Phytochemistry, 1974, Vol. 13, pp. 933 to 945. Pergamon Press. Printed in England.

INCORPORATION OF PHENYLALANINE AND EXAMINATION OF NORBELLADINES AS PRECURSORS OF THE MESEMBRINE ALKALOIDS*[1]

PETER W. JEFFS, HENRY F. CAMPBELL, DAVID S. FARRIER, GOURANGA GANGULI, NED H. MARTIN and GERADO MOLINA

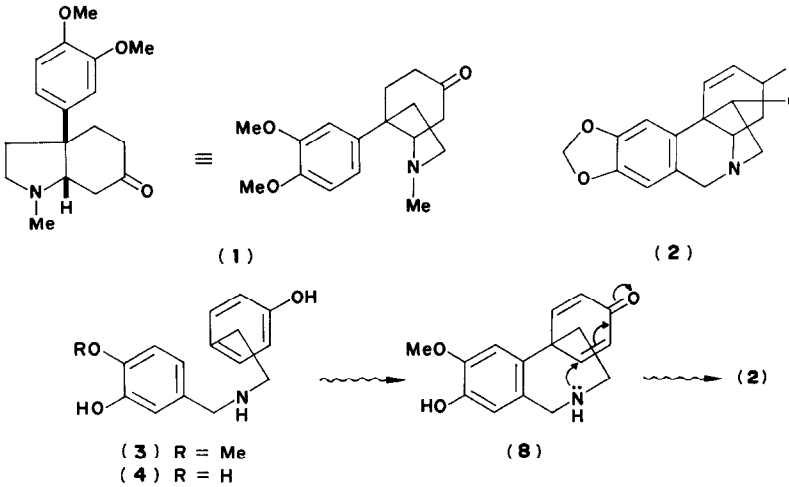
Paul M. Gross Chemical Laboratory, Duke University, Durham, NC 27706, U.S.A.

(*Received* 16 *September* 1973. *Accepted* 22 *October* 1973)

Key Word Index—*Sceletium strictum*; Aizoaceae; biosynthesis; mesembrine; phenylalanine.

Abstract—Experiments in which [2′,6′-^{3}H; 1′-^{14}C] phenylalanine and various labelled norbelladine and *O*-methylnorbelladine derivatives were examined as test precursors to the octahydroindole class of mesembrine alkaloids in *Sceletium strictum* are reported. The evidence presented leads to the conclusion that the mode of incorporation of phenylalanine differs from that found for the structurally analogous Amaryllidaceae alkaloids of the crinine group. Radiolabelled alkaloids which resulted from administering labelled norbelladine derivatives were shown to occur by fragmentation of the test precursors prior to incorporation.

EARLIER studies on the biosynthesis of mesembrine (**1**) have established that the basic skeleton of this alkaloid and its relatives is constructed from tyrosine and phenylalanine.[2] Each of these amino-acids is used separately to provide the C_6C_2–N unit and the aromatic C_6-fragment which together comprise the 3a-aryloctahydroindole nucleus of the mesembrine group.



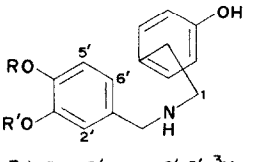
* Part V in the series "Sceletium Alkaloids." For Part IV see JEFFS, P. W., ARCHIE, W. C., HAWKS, R. L. and FARRIER, D. S. (1971) *J. Am. Chem. Soc.* **93**, 3752.

[1] Some of this work has been published previously in preliminary form; JEFFS, P. W., CAMPBELL, H. F., FARRIER, D. S. and MOLINA, G. A. (1971) *Chem. Commun.* 288.

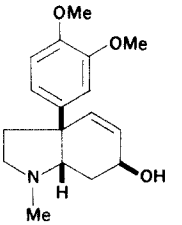
[2] JEFFS, P. W., ARCHIE, W. C., HAWKS, R. L. and FARRIER, D. S. (1971) *J. Am. Chem. Soc.* **93**, 3752.

Since the role of these two amino-acids as progenitors of the C_6–C_2–N and aromatic C_6-units in mesembrine is in direct parallel with the results obtained for the structurally similar Amaryllidaceae alkaloids of the crinine series, such as haemanthamine (**2**), further experiments on the biosynthesis were guided initially by consideration of a common pathway.

In the Amaryllidaceae, the extensive researches of Barton,[3] Battersby and Wildman and their coworkers[4] have established that biosynthesis of the crinine alkaloids of this family can be derived by the oxidative cyclization of the norbelladine derivative **3**. In this case the dienone **8**, which is formed, can undergo an intramolecular Michael addition to provide the crinine skeleton. The details of the formation of the pivotal 4′-*O*-methylnorbelladine from phenylalanine and tyrosine are well established and are known to involve the enzyme mediated selective 4′-*O*-methylation of norbelladine (**4**) as the penultimate step in the sequence.[5]



(**5**) R = R′ = H; 2′,6′-^{3}H.
(**6**) R = Me; R′ = H, 2′,6′-^{3}H.
(**7**) R = H; R′ = Me, 5′-^{3}H.
(**10**) R = Me; R′ = H, 1-^{14}C.
(**11**) R = H; R′ = Me, 1-^{14}C.



(**9**)

To test the intermediacy of norbelladine-type precursors in the biosynthesis of mesembrine, radiolabelled norbelladine (**5**), 4′-*O*-methylnorbelladine (**6**) and 3′-*O*-methylnorbelladine (**7**) were synthesized and administered separately to live *Sceletium strictum* plants by the methods which have been described previously.[2] After a period of 7–10 days the plants were harvested and the major alkaloid, mesembrenol (**9**),[6] was isolated and purified to constant activity. The results of these experiments, which are summarised in Table 1, were somewhat surprising in that 3′-*O*-methylnorbelladine appeared to be incorporated more efficiently than its 4′-*O*-methyl derivative. In addition, the level of incorporation of each of these test precursors was much lower than that of [*S*-methyl-^{14}C]methionine, which had been fed simultaneously as a control, and also significantly lower than the incorporation levels obtained previously with radiolabelled tyrosine and phenylalanine. The possibility that seasonal variation in alkaloid production was responsible for the low levels of incorporation was examined by a repeat feeding with [1-^{14}C] labelled *O*-methylnorbelladines (**10**) and (**11**). While the incorporation of each of these compounds was both substantially higher than the corresponding radio-isomers fed previously, the 3′-*O*-methyl isomer again provided the more efficient utilisation of the radiolabel (Table 1). Consideration of this incorporation data suggested

[3] BARTON, D. H. R., KIRBY, G. W., TAYLOR, J. B. and THOMAS, G. M. (1963) *J. Chem. Soc.* 4545.
[4] BATTERSBY, A. R., BINKS, R., BREUER, S. W., FALES, H. M., WILDMAN, W. C. and HIGHET, R. J. (1964) *J. Chem. Soc.* 1595.
[5] MANN, J., FALES, H. M. and MUDD, S. H. (1963) *J. Biol. Chem.* **238**, 3820.
[6] JEFFS, P. W., AHMANN, G., CAMPBELL, H. F., FARRIER, D. S., GANGULI and HAWKS, R. L. (1970) *J. Org. Chem.* **35**, 3512.

it was unlikely that the biosynthesis of the mesembrine alkaloids proceeds by a pathway involving an extension of the route to the crinine alkaloids.

