REACTIVITY OF PROPYLENE OXIDES TOWARDS DEOXYCYTIDINE AND IDENTIFICATION OF REACTION PRODUCTS

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SUMMARY

Deoxycytidine was reacted with four epoxides of varying alkylating rates: propylene oxide, glycidol, epichlorohydrin and trichloropropylene oxide. Deoxycytidine was chosen to compare the reactivities of these epoxides as all sites of possible alkylation, the oxygen and both nitrogens, are involved in base pairing in DNA. Reaction products were separated on HPLC. Products of the least and most reactive epoxides, propylene oxide and trichloropropylene oxide, were characterized by UV, IR, 360 MHz NMR and MS analysis. For the epichlorohydrin and glycidol reactions and all analytical separations, products were characterized by their HPLC retention times and UV spectra. While differences in reactivity among the epoxides towards specific nitrogen and oxygen sites were found, total reactivity correlated with Taft σ*-values of the substituent groups.

INTRODUCTION

Our research on structure-mutagenicity relationships for aliphatic epoxides [1—2] has prompted us to extend the studies available on interactions of propylene oxides with nucleosides [3—6]. We are interested in further developing methodology for the separation and identification of nucleoside

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Abbreviations: B, pyrimidine base; dCyd, deoxycytidine; DMSO, dimethylsulfoxide; EC, epichlorohydrin; GL, glycidol; HPLC, high pressure liquid chromatography; IS, internal standard; M, molecular ion; 3-methyl-dCyd, 3-methyldeoxycytidine; PO, propylene oxide; R, alkyl side chain; S, deoxysugar; TCPO, trichloropropylene oxide; TLC, thin-layer chromatography.

adducts with propylene oxides as a step towards elucidating their reactivity with DNA and understanding their mechanism of mutagenesis. In this study, deoxycytidine (dCyd) was selected for investigation since it has been indicated to have a wide range of reactivity among the propylene oxides of interest [6] and because it represents a nucleoside for which all sites of possible alkylation on the heterocycle, the N-3, N^4 and O^2, are involved in base pairing in DNA. It was the purpose of this investigation to identify alkylation products of dCyd with propylene oxide (PO) and trichloropropylene oxide (TCPO) by IR, NMR and MS spectra as well as by UV spectra to confirm that UV spectra can be used to determine the site of alkylation. The extent of alkylation by PO and TCPO was then compared with that of epichlorohydrin (EC) and glycidol (GL). Alkylation of dCyd by these four epoxides was correlated to Taft σ*-values [7] for the substituents, X = CH_3, CH_2OH, CH_2Cl and CCl_3, on

$$X-CH-CH_2.$$
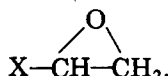
MATERIALS AND METHODS

Chemicals

The propylene oxides were purchased from Aldrich Chemical Co. (Milwaukee, WI) and dCyd, as well as 3-methyldeoxycytidine (3-methyl-dCyd) were purchased from Sigma Chemical Co. (St. Louis, MO). For ^{1}H-NMR, 100.00% gold label D_2O or 99.9% gold label DMSO-d_6 were purchased from Aldrich Chemical Co. Infrared quality potassium bromide was from Harshaw (Solon, OH).

Reactions

Preparative reactions were carried out with approx. 10 mg dCyd, accurately weighed, for each reaction to which 225 μl buffer (50 mM Tris—HCl, pH 7.4) and 25 μl of PO or TCPO were added per mg of dCyd. Reaction vials were placed in a shaking water bath at 37°C for 48 h. Comparative reactions with all four epoxides were also conducted with approx. 10 mg dCyd but the dCyd was dissolved in a solution of epoxide in 60% methanol/40% 0.1 M ammonium formate (pH 7.4) buffer. For every 1 mg of dCyd, 250 μl of buffer solution containing 300 μmol of epoxide was added. Reaction vials were placed in the shaking water bath at 37°C for 24 h.

Chromatography

Thin-layer chromatography (TLC) on prescored 2 × 10 cm 250 μ silica GF uniplates (Analtech, Newark, DE) was used to monitor reactions. Solvent systems used were: (A) n-butanol/ethanol/water (80:10:25), (B) isopropanol/ammonia/water (70:10:20) and (C) n-butanol/acetone/acetic acid/water (10:10:2:5).

