Biochem. J. (1972) 126, 893–900
Printed in Great Britain

Alkylation by Propylene Oxide of Deoxyribonucleic Acid, Adenine, Guanosine and Deoxyguanylic Acid

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(*Received* 15 *September* 1971)

1. Propylene oxide reacts with DNA in aqueous buffer solution at about neutral pH to yield two principal products, identified as 7-(2-hydroxypropyl)guanine and 3-(2-hydroxypropyl)adenine, which hydrolyse out of the alkylated DNA at neutral pH values at 37°C. 2. These products were obtained in quantity by reactions between propylene oxide and guanosine or adenine respectively. 3. The reactions between propylene oxide and adenine in acetic acid were parallel to those between dimethyl sulphate and adenine in neutral aqueous solution; the alkylated positions in adenine in order of decreasing reactivity were N-3, N-1 and N-9. A method for separating these alkyladenines is described. 4. Deoxyguanylic acid sodium salt was alkylated at N-7 by propylene oxide in neutral aqueous solution. 5. The nature of the side chain in the principal alkylation products was established by mass spectrometry, and the nature of the products is consistent with their formation by the bimolecular reaction mechanism.

Aliphatic epoxides include important biological alkylating agents; for example, ethylene oxide is a widely used chemosterilant; ethylene and propylene oxides are mutagenic for *Drosophila*, and are 'radiomimetic' agents (Loveless, 1966). However, extensive tests of the toxicity of either of these epoxides, administered by inhalation in several animal species, did not reveal any carcinogenic activity (Rowe *et al.*, 1956; Jacobson *et al.*, 1956); but certain aliphatic epoxides, including butadiene dioxide, are known to be carcinogenic (Hendry *et al.*, 1951; Van Duuren *et al.*, 1963).

Unlike many alkylating agents, the epoxides react with water without liberating acid; reaction with anions or tertiary amines in aqueous media liberates alkali. In general, these compounds can be classified as reacting typically by S_N2 attack of nucleophiles on the epoxide ring carbon atom; attack by amines or other reagents on an unsymmetrical olefine oxide is predicted to occur normally at the carbon atom bearing the greater number of free hydrogen atoms (Parker & Isaacs, 1959).

The characteristic biological actions of the aliphatic epoxides suggest that, as with other alkylating agents, DNA may be their significant target *in vivo*. However, few reports of the reactions of these agents with nucleic acids or nucleotides have appeared. Lawley & Wallick (1957) noted that ethylene oxide and propylene oxide resembled other alkylating agents in that they react with guanosine in water at the N-7 position; in this reaction the pH was not controlled and the alkali generated caused a further reaction now known to involve fission of the imidazole ring of the 7-substituted guanosines (Brookes & Lawley, 1961*a*; Haines *et al.*, 1962). Windmueller & Kaplan (1961) showed that ethylene oxide reacts with adenine nucleotides predominantly at the N-1 position, again showing that this epoxide behaved as a typical alkylating agent.

However, the findings reported by Pochon & Michelson (1967) might be considered to suggest that propylene oxide was atypical, in that no reaction with DNA or poly(G) could be detected in aqueous solution, whereas reaction was reported at N-1 of thymidine and uridine. The latter reactions would be expected to occur for alkylation in alkaline solutions, but the pH was not stated.

In an attempt to clarify these apparent contradictions, it was decided to reinvestigate the possible reaction between propylene oxide and DNA, assuming that the epoxide would in fact resemble other alkylating agents. From previous work with various alkylating agents (Lawley & Brookes, 1963), it was expected that 7-alkylguanine would be the major product and 3-alkyladenine the principal minor product. In order to identify these as products from DNA, it was necessary to isolate and characterize these compounds. It was already known that guanosine would react with propylene oxide to yield the appropriate guanine derivative (Lawley & Wallick, 1957) but this had not been isolated in quantity. The adenine derivative was expected to be the major product from alkylation of adenine by analogy with methylation of this base (Pal, 1962) but no procedure for isolation of substi-

tuted adenines in quantity from this reaction had been described in detail, and this problem was therefore investigated.

The nature of the side chain in the expected alkylation products was also of interest. As already mentioned, theory predicts that this would be the 2-hydroxypropyl substituent, CH_3–CHOH–CH_2–, rather than its hydroxyisopropyl isomer, CH_3–$CH(CH_2OH)$–. Although the close analogy of the u.v. spectra of the expected products and their methyl analogues would enable the site of attachment of the alkyl group to the purine ring to be established, other physical methods would be necessary to establish the configuration of the alkyl group. In the present case mass spectroscopy was effective.

