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STEROID EXCRETION IN A CASE OF 17α-HYDROXYLASE DEFICIENCY INVESTIGATED BY COMPUTERIZED GAS CHROMATOGRAPHY-MASS SPECTROMETRY *

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SUMMARY

The urinary steroids of a patient with congenital adrenal hyperplasia, hypertension and primary amenorrhea originated from a 17α-hydroxylase defect was investigated by on line-computerized gas chromatography-mass spectrometry using an open tubular capillary column. Steroids were detected, characterized or identified after methyloximetrimethylsilyl ether derivatization by a sequential analysis of mass spectra taken every 8 s and by computerized fragment ion current chromatography. No 17α-hydroxylated steroids were found among the 24 detected metabolites. The most important steroids for diagnostic purposes found in this urine are: 5β-pregnane-3α, 20α-diol, 5β-(and 5α-) pregnane-3α, 11β-20α-triols, 5β-(and 5α-) pregnane-3α, 11β, 20β, 21-tetrols, 11-keto-5β-pregnane-3α, 20α-diol, 3α, 11β, 21-trihydroxy-5β-pregnan-11-one (THB), 3α, 11β, 21-trihydroxy-5α-pregnan-11-one (*allo*-THB) and 3β, 11β, 21-trihydroxy-5α-pregnan-11-one (*allo*-3β-THB). Two metabolites with unusual structures: 11-keto-1,3,21-trihydroxy-steroids have been also found in this urine. A screening method is described for a rapid analysis of C_{21}-steroids in small amounts of urine which can be used for the diagnosis of inborn errors of corticosteroid biosynthesis.

Among the inborn errors of steroidogenesis, the 17α-hydroxylase defect is one of the less common (Edwards 1975). First investigated by Biglieri et al (1966) this defect was also shown to lead to adrenocortical hyperplasie through an increased secretion of ACTH. Increase of mineralocorticoids, principally of desoxycorticosterone, but also of corticosterone are responsible for the symptoms of hypertension exhibited by patients suffering from this defect. Perturbation of the steroidgenesis of androgens and oestrogens gives rise to primary amenorrhoea in females and also male pseudohermaphroditism.

Recently, the steroid profiles of a case of 17α-hydroxylase deficiency has been investigated by Honour et al (1978) using gel colum chromatography, gas chromatography and gas chromatography-mass spectrometry (GC-MS). In the present paper, a GC-MS urinary steroid profile from a case of congenital adrenocortical hyperplasia due to 17α-hydroxylase defect is investigated.

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MATERIALS AND METHODS

Clinical Details

The patient (S.R.M.) is female, age 19 years (genotype XY) and exhibited symptoms of primary amenorrhoea and hypertension. A 24 h urine specimen was collected having a total 17-ketosteroid value of 4.7 μg/24 h. Abdominal testicles were surgically removed.

Chemicals and Solvents were obtained from E. Merck, Darmstadt, GFR, unless otherwise stated in the text. A powerful silylating mixture comprising N,O-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA), trimethyl-chlorosilane (TMCS) and trimethylsilylimidazole (TMS), 3:2:3 was purchased from Serva Feinbiochemica GmbH & Co., Heidelberg, G.F.R.

Steroids

Reference steroids were obtained from Steraloids Inc., Wilton, N.H., U.S.A.

Urine Extraction and Hydrolysis were carried out as previously described (Ruiz and Arranz 1976). The pH-values of 5-10 ml aliquots of the urine were adjusted to 10.5-11.0 with NaOH and barium acetate solution added to precipitate interfering ions. After hydrolysis with 0.25 ml Helix pomatia glucuronidase/arylsulphatase (Boehringer Mannheim GmbH, Mannheim, G.F.R.) for 8 h at 60° the steroids were extracted with dichloromethane/ethyl acetate (50:50). Derivatization (methyloxime-trimethylsilyl ether) was carried out by adding 0.5 ml of a 3 % solution of methoxyamine hydrochloride (Eastman Kodak Inc., Rochester, U.S.A.) in dry pyridine and (17 mg/ml) leaving for 24 h at room temperature. After evaporation of pyridine under nitrogen, 200 μl of TMS reagent (BSTFA:TMCS: TMS, 3:2:3) were added and the mixture sonicated for a few minutes and maintained at 60° for one hour. Excess reagents were then removed by filtering through a small column (0.5 cm × 2 cm) of Sephadex LH-20 (Pharmacia, Uppsala/Sweden) swollen in chloroform: *n*-hexane 1:1. The first 2 ml eluted with the solvent were evaporated and dissolved in *n*-hexane for analysis by gas chromatography-mass spectrometry.

Computerized Gas Chromatography-Mass Spectrometry

An LKB 9000S GC-MS unit was employed, equipped with a 25 m open tubular capillary column coated with OV-101 (0.32 mm i.d., LKB Produkter, Bromma, Sweden). A *falling needle*-type solid injector (Chrompack, Netherlands) was used (van den Berg 1972) and modified before connexion to the capillary column with shrinkable teflon tubing. The operating conditicns were: column temperature: 240°, helium carrier gas flow rate: 1.7 ml/min, made up to 30 ml/min before the double-stage jet separator, separator temperature: 270°, ion source temperature: 290°, ionization voltage: 22.5 e. V., ionizing current 60 μA.

Repetitively scanned mass spectra (m/e 40-800, 4 s scan time) were recorded on magnetic disk every 8 s via an LKB 2130 data system connected on-line to the mass spectrometer. Each spectrum was assigned a retention time by the computer.

RESULTS

A computer-reconstructed total ion current chromatogram of a derivatized sample corresponding to 0.5 ml urine is illustrated in Fig. 1.

Each region of the chromatogram to be discussed below is indicated by (a)-(q). The mass spectra corresponding to each peak or shoulder were examined after subtraction of background spectra by the data system. The spectra thus obtained and their corresponding retention times (to 5α-cholestane t_R) were then examined by comparison with known compounds, or in some cases, the particular mass spectral fragments found allowed structural features to be inferred.

The following metabolites were detected and characterized via the screening chromatogram illustrated in Fig. 1.

Compound 1: A mass spectrum (spectrum 69) of peak *a* (spectra 69-71) showed a base peak at *m/e* 117 and additional ions at m/e 449 (M-15), 374 (M-90), 359 [M-(90 + 15)], 284 (M-2 × 90) and 269 [M-(2 × 90 + 15)] and its line diagram is presented in Fig. 2.

The fragmentation and t_R (0.92) agree with those of 5β-pregnane-3α, 20α-diol. The large *m/e* 117 ion corresponds to cleavage between C_{19} and C_{21}-steroids with a -CH [OSi-$(CH_3)_3$] CH_3 side chain; also, no molecular ion was observed and the ion corresponding to M-15 has approximately 5 % of the base peak intensity. A small amount of a second steroid *(compound 2)* with a spectrum similar to *compound 1* and a t_R value of 1.04 was detected in peak *b* (spectrum 75); the principal ion *m/e* 117 of this pregnanediol isomer is indicated in the mass chromatogram shown in Fig. 3, where the abundances of this ion some others, in spectra 70 to 120, are plotted by the computer.

