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Analysis of nucleosides using the atmospheric-pressure solids analysis probe for ionization

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ABSTRACT

Nucleosides are polar molecules and generally considered to be non-volatile. Therefore, electrospray ionization is the method of choice for analysis of these compounds by mass spectrometry. In this work, we demonstrate that the atmospheric-pressure solids analysis probe can be used to ionize modified and non-modified nucleosides. Analogously to atmospheric pressure chemical ionization, this ionization technique utilizes heat to vaporize the compounds and a corona discharge needle to ionize the vaporized analytes. The optimum source conditions were determined and the influence of temperature, the presence of cations and the nature of modification were investigated. It was found that nucleosides withstand the elevated temperature well and can be brought in the gas phase as neutral molecules. The generated fragmentations are comparable to those observed with electrospray ionization.

1. Introduction

Ambient desorption ionization methods have been extensively developed over the past decade and many variants are commercially available today [1]. Their main advantage is the facilitated sample introduction due to the fact that samples can very often be analyzed in a solid form without the need for sample preparation.

The atmospheric-pressure solids analysis probe (ASAP) is one of the ambient desorption methods derived from atmospheric-pressure chemical ionization (APCI). In ASAP the samples are first vaporized using heated nitrogen, followed by ionization by electrical discharge at a corona needle [2]. This has been shown to work well with polymers and apolar compounds like steroids.

With this work we would like to demonstrate that nucleosides, generally considered to be polar and non-volatile, can be ionized using ASAP.

Modified nucleosides are used as antiviral compounds and are also studied as biomarkers for tumors and mass spectrometry plays an important role in the analysis of these compounds [3]. Naturally occurring modified ribonucleosides are markers in phylogenetic studies [4] and the enzymes involved in their biosynthesis are well-studied [5]. Their identification and analysis are important in these research areas. Fast and selective detection methods for modified nucleosides are therefore needed in order to reduce screening time. With this work we wanted to investigate whether analysis of nucleoside analogs is possible using mass spectrometry with ASAP as ionization source.

2. Experimental

2.1. Instrumentation

A 3D quadrupole ion trap mass spectrometer (LCQ Duo, ThermoFinnigan, San Jose, CA) was used for all experiments. The transfer capillary temperature was set to 150 °C and the capillary voltage to 10 V. The optimum tube lens voltage was 0 V.

2.2. Ion source

The source consisted of a standard APCI source, in-house modified to a ASAP probe by placing a syringe needle (20G × 1.1/2″, BD Microlance 3, Becton Dickinson BV, The Netherlands) between the APCI heater and the corona discharge needle. The syringe needle was cut to length and kept in place using double-sided tape and attached to the bottom of the source housing (see photos in Fig. 1).

The corona discharge current was set to 4.0 μA, and the sheath gas flow rate to 60 arbitrary units.

Glass capillaries (length 25 mm, outer diameter 1.7 mm) were closed at one end by melting in a Bunsen burner flame. The sample was applied at the top of the glass capillary, which was subsequently placed over the syringe needle.

The temperature of the nitrogen stream heating the capillary with the sample was optimized for each compound and was typically in the range 300–400 °C. The glass capillaries were replaced after each data acquisition. Ion traces were generated by stepwise

