Journal of Chromatography, 234 (1982) 65–76
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM 14,213

STRUCTURE–RETENTION RELATIONSHIP OF STEROLS AND TRITERPENE ALCOHOLS IN GAS CHROMATOGRAPHY ON A GLASS CAPILLARY COLUMN

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(First received June 9th, 1981; revised manuscript received July 17th, 1981)

SUMMARY

The relative retention times and the methylene unit values of 168 acetate derivatives of sterols and triterpene alcohols, most of which are of higher plant origin, were determined on OV-1 and OV-17 glass capillary columns. Separation factors related to various types of double bond, alkyl substituent and other structural features were calculated from the retention data. These gas–liquid chromatographic retention characteristics are very useful for the identification and estimation of the structure of sterols and triterpene alcohols.

INTRODUCTION

Gas–liquid chromatography (GLC) is a vital tool for separating and identifying sterols and triterpene alcohols. A number of 4-desmethylsterols[1–16] have already been analyzed on several stationary phases and the correlation between the structure of these compounds and their retention data has been well discussed. Retention data on several stationary phases have also been reported for some 4-methylsterols[5,7–10,13], lanostane triterpene alcohols[2,4,8–10,13,16–18] and other tetracyclic triterpene alcohols[8–10,17,18] and pentacyclic triterpene alcohols[8–10,17–21]. Since 4-methylsterols and triterpene alcohols are closely related to 4-desmethylsterols, biogenetically as well as structurally, it seems of great value to study the GLC retention characteristics of large numbers of these compounds together with 4-desmethylsterols.

Such a study was therefore undertaken for a number of 4-methylsterols, tetra- and pentacyclic triterpene alcohols and 4-desmethylsterols. Although most previous work has been carried out on conventional packed columns, glass capillary columns were used in this study because such columns have recently been shown to have high resolving power even for the structurally closely related sterols and triterpene alcohols from natural sources[22–26]. Furthermore, it has also been shown that glass capillary columns can be used to differentiate C-24 epimeric 24-alkylsterols[27–29].

0021-9673/82/0000–0000/$02.75

The retention data ware expressed as the usual relative retention times, RRT, and as the methylene unit (MU) values[30,31], since the latter are independent of the operating temperature. Separation factors related to various types of double bond, alkyl substituent and other structural characteristics were calculated from the RRT data.

EXPERIMENTAL

A Shimadzu GC-4CM gas chromatograph equipped with a hydrogen flame ionization detector was used. Two support-coated open tubular (SCOT) glass capillary columns (30 m × 0.3 mm I.D.; Wako, Osaka, Japan) were coated either with OV-1 or OV-17 stationary phase. Sterol and triterpene alcohol samples as the acetate derivatives and internal standards were injected simultaneously as solutions in acetone. The sample size injected was adjusted so that the peak heights were comparable to that corresponding to 4–6 μg cholesterol acetate (*ca.* 60% of the full recorder response).

"Initial retention times"[32], based on the distances from the starting point of the solvent peak to the starting point of each sample peak on the chromatogram, instead of the usual retention times were determined since far less fluctuation in the former due to change in the amount of sample injected has been observed previously[32] and also in our preliminary experiments. The standard chart speed was 10 mm min. RRT was expressed relative to cholesterol acetate, and MU value was determined using *n*-dotriacontane (C_{32}), *n*-tritriacontane (C_{33}), *n*-tetratriacontane (C_{34}), *n*-hexatriacontane (C_{36}) and *n*-tetracontane (C_{40}) as the standard hydrocarbons. The reproducibility of the values of RRT and MU obtained from several runs on the same column was satisfactory for a given substance.

Acetylation was performed in acetic anhydride–pyridine (1:1) at room temperature overnight. Sterols and triterpene alcohols were obtained from three sources: gifts from individuals; isolated from plants[6,23–26,33–35], and derived from available samples.

RESULTS AND DISCUSSION

The two stationary phases used in this work, non-polar OV-1 (dimethyl silicone; McReynolds' constant, $\chi' = 16$ (ref. 36)) and slightly polar OV-17 (50% phenyl-50% methyl silicone; $\chi' = 119$ (ref. 36)), are the ones most frequently used for sterol analysis. The acetate derivatives of the compounds were chosen because they are derivatives commonly used for the purification of naturally occurring sterols and triterpene alcohols by argentation chromatography[16,23–26], are very stable and can be quantitatively prepared from the free alcohols. Analysis of such derivatives on a capillary column gave satisfactory results, and thus it was unnecessary to prepare other derivatives solely for GLC analysis. The free alcohols were unsuitable for GLC on a capillary column because they were eluted as broad peaks with considerable tailing.

Table I shows the RRT and the MU values for the acetate derivatives of 168 sterols and triterpene alcohols including 68 4-desmethylsterols, 36 4α-methylsterols, 42 tetracyclic triterpene alcohols and 22 pentacyclic triterpene alcohols, most of

TABLE I

RELATIVE RETENTION TIMES AND METHYLENE UNITS OF THE ACETATES OF STEROLS AND TRITERPENE ALCOHOLS ON OV-1 AND OV-17 COLUMNS

Values are relative to cholesterol acetate; those in parentheses are taken from ref. 27. Unless otherwise specified in this and in the subsequent Tables, the acetoxy group at C-3 of all the compounds was β-oriented, and if not carrying a Δ^5-bond or not otherwise mentioned, all the compounds have a 5α-configuration. Conditions: OV-1, column temp. 260°C, N_2 flow-rate 0.55 ml/min, splitting ratio 120:1, scavenger gas (N_2) flow-rate 50 ml/min, net retention times of standard substances, cholesterol acetate (16.14 min, number of theoretical plates 17,689), *n*-dotriacontane (17.02 min), *n*-tritriacontane (22.10 min), *n*-tetratriacontane (28.84 min) and *n*-hexatriacontane (48.96 min); OV-17, column temp. 260°C, N_2 flow-rate 0.60 ml/min, splitting ratio 100:1, scavenger gas flow-rate 70 ml/min, net retention times of standard substances, cholesterol acetate (15.60 min, theoretical plates 19,712), *n*-tetratriacontane (13.60 min), *n*-hexatriacontane (23.16 min) and *n*-tetracontane (65.86 min).

