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ANALYSIS OF STEROIDS BY HIGH-RESOLUTION GAS–LIQUID CHROMATOGRAPHY

II. APPLICATION TO URINARY SAMPLES*

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SUMMARY

Application of apolar glass capillary columns to steroid analysis by gas chromatography is described. Following a relatively simple clean-up procedure the isolated urinary steroids were separated in the gas phase as their methoxime trimethylsilyl derivatives. Total urinary steroid profiles are shown, being of diagnostic value in studying irregularities in steroid metabolism.

Special attention is paid to the identification of the separated steroids by retention indices and methylene unit values. In more detailed studies, structure elucidation by means of a gas chromatograph–mass spectrometer combination was of invaluable help, especially when dealing with previously unknown metabolites.

INTRODUCTION

For a long time the technique of steroid analysis by gas chromatography (GC) with capillary columns had no impact, because of the technical difficulties to be overcome. A major problem has been the availability and maintenance of columns coated with a uniform and thermostable layer of the stationary phase. Recently, considerable progress has been made with regard to the preparation of durable glass capillary columns[1]. Reliable and reproducible procedures have been given for the preparation of these columns coated with stationary phases of the dimethylpolysiloxane type. Also, preliminary results obtained with a more selective phase have been published[2].

On account of the intrinsically higher plate numbers, and the corresponding separation possibilities of capillary columns compared to packed ones, the use of this type of column is most attractive in the separation of complex mixtures. Additional advantages concern the identification of the individual components. Smaller amounts are detectable and, in the case of characterization with the aid of retention quantities, numerical values can be obtained with higher precision.

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In the coupled operation of a glass capillary column and a mass spectrometer the identification of steroids is favoured by a lower background signal. In particular the use of the mass fragmentometric method can notably lower the detection threshold[3] of, incidentally, even unresolved peaks. However, the need for higher chromatographic resolution remains in studies on epimeric steroids, substances giving rise to similar fragmentation patterns. Furthermore, the complexity of many steroid profiles still requires a higher degree of resolution for the identification of metabolites of diagnostic importance. The computer-assisted gas chromatography–mass spectrometry (GC–MS) analyses described by Reimendal and Sjövall[4] clearly illustrate this need.

In this article we shall describe the possibilities of glass capillary columns in steroid analysis. Two relatively simple procedures will be given for the isolation of steroids from urine. Identification of steroids by means of GC retention indices and on the basis of mass spectra will be shown and discussed.

EXPERIMENTAL

Pretreatment of urinary samples

Five millilitres of a 24-h urine sample were adjusted to pH 5.20 with 0.2 *M* HCl. The enzymatic hydrolysis was carried out with 0.2 ml of *Helix pomatia* enzyme solution (containing 20,000 Fishman units of β-glucuronidase and 160,000 Roy units of sulphatase) at 37° in a thermostatted bath over 24 h. In the case of infant urines usually a 72-h hydrolysis was carried out.

Following the hydrolysis two standard clean-up procedures have been used: a classical extraction method (I) and a steroid isolation method using the neutral resin Amberlite XAD-2 (II), first described by Bradlow[5].

Method I. Hydrolysis is followed by extraction with thrice 20 ml of distilled ethyl acetate. The combined extracts are washed twice with 20 ml of 0.1 *M* NaOH solution and twice with 20 ml of distilled water. Next the extract is dried over a few grams of anhydrous Na_2SO_4 and filtered off. By means of a Rotavap evaporator the solvent is removed and the residue is redissolved in 1.5 ml of ethyl acetate. From this an aliquot of 1 ml is introduced into a vial. The ethyl acetate is evaporated at 40° under nitrogen.

Methoxime trimethylsilyl (MO–TMS) derivatives are formed by addition of 100 μl of a 10% (w/w) solution of methoxylamine hydrochloride in pyridine. The reaction conditions are: MO derivatization for 15 min at 60°, followed by evaporation of the pyridine at 40° under a nitrogen flow; then 100 μl of N,O-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) (5:1) are added and the reaction proceeds for 2 h at 60°. Subsequently samples of 0.5–1.0 μl can be injected into the gas chromatograph.

Method II. The hydrolysed sample is pipetted on to a 10 × 0.5 cm I.D. column filled with 7 g Amberlite XAD-2 (particle size 50–100 μm). A flow-rate of about 0.5 ml/min is maintained. After the urine has passed through the column, it is washed with 5 ml of distilled water. Elution takes place with 15 ml of ethanol. Next the ethanol eluate is passed through an Amberlyst A-26 (particle size about 500 μm) column of the same dimensions, which is further eluted with an additional 5 ml of ethanol. Evaporation of this eluate at 40° under nitrogen yields a residue sufficiently

pure for gas–liquid chromatography (GLC). Steroid derivatives are prepared according to Method I.

For quantification purposes in our work 5α-cholestane or cholesteryl butyrate was added to the urine sample as internal standard before hydrolysis.

Gas chromatography

In this investigation glass capillary columns normally 20–30 m × 0.25 mm I.D. and 1 mm O.D., drawn from Pyrex glass tubes, have been used.

The columns were deactivated with a solution of benzyltriphenylphosphonium chloride in dichloromethane and coated afterwards with SE-30 or OV-101 in a static procedure as previously described[1].

