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ANALYTICAL CHEMICAL STUDIES ON STEROIDS

XXXVII. GAS CHROMATOGRAPHY OF 2,3-OXYGENATED ESTRATRIENES

TOSHIO NAMBARA, TAKEHIKO IWATA AND SEIJIRO HONMA
Pharmaceutical Institute, Tohoku University, Sendai (Japan)

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SUMMARY

Gas chromatography of the 2,3-oxygenated estratrienes was carried out employing 3% SE-30 as a stationary phase and their "steroid number (SN)" values were determined according to the proposed definition. The SN contributions of various oxygen functions at C-2 or C-3 were estimated by means of estra-1,3,5(10)-triene as a reference. The contribution of two functional groups on ring A and D was found to be in agreement with the summation of the values characteristic of each substituent. It was also demonstrated that the additivity rule is applicable to the 2,3-oxygenated estratrienes, when the SN contributions of the vicinal functional groups on ring A are regarded as a set.

INTRODUCTION

Several articles dealing with the correlation between the structures of steroids and their retention times in gas chromatography have already been reported. In 1962, VANDENHEUVEL AND HORNING introduced the concept of "steroid number (SN)", which is of use for characterization of the steroids in gas chromatography[1]. The SN contributions of representative functional groups have been determined with the neutral steroids, but not fully with the aromatic steroids. In connection with the biochemical problems concerning catechol estrogens it appeared to be of interest to us to examine the effect of various substituents on the SN with respect to the estratriene nucleus. The present paper describes the gas chromatographic behavior of the estratrienes having an oxygen function at C-2 and/or C-3.

EXPERIMENTAL

Materials

All the estratriene derivatives employed in this work were synthesized by established methods in this laboratory[2,3].

Preparation of derivatives

Trimethylsilyl (TMS) derivatives were prepared by treatment with hexamethyldisilazane and trimethylchlorosilane in pyridine in the manner described by SWEELEY *et al.*[4]. After evaporation of the solvent the residue was extracted with *n*-hexane, centrifuged and the supernatant was used. Trifluoroacetates (TFA) were prepared with trifluoroacetic anhydride and pyridine in tetrahydrofuran according to the procedure of VANDENHEUVEL *et al.*[5]. After evaporation of the solvent the residue was dissolved in tetrahydrofuran and injected into the gas chromatograph.

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 3% 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 218°. Nitrogen was used as the carrier gas at a flow rate of 55 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 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 was performed with 62 estratriene derivatives and their relative retention times (RRT) and SN were determined. As listed in Table I SN

TABLE I

RELATIVE RETENTION TIMES AND STEROID NUMBERS OF MONOSUBSTITUTED ESTRATIENES

Compound functional group	*RRT*	*SN*	*SN contribution of functional group*
Estra-1,3,5(10)-triene	0.13	19.0	
17-Oxo	0.23	21.3	2.3
16-Oxo	0.22	21.2	2.2
17β-OH	0.24	21.4	2.4
16β-OH	0.23	21.3	2.3
17β-OAc	0.32	22.6	3.6
17β-OTMS	0.28	22.0	3.0
17β-OTFA	0.18	20.4	1.4
3-OH	0.28	22.0	3.0
2-OH	0.28	22.0	3.0
3-OCH_3	0.25	21.6	2.6
2-OCH_3	0.23	21.3	2.3
3-OAc	0.38	23.2	4.2
2-OAc	0.34	22.8	3.8
3-OTMS	0.31	22.4	3.4
2-OTMS	0.28	22.0	3.0
3-OTFA	0.19	20.5	1.5
2-OTFA	0.17	20.1	1.1
Cholestane	1.00 (14.0 min)		
Androstane	0.13 (1.8 min)		

