0.553 mol) to reach a pH of 5.8–5.9. The resulting warm solution was treated in one portion with sodium tungstate dihydrate (5.0 g, 0.015 mol) and disodium ethylenediaminetetraacetic acid (1.0 g) dissolved together in 15 ml of warm (65–70°) water. Hydrogen peroxide (1.53 mol, 157 ml of a 30% solution) was then added dropwise with stirring over a 15-min period while maintaining the temperature between 40 and 55°. After the addition was complete, the reaction was kept at 50–55° for 1 hr to complete the epoxidation. The solution was then cooled to −5° over a 30-min period to initiate crystallization. After stirring for 2 hr at −5°, the product was filtered and the cake washed with cold propanol (four 50-ml portions). This salt when dried weighed 106 g, and was about 92% optically pure. To complete the resolution, the salt was dissolved in 770 ml of hot (75–80°) propanol. The slightly turbid solution was charcoal treated (2.5 g) and filtered while hot through a preheated funnel. To the hot filtrate was added 80 ml of warm (60–70°) water. Crystallization of the monohydrate began within a few minutes. After stirring the mixture at 0° for 2 hr the product was filtered, washed with cold propanol (three 25-ml portions), and dried *in vacuo* at 45°. The yield of phosphonomycin salt **6** was 90.1 g (32.5%): mp 132–134° dec; $[\alpha]^{25}{}^{\circ}{}_{405}$ −2.6° (*c* 5, H_2O) or +18.7° (*c* 3, DMF); Karl Fischer 6.6% (theory 6.5%); equiv wt 278.7 (theory 277.3).

Anal. Calcd for $C_{11}H_{13}NO_4P \cdot H_2O$: C, 47.64; H, 7.27; N, 5.05; P, 11.17. Found: C, 47.66; H, 7.00; N, 5.29; P, 11.09.

Registry No.—**3**, 25383-48-6; **4**, 25383-05-5; **5**, 25383-06-6; **6**, 25383-07-7; phosphonomycin, 23155-02-4.

Alkaloids of *Sceletium* Species.[1] III.[2] The Structures of Four New Alkaloids from *S. strictum*

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The isolation and structures of four new alkaloids, mesembrenol (**5**, R = Me; R′ = H), *O*-acetylmesembrenol (**5**, R = Me; R′ = Ac), 4′-*O*-demethylmesembrenol (**5**, R = R′ = H), and 4′-*O*-demethylmesembranol (**4**, R = H) are reported. The position of the phenolic hydroxyl in 4′-*O*-demethylmesembranol is determined by the application of a radioisotope dilution method. A discussion of the circular dichroism and nuclear magnetic resonance spectra of (+)-mesembrenone (**2**) and the nmr spectra of related alcohols mesembrenol and 6-epimesembrenol (**7**) is presented in providing information on the conformational preference of ring C in which it is shown that the equivalent forms of the half-chair, as represented in structures **2a**, **5a**, and **7a**, is preferred.

Certain *Sceletium* species (*Fam. Aizoaceae*) are used for the preparation of the drug known as *Channa* or *Kougoed*. Previous studies on *S. namaquense*, *S. tortuosum*, and *S. anatomicum* have led to the isolation and characterization of the alkaloids mesembrine (**1**), mesembrenone (**2**), and mesembranol (**4**, R = Me).[4]

Structural Studies.—In the course of a study of the biosynthesis of these alkaloids we have examined the major alkaloids of *Sceletium strictum* L. Bol.[5] Preliminary examination of the total alkaloid fraction by gas liquid chromatography (glpc) on several columns (see Experimental Section) showed it to contain one major component and several minor constituents. The major component proved to be a new alkaloid, mesembrenol (**5**, R = Me; R′ = H), $C_{17}H_{23}NO_3$, mp 145°, $[\alpha]D$ +90°, which could be isolated on occasions by crystallization of the total alkaloid fraction from acetone but was usually obtained only after chromatography over alumina. The infrared spectrum of mesembrenol shows absorption bands at 3630 and 3450 cm^{-1} characteristic of an alcoholic hydroxyl group and the presence of this group was substantiated by the formation of an O-acetyl derivative (**5**, R = Me; R′ = Ac). An N-methyl, two aromatic methoxyls, three aromatic hydrogens and two olefinic hydrogens signals are present in the nmr spectrum and a comparison with the spectra of mesembranol and mesembrine suggested it could be assigned as a member of the octahydroindole class of mesembrine-type alkaloids. This conclusion is supported by the mass spectrum which shows a molecular ion at *m/e* 289, and an intense peak at *m/e* 219. A detailed study of the mass spectra of the mesembrine alkaloids[6] has established that alkaloids of this ring system which possess a 3a-dimethoxyphenyl substituent all show a prominent peak at *m/e* 219 which is attributed to an ion of structure **6** (R = Me). The occurrence of the *m/e* 219 ion in the mass spectrum of mesembrenol implies that the double bond and hydroxyl group have to be situated in ring B and their placement as shown in structure **5** (R = Me, R′ = H) is provided by its oxidation to (±)-mesembrenone (**2**) with Jones reagent. The racemic nature of the product in this reaction is not exceptional and occurs as a consequence of the acidic conditions of the reaction which lead to the intervention of an equilibrium involving the protonated form of **2** and the symmetrical dienone **3**.

Elucidation of the remaining structural features of (+)-mesembrenol, namely, the stereochemistry of the C-6 hydroxyl and the absolute configuration was established by the hydrogenation of the new base to (−)-mesembranol (**4**, R = H; R′ = Me). The relative and absolute stereochemistry of the latter has been firmly established by a recent X-ray analysis of 6-epimesembranol methiodide.[2]

(1) Supported by the National Science Foundation Grant GB4361 and the National Institutes of Health through Grant AM13977-01 and a Research Career Program Award 1K04GM42342-01 to P. W. J.