Two instruments were used, *viz.* (1) A self-assembled gas chromatograph composed of Becker (Delft, The Netherlands) pressure controllers, Becker air thermostat covered with an asbestos lid through which an all-glass solid-state injection system[6] penetrates into the oven, a Becker flame ionization detector, and an amplifier constructed in our laboratory. By means of shrinkable PTFE tube the end of the column is connected with a steel capillary, 5 cm × 0.25 mm I.D., which ends in the flame tip. (2) A Hewlett-Packard (Avondale, Pa., U.S.A.) Type 5750 gas chromatograph. In this instrument the slightly modified all-glass solid-state injection system is horizontally positioned. A narrow-bore glass tube, the outer diameter of which tapers off to 1 mm, was used to adapt the detector junction to the capillary column. Nitrogen was used as carrier gas, the inlet pressure being controlled by a Becker pressure regulator.

Gas chromatography–mass spectrometry

The same columns were installed in a combination gas chromatograph–mass spectrometer. For this purpose an AEI (Elmsford, N.Y., U.S.A.) Model MS-12 mass spectrometer was equipped with a high-capacity pump and further modified as described by Leclercq and Leferink[7], enabling direct coupling with glass capillary columns. Operating conditions were: carrier gas (helium) flow-rate, 0.5–1 ml/min; oven temperature, 230 or 240°; accelerating voltage, 4 kV; trap current, 500 μA; electron-ionizing energy, 70 eV; source temperature, 250°.

RESULTS AND DISCUSSION

Quantitative aspects of sample clean-up

To quantify absolute losses during pretreatment [^{14}C]estriol and [^{14}C]dehydroepiandrosterone ([^{14}C]DHEA) were added to a urine sample and the recoveries of each step in Methods I and II were measured.

In Method I small differences between the [^{14}C]estriol and the [^{14}C]DHEA recovery were noticed in the alkali wash and evaporation steps used on the ethyl acetate extract. The acidic character of estriol accounts for the 4% loss on purifying the extract with alkali, whereas for DHEA only a 1% loss was measured. However, evaporation of the ethyl acetate under nitrogen at 40° caused a loss of 3% for the more volatile DHEA and only 1% for estriol. Overall recoveries following the pretreatment of ten samples together up to the derivatization step amounted to 91% ($\sigma = 2$) for estriol and 90% ($\sigma = 2$) for DHEA.

In Method II no activity of [^{14}C]estriol and [^{14}C]DHEA was measured in the urine after its passage through the Amberlite XAD-2 column. Washing the column with 5 ml distilled water caused losses of 0.2% for estriol and 1% for DHEA. Elution with 15 ml ethanol yielded 99% of the original estriol and 88% of the DHEA. On purifying the eluate on the Amberlyst A-26 column, the recoveries of estriol and DHEA in this step, including the wash with 5 ml ethanol, amounted to 84% and 98%, respectively. So, combining the Amberlite XAD-2 and the Amberlyst A-26 steps, an overall recovery of 83% for estriol and 86% for DHEA was measured.

These data illustrate the stronger bond between the more polar DHEA and the Amberlite XAD-2 matrix, while the estriol loss on the Amberlyst A-26 column can be explained by its acidic character.

Recovery after hydrolysis of the steroid conjugates is not included in our data; however, experiments with labelled conjugates under identical hydrolysis conditions indicate[8] a 90–100% yield of steroids liberated by the *Helix pomatia* enzyme. However, some conjugates are known that cannot be cleaved by *Helix pomatia* enzyme.

Blanks were prepared by performing the entire clean-up starting from 5 ml distilled water in lieu of urine. In the blank we identified dioctylphthalate (a plasticizer), squalene (of unknown origin), and cholesterol (from the enzyme).

Identification on the basis of chromatographic retention

According to the procedures described above, steroids present in several urine samples have been determined. Identification of the separated compounds can be based on a direct comparison of the numerical value of a retention parameter with a set of reference retention data. Three parameters will be discussed here, *viz.* relative retention, retention index and methylene unit value (*MU* value).

Relative retention. As no uniformity exists in the choice of reference compound, a comparison of relative retentions between different laboratories is troublesome. However, we have measured relative retentions for comparative reasons. Table I shows the relative retentions measured at 230° of a number of standard steroid-TMS derivatives with 5*α*-cholestane as the reference compound. Each figure represents an average value of ten runs performed on consecutive days with the self-assembled gas chromatograph.

Table I illustrates the degree of reproducibility of relative retention value when operating under strictly constant conditions. No relative retentions for steroids are reported in the literature to three decimal places; however, in our case, one can attach a value to the third decimal place. Moreover, the qualitative reliability of the combination used (the all-glass solid injection system and capillary column) is shown.

Retention index. The index concept, which means applying retention data based on the use of more than one standard, lends itself better to interlaboratory comparison of retention data. The Kováts index is well-known, but in practice when dealing with a complex mixture the addition of a series of consecutive *n*-alkanes to the sample might give rise to a partial or total overlap of these standards with some of the components in the mixture. Whether it is possible to use fewer standards depends on the linearity of the log plot of the *n*-alkanes. Groenendijk[9] has thoroughly examined the linearity of the log plot, both in the lower hydrocarbon range (C_1–C_9) and in the higher region (C_{18}–C_{32}). From his work it can be concluded that an unambiguous retention index system cannot be defined over a range of more than

TABLE I

RETENTIONS OF STANDARD STEROID TMS DERIVATIVES RELATIVE TO 5α-CHOLESTANE

Column: SE-30. Temperature: 230°.

