The Reaction of Propane Sultone with Guanosine, Adenosine, and Related Compounds (1)

B. M. Goldschmidt, K. Frenkel and B. L. Van Duuren

Laboratory of Organic Chemistry and Carcinogenesis,
Institute of Environmental Medicine, New York University Medical Center,
New York, New York 10016

Propane sultone (3-hydroxypropanesulfonic acid-γ-lactone) which is a monofunctional direct-acting alkylating carcinogen, has been shown to react irreversibly with DNA from various sources *in vitro*. It reacts with guanosine in DMSO to yield the N-7 alkyl nucleoside, while reaction with 2'-deoxyguanosine yields the corresponding N-7 alkyl purine. Evidence for the structure of these products and of one formed when adenosine and propane sultone react is presented.

Independent studies in several laboratories (2,3,4) recently reported on the carcinogenicity of propane sultone (3-hydroxypropanesulfonic acid-γ-lactone), I, in a variety of test systems. This compound has also been shown to be mutagenic to T4 bacteriophage (5).

The present study was undertaken as part of a broader program dealing with chemical structure and carcinogenicity relationships of a series of direct-acting alkylating agents. The earlier studies have focussed on epoxides (6) and chloro ethers (7).

The carcinogens β-propiolactone (8) and diepoxybutane (9) react at the N-7 position of guanine in both the nucleoside and polynucleotide. The carcinogen glycidaldehyde (6) has been shown to react with the N-1

and the amino group at C-2 on the pyrimidine ring of guanine to form a new five-membered ring (10). Alkylating agents can also react with the other bases and components of polynucleotides (11,12) but the relevance of any of these reactions to mutagenesis and carcinogenesis is still not clear.

Although propane sultone is a thiolactone, its chemical similarity to β-propiolactone (II), a well-known monofunctional carcinogenic alkylating agent, is evident. The rates of hydrolysis of compounds I and II, which is a measure of their potential reactivity with cellular nucleophiles are very similar (13,14). Both can react with nucleophiles *via* cleavage of the C-O bond and formation of a stable bond with the alkyl carbon. For these reasons we selected this compound for determination of carcinogenic activity (3). In this report it is shown that I reacts irreversibly with DNA *in vitro* and we have determined the structures of products formed when this carcinogen is allowed to react with guanosine and adenosine.

Samples of calf thymus DNA in phosphate/citrate buffer, pH 7.0, were allowed to react with propane sultone (I). After extensive dialysis, the transition melting point (Tm) of samples of DNA treated with I, untreated DNA, and DNA treated with β-propiolactone (II) were determined. The Tm of DNA treated with propane sultone or with β-propiolactone was lower than that of the control as shown in Figure 2. In addition, the λ max of DNA treated with alkylating agents was at 260 mμ, while that of the untreated DNA was at 257 mμ. The gradient density profile of the alkylated samples was considerably altered compared to the control. This infor-
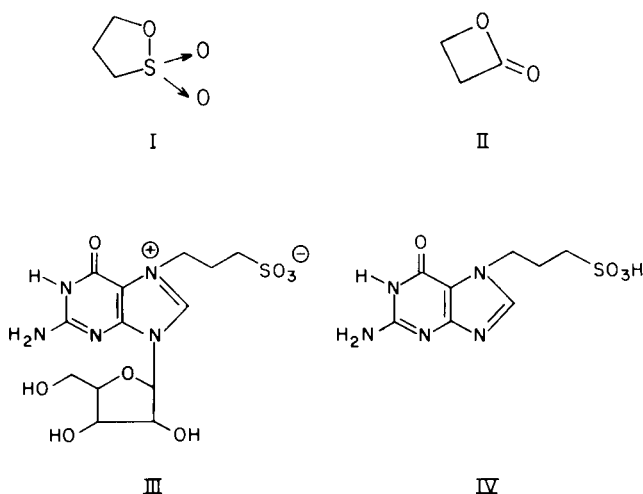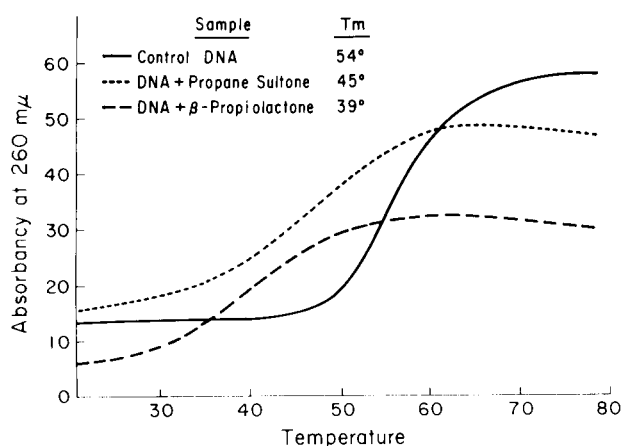

