Analytical chemical studies on steroids

Part XVI. Steroid number contribution for C/D-ring fusion

In previous papers the authors reported the gas-chromatographic separation of several pairs of the epimeric androstanes, whose structural difference exists only in C/D-ring fusion[1,2]. The use of a nonselective phase, *e.g.* methyl-silicone polymer, failed to differentiate distinctly the C-14-epimeric pairs, while C-13-epimers could be separated with relative ease on this column.

In 1962 VANDENHEUVEL AND HORNING introduced the concept of the so-called "steroid number" and determined the contribution values of various functional groups in the steroid nucleus[3]. With regard to the influence of ring juncture on the steroid number, the contribution for the A/B-ring system (5β/5α) was also estimated. However the effect of C/D-ring fusion has not yet been investigated in this respect. In this paper the authors wish to report the change in steroid number, due to the alteration of the usual C/D-*trans* fusion to 13α- and 14β-ring systems, for each of 10 pairs of epimers.

Materials

13α- and 14β-Androstane derivatives were prepared by the methods previously reported by the authors[3-8] and the usual C/D-*trans* steroids were obtained by the known procedures.

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 (3 m × 3 mm I. D.). The column was packed with 1.5 % SE-30 on a support of Chromosorb W (60–80 mesh). The detector and flash heater were kept at 250°, while the column was at 240°. Nitrogen was used as carrier gas at a flow rate of 75 ml/min.

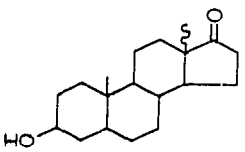
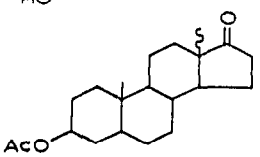
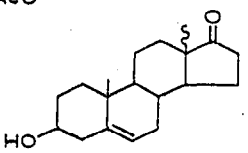
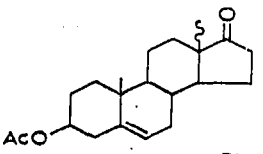
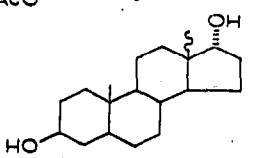
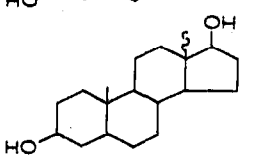
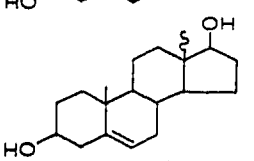
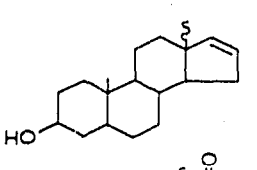
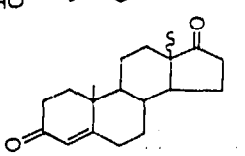
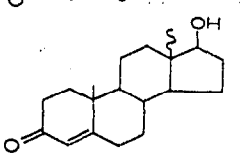
The relative retention time of each compound was measured using cholestane as a reference compound. According to the definition proposed by VANDENHEUVEL AND HORNING[3], a plot of log relative retention time against steroid number was made, whereby the values of androstane and cholestane were taken as 19 and 27, respectively.

Results and discussion

Gas chromatography of each of 10 pairs of C-13- and C-14-epimeric androstanes was carried out with a SE-30 column and the relative retention times were determined. The steroid numbers were obtained graphically from these observed values and the steroid number difference between each pair of epimers was calculated, respectively. As can be seen in Table I, the values due to the difference in C/D-ring fusion are almost constant with a mean of —0.6, and all the 13α-androstane derivatives are eluted before the corresponding C-13-isomers. On the other hand 14β-androstanes exhibit somewhat shorter or almost equal retention times compared with those of the corresponding C-14-epimers, and the steroid number contribution for 14β/14α is found to be —0.1 (see Table II). It is of particular interest that of the two C/D-*cis* steroids only a

