Biosynthesis of Mesembrine and Related Alkaloids.[1] The Amino Acid Precursors[2]

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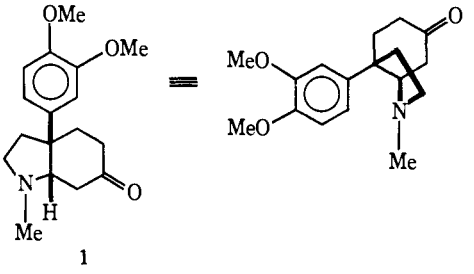
Abstract: The biosynthesis of mesembrine and related alkaloids occurring in *Sceletium strictum* L. Bol. has been investigated by administering L-[*S-methyl*-^{14}C]methionine, DL-[*2*-^{14}C]tyrosine, DL-[*3*-^{14}C]tyrosine, DL-[*ring*-^{14}C]-phenylalanine, and DL-[*3*-^{14}C]phenylalanine into the stems of live plants of this species. Chemical degradations of labeled alkaloids obtained in these experiments are used to establish that the aromatic ring in the mesembrine alkaloids is derived from the aromatic ring of phenylalanine but not tyrosine and that the perhydroindole moiety is derived from tyrosine and not phenylalanine. It was found that the *S*-methyl group of L-methionine provides the *O*- and *N*-methyl groups in these alkaloids.

Species of the *Sceletium* genera of the Aizoaceae family produce alkaloids, most of which belong to the octahydroindole class as exemplified in the structure of mesembrine (**1**). The structure patterns of this group contain a ring system which in principle may be constructed from a familiar C_6C_2N unit and an aromatic C_6 unit. The biosynthetic origin of the latter was of considerable interest since the occurrence of an isolated aromatic C_6 unit is certainly of rare, if not of unique, occurrence in the structures of natural products. This paper describes the work leading to the determination of the origin of both the C_6 unit and the C_6C_2N unit.



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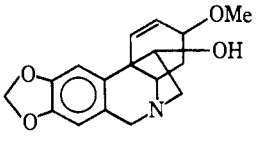

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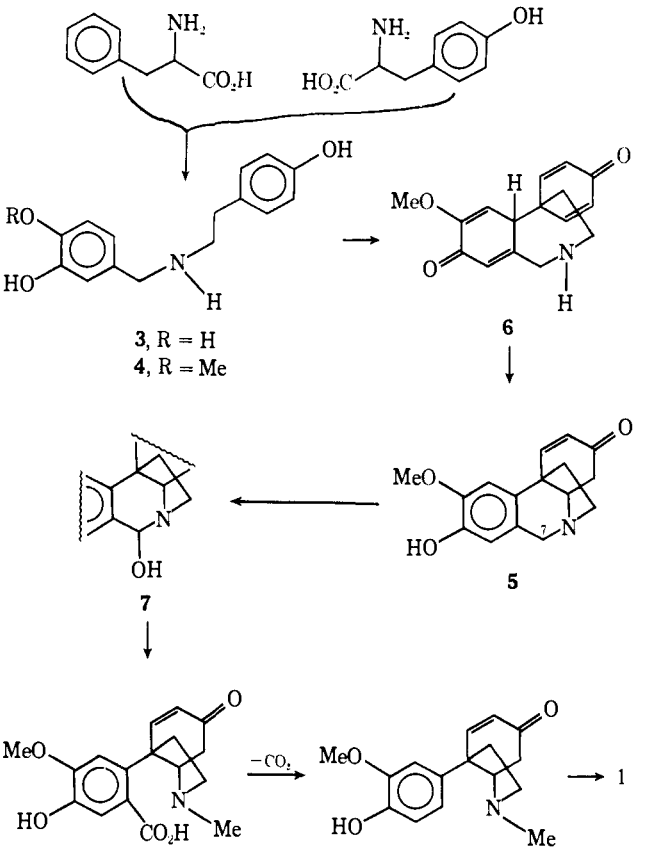
Although one scheme for the biosynthesis of mesembrine had been proposed,[4] our initial experiments were guided by the following alternative proposal. The close structural similarity of mesembrine and related alkaloids to the Amaryllidaceae alkaloids of the crinane class, *e.g.*, haemanthamine (**2**), prompted a consideration that biosynthesis of the two groups might follow a parallel course as summarized in Scheme I. Extensive

Scheme I. Proposed Biogenetic Pathway for Mesembrine



3, R = H
4, R = Me

6

5

7

investigations by a number of research groups[5] have established that the biosynthesis of haemanthamine involves the construction of the carbon skeleton from the amino acids phenylalanine and tyrosine, each of which follow separate pathways in providing the C_6C_1 and C_6C_2N units, respectively.

The further transformation of these two amino acids which leads to their conversion to the important intermediate norbelladine (**3**) has been demonstrated. Specific methylation of **3** to *O*-methylnorbelladine (**4**)

(1) Supported in part by the National Science Foundation (Grant No. GB4361) and by the National Institutes of Health (Grant No. 1R01 AM13977-02). A portion of this work has appeared as a preliminary communication: P. W. Jeffs, W. C. Archie, and D. S. Farrier, *J. Amer. Chem. Soc.*, **89**, 2509 (1967).

(2) (a) This paper is considered as part IV in the series Sceletium Alkaloids; (b) for part III, see P. W. Jeffs, G. Ahmann, H. F. Campbell, D. S. Farrier, G. Ganguli, and R. L. Hawks, *J. Org. Chem.*, **35**, 3512 (1970).

(3) (a) Career Development Awardee of the U. S. Public Health Service (No. 1K04 GM42342-02) from the Institute of General Medical Sciences; (b) NSF Undergraduate Research Participant 1965–1966; (c) NASA Fellow 1965–1968.

(4) K. Bodendorf and P. Kloss, *Arch. Pharm. (Weinheim, Ger.)*, **294**, 654 (1961).

(5) D. H. R. Barton, G. W. Kirby, J. B. Taylor, and G. M. Thomas, *J. Chem. Soc.*, 4545 (1963); A. R. Battersby, R. Binks, S. R. Breur, H. M. Fales, W C. Wildman, and R. J. Highet, *ibid.*, 1595 (1964); P. W. Jeffs, *Proc. Chem. Soc. London*, 80 (1962); J. Zulalian and R. J. Suhadolnik, *ibid.*, 422 (1964).

