CHROM. 6062

CORRELATION BETWEEN THE CHEMICAL STRUCTURE AND RETENTION BEHAVIOUR OF C_{19}-STEROIDS

T. FEHÉR AND L. BODROGI
First Department of Medicine, Semmelweis University Medical School, Budapest (Hungary)
(Received March 10th, 1972)

SUMMARY

Structure–chromatography relationships were studied by the determination of steroid-number values of hydroxyketo-, diketo-, hydroxydiketo- and triketoandrostanes and of their alkyl derivatives, and by measuring the group retention factors for the introduction or change of functional groups. In addition, the calculation of "group-number" values is proposed for the elucidation of chemical structure by gas chromatography.

INTRODUCTION

Although gas chromatography is a method of separation, it can also be used for the structural analysis of steroids with unknown functional group(s). In the elucidation of structure, the determination of steroid-number values according to VANDENHEUVEL AND HORNING[1], a parameter related to two reference substances, and the group retention factor analysis proposed by CLAYTON[2] and KNIGHTS AND THOMAS[3], can be used. The correlation found by MARTIN AND SYNGE[4] between the partition coefficient and functional groups of a compound, and the ΔR_M approach introduced by BATE-SMITH AND WESTALL[5] [$R_M = \log(1/R_F - 1)$] and applied to steroids by BUSH[6] substantiate the group retention factor concept.

The purpose of this communication is to present results on retention behaviour, steroid-number (SN) values and group retention factors (ΔR_M) of some C_{19}-steroids. In addition, for a more accurate analysis of structure–chromatography relationships, the introduction of the "group-number" (ΔSN) concept is proposed.

METHODS

Instrumental

A Pye-Unicam Series 104 gas–liquid chromatograph with a flame ionisation detector was used. Glass columns 7 ft. long by 4 mm I.D. were packed with 3 % SE-30 non-selective or 3 % QF-1 selective phase, both on Diatomite CQ, and operated isothermally at 220°. The carrier gas was nitrogen at a flow-rate of 60 ml/min. The detector was maintained at 260° and with an attenuation setting for analysis producing an f.s.d. for 10^{-10} A. The samples were injected in the liquid phase directly on

TABLE I

RELATIVE RETENTION TIME (RR_T) AND STEROID-NUMBER (SN) VALUES OF C_{19}-STEROIDS

No.	Compound	OH and/or oxo				Acetate			
		SE-30		QF-1		SE-30		QF-1	
		RR_T	SN	RR_T	SN	RR_T	SN	RR_T	SN
1	5α-Androstane	0.11	19.0	0.19	19.0				
2	5α-Cholestane	1.00	27.00	1.00	27.0				
3	3α-Hydroxy-5β-androstan-17-one	0.36	23.1	2.17	30.6			3.28	32.6
4	3α-Hydroxy-5α-androstan-17-one	0.39	23.5	2.07	30.4	0.52	24.5	3.15	32.4
5	3β-Hydroxy-5α-androstan-17-one	0.41	23.7	2.32	31.0				
6	3β-Hydroxy-androst-5-en-17-one	0.39	23.5	2.04	30.4	0.56	24.9		
7	17β-Hydroxy-5α-androstan-3-one	0.43	23.9	2.65	31.7	0.62	25.4	4.57	34.2
8	17β-Hydroxy-19-norandrost-4-en-3-one	0.49	24.1	3.57	33.1	0.61	25.4	5.65	35.3
9	17α-Methyl-17β-hydroxy-19-norandrost-4-en-3-one	0.50	24.5	3.75	33.3				
10	17α-Ethynyl-17β-hydroxy-19-norandrost-4-en-3-one	0.53	24.7	3.39	32.7				
11	17β-Hydroxy-androst-4-en-3-one	0.54	24.8	4.34	34.0	0.75	26.0		
12	17α-Methyl-17β-hydroxy-androst-4-en-3-one	0.60	25.2	4.38	34.0				
13	17β-Hydroxy-5β-androstan-3-one					0.53	24.7	4.26	33.9
14	17α-Methyl-17β-hydroxy-androsta-1,4-dien-3-one	0.64	25.4	5.46	35.1	0.85	26.5	6.22	35.6
15	Androst-4-en-3,17-dione	0.51	24.5	6.90	36.2				
16	Androsta-1,4-dien-3,17-dione	0.55	24.9						
17	11β-Hydroxy-androst-4-en-3,17-dione	0.86	26.4						
18	Androst-4-en-3,11,17-trione	0.61	25.3						

to the top of the column. The detector response was recorded on a Honeywell Electronic 194 Lab Chart Recorder at 0.1 in./min.