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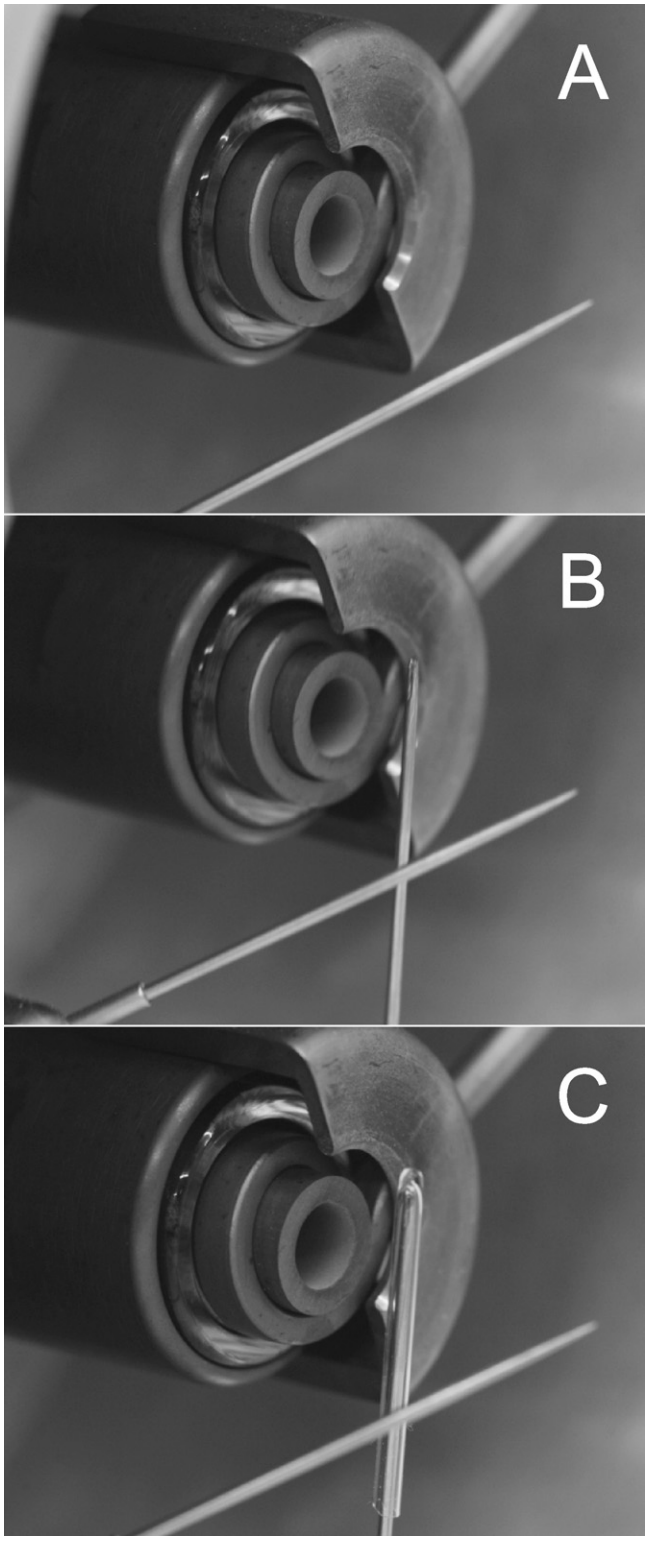

Fig. 1. Pictures of the converted atmospheric-pressure chemical ionization source into a atmospheric-pressure solids analysis probe. (A) Original source, (B) syringe needle mounted, and (C) glass capillary over the syringe needle.

increasing the temperature by 50 °C every 20 s while recording data in single ion monitoring mode.

For experiments using the standard electrospray ionization (ESI) source, the common settings (transfer capillary temperature, capillary voltage, tube lens voltage, trapping time) were kept identical. The ESI capillary voltage was set to 4.0 kV.

