Notes

CHROM. 3551

Gas–liquid chromatography of androst-16-enes as trimethylsilyl and chloromethyldimethylsilyl ethers

3α-Hydroxy-5α-androst-16-ene (androstenol) occurs in normal male and female urine[1,2] and is excreted in increased amounts in some endocrine diseases[3–6]. In an attempt to elucidate the possible physiological role of this compound and its isomers, a survey is being made of the urinary excretion of androstenol in cases of hirsutism, adrenal hyperplasia and adrenal tumour. Although a colorimetric method for androstenol is available[1], low-titre urines and the estimation of the "androstenol analogues" (3α-hydroxy-5β-androst-16-ene and 3β-hydroxyandrosta-5,16-diene)[7], demand a more sensitive gas–liquid chromatographic (GLC) method which will be published in detail elsewhere. The purpose of this communication is to describe the behaviour of the trimethylsilyl (TMS) ethers of one C_{18}- and some C_{19}-Δ^{16}-steroids on columns other than those described earlier[7], and to report on the separation of the chloromethyldimethylsilyl (CMDS) ethers.

TMS ethers were prepared either by the method previously described[7] or by adding 50 μl Trisil (Koch-Light Ltd., Colnbrook, Bucks., England) to the dry steroid (5–10 μg). After standing at room temperature for 5 min, excess reagent was evaporated *in vacuo* at room temperature, the residue dissolved in dry hexane (50–100 μl) and a portion (1 μl) introduced onto the GLC column by syringe or solid injection technique. The TMS ethers of the Δ^{16}-steroid series were found to be so volatile that they were easily lost by evaporation and were also readily hydrolysed. It was thought that the CMDS ethers might be more stable and more easily separated than the corresponding TMS ethers in view of earlier experiments with the CMDS ethers of the 17-oxosteroids[8].

Three glass columns have been used: (a) cyclohexanedimethanol succinate (CDMS)/JXR silicone gum (0.6/0.75 %, w/w) on 100–120 mesh Chromosorb W (5 ft. × 3.5 mm), (b) CDMS (1 %, w/w) on 100–120 mesh Gas Chrom Q (5 ft. × 3.5 mm) and (c) QF1 (5 %) on 100–120 mesh Chromosorb W (7 ft. × 3.5 mm). Steroids used were as follows: 3α-hydroxy-5α-androst-16-ene (androstenol, 3α,5α-), 3α-hydroxy-5β-androst-16-ene (3α,5β-), 3β-hydroxyandrosta-5,16-diene (3β,Δ^5-), 3β-hydroxy-5α-androst-16-ene (3β,5α-) and 3-hydroxyoestra-1,3,5(10),16-tetraene (oestratetraenol). The preparation and sources of these compounds have been given previously[9].

In general, TMS ethers can be run at temperatures in the range 165–175° and CMDS ethers in the range 190–200° with carrier gas flow rates of 50 ml/min (Table I and Fig. 1). The CMDS ethers were found to be more stable than the corresponding TMS ethers and could be left at room temperature for several days without appreciable hydrolysis occurring.

The TMS ethers of the four C_{19}-Δ^{16}-steroids were not well separated on any

TABLE I

RETENTION TIMES RELATIVE TO CHOLESTANE (= 1) OF TRIMETHYLSILYL (TMS) ETHERS RUN AT 170° AND CHLOROMETHYLDIMETHYLSILYL (CMDS) ETHERS RUN AT 195° OF C_{18}- AND C_{19}- Δ^{16}-STEROIDS ON GAS–LIQUID CHROMATOGRAPHIC COLUMNS

	Column (a) (*CDMS/JXR**)	*Column (b)* (*CDMS***)	*Column (c)* (*QF1****)
TMS ethers			
3α,5α-	0.09	0.15	0.24
3α,5β-	0.09	0.15	0.24
3β,Δ^5-	0.10	0.16	0.25
3β,5α-	0.10	0.16	0.25
Oestratetraenol	0.27	0.38	0.26
CMDS ethers			
3α,5α-	0.41	0.53	0.59
3α,5β-	0.51	0.70	0.63
3β,Δ^5-	0.62	0.87	0.73
3β,5α-	0.63	0.87	0.78
Oestratetraenol	0.96	1.72	0.95

* Cholestane time 94.5 and 34.3 min at 170° and 195° respectively; see text for composition of columns.