	Relative retention	*Standard deviation (%)*
Androsterone	0.393	0.12
Etiocholanolone	0.412	0.08
Dehydroepiandrosterone	0.479	0.13
11-Ketoetiocholanolone	0.502	0.16
Testosterone	0.643	0.30
Estradiol	0.675	0.09
11-Hydroxyandrosterone	0.710	0.20
11-Hydroxyetiocholanolone	0.743	0.17
allo-Pregnanediol	0.908	0.15
Pregnanediol	0.947	0.11
5α-Cholestane	1.000	—
Estriol	1.271	0.25

two carbon atoms in the region of C_1–C_9. However, within the accuracy of the measurements, there is no non-linearity in the part of the log plot that is of interest for steroids. This was confirmed by our experiments with a *n*-alkane mixture, including *n*-C_{24}, *n*-C_{28}, *n*-C_{30}, *n*-C_{32} and *n*-C_{36}, isothermally analysed at 250°. When two *n*-hydrocarbons are given their proper index values, the indices of the *n*-hydrocarbons inside and outside this region are, within the accuracy of the measurements, multiples of 100. With regard to the influence of the choice of the *n*-hydrocarbon standards, retention indices have been measured for the MO-TMS derivatives of some corticosteroid metabolites at 250° on SE-30 using the pairs C_{28}–C_{30}, C_{30}–C_{32} and C_{28}–C_{32} as standards. Table II shows the results. The indices marked with an asterisk represent extrapolated values.

As expected, the choice of the *n*-alkanes is not decisive for the retention index value. A linearization over a somewhat longer range reflects only relatively small differences in the index value. The indices above are calculated using the time of the edge of the residual solvent peak as the dead time.

The use of a solid-state injection system makes a methane injection impossible and so we tested the effect on the retention index value of the time of the edge of the reduced solvent peak as the dead time, and of a calculated dead time as proposed by Groenendijk and Van Kemenade[10]. This calculated dead time has been proposed as the basis of a new index system, the carbon index[11,12]. From our experiments with steroids it turned out that, apart from a factor 100, only non-significant differences between the Kováts index and the carbon index exist. So for the retention characterization of steroids there is no point in distinguishing between Kováts index and carbon index. The carbon index has the advantage that no solvent peak is needed.

For practical use, as shown in Table III, we express retention indices henceforth as integers, realizing that when precision is increased a decimal place can be significant. The work of Krupčík *et al.*[13] shows this.

TABLE II

RETENTION INDICES FOR SOME CORTICOSTEROID MO-TMS DERIVATIVES
Column: SE-30. Temperature: 250°.

*Compound***	*Standard*		
	n-C_{28}–*n*-C_{30}	*n*-C_{30}–*n*-C_{32}	*n*-C_{28}–*n*-C_{32}
n-C_{28}	2800.0	2799.2*	2800.0
THS	2880.3	2879.8*	2880.5
a-THS	2916.7	2916.3*	2916.9
THE	2978.2	2978.1*	2978.5
n-C_{30}	3000.0	3000.0	3000.4
a-THB	3024.4*	3024.5	3024.9
THF	3032.5*	3032.6	3033.0
a-THF	3043.1*	3043.3	3043.6
DOC	3066.3*	3066.5	3066.8
n-C_{32}	3199.2*	3200.0	3200.0

* These indices represent extrapolated values.
** For abbreviations, see Table V.

Methylene unit value

The *MU* values, also based on the retention behaviour of *n*-alkanes, are currently used for steroid identification in temperature-programmed analyses. In Table III the *MU* values of a number of MO-TMS derivatives of urinary steroids are given. They were measured on an OV-1 column. As standards *n*-C_{24}, *n*-C_{28}, *n*-C_{30} and *n*-C_{32} were used in a temperature-programmed analysis of 1°/min, starting at 220°. An extensive comparison of *MU* values determined with capillary columns in different laboratories cannot be made because of the limited use of capillary columns for steroid analysis until now. Recently, German and Horning[14] published *MU* values obtained with open-hole tubular columns and their values are also included in Table III. Also interesting is the comparison of *MU* values obtained with packed columns with those measured on capillary columns. Concerning the retention behaviour of steroids on SE-30 and OV-1 we can postulate that no distinction need be made between these two dimethylpolysiloxane phases. The *MU* values for steroids measured on the packed column are somewhat lower than the values measured on capillary columns. This indicates that values measured with one column type cannot blindly be used for the other type.

A good agreement is observed between our values on a capillary OV-1 column and the values reported by German and Horning[14] measured on a capillary column coated with SE-30.

As illustrated in Table III *MU* values determined by temperature programming are by no means identical to the isothermally measured retention indices divided by 100. It is clear that for the 17-ketosteroids considerable differences exist between both quantities. For the later-eluted corticosteroids the above-mentioned differences are largely reduced.

Steroid profiles

Fig. 1 shows the chromatogram of the isothermal analysis of steroids present in the urine of a normal adult male. The sample has been pretreated

TABLE III

MU VALUES FOR SOME URINARY STEROID MO-TMS DERIVATIVES AND RETENTION INDICES

(A) Capillary column OV-1; temperature programmed at 1°/min from 220°. (B) Capillary column SE-30; temperature programmed at 1°/min from 200° (ref. 14). (C) Packed column OV-1; temperature programmed at 1°/min from 180 or 200° (ref. 15). (D) Packed column SE-30; temperature programmed at 1°/min from 180° (ref. 14). (E) OV-1 column; 255°, isothermal.