Table I. Incorporation of Carbon-14 from Labeled Amino Acids into Mesembrine Alkaloids

Test precursor	No. of days	Month	Year	Carrier[d] Wt, mg	Carrier[d] % incorporation[a]
50 μCi of L-[*S-methyl*-^{14}C]methionine	3	July	1966	130	0.75
50 μCi of L-[*S-methyl*-^{14}C]methionine	14	July	1965	200	0.70
50 μCi of L-[*S-methyl*-^{14}C]methionine	10	Nov	1968		0.032[c]
50 μCi of L-[*S-methyl*-^{14}C]methionine	7	Feb	1968		0.002[b,c]
100 μCi of sodium formate-^{14}C	8	June	1966	342	>0.001
50 μCi of DL-[*ring*-^{14}C]phenylalanine	10	Oct	1965	250	0.09
50 μCi of DL-[*ring*-^{14}C]phenylalanine	22	Oct	1965	250	0.12
50 μCi of DL-[2-^{14}C]phenylalanine	10	July	1965	250	>0.001
50 μCi of DL-[3-^{14}C]phenylalanine	6	Aug	1968		0.001[c]
48 μCi of DL-[2-^{14}C]tyrosine	3	Aug	1966	200	0.122
52 μCi of DL-[2-^{14}C]tyrosine	5	May	1967	198	0.032
					0.32[c]
50 μCi DL-[3-^{14}C]tyrosine	10	July	1965	250	0.10

[a] In the cases where mesembrine was added as a carrier this figure may be regarded as a minimum since it was determined by the inverse isotope dilution method. It is assumed that amount of carrier is large in comparison to the amount of labeled alkaloid in the extract. [b] Precursor administered hydroponically to freshly cut shoots of *S. strictum*. [c] Mesembrenol. [d] Mesembrine.

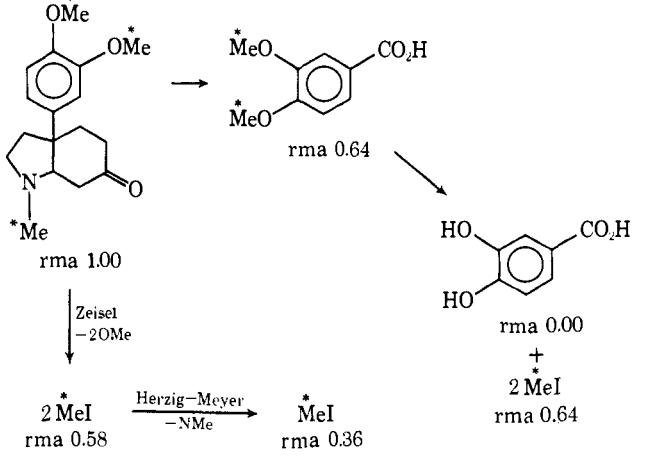
occurs, and this step is supported by the isolation of the enzyme mediating this reaction.[6] Although the subsequent steps utilized in the construction of the first intermediate possessing the crinane skeleton (*cf.* **5**) are as yet without direct experimental support, there is now an impressive body of evidence[7] available from biosynthetic studies on a wide variety of natural products to suggest that **5** is formed *via* a pathway which involves the intermediacy of the dienone **6**.

In a formal sense, conversion of the crinane skeleton to the mesembrine type involves the loss of the C-7 benzylic carbon. This can be envisioned to arise by hydroxylation at this position to afford the carbinolamine **7** which undergoes oxidation, either prior to or after *N*-methylation, to afford an amino acid which is in turn decarboxylated.[8] Although work to be described in later papers will show that these proposals require some modification, they serve as a useful basis for examining the roles which the aromatic amino acids tyrosine and phenylalanine play in the biosynthesis of this ring system.

Preliminary experiments with ^{14}C-methyl labeled methionine were undertaken to determine the most propitious time and method of administering labeled test precursors. Efficient incorporations were obtained by administering an aqueous solution of the radiolabeled amino acid directly into the stem by the technique which is described in detail in the Experimental Section. Plants were extracted after periods ranging from 3 to 21 days and inactive mesembrine was usually added to the crude alkaloid fraction as a carrier. Incorporation figures varied little with the duration of the feeding with this precursor over this time period. However, considerable differences in incorporation levels were observed with the time of year, the most efficient uptake being obtained during the months of April through July. Also some difference in levels of incorporation was noted in different alkaloids from the same feeding experiment. These results, together with those feeding experiments with radiolabeled phenylalanines and radiolabeled tyrosines, are presented in Table I.

Radioactive mesembrine isolated from a methionine feeding experiment was rigorously purified by preparative layer chromatography and by crystallization as the hydrochloride to constant radiochemical purity. Sequential *O*-methyl and *N*-methyl determination of this sample by the classical Zeisel–Hertzig–Meyer procedure showed that essentially all of the radioactivity was associated with the methyl groups. Furthermore, each methyl group appears to be equally labeled since the ratio of activity for the two methoxyls to the *N*-methyl group is very close to 2:1. Independent confirmation of these results was obtained by vigorous oxidation of the methionine-derived mesembrine to veratric acid, which was found to contain 64% of the original activity present in the alkaloid. All of the label in the veratric acid sample was accounted for in the two methoxyls as evidenced by its conversion by refluxing hydrobromic acid to inactive protocatechuic acid and radiolabeled methyl iodide. These results are summarized in Scheme II.

Scheme II. Degradation of Labeled Mesembrine Derived from L-[*S-methyl*-^{14}C]Methionine



Feeding experiments were undertaken with labeled phenylalanine and tyrosine in order to investigate the role which these two amino acids might play in the construction of the carbon skeleton of mesembrine. Of the three labeled phenylalanines in which the site of the ^{14}C was located at C-2, C-3, or uniformly on the carbons of

(6) H. M. Fales, J. Mann, and S. H. Mudd, *J. Amer. Chem. Soc.*, **85**, 2025 (1963); J. Mann, H. M. Fales, and S. H. Mudd, *J. Biol. Chem.*, **238**, 3820 (1963).

(7) For a summary, see A. R. Battersby, "Oxidative Coupling of Phenols," W. I. Taylor and A. R. Battersby, Ed., Marcel Dekker, New York, N. Y., 1967, p 119; A. I. Scott, *ibid.*, p 95.

(8) A similar biogenetic route has been suggested by Professor A. R. Battersby in ref 7.

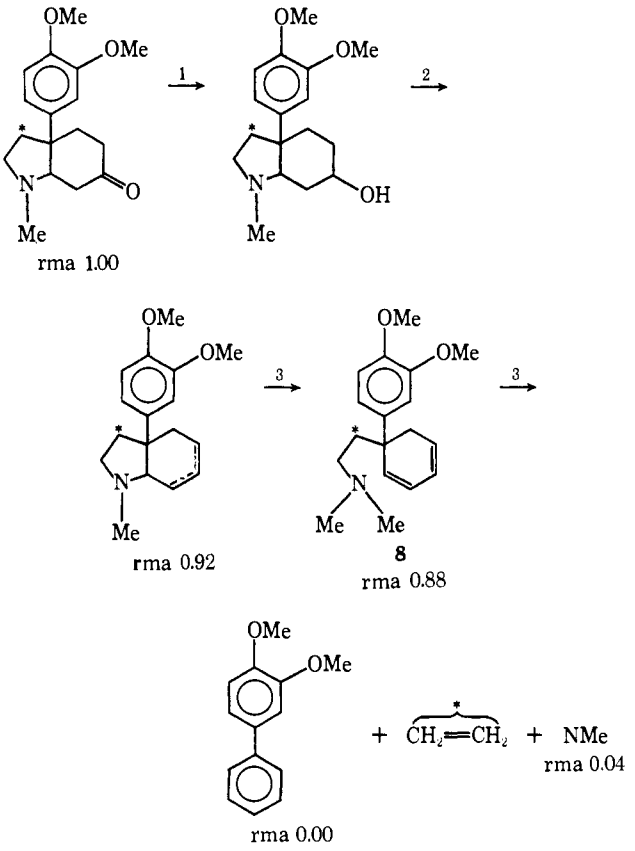
the aromatic ring, only the ring-labeled amino acid resulted in any significant incorporation of radioactivity into the alkaloid fraction. In contrast to these results, feeding experiments with both [2-^{14}C]- and [3^{14}C]tyrosines resulted in a significant incorporation of the label into the alkaloid fraction. From these results it may be concluded that phenylalanine is not converted to tyrosine in the biosynthesis of this alkaloid and it is clear that each of these amino acids follow separate metabolic routes in this *Sceletium* species. This is in keeping with the divergent pathways observed for these two amino acids in the Amaryllidaceae and in other higher plants.[9]