Steroids were chromatographed as free alcohols or acetates. The acetylation was carried out according to the general method of BUSH[6].

Parameters of separation and structure analysis

The relative retention time (RR_T) expresses the uncorrected retention time (R_T) of the steroid relative to 5α-cholestane. The steroid-number (SN) value was calculated according to VANDENHEUVEL AND HORNING[1] using 5α-cholestane (R_T = 1.0 on a log scale; SN = 27.0) and 5α-androstane (SN = 19.0) standards for measurements.

KNIGHTS AND THOMAS[3] demonstrated that the relative retention time of a steroid could be expressed in the form

$$\log RR_T = \Sigma \Delta R_M + \log RR_{Tn}$$

where ΔR_M, the group retention factor, is a change in log RR_T resulting from the introduction or chemical alteration of a functional group and RR_{Tn} is the relative retention time of the steroid nucleus. In order to conform to existing nomenclature[6,7], ΔR_{Mg} is a term of introduction *into*, and ΔR_{Mr} of a change of the functional group *on*, the nucleus. In the present work:

$$\Delta R_{Mg} = \Delta \log RR_T$$

where g = 2H, 11β-OH, 11-keto, 10-methyl or 17α-ethynyl,
and

$$\Delta R_{Mr} = \Delta \log RR_T$$

where r = OH into OAc conversion at the C-3 or C-17 position.

The precision of ΔR_M measurements is determined by that of the RR_T calculation. It depends on the temperature, flow-rate and amount of liquid phase. For this reason, the ΔR_M approach appears not to be useful in inter-laboratory comparisons of data.

It is suggested that a function calculated as a four-digit value by using two reference substances will characterize structure–chromatography relationships better. The SN value fulfils this requirement and, because of its logarithmic nature (similar to R_M), it is additive. Therefore, a change in SN will be a better index of the introduction or chemical alteration of a functional group and may be expressed in a manner analogous to the group retention factor as follows:

$$SN = \Sigma \Delta SN + SN_n$$

where SN_n is the steroid number of the nucleus and ΔSN is the "group-number" of a substituent. The symbol ΔSN_g is used to denote addition and ΔSN_r substitution of a group.

RESULTS AND DISCUSSION

The study was carried out on hydroxyketo-, diketo-, hydroxydiketo-, and triketoandrostanes and on their 10- and 17α-alkyl derivatives. Table I shows the

TABLE II

THE GROUP RETENTION FACTOR (ΔR_{Mg}) AND GROUP-NUMBER (ΔSN_g) CONTRIBUTIONS OF ADDITIONS TO C_{19}-STEROIDS

Addition	*Model*[a]	ΔR_{Mg}		ΔSN_g	
		SE-30	*QF-1*	*SE-30*	*QF-1*
2H (Δ^5)	6	+0.026	+0.056	+0.2	+0.6
2H (Δ^4)	11	+0.071	−0.212	−0.9	−2.3
2H (Δ^4)	16	+0.034		−0.4	
2H (11-keto)	18	−0.150		+1.1	
2H (17-keto)	11	−0.022	−0.203	+0.3	−1.8
11β-OH	15	−0.224		+1.9	
11-Keto	15	−0.074		+0.8	
17α-Ethynyl	8	−0.074	−0.022	+0.6	−0.4
17α-Methyl	8	−0.043	−0.022	+0.4	+0.2
17α-Methyl	11	−0.046	+0.006	+0.4	±0.0
10-Methyl	8	−0.080	+0.084	+0.7	+0.9

[a] For No. of model see first column of Table I.