Figure 1

Figure 2. Melting profiles of calf thymus DNA samples after treatment with propanesultone (I) or β-propiolactone (II) followed by dialysis. Initial optical densities for untreated DNA: 0.67, DNA + I: 0.66, and DNA + II: 0.71. Final optical densities 0.91 for control and 0.82 and 0.83 for DNA treated with I and II, respectively.

mation indicated the irreversible *in vitro* reaction of I with a polynucleotide.

As reported earlier (8), brief heating of aqueous solutions of II with guanosine followed by acid hydrolysis gave predominantly 7-guanine-3-propanoic acid. When these same procedures were used with I, guanine was the major product. However, when guanosine and I were allowed to react in dimethyl sulfoxide at 37° for 48 hours a high yield of compound III was obtained. Elemental analysis of III indicated that it consisted of I equivalent of guanosine and I equivalent of propane sulfonic acid. Purified III had uv maxima at 256 mμ in 0.1 N hydrochloric acid and at 265 mμ in 0.1 N sodium hydroxide. 7-Methylguanosine has a reported λ max at 256 mμ at pH 1.0 and at 265 mμ at pH 11 (15); since these λ max are similar to those of III this suggested then that III is probably 7-(3-propanesulfonic acid) guanosine. Additional evidence for this assignment was obtained by hydrolyzing III to yield IV. The uv spectrum of IV has λ max at 249 mμ in acid and 279 mμ in base. These were comparable to those of 7-guanine-3-propanoic acid, 250 mμ in acid and 280 mμ in base (5) suggesting that IV is 7-(3-propanesulfonic acid) guanine.

Examination of the nmr spectrum of III and IV substantiated these structural assignments. Compound III in deuterium oxide had peaks at 2.46 (pnt), 2.96 (m), 4.37 (m), 6.10 (db) and 9.23 (s) δ. The peaks at 2.46, 2.96 and 4,37 were assigned to the β, α, and γ hydrogens of the propanesulfonic acid chain respectively, while that at 6.10 δ was the H-I of the ribose and at 9.23 δ the C-8 aro-

matic proton of the purine. It has been shown that a downfield shift of the C-8 proton occurs when the N-7 of the imidazole moiety of guanosine is quaternized (16). It is also known that in aqueous acidic media at room temperature the exchange of the C-8 proton of 7-alkyl guanines is inhibited, while at higher temperatures in neutral or alkaline media the exchange is rapid (17). This was also found to be the case for compound III. Compound IV in 1% sodium deuteroxide/deuterium oxide showed four peaks - 2.32 (pnt), 2.84 (m), 4.48 (t) and 7.91 (s) δ, assignable to the β, α and γ hydrogens of the propane chain and the C-8 hydrogen of the purine. Additional evidence for these assignments comes from the nmr spectrum of 3-pyridiniumpropane sulfonic acid (V). This material was synthesized by allowing pyridine to react with propane sultone. In deuterium oxide the nmr spectrum of V had peaks at 2.42 (pnt), 2.92 (m), 4.78 (trp) and 7.9-9.1 (m). Here, the β, α and γ hydrogens of the propanesulfonic acid side chain were clearly discernable from the aromatic protons of the pyridine nucleus.

