Characterization of 3-oxo-Δ^4-steroids by gas chromatography of enol heptafluorobutyrates

Characterization of hydroxysteroids by esterification or etherification, followed by gas chromatography is well documented in the literature. Similar methods for the characterization of oxosteroids are not so widespread. CHAMBERLAIN AND THOMAS[1,2] have suggested reduction with lithium aluminium hydride while VANDENHEUVEL AND HORNING[3] have proposed derivative formation with N,N-dimethylhydrazine. Recently ZMIGROD AND LINDNER[4] have reported the gas chromatography of oxosteroids as ethylene thioketals. This note is intended to demonstrate the potential of enol heptafluorobutyration for the specific characterization of 3-oxo-Δ^4-steroids.

CLARK AND WOTIZ[5] first demonstrated that the heptafluorobutyrates of several hydroxysteroids were extremely sensitive to electron capture detection. Subsequently KNIGHTS[6] in discussions with WOTIZ and the present author pointed out that the conditions used for heptafluorobutyration would favour the formation of $\Delta^{3,5}$-enols from 3-oxo-Δ^4-steroids. Recent reports on heptafluorobutyration of hydroxysteroids have described milder conditions[7,8].

In the present study conditions similar to those first reported by CLARK AND WOTIZ[5] were used. The steroid (*ca.* 2 mg) was dissolved in benzene (0.1 ml) and heptafluorobutyric anhydride (0.1 ml) and heated in a stoppered tube at 65° for 30 min. Excess reagent and solvent were removed by heating *in vacuo* at 100° for 1–2 min. The residue was dissolved in acetone for gas chromatography.

The free steroids and their derivatives were chromatographed on 1% SE-30 at 190° and on 3% QF-1 at 240° using a Pye series 104 gas chromatograph equipped

TABLE I

ΔR_{MT} (HEPTAFLUOROBUTYRATION) VALUES FOR VARIOUS OXYSTEROIDS

	Compound	ΔR_{MT} *(heptafluorobutyration)*	
		3% QF-1 (240°)	*1% SE-30 (190°)*
1	Androst-4-ene-3,17-dione	−0.49	−0.15
2	Androst-4-ene-3,11,17-trione	−0.49	−0.14
3	Pregn-4-ene-3,20-dione	−0.41	−0.22
4	Cholest-4-en-3-one	−0.53	−0.19
5	17α-Acetoxyandrost-4-en-3-one	−0.47	−0.14
6	17β-Acetoxyandrost-4-en-3-one	−0.51	−0.14
7	20α-Hydroxypregn-4-en-3-one	−0.48	−0.16
8	20β-Hydroxypregn-4-en-3-one	−0.48	−0.23
9	17α-Hydroxyandrost-4-en-3-one	−0.55	−0.27
10	17β-Hydroxyandrost-4-en-3-one	−0.45	−0.19
11	3α-Hydroxy-5β-androstan-17-one	−0.03	−0.09
12	3α-Hydroxy-5α-androstan-17-one	−0.06	−0.13
13	3β-Hydroxy-5α-androstan-17-one	0.00	+0.02
14	3β-Hydroxycholest-5-ene	−0.01	−0.04
15	3β-Hydroxyandrost-5-en-17-one	+0.03	0.00
16	3β-Hydroxypregn-5-en-20-one	0.00	−0.02
17	3-Methyloestradiol	0.00	−0.06
18	Oestrone	−0.09	−0.14
19	Oestradiol	−0.13	−0.17

with a flame-ionization detector. Single peaks were obtained in all cases, with the exception of the oestrone and oestradiol derivatives which were formed in only 50% yield.

Table I lists the ΔR_{Mr}(heptafluorobutyration) values obtained on both columns. This parameter may be defined as the change in the logarithm of the retention time, produced by the esterification reaction[9,10].

On both columns the ΔR_{Mr} values for heptafluorobutyration of hydroxysteroids are small (compounds Nos. 11–17). Presumably the larger molecular weight of the derivative is approximately balanced by its increased volatility. ΔR_{Mr} values for 3-oxo-Δ^4-steroids are significantly higher (Nos. 1–6), although the effect is more marked with the selective column (QF-1). Phenolic hydroxyl groups (Nos. 18, 19) apparently behave as enols on SE-30 and as secondary hydroxyl groups on QF-1. The low ΔR_{Mr} value for hydroxyl groups means that the same ΔR_{Mr} value would be found for 3-oxo-Δ^4-steroids containing saturated hydroxyl groups (Nos. 7–10).

These results suggest a method for the specific characterization of 3-oxo-Δ^4-steroids. A ΔR_{Mr} (heptafluorobutyration) value on QF-1, of —0.55 to —0.40 would suggest the presence of this functional group. The extreme sensitivity of the enol heptafluorobutyrates compared with the corresponding derivatives of saturated hydroxyl groups, and their suitability for the analysis of biologically important steroids will be discussed elsewhere.

Acknowledgements

Part of this work was carried out in the M.R.C. Neuroendocrinology Research Unit at the University of Oxford. I wish to thank Prof. G. W. HARRIS for the facilities provided.

Department of Obstetrics and Gynaecology, J. CHAMBERLAIN
Charing Cross Hospital Medical School, London (Great Britain)

1 J. CHAMBERLAIN AND G. H. THOMAS, *Biochem. J.*, 86 (1963) 3P.
2 J. CHAMBERLAIN AND G. H. THOMAS, *Anal. Biochem.*, 8 (1964) 104.
3 W. J. A. VANDENHEUVEL AND E. C. HORNING, *Biochim. Biophys. Acta*, 74 (1963) 560.
4 A. ZMIGROD AND H. R. LINDNER, *Steroids*, 8 (1966) 119.
5 S. J. CLARK AND H. H. WOTIZ, *Steroids*, 2 (1963) 535.
6 B. A. KNIGHTS, personal communication, 1963.
7 G. CHARRANSOL AND H. H. WOTIZ, *2nd Intern. Congr. Hormonal Steroids, Milan; Excerpta Med. Intern. Congr. Ser.*, 111 (1966) 117.
8 K. NAKAGAWA, N. L. MCNIVEN, E. FORCHIELLI, A. VERMEULEN AND R. I. DORFMAN, *Steroids*, 7 (1966) 329.
9 I. E. BUSH, *The Chromatography of Steroids*, Pergamon, Oxford, 1961.
10 B. A. KNIGHTS AND G. H. THOMAS, *Nature*, 194 (1962) 833.

Received November 23rd, 1966