Characterization of the Products of Alkylation of 2′-Deoxyadenosine and 2′-Deoxyguanosine by Chloroethyl Ethyl Sulfide

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Alkylation of 2′-deoxyadenosine by 2-chloroethyl ethyl sulfide (CEES) in aqueous solutions at pH 6.0 and 25 °C led to two products. These have been isolated and characterized on the basis of mass spectrometry, nuclear magnetic resonance spectroscopy, and ultraviolet spectroscopy as 2′-deoxy-1-[2-(ethylthio)ethyl]adenosine and 2′-deoxy-N^6-[2-(ethylthio)ethyl]adenosine. The products formed from 2′-deoxyguanosine under these same conditions were identified as 2′-deoxy-7-[2-(ethylthio)ethyl]guanosine and 2′-deoxy-N^2-[2-(ethylthio)ethyl]guanosine, and the corresponding pair of deribosylated alkylated purines.

The mutagenic activity of mustard gas first implicated DNA as an important site of biological alkylation in 1946.[1] Subsequently, other alkylating agents also have been studied in an attempt to relate the chemical nature of DNA alkylation to the observed biological changes.[2,3] While cross-linking of DNA strands has been implicated as an important reaction for bifunctional agents such as mustard gas [bis(2-chloroethyl) sulfide],[4] different mechanisms have been proposed for compounds capable of only single site attack.

2-Chloroethyl ethyl sulfide is a monofunctional alkylating agent capable of both mutagenic and lethal effects in *E. coli*.[5] These different physiologic effects may reflect different molecular changes in the DNA and thus precise definition of the latter is important. Studies on other sulfur mustards have shown predominant alkylation of guanine and adenine bases in nucleic acids[16] and thus these bases have been emphasized in the present studies.

Another reason for study of CEES reactions is that it is a relatively large molecule which might be expected to participate in chemical reactions differing from those of the simpler, well-studied, methylating mutagens.[3,6-8] Identification of CEES adducts in DNA may provide a useful extension of earlier biological studies[5] as well as models for the alkylation reactions of bifunctional mustard gas. Some of the adducts formed may be short lived in vitro and this may be related to temporal changes in vivo.

Although acid hydrolysis of DNA and RNA has been employed in many of the earlier studies of alkylation of nucleic acids, it is now understood that some alkylation products will be destroyed by such conditions. Thus nucleoside adducts were chosen for characterization as reference compounds, since they can be released from polymers by enzymatic procedures instead of chemical hydrolysis (i.e., nucleoside monophosphates produced by deoxyribonuclease and nucleosides obtainable by subsequent digestion with alkaline phosphatase). Historically, nonaqueous solvents often have been used in alkylation studies of this kind to enhance yields, and pH values are sometimes varied. However, the more physiologically relevant aqueous medium was used in this study and the pH was held near the physiologic value. The structural studies reported here rely on spectroscopic techniques consistent with the limited amount of sample available.

Materials and Methods

2′-Deoxyadenosine (dA) and 2′-deoxyguanosine (dG) were obtained from Sigma Chemical Co. 2-Chloroethyl ethyl sulfide (CEES) was obtained from Chemical Procurement Laboratories. Sephadex G-10 was obtained from Pharmacia Fine Chemicals.

Nucleoside Alkylation. Solutions of deoxyadenosine (10 mg/mL) or deoxyguanosine (4 mg/mL) were prepared in deionized water at room temperature. Fifteen milliliters of the nucleoside solution and 40 μL of 2-chloroethyl ethyl sulfide were mixed at room temperature. The pH of the mixture was maintained at 6.0 while the reaction was shaken for about 1 hr. Little or no subsequent alkylation could be observed.

The reaction mixture was then applied to an 80 × 2.8 cm column of Sephadex G-10 and eluted with 50 mM ammonium formate, pH 6.5 at 27 °C. Twenty milliliter fractions were collected at a flow rate of 80 mL/h. Ultraviolet absorption of the column effluent was continuously measured with an LKB UV recorder set at a wavelength of 254 nm. Collection tubes were pooled for each component in the effluent and subjected to prolonged lyophilization. This resulted in sublimation of both water and ammonium formate from the sample, leaving the purified reaction products.

Ultraviolet Spectroscopy. Ultraviolet spectra were recorded using a Cary Model 14 recording spectrophotometer and a 1 cm light path. Spectra were measured at pH 7, 1, and 11.