Materials and Methods

Reaction between propylene oxide and adenine

Adenine (0.6g; 4mmol) was stirred with a mixture of acetic acid (10ml) and propylene oxide (Koch–Light Laboratories, Colnbrook, Bucks., U.K.) (1.2ml) for 16h at 37°C. The solution was then applied to a column (24cm × 3cm) of Dowex AG50W (X4; minus 400 mesh) eluted with 1M-HCl (1.2ml/min) and 14ml fractions were collected. After 2.5 litres had passed through the column, elution was continued with 2M-HCl until no further u.v.-absorbing material was eluted (4.5 litres total volume). The profile of u.v. absorption together with the values of E_{280}/E_{260} showed the presence of six components; the last of these was shown to be unchanged adenine.

Appropriate pooled fractions were evaporated. The hydrochlorides were obtained by dissolving the residues in methanol; the solution was filtered and ethyl acetate was added to the filtrate until a cloudy solution was obtained; these suspensions were left to crystallize at 0°C.

In one case sufficient material was obtained for elementary analysis, this being the major product (peak III) (Fig. 1), m.p. 198°C (corr.) (Found: C, 41.35; H, 5.34; N, 30.44; $C_8H_{12}ClN_5O$ requires C, 41.83; H, 5.26; N, 30.49%).

The u.v.-absorption spectra of the various products were determined, and the structural assignments of the positions of the alkyl substituents were made by comparison with appropriate known methylation products of adenine. In a parallel experiment in which dimethyl sulphate was used in place of propylene oxide, analogous methylation products were obtained, which were eluted in the same order from the column, and these were identified by comparison of their u.v. spectra and R_F values with those of known methyladenines.

Examination of 3-alkyladenine by mass spectrometry

The hydrochloride (peak III) (30mg) was converted into the free base by passing an aqueous solution (1ml) through a column (10cm × 1cm²) of Amberlite IR-45 (free base form) eluted with water; 10ml fractions were collected. Fractions 2–30, which contained the required base, were concentrated by evaporation under reduced pressure and the residue was recrystallized from ethanol (3ml) to yield colourless rectangular plates (6mg); m.p. (Kofler block, corr.) 227°C, disintegrating to yield small square plates, m.p. (decomp.) 268–272°C (Found: C, 49.22; H, 5.64; N, 36.11; $C_7H_{11}N_5O$ requires C, 49.73; H, 5.74; N, 36.25%).

Mass spectra were obtained with an A.E.I. MS-12 spectrometer by using a direct-insertion probe. The probe was inserted into the source at 100°C, the source temperature then being raised gradually to 230°C; an ionizing potential of 70eV was used.

As discussed in the Results and Discussion section the mass spectrum established the configuration of the alkyl group, and hence the structure 3-(*2-hydroxypropyl*)*adenine* for the compound in peak III.

Reaction between propylene oxide and guanosine

Guanosine (1.8mmol) in acetic acid (5ml) was stirred with propylene oxide (0.5ml) for 16h at 37°C. The solvent was then removed by evaporation under reduced pressure. The residue was dissolved in methanol and applied to a column (70cm × 3cm) of Sephadex LH-20 eluted with methanol and 15ml fractions were collected. Fractions 16–20 contained a product with spectra of a 7,9-disubstituted guanine ($\lambda_{max.}$ 283.5nm; irreversible change in alkali to $\lambda_{max.}$ 266.5nm); and fractions 22–28 a 7-substituted guanine ($\lambda_{max.}$ 283.5nm; reversible change in alkali to 280nm).

After evaporation of appropriate fractions and recrystallization from methanol, crystalline products were obtained that showed blue fluorescence on paper chromatograms under u.v. light (253.7nm). The 7,9-disubstituted product (light-blue fluorescence) was chromatographically homogeneous (R_F 0.16, with solvent propan-2-ol–aq. NH_3 (sp.gr. 0.88)–water, 85:2:13, by vol.) and was pure as judged by the occurrence of isosbestic points in the irreversible spectral change at pH9.6, at 240.5 and 276nm; but analyses were unsatisfactory in that the amount of C was slightly too high and N was too low (Found, C, 44.1; H, 5.88; N, 18.3; $C_{13}H_9N_5O_6,H_2O$ requires C, 43.5; H, 5.89; N, 19.5%). It was concluded that this product was contaminated with some of the material of the column.

