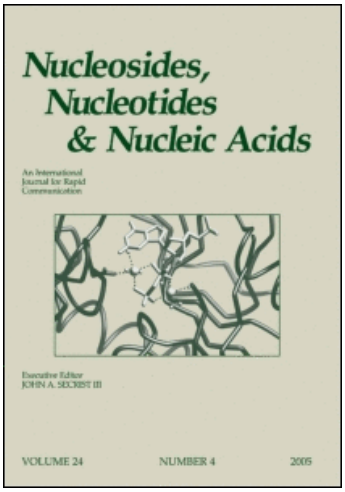

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REACTION OF EPICHLOROHYDRIN WITH ADENOSINE AND URIDINE- 5'-MONOPHOSPHATES

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ABSTRACT

The reaction of epichlorohydrin with 5'-AMP and 5'-UMP in aqueous buffer at pH 5.8 and 37°C gave two principal products for each nucleotide. These products are characterised as N^1-(3-chloro-2-hydroxypropyl) adenine, **1**; 3'-O-(3-chloro-2-hydroxypropyl)-5'-AMP, **2**; 3'-O-(3-chloro-2-hydroxypropyl)-5'-UMP, **3**; and N^3-(3- chloro-2-hydroxypropyl)-5'-UMP, **4**. The structure of the products are assigned using UV spectroscopy, ^1H and ^{13}C-NMR spectroscopy as well as FAB and high resolution mass spectrometry.

INTRODUCTION

Adducts of nucleic acids with carcinogens and mutagens are of interest due to their possible implications in the process of carcinogenesis and mutagensis.[1] Due to their high chemical reactivity, aliphatic epoxides are widely used chemical intermediates in industry and at the same time reported to be responsible for mutagenicity and production of chromosomal aberrations. Thus solutions of ethylene oxide produce mutations[2] in *S. typhimurium* TA 1535, reverse mutations in *Neurospora crassa*[3] and chromosomal aberrations in mammalian somatic cells.[4] Chemically ethylene oxide reacts with DNA to give N^7- hydroxyethylguanine.[5] Propylene oxide in a limited study has been reported to be carcinogenic in rats,[6] produces reverse mutations in *Neurospora crassa*[7] and chemically reacts with DNA at neutral pH to yield N^7-(2-hydroxypropyl) guanine and N^3-(2-hydroxypropyl) adenine.[8] Chloroethylene oxide and chloroacetaldehyde behave in their reactions with nucleic acid bases as bifunctional alkylating agents eventually yielding cyclic adducts.[9,10,11]

Epichlorohydrin is a widely used industrial chemical intermediate, it induces reverse mutations in *S. typhimurium* G 46 and TA 100 tester strains[12] and also induces chromosomal aberrations in bone marrow of IRC mice.[13] Information about the chemical reactivity of epichlorohydrin with nucleotides is lacking in the literature which should be of interest due to its mutagenic properties. The present communication describes the reaction of epichlorohydrin with 5′-AMP and 5′-UMP under mild aqueous conditions, the characterisation of new products obtained has been done using modern spectroscopic techniques.

