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CINNAMIC ACID INTERMEDIATES AS PRECURSORS TO MESEMBRINE AND SOME OBSERVATIONS ON THE LATE STAGES IN THE BIOSYNTHESIS OF THE MESEMBRINE ALKALOIDS*

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Key Word Index—*Sceletium strictum*; Aïzoaceae; biosynthesis; mesembrine; cinnamic acid pathway; late stages of alkaloid bioconversions.

Abstract—Experiments with various labelled cinnamic acid derivatives establish, in conjunction with previous work, that the incorporation of phenylalanine into the 3a-aryl octahydroindole ring system of the mesembrine alkaloids occurs via the intermediacy of cinnamic acid and 4′-hydroxycinnamic acid. The major pathway to the 3′,4′-dioxyaryl substituted alkaloids proceeds via 4′-hydroxydihydrocinnamic acid (4′-phloretic acid), and 3′4′-dioxygenated cinnamic acids are not involved as intermediates on this major pathway. In accord with this latter finding, the 3′-aryl oxygen substituent is introduced at a late state in the biosynthesis as evidenced by the bioconversion in *S. strictum* of sceletenone to mesembrenol and other related alkaloids. The late stages in the biosynthesis of the alkaloids are shown to involve the sequence: sceletenone, 4′-*O*-demethylmesembrenone, mesembrenone. Mesembrenone is converted to mesembrine, mesembrenol and mesembranol.

INTRODUCTION

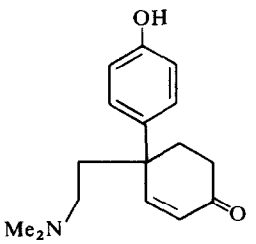
Our previous investigations of the biosynthesis of of sceletium alkaloids of the octahydroindole series, which are exemplified by the structure of the alkaloid mesembrine (**1**), have demonstrated that phenylalanine and tyrosine are involved in separate pathways as precursors to the aromatic ring and the C_6-C_2-N fragment, respectively [1]. Although these results parallel those observed for the structurally similar Amaryllidaceae alkaloids of the crinine (cf.**2**) group, further studies have demonstrated that the biosynthesis of the two classes of alkaloid are fundamentally different. This is evident from the significantly different manner observed for the incorporation of phenylalanine into these alkaloids (see Scheme 1). With the loss of the C-3 side-chain occurring upon incorporation of this amino acid into the mesembrine ring system, it is not possible to discern which of the original nuclear carbons of phenylalanine correspond to the C-1′ position in mesembrine. Incorporations [2] with phenylalanine-[2′,6′-$^3H_2$, 1′-^{14}C] (**3**) occur without loss of tritium in providing mesembrine labelled as indicated. This result indicates that the biosynthesis of the mesembrine alkaloids involves the formation of a ring A spirodienone intermediate (cf. **4**) as a necessary requirement to account for the retention of the tritium labels at the 2′,6′-position on the aromatic ring of the alkaloid. Such an observation rules out the possibility that the biosynthesis of the mesembrine alkaloids is proceeding via an extension of the crinine pathway since this would require the loss of 50% of the tritium label from the incorporation of the doubly labelled phenylalanine (**3**) (see Scheme 1).

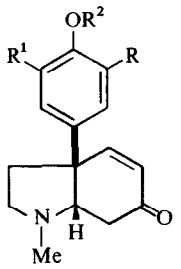
While it was still possible to reconcile these results by postulating a norbelladine type intermediate i.e. C_6—C_1—N—C_2—C_6, subsequent studies eliminated what were considered to be the logical contenders in this series [2]. In view of this previous lack of success in delineating any of the biosynthetic intermediates beyond tyrosine and phenylalanine, a systematic characterization of the immediate post-amino acid metabolites in the pathway to the mesembrine alkaloids offered the most promise for further progress. This paper deals with the results of an investigation of the biosynthetic intermediates derived from phenylalanine. In addition, the late stages of biosynthesis, after the basic octahydroindole skeleton of the alkaloids is formed, have been elucidated.

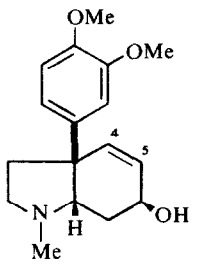
RESULTS AND DISCUSSION

The pathway for phenylalanine metabolism in relation to the biosynthesis of mesembrine and related alkaloids in *Sceletium* species assumes importance in view of the marked deviation of the biosynthesis of these alkaloids in comparison to the biosynthesis of the crinine bases. In the case of the latter alkaloids, Suhadolnik *et al.* [3] have presented evidence to show that phenylalanine is converted through the phenylpropanoid pathway to 3′,4′-dihydroxycinnamic acid, which in turn is transformed to the C_6—C_1 unit protocatechuic aldehyde. To test whether such a pathway was involved in the biosynthesis of mesembrine, cinnamic acid [2′-^{3}H, 1′-^{14}C] (**5**), 4′-hydroxycinnamic acid-[3′,5′-$^3H_2$] (**6**), 3′,4′-dihydroxycinnamic acid-[5′-^{3}H] (**7**) and ferulic acid-[5′-^{3}H] (**8**) were synthesized. The doubly labelled cinnamic acid (**4**) was obtained from (±)-phenylalanine-[2′-^{3}H] and (±)-phenylalanine-[1′-^{14}C], in separate experiments, by methylation with dimethyl sulphate and Hofmann elimination [4]. The tritium labelled cinnamic acids **6–8** were obtained from the corresponding tritiated benz-

* Part 8 in the series 'Sceletium alkaloids', For part 7 see (1976) *Org. Mass Spec.* **11**, 1.

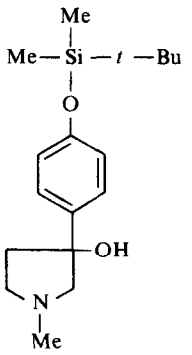






10 R = R^1 = R^2 = H
16 R = OMe, R^1 = R^2 = H
20 R = OMe, R^1 = ^{3}H, R^2 = H
21 R = OMe, R^1 = ^{3}H, R^2 = Me
22 R = OMe, R^1 = H. R^2 = $^3H_3$
24 R = R^1 = ^{3}H, R_2 = H

15
23 no 4,5-double bond.



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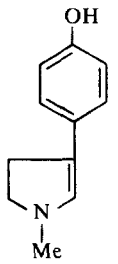

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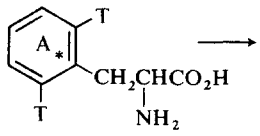
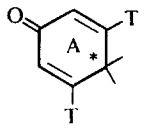
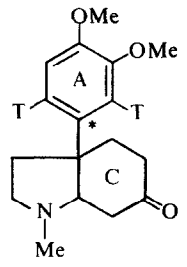
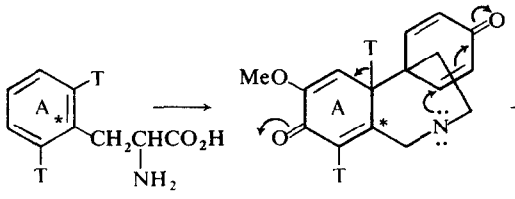
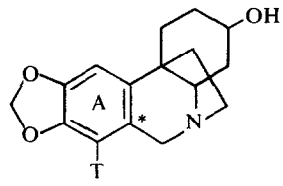






3 **4** **1**





T = ^{3}H
* = ^{14}C

crinine **2**

Scheme 1.

aldehydes by a malonic acid synthesis. Each of the labelled compounds were administered to *S. strictum* plants and the incorporations into mesembrenol (**15**), the major alkaloid, are recorded in Table 1.

The very low incorporations of the dioxyaryl cinnamic acids (**7**) and (**8**) was surprising in view of the reasonable levels of incorporation found for cinnamic and 4′-hydroxycinnamic acid. These latter results when viewed in conjunction with the occurrence of both the mono-oxyaryl alkaloids of the joubertiamine class (cf. **9**) and the mono-oxyaryl octahydroindole base sceletenone (**10**) in *Sceletium* species suggested that consideration be given to a pathway in which the 3′-aromatic oxygen function of the mesembrine alkaloids is introduced at a late stage in the biosynthesis [5]. We concentrated on the examination of 4′-hydroxycinnamic acid derivatives as potential precursors.

Tritium labelled 4′-hydroxycinnamaldehyde (**11**), 4′-hydroxycinnamyl alcohol (**12**),4′-hydroxydihydrocinnamic acid (phloretic acid) (**13**) and 4′-hydroxydihydrocinnamyl alcohol (**14**), prepared by standard procedures, were administered separately in a series of experiments to *S. strictum* plants. The results, which are summarized in Table 1, show that **13** was a good presursor, while the alcohol **12** gave only a modest incorporation of radioactivity. The remaining compounds, **11** and **14**, showed insignificant levels of incorporation.