An analysis of the spectra of peak *c* indicates the presence of a *compound* 3-silyl ether presenting a mass spectrum typical of 5-pregnene-3β, 20-diol (Fig. 4), spectrum 80, the molecular ion being present at *m/e* 462, a base peak *m/e* 117, an important diagnostic ion *m/e* 129 corresponding to the 3β-hydroxy-Δ^5-structure (Vihko, 1966) and fragments *m/e* 372 (M-90), 282 (M-2 × 90) and 267 [M-2 × × 90 + 15)], all of small intensity (5 % base peak). The t_R-value 1.10 confirms that the substance is 5-pregnene-3β, 20α-diol.

The other steroids are also present in peak *c*, one of them (*compound 4*, spectrum 85) with abundant ions at *m/e* 460 and 117 (Fig. 3) and the other (*compound 5*, spectrum 87) shows a prominent peak at *m/e* 117. *Compound 4* is probably a 5-pregnene-3, 16, 20-triol with an t_R-value of 1.17; the abundant ion at *m/e* 460 represents M-90.

The silyl ethers of the steroids of peak *d* (spectra89-100) indicate, as shown in the mass chromatogram of Fig. 3, the presence of steroids with t_R-values of 1.24 (compound 6, max. at spectrum 90), 1.28 (*compound 7*, spectrum 93) and 1.32 (*compound 8*, spectrum 96).

Most of the spectra of *compounds 6* (scan number 89-91) and *8* (spectrum 96) are contaminated with *compound 7*, one of the major steroids excreted in this urine. The spectrum of *compound 6* is reproduced in Fig. 5 (scan number 89), and has a base peack at *m/e* 460 (M-90), a pronounced fragment ion at *m/e* 117. Particularly typical of the spectrum of *compound 6* are the peaks at *m/e* 129, 141, 156, and 157, characteristic of 5-pregnene-3, 16, 20-triol (Gustafson et al 1969). The ions *m/e* 156/ /157 originate from ring D and the side chain without the trimethylsilanol and result probably from the cleavage of the bonds C_{14}/C_{15} and $C/_{13}/C_{17}$ (Eriksson et al 1970). The peak corresponding to the molecular ion is not found in the spectrum and that

corresponding to M-15 (*m/e* 535) has a very low abundance. *Compound 8* is present in very small amounts showing a spectrum with a base peak at *m/e* 117 and ions at *m/e* 141, 156 and 157, typical for 16, 20-dihydroxy-21-desoxysteroids. Absence of an ion at *m/e* 129 and presence of an ion at *m/e* 462 (M-90) indicates a typical spectral feature of a pregnane-3, 16, 20-triol. Zones corresponding to the molecular ion (M^+, 522) and M-15 (537) were not present. Its t_R-value was 1.32 which exclude for this compound a structure 5α(H)-3β, 16α, 20α (or 20β) corresponding to the isomers with higher retention time (Laatikainen 1970).

Compound 7 showed a mass spectrum typical of 11-ketopregnane-diol (Fig. 6) as its trimethylsilyl ether derivative.

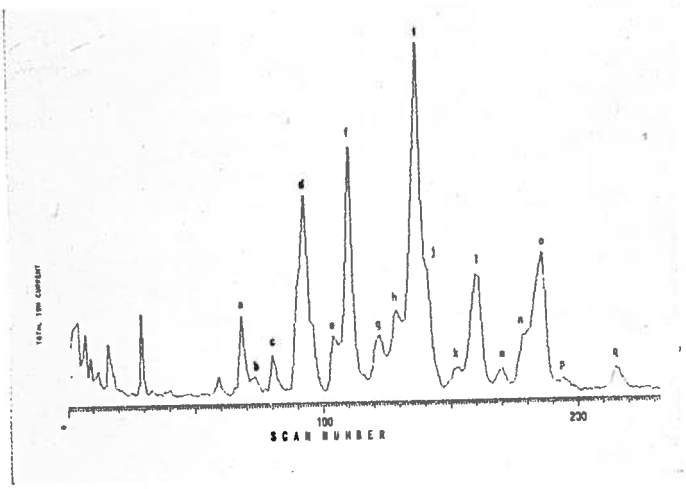


Fig. 1 — *Computer reconstructed total ion current chromatogram of a methyloxime-silyl-derivatized sample corresponding to 500 µl urine. Peaks or shoulders referred to in the text are indicated by (a) to (q). For experimental conditions see under* materials and methods

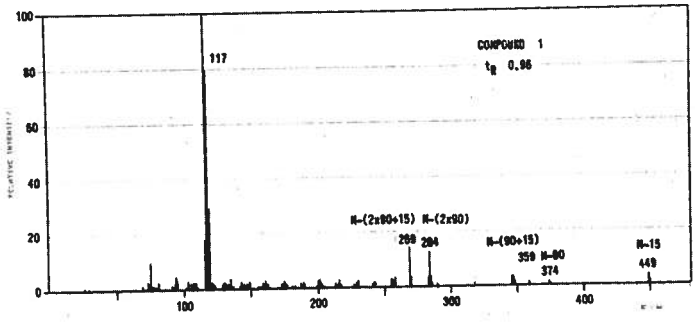


Fig. 2 — *Mass spectrum of the silyl ether of compound 1 identified as 5β-pregnane-3α, 20α-diol*

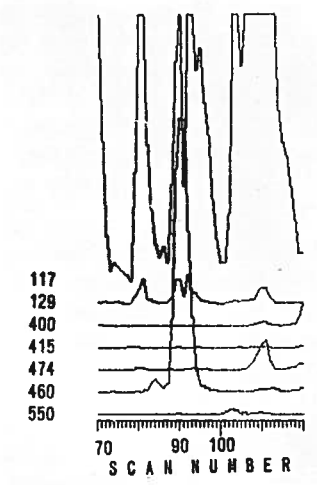


Fig. 3 — *Computer reconstructed ion-current chromatograms from spectra with scan numbers 70 to 120 for the* m/e *values 117, 129, 400, 415, 474, 460 and 550. Fragment-ion* m/e *117 is an abundant peak in steroid-silyl ethers with a 21-desoxy-21-hydroxy-side chain features*

Peaks were observed at *m/e* 478 (M^+, 15 % of the base peak intensity), 463 (M-15), 434 (M-44), 388 (M-90), 373 [M-(90 + 15)], 334 (base peak, M-144), 198 (M-2 × 90), 283 [M-(2 × 90 + 15)], 244 [M-(144 + 90)] and 117. The base peak at M-144 is commonly found in the mass spectra of 11-oxo-3α-hydroxy steroids and corresponds to the large M-72 peak found in the mass spectra of the underivatized 3α-hydroxy, 11-oxo steroids as in 3α-hydroxy-5β-androstane-11, 17-dione (Obermann et al 1971), but absent or negligible in the isomers with 3β/5β, 3β/5α or 3α/5α structures. It is therefore possible to conclude that this 11-keto compound has the 3α-hydroxy-5β structure. The presence of the 11-keto group is further confirmed by the non-formation of a methyloxime derivative, and by the presence of a peak at *m/e* 434 (M-44) observed in some 11-keto steroids and formed probably by C_{11} and C_{12} together with one oxygen and four hydrogen atoms (Eriksson 1970). *Compound 7* is therefore identified as 11-keto-5β-pregnane-3α, 20α-diol, the configuration at C_{20} being confirmed by the short retention time. Generally, the silyl ethers of 20α-hydroxy steroids are more polar than the 20β-epimers on a column of type SE-30 (similar in characteristics to OV-101), an exception being encountered with 11-oxygenated compounds. In such cases, the 20β-epimer is more polar where the configurations 3α-ol/5α(H)-, 3β-ol/5β(H)- or 3β-ol/5α(H) are present, as in the case of pregnane 3, 11β, 20, 21-tetrols (Eriksson 1974) and in 11-oxo-steroids with 3α-ol/5α(H) or 3α-ol/5β(H) structures as indicated by Eriksson (1970) for 3α, 20-dihydroxy-pregnane-11-one, and by Eriksson (1970) for 3α, 20-dihydroxy-pregnane-11-one, and by Setchell et al (1975) for 20α-/20β-cortolone.