RESULTS AND DISCUSSION

The reaction of 5′-AMP with epichlorohydrin was carried out in sodium acetate buffer at pH 5.8 and 37°C for 7 days, two new products of R_f 0.71 and 0.64 in solvent A, were detected on TLC over cellulose apart from unreacted starting material of R_f 0.18. The products were separated by partitioning over microcrystalline cellulose column using solvent A. The compound of R_f 0.71 was eluted in fractions 28-45 and its UV spectrum at pH 1 exhibited λ_{max} at 260 nm which was shifted to 270 nm at pH 12, the shift resembled that of an N^1 substituted adenine[14] rather than an N^1 substituted nucleoside or nucleotide.[15] The ^{1}H-NMR spectrum of the product was devoid of any ribose signals and exhibited signals due to adenine ring and alkyl side chain. Two singlets integrating for one proton each at δ 8.2 and 8.1 were assigned to H-8 and H-2 of adenine ring respectively. A multiplet at δ 3.58 for two protons was ascribed to methylene protons H-1 of the side chain. Another multiplet at δ 3.81 was ascribed to the methine proton H-2 and the multiplet at δ 3.52 for two protons was ascribable to methylene protons H-3 bearing the chlorine, these assignments agree with the values reported in the literature.[16] The evidence derived from the UV spectrum and ^{1}H-NMR led this compound to be characterised as N^1-(3-chloro-2-hydroxypropyl) adenine **1**, which was further confirmed by mass spectral analysis. FAB mass spectrum in the positive mode showed the molecular ion $[M^+]$ at m/z 227 and high resolution mass at m/z 227.0572 which corresponded with the molecular formula $C_8H_{10}ClN_5O$. The presence of the chlorine was confirmed by $[M+2]^+$ peak at m/z 229 which had a relative abundance of 35% of that of molecular ion due to the isotope ^{37}Cl. The peak matching analysis of important fragments also confirmed this assignment. Thus, high resolution mass showed an ion at m/z 192.6882 due to fragment $[M\text{-}Cl]^+$ and molecular formula $C_8H_{10}N_5O$ and an ion at m/z 178.0726 corresponding to ion $[M\text{-}CH_2Cl]^+$ and molecular formula $C_7H_8N_5O$.

The UV spectrum of the product R_f 0.64 was identical to that of 5′-AMP at pH 1 and 12 indicating an unsubstituted base moiety while paper electrophoresis

TABLE-1. ^{13}C-Chemical shifts (ppm) in the NMR spectra (D_2O) of some 3-chloro-2-hydroxypropyl derivatives of 5′- AMP and 5′-UMP.

Nucleotide derivative	Base carbons					Ribose carbons					Alkyl side chain		
	C-2	C-4	C-5	C-6	C-8	C-1′	C-2′	C- 3′	C-4′	C-5′	C-1	C-2	C-3
5′-AMP.2Na.	147.99	153.08	121.23	151.09	144.87	90.63	77.36	73.01	86.92	66.97	–	–	–
3′-(3-chloro-2-hydroxypropyl)-5′-AMP, **2**	148.34	152.00	121.76	150.20	144.84	90.33	76.91	88.11	88.24	66.84	65.20	72.80	47.00
5′-UMP.2Na.	168.99	154.54	105.26	144.33	–	85.98	72.37	76.53	85.90	67.10	–	–	–
3′-(3-chloro-2-hydroxypropyl)5′-UMP.Na_2, **3**	168.24	154.76	104.75	142.96	–	92.0	72.79	84.52	86.89	66.21	65.23	72.50	49.70
N^3-(3-chloro-2-hydroxypropyl)5′-UMP.Na_2, **4**	168.07	154.80	104.40	142.60	–	86.84	72.04	76.53	84.50	66.24	63.42	71.83	46.20

