Synthesis and PMR Spectra of 7-Hydroxyalkylguanosinium Acetates

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Received May 3, 1973

Guanosine and 9-methylguanine treated with epoxides in glacial acetic acid are hydroxyalkylated stereoselectively at the N_7 position of the guanine moiety. Previously unreported 7-(hydroxyalkyl)guanosinium acetates from the reactions of six epoxides with guanosine and 7-(hydroxyalkyl)-9-methylguaninium acetates from the reactions of two epoxides with 9-methyl guanine in glacial acetic acid have been prepared and characterized by their pmr spectra. By using an excess of epoxide, quantitative conversion of guanosine or 9-methylguanine to the corresponding 7-hydroxyalkylguanosinium or 7-hydroxyalkyl-9-methylguaninium acetate was achieved. Comparisons of the pmr spectra of the 7-(hydroxyalkyl)guanosinium acetates in DMSO-d_6 to the spectrum of guanosine reveal that the H_8 and amino group proton absorptions common to guanosine are shifted to a lower field, the absorptions of the H_1 proton is absent, and the coupling constant of the H'_1-H'_2 protons of the ribosyl group is decreased from about 5.7 ± 0.1 Hz in guanosine to about 3.5 ± 0.1 Hz in the products. The use of the pmr spectral features of 7-(hydroxyalkyl)-9-methylguaninium compounds in characterizing 7-hydroxyalkylguanosinium compounds is discussed. Evidence is presented which suggests that extensive delocalization of positive charge exists in both the pyrimidine and imidazole rings of N_7-hydroxyalkylated guanosine and N_7-hydroxyalkylated-9-methylguanine. The possible effects of charge delocalization upon the hydrogen bonding potential of 7-hydroxyalkylated guanine moieties in DNA is discussed.

Introduction.

The reactions of alkylating agents such as the nitrogen and sulfur mustards, aziridines, epoxides, β-lactones, alkylhalides, and alkylsulfonates with nucleic acids and their component nucleotides and nucleosides have received considerable attention (1,2). The high nucleophilic reactivity of N_7 of the guanine moiety in these biologically important molecules has been noted (3). As a part of a continuing study by this laboratory of the initial sites of reaction and comparative reactivities of carcinogenic alkylating agents with the nucleotides and nucleosides of DNA and RNA, the N_7-hydroxyalkylation of guanosine (I) by epoxides (II) and (III) has been investigated.

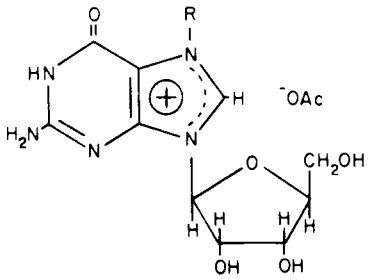
The N_7-hydroxyalkylation of guanosine in glacial acetic acid by 1,2-epoxyethane and 1,2,3,4-diepoxybutane has been described (4). However, the initially formed products from these reactions, which presumably were the corresponding 7-(hydroxyalkyl)guanosinium acetates (IVa and IVb), were not characterized. The products characterized were the 7-(hydroxyalkyl)guanines which resulted from the acid hydrolysis of the initially formed products. Some of the difficulties of isolating and characterizing the initially formed highly hydroscopic *N*-alkylated products of this type have been noted (5).

From a survey of the literature pertinent to the alkylation of guanosine it would appear that, other than some partial pmr spectral data for 7-methylguanosine (6), the pmr spectra of 7-alkylated guanines and 7-alkylated guanosines have not been reported. The low solubility of 7-alkylated guanines in nmr solvents makes it difficult to obtain useful pmr spectra of these compounds. Apparently, the fact that 7-alkylated guanosines and 7,9-disubstituted guanines are ionic compounds which are sufficiently soluble for obtaining useful single scan pmr spectra in nmr solvents such as deuterium oxide and DMSO-d_6 has not been considered. However, other workers have noted that the solubility of 7-methylguanosine in water is much greater than the solubility of guanosine (7).

The purpose of this report is to describe the preparation and pmr spectral characterization of the previously un-

