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Evaluation of liquid chromatography–thermospray mass spectrometry in the determination of some phenylglycidyl ether–2′-deoxynucleoside adducts

F. Lemière

Department of Chemistry, University of Antwerp (RUCA), Groenenborgerlaan 171, B-2020 Antwerp (Belgium)

E.L. Esmans*

Department of Chemistry, University of Antwerp (RUCA), Groenenborgerlaan 171, B-2020 Antwerp (Belgium) and Departments of Biology and Chemistry, University of Antwerp (UIA), Universiteitsplein 1, B-2610 Wilrijk (Belgium)

W. Van Dongen

Department of Chemistry, University of Antwerp (RUCA), Groenenborgerlaan 171, B-2020 Antwerp (Belgium)

E. Van den Eeckhout

College of Pharmacy, University of Ghent, Harelbekestraat 72, B-9000 Ghent (Belgium)

H. Van Onckelen

Department of Biology, University of Antwerp (UIA), Universiteitsplein 1, B-2610 Wilrijk (Belgium)

ABSTRACT

The adducts formed between 2′-deoxyadenosine (dAdo), 2′-deoxycytidine (dCyd) and 2′-deoxyuridine (dUrd) and phenyl glycidyl ether (PGE) were analysed by HPLC and LC–thermospray (TSP)-MS. Good results were obtained on a 10 RP Select B column (12.5 cm × 4 mm I.D.) using 0.1 *M* NH_4OAc–CH_3OH at a flow-rate of 0.8 ml/min. The mass spectra of the 2′-deoxynucleoside–PGE adducts, obtained under LC–TSP-MS conditions were all characterized by the presence of the protonated molecule $[MH]^+$ and $[BH + H]^+$ ions. The PGE–dCyd adduct underwent hydrolytic deamination to the corresponding PGE–dUrd adduct. There was an indication that this process of hydrolytic deamination also took place in the TSP interface. Localization of the alkylation site was possible in the PGE–dUrd adduct by the presence of an RDA rearrangement leading to a fragment ion at *m/z* 194. Preliminary sensitivity studies on PGE–dUrd showed a detection limit of 500 pg (signal-to-noise ratio = 2) in multiple ion monitoring at *m/z* 263 and 379.

* Corresponding author. Address for correspondence: Department of Chemistry, University of Antwerp (RUCA), Groenenborgerlaan 171, B-2020 Antwerp, Belgium.

INTRODUCTION

Epoxides are important chemicals which are frequently used in numerous industrial chemical

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processes. From a number of investigations it is clear that some of these epoxides, depending on the structure, are mutagenic. Therefore, the study of the interaction of these epoxides with DNA is warranted.

In the past, the interaction between these epoxides and DNA or nucleosides has been investigated mainly with the aid of HPLC and UV–Vis data. In a few instances chemical ionization mass spectrometry has been reported for the identification of adducts formed with small aliphatic epoxides [1,2], but the full potential of mass spectrometric techniques has certainly not been elaborated to its full extent.

EXPERIMENTAL

Instrumentation and materials

Off-line HPLC experiments were carried out on a Hewlett-Packard (Brussels, Belgium) Model 1090 liquid chromatograph equipped with an automatic injection system, a diode-array detector and a Hewlett-Packard Model 9000/300 data system. Separations were performed on a 5-μm particle size RP-18 column (15 cm × 4.6 mm I.D.) [Bio-Rad, Ghent (Eke), Belgium] and a 5-μm particle size LiChrospher 60 RP-Select B column (12.5 cm × 4 mm I.D.) (Merck, Darmstadt, Germany). HPLC spectro-grade methanol was purchased from Alltech (Applied Science Labs., Deerfield, IL, USA). Methylene dichloride, tetrahydrofuran (THF) and CCl_4 (Caldic, Hemiksem, Belgium) were distilled three times prior to use. 2′-Deoxyuridine (dUrd), 2′-deoxycytidine (dCyd) and 2′-deoxyadenosine (dAdo) were obtained from Janssen Chimica (Beerse, Belgium). Phenyl glycidyl ether was supplied by Fluka (Bornem, Belgium) and distilled prior to use. Ammonium acetate (analytical-reagent grade) was purchased from Janssen Chimica and a 0.1 *M* solution was prepared using Millipore Milli-Q purity water.

