fluoroborate electrolyte the only available base is the amine substrate, but in the methanol–potassium hydroxide electrolyte the much stronger bases, hydroxide ion and/or methoxide ion, are present at a combined concentration of 0.47 *M*. It is reasonable that the weaker base should attack the positions of higher positive charge density in a discriminating fashion and that the much stronger base should attack more randomly in a nondiscriminating reaction.

Support for this point of view can be mustered from other oxidation studies of *N,N*-dimethylbenzylamine. In both the oxidation with chlorine dioxide[5] at pH 8 and in the oxidation with potassium hexacyanoferrate (III) in 2 *M* potassium hydroxide[6] there is exhibited only a modest preference for attack at the methyl position. We have repeated the latter oxidation. The oxidation products obtained were benzaldehyde, *N*-methylbenzylamine, and *N*-benzyl-*N*-methylformamideand the ratio of methyl attack to benzyl attack was 2.8. The electrochemical dealkylation of *N,N*-dimethylbenzylamine at pH 12 at a glassy carbon electrode involves a mechanism similar to that shown in eq 1, and again there is no special preference for removal of the methyl group.[13] All of the above reactions are in the presence of a strong base where the proton transfer step would be expected to be nondiscriminating. By contrast, the photochemical oxidation of dimethylbenzylamine, where the strongest base present is the amine substrate, shows a very strong, albeit not quantitatively determined, preference for attack on the methyl group.[7]

Registry No.—*N*-Benzyl-*N*-methylformamide, 17105-71-4; *N*-methylbenzylamine, 103-67-3; formamide, 75-12-7; *N,N*-dimethylbenzylamine, 103-83-3.

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Peroxodisulfate Oxidation of Guanosine and Deoxyguanosine in Alkaline Aqueous Solution[1]

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The peroxodisulfate oxidation of the common nucleosides has been investigated. Only guanine nucleosides show appreciable reaction with peroxodisulfate in 1 *M* Na_2CO_3 solution at 40°. Rate *vs.* pH plots suggest that the guanosine anion is the kinetically significant reactant. No reaction between guanosine and peroxodisulfate was observed under neutral or mildly acidic conditions at 40°. Radical traps have no effect on either the rate or extent of peroxodisulfate disappearance or on the rate or extent of product formation. Products identified as a result of peroxodisulfate oxidation of guanosine were guanidine, urea, ribose, ribosylurea, and ribonic acid. Those products identified for deoxyguanosine oxidation were guanidine, urea, and deoxyribosylurea. Alkaline peroxodisulfate oxidation shows promise as a selective chemical method for the modification of polynucleotides at the site of guanine residues.

Our studies on the peroxodisulfate oxidation of the common nucleic acid bases[2] have shown that guanine reacts much more rapidly under all conditions than any other base. The relative rates of oxidation in 1 *N* KOH at 40° are as follows: adenine, 1; thymine, 5.5; uracil, 5.2; cytosine, 8.6; guanine, 338. We have further shown that the rates of these oxidations are dependent upon pH, since it is only the dianions of thymine and uracil and the monoanions of adenine and cytosine which react at a significant rate with peroxodisulfate ions. In contrast, both the mono- and dianions of guanine are reactive. It appeared probable, therefore, that of the nucleosides, only guanosine would be oxidized. We present evidence in this paper to substantiate this supposition. This suggests that peroxodisulfate ions can be used to modify polynucleotides, particularly polydeoxyribonucleotides, in a highly selective way.

Materials and Methods

Adenosine, uridine, thymidine, cytidine, guanosine, and deoxyguanosine were purchased from P & L Biochemicals Inc., Milwaukee, Wis. Guanidine hydrochloride was obtained from Heico, Inc., Delaware Water Gap, Pa. D-Ribose was purchased from Pfanstiehl Laboratories, Waukegan, Ill. Ribonolactone was obtained from the Sigma Chemical Co., St. Louis, Mo. 2-Deoxy-D-ribose and 8-hydroxyguanine were purchased from the Aldrich Chemical Company, Milwaukee, Wis. Potassium peroxodisulfate was a Baker Analyzed reagent, Phillipsburg, N. J., and was recrystallized from water for use in kinetic experiments. All other inorganic chemicals were Baker Analyzed reagents and were used without further purification. Ribosylurea was synthesized by the method of Benn and Jones[3] but was not isolated. Deoxyribosylurea was prepared by permanganate oxidation of deoxyguanosine by the method of Jones and Walker.[4] Ribonic acid was prepared by hydrolysis of ribonolactone in aqueous sodium hydroxide solution.

