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FIELD DESORPTION MASS SPECTROMETRY IN THE ANALYSIS OF A STEROID CONJUGATE, ESTRIOL-16α-GLUCURONIDE

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SUMMARY

Field desorption mass spectrometry was used for the first time in the analysis of a steroid conjugate, the estriol-16α-glucuronide, isolated from pregnancy urine. Two reference compounds were also investigated. The molecular ion peak of the underivatized conjugate was obtained indicating that the molecular weight of such compounds can easily be determined. Variation in the solvent composition causes some variation in the spectra obtained and complex ion formation was noted. Because of the ubiquity of sodium and potassium, ions containing these metallic elements and representing the sodium and potassium salts of the steroid glucuronic acid are seen in the spectra.

INTRODUCTION

Characterization of steroid conjugates isolated from biological materials has been hampered by the lack of suitable analytical techniques allowing accurate determination of their molecular weights. In mass spectrometry involving electron impact ionization (EI–MS), the molecular ion peaks of underivatized steroid conjugates are not obtained due to the unstability of the molecular ion although it has been possible to record peaks of the molecular ions of some derivatized steroid conjugates [1]. In addition, the abundance of fragment ion peaks giving little structure information in spectra obtained by EI–MS makes interpretation difficult and may preclude reliable identification of compounds.

The invention of more gentle ionization processes in mass spectrometry has provided new methods for the study of unstable organic compounds, the latest development being the introduction of field desorption mass spectrometry (FD–MS) by Beckey (cited from [2]). In the present study FD–MS was used for the first time in the analysis of a steroid conjugate, estriol-16α-glucuronide (3,17β-dihydroxy-1,3,5(10)-estratrien-16α-yl-β-D-glucopyranosiduronide) (E_3-16Gl), which had been isolated from pregnancy urine. The results demonstrate the potential usefulness of FD–MS for obtaining molecular ions in spectra of substances that do not give distinct molecular ion peaks when other ionization processes are used.

EXPERIMENTAL

Reference compounds

Reference estriol-16α-glucuronide was purchased from Ikapharm, Ramat-Gan, Israel, and from Sigma Chemical Company, St. Louis, Mo., U.S.A. The tritiated estriol-16α-glucuronide was from a batch which had been prepared in this laboratory [3].

Isolation of estriol-16α-glucuronide from pregnancy urine

A precipitate was prepared from a 20-l pool of third trimester pregnancy urine by adding ammonium sulfate (0·7 kg/l) as described by Cohen and Oran [4]. For further processing the precipitate was divided into smaller aliquots. Each was dissolved in 3 vol. of water and 25 vol. of methanol–acetone (1:1), and tritiated E_3-16Gl was added. The water–methanol–acetone suspension was stirred for 1·5 h and filtered, and the precipitate washed with methanol–acetone until it was colorless [4]. The water–methanol–acetone extract was taken to dryness and the residue dissolved in 500 ml of water. The steroid conjugates were extracted by percolating the 500-ml sample through a 1 kg Amberlite XAD-2 column according to the method of Bradlow [5]. The methanol fraction containing the conjugate was evaporated to dryness and the residue dissolved in urate–phosphate buffer and chromatographed on Sephadex G 25 [3, 6]. The fraction contain-