Thermospray (TSP) mass spectra were recorded on a VG-2000 quadrupole mass spectrometer equipped with a Waters HPLC system consisting of a Waters 600-MS pump, a Waters 700 satellite W 158 autosampler and a Waters 486 UV–Vis detector set at 260 nm. The source temperature was 250°C unless stated otherwise. The temperature of the thermospray capillary was *ca.* 200°C and was optimized on the solvent clusters. The repeller was operated between 175 and 180 V unless stated otherwise. The scan time was 1.0 s for a scan range from 100 to 550 u. In the multiple ion detection (MID) mode the dwell time was 100 ms. It should be noted that in the LC–TSP-MS set-up, the UV detector is installed between the HPLC column and the TSP interface. Therefore, the retention times found in the UV trace and the ion chromatograms are slightly different.

Synthesis of PGE–2′-deoxynucleoside compounds

A solution of 2 mg of a 2′-deoxynucleoside in 2 ml of methanol and 1 ml of a 1 *M* methanolic solution of phenyl glycidyl ether was stirred at 37°C in a Pierce Reacti-vial. After 24 h the solution was evaporated and the residue dissolved in 1 ml of water. The mixture was extracted twice with 4 ml of CCl_4 to remove the excess of PGE. The aqueous layer was evaporated under reduced pressure on a rotary evaporator. The residue was dissolved in 1 ml of water and used for HPLC and LC–MS analyses.

Semi-preparative synthesis of the adduct of PGE–dUrd

In order to investigate the detection limit, the PGE–dUrd-adduct was prepared and isolated by circular centrifugal thin-layer chromatography (TLC) as outlined below. The yield of the reaction between dUrd and PGE is low. Therefore, PGE was reacted with dCyd and the corresponding PGE–dCyd adduct was converted into the PGE–dUrd adduct by hydrolytic deamination.

A solution of 50 mg of dCyd in 50 ml of CH_3OH and 25 ml of a 1 *M* methanolic solution of PGE was stirred at 37°C. After 4 days, 10 ml of water were added and the mixture was heated under reflux for 24 h. The reaction mixture was evaporated, the residue dissolved in 25 ml of water and the solution extracted twice with 50 ml of CCl_4. The aqueous layer was lyophilized and the residue was dissolved in 1 ml of THF containing 3 drops of CH_3OH. This solution was purified by semi-preparative centrifugal TLC on

a Chromatotron (Harrison Research, Palo Alto, CA, USA) [Kieselgel 60 F_{254}, $CaSO_4$, 2-mm layer thickness, mobile phase THF–CH_2Cl_2 (40:60), flow-rate 7 ml/min). Three bands were collected: $R_F = 0.77$, 1-methoxy-2-hydroxy-3-phenoxypropane (methoxy-PGE); $R_F = 0.64$, PGE-diol; and $R_F = 0.38$, PGE–dUrd. Under these conditions, unreacted dCyd and PGE–dCyd have R_F values of almost zero and can be eluted by switching to 100% CH_3OH. Each PGE–dUrd fraction collected was evaporated to dryness and the residue dissolved in 0.5 ml of CH_2Cl_2 and purified a second time on the Chromatotron but using THF–CH_2Cl_2 (50:50). Two bands were collected: $R_F = 0.64$, PGE-diol; and $R_F = 0.14$, PGE–dUrd. The purity of the PGE–dUrd fraction was checked by TLC; a small amount of PGE-diol (<2%) was still present. At present a semi-preparative HPLC method using a poly(styrene–divinylbenzene) copolymer column (PRP1) in order to remove the last traces of PGE-diol is being investigated.

