Since polyribonucleotides are susceptible to base-catalyzed hydrolysis of the phosphodiester bonds, one might suppose that the peroxodisulfate oxidation would be chiefly useful for polydeoxyribonucleotides. However, the hydrolysis is quite slow at pH values suitable for the oxidation. For example, using the data of Bock,[29] one can calculate an approximate half-time of 850 hr for the hydrolysis of RNA at pH 9, 40°.

Acknowledgment. R. C. M. was a Frasch Foundation Fellow and a National Science Foundation Trainee. This work was also supported in part by an NSF Grant, GB-21267.

Registry No.—Guanosine, 118-00-3; deoxyguanosine, 961-07-9; peroxodisulfate ($K_2S_2O_8$), 7721-21-1.

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Sceletium Alkaloids. VI. Minor Alkaloids of *S. namaquense* and *S. strictum*

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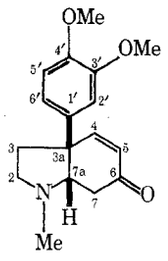
The structures of five new alkaloids are reported. *Sceletium* alkaloid A_4 (**3**) is a new type of *Sceletium* alkaloid containing a tetracyclic ring system and *N*-formyltortuosamine (**8**) is a ring C seco derivative of **3**. Three additional members of the 3a-aryloctahydroindole class are described by the structures of the phenolic base, 4′-*O*-demethylmesembrenone (**12**), Δ^7-mesembrenone (**7**), and sceletenone (**15**). The latter constitutes the prototype of a monooxyaryl member of this class. A unified biogenetic scheme which accounts for the origins of the various ring systems of the different classes of *Sceletium* alkaloids is presented.

Previous studies of various *Sceletium* species of the family *Aizoaceae* have provided a number of alkaloids. Most of the bases that have been characterized belong to a single group which are elaborated on the 3-aryl-*cis*-octahydroindoleskeleton as exemplified by mesembrenone (**1**).[2] A recent report has described the structures of three new *Sceletium* alkaloids based upon a different skeleton which is typified by the structure of joubertiamine (**2**).[3] The close structural similarity between the mesembrine and joubertiamine types suggests that they originate through a common biosynthetic pathway. While extensive studies[4] have been devoted to elucidating the biosynthetic route to the octahydroindole alkaloids of the mesembrine series, no clear understanding of the pathway by which these alkaloids are formed has yet emerged. In cognizance of this fact we have undertaken a study of the minor alkaloids of *S. strictum* and *S. namaquense* with the view that characterization of new structural types may prove helpful in revealing previously unsuspected biosynthetic relationships in this series. In this paper we describe two alkaloids which are representatives of new skeletal types and three additional examples of alkaloids based upon the 3-aryl-*cis*-octahydroindolenucleus.

Sceletium **Alkaloid A_4.** Popelak and coworkers in an earlier investigation of *S. tortuosum* had reported on the isolation of a crystalline alkaloid, sceletium A_4. Aside from a description of the physical properties, the data given were limited to an assignment of the molecular formula of the alkaloid as $C_{20}H_{24}N_2O_2$ and the suggestion that the alkaloid contained two methoxyl groups, probably in a veratryl chromophore, and an *N*-methyl group.[2] The occurrence of two nitrogen atoms in the molecular formula of sceletium A_4 led these authors to suggest that this alkaloid had to be placed in a different structural class from the other mesembrine alkaloids of known structure, which at the time of this observation consisted of three members of the 3-aryl-*cis*-octahydroindolegroup.

An investigation of the structure of sceletium A_4 was made possible when this alkaloid was encountered during a study of the nonphenolic alkaloid fraction of *S. namaquense*. After the removal of mesembrine and mesembrenone from this fraction, sceletium A_4 was obtained together with two other new alkaloids. The former was obtained as an optically active, crystalline base, mp 153–154°, $[\alpha]_D$ +131° (C_2H_5OH), which was assigned the molecular formula $C_{20}H_{24}N_2O_2$ from an accurate mass measurement of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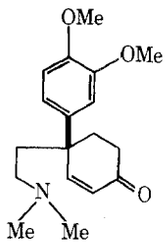
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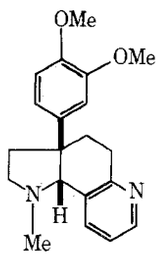
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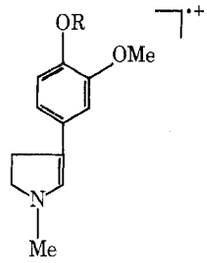
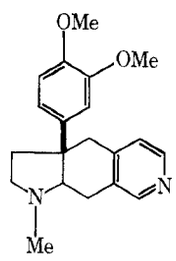

4, R = Me (*m/e* 219)
13, R = H (*m/e* 205)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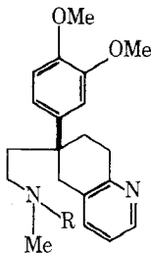
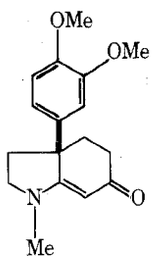

6, R = H
8, R = CHO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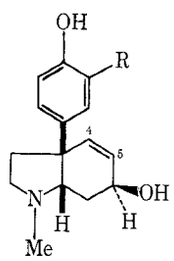
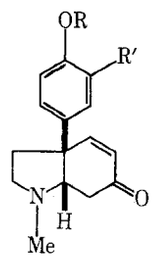

10, R = OMe (no 4,5 unsaturation)
11, R = OMe


12, R = H, R′ = OMe
15, R = R′ = H
16, R = Me, R′ = H

the molecular ion in the mass spectrum. The suspected identity of this compound with *Sceletium* alkaloid A_4 was confirmed by direct comparison with a sample provided by Dr. Popelak. Comprehensive spectral studies reported below led to the elucidation of its structure as represented in **3**.

The ^1H nmr spectrum of sceletium A_4 at 100 MHz exhibited a well-defined aromatic region consisting of an AMX pattern at δ 8.48 (J = 5.0 and 2.0 Hz), 7.56 (J = 7.8 and 2.0 Hz), and 7.15 (J = 7.8 and 5.0 Hz) and a typical three-proton pattern characteristic of the 3,4-dimethoxyphenyl group of the octahydroindole members of the mesembrine alkaloids. The only other assignable resonances in this spectrum were two *O*-methyl signals at δ 3.71 and 3.78, and an *N*-methyl signal at δ 2.34.

Some further clarification of the ^1H spectrum became apparent in the 220-MHz spectrum of the alkaloid and these assignments are collected in Table I.