The incorporation of radiolabel from **12** into mesembrenol was shown to be limited to the aromatic ring by the following experiment. Tritium labelled mesembrenol isolated from the 4′-hydroxycinnamyl alcohol feeding was mixed with mesembrenol biosynthetically labelled with carbon-14 in the aromatic ring from (±)-phenylalanine-[U-^{14}C] and was oxidized to veratric acid by the action of potassium ferricyanide in KOH. The resulting ^{3}H:^{14}C ratio of the veratric acid demonstrated, as expected, that within experimental error essentially all of the tritium (91%) in the mesembrenol was contained in the aromatic ring.

The relatively high incoporation of phloretic acid is noteworthy since it is not usually regarded as a common intermediate in the biosynthesis of phenylpropanoid derived compounds. However, Neish has shown that phloretic acid is incorporated with about the same efficiency as 4′-hydroxycinnamic acid in ligan biosynthesis [6] and this has been explained by assuming the existence of a pathway in which phloretic acid is converted to 4′-hydroxycinnamic acid. Two possible pathways may be invoked for the involvement of phloretic acid in the biosynthesis of the mesembrine alkaloids as shown in Scheme 2.

In order to differentiate the two pathways, a trapping experiment was devised. Phloretic acid-[3′,5′-$^{3}H_2$] was fed separately to *S. strictum* alone and in the presence of a 6 molar excess of 4′-hydroxycinnamic acid. If the phoretic acid is on the direct route in the synthesis of the alkaloids (path A), then the addition of the 4′-hydroxycinnamic acid should have little effect on the incorporation of phloretic acid into the alkaloids. Also, the 4′-hydroxycinnamic acid upon reisolation should be devoid of activity. However, if the phloretic acid is reconverted into 4′-hydroxycinnamic acid before being further transformed into the alkaloids (path B), then the incorporation of phloretic acid into the alkaloids should be greatly reduced or negligible, and the 4′-hydroxycinnamic acid should be radiolabelled.

In the event, the two feeding experiments resulted in incorporation values into **15** of 0.13% in the presence of 4′-hydroxycinnamic acid and 0.32% when fed alone. In the former experiment, reisolation and rigorous purifi-

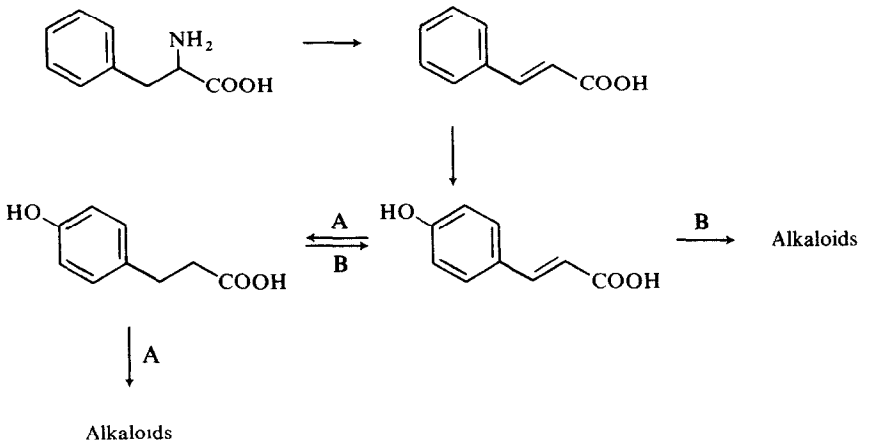
Table 1. Summary of feeding experiments

Compound	Activity FED, μCi (^{3}H ^{14}C ratio)	%Incorporation (^{3}H ^{14}C ratio)	Compound
Cinnamic acid-[2′-^{3}H, 1′-^{14}C] (**5**)	350(14.5:1)	0.06(13.3:1)	mesembrenol
4′-Hydroxycinnamic acid [3′5′-$^{3}H_2$] (**6**)	240	0.15*	mesembrenol
	350	0.03*	mesembrenol
3,4′-Dihydroxycinnamic acid-[5′-^{3}H] (**7**)	320	0.013	mesembrenol
	395	0.008	mesembrenol
Ferulic acid-[5′-^{3}H] (**8**)	320	0.003	mesembrenol
		0.058	4′-*O*-demethylmesembrenol
4′-Hydroxycinnamyl aldehyde-[3′,5′-$^{3}H_2$] (**11**)	165	<0.003*	mesembrenol
4′-Hydroxycinnamyl alcohol-[3′,5′-$^{3}H_2$] (**12**)	320	0.099*	mesembrenol
Phloretic acid-[3′,5′-$^{3}H_2$] (**13**)	310	0.13*	mesmbrenol
Phloretic acid-[3′,5′-$^{3}H_2$] (**13**) plus inactive 4′-hydroxycinnamic acid	310	0.032*	mesembrenol
		0.19	4′-hydroxycinnamic acid
4′-Hydroxydihydorcinnamyl alcohol-[3′,5′-$^{3}H_2$] (**14**)	291	0.024*	acid mesembrenol
Ferulic acid-[3′-*O*-methyl-^{14}C, 5′-^{3}H] (**17**)	340 (13.5:1)	0.06†(12.6:1)	mesembrenol
		0.15†(6.26:)	4′-*O*-demethylmesembrenone
		0.73†(12.15:1)	methyl ferulate
Phloretic acid-[3′,5′-$^{3}H_2$] (**13**)	303	2.26*	mesembrenol
	495	0.32	mesembrenol
Phloretic acid [3′,5′-$^{3}H_2$] (**13**) plus inactive ferulic acid	303	0.92*	mesembrenol
		0.38	ferulic acid
Mesembrenone-[4′-OC$^{3}H_3$] (**22**)	300	1.12§	mesembrenol
		0.58§	*N*-demethyl-*N*-formylmesembrenone
4′-*O*-Demethylmesembrenone-[5′-^{3}H] (**20**)	258	58.6§	mesembrenol
Mesembrenone-[5′-^{3}H] (**21**)	268	63.6§	mesembrenol
		3.4§	mesembrine
		0.54§	mesembranol
		<0.02§	4′-*O*-demethylmesembrenone
Sceletenone-[3′,5′-$^{3}H_2$] (**24**)	207	2.0§	mesembrenol

* Loss of 50% of tritium activity is necessitated by introduction of the second ring oxygen function

† Incorporations based on ^{14}C data.

§ Incorporations doubled on basis of feeding racemate.



Scheme 2.

cation of the 4′-hydroxycinnamic acid via conversion to the methyl ester demonstrated that it was radiolabeled to an extent corresponding to 0.19% incorporation.

These results do not allow a clear decision to be made concerning the two pathways illustrated in Scheme 2. Since, although the presence of 4′-hydroxycinnamic acid diminishes the incorporation of radioactivity from phloretic acid, the level of radiolabel in the alkaloid was still one-fourth that of the control. In addition, although the 4′-hydroxycinnamic acid is radiolabelled, the incorporation is rather small. The results are consistent with the existence of a facile equilibrium between 4′-hydroxycinnamic acid and phloretic acid, which could account for both the lowering of the incorporation of **13** in the presence of 4′-hydroxycinnamic acid and the occurence of radiolabel in the latter. Nevertheless, the possibility that a situation exists in which both pathways A and B were operating cannot be ruled out.

Although the incorporation of ferulic acid-[5′-^{3}H] into mesembrenol (**15**) was very low, the level of incorporation into the accompanying phenolic base 4′-*O*-demethylmesembrenone (**16**) at 0.05% approached that of 4′-hydroxycinnamic acid into mesembrenol. Further insight into the possible role of ferulic acid in the biosynthesis of the mesembrine alkaloids was sought with an experiment using doubly labelled ferulic acid [*O*-methyl-^{14}C,5′-^{3}H] (**17**). The doubly labelled ferulic acid (**17**), ^{3}H:^{14}C ratio of 13.5, was prepared from the corresponding ^{14}C-methyl labelled and 5′-^{3}H labelled vanillins, respectively, by a malonic ester synthesis in separate experiments. The two isotopically labelled ferulic acids synthesized in this way were mixed to give the doubly labelled sample.

The results obtained when **17** was administered to *S. strictum* paralleled those previously obtained. Based on the ^{14}C data, doubly labelled ferulic acid had an incorporation of 0.15% (^{3}H:^{14}C = 6.26:, 46% ^{3}H retention) into 4′-*O*-demethylmesembrenone (**16**) and of 0.1% (^{3}H:^{14}C = 12.6:1, 93% ^{3}H retention) into mesembrenol (**15**). The retention of 93% tritium in **15** from ferulic acid suggests intact incorporation of ferulic acid into this alkaloid. The loss of 54% of the tritium label in the phenolic base **16** may be accounted for by the potential lability of tritium adjacent to a phenolic hydroxyl group under the conditions used in the isolation of the alkaloids.

