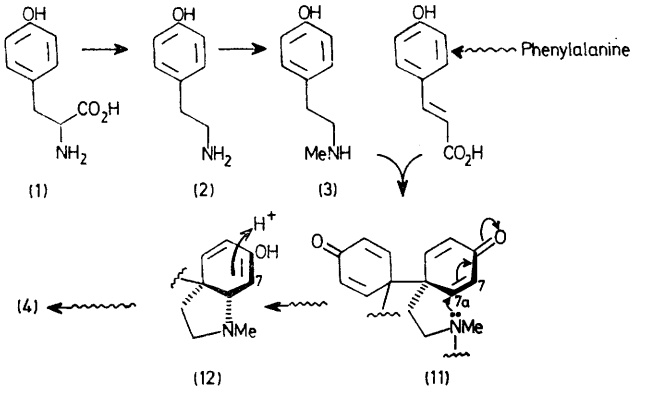
Post-Tyrosine Intermediates in the Biosynthesis of Mesembrine Alkaloids and Stereochemistry of Protonation at C-7 in the Formation of the Octahydroindole Skeleton

By PETER W. JEFFS,* DAVID B. JOHNSON, NED H. MARTIN, and BARBARA S. RAUCKMAN
(*Paul M. Gross Chemical Laboratory, Duke University, Durham, North Carolina* 27706)

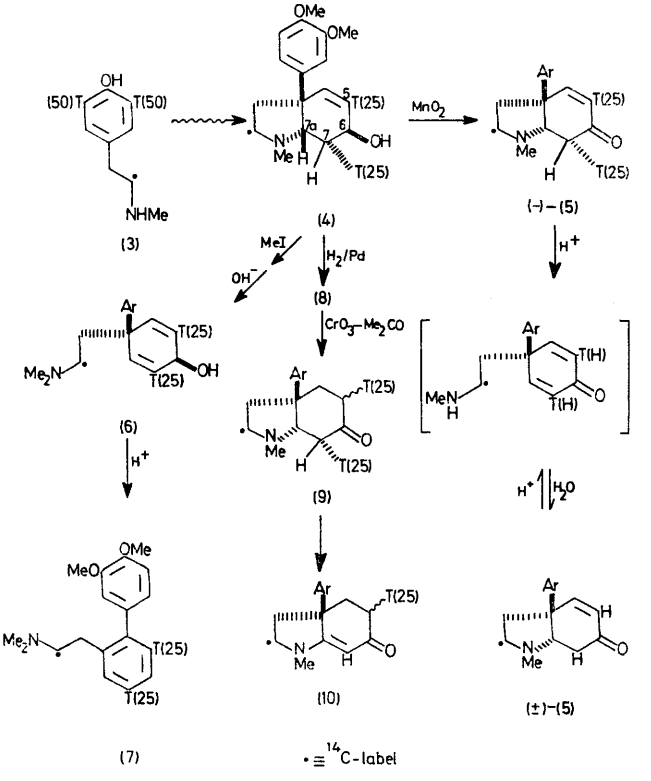
Summary The biosynthesis of (−)-mesembrenol is shown to involve the sequential intermediates, tyrosine → tyramine → *N*-methyltyramine, which when labelled at their symmetry equivalent 3,5-positions lead to a 50% loss of tritium leaving the remaining tritium equally distributed between the H-5 and H-7α positions; this result indicates that a stereospecific protonation at C-7 occurs from the β-face during the late stages of the biosynthesis.

THE previous evidence for tyrosine providing the entire C_6–C_2–N unit of the octahydroindole skeleton of the mesembrine alkaloids is based upon the incorporation of radioactivity at the predicted carbons from feeding experiments with [2-^{14}C] and [3-^{14}C]tyrosines.[1] Although these experiments with side-chain labelled compounds suggest the intact incorporation of this amino-acid, the contention that



the aromatic ring of tyrosine provides the C_6 hydroaromatic fragment of the alkaloid skeleton is not conclusively proven. Experiments with the double labelled samples reported in the Table now establish the intact incorporation of tyrosine and further define the sequence tyrosine (**1**) → tyramine (**2**) → *N*-methyltyramine (**3**) as a major pathway in the biosynthesis of the mesembrine alkaloids in *Sceletium strictum*.

An unexpected feature of the incorporation of compounds (**1**)—(**3**) is the loss of 50% of the original tritium. Location of the tritium at H-5 and/or H-7 positions in the labelled mesembrenol (**4**) obtained in these experiments was demonstrated by oxidation of (**4**) to (−)-mesembrenone (**5**) which on equilibration–racemization with 4N HCl at 25 °C for 7 days lost all tritium activity.† Conversion of mesembrenol to the dienol (**6**) on Hofman degradation, followed by subsequent acid-catalysed rearrangement of (**6**) to the 3,4-dimethoxybiphenyl (**7**)[2] proceeded with retention of 90% of the original tritium activity. Since the Hofmann elimination clearly involves the loss of the 7β-hydrogen through a *trans*-elimination in this system, the possible sites for the tritium are restricted to the H-5 and the H-7α positions. An equal distribution of the label between these two sites was demonstrated as follows. Catalytic hydrogenation of labelled mesembrenol to mesembranol (**8**) followed by oxidation of the latter by the Jones procedure[3] under carefully controlled conditions gave mesembrine (**9**) without significant loss of tritium. However, selective oxidation of mesembrine to the β-enaminoketone (**10**) with ethyl azodicarboxylate in CH_2Cl_2 solution was accompanied by a 55% loss of tritium as a consequence of removal of the 7α-hydrogen (tritium).[4]