** Cholestane time 51.6 and 18.1 min at 170° and 195° respectively.

*** Cholestane time 52.0 and 20.6 min at 170° and 195° respectively.

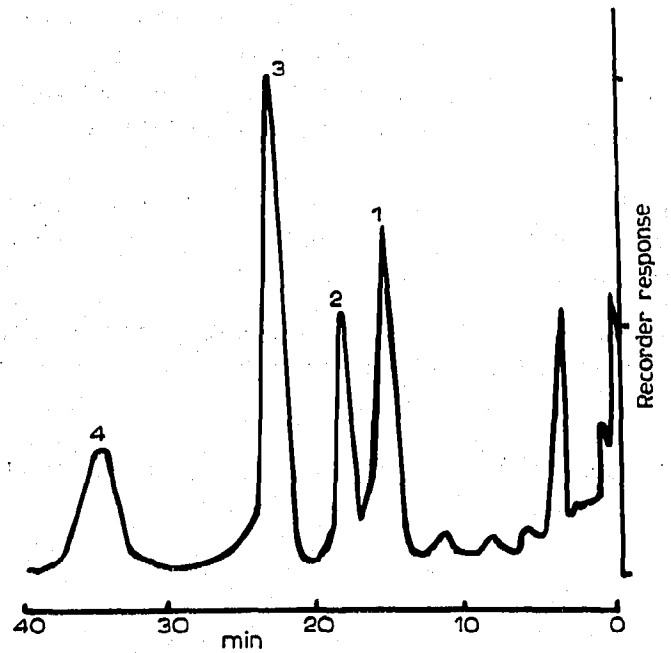

Fig. 1. GLC of the chloromethyldimethylsilyl ethers of Δ^{16}-steroids. (1) 3α-Hydroxy-5α-androst-16-ene (androstenol); (2) 3α-hydroxy-5β-androst-16-ene; (3) 3β-hydroxyandrosta-5,16-diene and 3β-hydroxy-5α-androst-16-ene; (4) 3-hydroxyoestra-1,3,5(10),16-tetraene. Column: CDMS/JXR (0.6/0.75%) on Chromosorb W (100–120 mesh) at 195° and 50 ml/min carrier gas flow rate.

phase tried. Oestratetraenol TMS ether, however, was easily resolved on columns (a) and (b). In contrast, the CMDS ethers were better separated although complete resolution was not achieved on any of the columns used. In keeping with earlier work, the 3α,5α- and 3α,5β-isomers were resolved by columns (a) and (b) while column (c) was needed for the resolution of the 3β,Δ^5- and 3β, 5α- compounds.

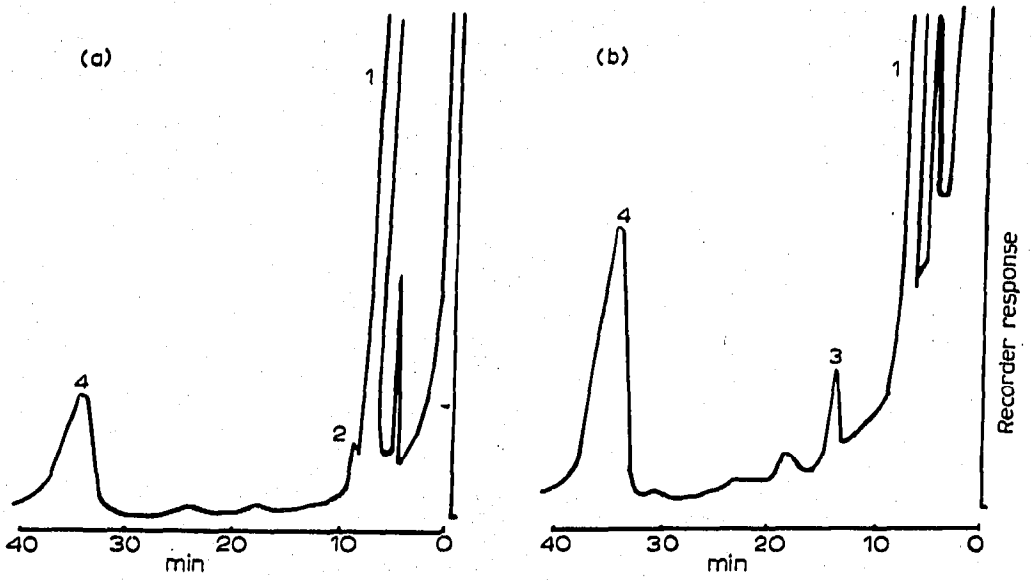

Fig. 2. GLC of the androstenol fraction obtained from alumina column chromatography of a normal female urine extract before (a) and after (b) chlorosilanisation (sensitivity twice that in Fig. 2a). (1) Unknown; (2) 3α-hydroxy-5α-androst-16-ene; (3) chloromethyldimethylsilyl ether of peak 2; (4) cholestane (internal standard). Conditions as for Fig. 1 but at 197°.

In many urines studied, GLC of the 'androstenol' fraction obtained from alumina chromatography (see ref. 7) revealed a large unidentified peak which was eluted just before the androstenol peak and often masked it. GLC of the extracts after chlorosilanisation, however, separated the peak due to the androstenol derivative and permitted quantitative estimation (Fig. 2).

A number of urines have now been processed by the method briefly described earlier[7] and the androstenol content measured by GLC of the free steroid, the TMS and CMDS ethers. Some of these results have been reported elsewhere[6].

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