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131. Shoji Hara, Tadashi Watabe, Yoshimasa Ike,*1 and Nobuo Ikekawa*2 : Systematic Analysis of Steroids. Ⅷ.*3 Molecular Structure of C_{19} and C_{18} Steroid Derivatives and Retention Times in Gas Chromatography.*4

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Systematic analyses were carried out by gas chromatography on about forty C_{19} and C_{18} synthetic steroids. A correlation between their retention time and molecular structure was examined and parameters were calculated for their functional groups.

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One of the most important uses of gas chromatography is the identification and determination of a substance from its retention time, but this is effective only in the case where a reference standard is available. Retention time of a given compound is usually presumed from experience, based on a large amount of data available for hitherto known, allied compounds. Clayton,[1] Knights, Thomas,[2] VandenHeuvel, Horning[3] and others[4] have already found additivity in the retention time in natural sterols and other steroids, and they have examined correlations between the retention time and structure. However, the number of different kind of parameters calculated for substituents is not large.

Also, the effects of functional groups of steroids on their retention time were discussed briefly in a previous paper.[5] In the present series, retention time of C_{19} and C_{18} series of steroids in gas chromatography was measured systematically, and the correlation between the retention time and the molecular structure was examined in order to use this information for aid in separation, purification, and structural determination of metabolic products of synthetic steroid hormones *in vivo*.

Experimental

A glass tube of 185 cm. in length and 4 mm. in internal diameter was used for the column. The liquid phase was 1.0% SE-30 and 1.5% QF-1, and the supports were Anakrom of 80~100 mesh and Chromosorb W of 60~80 mesh. The supports were treated first with acid and then with *ca.* 3% toluene solution of dimethyldichlorosilane. The apparatus used was Shimadzu GC-1C gas chromatograph provided with a hydrogen flame ionization detector. The column temperatures were 195° and 215° for SE-30 and QF-1, respectively. Retention time of cholestane used as reference standard was 17.45 min. on SE-30 and 3.50 min. on QF-1. Nitrogen was used as the carrier gas. The samples were made into *ca.* 1.0% solution in acetone and injected directly by the "on-column" technique.

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TABLE I. Relative Retention Time of C_{19} and C_{18} Steroids

No.	Steroid compound	1.0% SE-30		1.5% QF-1	
		RRT[a]	Log (RRT × 100)	RRT[a]	Log (RRT × 100)
1	Testosterone	0.51	1.71	4.12	2.61
2	Testosterone acetate	0.73	1.86	6.60	2.82
3	Testosterone propionate	1.05	2.02	7.80	2.89
4	Testosterone butyrate	1.38	2.14	9.66	2.98
5	17α-Methyl-testosterone	0.55	1.74	4.25	2.63
6	17α-Ethinyl-testosterone	0.65	1.81	3.91	2.59
7	5α-Androstan-3-one	0.36	1.56	4.21	2.63
8	17β-Hydroxy-5α-androstan-3-one	0.41	1.61	2.67	2.43
9	17β-Hydroxy-5α-androstan-3-one acetate	0.58	1.76	4.72	2.67
10	17β-Hydroxy-5α-androstan-3-one propionate	0.78	1.89	5.12	2.71
11	17β-Hydroxy-5α-androstan-3-one butyrate	0.82	1.91	6.30	2.80
12	17β-Hydroxy-17α-methyl-5α-androstan-3-one	0.45	1.65	2.82	2.45
13	5β-Androstan-3-one	0.35	1.54	4.51	2.65
14	17β-Hydroxy-5β-androstan-3-one	0.39	1.59	2.63	2.42
15	17β-Hydroxy-5β-androstan-3-one acetate	0.54	1.73	4.11	2.61
16	17β-Hydroxy-5β-androstan-3-one propionate	0.73	1.86	4.82	2.68
17	17β-Hydroxy-5β-androstan-3-one butyrate	1.12	2.05	5.93	2.77
18	17β-Hydroxy-17α-methyl-5β-androstan-3-one	0.32	1.51	2.58	2.41
19	3β-Hydroxy-5α-androstan-17-one	0.35	1.54	2.03	2.31
20	3β-Hydroxy-5α-androstan-17-one acetate	0.55	1.74	3.11	2.49
21	3α-Hydroxy-5α-androstan-17-one	0.36	1.56	2.04	2.31
22	3α-Hydroxy-5α-androstan-17-one acetate	0.47	1.67	3.57	2.55
23	3α-Hydroxy-5β-androstan-17-one	0.34	1.53	2.13	2.33
24	3α-Hydroxy-5β-androstan-17-one acetate	0.48	1.68	3.71	2.57
25	3β-Hydroxy-5β-androstan-17-one acetate	0.46	1.66	3.18	2.50
26	3β-Hydroxy-androst-5-en-17-one	0.35	1.54	2.11	2.32
27	3β-Hydroxy-androst-5-en-17-one acetate	0.54	1.73	3.30	2.52
28	Androst-4-ene-3,17-dione	0.47	1.67	6.60	2.82
29	5α-Androstane-3,17-dione	0.42	1.62	4.51	2.65
30	5β-Androstane-3,17-dione	0.38	1.58	4.21	2.62
31	19-Nortestosterone	0.42	1.62	3.40	2.53
32	19-Nortestosterone acetate	0.59	1.77	5.51	2.74
33	19-Nortestosterone propionate	1.15	2.06		
34	19-Nortestosterone butyrate	1.25	2.10	8.20	2.91
35	17α-Ethinyl-19-nortestosterone	0.54	1.73	3.31	2.52
36	A-Nortestosterone	0.31	1.49	2.93	2.47
37	A-Nortestosterone acetate	0.44	1.64	4.78	2.68
38	17β-Hydroxy-A-nor-5α-androstan-2-one	0.25	1.40	1.61	2.21
39	17β-Hydroxy-A-nor-5α-androstan-2-one acetate	0.36	1.56	2.66	2.42
40	17β-Hydroxy-17α-methyl-A-nor-5α-androstan-2-one	0.26	1.42	1.70	2.23
41	17β-Hydroxy-A-nor-5β-androstan-2-one	0.24	1.38	1.70	2.23
42	17β-Hydroxy-A-nor-5β-androstan-2-one acetate	0.35	1.54	2.54	2.40
43	Estrone	0.45	1.66		
44	Estradiol	0.48	1.68		
45	Estradiol 17-butyrate	1.81	2.26		
46	17α-Ethinyl-estradiol	0.61	1.79		
47	Estradiol 3-methyl ether	0.52	1.72		

