ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
21 (1999) 1053–1061

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

Mass spectrometric (HPLC/ESI–MS/MS) quantification of pyrimido[1,2-a]purin-10(3*H*)-one, a guanine adduct formed by reaction of malondialdehyde with DNA

Kristo Hakala [a,*], Seppo Auriola [b], Arto Koivisto [a], Harri Lönnberg [a]

[a] *Department of Chemistry, University of Turku, FIN-20014 Turku, Finland*
[b] *Department of Pharmaceutics, University of Kuopio, FIN-70211 Kuopio, Finland*

Received 19 January 1999; received in revised form 16 August 1999; accepted 30 August 1999

Abstract

A high performance liquid chromatography/electrospray ionization–tandem mass spectrometric (HPLC/ESI–MS/MS) method has been developed for quantification of pyrimido[1,2-a]purin-10(3*H*)-one adducts from DNA. The method is based on acid-catalyzed cleavage of the adducts from DNA and the use of [2,3a,10-$^{13}C_3$]pyrimido[1,2-a]purin-10(3*H*)-one as an internal standard in the analysis. For this purpose the latter compound was prepared. Rate constants for the acidcatalyzed cleavage of pyrimido[1,2-a]purin-10(3*H*)-one from the corresponding 2′-deoxyribonucleoside were determined, and its hydrolytic stability and possible formation by a cross reaction between guanine and [2,3a,10]pyrimido[1,2-a]purin-10(3*H*)-one were studied.

Keywords: DNA adduct; Malonaldehyde guanine; HPLC/ESI–MS/MS

1. Introduction

DNA of living organisms is susceptible to continuous structural modification by both xenobiotic compounds and endogenously produced metabolises. Cyclic adducts of nucleic acid bases produced by reactions with α,β-unsaturated aldehydes and dicarbonyl compounds constitute a well known example of such modifications [1]. One of the most abundant cyclic adducts is pyrimido[1,2-a]purin-10(3*H*)-one (**1**), obtained by the reaction of N^1 and N^2 of a guanine residue with malondialdehyde (MDA, propanedial) or acrolein (propenal) and subsequent dehydration [1–4]. Malondialdehyde is produced endogenously during lipid peroxidation [5–8]. Accordingly, the pyrimido[1,2-a]purin-10(3*H*)-one adduct is a possible indicator of an organism being exposed to oxidative stress.

Several analytical techniques, including high performance liquid chromatography (HPLC) combined with electrochemical detection [9], ^{32}P post labelling [10,11] and gas chromatography/mass spectrometry (GC/MS) [12–14], have been employed to quantify DNA adducts. Furthermore, liquid chromatography combined with elec-

* Corresponding author.

0731-7085/99/$ - see front matter
PII: S0731-7085(99)00228-9

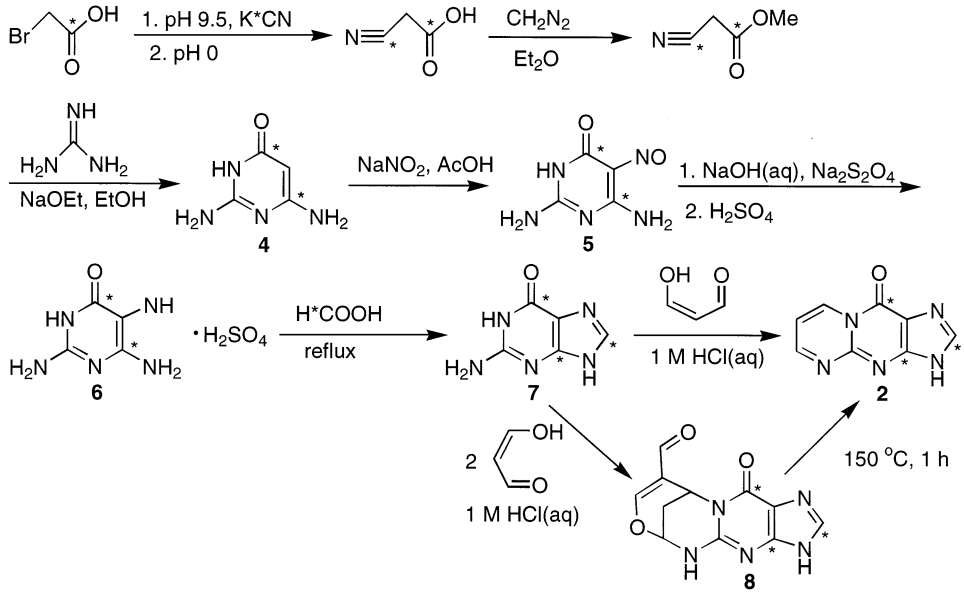

Fig. 1. The synthesis of [2,3a,10-$^{13}C_3$]pyrimido[1,2-a]purin-10(3*H*)-one (**2**).