From the ideas expressed earlier it was expected that phenyalanine would prove to be the progenitor of the aromatic C_6 unit and the incorporation results in Table I for the tyrosine and phenylalanine experiments lend support to this suggestion. Vigorous oxidation of mesembrine derived from DL-[*ring*-^{14}C]phenylalanine afforded veratric acid which was shown to contain all of the radioactivity present in the original alkaloid. This result indicates that specific incorporation of phenylalanine into mesembrine occurs in a manner which results in the aromatic ring of the alkaloid being derived from the aromatic nucleus of the amino acid. Lack of incorporation of label from the feeding experiments with DL-[2-^{14}C]phenylalanine and DL-[3-^{14}C]phenylalanine indicates that the entire side chain of this amino acid is lost in the conversion of phenylalanine to mesembrine.

Incorporation of the radiolabel into mesembrine from the feeding experiments with the [2-^{14}C]- and [3-^{14}C]phenylalanine (Table I) provides a strong indication that this amino acid is the precursor of the C_6C_2N unit. If the intact incorporation of each of these labeled tyrosines occurs in the manner shown in Scheme I the mesembrine should be labeled at the C-2 or C-3 carbons. To confirm this postulate a degradation scheme to isolate the carbons at these positions was required.

An indirect approach to the solution of this problem was available from a reaction involving the methine base **8** which was first described by Popelak and coworkers.[10] This compound, which is obtained from mesembrine in excellent yield by the reactions shown in Scheme III, on treatment with methyl iodide followed by base undergoes aromatization of ring B with the formation of ethylene and trimethylamine. When labeled mesembrine derived from [3-^{14}C]tyrosine was converted to **8** and subjected to a Hofmann degradation the 3,4-dimethoxbiphenyl and trimethylamine (trapped as tetramethylammonium iodide) produced from the reaction were virtually inactive (Scheme III). This result indicates that the label is contained on the ethylene produced in this reaction and restricts the sites of label in the mesembrine to the C-2 and C-3 carbons of the ethamine bridge.[11]

Scheme III. Degradation of Labeled Mesembrine Derived from [3-^{14}C]Tyrosine[a]

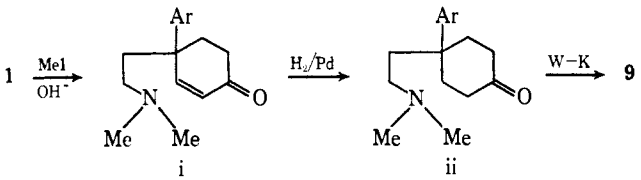


[a] 1, $NaBH_4$; 2, P_2O_5–toluene; 3, MeI–OH^-.

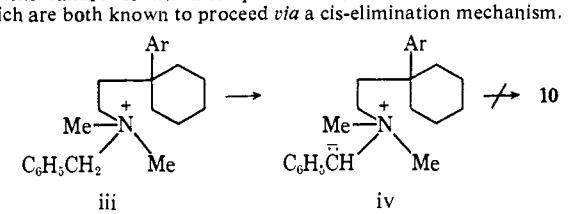
It appeared that it would be feasible to differentiate between the C-2 and C-3 carbons by conversion of the dimethylamino compound **9**[12] to the olefin **10** followed by cleavage to formaldehyde. Despite numerous attempts to effect the transformation of **9** to **10** by a variety of reactions under various conditions[13] none were successful.

A study of the chemistry of mesembrenol (**11**), which often occurs as the major alkaloid in *S. strictum*, provided a solution to the problem. The Hofmann degradation of **11** afforded the dienol **12** which without isola-

(12) Compound **9** is obtained in excellent overall yield (>85%) from mesembrine by the sequence of reactions involving intermediates i and ii.



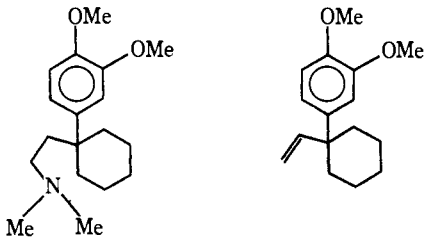
(13) The failure of the Hofmann elimination can be explained if one considers that abstraction of the β-hydrogen has to occur from a neopentyl carbon atom. Attempts to prepare **10** by pyrolysis of the *N*-oxide and by reactions of the ylide (iii), generated from *N*-benzylammonium salt (iv) with a variety of bases in different solvents, all failed to give this product. We have no obvious rationale to comprehend the reasons for the failure of the attempted eliminations with these reactions, which are both known to proceed *via* a cis-elimination mechanism.



(9) A. C. Neish, "Plant Biochemistry," J. Bonner and J. E. Varner, Ed., Academic Press, New York, N. Y., 1965, p 581; E. Leete, *J. Amer. Chem. Soc.*, **88**, 4218 (1966), and references cited therein.

(10) A. Popelak, E. Haak, G. Lettenbauer, and H. Springler, *Naturwissenschaften*, **47**, 156 (1960).

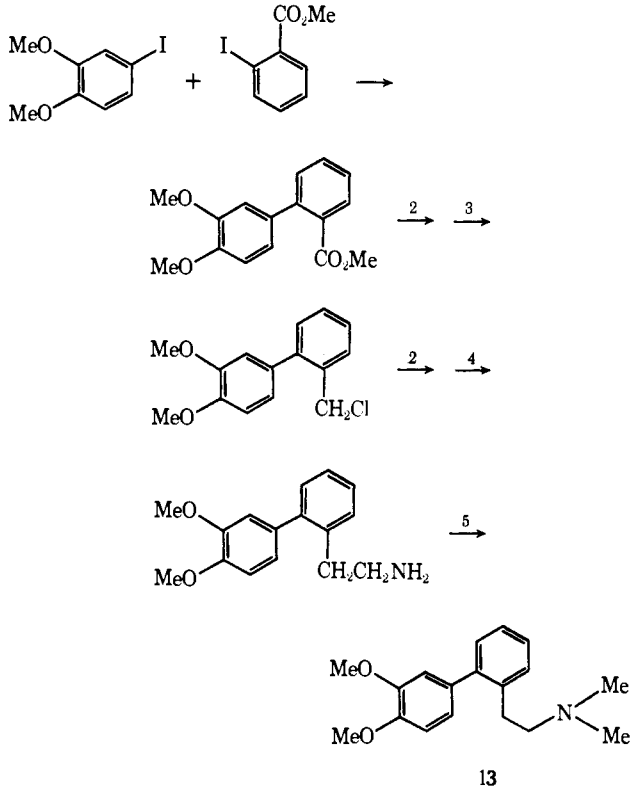
(11) This degradation scheme does not exclude the possibility of the label being located on the C-2 carbon in mesembrine, or both C-2 and C-3. However, these possibilities are most unlikely since they would require an unprecedented rearrangement of the labeled carbon in tyrosine and are in fact eliminated by subsequent studies reported in this paper.