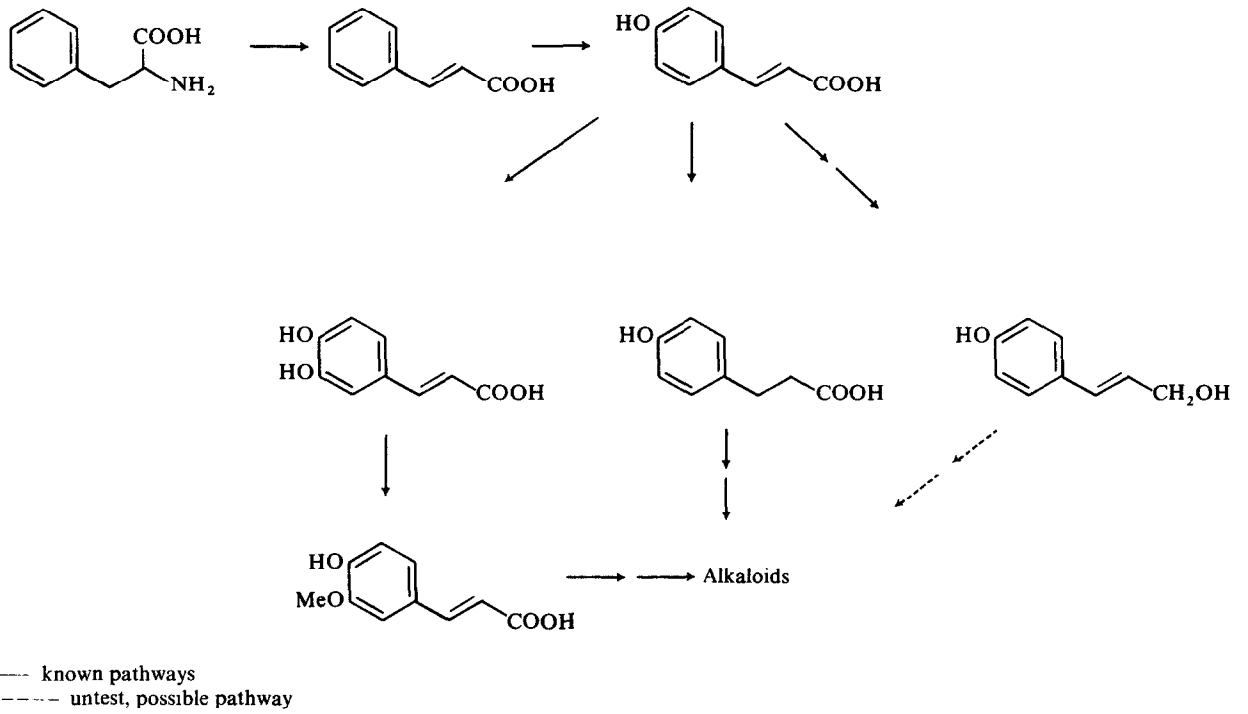
A highly radioactive component in the non-alkaloid fraction detected on TLC was isolated and characterized as the methyl ester of ferulic acid (^{3}H:^{14}C = 12.15:1, 90% ^{3}H retention) containing radioactivity corresponding to a 0.73% incorporation.

As a further check of the role of ferulic acid in the biosynthetic pathway, a trapping experiment involving the feeding of phloretic acid-[3′,5′-$^3H_2$] in the presence of an excess of inactive ferulic acid was performed. When radiolabelled phloretic acid (**13**) was administered to *S. strictum* plants alone, a 2.26% incorporation of label was observed into mesembrenol. In the presence of a 6 molar excess of ferulic acid, the incorporation of label from phloretic acid was lowered to 0.92% and was accompanied by a 0.32% incorporation of radioactivity into ferulic acid. Again, the incorporation of phloretic acid is lowered in the trapping experiment, but is still at a relatively high level demonstrating its direct incorporation into the alkaloids. The small amount of radioactivity found in the ferulic acid can be explained by the previously demonstrated dehydrogenation of phloretic acid back to 4′-hydroxycinnamic acid which is subsequently converted to ferulic acid.

The forgoing results, when considered in conjunction with later evidence (*vide infra*), lead to the conclusion that the incorporation of various precusors was consistent with the existence of a metabolic grid as represented in Scheme 3. Of the two pathways which have been established, the main pathway to the non-phenolic alkaloids is via phloretic acid with a minor pathway being represented via the ferulic acid route. In each experiment involving the feeding of ferulic acid, the ratio of phenolic alkaloids to non-phenolic alkaloids was much higher than normal. Since experiments dealing with the late stages of mesembrine alkaloid biosynthesis (*vide infra*) have shown that the non-phenolic bases are produced by *O*-methylation of phenolic alkaloids and are not, therefore, on a separate pathway, these findings may be explained by assuming competitive inhibition of the *O*-methyltransferase enzyme is occurring, presumably by binding of ferulic acid or methyl ferulate.

Late stages in the biosynthesis

On the premise that phenolic oxidative coupling is involved at some stage in the biosynthesis of the octa-



Scheme 3.

hydroindole skeleton of the mesembrine alkaloids, it is reasonable to expect that the first formed alkaloid would have a structure in which ring C is at the oxidation level of a Δ^4-6-ketone [2,8]. Two structures are suggested by the hypothesis: sceletenone (**10**) from the principal phloretic acid pathway and 4′-*O*-demethylmesembrenone (**16**) via the ferulic acid route.

It was a major point of interest to investigate the possible role of sceletenone as an intermediate in the biosynthesis of the 3′,4′-dioxyaryl alkaloids of the mesembrine series. To provide sufficient material for a biosynthetic study of the role of sceletenone(**10**), a synthesis of **10** was undertaken by a route modelled after the synthesis of mesembrenone by Curphy and Kim [9].

4-Bromophenol was silated with *t*-butyldimethylsilyl chloride to form the protected phenol using the procedure of Corey and Venkateswarlu [10]. Treatment of 4-bromophenol-*t*-butyldimethylsilyl ether with *n*-butyllithium in THF followed by condensation of the lithioderivative with *N*-methyl-3-pyrrolidone yielded the tertiary alcohol **18**. Refluxing **18** in 6N hydrochloric acid removed the silyl ether protecting group and dehydrated the alcohol to form the enamine **19**. Annelation of the the hydrochloride of the enamine **19** with *β*-chloromethylvinylketone provided (±)-sceletenone (**10**) in low yield. No attempt was made to improve the low yield (8%) of the last step of the synthesis since sufficient material for characterization and for the biosynthetic study was obtained from the first synthesis. The chromatographic and spectral properties of the synthetic alkaloid were identical to those of the natural sample providing a confirmation of the assigned structure.

Sceletenone was labelled by a base catalyzed exchange in ^3H_2O to give sceletenone-[3′,5′-$^3H_2$] (see Experimental). This compound was administered to *S. strictum* and after 12 days, isolation of the alkaloids gave (−)-mesembrenol which after rigorous purification retained a level of radioactivity corresponding to 2% incorporation. This result clearly established sceletonone as an intermediate in the biosynthesis of the 3′,4′-dioxyaryl alkaloids of the mesembrine series and indicates that the 3′-aromatic oxygen function is introduced at a late stage following the formation of the octahydroindole ring system.

To gain further insights into the late stages of the biosynthesis other radiolabelled alkaloids were synthesized. 4′-*O*-Demethylmesembrenone-[5′-^{3}H] (**20**), prepared by base catalyzed exchange of 4′-*O*-demethylmesembrenone, was converted to mesembrenone-[5′-^{3}H] (**21**) by methylation with diazomethane. Methylation of the phenolic alkaloid **16** with diazomethane-[^{3}H] afforded mesembrenone-[4′-*O*-methyl-^{3}H] (**22**). These were fed to *S. strictum* plants and the results are shown in Table 1.

The 1.1% incorporation of the *O*-methyl labelled mesembrenone into mesembrenol and the incorporation of mesembrenone-[5′-^{3}H] into mesembrine (**1**) and mesembranol (**23**) was in accord with a scheme in which the late stages of the biosynthesis of the mesembrine alkaloids involve the sequential reduction of the cyclohexenone chromophore.

The incorporations of **20** and **21** when fed to young 3-month-old plants resulted in spectacularly high incorporations into mesembrenol (Table 1) The results provide firm evidence for formation of the non-phenolic

alkaloids from the phenolic alkaloids. Also, since 4′-*O*-demethylmesembrenone isolated from the feedings of mesembrenone-[5′-^{3}H] to *S. strictum* was not radioactive, it would seem that *O*-demethylation does not appear to be an important biosynthetic process in the formation of phenolic alkaloids in this plant.

