Journal of Chromatography, 222 (1981) 475—477
Biomedical Applications
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 745

Note

Natural products chemistry
81*. Analysis of rat thymus steroids by liquid-gel chromatography and gas chromatography—mass spectrometry**

JOHANNES REISCH* and AHMED S. EL-SHARAKY

Institut für Pharmazeutische Chemie der Westfälischen Wilhelms-Universität, Hittorfstrasse 58—62, D-4400 Münster (G.F.R.)

(First received June 24th, 1980; revised manuscript received September 19th, 1980)

The presence of unidentified corticosteroids in rat thymus has been reported [1, 2]. In vivo and in vitro studies also showed the presence of α,β-unsaturated ketosteroids [3]. In the course of a gas chromatographic—mass spectrometric (GC—MS) study of steroids in thymus lipid extracts we identified 4-pregnene-3, 20-dione and 21-hydroxy-4-pregnene-3,20-dione in calf thymus [4].

In connection with studies of steroids in tymus tissues, a group isolation procedure has been developed [4, 5]. This is based on a combination of reversed- and straight-phase liquid-gel chromatography on hydroxyalkoxyalkyl Sephadex. The mixture of purified steroids was analysed by combined GC—MS methods.

MATERIALS AND METHODS

Thymus glands were obtained from 4-week-old male Wistar-strain rats. The total lipids were extracted from the tissues with chloroform—methanol (2:1) and purified as described before [6]. The extract was subjected to reversed-phase gel chromatography on a column of 18 g Lipidex-5000 (Packard-Becker, Groningen, The Netherlands) in chloroform—methanol—water (2:9:1) [4, 5]. The steroid-containing fraction was purified on a column of 7 g Lipidex-5000 in *n*-hexane—chloroform (9:1) [4, 5].

The steroids were analysed as O-methyloxime—trimethylsilyl ether (MO—TMS) derivatives [7]. Acid contaminants from the reaction with methoxy-

*For part 80, see J. Reisch, I. Mester, S.K. Kapoor, Zs. Rózsa and K. Szendrei, Justus Liebigs Ann. Chem., (1980) in press.
**This article is a part of the Doctoral Thesis of A.S.El-Sharaky (Münster 1980).

0378-4347/81/0000—0000/$02.50

amine · HCl were removed by chromatography through anion exchanger Amberlyst A-26 (Serva, Heidelberg, G.F.R.) [5].

For the gas—liquid chromatographic (GLC) analysis, the derivatized steroids were run on a 70 m × 0.25 mm I.D. glass capillary column coated with OV-101 programmed at a temperature of 180—280°C at a rate of 1°C/min. The methylene unit (MU) values were obtained with *n*-alkanes of even carbon numbers from C_{24} to C_{36}.

Mass spectra were obtained using a Varian MAT 44S gas chromatograph—mass spectrometer interfaced to a Spectra System-MAT 188 with spectrometer conditions: ion source temperature 200°C, separator temperature 250°C and an electron energy of 70 eV. The samples were run using the GC inlet.

For GC—MS analysis, all samples were run on a 25 m × 0.25 mm I.D. glass capillary column coated with SE-30 programmed at a temperature of 60—250°C at a rate of 10°C/min.

RESULTS AND DISCUSSION

The gas chromatogram of the isolated fraction showed two peaks with MU values and mass spectra consistent with those of authentic 4-pregnene-3,20-dione [4]. This steroid was recently reported to be present in calf thymus [4].

A second steroid was detected on a gas—liquid chromatogram with a MU value of 30.42 identical with that of authentic 11β-hydroxy-4-pregnene-3,20-dione. The MS investigation of the derivatized fraction on a GC—MS instrument using the GC inlet showed a substance with the parent ion M^+ = 460. The presence of the 20-oxo structure was indicated by an intense ion at *m/e* 100 (base peak) composed of C_{16}/C_{17} with substituents and additional hydrogen atom [8]. The presence of the TMS group was indicated by the fragment ion at *m/e* 370 due to loss of trimethylsilanol, $(CH_3)_3SiOH$. The fragment ions at *m/e* 143 and 240 are characteristic of 11β-hydroxy-steroid derivatives [9]. The identity of the component with authentic 11β-hydroxy-4-pregnene-3,20-dione was confirmed by direct comparison of their mass spectra (Fig. 1).

The detection of ketosteroids of unknown structures in calf [10] and rat thymus [1—3] has been reported. We found 4-pregnene-3,20-dione and 21-hydroxy-4-pregnene-3,20-dione in calf thymus [4]. The present report shows the presence of 4-pregnene-3,20-dione and 11β-hydroxy-4-pregnene-3,20-dione in rat thymus. The question regarding its source of biogenesis cannot be answered yet. The presence of the latter compound was, however, previously reported to be formed mainly by the adrenal tissues of different animals [11—13].

The binding of steroids to different thymus cell fractions was studied and it was found that 4-pregnene-3,20-dione binds to the greatest extent while 11β, 17-21-trihydroxy-4-pregnene-3,20-dione binds to the least extent [14]. This could indicate that the origin of the former is extra-thymic.

