylor, Thomas, Tsuda, Tsukamoto, Uyeo, Yajima, and Yanaihara, *J.*, 1954, 4622.
[17] Kondo and Uyeo, *Ber.*, 1935, **68**, 1756.
[18] Barton and Kirby, *Proc. Chem. Soc.*, 1960, 392.
[19] Schwinck and Adams, *Biochem. Biophys. Acta*, 1959, **36**, 102.
[20] Gamborg and Neish, *Canad. J. Biochem. Physiol.*, 1959, **37**, 1277.
[21] Suhadolnik, Fischer, and Zulalian, *J. Amer. Chem. Soc.*, 1962, **84**, 4348.

material was to be studied, but the successful outcome of the above approach made this unnecessary.

A possible pathway for the conversion of phenylalanine into the C_6–C_1 unit which is built into lycorine and its relatives is by way of cinnamic acid,[3] as for the phenolic cinnamic

TABLE 2.

Degradations of radioactive lycorine.

Lycorine and its degradation products	Precursors						
	[2-^{14}C]-Tyrosine Rel. activity	[3-^{14}C]-Tyrosine Rel. activity	[1-^{14}C]-Tyramine Rel. activity	[3-^{14}C]-Phenylalanine Rel. activity	[3-^{14}C]-Cinnamic acid Rel. activity	[1-^{14}C]-Norbelladine Rel. activity	[1 and 1′-^{14}C]-Norbelladine Rel. activity
Lycorine (XII)	1·00	1·00	1·00	1·00	1·00	1·00	1·00
Anhydromethine (XIII)	1·03	—	0·93	—	—	1·05	—
Diol (XIV)	1·05	1·02	0·98	0·99	—	1·03	1·04
Formaldehyde dimethone	0·99	0·00	0·98	0·00	—	0·97	0·77
Acidic lactam (XV; R = CO_2H)	0·01	1·04	0·00	1·02	—	0·00	0·23
Barium carbonate	—	—	—	0·00	—	—	0·00
Lactam (XV, R = H)	—	0·00	—	1·04	—	—	0·22
Phenanthridinium perchlorate (XVI)	—	—	—	0·95	—	—	0·23
Benzoic acid	—	—	—	0·97	—	—	0·21
N-Methylhydrastimide	—	—	—	—	0·97	—	—

acids.[22] This was tested by feeding sodium *trans*-[3-^{14}C]cinnamate to *N. bowdenii* plants; the precursor was prepared by Knoevenagel condensation of [*formyl*-^{14}C]benzaldehyde with malonic acid.[23] The available pure radioactive alkaloid was insufficient for complete degradation and it was therefore oxidised to hydrastic acid; [24] this was characterised as the corresponding *N*-methylimide, and it carried all the original activity (Table 2). An alternative pathway for the fission of phenylalanine by way of *threo*-(±)-3-phenylserine was tested, and eliminated, by Suhadolnik, Fischer, and Zulalian.[25] They further demonstrated the presence in Amaryllidaceae plants of phenylalanine deaminase, the enzyme which brings about the conversion of phenylalanine into *trans*-cinnamic acid. Incorporation of activity from *trans*-[3-^{14}C]-cinnamic and -hydroxycinnamic acid into hæmanthamine was also shown.[25]

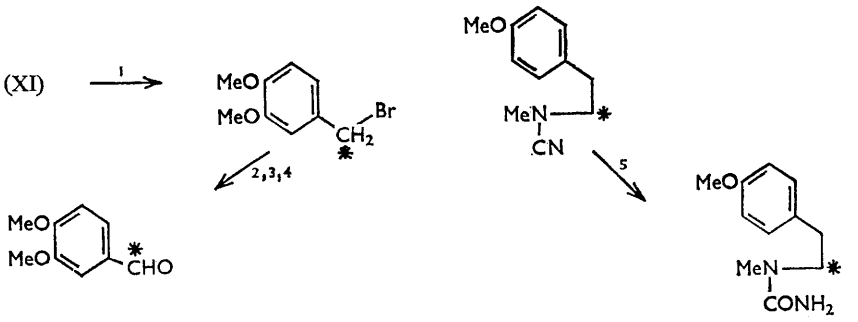
The nature of the C_6–C_1 unit, which is combined with the C_6–C_2 unit derivable from tyrosine, has been examined by several groups of workers. We find that [*carboxyl*-^{14}C]-3,4-dihydroxybenzoic acid is not significantly incorporated into lycorine and that the incorporation of generally tritium-labelled isovanillin into lycorine and norpluviine is of about this same very low order (Table 1). The latter result may, however, be a reflection of poor transfer of the aldehyde to the site of synthesis, because of its relatively non-polar nature.[26] In this connection, it is of interest that Barton and his co-workers [27] found that 3-hydroxy-4-methoxy-*N*-methylbenzylamine, labelled at both methyl groups, is incorporated into galanthamine and hæmanthamine (X) without randomisation but with loss of the *N*-methyl group. Experiments by the American workers also showed that tritiated 3,4-dihydroxybenzaldehyde is incorporated into lycorine, and that ring A and the two attached carbon atoms (7 and 11b) carry 91% of the original total activity.[25] Further information concerning the nature and formation of the C_6–C_1 unit has been derived from

[22] McCalla and Neish, *Canad. J. Biochem. Physiol.*, 1959, **37**, 537.
[23] Dalal and Dutt, *J. Indian Chem. Soc.*, 1932, **9**, 309.
[24] Warnhoff and Wildman, *J. Amer. Chem. Soc.*, 1957, **79**, 2192.
[25] Suhadolnik, Fischer, and Zulalian, *Biochem. Biophys. Res. Comm.*, 1963, **11**, 208; *Proc. Chem. Soc.*, 1963, 132.
[26] Davis, *Arch. Biochem. Biophys.*, 1958, **78**, 497.
[27] Barton, Kirby, Taylor, and Thomas, *Proc. Chem. Soc.*, 1961, 254; *ibid.*, 1962, 179; *J.*, 1963, 4545. We are grateful to Professor D. H. R. Barton for a copy of the last Paper prior to publication.

our own work and that of others on hæmanthamine and tazettine; the results will be considered in the paper on these alkaloids.

The foregoing experiments establish that phenylalanine and its close relatives provide ring A and the carbon atom at position 7 of lycorine (XII), whereas tyrosine and its equivalents provide the rest of the carbon skeleton. The two sets of precursors are clearly on separate pathways in the Amaryllidaceae. It seems probable that the nitrogen atom of lycorine is that originally present in tyrosine, and experiments to test this with ^{15}N-labelled materials are in hand. The evidence is also in keeping with phenylalanine's being deaminated to *trans*-cinnamic acid which, after hydroxylation, is cleaved to form 3,4-dihydroxybenzaldehyde. This may be combined with the C_6–C_2 unit or possibly be methylated to isovanillin before this step. Further evidence for this biosynthetic scheme is adduced below.