The second product (dark-blue fluorescence) was not obtained in an amount sufficient for analysis, but an identical base was obtained by hydrolysis of the first product (from fraction 18) with 1M-HCl (5ml, 1h, 100°C) and recrystallization from methanol as prisms, m.p. >300°C (Found: C, 45.3; H, 5.3; N, 33.1; $C_8H_{11}N_5O_2$ requires C, 45.9; H, 5.3; N, 33.5%).

The spectra of the product were as follows: at pH2, $\lambda_{max.}$ 249.5, 272nm ($10^{-3} \times \epsilon_{max.}$ 9.3, 6.5), $\lambda_{min.}$ 228.5nm; at pH7, $\lambda_{max.}$ 245, 283.5 ($10^{-3} \times \epsilon_{max.}$ 5.8, 7.3), $\lambda_{min.}$ 260; at pH12, $\lambda_{max.}$ 280 ($10^{-3} \times \epsilon_{max.}$ 6.9), $\lambda_{min.}$ 256.5. These spectra are diagnostic for 7-alkylguanines (Brookes & Lawley, 1961*a*).

The mass spectrum of the second product was determined, and this established the nature of the side chain, as discussed in the Results and Discussion section, thus enabling the identification of this product as 7-(2-*hydroxypropyl*)*guanine*. The first product from which it derives is therefore 7-(2-*hydroxypropyl*)-*guanosine*.

Reaction between propylene oxide and deoxyguanylate

Deoxyguanosine 5'-phosphate, sodium salt (1mmol) was dissolved in 2.5M-sodium acetate, pH5.2 (7ml), and propylene oxide (0.15ml) was added, the mixture being kept at 37°C. After 9 days a precipitate had formed (16mg), which had R_F and u.v.-absorption spectra identical with those of 7-(2-hydroxypropyl)guanine. The residual solution was applied to a column of Sephadex G-10 and eluted with dilute ammonia solution as for the supernatant from DNA treated with propylene oxide. Fractions 5–11 contained deoxyguanylic acid; fractions 12–18, 7-(2-hydroxypropyl)guanine; fractions 22–27 a small amount of guanine; and fractions 32–39 a trace of adenine, this last obviously an impurity in the original deoxyguanylic acid.

Reaction between propylene oxide and DNA

In a typical experiment, propylene oxide (0.28ml; 2.0mmol) was added to a solution of salmon sperm DNA (Mann Research Laboratories, New York, N.Y., U.S.A.) (10mM P) in sodium acetate (1.25M; pH5.8; 10ml) and the mixture was maintained at 37°C for 7 days. The pH, which had then risen to 7.3, was adjusted to 6.0 with acetic acid and the solution was heated to 100°C for 20min. After the solution had been cooled to 4°C, ethanol (2vol.) was added and a precipitate of polynucleotide material was removed by centrifugation.

The supernatant was applied to a column (30cm × 2.5cm) of Sephadex G-10 and eluted with aq. NH_3, pH10.4, 0.15ml/min, 15ml fractions being collected. The u.v. absorption of the fractions showed that fractions 4–8 contained polynucleotide material ($\lambda_{max.}$ 260nm); fractions 10–18, 7-alkylguanine, ($\lambda_{max.}$ 280nm); fractions 19–22, 3-alkyladenine ($\lambda_{max.}$ 273nm). The fractions that contained the products were pooled and those containing 3-alkyladenine were evaporated to a volume of 3ml; the absorption spectra were then redetermined with a Unicam SP.800 spectrophotometer.

As an alternative procedure for isolation of the principal reaction products, the whole reaction mixture, after 9 days at 37°C and at pH maintained around 6.8, was applied to a column (85cm × 1cm) of Sephadex G-10. Elution was done with ammonium formate (0.05M, adjusted to pH6.8); 2.9ml fractions were collected. The polynucleotide material was in fractions 14–21, 3-alkyladenine ($\lambda_{max.}$ 274nm) in fractions 37–40 and 7-alkylguanine ($\lambda_{max.}$ 283nm) in fractions 57–70. The use as eluting solvent of buffer at this pH instead of water reverses the order of elution of these products. This was advantageous for isolation and identification of the principal minor product, 3-alkyladenine, which was obtained in a smaller volume.

Identification of products from propylene oxide and DNA by mass spectrometry after paper chromatography

The products were isolated by chromatography of reaction mixtures of propylene oxide and DNA on Sephadex G-10 as described above. Then appropriate fractions were evaporated and the residues were applied to Whatman 3MM paper for chromatography in two dimensions, with propan-2-ol–aq. NH_3 (sp.gr. 0.88)–water (7:1:2, by vol.) and butan-1-ol–aq. NH_3 (sp.gr. 0.88)–water (85:2:13, by vol.) as solvents.

