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RETENTION INDICES OF STEROIDS AND THEIR GROUP INCREMENT WITH TEMPERATURE

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SUMMARY

   The steroid number (*SN*) was shown to be superior to the Kováts index for the expression of retention data for steroids because the former showed a smaller increment with temperature. *SN* values for 3-monosubstituted 5α-androstanes changed linearly with column temperature. The *SN* increments with temperature for 3-monosubstituted 5α-androstanes were almost the same as those for the 3,11,17-trisubstituted compounds. The additivity rule of the *SN* increment with temperature was valid on OV-1 and OV-17, but there were some deviations on polar XE-60 and cyclohexanedimethanol succinate stationary phases. It was characteristic that the trimethylsilyl and trifluoroacetate derivatives showed large negative increments on most columns, in spite of the small negative increment for the acetyl group.

INTRODUCTION

   It is accepted that the Kováts retention index[1] is a very suitable method for the expression of retention data. However, in general, this index varies with column temperature[1-6]. The temperature dependence of the Kováts retention index can be decreased by using a homologous basic compound as a reference compound instead of *n*-alkanes. WOODFORD AND VAN GENT[7] proposed the concept of carbon number and MIWA *et al.*[8] that of equivalent chain-length for fatty acids; VANDEN-HEUVEL and co-workers[9,10] introduced the steroid number for steroids; HEBGOOD AND HARRIS[11] applied the phenyl number to polyphenyl compounds. Nevertheless, in spite of the use of suitable reference compounds for individual classes of compounds, the indices are still not completely independent of temperature. Other approaches have been made by VANDENHEUVEL AND COURT[12] and TAKÁCS and co-workers[13,14]: the former introduced retention constants calculated from the relative retention times at two column temperatures, and the latter derived retention constants from Kováts retention indices. Although such constants are independent of temperature, they have the disadvantage that interlaboratory values may show considerable errors. It would be very convenient for the identification of steroids if a new index system could be devised to give a simple and reliable correlation of index values with column temperature. Based on the additivity rule for retention indices which has been applied to the identification of compounds[2,9,10,15,16], this paper describes the correlation of steroid number with column temperature, and investigates the additivity of functional group increments with temperature.

EXPERIMENTAL

Retention indices were determined by using a Shimadzu Model 4APTF gas chromatograph with a flame ionization detector. The columns were 1.5 m × 4 mm O.D. glass tubes packed with Gas-Chrom Q (100–120 mesh) coated with different stationary phases. The amounts of stationary phase coating the support were determined by Soxhlet extraction of the support after all analyses had been carried out. The steroid numbers (*SN*) of trisubstituted androstanes were obtained at 200°, 230°, 270° and 310° on OV-1 and OV-17 and at 235° and 250° on QF-1, XE-60 and cyclohexanedimethanol succinate (CHDMS). All of the hydroxy-steroids were of equatorial conformation. Acetate (Ac), trifluoroacetate (TFA) and trimethylsilyl ether (TMS) derivatives were prepared from the hydroxy-compounds.