Aromatic Intermediates.—In parallel with the foregoing work on the origin of the carbon skeleton of lycorine, experiments were undertaken to determine the nature of the intermediates. These experiments were designed to test the important proposal of Barton and Cohen[7] that a key step in the biosynthesis of the Amaryllidaceae alkaloids is the oxidative coupling of intermediates such as the phenols (VI; R′ = H or OH). Accordingly, [1-^{14}C]-norbelladine (VI; R = R′ = H) was prepared by condensation of 3,4-dihydroxybenzaldehyde with [1-^{14}C]tyramine; the resultant imine was then reduced catalytically over palladium. Chemical and radiochemical purity was achieved by chromatography of the products on buffered alumina, and the absence of labelled tyramine was firmly established. When this material was fed to " Twink " daffodils, it was incorporated well into lycorine (XII) and norpluviine (VII; R = H) (Table 1), and the former was degraded, by the route described above, to prove specific labelling at position 5 (Table 2). However, the possibility remains that the precursor (VI; R = R′ = H) may have been degraded and that perhaps a C_6–C_2 unit (*e.g.*, tyramine) was the actual unit incorporated into lycorine. Rigorous exclusion of this possibility demanded the preparation of doubly labelled norbelladine; for this, norbelladine labelled at position 1′ was prepared as follows. [*carbonyl*-^{14}C]3,4-Dimethoxybenzoyl chloride reacted with *O*-methyltyramine, and the resultant amide was reduced with lithium aluminium hydride to the tri-*O*-methyl derivative of the phenol (VI; R = R′ = H). Demethylation of this product with hot hydrochloric acid then yielded [1′-^{14}C]norbelladine (VI; R = R′ = H). This was mixed with the available [1-^{14}C]norbelladine (above) to give a doubly labelled [1- and 1′-^{14}C]norbelladine sample, and the amount of labelling at the two positions was determined by degradation. Methylation of the active material with diazomethane yielded belladine (XI) which was degraded as shown in the annexed scheme. In this way, 22% of the total activity was shown to be at position 1′ and 78% at position 1.



Reagents: 1 CNBr; 2 aq. NaOH; 3 MnO_2; 4 $NaHSO_3$; 5 HCl.

This doubly labelled material was incorporated into the alkaloids of *N. bowdenii* (Table 1), and the lycorine was degraded by the unambiguous route described above, to isolate the carbon atoms from positions 5 and 7. These contained, respectively (Table 2),

77 and 21% of the original activity of the lycorine, that is, the ratio is unchanged from that in the phenolic precursor (VI; R = R′ = H). Intact incorporation of this precursor into lycorine is thus established; similar work on belladine will be reported in a later Paper.

The foregoing experiments leave no doubt that the plants can construct lycorine (XII) from the phenolic base (VI; R = R′ = H), and further evidence that this system is a true intermediate was sought by dilution analysis. Thus, if substances of type (VI) are present in the living system, labelled tyrosine fed to the plants will cause their " pools " to become radioactive. A particular substance may then be isolated by using synthetic, non-radioactive material to act as a carrier for the biosynthetic product. Norbelladine itself (VI; R = R′ = H) is an unstable substance, and therefore our work was concentrated on the *O*-methyl ether (VI; R = Me, R′ = H). This is logical, since parallel work (below) had demonstrated the conversion of norpluviine (VII; R = H) into lycorine (XII). With this knowledge, one can infer that the immediate precursor of the pyrrolophenanthridine alkaloids is probably *O*-methylnorbelladine (VI; R = Me, R′ = H), as for hæmanthamine.[28] *O*-Methylnorbelladine, by phenol oxidation, would lead directly to norpluviine.

Synthetic *O*-methylnorbelladine was prepared by condensation of *O*-benzylisovanillin with *O*-benzyltyramine, and reduction of the Schiff's base with borohydride. Hydrogenolysis of the protecting benzyl groups afforded the product (VI; R = Me, R′ = H). The *O*-benzyltyramine is readily synthesised by condensation of 4-benzyloxybenzaldehyde with nitromethane (ammonium acetate method [29]) to give the nitrostyrene which is reduced to the required amine with lithium aliminium hydride.

" Twink " daffodils were fed with (±)-[2-^{14}C]tyrosine and, after a period of growth, the plants were worked up in the presence of inactive *O*-methylnorbelladine as carrier. Isolation of this base (66% recovery) gave radioactive material which held its activity after repeated crystallisation as the hydrochloride. This product was methylated with diazomethane to give belladine (XI) which was further fractionated by gas–liquid chromatography, and the final product was still radioactive. This result suggests that *O*-methylnorbelladine (VI; R = Me, R′ = H) is present in the daffodil plants, but the very small amount of the original activity found in this material (8×10^{-4} %) renders the experiment an indicative rather than a conclusive one.

It is of considerable interest in this connection that Mann, Fales, and Mudd [30] purified an enzyme from *N. bowdenii* bulbs which catalyses the methylation of norbelladine (VI; R = R′ = H) to yield *O*-methylnorbelladine (VI; R = Me, R′ = H).

Barton, Kirby, and their co-workers [27] showed, in independent studies, that singly and multiply labelled derivatives of norbelladine are incorporated intact into galanthamine and hæmanthamine, and they obtained a result by dilution analysis similar to that described above, indicating the presence of the *N*-methyl derivative of phenol (VI; R = Me, R′ = H) in " King Alfred " daffodils. Their researches and ours are thus complementary and in full agreement.

So far, no evidence has been presented to indicate when the hydroxyl group at position 2 of lycorine (XII) is introduced. This may occur by hydroxylation at the norbelladine stage (*i.e.*, *o*-hydroxylation of a phenol [31]), or later in the biosynthesis. In order to clarify this point, the phenol (VI; R = H, R′ = OH), labelled at position 1, was prepared from 3,4-dimethoxybenzaldehyde and [1-^{14}C]3,4-dimethoxyphenethylamine in a way similar to that used above for *O*-methylnorbelladine. Complete demethylation of the resultant tetra-*O*-methylhydroxynorbelladine (dimethyl ether of VI; R = Me, R′ = OMe) was achieved with hot hydrochloric acid. In trial experiments with non-radioactive materials, 3,4-dimethoxyphenylacetyl chloride was treated with 3,4-dimethoxybenzylamine to form the corresponding amide (XVIII). Reduction with lithium aluminium hydride gave the

[28] Barton, Kirby, and Taylor, *Proc. Chem. Soc.*, 1962, 340.
[29] Raiford and Fox, *J. Org. Chem.*, 1944, **9**, 170.
[30] Mann, Fales, and Mudd, *J. Amer. Chem. Soc.*, 1963, **85**, 2025.
[31] " Oxygenases," ed. Hayaishi, Academic Press, New York, 1962.

tetramethyl ether (dimethyl ether of VI; R = Me, R′ = OMe), identical with that obtained by the more convenient route by way of the Schiff's base above.