Analytical high pressure liquid chromatography (HPLC) on an Altex system (Berkeley, CA) with UV detection at 254 nm was carried out using a strong cation exchange column, Partisil SCX (4.6 mm × 25 cm) from Whatman (Clifton, NJ). The mobile phase was 0.1 M ammonium formate buffer

adjusted to pH 3.5 with formic acid. A flow rate gradient of 1.7 ml/min to 2.2 ml/min over 12 min was used and at 8 min the chart speed was decreased from 30 to 15 cm/min. Reaction solutions were diluted 1:5 with mobile phase buffer containing 0.02% pyridine as an internal standard and 20-μl aliquots were injected onto the column. All products are referred to as labelled in the Fig. 1 chromatograms.

For the PO and TCPO reactions, semi-preparative cation exchange HPLC was performed with a Whatman Partisil SCX Magnum 9 (10 mm × 50 cm) column using 0.08 M ammonium formate buffer (pH 3.5) at 3 ml/min. Corresponding product fractions were pooled from five, 0.35-ml injections of undiluted reaction mixture. The pooled fractions were freeze dried, redissolved in a small amount of water, and then purified using a Whatman Partisil ODS Magnum 9 column with a methanol/water gradient of 5—100% methanol over 25 min at a flow rate of 1.5 ml/min. Traces of buffer and unreacted material eluted within 25 min while products eluted at about 30 min. For IR analysis, approx. 20% of each product fraction from the reverse phase separation was mixed with 30 mg of KBr and freeze dried. The remaining product fraction was freeze dried directly and then used for MS and NMR analyses.

UV spectra

UV spectra were taken on a Hewlett Packard (Palo Alto, CA) Model 8450A UV/VIS spectrophotometer. UV spectra of products collected preparatively were measured at different pH-values by dilution with high purity water, 0.1 M citrate (pH 5) buffer, 0.1 M carbonate (pH 9) buffer, 0.1 M HCl or 0.1 M NaOH. For stability studies, spectra of those samples in HCl and NaOH were retaken after standing 24 h at room temperature. Spectra were also recorded for the latter samples after neutralization of the solutions. Samples collected from the analytical column were dilute and their UV spectra were obtained in the HPLC mobile phase as collected from the column. For all spectra, ratios of the absorbances at 254—280 nm were calculated.

IR spectra

IR spectra were obtained on a Perkin-Elmer (Norwalk, CN) Model 281 spectrometer equipped with a model 3600 Data Station. Freeze dried KBr samples were further dried under vacuum in a drying tube for 5 h at room temperature and then pressed in paper micro frames (2 × 10 mm aperture). Spectra of the isolated products as indicated were obtained by averaging 20 or 100 scans, as required to obtain satisfactory signal to noise ratios. The absorbance bands for the starting material (dCyd), 3-methyl-dCyd and the isolated products labelled as in Fig. 1 follow: dCyd, 3500—3100 cm^{-1} strong (s); 2941 shoulder (sh); 1667 s; 1613 s; 1470 medium (m); 1408 weak (w); 2369 w; 1299 s; 1219 m; 1099 s; 1053 s; 943 w; 901 w; 870 w; 794 s; 720 s. 3-methyl dCyd 3540—3140 s; 2950 sh; 1709 s; 1682 s; 1651 s; 1556 s; 1431 w; 1352 w; 1334 w; 1296 m; 1250 s; 1212 s; 1170 w; 1119—1096 s; 1057 s; 989 s; 816 w; 761 s; 666 w. PO-1 (20 scans averaged) 3500—3400 s;
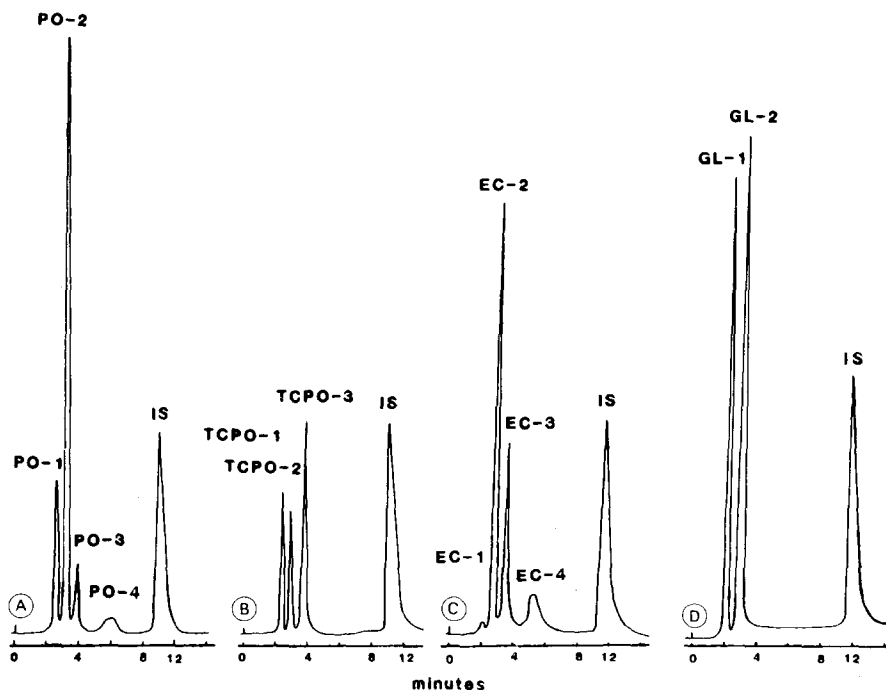
Fig. 1. Analytical cation exchange HPLC chromatograms of 24-h reactions: (A) deoxycytidine with propylene oxide; (B) deoxycytidine with trichloropropylene oxide; (C) deoxycytidine with epichlorohydrin; (D) deoxycytidine with glycidol.

