# Late Stages in the Biosynthesis of Mesembrine: Sceletenone as a Precursor to the *cis*-3a-(3,4-Dimethoxyphenyl)octahydroindole Alkaloids

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*Summary* Radiolabelled tracer experiments with sceletenone (**2**), 4′-*O*-demethylmesembrenone (**4**), and mesembrenones (**7**) and (**8**) in *Sceletium strictum* reveal the sequence of reactions involved in the late stages of the biosynthesis of mesembrine (**9**) and mesembrenol (**5**).

EARLIER experiments[1] have demonstrated the role of the amino-acids tyrosine and phenylalanine in providing the skeletal framework of the octahydroindole alkaloids of the mesembrine class. These results together with other evidence[2] have been interpreted as indicating the probable intermediacy of (**1**) which undergoes a Michael addition to generate the octahydroindole ring system. Since all members of this group with the exception of (**2**) have two aromatic oxygen substituents, a biogenetic pathway in which both aromatic oxygens are introduced at an early stage has been assumed. The recent isolation[3] of sceletenone and the occurrence of a series of structurally related alkaloids, *cf.* joubertiamine (**6**) from *S. joubertii*,[4] when considered in conjunction with a failure to observe reason-

able levels of incorporation of radiolabelled dioxygenated cinnamic acids into the mesembrine alkaloids, prompted a suggestion that the second aromatic oxygen in the latter series might be introduced at a late stage in the biosynthesis. Experimental evidence and information on the sequence of events involved in the late stages of formation of the mesembrine alkaloids is now provided.

TABLE

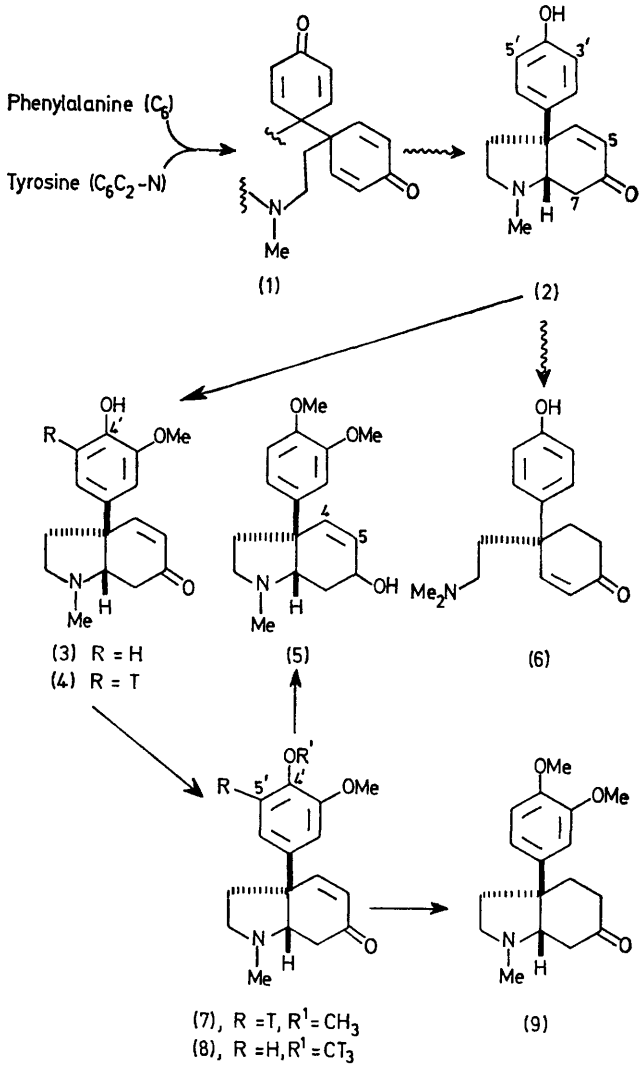
| Compound | Activity fed (μCi) | % Incorporation[b] | Alkaloid |
|---|---|---|---|
| (2) | 207 | 2·0[a] | (5) |
| (4) | 258 | 58·6 | (5) |
| (8) | 300 | 1·12 | (5) |
| (7) | 268 | 63·6 | (5) |
| | | 3·4 | (9) |
| | | 0·54 | mesembranol |
| | | <0·02 | (3) |

[a] Corrected for the loss of 50% of tritium required by the introduction of the second aryl oxygen function. [b] Incorporations doubled on basis of feeding the racemate.

(±)-[3′,5′-$^3$H]Sceletenone, prepared by the base-catalysed exchange of (±)-(2) with $^3H_2O$ in which the more labile tritium atoms introduced at the 5- and 7-positions were removed by subsequent back exchange, was administered to *S. strictum* plants. After 12 days, the plants were harvested and the alkaloids isolated and separated. The non-phenolic alkaloid fraction was diluted with inactive (−)-mesembrenol (5), and then purified by preparative layer chromatography to afford the alkaloid (5), which was crystallized to constant activity. The level of incorporation (see Table) indicates that sceletenone serves as an efficient precursor of (−)-mesembrenol and establishes that the 3′-aryl oxygen which appears ubiquitously in members of this class of alkaloid is introduced after the formation of the octahydroindole skeleton is complete.

Some information on the sequence of intermediates involved in the late stages of the biosynthesis of the mesembrine alkaloids was provided by incorporation studies with the radiolabelled alkaloids (±)-4′-*O*-demethyl[5′-$^3$H]mesembrenone (4), (±)-[5′-$^3$H]mesembrenone (7) and (±)-[4′-*O*-methyl-$^3$H]mesembrenone (8). The *O*-demethyl-mesembrenone (3) was labelled at the 5′-position with tritium by a procedure analogous to that described for sceletenone. Its incorporation into the dimethoxyphenyl alkaloid mesembrenol (5) occurred to the remarkable extent of 58% (see Table). The mesembrenones (7), prepared by methylation of 4′-*O*-demethyl[5′-$^3$H]mesembrenone with diazomethane, and (8), obtained by treatment of (±)-4′-*O*-demethyl-mesembrenone with tritiated diazomethane were administered separately to *S. strictum*. The incorporation of the ring-tritiated precursor (7) into mesembrenol again proved remarkably high at 62%, with lower levels appearing in mesembrine and mesembranol (5; no 4,5-double bond).

The negligible level of incorporation of radiolabel into the phenolic base (3) from (7) indicates that *O*-demethylation of mesembrenone does not occur to an appreciable extent




in this system. The results of incorporation of (±)-mesembrenone labelled in the 4′-*O*-methyl group, although confirming the existence of its biotransformation to mesembrenol, gave considerably lower levels of incorporation.†

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† The very different levels of incorporation of the two labelled mesembrenones (7) and (8) was unexpected. Although both test precursors were administered under identical conditions, the experiment with (7) was conducted with 3 month old plants whereas the lower incorporation was obtained with 2 year old plants.

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