When the [1-^{14}C]hydroxynorbelladine (VI; R = H, R′ = OH) was fed to " Deanna Durbin " and to " Twink " daffodils at the stage of growth which led to good incorporations of norbelladine (see above), there was an insignificant incorporation into both lycorine (XII) and norpluviine (VII; R = H). These results suggest strongly that hydroxylation occurs at a later stage in the biosynthesis of lycorine, and further support comes from the experiments described on the interconversions of the Amaryllidaceae alkaloids.

Modification of the Initial System.—The knowledge that the second hydroxyl group in ring C of lycorine is introduced late in the biosynthesis led to the working hypothesis that lycorine is formed by allylic oxidation of caranine (IX). Moreover, since there were indications from Sribney and Kirkwood's work [32] that a methylenedioxy-group arises in a living system from an *o*-methoxyphenol, a probable biosynthetic sequence was considered to be *O*-methylnorbelladine (VI; R = Me, R′ = H) ⟶ norpluviine (VII; R = H) ⟶ caranine (IX) ⟶ lycorine (XII). Further, we propose that norpluviine is the primary pyrrolophenanthridine alkaloid and that the many others are derived from it by oxidative changes, *e.g.*, galanthine (VIII; R = OMe, R′ = H), narcissidine (VIII; R = OH, R′ = OMe) and the hemiacetal and lactonic alkaloids.[4] * Preliminary studies (with Dr. D. A. Archer) have demonstrated the conversion of norpluviine into galanthine and narcissidine; details of these and similar experiments which are in progress will be reported later.

Norpluviine was tritiated by treatment with tritiated acetic acid and a palladium catalyst, and the tritiated norpluviine was purified to remove exchangeable tritium. This product was fed to " Deanna Durbin " plants and, after the usual period, the alkaloids were isolated. The norpluviine contained 57, and the lycorine 10·5%, of the original activity administered. When the feeding experiment was repeated on " Twink " daffodils at an earlier stage of development, the percentages were, respectively, 33 and 1·7. To show that the conversion is a one-way process, a parallel run was carried out in which tritiated lycorine, isolated from the foregoing experiments on " Deanna Durbin " plants, was fed to " Twink " daffodils. Less than 0·01% of the original activity fed was present in the isolated norpluviine whereas the lycorine from these plants contained 85%. It is thus established that hydroxylation of the completed skeleton can occur, at an allylic position in this case. Moreover, the demonstrated conversion is in keeping with the formation of the methylenedioxy-system of lycorine from the *o*-methoxyphenyl group of norpluviine, but does not yet conclusively prove this. Clear experimental proof that the methylenedioxy-group of hæmanthamine arises by cyclisation of an *o*-methoxyphenol has, however, been published by the Imperial College group (see ref. 8). Many other radioactive alkaloids are present in the mother-liquor from the isolation of lycorine and norpluviine, and these are being examined.

If the methylenedioxy-group of lycorine is derived from an *o*-methoxyphenol, then it must become labelled at the methylene group when [*methyl*-^{14}C]methionine is fed to the plants; [33] the reverse does not necessarily hold. This feeding experiment yielded active lycorine (XII) and norpluviine (VII; R = H) (Table 1). Hydrolysis of the former † gave formaldehyde (as dimethone) which carried 95% of the original activity of lycorine. The *O*-methyl group of norpluviine was shown by Zeisel's demethylation method to contain all the activity of the alkaloid.

These experiments interlock and are in keeping with the proposed order for formation, though further work is necessary to provide unambiguous proof; this is in progress.

* Professor D. H. R. Barton holds views similar to ours concerning the late steps for the formation of the hemiacetal and lactonic alkaloids (see ref. 8).

† We are grateful to Dr. P. W. Jeffs (University of Natal) for the experimental details.

[32] Sribney and Kirkwood, *Nature*, 1953, **171**, 931.

[33] For collected references see Battersby, *Quart. Rev.*, 1961, **15**, 259; Mothes and Schutte, *Angew. Chem.*, 1963, **75**, 265, 357.

Experimental

For general directions, see Battersby and Harper.[13] Melting points were determined on a Kofler hot-stage.

Radioactive Assay.—Activities were measured by scintillation counting in solution, with toluene or dioxan solutions (with naphthalene) of the scintillators. The efficiency of this method is generally 50—60% and it was determined for each sample by means of an internal standard of [1-^{14}C]hexadecane. Determinations of activity were made at least in duplicate. The values for the typical degradation described below can thus be expressed as disintegrations (dis.) for 100 sec. per mmole; these values have been converted into relative molar activities for the seven runs on lycorine recorded in Table 2.

Purity of Precursors and Isolated Alkaloids.—The labelled precursors synthesised as below were purified by recrystallisation and chromatography to a good state of chemical purity, and then fractionated by chromatography on Whatman No. 1 paper with the solvent system n-butanol–acetic acid–water (4 : 1 : 1) and autoradiographs were taken with *X*-ray film. The phenols were detected by spraying with diazotised *p*-nitroaniline. The radioactive area and the " phenolic spot " coincided, and generally no radiochemical impurities were present; those samples which were not completely pure at this stage were further purified (*e.g.*, by partition chromatography for hydroxynorbelladine) until radiochemical purity was achieved.

The isolated alkaloids were purified by recrystallisation, as the bases and as salts, until constant activity was achieved. Dilution with pure inactive alkaloid and further recrystallisation established radiochemical purity, as shown by the fact that activity was not lost in the course of the chemical transformations described below. The incorporations of activity (Table 1) were calculated [34] as (100 × total activity of isolated alkaloid) divided by (total activity of precursor fed). For this purpose, the final constant activity of the alkaloid per mg. was used and this was multiplied by the quantity of alkaloid isolated (in mg.) which was of good chemical purity.

Cultivation of Amaryllidaceae Plants, and Administration of Labelled Precursors.—The " Twink " and " Deanna Durbin " plants were grown (at Bristol) in pots in a greenhouse (for feeding in January or February) or in the open (for feeding in March or April). *Nerine bowdenii* plants were always grown in pots in the greenhouse in England, but in the open in Bethesda, U.S.A. When injection was used (p. 1597), the aqueous solution of the precursor (usually 0·5—1·0 ml.) was introduced into the hollow flower stem with a fine hypodermic needle, 3—7 days later a second injection (0·5—1·0 ml.) was made, and the plants were harvested 3—4 weeks after this.