2960—2950 sh; 1710 sh; 1660 s; 1630 s; 1450 w; 1380 w; 1269 m; 1210 w; 1090—1050 s; 990 sh; 940 w; 910 w; 835 sh; 800 m; 762 m; 710 w; TCPO-1 (100 scans averaged) 3520—3150 s; 2912 sh; 1708 sh; 1663 s; 1647 s; 1463 m; 1403 m; 1271 m; 1251 w; 1094—1050 s; 876 m; 801 s; 620 m. TCPO-3 (100 scans averaged) 3339—3100 s; 2930 sh; 1726 sh; 1665 s; 1593 s; 1533 w; 1468 w; 1404 w; 1356 w; 1280 m; 1098 w; 917 w; 877 w; 801 s; 761 sh; 656 m; 606 m.

NMR spectra

Proton NMR spectra were taken on a Bruker 360 MHz Fourier Transform spectrometer. Samples of 1—2 mg were dissolved in 99.9% DMSO-d_6. The shifts in ppm from tetramethylsilane, including the effect of decoupling and D_2O exchange, are listed.

TCPO-3, DMSO-d_6: δ 7.50 (d, J=8.4 Hz, 1, 6-H), 6.86 (broad, 1, NH), 6.18 (t, J=6.9, 1, 1'-H), 5.97 (d, J=8.4, 1, 5-H), 6.66 (t, J=6.5, 1, COH), 5.24 (d, J=4.1, 1, 3'-OH), 4.98 (t, J=5.5, 1, 5'-OH), 4.21 (q, J=3.0, 1, 3'-H), 4.15 (m, 1, CH), 3.75 (m, 1, 4'-H), 3.53 (m, 2, 5'-H), 3.4 (m, overlap with HDO CH$_2$), 2.05 (m, 2, 2'-H); decouple at 4.15: 6.65 (d, J=10, 1, COH) and simplified the multiplet at 3.4; decouple at 2.05: 4.21 (s, 1, 3'-H), 6.18 (s, 1, 1'-H); D_2O exchange: 7.47 (d, J=7.9, 1, 6-H), 6.16 (t, J=6.8, 1, 1'-H), 5.96 (d, J=8.3, 1, 5-H), 4.30 (q, J=3.4, 1, 3'-H), 4.14 (t, J=8.5, 1, CH), 3.75 (q,

J=3.4, 1, 4'-H), 3.52 (m, 2, 5'-H), 3.23-3.43 (m, 2, CH₂), 2.04 (m, 2, 2'-H); decouple at 3.43—3.33: simplified the multiplet at 4.14.