The reaction conditions under which propane sultone reacts with guanosine to yield III, were used in an attempt to prepare the analogous compound with 2′-deoxyguanosine. After purification of the reaction product, however, only IV was obtained. After alkylation the labile deoxy-sugar is apparently solvolyzed in the subsequent isolation or purification steps. This seems consistent with the literature reports that the ribose moiety of 7-alkyl guanosine is more reactive toward hydrolysis than the non-alkylated bases and deoxyguanosine itself is more reactive than guanosine (18).

Adenosine and propane sultone were allowed to react in DMSO and the structure of the isolated product, VI, was then determined. Elemental analysis of VI indicated that the ribose moiety was removed. This product has an ultraviolet spectrum with a λ max at 260 mμ in 0.1 N hydrochloric acid and 272 mμ in 0.1 N sodium hydroxide. The uv spectra of adenine substituted with alkyl groups at the N-3 position have virtually the same λ max in aqueous acid or base (12), while monoalkyl adenines with the substituents at the 6-amino position have a somewhat higher λ max in acid than VI (19). Since 1-methyladenine has uv λ max at 259 mμ in acid and 270 mμ in base, respectively (20), it was concluded that VI is 1-(3-propanesulfonic acid)adenine. The nmr spectrum of VI in basified deuterium oxide had the three peaks of the propane side chain at 2.27 (m), 2.95 (m) and 4.38 (trp) δ and the two aromatic protons of the adenine nucleus as singlets at 7.80 and 7.95 δ.

Attempts were then made to determine whether other nucleosides would react with I. 2′-Deoxyadenosine, 2′-deoxycytidine and thymidine were allowed to react with I

at 40° in DMSO for up to seven days. Tlc and uv analyses indicated that the order of reactivity was 2'-deoxyadenosine > 2'-deoxycytidine > thymidine. Under comparable conditions 2'-deoxyguanosine was more reactive than 2'-deoxyadenosine. These results are consistent with the reported reactivity of these nucleosides toward other alkylating agents, e.g. nitrogen mustards (12).

When guanosine, 5'-GMP or 5'-GTP were allowed to react with 1 in water/DMSO (1:1) each reacted with 1 as determined by tlc and uv spectra. When adenosine, 5'-AMP and 5'-ATP were allowed to react with 1 in water/DMSO (1:1) ATP was hydrolyzed, while AMP and adenosine failed to react. Only poly-G readily yielded an alkylated product when 1 was allowed to react with poly-G, poly-A or poly-U at 37° in the same solvent mixture and as determined by the same criteria.

Samples of herring sperm DNA were allowed to react with propane sultone. After dialysis the alkylated DNA was hydrolyzed in acid. The hydrolyzate was chromatographed on Sephadex, and the fractions were assayed by tlc and uv absorption in acid or base. Compound VI was shown to be present, while the initial dialyzate, after being concentrated and then chromatographed on Sephadex, showed IV and VI to be present.

EXPERIMENTAL

Ir spectra were obtained with a Perkin Elmer 421 infrared spectrophotometer. Samples were run as potassium bromide pellets. Nmr spectra were determined in deuterium oxide or deuterium oxide basified with sodium deuteroxide using a Varian A-60 spectrometer with sodium 2,2-dimethyl-2-silapentane 5-sulfonate as the internal standard. Ultraviolet spectra were obtained with a Cary 14 spectrophotometer. Microanalyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Michigan. Thin layer chromatography was carried out on 0.1 mm cellulose (C) or cellulose F (CF) plates (E. Merck AG.) using water or 0.01 N sodium acetate as the eluents, respectively. The nucleosides and the DNA used were obtained from Sigma Chemical Co.

Preparation of 7-(3-Propanesulfonic Acid)guanosine (III).