The leaf-feeding technique involved cutting off the end of a leaf under water, and then bending the leaf so that the cut end dipped to the bottom of a small glass tube containing the aqueous solution of labelled material (0·5—1·0 ml.). When most of the solution had been taken up by the plant, distilled water (0·5—1·0 ml.) was added to the tube, and this " washing in " was repeated several times. The leaf was then removed from the feeding-tube and the plants were harvested after 3—4 weeks.

Extraction and Separation of Alkaloids.—The whole plants (" Twink " or " Deanna Durbin ") were macerated with ethanol in a Waring blender, and the mixture was poured into a glass column containing a filter bed. Ethanol was allowed to percolate slowly through the plant material and periodic checks were made of the radioactivity of the issuing solution. The percolation was continued until the count fell to less than 2% of that at the start of the extraction, or until the solution from the column was no longer green, whichever took longer. Evaporation of the ethanolic solution left a residue which was partitioned between ether and an excess of 2N-sulphuric acid. The aqueous layer was extracted five times with ether, saturated with chloroform, adjusted to pH 10 with sodium carbonate, and extracted six times with equal volumes of chloroform. The organic extracts, without drying, were evaporated to *ca.* 20 ml., and lycorine and norpluviine crystallised. After the suspension had been kept at 4° overnight, the solid was collected, washed with chloroform, and dried. The chloroformic solution was examined as described below.

A solution of the solid in a slight excess of dilute hydrochloric acid was basified to >pH 12 with concentrated aqueous sodium hydroxide, and the precipitated lycorine was collected and washed with water. The filtrate was acidified with hydrochloric acid and basified with sodium

[34] Watkin and Neish, *Canad. J. Biochem.*, 1960, **38**, 559.

carbonate to precipitate norpluviine. Both alkaloids were purified by recrystallisation as the hydrochlorides from *ca.* 5N-hydrochloric acid. They were identified by m. p. and comparison of their infrared spectra (KBr disc) with authentic specimens.

The extraction was carried further only when [2-^{14}C]tyrosine was fed to " Twink " daffodils. In this case, inactive hæmanthamine (150 mg.) was added to the chloroform solution (above), which was then evaporated. A solution of the residue in 3% hydrochloric acid was extracted thrice with benzene, basified, and extracted thoroughly with chloroform. Evaporation of the combined extracts left a residue (600 mg.) which was chromatographed on basic alumina (51 g.). Ethyl acetate eluted caranine (53·5 mg.), m. p. 178—180°; total activity 5×10^5 dis./100 sec. Ethyl acetate containing 2—5% of ethanol eluted hæmanthamine (249 mg.), m. p. 202—205°; total activity 5×10^5 dis./100 sec.

N. bowdenni plants were extracted by the published method [12] save that, in the feeding experiments using [1- and 1′-^{14}C]norbelladine, phenolic and non-phenolic alkaloids were separated with alkali. This extraction gave alkaloids forming chloroform-soluble hydrochlorides (2·35 g.) and alkaloids forming chloroform-insoluble hydrochlorides (3·08 g.). The incorporation of activity was 6·37% into the total non-phenolic fraction, and 0·73% into the phenolic fraction.

Degradation of Lycorine.—The following sequence is that used for lycorine derived from [3-^{14}C[phenylalanine as precursor. The other samples of radioactive lycorine were degraded in a similar manner.

(*a*) 5,6-*Dihydro*-5-*methyl*-8,9-*methylenedioxy*-4-*vinylphenanthridine* [Lycorine anhydromethine, (XIII)]. The following method is much simpler and gives improved yields over earlier procedures.[15,16] A solution of lycorine (585 mg.; $2{\cdot}46 \times 10^5$ dis./100 sec./mmole), in methanol (25 ml.) and methyl iodide (1 ml.), was heated under reflux in the dark for 7 hr. The solution was evaporated to 10 ml., diluted with water (10 ml.), and shaken with freshly prepared moist silver oxide (*ca.* 1·3 g.) for 15 min. After removal of the solids by filtration, the solution was evaporated to dryness with the strict exclusion of carbon dioxide. The residue was heated at 100°/15 mm. for 25 min. and extracted with ether. Filtration removed a small amount of insoluble material, and the ether solution was evaporated to leave lycorine anhydromethine as a clear gum (479 mg.), sufficiently pure for the next stage. A portion was converted into the picrolonate, m. p. (from ethanol) 186·5—188° [lit.,[16] 180° (decomp.)].

(*b*) 5,6-*Dihydro*-4-(1,2-*dihydroxyethyl*)-5-*methyl*-8,9-*methylenedioxyphenanthridine* (XIV). The freshly-prepared anhydromethine (470 mg.), in peroxide-free dry ether (50 ml.), was treated with pyridine (5 ml.) and osmium tetroxide (0·6 g.). After 16 hr. at room temperature, the osmate complex was collected, washed with ether, and heated under reflux for 3 hr. with a solution of sodium sulphite (2·4 g.) in 1 : 1 aqueous ethanol (60 ml.). The suspension was filtered (Filtercel) and the filtrate evaporated until free from ethanol. The *diol* (XIV), which crystallised after the solution had been kept in the refrigerator overnight (270 mg.), crystallised from aqueous ethanol (charcoal), had m. p. 165—166° (Found: C, 68·1; H, 5·8. $C_{17}H_{17}NO_4$ requires C, 68·2; H, 5·75%) (Found: $2{\cdot}43 \times 10^5$ dis./100 sec./mmol.).

(*c*) 5,6-*Dihydro*-5-*methyl*-8,9-*methylenedioxy*-6-*oxophenanthridine*-4-*carboxylic acid* (XV; R = CO_2H). The foregoing, finely powdered diol (240 mg.) was suspended in an aqueous buffer (pH 5) made from 0·2N-acetic acid (24 ml.) and 0·2N-sodium acetate (56 ml.), and the suspension was shaken at room temperature with sodium metaperiodate (0·5 g.) for 6·5 hr. The colourless diol gradually dissolved and a yellow precipitate formed. This suspension was added gradually to a saturated aqueous solution of arsenious oxide (50 ml.). This solution was extracted once with ether, basified with potassium carbonate, and re-extracted thrice with ether. Evaporation of the combined ethereal solutions left a solid (A) which was treated as described below.

Dimedone (558 mg.) was added to the aqueous alkaline solution and, after 15 min., the solution was adjusted to pH 6 with hydrochloric acid; formaldehyde dimethone (180 mg.) separated, m. p. 193—194° (from aqueous ethanol) (Found: 0·0 dis./100 sec./mmol.).

