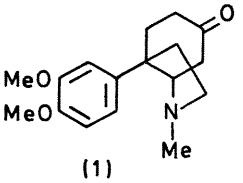
Biosynthesis of Mesembrine and Related Alkaloids, Mode of Incorporation of Phenylalanine, and Examination of Norbelladines as Precursors

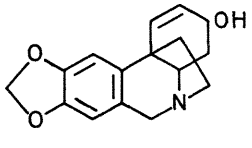
By P. W. JEFFS,* H. F. CAMPBELL, D. S. FARRIER, and G. MOLINA
(*Paul M. Gross Laboratory, Duke University, Durham, North Carolina* 27706)

Summary Evidence presented for the mode of incorporation of phenylalanine into the mesembrine alkaloids suggests the intermediacy of a bis-spirodienone.

PREVIOUS studies from this laboratory have demonstrated that tyrosine and phenylalanine follow separate metabolic pathways in providing the hydroaromatic C_6–C_2–N unit



(1)



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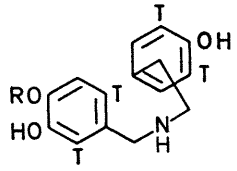
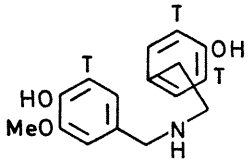


(5) R = H
(6) R = Me



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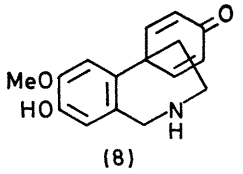

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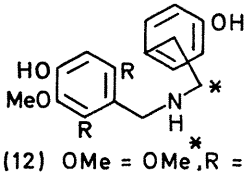
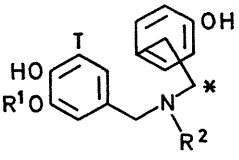
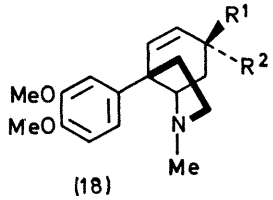


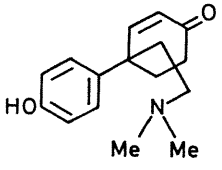
(12) OMe = OMe, R = H
(13) R = T
(14) OMe = OH, R = T



(15) $R^1 = R^2 = H$
(16) $R^1 = H, R^2 = Me$
(17) $R^1 = R^2 = Me$

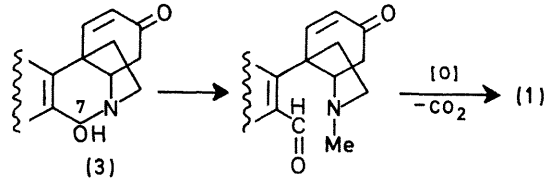


(18)



(19)

and the aromatic C_6 unit, respectively, from which the ring system of the mesembrine alkaloids [*cf.* mesembrine (**1**)][1] may be derived. In this respect they resemble the *Amaryllidaceae* alkaloids of the crinane class [*cf.* crinine (**2**)] with which they bear a close structural similarity. We decided to investigate if the mesembrine alkaloids of the octahydroindole family might be derived by a simple extension of the pathway operative in the established biosynthetic route to the crinane alkaloids. In principle, the conversion of the crinane system into the octahydroindole skeleton of the mesembrine family only requires the loss of the C-7 benzylic carbon atom; there are a number of ways in which this process can occur. Hydroxylation at C-7 of a crinane intermediate possessing the appropriate ring A oxygenation pattern to afford the carbinolamine (**3**), followed by the sequence (**3**) → (**4**) → (**1**) is one such route.[2]



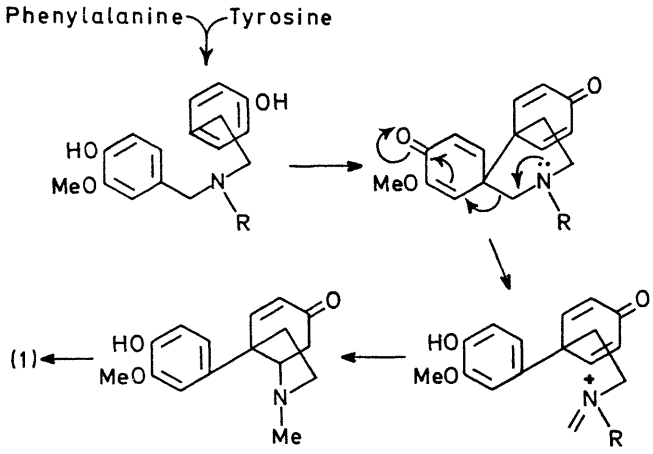
(3) (4)