Ultraviolet absorption spectra were measured using a Perkin-Elmer Model 202 spectrophotometer. Colorimetric measurements were carried out on a Klett-Summerson colorimeter.

Table I
Peroxodisulfate Oxidation of Guanosine and Deoxyguanosine. Nucleoside and Temperature Dependencies

Substrate	Concn,[a] M	Conditions	k_2', M^{-1} min^{-1} [b]	No. of expts
Guanosine[c]	0.01–0.05	1 N KOH, 25°	0.161 ± 0.015	11
Guanosine	0.01–0.05	1 M Na_2CO_3, 40°	0.630 ± 0.04	14
Guanosine	0.05	1 M Na_2CO_3, 50°	1.27	1
Deoxyguanosine[c]	0.05	1 M Na_2CO_3, 30°	0.330	1
Deoxyguanosine	0.03–0.05	1 M Na_2CO_3, 40°	0.678 ± 0.00	2
Deoxyguanosine	0.05	1 M Na_2CO_3, 50°	1.24	1

[a] [Substrate]/[$K_2S_2O_8$] = 10. [b] $k_2' = k\psi$/[substrate]. [c] Activation parameters: guanosine and deoxyguanosine E_a = 13.1 ± 0.4 kcal mol^{-1}, ΔS^* = −28 ± 2 cal mol^{-1} deg^{-1}.

Partition chromatography on paper was performed on Whatman 3 MM paper in the machine cut direction at 25° using an ascending technique. The solvent systems utilized were solvent I, 2-propanol–ammonium hydroxide (58%)–water (7:1:2 v/v); solvent II, ethyl acetate–formic acid (88%)/water (7:2:1 v/v); solvent III, 2-propanol–water (7:3 v/v). Dried chromatograms were sprayed with Ehrlich's reagent[5] for the detection of urea derivatives. Ribose and ribonic acid were visualized with a benzidine–periodate spray.[6] Guanidine, urea, urea derivatives, ribose, and ribonic acid were revealed using the nitroprusside–ferricyanide–hydroxide spray.[7] Ultraviolet-absorbing components were located on paper chromatograms illuminated with an ultraviolet light source.

The concentration of D-ribose was estimated using the colorimetric method of Nelson[8] with the modification that twice the recommended copper concentration was employed in each assay.

Acid-hydrolyzable ribose was determined as follows. An aliquot (5.0 ml) of alkaline guanosine (0.01 M)–peroxodisulfate (0.06 M) reaction mixture was withdrawn, neutralized with dilute hydrochloric acid, and diluted to a final volume of 10.0 ml. The resulting solution was placed in a 125-ml erlenmeyer flask containing 1 g of anion exchange resin (Bio-Rad AG1-X8, 200–400 mesh, chloride form). The mixture was agitated for 20 min at room temperature. After centrifugation of the resin, a 2.0-ml aliquot was withdrawn and mixed with 1 ml of 1.0 N HCl. The resulting solution was heated on a boiling water bath for 25 min, cooled to room temperature, mixed with 1 ml of 1.0 N KOH solution, and diluted to a final volume of 10.0 ml. An aliquot (1.0 ml) of the resulting solution was withdrawn, mixed with 1.0 ml of the modified Nelson's reagent, and developed as described.[8]

Guanidine was determined by the method of Marston[9] as presented by Snell and Snell.[10] Urea was determined by the method of Coulombe and Favreau.[11] In both cases, batchwise pretreatment of neutralized reaction aliquots with excess anion-exchange resin (Bio-Rad AG1-X8, 200–400 mesh, chloride form) to remove unreacted peroxodisulfate was required, since peroxodisulfate interfered with both determinations.

Peroxodisulfate was determined by a modification of the iodometric method of Kolthoff and Carr.[12,13] Kinetic data were collected as described.[2]

Results

Kinetics: Peroxodisulfate Disappearance. The kinetics of peroxodisulfate disappearance in reaction with guanosine were measured under pseudo-first-order conditions in 1.0 N KOH at 25° and in 1 M Na_2CO_3 solution at 40°. Those for deoxyguanosine were measured in 1 M Na_2CO_3 at 40°. Under all conditions, semilog plots of the disappearance of peroxodisulfate with time showed good linearity for at least 2 half-times, suggesting first-order decomposition of peroxodisulfate. Neither EDTA (1×10^{-4} M) nor acrylamide (5×10^{-4} M)[2,14,15] had any effect on the rate of peroxodisulfate disappearance in reactions run in 1 M Na_2CO_3 at 40°.