To a solution of the solid A in purified dioxan (14 ml.) was added N-potassium permanganate (6 ml.), and the mixture was kept at room temperature overnight. The precipitated manganese dioxide was dissolved by the addition of 2N-sulphuric acid and a slight excess of sodium pyrosulphite, and the dioxan was then evaporated. The acidic lactam (XV; R = CO_2H) then crystallised, m. p. 284—287° (94 mg.) (from methanol) [lit.,[17] 288° (decomp.)] (Found: C, 64·5; H, 3·9. Calc. for $C_{16}H_{11}NO_3$: C, 64·6; H, 3·7%) (Found: $2{\cdot}51 \times 10^5$ dis./100 sec./mmol.).

(*d*) 5-*Methyl*-8,9-*methylenedioxyphenanthridine*-6(5*H*)-*one* (XV; R = H). The foregoing active product was diluted with inactive acidic lactam (XV; R = CO_2H); the reported activities are corrected for this dilution. A solution of the lactam (159 mg.) in freshly distilled quinoline (10 ml.) was heated at the b. p. for 1 hr. with copper chromite [35] under carbon-dioxide-free nitrogen. The emerging gases were passed into saturated (at 20°) aqueous barium hydroxide solution which had previously been heated to 70°. The precipitated barium carbonate was collected, washed with water, and dried at 100° (Found: 0·0 dis./100 sec./mmol.).

The quinoline solution was filtered, the pad was well washed with ethyl acetate, and the filtrate was extracted thrice with 2N-hydrochloric acid, twice with 2N-sodium hydroxide, and washed with water. Evaporation of the dried solution left a solid which yielded the phenanthridinone (XV; R = H) (113 mg.), m. p. 243—247° (from methyl ethyl ketone) (lit.,[17] 238°) (Found: C, 71·3; H, 4·3. Calc. for $C_{15}H_{11}NO_3$: C, 71·15; H, 4·4%) (Found: $2·56 \times 10^5$ dis./100 sec./mmol.).

(*e*) 5-*Methyl*-8,9-*methylenedioxy*-6-*phenylphenanthridinium perchlorate* (XVI). A solution of the phenanthridinone (XV; R = H) (103 mg.) in dry dioxan (50 ml.) was treated with an ethereal solution of phenyl-lithium (*ca.* 5 equiv.). The ether was evaporated and the dioxan solution was heated in nitrogen under reflux for 2 hr. An excess of dilute hydrochloric acid was added to the cooled solution, the dioxan was evaporated, and the aqueous solution was extracted thrice with ethyl acetate, basified with sodium hydroxide, and extracted five times with ether. The base from the ether was dissolved in ethanol (10 ml.), and 20% aqueous perchloric acid (1·5 ml.) was added. When the ethanol had been evaporated, the *phenanthridinium perchlorate* crystallised (111 mg.), and was recrystallised (charcoal) from ethanol as yellow needles (101 mg.), m. p. 308—311° (Found: C, 61·0; H, 3·9; N, 3·4. $C_{21}H_{16}ClNO_6$ requires C, 60·8; H, 3·9; N, 3·4%) (Found: $2·33 \times 10^5$ dis./100 sec./mmol.).

(*f*) *Oxidation of the phenanthridinium perchlorate* (XVI). Aqueous N-potassium permanganate (48 ml.) was added to a solution of the perchlorate (93 mg.) in pyridine (30 ml.), the mixture was heated under reflux for 45 min., a further portion of the permanganate solution (12 ml.) was added, and the heating was continued for 1·25 hr. The manganese dioxide was dissolved by the addition of N-sulphuric acid and sodium pyrosulphite, and the resultant solution was extracted thrice with ether. Evaporation of the ether left a residue which was dissolved in the minimum volume of ether and the solution was poured into light petroleum (b. p. 40—60°). The clear solution was removed from the precipitated gum, and the residue from its evaporation was run in light petroleum (b. p. 40—60°) on to a column of silica gel; benzoic acid was eluted with light petroleum–ether (4 : 1) and sublimed before counting, m. p. 122—123° (Found: $2·38 \times 10^5$ dis./100 sec./mmol.).

5,6-*Dihydro*-5-*methyl*-8,9-*methylenedioxyphenanthridine Methiodide* (XVII).—A solution of the phenanthridinone (XV; R = H) (0·1 g.) in anhydrous dioxan (12 ml.) was added to dioxan (10 ml.) containing lithium aluminium hydride (385 mg.). The mixture was heated under reflux for 16 hr., cooled, and treated with a slight excess of water. The organic layer was decanted, the inorganic residue was thoroughly washed with ether, and the combined organic solutions were evaporated to dryness. Methyl iodide (0·8 ml.) was added to a solution of the residue in methanol (10 ml.), which was then heated under reflux (nitrogen) for 5 hr. and concentrated. The crystals crystallised from methanol to yield the *phenanthridine methiodide* (XVII), m. p. 231—236° (98 mg.) (Found: C, 50·7; H, 4·6; N, 3·6. $C_{16}H_{18}INO_2$ requires C, 50·3; H, 4·2; N, 3·7%).

trans-[3-^{14}C]*Cinnamic Acid*.—[*formyl*-^{14}C]Benzaldehyde (42 mg.; minimum activity 0·5 mc) was transferred, using pyridine (0·5 ml.), to a flask containing malonic acid (97 mg.) and anhydrous sodium sulphate (20 mg.). Piperidine (0·02 ml.) was added, and the mixture was heated at 100° for 2 hr. and then at 120° for 2·5 hr. Water (2·0 ml.) was added to the cooled mass, and the resultant solution was adjusted to pH 2 with hydrochloric acid. The precipitate of *trans*-(3-^{14}C]cinnamic acid was collected (44·9 mg., 77%), m. p. 136—137°, and recrystallised twice from aqueous methanol to afford the pure product used for the feeding experiments (37·9 mg.), m. p. 136—136·5° (Found: $6·7 \times 10^9$ dis./100 sec./mmol.).

4-*Benzyloxy*-β-*nitrostyrene*.—4-Benzyloxybenzaldehyde [36] (17·5 g.), nitromethane (15 ml.), and ammonium acetate (6 g.) were heated together under reflux with acetic acid (10 ml.) in a

[35] Adkins and Connor, *J. Amer. Chem. Soc.*, 1931, **53**, 1091.
[36] Bergmann and Sulzbacher, *J. Org. Chem.*, 1951, **16**, 84.

nitrogen atmosphere for 2 hr. The *nitrostyrene* crystallised from the cooled solution (15 g.), m. p. 122—123° (Found: C, 70·7; H, 5·3. $C_{15}H_{13}NO_3$ requires C, 70·6; H, 5·1%).