TABLE I

7-(Hydroxyalkyl)guanosinium Acetates



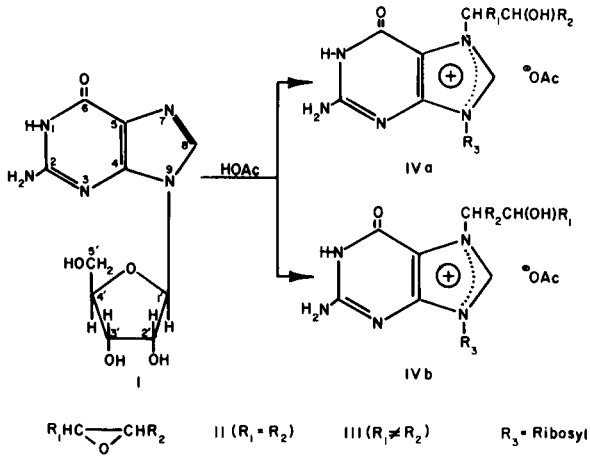
Compound	R	M.p. °C (a)	% Yield	Molecular Formula	Calcd., % C	H	N	Found, % C	H	N
VIII	CH_2CH_2OH	161-163	93	$C_{14}H_{21}N_5O_8$	43.41	5.46	18.08	43.31	5.54	18.04
XIa, XIb	$CH_2CH(OH)CH_3$, $CH(CH_3)CH_2OH$	101-105	88	$C_{15}H_{23}N_5O_8$	44.89	5.78	17.45	44.72	5.91	17.25
XII	$CH(CH_3)CH(OH)CH_3$	142-143	91	$C_{16}H_{25}N_5O_8$	46.26	6.07	16.86	45.92	6.41	16.67
XIV	$CH_2CH(OH)CH_2Cl$	128-131	94	$C_{15}H_{22}ClN_5O_8$	41.34	5.09	16.07	41.13	5.21	16.34
XV	$CH_2CH(OH)CH_2Br$	168-170	82	$C_{15}H_{22}BrN_5O_8$	37.51	4.62	14.58	37.35	4.41	14.36
XVI	$CH_2CH(OH)CH_2OH$	117-121	93	$C_{15}H_{23}N_5O_9$	43.17	5.55	16.78	43.19	5.30	16.72

(a) Indicates temperature range in which product appears to melt with the evolution of a vapor presumed to be glacial acetic acid. Products resolidified above the range given and gradually darkened with increasing temperature.

reported 7-(hydroxyalkyl)guanosinium acetates from the reactions of six epoxides with guanosine and the 7-(hydroxyalkyl)-9-methylguaninium acetates from the reaction of two epoxides with 9-methyl guanine in glacial acetic acid and to present evidence from the analyses of the pmr spectra of these compounds which indicates that the proton at the N_1 position of these products is involved in an ionization-exchange reaction at neutral *p*H.

Results and Discussion.

Guanosine (I) reacts with equally substituted epoxides (II, $R_1 = R_2$) and unequally substituted epoxides (III, $R_1 \neq R_2$) in glacial acetic acid to give 7-(hydroxyalkyl)-guanosinium acetates IVa and IVb. These products apparently result from attack of N_7 of guanosine at the ring carbons of the epoxides. Whereas the equally substituted epoxides react with guanosine to give only single geometric isomers of the 7-(hydroxyalkyl)guanosinium products, the unequally substituted epoxides may give mixtures of geometric isomers. However, it was found that unequally substituted epoxides reacted with guanosine to give, predominately, the isomer products resulting from attack of N_7 on the least substituted carbon of the epoxide ring.



Scheme 1

Products from the reactions of guanosine with 1,2-epoxyethane, 1,2-epoxypropane, 3-chloro-1,2-epoxypropane, 3-bromo-1,2-epoxypropane, 3-hydroxy-1,2-epoxypropane and *trans*-2,3-epoxybutane are indicated in Table I.

Analysis of the pmr spectra of the compounds in Table I revealed that only one of the unequally substituted epoxides, 1,2-epoxypropane, reacted with guanosine to give a detectable amount of the 7-(hydroxyalkyl)guanosinium acetate from reaction of N_7 of guanosine at the more highly substituted carbon of the epoxide ring. Analysis of the integrated areas of the H_8 and hydroxyalkyl group proton signals from the pmr spectrum of a mixture of IXa and IXb indicated a 7 to 1 ratio of isomer products. The limit of detection of isomer products by this method was estimated to be about one part in twenty parts. The other unequally substituted epoxides, 3-chloro-1,2-epoxypropane, 3-bromo-1,2-epoxypropane, and 3-hydroxy-1,2-epoxypropane, appear to give, within the limits of detection, only products resulting from reaction of N_7 of guanosine at the least substituted carbon of the epoxide ring.

The pmr spectra of 7-(hydroxyalkyl)guanosinium acetates in DMSO-d_6 are complicated by overlapping absorptions of the ribosyl and hydroxyalkyl group protons and by the methyl protons of the acetate anions. Spectra of these compounds were simplified by converting the acetates to the corresponding hydroxides. The assignment of proton absorptions was made by comparing the spectra of the compounds in Table I in DMSO-d_6 to the spectra of known compounds. The pmr spectra of the known compounds, guanosine (I), 7-methylguanosinium iodide (V), 7-methylguanosinium hydroxide (VI), and 7,9-dimethylguaninium iodide (VII), are shown in Figure 1; spectra of 7-(2-hydroxyethyl)guanosinium acetate (VIII), 7-(2-hydroxyethyl)guanosinium hydroxide (IX), and 7-(2-hydroxyethyl)-9-methylguaninium acetate (X) are shown in Figure 2; spectra of the 7-(hydroxyalkyl)guanosinium acetates from the reactions of 1,2-epoxypropane and *trans*-2,3-epoxybutane with guanosine, and the spectrum of the 7-(hydroxyalkyl)-9-methylguaninium acetate isomer mixture from the reaction of 1,2-epoxypropane with 9-methylguanine is shown in Figure 3.

