Journal of Chromatography, 420 (1987) 450–453
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

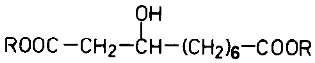
CHROMBIO. 3756

Letter to the Editor

Identification of 3-hydroxydecandioic acid dimethylate in human urine

Sir,

Some time ago we published refractive index values and mass spectral data for methylated acid compounds isolated from human urine samples separated by gas chromatography–mass spectrometry (GC–MS) [1]. Recently the compound numbered 179 in this paper was found in large amounts in the urine of a child suffering from a severe unknown metabolic disease [2] using GC–MS. In the meantime we have identified the compound to be the dimethylate of 3-hydroxydecandioic acid (1) detected previously in the urine of ketotic patients with unknown diseases [3–6]. In these publications the spectrum of compound 1 was published only in form of its trimethylsilylated derivative. The aim of this letter is to report the identification of compound 1 in form of its dimethylate 2.



$$\mathrm{ROOC{-}CH_2{-}\overset{OH}{\overset{|}{C}}H{-}(CH_2)_6{-}COOR}$$

1 $R = H$
2 $R = CH_3$

Compound 1 occurs in urine samples of healthy individuals only in trace amounts. Sometimes these traces can be detected after methylation to compound 2 only if selected-ion monitoring is applied. Purification of compound 2 was achieved by thin-layer chromatography (TLC) and isocratic reserved-phase high-performance liquid chromatographic (HPLC) separation.

EXPERIMENTAL

Hexane, 2-propanol and methanol were of HPLC grade from Rathburn (Walkerburn, U.K). N-Methyl-N-trimethylsilyltrifluoracetamide (MSTFA) was obtained from Fluka (Neu-Ulm, F.R.G.).

Extraction, methylation and TLC of 24-h urine samples were carried out as described before [1]. Further separation of the TLC fraction with R_F 0.46–0.55 was achieved by isocratic reversed-phase HPLC using a Rheodyne 7125 injector,

0378-4347/87/$03.50

a 250 mm×4.6 mm I.D. Nucleosil C_{18} column (5 μm), and LKB 2150 pump and a Spectra-Physics dual-channel UV detector, set at 254 nm. The mobile phase was methanol–water (3:2) with a flow-rate of 0.5 ml/min. Rechromatography of the peak corresponding to 2 was done on a 250 mm×7.1 mm I.D. Serva Si 100 column (3 μm), with hexane–2-propanol (9:1) as mobile phase at a flow-rate of 1.0 ml/min, using a Waters 6000A pump, a Waters U6K injector and a variable-wavelength Kontron Uvikon 722LC UV detector set at 225 nm.

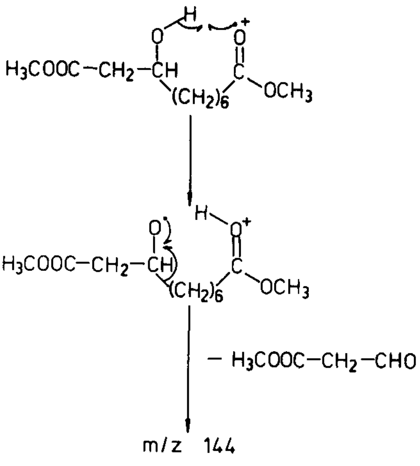
Trimethylsilylation of compound 2 was achieved using MSTFA at 60°C for 12 h.

Gas chromatography–mass spectrometry

All chromatographic enrichment steps were controlled by GC–MS using a Finnigan MAT 312 GC–MS system with a MAT SS 300 data system. The GC column was a 25 m×0.22 mm I.D. OV-101 wall-coated capillary column. The carrier gas was helium (2 ml/min). The temperature was programmed from 100°C to 200°C at 3°C/min, and from 200°C to 280°C at 5°C/min. The injection port temperature was 280°C, and the electron energy was 70 eV.