The downfield AMX pattern in the aromatic region was clearly indicative of a heteroaromatic ring and both the chemical shifts and coupling constants provided strong evidence in assigning this pattern to a 2,3-disubstituted pyridine system.[5] The ultraviolet spectrum of the alkaloid, with λ_{max} 225 nm (log ϵ 3.59), 267 (3.39), 273 (3.42) and 285 (3.10), supported the presence of this chromophore and in fact compared well with the uv of the model compound, 2-(3,4-dimethoxybenzyl)pyridine, which showed bands at λ_{max} 231 nm (log ϵ 3.27), 262 (3.23), 268 (3.19), and 282 (2.99). The small bathochromic shift observed in the $\pi \rightarrow \pi^*$ bands at 267 and 273 nm is accounted for by the known auxochromic effect of alkyl substituents on the pyridine nucleus.[6] Additional support for the presence of both benzenoid and pyridine chromophores in the alkaloid was provided by the occurrence of bands attributable to C=C and C=N stretching vibrations at 1508 and 1605 cm^{-1} in the infrared spectrum. Further information on the structure of *Sceletium* alkaloid A_4 was obtained by mass spectral studies. Preliminary examination of the low-resolution spectrum showed that aside from the molecular ion, and M − 1 and M − 15 fragments, ions of high abundance occurred in the high-mass range at *m/e* 296, 281, and 266. The high-resolution spectrum indicated that these ions occurred through the loss of ethylene and the loss of nitrogen containing fragments C_2H_5N and C_3H_8N, respectively. The loss of the latter was complemented by the appearance of C_3H_7N fragment of high abundance in the low-mass end of the spectrum. The occurrence of these fragments suggested that the structure of the alkaloid contained an *N*-methylpyrrolidine ring. The finding of an ion of elemental composition $C_{13}H_{17}NO_2$ at *m/e* 219 proved important. An ion of this composition, which has been assigned structure **4**, occurs in the spectra of all of the simple octahydroindole alkaloids containing a 3,4-dimethoxyphenyl substituent. Convincing evidence that the ion from the sceletium A_4 has the same structure as the isobaric fragment observed in the spectra of mesembrine and its derivatives was obtained from a comparison of the decomposition pathways in the mass spectra of sceletium A_4 and the mesembrine series by the use of metastable defocusing data.[7]

Analysis of the metastable spectrum of ion **4** from mesembrne and its analogs shows that it decomposes by two major pathways to give daughter ions at *m/e* 204 and 191, corresponding to the loss of a methyl radical and ethylene, respectively. Similarly, an identical fragmentation of the *m/e* 219 ion was observed in the metastable spectrum of sceletium A_4. These results when considered in conjunction

Table I
^1H Nmr Shift Assignments for 220-MHz Spectrum of *Sceletium* Alkaloid A_4

δ		Multiplicity	J, Hz	Assignment
1.91	1 H	m	$W_{1/2}$ = 13.0	H-3α
2.27	2 H	m		H-4α, H-3β
2.34	3 H	s		NMe
2.50	3 H	m		H-4β, H-2α, H-2β
2.94	1 H	m	$W_{1/2}$ = 16	H-5β
3.30	2 H	m		H-7a, H-5α
3.71	3 H	s		OMe
3.78	3 H	s		OMe
6.56	1 H	dd	8.0, 2.0	H-6′
6.65	1 H	d	2.0	H-2′
6.70	1 H	d	8.0	H-5′
7.15	1 H	dd	7.8, 5.0	Py H-3
7.56	1 H	dd	7.8, 2.0	Py H-4
8.48	1 H	dd	5.0, 2.0	Py H-2

with the previously cited spectral evidence for sceletium A_4 present a strong case for representing its *m/e* 219 fragment by structure **4**.

Combination of both partial structural units in **4** and the 2,3-disubstituted pyridine system leaves only two methylene groups unaccounted for in the moleculr formula of the alkaloid. Of the several possibilities, two structures, **3** and **5** appeared more likely on biogenetic grounds (*vide infra*). Since the alkaloid was available in very limited quantities, the possibility of a rigorous structure proof by chemical methods was precluded and therefore a single-crystal X-ray crystal structure analysis was undertaken in collaboration with Professor McPhail. The results of this analysis demonstrated that *Sceletium* alkaloid A_4 is correctly represented by structure **3**.[8]

Concurrent with our studies of sceletium A_4, Wiechers and collaborators have isolated partially racemic sceletium A_4 and a second alkaloid, tortuosamine ($C_{20}H_{26}N_2O_2$), from *S. tortuosum*. Since tortuosamine differed from sceletium A_4 by two hydrogens in the respective molecular formulas, a close structural relationship between the two alkaloids was suspected.

With the establishment of the structure of sceletium A_4 the South African workers were able to infer the structure of tortuosamine (**6**) which they confirmed through the conversion of sceletium A_4 to **6** by hydrogenolysis.[9]

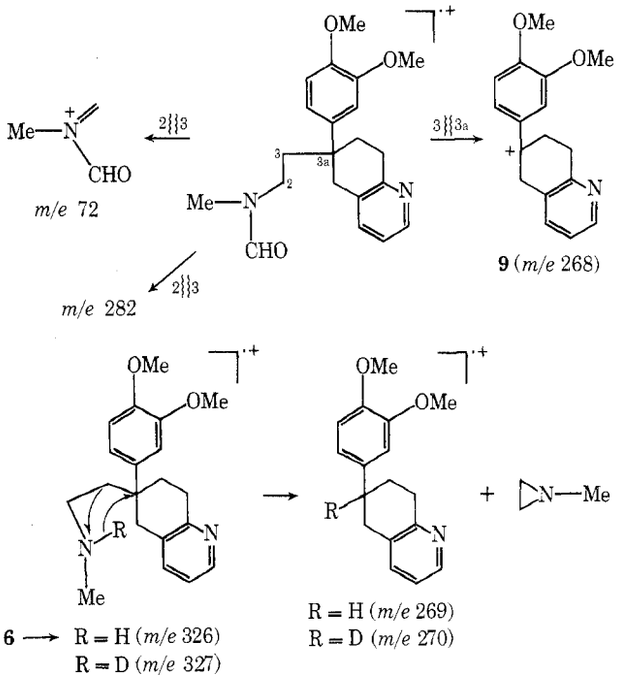
Δ^7-Mesembrenone and *N*-Formyltortuosamine. The late fractions from the column chromatographic separation of the ether-insoluble alkaloid fraction from *S. namaquense* afforded two pure alkaloids.

One of these proved to be a noncrystalline base of molecular formula $C_{17}H_{21}NO_3$. The spectral and chromatographic properties of this compound were identical with those of Δ^7-mesembrenone (**7**), which had previously been obtained by the reaction of mesembrine with diethyl azodicarboxylate.[10]

The second alkaloid from the late fractions was an optically active, noncrystalline base which was shown to have the molecular formula $C_{21}H_{26}N_2O_3$ by high-resolution mass spectrometry. The 250-MHz ^1H spectrum of the alkaloid exhibited a three-proton aromatic pattern centered at δ 6.83 and three low-field multiplets at 7.05, 7.42, and 8.32. The latter signals were shown to be mutually coupled by double-resonance studies and this information served to establish the presence of a 2,3-disubstituted pyridine ring. A strong carbonyl absorption at 1660 cm^{-1} in te infrared spectrum indicative of an amide carbonyl was supported by doubling of the signals associated with both an *N*-methyl (δ 2.66 and 2.70) and two aromatic *O*-methyl groups (δ 3.74, 3.75, 3.77, and 3.78). The temperature dependence of the 100-MHz spectrum showed not only the expected coalescence of the *N*-methyl and *O*-methyl signals at 82° but also resulted in the collapse of two lines centered at δ 7.86. The position and temperature-dependent behavior of the latter signal implied that the amide was present as an *N*-formyl group.