Compound 9 found in peak *e* gave a mass spectrum (spectrum 104, Fig. 7, upper part) very similar to that of the silyl ether of 5α-pregnane-3α, 11α, 20β-triol (Fig. 7, lower part) as well as of 11β-hydroxypregnanediol (5β-pregnane-3α, 11β, 20α-triol) published by Honour et al (1978). Peaks were found at *m/e* 552 (M^+; 4 % of the intensity of base peak), 537 (M-15; 4 %), 462 (M-90, 7 %), 447 [(M-(90 + 15)], 2 %), 435 (M-117; 8 %), 372 (M-2 × 90; 15 %), 282 (M-3 × 90; 8 %) and 117 (base peak). Its t_R of 1.40, its elution after 11-keto pregnanediol and before 5-pregnene-3β, 16α, 20α-triol (which has in a OV-101 column an t_R-value similar to 5α-pregnane-3α, 11β, 20α-triol (Honour et al 1978) give a tentative identification of this compound as 5β-pregnane-3α, 11β, 20α-triol.

The spectra from peak *f* indicate the presence of two *compounds 10* and *11*. The spectrum at scan number 111 corresponds to an t_R-value of 1.49 (Fig. 8) showing the typical ions of the mass spectra of the silyl ether of pregnane-3β, 16, 20-triol (Gustafsson 1970; Laatikainen 1970), but its base peak (*m/e* 117) differs from that (*m/e* 462; M-90) found for 5α-pregnane-3α, 16α, 20α-triol and 5β-pregnane-3β, 16α, 20α-triols. *Compound 10,* silyl ether has a mass spectrum with the molecular ion at *m/e* 552 and intense ions at *m/e* 537 (M-15), 462 (M-90), 447 [M-(90 + 15)], 435 (M-117), 372 (M-2 × 90), 282 (M-3 × 90), 141 and 156.

Besides the molecular ion at *m/e* 552 and M-15 at *m/e* 537, the fragment ions at *m/e* 141 and 156 indicate the structure 16, 20-dihydroxy-21-desoxy, which confirm the presence in peak *f* of a pregnane-3, 16, 20-triol isomer to that compound detected in peak *d (compound 8)*. The very abundant ion with *m/e* 117 observed in the mass chromatogram of Fig. 3 which represents in the spectrum of scan number 111 the base peak and the intense ions at *m/e* 282 (M-3 × 90), 372 (M-2 × 90) and 435 (M-117) made it possible to characterize the most important pregnanetriol in this mixture as pregnane-3, 11β, 20-triol. This steroid has a longer t_R-value than its epimer isolated in peak *e (compound 9)* and therefore corresponds to the 5α-(H)-configuration. As postulated by Honour *et al* (1978) these pregnane 3, 11, 20-triols are certainly formed by 21-dehydroxylation of the corresponding 5α-(H)-and 5β(H)-tetrahydrocorticosterones; because *allo*-THB is a much more important urinary metabolite than THB (Fig. 1) it is not surprising that the intensity of the ion *m/e* 117 in the mass chromatogram shown in Fig. 3 is at least 5 times greater in peak *j* (*compound 11, allo*-pregnane-3, 11, 20-triol) than in peak *e* (*compound 9,* pregnane-3, 11, 20-triol).

The spectra of peak *g* indicate the presence of the silyl ether mehyloxime of a compound (*compound 12,* t_R 1.63; spectrum 112 in Fig. 9) with a molecular ion at *m/e* 521, a base peak at *m/e* 188 and intense ions at *m/e* 490 (M-31), 175 and 103. The molecular mass corresponds to a dihydroxy diketonic steroid with an underivatized ketogroup (11-keto), whereas the ions 103, 175 and 188 indicate a 21-hydroxy-20-keto-structure. This compound is identified as 3α, 21-dihydroxy-5β-pregnane-11, 20-dione (THA), the 5β-configuration being assessed by its t_R-value (THA 1.63, *allo*-THA 1.67 on OV-1 (Setchell et al 1976). The steroids present in the following two peaks (*h* and *i*) have, as methyloxime-silyl ethers, mass spectra with molecular ions at *m/e* 595 and prominent peaks at 103, 172, 188, 189, 312 [M-(103 + 2 × 90)], 402 [M-(103 + 50)] and 492 (M-103) indicating a 21-hydroxy-20-one side-chain structure. The molecular ion at *m/e* 595 and the ions at *m/e* 564 (M-31) and 474 [M-(90-31)] and 384 [M-(2 × 90 + 31)] suggest a trihydroxy-pregnan-one with a derivatized keto--group (20-oxo). The spectra of the two compounds, *13* and *14,* are reproduced in Fig. 10 A, *compound 13,* upper part, spectra 129 of peak *h;* B, *compound 14,* spectrum 137 of peak *i*). These spectra are identical to those obtained for authentic THB and *allo*-THB-methyloxime-silyl ethers. Also, according to their t_R-values, the compounds 13 and 14 are THB and *allo*-THB, respectively.

A mass chromatogram reconstructed for the ions *m/e* 595 (M^+), 564 (M-31), 474 (M-90 + 31)], 384 [M-(2 × 90 + 31)], 312 [M-(2 × 90 + 103)], 188 and 175 is presented in Fig. 10 and allows a detailed analysis of both peaks, *h* and *i*. Both tetrahydro- and *allo*-tetrahydrocorticosterone give two chromatographic peaks (*syn*- and *anti*-geometric isomers) indicated in the mass chromatogram by the fragment *m/e* 564 in the spectra 129 (t_R 1.72) and 131 (t_R 1.75) for the 5β-compound (THB) and by the fragment *m/e* 595 in the spectra 135 (t_R 1.80) and 137 (t_R 1.82) for the 5α-isomer (*allo*-THB).

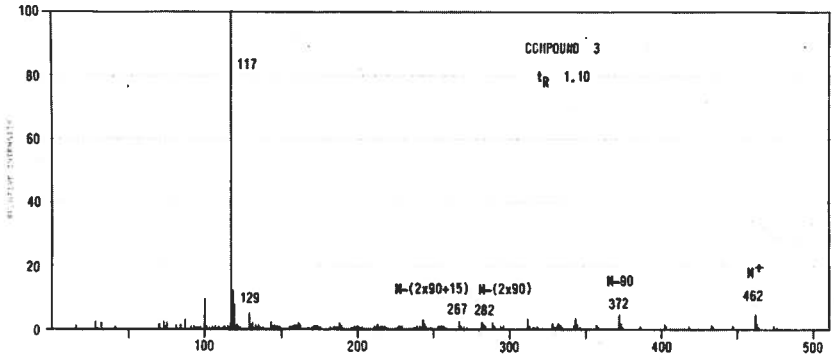


Fig. 4 — *Mass spectrum of the silyl ether of* compound 3 *(from peak c) identified as 5-pregnane-3α, 20α-diol*

