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Characterization of the products formed in the reaction between 1,3-butadienemonoxide and 2′-deoxyadenosine by liquid chromatography–continuous-flow fast atom bombardment mass spectrometry

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ABSTRACT

1,3-Butadiene, a widely produced industrial chemical, has recently been identified as a strong rodent carcinogen. Butadiene is metabolized to reactive 3,4-epoxy-1-butene (BM), which may bind to DNA. The reaction between 3,4-epoxy-1-butene and 2′-deoxyadenosine (dAdo) was studied. The reaction was carried out in trifluoroethanol–triethylamine and the reaction mixture was analysed by liquid chromatography–continuous-flow fast atom bombardment mass spectrometry. The recorded total-ion chromatogram showed four peaks. The spectra of the peaks exhibited a protonated molecule of BM adducts of dAdo (*m/z* 322), indicating formation of different isomers of the adducts. One alkylation site was shown to be at the exocyclic amino group of the 2′-deoxyadenosine.

INTRODUCTION

1,3-Butadiene (BD) is one of the most commonly produced petrochemicals in the world. Toxicological research has been focused on 3,4-epoxy-1-butene (BM), the main metabolite of butadiene catalysed by microsomes. It has been shown that BM is mutagenic, has carcinogenic activity in the mouse, is a substrate of human placental glutathione *S*-transferase and binds to haemoglobin [1–4]. Citti *et al.* [5] also reported binding to DNA *in vitro* and formation of two N-7 isomeric guanine adducts.

Of the numerous methods available for the detection and quantitation of DNA adducts in biological systems, the ^{32}P post-labelling assay of Randerath and co-workers [6,7] is perhaps the most widely utilized technique. However, the method is not inherently chemospecific and it does not reveal structural information about the modified nucleotides. This problem can be overcome by the use of mass spectrometry, the specificity of which is superior to that of most of the other analytical methods. Direct chemical desorption mass spectrometry (DCI-MS) [8] and tandem mass spectrometry [9] have been applied successfully to the characterization of the alkyldeoxynucleosides. However, owing to thermal decomposition of the compounds during the

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desorption process in DCI, the intensities of the protonated molecules are low and the intensities of the fragment ions are not reproducible. The recently introduced electrospray [10] and fast atom bombardment [11] techniques are gentler ionization methods than DCI. Furthermore, a liquid chromatograph can be connected easily with a mass spectrometer through electrospray or continuous-flow fast atom bombardment (CF-FAB). These techniques provide a very powerful tool for the identification of polar, thermally labile and non-volatile molecules. In this study we have applied HPLC–CF-FAB-MS to the characterization of the reaction products of 3,4-epoxy-1-butene and 2′-deoxyadenosine (dAdo).

EXPERIMENTAL

All the chemicals were from commercial sources: BM, trifluoroethanol (TFE) and triethylamine (TEA) were from Aldrich (Milwaukee, WI, USA) and dAdo was from Sigma (St. Louis, MO, USA). Distilled, deionized water (Milli-Q, Millipore, Finland) mixed with glycerol (99.5%, Prolabo, France) was used as an eluent in HPLC. Xenon (99.995%) used in FAB was from Aga (Finland).

The reaction was carried out in a trifluoroethanol–triethylamine (1:1) mixture by refluxing the reaction mixture at 50°C for 5 days. At the end of the reaction the sample was evaporated to dryness, dissolved in water and subjected to HPLC–CF-FAB-MS analysis.

The measurements were made with a Finnigan MAT 95 high-resolution mass spectrometer connected to an HPLC system by a Finnigan MAT Bio-Probe interface. The ionization chamber temperature was 50°C, the xenon particle energy 8 kV, the emission current 10 mA, the accelerating voltage 5 kV and the magnetic field scanning range m/z 70–500 (3 s per scan). In order to increase the evacuation speed of the ion source, a liquid nitrogen trap was used. The effluent deliver was done by using two high-pressure pumps (Waters 600 MS, Milford, MA, USA). The column was a Novapak C_{18} (15 cm × 4.6 mm I.D.), and samples were introduced into the LC system with a 10-μl loop. The reaction products were separated isocratically using water–glycerol (90:10) as a mobile phase at a flow-rate of 0.8 ml/min. The high viscosity of the mobile phase was compensated by using elevated temperature (57°C). The HPLC effluent was split after the column by a simple T-piece splitter [12], leaving a flow-rate of 6 μl/min to be directed to the mass spectrometer.