The foregoing spectral data suggested that the new alkaloid was represented by structure **8**. Further support for this proposal was obtained from the mass spectral fragmentation pattern, in which the key features were the observation of ions derived from the sequential loss of fragments of the *N*-formylethamine side chain giving rise to abundant ions at *m/e* 282 and 268 and a complementary ion *m/e* 72 at low mass. In relation to the *m/e* 268 fragment, to which we attribute structure **9**, it was of interest that the mass spectrum of tortuosamine[9] afforded a major fragment ion at *m/e* 269. This suggested that the genesis of the latter involved a hydrogen transfer process from the secondary nitrogen to the charge-bearing fragment. Proof of this pathway was obtained from the observation that tortuosamine *N*-d_1 exhibited a mass shift in this daughter ion fragment to *m/e* 270. The foregoing fragmentation pathways of tortuosamine and its *N*-formyl derivative are summarized in Scheme I.

Scheme I
Principal Mass Spectral Fragmentation Pathways of Tortuosamine (6), Tortuosamine-*N*-d_1, and *N*-Formyltortuosamine (8)



The structure of **8** was confirmed by its derivation from *Sceletium* alkaloid A_4, through hydrogenolysis to tortuosamine, and *N*-formylation of the latter in formic–acetic anhydride to give a product which was identical in its spectral and chromatographic properties with the natural base. A comparison of the CD spectra of the alkaloid and the synthetic product gave identical curves exhibiting a positive Cotton effect at 282 nm and a negative maximum at 266 nm; this served to identify *N*-formyltortuosamine and *Sceletium* alkaloid A_4 as belonging to the same chiral series. Whether the chirality of these alkaloids corresponds to that of the octahydroindroindole bases of the mesembrine series remains to be established.

4′-*O*-Demethylmesembrenone and Sceletenone. Previous investigation of the phenolic alkaloid fraction of *S. strictum* led to the isolation and characterization of the alkaloids 4′-*O*-demethylmesembranol (**10**) and 4′-*O*-demethylmesembrenol (**11**).[11] The presence of a third alkaloid, 4′-*O*-demethylmesembrenone (**12**) was detected initially from a radioscan of a tlc plate of the total phenolic alkaloids derived from a biosynthetic feeding experiment in which [*S-methyl*-^{14}C]methionine had been administered to live *S. strictum* plants. It was subsequently isolated by preparative tlc of the phenolic bases from *S. strictum* and also by a more extensive isolation procedure (*vide infra*) from the phenolic alkaloid fraction of *S. namaquense*.

4′-*O*-Demethylmesembrenone, $C_{16}H_{19}NO_3$, an optically inactive, oily base, exhibited bands at 3540 and 1668 cm^{-1} indicative of the presence of a phenolic hydroxyl and an α,β-unsaturated ketone group. Its mass spectrum displayed a fragmentation behavior analogous to that found for the alkaloids **10** and **11**, the most distinctive feature being the

Table II
^{13}C Nmr Shift Assignments of Membrenone (1) and Sceletenone (15)

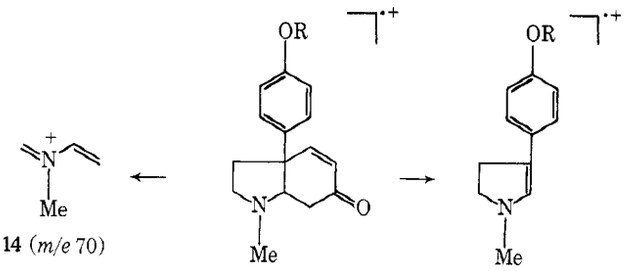
Compd	^{13}C shift, ppm															
	1′	2′	3′	4′	5′	6′	2	3a	3	4	5	6	7	7a	NMe	OMe
Mesembrenone (1)	135.2	110.9	148.6	147.7	110.0	118.8	56.2	50.9	38.3	153.3	126.2	196.9	38.6	73.8	40.1	55.8, 55.9
Sceletenone (15)	133.6	126.8	115.5	154.3	115.5	126.8	55.8	50.6	35.9	155.4	125.9	197.8	38.4	73.4	40.0	

occurrence of fragmentation pathways leading to the ions **13** (*m/e* 205) and **14** (*m/e* 70). The ^1H nmr spectrum of **12** resembled very closely that of mesembrenone and its relationship to this alkaloid was established by methylation to mesembrenone with diazomethane. These data reduce the structural possibilities to two, structure **12** and an isomer in which the position of the phenolic hydroxyl and *O*-methyl group in **12** are reversed.

A distinction between these two possible structures was made by a variation of the radiolabeling procedure which we had previously employed[11] in deducing the structures of **10** and **11**. On this occasion advantage was taken of the availability of biosynthetically labeled 4′-*O*-demethylmesembrenone containing ^{14}C labels in the *O*- and *N*-methyl groups. Methylation of the radiolabeled alkaloid afforded mesembrenone, which was oxidized to veratric acid, and the latter was selectively demethylated to isovanillic acid as previously described. A loss of 95% of the radiolabel occurred in the conversion of veratric acid to isovanillic acid and thereby established the aromatic substitution pattern of the alkaloid as depicted in structure **12**.

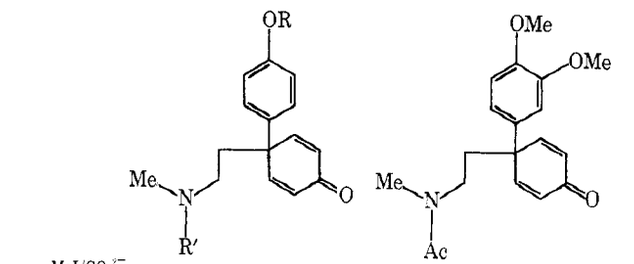
Examination of the phenolic alkaloid fraction of *S. namaquense* has revealed that is consists of a highly complex multicomponent mixture. Column chromatrography of the mixture over silica gel in chloroform–methanol afforded 240 fractions (see Experimental Section). Combination of fractions 148–164 followed by preparative tlc afforded (±)-4′-*O*-demethylmesembrenone and a new phenolic base, sceletenone (**15**), in impure form. High-pressure chromatography of the impure sceletenone over silica gel gave a sample which, although slightly contaminated, was used in the chemical studies described below. An analytically pure sample of sceletenone was obtained by further purification using high-pressure chromatography on phenyl–Corasil in water–acetonitrile (9:1). With the exception of the ^{13}C nmr spectrum, all of the crucial spectral data were obtained with this sample.

Sceletenone, $C_{15}H_{17}NO_2$, contained absorptions at 3592, 3300, and 1678 cm^{-1} indicative of a phenolic hydroxyl and an α,β-unsaturated ketone. The presence of a phenolic hydroxyl was confirmed by the methylation with diazomethane to give an *O*-methyl derivative **16**. The ^1H nmr spectra of both sceletenone and its *O*-methyl derivative contained an aromatic AA′BB′ pattern, two deshielded olefinic signals mutually coupled (J = 10.0 Hz), and also showed the typical small long-range coupling of the β hydrogen characteristic of the C-4 hydrogen in 6-keto-4-ene compounds in the mesembrine series. An *N*-methyl signal was also observed at δ 2.32. A further indication that sceletenone was based upon the octahydroindole skeleton was provided by the appearance of a C_4H_8N fragment (*m/e* 70) as the base peak in the mass-spectra of the alkaloid and its *O*-methyl derivative. The C_4H_8N fragment (**14**) occurs in high abundance in the mass spectra of all octahydroindole alkaloids of the mesembrine series and may be considered diagnostic for this class of alkaloids provided that it is accompanied by the appropriate 3-aryl-*N*-methylpyrrolidinium ion (*cf.* **4** or **13**) for the dioxyaryl members. Since sceletenone obviously possesses only a single aromatic oxygen function, the comparable ion, **17**, if present, would be expected at *m/e* 175. Indeed, this ion is found in the mass spectrum of sceletenone and its structure assignment is supported by the observation of a mass shift Δm = 14 in the comparable ion **18** in the mass spectrum of *O*-methylsceletenone.