The establishment of phloretic acid and sceletenone as intermediates in the biosynthesis of the mesembrine bases has provided a fresh insight to possible structures for biosynthetic intermediates between phloretic acid and the octahydroindole ring system of the mesembrine alkaloids.

EXPERIMENTAL

The general procedures and the methods of administering labelled test precursors are as [1] unless otherwise noted. Plants used in these studies were grown either in artificial medium in the Duke University Phytotron facility with conditions to give a 16 hr photoperiod with an 8 hr day temp. of 20° and a 16 hr temp. of 17° or in the Duke University Greenhouse. All radiolabelled synthetic intermediates were assayed for purity by TLC and subsequent radioscanning of the plate.

Counting methods. ^{14}C and ^{3}H were measured by liquid scintillation counting in 10 ml of 'cocktail' soln composed of 1 l. toluene, 5g 2,5-diphenyloxazole (PPO), 50 ml BBS-3 (Beckman solubilizer), and 4 ml of 4% $SnCl_2$ in 0.1 N HCl or in 10 ml of 3a70 Preblended Liquid Scintillation Cocktail (Research Products International Corp). Counting efficiencies for ^{14}C and ^{3}H were determined by external standardization with benzoic acid-{carboxyl-^{14}C} and hexadecane-{1,2-$^3H_2$} of known activity.

General procedure for purification of alkaloids. The plant extract, obtained as previously described, was taken up in 50 ml 10% HCl and extracted with Et_2O (4 × 35 ml) to remove nonbasic material. The acid layer was basified with N NaOH and extracted with $CHCl_3$ (2 × 35 ml) and with $CHCl_3$–MeOH (3:1) (2 × 35 ml). The combined organic layers were dried over Na_2SO_4, filtered, and evapd to give the nonphenolic alkaloids as a dark oil. These alkaloids were purified using repeated PLC on Si gel eluted with $CHCl_3$–MeOH or column chromatography on alumina (10 g, Neutral grade II) eluted with C_6H_6–EtOAc–MeOH. To achieve radiochemically pure samples, mesembrenol was repeatedly recrystallized from EtOAc or Me_2CO. A 10–20 mg sample of inactive (−)-mesembrenol was sometimes added to aid in the isolation. The aq. NaOH soln containing the phenolic alkaloids was acidified with conc HCl, rebasified with Na_2CO_3, and continuously extracted with $CHCl_3$ for 3 days. Evapn of the $CHCl_3$ soln gave the phenolic alkaloids as a dark oil. Purification of 4′-*O*-demethylmesembrenone was by repeated PLC on Si gel using $CHCl_3$–MeOH (9:1).

4-Hydroxybenzaldehyde-[3, 5-$^3H_2$]. Following Kirby's procedure [11,12] 4-hydroxybenzaldehyde-[3,5-$^3H_2$] was prepared by base-catalysed exchange in 75% yield. Using 500 mCi ^3H_2O (0.3 ml) and 2 mmol of aldehyde, the sp. act of the product was 12 mCi/mol.

4′-Hydroxycinnamic acid [3′, 5′-$^3H_2$] (6). DeBardeleben and Teng's malonic acid synthesis [13] was used to prepare radiolabelled 4′-hydroxycinnamic acid from 1 mmol of labelled 4-hydroxybenzaldehyde in 69% yield using 14 µl of aniline as a catalyst for the condensation sp. act. 11 mCi/mol

4′-Hydroxycinnamic acid methyl ester-[3′,5′-$^3H_2$]. The labelled acid **6** was methylated with CH_2N_2 and crystallized from H_2O to yield white needles (88.5 mg, sp. act. 10.3 mCi/mmol). Unlabelled material had mp 135–137° (lit. [14] mp 139–140°). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3580 (free OH), 3350 (bound OH), 1695 (ester C=O). NMR [14] (60 MHz, $CDCl_3$): δ3:80 (3H, *s*, Me), 6.28 (1H, *d*, $J_{2,3}$ = 16 Hz, C-2), 7.15 (4H, center of AA′ BB′ system, aromatic protons), 7.67 (1H, *d*, $J_{2,3}$ = 16 Hz, C-3)

4′-Hydroxycinnamyl alcohol-[3′,5′-$^3H_2$] (12). A 29.5 mg sample of the tritium labelled 4′-hydroxycinnamic acid ester was dissolved in 10 ml dry Et_2O and stirred under N_2. The reagent $LiAl(OEt)_3H$, prepared by adding 0.34 ml absolute EtOH dropwise to 2.2g $LiAlH_4$ in 100 ml dry Et_2O, was added in 0.5 ml vols once every hr for 4 hr. The reaction was cooled, quenched with H_2O, filtered through Celite and 10% aq. NH_4Cl added until a fine white ppt. appeared in the aq. layer. The aq. layer was repeatedly extracted with Et_2O. The combined Et_2O layers were washed with H_2O, dried over Na_2So_4, filtered, and evapd to give a white solid (19 mg, 78% yield, sp. act. 9.9 µCi/mmol). Unlabelled material had mp 121–123° (lit. [15] mp 124°). NMR (60 MHz, Me_2CO-d_6)· δ4.25 (2H, *d* $J_{1,2}$ = 5 Hz, C-1), 6.14 (1 H, *dt* $J_{1,2}$ = 5 Hz and $J_{2,3}$ = 16 Hz, C-2), 6.55 (1 H, *d*, $J_{2,3}$ = 16 Hz, G-3), 7.00 (4H, center of AA′ BB′ pattern, aromatic protons).

4′-Phloretic acid-[3′, 5′-$^3H_2$] (13). A 38 mg sample of the ^{3}H labelled acid **6** was hydrogenated in EtOH over 10% Pd/C. Recrystallization in H_2O yielded white needles (36 mg, 95% yield, sp. act. 11.0 mCi/mmol). Unlabelled material gave mp 130.5–132° NMR (100 MHz, $CDCl_3$/Me_2CO-d_6): δ 2.62 (2H, *t*, *J* = 7 Hz), 2.95 (2H, *t*, *J* = 7 Hz), 7.01 (4H, center of AA′ BB′ pattern, aromatic protons).

4′-Methoxymethylenoxycinnamic acid methyl ester-[3′,5′-$^3H_2$]. A 50 mg (0.31 mmol) sample of the ^{3}H labelled 4′-hydroxycinnamic acid ester in 5 ml dry DMF was added to 110 mg NaH (56% disp, washed with hexane). To the ice cold soln stirring under N_2, an excess, 1.5 ml, of chloromethyl methyl ether was was added. The reaction stirred in an ice bath for 0.5 hr and 1 hr at room temp. Et_2O was added, and the reaction was washed with a soln of Na_2CO_3. The wash was extracted with Et_2O, and the combined Et_2O layers were dried over Na_2SO_4, filtered, and evapd leaving a yellow oil (68 mg, 100% yield). Kugelrohr distillation (120°, 0.08 mm Hg) gave an analytical sample as a pale yellow oil. (Found: C, 64.61; H, 6.39. $C_{12}H_{14}O_4$ requires: C, 64.85; H, 6.35%). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1698 (COOMe) and 836 (*p*-disubstituted aromatic ring). NMR (60 MHz, $CDCl_3$)· δ 3.37 (3H, *s*, OMe), 3.68 (3H, *s*, COOMe), 5.07 (2H, *s*, OCH_2O), 6.20 (1H, *d*, $J_{2,3}$ = 16 Hz, C-2), 7.09 (4H, center of AA′BB′ pattern, aromatic protons), 7.55 (1H, *d*, $J_{2,3}$ = 16 Hz, C-3).

4′-Methoxymethylenoxycinnamyl alcohol-[3′, 5-$^3H_2$]. A 68 mg sample of the labelled 4′-methoxymethylenoxycinnamic acid ester was dissolved in 20 ml dry Et_2O and stirred under N_2. The reagent, $LiAl(OEt)H_3$, was added in 1 ml portions once every hr for 4 hr. The reaction was cooled, quenched with H_2O, filtered through Celite, and 10% aq. NH_4Cl was added until a fine white ppt. appeared in the aq. layer. The aq. layer was repeatedly extracted with Et_2O. The combined Et_2O layers were washed with H_2O, dried over Na_2SO, filtered and evapd to give 35 mg of a light yellow oil. The product had two spots on TLC; one spot had identical TLC behaviour with inactive 4′-methoxymethylenoxycinnamyl alcohol prepared in the same manner from inactive ester. Kugelrohr distillation (95–97°, 0.075 mm Hg) gave an analytical sample as a clear oil. (Found: C, 68.17; H, 7.04. $C_{11}H_{14}O_3$; requires C, 68.02; H, 7.27%). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3425 (bonded OH), no C=O, 838 (*p*-disubstituted aromatic ring). NMR (100 MHz, $CDCl_3$): δ 3.42 (3H, *s*, OMe), 4.19 (2H, *d*, $J_{1,2}$ = 5 Hz, C-1), 5.07 (2H, *s*, OCH_2O), 6.09 (1H, *d*, $J_{1,2}$ = 5 Hz and $J_{2,3}$ = 16 Hz, C-2), 6.43 (1H, *d*, $J_{2,3}$ = 16 Hz C-3), 6.96 (4H, center of AA′BB′ pattern, aromatic protons).