RESULTS AND DISCUSSION

If DNA is allowed to react with epoxides, covalent adducts are formed because the epoxides react with different nucleophilic sites on the purine and pyrimidine moieties. The assignment of the point of attachment on the heterocyclic moiety is certainly an important aspect of these studies and it has been shown that UV data on the isolated adducts can give an idea of the alkylation site [3]. However, it is our belief that assignment of the alkylation site based solely on the UV data can be risky and that other independent methodologies should be developed in order to obtain additional information.

In the past, the adducts formed between a series of 2′-deoxynucleosides and PGE have been analysed by HPLC and identified by fast atom bombardment (FAB) MS [4–8]. In view of experiments planned with calf thymus DNA, we started to investigate the possibilities of using LC–TSP-MS in this particular application.

As shown previously [5,6], the PGE–2′-deoxynucleoside adducts (see Fig. 1) can be analysed by reversed-phase chromatography on a standard 25 cm × 4.6 mm I.D. RP-18 column using different ratios of methanol and water (0.1 *M* $HCOONH_4$, pH 5.1) at a flow-rate of 1.8 ml/min. However, especially with the purine series, better results could be obtained on a poly(styrene–divinylbenzene) copolymer column. In most instances the total analysis time was *ca.* 40 min. Because LC–TSP-MS experiments require a flow-rate of 0.8–1 ml/min, we realized that under these conditions the analysis of a PGE–2-deoxynucleoside mixture on a 25-cm RP column would be impractical. Therefore, we decided to evaluate two short RP columns, *i.e.*, a 15 cm × 4.6 mm I.D. 5RP-18 and a 12.5 cm × 4 mm I.D. 5RP-8 Select B column. Both columns were mounted on an HP 1090 HPLC system equipped with a diode-array detector.

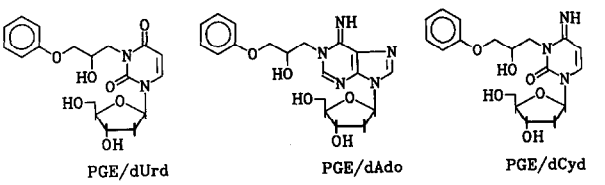

Fig. 1. Structures of PGE–2′-deoxynucleoside adducts.

5RP-18 column

In order to evaluate this column, the reaction mixture obtained from the interaction between dAdo and PGE was analysed using different ratios of CH_3OH and 0.1 *M* NH_4OAc (pH 5.1). Provided the excess of PGE present in the reaction mixture was removed by extraction with CCl_4, good results were obtained with CH_3OH–0.1 *M* NH_4OAc (45:55) (see Fig. 2).

Five compounds were detected with k' values

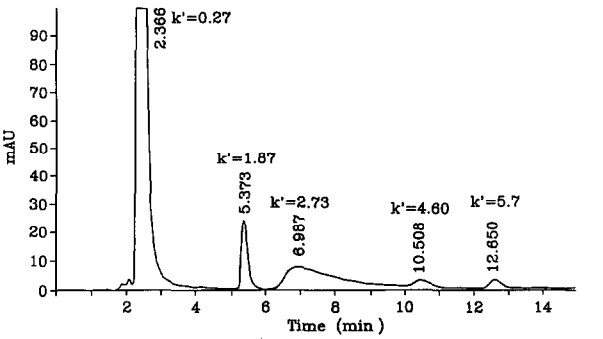

Fig. 2. HPLC of a PGE–dAdo mixture on a 5RP-18 column (detection at 260 nm) using CH_3OH–0.1 *M* NH_4OAc (45:55). Flow-rate, 0.8 ml/min.

of 0.27, 1.87, 2.73, 4.60 and 5.7. The last compound was identified as unreacted PGE, still present in small amounts after extraction. The compound with $k' = 0.27$ was unreacted dAdo. Identification was achieved with the aid of reference compounds and by examining the UV data. The UV spectra of the compounds with $k' = 1.87$ and 4.60 were very similar to that of PGE, suggesting a structural relationship. The compound with $k' = 2.73$, characterized by an asymmetric peak shape, was tentatively assigned to the PGE–dAdo adduct until further LC–TSP-MS data became available.