A strip of paper containing the spot, attached to a paper wick where necessary, was eluted downwards from a trough containing methanol, in a tank saturated with this solvent. The eluate was collected in a 100ml flask, and was concentrated under reduced pressure to a suitable small volume for application to the probe of the mass spectrometer. Elution was generally done overnight, to give a volume of about 20ml. All glassware was cleaned with chromic acid before use.

The efficiency of this procedure was checked by using the purines from a mild acid hydrolysate of DNA (5mg) obtained by the depurination procedure of Tamm *et al.* (1952), and chromatographed on Whatman 3MM paper with propan-2-ol–aq. NH_3 (sp.gr. 0.88)–water (7:1:2, by vol.) as solvent. Methanol (50ml) gave complete elution of both purines, as determined by the u.v. absorption of the eluate, by comparison with that obtained by using 0.1M-HCl (although of course a smaller volume of the latter sufficed).

Results and Discussion

Preparation and identification of 3-alkyladenine

The reaction between propylene oxide and adenine in acetic acid yielded various products whose order of elution and u.v. absorption spectra closely resembled those obtained from the reaction between dimethyl sulphate and adenine in neutral aqueous solution (Fig. 1 and Table 1). The latter were identified by comparison of their u.v. spectra with those of the

authentic methyladenines (Brookes & Lawley, 1960; Pal, 1962; Jones & Robins, 1963).

The order of reactivity of the various ring-N atoms of adenine towards either alkylating agent was N-3 > N-1 > N-9; no evidence for reaction at N-7 was found. These results therefore confirm and amplify those obtained by Pal (1962), who did not isolate the products as solids in quantity, but did identify them by their u.v.-absorption spectra. These results are also consistent with the known reactions of the 9-substituted adenines, which are alkylated mainly at N-1 (Brookes & Lawley, 1960; Jones & Robins, 1963), in that 1,9-disubstituted adenines were obtained; similarly, the occurrence of 3,7-disubstituted adenines is consistent with the known alkylation of 3-alkyladenine at N-7 (Jones & Robins, 1963). It should be noted that this pattern of alkylation of adenine is consistent also with the concept that in the neutral molecule of adenine in aqueous solution the proton on the imidazole ring is attached predominantly at the N-7 position, which is not alkylated to a detectable extent. The contrast between adenine and the 9-substituted adenines, which became alkylated at N-7 and N-3 (Lawley & Brookes, 1964) as well as at N-1, is thus confirmed again.

The finding that propylene oxide resembles dimethyl sulphate in some detail, with respect to the groups alkylated in adenine, supports the concept that it is reacting by a bimolecular mechanism, and that with both reagents the relative nucleophilicities of the various ring-N atoms in the adenine molecule determine the relative extents of reaction. This concept would clearly be supported if the configuration of the side chain introduced by propylene oxide was of the 'normal' configuration, i.e. CH_3–CHOH–CH_2–. For the principal product, the techniques of i.r., n.m.r. and mass spectroscopy were applied to investigate this question. The sole conclusive information was obtained by mass-spectroscopic examination of the base. (It should be noted that the use of hydrochlorides for this purpose should be avoided, since their volatility will generally be less than that of free bases, and the HCl liberated by their decomposition may react with the base so as to alter its mass spectrum.)

The major fragments in the mass spectrum (Fig. 2)