Mass Spectrometry. Electron impact mass spectra of trimethylsilylated samples[9,10] were recorded on a DuPont 21-110 instrument with ion source temperature 200 °C and ionizing voltage 70 eV. The chemical ionization mass spectra were obtained on a DuPont 491 instrument, using isobutane reagent gas at 200 °C.

Field desorption mass spectra were measured on a Varian CH 5 DF instrument operating under control of an Incos Model 2000 data system at nominal resolution of 1500. The magnet was scanned quadratically from *m/e* 900 to *m/e* 10 in 20 s.

Nuclear Magnetic Resonance. Ambient ^{1}H NMR spectra were recorded on Varian HA-100, JEOL-FX-100, and Varian HR-220 MHz NMR spectrometers equipped with fast Fourier transform units. Traces of *tert*-butyl alcohol and tetramethylsilane were added to the D_2O and Me_2SO-d_6 solutions, respectively, as internal standards. Up to 10 000 pulses were accumulated for each sample, using 8000 point data files. The guanine adduct, dGV, was dissolved in D_2O at pH 1.4. Otherwise, solutions were neutral. Deoxyguanine adducts were difficult to solubilize, hence NMR signals were not consistently strong.

Characterization of the Adducts

Alkylation of 2′-Deoxyadenosine. The profile of ultraviolet chromophores eluted from the Sephadex G-10 column is shown in Figure 1. Three compounds were detected, which are labeled dAI, dAII, and dAIII. On standing, product dAI

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Table I. The Chemical Shift Values of ^1H NMR Resonances of Deoxyadenosine and Deoxyguanosine Products in D_2O and Me_2SO-d_6

	dAI		dAIII		dGI		dGV	
	Me_2SO-d_6	D_2O	Me_2SO-d_6	D_2O	Me_2SO-d_6	D_2O	Me_2SO-d_6	D_2O
CH_3CH_2	1.15[a] (t,[b] J = 7.2[c])	1.18 (t, J = 7.2)	1.10 (t, J = 7.2)	1.15 (t, J = 7.2)	1.15 (t, J = 7.5)	1.21 (t, J = 7.3)	1.15 (t, J = 7.2)	1.16 (t, J = 7.2)
CH_3**CH_2**	2.53 (q, J = 7.2)	2.57 (q, J = 7.2)	~2.50	2.54 (q, J = 7.2)	*d*	2.51 (q, J = 7.1)	*d*	2.50 (q, J = 7.2)
-**SCH_2**CH_2-	2.85 (t, J = 6.5)	3.08 (t, J = 6.2)	3.05 (t, J = 6.5)	3.14 (t, J = 6.5)	2.99 (t, J = 6.5)	3.09 (t, J = 6.1)	2.92 (t, J = 6.5)	3.02 (t, J = 6.5)
-CH_2**CH_2**N-	4.14 (t, J = 6.5)	4.54 (t, J = 6.4)	4.44 (t, J = 6.4)	4.70 (t, J = 6.5)	4.29 (t, J = 6.5)	*d*	4.03 (t, J = 6.5)	4.53 (t, J = 6.5)
H-C-1′	6.18 (pt, J = 6.6, 6.6)	6.52 (pt, J = 6.6, 6.6)	~6.46	*d*	*d*	6.42 (t, J = 5.6)		
H_2-C-2′	~2.30 (m)	~2.76 (m)	*d*	*d*	*d*	*d*		
H-C-3′	4.33 (m)	4.7 (m)	*d*	*d*	*d*	*d*		
H-C-4′	3.81 (m)	~4.18 (m)	*d*	*d*	*d*	*d*		
H_2-C-5′	3.50 (m)	3.80 (m)	*d*	*d*	*d*	*d*		
H-C-2 (dA) or H_2-N-2 (dG)	8.06 (s)	8.48 (s)	~7.9 (s)	*d*	6.19 (s)		6.06 (s)	
H-C-8	8.12 (s)	8.52 (s)	~8.34 (s)	*d*	7.88 (s)		7.85 (s)	8.62 (s)

[a] All chemical shifts values are in ppm from Me_3Si in Me_2SO-d_6 or from DSS in D_2O. [b] Abbreviation used as: t = triplet, q = quartet, pt = pseudotriplet, m = multiplet, s = singlet. [c] Coupling constant, in Hz. [d] These resonances are either too broad or too weak, or shaded by other peaks due to impurities, limited sample solubility, or limited sample.

was observed to be converted slowly to dAIII.