While the structures of the intermediates beyond the phenylalanine → *p*-hydroxycinnamic acid[5] and tyrosine → *N*-methyltyramine pathway are unknown, the intermediacy of a bis-spirodienone which may be represented by the generalized structure (**11**) has been implicated.[6] With this

† In this and all subsequent experiments involving removal of tritium from the H-5 and H-7 position the degradations were carried out either with the appropriate deuteriated analogues or in deuteriated solvent media. The positions in which deuterium was lost or gained, as appropriate, were established by ^{1}H n.m.r. and/or ^{13}C n.m.r. spectroscopy and by mass spectrometry.

TABLE

Test precursor	$^3H:^{14}C$ Ratio Precursor	Mesembrenol	% Tritium lost	% Incorporation[a] Mesembrenol
[L-3,5-^{3}H; *U*-^{14}C]Tyrosine (**1**)	15·7[b]	8·61	46·0	0·60
[3,5-^{3}H; 1-^{14}C]Tyramine (**2**)	12·0	5·73	52·8	1·25
N-Methyl-[3,5-^{3}H; 1-^{14}C]tyramine (**3**)	17·8	9·28	48·0	0·10
[^{14}C-methyl]-*N*-Methyl-[3,5-^{3}H]tyramine ..	22·8	12·0	47·5	0·06

[a] Based upon ^{14}C-incorporation.
[b] Corrected for the expected loss of 1/9 of the uniform ^{14}C-label through the loss of the carboxyl carbon during incorporation.

in mind two important points are shown by our results. One is the occurrence of a stereospecific protonation at C-7 from the β-face of the 6,7- double bond on an enolate or its equivalent enol (**12**). Such a result is consistent with an internal conjugate addition of the nitrogen on C-7a at the 7a *si*, 7 *re* face (mesembrine numbering) of a dienone such as (**11**) followed by a stereospecific β-protonation of the resulting enolate at C-7 at the 6 *re*, 7 *re* face.

Secondly, the loss of 50% of the tritium from the aromatic C_6–C_2-N fragments (**1**)—(**3**) and the equi-distribution of the remaining activity between the 5,7-positions of mesembrenol is an intriguing result. We believe that the best explanation of this result is to postulate that substitution has to occur at one of the symmetry equivalent 3,5-positions in an aromatic ring of the *N*-methyltyramine derived fragment by some unknown group X (not hydrogen). This group X is then required to undergo substitution by hydrogen resulting in a 50% loss of tritium prior to formation of a dienone. On the other hand, if one assumes that a dienone, *cf.* (**11**) or similar structure, is formed before any loss of tritium occurs, the 3,5-positions are now enantiotopic and therefore clearly distinguishable by an enzyme mediated reaction involving tritium substitution in these positions. Should the latter occur then, as a consequence of the stereospecificity of the internal conjugate addition of the nitrogen to the C-7a carbon, the resulting mesembrine alkaloids would be expected to contain tritium either at the H-5 or H-7α position but not both. The result has provided further impetus for the search for post-*N*-methyltyramine derived intermediates containing an aromatic nucleus.

We thank the National Institutes of Health for a grant and for a Career Development Award to P.W.J., and the National Science Foundation for Grants to Duke University for support of the Phytotron Facility.

(*Received*, 6*th October* 1975; *Com.* 1138.)

[1] P. W. Jeffs, W. C. Archie, R. L. Hawks, and D. S. Farrier, *J. Amer. Chem. Soc.*, 1971, **93**, 3752.
[2] P. W. Jeffs, G. Ahmann, H. F. Campbell, D. S. Farrier, G. Ganguli, and R. L. Hawks, *J. Org. Chem.*, 1970, **35**, 3512.
[3] For an example of a modified Jones oxidation which may be carried out without enolization occurring see, W. S. Briggs and C. Djerassi, *J. Org. Chem.*, 1968, **33**, 1612; J. Fishman, *J. Amer. Chem. Soc.*, 1965, **87**, 3456.
[4] Experiments with [$^2H_4$]-5,5,7,7-mesembrine show that ethyl azodicarboxylate effects the conversion of this compound to the [$^2H_2$]-5,5 analogue of (**10**). The source of the hydrogen at C-7 in the latter is unknown, however, it does not affect the conclusions derived from the degradation scheme. For details of the oxidation of unlabelled mesembrine see, P. W. Jeffs, H. F. Campbell, and R. L. Hawks, *Chem. Comm.*, 1971, 1338.
[5] P. W. Jeffs and J. M. Karle, in preparation.
[6] P. W. Jeffs, H. F. Campbell, D. S. Farrier, G. Ganguli, N. H. Martin, and G. Molina, *Phytochem.*, 1974, **13**, 933; P. W. Jeffs, T. Capps, D. B. Johnson, J. M. Karle, and B. S. Rauckman, *J. Org. Chem.*, 1974, **34**, 2703.