trospray ionization mass spectrometry (HPLC/ESI–MS or HPLC/ESI–MS/MS) has been applied to identification, but not quantification, of **1** as a constituent of human liver DNA [15]. We now report on the synthesis of [2,3a,10-$^{13}C_3$]pyrimido[1,2-a]purin-10(3H)-one (**2**, Fig. 1) and the use of this compound as an internal standard in the quantification of **1** from calf thymus DNA treated with MDA. The obvious advantage of this method is that it allows, in contrast to the other methods employed, analysis of DNA adducts without further derivatization. Similar LC/ESI–MS/MS methods based on the use of an isotopically modified adduct as an internal standard have been introduced earlier for quantification of N^2,3-ethenoguanine [16], 1,N^2-ethenoguanine [17], 5,6,7,9-tetrahydro-7-hydroxy-9-oxoimidazo[1,2-α]purine [17] and 8-hydroxy-2′-deoxyguanosine [18] from DNA. To demonstrate that **1** can be removed from DNA by simple acid-catalyzed depurination as readily as unmodified purine bases, the kinetics of acid-catalyzed hydrolysis of 3-(2-deoxy-β-D-erythro-pento furanosyl)pyrimido[1,2-a]purin-10-one (**3**, Fig. 2) have been studied. Moreover, it is shown that the depurination can be carried out in the presence of **2** without conversion of guanine to **1**.

2. Experimental

2.1. General

The UV spectra were recorded on a Perkin–Elmer Lambda 12 UV–Vis spectrometer. An online HPLC/ESI–MS or HPLC/ESI–MS/MS was applied to record the positive ion mass spectra. The measurements were carried on either a LCQ quadrupole ion trap mass spectrometer equipped with an ESI ion source (Finnigan MAT), a Rheos 4000 HPLC pump (Flux instruments) and a LaChrom L-7200 injector (Merck–Hitachi), or on a Perkin–Elmer Sciex API 365 triple quadrupole LC/MS/MS equipped with a PE 200 Micro pump

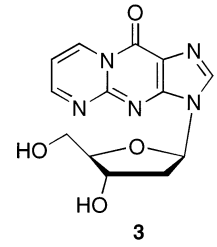

Fig. 2. The structure of 3-(2-deoxy-β-D-erythro-pentofuranosyl)pyrimido[1,2-a]purin-10-one.

and a PE Series 200 Autosampler. On applying the former apparatus, the spray was stabilized using nitrogen sheat gas flow (value 90) and nitrogen auxiliary gas flow (value 3). The spray needle potential was set to 5.9 kV, capillary voltage to 19 V and tube lens offset to 25 V. The stainless steel inlet capillary was heated to 200°C. With the triple quadrupole LC/MS/MS, the spray was stabilized using purified air nebulizer gas flow (value 8) and nitrogen curtain gas flow (value 10). The spray needle potential was set to 5.2 kV, orifice voltage to 46 V and ring voltage to 180 V. The nitrogen collision gas was set to value 4 and collision gas energy was 42.5 V. The nitrogen auxiliary gas flow (7000 cm^3 min^{-1}) was heated to 285°C. With both applications, the column was Jones chromatography 100-2.1 Genesis C18 4 μ.

2.2. Pyrimido[1,2-a]purin-10(3H)-one (**1**), and [2,3a,10-$^{13}C_3$]pyrimido[1,2-a]purin-10(3H)-one (**2**)

[1-^{13}C]Bromoacetic acid (Isotec Inc.) was converted to [1,3-$^{13}C_2$]cyanoacetic acid (**4**) by treating it with potassium [^{13}C]cyanide in aqueous methanol [19,20]. Esterification with diazomethane and subsequent cyclization with diguanidine then gave 2,4-diamino-[4,6-$^{13}C_2$]pyrimidin-6(1*H*)one (**5**), which was further nitrosoated and reduced to 2,4,5-triamino-[4,6-$^{13}C_2$]pyrimidin-6(1*H*)one, isolated as its sulfate salt **6** [19,21]. The latter compound was converted to [4,6,8$^{13}C_3$]guanine (**7**) as follows. Complex **6** (0.48 mmol, 73.8 mg) was refluxed in 0.50 ml of [^{13}C]formic acid (Isotec Inc.) for 3 days. The reaction was followed by RP HPLC on a Hypersil®ODS (5 μm) column using 2 mmol l^{-1} aqueous ammonium acetate–acetonitrile–formic acid (98.1:0.9:1, v/v/v) as an eluent. After the reaction was complete, unreacted formic acid was removed by repeated coevaporations with ethanol, and the residue was purified by semipreparative HPLC (LiChrospher®100 RP-18, 5 μm) using the eluent indicated above. Yield 90%. [4,6,8$^{13}C_3$]Guanine was finally converted to **2** by treatment with MDA obtained by acid-catalyzed hydrolysis of 1,1,3,3-tetramethoxypropane [3,22]. The product was purified by semipreparative HPLC on a LiChrospher®100 RP-18 (5 μm) column. The initial purification was carried out using 5 mmol l^{-1} aqueous ammonium acetate–acetonitrile (93.7:6.3, v/v) as an eluent. The crude product was then further purified using 2 mmol l^{-1} aqueous ammonium acetate–acetonitrile (95:5, v/v) as an eluent. Yield 4.3%. UV λ_{max} = 253 and 318 nm. MS: M + 1 = 191. Pyrimido[1,2-a]purin-10(3*H*)-one (**1**) was prepared similarly from guanine.