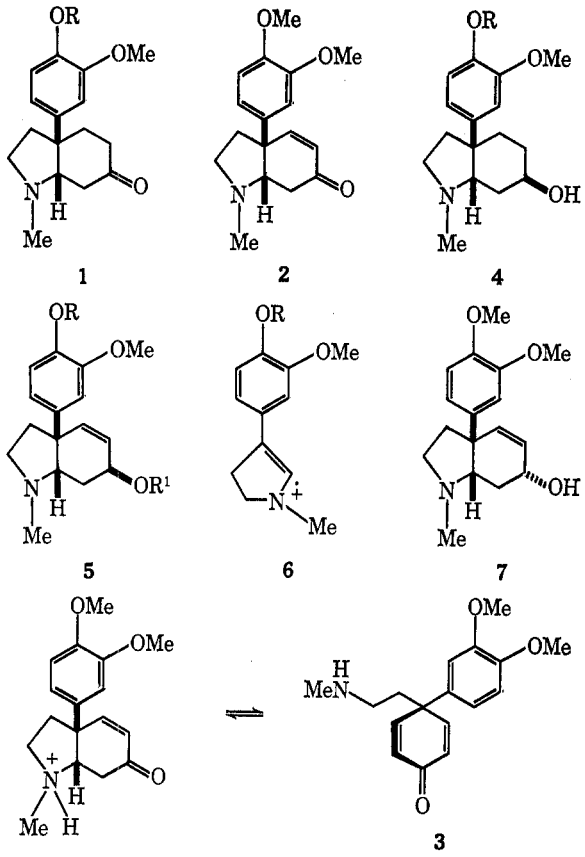
(2) See P. Coggon, D. S. Farrier, P. W. Jeffs, and A. T. McPhail, *J. Chem. Soc. A*, in press, for paper II in this series.

(3) (a) National Science Foundation Undergraduate Research Participant, 1967; (b) National DefenseE ducation Act Fellow, 1966–1969, Du Pont Fellow, 1969–1970; (c) National Aeronautics and Space Administration Act Fellow, 1965–1968.

(4) For a review, see A. Popelak and G. Lettenbauer, "The Alkaloids," Vol. IX, R. H. F. Manske, Ed., Academic Press, New York, N. Y., 1968, p 467.

(5) Identified by Dr. L. Bolus, Bolus Herbarium, University of Cape Town, South Africa, through the courtesy of Mr. Herre, Stellenbosch, South Africa.

(6) P. W. Jeffs and N. Martin, unpublished observations.



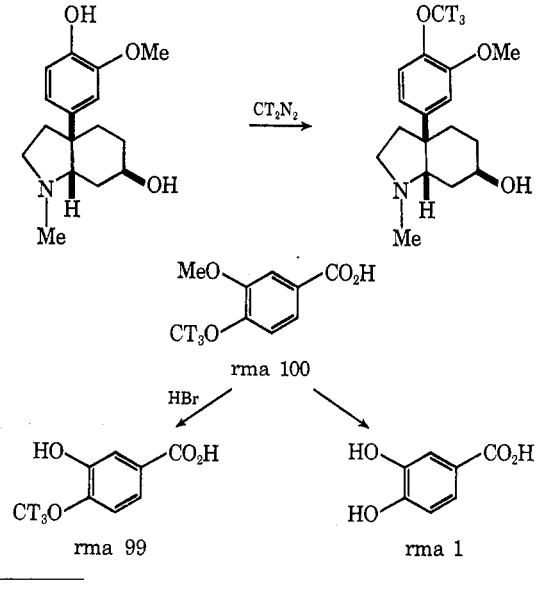
The presence of the known alkaloids mesembrenone, mesembrine, and mesembranol was indicated by glpc analysis and each alkaloid could be isolated by chromatography over alumina. In addition, O-acetylmesembrenol (**5**, R = Me; R′ = Ac) was obtained on occasions and in one instance proved to be the major alkaloid. The more polar fractions from the chromatographic separation gave a crystalline product, mp 200–203° which consisted of a mixture of two new bases (glpc). The phenolic nature of these two new alkaloids was evident by their solubility in sodium hydroxide solution and this was used to advantage in separating them from nonphenolic bases. Attempts to separate the phenolic alkaloids by column chromatography were unsuccessful, however, fractional crystallization of the mixture from methanol gave a pure compound, mp 219.5–220°. The mass spectrum of this compound has a parent ion at *m/e* 275 corresponding to the molecular formula $C_{16}H_4NO_3$ and an intense peak at *m/e* 205. The latter is 14 mass units less than ion **6** (R = Me) found in the mass spectra of mesembrine-type alkaloids possessing a dimethoxyphenyl ring; this fact, in conjunction with its phenolic character suggested that this alkaloid is an O-demethylmesembrenol. This was substantiated by the methylation of the phenolic base to (+)-mesembrenol on treatment with diazomethane and its structure and stereochemistry is therefore defined with the exception of the location of the phenolic hydroxyl group.

Catalytic hydrogenation of the phenolic alkaloid mixture affords a single compound, mp 201°, which is homogeneous by glpc and corresponds to the second component in the original mixture. Alternatively, preparative layer chromatography of mother liquor fractions of the phenolic alkaloid fraction which were enriched in this second component led to its isolation in pure form. The mass spectrum of this compound shows a parent ion at *m/e* 277 corresponding to $C_{16}H_{23}NO_3$ and it is converted to (−)-mesembranol with diazomethane. These results establish that the two phenolic alkaloids are an O-demethylmesembranol and an O-demethylmesembrenol in which the phenolic hydroxyl is located at the same site in both compounds.

In principle, a distinction between the two possible structures 4′-O-demethylmesembranol (**4**, R = H) and 3′-O-demethylmesembranol for the phenolic alkaloid, mp 201°, may be made on the basis of either of two criteria: (1) the number of deuteria introduced into the aromatic ring under conditions of base or acid catalyzed exchange, or (2) ethylation of the phenolic hydroxyl followed by drastic oxidation of the product to a substituted benzoic acid. Attempts to effect deuterium exchange under basic conditions gave equivocal results and the small amount of material available precluded the use of ethylation and oxidative degradation because of the low yield expected in this reaction. To circumvent these difficulties, application of a radio–dilution method was used to locate the site of the phenolic hydroxyl group.

A few milligrams of the phenolic alkaloid, which had been previously equilibrated with tritium oxide, was treated with diazomethane-T_2 and the product isolated by dilution with inactive mesembranol. Vigorous oxidation of the radioactive mesembranol afforded radio-labeled veratric acid which was isolated from the oxidation by adding inactive acid as a carrier. Treatment of the labeled veratric acid with hydrobromic acid under carefully controlled conditions gave isovanillic acid,[7] which in turn was converted to protocatechuic acid. No loss of label occurred in the conversion of veratric acid to isovanillic acid whereas the protocatechuic acid isolated was essentially inactive. Similarly, the transformation of labeled veratric acid directly to protocatechuic acid could also be achieved with hydrogen bromide under the appropriate conditions (see Experimental Section). A summary of this degradation scheme is presented in Chart I, and the results clearly demonstrate that all of the tritium label

CHART I



(7) A. Lovecy, R. Robinson, and S. Sugasawa, *J. Chem. Soc.*, 818 (1930).