Analogous conditions were applied to the analysis of the PGE–dUrd and PGE–dCyd reaction mixtures. In all these instances CH_3OH–0.1 *M* NH_4OAc (55:45) gave a baseline separation of all compounds together with a short analysis time. With the PGE–dUrd reaction mixture again five compounds were detected with k' values of 0.16, 1.81, 2.19, 5.28 and 7.30. The compound with $k' = 0.16$ was identified as dUrd. For the k' value of 2.19 (retention time 5.79 min), the PGE–dUrd adduct was assigned from the UV data. Almost the same features were observed with the PGE–dCyd reaction mixtures: five compounds with k' values of 0.12, 1.82, 2.93 (broad) 5.27 and 7.16 were found. However, an extra component eluted after 6.02 min ($k' = 2.21$). From preliminary inspection of the UV data for the different compounds present in the mixture, the products with k' values of 2.21 and 2.93 could be PGE–2′-deoxynucleoside adducts.

5RP-8 Select B column

Asymmetric peaks on an RP column are often the result of interactions of basic components with residual silanol groups or can be due to pH-dependent equilibrium phenomena. These asymmetric broadened peaks were observed in the chromatograms of PGE–dAdo and PGE–dCyd reaction mixtures when analysed on a 5RP-18 column. Therefore, all the analyses described above were repeated on a 5RP-8 Select B column using CH_3OH–0.1 *M* NH_4OAc (45:55). As shown in Fig. 3, all asymmetric peaks were absent under these chromatographic conditions.

More detailed examination of the results obtained for the PGE–dCyd reaction mixture showed partial resolution of the compounds eluting around 3.5 min. As the corresponding UV data were identical, we assumed that under these conditions both epimers were separated. This assumption was confirmed later by the LC–TSP-MS data.

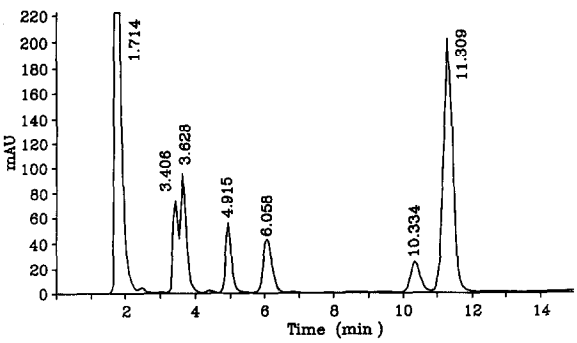

Fig. 3. HPLC of a PGE–dCyd mixture on a 5RP-8 Select B column (detection at 260 nm) using CH_3OH–0.1 *M* NH_4OAc (45:55). Flow-rate, 0.8 ml/min.

LC–TSP-MS

The mixtures described above were analysed by LC–TSP-MS using CH_3OH–NH_4OAc (40:60) (pH 5.1) on a 5RP-8 Select B column at a flow-rate of 0.8 ml/min. The instrument was tuned as such that the intensity of the solvent clusters was optimized. The optimum temperature of the thermospray capillary was usually around 200°C. The source temperature was 250°C unless stated otherwise.