9 10

tion was smoothly rearranged in 1 *N* hydrochloric acid to the biphenyl system **13**. The structure of **13** was confirmed by a straightforward synthesis according to the flow diagram shown in Scheme IV. A second Hofmann

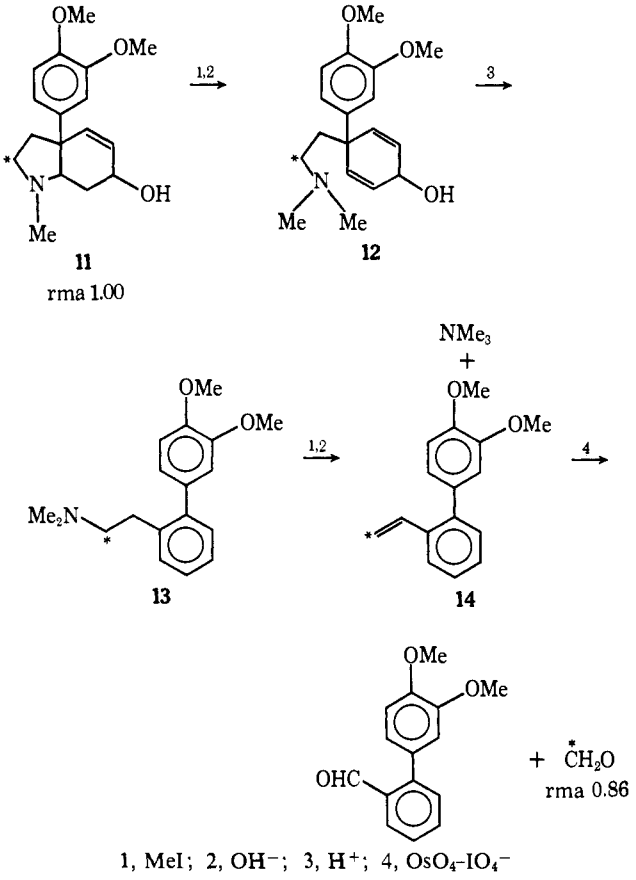
Scheme IV. Synthesis of 3,4-Dimethoxy-2′-(2-dimethylaminoethyl)biphenyl (**13**)[a]



[a] 1, Cu/225°; 2, $LiAlH_4$; 3, $SOCl_2$; 4, KCN–DMSO; 5, HCO_2H, HCHO.

degradation on **13** afforded the styrene **14** which on cleavage with osmium tetroxide–periodate in aqueous dioxane gave a quantitative yield of formaldehyde, which was isolated as its dimedone adduct. When a sample of mesembrenol derived from a feeding experiment with [2-^{14}C]tyrosine was degraded in this manner the radioactive label was shown to be located to greater than 86% on the C-2 carbon atom. This result provides a clear indication that tyrosine is incorporated intact into the octahydroindole skeleton of these alkaloids.

In summary, the biosynthesis of mesembrine and related octahydroindole alkaloids involves the utilization of the two aromatic amino acids, phenylalanine and tyrosine, for the construction of the basic ring system in this family. It is established that the aromatic ring of phenylalanine provides the aromatic C_6 unit present as the 3,4-dimethoxyphenyl ring and that tyrosine is utilized for the elaboration of the C_6C_2N unit which constitutes the octahydroindole moiety. The carbons of the *O*- and *N*-methyl groups are provided by the *S*-methyl of methionine, presumably *via* the agency of the ubiquitous biological transmethylating agent *S*-adenosylmethionine.



rma 1.00

rma 0.86

1, MeI; 2, OH^-; 3, H^+; 4, OsO_4–IO_4^-

Experimental Section

General Procedures. Radioactive samples were counted with a Nuclear-Chicago Unilux I liquid scintillation system, Model 6850. Ir spectra were measured with Perkin-Elmer Models 137 and 237 Infracord recording spectrophotometers. Pmr spectra were determined with a Varian A-60 spectrometer, and all chemical shifts are reported in δ units relative to tetramethylsilane. Melting points were taken on a Thomas Hoover capillary apparatus and are corrected. Glpc analyses were carried out on an F & M Model 402 high efficiency gas chromatograph using 8 ft × 0.125 in. glass columns packed with 4% SE 30 or 4% Carbowax 20M on Aeropak 30. The liquid phase and column temperatures are indicated where applicable.

Commercial radiochemicals were purchased from New England Nuclear Corporation and Nuclear Chicago and were supplied with quality control data sheets asserting radiochemical purity in the range 97.5–99%. Chromatographic materials were obtained from E. Merck (AG), Darmstadt, Germany. Reagents and solvents used were of reagent quality, and all solvents were redistilled. The removal of solvents was accomplished under reduced pressure on a Büchi rotary evaporator at steam bath temperatures unless otherwise noted. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn., and Janssen Pharmaceutica, Beerse, Belgium.

Counting Methods. Carbon-14 activities were measured by liquid scintillation counting in 10 ml of "DAM-water" stock solution which was prepared by admixture of 1200 ml of *p*-dioxane, 200 ml of anisole, 200 ml of 1,2-dimethoxyethane, 178 ml of water, 9.6 g of 2,5-diphenyloxazole (PPO), and 0.24 g of *p*-bis[2-(5-phenyloxazolyl)]benzene (POPOP). This scintillation solution was found to be generally applicable for both organic compounds and water-soluble organic salts. This solution gave an efficiency of 70% as determined by external standardization with [*carboxyl*-^{14}C]benzoic acid. Activities were determined by the balance point counting procedure, uniform quenching being assumed.

Activity determinations were generally made at least in duplicate, and counting times were adjusted to maintain a maximum counting error of 5%. Quantitative sample weighings were accomplished with a Cahn electrobalance, Model M-10.

Administration of Labeled Precursors. Feeding experiments were carried out with 1–2-year old plants of *Sceletium strictum* L. Bol (family *Aizoaceae*) maintained in a greenhouse. The precursors were dissolved in *ca.* 100–200 μl of H_2O and transferred with a syringe to capillary tubes which had been drawn to a sharp point. The tip of the tubes was then allowed to pierce the woody stem above the roots to a depth of 1–2 mm, and held in juxtaposition by taping to small sticks imbedded in the soil. When the solution had been taken up by the plant, an equal volume of distilled water was added to the tube, this process being repeated several times. Generally, four to six plants were used in each experiment.

Quantitative glpc assay of sectionalized portions of mature plants indicates alkaloid concentration is greatest in the woody stem area and decreases in the order root ≫ green stem > leaf.

