ANALYTICAL CHEMICAL STUDIES ON STEROIDS

PART IX. GAS CHROMATOGRAPHIC SEPARATION OF C-14-EPIMERIC 5α-ANDROSTANES

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Numerous articles dealing with the analysis of steroids by gas chromatography have been published since the first success of HORNING and his coworkers[1]. The correlation between the structure of steroids and their retention time has been widely investigated, and in consequence the contribution of structural differences in the steroid skeleton, as well as functional groups, to the retention time can be determined in terms of the so-called steroid number[2,3].

During the course of our studies on the stereochemistry of ring D in 14β-steroids, characterization of the two C-14-epimeric 5α-androstanes, which would possibly be produced from the Δ^{14}-compound by catalytic hydrogenation, was required. However, successful separation of the two C-14-epimers by means of gas chromatography has not yet been reported. In this paper, the gas chromatographic separation of C-14-epimeric 5α-androstanes using several types of column, and the effect of substituents in ring D on the separation of each pair of epimers with respective liquid phases, are described.

EXPERIMENTAL

Materials

14β-Androstanes were prepared by the methods previously reported by one (T.N.) of the authors[4-7] and the others by the known procedures.

Gas chromatography

The apparatus used for this work was a Shimadzu Model GC-1B gas chromatograph equipped with a hydrogen flame ionization detector and a U-shaped stainless steel column (1.5 or 2.25 m × 4 mm I.D.). The column was packed with either 1 % neopentyl glycol succinate (NGS) or 1 % cyanoethyl methyl silicone polymer (CNSi) on a support of Anakrom (90–100 mesh), or 1.5 % methyl phenyl silicone polymer (SE-52) or 3 % methyl fluoralkyl silicone polymer (QF-1) on a support of Gas-Chrom P (80–100 mesh). A 2 μl aliquot of a 1 % solution of steroid dissolved in acetone or ether was injected into the sample chamber.

RESULTS AND DISCUSSION

Gas chromatography of 10 pairs of C-14-epimeric 5α-androstanes was carried out with either the selective or nonselective liquid phase mentioned above.

Almost all the compounds, except in a few instances, gave a single peak without decomposition. During gas chromatography the 16β-acetoxy derivative (VI) in the 14β-series was susceptible to thermal degradation resulting in the formation of the Δ^{16}-compound, which was identified by comparison with the retention time of an authentic sample. It is of interest that in 14β-steroids the 16β-bond, which may have quasiaxial character, should be eliminated with relative ease to provide the more stable unsaturated compound. In addition, at the elevated column temperature, thermally unstable C-17-enol acetates (VIII) in both the 14α- and 14β-series did not survive and were transformed into 17-oxo derivatives. In the present work, therefore, gas chromatography was performed at a column temperature of 220°. The relative retention times hereby observed, using cholestane as a reference compound, are given in Table I.

Two pairs of epimers having no oxygen function in ring D, namely 5α-androst-16-en-3β-ols (IX) and 5α-androstan-3β-ols (X), did not show satisfactory separation with any kind of stationary phase. It is generally believed that one of the chief advantages of a nonselective phase such as a methyl silicone polymer lies in its effectiveness with different molecular shapes, and this generalization is evident from the successful separation of C-5-epimers. In the case of C-14-epimeric steroids, however, the difference in the C/D-ring juncture was not significantly reflected in the retention times, which were almost the same for both C-14-epimers, when SE-52 was used as the stationary phase.