a) Relative retention time (cholestane=1 : 17.45 min. on SE-30, 3.50 min. on QF-1)

Table II. ⊿Log RRT of Converted Functional Group of Steroid

Converted functional group	No.	Root compound (abbreviation)[a]	1.0% SE-30 ⊿Log (RRT × 100)	1.5% QF-1 ⊿Log (RRT × 100)
17β-OH to 17β-OAc	2～1	A^4-17β-ol-3-one	0.15	0.21
	9～8	αA-17β-ol-3-one	0.15	0.24
	15～14	βA-17β-ol-3-one	0.14	0.19
	32～31	19-nor-A^4-17β-ol-3-one	0.15	0.21
	37～36	A-nor-A^3-17β-ol-2-one	0.15	0.21
	39～38	A-nor-αA-17β-ol-2-one	0.16	0.21
	42～41	A-nor-βA-17β-ol-2-one	0.16	0.17
17β-OH to 17β-OPr	3～1	A^4-17β-ol-3-one	0.31	0.28
	10～8	αA-17β-ol-3-one	0.28	0.28
	16～14	βA-17β-ol-3-one	0.27	0.26
	33～31	19-nor-A^4-17β-ol-3-one	0.44	
17β-OH to 17β-OBu	4～1	A^4-17β-ol-3-one	0.43	0.37
	11～8	αA-17β-ol-3-one	0.30	0.37
	17～14	βA-17β-ol-3-one	0.46	0.35
	34～31	19-nor-A^4-17β-ol-3-one	0.48	0.38
	45～44	$E^{1,3,5(10)}$-3,17β-ol	0.58	
3β-OH (5α-H) to 3β-OAc	20～19	αA-3β-ol-17-one	0.20	0.18
3α-OH (5β-H) to 3α-OAc	24～23	βA-3α-ol-17-one	0.15	0.24
3α-OH (5α-H) to 3α-OAc	22～21	αA-3α-ol-17-one	0.11	0.24
3α-OH to 3-OMe	47～44	$E^{1,3,5(10)}$-3,17β-ol	0.04	
3β-OH (5α-H) to C=O	29～19	αA-3β-ol-17-one	0.08	0.34
3α-OH (5β-H) to C=O	30～23	βA-3α-ol-17-one	0.05	0.29
3α-OH (5α-H) to C=O	29～21	αA-3α-ol-17-one	0.06	0.34
17β-OH to C=O	28～1	A^4-17β-ol-3-one	−0.04	0.21
	29～8	αA-17β-ol-3-one	0.01	0.22
	30～14	βA-17β-ol-3-one	−0.01	0.20
Δ^4 to 4,5α-H	8～1	A^4-17β-ol-3-one	−0.10	−0.18
	9～2	17β-AcO-A^4-3-one	−0.10	−0.15
	10～3	17β-PrO-A^4-3-one	−0.13	−0.18
	11～4	17β-BuO-A^4-3-one		−0.18
	12～5	17α-Me-A^4-17β-ol-3-one	−0.09	−0.18
Δ^4 to 4,5β-H	14～1	A^4-17β-ol-3-one	−0.12	−0.19
	15～2	17β-AcO-A^4-3-one	−0.13	−0.21
	16～3	17β-PrO-A^4-3-one	−0.16	−0.21
	17～4	17β-BuO-A^4-3-one	−0.09	−0.21
	18～5	17α-Me-A^4-17β-ol-3-one	−0.23	−0.22
10β-Me	1～31	19-nor-A^4-17β-ol-3-one	0.09	0.08
	2～32	17β-AcO-19-nor-A^4-3-one	0.09	0.08
	3～33	17β-PrO-19-nor-A^4-3-one	0.04	
	4～34	17β-BuO-19-nor-A^4-3-one	0.04	0.07
	6～35	17α-Ethin-19-nor-A^4-17β-ol-3-one	0.08	0.07
17α-Me	5～1	A^4-17β-ol-3-one	0.03	0.02
	12～8	αA-17β-ol-3-one	0.04	0.02
	40～38	A-nor-αA-17β-ol-2-one	0.02	0.02
17α-C≡CH	6～1	A^4-17β-ol-3-one	0.10	−0.02
	35～31	19-nor-A^4-17β-ol-3-one	0.11	−0.01
	46～44	$E^{1,3,5(10)}$-3,17β-ol	0.11	
Ring A to A-nor (A^3)	36～1	A^4-17β-ol-3-one	−0.22	−0.14
	37～2	17β-AcO-A^4-3-one	−0.22	−0.14
Ring A to A-nor (αA)	38～8	αA-17β-ol-3-one	−0.21	−0.22
	39～9	17β-AcO-αA-3-one	−0.20	−0.25
	40～12	17α-Me-αA-17β-ol-3-one	−0.23	−0.22
Ring A to A-nor (βA)	41～14	βA-17β-ol-3-one	−0.21	−0.19
	42～15	17β-AcO-βA-3-one	−0.19	−0.21