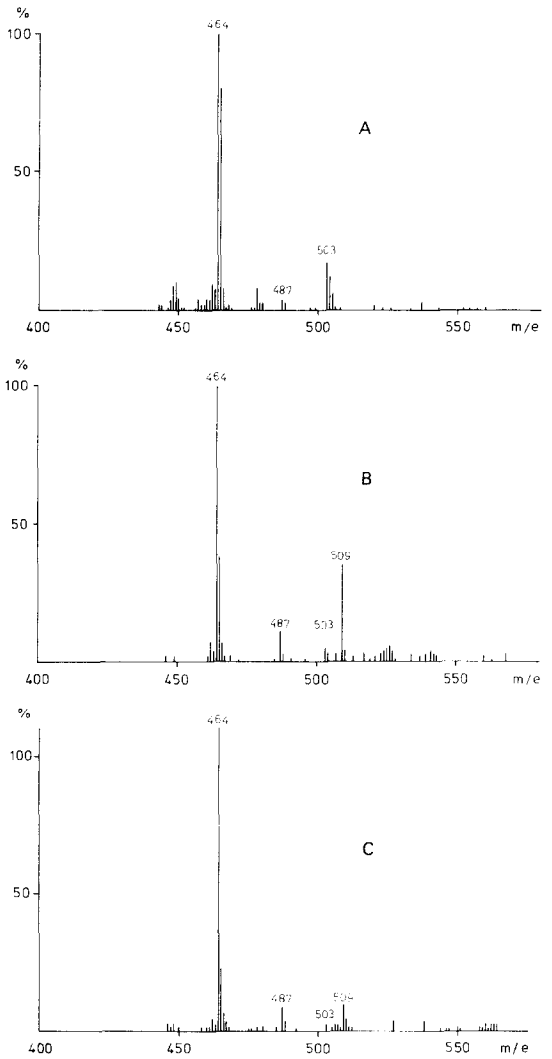


Fig. 1. Field desorption mass spectra of estriol-16α-glucuronic acid obtained from Sigma Chemical Company (1A) and from Ikapharm (1B), and of estriol-16α-glucuronide isolated from pregnancy urine (1C). The compounds were dissolved in ethanol–water (90% ethanol v/v).

ing E_3-16Gl was then applied in chloroform–methanol (1:1) to a 16 g Sephadex LH-20 column [3, 6]. Before chromatography on Sephadex LH-20 the samples were divided into smaller aliquots to dissolve them in the eluting solvent (chloroform–methanol). Chromatography on Sephadex LH-20 was controlled by monitoring the behaviour of the [^{3}H]-E_3-16Gl. Both chromatographic procedures were repeated several times until all pigments had disappeared from the fraction containing the E_3-16Gl. Finally the combined fractions were submitted to the crystallization procedure described by Elce *et al.*[7].

Field desorption mass spectrometry

A varian MAT CH5 DF instrument equipped with a field desorption/field ionization/electron impact combination ion source was used. The tungsten wire activated with benzonitrile is dipped in the solution in which the compound to be investigated is dissolved. In this work various concentrations of ethanol in water were used to dissolve the steroid conjugates. The spectra were taken at 15 mA heating current of the tungsten wire (field ion emitter). The ion source temperature was 65°C and the accelerating voltage 3 kV. The extraction plate had a voltage of −6·5 kV. The scanning speed was 13 amu/s, the paper speed 4 cm/s. A 100 cycles filter was used and the resolution of the instrument was kept at m/Δm = 500–600 (10% valley). Each compound was analyzed several times.

RESULTS AND DISCUSSION

The three samples of E_3-16Gl investigated were the sample isolated from pregnancy urine, the Ikapharm and the Sigma reference standards. It was found that different spectra were obtained depending among other things on the relation between ethanol and water in the sample. With ethanol concentrations higher than 70% the type of spectra shown in Fig. 1 were

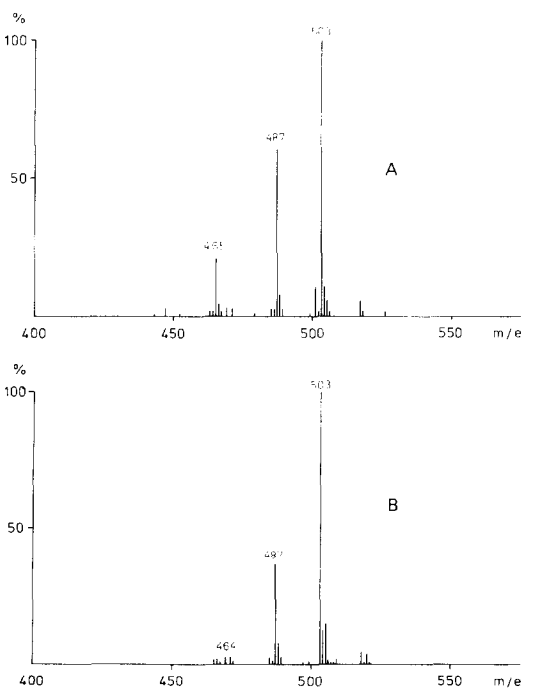
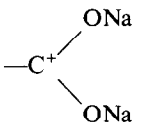


Fig. 2. Field desorption mass spectra of estriol-16α-glucuronide obtained from Ikapharm (2A) and isolated from pregnancy urine (2B). The compounds were dissolved in ethanol–water (50% ethanol v/v).

obtained. However, when the ethanol concentration decreased until the solution became opalescent (45–55% ethanol) the type of spectra seen in Fig. 2 were obtained.