14 (*m/e* 70)

sceletenone R = H (*m/e* 243) **17**, R = H (*m/e* 175)
R = Me (*m/e* 257) **18**, R = Me (*m/e* 189)



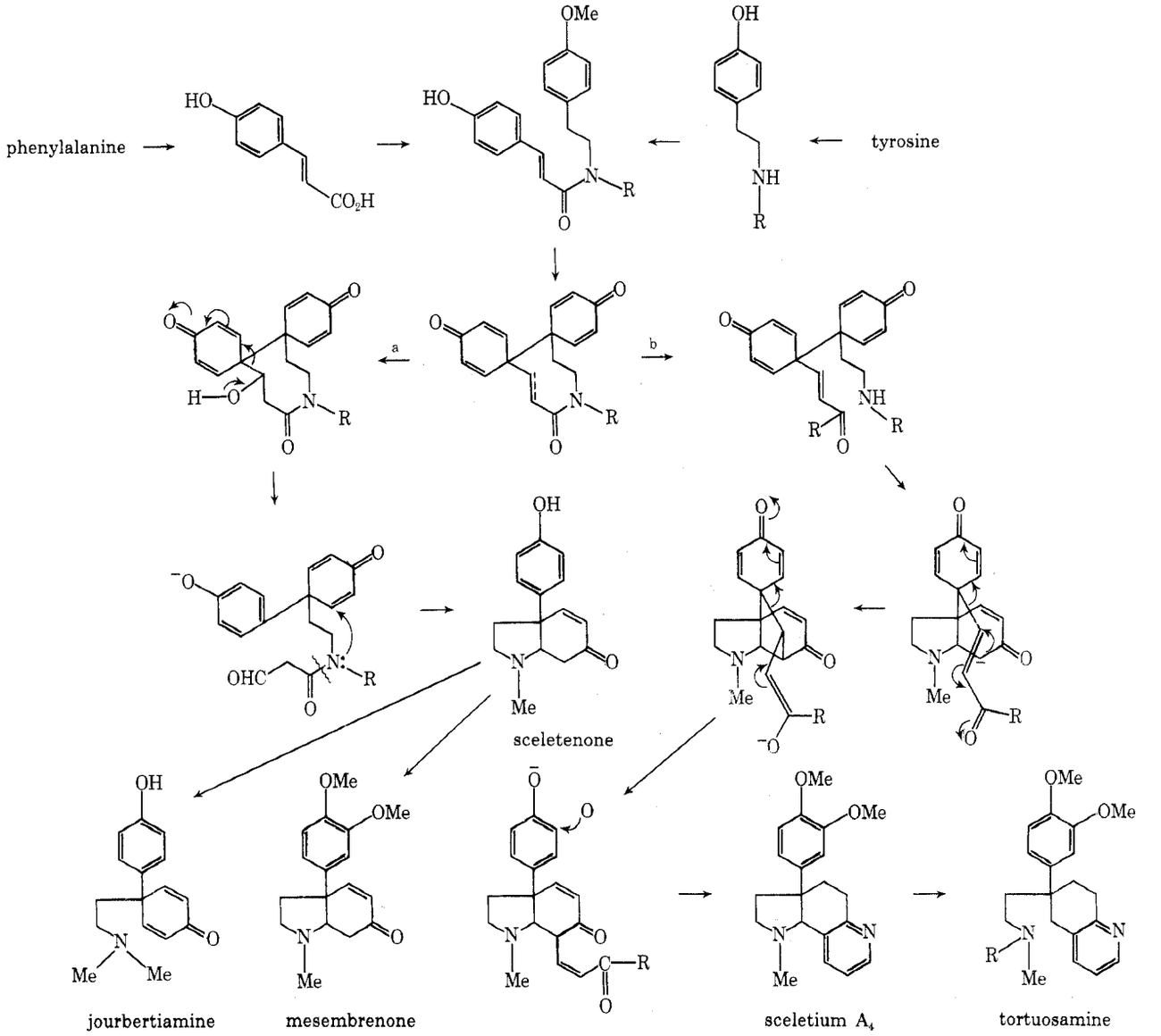
15 $\xrightarrow{MeI/CO_3^{2-}}$ **19**, R = H, R′ = Me **21**

15 $\xrightarrow{Ac_2O/py}$ **20**, R = R′ = Ac

Compelling evidence for the structure of sceletenone was obtained by comparison of its ^{13}C nmr spectra with that of mesembrenone. The assignments of the carbon resonances (Table II) for the latter were made after a detailed examination of the ^{13}C spectra of a series of octahydroindole alkaloids of the mesembrine class. A comparison of the carbon shifts of the two alkaloids shows a very close correspondence in all signals with the exception of aromatic carbon resonances. The latter reflect the different aromatic substitution patterns and the values observed in each case are in fact found to be in good agreement with the calculated shifts derived from the substituent parameters of Levy and coworkers.[12] We have found that ^{13}C shifts are a very sensitive indicator of structural change in this series and therefore the close correspondence in chemical shifts of the nonaromatic carbons constitutes firm evidence for the proposed structure of sceletenone as **15**.

Some additional evidence supporting the proposed structure has been obtained from reactions of sceletenone involving a Hofmann degradation and its behavior upon acetylation. In the case of the former reaction, the methiodide of **15** underwent a facile elimination on basification with aqueous sodium carbonate solution. The ease of this elimination is in keeping with the presence of a β-amino ketone structural fragement and is paralleled by the equally facile Hofmann reaction of the ketones, mesembrine and mesem-

Scheme II
A Unified Scheme for the Biogenesis of the Various Classes of *Sceletium* Alkaloids



brenone. The structure of the product as the dienone **19** is supported by its spectral properties, with bands in the ultraviolet spectrum at 232 (log ϵ 4.24) and 272 nm (3.25) and a low-frequency carbonyl at 1655 cm^{-1} in the infrared spectrum. The mass spectrum of the Hofmann product exhibited ions at *m/e* 58 and 72, characteristic for an *N,N*-dimethylaminoethyl side chain, and an M − CO fragment at *m/e* 215 providing strong support for the proposed structure of this compound.

Acetylation of sceletenone in acetic anhydride–pyridine results in the formation of an *O,N*-diacetate. The assignment of the structure of this product as **20** is in full accord with its spectral properties, which compare well (see Experimental Section) with those obtained for the model compound **21** derived from a similar acetylation of mesembrenone.

The formation of the dienones **19** and **20** is in accord with the known propensity of β-amino ketones to undergo β-elimination under acetylation conditions.

Biogenetic Relationships. Although it is possible that sceletium A_4 and related bases such as tortuosamine and its derivatives are biosynthesized by a process which simply involves heteroannelation of mesembrine, another and more attractive hypothetical pathway is presented in Scheme II. The utilization of phenylalanine and tyrosine in the manner indicated in this scheme is consistent with the results of labeling experiments[4] insofar that they indicate that (1) these two mino acids furnish the aromatic ring and the C_6–C_2–N units in mesembrine, (2) the mode of incorporation of phenylalaninnine probably requires a ring A spirodienone intermediate, and (3) all likely Ar–C_1–N–C_2–Ar (norbelladines) candidates may be excluded from consideration.