Control experiments showed no more than 4% decomposition of peroxodisulfate over a 24-hr period in 1 M Na_2CO_3 at 40°. The extent of peroxodisulfate decomposition in the presence of adenosine, cytidine, uridine, or thymidine under the same reaction conditions never exceeded that observed for the maximum peroxodisulfate blank.

A first-order rate constant of 1.6×10^{-5} min^{-1} for the thermal decomposition of peroxodisulfate in aqueous solution at 40° was calculated using the data of Hakoila.[16] The first-order rate constant calculated for 4% decomposition of peroxodisulfate in 1 M Na_2CO_3 at 40° after 24 hr is 2.9×10^{-5} min^{-1}. If the difference between the calculated and our observed-first-order rate constant were attributable to reaction between the nonreactive nucleosides and peroxodisulfate, then the maximum calculated-second-order rate constant for reaction with nucleosides other than guanosine or deoxyguanosine is 1.2×10^{-3} M^{-1} min^{-1} under the conditions employed here. Values for the apparent-second-order rate constants (k_2') for guanosine and deoxyguanosine (Table I) are at least 500 times this value.

Nucleoside Dependence. Table I shows that values for the apparent-second-order rate constants (k_2') for guanosine oxidation in 1 N KOH at 25° and 1 M Na_2CO_3 at 40° are reasonably constant over the fivefold range of nucleoside concentration employed. Thus, the rate law which describes guanosine and deoxyguanosine oxidation is given by $-d[S_2O_8^{2-}]/dt = k_2'[\text{nucleoside}]_{tot}[S_2O_8^{2-}]$. The rate law held for a minimum of 2 half-times for both nucleosides. Second-order rate constants for guanosine and deoxyguanosine oxidation in 1 M Na_2CO_3 at 40° are virtually identical. The observed variation in k_2' with temperature together with the calculated activation parameters are also presented in Table I.

pH Dependence. Values for the apparent-second-order rate constant (k_2') for guanosine (0.01 M) oxidation at 25° showed no significant variation over the range of hydroxide ion concentrations 0.025–1.0 N at constant ionic strength (μ = 1.0, KCl).

Figure 1 shows the effect of varying pH on the apparent-second-order rate constant for the peroxodisulfate oxidation of guanosine at lower pH values, 40°, μ = 1.21. That point on the plot at which the value of k_2' is half-maximal corresponds to a pH of 9, in reasonable agreement with a pK_a for guanosine of 9.1 at 40° calculated from the heat of ionization presented by Izatt and Christensen.[17] These data indicate that it is only the guanosine anion which undergoes reaction with peroxodisulfate. The dianion of guanine is about 30 times more reactive with peroxodisulfate than the monoanion of guanosine.[2]

Ionic Strength Dependence. The peroxodisulfate oxidation of guanosine is subject to a positive salt effect consistent with a bimolecular reaction between ionic species of similar charge. Measurements at two ionic strengths

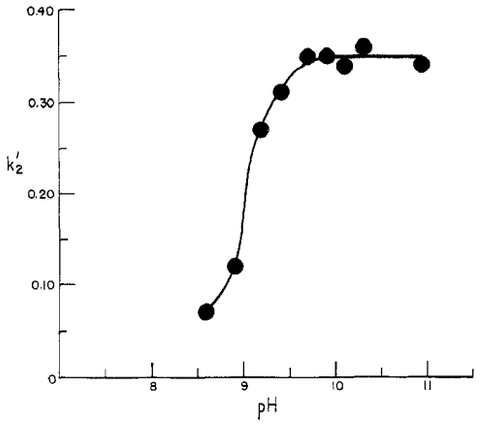

Figure 1. The peroxodisulfate oxidation of guanosine at 40°: pH dependence, [guanosine] 0.010 *M*, [$K_2S_2O_8$] 0.001 *M*, sodium carbonate–sodium bicarbonate buffer, μ = 1.21.

showed the following results: μ = 1.21, k_2' = 0.35; μ = 3.0, k_2' = 0.64 (pH 11, 40°).