TCPO-1, DMSO-d_6: 7.91 (d, J=8.0, 1, 6-H), 7.5 (broad, 1, NH), 6.81 (t, J=5.1, 1, COH), 6.18 (t, J=6.0, 1, 1'-H), 5.80 (d, J=8.2, 1, 5-H), 5.26 (d, J=4.2, 1, 3'-OH), 5.04 (t, J=4.9, 1, 5'-H), 4.44 (m, 2, CH₂), 4.24 (m, 1, 3'-H), 4.03 (m, 1, CH), 3.80 (m, 1, 4'-H), 3.57 (m, 2, 5'-H), 2.10 (m, 2, 2'-H), D₂O exchange: 7.90 (d, J=8.0, 1, 6-H), 6.16 (t, J=5.8, 1, 1'-H), 5.75 (d, J=8.0, 1, 5-H), 4.42 (m, 2, CH₂), 4.22 (q, J=2.6, 1, 3'-H), 4.02 (t, J=9.4, 1, CH), 3.80 (q, J=3.8, 1, 4'-H), 3.5 (m, overlap with HDO, 5'-H), 2.13 (m, 2, 2'-H); decouple at 4.02: 4.44 (d, J=7.5, 2, CH₂); decouple at 2.12: 6.18 (s, 1, 1'-H), 4.24 (s, 1, 3'-H).

PO-1, DMSO-d_6: 7.88 (d, J=8.2, 1, 6-H), 6.16 (t, J=6.6, 1, 1'-H. 5.73 (d, J=8.1, 1, 5-H), ~5 (broad, 4, NH + 3'-OH + 5'-OH + COH), 4.22 (m, 1, 3'-H), 3.87 (m, 1, CH), 3.79 (q, J=3.2, 1, 4'-H), 3.61 (m, 2, CH₂), 3.56 (m, 2, 5'-H), 2.10 (m, 2, 2'-H), 1.00 (d, J=5.9, 3, CH₃); D₂O exchange: 7.84 (d, J=8.1, 1, 6-H), 6.13 (t, J=6.5, 1, 1'-H), 5.72 (d, J=8.1, 1, 5-H), 4.20 (m, 1, 3'-H), 3.85 (m, 1, CH), 3.78 (q, J=3.7, 1, 4'-H), 3.60 (m, 2, CH₂), 3.54 (t, J=4.8, 2, 5'-H), 2.07 (m, 2, 2'-H), 0.99 (d, J=5.7, 3, CH₃); decouple at 0.99: simplified the multiplet at 3.85; decouple at 3.78: 4.20 (t, J=3.7, 1, 3'-H), 3.54 (d, J=5.5, 2, 5'-H); decouple at 3.85: simplified the multiplet at 3.60.

PO-2, DMSO-d_6: 8.5 (broad, 2, NH₂), 7.82 (d, J=7.4, 1, 6-H), 6.15 (t, J=6.8, 1, 1'-H), 5.75 (d, J=7.5, 1, 5-H), 4.21 (m, 1, 3'-H), 3.76 (q, J=3.3, 1, 4'-H), 3.55 (m, 2, 5'-H), 1.94 (m, 2, 2'-H); in D₂O 7.87 (d, J=7.6, 1, 6-H), 6.25 (t, J=7.2, 1, 1'-H), 6.08 (d, J=6.1, 1, 5-H), 4.42 (q, J=3.6, 1, 3'-H), 4.05 (q, J=3.9, 1, 4'-H), 3.83 (m, 1, 5'-H), 3.74 (m, 1, 5'-H), 2.43 (m, 1, 2'-H), 2.30 (m, 1, 2'-H).

3-Methyl-dCyd, DMSO-d_6: 7.43 (d, J=8.0, 1, 6-H), 6.18 (s, 1, NH), 6.15 (t, J=6.6, 1, 1'-H), 5.88 (d, J=8.2, 1, 5-H), 4.21 (m, 1, 3'-H), 3.76 (q, J=3.1, 1, 4'-H), 3.54 (m, 2, 5'-H), 2.22 (m, 2, 2'-H).

Mass spectra

Electron ionization mass spectra were obtained on a Finnigan Model 4023 GC/MS instrument at 70 eV. Freeze dried samples were introduced by direct probe. Only *m/e*-values greater than 99 and above 1% relative intensity are listed except as noted. *M/e* (relative intensity):

TCPO-3, background subtracted, 265 (1.0), 236 (2.3) B-Cl, 155 (4.6) BH₂-CCl₃, 152 (1.6), 126 (2.0) 125 (6.6) BH₂-CCl₃CHO, 119 (1.1), 118 (9.6), 117 (98) S, 113 (1.5) BH₂-R, 110 (1.7), 99 (16) S-18.