*Compound**	*MU value*				*Retention index*
	A	*B*	*C*	*D*	*E*
A	25.32	25.30	25.01	25.01	2563
E	25.44	25.45	25.22	25.22	2575
DHEA	25.96	25.99	25.63	25.63	2625
11KA	—	26.33	26.00	26.00	—
11KE	26.32	26.36	26.10	26.10	2656
11HA	27.21	27.19	26.93	26.93	2736
11HE	27.34	27.32	27.12	27.12	2744
16DHEA	27.68	27.74	27.32	27.43	—
PD	27.85	27.86	27.58	27.64	2802
PT	28.20	28.19	28.00	28.00	2832
A'T	28.65	28.59	—	28.40	—
THE	29.77	29.77	29.60	29.65	2982
THA	29.89	29.88	29.71	29.79	2992
THB	30.10	30.09	29.94	29.94	3012
a-THB	30.28	30.26	30.11	30.11	3030
THF	30.39	30.38	30.23	30.27	3038
a-THF	30.49	30.49	30.36	30.36	3050
CL	30.67	30.65	30.50	30.48	3068
βCL	30.95	30.95	30.79	30.80	3096
βC	—	30.97	30.79	30.80	—
CH	31.20	31.22	30.79	31.01	3124
C	31.45	31.42	31.22	31.22	3138

* For abbreviations, see Table V.

according to Method II. Apart from the minor peaks, several well-separated metabolites are recognized in the series of 17-ketosteroids and corticosteroids. Androsterone, etiocholanolone, dehydroepiandrosterone, 11-ketoetiocholanolone, 11β-hydroxyandrosterone, and 11β-hydroxyetiocholanolone are the most important 17-ketosteroids detected. THE, THF, *allo*-THF, the cortols, and cortolones are the main metabolites of cortisol. Pregnanediol and pregnanetriol also appear as relatively large peaks.

In order to calculate retention indices in isothermal runs, a second analysis is carried out with the *n*-alkanes C_{24}, C_{28} and C_{32} applied on the glass needle of the injection system, as well as the sample. In temperature-programmed runs these standards serve for the *MU* value calculation.

An example is given in Fig. 2, showing the total urinary steroid profile of a twelve-year-old normal boy, where the *n*-alkanes are also included. This sample has been pretreated according to the ethyl acetate extraction method. The amounts of some steroids in this sample have been assessed with cholesteryl butyrate as internal standard. In fact only semiquantitative information is provided because flame

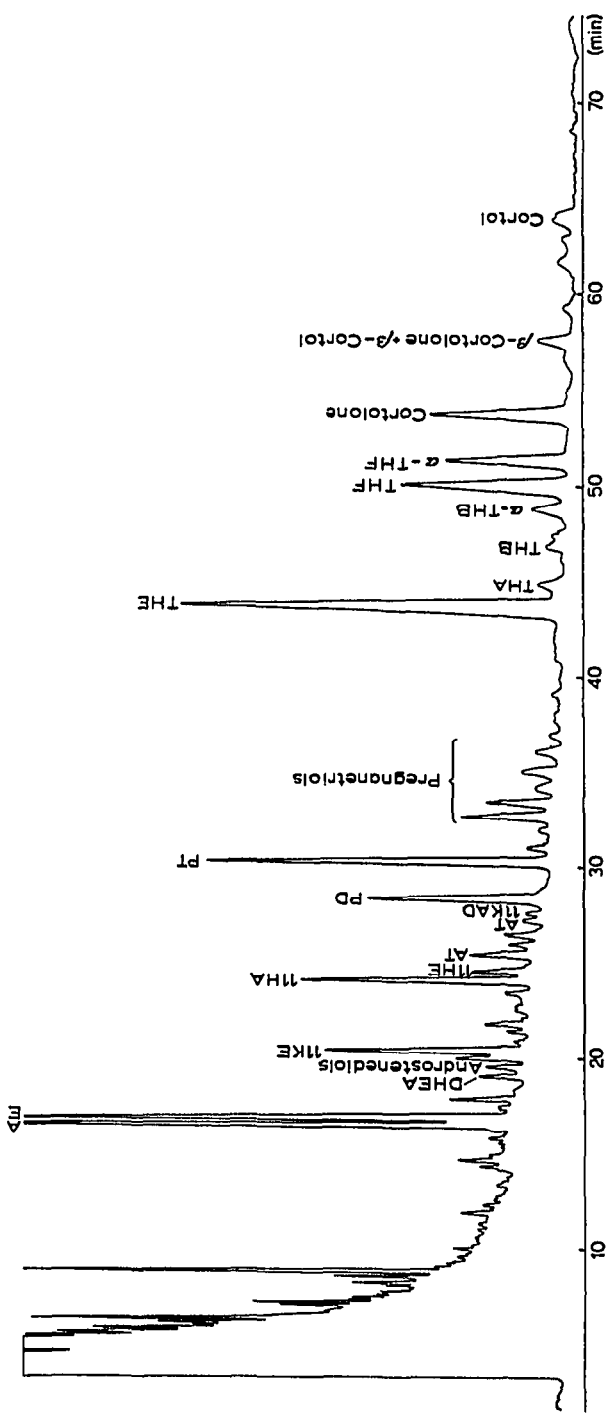

Fig. 1. Total urinary steroid profile of a normal adult male as MO-TMS derivatives. Isothermal analysis at 250° in a 30 m × 0.25 mm I.D. glass capillary column coated with SE-30. Carrier gas, nitrogen. Inlet pressure, 0.6 atp. 11KAD = Androstanediol-11-one; AT = androstanetriol. For further abbreviations, see Table V.