All three compounds were converted to trimethylsilyl derivatives whose electron impact mass spectra were measured. The mass spectrum of trimethylsilylated dAII was identical to that of the trimethylsilyl derivative of the starting material, deoxyadenosine, with a molecular ion peak at *m/e* 467. The spectra of the derivatives of both dAI and dAIII contain molecular ion peaks at *m/e* 555, an increment of 88 mass units relative to the derivatized starting material. This molecular weight is consistent with the presence of three trimethylsilyl groups and one ethylthioethylene moiety on each nucleoside molecule. The presence of three sites for silylation on both of the product molecules provides evidence that alkylation has occurred on the base, rather than on the sugar moiety. This is further confirmed by electron impact induced cleavage of bis(trimethylsilyl)deoxyribose, leading to a B + H peak[9] at *m/e* 295. Alkylation at N-7 leading to a quaternary ammonium center is also ruled out, since presence of the latter structure is known to cause oxidation at position 8 during the derivation reaction.[11] The masses of molecular ions and fragment ions observed exclude 8-oxo or ring-opened analogues. Both M-15 and M-88 peaks are present (*m/e* 540 and 467), corresponding to loss of CH_3 and C_4H_8S from the molecular ion. The base peak in each spectrum occurs at *m/e* 207 and represents ions formed by the loss of both the bis(trimethylsilyl)deoxyribose moiety (accompanied by transfer of a hydrogen atom) and the ethylthioethylene moiety (accompanied by hydrogen transfer). A number of other peaks in the spectrum, e.g., *m/e* 217, are characteristic of trimethylsilylated deoxyribose.[9]

Although generally similar, the two spectra are distinguishable by two interesting peaks at *m/e* 480 and 220, which are both prominent in the spectrum of dAIII. These fragment ions arise by cleavage of $C_2H_5SCH_2$ from the alkyl side chain, the former from the molecular ion and the latter accompanying the loss of silylated deoxyribose with hydrogen transfer. This α cleavage is associated with the structure alkylated at N^6 (*vide infra*) and presumably reflects the influence of the charged exocyclic (aliphatic) nitrogen as compared to the highly conjugated N-1 site.

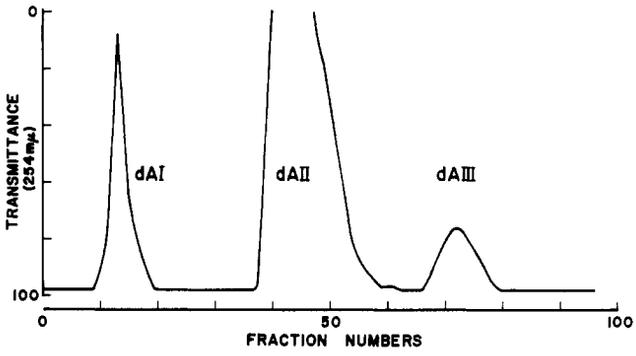


Figure 1. Chromophoric profile of the product mixture from the alkylation of deoxyadenosine.

The molecular weight of dAI was confirmed by observation of an M + 1 base peak at *m/e* 556 in the chemical ionization mass spectrum of the trimethylsilyl derivative and of an M + 1 base peak at *m/e* 340 in the field desorption spectrum of the underivatized adduct.