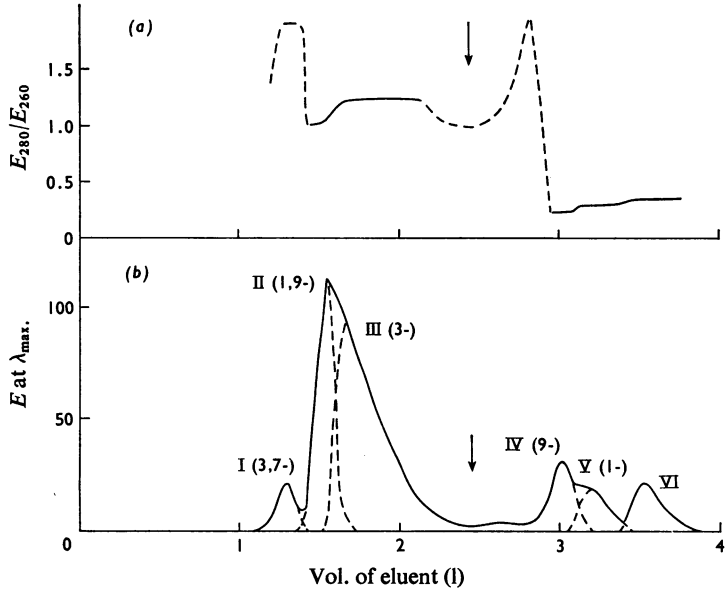


Fig. 1. *Chromatography on Dowex* 50 (H^+ *form) of the products of reaction between propylene oxide and adenine*
Adenine (0.6g) was stirred for 16h at 37°C in acetic acid (10ml) that contained propylene oxide (1.2ml); the residue after evaporation of the mixture under reduced pressure was dissolved in 1M-HCl and applied to a column (24cm × 3cm) of Dowex AG50W (X4; minus 400 mesh) that was eluted with 1M-HCl; 14ml fractions were collected; from the point shown by the arrow, elution was continued with 2M-HCl. The various products (peaks I–V) were identified by their absorption spectra (Table 1) after isolation as hydrochlorides; peak VI contained adenine. The numbers indicate the positions of the alkyl substituents, e.g. I (3,7-) was identified as 3,7-di(hydroxypropyl)adenine, (*a*) E_{280}/E_{260} ratio; ---- indicates values for fractions between those in which the various products were eluted; (*b*) E at $\lambda_{max.}$; the values for $\lambda_{max.}$ and E_{280}/E_{260} for the purified products are shown in Table 1.

Table 1. *Properties of products from adenine and propylene oxide isolated as hydrochlorides after chromatography on Dowex 50 (H^+ form), (Fig. 1), numbered in order of elution, compared with those of corresponding products from adenine and dimethyl sulphate*

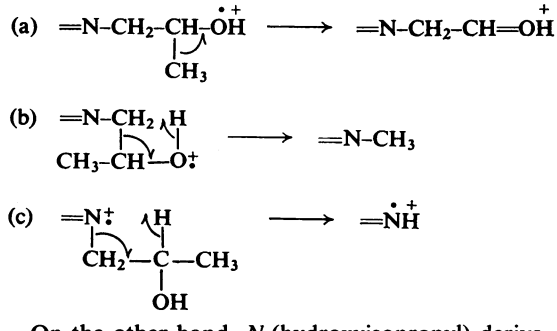
Solvent (1), methanol–conc. HCl–water, (7:2:1), by vol.); (2), butan-1-ol–aq. NH_3 (sp.gr. 0.88)–water (85:2:13, by vol.). Values in parentheses are $10^{-3} \times \epsilon$ at $\lambda_{max.}$. R_{ad} is the R_F relative to adenine.

Product	Position(s) of alkyl substituents	R_{ad} Solvent (1)	R_{ad} Solvent (2)	pK_a	$\lambda_{max.}$ (nm) (pH2)	E_{280}/E_{260} (pH2)	$\lambda_{max.}$ (nm) (pH12)	E_{280}/E_{260} (pH12)
(a) Products from propylene oxide								
I	3,7-	2.3	1.3	—	277.5	1.7	281	2.0
II	1,9-	2.5	1.6	—	260	0.27	260	0.37
							268*	0.68*
III	3-	2.0	1.3	6.0	274 (18.6)	1.44	273 (13.4)	1.55
IV	9-	1.7	1.4	—	259	0.22	261	0.23
V	1-	1.7	1.1	7.2	258.5	0.28	271†	1.08†
(b) Products from dimethyl sulphate								
	3,7-	2.0	0.5		276.5 (15.7)	1.5	279.5	1.8
	1,9-	1.8	1.1		261 (14.0)	0.30	261	0.43
							267*	0.73*
	3-	1.5	1.1		274 (15.9)	1.2	273	1.4
	9-	1.4	1.5		261 (14.0)	0.34	262.5	0.24
	1-	1.3	0.9		259 (11.7)	0.31	270†	0.90†

* After 18h at 37°C.
† At pH9.6.

of the 3-(hydroxypropyl)adenine formed by the loss of part, or the whole, of the hydroxypropyl group from the molecular ion, *m/e* 193 (M^+, 20%, relative to the most abundant peak at *m/e* 135), were: (a) *m/e* 178 (M^+–CH_3, 21%): (b) *m/e* 149 (M^+–CH_3CHO, 70%), corresponding metastable peak *m** at 115.0; (c) *m/e* 135 [M^+–$(CH_3)_2CO$], *m** at 94.4.