The transformation of phenylalanine to hydroxylated cinnamic acids is a well-established metabolic pathway in higher plants[13] and the combination of *p*-hydroxycinnamic acid with a tyrosine Ar–C_2–N unit to provide a cinnamic acid amide has precedent in the biosynthesis of colchicine.[14] The intervention of a similar Ar–C_3–N–C_2–Ar intermediate in the biosynthesis of mesembrine and *Sceletium* alkaloid A_4 can readily account for the genesis of both ring systems by the route depicted in Scheme II. The postulation of the late-stage aromatic oxygenation for the introduction of the second aromatic oxygen function, which is present in the majority of the alkaloids of this family, although arbitrary, has the advantage that it is possible to in-

clude both the monooxyaryl alkaloids of the joubertiamine series and sceletenone in a single unified scheme.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Infrared spectra were obtained for solutions in $CHCl_3$ on Perkin-Elmer Models 137, 237, and 621 spectrophotometers. Ultraviolet spectra were measured in 95% ethanol on a Beckman DBG or Cary 14 spectrophotometer. Proton nuclear magnetic resonance (nmr) spectra were recorded at 60 MHz on a Varian A-60, at 90 MHz on a Bruker HFX-90, at 100 MHz on a JEOL MH-100, at 220 MHz on a Varian HR-220, and at 250 MHz on a spectrometer constructed at the Carnegie-Mellon Institute. Carbon-13 spectra were recorded at 22.63 MHz on the Bruker HFX-90. Tetramethylsilane was used as an internal reference in deuteriochloroform as a solvent unless otherwise noted. Low-resolution mass spectra were determined on a Du Pont 21-490 instrument. Radioactive samples were counted on a Beckman LS 150 as previously described.[4] CD spectra were obtained on a Jasco ORD/CD spectropolarmeter. Gas–liquid chromatography (glc) was carried out on a Hewlett-Packard 402 instrument with 8 ft × 0.125 in. glass columns packed with 3% OV-17 or 3% SE-30 on Gas-Chrom Q (100–120 mesh). High-pressure liquid chromatography (hlpc) was performed on a Waters ALC-202 instrument.

Extraction of Alkaloids from *Sceletium namaquense*. Several methods were employed in the extraction of the alkaloid fraction from this species; that described is representative of one of the more satisfactory procedures.

Dried plant material of *S. namaquense* (3.5 kg) was placed in a Soxhlet and extracted with 15 l. of 95% ethanol for 17 hr. The extracted material was transferred in portions to a blender and macerated with a further 10 l. of 95% ethanol. The combined ethanol extracts were concentrated to *ca.* 2.5 l. and acidified with 5% tartaric acid. The aqueous acidic solution was extracted with ether (5 × 300 ml) and the ether extract was discarded. After basification of the aqueous phase with Na_2CO_3 the solution was extracted successively with $CHCl_3$ (10 × 500 ml) and $CHCl_3$–MeOH (4:1) (3 × 100 ml). The $CHCl_3$ extracts were combined, the solvent was concentrated to *ca.* 1.5 l., and the solution was filtered to remove small quantities of insoluble fatty impurities. The $CHCl_3$ filtrate was extracted with 1 *N* NaOH (3 × 100 ml) and washed thoroughly with water. Evaporation of the $CHCl_3$ solution gave 120 g of nonphenolic alkaloids. The phenolic alkaloids (20 g) were recovered from the NaOH solution by adjusting to pH 9 (with CO_2) and extraction into $CHCl_3$ (5 × 100 ml).

Isolation of Nonphenolic Alkaloids. The nonphenolic alkaloid fraction (120 g) was extracted with 3 × 500 ml of boiling ether to remove 60 g of ether-soluble alkaloids containing largely mesembrine and mesembrenone. The ether-insoluble residue was dissolved in 1200 ml of $CHCl_3$–MeOH (3:1) and filtered through a column (4 ft × 3 in.) containing 1400 g of silica gel (170–200 mesh). Evaporation of the total eluate gave 50 g of alkaloidal material. A portion (20 g) of this fraction was dissolved in $CHCl_3$ (100 ml) and chromatographed over neutral alumina (1400 g, activity 4) contained in a 4 ft × 3 in. column. The column was eluted with 3 l. of solvent using a linear gradient of $CHCl_3$–$CHCl_3$/MeOH (4:1) and 15-ml fractions were collected: fractions 1–120, nonalkaloidal material (3.10 g); 121–143, mesembrine (1.63 g); 144–175, mesembrenone (2.0 g); 176–205, mesembrenone and *Sceletium* alkaloid A_4 (0.65 g); 206–215, unidentified alkaloids (1.45 g); 216–225, *N*-formyltortuosamine and unidentified alkaloids (3.115 g); 226–235, Δ^7-mesembrenone and other alkaloids (1.114 g); and four further fractions (236–330) containing mesembranol (2.3 g) and an unresolved mixture of polar components (5.852 g).

Sceletium Alkaloid A_4 (3). Fraction 176–205 (0.65 g) was rechromatographed over neutral alumina (30 g, activity 3) using two successive linear solvent gradients of benzene–benzene/EtAc (1:1) and EtAc/MeOH (1:1)–MeOH. Mesembrenone was eluted in the early fractions and sceletium A_4 in the late fractions. Sceletium A_4 crystallized from EtAc as prisms: mp 153–154.5°; ir 1605 (C=N–) and 1580 cm^{-1} (aromatic ring C=); uv max 225 nm (log ϵ 3.59), 267 (3.39), 273 (3.42, 285 (3.10); CD (95% EtOH) $[\theta]_{278}$ +6310°, $[\theta]_{274}$ +5550°, $[\theta]_{247}$ −1879°; nmr, see Table I; mass spectrum *m/e* (rel intensity) 324 (100, M^+), 323, (78, M − 1), 309 (45, M − CH_3), 296 (30, M − C_2H_4), 281 (17, M − C_2H_5N), 266 (56, M − C_3H_8H), 219 (12, $C_{13}H_{17}NO_2$), 57 (15, C_3H_7N).

Anal. Calcd for $C_{20}H_{24}N_2O_2$: *m/e* 324.1837. Found: *m/e* 324.1832.

***N*-Formyltortuosamine (8).** Fractions 216–225 (3.11 g) were subjected to successive preparative tlc separation on silica gel in $CHCl_3$–MeOH (9:1), alumina in $CHCL_3$–MeOH (97:3), and finally alumina in $CHCl_3$ to give *N*-formyltortuosamine (8), homogeneous by tlc and glc, as an oil: ir 1660 cm^{-1} (amide C=O); uv max 220, 271, and 277 nm; 250-MHz proton nmr δ 2.66 and 2.70 (two s, 3 H, NCH_3 for each conformer), 3.74, 3.75, 2.77, and 3.78 (four s, 6 H, OMe), 6.88 (m, 3 H, aromatics), 7.0 (m, 1 H, pyr-H_3), 7.42 (apparent t, 1 H, pyr-H_4), 7.81 (two s, 1 H, NCHO for each conformer), 8.32 (m, 1 H, pyr-H_2); 100-Mz proton nmr (120°) δ 2.74 (NCH_3), 3.84, 3.86 (two s, 6 H, OMe), 6.90 (m, 3 H, aromatics), 7.00 (dd, 1 H, J = 8.0, 5.0 Hz, pyr-H_3), 7.42 (dd, 1 H, J = 8.0, 2.0 Hz, pyr-H_4), 7.80 (s, 1 H, NCHO), 8.32 (dd, 1 H, J = 5.0, 2.0 Hz, pyr-H_2); CD (95% EtOH) $[\theta]_{282}$ +12,000°, $[\theta]_{266}$ −6380°; mass spectrum *m/e* (rel intensity) 354 (30, M^+), 282 (100, M − C_3H_6NO), 269 (32, M − C_4H_7NO), 268 (57, M − C_4H_8NO), 151 (30, $C_9H_{11}O_2$), 130 (31, C_9H_8N), 118 (30, C_8H_8N), 107 (28, C_7H_9N), 72 (10, C_2H_6NO), 57 (5.4, C_3H_7N), 55 (16, C_3H_5N).