2.3. 3-(2-Deoxy-β-D-erythro-pentofuranosyl)-pyrimido[1,2-a]purin-10-one (**3**)

Complex **3** was prepared by treating 2′-deoxyguanosine with MDA as described previously [4], and purifying the product by semipreparative RP HPLC on a LiChrospher®100 RP-18 (5 μm) column, the eluent being water–acetonitrile (91.5:8.5, v/v). ^{1}H NMR (400 MHz, DMSO) δ = 9.35 (dd, $J_{8,6}$ = 2 26 Hz, $J_{8,7}$ = 7.13 Hz, H8), 9.06 (dd, $J_{6,7}$ = 3.82 Hz, $J_{6,8}$ = 2.20 Hz, H6), 8.50 (s, 2H), 7.31 (dd, $J_{7,6}$ = 3.84 Hz, $J_{7,8}$ = 7 19 Hz, H7), 6.44 (dd, $J_{1',2'}$ = 7.31 Hz, $J_{1',2''}$ = 6.28 Hz, H1′], 5.36 (d, J = 4.16 Hz, 3′-OH), 5.04 (t, J = 5.60 Hz, 5′-OH), 4.43 (m, H3′), 3.90 (dt, $J_{4',5'} = J_{4',5''}$ = 4.36 Hz, $J_{4',3'}$ = 2.68 Hz, H4′), 3.61–3.67 (m, H5′), 3.52–3.57 (m, H5″), 2.65–2.72 (m, H2′), 2.31–2.37 (m, H2″). FAB + MS; m/z: 304 [M + H^+], 326 [M + Na^+], 188 [1 + H^+]. UV; λ_{max} = 250 and 319 nm.

2.4. Kinetic measurements with monomeric nucleosides

Pseudo first-order rate constants for the hydrolysis of 3-(2-deoxy-β-D-erythro-pentofuranosyl) pyrimido[1,2-a]purin-10-one, 2′deoxyguanosine and 2′-deoxyadenosine were determined in formic acid–sodium formate buffers at 80°C. The reactions were started by adding solid nucleoside to the pre-thermostated reaction solution, the initial substrate concentration being in the order of 0.1 mmol l^{-1} and the total volume of reaction solution 2 ml. In total 10–15 aliquots of 60 μl were withdrawn at appropriate intervals during two half-lives of the reaction. The reaction was stopped by cooling the sample tubes on an

ice-bath and immediately neutralizing the buffer acid with a calculated amount of aqueous sodium acetate. The composition of the aliquots was determined by RP HPLC on a Hypersil®ODS (5 μm) column using 5 mmol l^{-1} aqueous ammonium acetate–acetonitrile as an eluent. With 2′-deoxyguanosine the contents was 97.5:2.5 (v/v), with 2′-deoxyadenosine 96:4 (v/v), and with **3** 94:6 (v/v). The reaction products, viz. guanine, adenine and **1** obtained from 2′-deoxyguanosine, 2′-deoxyadenosine and **3**, respectively, were identified by spiking with authentic samples. The first-order rate constants were calculated by applying the integrated first-order rate equation to the signal area of the starting material.

*2.5. Stability of pyrimido[1,2-a]purin-10(3H)-one (**1**) and its [2,3a,10-$^{13}C_3$]-analog (**2**)*

The hydrolytic stability of pyrimido[1,2-a]purin-10(3*H*)-one (**1**) was studied in formic acid–sodium formate buffers at 80°C by the HPLC technique described above. The conversion of **1** into guanine was observed to be two orders of magnitude slower than its formation from **3**.

To study the possible formation of **1** by cross reaction between guanine and **2** under the conditions used to depurinate DNA, the following experiment was carried out. Complex **2** (12 μg) was incubated at 90°C in a formic acid–sodium formate buffer (1 ml, 0.12 mol l^{-1}, pH 3.3) containing a large excess of adenine, guanine and cytidine (300 μg each). Aliquots were withdrawn at 0.5 h interval, and **1** and **2** were quantified by the LC/ESI–MS/MS method described below. Upon 2 h incubation less than 1% of **2** was converted to **1**.