To test the validity of such a scheme the tritiated compounds, (**5**), (**6**), and (**7**) were fed to *Sceletium strictum* L. Bol. under identical conditions. Compounds (**5**) and (**6**) are biosynthetic intermediates in the pathway to the crinane system in which the sequence (**5**) → (**6**) → (**8**) is known to occur.[3] Since the presence of a 3′-*O*-methyl group in (**7**) will prevent its conversion into (**8**), information on the efficiency of incorporation of (**7**) when compared to that of (**5**) and (**6**) is potentially useful in determining whether the biosynthesis of (**1**) proceeds *via* a crinane type intermediate.[4] To the extent that specific radiochemical yields data are reliable, the results, shown in the Table do not support this possibility. They suggest that 3′-*O*-methylnorbelladine (**7**) is the more efficient precursor and virtually exclude (**6**) as a possible biosynthetic intermediate. Unfortunately, the activities of the alkaloids obtained from these experiments were too low to permit degradations to determine the sites of labelling. However, the incorporation of radioactivity into the alkaloids from the 3′-*O*-methylnorbelladine feeding experiment suggested the possibility of an attractive alternative biogenetic route (see Scheme 1). In support of Scheme 1, ample precedent exists for the fragmentation of substituted aminomethyldienones in the chemistry[6] and biosynthesis[7] of alkaloids.†

Before examining this scheme, *o*-tritio-DL-phenylalanine was prepared by reduction of 2′-bromo-DL-phenylalanine with tritium gas over palladium.‡ After rigorous purification of the tritiated amino-acid, it was mixed with DL-[1′-^{14}C]phenylalanine to give the doubly labelled compounds (**9**). Symmetry considerations permit this doubly labelled compound to be viewed as containing equal amounts

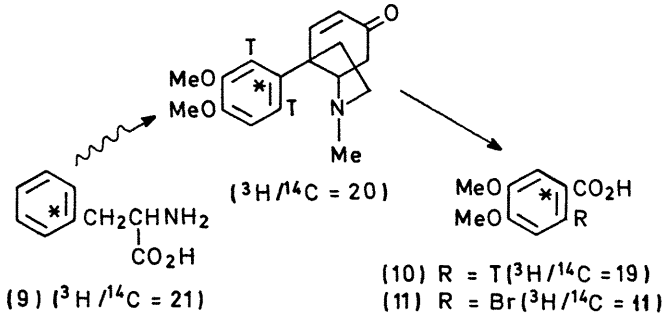
† We thank a referee for the suggestion that reference be made to these pertinent examples.
‡ We thank Dr. John W. Daly, National Institutes of Health, Bethesda, Maryland, for a gift of 2′-bromophenylalanine and for details of its conversion into *o*-tritio-phenylalanine.

of tritium at the equivalent 2′,6′-positions. When (**9**) was administered to *S. strictum* plants radioactive mesembrine and mesembrenone (**18**; $R^1=R^2=O$) were subsequently isolated and each was found to contain virtually the same $^3H/^{14}C$ ratio as the phenylalanine. This establishes that



SCHEME 1

there is no loss of tritium from the 2′,6′-positions of phenylalanine during its conversion into these alkaloids and thereby rules out the intervention of a crinane type of in complete consonance with the route suggested in Scheme 1 and further experiments were undertaken in an attempt to identify the other intermediates involved.



SCHEME 2

In a series of double labelling experiments, 3′-*O*-methylnorbelladine (**12**) and (**13**), norbelladine (**14**) and (**15**), *N*-methylnorbelladine (**16**), and 3′-*ON*-dimethylnorbelladine (**17**) were each tested as possible precursors. In the event, although the alkaloids derived from norbelladine and 3′-*O*-methylnorbelladine were radioactive neither of these compounds were incorporated intact (see Table).§ In the case of the *N*-methyl compounds (**16**) and (**17**), no significant incorporation of radioactivity into the alkaloids was observed.