TABLE 1. INCORPORATION OF RADIOLABELLED TEST PRECURSORS INTO ALKALOIDS OF *Sceletium strictum*

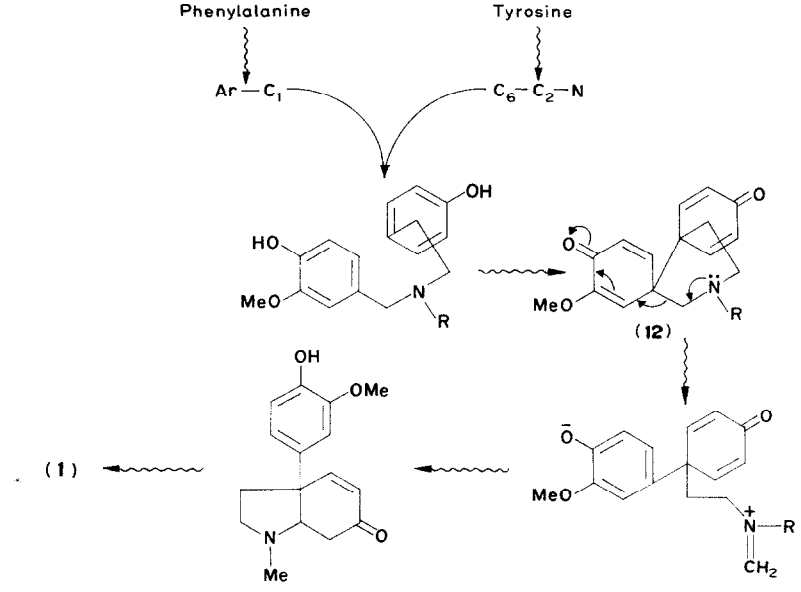
Precursor	Sp. act.* mCi/mmol	^{3}H-^{14}C	Alkaloid(s)	Sp. act.* μCi/mmol	^{3}H-^{14}C
[$^{3}H_4$]norbelladine (**5**)	5·10	—	Mesembrenol	0·30	—
4′-*O*-methyl[$^{3}H_4$]norbelladine (**6**)	5·23	—	Mesembrenol	0·07	—
3′-*O*-methyl[$^{3}H_3$]norbelladine (**7**)	6·36	—	Mesembrenol	0·209	—
4′-*O*-methyl[1-^{14}C]norbelladine (**10**)	0·902	—	Mesembrenol	0·039	—
3′-*O*-methyl[1-^{14}C]norbelladine (**11**)	2·04	—	Mesembrenol	0·208	—
DL-[2′,6′-^{3}H,1′-^{14}C]phenylalanine (**15**)	3·35	21·0	Mesembrine	0·012‡	20·2
			Mesembrenone	0·009‡	21·0
3′-*O*-methyl[3′-*O*-methyl-^{14}C,1-^{14}C]norbelladine (**18**)	1·64	2·45†	Mesembrenol	0·072	0·27†
			Mesembrenone	0·012	
[5′-^{3}H,1-^{14}C]norbelladine (**19**)	1·20	9·1	Mesembrenol	0·006‡	1·18
			Mesembrine		0·41
			Mesembrenone		1·0
3′-*O*-methyl[2′,6′-^{3}H,1-^{14}C]norbelladine (**20**)	0·380	5·12	Mesembrenol	0·20	0·51
N-methyl[2′,6′-^{3}H,1-^{14}C]norbelladine (**21**)	0·896	21·2	Mesembrine	Inactive	—
N-methyl[5′-^{3}H,1-^{14}C]norbelladine (**22**)	0·96	9·92	Mesembrine	Inactive	—
			Mesembrenone	Inactive	
3′-*O*,*N*-dimethyl[5-^{3}H,1-^{14}C]norbelladine (**23**)	0·168	16·6	Mesembrine	Inactive	—
			Mesembrenone	Inactive	
3′-deoxy[3,5′-^{3}H,1-^{14}C]norbelladine (**25**)	0·80	18·5	Mesembrine	Inactive	—
3′-deoxy-*N*-methyl 3′,5′-^{3}H,1-^{14}C nobelladine (**26**)	0·82	19·5	Mesembrine	Inactive	—

* For doubly labelled samples the value quoted is for the ^{14}C isotope.
† This value represents the ratio of ^{14}C labels in the methoxyl group to that at the C-1 atom.
‡ This figure represents % incorporation of radioactivity into the specified alkaloid.

If 3′-*O*-methylnorbelladine is a true precursor to the mesembrine series then the formation of the dienone **8**, which occurs as a necessary intermediate in the formation of crinine alkaloids, is precluded, and the *bis*-spirodienone **12** becomes a reasonable alternative. This intermediate appeared particularly attractive since not only is its genesis from 3′-*O*-methylnorbelladine readily derived but, also, its subsequent conversion to the mesembrine series can be visualized to occur by a simple fragmentation-aromatisation reaction of the *p*-aminomethyldienone system (Scheme 1). Precedent for this kind of fragmentation of *p*-methylaminodienones exists in the biosynthesis of the erythrina alkaloids, where the conversion of **13–14** has been demonstrated.[7] Also, a laboratory analogy for this reaction has been utilised in an elegant synthesis of protostephanine.[8]

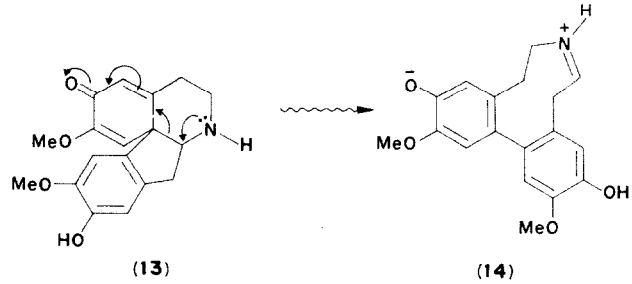
Because of the low levels of incorporation experienced with the norbelladine derivatives cited above, we felt that the question of the possible intermediacy of a *bis*-spirodienone intermediate could be approached more effectively, at least in the initial phase, by a study of the mode of incorporation of the aromatic ring of phenylalanine. The first objective was to determine in an unequivocal manner which of the nuclear carbons of the aromatic ring of phenylalanine corresponded to the C-1′ position in the aromatic ring of the mesembrine alkaloids. Since the loss of the C-3 side-chain occurs during the

[7] BARTON, D. H. R., JAMES, R., KIRBY, G. W., TURNER, D. W. and WIDDOWSON, D. A. (1968) *J. Chem. Soc.* **C**, 1529.
[8] BATTERSBY, A. R., BHATUAGAR, A. K., HACKETT, P., THORBER, C. W. and STAUNTON, J. (1968) *Chem. Commun.* 1214.



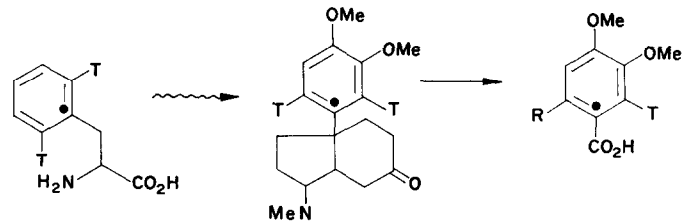
SCHEME 1.

incorporation of this amino acid into the alkaloid skeleton, it is not possible to derive this information by inference. To overcome this problem DL-[2,6-^{3}H]phenylalanine was prepared by reduction of DL-2′-bromophenylalanine with tritium gas over palladium. Combination of this tritiated product with DL-[1-^{14}C]phenylalanine gave the doubly labelled compound **15**, which from symmetry consideration may be viewed as containing equal amounts of tritium at the equivalent 2′,6′-positions. When this compound was administered to *S. strictum* and the plants extracted after 9 days, a radioscan of a thin layer chromatoplate of the crude alkaloid fraction showed that the majority of the activity was associated with mesembrine and mesembrenone. Purification of these two alkaloids after addition of carrier showed that each alkaloid possessed the same tritium to C-14 ratio as that of the administered phenylalanine.



It is clear from this result that the biosynthesis of mesembrine cannot involve an intermediate of the crinine-type otherwise a 50% loss of tritium would have occurred from **15** as a consequence of its conversion to the dienone **8**, which is an obligatory intermediate on the crinine pathway. The location of the tritium labels at the 2′,6′-positions in mesembrine derived from **15** was established by oxidation of the alkaloid to veratric

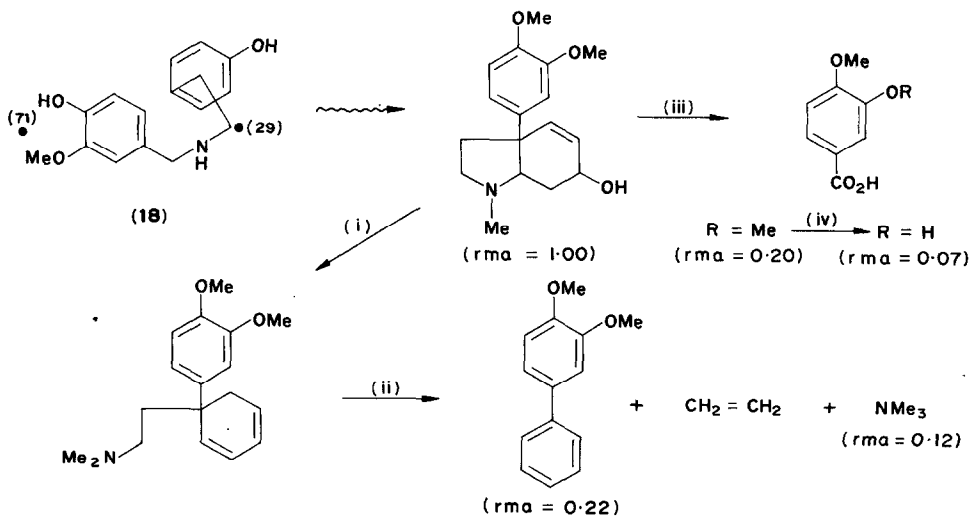
acid **16** with a large excess of potassium ferricyanide[9] and subsequent conversion of the latter to 6-bromoveratric acid. The $^3H:^{14}C$ ratio remained essentially unchanged in the conversion of mesembrine to veratric acid while 47% of the tritium was lost on conversion to 6-bromoveratric acid (**17**). Several attempts to introduce an iodine substituent in the 2-position of 6-bromoveratric acid through an aryl thallation reaction were unsuccessful and although direct proof that the second tritium in the mesembrine from this experiment is indeed located at the 2′-position was not obtained, its assignment to this location can be made with some degree of confidence.