2.6. Depurination of calf thymus DNA

To verify that 4 h incubation of calf thymus DNA in formic acid–sodium formate buffer (0.2 mol l^{-1}, pH 3.3) at 80°C is sufficient to result in quantitative depurination, the following experiment was carried out. A solution of calf thymus DNA (0.5 mg ml^{-1}) was prepared in distilled water and divided in two parts. One of these samples (sample A) was directly subjected to acid-catalyzed depurination (4.5 h in formic acid–sodium formate buffer (0.2 mol l^{-1}, pH 3.3, 80°C). The other sample (sample B) was first denaturated by heating in Tris buffer (1 mmol l^{-1}, pH 7.2) and digested to a mixture of nucleosides by successive treatments with nuclease P_1 (Sigma, N-8630, penicillum citrinum), phosphodiesterase I (Sigma, P-6903, bovine intestinal mucosa) and alkaline phosphatase (Sigma, P-4252, bacteria *Escherichia coli*) according to the protocol of Crain [22]. The reaction mixture was lyophilized, dissolved in formic acid–sodium formate buffer (0.2 mol l^{-1}, pH 3.3), and then incubated at 80°C for 4.5 h, i.e. a period known to be sufficiently long to result in complete depurination of monomeric purine nucleosides. Both reaction mixtures, A and B, were finally filtrated through a 0.2 μm membrane, and the released purine bases were quantified by RP HPLC, as described above. The amount of purine bases in both samples were equal within the limits of experimental errors, indicating that the calf thymus DNA was completely depurinated even if it was not digested to nucleosides prior to the acid-catalyzed hydrolysis.

2.7. Modification of calf thymus DNAwith MDA and release of thepyrimido[1,2-a]purin-10(3H)-one bases

Calf thymus DNA was modified by reacting it with MDA essentially as described previously [9]. Accordingly, to 0.5 ml samples of aqueous solution of DNA (1 mg ml^{-1}), from 1 to 30 μl of MDA solution were added. The MDA solution was prepared by hydrolyzing freshly distilled 1,1,3,3-tetramethoxypropane (0.2 g) in aqueous hydrogen chloride (10 ml, 0.1 mol l^{-1}) at 40°C for 45 min, after which aqueous ammonium acetate (0.1 g in 8 ml) was added. The DNA samples were then incubated for 0.5 h at 37°C. Aqueous ammonium acetate (130 μl, 2 mol l^{-1}) was added, and the DNA was precipitated with cold ethanol (7 ml) and kept in the cold for 3 h. Finally, the precipitate was collected by centrifugation, washed thoroughly with cold ethanol and lyophilized. The modified DNA was stored at 4°C.

Table 1
Pseudo-first-order rate constants for the hydrolysis of the *N*-glycosidic bond of 3-(2-deoxy-β-D-erythro-pentofuranosyl)pyrimido[1,2-a]purin-10-one (**3**), 2-deoxyguanosine (dG) and 2′-deoxyadenosine (dA) in formic acid–sodium formate buffers at 80°C[a]

Compound	[HCOOH] (mol l^{-1})	[HCOONa] (mol l^{-1})	k (10^{-4} s^{-1})
3	0.045	0.005	14.3 ± 0.5
	0.015	0.005	6.8 ± 0.2
	0.005	0.005	3.1 ± 0.1
dG	0.015	0.005	6.2 ± 0.2
dA	0.015	0.005	3.1 ± 0.1

[a] The pseudo-first-order rate constant for the subsequent decomposition of pyrimido[1,2-a]purin10(3*H*)-one (**1**) from **3** was $(1.19 \pm 0.02) \times 10^{-5}$ s^{-1} in the buffer containing 0.045 mol l^{-1} formic acid and 0.005 mol l^{-1} sodium formate.

To each modified DNA sample, 10 ng of **2** was added as an internal standard. The samples were incubated in formic acid–sodium formate buffer (0.2 mol l^{-1}, pH 3.3) at 90°C for 70 min. Aqueous ammonium acetate (100 μl, 0.5 mol l^{-1}) was added, the samples were cooled to 0°C, filtered through a 0.2 μm membrane, and concentrated to a final volume of 300 μl. The samples were subjected to RP HPLC on a LiChrospher®100 RP-18 (5 μm) column using 2 mmol l^{-1} aqueous ammonium acetate–acetonitrile (98.1:1.9, v/v) as an eluent (flow rate 1 ml min^{-1}). The retention time of **1** and **2** were 19.8 min, while those of unmodified nucleic acid bases fell in the range 2.5–11 min. Finally, the samples were evaporated to dryness and stored at 4°C. To verify that the presence of **2** during depurination does not result in additional formation of **1**, some samples were also prepared by omitting the internal standard.

