Notes

CHROM. 3431

Analytical chemical studies on steroids

Part XIX. Gas chromatographic separation of isomeric 16- and 17-oxosteroids

In the previous publications of this series, the authors reported the gas chromatography of C-13 and C-14 epimeric androstanes[1–3]. The occurrence of 16-oxosteroids as the natural products prompted us to explore the gas chromatographic separation of the usual 17-oxo compounds and their corresponding 16-isomers. The present paper deals with the gas chromatographic behavior of five pairs of isomeric oxosteroids and their O-trimethylsilyl oxime derivatives.

Experimental

Materials. Almost all the samples were prepared by the methods described in the preceding papers[1–3] and the others by known procedures.

Preparation of derivatives. To a solution of the sample (about 1 mg) in pyridine (0.5 ml) was added hydroxylamine hydrochloride (about 1.5 mg), and the resulting solution was heated at 70–80° for 1 h. This solution was further treated with hexamethyldisilazane (0.2 ml) and trimethylchlorosilane (0.1 ml) in the manner described by SWEELY *et al.*[4] After evaporation of the solvent the residue was extracted with *n*-hexane (0.5 ml), centrifuged and the supernatant was used for gas chromatography.

Gas chromatography. The apparatus used for this work was a Shimadzu Model GC-1C gas chromatograph equipped with a hydrogen flame ionization detector and a U-shaped stainless steel column (1.875 m × 3 mm I.D.). The column was packed with either 1.5 % SE-30 on a support of Chromosorb W (60–80 mesh) or 2 % OV-17 on Shimalite W (60–80 mesh). The temperatures of column, detector and injection chamber were kept at 220°, 280° and 250°, respectively. Nitrogen was used as carrier gas at a flow rate of 60 (for SE-30) or 80 ml/min (for OV-17).

Results and discussion

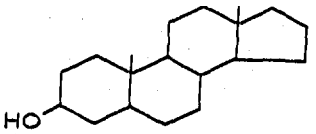
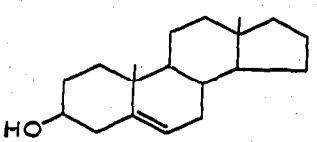
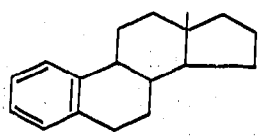
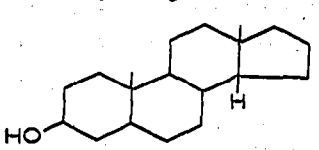
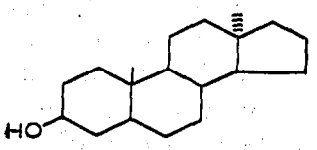
Gas chromatography of five pairs of isomeric 16- and 17-oxosteroids was carried out with 1.5 % SE-30 and 2 % OV-17. The relative retention times observed, using cholestane as a reference compound, are given in Table I.

When the oxosteroid itself was applied, satisfactory separation of isomeric 16- and 17-oxosteroids could not be attained on any kind of stationary phase. It is generally believed that one of the chief advantages of a non-selective phase lies in its effectiveness with different molecular shapes. Therefore, transformation of these oxosteroids into suitable derivatives was undertaken to accentuate the existing positional difference. In gas chromatographic work it is desirable that the derivative should be formed quantitatively and, in addition, the retention time of the product should be different from that of the starting material. Some pertinent carbonyl reagents, such as

TABLE I

RELATIVE RETENTION TIMES OF ISOMERIC 16- AND 17-OXOSTEROIDS

Conditions: stainless steel column (1.875 m × 3 mm I.D.); 1.5% SE-30 on Chromosorb W (60–80 mesh) or 2% OV-17 on Shimalite W (60–80 mesh); N_2 flow rate, 60 or 80 ml/min; column temp., 220°; detector temp., 280°; injection chamber temp., 250°.

Compound structure		*Position of keto group*	*Free*[a]		*NOTMS derivative*[b]	
			1.5% SE-30	*2% OV-17*	*1.5% SE-30*	*2% OV-17*
	(I)	16	0.42	0.90	0.86	0.95
		17	0.42	0.91	0.82	0.80
	(II)	16	0.40	0.90	0.85	0.96
		17	0.39	0.89	0.79	0.80
	(III)	16	0.21	0.43	0.36	0.59
		17	0.22	0.43	0.34	0.49
	(IV)	16	0.43	0.91	0.70	0.75
		17	0.41	0.85	0.81	0.79
	(V)	16	0.40	0.79	0.68	0.73
		17	0.39	0.68	0.58	0.53
Cholestane			1.00 (13.2 min)	1.00 (10.8 min)	1.00 (13.2 min)	1.00 (10.8 min)

[a] Sample without any pretreatment.
[b] Trimethylsilylated product of the oxime.

dimethylhydrazine and methoxyamine, have already been proposed for this purpose[5,6]. However, these techniques were still found to be unsatisfactory for the complete separation of these isomers, and hence introduction of the more accentuated substituent to the carbonyl group appeared to be necessary in order to increase the separation factors. Considering the steric interaction with the bulky reagent, the two-step method was devised for the preparation of the derivatives. Thin-layer chromatographic inspection demonstrated that formation of the oxime with hydroxylamine proceeded readily under the above-mentioned conditions. The procedure of SWEELEY *et al.* being then employed, subsequent O-trimethylsilylation of the oxime was also accomplished with relative ease.

