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PREPARATION OF ELECTROPHORIC DERIVATIVES OF N7-(2-HYDROXYETHYL)GUANINE, AN ETHYLENE OXIDE DNA ADDUCT

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SUMMARY

Ethylene oxide, a potential human carcinogen, mainly damages DNA by reacting at guanine sites to form N7-(2-hydroxyethyl)guanine. In order to determine this DNA adduct with high sensitivity by gas chromatography, we have prepared, for comparison purposes, four electrophoric derivatives. The two that are most promising to date are bis- and tris-pentafluorobenzyl products prepared by first chemically transforming the N7-(2-hydroxyethyl)guanine to a corresponding xanthine, and then reacting the latter with pentafluorobenzyl bromide. These two derivatives are obtained in good yields and give molar responses of 0.6 and 0.5, respectively, relative to that of lindane by gas chromatography with electron-capture detection.

INTRODUCTION

Ethylene oxide is considered to be a potential human carcinogen[1] and is weakly mutagenic[2]. Its genotoxic effects and their reference to human cancer have been reviewed[3,4]. It causes cancer in exposed animals (see, *e.g.*, ref. 5). This chemical is widely used: approximately $2.5 \cdot 10^6$ tons per year are consumed in Western Europe, and about $8 \cdot 10^5$ tons are produced annually in the U.S.A. where roughly 150 000 workers deal with it daily in various industrial processes[4] (1986 data). For example it is commonly used in hospitals to sterilize medical equipment[6].

As an alkylating agent, ethylene oxide reacts with both proteins and DNA. Hemoglobin adducts of ethylene oxide have been measured to monitor animal or human exposure to this chemical[7–10]. The reaction of ethylene oxide with DNA is probably the reason for its carcinogenic and mutagenic effects. N7-(2-Hydroxyethyl)-guanine (**1**) is the main product when it reacts with DNA[7]. Other, less abundant adducts are O^6-(2-hydroxyethyl)guanine, and N1, N3 and N7 adenine products[11].

Our approach to quantifying a DNA adduct such as that from ethylene oxide is to isolate the adduct as a modified nucleobase, derivatize it with an electrophore, and quantify the product by gas (GC) or liquid (LC) chromatography with detection by electron-capture negative-ion mass spectrometry (ECNI-MS). Such methodology is attractive because of its potential for high sensitivity and specificity, which is required for measurement of DNA adducts in human samples. Towards this general goal, we

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have been optimizing appropriate electrophoric derivatives, sample preparation steps and instrumental conditions (see, *e.g.*, ref. 12). It is useful to form a pentafluorobenzyl derivative of an alkyl and related DNA adduct that can be isolated as a modified nucleobase, because of the excellent GC–ECNI-MS properties of such a derivative. Prior work has included the development of a sensitive electrophoric derivative of O^6-(2-hydroxyethyl)guanine[13]. Here we report the preparation of such a derivative of N7-(2-hydroxyethyl)guanine.

EXPERIMENTAL

Materials

N7-(2-Hydroxyethyl)guanine was purchased from Chemical Science Labs. (Lenexa, KS, U.S.A.). N7-Methylxanthine was purchased from Sigma (St. Louis, MO, U.S.A.). Pentafluorobenzyl bromide (PFBzBr), potassium carbonate, chloroacetaldehyde, diethyl ether, methyl iodide and tetrabutylammonium hydrogen sulphate were from Aldrich (Milwaukee, WI, U.S.A.). Organic solvents were from American Scientific (Boston, MA, U.S.A.). They were distilled and dried over molecular sieves prior to use. Preparative and analytical thin-layer chromatography (TLC) separations were performed with GHLF silica gel Uniplates with fluorescence indicator (Analtech, Newark, DE, U.S.A.). Flash column chromatography was performed with silica gel 60 (200–300 mesh) from (EM Science, NJ, U.S.A.).

All compositions were v/v unless indicated otherwise.