Isolation and Purification of Alkaloids. A. Crude Extract. Whole plants fresh from the feeding experiments were harvested and excess soil was removed from the roots under a spray of water. The plants were macerated with 250–300 ml of EtOH in a Waring Blendor for 10 min (the solution temperature reached 60° during this process). The homogenate was filtered on a Büchner funnel to collect a woody filter cake which was further extracted with EtOH for 8–12 hr in a Soxhlet extractor. Omission of this last step considerably reduces the yield of alkaloids. The extract and the original filtrate were combined and the EtOH was removed on the Rotovac. The gummy residue was taken up in 100 ml of $CHCl_3$ which was extracted with three 50-ml portions of a saturated Na_2CO_3 solution. The green $CHCl_3$ solution was next extracted with three 100-ml portions of 5% HCl. The emulsion often appearing in the aqueous layer could be reduced to a filterable coagulate by gentle agitation under reduced pressure (rotary evaporator). The combined aqueous extracts were basified with saturated Na_2CO_3 solution, and then extracted four times with $CHCl_3$ (50–100-ml portions). The combined extracts were dried (K_2CO_3 or $MgSO_4$), filtered, and concentrated to provide the crude alkaloid fraction which amounted to 0.4–0.6% of the wet weight of the plants. The weight of the dried filter cake from above was *ca.* 10% that of the intact plants.

B. Mesembrine (1). Mesembrine, which occurs to the extent of *ca.* 1% in the alkaloid extract, was isolated as its hydrochloride (usually 80–120 mg) from MeOH–Et_2O when inactive mesembrine (generally 250 mg) had been added at the final $CHCl_3$ extraction step in the process described above.

The mesembrine hydrochloride thus isolated required several recrystallizations to achieve a state of radiochemical purity, at which point it could be calculated that the original material, though chemically quite pure (tlc, see below), was only *ca.* 5% radiochemically pure. Radiochemically pure mesembrine hydrochloride was therefore obtained as follows. The crude hydrochloride was converted to the free base (Et_2O extraction from Na_2CO_3 solution) and applied to a preparative layer chromatography plate (2 mm, 1:1 silica gel HR–HF_{254}) which was developed with 4:8:1.5 heptane–$CHCl_3$–MeOH. The mesembrine band (R_f 0.4) was scraped off and extracted in a Soxhlet with C_6H_6. After removal of the solvent, the residue was taken up in Et_2O, the hydrochloride formed, and crystals were obtained from MeOH–Et_2O (*ca.* 1:10); mp 206–208° (lit.[14] mp 205–207°); ir (free base, CCl_4) 1725 cm^{-1} (C=O). Recrystallization did not alter the molar activity.

A portion of the alkaloid extract (diluted with carrier) derived from the [*ring labeled*-^{14}C]phenylalanine feeding was subjected to tlc on silica gel H using the same solvent system employed above. Autoradiography with X-ray film (Ansco nonscreen, contact time 522 hr) revealed several radioactive spots in addition to mesembrine.

C. Mesembrenol (11). Glpc studies (Carbowax, 250°) with undiluted alkaloid extracts from *S. strictum* revealed that mesembrenol is generally the major alkaloid (*ca.* 70–90%). In view of these results, isolation studies from the later feeding experiments centered about mesembrenol which could readily be isolated, along with some of its cogeners, in the following manner.

The radioactive alkaloid extract was chromatographed on neutral alumina oxide (activity III), the fractions being monitored for alkaloid distribution by glpc (Carbowax, 250°), or ascending chromatography on ChromAr 500[15] (4:1:1 MeOH-$CHCl_3$-C_6H_6; R_f values follow). Elution with C_6H_6 afforded mesembrine (R_f 0.85) followed by mesembrenol (0.73) which was totally removed with 10–20% EtOAc in C_6H_6. The solvents were evaporated and mesembrenol subsequently obtained by crystallization from EtOAc (100–200 mg). One or two recrystallizations from the same solvent afforded radiochemically pure material, mp 142-142.5°. Per cent incorporations were determined by inverse isotopic dilution with mesembrenol carrier to determine the amount of mesembrenol remaining in the mother liquors.

Zeisel–Herzig–Meyer Demethylations. A. Mesembrine. A Pregl-Lieb apparatus was modified[16] for sequential *O*- and *N*-methyl microdeterminations so that the reaction flask (B) preceding the washer–receiver train was extended through a Claisen head adapter to another 5-ml reaction flask (A). The washer was charged with 25% NaOAc and 5% $CdSO_4$ (10:1). The receiver was charged with 10% ethanolic NMe_3 and cooled in a Dry Ice–acetone bath.

To flask A was added 30 mg (92 μmol) of mesembrine hydrochloride (236 dpm/mmol) which was dissolved in 0.1 ml of acetic anhydride. To this was added a crystal of phenol, a small square of aluminum foil, and 2 ml of freshly distilled HI (sp gr 1.7). A stream of carrier N_2 (dried by H_2SO_4 purge) was adjusted to a flow rate of one bubble/sec. The solution was heated at reflux for 60 min and then all but 0.5 ml allowed to distill into flask B over 10 min. This completes the O-demethylation process. The receiver was removed and upon warming to room temperature afforded 29.7 mg (148 μmol; 81% yield) of NMe_4I. A fresh receiver was attached, and to the cooled residue in A was added 10 mg of NH_4I and 3 drops of 3.4% chloroauric acid (catalyst). The remainder of the residue was distilled into flask B and the residual salt pyrolyzed for 90 min. The receiver was then removed, and upon warming to room temperature afforded 5.6 mg (28 μmol, 30.5% yield) of NMe_4I (from the *N*-Me group).

Accurately weighed portions of each NMe_4I collection were dissolved in water (10 ml) and passed through a thoroughly washed Dowex AG 1-X8 anion exchange column (chloride form) with 95% EtOH (100 ml). The eluate was taken to dryness and dissolved in the scintillation solution for counting. The results from this experiment and six additional determinations on mesembrine hydrochloride derived from the methionine feedings are given in Table II.

Table II.

	% yield		% label		
	O-Me	*N*-Me	*O*-Me	*N*-Me	Total
Av	83 ± 7	73. ± .8	46 ± 7	30 ± 5	81 ± 9

Degradation of Radioactive Mesembrine (1). The following degradation reactions were carried out on radioactive mesembrine derived from the amino acid feeding experiments listed in Table I. The physical constants and properties of the radioactive substances, in so far as they were observed, were identical with the more extensive observations achieved with the inactive materials; therefore, physical and spectral observations for new compounds obtained from the pilot reactions with "cold" materials are reported in the experimental details given below for the reactions carried out with radioactive compounds.

A. Mesembranols. Mesembrine, as the free base (190 mg, 0.657 mmol; 48 dpm/μmol), was dissolved in 2.5 ml of MeOH and added to a rapidly stirred solution of $NaBH_4$ (250 mg, 6.6 mmol) in 2 ml of cold MeOH. The mixture was refluxed for 30 min, then concentrated on the rotovac to *ca.* 1 ml. The residue was diluted with 5% NaOH (10 ml) and the product extracted into two 20-ml portions of Et_2O. The Et_2O was evaporated and residual water removed by azeotropic distillation with benzene. There was obtained 182.6 mg (0.625 mmol; 95.5% yield) of an oil, which, according to previous experiments, is composed of 80% epimesembranol and 20% mesembranol. The infrared spectrum showed no carbonyl absorption and was identical with those obtained in several pilot experiments with inactive material.