TABLE III

THE GROUP RETENTION FACTOR (ΔR_{Mr}) AND GROUP-NUMBER (ΔSN_r) CONTRIBUTIONS OF SUBSTITUTIONS ON C_{19}-STEROIDS

OH into OAc conversion	*Model*[a]	ΔR_{Mr}		ΔSN_r	
		SE-30	*QF-1*	*SE-30*	*QF-1*
5β(H),3α-OH → 3α-OAc	3		+0.180		+2.0
5α(H),3α-OH → 3α-OAc	4	−0.121	+0.183	+1.0	+2.0
Δ^5-3β-OH → 3β-OAc	5	−0.150		+1.4	
5α(H),17β-OH → 17β-OAc	7	−0.152	+0.237	+1.5	+2.5
Δ^4-17β-OH → 17β-OAc	8	−0.138	+0.199	+1.3	+2.2
	11	−0.143		+1.2	
$\Delta^{1,4}$-17β-OH → 17β-OAc	14	−0.122	+0.057	+1.1	+0.5

[a] For No. of model, see first column of Table I.

relative retention time and steroid-number values. Steroids containing a free hydroxyl or ketone group exhibited an SN value between 23 and 27 on SE-30 non-selective phase, and between 30 and 36 on QF-1 selective phase. Values of SN for the acetates exceeded those of the parent hydroxysteroids.

The Δ_{Mg} and ΔSN_g values for the addition of 2H, 11β-OH, 11-oxo, 10-methyl or 17α-alkyl groups are presented in Table II. According to the observations, the ΔR_{Mg} and ΔSN_g values for reduction of double-bond and ketone groups were determined not only by the position of the function under study but also by the other constituents of the molecule and by the liquid phase. The introduction of an oxygen function or an alkyl substituent resulted in a negative ΔR_{Mg} and positive ΔSN_g contribution, with some exceptions on QF-1.

The ΔR_{Mr} and ΔSN_r values for the acetylation of C-3 or C-17 hydroxyl groups are presented in Table III. The selectivity of the two phases was found to be approximately the same for C-3 and C-17 acetylation. Although the elution patterns showed

a greater affinity of both liquid phases for acetates than for the parent hydroxysteroids, the ΔR_{Mr} contribution to this change was found to be negative on SE-30 and positive on QF-1. However, the ΔSN_r values for acetylation were invariably positive and covered a greater range of values than did the ΔR_{Mr} values.

The ΔR_M contributions of addition or change of a substituent observed by us were similar to those reported by other workers[8-10] for functional groups of androstanes and pregnanes. From the patterns shown in Tables II and III, it can be seen that the ΔR_{Mg} and ΔR_{Mr} values are lower by one order of magnitude than the ΔSN_g and ΔSN_r values.

Values for the group retention factor between 0.05 and 0.01 can be measured with a precision that makes the analysis uncertain. The group-number values proposed in this paper do not approach the order of the methodological error. For this reason, changes in the structure of a steroid can be more adequately characterized if simultaneous measurements of group retention factors and group-numbers are carried out by the gas chromatographic approach.

REFERENCES

1 W. J. A. VANDENHEUVEL AND E. C. HORNING, *Biochem. Biophys. Res. Commun.*, 3 (1960) 356.
2 R. B. CLAYTON, *Biochemistry*, 1 (1962) 357.
3 B. A. KNIGHTS AND G. H. THOMAS, *Anal. Chem.*, 34 (1962) 1046.
4 A. J. P. MARTIN AND R. L. M. SYNGE, *Biochem. J.*, 35 (1941) 1354.
5 E. C. BATE-SMITH AND R. G. WESTALL, *Biochim. Biophys. Acta*, 4 (1950) 427.
6 I. E. BUSH, *The Chromatography of Steroids*, Pergamon Press, Oxford, 1961, p. 358.
7 T. FEHÉR, *J. Chromatogr.*, 19 (1965) 551.
8 J. CHAMBERLAIN, B. A. KNIGHTS AND G. H. THOMAS, *J. Endocrinol.*, 26 (1963) 367.
9 R. J. HEITZMAN AND G. H. THOMAS, *J. Endocrinol.*, 33 (1965) 455.
10 G. H. THOMAS, in J. K. GRANT (Editor), *The Gas–Liquid Chromatography of Steroids*, Cambridge University Press, Cambridge, 1967, p. 129.