TCPO-1, background subtracted, 238 (3.1), 237 (1.7), 236 (1.3) B-Cl, 201 (1.1), 155 (7.2) BH₂-CCl₃, 151 (1.3), 148 (1.2), 147 (1.4), 146 (11), 137 (2.0), 131 (2.4), 126 (2.8) BH₂-CCl₃CHO, 125 (7.5) BH-CCl₃CHO, 120 (1.9), 119 (7.9), 118 (12), 17 (100)S, 113 (3.9) BH₂-R, 108 (1.3), 103 (1.4), 99 (20)S-18.

PO-1, 242 (0.5) M-CH₃CO, 229 (1.4) MH₂-R, 212 (0.25) MH₂-OR 197 (1.3) B+28, 193 (0.3), 179 (2.4), 171 (4.5), 170 (0.4) B+2, 169 (0.3) B+1,

155 (3) BH_2-CH_3, 154 (1.9), 153 (8.1) BH_2-18, 137 (1.0), 130 (1.4), 127 (2.4), 126 (33) BH_2-CH_3CHO, 125 (1.6) BH-CH_3CHO, 118 (4.5), 117 (91)S, 114 (3), 113 (50) BH_2-R, 112 (3), 111 (1.4), 110 (1.4), 108 (3), 100 (4), 99 (50)S-18.

RESULTS AND DISCUSSION

UV spectral results

The UV peak maxima and absorbance ratios for fractions collected on the analytical HPLC system are listed in Table I. Peak maxima combined with absorbance ratios can be taken as the signature of most compounds and are powerful tools in identification of HPLC peaks [8,9]. Products with the same HPLC retention times had the same UV peak maxima and similar 254/280 nm absorbance ratios. The UV peak maximum for each site of dCyd alkylation is unique and therefore indicative of the position of alkylation [10]. Our results, similar to this review of the literature, show little variation in the peak maxima with a change in the attached alkyl group. All products eluting at 2 min, labelled 1, are consistent with O^2 alkylation, those eluting at 4 min, labelled 3, are consistent with N-3 alkylation and the late eluting products, labelled 4, are consistent with N^4 alkylation. Unreacted dCyd, labelled 2, eluted at 3 min.

In addition to peak maxima and absorbance ratios, O^2 and N-3 alkylated dCyd products can be characterized by their differences in stability. It has been noted [11,12] that O^2-alkyl dCyd is less stable in alkali and acid than

TABLE I

HPLC RETENTION TIMES AND UV SPECTRA OF FRACTIONS IN FIG. 1.

Fraction	Peak maximum[a]	254/280 nm Absorbance ratio	Retention times (min)
PO-1	263	2.29	2
PO-2	277	0.40	3
PO-3	282	0.31	4
PO-4	294	0.26	6
TCPO-1	263	2.09	2
TCPO-2	277	0.37	3
TCPO-3	281	0.26	4
EC-1	264	1.94	2
EC-2	276	0.40	3
EC-3	281	0.35	4
EC-4	293	0.25	5.5
Gl-1	263	2.28	2
Gl-2	278	0.37	3

[a]Taken in the HPLC buffer (pH 3.5).

TABLE II

UV DATA FOR PEAKS IN FIG. 1

Peak maxima and 254/280 nm absorbance ratios

Sample	0.1 M HCl	pH 5	H_2O	pH 9	0.01 M NaOH	0.1 M NaOH	Approx. pKa
TCPO-1	265[a](1.9)[b]	—	263 (2.0)	266 (1.1)	266 (1.1)	268 (0.98)	~9
TCPO-3	278 (0.54)	—	280 (0.4)	269 (0.95)	268 (0.96)	268 (0.97)	~9
PO-1	263 (1.8)	—	263 (2.2)	263 (1.7)	—	264 (1.3)	~9
PO-2[c]	276 (0.50)	272 (0.80)	271 (0.9)	—	—	268 (0.96)	~5