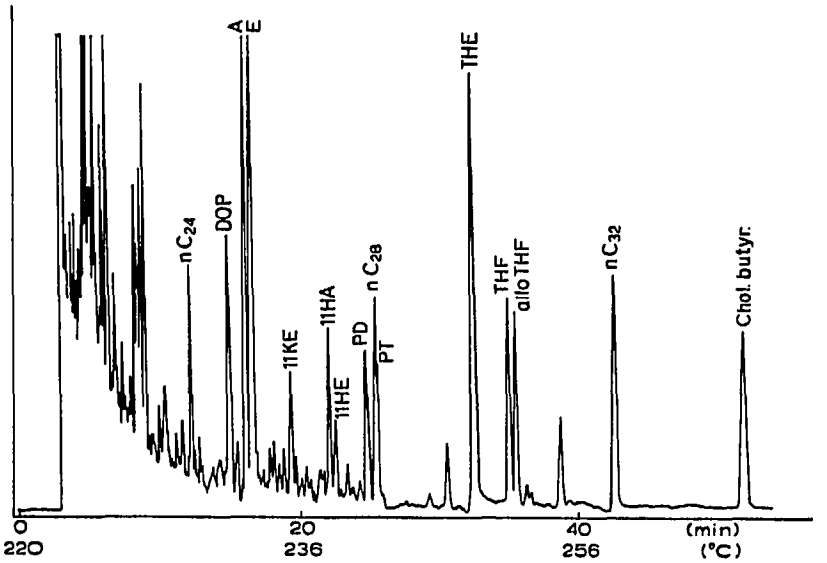

Fig. 2. Total urinary steroid profile of a normal 12-year-old boy as MO-TMS derivatives, *n*-alkanes and cholesteryl butyrate included. Temperature-programmed analysis at 220° plus 0.8°/min in a 24 m × 0.25 mm I.D. glass capillary column coated with OV-101. Carrier gas, nitrogen. Inlet pressure, 0.7 atp. For abbreviations, see Table V.

response factors were not taken into consideration. Table IV summarizes the values calculated on the basis of the derivatized compounds.

The steroid profile of an adult male with a 21-hydroxylase deficiency is given in Fig. 3. In this characteristic pattern the quantities of pregnanetriol and pregnanetriolone directly point to the enzyme defect.

In Fig. 4 the total steroid profile of a 45-year-old male with Cushing's syndrome is shown. The large peak of etiocholanolone and the high excretion of the cortisol metabolites THF and THE are indicative of the syndrome.

Quantitative and qualitative differences in steroid patterns obtained from

TABLE IV

MAJOR URINARY STEROIDS EXCRETED BY A NORMAL 12-YEAR-OLD BOY

Steroid	*Amount (mg per 24 h)*
Androsterone	1.9
Etiocholanolone	2.2
11-Ketoetiocholanolone	0.6
11-Hydroxyetiocholanolone	0.3
11-Hydroxyandrosterone	0.7
Pregnanediol	0.4
Pregnanetriol	0.6
Tetrahydrocortisone	2.8
Tetrahydrocortisol	1.3
allo-Tetrahydrocortisol	1.3