To 2.0 g. of guanosine (6.8 mmoles) in 50 ml. of DMSO were added 5.0 ml. (57 mmoles) of propanesultone (Aldrich Chemical Co.) and the resulting solution was stirred for two days at 37°. The mixture was filtered and the precipitate discarded as it had no uv absorption. Absolute ethanol was added to the clear filtrate until a copious precipitate formed, and this was allowed to stand at 4° for several hours and filtered. The solid was washed with a small quantity of absolute ethanol, and 2.5 g. (> 90% yield of crude product) of III was obtained. This product was recrystallized twice from water/ethanol at room temperature. The ir spectrum had bands at 3400, 1720, 1700, 1672, 1642, 1612, 1525, 1396, 1200, 1180 and 1035 cm⁻¹. In 0.1 N hydrochloric acid, III had λ max at 256 mμ (ε 13,000), while in 0.1 N sodium hydroxide it had λ max at 265.5 mμ (ε 10,600). When heated the substance started to decompose at 180°. The tlc on CF showed a single fluorescent spot, R_f 0.79.

Anal. Calcd. for $C_{13}H_{19}N_5O_8S$: C, 38.51; H, 4.72; N, 17.27;

S, 7.91. Found: C, 38.35; H, 4.62; N, 16.85; S, 7.51.

Preparation of 7-(3-Propanesulfonic Acid)guanine (IV).

To 2.0 g. (7.0 mmoles) of 2'-deoxyguanosine in 25 ml. of DMSO, 1.0 ml. (11 mmoles) of propanesultone were added. The resulting solution was kept at 37° for 24 hours. The precipitate which formed was discarded as in the preparation of III above. Absolute ethanol was added to the clear filtrate to precipitate the product. The solid was then dissolved in 2 N sodium hydroxide and allowed to stand at 4° overnight. The solution was acidified to pH 7-8 and the resulting precipitate also discarded. Addition of absolute ethanol to the filtrate led to the precipitation of a product, IV, (yield ≈ 70%), which was then recrystallized from 1% hydrochloric acid-ethanol. Compound IV was also prepared by allowing guanosine or 2'-deoxyguanosine or guanine to react with propanesultone in DMSO at 90° for 30 minutes, 15 minutes, 2 hours, respectively. Similarly, compound III could be converted to IV by heating for 5 minutes at 90° in 2 N sodium hydroxide. The ir spectrum of IV had bands at 1721, 1670, 1473, 1222, 1159, 1140 and 1038 cm⁻¹. The uv spectrum of IV in 0.1 N hydrochloric acid had λ max at 249 mμ (ε 11,700), while in 0.1 N sodium hydroxide it had λ max at 279 mμ (ε 7,600). A heated sample failed to melt below 300°. The tlc of purified IV on CF showed a single fluorescent spot, R_f 0.83.

Anal. Calcd. for $C_8H_{11}N_5O_4S$: C, 35.16; H, 4.03; N, 25.64; S, 11.72. Found: C, 35.16; H, 4.04; N, 25.64; S, 11.83.

Preparation of 3-Pyridiniumpropanesulfonic Acid (V).

To 2.0 ml. of pyridine (25 mmoles) in 30 ml. of DMSO was added 2.5 ml. of propanesultone (29 mmoles). The solution was stirred at room temperature for 0.5 hour and a precipitate formed. Addition of acetone to the mother-liquor resulted in a second precipitate. They were combined and recrystallized from water-acetone. The ir spectrum of this compound had bands at 1625, 1485, 1239, 1218, 1195, 1168, 1140 and 1035 cm⁻¹. The uv spectrum in 0.1 N hydrochloric acid had λ max at 259 mμ (ε 4,450) and in 0.1 N sodium hydroxide λ max at 259 mμ (ε 4,700) with shoulders at 254 and 264 mμ in both acid and base. The sample melted at 278-280°. The tlc on C of the purified substance showed a single fluorescent spot, R_f 0.95.