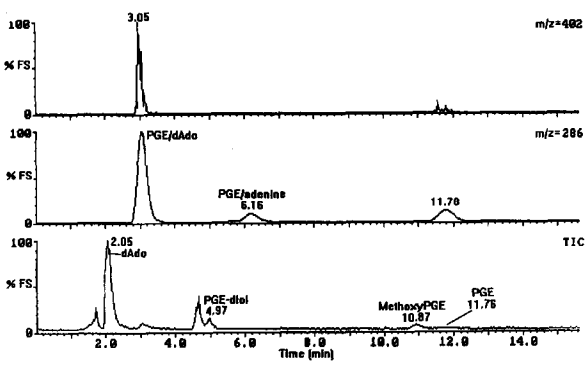

Fig. 4. LC–TSP-MS of a PGE–dAdo on a 5RP-8 Select B column using CH_3OH–0.1 *M* NH_4OAc (40:60). Flow-rate, 0.8 ml/min. $[MH]^+ = m/z$ 402; $[BH + H]^+ = m/z$ 286.

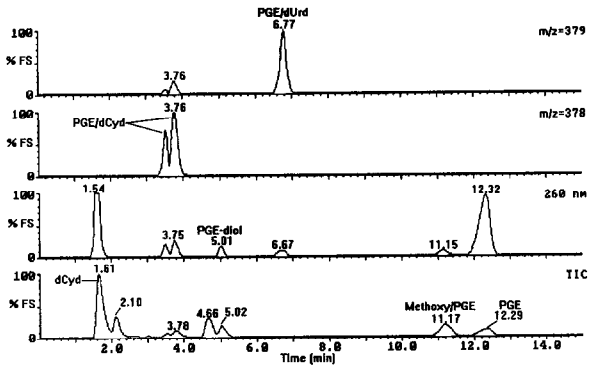

Fig. 5. LC–TSP-MS of a PGE–dCyd mixture on a 5RP-8 Select B column using CH_3OH–0.1 M NH_4OAc (45:55). Flow-rate, 0.8 ml/min.

Analyses of the PGE–dAdo reaction mixture (see Fig. 4) confirmed the presence of unreacted dAdo ($t_r = 2.05$ min) and a PGE–dAdo adduct ($t_r = 3.05$ min). Unreacted dAdo was characterized by the presence of a protonated molecule $[MH]^+$ at m/z 252 (23%) and an $[M + Na]^+$ adduct at m/z 274 (9%). Other ions present were $[BH + H]^+$ (m/z 136; 100%), $[BH + Na]^+$ (m/z 158; 7%) and m/z 117 ($[S]^+$; 8%). The PGE–dAdo adduct was localized with the aid of the reconstructed ion chromatogram for m/z 402 ($[MH]^+$) and its mass spectrum was characterized by m/z 402 (7%), 286 ($[BH + H]$; 100%), 268 ($[BH + H]^+ - H_2O$; 92%), 366 ($[MH]^+ - 2H_2O$; 8%) and 348 ($[MH]^+ - 3H_2O$; 3%). If the reconstructed ion chromatogram for m/z 286 was selected, another compound with $t_r = 6.16$ min responded. Its mass spectrum was characterized by ions at m/z 286 ($[MH]^+$; 100%), 268 ($[MH - H_2O]^+$; 12%) and 136 ($[MH - PGE]^+$) and therefore identified as a PGE–adenine adduct. Whether this compound was formed by a depurination reaction or by reaction of PGE with a minor amount of adenine present in dAdo has not been clarified. All other compounds were PGE or PGE-derived: $t_r = 4.97$ (PGE-diol), 10.87 (monomethoxy-PGE derivative) and 11.76 (PGE). However, as can be seen in Fig. 4, a second compound characterized by m/z 402 and 286 is present. This compound co-elutes with PGE. This means there are two PGE–dAdo adducts present in the reaction mixture. They are monoalkylated at different positions on the heterocyclic moiety.