O-*Benzyltyramine*.—A solution of the nitrostyrene (13 g.), in anhydrous tetrahydrofuran (50 ml.), was added during 1 hr. to lithium aluminium hydride (5 g.) in tetrahydrofuran (50 ml.). The mixture was heated under reflux for 2·5 hr., cooled, and treated slowly with a slight excess of saturated aqueous Rochelle salt. The organic solution was decanted from the coagulated solids, which were washed with ether (2 × 100 ml.). Evaporation of the combined organic solutions left a residue which was dissolved in dilute hydrochloric acid and the solution was filtered. Extraction of the filtrate thrice with ethyl acetate was followed by basification and further extraction thrice with ether. The latter extracts gave O-*benzyltyramine*, which was purified as the hydrochloride, m. p. 210—215° (from water) (4·4 g.) (Found: C, 68·25; H, 7·2. $C_{15}H_{18}ClNO$ requires C, 68·3; H, 6·8%).

4-*Benzyloxy*-N-(3-*benzyloxy*-4-*methoxybenzyl*)*phenethylamine*.—3-Benzyloxy-4-methoxybenzaldehyde (1·04 g.) and *O*-benzyltyramine hydrochloride (1·12 g.) were dissolved in ethanol (120 ml.) and treated with aqueous N-sodium hydroxide (4·25 ml., 1 equiv.). The solution was evaporated and, after the residue had been heated *in vacuo* at 120° for 10 min., it was dissolved in methanol (50 ml.). Sodium borohydride (0·67 g.) was added, and the solution was kept overnight, treated with an excess of aqueous hydrochloric acid, and evaporated to remove the methanol. Crystals separated, which were recrystallised from aqueous ethanol, to give 4-*benzyloxy*-N-(3-*benzyloxy*-4-*methoxybenzyl*)*phenethylamine hydrochloride* (0·88 g.), m. p. 126—128° (Found: C, 71·6; H, 6·6. $C_{30}H_{32}ClNO_3,H_2O$ requires C, 70·9; H, 6·7%).

4-*Hydroxy*-N-(3-*hydroxy*-4-*methoxybenzyl*)*phenethylamine* (O-*Methylnorbelladine*: VI; R = Me, R′ = H).—A solution of the previous product (547 mg.) in ethanol (60 ml.) containing 2N-hydrochloric acid (1 ml.) was shaken with hydrogen and 10% palladium–charcoal (180 mg.). Uptake (53·5 ml.) was complete in 25 min. The solution was filtered, evaporated to dryness, and the residue recrystallised from ethanol–ethyl acetate, to give the phenethylamine (VI; R = Me R′ = H) as the *hydrochloride* (237 mg.), m. p. 209—211° (Found: C, 61·8; H, 6·4. $C_{16}H_{20}ClNO_3$ requires C, 62·0; H, 6·45%).

[1′-^{14}C]*Norbelladine* (VI; R = R′ = H) [with Dr. R. J. Highet].—A solution of 4-methoxy-β-nitrostyrene (18·5 g.) in anhydrous tetrahydrofuran was added to a suspension of lithium aluminium hydride (4 g.) in the same solvent. After the mixture had been heated under reflux for 3 days, it was treated with water (12 ml.), stirred for 1 hr., and filtered, the filter-pad being washed thrice with ether. The combined organic solutions were extracted twice with an excess of N-hydrochloric acid, and the aqueous extracts were basified and extracted twice with chloroform. Evaporation of the chloroform extracts left a residue (10·3 g.), which was treated with a slight excess of concentrated hydrochloric acid to give a crystalline hydrochloride. Recrystallisation from ethanol yielded 4-methoxyphenethylamine hydrochloride (5·3 g.), m. p. 201—208° raised by one recrystallisation to 209—211° (lit.,[37] 207°) (Found: C, 58·0; H, 7·5. Calc. for $C_9H_{14}ClNO$: C, 57·6; H, 7·5%).

A solution of [*carboxyl*-^{14}C]3,4-dimethoxybenzoic acid (8·8 mg., total activity 6 × 10^9 dis./100 sec.) in benzene (10 ml.) was heated under reflux with thionyl chloride (4 drops) and a 10% solution of pyridine in benzene (0·01 ml.) for 90 min.; the cooled solution was washed with water, N-sodium hydrogen carbonate, and water. 4-Methoxyphenethylamine hydrochloride (11·5 mg.) was added to the vigorously stirred benzene solution and 0·1N-sodium hydroxide (2 equiv.) was added during 40 min. After the mixture had been stirred for 1 hr., the benzene layer was separated, and washed with water, dilute hydrochloric acid, and water. The benzene solution of neutral material carried 4·01 × 10^9 dis./100 sec.; total activity (68%).

In trial runs with inactive materials, the N-(3,4-*dimethoxybenzyl*)-4-*methoxyphenylacetamide* was crystallised from ethanol, m. p. 157—158° (Found: C, 68·6; H, 6·6. $C_{18}H_{21}NO_4$ requires C, 68·55; H, 6·7%), $\lambda_{max.}$ (in EtOH) 257, 284, and 278 mμ (log ε 4·18, 3·92, and 3·89).

The benzene solution containing the radioactive amide was freeze-dried, and the residue was sealed in a tube with lithium aluminium hydride (10 mg.) and anhydrous tetrahydrofuran (3 ml.). The mixture was heated at 75° for 3 days and then stirred whilst water (1 drop) was added. The suspension was stirred for 1 hr., centrifuged, and the solution extracted twice with an excess of dilute hydrochloric acid and once with water. The combined aqueous layers were made strongly alkaline with sodium hydroxide and extracted twice with benzene. Total activity of

[37] Rosenmund, *Ber.*, 1909, **42**, 4782.

the benzene extracts was $2{\cdot}67 \times 10^9$ dis./100 sec. The residue from evaporation of the benzene was heated in a sealed tube with concentrated hydrochloric acid (2 ml.) for 2 hr. at 180° and the cooled solution was adjusted to pH 8·9 with potassium hydrogen carbonate. This solution was stirred with neutral alumina (1 g.) and the solid was packed into a column. Elution with 0·1N-potassium hydrogen carbonate removed a small active fraction, and the norbelladine was eluted with 0·1N-acetic acid; total activity $1{\cdot}42 \times 10^9$ dis./100 sec. An excess of N-hydrochloric acid was added to this solution before it was concentrated to low volume for the work below. Chromatography of this material on Whatman No. 1 paper in n-butanol–acetic acid–water (4 : 1 : 1) showed a single spot on the autoradiograph, R_F 0·61, indistinguishable from the inactive norbelladine prepared on a larger scale and further characterised as norballadine *picrate*, plates from water, m. p. 102—117° (Found: C, 49·9; H, 4·4; N, 11·1. $C_{21}H_{20}N_4O_{10},H_2O$ requires C, 49·8; H, 4·4; N, 11·1%). The anhydrous form was obtained when the monohydrate was dried at 100° (Found: C, 51·4; H, 4·2. $C_{21}H_{20}N_4O_{10}$ requires C, 51·6; H, 4·1%).