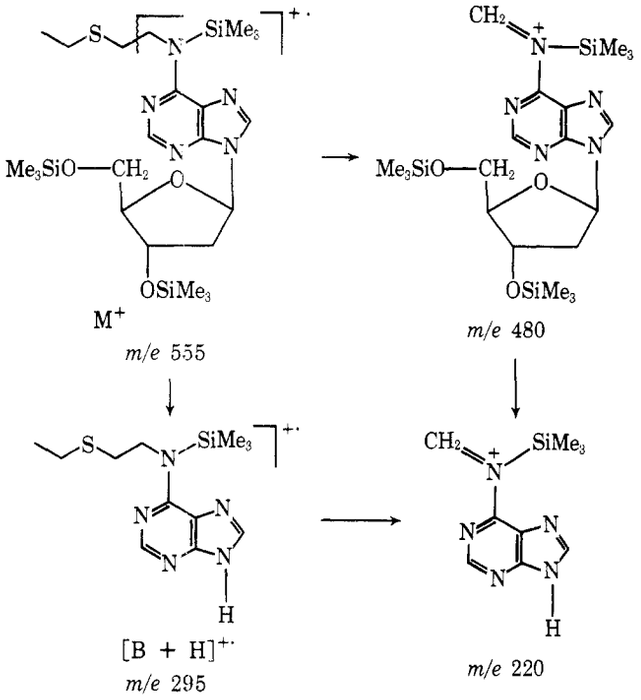
The chemical shift values (in ppm) of the proton resonances of compounds dAI and dAIII in D_2O and Me_2SO-d_6 are listed in Table I. The NMR spectra of both adducts contain four more signals than the spectrum of the starting material, 2′-deoxyadenosine. These occur as triplets at 1.15, 2.85, and 4.14 ppm and a quartet centered at 2.53 ppm (half of which is buried in the Me_2SO-d_6 peak from the solvent). Two triplets and the quartet integrate to about two protons, while the third triplet represents three protons. The position and size of these signals suggests that both dAI and dAIII contain one -CH_2CH_2- group and one CH_2CH_3 group and that one ethylthioethylene moiety has been added to each nucleoside by the alkylation reaction. No direct evidence could be obtained for the sites of alkylation. However, alkylation at N-7 or C-8 is excluded by the persistence of H-8 resonance in D_2O.[12]

The site of alkylation on the purine base was assigned primarily by ultraviolet spectroscopy. Table II shows the ultraviolet spectra of the three compounds isolated from the re-

Table II. Maximum Absorption (nm) in Ultraviolet Spectra of Products[a]

compd	registry no.	pH 7	pH 1	pH 11
2′-deoxy-1-[2-(ethylthio)ethyl]adenosine (dAI)	66792-45-8	260	260	260 (268)
2′-deoxyadenosine (dAII)	958-09-8	259	257	259
2′-deoxy-N^6-[2-(ethylthio)ethyl]adenosine (dAIII)	66792-46-9	262	263	268
2′-deoxy-7-[2-(ethylthio)ethyl]guanosine (dGI)	66901-78-8	250, 284	253 (280)	267
2′-deoxyguanosine (dGII)	961-07-9	255 (270)	255	258–268
2′-deoxy-N^2-[2-(ethylthio)ethyl]guanosine (dGIII)	66792-47-0	253.5 (280)	257 (285)	258 (274)
N^2-[2-(ethylthio)ethyl]guanine (dGIV)	66792-48-1	247, 278	251, 280	274
7-[2-(ethylthio)ethyl]guanine (dGV)	693-07-2	283, 245	250, 276	280, 240

[a] Values for reference compounds[8,13,14,19,29–31] removed at referee's request.