(14) E. Smith, N. Hosansky, M. Shamma, and J. B. Moss, *Chem. Ind. (London)*, 402 (1961).

(15) Mallinckrodt product: 70% silicic acid supported on 30% glass fiber sheets 500 μ thick.

(16) The apparatus used corresponds closely to that described by R. Belcher, "Submicro Methods of Organic Analysis," Elsevier, New York, N. Y., 1966, p 102.

B. Isomeric Mesembrenes. The above mixture of epimeric mesembranols (182 mg, 0.625 mmol) was dissolved in 15 ml of dry toluene (stored over sodium) containing an excess (*ca.* 200 mg) of P_2O_5. The solution was stirred at reflux for 3 hr, then cooled to room temperature. Water (3 ml) was cautiously added dropwise from a syringe (exothermic reaction) with continued stirring. The solution was basified with saturated Na_2CO_3 (5 ml), diluted with Et_2O (10 ml), and partitioned. The aqueous phase was separated and extracted with three 10-ml portions of ether. The combined organic phases were washed with two 10-ml portions of water and evaporated on the rotary evaporator (EtOH was added to aid in the removal of toluene as a low boiling azeotrope). There was obtained 103 mg (61% yield) of the isomeric mesembrenes as a pale yellow oil (44 dpm/μmol): pmr (CCl_4) δ 5.5–5.9 (2 H, –CH=CH–); the ir spectra (neat) showed no OH absorption in the region 3700–3100 cm^{-1}.

This material darkens upon standing for 2–3 days and in the present experiment was used immediately in the next step.

C. Mesembrene Methine (8). A solution containing 90 mg (0.329 mmol; 44 dpm/μmol) of the mixture of mesembrenes and 4 ml of CH_3I in 10 ml of Me_2CO was refluxed with stirring for 4 hr. The solvent was evaporated and the methiodide dissolved in 2 ml of H_2O and treated with an excess of freshly prepared Ag_2O. The solid was removed by filtration through a sintered glass funnel and washed with 10 ml of H_2O. Water was removed from the filtrate by continuous azeotropic distillation with benzene. The residue was transferred with NaOH to a glass tube sealed at one end, and then dried under a stream of N_2. The tube was partially inserted into an inclined heating block and the quaternary hydroxide pyrolyzed under reduced pressure. A light yellow, mobile oil was collected at 140–150° (0.3–0.35 mm) in the cool portion of the tube and amounted to 85.8 mg (0.3 mmol; 42 dpm/μmol) of **8** (91% yield): ir (neat) 3040 and 1675 cm^{-1} (weak, CH=CH); pmr ($CDCl_3$) δ 5.8–6.0 (–CH=CHCH=CH–).

D. 3,4-Dimethoxybiphenyl. The diene (85 mg) from above was converted to the methiodide, then treated with Ag_2O, and the quaternary hydroxide transferred to a pyrolysis tube as described previously. The salt was pyrolyzed at 180° (0.3–0.35 mm), gaseous decomposition products being collected by passage first through trap A (−50°, Dry Ice–isopentane), then through trap B (−195°, liquid N_2).

3,4-Dimethoxybiphenyl was collected as a distillate in the cool part of the pyrolysis tube (60 mg, 94.5% yield) and crystallized from MeOH–H_2O as white needles: mp 69.5° [lit.[17] mp 70.5°]; mmp with authentic material 70.0–70.5°; uv max (EtOH) 210, 262, 285 mμ (shoulder); the ir spectrum obtained in CCl_4 was identical with that obtained from a synthetic sample. This product contained no activity.

Anal. Calcd for $C_{14}H_{14}O_2$: C, 78.48; H, 6.59. Found: C, 78.88; H, 6.86.

Trap A was warmed to room temperature while sweeping the contents with a slow stream of nitrogen through a 10% ethanolic CH_3I solution (cooled in a Dry Ice–acetone bath). The EtOH solution was warmed to room temperature, and upon concentration under a stream of N_2 afforded 5 mg of NMe_4I: mp >325°; ir (KBr) identical with a spectrum of authentic NMe_4I. This material was recrystallized from 1:1 EtOH–H_2O and an accurately weighed portion was counted after anion exchange (see Zeisel determinations above). It showed a near background count rate (0.019 dpm/μmol) corresponding to 4% of the activity of the original mesembrine.

The contents of trap B (none apparent by visual observation) were likewise swept into a Br_2–CCl_4 solution at room temperature. A trace of oily material (*ca.* 0.5 mg) remained after removal of Br_2 and CCl_4 under nitrogen.

This material was presumably dibromoethylene; however, neither its composition nor purity was ascertained, but it did show some activity on counting.

E. Permanganate Oxidation to Veratric Acid. This reaction was first reported by Popelak, *et al.*,[10] but no experimental details were given.

Mesembrine hydrochloride (106 mg, 0.325 mmol; 33 dpm/μmol) derived from the DL-[*ring labeled*-^{14}C]phenylalanine feeding was dissolved in 25 ml of 10% KOH and the turbid solution brought to 100°. Vigorous magnetic stirring was maintained while *ca.* 20 ml of 5% $KMnO_4$ was added dropwise during 45 min, at which time spot testing showed the presence of unchanged permanganate persisting for several minutes. The reaction mixture was maintained at 100° for an additional 1.75 hr, then cooled, and an excess of dilute $NaHSO_3$ added followed by addition of 10% HCl (200 ml) to give a colorless, clear solution. The acidic solution was continuously extracted with benzene for 8 hr. The benzene was reduced to 10 ml and organic acids were removed by extraction with two 25-ml portions of dilute Na_2CO_3, acidification with HCl, and reextraction with three 25-ml portions of Et_2O. The combined Et_2O extracts were evaporated, and white needles of veratric acid obtained by slow crystallization of the residue from *ca.* 1:5 MeOH–H_2O (5.1 mg, 0.028 mmol; 33 dpm/μmol) (8.6% yield): mp 180–182° sub [lit.[18] mp 179–181.5°]; mmp 182–183° sub; ir (micro KBr) 1670 cm^{-1} (C=O), spectrum identical with an authentic specimen.

Commencing with mesembrine hydrochloride derived from a [*methyl*-^{14}C]methionine feeding (201 mg after dilution; 97.3 dpm/μmol), a similar reaction afforded 14 mg of veratric acid (12% yield), mp 181–182°, which was twice recrystallized without loss of activity (62 dpm/μmol; 64% of original mesembrine activity).

F. Demethylation of Veratric Acid to Protocatechuic Acid. The demethylation was accomplished in the modified Pregl-Lieb apparatus described above. A mixture of 13.9 mg of veratric acid (ultimately derived from the [*methyl*-^{14}C]methionine feeding; diluted with carrier to give a constant activity of 19 dpm/μmol), a small piece of aluminum foil, and 0.9 ml of HI (sp gr 1.7) was heated to gentle reflux with stirring for 70 min. A stream of predried N_2 carried radioactive MeI through a 25% NaOAc purge solution and into 10% ethanolic NMe_3 (cooled in a Dry Ice–acetone bath). The HI solution was taken to dryness under a stream of N_2 and the residue sublimed at 150–160° (0.17 mm) to give 5 mg of protocatechuic acid: mp 198.5–199°; ir (Nujol) 3650–2400 cm^{-1} (OH), 1675 cm^{-1} (C=O). This material was virtually inactive.