Anal. Calcd. for $C_8H_{11}NO_3S$: C, 47.76; H, 5.47; N, 7.00; S, 15.92. Found: C, 47.62; H, 5.42; N, 6.92; S, 15.75.

Preparation of 1-(3-Propanesulfonic Acid)adenine (VI).

To 2.0 g. of adenosine (7.5 mmoles) dissolved in 20 ml. of DMSO, 4.0 ml. (44 mmoles) of propanesultone was added. The solution was stirred at 37° for 24 hours and at room temperature for an additional 24 hours. The precipitate was discarded and absolute ethanol was added to the clear filtrate. The precipitate which formed was collected and washed several times with absolute ethanol. The solid was then redissolved in DMSO and reprecipitated by the addition of absolute ethanol. The precipitate was dissolved in 2 N sodium hydroxide and allowed to stand for several hours. Acidification with hydrochloric acid to pH 4.0 followed by the addition of ethanol reprecipitated the product. This was recrystallized from water twice to yield ≈ 12% pure VI, m.p. 332°. A tlc on CF of the pure sample showed a single fluorescent spot, R_f 0.75. The ir spectrum had bands at 3440, 3050, 1690, 1640, 1580, 1418, 1240, 1208, 1162 and 1040 cm⁻¹. The uv spectrum of VI in 0.1 N hydrochloric acid had a λ max at 260 mμ (ε 13,000) and 207 mμ (ε 28,500), while in 0.1 N sodium hydroxide it had λ max at 272 mμ (ε 13,000) and 211 mμ (ε 17,000).

Anal. Calcd. for $C_8H_{11}N_5O_3S$: C, 37.35; H, 4.31; N, 27.22;

S, 12.46. Found: C, 36.68; H, 4.22; N, 27.08; S, 12.17.

DNA Melting Curves (Tm).

Ten ml. solutions of calf thymus DNA (0.1 mg./ml.) in M phosphate/0.1 M citrate buffer, pH 7.0, were treated with 0.5 ml. of I or II and 1.0 ml. of DMSO. After 24 hours of stirring at $37°$ the samples were dialyzed at $4°$ against 0.01 M phosphate/.001 M citrate buffer for five days. The melting profiles on DNA samples in the dialysis buffer were obtained on a Gilford Spectrophotometer by monitoring the absorbancy at 260 mμ. The temperature was increased at a rate of $2°$/minute until the optical density remained nearly constant at above $90°$. The results are shown in Figure 2.

Buoyant Density Determinations.

Buoyant densities of DNA treated with I were determined in a Beckman Model E ultracentrifuge using an ultraviolet optical system. Ten ml. samples of calf thymus DNA, 0.1 mg./ml., in M phosphate/0.1 M citrate buffer, pH 7.0, to which 1.0 ml. of DMSO had been added, were treated with 0.20, 0.30, and 0.50 ml. of I, respectively. The samples were stirred at $37°$ for 18 hours and following dialysis at $4°$ the DNA ($\approx$ 1.58 μg./ml.) in 0.01 M phosphate/0.001 M citrate buffer. The DNA in cesium chloride (density, 1.7 g./ml.) was centrifuged at 44,770 rpm for 20 hours at $25°$.

The buoyant density of an untreated DNA sample was 1.699, that treated with 0.20 ml. of I had a buoyant density of 1.705, the sample treated with 0.30 ml. of I had a very broad band, while the DNA treated with 0.50 ml. of I was largely fragmented, i.e. no real band was observed at the highest concentration.

Alkylation and Degradation of DNA.