RESULTS AND DISCUSSION

3-Monosubstituted 5α-androstanes

KOVÁTS retention indices (*RI*) of 3-monosubstituted 5α-androstanes on OV-1 and CHDMS are shown in Fig. 1. The *RI* values changed linearly with column temperature on both non-polar and polar columns; the $\partial RI/\partial T$ values were 1.1–1.9 and 1.9–3.7 units per degree, respectively, and the order of the changes was TFA < TMS < Ac < OH < CO. Such large changes with temperature may give rise to considerable errors in interlaboratory *RI* values. Therefore, the steroid number (*SN*) concept should be superior to the *RI* system. The relationships between *SN* and column temperature on OV-1, OV-17, QF-1, XE-60 and CHDMS are plotted in Fig. 2. On the QF-1, XE-60 and CHDMS columns, the *SN* values were lower at low loading than at high loading; on the OV-1 column, the reverse occurred. These results are caused by the non-polarity of the surface of the silanized diatomaceous support (Gas-Chrom Q).
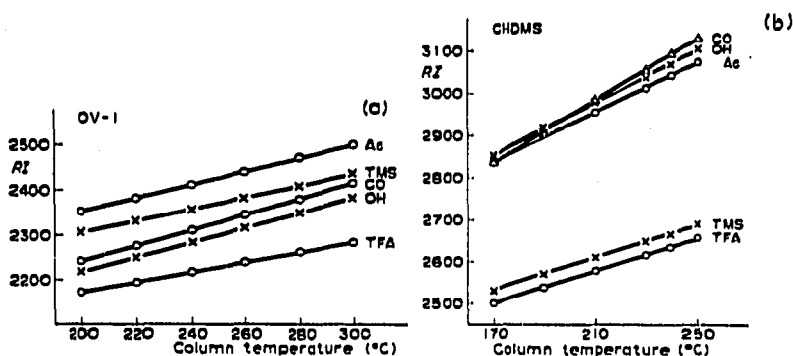

Fig. 1. Relationship between the KOVÁTS retention indices (*RI*) of 3-substituted 5α-androstanes and column temperature. (a) On OV-1; (b) on CHDMS.

All of the compounds showed a linear relationship between *SN* and column temperature on each of the five columns, *i.e.*, $SN = a + bt$. Therefore, *SN* defined at two column temperatures is independent of temperature. As seen in Table I,
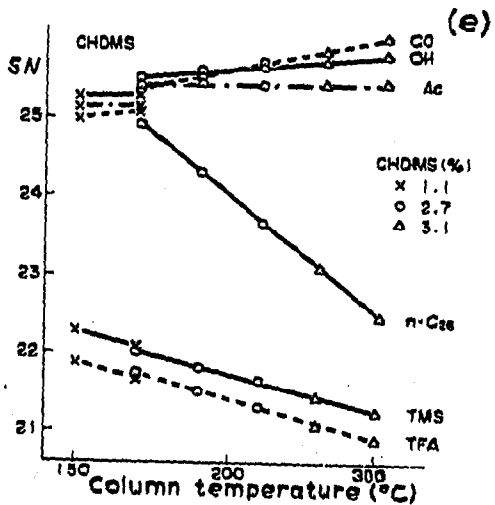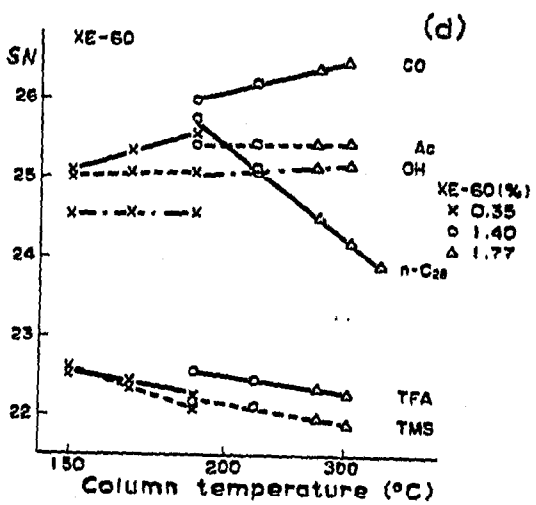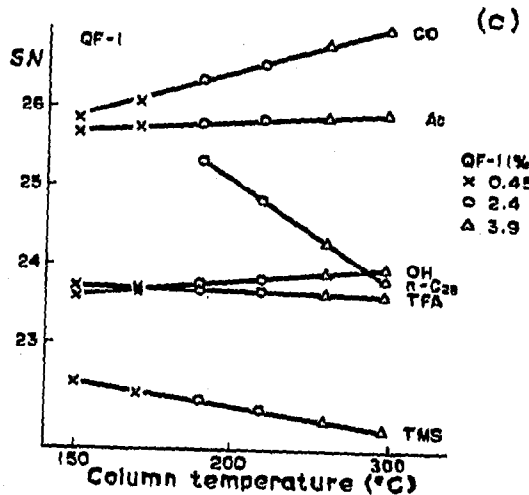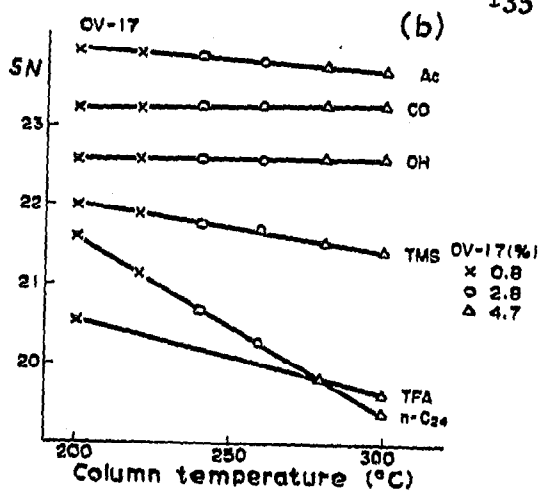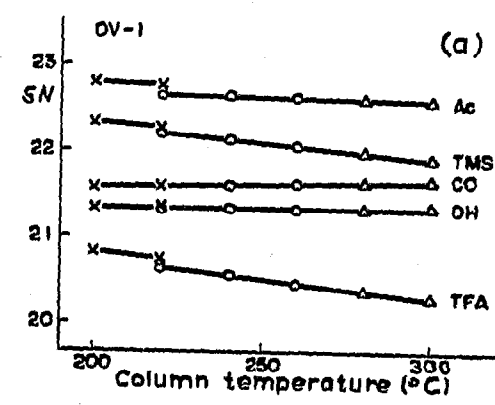
Fig. 2. Relationship between the steroid numbers (SN) of 3-substituted 5α-androstanes and column temperature. (a) On OV-1; (b) on OV-17; (c) on QF-1; (d) on XE-60; (e) on CHDMS.