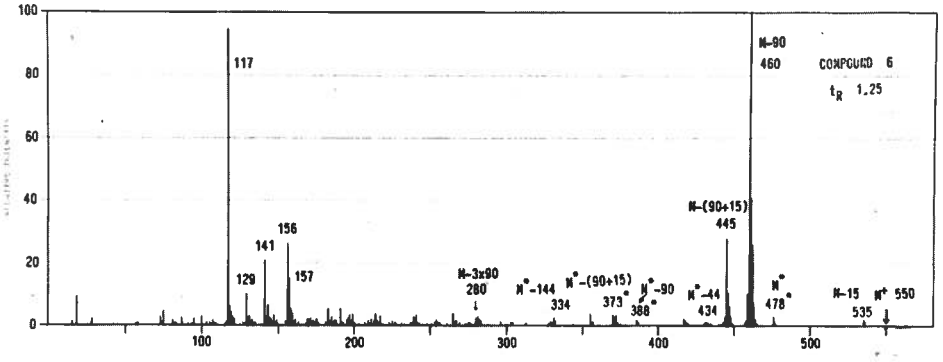


Fig. 5 — *Mass spectrum of the silyl ether of* compound 6 *characterized as 5-pregnane-3,16,20-triol. Fragments indicated by an asterisk belong to* compound 7 *(See Fig. 6)*

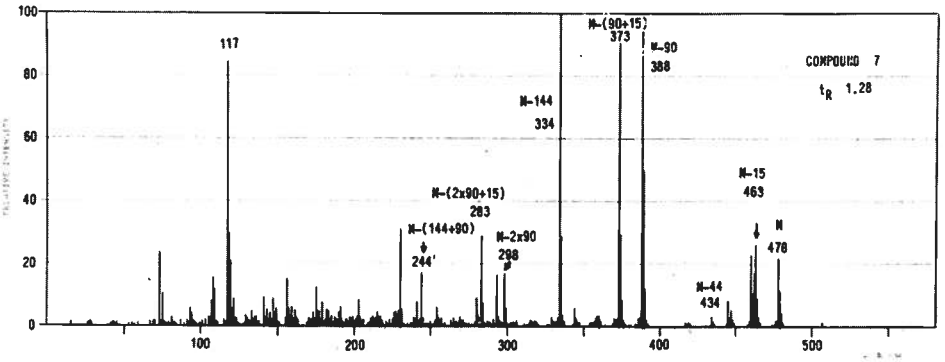


Fig. 6 — *Mass spectrum of the silyl ether of* compound 7 *identified as 11-keto-5β-pregnane-3α, 20α-diol*

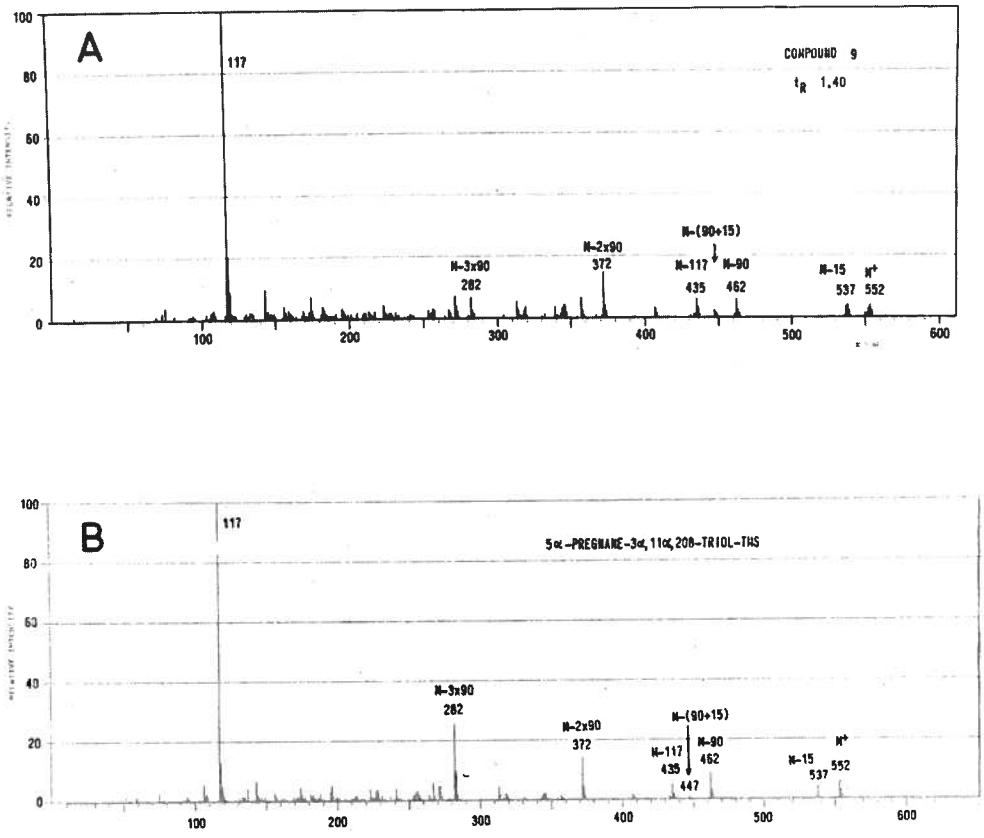


Fig. 7 — *Mass spectrum of the silyl ether of compound 9 (A) and authentic 5α-pregnane-3α, 11α.20β-triol (B).* Compound *9 was identified as 5β-pregnane-3, 11β, 20α-triol*

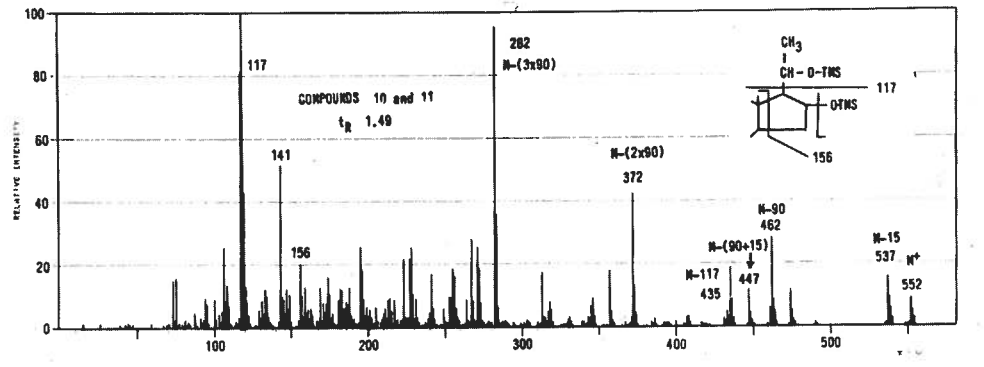


Fig. 8 — *Mass spectrum of a mixture of the silyl ethers of* compound 10 *and* 11 *characterized as pregnane-3, 16, 20-triol and* allo*-pregnane-3, 11, 20-triol*