Anal. Calcd for $C_{21}H_{26}N_2O_3$: *m/e* 354.1943. Found: *m/e* 354.1934.

Hydrogenolysis of Sceletium A_4. The alkaloid **3** (94 mg) was dissolved in water (3 ml) containing 50 μl of 12 *N* HCl, and mixed with 10% palladium on carbon catalyst. The mixture was stirred at 55° for 42 hr until the hydrogenolysis was complete. Chromatography on silica gel plates (made up in 5% K_2CO_3 solution) in $CHCl_3$–MeOH (3:2) gave tortuosamine (**6**) as a colorless oil: ir (neat) 3280 (NH), 1600 1580 cm^{-1}; mass spectrum *m/e* (rel intensity) 326 (17, M^+), 269 (100, M − C_3H_7N), 268 (66, M − C_3H_8N), 254 (8, M − $C_4H_{10}N$), 132 (13, $C_9H_{10}N$), 57 (13, C_3H_7H), and 55 (12, C_3H_5N). Tortuosamine-*N*-d_1 showed M^+ 327 and Δm 269 → 270, Δm 268 → 269 (it was necessary to equilibrate the source with D_2O in order to obtain this spectrum); nmr (60 Mz) δ 1.27 (s, 1 H, NH), 2.29 (s, 3 H, NMe), 3.80 and 3.83 (s, 3 H, OCH_3), 6.79 (m, 3 H, phenyl H), 7.06 (dd, 1 H, J = 5.0 and 8.0 Hz, pyr-H_3), 7.48 (dd, 1 H, J = 8.0, 2.0 Hz, pyr-H_4), 8.40 (dd, 1 H, J = 5.0, 2.0 Hz, pyr-H_2). The above spectral data agree well with those reported for tortuosamine.[9]

Anal. Calcd for $C_{20}H_{26}N_2O_2$: *m/e* 326.1994. Found: *m/e* 326.1990.

N-Formylation of Tortuosamine. Tortuosamine (50 mg) was stirred at 0° with 2 ml of acetic–formic anhydride for 6 hr. Basification of the reaction mixture with saturated Na_2CO_3 solution followed by extraction with $CHCl_3$ afforded *N*-formyltortuosamine (40 mg). The chromatographic (tlc and glc) and spectral data (nmr, CD, and ir) were identical with those of the natural base.

Treatment of *N*-formyltortuosamine (10 mg) under reflux with 1 *N* HCl for 2 hr gave tortuosamine as the major product, mass spectrum *m/e* 326 (M^+).

(−)-Δ^7-Mesembrenone (7). Preparative tlc of fractions 226–235 (1.114 g) on silica gel in $CHCl_3$–MeOH (9:1) afforded 240 mg of a component of R_f value 0.35. Rechromatography of this component on alumina (tlc) in $CHCl_3$–MeOH (97:3) gave a band (R_f 0.46, 90 mg) which was further purified by tlc on alumina in $CHCl_3$ to give 40 mg of (−)-Δ^7-mesembrenone (**7**) as an oil. The spectral (mass spectrum, ir, nmr, uv, and CD) and chromatographic data (glc and tlc) of this compound were identical with those of an authentic sample prepared from mesembrine.[10]

Phenolic Alkaloids. Isolation of 4′-*O*-Demethyl[3′-*O*-*methyl*-^{14}C,*N*-*methyl*-^{14}C]mesembrenone. The phenolic alkaloid fraction derived from an experiment in which the alkaloids had been obtained from *S. strictum* plants to which [*S-methyl*-^{14}C]methionine had been administered was subjected to preparative tlc on silica gel H. Double development with $CHCl_3$–MeOH (3:1) gave 4′-*O*-demethylmesembrenol (**10**, R_f 0.62) and 4′-*O*-demethylmesembranol (R_f 0.38). A radioscan of the plate showed a single radioactive component at R_f 0.65 which on recovery from the plate gave labeled 4′-*O*-demethylmesembrenone (**12**) as an oil. The same alkaloid was subsequently isolated by preparative tlc of the column fractions 148–164 from the phenolic alkaloid fraction of *S. namaquense*. 4′-*O*-Demethylmesembrenone showed the following spectral properties: ir 3540 (OH), 1678 (O=O), 1612, 1605, 1510 cm^{-1}; nmr (100 MHz) δ 2.10–2.80 (m, 4 H), 2.33 (s, 3 H, NMe), 2.59 (br m, 8 H), 3.01 (t, 1 H, H-7a), 3.33 (m, 1 H, H-2), 3.91 (s, 3 H, OMe), 5.40–5.70 (br s, 1 H, OH), 6.12 (d, 1 H, J = 10.0 Hz, H-5), 6.75 (dd, 1 H, J = 10.0 and 2.0 Hz, H-4), 6.90 (m, 3 H, phenyl H); uv max 220 nm (log ϵ 4.04), 280 (3.59); uv max (0.1 *N* NaOH) 213 nm (log ϵ 4.04), 244 (4.0), 292 (3.69); mass spectrum *m/e* (rel intensity) 273 (44, M^+), 258 (5.3, M − CH_3), 205 (21), 70 (100, C_4H_8N).

Anal. Calcd for $C_{16}H_{19}NO_3$: *m/e* 273.1365. Found: *m/e* 273.1361.

Methylation of 4′-*O*-Demethyl[3′-*O*-*methyl*-^{14}C,*N*-*methyl*-^{14}C]mesembrenone. Radioactive 4′-*O*-demethylmesembrenone 92.5 mg, 2.63 μCi/mmol) was dissolved in 2 ml of MeOH and the

solution was cooled to −78°. An excess of diazomethane was added to the solution and the solution was allowed to stand at 0° for 48 hr. Mesembrenone (97.5 mg) was added to the reaction mixture and the solvent was evaporated. The residue was treated with 2-propanol–ether containing HCl and the mesembrenone hydrochloride was crystallized several times from this solvent to give 52 mg of material of constant activity (4.03×10^{-2} μCi/mmol).

Oxidation of [3′-*O*-methyl-^{14}C,*N*-methyl-^{14}C]Mesembrenone. Radiolabeled mesembrenone hydrochloride (52 mg) from the above experiment was mixed with inactive mesembrenone hydrochloride (58 mg) and added to a solution of $K_3Fe(CN)_6$ (33 g) and KOH (5.8 g) in 35 ml of H_2O. The solution was brought to reflux, and during the course of 72 hr three further additions of the above quantities of $K_3Fe(CN)_6$ and KOH were made. The reaction mixture was filtered and the filtrate was acidified with 50% H_2SO_4 before extracting continuously with Et_2O for 72 hr. The Et_2O extract was washed successively with 1 *N* HCl and water. Extraction of the ether solution with saturated Na_2CO_3 and recovery of the acidic fraction by acidification of the $NaCO_3$ solution followed by reextraction into ether gave 40 mg of [3′-*O*-methyl-^{14}C]veratric acid. Several recrystallizations from H_2O–MeOH (5:1) gave a radiochemically pure sample, mp 180–182° (9.2×10^{-3} μCi/mmol).