Synthesis

N^2,3-Etheno-N7-(2-hydroxyethyl)guanine (**2**). A mixture of N7-(2-hydroxyethyl)guanine (30 mg, 0.15 mmol) and chloroacetaldehyde diethyl ether (7 ml, 46 mmol) in 30 ml of water was heated at 80°C for 15 h. The reaction turned clear and homogenous after 6 h. Formation of product was monitored by TLC (fluorescence spot). The residue after rotary evaporation was further evaporated with 10 ml of benzene. The crude product was purified by flash column chromatography using methanol–dichloromethane (15:85), yielding 25 mg (80%) of product. ^{1}H NMR (300 MHz, $C^2H_3O^2H$) δ 8.32 (s, 1H), 8.0 (d, 1H), 7.65 (d, 1H), 4.59 (t, 2H), 3.56 (t, 2H).

N1-Pentafluorobenzyl-N^2,3-etheno-N7-(2-hydroxyethyl)guanine (**3**). To a stirred suspension of **2** (10 mg, 0.046 mmol) in 5 ml of acetonitrile–acetone (4:1) were added 12 mg (0.085 mmol) of potassium carbonate. The reaction mixture was stirred for 3 min and pentafluorobenzyl bromide (0.1 ml, 0.65 mmol) was added. The reaction mixture was stirred at room temperature for 20 h and filtered. The filtrate was vacuum evaporated and the crude product was purified by preparative TLC using methanol–chloroform (1:10), to yield 7.3 mg (45%) of product. ^{1}H NMR ($C^2H_3O^2H$ δ 8.01 (s, 1H), 7.65 (d, 1H), 7.35 (d, 1H), 5.49 (s, 2H), 4.59 (t, 2H), 3.95 (t, 2H).

N7-(2-Hydroxyethyl)xanthine (**4**). N7-(2-Hydroxyethyl)guanine (20 mg, 0.102 mmol) was dissolved in 1 *M* hydrochloric acid (1.5 ml) and the solution was cooled to 0°C. A cold solution of sodium nitrite (105 mg, 1.52 mmol) in 0.8 ml 1 *M* hydrochloric acid was added dropwise and the resulting solution was stirred at room temperature for 30 min and continued at 80°C overnight. TLC indicated the disappearance of starting material and appearance of a new product with higher R_F value using *n*-butanol–acetic acid–water (10:0.5:2). The reaction mixture was neutralized with sodi-

um hydroxide and cooled to 0°C. The precipitated product was isolated by centrifugation, washed three times with cold water and once with hexane. It was vacuum dried yielding product as a white powder (14 mg, 70%). MS (electron impact, EI): 196 (M^+), UV: λ_{max} 260, 228 nm (1 *M* HCl). ^{1}H NMR (300 MHz, $^2H_2O/^2HCl$) δ 8.75 (s, 1H), 4.65 (t, 2H), 4.01 (t, 2H).

1,3-Bis-(pentafluorobenzyl)-N7-(2-hydroxyethyl)xanthine (**5**). To a stirred suspension of potassium carbonate (46 mg, 0.33 mmol) in dry acetone–acetonitrile (1:1, 1 ml) at room temperature was added 6.5 mg (0.033 mmol) of N7-(2-hydroxyethyl)xanthine. Pentafluorobenzyl bromide (0.49 ml, 3.3 mmol) was added followed by continued stirring for 24 h. TLC using ethyl acetate–hexane (85:15), showed the product at R_F = 0.54. The potassium carbonate was removed by filtration and washed with acetone–acetonitrile (1:1). Evaporation of the solvent yielded a yellow solid which was purified by flash column chromatography using ethyl acetate–hexane (80:20), followed by evaporation. Hexane addition and overnight storage in the refrigerator gave, after filtration and drying, 9.5 mg (52%) of white solid. ^{1}H NMR (C^2HCl_3, 300 MHz) δ 7.67 (s, 1H), 5.29 (s, 2H), 5.40 (s, 2H), 4.45 (t, 2H), 3.95 (t, 2H).