RESULTS AND DISCUSSION

Fig. 1 shows a background-subtracted total-ion chromatogram of the reaction mixture recorded by HPLC–CF-FAB-MS with whole-mass-range scanning. The spectra of each of the five peaks detected are presented in Fig. 2. Although the spectra are affected by interference from the background ions, they are still indicative of the

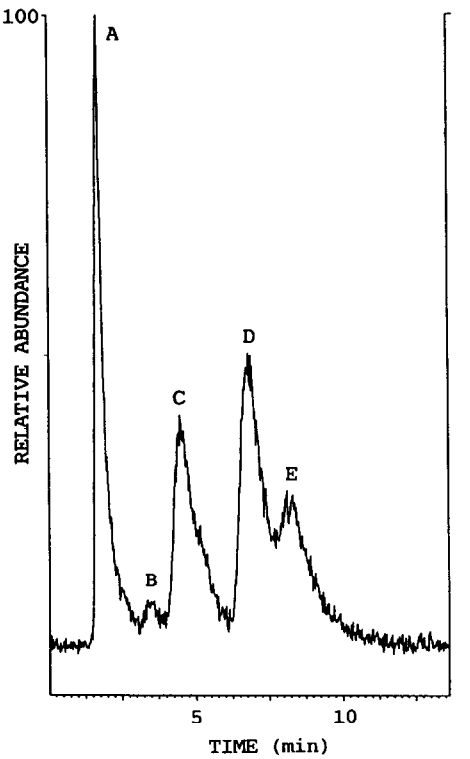
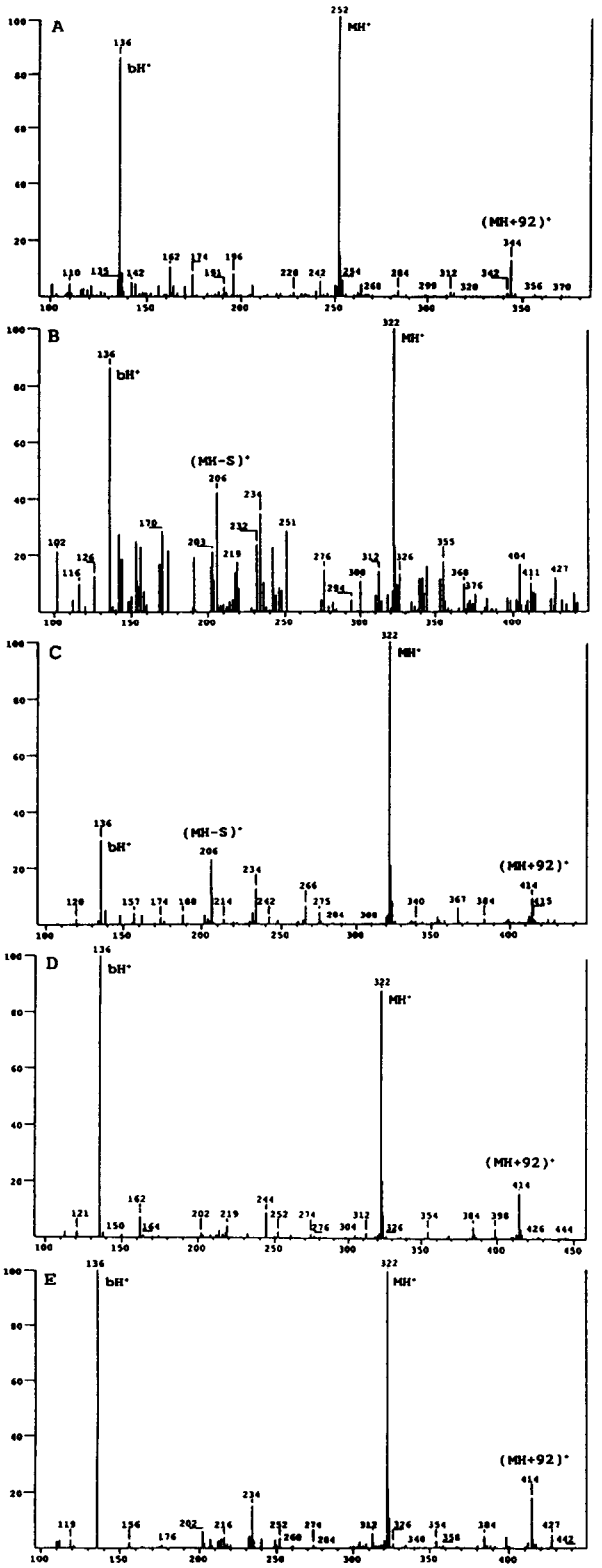