The total ion chromatogram obtained for the PGE–dCyd reaction mixture is shown in Fig. 5. PGE–dCyd eluted after 3.75 min and its mass spectrum was characterized by ions at m/z 378 ($[MH]^+$; 2%), 262 ($[BH + H]^+$; 23%) and 244 ($[BH + H]^+ - H_2O$; 23%) (see Fig. 7). When the chromatogram depicted in Fig. 5 was examined more closely, it was observed that two unresolved compounds were eluting around 3.75 min. The spectra of both compounds were identical. This led us to the conclusion that the two epimers of the PGE–dCyd adduct were partially resolved. Other known components, PGE-diol ($t_r = 5.01$ min), methoxy-PGE ($t_r = 11.15$ min) and PGE ($t_r = 12.32$ min), could easily be assigned from the mass spectral data. Unreacted dCyd had a retention time of 1.54 min.

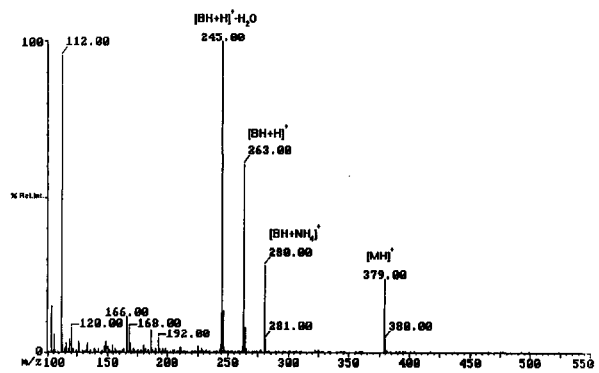

Fig. 6. Mass spectrum of the PGE–dUrd adduct in the PGE–dCyd mixture ($t_R = 6.77$ min).

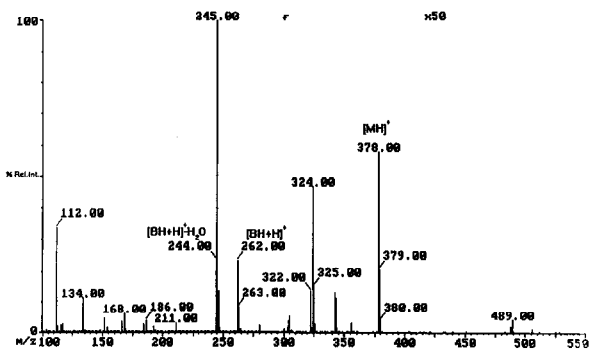

Fig. 7. Mass spectrum of the PGE–dCyd adduct in the PGE–dCyd mixture ($t_R = 3.75$ min).

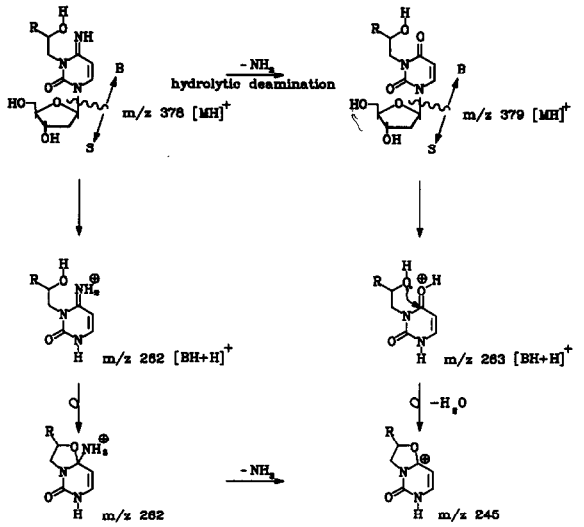

Fig. 8. Possible pathways for the formation of m/z 245 in the TSP-MS of PGE–dCyd.