TABLE I

$\partial SN/\partial T$ VALUES FOR 3-MONOSUBSTITUTED 5α-ANDROSTANES

Stationary phase	$\partial SN/\partial T \times 10^4$				
	CO	OH	Ac	TMS	TFA
OV-1	9	0	—10	—40	—47
OV-17	11	6	—24	—56	—90
QF-1	116	38	23	—58	—7
XE-60	104	36	12	—60	—58
CHDMS	78	45	10	—89	—98

$\partial SN/\partial T$ values are smaller than $\partial RI/\partial T$ values. Carbonyl and hydroxyl groups exhibited very small temperature dependences on OV-1 and OV-17; while the acetoxy group showed only a negligibly small change on all columns. The carbonyl group had characteristically very large positive values on QF-1, XE-60 and CHDMS columns, but the hydroxyl group showed moderate values. TMS and TFA groups gave high negative values on all of the stationary phases studied.

The use of TMS and TFA derivatives to lower the retention of a hydroxy-compound is recognized in analytical work. The above results suggest that the effectiveness of these derivatives for separation of the peaks could be increased by changing the column temperature, even by raising the column temperature.

3,11,17-Trisubstituted 5α-androstanes

Calculation of the SN increment with temperature for 3,11,17-trisubstituted 5α-androstanes having the same substituent at all three positions was carried out on the assumption that the contributions of the substituents at each position are the same. The substituents studied were CO, OH, Ac, TFA and TMS, and the results are given in Table II. The results were almost the same as those for the monosubstituted androstanes, proving that the above assumption is valid.