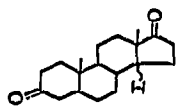
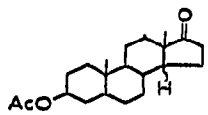
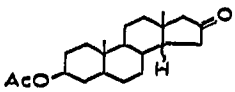
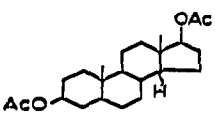
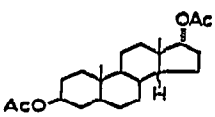
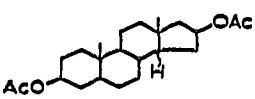
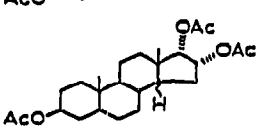
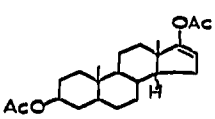
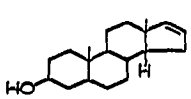
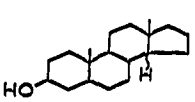
The preliminary work showed the desirability of using a selective liquid phase for this purpose. Of the selective phases so far examined, NGS exhibited the highest degree of stereoselectivity for the pairs of epimers. In particular, the presence of acetoxyl groups at C-16 and/or C-17 appeared to be favorable for distinct resolution with this column as can be seen in the results obtained with 17α-, 16β-acetoxy (V, VI) and 16α,17α-diacetoxy derivatives (VII). It is sufficiently substantiated that QF-1 is a useful stationary liquid for separation of alcohols, ketones, and esters. Fine resolution was achieved with this phase, particularly in the case of the two pairs having an oxo-group at C-16 or C-17, *viz.* 5α-androstane-3,17-diones (I) and 3β-acetoxy-5α-androstan-16-ones (III). The column packed with CNSi-phase, however, proved to be effective only for the two epimeric 16α,17α-diacetoxy derivatives (VII).

Observations that 5β-H steroids are eluted before the corresponding 5α-H epimers have been made in many instances[1-3,8]. On the other hand, the reversed elution order of A/B-*cis,trans* epimers has also been reported on separation of C_{19}-steroids with a selective liquid phase[2,9]. With respect to C/D-ring fusion, the retention time of 14β-androstanes was found to be somewhat shorter than that of the epimers, with only one exception, *viz.* the 17α-acetoxy derivatives (V). At the present time, any plausible explanation for the elution order is not available and must await further knowledge about the spatial arrangement of the functional groups in the steroid nucleus. Also, it is hoped that conversion into suitable derivatives, which may accentuate the existing stereochemical differences, will make the distinct separation of C-14-epimers possible.

TABLE I

RELATIVE RETENTION TIMES OF C-14-EPIMERIC 5α-ANDROSTANES*

Conditions: stainless steel column (1.5 or 2.25 m × 4 mm I.D.); packing 1% NGS or 1% CNSi on Anakrom (90–100 mesh), 1.5% SE-52 or 3% QF-1 on Gas-Chrom P (80–100 mesh); N_2 flow rate 30 ml/min; column temp. 220°; detector temp. 240°; flash heater temp. 250°.

Compound structure	*Configuration of C-14-H*		*Column stationary phase and length*			
			1.5% SE-52 1.5 m	*1% NGS 1.5 m*	*1% CNSi 1.5 m*	*3% QF-1 2.25 m*
	(I)	β	0.47	4.59	0.75	*4.45*
		α	0.50	4.73	0.77	*4.72*
	(II)	β	0.63	4.08	0.88	3.69
		α	0.66	3.92	0.90	3.76
	(III)	β	0.70	3.88	—	*3.74*
		α	0.69	3.97	—	*3.90*
	(IV)	β	0.93	4.05	1.21	3.68
		α	0.98	3.92	1.20	3.68
	(V)	β	0.95	*3.90*	*1.18*	3.48
		α	0.90	*3.72*	*1.11*	3.42
	(VI)	β	0.97	*3.77*	1.20	*3.60*
		α**	0.99	*3.94*	1.24	*3.71*
	(VII)	β	*2.06*	*11.9*	*2.73*	—
		α	*2.23*	*14.5*	*3.12*	—
	(VIII)	β**	0.82	3.57	1.01	3.02
		α**	0.88	3.59	1.03	3.06
	(IX)	β	0.17	0.71	0.22	0.43
		α	0.17	0.74	0.22	0.43
	(X)	β	0.20	0.70	0.25	0.46
		α	0.20	0.70	0.25	0.48
Cholestane			1.00 (12.4 min)	1.00 (3.5 min)	1.00 (12.9 min)	1.00 (7.6 min)

* Values for pairs of epimers whose mixture exhibited recognizably separated peaks are printed in italics.

** The values for VIα and VIIIα,β indicate the relative retention time of a main peak accompanied by minor decomposition products.

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SUMMARY

Gas chromatographic separation of 10 pairs of C-14-epimeric 5α-androstanes was examined using SE-52, NGS, CNSi and QF-1 as stationary phases. The effect of the substituents in ring D on the separation of each pair of epimers with the respective liquid phase is described.

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