1,3-Bis-(pentafluorobenzyl)-N7-(2-methoxyethyl)xanthine (**7**). To a stirred solution of **5** (4 mg, 0.007 mmol) in dichloromethane (1.4 ml) was added 0.7 ml of 1 *M* potassium hydroxide followed by $(C_4H_9)_4NHSO_4$ (12.2 mg, 0.035 mmol). After the resulting solution was stirred at room temperature for 5 min, methyl iodide (0.028 ml, 0.45 mmol) was added and stirring was continued for 18 h. TLC using ethyl acetate hexane (1:1), indicated the disappearence of starting material and formation of a new product (R_F = 0.4). The organic layer was separated, the aqueous layer extracted three times with dichloromethane and the combined dichloromethane fractions were washed with 5% hydrochloric acid, brine, and dried over anhydrous sodium sulfate. The filtered solution was evaporated *in vacuo* to yield a crude product which was purified by flash column chromatography using ethyl acetate–hexane (1:1), yielding a white solid (3.7 mg, 90%). ^{1}H NMR (300 MHz, C^2HCl_3: δ 7.68 (s, 1H), 5.04 (s, 2H), 5.25 (s, 2H), 4.25 (t, 2H), 3.79 (t, 2H), 3.25 (s, 3H).

1,3-Bis-(pentafluorobenzyl)-N7-(2-[pentafluorobenzyloxy]ethyl)xanthine (**6**). To a stirred solution of **5** (2 mg, 0.0035 mmol) in dichloromethane (0.6 ml) was added 0.3 ml of 1 *M* potassium hydroxide, $(C_4H_9)_4NHSO_4$ (6.1 mg, 0.018 mmol) and pentafluorobenzyl bromide (0.01 ml, 0.07 mmol). After stirring at room temperature for 20 h, the reaction mixture was worked up the same as for **5**. Flash column chromatography using ethyl acetate–hexane (3:7), yielded a yellow oil (1.2 mg, 47%). ^{1}H NMR (300 MHz, C^2HCl_3) δ 7.58 (s, 1H), 5.45 (s, 2H), 5.25 (s, 2H), 4.64 (s, 2H), 4.45 (t, 2H), 3.80 (t, 2H). MS (EI) *m/z* 735 (M^+), 554 (M − PFBz), 181 (PFBz).

Compound **6** was also synthesized as follows: to a stirred solution of **4** (2.4 mg, 0.012 mmol) in dichloromethane (0.4 ml), 1 *M* potassium hydroxide (0.2 ml), $(C_4H_9)_4NHSO_4$ (22 mg, 0.073 mmol) and pentafluorobenzyl bromide was added. The work-up procedure was the same for **6**, giving 8 mg (80%) of product after flash column chromatography.

1,3-Bis-(pentafluorobenzyl)-N7-methylxanthine (**10**). To a stirred solution of N7-methylxanthine (6.5, 0.039 mmol) in 5 ml of acetonitrile–acetone (1:1), was added 46 mg (0.33 mmol) of potassium carbonate. The reaction mixture was stirred for 1 min and pentafluorobenzyl bromide (0.25 ml, 1.4 mmol) was added. The reaction was stirred for 24 h at room temperature and filtered. The filtrate was rotary-evaporated

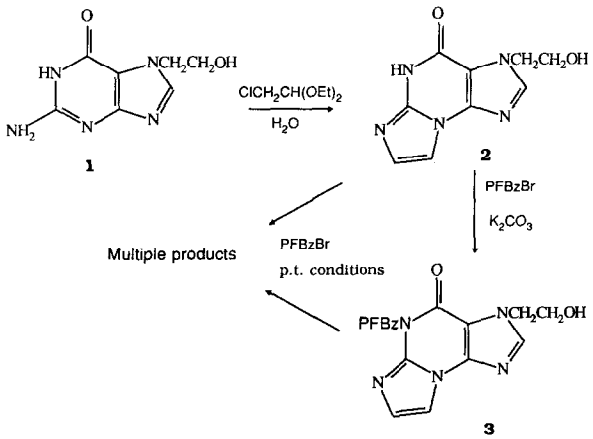
and the crude product was purified by flash column chromatography using ethyl acetate–hexane (5:3), to yield 11.5 mg (55%) of product. ^{1}H NMR (300 MHz, $^2HCl_3$): δ 7.5 (s, 1H), 5.39 (s, 2H), 5.28 (s, 2H), 3.95, (s, 3H).