TABLE II

$\partial SN/\partial T$ VALUES FOR 3,11,17-TRISUBSTITUTED 5α-ANDROSTANES HAVING THE SAME SUBSTITUENTS

Stationary phase	$\partial SN/\partial T \times 10^4$				
	CO	OH	Ac	TMS	TFA
OV-1	17	13	—25	—32	—51
OV-17	12	14	—27	—52	—89
QF-1	47	36	—4	—53	—51
XE-60	100	64	0	—58	—36
CHDMS	67	47	22	—78	—84

Additivity of SN increments with temperature for 3,11,17-substituted 5α-androstanes

The additivity of $\partial SN/\partial T$ values was studied with 3,11,17-trisubstituted 5α-androstanes in which CO and/or OH, Ac, TFA or TMS groups were substituted. Five stationary phases, OV-1, OV-17, QF-1, XE-60 and CHDMS, were examined. The

calculation of $\partial SN/\partial T$ values for steroids with mixed substituents was carried out as follows. The difference between the observed value and the product of the number of substituents and one third of the $\partial SN/\partial T$ value for the steroid having three identical substituents was divided by the number of the substituents in the compound under consideration. The results are shown in Tables III and IV for non-polar OV-1 and OV-17, respectively. The $\partial SN/\partial T$ values for the groups are almost the same as those for the monosubstituted compounds and the additivity of $\partial SN/\partial T$ for the groups holds satisfactorily, even for TFA or TMS derivatives, which have very high volatilities. The calculated values for CO were almost constant.

TABLE III

$\partial SN/\partial T$ VALUES FOR 3,11,17-TRISUBSTITUTED 5α-ANDROSTANES ON OV-1

Compound No.	Substituents in 5α-androstane			SN (200°)	Found	$\partial SN/\partial T \times 10^4$				
	3-	11-	17-			CO	OH	Ac	TFA	TMS
1	CO	CO	CO	24.35	52	17				
2	OH	CO	CO	24.22	43	15	8			
3	OH	CO	OH	24.81	40	13	11			
4	OH	OH	OH	25.47	40		13			
5	Ac	CO	CO	25.77	3	14		-32		
6	Ac	CO	Ac	27.56	-21	28		-19		
7	Ac	Ac	Ac	28.78	-74			-25		
8	TFA	CO	CO	23.65	-24	14			-50	
9	TFA	CO	TFA	23.35	-84	19			-51	
10	TFA	TFA	TFA	22.26	-154				-51	
11	TMS	CO	CO	25.23	-7	13				-42
12	TMS	CO	TMS	26.83	-66	-1				-42
13	TMS	TMS	TMS	26.65	-97					-32

TABLE IV

$\partial SN/\partial T$ VALUES FOR 3,11,17-TRISUBSTITUTED 5α-ANDROSTANES ON OV-17

Compound No.	Substituents in 5α-androstane			SN (200°)	Found	$\partial SN/\partial T \times 10^4$				
	3-	11-	17-			CO	OH	Ac	TFA	TMS
1	CO	CO	CO	28.66	36	12				
2	OH	CO	CO	28.08	32	9	8			
3	OH	CO	OH	28.59	45	18	17			
4	OH	OH	OH	29.26	41		14			
5	Ac	CO	CO	29.46	-7	10		-31		
6	Ac	CO	Ac	30.82	-32	21		-22		
7	Ac	Ac	Ac	31.74	-80			-27		
8	TFA	CO	CO	26.14	-80	-10			-104	
9	TFA	CO	TFA	24.36	-186	-8			-99	
10	TFA	TFA	TFA	21.56	-266				-89	
11	TMS	CO	CO	27.44	-34	9				-58
12	TMS	CO	TMS	26.90	-83	20				-48
13	TMS	TMS	TMS	25.64	-155					-52

The results on QF-1, which has a strong affinity for the CO group, are given in Table V. Comparing the $\partial SN/\partial T$ values of identically substituted trisubstituted derivatives with those of the monosubstituted derivatives for CO and TFA compounds, the former were considerably lower than the latter. However, except for the diacetate (compound 7) and mono-TMS (compound 11) derivatives, the additivity rule is valid on non-polar stationary phases.