Peak maxima and 254/280 nm absorbance ratios

After 24 h in HCl, H_2O or NaOH

Sample	0.1 M HCl	add NaOH (1:1)[d]	0.1 M NaOH	add HCl (1:1)[d]
TCPO-1	266 (1.0)	268 (1.0)	266 (1.0)	268 (1.0)
TCPO-3	279 (0.5)	268 (1.0)	269 (1.0)	278 (0.8)
PO-1	263 (1.0)	264 (1.3)	264 (1.5)	265 (1.3)
PO-2	277 (0.5)	269 (0.97)	269 (0.95)	269 (0.97)

[a]Peak maxima (nm).
[b]Absorbance ratio in parenthesis.
[c]Unreacted dCD.
[d]NaOH or HCl (0.1 M, 0.5 ml) added to 0.5 ml solution in a 1-ml cuvette.

3-alkyl dCyd. The products isolated semi-preparatively, PO-1, TCPO-1 and TCPO-3, were subjected to acid and base treatment. It is apparent upon examination of Table II that both 0.1 M HCl and 0.1 M NaOH treatment irreversibly alters PO-1 and TCPO-1 while TCPO-3 and the unreacted PO-2 remains intact. This is consistent with identification of both PO-1 and TCPO-1 as O^2-alkyl dCyd and TCPO-3 as 3-alkyl dCyd. The spectra of PO-1, TCPO-1 and TCPO-3 in 0.1 M NaOH and 0.1 M HCl are given in Fig. 2.

The measurement of UV peak maxima and absorbance ratios of PO-1, PO-2, TCPO-1 and TCPO-3 at several pH values, as indicated on Table II, allowed for an approximate determination of the pKa [13]. The O^2 and N-3 alkylated products, PO-1, TCPO-1 and TCPO-3 exhibit a pKa around 9 which is indicative of an imino structure [11,14,15]. The N^4-alkylated products and unreacted dCyd, PO-2, which have the amino structure, exhibit a lower pKa around 4 [10].

IR, NMR and MS results

To confirm the validity of product identification by UV peak maxima, additional spectral information was obtained for the major products from the least and most reactive epoxides, PO and TCPO. All the spectral information, as discussed below, supports the UV results that products with the same HPLC retention times are alkylated at the same site of dCyd. In the IR spectra the N-3 alkylated product, TCPO-3, is distinguished from the O^2 alkylated products (TCPO-1 and PO-1) by the unique band at 1535 cm^{-1}. This is similar to the band at 1556 cm^{-1} in the 3-methyl-dCyd spectrum. The products, PO-1, TCPO-1 and TCPO-3, have an absorbance band in the 1708—1726 cm^{-1} range which would be consistent with an imino structure [16,17]. This band is not present in the amino starting material. Both of the TCPO products show a strong C-Cl absorption at 801 cm^{-1} [18].

The differences between O^2 and N-3 alkylation products are also evident in the 360 MHz NMR spectra. Proton assignments in the spectra of unreacted dCyd (PO-2) and 3-methyl-dCyd were made according to existing literature [19—22] which was extended to the interpretation of the reaction products' spectra. Decoupling data was also used in the interpretation of the DMSO-d$_6$ and D$_2$O exchange spectra of the reaction products. The chemical shifts for the pyrimidine and alkyl adduct protons are listed in Table III. For both 3-methyl-dCyd and TCPO-3, the 6-H is shifted to 7.4 ppm from the usual 7.9 ppm found in dCyd, PO-2, PO-1 and TCPO-1. In comparing the two TCPO alkylated products, the CH$_2$ of TCPO-3 is found further upfield than the CH$_2$ of TCPO-1, as would be expected for a CH$_2$ on a nitrogen atom compared with a CH$_2$ on an oxygen atom [23]. The alkyl adduct protons for both TCPO products are found further upfield than for the PO product due to the deshielding effect of the chlorines [24].