An *N*-(2-hydroxypropyl) derivative would readily give these fragments by the mechanisms:



On the other hand, *N*-(hydroxyisopropyl) derivatives, which would be formed by attack of the basic moiety on the secondary carbon atom of propylene oxide, would give peaks at *m/e* 163 (M^+–CH_2O) and 162 (M^+–CH_2OH) by processes analogous with (a) and (b). These peaks were in fact absent from the spectrum (Fig. 2) and this structural assignment is therefore eliminated.

Preparation and identification of 7-alkylguanine

To obtain the required 7-(hydroxypropyl)guanine, guanosine was treated with propylene oxide, and the products were separated on Sephadex LH-20. Evidently some conversion of the product 7-(hydroxypropyl)guanosine had occurred during the reaction, since in addition to this product, the base 7-(hydroxypropyl)guanine was found. The 7-substituted nucleoside thus showed the property characteristic of this type of derivative in that it was more susceptible to acid hydrolysis than guanosine itself. Similarly, when deoxyguanylic acid (sodium salt) was treated with propylene oxide in neutral aqueous solution, the initially formed 7-substituted deoxynucleotide hydrolysed to yield the free base, as found for several analogous derivatives (Lawley, 1957; Lawley & Brookes, 1963). The rate of reaction of propylene oxide in aqueous medium was not determined in the present work, but, as judged by the increase in pH during the reaction, the half-life was at least several hours; hydrolysis of the alkylated nucleotide would therefore be appreciable before alkylation was complete.

The rates of hydrolysis of the 7-(hydroxypropyl)-guanosine were determined in 1M-HCl at 37°C ($t_{\frac{1}{2}}$ 6.2h) and at 50°C ($t_{\frac{1}{2}}$ 1.2h); for comparison, 7-methylguanosine at 37°C has $t_{\frac{1}{2}}$ 7.1h (Lawley & Brookes, 1963). Also characteristic of a 7,9-disubstituted guanine was the observed ring-fission reaction

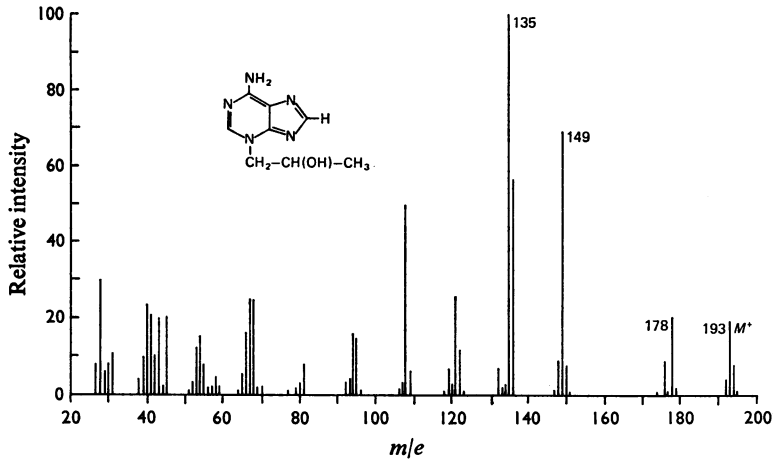


Fig. 2. *Mass spectrum of* 3-(2-*hydroxypropyl*)*adenine*

For experimental details see the text.

in mildly alkaline solution; at pH 9.6, 37°C, the half-life was 0.46h.

The configuration of the side chain of the base 7-(hydroxypropyl)guanine was investigated by mass spectrometry. This showed (Fig. 3) the molecular ion, *m/e* 209 (M^+, 40%) and major fragments, of the type previously designated (a), *m/e* 194 (M^+–CH_3, 3%); (b), *m/e* 165 (M^+–CH_3CHO, 100%), *m** at 130.3; and (c), *m/e* 151 (M^+–$(CH_3)_2CO$, 35%), *m** at 109.1. The structure of a 2-hydroxypropyl derivative is thus favoured, as for the 3-(2-hydroxypropyl)-adenine. The 7-(hydroxyisopropyl) alternative is contra-indicated; the prediction from this structure that peaks at *m/e* 179 and 178 would be strong was not supported, although weak peaks at these values were found.

Reaction of propylene oxide with DNA

Since propylene oxide was expected to react feebly in aqueous solutions, the nature of the principal reaction products was investigated by using a method first devised for the study of methylation of DNA by comparatively unreactive agents, *N*-methyl-*N*-nitrosourethane and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (Lawley, 1968). This was designed to permit the use of a relatively large amount of DNA, and thus the expected relatively low amounts of products obtained could be detected and identified by u.v. absorption. The principle is that the products are hydrolysed out of the alkylated DNA at neutral pH values, leaving the unchanged purines attached to the macromolecule. The alkylpurines can then be obtained by exclusion chromatography, by using in this case Sephadex G-10, which also effects their separation, because of their differential absorption to this column material.