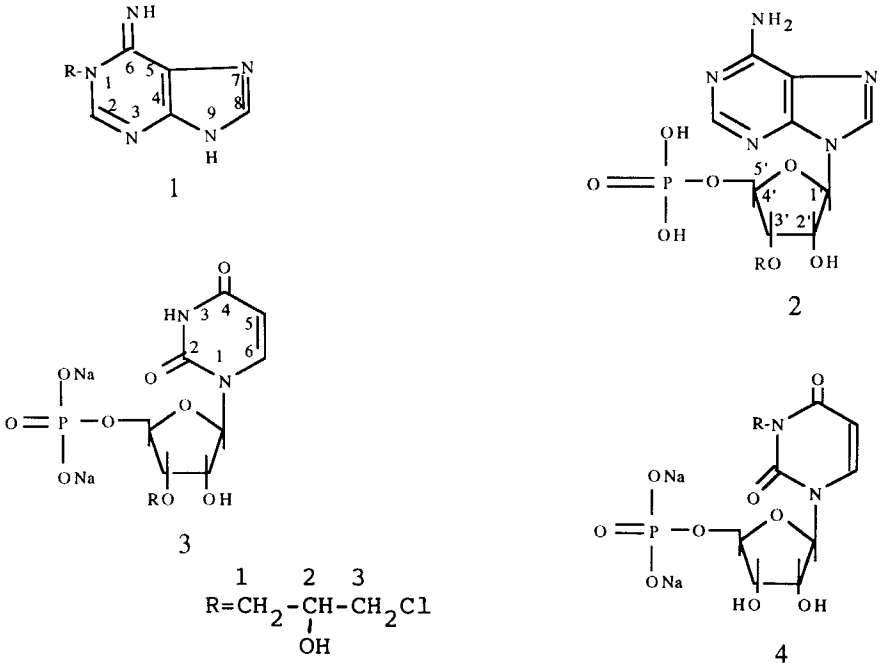

FIG-1.

also suggested an unsubstituted phosphate. The ^{1}H-NMR spectrum of the product exhibited the H-8 and H-2 signals of the adenine base at δ 8.26 and 8.17 respectively which were assigned on the basis of literature values.[17] The anomeric proton H-1′ was located upfield as compared to that of unsubstituted 5′-AMP indicating substitution of 3′-OH group[18] by epichlorohydrin. The protons of the alkyl group appeared at δ 4.12, a multiplet for two methylene protons ascribed to H-1, a multiplet at δ 3.93 for 1H, ascribed to methine H-2 and another multiplet for 2H at δ 3.62 ascribed to methylene protons H-3 which bears the chlorine. The product was therefore assigned as 3′-O-(3-chloro-2-hydroxypropyl)-5′-AMP, **2**. The structure is confirmed by FAB (negative ion) and high resolution mass spectrometry. The FAB (negative ion) MS exhibited $[M-H]^-$ at m/z 439 and high resolution mass at m/z 439.0662 corresponding to exact molecular formula, $C_{13}H_{19}ClN_5O_8P$. Another peak at m/z 441 with relative abundance of 35% of that of molecular ion was observed which corresponds to the ion $[M-H+2]^-$ and molecular formula $C_{13}H_{19}{}^{37}ClN_5O_8P$. Peak matching analysis of ions $[M-Cl]^-$ and $[M-CH_2Cl]^-$ were also in complete agreement with the assigned structure. In order to further confirm the site of reaction at the ribose moiety use of ^{13}C-NMR was

made as the substitution at ribose hydroxyls leads to shifts of larger magnitudes for the substituted carbon atom.[19,20] The ^{13}C-NMR of **2** indeed exhibited a pronounced downfield shift of C-3′ to the extent of 15 ppm (Table-1), confirming the structural assignment.

The reaction of 5′-UMP with epichlorohydrin under similar conditions showed the formation of two new products of R_f 0.77 and 0.73 on TLC over cellulose in solvent A. The product of R_f 0.77 was obtained in fractions 23-41 on partition column chromatography over cellulose using solvent A. The UV spectrum at pH 1 and 12 as well as electrophoretic mobility in buffer I and II were identical to 5′-UMP indicating an unsubstituted base as well as phosphate moieties. The ^{1}H-NMR spectrum in D_2O showed signals for H-6 and H-5 of the base at δ 7.94 and 5.96 respectively as sharp doublets of 8 Hz each, while a doublet of 4.3 Hz for anomeric proton H-1′ was located at δ 5.94. As compared to the anomeric proton of 5′-UMP the compound showed an upfield shift indicating substitution of 3′-hydroxyl of ribose.[18] The protons of alkyl group were located at δ 4.11, 3.94 and 3.64 as multiplets integrating for 2H, 1H and 2H and were assigned to H-1, H-2 and H-3 protons respectively. FAB (negative ion) MS exhibited $[M-H]^-$ at m/z 459 and high resolution MS exhibited exact mass at 459.0126 corresponding to the correct molecular formula $C_{12}H_{15}ClN_2Na_2O_{10}P$. The presence of $[M-H+2]^-$ peak at m/z 461 with relative abundance of 35% of that of molecular ion also confirmed the presence of chlorine. The peak matching analysis of other fragments $[M-H-Cl]^-$ at m/z 424 and $[M-H-CH_2Cl]^-$ at m/z 410 completely supported the structure of the product as 3′-O-(3-chloro-2-hydroxypropyl)-5′-UMP, disodium salt **3**. The location of the substitution at the 3′-hydroxyl of ribose was further confirmed by ^{13}C-NMR spectrum (Table-1). As expected the 3′-carbon of ribose was shifted to about 8 ppm downfield as compared to that of 5′-UMP.