The intermediate base 4-methoxy-*N*-(3,4-dimethoxybenzyl)phenethylamine (XI; NMe = NH) was isolated as the *hydrochloride* in trial experiments with inactive materials. It crystallised from ethanol–ethyl acetate, m. p. 222—223° (Found: C, 64·0; H, 7·1. $C_{18}H_{24}ClNO_3$ requires C, 64·0; H, 7·1%), $\lambda_{max.}$ (in EtOH) 225, 278, and 283 mμ (log ε 4·31, 3·73, and 3·69).

[1-^{14}C]*Norbelladine* (VI; R = R′ = H).—Inactive tyramine (40 mg.) was added to a solution of [1-^{14}C]tyramine hydrochloride (0·1 mc; 4·25 mc/mmole) in ethanol and 3,4-dihydroxybenzaldehyde (40 mg.) was then added. The solution was heated under reflux for 20 min., evaporated to dryness, and the orange crystalline Schiff's base (see below) was dissolved in acetic acid (5 ml.) and shaken overnight with hydrogen and 10% palladised charcoal (40 mg.). After filtering off the catalyst, the solution was evaporated to dryness, and the residue, in water (*ca.* 10 ml.), was treated with basic alumina (10 g.). The pH of the suspension was rapidly adjusted to 8·4 with acetic acid and ammonia, and the solid was packed into a column. Water (300 ml.) was passed through the column to remove the unchanged tyramine, and the [1-^{14}C]-norbelladine was eluted with 0·2N-acetic acid. The homogeneity of this fraction was demonstrated by paper chromatography and autoradiography, as above. The single active spot (R_F 0·61) corresponded to the inactive norbelladine, and was clearly distinguishable from the spot corresponding to active tyramine (R_F 0·57). It was demonstrated that a mixture of active norbelladine and tyramine was separated by the solvent used (see above).

In trial experiments with inactive materials, the Schiff's base N-(3,4-*dihydroxybenzylidene*)-4-*hydroxyphenethylamine* was recrystallised several times from ethanol–benzene, m. p. 229—232° [Found: C, 70·0; H, 5·9; Equiv. (perchloric acid in acetic acid), 258. $C_{15}H_{15}NO_3$ requires C, 70·0; H, 5·9%; Equiv. 257], $\lambda_{max.}$ (in EtOH) 275, 308, 401 mμ (log ε 4·05, 3·87, 3·93).

[1- *and* 1′-^{14}C]*Norbelladine* (VI; R = R′ = H) *and its Degradation*.—A solution of [1-^{14}C]-norbelladine (specific activity *ca.* 4·15 mc/mmole) in N-hydrochloric acid was mixed with a similar solution of [1′-^{14}C]norbelladine (specific activity *ca.* 33 mc/mmole) and then concentrated to 2 ml. This was the stock solution used in feeding experiments. An accurate aliquot (22 μl.) of this solution was added to a solution of inactive norbelladine picrate (700 mg.) in hot water and the picrate, which separated on cooling, was recrystallised to constant activity from water. The final specific activity was 96% of that calculated from the dilutions involved.

A further aliquot of the stock solution (total activity *ca.* $1{\cdot}3 \times 10^7$ dis./100 sec.) was added to a methanolic solution of norbelladine (1·12 g.; see below) and treated with a large excess of ethereal diazomethane (from 20 g. of *N*-methyl-*N*-nitroso-*N*′-nitroguanidine). After 3 days, the solution was evaporated and the residue fractionated by chromatography on alumina. Benzene eluted belladine (XI) which was crystallised to constant activity ($4{\cdot}84 \times 10^5$ dis./100 sec./mmole) as the hydrochloride (0·626 g.). The free base was recovered from this hydrochloride, dissolved in chloroform, and treated overnight with cyanogen bromide (1·5 g.). The residue recovered by evaporation was kept for 1 hr. in solution in dioxan (10 ml.) and 10% aqueous sodium hydroxide (20 ml.), to convert the 3,4-dimethoxybenzyl bromide into the corresponding alcohol. Extraction of the solution with chloroform yielded an oil which, in chloroform, was treated with manganese dioxide (3 g.), and after 20 min. no 3,4-dimethoxybenzyl alcohol remained (v.p.c.). The solution was filtered, washed with dilute sulphuric acid, and evaporated. The residue was partitioned between a large excess of saturated sodium hydrogen sulphite with ethanol (5 ml.) and chloroform. The aqueous layer was basified with sodium hydroxide and extracted with benzene, to afford crude 3,4-dimethoxybenzaldehyde

(115 mg.). This was converted into its semicarbazone and recrystallised to constant activity ($1{\cdot}15 \times 10^5$ dis./100 sec./mmole, 21·8% of total), m. p. 177°.

The chloroform solution contained crude *N*-cyano-*N*-methyl-4-methoxyphenethylamine, which was converted, by being heated under reflux overnight with 9N-sulphuric acid and sufficient ethanol to effect solution, into N-*methyl*-N-(4-*methoxyphenethyl*)*urea*. The acidic solution was extracted with chloroform to remove neutral impurities, basified, and extracted again with chloroform. Evaporation of the latter extracts and crystallisation of the residue from ethanol–ethyl acetate gave the pure urea, m. p. 157—158° ($3{\cdot}78 \times 10^5$ dis./100 sec./mmole, 78·2% of total activity). (Found: C, 63·5; H, 7·7; N, 13·2. $C_{11}H_{16}N_2O_2$ requires C, 63·45; H, 7·45; N, 13·45%).

A sample of this urea was synthesised, for comparison, by treatment of *N*-methyl-4-methoxyphenethylamine (190 mg.) in acetic acid (10 ml.) with potassium cyanate (200 mg.). After 30 min., the mixture was diluted with water and extracted with chloroform. Crystallisation of the extracted material, as above, gave the same urea (48 mg.) (m. p., mixed m. p., and infrared spectrum).

The norbelladine used above was obtained by extracting a solution of the pure picrate in 10% acetic acid with ether until it was free from picric acid. Evaporation of the solution to dryness then gave norbelladine, together with some acetic acid.