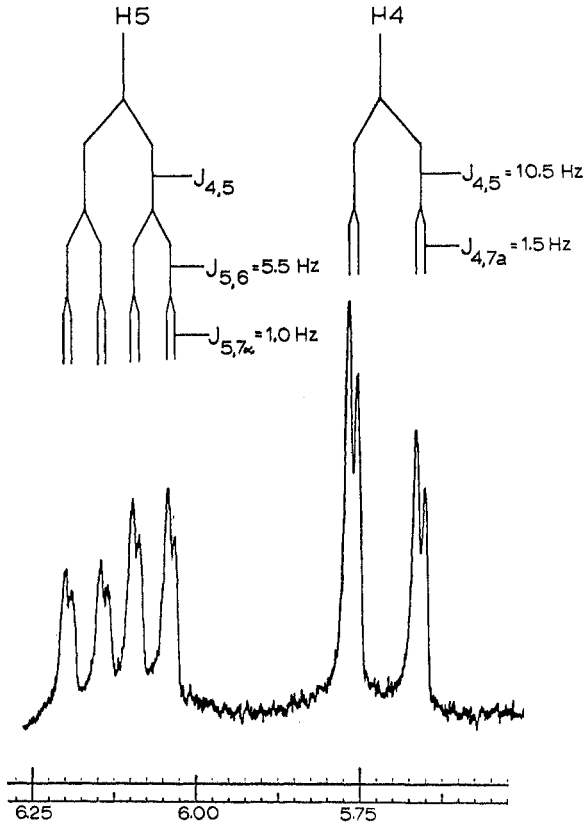
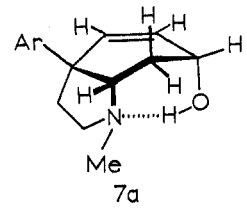


Figure 1.—Nmr spectrum of olefinic hydrogen region of 6-epimesembrenol.

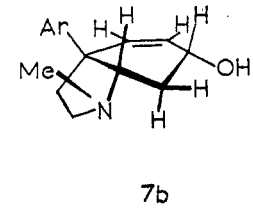
in the veratric acid obtained from the oxidation reaction is located in the 4-O-methyl group of the acid. In view of the demonstrated relation between the phenolic bases, the location of the tritiomethyl group in the veratric acid derived from one serves to establish the structures of both alkaloids as 4′-O-demethylmesembranol (**4**, R = H) and 4′-O-demethylmesembrenol (**5**, R = R′ = H), respectively.

Nmr and CD Spectral Studies.—Previous studies[8] of mesembranol, 6-epimesembranol, and the related ketone, mesembrine, have shown that the preferred ground-state conformations of these alkaloids are those in which ring B exists in the particular chair conformation in which the aryl substituent occupies the quasi-axial position. In view of this rather unexpected finding it was appropriate to examine the conformational features of the unsaturated analogs mesembrenol, 6-epimesembrenol (**7**) and mesembrenone.

The 6-epimesembrenol required for this study was obtained by reduction of (±)-mesembrenone with lithium aluminum hydride in tetrahydrofuran. In our hands this reduction afforded the 6-epi alcohol **7** as the major product together with mesembrenol. The latter, which was obtained in its racemic form, mp 124°, was identified by spectral comparisons with those obtained for the optically active form.[9] 6-Epimesembrenol was characterized from its nmr spectrum (*vide infra*) and by its oxidation to mesembrenone with manganese dioxide. The ir spectrum of **7** in carbon tetrachloride solution shows no absorption attributable to a free OH-stretching mode but instead a strong broad, concentration-independent, bonded OH absorption occurs at 3385 cm^{-1} over the concentration range 1.78 × 10^{-2} M to 1.78 × 10^{-3} M. Since no trace of any free OH-stretching absorption band was detected, 6-epimesembrenol must exist almost exclusively in the intramolecularly hydrogen bonded N–HO half-chair conformation **7a**. The existence of any significant concentration of the alternate form **7b** therefore may be confidently excluded from consideration.



7a



7b

The semirigid nature for conformation **7a** of 6-epimesembrenol provides a convenient model for an nmr study the results of which might be expected to afford information useful in diagnosing the conformational features of mesembrenol and mesembrenone.

Examination of the nmr spectrum of 6-epimesembrenol shows the following features which are pertinent to the conformational aspects of its structure. The signals from the two olefinic hydrogens appear (see Figure 1) as a 4-line pattern (C-4 H) and an 8-line (C-5 H) pattern centered at δ 5.73 and 6.12, respectively. The magnitudes of $J_{5,6}$ = 5.5 Hz and $J_{4,6}$ = 0 Hz (first order analysis) are fully consistent with conformation **7a**. Examination of a Drieding model shows that in this conformation the value of the dihedral angle which C-6 H makes with the hydrogens at C-4 and C-5 approaches 0°, resulting in a situation which is known to give rise to maximum vicinal and minimum allylic coupling.[10] On the other hand **7b** would be predicted to give rise to a small $J_{5,6}$ and a maximum $J_{4,6}$ in view of the 90° dihedral angle relationship of the C-6 H with the hydrogens at C-4 and C-5 present in this conformation.

Double resonance (nmdr) experiments show that the fine splitting observed in the C-4 H signal is due to long range coupling to the C-7a hydrogen signal which is located at δ 2.55. Since in this latter decoupling experiment the 8-line pattern of the C-5 hydrogen resonance signal remains unchanged, the fine splitting present in it has to be ascribed to coupling to one of the C-7 hydrogens. In view of the known stereospecificity[10] of ^4J couplings, this is most reasonably assigned to the C-7α hydrogen in which its stereochemical relation to the C-5 hydrogen is a distorted "W" arrangement.

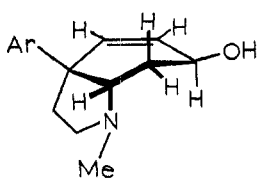
A similar analysis of the nmr spectrum of mesembrenol provides clear evidence that it too exists in the analogous ring C half-chair conformation **5a** like that found in the 6-epi alcohol. This occurs despite the fact that it lacks the advantage of the stabilizing effect which the intramolecular hydrogen bond undoubtedly confers on this conformation in 6-epimesembrenol. Inspection of the olefinic hydrogen pattern shows only a small splitting between the C-5 and C-6 hydrogen sig-

(8) P. W. Jeffs, R. L. Hawks, and D. S. Farrier, *J. Amer. Chem. Soc.*, **91**, 3831 (1969).