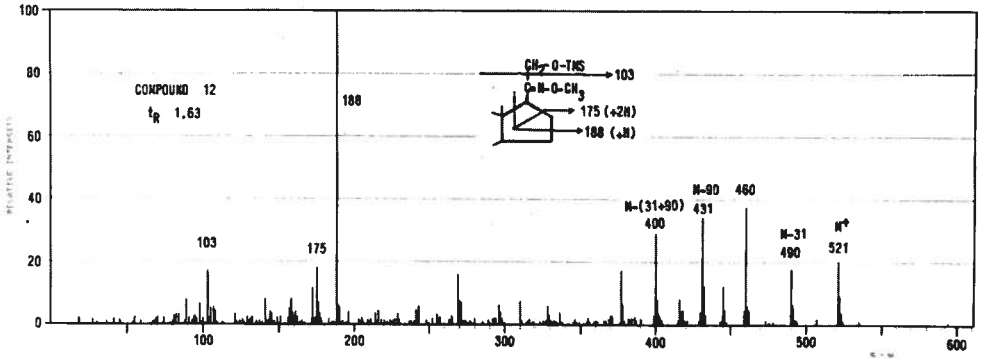
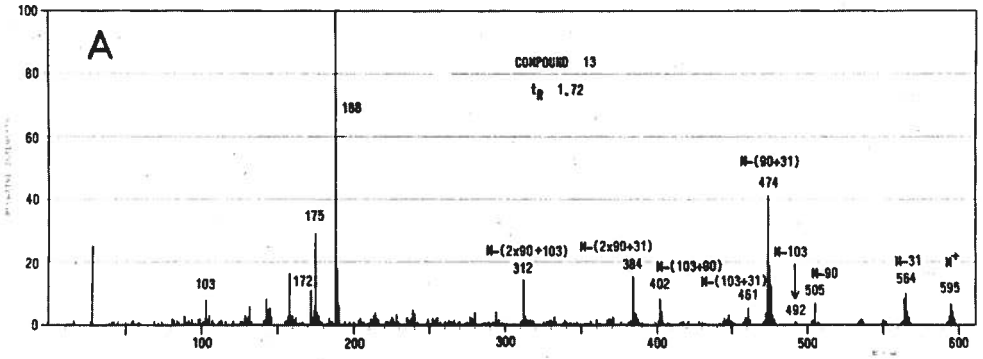


Fig. 9 — *Mass spectrum of the methyloxime-silyl derivative of* compound 12 *(spectrum 122) identified as 3α, 21-dihydroxy-5β-pregnane-11, 20-dione (TH-A)*



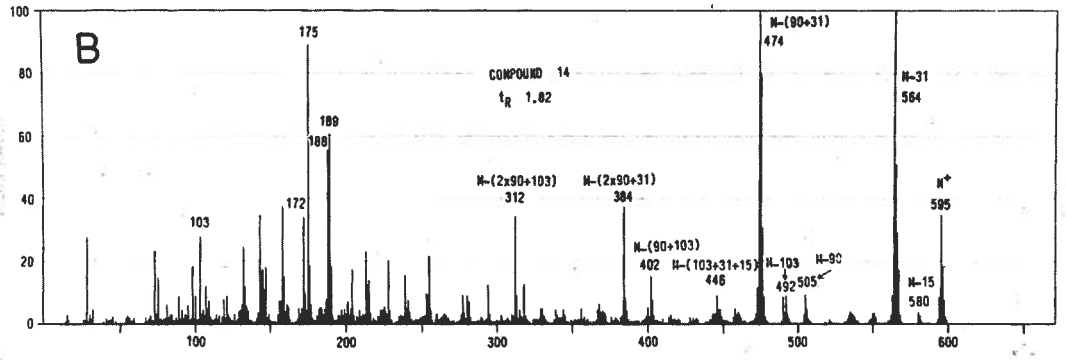


Fig. 10 — *Mass spectrum of the methyloxime-silyl derivatives of* compound 13 *(A, scan number 129) and* 14 *(B, scan number 137) identified as 3α, 11β, 21-trihydroxy-5β-pregnane-20-one (TH-B) and 3α, 11β, 21-trihydroxy-5α-pregnane-20-one (*allo *TH-B) respectively*

The presence of a further isomer is indicated in the mass chromatogram (Fig. 11) in the region reconstructed from the spectra 156 to 163 (peak at 160, t_R 2.11); this steroid, whose spectrum is discussed later (*compound 16*, figure 12) had a t_R-value similar to that of the methyloxime-silyl of 3β, 11β, 21-trihydroxy-5α-pregnan-20-one (*allo*-3β-THB).

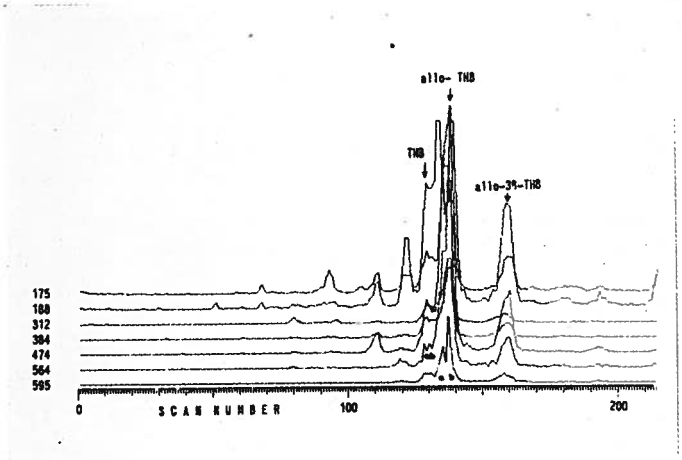


Fig. 11 — *Fragment ion current chromatograms constructed by the computer in the screening analysis of the methyloxime-silyl derivatives of the urinary steroids;* m/e *values are typical of the derivatives of trihydroxy-pregnan-one (isomeric tetrahydrocorticosterones).* Syn- *and* anti-*geometric isomers are shown for the fragments* m/e 564 *and* 595 *and indicated by* a *and* b, *in each case*

The methyloxime-silyl ether of *compound 15* detected in the shoulder *j* (spectrum 142, t_R 1.88) gave a mass spectrum (Fig. 12) with a molecular ion at *m/e* 566 characteristic of pregnanetriol-one without oxime derivatization of the keto-group. This fact and the presence of a small ion at *m/e* 522 corresponding to M-44 indicates an 11-keto group in the molecule. Both spectrum and retention time of this steroid are quite similar to those of the silyl ether of 1,3,20-trihydroxy-5ξ-pregnan-11-one, a steroid isolated in urine of a patient with 17α-hydroxylase deficiency by Honour et al (1978). In the spectrum of Fig. 10 and in those published by these authors intense ions are present at *m/e* 566 (M^+), 555 (M-15), 476 (M-90), 461 [M-(90 + 15)], 386 (M-2 × 90), 271, 209, 196 and 147. The absence in our spectrum of the ion *m/e* 117 (base peak in that published by Honour et al 1978) can be explained by the background subtraction (using spectrum 117, which presents a strong *m/e* 117 value, as indicated in the mass chromatogram (included in the Fig.)). The ions *m/e* 196, 209 and 271 are very typical for the presence of a 1β,3-hydroxy-11-keto-structure in C_{21}-steroids (Shackleton and Snodgrass 1974, Honour et al 1978). The chromatographic peak *k* was found to contain minor amounts of one or more compounds presenting *m/e* 117 in the mass spectra (see insert in Fig. 12, scan number 151-153). Owing to the very small amounts, it was not possible to carry out further characterization.