2.8. HPLC/ESI–MS or HPLC/ESI–MS/MS analyses

The samples containing **1** and **2**, and traces of the unmodified bases, were dissolved in 60 μl of water. Samples of either 10 or 20 μl were injected to LC/ESI–MS. On using the LCQ quadrupole ion trap mass spectrometer, the following gradient elusion (Jones chromatography 100-2.1 Genesis C18 4 μm) was applied: from 0 to 2 min isocratically water–methanol (95:5, v/v), and then a linear gradient to water–methanol (55:45, v/v) in 10 min. The flow rate was 100 μl min^{-1}. Quantification of **1** was accomplished by using 500 ms collection time of ions in the trap and comparing the selected ion monitoring (SIM) peak areas of **1** ($M + H^+$, m/z 188) and **2** ($M + H^+$, m/z 191). On using the triple quadrupole LC/MS/MS, a linear gradient from water–methanol (95:5, v/v) to water–methanol (30:70, v/v) in 16.5 min was applied (Jones chromatography 100-2.1 Genesis C18 4 μm). The molecules were measured by MRM scan using 200 ms dwell time and splitter ion m/z 106.0 for **1** and m/z 107.0 for **2**.

The calibration curve was constructed using samples prepared from authentic **1** and **2**. The measurement were carried out on the triple quadrupole LC/MS/MS spectrometer, as indicated above.

3. Results and discussion

3.1. Acid-catalyzed hydrolysis of 3-(2-deoxy-β-D-erythro-pentofuranosyl)pyrimido[1,2-a]purin-10-one (3)

The hydrolysis of 3-(2-deoxy-β-D-erythro-pentofuranosyl)pyrimido[1,2-a]purin-10-one [4] (**3**) was followed by analyzing the compositions of aliquots withdrawn at appropriate intervals by RP HPLC. The only reaction detected was the cleavage of the *N*-glycosidic bond, i.e. the conversion of **3** to **1**. Table 1 records the first-order rate constant obtained. At pH 3.3 and 80°C, i.e. under conditions similar to those used subsequently to depurinate DNA, the hydrolysis was slightly faster than that of unmodified 2′-deoxyguanosine or 2′-deoxyadenosine, and hence much faster than that of pyrimidine nucleosides [23]. In the pH range studied, pyrimido[1,2-a]purin-10(3*H*)-one was observed to be hydrolytically two orders of magnitude more stable than the starting nucleoside **3**. Accordingly, 1.5 h incubation at pH 3.3 and 80°C was sufficient to result in virtually complete hydrolysis of **3** to **1**, and during this