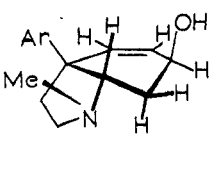
(9) Bodendorf and Krieger [*Arch. Pharm. (Weinheim)*, **290**, 441 (1957)] have reported that the reduction of (+)-mesembrenone with $LiAlH_4$ in ether affords (+)-mesembrenol, mp 117°, as the only product in 60% yield. Their results were reported without any implications of stereochemical detail.

(10) For a recent summary, see S. Sternhell, *Quart. Rev. (London)*, **23**, 236 (1969).

nals in the spectrum (Figure 2), and the appearance of this pattern as a quartet of uneven triplets may be accounted for by the fortuitous near equivalence in the line separations in the spectrum which originate from the four coupling constants $J_{5,6}$, $J_{4,6}$, $J_{4,7a}$, and $J_{5,7\alpha}$. The small and similar values of $J_{5,6}$ and $J_{4,6}$ are best accommodated by conformation **5a** in which the dihedral angle between the C-6 and the C-4 hydrogens is ~90°. The alternative conformation **5b** would be anticipated to give rise to quite different values for these couplings. Furthermore, it was possible to demonstrate that the long range coupling of the hydrogens at C-4 and C-7a found in the epi alcohol **7a** also exists in an analogous manner in mesembrenol from the observation that the upper pair of triplets resulting from the C-4 hydrogen signal could be collapsed to a double doublet by a nmdr experiment involving irradiation at the resonance position of the C-7a hydrogen at δ 2.33. The lower doublet of triplets of the C-5 hydrogen signal in this pattern remained essentially unchanged in this experiment and its multiplicity may be accounted for by the presence of a small long-range coupling of the C-5 hydrogen to the C-7α hydrogen which is similar in magnitude to $J_{5,6}$.



5a



5b

In approaching the question of the conformation of mesembrenone, consideration of the magnitude of $J_{5,6}$ so useful in the case of the mesembrenols is not available and an alternative to the nmr method was sought. Fortunately, the application of circular dichroism (CD) provides a potential means of ascertaining the preferred conformation. Previous studies have shown that the chirality in an optically active nonplanar cyclohexenone may be correlated with the sign of the Cotton effect arising from the n → π* or π → π* transition of the enone chromophore. Since mesembrenone is obtained as the racemate in the usual isolation procedure it was necessary to devise conditions for preparing optically active mesembrenone of known absolute configuration for CD studies.

Oxidation of (+)-mesembrenol offered a simple route to the desired product. In devising this reaction due cognizance had to be taken of the expected ease of racemization of the product under anything but neutral conditions; the use of the neutral oxidant manganese dioxide appeared to fulfill these requirements. (+)-Mesembrenone (2) was obtained when a solution of (+)-mesembrenol in carefully purified chloroform was stirred over manganese dioxide and the reaction terminated as soon as the oxidation was complete as evidenced by glpc analysis. The optical purity of the product was ascertained by converting it to mesembrine. A comparison of its ord spectrum with that of natural mesembrine indicated that the mesembrenone was 80% optically pure.[11]

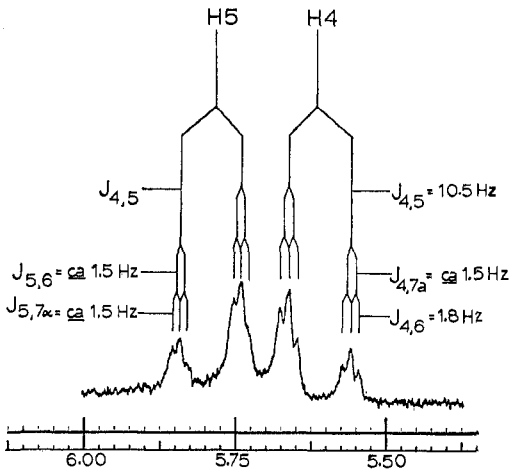
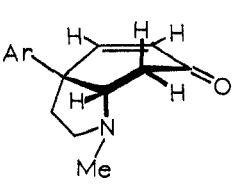


Figure 2.—Nmr spectrum of olefinic hydrogen region of mesembrenol.

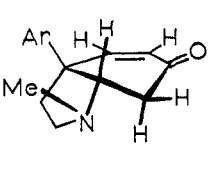
The experimental conditions for the preparation of (+)-mesembrenone are quite critical and even small deviations led to a product which was either completely or essentially racemic. In this connection it is pertinent to mention that some measure of the ease with which (+)-2 undergoes racemization may be gathered from the observations that (+)-2 in ethanol was racemized on standing at room temperature overnight or by brief heating to ~ 50° for 5 min.

The CD spectrum of (+)-mesembrenone recorded in chloroform showed a negative maximum at 334 mμ attributable to the Cotton effect resulting from n → π* transition of the enone chromophore. It is interesting to note that observation of this band in the electronic absorption spectrum is precluded by virtue of being submerged beneath the 'tail' resulting from the intense band at 278 mμ associated with the veratrole chromophore.

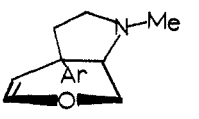
A negative Cotton effect for the n → π* transition of the enone chromophore in a structure possessing the absolute stereochemistry represented by structure **2** necessitates that the direction of chirality be as in **2a**.[12] This can be seen more clearly from a comparison of the octant projections of **2a** and **2b** from which it is obvious



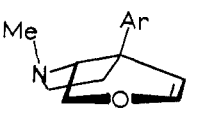
2a



2b



2a



2b

(11) This value should be regarded as the minimum value for optical purity of the mesembrenone since, in view of the ease with which this compound undergoes racemization, it is conceivable that some racemization occurred during its hydrogenation to mesembrine. Attempts to minimize this possibility were made by carrying out the reaction at 0°, with an excess of catalyst to ensure rapid reduction (15-min catalyst contact).

(12) W. B. Whalley, *Chem. Ind. (London)*, 1024 (1962). G. Snatzke, *Tetrahedron*, **21**, 413 (1965); 421 (1965); 439 (1965).

