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THE BIOSYNTHESIS OF *SCELETIUM* ALKALOIDS IN *SCELETIUM SUBVELUTINUM* L. BOLUS

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Summary: Six *Sceletium* (Mesembrine) alkaloids (1)-(6) are identified, together with *N,N*-dimethyltyramine (10) as constituents of *Sceletium subvelutinum*. The alkaloids (1)-(6) incorporate label from radioactive tyramine (8) and 4-hydroxyphenylpropionic acid (12) as expected; notably [3,5-^{3}H]-4-hydroxydihydrocinnamaldehyde [as (13)] is a more efficient alkaloid precursor than the acid (12). Preliminary evidence locates the amine (16) potentially as a key precursor for *Sceletium* alkaloids; (14) is less efficiently incorporated.

Plants of the *Sceletium* genus elaborate a small, unique group of alkaloids exemplified by joubertiamine (4) and mesembrine (7)[1]. The biosynthetic origins lie in the amino acids tyrosine[2] *via* tyramine (8) and *N*-methyltyramine (9)[1,3] [ring B and attached C_2-N fragment, see (1)] and phenylalanine (11)[2,4], by way of 4-hydroxyphenylpropionic acid (12) and closely related compounds[5] [ring A with complete loss of the side chain present in phenylalanine, see (1)]. Late stages of biosynthesis involve formation of the heterocyclic ring seen in (7) and, as a major pathway, subsequent introduction of the second aromatic oxygen substituent seen in (7) rather than at an earlier stage. Intermediates more complex than (9) and (12) have not been identified in spite of attempts to do so[1]; compounds of type Ar-C_2-N-C_1-Ar, which are intermediates in the biosynthesis of Amaryllidaceae alkaloids, are notably not implicated[5]. We report here results which show that biosynthesis proceeds from (12) by way of the aldehyde (13) and we tentatively identify the amine (16) as a key biosynthetic intermediate in a study with the alkaloids of *Sceletium subvelutinum* L. Bolus.

The alkaloids produced by *S. subvelutinum*, grown in a greenhouse in Leeds (summer 1987, 1988), were isolated and separated on silica followed by h.p.l.c. (Technicol Spherisorb; 1:1 MeOH, $CHCl_3$ + conc. NH_3) to give in order of increasing polarity: (1),(3),(5),(2),(4),(6). The structures of the alkaloids, joubertiamine (4) and (1)-(3), (5) and (6) were deduced from the 400MHz ^{1}H n.m.r. spectra and characteristic mass spectra of the compounds (*cf.* refs. 6 and 8). The alkaloids (3) and (5) had previously been isolated from this plant [6]; (3) was found to have the normal (relevant) stereochemistry [see (7)] associated with *Sceletium* alkaloids. It is reasonable to assume that the other alkaloids from *S. subvelutinum* are therefore of the same absolute stereochemistry. *N,N*-Dimethyltyramine (hordenine) (10) was also found by us to be a constituent of *S. subvelutinum* (direct comparison with synthetic material[7]) as it is in *S. joubertii* along with (2), (4), and (6)[8].

The condensation of amines with aldehydes is now seen to be a key step in the biosynthesis of numerous alkaloids with richly varied structures[9]. It follows that 4-hydroxydihydrocinnamaldehyde (13) could be an intermediate beyond the corresponding acid (12) in the biosynthesis of *Sceletium* alkaloids. [3,5-^{3}H]-4-Hydroxydihydrocinnamaldehyde [as (13)] was prepared by tritiation[10] of (12) (labelling sites checked through initial deuteriation) to give

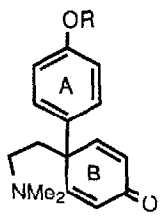
Table: Incorporation of Precursors into *S. subvelutinum* alkaloids.

With the exception of entries in the last row, all entries are % specific incorporations

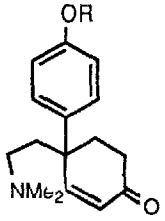
	[3,5-^{3}H](13)	[3,5-^{3}H](12)	[7-^{14}C](8)	[^{3}H](16)	[^{3}H](14)
(1)	0.17	–	–	1.12	0.02
(2)	0.76	0.01	0.01	0.08	0.035
(3)	0.055	0.003	0.003	0.02	0.02
(4)	0.62	0.004	0.01	0.1	0.04
(5)	0.08	–	–	–	0.025
(6)	0.07	0.005	0.02	0.02	0.025
(10)	$< 10^{-3}$	$< 10^{-3}$	0.01	$< 10^{-3}$	$< 10^{-3}$
% total incorp.	0.07	0.006	0.012	0.2	0.017

[3,5-^{3}H]-4-hydroxyphenylpropionic acid [as(12)]. This material was then converted into the required labelled aldehyde. This aldehyde, as its water-soluble bisulphite addition compound (*cf.* ref.11), was fed to *S. subvelutinum* in parallel with a mixture of [3,5-^{3}H]-4-hydroxyphenylpropionic acid [as (12)] and [7-^{14}C]tyramine. Incorporation of radioactivity into the alkaloids (1)-(6) was as shown in the Table. It is clear that the aldehyde (13) is a better precursor for the alkaloids than are the acid (12) and tyramine (8). The small amounts of alkaloid available precluded degradation to establish the specificity of labelling and, in addition, inevitable loss of the side-chain during the course of biosynthesis prevented us from labelling the aldehyde function with tritium to prove that the aldehyde (13) is implicated in biosynthesis without oxidation to the acid (12) (*cf.* refs. 9a and 11) but in any case the significantly higher incorporation of (13) compared to (12) argues against this. Further, the *N,N*-dimethyltyramine (10) isolated in these experiments provides an independent check on the random incorporation of tritium through *de novo* synthesis of tyrosine and C_1-metabolism. In all cases (Table) the incorporation of tritium label was reassuringly, negligible. In conclusion, the results point strongly to the implication of the aldehyde (13) as a biosynthetic intermediate lying after the acid (12).