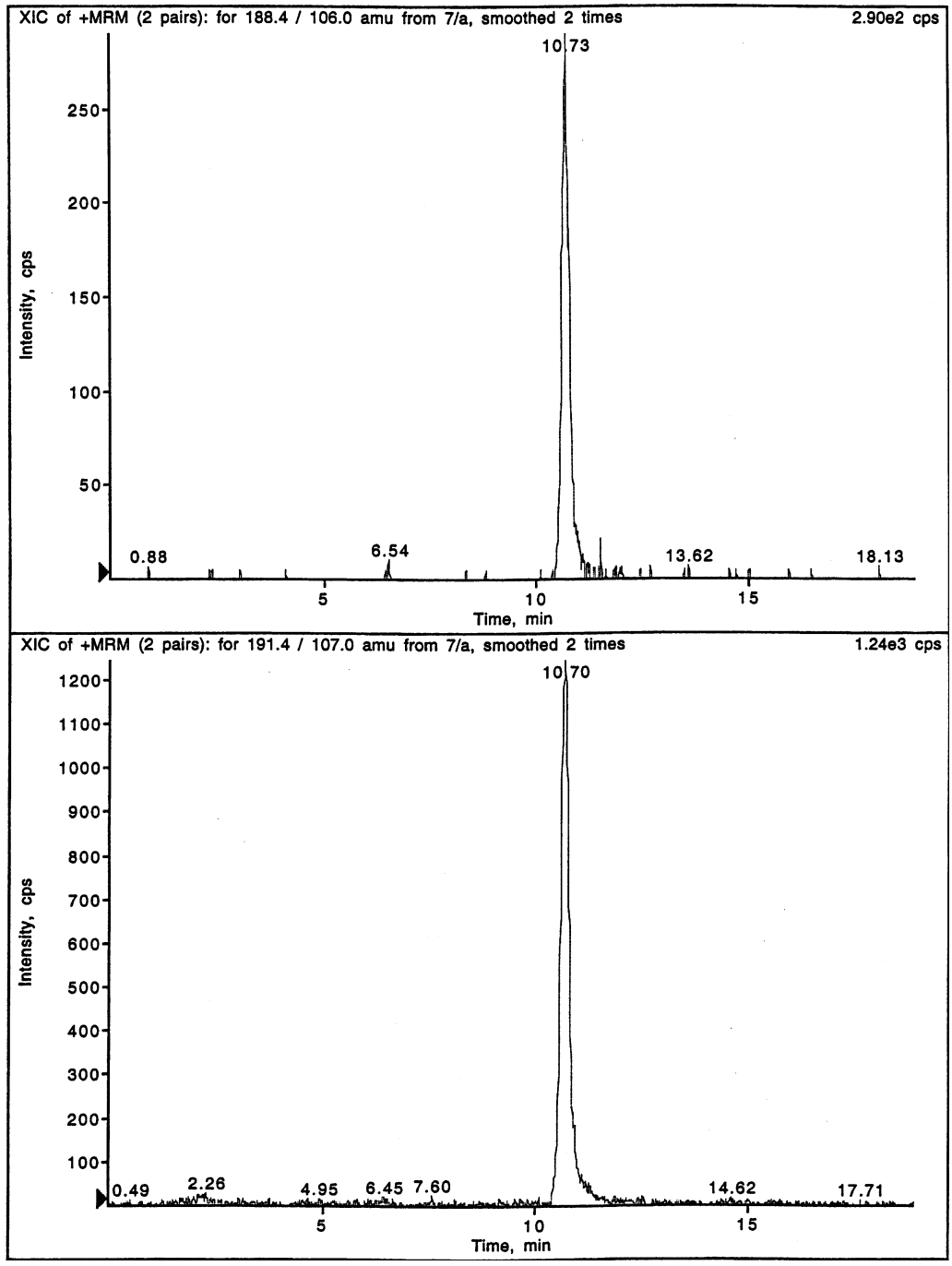

Fig. 3. An example of calibration curve measurement. On using the triple quadrupole LC/MS/MS, a linear gradient from 5 to 70% methanol in water in 16.5 min was applied (Jones chromatography 100-2.1 Genesis C18 4 μm). The molecules were measured by MRM scan using 200 ms dwell time and splitter ion m/z 106.0 for **1** and m/z 107.0 for **2**. The upper picture shows the chromatogram of 100 pg/inj. of **1**, and the lower 500 pg/inj. of **2**.

period less than 2% of released **1** was converted to guanine.

We have shown previously [24] that the acid-catalyzed depurination may be retarded by a factor of **5** on going from monomeric nucleosides to polynucleotides. To verify that 4.5 h incubation at pH 3.3 and 80°C (or 70 min at 90°C) is sufficient for quantitative removal of guanine residues, and hence also **1**, from DNA, the following experiment was carried out. A sample of calf thymus DNA was on one hand subjected to acid-catalyzed depurination and, on the other hand, digested enzymatically to nucleosides by the method of Crain [22]. The nucleoside mixture was then depurinated under the conditions used to depurinate the DNA sample. RP HPLC analysis of the two hydrolyzates indicated that equal amounts of guanine were obtained by both methods. Since it was additionally known that **1** is not markedly destroyed under the conditions required for virtually complete depurination of DNA, the acid-catalyzed hydrolysis in formic acid–sodium formate buffer was selected as the method for release of **1** from DNA.

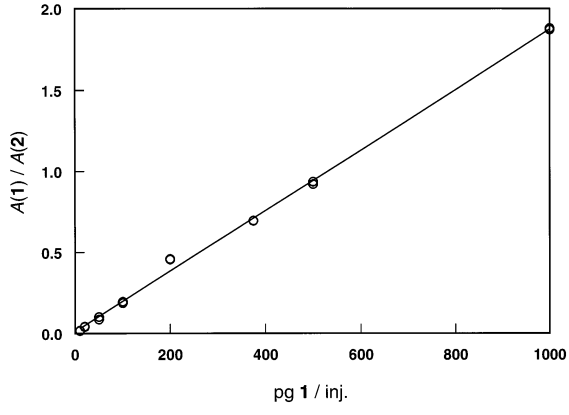

Fig. 4. Calibration curve for quantification of pyrimido[1,2-a]purin-10(3*H*)-one (**1**). The ratio of the signal areas of **1** and its isotopically modified analog **2**, employed as an internal standard, plotted against the mass of **1** injected. All standard solutions have been measured twice. Slope $(1.86 \pm 0.02) \times 10^{-3}$ pg^{-1}, intercept $(1 \pm 1) \times 10^{-2}$, correlation coefficient (r) 0.998.

3.2. Synthesis and stability of [2,3a,10-$^{13}C_3$]pyrimido[1,2-a]purin-10(3H)-one (2)

Complex **2** was obtained by treating [4,6,8-$^{13}C_3$]guanine (**7**) with MDA [3,21]. [4,6,8-$^{13}C_3$]Guanine was prepared in principle as described previously [19] for [4,5,6,8-$^{13}C_4$]guanine (Fig. 1). However, the sulfate salt of 2,4,5-triamino-[4,6-$^{13}C_2$]pyrimidin-6(1*H*)-one (**6**) was cyclized to **7** by refluxing it in [^{13}C]formic acid for 3 days, instead of treating with morpholinium formate. This simple transformation gave an almost quantitative yield. The final product was characterized by RP HPLC, and UV and mass spectroscopy.