Isotopic Dilution Analysis.—Eight " Twink " daffodil plants were fed as usual with an aqueous solution of (±)-[2-^{14}C]tyrosine (0·1 mc) and after 20 days the plants were extracted as above. The chloroform-insoluble alkaloids were collected, and half the remaining chloroform solution was shaken with an aqueous solution of *O*-methylnorbelladine (156 mg.) in N-sodium hydroxide (20 ml.). Three more extractions of the organic layer were carried out with dilute sodium hydroxide. The combined alkaline solutions were quickly washed once with chloroform, acidified, and finally made alkaline with potassium carbonate. Extraction four times with chloroform–propan-2-ol (3 : 1) afforded crude *O*-methylnorbelladine, which was converted into the hydrochloride and purified by multiple recrystallisation from dilute hydrochloric acid and ethanol–ethyl acetate.

This hydrochloride, in methanol, was methylated with a large excess of diazomethane by the method used above for the conversion of [1- and 1′-^{14}C]norbelladine into belladine. The resultant belladine was fractionated on a Gaschrom P column carrying 1% SE-30, 5·4 mm. diam., 9 ft. long. At 181° and 15 p.s.i. inlet pressure, the retention time was 11·2 min. The belladine from the v.p.c. column was trapped (cold trap) and counted (Table 1).

3,4-*Dimethoxy*-N-(3,4-*dimethoxybenzyl*)*phenethylamine* (Di-*O*-methyl ether of VI; R = Me, R′ = OMe).—(*a*) *Schiff's base route.* A solution of [2-^{14}C]3,4-dimethoxyphenethylamine hydrochloride [38] (50·5 mg.) and 3,4-dimethoxybenzaldehyde (56 mg.) in ethanol (5 ml.) was treated with N-sodium hydroxide (1 equiv.) and evaporated to dryness. The residue was heated at 120° for 10 min. *in vacuo*, cooled, and reduced in methanolic solution, with sodium borohydride (69 mg.). After being kept overnight, the solution was acidified, evaporated to remove the methanol, basified, and extracted thrice with ether. Evaporation of the ethereal extracts left a solid (62 mg.) which recrystallised from di-isopropyl ether to afford the *phenethylamine* (di-*O*-methyl ether of VI; R = Me, R′ = OMe), m. p. 86—88° (Found: C, 68·9; H, 7·45; N, 4·4. $C_{19}H_{25}NO_4$ requires C, 68·9; H, 7·6; N, 4·2%).

(*b*) *Amide route.* 3,4-Dimethoxyphenylacetic acid (182 mg.) and thionyl chloride (3 ml.) were heated on a steam-bath for 30 min., and the excess of thionyl chloride was evaporated. A solution of the residue in anhydrous ether (2 ml.) was added to 3,4-dimethoxybenzylamine [39] (523 mg.) in ether (3 ml.), and the mixture was then partitioned between ethyl acetate and dilute hydrochloric acid. After the aqueous layer had been further extracted with ethyl acetate, the combined organic extracts were evaporated and the residue recrystallised from ethyl acetate, to give N-(3,4-*dimethoxybenzyl*)-3,4-*dimethoxyphenylacetamide* (XVIII), m. p. 127—129° (244 mg.) (Found: C, 66·3; H, 6·4; N, 4·2. $C_{19}H_{23}NO_5$ requires C, 66·1; H, 6·7; N, 4·1%).

A solution of the amide (244 mg.) in anhydrous tetrahydrofuran (100 ml.) was added to lithium aluminium hydride (1·21 g.) in tetrahydrofuran, and the mixture was heated under reflux for 16 hr. Rochelle salt solution (100 ml.; 30% w/v) was added to the cooled mixture, and the organic layer was decanted and evaporated. The residue, in ethyl acetate, was extracted twice with dilute hydrochloric acid, and the aqueous solution was basified and extracted

[38] Battersby, Binks, Francis, McCaldin, and Ramuz, *J.*, 1964, in the press.
[39] Juliusberg, *Ber.*, 1907, **40**, 120.

thrice with ether. Evaporation of the dried ethereal extracts gave the amine (di-*O*-methyl ether of VI; R = Me, R = OMe) (106 mg.), identical (m. p., mixed m. p., and infrared spectrum) with that obtained under (*a*) above.

[1-^{14}C]3,4-*Dihydroxy*-N-(3,4-*dihydroxybenzyl*)*phenethylamine* (*Hydroxynorbelladine* VI; R = H, R′ = OH).—The foregoing tetramethoxy-ether, from (*a*) (48 mg.) was heated with AnalaR concentrated hydrochloric acid in an evacuated sealed tube at 160° for 30 min. Crystals separated from the cooled solution, and these were purified by repeated recrystallisation from dilute hydrochloric acid, to give [1-^{14}C]*hydroxynorbelladine hydrochloride*, m. p. 185—185·5° (Found: C, 57·9; H, 5·95; N, 4·9. $C_{15}H_{18}ClNO_4$ requires C, 57·8; H, 5·8; N, 4·5%). Purity was checked by autoradiography and, in one preparation where this was not satisfactory, the sample was further purified by partition chromatography on a column of Whatman No. 1 cellulose powder with the solvent system n-butanol–acetic acid–water (4 : 1 : 1).

Hydrolysis of Lycorine and Demethylation of Norpluviine.—These alkaloids were obtained from plants fed with [*methyl*-^{14}C]*methionine* (Table 1). A solution of lycorine hydrochloride (20 mg.) (Found: $1{\cdot}72 \times 10^5$ dis./100 sec./mmol.) in water was added to phosphoric acid (40 ml.) and heated for 100 min. on a steam-bath under nitrogen (experimental details kindly supplied by Dr. P. W. Jeffs, University of Natal). The emerging gases were passed through a solution of dimedone (79 mg.) in water (25 ml.) made alkaline with potassium carbonate. At this stage the apparatus was set for steam-distillation, and 100 ml. of distillate was collected in the solution of dimedone. After 30 min., this solution was acidified (pH 3), and the precipitated formaldehyde dimethone was collected (14·1 mg.) and recrystallised (charcoal) from aqueous ethanol, m. p. 193—195° (Found: $1{\cdot}63 \times 10^5$ dis./100 sec./mmole).

Norpluviine (20·5 mg.) (Found: $3{\cdot}17 \times 10^5$ dis./100 sec./mmole) was dissolved in a few drops of a solution of phenol in acetic anhydride (70%, w/v) and was added to purified hydriodic acid (6 ml.; constant boiling). This solution was heated under reflux for 1 hr. in a stream of nitrogen which was then passed first through a 10% solution of potassium antimony tartrate and then into a 5% ethanolic solution of triethylamine [40] (7 ml.) at −76°. After the triethylamine solution had been kept at room temperature overnight, it was evaporated to dryness to leave triethylamine methiodide (15·7 mg.) (Found: $3{\cdot}2 \times 10^5$ dis./100 sec./mmole).

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[40] Brown and Byerrum, *J. Amer. Chem. Soc.*, 1952, **74**, 1523.