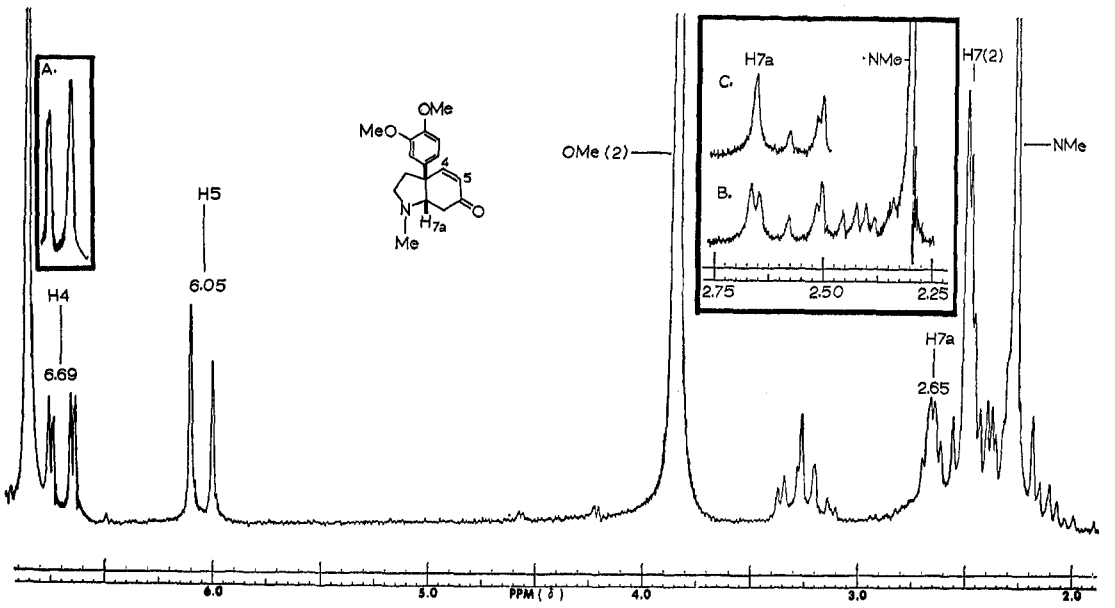


Figure 3.—Nmr spectra of mesembrenone and 5,7,7-d_3 mesembrenone with associated spin decoupling studies. A. Decoupling of C-4 hydrogen resonance by irradiation at C-7a hydrogen resonance frequency (δ 2.65). B. C-7 hydrogen signal in 5,7,7-d_3-mesembrenone. C. Decoupling of C-7a hydrogen signal in 5,7,7-d_3-mesembrenone by irradiation of the C-4 hydrogen resonance (δ 6.69).

that Cotton effects of opposite sign are predicted for these two conformations. A correlation exists[13] between the magnitude of the CD maximum and the deviation from coplanarity in the enone chromophore and the value of $[\theta] = -5060°$ calculated for optically pure (+)-mesembrenone is slightly larger than the molecular ellipticity values found for Δ^4-3-keto steroids[14] which range from $[\theta] = -4290$ to $-4719°$. In consonance with these observations, a comparison of Dreiding models of mesembrenone and the steroid systems shows that the cyclohexenone ring in the alkaloid has a somewhat larger degree of deformation from coplanarity.

Some further support for the above conformational assignment is available from the nmr spectrum of mesembrenone (Figure 3) which shows that the C-4 proton is long-range coupled just as in the case of the alcohols **4a** and **7a**. Nmdr studies demonstrate that the hydrogen involved in this long-range coupling is located at δ 2.65 (A). Assignment of this signal to the C-7a hydrogen resonance can be made from a comparison of its appearance in the mesembrenone spectrum where it occurs as a rough quartet to its appearance in the 5,7,7-d_3-mesembrenone spectrum in which it is a simple doublet (B). The doublet nature in the 5,7,7-d_3-mesembrenone spectrum is shown to be due to its long-range coupling to the C-4 hydrogen by the appropriate nmdr experiment (C). The long-range couplings of the C-4 and C-7a hydrogens and the C-5 and C-7α hydrogens in the mesembrenols is therefore paralleled in mesembrenone. This fact, together with the similarity in magnitude of the long-range coupling constants in these three compounds provides corroborative evidence for the conformation of mesembrenone as **2a**.

In summary, the spectral evidence indicates that the preferred ground-state conformations of mesembrenol and 6-epimesembrenol correspond to the equivalent half-chair ring C conformation as represented by structures **5a** and **7a**, respectively. Similarly, mesembrenone adopts the analogous ring C half-chair conformation rather than the alternative form **2b**.

Experimental Section

Melting points were taken on a Thomas–Hoover capillary apparatus and are corrected. Infrared spectra were recorded on Perkin-Elmer Models 137, 237, and 621 recording spectrophotometers. The nmr spectra were recorded at 60 MHz on a Varian A60 and at 100 MHz on a Varian HA 100 nmr spectrometer.[15] Glpc analyses were carried out on an F and M Model 402 high efficiency gas chromatograph with dual flame ionization detectors. Mass spectra[16] were obtained on an MS-902 mass spectrometer using a direct inlet system and operated with an ionization energy of 70 eV. CD and ORD spectra were obtained on a Jasco ORD–CD spectropolarimeter. Radioactive samples, dissolved in 10 ml of a stock solution prepared by mixing 1.2 l. of *p*-dioxane, 200 ml of 1,2-dimethoxyethane, 178 ml of water, 9.6 g of diphenyloxazole, and 0.24 g of *p*-bis[2-(5-phenyloxazolyl)]benzene, were counted on a Nuclear Chicago Unilux 1 liquid scintillation system.

Isolation of the alkaloids of *S. strictum* has been carried out in several different ways; the procedure described below is representative.

Three-year-old plants of *Sceletium strictum* L. bol. grown from seed were harvested in May and homogenized with 95% ethanol (4 l. in a Waring Blendor and the resulting suspension was heated on a steam bath for 1 hr prior to standing overnight. The solution was filtered and the filter cake (dry weight 151 g) extracted in a Soxhlet with CH_3OH. The combined extracts were concentrated to ~600 ml *in vacuo* and the resulting solution was treated with excess Na_2CO_3 before extracting with $CHCl_3$ (five 400-ml portions). After the $CHCl_3$ solution had been concentrated to ~1 l. it was extracted with 1 *N* HCl (six 300-ml portions). The combined HCl extracts were basified and reextracted with $CHCl_3$ (seven 200-ml portions). Removal of the $CHCl_3$ left 3.9 g of crude alkaloids. A further 0.12 g of alkaloid fraction was obtained by a repetition of the extraction procedure using

(13) G. Snatzke, "Optical Rotatory Dispersion and Circular Dichroism in Organic Chemistry," G. Snatzke, Ed., Heyden and Son, London, 1967, p 208.

(14) L. Velluz and M. Legrand, *Angew. Chem.*, **73**, 603 (1961).