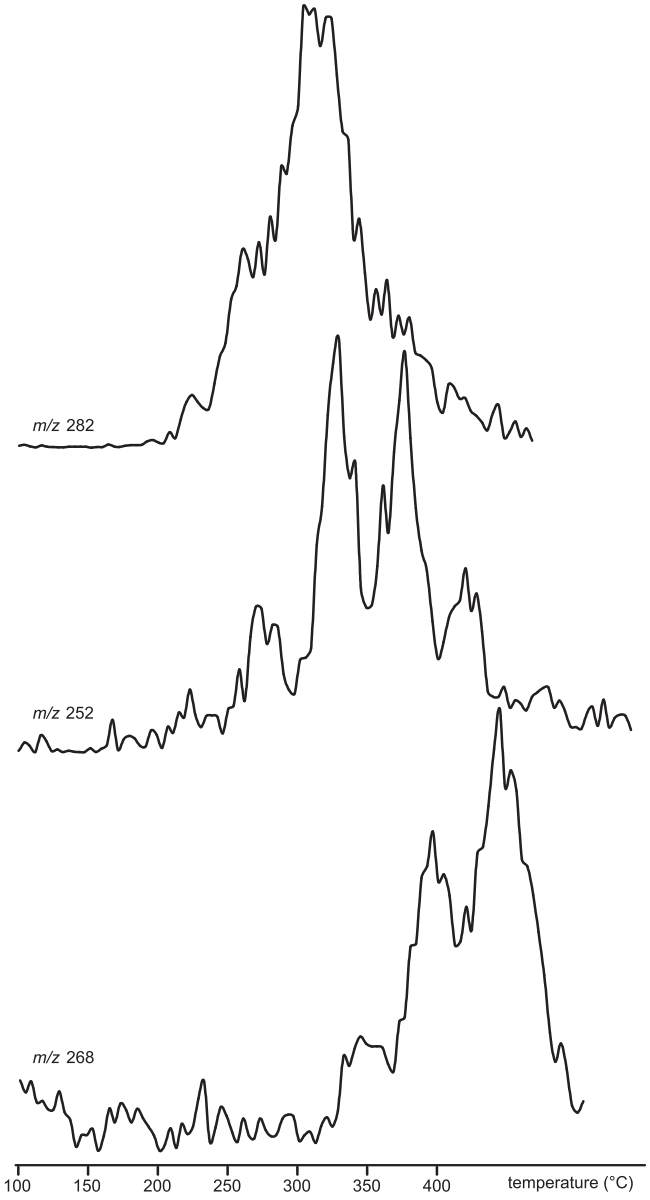

Fig. 2. Extracted ion traces for the m/z corresponding to the $[M+H]^+$ of adenosine (m/z 268) 2′-deoxyadenosine (m/z 252) and 1-methyladenosine (m/z 282) as a function of temperature. The maximum ion intensities are 2.8×10^4, 1.7×10^4 and 1.6×10^6 for adenosine, 2′-deoxyadenosine and 1-methyladenosine respectively.

2.3. Chemicals

Adenosine, 2′-deoxyadenosine, uridine and 2′-deoxyuridine were purchased from Pharma Waldhof (Düsseldorf, Germany), 1-methyladenosine was obtained from Sigma–Aldrich. 2′-Deoxy-3′-trityl-N^5-benzoylcytidine and 5′-*O*-*tert*-butyldimethylsilyl thymidine (TBDMS thymidine) were synthesized in our laboratory as part of ongoing research projects [6]. Sodium acetate and potassium acetate were obtained from Merck (Darmstadt, Germany).

2.4. Sample preparation

All nucleoside samples were dissolved to a final concentration of 30 μM. 1 μL (30 pmol) was applied on the capillary for each experiment. For the ESI experiments the samples were diluted in such a way that 30 pmol sample was consumed for the acquisition of ten spectra. Where stated, sodium and potassium ions were added to the nucleoside solution in a concentration of 1 mM.

For samples delivered on a silicagel thin layer chromatography (TLC) plate the silicagel of the spots was scraped off and suspended in 100 μL acetonitrile. After centrifugation on a mini centrifuge at 2000 × *g* (PicoFuge, Stratagene, La Jolla, CA), 1 μL of the supernatant was applied on the glass capillary.

3. Results and discussion

3.1. Optimization of the ionization conditions

The APCI heater was retracted as far as possible in order to have the necessary space to mount the syringe needle with the capillary. No other adjustments were needed and no improvements could be obtained by shifting the position of the heater, capillary or corona needle.

Although considered to be non-volatile, nucleosides have been analyzed in our laboratory in the past using electron impact and chemical ionization [7]. In these sources the samples are vaporized by the very low air pressure (10^{-8}–10^{-10} kPa) and the applied heat (100–200 °C). These ionization methods were not soft (compared to ESI) and even with chemical ionization it was often difficult to observe the molecular ion peak.

This led us to experiments in which we wanted to investigate whether evaporation was possible under atmospheric pressure and whether ASAP was a suitable ionization method for nucleosides.

For adenosine and 2′-deoxyadenosine melting points of respectively 234 and 188 °C have been reported in the literature [8] which were starting points for setting the temperature of the APCI heater. Once a suitable working temperature was found, a temperature gradient chromatogram was recorded by stepping the temperature manually by 50 °C every 20 s. The heater response was such that the temperature was reached in 5 or 10 s and a small overshoot of 10–20 °C was observed. As can be seen from the extracted ion traces in Fig. 2, the molecules start to be desorbed at 350 and 300 °C for adenosine and 2′-deoxyadenosine respectively, as expected from their melting points. When the temperature is kept constant, a decay of the ion current in function of time is observed. At higher temperatures the decrease of the ion current is caused by the depletion of the sample.