Fifty mg. of herring sperm DNA was dissolved in 40 ml. of 0.01 M phosphate/0.001 M citrate, pH 7.0, and then 10 ml. of M phosphate/0.1 M citrate was added. Propanesultone, 0.5 ml., was added to this stirred solution at $37°$. After three hours, the solution was dialyzed as described above. The purified DNA was acidified with 2 N hydrochloric acid to pH 1.0 and heated on a steam bath for 1 hour. After cooling and concentrating via low temperature vacuum distillation of the solvent, the mixture was chromatographed on Sephadex G-10 using 0.02 M citrate/0.02 M phosphate, pH 3.5, as the eluent. The dialyzate was concentrated and chromatographed in the same manner as the hydrolyzed DNA. Comparative chromatograms were run with untreated DNA. Compounds IV and VI were separated from the unreacted purines, pyrimidines, sugar, etc. on the Sephadex column and detected in the eluents by tlc and uv analysis.

Acknowledgement.

REFERENCES

(1) A preliminary account of this work was presented at the May 1972 meeting of the American Association for Cancer Research. *Proc. 63rd Ann. Meet. Am. Assoc. Cancer Res.*, p. 94, Boston, Mass. (1972). This work formed part of the Ph.D. thesis presented by K. Frenkel at New York University in 1974.

(2) H. Druckrey, H. Kruse, R. Preussmann, S. Ivankovic, Ch. Landschütz and J. Gimmy, *Z. Krebsforsch.*, **75**, 69 (1970).

(3) B. L. Van Duuren, S. Melchionne, R. Blair, B. M. Goldschmidt and C. Katz, *J. Nat. Cancer Inst.*, **46**, 143 (1971).

(4) B. Ulland, M. Finkelstein, E. K. Weisburger, J. M. Rice and J. M. Weisburger, *Nature*, **230**, 460 (1971).

(5) T. H. Corbett, C. Heidelberger and W. F. Dove, *Mol. Pharmacol.*, **6**, 667 (1970).

(6) B. L. Van Duuren, *Ann. N. Y. Acad. Sci.*, **163**, (Art. 2), p. 633-651, Ed. M. Krauss (1969) and earlier references cited therein.

(7) B. L. Van Duuren, A. Sivak, B. M. Goldschmidt, C. Katz and S. Melchionne, *J. Nat. Cancer Inst.*, **43**, 481 (1969).

(8) J. J. Roberts and G. P. Warwick, *Biochem. Pharmacol.*, **12**, 1441 (1963); N. H. Colburn and R. K. Boutwell, *Cancer Res.*, **28**, 642 (1968).

(9) P. Brookes and P. D. Lawley, *J. Chem. Soc.*, 3923 (1961); P. D. Lawley and P. Brookes, *J. Mol. Biol.*, **25**, 143 (1967).

(10) B. M. Goldschmidt, T. P. Blazej and B. L. Van Duuren, *Tetrahedron Letters*, No. 13, 1583 (1968).

(11) G. P. Warwick, *Cancer Res.*, **23**, 1315 (1963).

(12) C. C. Price, G. M. Gaucher, P. Konero, R. Shibakawa, J. R. Sowa and J. Yamaguchi, *Biochem. Biophys. Acta*, **166**, 327 (1968).

(13) F. A. Long and M. Purchase, *J. Am. Chem. Soc.*, **72**, 3267 (1950).

(14) A. Mori, M. Nagayama and H. Manderi, *Bull. Chem. Soc. Japan*, **44**, 1669 (1971).

(15) J. W. Jones and R. K. Robins, *J. Am. Chem. Soc.*, **85**, 193 (1963).

(16) R. Roe, Jr., J. S. Paul and P. O'B. Montgomery, Jr., *J. Heterocyclic Chem.*, **10**, 849 (1973).

(17) M. Tomasz, *Biochem. Biophys. Acta*, **199**, 18 (1970).

(18) J. A. Zoltewicz, D. F. Clark, T. W. Sharpless and G. Grahe, *J. Am. Chem. Soc.*, **92**, 1741 (1970).

(19) A. D. Broom, L. B. Townsend, J. W. Jones and R. K. Robins, *Biochem.*, **3**, 494 (1964).

(20) P. Brookes and P. D. Lawley, *J. Chem. Soc.*, 539 (1960).