Identification of Products. Products characterized in the peroxodisulfate oxidation of guanosine in 1 *M* Na_2CO_3 at 40° were guanidine, urea, ribose, ribosylurea, and ribonic acid. Those for deoxyguanosine were guanidine, urea, and deoxyribosylurea. Guanidine was identified by its chromatographic mobility (R_f's solvents I, II, III: 0.52, 0.57, 0.62), its color development with the nitroprusside–ferricyanide–hydroxide spray,[7] and isolation as its crystalline picrate.[4] Urea was identified by its chromatographic mobility (R_f's solvents I, II, III: 0.58, 0.73, 0.63) and color development with Ehrlich's reagent.[5] Ribose was characterized by its chromatographic mobility (R_f's solvents I, II, III: 0.58, 0.30, 0.67) and by its color development with the benzidine–periodate and nitroprusside–ferricyanide–hydroxide spray (white on yellow background after 5 hr). Ribonic acid (sodium salt) was confirmed by its chromatographic mobility (R_f's solvents I, III: 0.29, 0.38) and color development with the benzidine–periodate spray. Ribosylurea was detected by its chromatographic mobility (R_f's solvents I, II, III: 0.41, 0.24, 0.48) and color development with Ehrlich's reagent and the benzidine–periodate spray. Deoxyribosylurea was confirmed by its chromatographic mobility (R_f solvent III: 0.48) and color development with Ehrlich's reagent.

In addition to the products mentioned, the oxidation of guanosine by excess peroxodisulfate in 1 *M* Na_2CO_3 solution at 40° produces a compound which is detectable chromatographically as a dark spot when dried chromatograms are viewed with an ultraviolet light source. A similar but not identical material is formed in the oxidation of deoxyguanosine. Both the ultraviolet-absorbing component produced as a result of guanosine oxidation (R_f's solvents I, II, III: 0.10, 0.07, 0.24) and that produced as a result of deoxyguanosine oxidation (R_f solvent III: 0.27) can be detected as pink spots when dried chromatograms are sprayed with the nitroprusside–ferricyanide–hydroxide spray.

The ultraviolet-absorption spectrum for both materials was measured following elution from paper chromatograms. The spectra for both materials were identical at three pH values: λ_{max} (pH 1) 210, λ_{max} (pH 6.8) 230 (shoulder), λ_{max} (pH 14) 230.

The oxidation of 8-hydroxyguanine by 2 equiv of peroxodisulfate in 1 *M* Na_2CO_3 at 40° produces a material (R_f solvent III: 0.17) with the same ultraviolet-absorption maxima at the same pH values which also develops a pink coloration with the nitroprusside–ferricyanide–hydroxide spray.

Kinetics of Ribose Liberation from Guanosine. Figure 2 shows the change in free ribose concentration with time as a result of peroxodisulfate oxidation of guanosine in 1 *M* Na_2CO_3 solution at 40°. Data on the change of ribose concentration as a function of increasing initial peroxodisulfate:guanosine ratios are included.

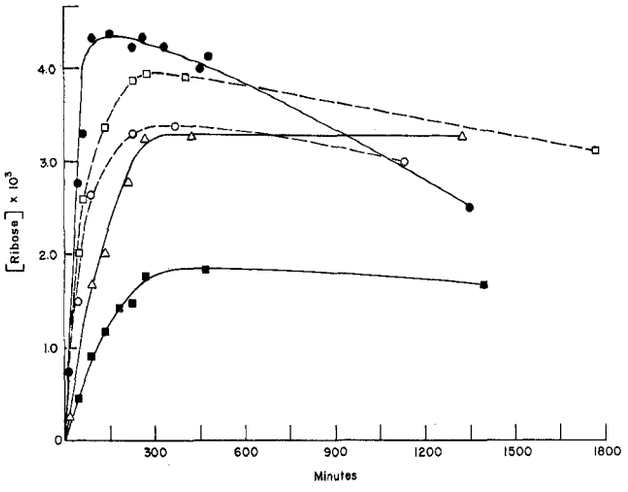

Figure 2. Ribose liberation during the oxidation of guanosine, [guanosine] 0.010 *M*, 1 *M* Na_2CO_3, 40°. Molar ratios peroxodisulfate:guanosine: ■, 1:1; △, 2:1; ○, 3:1; □, 4:1; ●, 6:1.