Conversion of PGE–dCyd into PGE–dUrd

In a study published by Solomon and co-workers [1,2], it was found that the adduct formed between propylene oxide and dCyd had a limited lifetime in aqueous solution and was converted into the corresponding dUrd adduct by hydrolytic deamination. Such observations were also made here with the PGE–dCyd adduct. Indeed,

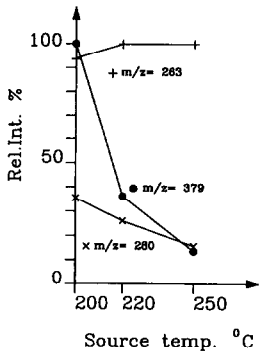

Fig. 10. Influence of the source temperature on the intensity of some diagnostic ions in the mass spectrum of PGE–dUrd. $[MH]^+ = m/z$ 379; $[BH + H]^+ = m/z$ 263; $[BH + NH_4]^+ = m/z$ 280.

when the LC–TSP-MS analysis of the PGE–dCyd reaction mixture was investigated further for the presence of ions typical of a PGE–dUrd adduct, a signal was observed in the reconstructed ion chromatograms not only at 6.77 min but also at 3.76 min, the retention time of the PGE–dCyd adduct (Fig. 5). The mass spectrum of the compound eluting at 6.77 min, depicted in Fig. 6, is characterized by ions at m/z 379 ($[MH]^+$), 263 ($[BH + H]^+$), 280 ($[BH + NH_4]^+$) and 245 ($[BH + H]^+ - H_2O$), which unequivoc-

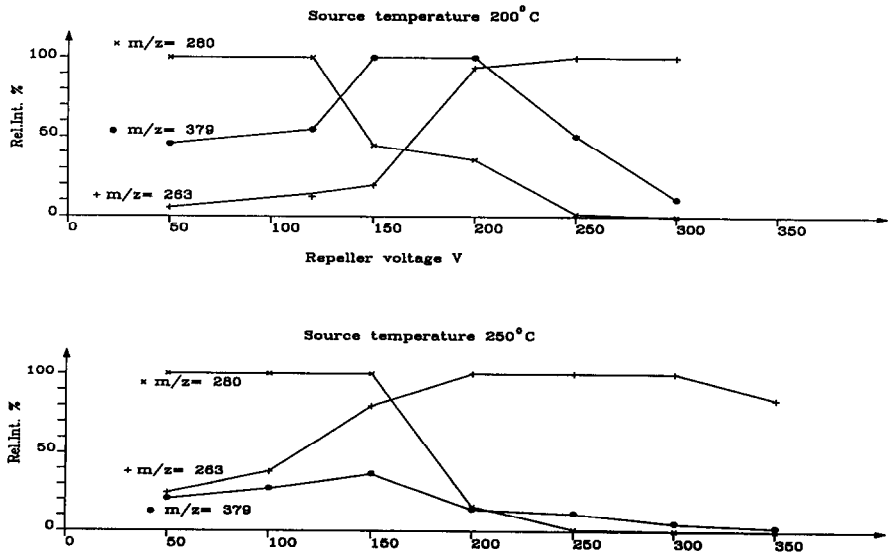

Fig. 9. Influence of the repeller voltage on the intensity of some diagnostic ions in the mass spectrum of PGE–dUrd. $[MH]^+ = m/z$ 379; $[BH + H]^+ = m/z$ 263; $[BH + NH_4]^+ = m/z$ 280.