Demethylation of [3′-*O*-methyl-^{14}C]Veratric Acid to Isovanillic Acid. A suspension of labeled veratric acid (19.5 mg) and carrier (19.5 mg) in 1.0 ml of 48% HBr was dissolved by gently refluxing the solution. Immediately after a copious precipitate had formed, the reaction mixture was quickly cooled and filtered. Examination of the precipitate by tlc showed some veratric acid remaining; so the mixture was methylated with CH_2N_2 and extracted with 2% NaOH. Upon acidification and extraction with $CHCl_3$, tlc examination of the $CHCl_3$ extract showed partial hydrolysis; so the ester was hydrolyzed with 10% NaOH on a steam bath for 30 min. After acidification and extraction with $CHCl_3$, a pink solid was obtained which was crystallized from EtOH–H_2O to give isovanillic acid, mp 248–249.5°. Counting of this sample showed that it contained only 8% of the activity (4.08×10^{-4} μCi/mmol) of the veratric acid from which it was derived.

Isolation of Sceletenone (15) from *S. namaquense*. The crude phenolic fraction from *S. namaquense* was chromatographed over silica gel (1200 g) using a linear gradient of $CHCl_3$–MeOH (20:1, 2 l.) against $CHCl_3$–MeOH (3:1, 2 l.) followed by $CHCl_3$–MeOH (3:1, 1 l.) against $CHCl_3$–MeOH (1:1, 1 l.). A total of 260 25-ml fractions was collected to give a total of 4.90 g.

Preparative tlc of fractions 148–164 (1.86 g) on silica gel in $CHCl_3$–MeOH (9:1) gave 450 mg of a component, R_f 0.3. Further purification of this material by high-pressure liquid chromatography on a 6 ft × 0.375 in. column of silica gel H in $CHCl_3$–MeOH (20:1) at 300 psi gave 277 mg of sceletenone (**15**) containing <5% of a higher molecular weight impurity (by mass spectral analysis). A sample (20 mg) of this material was further purified by high-pressure liquid chromatography on a 6 ft × 0.125 in. column of phenylcorasil in H_2O–CH_3CN (9:1), giving 12 mg of an oil: ir 3592, 3300 (OH, ratio of intensity of the former to the latter peak increased on dilution), and 1678 cm^{-1} (–C=O); uv max ~230 nm (ε 7570) and 279 (1240); uv max (NaOH–EtOH) ~230, ~240, and 285 nm; 100-MHz proton nmr δ 2.0–2.7 (m, 6 H), 2.32 (s, 3 H, NMe), 3.30 (m, 1 H), 6.09 (d, 1 H, J = 10.0 Hz, H-5), 6.74 (dd, 1 H, J = 10.0 and 2.0 Hz, H-4), 7.02 (center of AA′BB′ pattern, 4 H, aromatic hydrogens); carbon-13 nmr ($CHCl_3$) 35.9, 38.4, 40.0, 50.6, 55.8, 73.4, 115.3, 115.5, 125.0, 127.4, 154.3, 155.4, and 197.8 ppm (see Table II for assignments); mass spectrum *m/e* (rel intensity) 243 (53, M$^+$), 215 (7, M − CO), 175 (13, $C_{11}H_{13}NO$), 70 (100, C_4H_8N).

Anal. Calcd for $C_{15}H_{17}NO_2$: *m/e* 243.1259. Found: *m/e* 243.1255.

Hofmann Degradation of Sceletenone. Sceletenone (25 mg) was dissolved in 0.5 ml of acetone and CH_3I (0.5 ml) was added. The reaction mixture was allowed to stand overnight. The solid residue obtained on evaporation of the solvent was dissolved in 5 ml of H_2O, basified with saturated Na_2CO_3 solution, dried over $MgSO_4$, filtered, and evaporated *in vacuo*, leaving a light yellow oil. The oil was chromatographed on silica gel with 15% MeOH in $CHCl_3$, giving 6 mg of sceletenone and 5 mg of the dienone **19** as a light yellow oil: ir 3590 (OH), 1655, and 1611 cm^{-1} (dienone); uv max (MeOH) 232 nm (ε 17,500) and 272 (1770); mass spectrum *m/e* (rel intensity) 257 (56, M$^+$). 72 (69, $C_4H_{10}N$), 58 (100, C_3H_8N).

Anal. Calcd for $C_{16}H_{19}NO_2$: *m/e* 257.1416. Found: *m/e* 257.1415.

Acetylation of Sceletenone. A 10-mg sample of sceletenone was dissolved in 1.5 ml of pyridine. Five drops of acetic anhydride was added, and the reaction was stirred overnight at room temperature under a N_2 atmosphere. The excess pyridine and acetic anhydride were removed by vacuum distillation, giving a yellow oil: ir no OH, 1750 (ester), 1660 (dieone + amide), 1627 cm^{-1} (dienone); uv 225 nm (ε 14,800), 277 (4300); 90-MHz proton nmr δ 2.2–2.4 (m, 2 H, H-3), 3.15–3.4 (m, 2 H, H-2), 2.06 (s, 3 H, NAc), 2.31 (s, 3 H, OAc), 2.98 (s, 3 H, NMe), 6.66 (center of AA′BB′ pattern, 4 H, dienone protons), 7.05 (center of AA′BB′ pattern, 4 H, aromatic protons); mass spectrum *m/e* (rel intensity) 327 (27, M$^+$), 285 (11, M − C_2H_2O), 254 (42, M − C_3H_7NO), 212 [96, M − (C_2H_2O + C_3H_7NO)], 199 [18, M − (C_2H_2O + C_4H_8NO)], 185 [46, M − (C_2H_2O + $C_5H_{10}NO$)], 100 (100, $C_5H_{10}NO$), 86 (42, C_4H_8NO).

Anal. Calcd for $C_{19}H_{21}NO_4$: *m/e* 327.1470. Found: *m/e* 327.1464.

Acetylation of Mesembrenone. A 220-mg sample of mesembrenone was dissolved in 25 ml of pyridine under N_2. Acetic anhydride (5 ml) was added, and the solution was stirred for 24 hr. The excess pyridine and acetic anhydride was removed by vacuum distillation. The dark yellow oil was put through a short alumina column (activity II) with $CHCl_3$ and then 2.5% MeOH in $CHCl_3$, resulting in a yellow oil: 230 mg; ir 1658 (dienone + amide), 1640, and 1620 cm^{-1} (dienone); uv (MeOH) 228 nm (ε 13,900), and 278 (3780); 100-MHz proton nmr δ 2.18–2.48 (m, 2 H, H-3), 3.2–3.44 (m, 2 H, H-2), 2.07 and 2.09 (two s, 3 H, NAc for each conformer), 2.98 and 3.03 (two s, 3 H, NMe), 3.92 (s, 6 H, OMe), 6.73 (center of AA′BB′ pattern, 4 H, dienone protons), 6.8–7.05 (m, 3 H, aromatic protons); mass spectrum *m/e* (rel intensity) 329 (30, M$^+$), 256 (30, M − C_3H_7NO), 243 (11, M − C_4H_8NO), 229 (52, M − $C_5H_{10}NO$), 100 (100, $C_5H_{10}NO$), 86 (17, C_4H_8NO).