The product of R_f 0.73 in solvent A was obtained in fractions 52-81 on cellulose column chromatography. The UV spectrum of this product exhibited a characteristic shift of λ_{max} from 260 nm at pH 1 to 264 nm at pH 12 indicating substitution at N^3-position of the uridine.[21] The paper electrophoresis in buffers I and II did not show phosphate esterification. the ^{1}H-NMR spectrum exhibited signals for H-5 and H-6 protons as 8 Hz doublets at δ 5.98 and 8.01 respectively while the anomeric proton H-1′ appeared at δ 6.02 as a sharp doublet of 4.3 Hz, identical to that of 5′-UMP indicating an unsubstituted ribose. The protons of alkyl side chain resonated at δ 4.01 a multiplet for H-1, the methine proton H-2 at δ 3.91 and the halogen bearing methylene protons H-3 at δ 3.53. the product was therefore assinged as N^3-(3-chloro-2-hydroxypropyl)-5′-UMP as the disodium salt

4. The assignment was further confirmed by FAB (negative ion) MS, which exhibited $[M-H]^-$ at m/z 459, high resolution MS gave exact mass at m/z 459.0126 corresponding to exact molecular formula $C_{12}H_{15}ClN_2Na_2O_{10}P$. The signal for $[M-H+2]^-$ due to ^{37}Cl was localed at m/z 461 whose relative abundance was 35% of that of molecular ion. The peak matching of other fragments also corresponded with the structure. The ^{13}C-NMR spectrum (Table-1) also showed an unsubstituted ribose moiety. The results of this study show that epichlorohydrin reacts with 5′-AMP and 5′-UMP, under the reported reaction conditions as an alkylating agent principally through opening of epoxide ring as has been reported for other epoxides.[5,14] The results described in this publication deserve comments, particularly the formation of 3′-O-alkyl derivatives of 5′-AMP and 5′-UMP. Martin *et.al.*[18] have reported the formation of both 2′-O- and 3′-O- methyl derivatives of adenosine and cytidine by using diazomethane in hot 1,2-dimethoxyethane, the yield of 2′-O-isomer being three times greater than that of 3′-O-isomer. It is known that alkylation of nucleosides is dependent upon the reaction conditions used such as nature of alkylating agent, pH of the reaction, nature of the solvent etc. and therefore the absence of 2′-O-alkylation may possibly be attributed to the reaction conditions employed during the present studies. The formation of N^1-alkylated adenine during the reaction reflects a greater sensitivity of purine nucleotides towards depurination as compared to pyrimidine nucleotides. In the literature[22] methylation of 5′-AMP with dimethyl sulfate at pH 4.5 and 6.8-7.4 has been reported to give N^1-methyl adenine as one of the products and therefore the isolation of N^1-alkylated adenine **1** as one of the products may be a result of depurination of 5′-AMP. It is expected that the reaction of 5′-UMP should also result into the formation of a disubstituted product containing alkyl groups both at the base and ribose, but such a product was not detected, however formation of such a derivative cannot be completely ruled out.