Fig. 1. Background-subtracted total-ion chromatogram of the reaction mixture recorded by HPLC–CF-FAB-MS.



structure. All the spectra contain an abundant protonated molecule and also glycerol adduct ion. The spectrum of peak A exhibits an abundant protonated molecule of dAdo (m/z 252) and an ion at m/z 136 (bH^+) formed by the cleavage of the glycosidic bond with hydrogen transfer. The spectra of the other peaks (B–E) exhibit abundant protonated molecules of BM adducts of dAdo (m/z 322), indicating the formation of isomeric adducts.

The spectra of the peaks B and C exhibit an abundant ion m/z 206, formed by the cleavage of the glycosidic bond with a hydrogen transfer. The ion m/z 206 decomposes further by the loss of BM adduct, producing the ion m/z 136 (bH^+). The presence of the ion m/z 206 in the spectra of B and C indicates that the alkylation site is on the base moiety. Unfortunately, the spectra are affected by interference from the background ions so that the exact site of alkylation cannot be determined. Based on the reaction conditions shown in Fig. 3 (aprotic, basic) the most probable alkylation site is the exocyclic amino group of dAdo, *i.e.* the N^6 position.

In the case of D and E it is not obvious that the alkylation site is N^6 of dAdo, since the spectra do not exhibit the characteristic ion m/z 206. Therefore we are suggesting that the alkylation site could be N-3 of dAdo. This makes possible rearrangement and simultaneous loss of BM and sugar. The chemistry of expoxides does not support the modification of sugar hydroxyls.

The results suggest that N^6 isomers can be separated from the other isomers and identified. Based on the nature of the starting materials and the reaction chemistry of epoxides, an isomeric pair of diastereomers of N^6-dAdo adducts is expected. So, we realize that we were not able to separate all the four N^6 isomers of BM and dAdo adducts. Our future research will be focused on the separation of all N^6 isomers and their use as standards in a post-labelling assay.

Fig. 2. The FAB spectra of 2′-deoxyadenosine (A) and 3,4-epoxy-1-butene 2′-deoxyadenosine adducts (B–E).

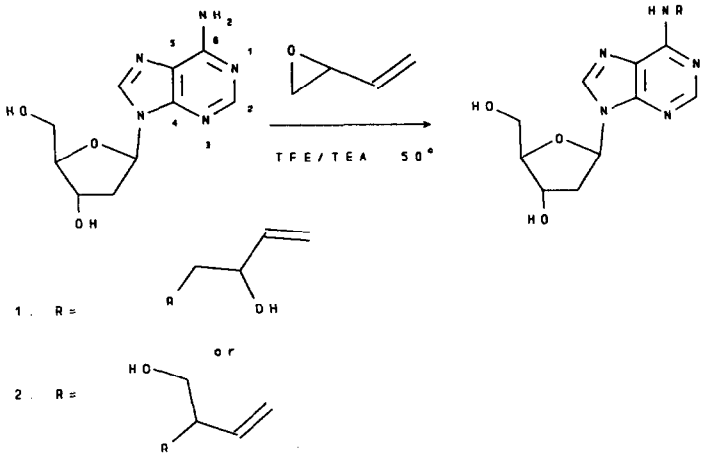


Fig. 3. Reaction of 2′ deoxyadenosine and 3,4-epoxy-1-butene.

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