(15) $^3H:^{14}C = 21$ $^3H:^{14}C = 20$ (16) R = T, $^3H:^{14}C = 19$

(17) R = Br, $^3H:^{14}C = 11$

Thus, these results are fully consonant with the proposed pathway outlined in Scheme 1. In particular, the retention of both tritium labels at their original sites implies, (i) that bond formation (presumably through phenol oxidative coupling) occurs at the C-1′ position of an aromatic ring in an intermediate derived from phenylalanine, and (ii) the logical consequence of the former is to provide a ring A spirodienone which in order to retain both tritiums at their original sites must undergo aromatisation of ring A by a process which is at least mechanistically analogous to the fragmentation pathway portrayed in Scheme 1.

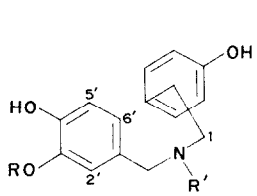


(i) H_2–Pt; P_2O_5–toluene; MeI–$\bar{O}$H. (ii) MeI–$\bar{O}$H (iii) $KMnO_4$. (iv) HBr.

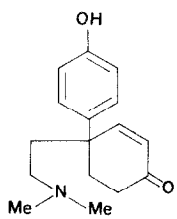
SCHEME 2.

[9] HAWORTH, R. D. and DE SILVA, L. B. (1951) *J. Chem. Soc.*, 3511. For a more recent application in the alkaloid field see LEETE, E. and NEMETH, P. E. (1960) *J. Am. Chem. Soc.* **82**, 6056.

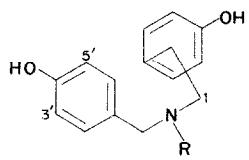
Since the results of the phenylalanine experiment appeared to provide support for the modified scheme, we sought to extend the investigation of the possible role of the various norbelladines in the biosynthesis of mesembrine. Rather than use the labelling pattern selected previously for 3′-*O*-methylnorbelladine a more decisive test of the ideas presented in Scheme 1 could be made by testing its bioconversion to mesembrine if it were labelled at the *O*-methyl and C-1 carbons with ^{14}C in a known ratio. Utilisation of this labelled precursor in the manner envisioned should give rise to mesembrine alkaloids containing ^{14}C labels at the 3′-*O*-methyl and C-2 carbons. Compound **18** (3′-*O*-methyl-^{14}C:1-^{14}C ratio = 71:24) was synthesized and fed to *S. strictum*. The majority of the activity of the alkaloid fraction from this feeding experiment was associated with mesembrenol and the necessary degradation experiments, which are discussed, are depicted in Scheme 2. When the mesembrenol was oxidized to veratric acid only 20% (instead of the expected 71%) of the relative molar activity (rma) was present in the acid. This result was corroborated by an independent series of reactions in which the radio-labelled mesembrenol was reduced to mesembranol and then converted to 3,4-dimethoxybiphenyl (0·22 rma) by a series of reactions which have been previously described.[2,10] The latter sequence showed that 0·12 rma was present in the *N*-methyl group. Furthermore, selective demethylation of the radiolabelled veratric acid to isovanillic acid, which could be achieved with HBr under carefully controlled conditions, showed that the label was distributed between both methoxyl groups. It was evident from this information that 3′-*O*-methylnorbelladine is not incorporated intact into the mesembrine ring system. Scatter of the activity between the two methoxyls and the *N*-methyl group indicated that the C-1 pool was being labelled, presumably through *O*-demethylation of the 3′-*O*-methyl group of the test precursor or one of its transformation products.



(19) R = R′ = H, 2′,6′-^{3}H; 1-^{14}C
(20) R = Me, R′ = H, 5′-^{3}H; 1-^{14}C
(21) R = H, R′ = Me, 2′,6′-^{3}H; 1-^{14}C
(22) R = H, R′ = Me, 5′-^{3}H, 1-^{14}C
(23) R = R′ = Me, 5′-^{3}H; 1-^{14}C



(24)



(25) R = H, 3′, 5′-^{3}H; 1-^{14}C
(26) R = Me, 3′, 5′-^{3}H; 1-^{14}C

Confirmation that 3′-*O*-methylnorbelladine was not simply undergoing *O*-demethylation to norbelladine before incorporation was obtained by the observation that both norbelladine and its 3′-*O*-methyl derivative, containing ^{3}H and ^{14}C-labels as indicated in structures **19** and **20**, respectively, when fed to *S. strictum* plants resulted in mesembrine alkaloids in which the ^{3}H:^{14}C ratios were markedly different in each case from that of the test precursors (see Table 1). Obviously both norbelladine and its 3′-*O*-methyl derivative suffer cleavage prior to incorporation into the alkaloid ring system. Thus the hypothesis outline in Scheme 1 is incorrect despite the results of the phenylalanine experiments.

[10] POPELAK, A., HAACK, E., LETTENBAUER, G. and SPINGLER, H. (1960) *Naturwissenschaften* **47**, 156.

Despite these discouraging results with the norbelladine series, it seemed worthwhile to examine two further examples of this ring system as possible precursors to the mesembrine alkaloids. Previous studies by Barton and coworkers[3] on the biosynthesis of the Amaryllidaceae alkaloid galanthamine in a *Narcissus* species of daffodil have demonstrated that whereas norbelladine was not incorporated into this alkaloid, *N*-methylnorbelladine proved to be a good precursor. Obviously this plant is unable to convert norbelladine to its *N*-methyl derivative.[11] Therefore to cover the possibility of this contingency in *S. strictum* it was necessary to examine *N*-methyl and 3′-*O*,*N*-dimethylnorbelladines as possible biosynthetic intermediates in the mesembrine series. The two doubly labelled radioisomers of *N*-methylnorbelladine **21** and **22** and the doubly labelled 3′-*O*,*N*-dimethylnorbelladine **23** were synthesized and then administered in separate experiments to *S. strictum* plants. A radioscan of the TLC plate of the crude alkaloid fraction from each of the three experiments showed only a single radioactive zone, in each case, which exhibited ^{3}H:^{14}C ratios similar to the corresponding values of the respective test precursors. The R_f value of the radioactive component corresponded approximately with the R_f values of mesembrine and mesembrenone. However, subsequent dilution experiments with cold mesembrine and mesembrenone demonstrated that the radioactivity was not associated with either of these alkaloids. Since the sub-milligram amounts of the radioactive metabolite precluded attempts to carry out structural investigations, the possibility that it was an aberrant product derived from the test precursors through simple *O*-methylation, or *O*-demethylation and *O*-methylation at a different site, was examined by chromatographic comparisons with all six *O*,*N*-dimethyl- and *O*,*O*,*N*-trimethylnorbelladines and belladine. Of these compounds, only belladine had comparable chromatographic properties to that of the radioactive component. However, the non-identity of the radiolabelled metabolite and belladine was demonstrated by the isotope dilution method. In our experience all radiolabelled precursors which are incorporated intact have resulted in a distribution of activity among all the alkaloids of *S. strictum*. It would appear that this test precursor is being converted to a single metabolite as a result of the occurrence of an aberrant pathway (*vide infra*).