An examination of the pmr spectra in Figure 1 reveals that the absorption of H_1 is present in the spectra of guanosine (I), 7-methylguanosinium iodide (V), and 7,9-dimethylguaninium iodide (VII). However, the H_1 absorption is absent in the spectrum of 7-methyl guanosinium hydroxide (VI). The chemical shifts of the protons at the 1,8 and of the amino group protons at position 2 of 7-methylguanosinium iodide (V) and 7,9-dimethylguaninium iodide (VII) are dramatically shifted downfield by about 0.8, 1.2, and 0.6 ppm respectively from their values in guanosine, the ring protons of the ribosyl group of V and VI are shifted by less than 0.1 ppm from their positions in guanosine, the hydroxyl group signals are collapsed into a broad singlet, and the coupling constant of the H'_1-H'_2 protons of the ribosyl group is decreased from about 5.7 ± 0.1 Hz in guanosine (I) to about 3.5 ± 0.1 Hz in 7-methylguanosinium iodide (V) and 7-methylguanosinium hydroxide (VI). In the pmr spectrum of 7-methylguanosinium hydroxide (VI), H_8 is shifted downfield by about 1.1 ppm, and the amino group protons are shifted downfield by about 0.04 ppm from their positions in guanosine. The absence of a signal for the H_1 proton in the spectra

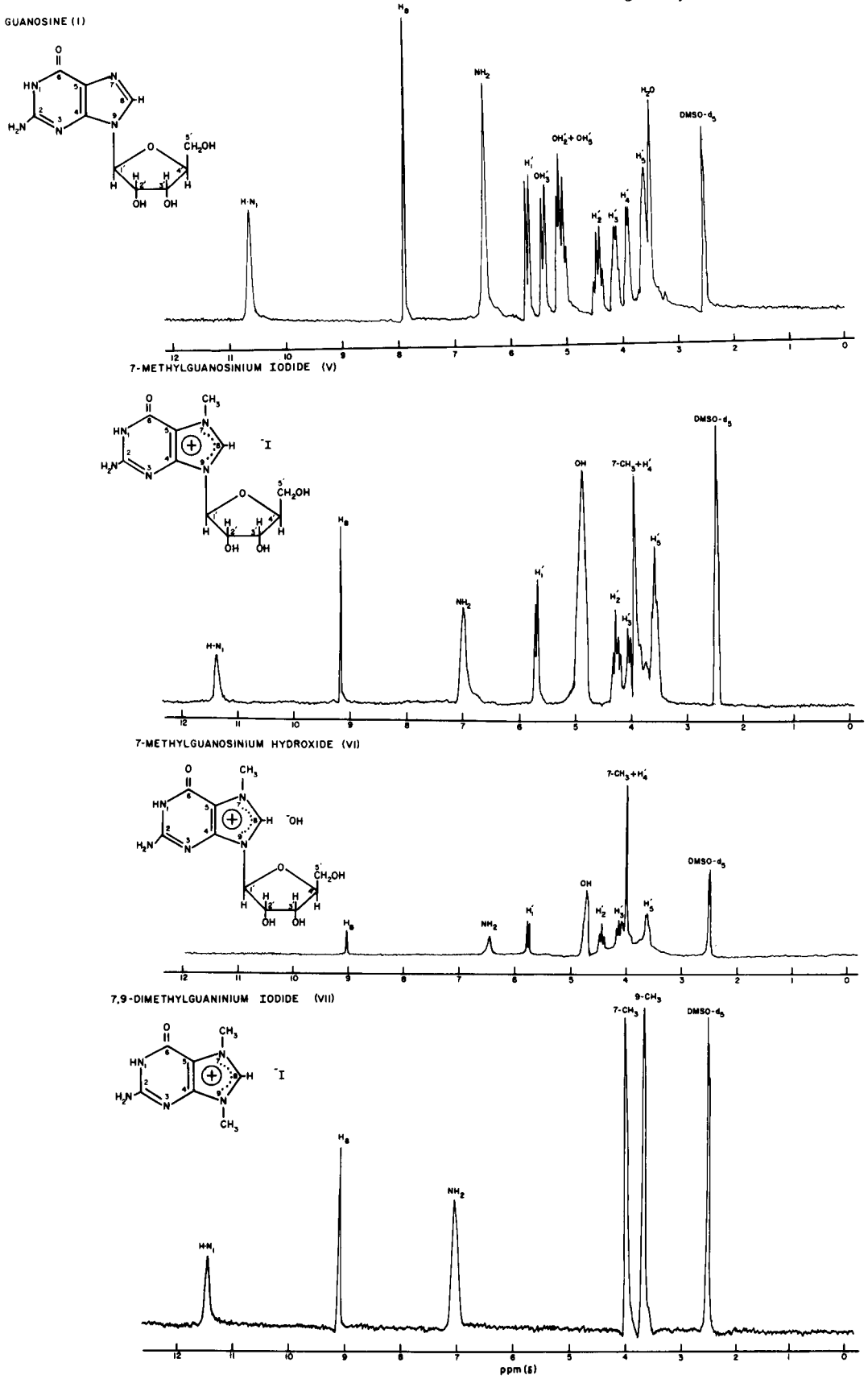


Figure 1. Pmr spectra of guanosine (I), 7-methylguanosinium iodide (V), 7-methylguanosinium hydroxide (VI), and 7,9-dimethylguaninium iodide (VII) in DMSO-d_6.