As mentioned above, the isotopically unmodified pyrimido[1,2-a]purin-10(3*H*)-one was observed to be hydrolytically virtually stable under the conditions needed to remove it from DNA. One might, however, speculate that **1** could be formed by a cross reaction between guanine and **2** and **1** upon depurination of DNA in the presence of **2**. To exclude this possibility, **2** was treated under the depurination conditions with a large excess of unmodified nucleic acid bases. HPLC/ESI–MS/MS analysis of the aliquots withdrawn from the reaction mixture indicated that less than 1% of **2** was converted to **1** under conditions needed to achieve complete depurination of DNA. Taking into account that the amount of **2** used as an internal standard in the MS analyses is only of the same order as the amount of **1** to be quantified, this cross reaction is not a source of marked experimental errors.

3.3. Mass spectrometric analyses

As an initial experiment, a calibration curve for the HPLC/ESI–MS analysis was determined with the aid of artificially prepared samples. Accordingly, samples containing 1.0, 2.0, 5.0, 10.0, 20.0, 37.5, 50.0 and 100 ng of **1** and 50 ng of **2** in 1 ml of water were prepared, and 10 μl aliquots of these samples were subjected to the HPLC/ESI–MS/MS analysis on a Perkin–Elmer Sciex API 365 triple quadrupole LC/MS/MS (Fig. 3). Signal areas for **1** (M + 1 m/z 188, splitter ion m/z 106.0) and **2** (M + 1 m/z 191, splitter ion m/z 107.0)

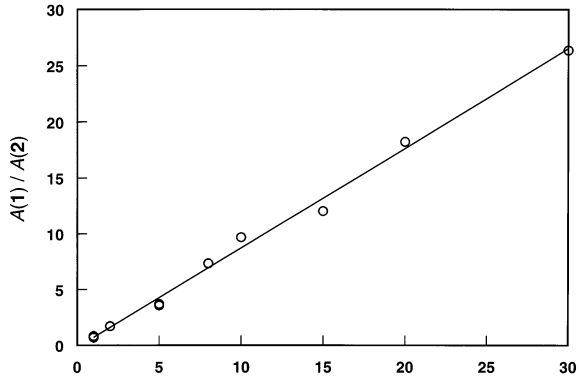

Fig. 5. Formation of pyrimido[1,2-a]purin-10(3*H*)-one (**1**) adducts upon treatment of calf thymus DNA with MDA. The amount of **1** isolated from 0.5 mg of DNA plotted against the concentration of MDA used to modify the DNA. The DNA samples modified by 1, 5 and 8 μl of MDA solution were measured twice. Slope $(8.90 \pm 0.23) \times 10^{-3}$ μl^{-1}, intercept $(-1.8 \pm 3) \times 10^{-1}$, correlation coefficient (r) 0.997.

were measured. Fig. 4 shows the dependence of the ratio of these two areas, $A(\mathbf{1})/A(\mathbf{2})$, on the mass concentration of **1**. As seen, $A(\mathbf{1})/A(\mathbf{2})$ is strictly proportional to the amount of **1** in the sample.

Calf thymus DNA (1 mg ml^{-1}) was then modified by treating it with various amounts of MDA in aqueous ammonium acetate for 30 min at 37°C. After this treatment, DNA was precipitated with cold ethanol, washed with ethanol and lyophilized. The modified DNA samples were then dissolved in formic acid–sodium formate buffer (pH 3.3), a known amount of **2** was added as an internal standard, and the samples were incubated at 90°C for 70 min to achieve complete depurination. The hydrolyzate was passed through a membrane filter and the unmodified nucleic acid bases were removed by RP HPLC. The samples were then subjected to HPLC/ESI–MS or HPLC/ESI–MS/MS analysis on two different spectrometers: a Finnigan MAT LCQ quadrupole ion trap mass specrometer or a Perkin–Elmer Sciex API 365 triple quadrupole mass spectrometer. The ratio $A(\mathbf{1})/A(\mathbf{2})$ was determined for each sample by comparing the selected ion monitoring peak areas of **1** and **2**. Fig. 5 shows the results obtained by the Finnigan MAT LCQ quadrupole ion trap mass specrometer. Other analyses were made by the Perkin–Elmer Sciex API 365 triple quadrupole LC/MS/MS mass specrometer, using MS/MS analysis, because it was much more sensitive. As seen $A(\mathbf{1})/A(\mathbf{2})$ increases linearly with the amount of MDA used to modify the calf thymus DNA. To check the reproducibility of the method, samples made by adding 1.0, 5.0 and 8.0 μl of MDA solution to DNA were prepared as duplicate. The results differed by ± 8, ± 3 and $\sim 0\%$, respectively, indicating good reproducibility. It was also checked that the area $A(\mathbf{1})$ remained unchanged within the limits of experimental errors when depurination of DNA was carried out in the absence of **2**. Accordingly, no cross reaction between **2** and the DNA guanine moieties could be detected.