The NMe_4I collected from the trimethylamine trap (7.8 mg) was counted after anion exchange (see above) and possessed all of the original activity (9.6 μCi/mmol/methoxyl).

Protocatechuic acid (3.9 dpm/μmol) was obtained in a similar manner by demethylation of veratric acid (3.9 dpm/μmol) ultimately derived from the [*ring labeled*-^{14}C]phenylalanine feeding.

Conversion of Mesembrenol to 3′,4′-Dimethoxy-2-(β-dimethylaminoethyl)biphenyl (13). Mesembrenol (15 mg) derived from administering [2-^{14}C]tyrosine to *S. strictum* was diluted with inactive mesembrenol (80 mg) and refluxed in 3 ml of Me_2CO containing MeI (3 ml) for 6 hr. The solvent was removed, the residue treated with a solution containing 10 g of NaOH in 50 ml of MeOH–H_2O (1:1), and the mixture heated under reflux for 40 hr. The reaction mixture was cooled and extracted with three 15-ml portions of $CHCl_3$, and the combined $CHCl_3$ extract was washed with H_2O (15 ml) and then shaken with 10% HCl (5 ml) for 5 min. An excess of Na_2CO_3 was added until the solution was basic and the Et_2O layer separated and dried over anhydrous $MgSO_4$. Removal of the solvent *in vacuo* afforded the dimethylamino compound **13** which was identical in its glpc and spectral properties with that of a synthetic sample. The methiodide prepared in refluxing Me_2CO was recrystallized to constant activity, mp 212–215° (1.17 μCi/mmol).

Hofmann Elimination of the Biphenylamine 13. The labeled methiodide of **13** from above was dissolved in 5 ml of MeOH and 5 ml of 10 *N* NaOH solution added. The solution was refluxed for 5.5 hr, cooled and extracted with three 8-ml portions of Et_2O, and this washed with H_2O (8 ml) and dried over $MgSO_4$. Removal of the solvent yielded an oil (86 mg) which was chromatographically (glpc) identical with the styrene **14** obtained from "cold" material: pmr δ 7.23 (m, 4 H, Ar ring), 6.83 (br s, 3 H, Ar ring), 6.74 (4 line X part, 1 H, $-CH{=}CH_2$, J = 10.5, 17 Hz), 5.61 (dd, 1 H, $-CH{=}CH_cH_t$, J = 2, 17 Hz), (dd, 1 H, $-CH{=}CH_cH_t$, J = 2, 10.5 Hz), 3.98 and 3.88 (s, 3 H, OCH_3). A sample was collected by glpc on 8 ft × 0.25 in. 4% SE 30, 212°, 30 ml/min, retention time 3.0 min.

Anal. Calcd for $C_{16}H_{16}O_2$: *m/e* 240.1150. Found: *m/e* 240.1161.

Cleavage of the Styrene 14. The oily styrene **14** (28 mg) was dissolved in 5 ml of anhydrous Et_2O containing 1 ml of pyridine and the solution stirred for 48 hr with 35 mg of OsO_4. The solvent was removed *in vacuo* and the residue dissolved in 5 ml of $CHCl_3$ and cooled to 0°. A stream of H_2S was passed into the solution, the precipitate was filtered off, and the solvent removed from the filtrate *in vacuo*. Aqueous dioxane (1:1; 5 ml) was added to the residue and the solution stirred during the addition of 200 mg of

(17) G. Allen and J. M. Bruce, *J. Chem. Soc.*, 1757 (1963).

(18) H. Houben and W. Fischer, *Ber.*, 60, 1759 (1927).

$NaIO_4$. After 90 min the stirred solution was extracted with two 8-ml portions of Et_2O. Treatment of the aqueous phase with 40 mg of dimedone afforded a precipitate of the dimedone–formaldehyde after 10 min. Several recrystallizations of the product from 95% aqueous EtOH afforded 2 mg, mp 190–191° (lit.[19] mp 193–194°) (1.00 μCi/mmol).

3′,4′-Dimethoxy-2-biphenylcarboxylic Acid. Methyl 2-iodobenzoate (82 g) was mixed with 4-iodoveratrole (118 g) and copper powder (155 g). This mixture was heated slowly to 225° in a Woods metal bath and maintained at this temperature 4 hr with occasional stirring. The melt was cooled, dissolved in 300 ml of benzene, and filtered to remove copper. The solvent was removed on a rotary evaporator and the residue placed on an alumina column (act. 1, 1 kg). Elution with hexane removed starting materials and continued elution with benzene gave two products in a 1:1 ratio (glpc). The mixture was dissolved in 100 ml of methanol and 10 g of NaOH added. This solution was refluxed 1 hr, cooled, extracted twice with ether, and neutralized with HCl. On further cooling, light yellow crystals precipitated (25.5 g) which were collected by filtration and dissolved in a solution of 200 ml of acetic acid and 200 ml of acetic anhydride. This was refluxed 1.5 hr and then concentrated to 150 ml. On cooling, diphenic anhydride precipitated (6 g, mp 222–227°). The mother liquors from this crystallization were concentrated to 100 ml and added to 100 ml of H_2O. On cooling, 3′,4′-dimethoxy-2-biphenylcarboxylic acid (6.5 g) was deposited as prisms: mp 162–165°; ir (Nujol) 1720 cm^{-1}; pmr δ 7.47 (m, 4 H, Ar ring), 6.94 (br s, 3 H, Ar ring), and 3.77 (s, 6 H, OCH_3).

Anal. Calcd for $C_{15}H_{14}O_4$: *m/e* 258.0892. Found: *m/e* 258.0894.

Anal. Calcd for $C_{15}H_{14}O_4$: C, 69.77; H, 5.42. Found: C, 70.07; H, 5.52.

Methyl 3′,4′-Dimethoxy-2-biphenylcarboxylate. The biphenylcarboxylic acid (6.1 g) in 50 ml of MeOH was added to a suspension of Ag_2O (prepared from 11 g of $AgNO_3$) in 75 ml of MeOH. This solution was stirred 1 hr at 25°. Methyl iodide (10 ml) was added directly to the reaction mixture and stirring continued for 1 hr at 25°. The mixture was filtered and filtrate evaporated to yield an oil which was dissolved in 100 ml of Et_2O and extracted with two 25-ml portions of a 10% Na_2CO_3 solution. The ether layer was dried over $MgSO_4$, filtered, and evaporated to yield the ester as an oil (5.0 g): pmr δ 7.42 (m, 4 H, Ar ring), 6.88 (br s, 3 H, Ar ring), 3.86 and 3.84 (s, 3 H, OCH_3), and 3.62 (s, 3 H, $COOCH_3$). A sample was collected by glpc on the SE 30 column, 220°, 30 ml/min, retention time 3.92 min, for analysis.

Anal. Calcd for $C_{16}H_{16}O_4$: *m/e* 272.1049. Found: *m/e* 272.1047.