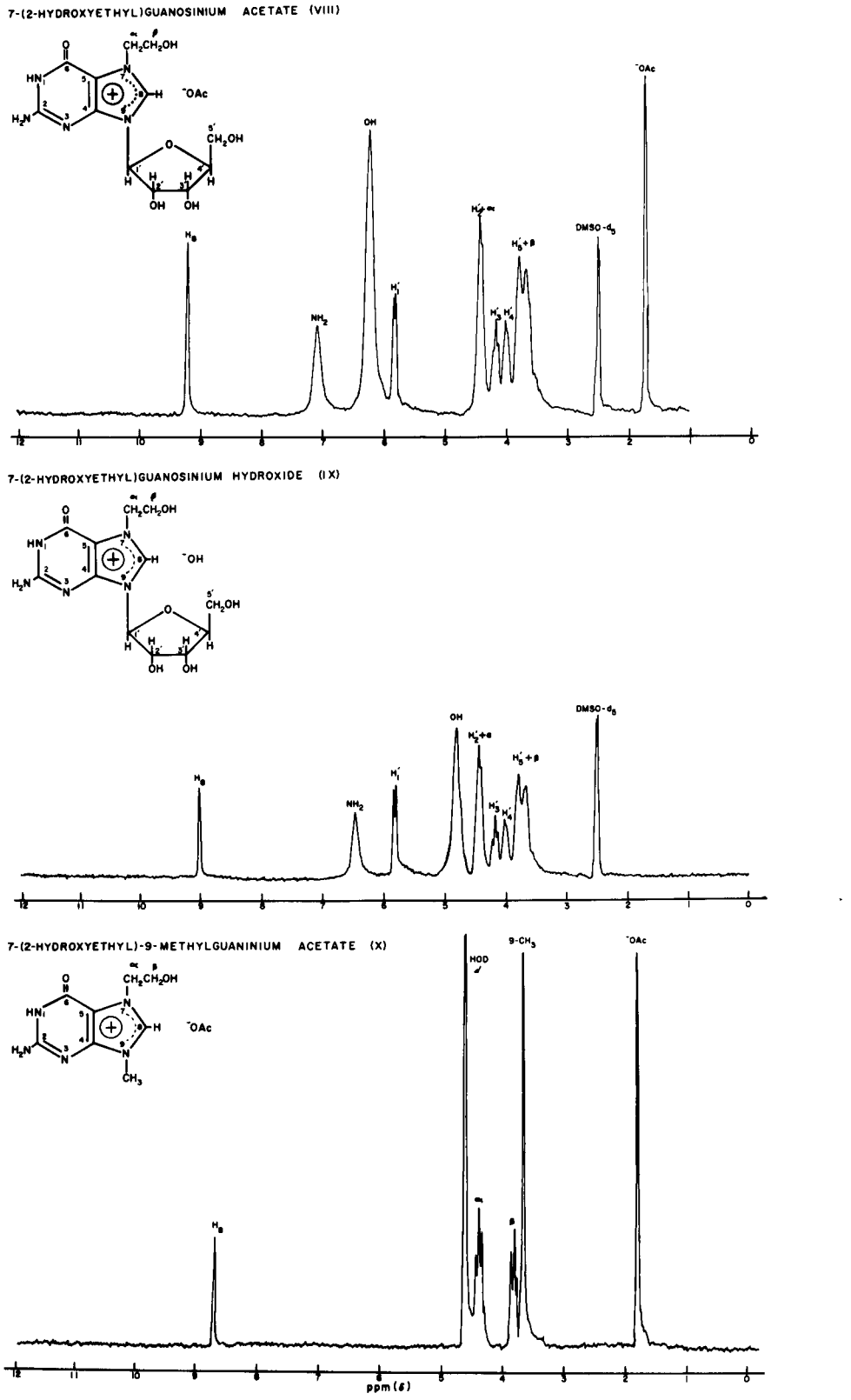
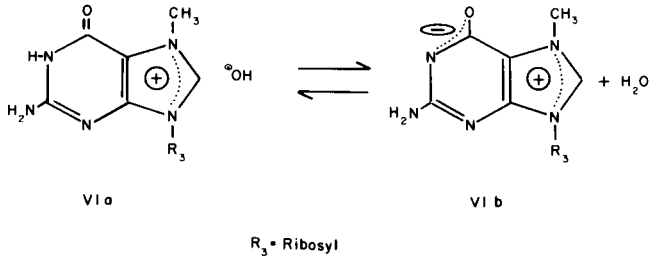


Figure 2. Pmr spectra of 7-(2-hydroxyethyl)guanosinium acetate (VIII), 7-(2-hydroxyethyl)guanosinium hydroxide (IX) and 7-(2-hydroxyethyl)-9-methylguaninium acetate (X) in DMSO-d_6.

of 7-methylguanosinium hydroxide (VI), and the differences in the chemical shifts of the H_8 and the amino group protons is taken as evidence for an exchange process associated with an equilibrium between structures VIa and VIb.