The last remaining possibility that appeared worthwhile investigating in terms of a C_6–C_1–N–C_2–C_6 precursor to the mesembrine series was prompted by a report of the isolation of joubertiamine **24** and several related alkaloids from *S. joubertii*.[12] The single oxygen function on the aromatic ring in a ring system which is obviously biogenetically related to the octahydroindole skeleton of the mesembrine alkaloids suggested consideration be given to the possibility that one of the aromatic oxygen functions in the mesembrine series is introduced at a late stage in the pathway.[13] 3′-Deoxynorbelladine and 3′-deoxy-*N*-methylnorbelladine were synthesized to provide the labelling pattern as indicated in **25** and **26** respectively. When these compounds were administered independently to *S. strictum* and the alkaloid fraction subsequently isolated, examination of the radiochromatogram indicated the presence of a single radiolabelled component in each case. Although it was not possible to identify either of these metabolites, the suggestion that they occurred as a result of an aberrant process was supported by the large

[11] More recently a report has been made that 4′-*O*-methylnorbelladine can serve as a precursor to galanthamine in *Leucojum aestivum* [FUGANTI, C. (1969) *Chim. Ind.* (*Milan*) **51**, 1254].

[12] ARNDT, R. R. and KRUGER, P. E. J. (1970) *Tetrahedron Letters* 3237.

[13] A recent example of such a process has been reported, see COLLINS, J. F., DONNELLY, W. J., GRUNDON, M. F., HARRISON, D. M. and SPYROPOULOS, C. G. (1972) *Chem. Commun.* 1029.

difference observed in their ^3H:^{14}C ratios from that of the administered test precursors (see Experimental) and the demonstration of their non-identity with any of the known alkaloids of the plant.

Thus, the evidence described clearly demonstrates that despite the strong structural similarity between the crinine and mesembrine alkaloids and derivation of the basic skeletons of both from tyrosine and phenylalanine to provide analogous structural entities, the metabolic pathways involved in the biosynthesis of the alkaloid families are fundamentally different. This is evident in the mesembrine series both from the manner in which phenylalanine is incorporated, which must surely implicate a ring A-spirodienone intermediate, and by the elimination of the logical precursors based upon an $Ar–C_1–\acute{N}–C_2–C_6$ structural unit.

In retrospect, such differences in the metabolic pathways to the crinine and mesembrine alkaloids are not entirely unexpected in view of the widely different phylogenetic origins of the Amaryllidaceae and Aizoaceae families.

In comparison with other similar studies reported on biosynthesis of alkaloids, the studies reported in this account indicate that *S. strictum* exhibits a remarkable versatility in its ability to metabolise unnatural substrates in various ways. Some of these aberrant pathways with radiolabelled compounds lead to incorporation of significant levels of radioactivity in the alkaloids of this species. This presents potential pitfalls for interpretation of the results which are only avoided by carefully selected experiments with doubly labelled test precursors in which labels are judiciously positioned. Much of the foregoing results reported herein would have led to erroneous conclusions with singly labelled substrates.

EXPERIMENTAL

The general procedures and the methods of administering labelled test precursors are as described previously[2] unless otherwise noted. Plants used in these studies were grown in an artificial medium in the Duke University Phytotron facility with conditions controlled to give a 16 hr photo period with an 8 hr day temp. of 20° and a 16 hr temp. of 17°. All radiolabelled synthetic intermediates were assayed for purity by TLC and subsequent radioscanning of the plate.

[$^3H_4$]*Norbelladine* (**5**). Tyramine (225 mg) was heated at 100° for 2·5 hr in 1·0 ml ^3H_2O [120 mCi] to which 0·16 ml $SOCl_2$ had been previously added. [3,5-^{3}H]Tyramine (200 mg : 2·05 mCi/mmol) was obtained by dilution of the reaction mixture with H_2O, basification with NH_4OH and repeated extraction with $CHCl_3$ followed by crystallisation from C_6H_6–MeOH. A portion of the sample was diluted with carrier and converted to 3,5-dibromotyramine hydrobromide, m.p. 267·5–268·5° [lit.[14] m.p. 270°] which showed less than 0·5% of the activity of the starting material. *O,O*-Dibenzyl[2,6-^{3}H]protocatechuic aldehyde (120 mg : 2·97 mCi/mmol), obtained from [2,6-^{3}H]isovanillin,[15] was combined with tyramine (50 mg : 2·05 mCi/mmol) and the mixture dissolved in 10 ml MeOH under reflux for 30 min. The soln on cooling deposited the imine, m.p. 160–162° after recrystallization from MeOH. A soln of the imine (145 mg) in MeOH (20 ml) was stirred with H_2 over 10% Pd/C (65 mg) in 5 ml MeOH until there was no further uptake of H_2. To this soln was added 0·1 ml conc. HCl and the hydrogenation continued for a further 15 min until the uptake of H_2 ceased. After filtration of the soln, the solvent was removed and the residue crystallized from MeOH–acetone to give norbelladine hydrochloride (20 mg : 5·09 mCi/mmol), m.p. 192–195° dec. (lit.[3] m.p. 175–176°).

4′-O-*Methyl*[$^3H_4$]*norbelladine* (**6**). [3′,5′-^{3}H]Tyramine (25 mg : 2·05 mCi/mmol) and [2,6-^{3}H]isovanillin (28·9 mg : 3·05 mCi/mmol) were mixed in 1 ml MeOH–EtOH (1 : 1). After standing for 31 hr, the soln was hydrogenated (2 hr) in 15 ml EtOH containing 20 mg 10% Pd/C. After removing the catalyst on celite, the soln was concentrated and treated with ethanolic HCl. The hydrochloride of **6** was obtained from EtOH–Et_2O as tan crystals (38·4 mg : 5·53 mCi/mmol). Two recrystallizations from EtOH–Et_2O gave radio chemically pure material (5·33 mCi/mmol), m.p. 206–207° (lit.[16] 205–207°).

[14] ZEYNEK, R. (1921) *Z. Phys. Chem.* **114**, 283.
[15] KIRBY, G. W. and ONGUKOYA, L. (1965) *J. Chem. Soc.* 6914.
[16] KIRBY, G. W. and TIWARI, H. P. (1960) *J. Chem. Soc.* 676.

3′-O-*Methyl*[$^3H_3$]*norbelladine* (**7**). A soln [5-^{3}H]vanillin (28·9 mg, 0·19 mmol : 4·21 mCi/mmol) and [4,6-$^3H_2$]tyramine (25 mg, 0·18 mmol : 2·05 mCi/mmol) in MeOH (1·0 ml) was allowed to stand at room temp. for 22 hr. The imine (43·5 mg: 88·3% yield; 6·34 mCi/mmol), which separated as gold crystals, was collected and washed with MeOH (1 ml). The imine was converted to the hydrochloride of **7** as described above to give crystals, m.p. 176–177° (lit.[16] m.p. 170–171°) (34·5 mg: 68·5% yield) after crystallization from EtOH–Et_2O (6·36 mCi/mmole) (Found: C, 62·61; H, 6·42; N, 4·27. Calc. for $C_{16}H_{20}NO_3Cl$: C, 62·03; H, 6·51; 4·52%). The free base regenerated from the hydrochloride crystallized from EtOAc to give prisms m.p. 143–143·5° (Found: C, 70·26; H, 6·83; N, 5·12. $C_{16}H_{19}NO_3$ requires: C, 70·31; H, 7·01; N, 5·12%). In later experiments it was sometimes found that catalytic reduction of the imine double bond caused cleavage at this site. The borohydride reduction, described below is therefore recommended over the procedure used above.

4′-O-*Methyl*[1-^{14}C]*norbelladine* (**10**). This material was prepared from [1-^{14}C]tyramine hydrobromide (4·1 mg : 100 mCi) and isovanillin (20 mg) according to the lit. procedure to give 18 mg of norbelladine as its hydrochloride (0·902 μCi/mmol).

3′-O-*Methyl*[1-^{14}C]*norbelladine* (**11**). A mixture of vanillin (20 mg, 0·131 mmol), Na_2CO_3 (25 mg, 0·236 mmol), and [1-^{14}C]tyramine hydrobromide (4·1 mg : 0·0187 mmol; 0·10 mCi) in MeOH (0·5 ml) was briefly warmed with shaking and then allowed to stand for 2 days at room temp. The soln was diluted with 10 ml MeOH and stirred with an excess of KBH_4 (*ca* 50 mg) until effervescence ceased. H_2O (5 ml) was added and the MeOH evaporated. Enough conc. HCl was then added cautiously to the aq. residue to give an acidic soln and 19 mg of inactive **11** added. The soln (*ca* 5 ml) was transferred to a Gould extractor,[17] basified with Na_2CO_3 and continuously extracted with $CHCl_3$. The $CHCl_3$ extract was evaporated and the residue was taken up in 10% HCl (*ca* 5 ml) and washed 1 × with Et_2O (10 ml). The acidic soln was neutralized with Na_2CO_3 and extracted with EtOAc (2 × 10 ml). After evaporation of the EtOAc the hydrochloride of **11** was prepared in EtOH and addition of Et_2O gave crystals of 3′-*O*-methyl[1-^{14}C]norbelladine hydrochloride (14 mg: 639 μCi/mmol). This procedure was repeated several times to furnish additional quantities of **11** of higher specific activities.