The isomeric O-trimethylsilyl oxime derivatives thus prepared gave a single peak exhibiting different retention times on either SE-30 or OV-17. The complete

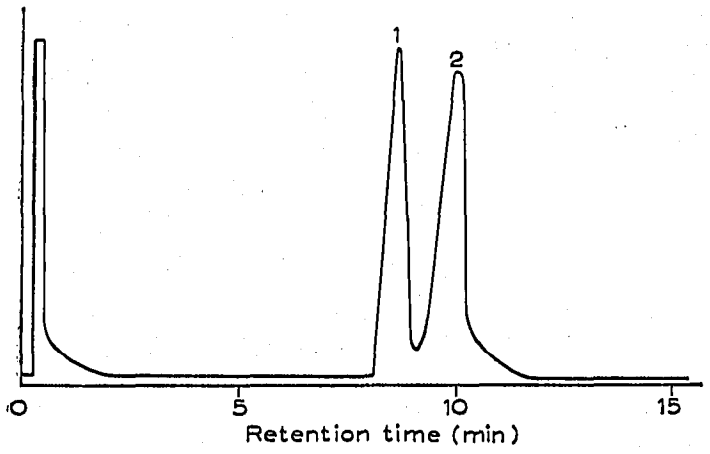


Fig. 1. Gas chromatogram of the O-trimethylsilyl oxime derivatives. 1 = 3β-Hydroxyandrostan-17-one; 2 = 3β-hydroxyandrostan-16-one. Column: 2% OV-17 (on Chromosorb W).

separation of the isomers was obtained with the latter of these two stationary liquids. A typical gas chromatogram is illustrated in Fig. 1. It is of particular interest that in almost all cases the 17-oxosteroid was eluted before the corresponding 16-isomer with only one exception, the 14β-series. This technique appears to offer a means of magnifying the existing slight difference in an arrangement of the functional groups on the steroid nucleus.

In addition the steroid numbers[7] were determined of the usual C/D-*trans* steroids having ring D ketone and their O-trimethylsilyl oxime derivatives. As shown in Table II, steroid number contributions (ΔSN) for the O-trimethylsilyl oxime of 16- and 17-ketones were found to be 2.1 and 1.9, respectively. The constancy of the values observed here may be useful in distinguishing the position of the ketone.

TABLE II

STEROID NUMBER CONTRIBUTION FOR O-TRIMETHYLSILYL OXIME

Compound No.	*Position of keto group*	*SN*[a]		ΔSN[a]
		Parent compound	*NOTMS derivative*	
I	16	24.3[b]	26.4	2.1
	17	24.3[b]	26.2	1.9
II	16	24.3[b]	26.4	2.1
	17	24.3[b]	26.2	1.9
III	16	21.1[c]	23.2	2.1
	17	21.1[c]	23.0	1.9

[a] *SN* = Steroid number (retention time: cholestane 13.2 min, androstane 1.6 min); ΔSN = steroid number contribution for O-trimethylsilyl oxime.
[b] Trimethylsilylated product.
[c] Sample without any pretreatment.

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Gas chromatographic separation of pyridine homologues, chloroanilines and toluidines

During the course of studies on the separation of isomers by solvent extraction, GLC techniques have been used for quantitative analysis of the isomers present in the solvent phases. Several liquid phases have been studied and it has been found that glycerol, diglycerol and polyphenyl ether (5 ring-OS-124) give good resolution of pyridine bases, *o*- and *p*-chloroanilines and *o*- and *p*-toluidines.

Glycerol and diglycerol have previously been used[1,2] for the analysis of pyridine bases but have not been reported for *o*- and *p*-chloroanilines or *o*- and *p*-toluidines. Of these two stationary phases, diglycerol gave very good results. It was found with glycerol that, whilst it gave good resolution, its efficiency decreased with time for the chloroanilines and the toluidines. The resolution obtained for *o*- and *p*-chloroanilines and for *o*- and *p*-toluidines is given in Table I.

It is believed that the use of polyphenyl ether for the separation of pyridine bases, *o*- and *p*-chloroaniline and *o*- and *p*-toluidines has not previously been reported.

Experimental

Instrumentation. The Pye "Series 104" Dual Flame Ionisation, Temperature Programmed Chromatograph Model 24 was used.