TABLE I

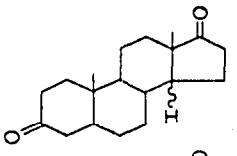
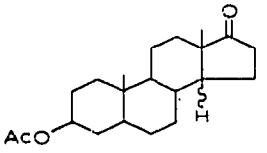
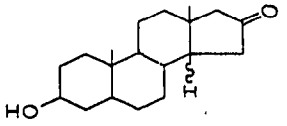
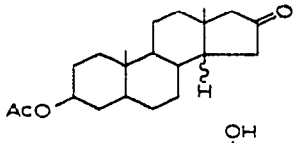
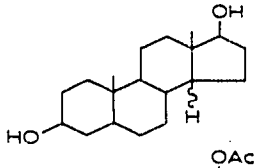
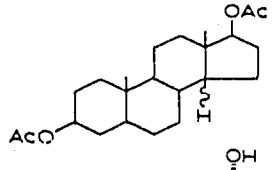
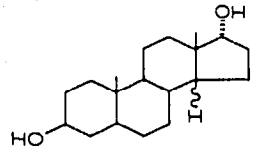
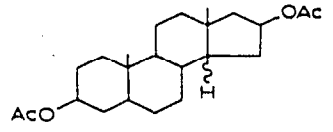
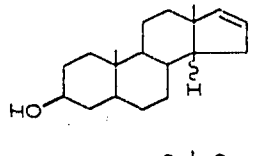
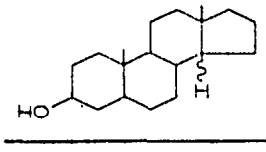
STEROID NUMBERS OF C-13-EPIMERIC ANDROSTANE DERIVATIVES

Compound structure	*Configuration of C-13-CH_3*	*RRT**	*SN**	*ΔSN**
	α	0.358	23.0	—0.6
	β	0.424	23.6	
	α	0.510	24.4	—0.6
	β	0.606	25.0	
	α	0.348	22.9	—0.6
	β	0.414	23.5	
	α	0.419	23.6	—0.6
	β	0.484	24.2	
	α	0.410	23.4	—0.5
	β	0.444	23.9	
	α	0.383	23.2	—0.6
	β	0.438	23.8	
	α	0.378	23.2	—0.6
	β	0.434	23.8	
	α	0.202	20.7	—0.6
	β	0.227	21.3	
	α	0.444	23.9	—0.6
	β	0.530	24.5	
	α	0.494	24.2	—0.6
	β	0.575	24.8	

* RRT = Relative retention time (cholestane 19.8 min); SN = steroid number (androstane 2.6 min); ΔSN = steroid number contribution for C/D-*cis*.

TABLE I

STEROID NUMBERS OF C-14-EPIMERIC ANDROSTANE DERIVATIVES

Compound structure	*Configuration of C-14-H*	*RRT**	*SN**	*ΔSN**
	α	0.459	24.0	—0.1
	β	0.454	23.9	
	α	0.606	25.0	—0.1
	β	0.590	24.9	
	α	0.444	23.9	—0.1
	β	0.438	23.8	
	α	0.611	25.2	—0.1
	β	0.609	25.1	
	α	0.438	23.8	0.0
	β	0.438	23.8	
	α	0.868	26.5	—0.1
	β	0.863	26.4	
	α	0.444	23.9	0.0
	β	0.444	23.9	
	α	0.878	26.6	—0.1
	β	0.873	26.5	
	α	0.227	21.3	—0.1
	β	0.217	21.2	
	α	0.237	21.4	—0.1
	β	0.232	21.3	

* RRT = Relative retention time (cholestane 19.8 min); SN = steroid number (androstane 2.6 min); ΔSN = steroid number contribution for C/D-*cis*.

set of C-13-epimers can be distinctly separated from each other on the nonselective phase, although no plausible explanation is at present available for this chromatographic behavior. In addition it was found interesting that there is a relatively high degree of constancy of the observed values in both series. The contribution values for C/D-*cis* ring fusion thus established may be helpful to discriminate the structural difference in the steroid skeleton by means of gas chromatography.

Acknowledgements

The authors thank Miss HARUKO IKEDA for technical assistance. The present work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education (Japan), which is gratefully acknowledged.

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Received July 31st, 1967

J. Chromatog., 31 (1967) 535–538

Conversion of chlorpromazine sulfoxide to chlorpromazine by use of metals in acid solution

Previous work from this laboratory dealt with the conversion of some phenothiazine derivatives to the corresponding sulfoxides[1]. For example chlorpromazine sulfoxide[2], one of the major metabolites of chlorpromazine is the resulting product of the oxidation of the sulphur in the phenothiazine nucleus.

In routine work in the forensic laboratory the sulfoxides are normally extracted from the body tissues and from urine. Due to the similarity of the ultraviolet spectra of the various sulfoxides, difficulties are always encountered in trying to establish the identity of any particular sulfoxide. Because of this, work was commenced to study the conversion of any particular sulfoxide to the original parent phenothiazine.

Materials

Samples of chemically pure chlorpromazine and of chemically pure chlorpromazine sulfoxide were obtained from Smith, Kline and French laboratories, and were used throughout the experimental work.