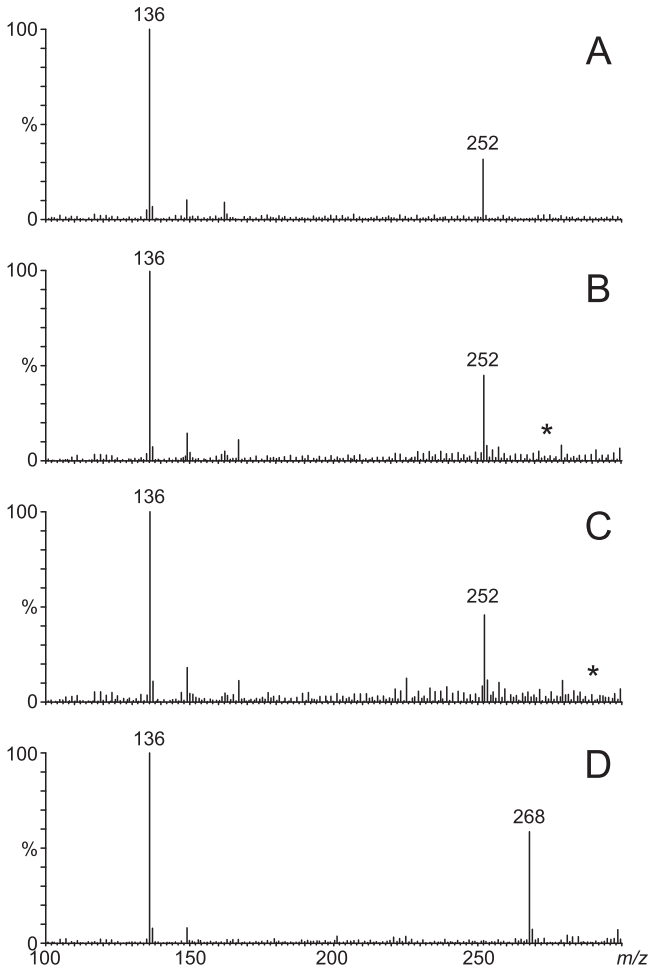

Fig. 3. Spectra obtained with ASAP in positive ionization mode from 30 pmol (A) 2′-deoxyadenosine, (B) 2′-deoxyadenosine in the presence of 1 nmol sodium, (C) 2′-deoxyadenosine in the presence of 1 nmol potassium and (D) adenosine. Asterisks indicate possible positions of cation adducts.

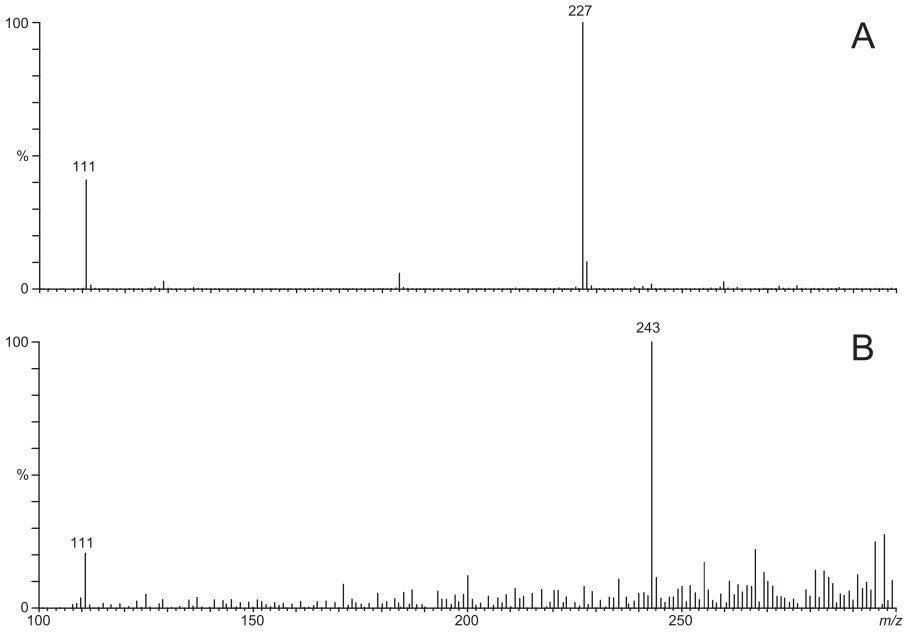

Fig. 4. Spectra obtained with ASAP in negative ionization mode from 30 pmol (A) 2′-deoxyuridine and (B) uridine.