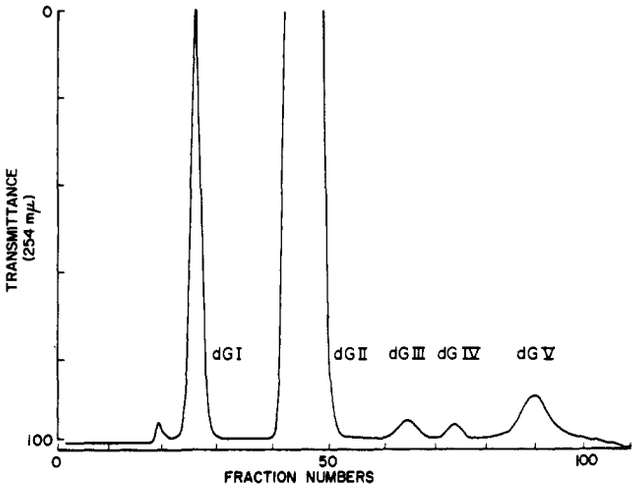


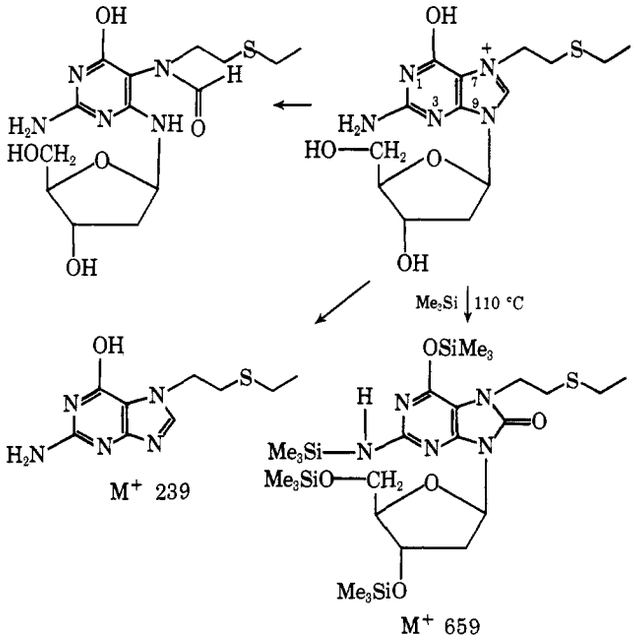
Figure 2. Chromophoric profile of the product mixture from the alkylation of deoxyguanosine.

action of deoxyadenosine with chloroethyl ethyl sulfide and references to published values for isomeric monomethylated and monoethylated deoxyadenosine and adenosine. pK_A values and corresponding ultraviolet absorption spectra for alkylated purine nucleosides provide no information about the nature of nonchromophoric substituents but rather reflect primarily the site of alkylation on the purine.[14,19,20] On the basis of the values in Table II, product dAI was assigned as 2′-deoxy-1-[2-(ethylthio)ethyl]adenosine and product dAIII was assigned as 2′-deoxy-N^6-[2-(ethylthio)ethyl]adenosine. Compound dAII is unreacted deoxyadenosine. The observed spontaneous conversion of dAI to dAIII further supports these assignments, since isomerization of N-1 alkylated adenosine to N^6-alkylated adenosine is well documented.[15]

Alkylation of 2′-Deoxyguanosine. The profile of ultraviolet chromophores eluted from the Sephadex G-10 column is shown in Figure 2. The five compounds labeled dGI through V were collected and characterized. The material in peak dGI was spontaneously converted to that in peak dGV on standing for several days.

Peak dGII was found to be unreacted starting material, deoxyguanosine, based on its UV spectra (Table II), the mass spectrum of its tetrakis(trimethylsilyl) derivative (M^+ 555), and the elution from the column coincident with authentic material.

Peak dGI was characterized by UV and NMR spectroscopy and by several kinds of mass spectrometry. The UV spectra obtained at pH 1, 7, and 11 (Table II) suggest that the dGI adduct is a 7-alkylated deoxyguanosine. Field desorption spectra measured on an underivatized dGI sample did not contain peaks corresponding to nucleoside molecular ions, rather a mono(ethylthioethyl)guanine was identified, with a base peak at *m/e* 239. An electron impact mass spectrum of the trimethylsilyl derivative of dGI contained a molecular ion peak at *m/e* 659, accompanied by an M − 15 peak at *m/e* 644. This is consistent with the structure shown in the scheme, in which the alkylated nucleoside carries four trimethylsilyl groups and an 8-oxo group. Such oxidation is well documented at C-8 in trimethylsilylation of 7-alkylated guanosine[10,11] and confirms N-7 as the site of alkylation. In addition to the ex-



pected B + H peak at *m/e* 399, intense peaks at *m/e* 383 and 368 confirm the presence of (ethylthioethyl)guanine in the sample before silylation, presumably formed by deribosylation after chromatography.

Nuclear magnetic resonance spectra of dGI also supported the identification of this adduct as 2′-deoxy-7-[2-(ethylthio)-ethyl]guanosine. Resonances in both D_2O and Me_2SO-d_6 are shown in Table I. One methyl and three methylene groups in the ethylthioethylene side chain are discernable as in the two deoxyadenosine adducts discussed above. In Me_2SO-d_6 protons on N^2 and C-8 were detected eliminating these as positions of substitution. The 1′-carbohydrate proton was characterized in the spectrum run in D_2O. The extended times (up to 12 h) employed in the Fourier transform technique, necessitated by limited sample solubility, had the disadvantage of permitting substantial deribosylation to take place during the measurements. As these proceeded, the initially clear D_2O solution became cloudy and a new set of signals appeared, which were similar to those from dGV. This facile deribosylation is considered characteristic of 7-alkylated guanosines.[14,19]

Alkylation at N-7 in guanosines is also known to promote opening of the imidazole ring with the addition of the elements of water, as shown in the scheme above.[14,19,21] This reaction is usually promoted by alkali. Indeed, when the product mixture from the alkylation of deoxyguanosine by CEES was adjusted to pH 7 before application to the Sephadex column, dGI was isolated in which the imidazole ring was opened. The ring-opened and ring-closed N-7 adducts had similar retention factors on the column, both eluting ahead of unreacted 2′-deoxyguanosine.