RESULTS AND DISCUSSION

It is attractive to determine alkyl and related DNA adducts by GC–ECNI–MS as their pentafluorobenzyl derivatives, because such derivatives generally are formed easily in high yield, chemically and physically stable, and detected readily with high sensitivity by this technique[13]. Thus we sought such a derivative for N7-(2-hydroxyethyl)guanine, an ethylene oxide DNA adduct.

Unfortunately, the derivatization procedures that we used previously to form pentafluorobenzyl derivatives of a series of pyrimidines and purines[13–15] including O^6-(2-hydroxyethyl)guanine[13], were not successful when applied to N7-(2-hydroxyethyl)guanine (**1**). Basically we either recovered unreacted starting material or obtained a complex mixture of products, based on analysis by TLC or HPLC. It appeared that starting material was recovered because it failed to dissolve in the reaction mixture. Efforts to overcome this latter problem by conducting the reactions on smaller amounts of **1**, or starting with **1** predissolved in aqueous acid or base, gave similarly poor results.

Faced with this difficulty, we adopted a strategy of chemically transforming the N7-(2-hydroxyethyl)guanine prior to derivatizing it with pentafluorobenzyl bromide (PFBzBr). One of two approaches that we investigated in parallel is shown in Scheme 1. Following a known procedure[16], which is a modification of the procedure of Oesch and Doerjer[17], we subjected **1** to a cyclocondensation reaction with chloroacetaldehyde diethyl ether in water at 80°C. This gave a high yield (see Table I) of N^2,3-etheno-N7-(2-hydroxyethyl)guanine (**2**), a fluorescent compound. This product in turn could be pentafluorobenzylated under mild conditions (potassium carbonate) to form **3**. However, further derivatization of **3** with PFBzBr under phase transfer con-



Scheme 1.

TABLE I

PREPARATION AND GC–ECD PROPERTIES OF DERIVATIZED PURINES

Reaction	*Product*		
	% Yield (preparative)	*Retention time (min)*[a]	*Response*[b]
1 → **2**	80%	–	–
2 → **3**	45%	7.9	0.06
1 → **4**	70%	–	–
4 → **5**	52%	8.0	0.6
4 → **6**	80%	10.7	0.5
5 → **6**	47%	10.7	0.5
5 → **7**	90%	7.3	0.05
9 → **10**	49%	7.2	0.1

[a] For GC conditions, see Fig. 1.

[b] Molar response by GC–ECD based on a comparison of the peak area relative to the peak area relative to that of lindane.