EXPERIMENTAL

5′-AMP and 5′-UMP disodium salts were from BDH (England). Partition column chromatography over microcrystalline cellulose was carried out in a glass column (50x1.5 cm), 5 ml fractions were collected using solvent A, (ethanol:0.5 M, ammonium formate pH 7.4; 7:3 v/v). For ascertaining the purity of products thin layer precoated cellulose plates (E. Merck) were used, solvents being A; solvent B (isopropanol:ammonium hydroxide:water; 7:1:2 v/v) and solvent C (n-propanol:ammonium hydroxide:water; 55:10:35 v/v). UV spectra were run on a Unicam SP-500 spectrometer. ^1H and ^{13}C-NMR spectra were determined on Bruker AM-300 spectrometer as 5 mg/0.5 ml solutions in D_2O. For ^{13}C-NMR, DSS

was used as the internal standard. Paper electrophoreses was carried out on Whatman No. 1 strips (43.5x8.8 cm) at 40 volts and 18 Amps. for two hours, using buffer I, 0.05 M, phosphate, pH 8.5 and buffer II, 0.1 M acetate pH 4.3. FAB-MS and peak matching analysis were recorded on Finnigan MAT-312 mass spectrometer connected to PDP 11/34 computer system.

N^1-(3-chloro-2-hydroxypropyl) adenine, **1**. 5'-AMP (182 mg) was incubated with epichlorohydrin (0.16 ml) in sodium acetate buffer (5 ml) pH 5.8 at 37° for 7 days. The reaction mixture was concentrated to dryness under vacuum below 40°C, dissolved in solvent A (3 ml) and loaded on cellulose column. The fractions were monitored on cellulose TLC and the product, which appeared in fractions 28-45 was pooled, solvent was evaporated under vacuum and dried over P_2O_5, to yield an amorphous powder (42 mg), R_f solvent A, 0.71, solvent B, 0.58 and solvent C, 0.62. UV, pH 1, λ_{max} 260, λ_{min} 230 nm; pH 12 λ_{max} 270, λ_{min} 232 nm. ^{1}H-NMR (300 MHz): δ 3.58 (m, 2H, -CH_2); 3.81 (m, 1H, -CH-); 3.52 (m, 2H, -CH_2Cl); 8.10 (s, 1H, H-2); 8.20 (s, 1H, H-8). FAB (positive ion) MS: m/z 227 $[M]^+$; 229 [M+2, 35% of M^+], 192, 178. High resolution mass: $C_8H_{10}ClN_5O$, calcd. 227.0572 found 227.0574; $[M\text{-}Cl]^+$ $C_8H_{10}N_5O$, calcd. 192.0882, found 192.0885; $[M\text{-}CH_2Cl]^+$, $C_7H_8N_5O$, calcd. 178.0726 found 178.0729.

3'-O-(3-chloro-2-hydroxypropyl)-5'-AMP, **2**. The product which appeared in fractions (60-84) was pooled, solvent was evaporated *in vacuo* below 40°C to get an amorphous hygroscopic powder (48 mg); R_f solvent A 0.64, solvent B 0.46 and solvent C 0.52. UV: pH 1 λ_{max} 260 nm λ_{min} 230 nm; pH 12 λ_{max} 260 nm and λ_{min} 230 nm. Paper electrophoresis buffer I 8.1 cm (AMP 8.1 cm), buffer II 5.1 cm (AMP 5.1 cm). ^{1}H-NMR, δ = 4.12 (m, 2H, -CH_2), 3.93 (m, 1H, -CH-), 3.62 (m, 2H, -CH_2Cl), 4.11 (m, 2H, H-5'), 5.91 (d, J = 4.8 Hz, 1H, H-1'), 8.26 (s, 1H, H-8), 8.17 (s, 1H, H-2). FAB (negative ion) MS: m/z 439 $[M]^-$, 441 [M+2, 35% of M^-]. High resolution mass, $C_{13}H_{19}ClN_5O_8P$. calcd. 439.0662 found 439.0660. The ion $[M\text{-}Cl]^-$ $C_{13}H_{19}N_5O_8P$ calcd. 404.0973, found 404.0971 and [M-CH_2Cl], $C_{12}H_{17}N_5O_8P$ calcd. 390.0813 found 390.0815.