Ultraviolet spectroscopic measurements on the base-treated dGI adduct confirmed its ring-opened structure (Table II). The mass spectrum of a trimethylsilyl derivative of this material was measured using electron impact ionization. The molecular weight of 661 daltons is consistent with a tetrakis-(trimethylsilyl) derivative of deoxyguanosine carrying one ethylthioethyl group, in which the five-membered imidazole ring has been opened with the addition of the elements of water. Electron impact induced loss of the sugar moiety leads to B^+ ions and B^+ – 15 ions (*m/e* 400 and 385, respectively), confirming that the ethylthioethylene group is attached to the base. However, the alkyl group is cleaved as readily as the sugar, leading to M – 88 (*m/e* 573) and B + H (*m/e* 313) ions.

The chemical ionization mass spectrum of the trimethylsilyl derivative of the base-treated dGI sample was also measured. The protonated molecular ion peak at *m/e* 662 is the base peak in the spectrum and is consistent with the assignment of the sample as a tetrakis(trimethylsilyl)ethylthioethyldeoxyguanosine in which the imidazole ring has been opened with the addition of water. B + 2 H ions of mass 402 also contribute an intense peak to the spectrum.

The electron impact mass spectrum of trimethylsilylated material from peak dGIII contains molecular ions and M – 15 peaks at *m/e* 643 and 628, respectively, consistent with the tetrakis(trimethylsilyl) derivative of an adduct of deoxyguanosine carrying one 2-(ethylthio)ethyl group. Alkylation at positions 1 and O^6 is eliminated by the attachment of four trimethylsilyl groups. The trimethylsilylation conditions used in this study put only one trimethylsilyl group on the primary amine at the N^2 position in deoxyguanosine. However, under these same conditions, one trimethylsilyl group is also attached to a secondary amine such as 2′-deoxy-N^2-(methyl)-guanosine. Thus alkylation at N^2 is not eliminated as a possibility by formation of the tetrakis(trimethylsilyl) derivative.

Titration curves were particularly useful in the characterization of peak dGIII. Ionization was determined as a function of pH by calculating extinction ratios of UV absorption at 270/250 and 280/260 nm. A second pK_A inflection, around pH 9.5, indicates that the monoalkylated deoxyguanosine eluted at peak dGIII is substituted at position 8 or N^2 and not at 1 or O^6.[14,22]

The final assignment of dGIII as 2′-deoxy-N^2-[2-(ethylthio)ethyl]guanosine was based on the maxima and shapes[20] of complete ultraviolet spectra scanned at pH 1, 7, and 11. These maxima are reported in Table II and are clearly distinguishable from those characteristic of deoxyguanosine or guanosine alkylated at other positions, including C-8.

The electron impact mass spectrum of trimethylsilylated peak dGIV contained M^+ and $(M - 15)^+$ peaks at *m/e* 455 and 440, consistent with the structure of a tris(trimethylsilyl) derivative of a deribosylated mono(ethylthioethyl)guanine. Characteristics of UV spectra of dGIV obtained at several pH values are presented in Table II. The maxima and overall shape of the absorption curves[20] match those of N^2-alkylguanines and are distinctive from guanines alkylated at other positions. Thus dGIV is identified as N^2-[2-(ethylthio)-ethyl]guanine by its UV spectra and by its relationship to dGIII.

The electron impact mass spectrum of trimethylsilylated dGV contains M^+ and $(M - 15)^+$ ions at *m/e* 383 and 368, suggesting that the sample is a bis(trimethylsilyl)monoalkylguanine derivative.

Table I shows chemical shift values from the ^1H NMR spectrum of dGV. Protons are detected for equimolar amounts of ethylthioethylene and guanine, but ribosyl protons are absent. The sample had very limited solubility in water at pH 7, and the measurements in Table I were made at pH 1.4. The C^8–H chemical shift is very close to that of C^8–H in guanine measured under similar conditions (8.53 ppm). One methylene group in the side chain is moved downfield about 0.23 ppm, confirming the assignment of the purine-bonded methylene signal. Other proton signals are unaffected by the pH change.