(15) Obtained at North Carolina State University, Raleigh, and at the Environmental Health Laboratories, U. S. Public Health Service, Research Triangle Park, through the courtesy of Dr. Zelman Gaibel.

(16) Recorded through the cooperation of the Research Triangle Mass Spectrometry Center which is sponsored by a Special Facilities Grant No. FR-0330-01, National Institutes of Health.

$CHCl_3$:CH_3OH (3:1) to afford a total alkaloid fraction of 4.02 g (2.6% based on dry weight).

A sample of the crude alkaloid fraction was analyzed by glpc on two columns and the results are shown in Table I in which the relative retention times for identifiable alkaloids are given with respect to mesembrenol.

TABLE I

Alkaloid	SE-30[a]	Carbowax 20M[b]
Mesembrine	1.11	0.76
Mesembrenone	1.17	1.00
Mesembranol	1.00	0.87
Mesembrenol	1.00	1.00
O-Acetylmesembrenol	1.41	0.71
4′-O-Demethylmesembrenol	0.94	1.55
4′-O-Demethylmesembranol	0.94	1.65

[a] Glass column (8 ft × 0.25 in.) containing 3% SE 30 on Aeropak 30 (100–120 mesh) at a column temperature of 220°. [b] Glass column (8 ft × 0.25 in.) containing 4% Carbowax 20M on Aeropak 30 (100–120 mesh) as column temperature of 250°.

Chromatography of the crude alkaloid fraction (4.02 g) over neutral alumina (200 g, activity II) using a linear gradient of benzene (2 l.) against ethyl acetate (2 l.), 300 ml of ethyl acetate–C_2H_5OH (4:1) and finally 500 ml of CH_3OH. A total of 360 15-ml fractions were collected and the components of these fractions were analyzed by glpc using both Carbowax 20M and SE-30 columns under conditions specified in Table I. Fractions were combined as follows on the basis of the glpc results: 1–60, nonalkaloidal material (14 mg); 61–72, unidentified component (7 mg); 73–84, mesembrenone (32 mg); 85–87, mixture (1:1) mesembrine–mesembrenone (13 mg); 88–140, mesembrine (101 mg); 141–170, mesembrenol (285 mg); 171–326, mesembrenol–mesembranol (90:10) (871 mg); 327–360 mesembrenol, mesembranol, 4′-O-demethylmesembrenol, and 4′-*O*-demethylmesembranol (2.62 g).

The material from the combined fractions 327–360 was partitioned between $CHCl_3$ and 10% NaOH solution, the organic layer separated and the nonphenolic alkaloids (1.33 g) recovered from the $CHCl_3$. Glpc analysis of this material showed several unidentified minor components and peaks attributable to mesembranol and mesembrenol. The crude phenolic alkaloid fraction (0.75 g) consisted of two major components (glpc) and several minor alkaloids (<10%). (+)-**Mesembrenol** was purified by recrystallization from ethyl acetate or acetone to give colorless prisms: mp 140°; $[\alpha]^{25}D$ +91° (*c* 0.0176, $CHCl_3$); $\nu_{max}^{CHCl_3}$ 3630, 3450 cm^{-1} (OH), ratio of the intensity of the former to the latter peak increased on dilution; λ_{max}^{EtOH} 230 mμ (ε 9210), 279 (3515), 284 (2990); mass spectrum *m/e* 289 (M^+) (8.8), 219 (54), 70 (100); nmr δ 6.84 (m, 3 H, aromatic hydrogens), 5.70 (2 H, center quartet of triplets, H-4 and H-5), 4.30 (m, 1 H, C-6 H), 3.76 and 3.80 (s, each 3 H, OCH_3), and 2.30 (s, 3 H, NCH_3).

Anal. Calcd for $C_{17}H_{23}NO_3$: C, 70.56; H, 8.01; N, 4.84. Found: C, 70.48; H, 7.99; N, 4.83.

The **O-acetyl** derivative prepared in the usual way was obtained as an oil which was purified by chromatography in benzene over alumina (activity II) to afford an oil: ν_{max} 1740 (C=O), 1235 cm^{-1}; nmr δ 6.92 (m, 3 H, aromatic H), 5.80 (s, 2 H, H-4 and H-5), 5.92 and 3.86 (s, each 3 H, OCH_3), and 2.05 (s, 3 H, NCH_3).

A sample was purified by glpc for analysis using an 8-ft SE-30 column at 220°. The spectral and chromatographic properties of this sample were identical with a compound which was isolated from six-month-old *S. strictum* plants.

Anal. Calcd for $C_{19}H_{25}NO_4$: *m/e* 331.1783. Found: *m/e* 331.1777.

Chromic Acid Oxidation of (+)-Mesembrenol.—Mesembrenol (54 mg) was dissolved in 10 ml of acetone and the solution cooled to −10°. Chromic acid[17] (3 drops) was added to the solution of mesembrenol which was stirred and maintained at −10° for 20 min after the addition was complete. The excess chromic acid was destroyed by the addition of ~2 ml of a 1:1 mixture of isopropanol–acetone and the solvents removed *in vacuo*. The residue obtained was dissolved in 10 ml of water and basified with Na_2CO_3, and the aqueous solution extracted with $CHCl_3$. The crude product recovered from the $CHCl_3$ extract showed a single component on glpc analysis which corresponded to mesembrenone. Chromatography of the product over neutral alumina (activity III 20g) in 9:1 benzene–ethyl acetate afforded 35 mg of pure (±)-mesembrenone, $[\alpha]_{300}$ 0°, in the first 150 ml of solvent. The hydrochloride prepared from this sample crystallized from methanol–ether as plates, mp 190–195° (dec), which proved identical with an authentic sample of (±)-mesembrenone hydrochloride.

Manganese Dioxide Oxidation of (+)-Mesembrenol.—(+)-Mesembrenol (25.2 mg) was dissolved in 5 ml of $CHCl_3$ (which had been purified immediately before use by two passes through a column of basic alumina) and the solution cooled to 0° in an ice bath. Manganese dioxide (77 mg) was added to the solution and stirring continued for 25 hr at which point glpc analysis of the reaction mixture showed a 55% conversion of the mesembrenol to mesembrenone. An additional 75 mg of MnO_2 was then added and stirring continued for 1.5 hr at which time the mesembrenol remaining constituted less than 1% of the reaction mixture by glpc analysis. The reaction mixture was filtered to remove MnO_2 and the solvent was removed by a nitrogen stream at room temperature. Drying was completed at 25° *in vacuo* to yield (+)-mesembrenone (23 mg) as an oil: CD $[\theta]_{395}$ 0°, $[\theta]_{334}$ −4060°, $[\theta]_{310}$ 0° (*c* 1.072 mg/ml, ethanol). A significant decrease in the negative maxima in the CD spectrum was observed when ethanolic solutions were allowed to stand at room temperature. One sample which was allowed to stand for 24 hr was completely racemized. Similarly, an oxidation reaction carried out as specified above gave (+)-mesembrenone of very low optical purity (~5%) when removal of the $CHCl_3$ solvent was carried out at 50° on a Rotovac.