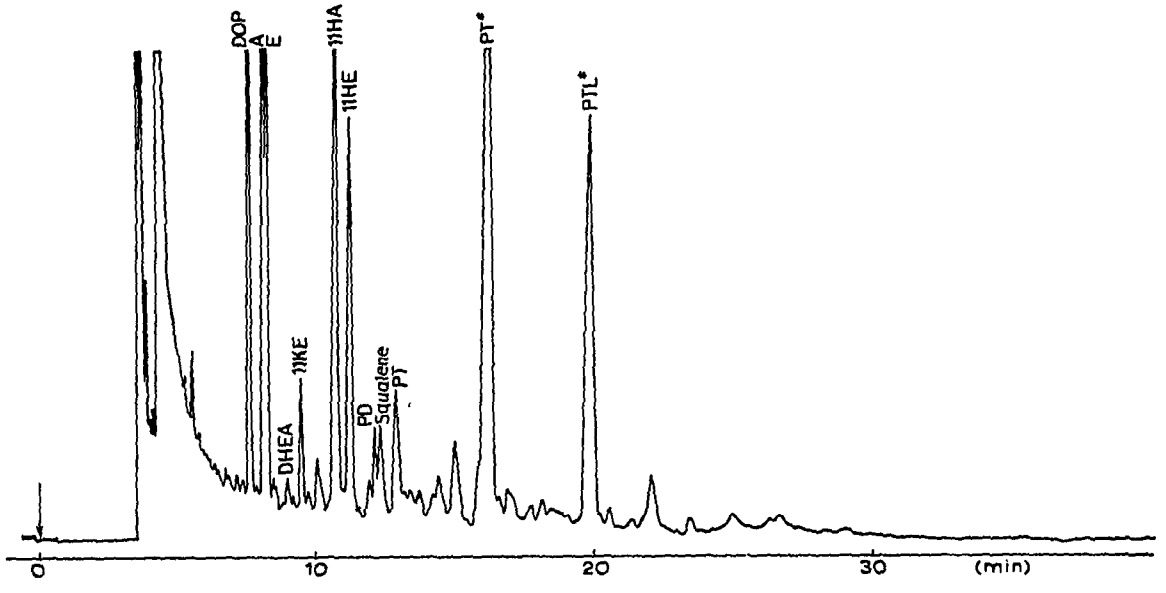

Fig. 3. Total urinary steroid profile of a 16-year-old boy with 21-hydroxylase deficiency as MO-TMS derivatives. Isothermal analysis at 250° in the same column as in Fig. 2. Carrier gas, nitrogen. Inlet pressure, 0.8 atp. For abbreviations, see Table V. The asterisk means that the 17α-hydroxy group is free.

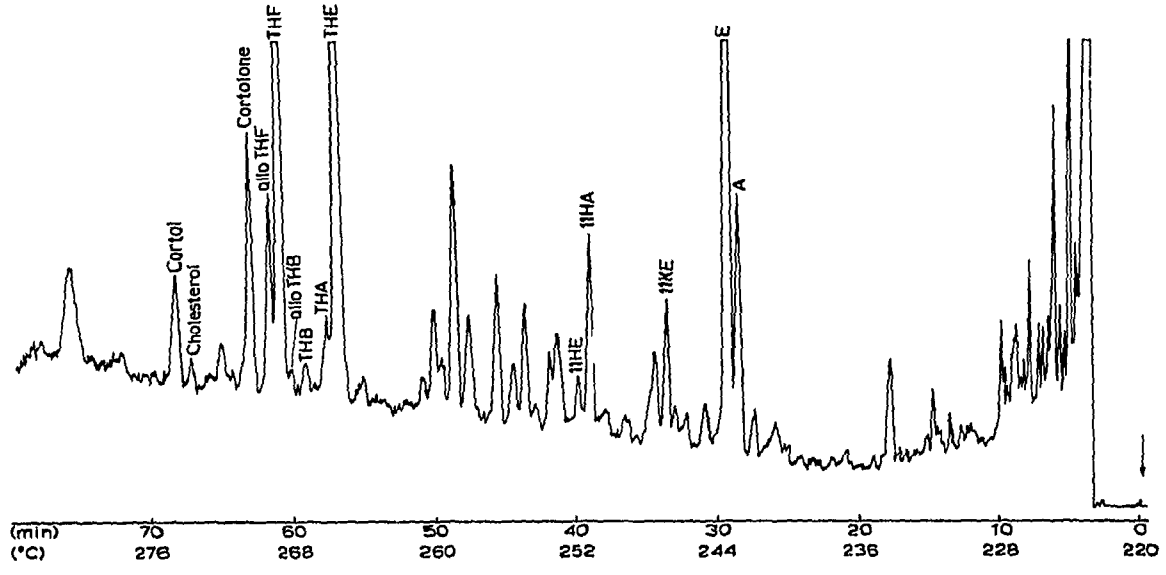

Fig. 4. Total urinary steroid profile of a 45-year-old male with Cushing's syndrome as MO-TMS derivatives. Temperature-programmed analysis at 200° plus 0.8°/min in a 30 m × 0.25 mm glass capillary column coated with SE-30. Carrier gas, nitrogen. Inlet pressure, 0.5 atp. For abbreviations, see Table V.

urines in pathological cases clearly illustrate the potentiality of the profile approach in steroid methodology. Irregularities in the pathways of steroid metabolism quite often give rise to characteristic changes in the urinary steroid profile. Thus typical patterns are obtained in cases of 21-hydroxylase deficiency, 3β-dehydrogenase isomerase deficiency, 11β-hydroxylase deficiency, and 17α-hydroxylase deficiency.

In a separate study steroids present in the urines of newborn infants have been studied. As is well known, these compounds differ markedly from the main urinary steroids of the adult human. Urinary samples were pretreated as described earlier, except for a 72-h hydrolysis necessary because of the stable character of some sulphate conjugates. Initially TMS derivatives were prepared. Although a much better separation was obtained compared to analyses on packed columns described earlier[16,17], the large number of epimeric structures with very similar retention times hindered the identification of the components in the series of pregnenetriols, androstenetetrols, and androstenetriolones. Later MO-TMS derivatives were used, also in order to stabilize the structures of possibly occurring steroids with dihydroxyacetone side chains. Moreover, the interpretation of mass spectra is facilitated in the case of MO-TMS derivatives. A chromatogram is given in Fig. 5.