The spectra taken from the chromatographic peak *l* show a mixture of two compounds, *16* and *17*. The spectrum 160 presented in Fig. 13 (t_R = 2.11) shows a *m/e* ion 595 corresponding to the molecular ion of a pregnanetriolone methyloxime silyl ether *(compound 16)* similar to that found for THB and *allo*-THB (*compounds 13*

and *14,* Fig. 9); the characteristic peaks are found at *m/e* 580 (M-15), 564 (M-31), 505 (M-90), 474 [M-(31 + 90)], 461 [M-(31 + 103)], 402 [M-(90 + 103)], 384 [M-(31 + 2 × 90)], 188 (base peak of this compound, in the mixed spectra, 78 % abundance), 175, 172 and 103. *Compound 16* is identified as *allo*-3β-THB. The peaks corresponding to *compound 17* indicate a pregnane-triolone silyl ether, with underivatized ketone (11-keto) with a base peak at *m/e* 373 [M-(90 + 103)] and prominent fragment ions at *m/e* 463 (M-103) and 283 M-(2 × 90 + 103) characteristic of a *bis*-silyl-ether of a 20,21-dihydroxy-11-keto-pregnanesteroid (Setchell et al 1976).

At least two steroids were present in peak *m* (spectrum 170 t_R 2.24, Fig. 14, *compounds 18* and *19*). *Compound 18* has a *tetrakis* (trimethylsiloxy)-pregnane structure indicated in the spectrum by a molecular ion of *m/e* 640 and fragment ions at *m/e* 625 (M-15), 550 (M-90), 537 (M-103), 460 (M-2 × 90) and 103. *Compound 19* gave a prominent ion at *m/e* 117 (base peak), found in the spectra of 21-desoxy-20-hydroxy-17α-desoxy-C_{21}-steroids, and ions 196, 209 and 271, typical fragments of 1,3-dihydroxy-11-oxo-steroid-silylethers. Its identification remain uncertain. Two other peaks in this spectrum (*m/e* 463 and 283) belong perhaps to a third compound. The minor amounts of the steroids of peak *m* make an identification impossible under the experimental conditions employed in this screening.

The *compounds 20* and *21* detected in the peaks *n* and *o,* respectively, are isomeric pregnanetetrols, as indicated by their spectra presented in Fig. 15 (upper part, A, *compound 21,* spectrum 188, t_R 2.36; lower part, B, *compound 22,* spectrum 185, t_R 242). Both compounds gave a molecular ion at *m/e* 640 and other fragments at 635 (M-15), 550 (M-90), 537 (M-103), 460 (M-2 × 90), 447 [M-(90 + 103)], 357 [M-(2 × 90 + 103)], 267 [base peak, M-(3 × 90 + 103)], 129 and 117. These spectra are similar to those found for isomeric pregnane-3, 11β,20,21-tetrols as silyl ethers (Eriksson 1971). *Compounds 20* and *21* are identified according to their spectra and t_R-values as 5β-pregnane-3α, 11β,20β,21-tetrol and 5α-pregnane-3α 11β, 20β, 21-tetrol respectively.

Small amounts of two steroids *(compounds 22* and *23)* were found in peak *p,* a pregnanetriolone (M^+, 595) and a pregnanetetrol (M^+ 640, not found in the spectra). As indicated in Fig. 16 (spectrum 195, t_R 2.55), the first steroid gave fragments at *m/e* 595 (M^+), 564 (M-31), 474 [M-(31 + 90)], 402 [M-(90 + 103)], 188 and 175, characteristic of a 21-hydroxy-20-oxo-steroid. The fragments indicated in the spectrum by an asterisk *m/e* 117, 141, 156, 157, 460 (M-2 × 90), 535 [M-(90 + 15)] and 550 (M-90) belong to *compound 23* and indicate for this steroid a 21-desoxy-16,20-dihydroxy structure.

Finally, the last metabolite *(compound 24)* detected during this screening (peak *q*, t_R 2.80, spectrum 215, Fig. 17) gave a molecular ion at *m/e* 683 corresponding to a pregnanetetrolone, methyloxime trimethylsilyl ether, *m/e* 103 (primary silyl group), 175 and 188 (characteristic of a 21-hydroxy-20-keto-structure and ions *m/e* 652 (M-31), 580 (M-103), 562 [M-(31 + 90)], 490 [M-90 + 103)], 472 [M-2 × 90 + + 31)], 400 [M-(2 × 90 + 103)], and 382 [M-(3 × 90 + 31)]. Absence of the ion *m/e* 276 in the spectrum that no 16,21-hydroxy-20-oxo-structure is present; the positions of the two other hydroxyl groups were not determined.

In *Table 1* the t_R-values are summarized together with the principal peaks of diagnostic importance found in each spectrum and the final identification of characterization of each one of the 24 metabolites detected in this study.

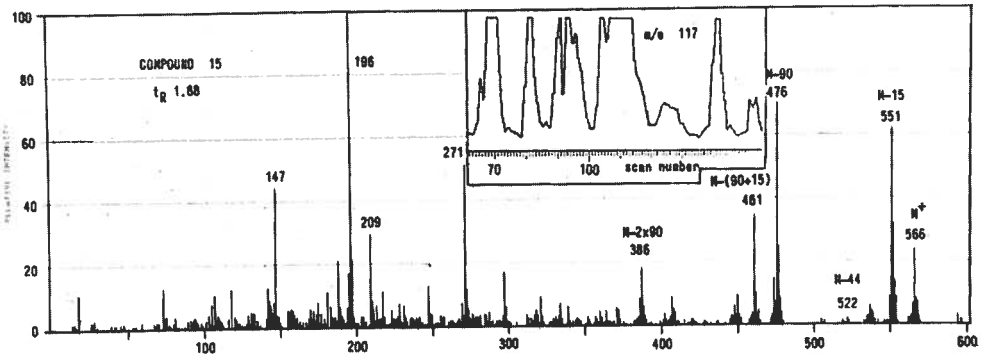


Fig. 12 — *Mass spectrum of the silyl ether derivative of* compound 15 *(scan number 142) characterized as 11-keto-pregnane-1,3,20-triol. This spectrum was taken after background subtraction of that of scan number 117, which explains the absence of peak m/e 117. The insert show the fragment in current chromatogram of m/e 117 obtained for spectra of scan number 62-155 in the analysis of the methyloxime-silyl derivatives of the urinary steroids. This mass chromatogram indicates for the spectrum of scan number 142 an abundant fragment ion of m/e 117*

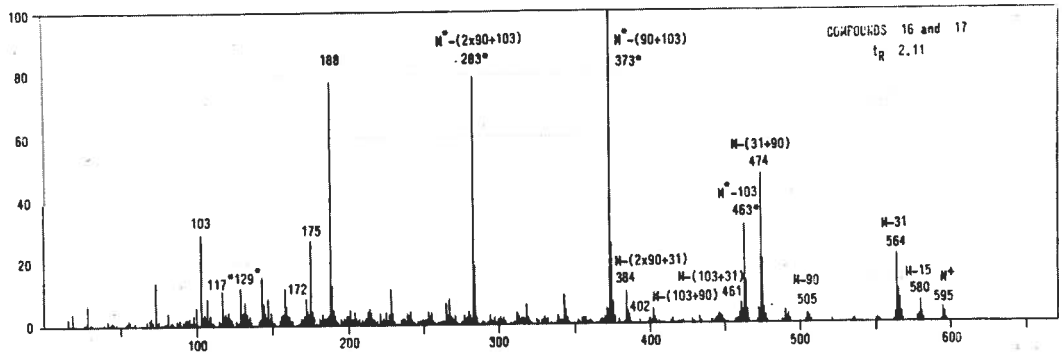


Fig. 13 — *Mass spectrum of the unresolved mixture of the methyloxime silyl ether derivatives of* compounds 16 *and* 17 *(scan number 160). The metabolites were identified as 3β, 11β, 21-trihydroxy-5α-pregnane-20-one* (allo-3β-*THB) and 11-keto-pregnane-3,20,21-triol. The fragments of* compound 17 *are indicated by an asterisk*