The spectra recorded in positive ionization mode show signals for the expected protonated molecules and also for the protonated bases (*m*/*z* 136) released from the molecules (Fig. 3). Therefore, the spectra were compared with those obtained with a standard ESI source and by consuming the same amount of sample. In both cases the total ion yield as well as the ratio of the molecular ion to the released base were comparable, suggesting that the fragmentation occurs after the ionization, *e.g.*, the transfer capillary or the transfer multipole.

3.2. Interference of cations in solution

Next, the influence of the presence of sodium and potassium on the sensitivity was checked. These ions form cation adducts and reduce the signal intensity in ESI. To the 30 μM nucleoside solutions 1 mM sodium or potassium were added. In all cases no cation adducts were detected and the ion intensity was only slightly reduced (Fig. 3, panels B and C). For example, the base peak intensities were 8.5×10^5, 7.9×10^5 and 7.0×10^5 for 2′-deoxyadenosine without addition of cations, with 1 mM sodium and 1 mM potassium respectively. The same solutions did not give useful spectra with ESI (data not shown).

These results indicate that neutral molecules are vaporized preferentially rather than the cationized molecules and that ionization occurs at the corona discharge needle. Indeed, switching off the high voltage of the corona needle causes the signal to drop completely.

The addition of acetic acid (1% v/v) or formic acid (0.1% v/v) to the sample solution did not generate any substantial change in the ionization efficiency.

3.3. Negative ionization mode

Two pyrimidine base containing nucleosides (uridine and 2′-deoxyuridine) were selected for analysis in negative ionization mode. The spectra are shown in Fig. 4. Similarly as in positive ionization mode base release occurs and the presence of the uracil base anion peak at *m*/*z* 111 can be observed.

3.4. Modified nucleosides

The behavior in the ASAP of modified nucleosides was also investigated.

Up to now, more than one hundred modified nucleosides have been detected and identified in living organisms [9]. Modified nucleosides can also be considered as biomarkers for tumors and can be detected in urine [10]. Synthesis of modified nucleosides involves many reaction steps, accompanied with protection and deprotection of functional groups. The protective groups are in general apolar moieties (*e.g.*, trityl, benzoyl, isobutyryl, silyl) which make the compounds more apolar and hence better suited for ionization with ASAP. However, the protecting groups are generally more labile and significant fragmentation could be expected. Because it was impossible to analyze all possible known modifications only a small selection was analyzed and representative examples are shown here.