Scheme 2

Although the downfield shift in the H_8 absorption in the N_7-alkylated guanosines with respect to guanosine might have been expected as a consequence of the deshielding influence of the positive charge in the imidazole ring, the relatively large downfield shifts of H_1 and of the amino group protons at position 2 of the pyrimidine ring of the iodides V and VII were unexpected. Clearly, the electronic properties of the purine ring are markedly changed upon methylation at N_7. Extensive delocalization of the positive charge in the iodide derivatives could account for the relatively large downfield shifts of the H_1 and of the amino group protons. In the presence of a basic counter anion such as the hydroxide ion in VI an equilibrium between VIa and VIb would be expected. The removal of the H_1 proton of VIa could lead to stabilization of the positive charge. In VIb, H_8 and the amino group protons would be less deshielded than in VIa. Presumably, the decrease in the H'_1-H'_2 coupling constant upon the N_7-methylation of guanosine is associated with either the effect of the positive charge in the imidazole ring or in a change in the orientation of the purine ring with respect to the ribosyl group due to rotation about the N_9-C_1' bond. It has been recently suggested on the basis of nmr studies of N_7-protonated 5'-guanosine monophosphate that the equilibrium ratio of the syn to the anti conformers increases with a lowering of the pD (8).

An examination of the pmr spectrum of 7-(2-hydroxyethyl)guanosinium acetate (VIII) in Figure 2 reveals that the absorptions of H_8, the amino group protons at position 2 and H'_1 have essentially the same chemical shift values as the corresponding protons of 7-methylguanosinium iodide (V) in Figure 1. However, the absorption of H_1 is absent in the spectrum of VIII. Presumably, the absence of an H_1 signal in VIII is due to a rapid exchange involving the H_1 proton and the acetate counter ion. The spectrum of 7-(2-hydroxyethyl)guanosinium hydroxide (IX) resembles the spectrum of 7-methylguanosinium hydroxide (VI). The absorption of the methylene protons of IX were assigned as indicated in Figure 2 on the basis of peak area integrations and comparisons of the spectrum of IX with the spectra of guanosine (I), 7-methylguanosinium iodide (V), and 7-(2-hydroxyethyl)-9-methylguaninium acetate (X). These assignments are consistent with the expectation that the methylene protons nearest N_7 in IX would occur at a lower field than the methyl protons of V. Integration of peak areas and the presence of sharp singlets for the H_8 protons in the spectra of VIII and X indicate that the N_7-hydroxyalkylation of guanosine and of 9-methylguanine are highly stereoselective processes. The spectrum of X was taken immediately after the deuterium oxide solution was prepared. The H_8 signal in the spectrum of X in deuterium oxide was observed to diminish with time and to be completely absent after 12 hours of contact with deuterium oxide at ambient temperature; the H_1, amino group and hydroxyl protons were exchanged immediately.

Spectra of the products of the reactions of 1,2-epoxypropane and *trans*-2,3-epoxybutane with guanosine (XIa, XIb, and XII) and of the reaction of 1,2-epoxypropane with 9-methylguanine (XIIIa and XIIIb) in glacial acetic acid are shown in Figure 3. Since *trans*-2,3-epoxybutane is equally substituted, only one N_7-hydroxyalkylated product is possible; however, 1,2-epoxypropane would be expected to give two N_7-hydroxyalkylated products.

By comparing the pmr spectra of XIa and XIb with XII and XIIIa and XIIIb the proton absorption in Figure 3 were assigned. Integration of the H_8 and methyl signals in the spectrum of XIa and XIb indicates an isomer distribution of about seven to one; on the basis of the chemical shifts of the methyl groups in the spectra of these products and stereochemical considerations, the major product from the reaction of the unequally substituted epoxide, 1,2-epoxypropane with guanosine, is identified as the product resulting from reaction of the least substituted carbon of 1,2-epoxypropane with N_7 of guanosine. The signal associated with the H_1 proton is not observed due to the chemical exchange. Integration of the signals in the spectrum of the products from the reaction of 1,2-epoxypropane with 9-methylguanine in glacial acetic acid indicates an identical (seven to one) distribution of isomer products as was obtained in the reaction of 1,2-epoxypropane with guanosine.