Summary of feeding results with Sceletium strictum

Precursor	Labelling pattern (ratio)	Alkaloids	Labelling pattern (ratio)	Specific radiochemical yield × 10^3 (%)	% Incorporation
Norbelladine (**5**)		mesembrenol		6·1	
4′-*O*-Methylnorbelladine (**6**)		mesembrenol		1·6	
3′-*O*-Methylnorbelladine (**7**)		mesembrenol		3·3	
DL-Phenylalanine (**9**)	2′,6′-T/1-^{14}C(21)	mesembrine	2,6-T/1-^{14}C(20)		0·012[a]
		mesembrenone	2,6-T/1-^{14}C(21)		
3′-*O*-Methylnorbelladine (**12**)	3′-OMe-^{14}C/1-^{14}C(2·45)	mesembrenol	3′-OMe-^{14}C/1-^{14}C(0·27)		
3′-*O*-Methylnorbelladine (**13**)	2′,6′-T/1-^{14}C(5·12)	mesembrenol	T/^{14}C=(0·51)		
Norbelladine (**14**)	2′,6′-T/1-^{14}C(8·0)	mesembrenol	T/^{14}C=(0·83)		0·005[a]
Norbelladine (**15**)	5′-T/1-^{14}C(9·1)	mesembrine	T/^{14}C=(1·1)		0·006[a]
		mesembrenone	T/^{14}C=(1·2)		
N-Methylnorbelladine (**16**)	5′-T/1-^{14}C(9·9)	mesembrine	inactive		
3′-*ON*-Dimethylnorbelladine (**17**)	5′-T/1-^{14}C(17)	mesembrine	inactive		

[a] Values based on ^{14}C.

intermediate, which if formed by the sequence phenylalanine → (**5**) → (**6**) → (**8**) → (**1**) would require 50% loss of tritium.[4] The positions of the tritium labels in the mesembrine derived from this experiment were established by its oxidation to the labelled veratric acid (**10**), which on conversion into 6-bromoveratric acid (**11**) resulted in a 50% reduction in the $^3H/^{14}C$ ratio. Since both of the original tritiums are retained in the biosynthetic sequence leading to mesembrine, the location of one at the C-6 position by this degradation procedure permits an assignment of the position of the second tritium to be made (Scheme 2) with some confidence. The mode of incorporation of (**9**) is thus

These results exclude the norbelladine system as an intermediate in the biosynthesis of the mesembrine alkaloids, showing that Scheme 1 requires modification. However, the intervention of a bis-spirodienone intermediate, formed from a combination of two molecules, one derived from tyrosine and the other from phenylalanine, is obviously required in the biosynthesis of these alkaloids. Furthermore, this intermediate, irrespective of its actual structure, must undergo aromatization by a pathway which is at least formally analogous to that presented to account for the results reported in the double labelling experiment with phenylalanine.

§ Since these experiments indicate that both norbelladine and 3′-*O*-methylnorbelladine undergo cleavage before incorporation, the conclusions presented earlier from the experiments with the tritium labelled compounds (**5**), (**6**), and (**7**) are invalidated and the superior incorporation of activity from (**7**) must have been fortuitous.

The recent report[8] of a new structural type of Sceletium alkaloid, represented by joubertiamine (**19**), suggests several common alternative routes in which the mesembrine alkaloids and the joubertiamine type may both be incorporated.

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[1] P. W. Jeffs, W. C. Archie, and D. S. Farrier, *J. Amer. Chem. Soc.*, 1967, **89**, 2509; P. W. Jeffs, W. C. Archie, R. L. Hawks, and D. S. Farrier, *ibid.*, in the press.

[2] *Cf.* benzylic hydroxylation of haemanthamine to haemanthidine in *Sprekelia formosissima*, H. M. Fales and W. C. Wildman, *J. Amer. Chem. Soc.*, 1964, **86**, 294.

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

[4] For the synthesis of (**6**) and a discussion of the fate of the tritium atoms during its incorporation into *Amaryllidaceae* alkaloids of the lycorine type see, G. W. Kirby and H. P. Tiwari, *Chem. Comm.*, 1966, 676.

[5] For a definition see, J. R. Gear and I. D. Spenser, *Canad. J. Chem.*, 1963, **41**, 783.

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[7] D. H. R. Barton, R. James, G. W. Kirby, D. W. Turner, and D. A. Widdowson, *J. Chem. Soc.* (*C*), 1968, 1529.

[8] R. R. Arndt and P. E. J. Kruger, *Tetrahedron Letters*, 1970, 3237.