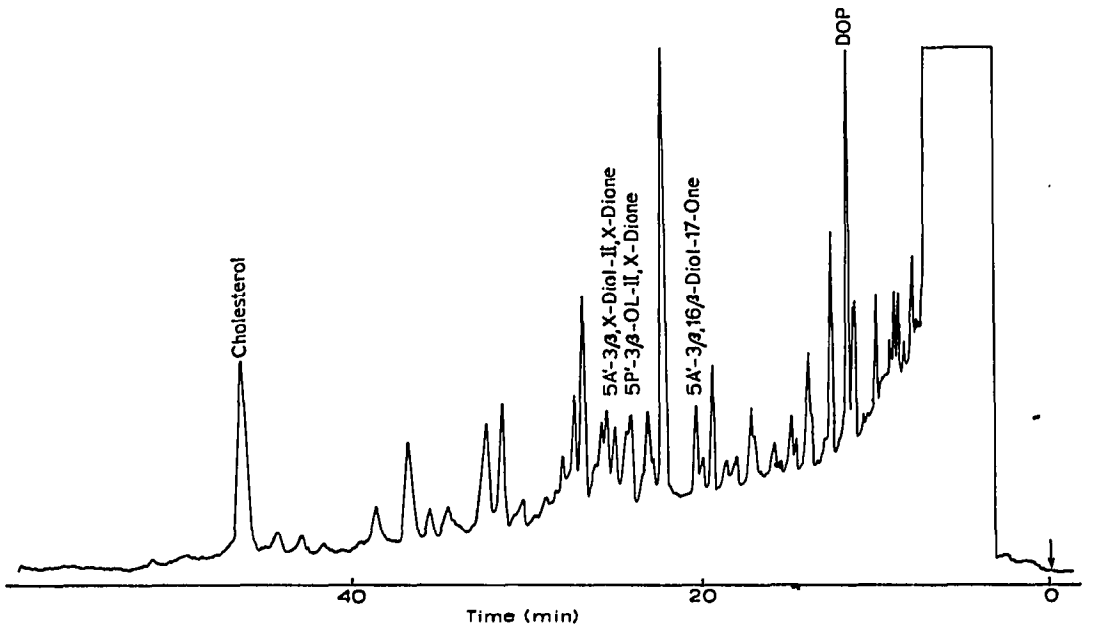

Fig. 5. Total urinary steroid profile of a twin baby (age three days) as MO-TMS derivatives. Isotherma analysis at 250° in a 20 m × 0.25 mm I. D. glas capillary column coated with SE-30. Carrier gas, nitrogen. For abbreviations, see Table V. X means unknown position of the substituent.

Mass spectra

On studying total urinary steroid profiles, mass spectra confirmed the identity of peaks established before on the basis of retention indices. With the aid of the GC–MS combination special attention has been paid to the steroid profiles obtained from the urine of a triplet at intervals up to the age of one month. A striking similarity in steroid profiles of the three babies was noticed, both from the quantitative and

the qualitative point of view, while the excreted amounts decreased significantly with increasing age. In contrast to the profiles of most newborns, with generally 5-androstene-3β,16α-diol-17-one, 5-androstene-3β,17-diol-16-one, 5-androstene-3β,16α,17β-triol and 5-pregnene-3β,16α-diol-20-one as the quantitatively most important steroids, several saturated steroids have been identified, *viz. allo*-pregnanediol, pregnanediol, 5α-pregnane-3α,16α-diol-20-one, 5β-pregnane-3α,16α-diol-20-one, 5ξ-pregnane-3ξ, 16ξ,21ξ-triol and two pregnanetriolones with unknown configurations[18].

Persilylation of steroids

At present no uniformity exists in the level of steroid silylation. Whether or not the sterically hindered hydroxyl groups are also derivatized has a pronounced influence on the retention behaviour of the steroid derivative. When using packed columns, it is favourable from the point of view of separation to preserve the 11β-hydroxyl and tertiary 17α-hydroxyl group and make use of the separation conditions based on the solute–solvent interactions due to the free hydroxyl groups. On preparing this type of derivative one has to ensure that the excess of methoxylamine present in the reaction mixture after MO derivatization, like H^+, does not exert catalytic activity upon the silylation reaction. Usually an extraction with ethyl acetate is carried out, followed by washing with bicarbonate solution. However, with regard to decreasing column adsorption effects and the gain of thermal stability persilylation is attractive, particularly when dealing with (sub)nanogram amounts of steroids.

By their higher resolving power capillary columns allow full silylation. Moreover no extraction of the MO derivatives is necessary, thus preventing extra steps in the pretreatment, and the retention times of the polyhydroxylated corticosteroids are considerably shortened.

Fig. 6 represents the *MU* values of fully silylated steroids and those of the corresponding compounds with free 11β-hydroxyl and tertiary 17α-hydroxyl groups. The data are taken from the literature[16].