The mass spectra were useful in distinguishing between abnormal and normal product formation despite the fact that the products were marginally volatile for MS analysis, as is the case with dCyd [25]. Chemical ionization MS or derivitization with *N,O*-bis(trimethylsilyl)acetamide/trimethylchloro-silane/pyridine (100:1:10) did not yield additional information over the
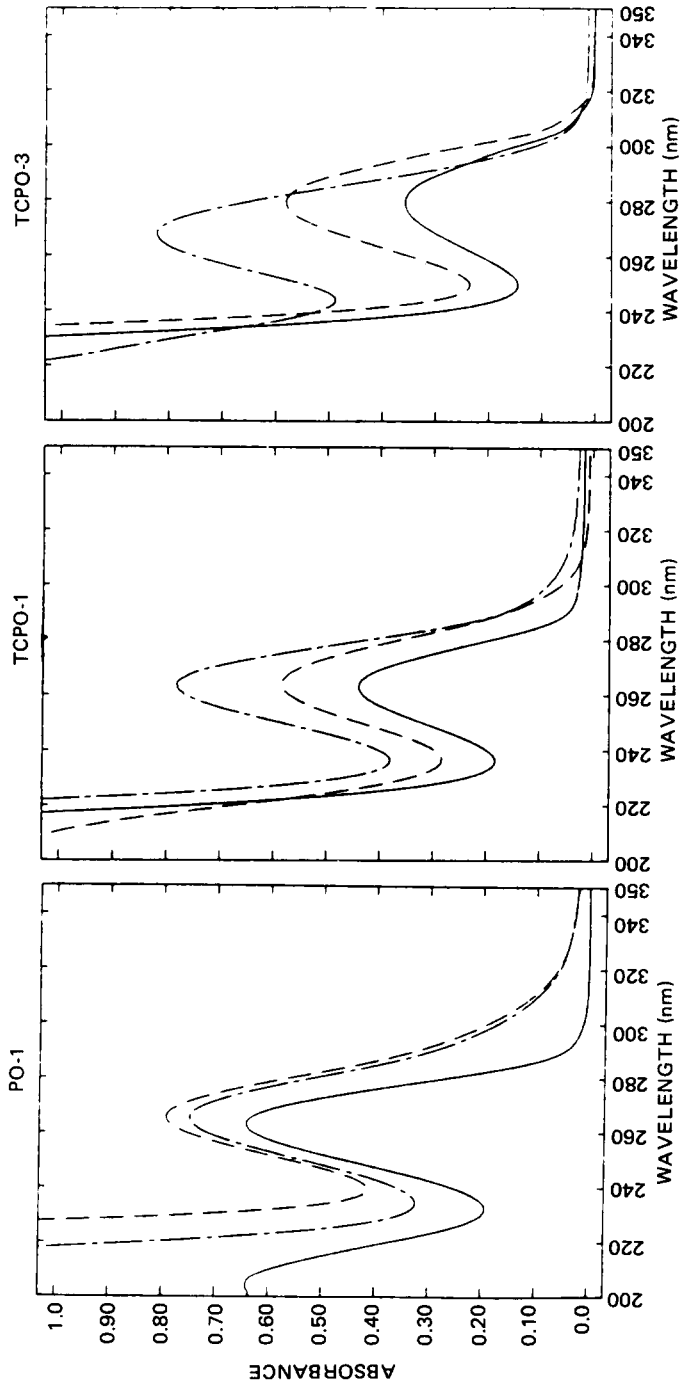
Fig. 2. UV spectra of PO-1, TCPO-1 and TCPO-3 at neutrality (———), in 0.1 M HCl (- - -) and in 0.1 M NaOH (— - —).

TABLE III

CHEMICAL SHIFTS FOR PYRIMIDINE AND ALKYL PROTONS[a]

Compound	Solvent	Pyrimidine protons			Alkyl protons			
		NH	C6	C5	CH$_2$	CH	COH	CH$_3$
TCPO-1	DMSO-d_6	~7.5 broad	7.91,d J=8.0	5.80,d J=8.2	4.44,m	4.03,m	6.81,t J=5.1	—
	D$_2$O Exchange	—	7.90,d J=8.0	5.75,d J=8.0	4.42,m	4.02,m	—	—
TCPO-3	DMSO-d_6	~6.9 broad	7.50,d J=8.4	5.97,d J=8.4	~3.4,m[b]	4.15,m	6.65,d J=6.5	—
	D$_2$O Exchange	—	7.47,d J=8.4	5.96,d J=8.4	3.33,m	4.14,m	—	—
PO-1	DMSO-d_6	~5.0 broad	7.88,d J=8.2	5.73,d J=8.1	3.61,m	3.87,m	~5.0 broad	1.00,d J=5.9
	D$_2$O Exchange	—	7.84,d J=8.1	5.72,d J=8.1	3.61,m	3.85,m	—	0.99,d J=5.7
PO-2[c]	DMSO-d_6	~8.5 broad (NH$_2$)	7.82,d J=7.4	5.75,d J=7.5	—	—	—	—
	D$_2$O	—	7.87,d J=7.6	6.08,d J=6.1	—	—	—	—
3-Methyl-dCyd	DMSO-d_6	~6.2	7.43,d J=8.0	5.88	—	—	—	3.21,s
dCyd	DMSO-d_6	7.12,d broad (NH$_2$)	7.79,d J=7.5	5.72,d J=7.4	—	—	—	—