Anal. Calcd for $C_{19}H_{23}NO_4$: *m/e* 329.1627. Found: *m/e* 329.1632.

***O*-Methyl Derivative of Sceletenone.** An excess of an ethereal solution of diazomethane was added to 25 mg of sceletenone in 5 ml of CH_3OH at 40°. After 48 hr, the solvent was removed *in vacuo* and the oily residue was subjected to tlc on silica gel using $CHCl_3$–MeOH (20:1). The resulting oil was further purified *via* an acid–base extraction to afford a pale yellow oil: 6 mg; ir no OH, 1680 cm^{-1} (C=O); 250-MHz proton nmr δ 2.2–2.7 (m, 6 H), 2.32 (s, 3 H, NMe), 3.25 (m, 1 H), 3.79 (s, 3 H, OMe), 6.10 (d, 1 H, J = 10.0 Hz, H-5), 6.70 (dd, 1 H, J = 10.0, 1.5 Hz, H-4), 6.88 (center of AA′BB′ pattern, 4 H, aromatic protons); mass spectrum *m/e* (rel intensity) 257 (100, M$^+$), 229 (9, M − CO), 189 (14, $C_{12}H_{15}NO$), 70 (82, C_4H_8N).

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

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Registry No. —**1**, 25516-12-5; **3**, 35135-35-4; **6**, 51934-13-5; **7**, 35714-44-4; **8**, 51934-14-6; **12**, 51934-30-6; **15**, 51934-31-7; **16**, 51934-32-8; **19**, 28564-22-9; **20**, 51934-33-9; **21**, 51934-34-0; veratric acid, 93-07-2; isovanillic acid, 645-08-9.

References and Notes

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A Study of the Structures of Some Benzo-1,2,3-triazinium Betaines

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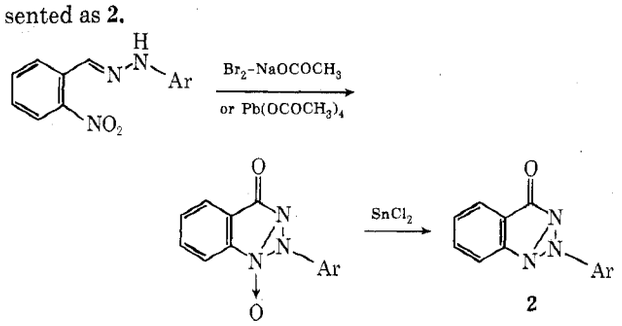
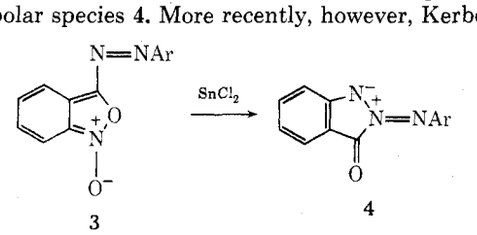
Methylation, phenylation, *p*-bromophenylation, and *p*-methylphenylation of 4(3*H*)-benzo-1,2,3-triazinone with dimethyl sulfate, diphenyl-, di-*p*-bromophenyl-, and di-*p*-tolyliodonium chloride results in formation of the N_2-substituted benzo-1,2,3-triazinium betaines **8** and **6a–c**, respectively. These latter compounds are identical with the products obtained by stannous chloride reduction of the betaines **10** and **5a–c** formed by oxidative cyclization of *o*-nitrobenzaldehyde methyl-, phenyl-, *p*-bromophenyl-, and *p*-tolylhydrazone, respectively, with lead(IV) acetate. Examination of the ir, nmr, uv, and mass spectra of the two classes of betaines **5** and **6** reveals that, while ir and nmr techniques afford little definitive evidence on structure, uv and mass spectroscopy can be used both for confirmation of structure and to distinguish between the two types of betaines.

Mild oxidation of *o*-nitrobenzaldehyde arylhydrazones with either bromine–sodium acetate or lead(IV) acetate results in the overall loss of two hydrogen atoms and production of a class of *N*-aryl heterocycles, the structure of which has been the subject of uncertainty and some controversy for the past 50 years. These oxidation products were first prepared and investigated by Chattaway,[1-6] who described them as "isodiazomethanes" and formulated them as the triaziridine derivatives **1**. Structural assignment was based entirely on evidence from degradation studies; in particular, Chattaway showed that reduction of "**1**" with stannous chloride resulted in the loss of a single oxygen atom and formation of a second class of compounds which he represented as **2**.



1 2

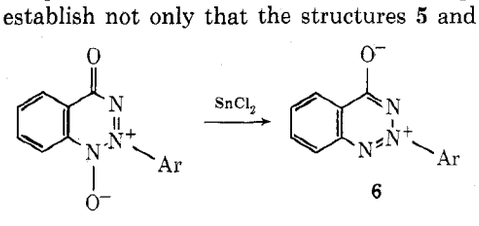
In a later reinvestigation of this work, Gibson,[7] partly on the basis of mechanistic reasoning and partly as a result of spectroscopic (uv, ir) studies, suggested that the initial oxidation products formulated by Chattaway as **1** could be better represented as the isomeric phenylazoanthranil *N*-oxides **3**, and the stannous chloride reduction products as the dipolar species **4**. More recently, however, Kerber[8] has



3 4

challenged Gibson's assignments and the evidence on which they were based. Kerber pointed out that Gibson's spectroscopic data were probably not consistent with structure **4**, and that structure **3** was improbable inasmuch as anthranil *N*-oxides are a rare, if not unknown, class of heterocycle, and proposed the triazinium betaine structures **5** and **6** for the oxidation and reduction products, respectively. Kerber's assignments, like those of Gibson, were based partly on mechanistic reasoning and partly on spectroscopic (ir, uv, nmr, mass spectral) evidence.

Heterocyclic betaines have been a subject of interest in this department for some years,[9] and within this context unsuccessful attempts were made some time ago to obtain definitive evidence for the structure of the dipolar species obtained from the oxidation of *o*-nitrobenzaldehyde arylhydrazones.[10] In the present paper we report the results of a further chemical and spectroscopic investigation of these compounds and their derived reduction products which establish not only that the structures **5** and **6** proposed by Kerber for the two classes of heterocycles are correct, but that the two structural types can be clearly differentiated on the bases of their uv and mass spectra.



5 6

Discussion

At the outset of the present study Kerber's structure **5** for the products of oxidative cyclization of *o*-nitrobenzaldehyde arylhydrazones was assumed to be correct and to be compatible with a plausible mechanism for the overall reaction (Scheme I). Consequently, attention was concentrated on the development of procedures whereby compounds of the type **5** and/or **6** could be synthesized by an alternative route to that used by Chattaway. The simpler of the two series of betaines, *i.e.*, **6**, was investigated initially in the hope that, were an alternative synthesis of these compounds to be devised, it might then prove possible to effect specific N_1-oxidation to give **5**.

In 1968, Wagner and Gentzsch reported[11] that treatment of 4(3*H*)-benzo-1,2,3-triazinone (**7**) with dialkyl sulfates in base gave a mixture of products, namely the O- and N_3-al-