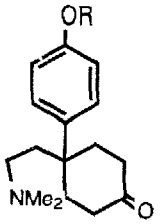
It may readily be hypothesised that condensation of the aldehyde (13) with *N*-methyltyramine (9) leads through an imminium salt (15) to the amine (16) as an intermediate in *Sceletium* biosynthesis. We have examined *N*-(3-(4-hydroxy-[3,5-^{3}H]-phenyl)propyl)-*N*-methyltyramine [as(16)] and similarly labelled (14) as alkaloid precursors in *S. subvelutinum* and find that (16) is an efficient alkaloid precursor (Table); incorporation into (10) was again negligible. Tritiated (14) was utilized much less well consistent with the conclusion[1,3] that *N*-methyltyramine (9) is an intermediate after tyramine. We conclude tentatively that the biosynthesis of the alkaloids of *S. subvelutinum* is as shown in the Scheme; the dienone (17) must be of the type shown since tritium label at C-2' and C-6' of (11) is retained during biotransformation into mesembrine (7) and mesembrinone[4]. Work is necessarily in hand to feed the amine (16) doubly labelled to establish rigorously that (16), now seen as potentially a key intermediate in *Sceletium* alkaloid biosynthesis, is an intact precursor. It should be noted that negative results have been obtained with (16) and similar compounds[1]. This was attributed to problems arising from the poor solubility of these compounds in water; we used aqueous ethanol solutions which had little apparent effect on the plants.



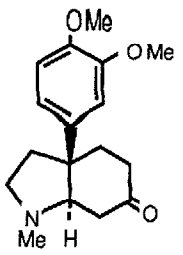
(1) R = Me
(2) R = H



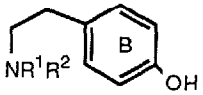
(3) R = Me
(4) R = H



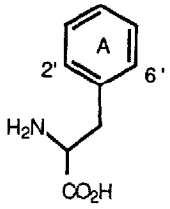
(5) R = Me
(6) R = H



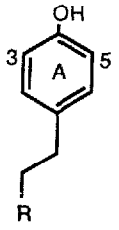
(7)



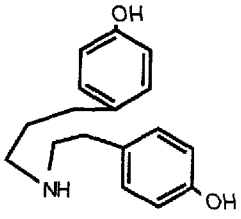
(8) $R^1 = R^2 = H$
(9) R^1 = Me, R^2 = H
(10) $R^1 = R^2$ = Me



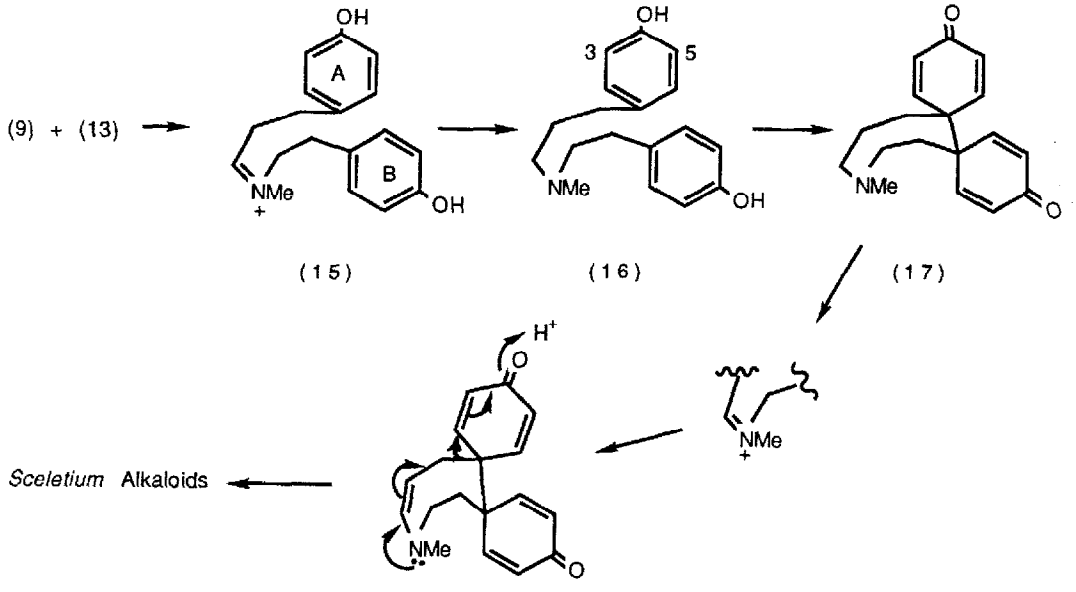
(11)



(12) R = CO_2H
(13) R = CHO



(14)



Scheme

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