Analysis of the pmr spectra of products from the reactions of guanosine with 3-chloro-1,2-epoxypropane (XIV), 3-bromo-1,2-epoxypropane (XV) and 3-hydroxy-1,2-epoxypropane indicated that within the limits of

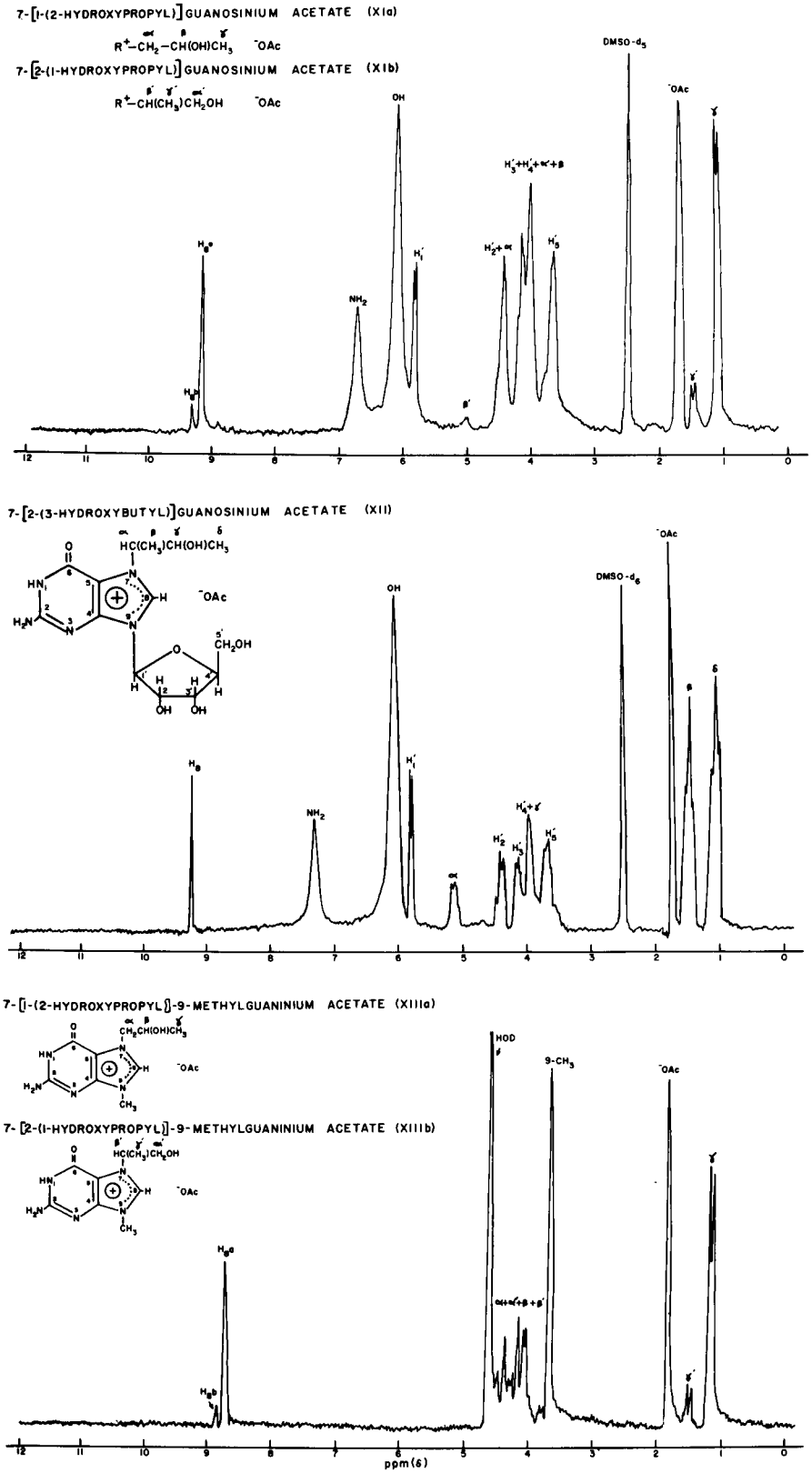


Figure 3. Pmr spectra of a mixture of 7-[1-(2-hydroxypropyl)]guanosinium acetate and 7-[2-(1-hydroxypropyl)]-guanosinium acetate isomers (XIa and XIb) in a ratio of about 7 to 1 respectively, 7-[2-(3-hydroxybutyl)]guanosinium acetate (XII) and a mixture of 7-[1-(2-hydroxypropyl)]-9-methylguaninium acetate and 7-[2-(1-hydroxypropyl)]-9-methylguaninium acetate isomers (XIIIa and XIIIb) in a ratio of about 7 to 1 in DMSO-d_6.

detection only single 7-hydroxyalkyl guanosinium acetates arising from reaction of N_7 of guanosine with the least substituted carbon of the epoxide rings were formed. This result was unexpected since these epoxides are unequally substituted and would be expected to give isomer products. Presumably, the combination of steric and electronic factors make the reactions of guanosine with these unsymmetrically substituted epoxides highly stereoselective at the least substituted carbon of the epoxide ring. This finding is consistent with the results of studies of acid catalyzed ring opening reactions of 1,2-epoxypropane with 3-chloro-1,2-epoxypropane and 3-hydroxy-1,2-epoxypropane (9).