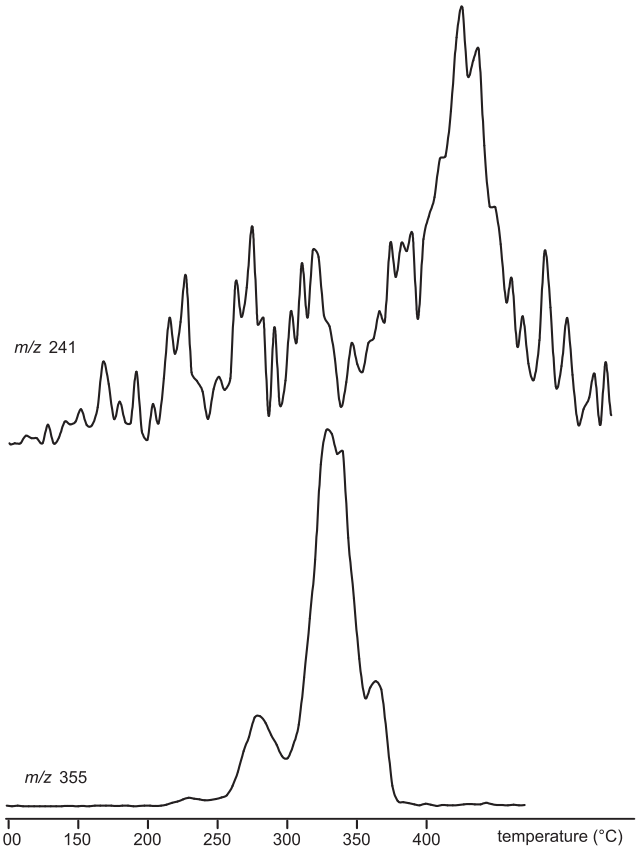

Fig. 5. Extracted ion traces for thymidine (*m*/*z* 241) and 5′-TBDMS-thymidine (*m*/*z* 355) as a function of temperature. The maximum ion intensities are 3.8×10^3 and 2.5×10^5 for thymidine and 5′-TBDMS-thymidine respectively.

Influence of methylation was investigated by comparison of the behavior of 1-methyladenosine with adenosine. The evaporation temperature was lower than for adenosine which can be linked to the less polar character of 1-methyladenosine and to its lower melting point (215 °C [11]) (Fig. 2).

Examples of intermediates in the synthesis of modified nucleosides are shown in Figs. 5 and 6. The TBDMS thymidine yields very high signals (Fig. 5) compared to the non-modified nucleoside. This is not so surprising as silyl groups cause the compound to be more apolar and more volatile. In the past, silyl groups were used to derivatize nucleosides for analysis by fast atom

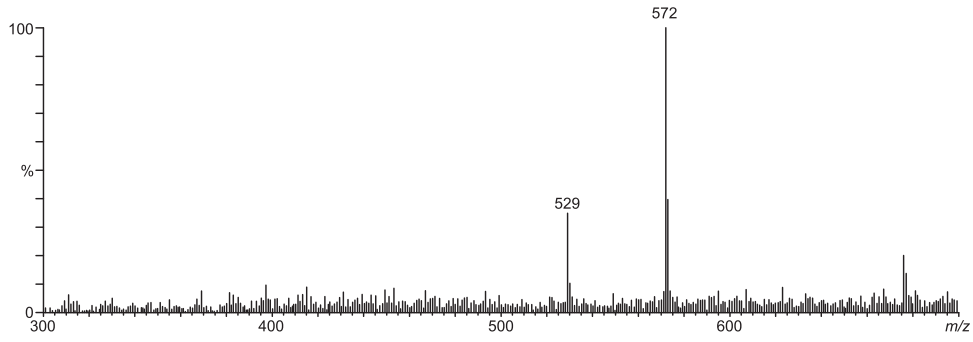

Fig. 6. Spectrum for 2′-deoxy-3′-trityl-N^5-benzoylcytidine obtained by ASAP in negative ionization mode. The sample was eluted from a TLC plate.

bombardment mass spectrometry or gas chromatography. Also, almost no fragmentation was observed (data not shown). The 2′-deoxy-3′-trityl-N^5-benzoylcytidine, a protected intermediate during synthesis of modified nucleosides and oligonucleotides appears to be very stable under conditions for ionization with ASAP (Fig. 6). In the negative ion spectrum obtained from elution of a silicagel TLC plate a fragment is observed at m/z 529 which corresponds to the loss of HNCO (43u) and can be rationalized by a retro Diels–Alder rearrangement as often found in the spectra in negative ionization mode of pyrimidines [12]. The trityl group is often lost during the ionization step but here we obtained good quality spectra in order to support the chemical synthesis experiments in our laboratory.

4. Conclusions

The applicability of using ASAP for the analysis has been investigated. It was possible to obtain spectra in positive and in negative ionization mode for non-modified and modified nucleosides. The optimum temperature for evaporation of the nucleosides is related to the melting point for the compounds that we analyzed. The evaporation is not hampered in the presence cation concentrations of 1 mM and clearly less interference on the quality of the spectra has been observed compared to ESI.

For synthetic modified nucleosides containing apolar protecting groups the protonated or deprotonated molecules were detected. The observed fragmentation was comparable to ESI, suggesting that the fragmentation is not primarily caused by the applied heat. Silylated compounds needed lower operating temperatures and showed higher ion currents than the non-modified nucleosides.

The short sample preparation and sample introduction time, together with the low cross-contamination risk makes ASAP a valuable ionization method for analysis of nucleosides using mass spectrometry.

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