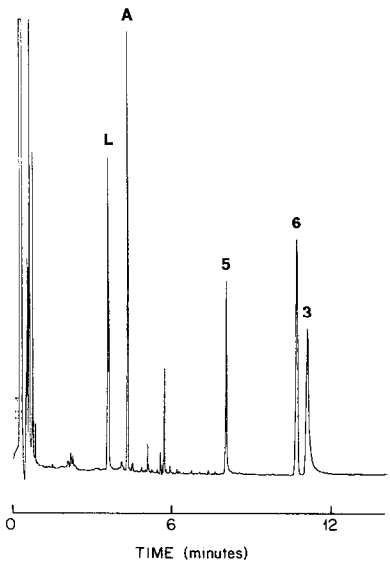


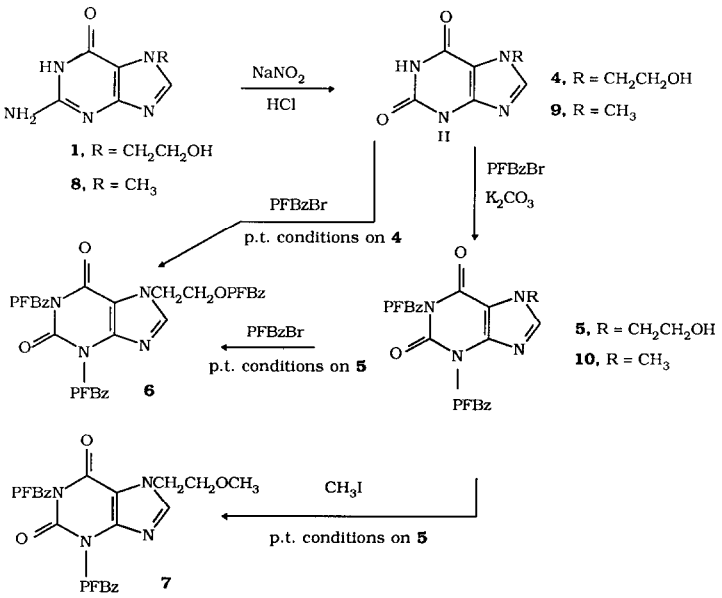
Fig. 1. GC–ECD chromatogram of lindane (L, 6.6 pg), aldrin (A, 6.6); **3** (73.6); **5** (2.5); and **6** (2.7). A 1-μl solution of these compounds in toluene was on-column injected onto a 15 m × 0.321 mm Ultra cross-linked 5% phenyl methyl silicone fused-silica capillary column, 0.17 μm film thickness (Hewlett-Packard, Palo Alto, CA, U.S.A.); injector 50°C; column 120°C. The injector was programmed immediately at a setting of 180°C/min up to 260°C, and the column was programmed, after a 2 min hold, at 80°C/min up to 260°C. The carrier gas was helium at 6 ml/min and the make-up gas was nitrogen at 24 ml/min.

ditions, or of **2** directly, gave a complex mixture of products. Although **3** itself could be determined by GC–electron-capture detection (ECD) (Fig. 1, Table I), it gave a tailed GC peak. Further, its inherent fluorescence was considered a drawback as well, since it therefore would tend to decompose upon exposure to light.

The second type of chemical transformation that we investigated is shown in Scheme 2. Using reaction conditions similar to those employed by others to convert guanine to xanthine[18], we treated 1 with nitrous acid to form N7-(2-hydroxyethyl)-xanthine (**4**), in a 70% yield. Further reaction of **4** with PFBzBr under mild conditions gave **5**. The fully pentafluorobenzylated product **6** could be obtained by reacting either **4** or **5** with PFBzBr under phase transfer conditions. The better of the two pathways is the direct conversion of **4** to **6**, since this saves a step and also gives a slightly higher yield than the two step pathway. We also methylated the hydroxy group of **5** with methyl iodide under phase transfer conditions, forming **7**.

To help establish the generality of the nitrous acid-pentafluorobenzylation reaction sequence (shown in Scheme 2) for similar N7-substituted guanines, and also to provide a related product for comparison purposes, we subjected N7-methylguanine (**8**), to two of the same reactions. This work is summarized as well in Scheme 2, showing the successive formation of N7-methylxanthine (**9**) and then N1,N3-bis-(pentafluorobenzyl)-N7-methylxanthine (**10**).

Table I summarizes the yields for above reactions, along with the retention times and molar responses of the appropriate products of GC–ECD. Basically the behavior of the compounds by GC–ECD is good, as revealed by the moderate retention times and, as shown in Fig. 1, relatively sharp peaks. Noteworthy is the good behavior of the pentafluorbenzyl derivative **5**, in spite of its free, primary hydroxyl group. This makes it puzzling why **7**, the corresponding methyl ether, gives a much



Scheme 2.

lower response. For **10** as well, the related N7-methyl derivative, the response is also low. Whether these differences in response reflect variations in the losses of the compounds in the GC–ECD, or inherent differences in their electrophoricity, is not clear. In studies of other structurally complex, strong electrophores by GC–ECD, we have encountered similar, complex results of this type[19].

Although it is difficult to prove, we believe that, in the present study, the variations in response reflect differences in the recoveries of these compounds in the GC–ECD. There are two considerations that encourage this hypothesis. First is that the compounds probably all undergo electron capture by the same mechanism: dissociative electron capture forming a parent anion and pentafluorobenzyl radical, based on our studies of related derivatives by ECNI-MS[13]. Secondly, we have observed that surface effects producing losses of structurally complex, strong electrophores in a GC–ECD can be highly specific[19–21].