4′-Methoxymethylenoxycinnamyl aldehyde-[3′, 5-$^3H_2$]. Labelled 4′-methoxymethylenoxycinnamyl alcohol (35 mg) from the previous expt and 350 mg of active MnO_2 were stirred under N_2 at room temp. in 10 ml $CHCl_3$ which had been dried by passing through an alumina column. After 2.5 hr, the reaction was filtered through Celite, and the solvent was evapd to yield a yellow oil (17.8 mg, sp. act 6.9 mCi/mmol). The product had one spot on TLC which was identical to inactive 4′-methoxymethylenoxycinnamyl aldehyde prepared in the same manner from unlabelled alcohol (85% yield). Kugelrohr distillation (117–120°, 0.35 mm Hg) of the unlabelled aldehyde resulted in a pale yellow oily solid. (Found: C, 68.94; H, 6.26. $C_{11}H_{12}O_3$ requires: C, 68.74; H, 6.29%). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: No OH, 1670 (CHO). NMR (60 MHz, $CDCl_3$): δ 3.60 (3H, *s*, OMe), 5.19 (2H, *s*, OCH_2O), 6.55 (1H, *dd*, $J_{1,2}$ = 8 Hz and $J_{2,3}$ = 16 Hz,

C-2), 7.25 (4H, center of AA'BB' pattern, aromatic protons), 7.39 (1H, *d*, $J_{2,3}$ = 16Hz C-3), 10.08 (1H, *d*, $J_{1,2}$ = 8 Hz, CHO).

4'-Hydroxycinnamyl aldehyde-[3', 5'-$^3H_2$] (**11**). The aldehyde (17 mg) from the previous expt was stirred in 2 N H_2SO_4 for 3 hr. The acid soln was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, dried over Na_2SO_4, filtered and evapd to give a yellow solid (7.7 mg, 61% yield). Recrystallization in C_6H_6 gave light yellow needles (sp. act. 3.5 mCi/mmol). Unlabelled material had mp 139.5–140° (lit. [16] mp 140°). NMR (100 MHz, $CDCl_3/Me_2CO$-d_6): δ 6.59 (1H, *dd*, $J_{1,2}$ = 8 Hz and $J_{2,3}$ = 16 Hz, C-2), 7.24 (4H, center of AA'BB' pattern, aromatic protons), 7.49 (1H, *d*, $J_{2,3}$ = 16 Hz, C-3), 8.74 (1H, *bs*, OH), 9.68 (1H, *d*, $J_{1,2}$ = 8 Hz, CHO).

4'-Hydroxydihydrocinnamyl alcohol-[3', 5'-$^3H_2$] (**14**). A soln of 40 mg $LiAlH_4$ in 8 ml freshly distilled THF was stirred at room temp. under N_2. Phloretic acid-[^{3}H] (**13**) (21 mg) in 2 ml THF was added dropwise. After stirring at 25° for 3 hr, the reaction was cooled in an ice bath, quenched with 10% aq. NH_4Cl, and filtered through Celite. The THF layer was separated and the aq. layer was extracted with $CHCl_3$. The combined organic layers were dried over Na_2SO_4, filtered, and evapd to give 20.6 mg of the hydroxy acid **14** as a pale yellow oil. Sp. act. 7.3 mCi/mmol. NMR (100 MHz, $CDCl_3/Me_2CO$-d_6): δ 1.80 (2H, *m*, C-2), (2H, *t*, $J_{2,3}$ = 7.5 Hz, C-3), 3.61 (2H, *t*, $J_{1,2}$ = 6.5 Hz, C-1), 6.84 (4H, center of AA'BB' pattern, aromatic protons).

Vanillin-[5-^3H]. Prepared by a base catalyzed exchange according to the procedure of refs [11, 12] in 80% yield. The sp. act. of the product from a reaction using 100 mCi ^3H_2O (0.02 ml) and 0.3 mmol vanillin was 7.95 mCi/mmol.

Ferulic acid-[5'-^3H] (**8**). Prepared by a malonic acid synthesis as in [13] from 0.26 mmole vanillin-[5-^{3}H] in 41% yield using 7 µl piperidine as a catalyst for the condensation (sp. act 6.1 mCi/mmol).

4-Benzylvanillin-[3-O-methyl-^{14}C]. Prepared as in refs [17, 18] from 40 mg 4-benzylvanillin and 7.3 mg methyl iodide-[^{14}C] (250 µl) in 82% yield (sp. act 1.98 mCi/mmol).

Vanillin-[O-methyl-^{14}C]. 4-Benzylvanillin-[3-*O*-methyl-^{14}C] (35 mg was dissolved in conc HCl–glacial HOAc (1:3) and heated for 50 min on a steam bath. The reaction was allowed to cool, poured into H_2O, basified with Na_2CO_3, extracted with $CHCl_3$, dried over Na_2SO_4, filtered and evapd to give 30 mg of a yellow solid. The solid was sublimed (50°, 1.5 mm Hg) to give the desired product as white needles (10 mg, 45% yield, sp. act. 1.72 mCi/mmol).

Ferulic acid-[3'-O-methyl-^{14}C]. Prepared by a malonic acid synthesis as in ref. [13] from 10 mg vanillin-[3-*O*-methyl-^{14}C] and 10 mg inactive vanillin in 43% yield using 5 µl of piperdine as a catylst for the condensation (sp. act. 0.55 mCi/mmol).

Cinnamic acid-[2-^3H, 1'-^{14}C] (**5**). In a modification of the method of ref. [4], dimethylsulfate (*ca* 0.12 ml, *ca* 1 mmol) was added to a stirring soln of (±)-phenylalanine (16.5 mg, 0.1 mmol) in 3N NaOH (2.5 ml). After 45 min additional dimethylsulfate (0.12 ml) was added. After a further 45 min the basic soln was heated *in vaccuo* on a steam bath for 30 min. The white solid residue was treated with 10% aq. HCl (3 ml) to pH 2, cooled and filtered to afford cinnamic acid as white needles, 11.5 mg (78% yield), mp 135–136°. By repeating the reaction with (±)-phenylalanine-[2'-^{3}H] (4 mg, ~3.3 mCi) and adding 17 mg of inactive cinnamic acid to the product, cinnamic acid-[2'-^{3}H] (18.5 mg, sp. act. 9.1 mCi/mmol) was obtained. A sample of cinnamic acid-[1'-^{14}C] (4.3 mg, sp. act. 0.72 mCi/mmol) was prepared in corresponding manner form (±)-phenylalanine-[1'-^{14}C].

3,4-Dihydroxybenzaldehyde-[5-^3H]. $AlCl_3$ (194 mg, 1.31 mmol) was placed in a small flame-dried, N_2 filled flask with a condenser, drying tube and N_2 inlet. A soln of 80 mg (0.53 mmol) vanillin-[5-^{3}H] in 1.5 ml of CH_2Cl_2 (dried over molecular sieves) was added slowly with stirring. After cooling to 10° 0.45 ml (1.3 mmol) of Py was added, and the reaction was warmed to room temp. and then refluxed at 45–50° for 24 hr under N_2. The reaction was cooled below 25° and hydrolyzed with 15–20% HCl until acidic to Congo Red. The lower phase (CH_2Cl_2) contained the vanillin, and it was extracted with more dil. HCl. The aq. extracts were combined and extracted with Et_2O. The Et_2O containing the dihydroxyaldehyde was evapd under N_2 yielding 65 mg (92% yield) of the brownish-purple residue. The product was not worked up due to its facile oxidation, but it was converted immediately in the malonic acid condesation to form 3',4'-dihydroxycinnamic acid.

3',4-Dihydroxycinnamic acid-[5-^3H] (**7**). The aldehyde (64.6 mg, 0.47 mmol), 104 mg (1 mmol) malonic acid, 1 ml of Py and 7 µl aniline were mixed, and the malonic acid condensation procedure for ferulic acid was followed with the following exception. The acidified Py-H_2O mixture was extracted with Et_2O instead of EtOAc. The Et_2O was removed, and the residue slurried in Na_2CO_3 soln (pH 7), filtered and acidified to yield brown crystals which after crystallization from H_2O gave the pure acid **6** (20 mg. sp. act. 6.5 mCi/mmol).