It is not known whether active sites for 11β-hydroxylation of 4-pregnene-3, 20-dione to 11β-hydroxy-4-pregnene-3,20-dione are present, which could explain the presence of the latter compound in rat thymus. It was shown that the corticoid content of rat thymus decreased in adrenalectomized animals [1, 2]. This could indicate also an extra-thymic origin of 11β-hydroxy-4-pregnene-3,20-dione.

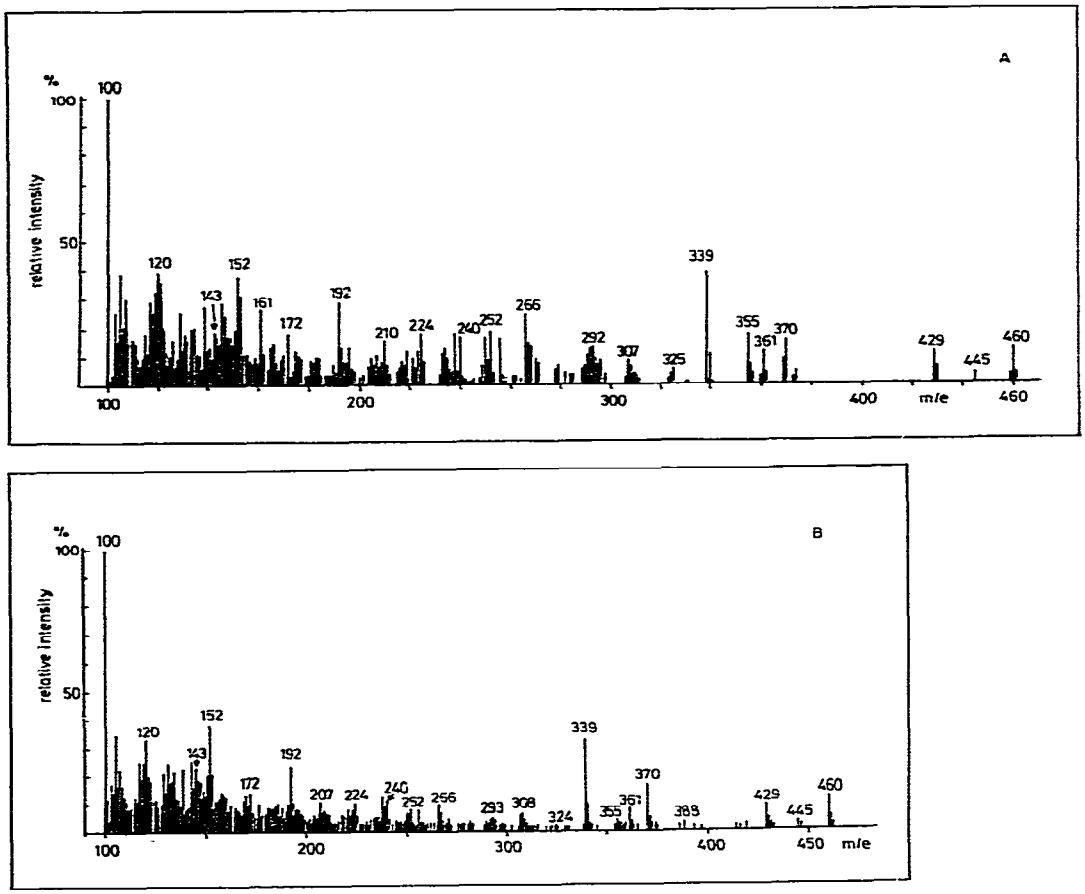

Fig. 1. Mass spectra of the MO—TMS derivatives of 11β-hydroxy-4-pregnene-3,20-dione from rat thymus (A) and authentic compound (B).

REFERENCES

1 M. Negeon-Dureuil, M. Rabinowicz and A.R. Ratsimamanga, C.R. Soc. Biol., 146 (1952) 1929.
2 T. Rahandraha and A.R. Ratsimamanga, Bull. Soc. Chim. Biol., 35 (1953) 301.
3 A.D. Abraham, Rev. Roum. Endocrinol., 8 (1971) 83.
4 J. Reisch and A.S.El-Sharaky, Hoppe-Seyler's Z. Physiol. Chem., 361 (1980) 791.
5 M. Axelson, G. Schumacher and J. Sjövall, J. Chromatogr. Sci., 12 (1974) 535.
6 J. Folch, M. Lees and G.H. Sloane Stanley, J. Biol. Chem., 226 (1957) 497.
7 W.L. Gardiener and E.C. Horning, Biochim. Biophys. Acta, 115 (1966) 524.
8 C.J.W. Brooks and D.J. Harvey, Steroids, 15 (1970) 283.
9 P. Vouros and D.J. Harvey, J. Chem. Soc., Perkin Trans., 1 (1973) 727.
10 W. Henning, H. Möllmann, J. Reisch, H. Kindler and H. Alfes, Z. Zellforsch., 142 (1973) 465.
11 M. Holzbauer and H.M. Newport, J. Physiol. (London), 200 (1965) 821.
12 N.A. Yudaev and S.A. Afinogenova, Endocrinol. Exp., 9 (1975) 114.
13 M. Weiss and V.L. Ford, Comp. Biochem. Physiol., B57 (1977) 15.
14 W.K. Brunkhorst and E.I. Hess, Biochim. Biophys. Acta, 82 (1964) 385.