The methodology described above allows reliable quantification of **1** at the level of 10 pa/injection (LOQ, with RSD value of 9%), which means that 50 fmol of the adduct may be quantified. Assuming that one base out of 10^6 guanine residues would be modified, less than 100 μg of DNA is required for quantification. The LOD was measured to be **2** pg/inj. with $S/N > 3$. Accordingly, the method appears to meet the requirements of screening of biological samples.

References

[1] L.J. Marnett, in: K. Hemminki, A. Dipple, D.E.G. Shuker, F.F. Kadlubar, D. Segerbäck, H. Bartsch (Eds.), DNA Adducts: Identification and Biological Significance, IARC, Lyon, 1994, pp. 151–163.
[2] A.K. Basu, S.M. O'Hara, P. Valladier, K. Stone, O. Mols, L.J. Marnett, Chem. Res. Toxicol. 1 (1988) 53–59.
[3] H. Seto, T. Takesue, T. Ikemura, Bull. Chem. Soc. Jpn. 58 (1985) 3431–3435.
[4] H. Seto, T. Okuda, T. Takesue, T. Ikemura, Bull. Soc. Chem. Jpn. 56 (1983) 1799–1802.
[5] D.R. Janero, Free Radic. Biol. Med. 9 (1990) 515–540.
[6] H. Esterbauer, P. Eckl, A. Ortner, Mutat. Res. Rev. Genet. Toxicol. 238 (1990) 223–233.
[7] H. Esterbauer in Free Radicals, in: G. Pli, K.H. Cheeseman, M.U. Dianzani, T.F. Slater (Eds.), Liver Injury, IRL Press, Oxford, 1985, pp. 29–47.
[8] H.H. Draper, M. Hadley, Xenobiotica 20 (1990) 901–907.
[9] Y. Goda, L.J. Marnett, Chem. Res. Toxicol. 4 (1991) 520–524.

[10] C.E. Vaca, J.-L. Fang, M. Mutanen, L. Valsta, Carcinogenesis 16 (1995) 1847–1851.
[11] C.A. Vaca, P. Vodicka, K. Hemminki, Carcinogenesis 13 (1992) 593–599.
[12] C.A. Rouzer, A.K. Chaudhary, M. Nokubo, D.M. Ferguson, G.R. Reddy, I.A. Blair, L.J. Marnett, Chem. Res. Toxicol. 10 (1997) 181–188.
[13] A.K. Chaudhary, M. Nokubo, G.R. Reddy, S.N. Yeola, J.D. Morrow, I.A. Blair, L.J. Marnett, Science 265 (1994) 1580–1582.
[14] A.K. Caudhary, M. Nokubo, L.J. Marnett, I.A. Blair, Biol. Mass Spectrom. 23 (1994) 457–464.
[15] A.K. Chaudhary, M. Nokubo, T.D. Oglesby, L.J. Marnett, I.A. Blair, J. Mass Spectrom. 30 (1995) 1157–1166.
[16] J.B. Fenn, M. Mann, C.K. Meng, S.F. Wong, C.M. Whitehouse, Science 246 (1989) 64–71.
[17] M. Müller, F.J. Belas, I.A. Blair, F.P. Guengerich, Chem. Res. Toxicol. 10 (1997) 242–247.
[18] J. Serrano, C.M. Palmeira, K.B. Wallace, D.W. Kuehl, Rapid Commun. Mass Spectrom. 10 (1996) 1789–1791.
[19] N. Scheller, R. Sangaiah, A. Ranasinghe, V. Amarnath, A. Gold, J.A. Swenberg, Chem. Res. Toxicol. 8 (1995) 333–337.
[20] R.A. Lazarus, M.A. Sulewski, S.J.J. Benkovic, Label. Compd. Radiopharm. 19 (1982) 1189–1195.
[21] H. Seto, T. Seto, T. Ohkubo, I. Saitoh, Chem. Pharm. Bull. 39 (1991) 515–517.
[22] F.P. Crain, Methods Enzymol. 193 (1990) 782–790.
[23] M. Oivanen, J. Hovinen, P. Lehikoinen, H. Lönnberg, Trends Org. Chem. 4 (1993) 397–412.
[24] H. Hakala, M. Oivanen, E. Saloniemi, A. Guzaev, H. Lönnberg, J. Phys. Org. Chem. 5 (1992) 824–828.