Mesembrenone-[4'-OC$^3H_3$] (**22**). 4'-*O*-Demethylmesembrenone (13 mg) was placed in a 10 ml round-bottomed flask equipped with a rubber septum. Two drops of ^3H_2O (5 Ci/ml) and 4 drops dry dioxane were added. The mixture was warmed until homogeneous, 8 ml of a dry ethereal CH_2N_2 soln, and another 1 ml dioxane were added, and the soln was stored at 0° for 5 days. After addition of HOAc to the reaction, the aq. layer was basified with satd Na_2CO_3 soln, extracted with $CHCl_3$, dried over Na_2SO_4, filtered and evapd, yielding a dark oil. The oil was separated on prewashed commercial Si gel TLC using $CHCl_3$–MeOH (19:1) to give **22** (3 mg) as yellow oil (sp. act. 32.2 mCi/mmol).

4'-O-Demethylmesembrenone-[5'-^3H] (**20**). Following a procedure described in ref. [18] a soln of 95 mg 4'-*O*-demethylmesembrenone, 50 mg dry Na formate, 1 ml dry DMF, 0.1 ml ^3H_2O (500 mCi), and 0.1 ml of H_2O was heated in a sealed tube under N_2 in an oil bath at 100± 5° for 7 days. The tube was then opened, and the contents were diluted with aq. Na_2CO_3 and extracted with $CHCl_3$. The $CHCl_3$ layer was dried over Na_2SO_4, filtered and evapd using vacuum distillation to remove the remaining DMF. The residue was stirred *ca* 18 hr under N_2 with 140 mg K *t*-butoxide in 2 ml H_2O. An excess of solid CO_2 was added to the soln, and the liberated phenol was extracted into $CHCl_3$, dried over Na_2SO_4, filtered and evapd. TLC of the residue on Si gel using $CHCl_3$–MeOH (9:1) resulted in 30 mg of desired product **20** as a pale yellow solid (sp. act. 7.1 mCi/mmol).

Mesembrenone-[5'-^3H] (**21**). A 20 mg sample of **20** was methylated with CH_2N_2. The resulting yellow oil was purified by TLC on Si gel using $CHCl_3$–MeOH (19:1) to give **21** as a white oil (13.8 mg, sp. act. 6.55 mCi/mmol).

4-Bromophenol-t-butyldimethylsilyl ether. Following the general procedure of ref. [10], 1.73 g (10 mmol) *p*-bromophenol, 2.00 g (3 equiv) imidazole, and 1.82 g (1.2) equiv *t*-butyldimethylsilyl chloride in 4 ml dry DMF were stirred *ca* 18 hr under N_2 at 40°. The reaction was worked up by adding $CHCl_3$, washing with H_2O, drying the organic layer over Na_2SO_4, filtering, evaporating the $CHCl_3$ and vacuum distilling the remaining DMF. The residue was column chromatographed on neutral alumina (Act II, 30 g) eluting with C_6H_6 to yield 2.33 g of a colorless liquid (81% yield). An analytical sample was prepared by Kugelrohr distillation (55°, 0.05 mm Hg). (Found: C, 49.92; H, 6.71. $C_{12}H_{19}BrOSi$ requires C, 50.17; H, 6.67%). IR $\nu_{max}^{CHCl_3}$. cm^{-1}: No OH, 1255 ($SiMe_2$). NMR (100 MHz, $CDCl_3$): δ 0.17 (6H, *s*, SiMe), 0.98 (9H, *s*, CMe), 7.13 (4H, center of AA'BB' pattern, aromatic protons).

1-Methyl-3-(4-(t-butyldimethyl ether) phenyl)-3-pyrrolidinol (**18**). A 5.74 g (0.02 mol) sample of the silyl ether in 20 ml dry THF was cooled in a dry ice–Me_2CO bath and kept under N_2. Over a 20 min period 8.7 ml (0.02 ml) of *n*-butyllithium (2.3 M soln in hexane) were added dropwise. After 40 min 1.85 ml (*ca* 0.02 mol) of *N*-methylpyrrolidone [19] in 2 ml dry THF were added dropwise. After an additional 45 min, the soln was allowed to warm up to room temp. and quenched with H_2O (10 ml) before extraction with $CHCl_3$. The $CHCl_3$ layer was dried over Na_2SO_4, filtered and evapd to give 5.3 g of crude product. The crude product was dissolved in Et_2O, extracted with N HCl, basified with Na_2CO_3 and continuously extracted with $CHCl_3$.

Evapn of the $CHCl_3$ soln gave 2.87 g of the product as a dark oil (47% yield). Kugelrohr distillation (100°, 0.1 mm Hg) resulted in a pale yellow oil. (Found: C, 66.83; H, 9.54; N, 4.75. $C_{17}H_{29}NO_2Si$ requires:C, 66.41; H, 9.50, N, 4.56%). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3495 (free OH), 1252 ($SiMe_2$). NMR (100 MHz, $CDCl_3$): δ 0.20 (6H, *s*, SiMe), 1.00 (9H, *s*, CMe), 2.2–3.2 (6H, *m*, CH_2), 2.49 (3H, *s*, NMe), 7.05 (4H, center of AA'BB' pattern aromatic protons).

1-Methyl-3-(4-hydroxyphenyl-1-pyrroline (**19**). Following the method of ref. [9], 1.38 g (4.5 mmol) of the pyrrolidinol (**19**) were refluxed in 50 ml of 6 N HCl for 2 hr. The soln was cooled and basified with Na_2CO_3, at a temp. below 25°. The aq. soln was then extracted with $CHCl_3$, the $CHCl_3$ dried over Na_2SO_4, filtered and evapd to yield 0.61 g of product as an orange oily solid (81% [illegible] 70ev *m/e*: M^+ 175.0995, $C_{11}H_{13}NO$ requires [illegible] IR ν_{max} cm^{-1}: 1662 and 1620 (C=C). NMR (100 MHz, $CDCl_3$–CD_3OD): δ 2.4–3.2 (4H, *m*, CH_2), 2.63 (3H, *s*, NMe), 6.25 (1H, *s*, vinyl proton), 6.94 (4H, center of AA'BB' pattern, aromatic protons).

Sceletenone (**10**). Following the method of ref. [20], 0.85 g (5.1 mmol) of the pyrroline (**22**) was dissolved in dry absolute EtOH and satd with dry HCl gas. Removal of the EtOH provided a gummy HCl salt which was dissolved in 25 ml dry MeCN. Freshly made β-chloromethylvinylketone mixture (2 ml) was added, and the soln refluxed under N_2 for 18 hr. The reaction was cooled, poured into 100 ml of N HCl and extracted with Et_2O to remove nonbasic materials. The aq. soln was extracted with $CHCl_3$ which was dried over Na_2SO_4, filtered, and evapd. The resulting dark oil was chromatographed twice on Si gel TLC eluted with $CHCl_3$–MeOH (9:1) to yield (±)-sceletenone (**10**), 107 mg (8% yield), oil MS (probe) 70 eV *m/e* (rel. int): 243 (M^+; 60), 215(M-CO; 8) 175($C_{11}H_{13}NO$; 10), 70 (C_4H_8N; 100); M^+ 243.1255, $C_{15}H_{17}NO_2$ requires 243.1259. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3592 (free OH), 3300 bonded OH), 1678 (C=O). NMR (100 MHz, $CDCl_3$): 2.0–2.7(6H, *m*), 2.32(3H, *s*, NMe), 3.3 (1H, *m*, C-7a), 6.09 (1H, *d*, J = 10 Hz, C-5), 6.74 (1H, *dd*, J = 10 and 2 Hz, C-4), 7.02 (4H center of AA'BB' pattern aromatic protons). The spectral and chromatographic behavior (GLC: OV-17, 250°; TLC: Si gel, $CHCl_3$–MeOH, 9·1) of the synthetic sample was identical to that of the naturally occurring material.

Sceletenone-[3', 5'-$^3H_2$] (**24**). Prepared in the same manner as 4'-*O*-demethylmesembrenone-[5'-^{3}H]. A 32 mg sample of sceletenone yielded 16 mg of the labelled alkaloid as a pale yellow oil (sp. act. 6.6 mCi/mmol).

Feeding experiments with labelled precusors. (1). *Cinnamic acid-[2'-^3H,1'-^{14}C]* (**5**). A mixture (^{3}H: 328 μCi and ^{14}C: 22.5 μCi, ^{3}H: ^{14}C = 14.5:1) of labelled cinnamic acids (**5**) was neutralized with $NaHCO_3$ and fed to 3 *S. strictum* plants which were maintained in the Phytotron for 12 days before harvesting. The crude alkaloid fraction (27.2 mg) was combined with mesembrenol (18 mg) and mesembrenone (22 mg). Chromatography of the alkaloid fraction on Al_2O_3 (20 g, Act II) in C_6H_6 (1–35), C_6H_6–EtOAc (3:1) (36–70) and EtOAc–MeOH (93:7) (71–90) gave mesembrenone (15 mg: 15–45) and mesembrenol (50 mg: 55–80). The mesembrenol was purified by PLC on Si gel to give 13.4 mg which was recrystallized from EtOAc to constant activity (9.9 mg, sp. act. ^{3}H 0.011 μCi/mg, ^{14}C 0.008 μCi/mg; ^{3}H: ^{14}C = 13.3:1) corresponding to 0.06% incorporation of **5**.