3'-O-(3-chloro-2-hydroxypropyl)-5'-UMP, Disodium salt, **3**. 5'-UMP (184 mg) was reacted with epichlorohydrin (0.16 ml) in 1.25 M sodium acetate buffer pH 5.8 (5 ml) for 7 days at 37°C. The crude reaction mixture on cellulose TLC showed the formation of two new products. The reaction mixture was evaporated to dryness *in vacuo* below 40°C, dissolved in solvent A (5 ml) and chromatographed over microcrystalline cellulose column using solvent A. The first product appeared in fractions 23-41. The fractions were pooled and evaporated to dryness to yield an

amorphous powder (42 mg), Rf solvent A 0.77, solvent B 0.43 and solvent C 0.68. UV: pH 1, λ_{max} 260 nm, pH 12 λ_{max} 260 nm. Paper electrophoresis buffer I 9.4 cm (5'-UMP, 9.4 cm). ^{1}H-NMR (300 MHz): δ 4.11 (m, 2H, -CH_2), 3.94 (m, 1H, -CH), 3.64 (m, 2H, CH_2-Cl), 5.94 (d, J = 4.3 Hz, 1H, H-1'), 5.96 (d, J = 8.1 Hz, 1H, H-5), 7.94 (d, J = 8.1 Hz, 1H, H-6). FAB (negative ion) MS: m/z 459 $[M-H]^-$, 461 [M-H+2, 35% of $M-H^-$]. High resolution mass $C_{12}H_{15}ClN_2Na_2O_{10}P$, calcd. 459.0126 found 459.0122. The ion $[M-H-Cl]^-$ $C_{12}H_{15}N_2Na_2O_{10}P$, calcd. 424.0262 found 424.0260 and the ion $[M-H-CH_2Cl]^-$ $C_{11}H_{13}N_2Na_2O_{10}P$, calcd. 410.0105 found 410.0103.

N^3-(3-chloro-2-hydroxypropyl)-5'-UMP-disodium salt, **4**. The fractions (52-81) containing the product were pooled and worked up in the manner described above to get an amorphous powder (52 mg), Rf solvent A 0.73, solvent B 0.40 and solvent C 0.64. UV: pH 1 λ_{max} 260 nm, pH 12 λ_{max} 265 nm. Paper electrophoresis buffer I 8.8 cm (5'-UMP 8.8 cm), buffer II 3.2 cm, (5'-UMP 3.2 cm). ^{1}H-NMR (300 MHz): δ 4.01 (m, 2H, -CH_2), 3.91 (m, 1H, -CH), 3.53 (m, 2H, -CH_2Cl), 4.16 (dd, 2H, H-5'), 4.27 (m, 1H, H- 4'), 6.02 (d, J = 4.3 Hz, 1H, H-1'), 5.98 (d, J = 8.0 Hz, 1H, H-5), 8.01 (d, J = 8.0 Hz, 1H, H-6). FAB (negative ion) MS: m/z 459 $[M-H]^-$, 461 [M-H+2, 35% of $M-H^-$], 424, 410. High resolution MS: $C_{12}H_{15}ClN_2Na_2O_{10}P$, calcd. 459.0126 found 459.0122. The ion $[M-H-Cl^-]$ $C_{12}H_{15}N_2Na_2O_{10}P$, calcd. 424.0262 found 424.0260 and the ion $[M-H-CH_2Cl]^-$ $C_{11}H_{13}N_2Na_2O_{10}P$, calcd. 410.0105 found 410.0103.

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