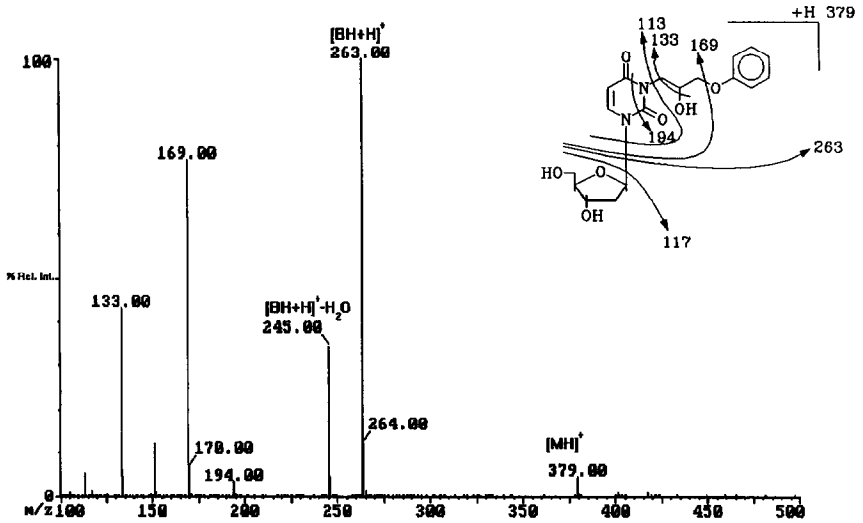

Fig. 11. Mass spectrum of the PGE–dUrd adduct. Repeller voltage, 300 V; source temperature, 250°C.

ally confirm the presence of PGE–dUrd in the reaction mixture.

When the mass spectrum of the PGE–dCyd adduct (see Fig. 7) was examined more closely, an intense ion at m/z 245 was observed and the relative abundance of the m/z 379 and 263 ions was higher than could be explained in terms of natural isotope abundance. Therefore, this phenomenon must be the result of a hydrolytic deamination of the PGE–dCyd adduct in the TSP interface.

Some diagnostic ions observed in the TSP mass spectrum of the PGE–dCyd adduct can be rationalized by Fig. 8. The fragment ion at m/z 244 can only be the result of the elimination of a molecule of H_2O from the $[BH + H]^+$ ion of the PGE–dCyd adduct. Whether the ion at m/z 245 is formed by elimination of NH_3 from the m/z 262 ion and/or by loss of H_2O from the m/z 263 ion is not known and should be resolved with the aid of isotopically labelled compounds.

Influence of repeller voltage

As it has been shown that the voltage applied on the repeller electrode can influence the TSP mass spectra, the mass spectrum of the PGE–dUrd adduct was recorded at fixed source temperatures (200 and 250°C) (see Fig. 9) but at different repeller voltages. The intensities of $[MH]^+$ (m/z 379), $[BH + H]^+$ (m/z 263) and $[BH + NH_4]^+$ (m/z 280) are shown in Fig. 9. At low voltage (<150 V) $[BH + NH_4]^+$ predominates. Repeller voltages between 150 and 200 V gave optimum intensities for the molecular ion at m/z 379 $[MH]^+$ and the protonated base moiety at m/z 263 $[BH + H]^+$. Higher voltages (>200 V) resulted in increased fragmentation.

Influence of source temperature

The effect of the source temperature was evaluated at a constant repeller voltage of 200 V at 200, 220 and 250°C (see Fig. 10). As expected, the intensity of $[MH]^+$ rapidly diminished in favour of the $[BH + H]^+$ ion at m/z 263.

Alkylation site

In order to investigate whether LC–TSP-MS could give information about the alkylation site, the PGE–dUrd adduct was analyzed at a high repeller voltage and high source temperature (see Fig. 11). As already shown by Claereboudt [8], some daughter ions in the FAB tandem mass spectra of PGE–pyrimidine nucleoside adducts can be used for localization of the PGE moiety on the pyrimidine nucleus. Fig. 11 shows the LC–TSP-MS of PGE–dUrd (300 V; 250°C). An important ion that is diagnostic for the alkylation site was found at m/z 194, which can be explained by a retro Diels-Alder (RDA) rearrangement of the m/z 263 ion. Although these results

are very preliminary, the present data justify a deeper study.

Sensitivity

The purified PGE–dUrd adduct was used for sensitivity studies. Full-scan spectra (positive-ion mode, capillary temperature 205°C, source temperature 200°C) were obtained for 20 ng injected on-column. Using multiple-ion detection (m/z 263 and 379) 500 pg were detected at a signal-to-noise ratio of 2. In the near future, sensitivity will be evaluated in the negative-ion mode.

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