(2). *4'-Hydroxycinnamic acid-[3', 5'-$^3H_2$]* (**6**). Compound **6** (9.6 mg, 32 μCi/mg, 310 μCi) was dissolved in 0.5 ml M $NaHCO_3$ soln (pH 7.5) and fed to 4 plants for 23 days. Due to the spillage of one feeding tube *ca* 25% of the radioactive material was lost, and only 240 μCi were fed. The extraction procedure gave 31 mg (3.7% relative plant extract) of crude alkaloids with 4'-*O*-demethylmesembranol, mesembrenol, mesembrenone and mesembrine (2:2:1:1) mixture by GLC and TLC. Radioactivity occurred in several of the alkaloid zones indicating incorporation. The non-phenolic alkaloids (20 mg) were separated, diluted with inactive alkaloids, and chromatographed on alumina with the series of solvent elution as described before. Recrystallization of mesembrenol to constant activity gave crystals corresponding to 0.15% incorporation. Sp. act. 5×10^{-3} μCi/mg. A repeat feeding was done 10 months later using 11 mg (350 μCi) in M $NaHCO_3$ soln fed to 2 plants for 16 days. The incorporation was determined to be 0.05% by recrystallization of mesembrenol (sp. act. 3.5×10^{-3} μCi/mg).

(3). *3',4'-Dihydroxycinnamic acid-[5'-^3H]* (**7**). Compound **7** (8.8 mg, 36.3 μCi/mg, 320 μCi) was dissolved in 0.25 ml M $NaHCO_3$ soln and 0.25 ml 1% Tween 20 (pH 7) and fed to 5 plants for 15 days. The Na salt of this compound tended to decompose in the feeding tubes. Also, the plants appeared sickly after 15 days, and this experiment was terminated. The usual extraction procedure gave 45 mg (3.6% relative crude plant extract). GLC and TLC indicated mesembranol and mesembrenol to be the predominant alkaloids with some 4'-*O*-demethylmesembranol. The non-phenolic alkaloids (32 mg) were diluted with the inactive alkaloids, chromatographed and isolated. Recrystallization of the mesembrenol fraction from EtOAc gave material of constant activity (sp. act. 2.35×10^{-3} μCi/mg, 0.0125% incorporation). A repeat experiment was done 13 months later using 11 mg (395 μCi) dissolved in 0.3 ml M $NaHCO_3$ soln fed to 2 plants for 21 days. Mesembrenol was isolated and purified to constant activity (sp. act. 1.3×10^{-3} μCi/mg, 0.0084% incorporation).

(4) *Ferulic acid-[5'-^3H]* (**8**). Ferulic acid-[5'-^{3}H] (8.7 mg, 37.2 μCi/mg; 320 μCi) was dissolved in 0.25 ml M $NaHCO_3$ soln fed to 2 plants for 21 days. Mesembrenol was isolated 23 days. Extraction of the alkaloids gave 37 mg (3.7% relative plant extract) of crude alkaloid. GLC and TLC data indicated 4'-*O*-demethylmesembrenol 4'-*O*-demethylmesembranol and 4'-*O*-demethylmesembrenone and very little non-phenolic alkaloids to be present. The non-phenolic alkaloids (13.3 mg) were separated, diluted with 150 mg mesembrenone and mesembrine, 20 mg mesembrenol and 40 mg mesembranol, and chromatographed on alumina (Act II) as before. Mesembrenol was crystallized to constant activity from EtOAc (sp. act. 1×10^{-4} μCi/mg. 0.0003% incorporation). The phenolic alkaloid fraction was treated with CH_2N_2 and mesembrenol (20 mg) added. Purification by column chromatography, and recrystallization in EtOAc gave mesembrenol (sp. act. 7×10^{-3} μCi/mg, 0 058% incorporation).

(5) *4'-Hydroxycinnamyl alcohol-[3', 5-$^3H_2$]* (**12**) A 4.88 mg (320 μCi) sample of **12** dissolved in 1.5 ml 2% Tween soln was fed to 4 plants in the Phytotron for 13 days. The feeding yielded an alkaloid fraction of 108 mg. Inactive mesembrenol (18.5 mg) was added to 55.3 mg of the alkaloid fraction and the mesembrenol crystallized to constant activity (26.5 mg, 0.06 μCi/mg) corresponding to a 0.099% incorporation of **12**.

Degradation of mesembrenol to veratric acid. A modification of the method of [21] was used. The mesembrenol-[5'-^{3}H] obtained from the feeding of **12** and the ^{14}C-labelled mesembrenol obtained from feeding phenylalanine-[^{14}C(U)] were mixed to give 25 mg of doubly labelled mesembrenol (^{3}H: ^{14}C = 36.97:1). A soln of the doubly labelled mesembrenol, 180 mg KOH, 1g potassium ferricyanide and 3 ml H_2O was heated in an oil bath with stirring. After 24 hr another 180 mg KOH and 1g potassium ferricyanide were added. After another 24 hr similar amounts of KOH and potassium ferricyanide were added and 20 hr later the soln was cooled, filtered through Celite and acidified with a soln of 50% conc H_2SO_4. The acidic soln was extracted with $CHCl_3$ which was then dried over Na_2SO_4, filtered and evapd. PLC of the residue (5 mg) on Si gel eluted with $CHCl_3$–MeOH (9:1) gave 2 mg veratric acid as a light yellow solid (^{3}H: ^{14}C = 34.9: ^{3}H retention = 94%), mp 179–81°. MS (probe) 70 eV *m/e* (rel. int.): 166 (M^+, 100).

(6) *4'-Hydroxycinnamyl aldehyde-[3', 5'-$^3H_2$]* (**11**). A 6.8 mg (165 μCi) sample of **11** dissolved in 1.6 ml 5% Tween soln was to 5 plants in the Phytotron for 13 days. The feeding yielded an an alkaloid fraction of 219 mg. Since during repeated recrystallization the isolated mesembrenol did not reach radiochemical purity, the mesembrenol was converted to the hydrochloride salt of mesembrenone. Inactive mesembrenol (20 mg) was added to 50 mg of the alkaloid fraction from the feeding of **11** to give *ca* 28 mg of mesembrenol-[5'-^{3}H]. The mesembrenol-[5'-^{3}H] was dissolved in 10 ml of Me_2CO and cooled to −10°. Chromic acid

(3 drops) was added and the stirring soln maintained at −10° for 20 min. Excess chromic acid was destroyed by the addition of 2 ml *iso*-PrOH–Me_2CO (1:1) and the solvents were removed *in vacuo*. The residue was dissolved in 10 ml H_2O, basified with Na_2SO_4, filtered and evapd. PLC of the residue (5 mg) on Si gel short alumina column (neutral, Act III) using C_6H_6–EtAc (9:1) Evapn of the solvent gave 13 mg of a clear oil which had the identical TLC behaviour of an authentic sample of mesembrenone. The HCl salt showed negligible activity.

(7) *4'-Phloretic acid-[3', 5'-$^3H_2$]* (**13**). A 4.49 mg (310 μCi) sample of **13** dissolved in 0.8 ml H_2O was fed to 3 plants in the Phytotron. After 7 days isolation gave an alkaloid fraction of 90.5 mg. Inactive mesembrenol (18 mg) was added to 51 mg of the alkaloid fraction to give mesembrenol (30 mg, 0.0066 μCi/mg) corresponding to a 0.13% incorporation of **13**.

(8). *4'-Phloretic acid-[3', 5-$^3H_2$]* (**13**) *with inactive 4'-hydroxycinnamic acid.* A 4.45 mg (310 μCi) sample of **13** and 30 mg of inactive 4'-hydroxycinnamic acid (as its $NaHCO_3$ salt) dissolved in H_2O, pH = 7.4, were fed to 4 plants in the Phytotron. After 7 days, the plants were extracted to yield an alkaloid fraction of 56 mg. Inactive mesembrenol (20 mg) was added to the alkaloid fraction to give mesembrenol (26 mg, 0.0019 μCi/mg) corresponding to a 0.032% incorporation of **13**.