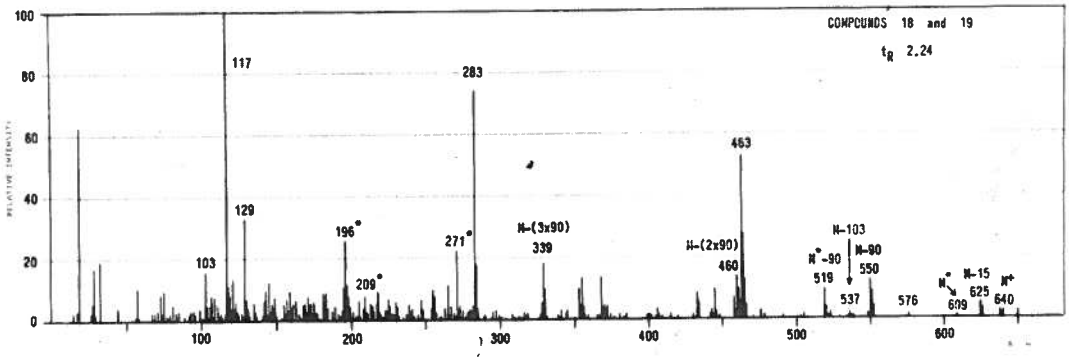
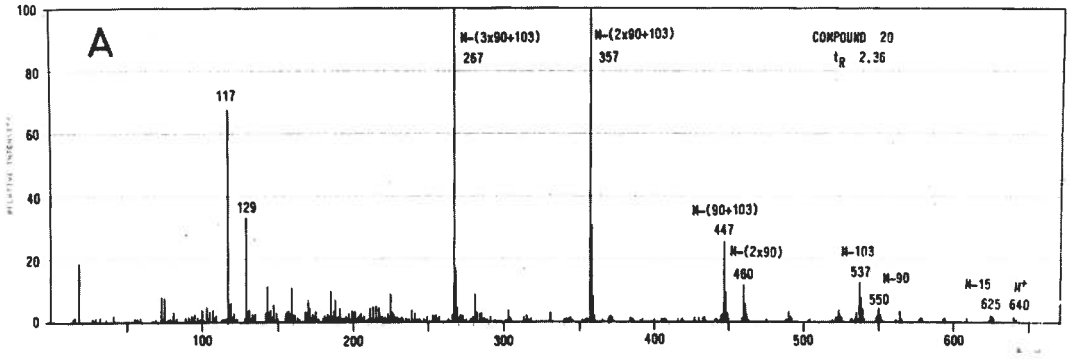


Fig. 14 — *Mass spectrum (scan number 170) of the unresolved mixture of the silyl ether derivatives of 3β,21-dihydroxy-5α-pregnan-20-one* (allo-3β-*THB) and 11-keto-pregnan-3.20,21-triol. The fragments of 1,3,20-triol (isomer of compound 15)*



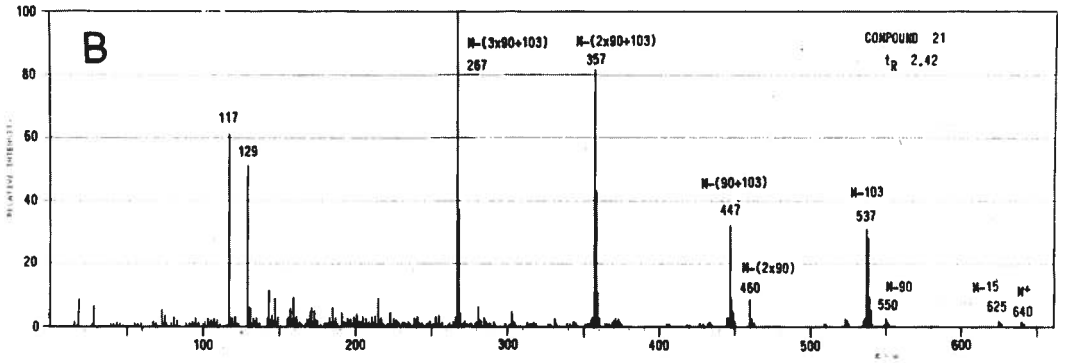


Fig. 15 — *Mass spectra of* compounds 20 *and* 21 *as silyl ethers (scan number A, 180; B, 185), identified as 5β-pregnane-3α, 11β, 20β, 21-tetrol (A) and 5α-pregnane-3α, 11β, 20β, 21-tetrol (B)*

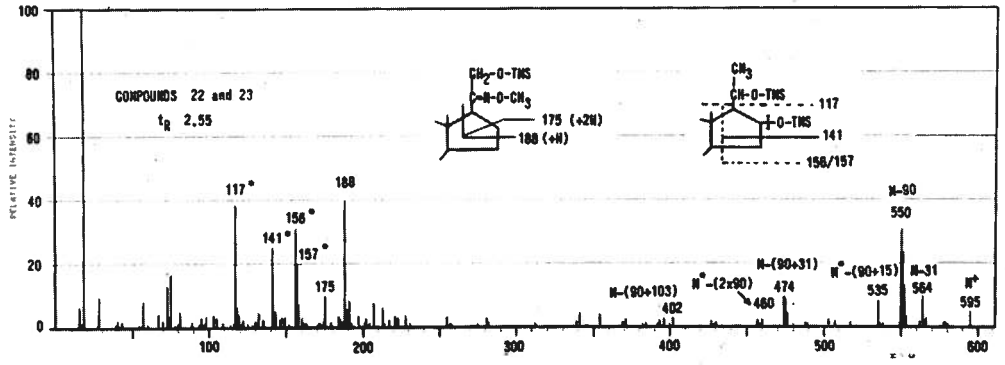


Fig. 16 — *Mass spectrum (scan number 195) of the unresolved mixture of the methyloxime silyl ether derivatives of* compounds 22 *and* 23 *characterized as 20-keto-pregnanetriol and pregnane-3,x, 16,20-tetrol respectively*