As an additional aid to identification of the principal products, it has been found that their order of elution is characteristic and, for methyl and ethyl derivatives, independent of the nature of the alkyl group, but can vary when the solvent is varied. In the present case, the order of elution of products after the polynucleotide residue was found to be, with dilute ammonia at around pH 10 as described previously (Lawley, 1968), 7-alkylguanine then 3-alkyladenine. But when the neutral solvent, 0.05M-ammonium formate adjusted to pH 6.8, was used, this order was reversed, as found for the methyl and ethyl homologues (P. D. Lawley & D. J. Orr, unpublished work).

The products are eluted in reasonably small volumes by this method, and their identification by u.v. absorption, even if present in small amounts, is thus facilitated. Fig. 4 compares the u.v. spectra of the products from DNA and propylene oxide with those of the authentic alkylpurines. For the 3-alkyladenine the pH of the solutions was also varied in the range 5–7 and the spectroscopically derived value of pK_a' of 5.9 was in agreement with that found for 3-(2-hydroxypropyl)adenine obtained by alkylation of adenine.

The products from DNA were also resubjected to chromatography on paper, then eluted and examined by mass spectrometry. The spectra were identical with those of the corresponding authentic bases, and therefore these products were 2-hydroxypropyl derivatives. It is thus confirmed that mass spectrometry is a valuable addition to the techniques available for identification of products of DNA alkylation.

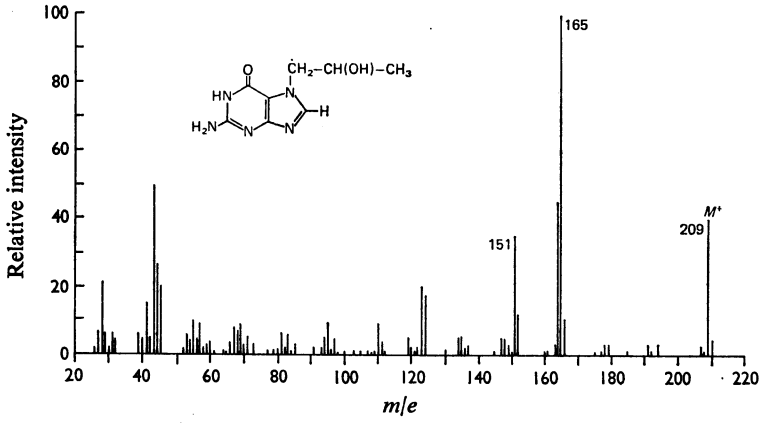


Fig. 3. *Mass spectrum of* 7-(2-*hydroxypropyl*)*guanine*

For experimental details see the text.

The technique had been applied to studies of this type by Lee & Lijinsky (1966) and Lijinsky *et al.* (1968) (nucleic acid methylation) and by Walles (1970) (reaction of DNA with isopropyl methanesulphonate).

A question that arose during the present work with this technique concerned the method of elution of the alkylated bases from paper chromatograms. It was found that elution with methanol, a suitably volatile solvent, was satisfactory, even with the relatively insoluble bases such as guanine and its derivatives. Since it was likely that these extracts would contain contaminants from the paper, it was advantageous to insert the probe into the source of the mass spectrometer at a temperature below that required to volatilize the compound, and then gradually raise the temperature. The contaminants were thus volatilized before the required spectrum appeared.

Having identified the principal products from alkylation of DNA by propylene oxide, it remained to measure their relative yields. Elution of the products from Sephadex G-10 with 0.05M-ammonium formate, pH6.8, gave well defined peaks of u.v. absorption characteristic of these products, and by summation of the values of $E_{max.}$ for the appropriate fractions, the molar ratio of the products 3-alkyladenine and 7-alkylguanine was found to be 1:5. The yield of products was low relative to the relatively high concentration of reagents used. For example, by using 16mM-DNA P with 230mM-propylene oxide, the extent of reaction to give 3-alkyladenine was 6.2mmol of 2-hydroxypropyl/mol of DNA P, and of 7-alkylguanine was 33mmol/mol of DNA P. It should be noted that a relatively high concentration of buffer (in this case, sodium acetate, about 1.5M) was required to maintain the pH in the range 5.8–7, and that a considerable amount of propylene oxide reacted with acetate ion, since the pH increased during the reaction.