Taking into account both the reaction yields and the GC–ECD behavior of the compounds, two emerge as the best ones for the trace determination of N7-(2-hydroxyethyl)guanine by GC–ECD or GC–ECNI–MS: **5** and **6**. They are comparable in terms of ease of formation as well as GC–ECD properties. Whether they will both continue to perform similarly at lower trace levels will be defined in our future studies, including an extension of the method to the determination of N7-(2-hydroxyethyl)guanine in biological samples.

The results presented here are relevant to N7-guanine adducts derived from exposure of DNA to other chemicals of industrial importance besides ethylene oxide. Ethene is metabolized to ethylene oxide, and, not surprising then, also gives rise to hydroxyethylations of nucleophilic sites on protein and DNA[7]. One of the DNA adducts arising from *in vivo* exposure to vinyl chloride, a known human carcinogen, is N7-(2-oxoethyl)guanine[2]. Potentially this adduct could be reduced to the corresponding alcohol for subsequent derivatization by our technique. The carcinogen styrene, via metabolism to styrene-7,8-epoxide, predominantly reacts like ethylene oxide at the N7 position of guanine producing a mixture of positional and diastereomeric hydroxyphenethyl adducts[22]. These products should also be amenable to our procedures.

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REFERENCES

1 P. J. Landrigan, T. J. Munhardt, J. Gordon, J. A. Lipscomb, J. R. Burg, L. F. Mazzuckelli, T. R. Lewis and R. A. Lemen, *Am. J. Ind. Med.*, 6 (1984) 103.
2 B. Singer and D. Grunberger, *Molecular Biology of Mutagens and Carcinogens*, Plenum Press, New York, 1983.
3 L. Ehrenberg and S. Hussain, *Mutat. Res.*, 86 (1981) 1.
4 A. Kolman, M. Naslund and C. J. Calleman, *Carcinogen.*, 1 (1986) 1245.
5 W. M. Snellings, C. S. Weil and R. R. Maronpot, *Toxicol. Appl. Pharmacol.*, 75 (1984) 105.
6 M. Sun, *Science (Washington, D.C.)*, 231 (1986) 448.
7 D. Sergerback, *Chem. Biol. Interact.*, 45 (1983) 139.

8 S. Osterman-Golkar, P. B. Farmer, D. Segerback, E. Bailey, C. J. Calleman, K. Svensson and L. Ehrenberg, *Teratogen., Carcinogen, Mutagen.*, 3 (1983) 395.
9 P. B. Farmer, E. Bailey, S. M. Gorf, M. Tornquist, S. Osterman-Golkar, A. Kautiainen and D. P. Lewis-Enright, *Carcinogen.*, 7 (1986) 637.
10 M. Tornquist, J. Mowrer, S. Jensen and L. Ehrenberg, *Anal. Biochem.*, 154 (1986) 255.
11 D. Segerback, *Thesis*, University of Stockholm, Stockholm, 1985.
12 R. S. Annan, G. Kresbach, R. Giese and P. Vouros, *J. Chromatogr.*, 465 (1989) 285.
13 M. Saha, R. S. Annan, G. M. Kresbach, F. Vouros and R. W. Giese, *Biomed. Environ. Mass Spectrom.*, in press.
14 O. Minnetian, S. Saha and R. W. Giese, *J. Chromatogr.*, 410 (1987) 453.
15 J. Adams, M. David and R. W. Giese, *Anal. Chem.*, 58 (1986) 345.
16 N. Fedtke and J. Swenberg, personal communication.
17 F. Oesch and G. Doerjer, *Carcinogen.*, 3 (1982) 663.
18 E. Shaw, *J. Org. Chem.*, 27 (1962) 883.
19 A. Sentissi, M. Joppich, K. O'Connell, A. Nazareth and R. W. Giese, *Anal. Chem.*, 56 (1984) 2512.
20 A. Nazareth, K. O'Connell, A. Sentissi and R. W. Giese, *J. Chromatogr.*, 314 (1984) 219.
21 A. Sentissi, K. O'Connell and R. W. Giese, *J. Chromatogr.*, 331 (1985) 396.
22 S.-F. Liu, S. M. Rappaport, J. Rasmussen and W. J. Bodell, *Carcinogen.*, 9 (1988) 1401; and references cited therein.