The optical purity of the sample of (+)-mesembrenone, $[\theta]_{334}$ −4060°, was established by its catalytic reduction to (+)-mesembrine as follows. A sample (20 mg) of (+)-mesembrenone was dissolved in 2 ml of ethyl acetate, which had been purified by passing through a column of basic alumina (activity I) immediately before use, and introduced into a stirred suspension of 40 mg of 10% palladium on carbon in 3 ml of ethyl acetate which had been cooled to 0°. The mixture was stirred under a hydrogen atmosphere at 1 atm and the progress of the reaction followed by glpc analysis; reduction was found to be complete in 10 min. After removal of the catalyst the usual work-up afforded (+)-mesembrine (~19 mg) as an oil. A small sample of this material was converted to the hydrochloride, mp 204–207°, and an independent comparison of the optical purity of the free base and the hydrochloride was made by a comparison of the molecular ellipticities of natural (+)-mesembrine and its hydrochloride. The average value obtained for the optical purity of the (+)-mesembrine from the hydrogenation of (+)-mesembrenol was 80%.

Catalytic Hydrogenation of Mesembrenol.—A solution of mesembrenol (26 mg) in 10 ml of CH_3OH was stirred over PtO_2 (4 mg) in an atmosphere of hydrogen until no more hydrogen was absorbed. After filtering the solution free of the catalyst, the solvent was removed to leave a residue which showed a single component on glpc corresponding to mesembranol. Crystallization of the residue from acetone gave (−)-mesembrenol as white prisms, mp 145–145.5°. This material was shown to be identical in every respect with an authentic sample of (−)-mesembranol by comparison of melting point, mixture melting point, tlc, glpc, and mass spectrum. The CD spectrum showed a plain negative curve identical with that of natural (−)-mesembranol.

4′-O-Demethylmesembrenol.—The crude phenolic alkaloid fraction, obtained either from the total alkaloid fraction by extraction with NaOH solution or from the NaOH soluble fraction of material enriched in the phenolic alkaloids *via* chromatography, crystallized from ethyl acetate as prisms, mp 200–203°. Glpc analysis of this material showed it was a two-component mixture. A sample (30 mg) of this mixture of CH_3OH (2 ml) was treated with an excess of an ethereal solution of CH_2N_2. Removal of the solvent and excess CH_2N_2 left a solid which on analysis by glpc showed two peaks corresponding to mesembranol and mesembrenol. The identity of the methylation products was confirmed by chromatographic separation over alumina, the fraction eluted with benzene–$CHCl_3$ (3:1) being identical with authentic (+)-mesembrenol while the later fraction eluted with benzene–$CHCl_3$ (1:3) was identical with authentic (−)-mesembranol.

Repeated fractional crystallization of the phenolic alkaloid mixture from CH_3OH afforded 4′-*O*-demethylmesembrenol as colorless prisms: mp 219–220°; $[\alpha]^{25}_{300}$ +533° (*c* 0.83 mg/ml, C_2H_5OH); λ_{max}^{EtOH} 225 mμ (log ε 3.44), 280 (3.44); $\lambda_{max}^{0.1\ N\ NaOH}$ 246

(17) A. Bowers, T. G. Halsall, E. R. H. Jones, and A. J. Lemin, *J. Chem. Soc.*, 2548 (1953).

(3.96), 295 (3.59); mass spectrum *m/e* 275 (M^+) (22), 205 (65), 70 (100), and 45 (90).

Anal. Calcd for $C_{16}H_{21}NO_5$: *m/e* 275.1521. Found: *m/e* 275.1540.

A solution of the phenolic alkaloid (~3 mg) in 1 ml of CH_3OH on treatment with excess ethereal CH_2N_2 afforded (+)-mesembrenol, mp 143–144°, identified by chromatographic and spectral comparisons with an authentic sample.

4′-*O*-Demethylmesembranol. A.—Mother liquors from the solution from which 4′-*O*-demethylmesembrenol had been obtained were subjected to preparative layer chromatography on silica gel H using $CHCl_3$–CH_3OH (3:1) and applying a double-pass technique. The two bands which appeared at R_f 0.61 and 0.32 gave 4′-*O*-demethylmesembrenol, mp 219°, and 4′-*O*-demethylmesembranol respectively. The latter crystallized from CH_3OH as prisms: mp 201°; $[\alpha]^{25}_{D}$ −199° (*c* 0.71, C_2H_5OH); λ_{max}^{EtOH} 229 (3.62), 280 (3.38); $\lambda_{max}^{0.1\ N\ NaOH}$ 245 (3.89), 294 (3.53; mass spectrum *m/e*, 277 (60), 205 (89), 204 (66), 70 (100).

Anal. Calcd for $C_{16}H_{23}NO_5$: *m/e* 277.1678. Found: *m/e* 277.1680.

B.—A mixture of the phenolic alkaloids (14 mg) in 10 ml of CH_3OH was stirred under an atmosphere of hydrogen in the presence of 10% palladium on carbon (10 mg) for 3 hr. The catalyst was filtered off and the filtrate concentrated to afford crystals (~6 mg), mp 201°, which were identical by chromatographic (tlc, glpc) and mass spectral comparisons with the 4′-*O*-demethylmesembranol obtained above.

Tritiomethylation of 4′-*O*-Demethylmesembranol.—To ~5 mg of 4′-O-demethylmesembranol in 0.2 ml of CH_3OH, 0.2 ml of tritiated water [activity 1 Ci/g] was added. This solution was mixed with a solution of CH_2N_2 in 10 ml of tetrahydrofuran containing 0.2 ml of tritiated water [20 μCi/g] and the mixture was kept for 10 days at 0–5°. Inactive mesembranol (12 mg) was added to the reaction mixture as a carrier and the product obtained after removal of the solvents was purified by chromatography over alumina to afford [*4′-O-methyl-³H*]-mesembranol of high specific activity, 43.6 μCi/mmol.