a) Abbreviation A: androstane αA: 5α-androstane βA: 5β-androstane E: estrane AcO: acetoxy PrO: propionyloxy BuO: butyryloxy

Results and Discussion

The samples used for the analyses were about 40 steroids; androgen series C_{19} compounds with fundamental skeletons of testosterone, 17β-hydroxyandrostan-3-one (5α and 5β isomers), 3α- and 3β-hydroxyandrostan-17-one (5α and 5β isomers), and 3β-hydroxyandrost-5-en-17-one, and C_{18} steroid derivatives including 19-nortestosterone, A-norandrostan-2-one, and estrone. These were analyzed simultaneously as a mixture, and the retention time of each compound was converted into the relative retention time with cholestane as the standard. Experimental values obtained in which the relative retention time agreed within a range of 0.02 were averaged and these values are listed in Table I, which also gives the logarithmic values of one hundred times the relative retention time.

A parameter was calculated for each of the functional groups from the values listed in Table I by selecting a pair of compounds having the common fundamental skeleton. The values of these parameters are given in Table II. In general, the values obtained for a given functional group are approximately the same. When the 4-ene series steroids are reduced to 5α or 5β, and when 17β-hydroxyl group is oxidized to a carbonyl group, their parameters on QF-1 are larger than on SE-30, while the parameters on SE-30 become larger than on QF-1 upon the introduction of a methyl or ethinyl group into 17α-position.

An example of the establishment of an additivity between the retention time of steroids and each parameter is as follows. Taking testosterone as the fundamental skeleton, calculation of the relative retention time of 17β-hydroxy-17α-methyl-5α-androstan-3-one in 1.0% SE-30 gives the following values, using the parameter (mean value, the following the same) for the methyl group, and parameter for reduction of 4-ene to 4,5α-H.

log(RRT×100) for testosterone	1.71
Δ log(RRT×100) for change of 17α-H to 17α-methyl	0.03
Δ log(RRT×100) for change of Δ^4 to 4,5α-H	−0.10
Calculated log(RRT×100) for 17β-hydroxy-17α-methyl-5α-androstan-3-one	1.64
Found log(RRT×100) for 17β-hydroxy-17α-methyl-5α-androstan-3-one	1.65

Similarly, the relative retention time of 17β-acetoxy-A-nor-androst-3(5)-en-2-one is calculated in the following manner, using the parameter for change of ring A to A-nor and that for acetylation of 17β-hydroxyl group.

log(RRT×100) for testosterone	1.71
Δ log(RRT×100) for change of ring A to A-nor	−0.21
Δ log(RRT×100) for change of 17β-hydroxy to 17β-acetoxy	0.15
Calculated log(RRT×100) for A-nortestosterone acetate	1.65
Found log(RRT×100) for A-nortestosterone acetate	1.64

These calculated values show good agreement with the experimental values. If a large number of parameters for functional groups are measured, these values can be utilized as a means for structural determination of synthetic steroids and their metabolic products *in vivo*. Likewise, the retention time of a compound can be more or less accurately predicted if the structure of this compound can be indicated. Such data have already been used for the structural determination and identification of the metabolic products of several kinds of synthetic steroids.[6]

6) T. Watabe, S. Yagishita, S. Hara, to be published.