DL-[2′,6′-^{3}H, 1′-^{14}C]*Phenylalanine* (**15**). 2′-Bromo-DL-phenylalanine (20 mg) in 1·0 ml 1·5 N KOH was reduced with tritium gas (3 Ci) in the presence of 10 mg 10% Pd/C for 2 hr.[18] The reduction was completed with H_2 for an additional 1 hr and the product was distilled under reduced pressure to remove labile tritium. The residue (10 mg: 16·5 Ci/mmol) was dissolved in 10 ml H_2O and stored at −15°. A sample (0·1 ml) from the solution of the labelled phenylalanine was removed and 9 mg inactive DL-phenylalanine added. The soln was acidified with dil. HCl and then lyophilized to a solid which was crystallized 2 × from EtOH to give the pure hydrochloride of DL-[2′,6′-^{3}H]phenylalanine. A mixture of this tritium labelled material (0·234 mg, 1·05 mCi) with DL-[1′-^{14}C]phenylalanine (2·4 mg, 0·05 mCi) was dissolved in 0·5 ml 0·1 N HCl to give the double labelled amino-acid[15] which possessed $^3H : {}^{14}C = 21·0$.

3′-O-*Methyl*[3′-O-*methyl*-^{14}C, 1-^{14}C]*norbelladine* (**18**). Radiolabelled MeI (11·9 mg : 0·084 mmol; 250 μCi) was transferred on a vacuum line to a soln containing 4-benzyloxy-3-hydroxybenzaldehyde (57·5 mg, 0·252 mmol) and anhyd. K_2CO_3 (55 mg) in dry acetone (4 ml). The contents were sealed *in vacuo* and heated at 65° for 96 hr with stirring. After cooling (liq. N_2), the seal was broken and 2 ml inactive MeI added. The soln was refluxed for 8 hr to complete methylation and the contents then filtered through cotton to remove solid K_2CO_3 which was washed with acetone. The filtrate was concentrated under vacuum to afford *O*-benzyl[methyl-^{14}C]vanillin. This was mixed with *O*-benzyltyramine hydrochloride (75 mg, 0·284 mmol), and K_2CO_3 (65 mg, 0·47 mmol) in MeOH (1 ml) and allowed to stand at room temp. for 12 hr. The solid imine which precipitated was collected and washed successively with 2 ml portions of cold EtOH and H_2O. The imine (60 mg) was next reduced with an excess of KBH_4 (170 mg, 3·15 mmol) in MeOH (12 ml) at room temp. After standing for 2 hr, H_2O (2 ml) was added and the MeOH removed. The aq. residue was extracted continuously with $CHCl_3$ in a Gould extractor, and the $CHCl_3$ subsequently removed *in vacuo*. The residue provided crystals of the dibenzyloxy amine hydrochloride (53 mg; 80% yield; 1·59 mCi/mmol) upon treatment with ethanolic HCl. This material was hydrogenated in 5·5 ml EtOH containing 10% Pd/C (5 mg) to give 3′-*O*-methyl[methyl-^{14}C]norbelladine hydrochloride. Recrystallisation from EtOH-Et_2O afforded 25 mg of crystalline material which was radiochemically pure by autoradiography (1·52 mCi/mmol). The title compound was prepared by mixing accurately weighed portions of the hydrochlorides of the above compound and 3′-*O*-methyl[1-^{14}C]norbelladine in H_2O (0·5 ml) prior to feeding. The activity of the composite was thus 1·64 mCi/mmol, and the methoxyl to C-1 activity ratio was set at 2·45.

[5′-^{3}H, 1-^{14}C]*Norbelladine* (**19**). (a) [5′-^{3}H]Norbelladine. 3-Benzyloxy-4-hydroxybenzaldehyde (210 mg, 0·92 mM) was heated at 100° for 96 hr in 0·3 ml ^3H_2O (0·25 Ci) to which sodium *t*-butoxide (50 mg, 0·52 mM) had been previously added. Recovery and purification of the product gave 143 mg (63%) of the [5-^{3}H]-aldehyde (6·54 mCi/mM). A mixture of this aldehyde (70 mg, 0·3 mM) and tyramine (46 mg, 0·3 mM) was dissolved in MeOH (5 ml) and the soln refluxed for 5 hr. The imine was not isolated but hydrogenated

[17] GOULD, B. S. (1943) *Science* **98**, 546.

[18] The catalytic tritiation was carried out by New England Nuclear, Boston, Mass. according to directions kindly supplied by Drs. J. W. Daly and G. Guroff, National Institutes of Health.

directly to [5′-^{3}H]norbelladine hydrochloride (67 mg, 4·5 mCi/mM) as indicated previously. (b) [1-^{14}C]-Norbelladine. A soln of [1-^{14}C]tyramine hydrobromide (9·3 mg, 0·043 mM, 0·143 mCi), 3,4-dibenzyloxybenzaldehyde (205 mg, 0·645 mM) and K_2CO_3 (16 mg, 0·116 mM) in 25 ml MeOH was refluxed for 17 hr. After cooling the soln, 200 mg KBH_4 was added and the mixture stirred for 3 hr. The soln was acidified with HOAc and the product isolated. Chromatography over alumina (act III) and then rechromatography over alumina (act II) in $CHCl_3$ was necessary to remove traces of contaminating tyramine from the 3′,4′-dibenzyloxy[1-^{14}C]norbelladine (37 mg, 1·2 mCi/mM). This sample was combined with 36 mg of inactive material and hydrogenolysed with 10 mg Pd/C in MeOH (5 ml) containing 3 drops conc. HCl to give [1-^{14}C]norbelladine hydrochloride (48 mg, 56 μCi/mM). The radiochemical purity of this sample was established by TLC on silica gel H in MeOH–$CHCl_3$–NH_4OH (10 : 9 : 1) followed by a radioscan of the plate. Tyramine, which was previously shown to be well separated from norbelladine in this solvent system, was absent. The doubly labelled sample [5′-^{3}H, 1-^{14}C]norbelladine was obtained by mixing the two singly labelled compounds to give ^{3}H:^{14}C = 9·1.

3′-*O*-*Methyl*[2′,6′-^{3}H,1-^{14}C]*norbelladine* (**20**). A mixture of *O*-benzyltyramine hydrochloride (210 mg, 0·79 mmol), 3-hydroxy-4-benzyloxybenzaldehyde (194 mg, 0·85 mmol), and 220 mg (1·7 mmol) of K_2CO_3 were refluxed for 1 hr in MeOH. The soln was cooled to room temp. and KBH_4 (200 mg, 3·7 mmol) was added with stirring. After 2 hr the reaction mixture was diluted with an equal vol. H_2O and the solvents removed on the rotovac. The residue was acidified with dil. HCl, then brought to pH 8 with Na_2CO_3. The resulting suspension was continuously extracted with EtOAc. Evaporation of EtOAc and treatment of the residual oil with ethanolic HCl afforded 4-benzyloxy-*N*-(3-hydroxy-4-benzyloxybenzyl)phenethylamine as its hydrochloride which formed plates upon recrystallization from EtOH (250 mg, 66% yield), m.p. 204–206°. (Found: C, 73·29; H, 6·25; N, 3·05. $C_{29}H_{29}NO_3$ requires: C, 73·17; H, 6·35; N, 2·94%). In another experiment, the amine was isolated as its free base in 76% yield, m.p. 125·5–126·5°; IR ($CHCl_3$): 3550 cm^{-1} (sharp, OH); NMR ($\delta_{TMS}^{CDCl_3}$): 7·38 (*s*, 10 H), 7·25–6·25 (complex, 7 H), 5·03 (*bs*, 4 H), 3·85 (broad signal, 2 H), 3·67 (*s*, 2 H), 2·78 (*s*, 4 H); MS: *m/e* 439 (M^+). The amine (64 mg) was tritiated under basic conditions in 0·25 ml (250 mCi) tritiated H_2O. After addition of 12·5 mg of carrier on completion of the exchange, the crude product was chromatographed on neutral alumina (activity V) in $CHCl_3$ to give the pure tritiated amine (30·8 mg; 5·29 mCi/mmol). This material was then dissolved in MeOH–Et_2O (5 ml, 1 : 1) and diluted with an equal vol. of freshly distilled ethereal CH_2N_2. The soln was allowed to stand at −25° for 7 days, then at 10° for 19 days, and at room temp. for 4 days. The solvents were removed by slow evaporation *in vacuo* to obtain an oil which solidified. Treatment of the solid with ethanolic HCl and crystallisation from EtOH provided 19 mg of the hydrochloride of 4-benzyloxy-*N*-(3-methoxy-4-benzyloxy[2,6-^{3}H]benzylphenethylamine) (55% yield; 5·34 mCi/mmol) as crystals. Hydrogenolysis of this material was carried out as previously described to afford 3′-*O*-methy[2′,6′-^{3}H]norbelladine (10 mg, 4·82 mCi/mmol). The radiochemical purity of this material was ascertained when the single spot on the papergram contained 99·8% of the total activity of the strip (liquid scintillation counting of cut sections). The double isotope labelled 3′-*O*-methylnorbelladine (**20**) was then prepared by mixing weighed quantities of the above material with 3′-*O*-methyl[1-^{14}C]norbelladine which had been prepared as previously described. The calculated and observed ^{3}H : ^{14}C ratio was 5·17 and 5·12, respectively.