All three spectra in Fig. 1 show the ions m/e 464, 487 and 503. The ion m/e 464 is the molecular ion of E_3-16Gl (mol. wt. 464·5). The ion m/e 487 is probably M + 1 of the sodium salt of E_3-16Gl and the ion m/e 503 is probably the M + 1 ion of the potassium salt. The ion m/e 509 is suggested to be a complex ion of similar nature as found by Schulten and Beckey [2] for sodium acetate, i.e. another sodium atom is attached to the carboxylic acid group of the glucuronic acid moiety:



This ion was not seen in any of the spectra of the Sigma preparation and it was especially pronounced for the Ikapharm standard (Fig. 1). The spectra obtained for the isolated urinary compound resembled always more the spectra of the Ikapharm standard than those of the Sigma standard. There were no significant fragments below m/e 400 in the three spectra shown in Fig. 1.

The spectra shown in Fig. 2 were obtained with solutions of the compounds which contained so much water that they were opalescent because of some insoluble material. In these spectra the M + 1 ions of the sodium and especially of the potassium salts of the E_3-16Gl were the most significant ions. The Ikapharm standard showed the M + 1 ion for the free acid, but the spectrum of the compound from pregnancy urine had only a very small molecular ion of the free acid. In some other spectra the M + 1 ion for the sodium salt was the most prominent ion. Thus a great variation occurred with regard to the relative intensities of the molecular or the M + 1 ions of the free acid and of the sodium and potassium salts. This variation was not only due to the variation in the relative amounts of water and ethanol in the solution, but also to other factors such as the temperature of the tungsten wire at the time when the spectrum was taken. A more detailed analysis of this phenomenon was not carried out in these preliminary studies.

In one spectrum from the sample obtained from pregnancy urine a number of ions were seen also in the lower mass region (Fig. 3). The ion m/e 288 is the molecular ion of estriol. The ion m/e 272 is most likely the estriol molecule with loss of the 16-hydroxyl.

In summary, the present report deals with a typical steroid conjugate which is unstable under electron impact conditions. Useful mass spectra of underivatized steroid conjugates have not been obtained by conventional MS methods, because the molecular ions could not be recorded. The new FD–MS technique provided spectra with intense molecular ions of E_3-16Gl. Thus it can be concluded that FD–MS offers a useful means for the determination of the molecular weight of steroid conjugates, and, in combination with other techniques, for their identification.

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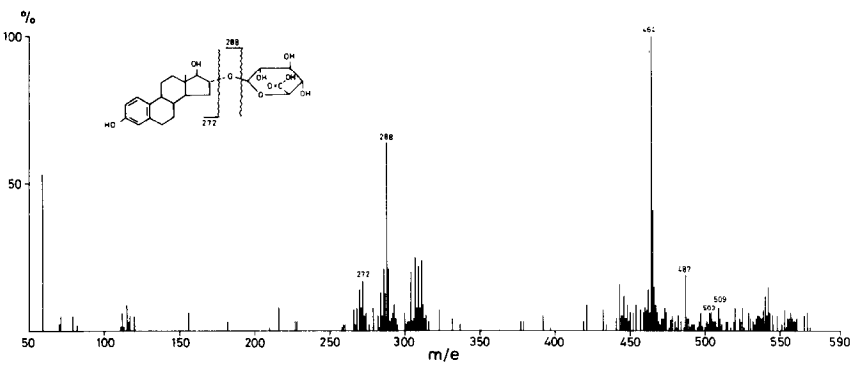

Fig. 3. Field desorption mass spectrum of estriol-16α-glucuronide isolated from pregnancy urine.

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