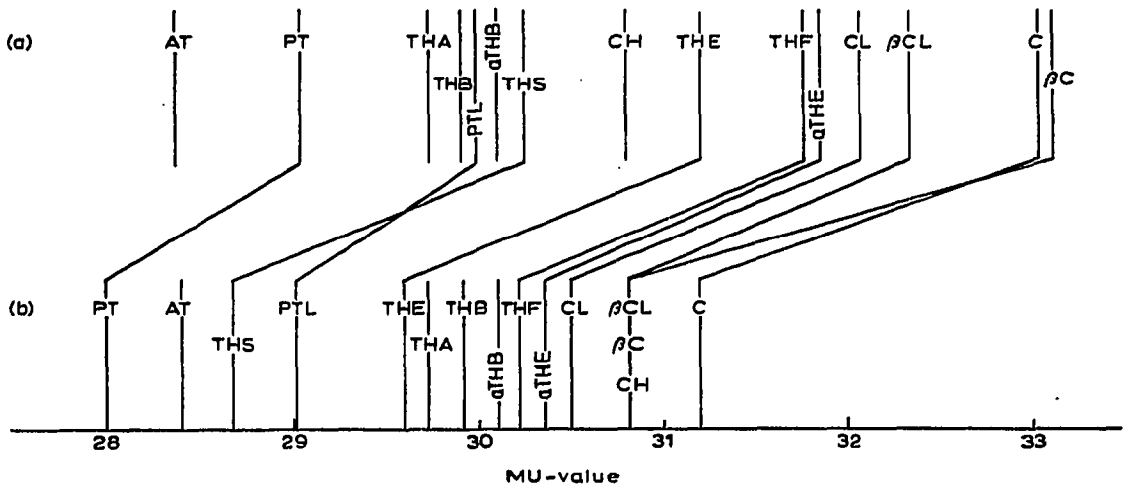

Fig. 6. Shift in *MU* values of partly (17α- and 11β-hydroxy groups free) silylated steroids (a) to fully silylated steroids (b). For abbreviations, see Table V.

TABLE V

ABBREVIATIONS OF THE NAMES OF THE STEROIDS IN THIS STUDY

Abbreviations: A = androstane; P = pregnane; A′ = androstene; P′ = pregnene; E = estratriene; C′ = cholestene.

Trivial name	*Systematic name*	*Abbreviation*
Androsterone	3α-Hydroxy-5α-A-17-one	A
Etiocholanolone	3α-Hydroxy-5β-A-17-one	E
11-Ketoandrosterone	3α-Hydroxy-5α-A-11,17-dione	11KA
11-Ketoetiocholanolone	3α-Hydroxy-5β-A-11,17-dione	11KE
11-Hydroxyandrosterone	3α,11β-Dihydroxy-5α-A-17-one	11HA
11-Hydroxyetiocholanolone	3α,11β-Dihydroxy-5β-A-17-one	11HE
Dehydroepiandrosterone	3β-Hydroxy-5-A′-17-one	DHEA
Hydroxydehydroepiandrosterone	3β,16α-Dihydroxy-5-A′-17-one	16DHEA
Testosterone	17β-Hydroxy-4-A′-3-one	T
Pregnanolone(5β)	3β-Hydroxy-5β-P-20-one	PN
Pregnanolone(5α)	3β-Hydroxy-5α-P-20-one	PN
Pregnanediol	5β-P-3α,20α-diol	PD
allo-Pregnanediol	5α-P-3α,20α-diol	*a*-PD
Pregnanetriol	5β-P-3α,17α,20α-triol	PT
Pregnanetriolone	3α,17α,20α-Trihydroxy-5β-P-11-one	PTL
Androstenetriol	5-A′-3α,16β,17α-triol	A′T
Estrone	3-Hydroxy-1,3,5(10)-E-17-one	E_I
Estradiol	1,3,5(10)-E-3,17β-diol	E_{II}
Estriol	1,3,5(10)-E-3,16α,17β-triol	E_{III}
Desoxycorticosterone	21-Hydroxy-4-P′-3,20-dione	DOC
THDOC	3α,21-Dihydroxy-5β-P-20-one	THDOC
THA	3α,21-Dihydroxy-5β-P-11,20-dione	THA
THB	3α,11β,21-Trihydroxy-5β-P-20-one	THB
allo-THB	3α,11β,21-Trihydroxy-5α-P-20-one	*a*-THB
THS	3α,17α,21-Trihydroxy-5β-P-20-one	THS
THE	3α,17α,21-Trihydroxy-5β-P-11,20-dione	THE
THF	3α,11β,17α,21-Tetrahydroxy-5β-P-20-one	THF
allo-THF	3α,11β,17α,21-Tetrahydroxy-5β-P-20-one	*a*-THF
Cortolone	3α,17α,20α,21-Tetrahydroxy-5β-P-20-one	CL
β-Cortolone	3α,17α,20β,21-Tetrahydroxy-5β-P-20-one	βCL
Cortol	5β-P-3α,11β,17α,20α,21-pentol	C
β-Cortol	5β-P-3α,11β,17α,20β,21-pentol	βC
Cholesterol	5-C′-3β-ol	CH
Dioctylphthalate		DOP

CONCLUSIONS

It has been shown that glass capillary columns can be used successfully in the separation of urinary steroids. Our experiments showed that sufficiently deactivated and well-coated columns can be used for periods from several months to half a year without notable deterioration. The available resolving power eliminates the need for intensive prefractionation. Thus urinary samples only need enzymatic hydrolysis, isolation of the steroids, extract purification and concentration, and finally derivatization. The steroid profiles obtained are most suitable for the tracing of disorders in steroid metabolism. The interpretation of the profiles is favoured by the fact that steroid profiles determined in different laboratories show a fair resemblance.

Though several minor peaks will remain unidentified (compound noise), a

great number of steroid metabolites can be characterized on the basis of retention indices and *MU* values. In this respect, one has to take into account the relation between precision of data and resolution of the chromatographic system.

In more detailed studies, as exemplified in our work on the group of steroids excreted by newborn infants, the availability of a GC–MS combination is of invaluable help when dealing with metabolites of unknown structure.

Thus, concerning the large-scale applicability of the technique described, we feel that for routine steroid screening identification with the aid of retention data is sufficient. However, the availability of a GC–MS combination, possibly of a GC–MS–computer combination, in a larger research centre, is an absolute requisite.

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