The UV characteristics of dGV are listed in Table II. The maxima and also the shapes[20] of the UV curves are identical with those of authentic 7-alkylguanines and distinct from guanines alkylated at other positions. Thus, dGV is identified as 7-[2-(ethylthio)ethyl]guanine.

Discussion

Deoxyadenosine and adenosine have been shown to be methylated by a variety of agents primarily at N-1.[3,6,7,14] Although positions N-3 and N-7 are also considered active, only the latter has been found to be substituted as a product of nucleoside methylation and that only rarely.[6,14,18] Although direct methylation is considered not to occur at exocyclic N^6,[7] mutagens that transfer alkyl groups larger than methyl have been observed to alkylate the N^6 position of adenosine.[7,8] Thus, the products formed by the reaction of CEES and deoxyadenosine are consistent with the general chemistry of adenosine nucleosides.

The alkylation reactions of two compounds closely related to CEES, bis(2-chloroethyl) sulfide and 2-hydroxyethyl 2-chloroethyl sulfide, have been studied by Shooter et al.[16] and Lawley et al.[17] Polyadenylic acid, bacteriophage DNA, and bacteriophage RNA were the substrates. Alkylated polymers were hydrolyzed by combinations of enzymatic and chemical steps, and the resulting modified adenines and adenylic acids were characterized by their chromatographic mobility and ultraviolet spectra. Adenine and adenylate adducts were reported to be alkylated at N-1, N-3, and N^6. A major difference between the results of Shooter and Lawley and those reported here is the recovery by the earlier workers of 3-alkylated adenine from polymers. This difference between the reactivity of polymers and adenine nucleosides appears to be general.[14,18]

Since authentic standards were not available for co-chromatography with some of the polymer-derived products in the earlier studies, the present characterization of deoxyadenosines alkylated at N-1 and N^6 by closely related CEES strengthens previously reported structure assignments.

Deoxyguanosine and guanosine are alkylated most readily at position 7; however, adducts of these nucleosides carrying alkyl groups at 1, N^2, and O^6 [14,23,29] also have been characterized. Guanine adducts alkylated at 3, 7, O^6 and N^2 have been isolated from polyguanylic acid, DNA, and RNA.[10,14,23-28] In particular, bis(2-chloroethyl) sulfide and 2-chloroethyl 2-hydroxyethyl sulfide have been shown to alkylate guanine at N-7 in DNA and RNA in vivo and in vitro.[4,16,17] No guanine positions other than N-7 have been reported to be alkylated by these two sulfur mustards. Thus, the alkylation at N-7 of deoxyguanosine by CEES was anticipated. Alkylation by CEES at N^2 is of greater interest because all the N^2-mono adducts found heretofore in nucleosides and polymers (i.e., excluding guanine itself) have been formed from epoxides of massive polycyclic hydrocarbons.[23-26] The present work demonstrates that a medium sized substituent also can be attached to N^2, and, presumably, by an alkylation mechanism different from that of the epoxides.[3,32] It seems likely that N^2-alkylation of nucleosides and nucleic acids is more general than previously recognized. The 2′-deoxy-7-[2-(ethylthio)ethyl]guanosine and N^2-[2-(ethylthio)ethyl]-guanine adducts reported here are unstable in acid and would have been destroyed by the acid hydrolysis of polymers used by some previous investigators.

The products characterized here can be used as standards for characterization of nucleoside ^{35}S adducts from CEES-alkylated deoxyribonucleic acid.

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Registry No.—CEES, 693-07-2.