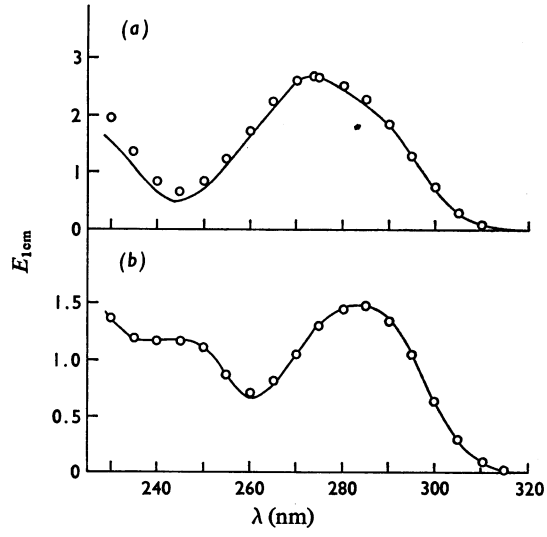


Fig. 4. *U.v.-absorption spectra of products from the action of propylene oxide on salmon sperm DNA in neutral aqueous solution*

The products were obtained by chromatography of the reaction mixture, after 9 days at 37°C, on Sephadex G-10 that was eluted with 0.05M-ammonium formate, as described in detail in the text. (*a*), ○, fraction 39; ——, 3-(2-hydroxypropyl)-adenine (0.28mM); (*b*), ○, fraction 62; ——, 7-(2-hydroxypropyl)guanine (0.19mM).

The findings that propylene oxide does alkylate

DNA *in vitro* at neutral pH are clearly consistent with the view that alkylation of DNA *in vivo* could account for the 'radiomimetic' action of this agent, and probably that of its analogues. The relatively low extents of reaction observed may be contrasted, for example, with those achieved with sulphur mustards, where in aqueous solution most of the reagent can combine with DNA (Brookes & Lawley, 1961*b*). It is therefore possible that these differences in reactivity could be consistent with the relatively low toxicity of propylene oxide; e.g. Smyth *et al.* (1941) reported that the LD_{50} for the rat (single dose by stomach tube as aqueous solution) was 1.14g/kg. However, because of the apparent lack of information on extents of alkylation by epoxides *in vivo* any correlation with toxicity must at present be tentative.

It would also be premature to attempt to correlate the present results in any detail with the mutagenic action of propylene oxide. However, the question whether this agent reacts in part through the S_N1 mechanism is relevant in this connexion. The evidence from the present work, although limited by the low extents of reaction achieved, is consistent with the operation of the bimolecular S_N2 mechanism of alkylation in two respects. First, 2-hydroxypropyl derivatives were found, in accord with the predictions for bimolecular reaction. Asymmetric epoxides reacting through the S_N1 mechanism, on the other hand, are predicted to generate a partial positive charge on the carbon atom carrying the alkyl substituent (Parker & Isaacs, 1959), and in the present case this would lead to formation of hydroxyisopropyl derivatives. Secondly, the major products of alkylation of DNA resulted from reaction of the most nucleophilic centres, N-7 of guanine and N-3 of adenine. When the residual polynucleotide was subjected to mild acid hydrolysis with 0.1M-HCl at 70°C and examined by paper chromatography (Lawley & Thatcher, 1970), only unchanged purines, guanine and adenine, were detected. No fluorescent product with properties consistent with those of an O^6-alkylguanine was found.

A classification of alkylating mutagens is suggested by the work of Loveless (1969), in that, for certain potent mutagens, such as *N*-methyl-*N*-nitrosourea and ethyl methanesulphonate, ability to induce mutation by the transition mechanism is associated with alkylation of guanine residues in DNA at the O-6 position. This reactivity has in turn been positively correlated with alkylation by the S_N1 mechanism (Lawley & Thatcher, 1970). Further, positive correlation between mutational efficiency and ability to react through the S_N1 mechanism has been deduced independently, from studies with a series of alkyl methanesulphonates as mutagens for barley (Osterman-Golkar *et al.*, 1970).

The present work indicates that propylene oxide is unlikely to be in the category of S_N1 alkylating agents, but the limitations of the techniques used do not permit this possibility to be entirely excluded.

This investigation was supported by grants to the Chester Beatty Research Institute, Institute of Cancer Research, from the Medical Research Council and the Cancer Research Campaign. We thank Dr. P. Brookes for reviving our interest in epoxides, and Professor A. B. Foster for his interest in the mass spectrometry of the compounds involved; in this area Mr. M. H. Baker provided skilled technical assistance. The AEI MS-12 mass spectrometer was purchased on a special grant (G969/189/C) from the Medical Research Council.

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