TABLE II

RELATIVE RETENTION TIMES AND STEROID NUMBERS OF DISUBSTITUTED ESTRATRIENES

Compound functional group	*RRT*	*SN*		*ΔSN observed*
		Expected[a]	*Observed*	
17-Oxo				
3-OH	0.51	24.3	24.4	0
2-OH	0.51	24.3	24.4	
3-OAc	0.65	25.5	25.3	0.3
2-OAc	0.60	25.1	25.0	
3-OTMS	0.55	24.7	24.7	0.5
2-OTMS	0.48	24.3	24.2	
3-OTFA	0.33	22.8	22.7	0.4
2-OTFA	0.30	22.4	22.3	
17β-OH				
3-OH	0.55	24.4	24.7	0
2-OH	0.55	24.4	24.7	
3-OCH_3	0.47	24.0	24.1	0.3
2-OCH_3	0.44	23.7	23.8	
17β-OAc				
3-OH	0.74	25.6	25.8	0
2-OH	0.74	25.6	25.8	
3-OCH_3	0.65	25.2	25.3	0.3
2-OCH_3	0.60	24.9	25.0	
3-OAc	0.99	26.8	27.0	0.3
2-OAc	0.93	26.4	26.7	
3-OTMS	0.81	26.0	26.2	0.4
2-OTMS	0.73	25.6	25.8	
3-OTFA	0.46	24.1	24.0	0.3
2-OTFA	0.43	23.7	23.7	
17β-OTMS				
3-OCH_3	0.57	24.6	24.8	0.2
2-OCH_3	0.54	24.3	24.6	
3-OTMS	0.72	25.4	25.7	0.5
2-OTMS	0.63	25.0	25.2	
17β-OTFA				
3-OCH_3	0.36	23.0	23.0	0.2
2-OCH_3	0.34	22.7	22.8	
3-OTFA	0.28	21.9	22.0	0.3
2-OTFA	0.26	21.5	21.7	
Cholestane	1.00 (14.0 min)			
Androstane	0.13 (1.8 min)			

[a] The values used in these calculations were obtained from Table I.

contributions of the functional groups at C-17 or C-16 were estimated with the monosubstituted compounds by subtracting the value of estra-1,3,5(10)-triene, respectively. Some of these functional group values are in good accordance with those which have already been reported for the saturated steroids[1]. This finding is of particular interest in suggesting that a structural alteration far removed from the site of the functional group exerts no significant influence and the angular distortion due to the distant part of a molecule may be of subtle nature. In addition, the SN contribution of an aromatic ring A was found to be 1.0.

The SN contributions of the oxygen functions such as hydroxyl, methoxyl, acetoxyl, trimethylsilyloxyl and trifluoroacetoxyl groups at C-2 or C-3 were deter-

mined in a similar fashion employing estra-1,3,5(10)-triene as a reference. Table I also contains SN and contribution values of these functional groups. With respect to the free hydroxylic compounds no difference can be seen between the two positional isomers. However, it seems likely that a more distinct resolution could be attained, when a sufficiently bulky group is introduced to accentuate the existing difference. It is also apparent from Table II that the contribution values of the two functional groups on ring A and D are in agreement with the summation of the values characteristic of each substituent. The additive relationship will be of great use for characterization of these oxygenated steroids, which include the important estrogen metabolites. The constancy of SN difference (Δ SN) between a pair of positional isomers is evidently observed irrespective of the nature of the C-17 substituent.

With the 2,3-dioxygenated compounds the experimental SN values are not necessarily identical with the summation of values related to the steroid skeleton and the functional groups (see Table III). BROOKS *et al.* examined the effect of the functional groups on the gas chromatographic behavior of the estratrienes, including several 2,3-oxygenated derivatives, and in consequence observed that the adjacent functional groups modify the retention effects of each other[6]. The marked deviation from the expected value may be ascribable to the intramolecular interaction of the vicinal substituents on ring A. So far as examined, however, the SN contribution of both substituents on ring A appears to be almost constant. This finding implies that the SN contribution of the adjacent functional groups can be regarded as a set, and

TABLE III

RELATIVE RETENTION TIMES AND STEROID NUMBERS OF 2,3-OXYGENATED ESTRATRIENES[a]

Compound functional group			*RRT*	*SN*		*SN contribution of functional groups at C-2 and C-3*
C-2	*C-3*	*C-17*		*Expected*[a]	*Observed*	
OH	OCH_3	β-OH	0.79	27.0	26.1	4.7
		Oxo	0.75	26.9	25.9	4.6
OCH_3	OH	β-OH	0.74	26.7	25.8	4.4
		Oxo	0.70	26.6	25.6	4.3
OCH_3	OTMS	β-OTMS	1.00	27.7	27.0	5.0
		Oxo	0.79	27.0	26.1	4.8
OTMS	OCH_3	β-OTMS	0.98	27.6	26.9	4.9
		Oxo	0.75	26.9	25.9	4.6
OTFA	OCH_3	β-OTFA	0.44	24.1	23.8	3.4
		Oxo	0.50	25.0	24.3	3.0
OCH_3	OTFA	β-OTFA	0.43	24.2	23.7	3.3
		Oxo	0.50	25.1	24.3	3.0
OCH_3	OAc	β-OAc	1.44	29.1	28.4	5.8
OAc	OCH_3	β-OAc	1.48	29.0	28.5	5.9
Cholestane			1.00 (14.0 min)			
Androstane			0.13 (1.8 min)			

[a] The values used in these calculations were obtained from Table I.

that the additivity rule is also applicable for the 2,3-oxygenated estratrienes.

It is hoped that the present results may help to identify metabolites derived from the modified steroids as well as the naturally occurring estrogens.

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