Isolation and purification of 4'-hydroxycinnamic acid. The Et_2O layer obtained in the previous experiment containing the nonbasic compounds was evapd to give 224 mg of a green residue. The residue was dissolved in $CHCl_3$ and extracted with 50% satd $NaHCO_3$ soln. The aq. layer was acidified with dropwise addition of conc HCl. Repeated extraction of the acidic soln with Et_2O yielded 15.1 mg of a yellow oil. PLC of the oil on K_2CO_3–Si gel (1:19) using $CHCl_3$–MeOH (1:1) and elution of a band, $R_f \sim 0.3$, gave 3.5 mg of a yellow oil. The oil was diluted with 7 mg of inactive 4'-hydroxycinnamic acid and repeatedly recrystallized to radiochemical purity (0.023 μCi/mg) corresponding to a 0.24% incorporation of **13**. A sample of 4 mg 4'-hydroxycinnamic acid from the feeding of **13** was methylated with CH_2N_2. PLC of the resulting oil on K_2CO_3–Si gel (1:19) using $CHCl_3$–Me_2CO (3:1) yielded the methyl ester as a white solid (0.019 μCi/mg) corresponding to a 0.19% incorporation of **13** into 4'-hydroxycinnamic acid.

(9) *4-Hydroxydihydrocinnamyl alcohol-[3', 5'-$^3H_2$]* (**14**). A 6.06 mg (291 μCi) sample of **14** dissolved in 1.1 ml 2% Tween was fed to 4 plants in the greenhouse for 12 days. The plants were not watered during the feeding. Extraction of the plants gave 56 mg of non-phenolic alkaloids. Inactive mesembrenol (20 mg) was was added to the non-phenolic alkaloid fraction to give mesembrenol (26.5 mg, 0.0021 μCi/mg) corresponding to a 0.024% incorporation of **14**.

(10) *Ferulic acid-[3'-O-methyl-^{14}C, 5'-^3H]* (17) An 18 mg mixture (^{3}H:316.1 μCi and ^{14}C:23.45 μCi, ^{3}H:^{14}C = 13.5:1) of labelled ferulic acid samples (as the $NaHCO_3$ salts) dissolved in 1.5 ml H_2O was fed to 6 plants in the Phytotron. The plants were not watered during the feeding period of 10 days. Isolation of the alkaloids gave 511 mg of non-phenolic bases and 93 mg of phenolic alkaloids. Inactive mesembrenol (10 mg) was added to the non-phenolic alkaloid fraction to give mesembrenol (1.3 mg, ^{3}H: 0.0032 μCi/mg, ^{14}C: 0.003 μCi/mg) corresponding to a 0.01% incorporation (^{3}H:^{14}C = 12.6:1. 93% ^{3}H retention) of ferulic acid. The phenolic fraction gave 4'-*O*-demethylmesembrenone (2 mg. ^{3}H: 0.104 μCi/mg, ^{14}C: 0.017 μCi/mg) corresponding to 0.15% incorporation (^{3}H:^{14}C = 6.26:1, 46% ^{3}H retention) of ferulic acid.

Isolation of ferulic acid methyl ester. The ferulic acid methyl ester was isolated from a band, $R_f = 0.8$ by PLC on Si gel eluted with $CHCl_3$–MeOH (9:1) of the phenolic alkaloid fraction obtained from the ferulic acid feeding. The compound was identified by MS and by recrystallization with inactive ferulic acid methyl ester without any accompanying change in radioactivity. A 10 mg sample of the ester (^{3}H:0.18 μCi/mg, ^{4}C:0.016 μCi/mg) corresponding to 0.73% incorporation (^{3}H:^{14}C = 12.15:1, 90.0% ^{3}H retention) of **17** was isolated. MS (probe) 70eV *m/e* (rel. int.) : 208 (M^+, 100), 177 (M-OMe, 84), 145 (M-$C_2H_7O_2$, 58).

(11) *4'-Phloretic acid-[3', 5'-$^3H_2$]* (**13**). A 4.92 mg (303 μCi) sample of **13** dissolved in 0.8 ml H_2O was fed to 6 plants in the greenhouse for 14 days. Extraction of the plants yielded 320 mg of non-phenolic alkaloids which afforded 82 mg of mesembrenol (0.042 μCi/mg) corresponding to a 2.26% incorporation of **13**.

(12) *4'-Phloretic acid-[3', 5'-$^3H_2$]* (**13**) *and inactive ferulic acid.* A 4.93 mg (303 μCi) sample of **13** and 30 mg of inactive ferulic acid (as its $NaHCO_3$ salt) dissolved in 1 ml H_2O, pH = 7.8, were fed to 6 plants in the greenhouse for 14 days. The feeding yielded 237 mg of non-phenolic alkaloids and 68.5 mg of mesembrenol (0.021 μCi/mg) corresponding to a 0.92% incorporation of **13**.

Isolation of ferulic acid-[5'-^3H] (**8**). The Et_2O layer obtained in the previous experiment containing the nonbasic compounds was extracted with a 50% satd $NaHCO_3$ soln. The aq. layer was acidified with conc HCl. Continuous extraction of the acidic soln with $CHCl_3$ for 3 days yielded 70 mg of a yellow–brown residue. PLC of the residue on K_2CO_3–Si gel (1:19) eluting with $CHCl_3$–MeOH (1:1) resulted in 9.6 mg of crude ferulic acid. This was dissolved in MeOH and treated with CH_2N_2 in Et_2O. The product was run 3× on PLC using K_2CO_3-Si gel (1:19) eluted with $CHCl_3$–Me_2CO (6:1) and then recrystallized from H_2O to give methyl ferulate (1 mg, 0.02 μCi/mg) corresponding to a 0.38% incorporation of **13**

(13) *Mesembrenone-[4'-OCT$^3H_3$]* (**22**). A 2.38 mg (300 μCi) sample of **22** (as the HCl salt) dissolved in 1 ml H_2O (pH = 3) was fed to 6 plants in the Phytotron for 10 days. Isolation of the alkaloids gave 291 mg of non-phenolic bases. Inactive mesembrenol (10 mg) was added to the non-phenolic fraction which was purified to give mesembrenol (20 mg. 0.085 μCi/mg) corresponding to a 1.12% incorporation of **22**. The non-phenolic alkaloid fraction also gave a new crystalline alkaloid, *N*-demethyl-*N*-formylmesembrenone [22] (19.5 mg, 0.044 μCi/mg) corresponding to a 0.58% incorporation of **22**

(14)-O-*Demethylmesembrenone-[5'-^3H]* (**20**). A 9.9 mg (258 μCi) sample of **20** (as its HCl salt) dissolved in 1 ml H_2O (pH = 4) was fed to 6 plants in the greenhouse for 14 days. The feeding yielded 393 mg of non-phenolic alkaloids and to give mesembrenol (113 mg, 0.67 μCi/mg) corresponding to a 58.6% incorporation of **20**.

(15) *Mesembrenone-[5'-^3H]* (**21**). A 11.9 mg (268 μCi) sample of **21** (as its HCl salt) dissolved in 1.5 ml H_2O (pH = 5) was fed to 6 plants in the greenhouse for 14 days. Extraction and isolation of the alkaloid fraction yielded 284 mg of non-phenolic alkaloids and 101 mg of phenolic alkaloids. Purification of the non-phenolic alkaloid fraction gave mesembrenol (73 mg, 1.17 μCi/mg, 63.6% incorporation of **21**) and mesembranol (37.5 mg, 0.19 μCi/mg, 0.54% incorporation). 4'-*O*-Demethylmesembrenone (14 mg) was added to the crude phenolic fraction which was purified to give 4'-*O*-demethylmesembrenone (<0.0024 μCi/mg, <0.021% incorporation).

Purification of mesembrine-[5'-^3H] (1) as its reduction product mesembrenol-[5'-^3H]. A mixture of mesembrine and mesembrenone, (62.5 mg), obtained from the initial TLC of the non-phenolic alkaloids in the previous experiment was dissolved in 10 ml EtOH and treated with 300 mg $NaBH_4$. The mixture was refluxed for 30 min and allowed to cool. The solvent as removed and the solid residue was dissolved in H_2O and extracted with $CHCl_3$. The layer was dried over Na_2SO_4, filtered and evapd to give a yellow oil. PLC on Si gel eluting 3× with $CHCl_3$–MeOH (19:1) gave mesembrenol (7.1 mg, 0.26 μCi/mg; 3.4% incorporation).

(16) *Sceletenone-[3', 5'-$^3H_2$]* (**24**). An 8.6 mg (207 μCi) sample of radiolabelled sceletenone (as its HCl salt) dissolved in 1.5 ml H_2O (pH = 6) was fed to 4 plants in the greenhouse. After 12 days the plants were harvested and extracted to give 122 mg of non-phenolic alkaloids. Inactive mesembrenol (12 mg) was added to the non-phenolic alkaloid fraction which was purified to give mesembrenol (23.3 mg, 0.18 μCi/mg, 2% incorporation).

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