N-*Methyl*[2′,6′-^{3}H,1-^{14}C]*norbelladine*(**21**). 3-Hydroxy-4-benzyloxybenzaldehyde (1·00 g, 4·39 mmol) was dissolved in 40 ml EtOH in a flask fitted with a dry ice–acetone condenser. An excess of gaseous $MeNH_2$ was passed into the soln and the reaction mixture was stirred at room temp. for 2 hr. KBH_4 (550 mg) was added and stirring was continued for an additional 4 hr. The ethanol was evaporated under reduced pressure and H_2O was added to the residue followed by adjustment of the pH to 8 by addition of 10% HCl. The aq. suspension was extracted with $CHCl_3$ and the $CHCl_3$ soln was dried. The $CHCl_3$ was evaporated leaving behind 1·06 g (99% yield) of a grey solid. This material was shown to be the desired secondary amine (**31**) by its spectral properties: IR ($CHCl_3$), 3650 cm^{-1} and 3550 cm^{-1}; NMR ($CDCl_3$), δ 7·35 (*s*, 5H), δ 6·78 (*m*, 3H), δ 5·01 (*s*, 5H), δ 3·53 (*s*, 2H), δ 2·30 (*s*, 3H); ($CDCl_3$ + D_2O), δ 5·01 (*s*, 2H), remainder of spectrum was unchanged. The secondary amine (549 mg, 2·26 mmol) was dissolved in 20 ml dry C_6H_6 and 294 mg (1·13 mmol) of 4-benzyloxyphenylacetyl chloride (**25**) in 15 ml dry C_6H_6 was added dropwise with stirring. The reaction mixture was stirred at room temp. for 6 hr and then filtered to remove solid material. The C_6H_6 filtrate was washed with 10% HCl (3×), $NaHCO_3$ soln (3×), and H_2O (5×) and then dried. Evaporation of the C_6H_6 resulted in 518 mg (98% yield of a clear oil. This material was shown to be the desired amide by its spectral properties: IR ($CHCl_3$), 3550 cm^{-1} and 1640 cm^{-1}; NMR $\delta_{TMS}^{CDCl_3}$ 7·28 (*s*, 10H), 6·80 (*m*, 7H), 4·92 (*s*, 2H), δ 4·90 (*s*, 2H), δ 4·40 (*s*, 1H), 4·28 (*s*, 1H), 3·60 (*s*, 2H), 2·82 (*s*, 1·5H), and 2·72 (*s*, 1·5H). The tertiary amide (315 mg, 0·675 mmol) dissolved in 10 ml dry C_6H_6 was added dropwise to a refluxing suspension of 250 mg $LiAlH_4$ in 20 ml Et_2O and refluxing was continued for 10 hr. The reaction was quenched by dropwise addition of 10% NH_4Cl and the reaction mixture was filtered to remove the grey pasty solid. The organic and aqueous layers in the filtrate were separated and the aq. phase was extracted with Et_2O. The combined Et_2O solns were dried. The solvent was evaporated under reduced pressure leaving behind 263 mg (86% yield) of a light yellow solid. Recrystallization from EtOH gave 4-benzyloxy-*N*-(3-hydroxy-4-benzyloxybenzyl)-*N*-methylphenylethylamine as needles, m.p. 98–99°; NMR, $\delta_{TMS}^{CDCl_3}$ 7·28 (*s*, 10H),

6·80 (*m*, 7H), 4·96 (*s*, 4H), 3·40 (*s*, 2H), 2·64 (*s*, 4H) and 2·20 (*s*, 3H) (Found: C, 79·65; H, 6·74; N, 3·06. $C_{30}H_{31}NO_3$ requires: C, 79·47; H, 6·84; N, 3·09%). Tritiation of the above amine (170 mg) in (0·2 ml, ^3H_2O 500 mCi) under standard conditions provided the desired tritiated base (96 mg) which was hydrogenolysed to *N*-methyl-[2′,6′-^{4}H]norbelladine hydrochloride (60 mg, 21·4 mCi/mmol), m.p. 206–207° (lit.[3] m.p. 207–208°). The doubly labelled sample **21** was prepared mixing the tritiated material with *N*-methyl-[1-^{14}C]norbelladine, which was prepared from *p*-benzyloxyphenyl-acetic acid labelled with ^{14}C in the carboxyl group, to provide *N*-methyl-[2,6-^{3}H,1-^{14}C]norbelladine (**21**) with ^{3}H:^{14}C = 21·2.

N-*Methyl*-[5′-^{3}H,1-^{14}C]*norbelladine* (**22**). 3-Benzyloxy-4-hydroxy-5-tritiobenzaldehyde (70 mg, 6·42 mCi/mmol) was carried through the analogous synthetic sequence described above (with appropriate dilutions to obtain sufficient material) to yield *N*-methyl-[5′-^{3}H]norbelladine (36 mg, 2·15 mCi/mmol). Admixture of this material with the ^{14}C-labelled compound provided the doubly labelled *N*-methyl norbelladine (**22**) with ^{3}H:^{14}C = 9·92. In a pilot experiment with non-radioactive material the intermediate *4-benzyloxy*-N-(*3-benzyloxy-4-hydroxybenzyl*)-N-*methylphenethylamine* was characterized as a crystalline hydrochloride, m.p. 154–155°, NMR $\delta_{TMS}^{CDCl_3}$ 7·28 (*s*, 10H), 6·82 (*m*, 7H), 4·90 (*s*, 4H), 3·40 (*s*, 2H), 2·64 (*s*, 4H) and 2·20 (*s*, 3H) (Found: C, 73·43; H, 6·72; N, 2·77. $C_{30}H_{32}NO_3Cl$ requires: C, 73·54; H, 6·54; N, 2·86%).

3′-O,N-*Dimethyl*[5′-^{3}H,1-^{14}C]*norbelladine* (**23**). Condensation of vanillin (3·0 g) with $MeNH_2$ followed by reduction of the product with KBH_4 afforded *N*-(3-methoxy-4-hydroxybenzyl)-*N*-methylamine which was characterized by its spectral properties: IR ($CHCl_3$) 3650 cm^{-1}, 3550 cm^{-1}; NMR ($CDCl_3$) δ 6·82 (*m*, 3H), δ 5·58 (*s*, 2H), δ 3·70 (*s*, 5H) and δ 2·42 (*s*, 3H). This amine (257 mg 1·54 mmol) was reacted with 4-benzyloxyphenacetyl chloride (200 mg, 0·768 mmol) to give the amide (270 mg); IR 3550 cm^{-1} and 1640 cm^{-1}. Reduction of the amide (199 mg) with $LiAlH_4$ (50 mg) using the procedure described in the previous experiment gave *4-benzyloxy-N-(3-methoxy-4-hydroxybenzyl)-N-methylphenethylamine* (135 mg) which crystallized from ethanol, m.p. 145–146°: NMR, $\delta_{TMS}^{CDCl_3}$ 7·28 (*s*, 5H), 6·83 (*m*, 7H), 4·98 (*s*, 2H), 3·75 (*s*, 3H), 3·42 (*s*, 2H), 2·66 (*s*, 4H) and 2·22 (*s*, 3H) (Found: C, 76·58; H, 7·01; N, 3·96. $C_{24}H_{27}NO_3$ requires: C, 76·39; H, 7·16; N, 3·71%). The above amine (200 mg) in 0·2 ml of ^3H_2O (500 mCi) and 0·6 ml dimethylformamide was heated in N_2 in a sealed tube at 100° for 90 hr. On cooling the reaction mixture, crystals of the tritiated material (142 mg) were obtained and two crystallizations from EtOH afforded the pure [5′-^{3}H]amine (117 mg, 5·16 mCi/mmol). Hydrogenolysis of this amine (82 mg) afforded the hydrochloride 3′-*O*,*N*-dimethyl-[5′-^{3}H]norbelladine (62 mg, 4·90 mCi/mmol), m.p. 184–186° from EtOH (Found: C, 71·15; H, 7·30; N, 4·75. $C_{17}H_{21}NO_3$ requires: C, 71·05; H, 7·4; N, 4·9%). A repetition of the synthesis employing 4-benzyloxyphenacetyl chloride derived from ^{14}C-carboxyl labelled 4-benzyloxyphenylacetic acid (150 mg, 166 μCi/mmol) gave 3′-*O*,*N*-dimethyl[1-^{14}C]norbelladine hydrochloride (118 mg, 168 μCi/mmol), m.p. 184–186°. Admixture of appropriate quantities the two labelled 3′-*O*,*N*-dimethylnorbelladine samples provided the doubly labelled compound **23** ^{3}H:^{14}C = 16·6.