RESULTS AND DISCUSSION

Full MS data for compound 2 are given in Fig. 1. Ions of mass 103 accompanied by peaks of mass 61 and 71 are typical for the presence of a 3-hydroxy acid methylate [7]. The loss of 102 mass units from the molecular ion is typical for the dimethylate of a 3-hydroxy dicarboxylic acid: the hydrogen of the OH group is transferred to the charged carboxylic group at the other end of the molecule. The radical thus produced is cleaved by loss of $H_3COOC–CH_2–CHO$ [8].



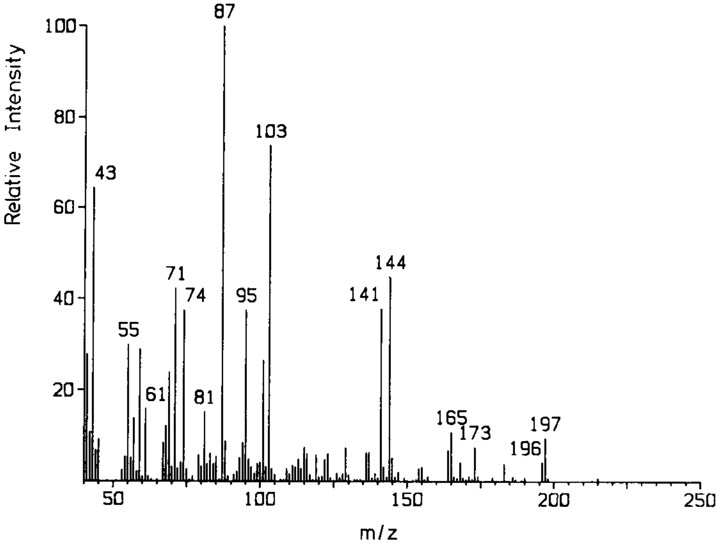


Fig. 1. Mass spectrum of 3-hydroxydecandioic acid dimethylate 2.

The ion of mass 141 — another of the key ions of this compound — results by loss of OCH_3 and $H_2C{=}C{=}O$ followed by elimination of CH_3OH. Confirmation of the deduced structure was obtained by trimethylsilylation [3].

Unfortunately the molecular ion of the original compound 2 cannot be detected (Fig. 1). In our first paper [1], compound 2 was assumed to have a mass of 228, and possibly the molecular formula $C_{11}H_{16}O_5$. Now this must be revised: the molecular mass is 246, and the molecular formula $C_{12}H_{22}O_5$.

ACKNOWLEDGEMENTS

We thank the Robert Pfleger-Stiftung in Bamberg and the Fonds der Chemischen Industrie for financial support. We also thank Michael Glaessner for running the mass spectra and Dietrich Laatsch for providing the glass capillary columns.

Lehrstuhl Organische Chemie I,
Universität Bayreuth,
Postfach 10 12 51,
D-8580 Bayreuth (F.R.G.)

V. PUCHTA
G. SPITELLER*

1 M. Spiteller and G. Spiteller, J. Chromatogr., 164 (1979) 253.
2 E. Jellum, personal communication.
3 J.-S. Svendsen, L.K. Sydnes and J.E. Pettersen, Anal. Chem. Symp. Ser., 21 (1984) 223.
4 J. Greter, S. Lindstedt, H. Seeman and G. Steen, Clin. Chem., 26 (1980) 261.
5 J. Greter, S. Linstedt and G. Steen, Adv. Mass Spectrom., 8B (1980) 1362.
6 T. Kuhara and J. Matsumoto, Anal. Chem. Symp. Ser., 7 (1981) 155.
7 J. Pfordt and G. Spiteller, Liebigs Ann. Chem., (1980) 175.
8 R.E. Wolff, M. Greff and J.A. McCloskey, in Advances in Mass Spectrometry, Vol. 4, Inst. of Petroleum, London, 1968, p. 193.

(Received April 13th, 1987)