$KMnO_4$ Oxidation of [*4′-O-Methyl-³H*]-Mesembranol.—The tritium-labeled alcohol from the above experiment was dissolved in a mixture of dioxane (5 ml) and 5% aqueous Na_2CO_3 (15 ml). Potassium permanganate (3%) was added dropwise to this solution at reflux until the clear supernatant layer was distinctly pink. The refluxing was continued for a total of 70 min and the mixture cooled and treated with solid $NaHSO_3$ until clear. Veratric acid (25 mg) was added as a carrier and the solution acidified with concentrated hydrochloric acid before extracting with $CHCl_3$ (five 20-ml portions). A Na_2CO_3 extraction of the $CHCl_3$ concentrate, followed by acidification of the aqueous extract and reextraction with $CHCl_3$ (three 60-ml portions) and ether (six 60-ml portions), gave, on removal of solvents, a solid residue. Crystallization of this residue from C_2H_5OH gave veratric acid (14 mg), mp 180–182°, which was radioactive. Two recrystallizations gave radiochemically pure material 3.06×10^{-1} μCi/mmol.

Sequential Demethylation of Radiolabeled Veratric Acid.—A suspension of the labeled veratric acid (14 mg) in 0.1 ml of 48% HBr was heated under reflux. A clear solution was obtained after 2.5 min and heating was continued for a further 3 min until a solid precipitated. The solution was cooled, diluted with an equal volume of water, and filtered. The residue was washed with three 0.5-ml portions of water and dried to give 8 mg of isovanillic acid, mp 243–247°, which was radioactive (3.03×10^{-1} μCi/mmol). This sample was mixed with 0.5 ml of 48% HBr and heated under gentle reflux for 25 min, during which time an additional 0.2 ml of HBr was added after 15 min. On cooling the solution to room temperature a crystalline precipitate of protocatechuic acid formed which was filtered and washed with water to give ~3.5 mg, mp 194–196°. This sample showed only weak activity (3.00×10^{-2} μCi/mmol). Insufficient material was available to attempt to crystallize this sample to constant activity.

Demethylation of [*4′-O-Methyl-³H*]-Vanillic Acid to Protocatechuic Acid.—Radio-labeled veratric acid (3.06×10^{-1} μCi/mmol ~2 mg, was heated under gentle reflux with 0.8 ml of 48% HBr for 35 min. The sample was cooled and the crystalline protocatechuic acid was filtered off and washed thoroughly with water. A sample of this material (mp 196–198°) showed only very low activity (2.86×10^{-3} μCi/mmol).

$LiAlH_4$ Reduction of (±)-Mesembrenone.—(±)-Mesembrenone (2.10 g) in 25 ml of dry tetrahydrofuran was added dropwise to a stirred solution of 3.0 g $LiAlH_4$ in 60 ml of tetrahydrofuran. Stirring was continued for 2 hr and the reaction was quenched with 10% aqueous NH_4Cl. Work-up of the reaction mixture yielded a yellow oil (2.03 g) which, by gas chromatographic analysis, was shown to be a mixture of 6-epimesembrenol and mesembrenol in a 65:35 ratio.

The oil was dissolved in benzene and placed on a dry-packed alumina column (Woelm grade II, 75 cm × 1.6 cm). Elution was carried out by a gradient solvent system, benzene–25% benzene in ethyl acetate (2.5 l.). A total of 250 20-ml fractions were collected. The progress of the column was monitored by glpc analysis on a Carbowax 20M column operated as indicated in Table I. Fractions 15–85 contained (±)-6-epimesembrenol (1.1 g), 86–96 contained mixtures of both alcohols (~400 mg), and 97–250 contained (±)-mesembrenol. **(±)-6-Epimesembrenol**, an oil, exhibited the following spectral properties: $\gamma_{max}^{CCl_4}$ 3385 cm^{-1} unchanged over concentration range 1.78×10^{-2} *M* to 1.78×10^{-3} *M*; mass spectrum *m/e* 289 (M^+) (12), 250 (21), 219 (24), 144 (64), 70 (100); nmr δ (m, 3 H, aromatic hydrogens), 6.12 (8-line pattern, 1 H, H-4, $J_{4,5}$ = 10.5, $J_{5,6}$ = 5.5, $J_{5,7\alpha}$ = 1.0 Hz), 5.73 (d, 1 H, H-5, $J_{4,5}$ = 10.5, $J_{4,7a}$ 1.5 Hz), 4.00 (m, 1 H, C-6), 3.82 and 3.80 (m, each 3 H, OCH_3 aromatic), and 2.40 (s, 3 H, NCH_3). A sample of the alcohol **7** (10 mg) in $CHCl_3$ afforded (±)-mesembrenone on stirring with MnO_2 (100 mg) for 4 hr. The spectral and chromatographic properties of the product were identical with an authentic sample of mesembrenone. (±)-6-Epimesembrenol was purified for analysis by glpc.

Anal. Calcd for $C_{17}H_{23}NO_5$: *m/e* 289.1678. Found: *m/e* 289.1675.

(±)-Mesembrenol crystallized from acetone as prisms, mp 122–124.5° (lit.[9] mp 117°). Its spectral properties and chromatographic properties were identical in every respect with that of natural (+)-mesembrenol.

Anal. Calcd for $C_{17}H_{23}NO_5$: *m/e* 289.1678. Found: *m/e* 289.1675.

Deuteration of (±)-Mesembrenone.—(±)-Mesembrenone (90 mg) was dissolved in 5 ml of dry dioxane to which 200 mg of sodium was added. Deuterium oxide (5 ml) was next added dropwise and the solution refluxed 18 hr. The dioxane and deuterium oxide were then removed by distillation *in vacuo*, and the residue recharged with 5 ml of dioxane, 200 mg of sodium, and 5 ml of deuterium oxide as above. Reflux was initiated and continued for 10 hr and the solvents were removed as before. The residue was taken up in $CHCl_3$. This was washed once with 2 ml of water and dried over anhydrous $MgSO_4$. The drying agent was then filtered off, the $CHCl_3$ removed from the residue in a nitrogen stream, and final drying accomplished *in vacuo*. The light yellow oil weighed 43 mg. Glpc analysis was consistent with mesembrenone. Nmr indicated that deuteration at C-5 and C-7 (**2**) was complete.

Registry No.—**2**, 25516-12-5; **4** (R = H), 25516-13-6; **5** (R = R′ = H), 25516-14-7; **5** (R = Me; R′ = H), 25516-15-8; **5** (R = Me; R′ = Ac), 25516-16-9; **7**, 25568-56-3.

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