3′-*Deoxy*[3′,5′-^{3}H,1-^{14}C]*norbelladine* (**25**). Tyramine hydrochloride was condensed with 4-hydroxy-[3,5-^{3}H]-benzaldehyde and the product reduced with KBH_4 according to the procedures described earlier. A repeat of the procedure employing [1-^{14}C]tyramine and admixture of the hydrochlorides of ^{3}H-(**24**) (45 mg, 4·7 mCi/mmol) and ^{14}C-(**24**) (7·7 mg, 0·8 mCi/mmol) gave the title compound with ^{3}H:^{14}C = 18·5. An experiment with unlabelled material afforded 3′-deoxynorbelladine hydrochloride, m.p. 241–242° $\delta_{TMS(ext)}^{D_2O}$ 3·20 (*m*, 4H), 4·23 (*s*, 2H), 6·9–7·6 (*m*, 8H); MS (free base) Calc. *m/e* for $C_{15}H_{17}NO_2$ 243·1259; Found *m/e* 243·1265.

3′-*Deoxy*-N-*methyl*-[3′,5′-^{3}H,1-^{14}C]*norbelladine* (**26**). Methylamine was bubbled for 2 hr into a stirred soln of 4-hydroxybenzaldehyde (2·44 g, 20·0 mmol) in MeOH (25 ml) in a flask fitted with a dry ice–acetone condenser. Then KBH_4 (700 mg, 13·0 mmol) was added and the mixture was stirred overnight at room temp. After 15 hr, excess $MeNH_2$ was removed *in vacuo*. After acidification with conc. HCl and filtration of the precipitated inorganic salts, the solvent was removed *in vacuo* to leave orange crystals. Recrystallization from abs. EtOH gave pale tan plates, 2·17 g (79%): m.p. 191·5–192°; NMR $\delta_{TMS(ext)}^{D_2O}$ 2·73 (*s*, 3H), 4·13 (*s*, 2H), 6·99 (*d*, 2H, *J* 8 Hz), 7·42 (*d*, 2H, *J* 8 Hz) (Found: C, 55·49; H, 7·07; N, 7·99. $C_8H_{12}N$ requires: C, 55·41; H, 6·93; N, 8·02%). Hexamethyldisilazane (1·5 ml) was added to the amine hydrochloride (244 mg, 1·43 mmol) in a flame dried flask and the mixture was refluxed 1·5 hr, at which time soln was complete. Removal of solvent *in vacuo* left a pale brown oil, 334 g (96%), which was used immediately without purification as follows: A soln of 4-benzyloxyphenacetyl chloride (231 mg, 0·89 mmol) in anhyd. C_6H_6 (3 ml) was added dropwise to a stirred soln of the amine (334 mg, 1·72 mmol) in anhyd. C_6H_6 (3 ml) in a flame dried flask in a N_2 atmosphere. The reaction mixture was stirred at room temp. overnight. The mixture was then filtered, diluted with EtOAc (30 ml) and washed with 1 N HCl (2 × 20 ml) and satd aq. Na_2CO_3 (2 × 20 ml). After drying, solvent was removed to render a pale yellow oil. This was dissolved in 75% aq. EtOH (20 ml) and refluxed in N_2 1·5 hr. H_2O (50 ml) was added, and the cloudy mixture was extracted with $CHCl_3$ (3 × 30 ml) and the extracts dried. Evaporation of the solvent afforded *N*-(*4-benzyloxyphenacetyl*)-*4-hydroxybenzylmethylamine* as a frothy solid, 272 mg (85%): IR ($CHCl_3$), 1615 cm^{-1} (C=C), 1630 cm^{-1} (C=O); $\delta_{TMS}^{CDCl_3}$ 2·94 (*d*, 3H), 3·77 (*s*, 2H), 4·53 (*d*, 2H), 5·07 (*s*, 2H), 6·7–7·4 (*m*, 8H), 7·40 (*s*, 5H) (Found: *m/e* 361·1672. $C_{23}H_{23}NO_3$ requires: *m/e* 361·1678). Reduction of the amide (228 mg) with $LiAlH_4$ in THF afforded *N*-(4-hydroxybenzyl)-*N*-methyl-2-(4-benzyloxyphenyl)ethylamine (206 mg) as a pale oil; NMR, $\delta_{TMS}^{CDCl_3}$ 2·33 (*s*, 3H), 2·72 (*s*, 4H), 3·57 (*s*, 2H), 5·06 (*s*, 2H), 6·6–7·4 (*m*, 8H) 7·4 (*s*, 5H) (Found: *m/e* 347·1880.

$C_{22}H_{25}NO_2$ requires: *m/e* 347·1885). Hydrogenolysis of the *O*-benzylamine gave the title compound as an amorphous solid from EtOH–Et_2O (Found: *m/e* 257·1410. $C_{16}H_{19}NO_2$ requires: 257·1416). 3′-Deoxy-*N*-methyl[3′,5′-^{3}H]norbelladine was obtained by subjecting *N*-(4-benzyloxyphenacetyl)-4-hydroxybenzylmethylamine (110 mg) to exchange in ^3H_2O (0·10 ml, 250 mCi) in DMF (0·3 ml). After purification of the tritiated material, it was hydrogenolysed and the product recrystallized (×2) from EtOH–Et_2O to give the pure 3′-Deoxy[3′-5′-^{3}H]-*N*-methylnorbelladine (55 mg, 2·93 mCi/mmol). Admixture of this sample with ^{14}C-labelled material (0·82 mCi/mmol) prepared from ^{14}C-carboxyl¶ labelled 4-benzyloxyphenylacetic acid gave double labelled compound **25** with $^3H : ^{14}C = 19·5$.

Isolation and purification of alkaloids derived from experiments with radiolabelled precursors. The crude alkaloid extract, obtained as previously described, was examined on a silica gel TLC plate in $CHCl_3$–MeOH (3 : 1) against standards of known alkaloids, and radioscanned to determine the amount and distribution of radioactivity amongst the alkaloids. For additional verification, the active zones were scraped off the TLC plate, the compound extracted and then examined by GLC on SE-30 and Carbowax 20 M columns. In all cases where incorporation of activity into the alkaloids occurred the radio-label was distributed among several alkaloids. In feeding experiments where incorporation of activity was associated with the alkaloid fraction, most resided in mesembrenol, mesembrenone and mesembrine. In such cases, the crude alkaloid fraction (*ca* 20–50 mg) was combined with inactive mesembrenol, mesembrenone and mesembrine and the mixture chromatographed on neutral alumina (act 2, 20–30 g) in C_6H_6–EtOAc–MeOH. To achieve radioachemically pure samples, mesembrenol was crystallized from EtAc, m.p. 144–145° and mesembrenone and mesembrine were crystallized as their hydrochlorides from propan-2-ol and MeOH–Et_2O, respectively.

Feeding experiments and degradation of radiolabelled alkaloids. Experiments with the feeding of the test precursors **5**, **6**, **7**, **10** and **11** since they did not involve degradation of the derived alkaloids are adequately described by the results reported in Table 1.