On XE-60, the $\partial SN/\partial T$ values for trisubstituted 5α-androstanes having identical substituents were the same as those for the monosubstituted compounds (Table VI). For the compounds having two CO groups, the calculated increments for the

TABLE V

$\partial SN/\partial T$ VALUES FOR 3,11,17-TRISUBSTITUTED 5α-ANDROSTANES ON QF-1

Compound No.	Substituents in 5α-androstane			SN (235°)	Found	$\partial SN/\partial T \times 10^4$				
	3-	11-	17-			CO	OH	Ac	TFA	TMS
1	CO	CO	CO	36.77	146	49				
2	OH	CO	CO	33.99	113	44	15			
3	OH	CO	OH	32.78	113	41	32			
4	OH	OH	OH	32.36	107		36			
5	Ac	CO	CO	35.96	113	59		15		
6	Ac	CO	Ac	36.89	−33	−25		−41		
7	Ac	Ac	Ac	37.30	−13			−4		
8	TFA	CO	CO	33.76	40	46			−58	
9	TFA	CO	TFA	32.62	−47	55			−48	
10	TFA	TFA	TFA	31.65	−153				−51	
11	TMS	CO	CO	32.14	0	27				−98
12	TMS	CO	TMS	29.68	−27	79				−38
13	TMS	TMS	TMS	25.64	−160					−53

TABLE VI

$\partial SN/\partial T$ VALUES FOR 3,11,17-TRISUBSTITUTED 5α-ANDROSTANES ON XE-60

Compound No.	Substituents in 5α-androstane			SN (235°)	Found	$\partial SN/\partial T \times 10^4$				
	3-	11-	17-			CO	OH	Ac	TFA	TMS
1	CO	CO	CO	36.83	300	100				
2	OH	CO	CO	36.89	173	55	−27			
3	OH	CO	OH	36.15	220	92	60			
4	OH	OH	OH	36.47	193		64			
5	Ac	CO	CO	35.77	200	100		0		
6	Ac	CO	Ac	36.03	60	60		−40		
7	Ac	Ac	Ac	36.12	0			0		
8	TFA	CO	CO	33.04	93	65			−107	
9	TFA	CO	TFA	30.83	−13	59			−57	
10	TFA	TFA	TFA	28.53	−107				−36	
11	TMS	CO	CO	32.29	87	73				−113
12	TMS	CO	TMS	29.26	−20	96				−60
13	TMS	TMS	TMS	25.28	−173					−58

TABLE VII

$\partial SN/\partial T$ VALUES FOR 3,11,17-TRISUBSTITUTED 5α-ANDROSTANES ON CHDMS

Compound No.	Substituents in 5α-androstane			SN (235°)	Found	$\partial SN/\partial T \times 10^4$				
	3-	11-	17-			CO	OH	Ac	TFA	TMS
1	CO	CO	CO	36.27	200	67				
2	OH	CO	CO	36.03	166	59	32			
3	OH	CO	OH	37.85	127	33	30			
4	OH	OH	OH	39.32	140		47			
5	Ac	CO	CO	35.55	180	79		46		
6	Ac	CO	Ac	35.80	100	56		17		
7	Ac	Ac	Ac	35.92	67			22		
8	TFA	CO	CO	31.18	213	149			79	
9	TFA	CO	TFA	27.52	40	208			−14	
10	TFA	TFA	TFA	24.12	−253				−84	
11	TMS	CO	CO	31.24	213	146				79
12	TMS	CO	TMS	27.50	20	176				−24
13	TMS	TMS	TMS	23.16	−233					−78

third substituent were very small, except for Ac. With compounds having one CO and two other substituents, the additivity rule was valid except for Ac.

On the polyester CHDMS stationary phase, the increments calculated for trisubstituted derivatives with identical substituents were the same as those for the monosubstituted compounds (Table VII). With the OH and Ac derivatives, the additivity rule held, although with some deviations. However, with the TFA and TMS derivatives, very high values for CO resulted, and higher values were assigned to TFA and TMS with increasing number of CO groups. CO values showed the reverse tendency.

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