3′,4′-Dimethoxy-2-biphenylmethanol. A solution containing methyl 3′,4′-dimethoxy-2′-biphenylcarboxylate (5.6 g) in 100 ml of anhydrous ether was added slowly to $LiAlH_4$ (2.0 g) in 100 ml of dry Et_2O. The solution was stirred at 25° for 30 min and then brought to reflux for 45 min. Water was added and the water layer was separated and extracted with two 100-ml portions of Et_2O. The combined Et_2O extracts were washed with H_2O (50 ml) and dried over $MgSO_4$. The ether solution was filtered and the solvent removed *in vacuo* to yield the alcohol (4.4 g) as an oil: ir (neat) 3500 cm^{-1} (OH); pmr δ 7.34 (m, 4 H, Ar ring), 6.92 (br s, 3 H, Ar ring), 4.55 (s, 2 H, $-CH_2-$), 3.86 and 3.83 (s, 3 H, OCH_3), and 2.39 (s, 1 H, OH). A sample was collected from the SE 30 column at 230°, 30 ml/min, retention time 3.20, for analysis.

Anal. Calcd for $C_{15}H_{16}O_3$: *m/e* 244.1099. Found: *m/e* 244.1098.

2′-Chloromethyl-3,4-dimethoxybiphenyl. A dry benzene solution (50 ml) containing 3′,4′-dimethoxy-2-biphenylmethanol (4.4 g) was added slowly to a stirred solution containing 5 ml of $SOCl_2$ and 5 drops of pyridine in 100 ml of benzene. This solution was then refluxed 1.5 hr, cooled, and poured into 100 g of ice. The benzene layer was separated and washed twice with dilute $NaHCO_3$ solution and then dried over Na_2SO_4. The usual work-up gave the chloromethyl compound (4.5 g) as an oil: pmr δ 7.36 (m, 4 H, Ar ring), 6.97 (br s, 3 H, Ar ring), 4.55 (s, 2 H, $-CH_2Cl$), 3.93 and 3.91 (s, 3 H, OCH_3). An analytical sample was prepared by glpc on the SE 30 column, 230°, 30 ml/min, retention time 2.80 min.

Anal. Calcd for $C_{15}H_{15}O_2Cl$: *m/e* 262.0761. Found: *m/e* 262.0765.

2′-Cyanomethyl-3,4-dimethoxybiphenyl. Freshly distilled DMSO (50 ml) containing 2′-chloromethyl-3,4-dimethoxybiphenyl (4.5 g) was added at 25° to a DMSO solution (50 ml) containing NaCN (2 g). The solution was stirred at 85° for 3 hr, cooled, and poured into 300 ml of H_2O to give a milky suspension. The suspension was extracted with three 100-ml portions of Et_2O; this was washed with H_2O (100 ml) and dried over Na_2SO_4. The cyanomethyl compound was obtained from the ether as an oil (4.5 g): ir (neat) 2600 cm^{-1} (CN); pmr δ 7.37 (m, 4 H, Ar ring), 6.88 (m, 3 H, Ar ring), 3.93 and 3.90 (s, 3 H, OCH_3), and 3.63 (s, 2 H, $-CH_2CN$); purified for analysis by glpc on the SE 30 column at 230°, 30 ml/min, retention time 2.92 min.

Anal. Calcd for $C_{16}H_{15}NO_2$: *m/e* 253.1103. Found: *m/e* 253.1106.

2′-(2-Aminoethyl)-3,4-dimethoxybiphenyl. A solution of 2′-cyanomethyl-3,4-dimethoxybiphenyl (4.5 g) in 50 ml of THF was added dropwise to a stirred solution of $LiAlH_4$ (3.0 g) in 50 ml of THF. The solution was stirred at 25° for 30 min, then refluxed 1 hr. Excess $LiAlH_4$ was quenched with wet Et_2O. The Et_2O layer was separated and the gelatinous aqueous layer extracted with three 50-ml portions of Et_2O. The combined Et_2O solutions were then extracted with two 50-ml portions of 10% HCl and the aqueous layers neutralized with NaOH and extracted with two 50-ml portions of Et_2O. The ether solution from the acid extraction yielded the starting nitrile (1.4 g). The ether layers from the neutralized aqueous extraction were combined and dried over $MgSO_4$. The amine was obtained as an oil (2.2 g) from this extract and exhibited the following spectral properties: ir (neat) 3350 cm^{-1}, 1640 cm^{-1} (NH_2); pmr δ 7.35 (s, 4 H, Ar ring), 6.89 (s, 3 H, Ar ring), 3.92 and 3.88 (s, 3 H, OCH_3), and 2.78 (br s, 4 H, $-CH_2CH_2-$). An analytical specimen was prepared by glpc on the SE 30 column at 250°, 30 ml/min, retention time 2.88 min.

Anal. Calcd for $C_{16}H_{19}NO_2$: *m/e* 257.1416. Found: *m/e* 257.1412.

3,4-Dimethoxy-2′-(2-dimethylaminoethyl)biphenyl (13). 2′-(2-Aminoethyl)-3,4-dimethoxybiphenyl (1.8 g) was dissolved in a 50-ml solution of 1:1 formic acid–formaldehyde (38%). The solution was heated 2 hr at 100° and after cooling 75 ml of HCl (10%) was added and the aqueous layer extracted with three 50-ml portions of Et_2O. The aqueous layer was neutralized with saturated Na_2CO_3 solution and then extracted with three 50-ml portions of Et_2O. The combined ether extracts were dried over Na_2SO_4 and filtered, and the ether filtrate was concentrated *in vacuo*, to leave the oily amine **13** (1.5 g): pmr δ 7.23 (s, 4 H, Ar ring), 6.86 (m, 3 H, Ar ring), 3.90 and 3.87 (s, 3 H, OCH_3), 2.60 (A_2B_2 m, 4 H, $-CH_2CH_2-$), and 2.18 (s, 6 H, NCH_3). A sample was collected by glpc for analysis, SE 30, 250°, 30 ml/min, retention time 2.00 min.

Anal. Calcd for $C_{18}H_{23}NO$: *m/e* 285.1729. Found: *m/e* 285.1731.

The methiodide of **13**, prepared in refluxing the Me_2CO and MeI, was crystallized from acetone, mp 212–215°.

Anal. Calcd for $C_{19}H_{26}NO_2I$: C, 53.40; H, 6.09. Found: C, 53.10; H, 6.10.

The hydrochloride of **13**, prepared in ether–ethanol containing concentrated HCl, was crystallized from ethanol, mp 179–180°.

Anal. Calcd for $C_{18}H_{24}NO_2Cl$: C, 67.40; H, 7.49. Found: C, 67.30; H, 7.50.

Acknowledgments. We are deeply indebted to Mr. Hans Herre, Stellenbosch, South Africa, for supplying seeds of *Sceletium strictum*, and to Don Blake and Newton McQuay of the Department of Botany for care and advice on the cultivation of the plants. Our grateful thanks are extended to Professor W. C. Wildman, Iowa State, and Dr. A. Popelak, C. F. Boehringer and Son, Mannheim, Germany, for generous gifts of mesembrine.

(19) M. V. Ionescu and V. N. Georgescu, *Bull. Soc. Chim. Fr.*, **41**, 705 (1927).