Watson and Crick (10) have proposed a mechanism whereby changes in the tautomeric structures of the purine and pyrimidine bases of DNA could result in modification of base pairing which could lead to errors in DNA replications. Lawley and Brookes (11) have examined the uv spectra of 7,9-disubstituted guanines and 7-methylguanosine and have presented experimental evidence supporting the view that the modification of the base pairing of guanine to cytosine would be expected when the guanine moiety is alkylated at the N_7 position. By comparing the uv spectral changes with *p*H of solutions of 7,9-disubstituted guanines to 7- and 9-monosubstituted guanines, evidence was obtained indicating that the proton at the N_1-position of the disubstituted guanines was ionized to a much greater extent than for 9-monosubstituted guanines. The pK_a associated with the ionization of the N_1 proton is about 7.3 ± 0.1 *p*H units in 7,9-disubstituted guanines as compared to a value of about 9.2 *p*H units for this proton in guanosine. These results indicate that the H_1 proton of 7,9-disubstituted guanines ionizes readily at neutral *p*H and that these compounds exist as equilibrium mixtures of the associated (VIa) and disassociated betaine (VIb) forms in neutral aqueous solution. On the basis of this evidence, the hypothesis was made that the effect of alkylation of DNA at physiological *p*H would be the ionization of the H_1 proton with subsequent weakening of the hydrogen bonding between the guanine and cytosine bases. The ionized form of the 7-alkylated guanine moiety would then resemble the adenine moiety with respect to its potential for hydrogen bonding. Since adenine pairs with thymine, the net result in DNA replication would be the change from a guanine to an adenine moiety in one strand of DNA and a change of a cytosine to a thymine moiety on the complementary strand. The modified strands could then serve as templates during replication of modified DNA (12).

The results of the present study are among the first experimental findings obtained by application of nmr techniques which demonstrate that the tendency of the H_1 proton of the guanine moiety to ionize is increased upon alkylation at the N_7 position.

EXPERIMENTAL

Proton nmr spectra were recorded on a Jeolco PS-100 nmr spectrometer. Solutions contained 10% or less by weight of the 7-hydroxyalkyl guanosine derivatives in DMSO-d_6 or in deuterium oxide. Chemical shifts are in δ units, ppm. Guanosine, epoxides, 7-methylguanosinium hydroxide, and common laboratory reagents were obtained from commercial sources. The epoxides were freshly distilled prior to use. 7-Methylguanosinium iodide and 7,9-dimethylguaninium iodide were prepared by the methylation of guanosine and 9-methylguanine according to a published procedure (7), 9-methylguanine was prepared by a reported procedure (13). Melting point ranges were obtained with a Mettler FP-1 melting point apparatus. Elemental analyses were provided by Atlantic Microlab, Inc., Atlanta, Georgia.

General Procedure for N_7-Hydroxyalkylations of Guanosine by Epoxides.

In a 100 ml. three necked flask equipped with a thermometer and an addition funnel was placed 50 ml. of glacial acetic acid and 0.1 mole of the epoxide. The acetic acid-epoxide solution was stirred and heated in a water bath to 50-55° by a heating type magnetic stirrer. To the stirred solution, 2.8 g. (0.01 mole) of guanosine was added portionwise to the reaction mixture over a 1 to 3 hour period depending upon the reactivity of the epoxide. Upon the addition of guanosine, the glacial acetic acid solution became turbid; the turbidity faded to give a clear solution as the reaction progressed. After the last addition of the guanosine, stirring and heating were continued until the reaction was complete. Additional epoxide was added to shorten reaction times of the less reactive epoxides. Progress of the reaction was followed by tlc analysis of portions of the reaction mixture. The 7-hydroxyalkyl guanosinium products and unreacted guanosine were detected on Mallinckrodt Chromar 7GF precoated tlc plates by uv light (2730Å). Distilled water was used as the developing solvent. Reaction products gave a blue fluorescence, whereas the unreacted guanosine showed a dark absorption under uv light. The R_f values of the 7-hydroxyalkylguanosinium products were lower than for guanosine (R_f = 0.85 ± 0.03). Upon completion of the reaction as evidence by the absence of unreacted guanosine, approximately 200 ml. anhydrous acetone was added to the reaction mixture until the reaction mixture became turbid. Anhydrous diethyl ether (800 ml.) was added to the acetone solution to cause precipitation of the 7-hydroxyalkyl guanosinium acetate. The supernatant ether solution was decanted from the solid reaction product. An additional 200 ml. of anhydrous ether was added to the products which was then filtered under a nitrogen atmosphere. The solid was washed with ether to remove excess epoxide, glacial acetic acid and the hydroxyacetate ester byproduct. The 7-(hydroxyalkyl)guanosinium acetate products were dried at room temperature under reduced pressure.

Conversion of Acetates to Hydroxides.

7-Hydroxyalkylguanosinium acetates were converted to the corresponding hydroxides by repeated dissolving of the acetates in water to obtain saturated solutions which were added to an equal volume mixture of anhydrous acetone and ether to precipitate the hydroxides. This procedure was repeated from four to six times to effect the complete conversion of the acetates to the hydroxides. The hydroxides were isolated and dried as

described in the procedure for obtaining the acetates. The absence of acetate methyl proton absorptions in the pmr spectra was taken as evidence of complete conversion of the acetates to the hydroxides. Additional evidence for the complete conversion of the acetates to the hydroxides was provided by agreement of the elemental composition of the hydroxides.