References and Notes

(1) C. Auerbach and J. M. Robson, *Nature (London)*, **157**, 302 (1946).
(2) D. G. Fahmy and M. J. Fahmy, *J. Genet.*, **54**, 146 (1956).
(3) W. C. J. Ross, "Biological Alkylating Agents", Butterworths, London, 1962; E. C. Miller and J. A. Miller, *Pharm. Rev.*, **18**, 805 (1966); A. E. Pegg, *Adv. Cancer Res.*, **25**, 195 (1977).
(4) P. Brookes and P. D. Lawley, *Biochem. J.*, **80**, 496 (1961).
(5) R. M. Gilbert, S. Rowland, C. L. Davison, and B. Papirmeister, *Mutat. Res.*, **28**, 257 (1975).
(6) J. W. Jones and R. K. Robins, *J. Am. Chem. Soc.*, **85**, 193 (1963).
(7) B. Singer, L. Sun, and H. Fraenkel-Conrat, *Biochemistry*, **13**, 1913 (1974).
(8) A. Dipple, P. Brookes, D. S. Mackintosh, and M. P. Rayman, *Biochemistry*, **10**, 4323 (1971).
(9) J. A. McCloskey in "Basic Principles in Nucleic Acid Chemistry", Vol. 1, P. O. P. Ts'o, Ed., Academic Press, New York, N.Y., 1974, pp 209–309.
(10) D. L. Ludlum, B. S. Kramer, J. Wang, and C. Fenselau, *Biochemistry*, **14**, 5480 (1975).
(11) D. L. Von Minden, R. N. Stillwell, W. A. Koenig, K. J. Lyman, and J. A. McCloskey, *Anal. Biochem.*, **50**, 110 (1972).
(12) P. O. P. Ts'o, N. S. Kondo, R. K. Robins, and A. D. Broom, *J. Am. Chem. Soc.*, **91**, 5625 (1969).
(13) D. Voet, W. B. Gratzer, R. A. Cox, and P. Doty, *Biopolymers*, **1**, 193 (1963).
(14) B. Singer in "Progress in Nucleic Acid Research and Molecular Biology", Vol. 15, W. Cohn, Ed., Academic Press, New York, N.Y., 1975, pp 219–284.
(15) M. H. Wilson and J. A. McCloskey, *J. Org. Chem.*, **38**, 2247 (1973).
(16) K. V. Shooter, P. A. Edwards, and P. D. Lawley, *Biochem. J.*, **125**, 829 (1971).
(17) P. D. Lawley, J. H. Lethbridge, P. A. Edwards, and K. V. Shooter, *J. Mol. Biol.*, **39**, 181 (1969).
(18) P. D. Lawley and P. Brookes, *Biochem. J.*, **89**, 127 (1963).
(19) R. Shapiro, ref 14, Vol. 8, 1973, pp 73–112.
(20) R. H. Hall, "The Modified Nucleosides in Nucleic Acids", Columbia University Press, New York, N.Y., 1971.
(21) J. A. Haines, C. B. Reese, and L. Todd, *J. Chem. Soc.*, 5281 (1962).
(22) W. E. Cohn, *J. Am. Chem. Soc.*, **73**, 1539 (1951).
(23) A. Dipple, P. Brookes, P. S. Mackintosh, and M. P. Rayman, *Biochemistry*, **10**, 4323 (1971).
(24) A. M. Jeffrey, S. H. Blobstein, I. B. Weinstein, F. A. Beland, R. G. Harvey, H. Kasai, and K. Nakanishi, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 2311 (1976).
(25) A. M. Jeffrey, K. W. Jennette, S. H. Blobstein, I. B. Weinstein, F. A. Beland, R. G. Harvey, H. Kasai, I. Miura, and K. Nakanishi, *J. Am. Chem. Soc.*, **98**, 5714 (1976).
(26) M. Koreeda, P. D. Moore, H. Yagi, H. J. Yeh, and D. M. Jerina, *J. Am. Chem. Soc.*, **98**, 6720 (1976).
(27) J. M. Essigmann, R. G. Croy, A. M. Nadzan, W. F. Busby, V. N. Reinhold, G. Buchi, and G. N. Wogan, *Proc. Natl. Acad. Sci. U.S.A.*, **74**, 1870 (1977).
(28) P. D. Lawley and S. A. Shah, *Biochem. J.*, **128**, 117 (1972).
(29) P. B. Farmer, A. B. Foster, M. Jarman, and M. J. Tisdale, *Biochem. J.*, **135**, 203 (1973).
(30) R. E. Holmes and R. K. Robins, *J. Org. Chem.*, **28**, 8483 (1963).
(31) P. D. Lawley, D. J. Orr, and M. Jarman, *Biochem. J.*, **145**, 73 (1975).
(32) H. Yagi, O. Hernandez, and J. M. Jerina, *J. Am. Chem. Soc.*, **97**, 6881 (1975).
(33) W. C. Ross, "Biological Alkylating Agents", Butterworths, London, 1962, p 11.