[a]360 MHz NMR shifts from TMS; m, multiplet; q, quadruplet; t, triplet; d, doublet; s, singlet.
[b]Overlapping with water peak.
[c]Unreacted dCyd.

electron ionization spectra obtained and also were not satisfactory for the complete characterization of the dCyd derivatives. However, masses commonly found in nucleoside mass spectra from fragmentation of the molecular ion (M), pyrimidine base (B) and deoxysugar (S) moieties, such as $B+2$, $B+28$ and S-18 [25–27] are found in addition to masses expected for alkylated dCyd. Losses of alkyl side chain fragments from B are useful in distinguishing between normal (attachment at the least substituted carbon atom of the epoxide) and abnormal alkylation products. Similar to adenine alkylated by PO [3], normal alkylation allows for losses of CH_3, CCl_3, CH_3CHO and CCl_3CHO from the side chain while from the abnormal product losses of CH_2O and CH_2OH are expected. For the dCyd reaction products with PO and TCPO, the masses expected from normal product formation, m/e 155 and 125, are present. No evidence was found for abnormal alkylation which would result in masses of m/e 139 and 140 for PO-1 and masses m/e 240 and 241 for the TCPO products.

Comparative reactivity studies

For dCyd reacted with PO, GL, EC and TCPO, the differences in product yields among the epoxides were evident on the analytical system (Fig. 1 and Table IV). In contrast with previous findings [6] of alkylation at the same site of DNA bases (N-3 for dCyd) by different epoxides, we report differences in reactivity towards sites on dCyd for the epoxides tested. As shown in Table IV, only the more reactive compounds, EC and TCPO, alkylate predominantly at the N-3 position of dCyd. However, although differences in reactivity are present, it was total reactivity (represented by the ratio of remaining unreacted material, peak 2, to internal standard) that correlated to Taft $\sigma*$-electron withdrawing values [7] for the substituents.

TABLE IV

PEAK HEIGHT RATIOS AND TAFT $\sigma*$-VALUES

Reaction	Peak height ratios[a]				Taft $\sigma*$-values[b]
	1 : IS[c]	2 : IS	3 : IS	4 : IS	
dCyd/PO	0.72 ± 0.1	2.9 ± 0.3	0.28 ± 0.03	0.09 ± 0.06	0.00
dCyd/GL	2.0 ± 0.06	2.4 ± 0.1	—	—	0.56
dCyd/EC	0.04 ± 0.006	2.0 ± 0.06	0.87 ± 0.03	0.17 ± 0.006	1.05
dCyd/TCPO	0.70 ± 0.1	0.6 ± 0.2	1.2 ± 0.4	—	2.65

[a]Peak height ratios of HPLC peaks in Fig. 1 to internal standard average of 3 separate reactions $\pm$ S.D.
[b]Taft $\sigma*$-values for substituents on the epoxides: $X=CH_3$, $HOCH_2$, $ClCH_2$ and Cl_3C on X-CH-CH$_2$ from Ref. 7.
        \/
         O
[c]Internal standard.

The correlation of total reactivity to Taft $\sigma *$-values was excellent ($r = 0.9999$) and is comparable to our previous correlation [28] with nicotinamide ($r = 0.9438$) and p-nitrobenzyl pyridine ($r = 0.9474$) reactivities. Deoxycytidine alkylation may be interesting to pursue in other alkylation studies since O^2-ethylcytosine has been found to persist in alkylated DNA in vivo [29,30], and the more extensively studied formation and persistence of O^6-alkylguanine has not always correlated with toxicity [31—35]. In fact, for alkylated poly(deoxycytidylic-deoxyguanylic) acid, most misincorporations during replication have been found opposite modified cytosine residues [36].

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