Table 1

metabolite	t_R [1]	M^+	principal ions *m/e*	characterized or identified as
1	0.96	—*	117(b.p.)	5β-pregnane-3α, 20α-diol
2	1.04	—*	117(b.p.)	5ξ-pregnane-3, 20-diol
3	1.10	462	117(b.p.)	5-pregnane-3β, 20α-diol
4	1.17	—*	117, 460	5-pregnane-3β, 16, 20-triol
5	1.20	—*	117	unidentified
6	1.25	—*	117, 129, 141, 156, 157, 445, 460(b.p.)	5-pregnane-3β, 16, 20-triol
7	1.28	478	117, 334(b.p.), 373, 338, 434	11-keto-5β-pregnane-3α, 20α-diol
8	1.32	—*	117(b.p.), 141, 156, 157, 462	5ξ-pregnane-3, 16, 20-triol
9	1.40	552	117(b.p.), 435	5β-pregnane-3α, 11β, 20α-triol
10	1.49	552	117, 141, 156	5ξ-pregnane-3, 16, 20-triol
11	1.49	552	117(b.p.), 435	5α-pregnane-3α, 11β, 20α-triol
12	1.63	521	103, 175, 188(b.p.)	TH-A
13	1.72/1.75	595	103, 175, 188(b.p.), 312, 474, 564	TH-B *(syn/anti)*
14	1.80/1.82	595	103, 175, 188, 312, 474(b.p.), 564	*allo*-THB *(syn/anti)*
15	1.88	566	117, 196, 209, 271	11-keto-pregnane-1, 3, 20-triol
16	2.11	595	103, 175, 188, 474, 564	*allo*-3β-THB
17	2.11	—*	117, 129, 283, 373, 463	11-keto-pregnane-3, 20, 21-triol
18	2.24	640	103, 129, 460, 537, 550, 635	pregnane-3, x, 20, 21-tetrol
19	2.24	566	117(b.p.), 196, 209, 271	11-keto-pregnane-1, 3, 20-triol
20	2.36	640	117, 129, 267, 357(b.p.), 447, 537, 550, 525	5β-pregnane-3α, 11β, 20β, 21-tetrol
21	2.42	640	117, 129, 267(b.p.), 357, 447, 537, 550, 525	5α-pregnane-3α, 11β, 20β, 21-tetrol
22	2.55	595	175, 188, 474, 564	20-keto-pregnanetriol
23	2.55	640	117, 141, 156, 157, 460, 550	pregnane-3,x,16,20-tetrol
24	2.80	683	103, 175, 188(b.p.), 382, 400, 472, 562, 652	20-keto-pregnane-3,x,y,21-tetrol

[1] relative retention time to 5α-cholestane
* molecular ion was not detected, b.p. indicates base peak

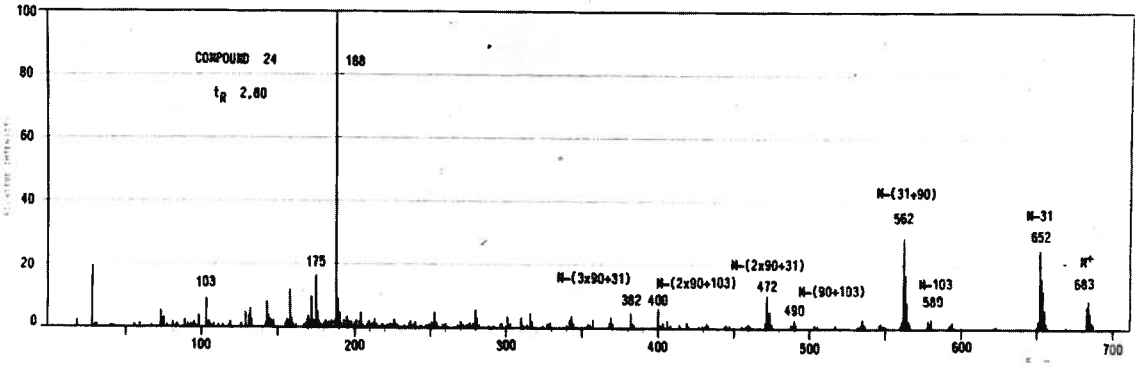


Fig. 17 — *Mass spectrum (scan number 215) of the silyl ether of* compound 24, *characterized as 20-keto-pregnane-3,x,y,21-tetrol*

DISCUSSION

Urinary steroid profile analysis by computerised GC-MS using an open tubular capillary column revealed an abnormal excretion pattern in the case of the 17α-hydroxylase deficiency investigated. None of the 17-hydroxylated C_{21}-steroids found as major metabolites in normal urine (Setchell et al 1976) e. g. tetrahydrocortisol (THF) and tetrahydrocortisone (THE) were found on examining repetitively scanned spectra from injection of a hydrolyzed urine extract (MO-TMS derivative). Furthermore, 18-hydroxylated steroids were not detected using the procedure adopted here, although under the reaction conditions employed, 18-hydroxy-corticosterone was fully derivatized. Tetrahydro-18-hydroxylated compounds have been found in normal urine (Ulick et al 1958; Ulick and Vetter 1965) and in case of 17α-hydroxylase deficiency (Honour et al 1978) but their detection in complicated by the fact that two forms are possible-free and 18-20 cyclic ketal-(Shackleton and Honour 1977; Honour and Shackleton 1977). The lack of 18-hydroxylated compounds in the present investigation could be due to sensitivity of the procedure adopted, or the patient has an additional 18-hydroxylase deficiency, as found recently in a patient with complete male pseudo-hermaphrodism and hyperaldosteronism (Waldhäusl et al 1978). It is therefore not possible to exclude the latter possibility.

Tetrahydro-DOC was not detected in the present study, in agreement with the findings of Honour et al (1978). The absence is probably explained by a 21-dehydroxylation process causing 21-dehydroxylated steroids to be formed. The latter compounds e. g. 5β-pregnane-3α,20α-diol, 11-ketopregnanediol and pregnane-3,11,20-triols are found in fairly large amounts in the present case. The 21-dehydroxylation has been demonstrated in the faecal flora of rats (Eriksson et al 1969) and humans (Bokkenheuser et al 1976; Kelly et al 1977).

The two metabolites are assigned the structural features of a 1,3,20-trihydroxy-11-keto-steroid, one of them identified as an 11-keto-pregnane-1,3,20-triol (molecular weight 566). Compounds with such a structure have been detected or identified by Honour et al (1978) in urine of a patient with 17α-hydroxylase defect.

The two principal metabolites among the pregnanetetrols detected in this urine were identified as 5β-pregnane-3α,11β,20β,21-tetrol and 5α-pregnane-3α,11β,20β, 21-tetrol. They were present in the ratio 5β/5α 1:2, similar to that found in the urine of a healthy man after ingestion of corticosterone (Exley 1965).

The procedure found here is a rapid one, no pre-fractionation on Sephadex columns having been carried out, *i.e.* a total steroid profile is obtained in one step, allowing a sensitive means of identifying most of the urinary steroid metabolites. It is therefore especially suitable for the investigation of metabolic defects where only small volumes of urine may be available.

Acknowledgements

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RESUMO

Os esteróides urinários num doente com hiperplasia suprarrenal congénita, hipertensão e amenorreia primária causadas por um defeito enzimático em 17α-hidroxilase foram investigados por cromatografia gasosa em coluna capilar associada à espectrometria de massa em sistema computarizado *on line*.

Os esteróides foram detectados, caracterizados ou identificados por análise seqüencial dos espectros de massa tomados a cada 8 segundos e por cromatografia de massa computarizada, após formação de derivados metiloximas e éteres trimetilsilícicos.

Não foram encontrados corticosteróides com hidroxilação em posição 17α. Os mais importantes esteróides de valor diagnóstico encontrados nesta urina foram 5β-pregnano-3α, 20α-diol, 5β-(e 5α-)pregnano-3α,11β,20α-tri6is, 5β-(e 5α-) pregnano-3α, 11β, 20β, 21-tetróis, 3α, 20α-dihidroxi-5β-pregnan-11-ona, 3α, 11β,21-trihidroxi-5β-pregnan-11-ona (THB), 3α, 11β, 21-trihidroxi-5α-pregnan-11-ona (*allo*-THB) e 3β, 11β,21-trihidroxi-5α-pregnan-11-ona (allo-3β-THB). Também foram encontrados dois esteróides incomuns apresentando uma estrutura 1,3,20-trihidroxi-11-cetónica.

O método *screening* descrito permite uma análise rápida de esteróides em C_{21} em pequena quantidade de urina muito útil no diagnóstico de erros inatos da biossíntese de corticosteróides.

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