The data of Figure 2 show that the concentration of ribose initially increases with time during the peroxodisulfate oxidation of guanosine. The initial rate of ribose liberation is directly proportional to the initial peroxodisulfate:guanosine ratio. Ribose concentration approaches a maximum value and remains relatively constant over the time periods investigated when the initial peroxodisulfate:guanosine ratio is less than 4. At an initial peroxodisulfate:guanosine ratio of 6, the concentration of ribose declines from its maximum value. The maximum observed ribose concentration in these experiments was generally in the range of 0.35×10^{-2} to 0.45×10^{-2} *M* or approximately 40% of theoretical.

Kinetics of Peroxodisulfate Oxidation of D-Ribose and 2-Deoxy-D-ribose. The oxidations of D-ribose and 2-deoxy-D-ribose under these conditions are complex and cannot be fully described here. The rate of peroxodisulfate disappearance increases with time following an "induction period" of variable length. The rate of peroxodisulfate disappearance approaches first-order dependence on peroxodisulfate concentration following the observed lag. Neither EDTA nor acrylamide had any significant effect on the duration of the observed lag or on the rate of peroxodisulfate disappearance following the lag. The lag in peroxodisulfate disappearance in the reaction with D-ribose could be eliminated by allowing solutions of D-ribose in 1 *M* Na_2CO_3 to incubate at 40° for 20–30 min prior to the introduction of peroxodisulfate. Values for the apparent-second-order rate constants (k_2') for the oxidation of both sugars increased with decreasing sugar concentration in 1 *M* Na_2CO_3 at 40°. All k_2' values were calculated from linear regions of semilog plots of peroxodisulfate concentration *vs.* time. The variation in values of the apparent-second-order rate constant with decreasing sugar concentration is illustrated by the following data: [D-ribose] 0.100, $k_2' = 0.42 \pm 0.04$ M^{-1} min^{-1}; [D-ribose] 0.0500, $k_2' = 0.59 \pm 0.04$ M^{-1} min^{-1}; [D-ribose] 0.0100, $k_2' = 1.1 \pm 0.02$ M^{-1} min^{-1}; [2-deoxy-D-ribose] 0.100, $k_2' = 0.14 \pm 0.02$ M^{-1} min^{-1}; [2-deoxy-D-ribose] 0.0500, $k_2' = 0.362$ M^{-1} min^{-1}; [2-deoxy-D-ribose] 0.0050, $k_2' = 1.2$ M^{-1} min^{-1}. Ribose was oxidized more rapidly than deoxyribose under identical experimental conditions.

Table II
Products and Observed Stoichiometry[a]

Time, hr	[EDTA]	[Acrylamide]	Mol $S_2O_8^{2-}$/mol substrate	Mol ribose/mol substrate	Total mol urea/mol substrate	Mol guanidine/mol substrate	Mol acid-hydrolyzable ribose/mol substrate
			Guanosine				
3			1.9	0.4			
3	10^{-4}		1.9				
3	10^{-4}	0.005	2.0				
24			4.0	0.22	<0.1	0.96	0.13
24	10^{-4}		4.0	0.23	<0.1	0.96	0.08
24	10^{-4}	0.005	3.9	0.23	<0.1	0.94	0.10
			Deoxyguanosine				
3			1.9				
24			3.4		<0.1	0.87	

[a] General conditions: [nucleoside] 0.010 *M*, [$S_2O_8^{2-}$] 0.060 *M*, 1 *M* Na_2CO_3, 40°.

Stoichiometry of Ribose Oxidation. D-Ribose (0.0050 *M*) oxidation in the presence of excess peroxodisulfate (0.010 *M*) was monitored both by measurement of the disappearance of peroxodisulfate and D-ribose with time over 20-hr period. The results showed that 1.8 mol of peroxodisulfate was consumed per mole of ribose. The concentration of reducing sugar at the end of this same period was 0.075×10^{-2} *M* or 15% of the original. Paper chromatography of 1% ribose solutions in 1 *M* Na_2CO_3 in the presence of 2 equiv of $K_2S_2O_8$ showed the presence of ribonic acid and unchanged ribose after 20-hr incubation at 40°. The possible formation of products of the uronic or saccharic acid type was not investigated. Vasudeva, *et al.*,[18] report the formation of both gluconic and glucuronic acids as well as some formaldehyde and formic acid by the peroxodisulfate oxidation of glucose at higher temperatures where free-radical pathways probably predominate.