Incorporation of DL-[2′,6′-^{3}H,1′-^{14}C]*phenylalanine* (**15**). Radioscanning of a TLC chromatoplate of a portion of the crude alkaloid fraction (20 mg) derived from administering the doubly labelled amino-acid (**15**), $^3H : ^{14}C = 21·0$, showed that the major region of activity was present in a region corresponding to the R_f values of mesembrine and mesembrenone. Addition of inactive samples of mesembrine (311 mg) and mesembrenone (111 mg) to the 20 mg of crude alkaloid and subsequent column chromatography gave after several crystallizations of the hydrochlorides, mesembrine ($^3H : ^{14}C = 20·2$) and mesembrenone ($^3H : ^{14}C = 21·0$).

Ferricyanide oxidation of radioactive mesembrine to veratric acid. Mesembrine (280 mg $^3H : ^{14}C = 20·2$) derived from the feeding of **15**, 17·5 g KOH, 100 g $K_3Fe(CN)_6$, and 200 ml H_2O were stirred on a steam bath for 22 hr. The same amount of KOH and ferricyanide were added and the reaction was continued for 23 hr after which another equal portion of KOH and ferricyanide were added. After 24 hr of heating, the reaction mixture was filtered and acidified with 50% H_2SO_4. Continuous extraction with Et_2O for 3 days resulted in 138 mg of a light yellow solid. Recrystallization of this solid from 1 : 5 MeOH–H_2O yielded veratric acid (75 mg, 42% yield) m.p. 179–182° ($^3Hi : ^{14}C = 19·1$).

Bromination of veratric acid to 6-bromoveratric acid. The doubly labelled veratric acid (42·5 mg) from the previous experiment was dissolved in 6·5 ml H_2O at 90° and 0·02 ml Br_2 in 3 ml H_2O was added dropwise over 10 min with stirring. Upon cooling, crystallization occurred to give 39 mg (64% yield) of light tan crystals which were recrystallized (×2) to give 6-bromoveratric acid, m.p. 183–184° ($^3H : ^{14}C = 10·9$).

Attempts to prepare 2-iodo-6-bromoveratric acid. 6-Bromoveratric acid (88 mg, 0·337 mmol) was dissolved in 3 ml of acetonitrile and 223 mg (0·411 mmol) of thallium (**3**) trifluoroacetate was added. Stirring at room temperature for 24 hr and refluxing for 22 hr were tried in separate experiments followed by cooling and adding 392 mg (2·36 mmol) KI in 2 ml H_2O. After 15 min at room temp., 230 mg sodium metabisulfate was added and stirring was continued for 10 min followed by filtration. After the usual work up, starting material was recovered.

Degradations of mesembrine derived from 3′-O-*Methyl*[3′-O-*methyl*-^{14}C,1-^{14}C]*norbelladine* (**18**). (a) Radiolabelled mesembrenol (137 mg, 0·073 μCi/mmol) derived from *S. strictum* to which the test precursor **18** had been administered was hydrogenated in EtOH (5 ml) containing PtO_2 (17·5 mg). The mesembranol (0·082 μCi/mmol) when subjected to dehydration and two successive Hofmann degradations as previously described[2] gave 3,4-dimethoxybiphenyl m.p. 69–70° (0·0166 μCi/mmol) and trimethylamine (0·005 μCi/mmol).* (b) Radiolabelled mesembrenol was combined with inactive carrier to give 163 mg (0·557 mmol, 0·043 μCi/mmol) and dissolved in dioxane (5 ml). After adding 10 ml 10% Na_2CO_3 soln, the mixture was stirred and brought to 100°. During 1 hr, 47 ml 5% $KMnO_4$ was added dropwise. Thereafter, the purple color of unreduced permanganate persisted for several min. The soln was allowed to stir an additional 10 min, then cooled (ice bath), and decolorized with excess $NaHSO_3$. After acidification with conc. HCl (added dropwise with stirring), the solution was continuously extracted with C_6H_6 for 8 hr. The C_6H_6 extract was processed to provide 8 mg veratric acid from MeOH–H_2O, m.p. 180–182°. Upon recrystallization, the veratric acid (0·008 μCi/mmol) showed one spot (R_f 0·61) by TLC on silica gel G with 90 : 25 : 4 C_6H_6–dioxane–AcOH). (c) *Demethylation of veratric acid to isovanillic acid.* The above veratric acid (8 mg; 0·008 μCi/mmol) was

* The low activity of this sample measured as $NMe_4^+I^-$ introduces the possibility of considerable error in the quoted activity.

suspended in 0·1 ml 48% HBr. After heating at incipient boil with intermittent shaking for 6 min the material dissolved. Heating was continued for 3 min at which time crystals began to separate. The soln was then cooled, diluted with an equal vol. H_2O and filtered to give a solid residue. TLC analysis of a portion of this substance using 90 : 25 : 4 C_6H_6–dioxane–AcOH[19] revealed this material was composed of roughly 80% isovanillic acid (R_f 0·5) with ~19% veratric acid (R_f 0·62) and ~1% vanillic acid (R_f 0·55). The desired isovanillic acid component was separated by TLC in the same manner from the *ca* 5 mg product and counted as crude crystals (0·0031 μCi/mmol); too little material remained to be recrystallized.

Incorporation of [5'-^{3}H, 1-^{14}C]-*Norbelladine* (**19**) and 3'-O-*Methyl*[2',6'-3H,1-^{14}C]*norbelladine* (**20**). A TLC radioscan of each of the crude alkaloid fractions resulting from feeding **19** and **20** showed that radioactivity was associated with several zones corresponding to known alkaloids. Preparative TLC separation of the alkaloids derived from administering **19** provided mesembrenol and a mixture of mesembrine and mesembrenone (22 mg). The mesembrenol and the mesembrine–mesembrenone fraction were diluted with known amount of carrier and rechromatographed on alumina. Each of the alkaloids was eventually obtained radiochemically pure after several recrystallizations. The values of ^{3}H:^{14}C ratio obtained are recorded in Table 1. The crude alkaloid fraction derived from the feeding experiment with **19** was chromatographed (×2) on alumina and the mesembrenol isolated and purified by crystallization (×3) to constant activity (see Table 1).

Feeding experiments with doubly labelled N-*methylnorbelladines* **21**, **22** *and* **23**. The radiolabelled compounds **21**, **22** and **23** were administered to *S. strictum* plants; their hydrochlorides in 1% aq. soln of Tween 20. A TLC radioscan of the crude alkaloid fraction derived from the experiment with compound **21** showed two zones of activity at R_f 0·76 and 0·83. Addition of mesembrine (320 mg, R_f 0·83) and mesembrenone (115 mg, R_f 0·76) to the crude alkaloid fraction and subsequent chromatography over alumina afforded mesembrine and mesembrenone devoid of radioactivity. The two radioactive components were eluted in a MeOH wash of the alumina column and were not associated with any of the known alkaloids. Belladine hydrochloride[20] was added to a small portion of the crude alkaloid extract. Four recrystallisation followed by two preparative TLC separations produced a progressive decrease in activity of the belladine sample indicating the radioactive component (^{3}H : ^{14}C = 48·7) was not associated with belladine. Exactly parallel results were obtained with the feeding experiments with compounds **22** and **23**.

Feeding experiments with doubly labelled 3'-*deoxynorbelladines* **25** *and* **26**. Compounds **25** and **26** were administered in *ca* 1 ml 5% aq. Tween 20 to *S. strictum*. A TLC radioscan of the crude alkaloid fraction showed single zones of activity at R_f 0·82 and 0·84, respectively. The radioactive component(s) was removed from the TLC plate and examined by GLC and also counted. The GLC showed the same two components in each case, neither corresponding to any known mesembrine alkaloid. The ^{3}H : ^{14}C ratio in the material derived from **25** showed a large increase *ca* 500 while the ratio in the unknown material from **26** also showed a very different ratio, *ca* 200 from that of the starting precursor.

Acknowledgements—We are indebted to the National Institutes of Health for support through grants (AM 13977 and GM 19251) and for a Career Development Award to PWJ (GM 42342) and a predoctoral fellowship to GM. DSF would like to thank the National Aeronautics and Space Administration for fellowship support and HFC and NHM are indebted for support provided through the award of National Defense Act IV fellowships. We are also grateful to the National Science Foundation for grants (GB 7153 and G 19634) to Duke University for support of the Phytotron facility.

[19] PASTUSKA, G. (1961) *Z. Anal. Chem.* **179**, 355.
[20] BARTON, D. H. R. and KIRBY, G. W. (1962) *J. Chem. Soc.*, 806.