2-(Hydroxyethyl)guanosinium Acetate (VIII).

Gaseous 1,2-epoxyethane was bubbled into a slurry containing 100 ml. of glacial acetic acid and 0.01 mole of guanosine at room temperature until the solution cleared. Completeness of reaction was checked by tlc. Additional 1,2-epoxyethane was bubbled into the reaction mixture until no unreacted guanosine could be detected. The product VIII was isolated as described in the general procedure; pmr (DMSO-d_6): δ 1.76 (s, 3H), 3.7 (m, 4H), 4.00 (m, 1H), 4.18 (m, 1H), 4.41 (m, 3H), 5.81 (d, 1H, J, 3.5 Hz), 6.20 (m, 4H), 7.07 (s, 2H), 9.18 (s, 1H).

2-(Hydroxyethyl)guanosinium Hydroxide (IX).

Compound VIII was converted to the corresponding hydroxide IX as previously described: m.p. 97-99°; pmr (DMSO-d_6): δ 3.7 (m, 4H), 4.00 (m, 1H), 4.16 (m, 1H), 4.4 (m, 3H), 5.79 (d, 1H, J, 3.5 Hz), 6.45 (s, 2H), 9.01 (s, 1H).

Anal. Calcd. for $C_{12}H_{19}N_5O_7$: C, 41.74; H, 5.55; N, 20.28. Found: C, 41.99; H, 5.70; N, 20.42.

7-(2-Hydroxyethyl)-9-methylguaninium Acetate (X).

1,2-Epoxyethane gas was bubbled into a slurry containing 0.50 g. (0.0003 mole) of 9-methylguanine and 30 ml. of glacial acetic acid as previously described. The yield of product X was 0.76 g. (93%), m.p. 160-163°; pmr (deuterium oxide): δ 1.80 (s, 3H), 3.64 (s, 3H), 3.81 (t, 2H), 4.39 (t, 2H), 8.68 (s, 1H).

Anal. Calcd. for $C_{10}H_{15}N_5O_4$: C, 44.63; H, 5.57; N, 26.02. Found: C, 44.37; H, 5.62; N, 25.84.

7-[1-(2-Hydroxypropyl)]-9-methylguaninium Acetate (XIIIa) and 7-[2-(1-Hydroxypropyl)]-9-methylguaninium Acetate (XIIIb).

A mixture of XIIIa and XIIIb was obtained by the addition of 5 ml. (0.071 mole) of 1,2-epoxypropane to a slurry containing 0.50 g. (0.003 mole) of 9-methylguanine in 30 ml. of glacial acetic acid. The yield of XIIIa and XIIIb isolated as described in the general proceudre was 0.77 g. (89.7%) m.p. $> 238°$ dec. The pmr spectrum of a mixture of XIIIa and XIIIb in deuterium oxide is shown in Figure 3.

Anal. Calcd. for $C_{11}H_{17}N_5O_4$: C, 46.64; H, 6.05; N, 24.72. Found: C, 46.35; H, 6.15; N, 24.77.

7-[1-(2-Hydroxy-3-chloropropyl)]guanosinium Acetate (XIV).

Compound XIV was prepared and isolated as described in the general procedure; pmr (DMSO-d_6): δ 1.81 (s, 3H), 3.72 (m, 4H), 4.02 (m, 1H), 4.18 (m, 2H), 4.4 (m, 3H), 5.78 (d, 1H, J, 3.5 Hz), 6.5 (s, 4H), 7.42 (s, 2H), 9.23 (s, 1H).

7-[1-(2-Hydroxy-3-bromopropyl)]guanosinium Acetate (XV).

Compound XV was prepared and isolated as described in the general procedure; pmr (DMSO-d_6): δ 1.80 (s, 3H), 3.64 (m, 4H), 3.99 (m, 1H), 4.17 (m, 2H), 4.4 (m, 3H), 5.79 (d, 1H, J, 3.5 Hz), 6.5 (s, 4H), 7.43 (s, 2H), 9.21 (s, 1H).

7-[1-(2,3-Dihydroxylpropyl)]guanosinium Acetate (XVI).

Compound XVI was prepared and isolated as described in the general procedure; pmr (DMSO-d_6): δ 1.78 (s, 3H), 3.26 (m, 2H), 3.6 (m, 2H), 3.9 (m, 1H), 4.1 (m, 1H), 4.4 (m, 3H), 5.81 (d, 1H, J, 3.5 Hz), 6.2 (s, 5H), 7.3 (s, 2H), 9.18 (s, 1H).

Acknowledgements.

This investigation was supported in part by National Institutes of Health Grant CA11479-04, Damon Runyon Memorial Fund for Cancer Research DRG-355-0, the Southwestern Medical Foundation and National Institutes of Health Career Development Award (JSP) CA-23087-05 and the Robert A. Welch Foundation.

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