Stoichiometry of Deoxyribose Oxidation. The measurement of peroxodisulfate (0.010 *M*) disappearance in the reaction with 2-deoxy-D-ribose (0.0050 *M*) showed that 1.2 equiv of peroxodisulfate were consumed per mole of deoxyribose over a 24-hr period. One equivalent of $K_2S_2O_8$ was consumed during the first 9-hr incubation in 1 *M* Na_2CO_3 at 40°.

Stoichiometry of Guanosine and Deoxyguanosine Oxidation with Excess Peroxodisulfate. Table II presents a summary of the quantitative determinations of products for guanosine and deoxyguanosine oxidations in 1 *M* Na_2CO_3 at 40°. The effect of EDTA and acrylamide is included. The conditions for these determinations were adjusted to permit exhaustive oxidation of the nucleosides over a 24-hr period. Peroxodisulfate was present in sixfold molar excess.

The data indicate that 2 equiv of peroxodisulfate is consumed by both guanosine and deoxyguanosine during the first 3 hr of reaction in 1 *M* Na_2CO_3 at 40°. Four equivalents of peroxodisulfate is consumed over a 24-hr period in reaction with guanosine while 3.4 equiv is consumed in reaction with deoxyguanosine under the same conditions. Neither EDTA nor acrylamide had any effect on the initial rate of peroxodisulfate consumption or on the overall consumption of peroxodisulfate over the time period investigated.

Following a 24-hr oxidation under these conditions, the unoxidized carbohydrates are distributed as follows: free ribose, 20%; acid-hydrolyzable ribose (presumably a glycoside), 10%.

The molar ratio of urea produced per mole of substrate oxidized is less than 0.1 for both guanosine and deoxyguanosine. This value represents total urea in both cases and as such is a measure of both the free urea and the ureidoglycosides described as products of these reactions. No additional urea formation was observed following acid hydrolysis of the reaction products by the method employed for the detection of acid-hydrolyzable ribose. Since the total urea concentration is less than the acid-hydrolyzable ribose concentration, the amount of acid-hydrolyzable ribose cannot be an accurate measure of the ribosylurea content of the product mixtures using these analytical methods.

The molar ratio of guanidine produced per mole of both guanosine and deoxyguanosine oxidized approaches 1 over a 24-hr period.

EDTA and acrylamide had no effect on the extent of product formation in the peroxodisulfate oxidation of guanosine under these reaction conditions. The effect of EDTA and acrylamide on the peroxodisulfate oxidation of deoxyguanosine was not investigated.

Discussion

The kinetic dependencies, the lack of effect of radical traps, and the magnitude of the activation parameters all suggest that the peroxodisulfate oxidations described here, like those of the free bases,[2] do not involve any significant free-radical contribution.

A number of reactions of nucleic acid purines and pyrimidines which lead to ring opening have been investigated from the point of view of selectivity. Kochetkov and Budovskii[19] should be consulted for a general review of the chemistry involved and Shapiro[20] for the case of guanine. Potassium permanganate[21] and osmium tetroxide[22] are reasonably specific for thymine residues. *m*-Chloroperoxybenzoic acid,[23] while yielding *N*-oxides of cytosine and adenine in weakly acidic solution, gives ring cleavage of guanine, uracil, and thymine in the alkaline range. Hydrogen peroxide in alkaline solution selectively attacks uracil residues[24] as does hydrazine.[25] Hydroxylamine reacts selectively with cytosine residues at pH 6 and with uracil residues at pH 10.[26] The dye-sensitized photooxidation of guanine also results in a significant reaction with thymine.[27] Oxidation by peroxodisulfate appears to be among the most specific of these reactions. The results of our investigations on the peroxodisulfate oxidation of the common nucleosides in 1 *M* sodium carbonate solution show that guanosine and deoxyguanosine are at least 500 times more reactive than any other nucleoside. This specificity suggests that peroxodisulfate ions could be a useful reagent for the selective alteration of polynucleotides. For example, peroxodisulfate removal of guanine residues followed by base- or amine-catalyzed β-elimination[28] should lead to chain cleavage at guanine sites in a highly selective way.

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°.

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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