Acetate	*Position of double bond**	*OV-1*		*OV-17*	
		RRT	*MU*	*RRT*	*MU*
I. 4-Desmethylsterol (cholestane group)					
C_{26} sterol					
24-Nor-5,22-cholestadienol	5,22*E*	0.65	30.26	0.66	32.96
C_{27} sterol					
Cholestanol	—	1.03	31.93	1.02	34.61
Epicholestanol (cholestan-3α-ol)	—	0.92	31.51	0.89	34.09
Coprostanol (5β-cholestanol)	—	0.86	31.25	0.82	33.77
Epicoprostanol (5β-cholestan-3α-ol)	—	0.89	31.38	0.84	33.87
20-Isocholesterol	5	0.91	31.47	0.89	34.09
Cholesterol	5	1.00	31.82	1.00	34.53
22-Dehydrocholesterol	5,22*Z*	0.88	31.34	0.90	34.13
22-Dehydrocholesterol	5,22*E*	0.92	31.51	0.93	34.25
Desmosterol	5,24(25)	1.09	32.14	1.21	35.24
27-Nor-5,22-ergostadienol (24*R*/β)	5,22*E*	0.89	31.38	0.89	34.09
7-Cholestenol	7	1.13	32.28	1.18	35.15
27-Nor-7,22-ergostadienol (24*R*,β)	7,22*E*	1.01	31.86	1.06	34.75
24-Dihydrozymosterol	8	1.07	32.07	1.06	34.75
Zymosterol	8,24(25)	1.16	32.37	1.28	35.45
C_{28} sterol					
Campestanol (24*R*/α)	—	1.33 (1.329)	32.89	1.33 (1.334)	35.61
Ergostanol (24*S*/β)	—	1.33 (1.325)	32.87	1.33 (1.329)	35.60
24-Methylcoprostanol (24*R,S*)	—	1.11	32.21	1.07	34.78
Pollinastanol	9:19-Cyclo	1.17	32.40	1.20	35.21
24-Dehydropollinastanol	9:19-Cyclo,24(25)	1.27	32.72	1.44	35.90
Campesterol (24*R*/α)	5	1.29 (1.293)	32.78	1.31 (1.312)	35.55
22-Dihydrobrassicasterol (24*S*/β)	5	1.29 (1.286)	32.76	1.31 (1.307)	35.54
Brassicasterol (24*S*/β)	5,22*E*	1.12	32.24	1.14	35.02
5,23-Ergostadienol	5,23*E*	1.26	32.69	1.35	35.65
24-Methylenecholesterol	5,24(28)	1.27	32.72	1.35	35.65
5,24-Ergostadienol	5,24(25)	1.46	33.23	1.63	36.36
5,25-Ergostadienol (24*S*/β)	5,25(27)	1.23	32.60	1.32	35.57
5,7-Ergostadienol (24*S*/β)	5,7	1.46	33.23	1.54	36.15
Ergosterol (24*R*/β)	5,7,22*E*	1.26	32.69	1.35	35.65
5,8,22-Ergostatrienol (24*R*/β)	5,8,22*E*	1.19	32.46	1.22	35.27
7-Ergostenol (24*S*/β)	7	1.46	33.23	1.55	36.17

(Continued on p. 68)

TABLE I *(continued)*

Acetate	*Position of double bond**	*OV-1*		*OV-17*	
		RRT	*MU*	*RRT*	*MU*
7,22-Ergostadienol (24*R*/β)	7,22*E*	1.26	32.69	1.36	35.68
7,24(28)-Ergostadienol	7,24(28)	1.43	33.16	1.61	36.32
Fecosterol	8,24(28)	1.36	32.97	1.43	35.87
C_{29} sterol					
Stigmastanol (24*R*/α)	–	1.65	33.69	1.65	36.41
		(1.648)		(1.651)	
5-Dihydroclionasterol (24*S*/β)	–	1.64	33.67	1.64	36.39
		(1.640)		(1.642)	
24-Ethylcoprostanol (24*R*/α)	–	1.38	33.03	1.32	35.57
22-Stigmastenol (24*S*/α)	22*E*	1.44	33.18	1.45	35.92
24-Ethyl-22-coprostenol (24*S*, α)	22*E*	1.21	32.53	1.16	35.09
Sitosterol (24*R*/α)	5	1.60	33.57	1.63	36.37
		(1.597)		(1.634)	
Clionasterol (24*S*/β)	5	1.59	33.55	1.63	36.35
		(1.588)		(1.626)	
Stigmasterol (24*S* α)	5,22*E*	1.40	33.07	1.43	35.87
		(1.397)		(1.431)	
Poriferasterol (24*R*·β)	5,22*E*	1.40	33.09	1.44	35.90
		(1.404)		(1.439)	
5,23-Stigmastadienol	5,23*E*	1.53	33.41	1.62	36.34
23,24-Dimethyl-5,22-cholestadienol (24*R*/β)	5,22*E*	1.36	32.97	1.37	35.71
5,24-Stigmastadienol	5,24(25)	1.78	33.97	1.95	37.04
28-Isofucosterol	5,24(28)*Z*	1.67	33.74	1.81	36.76
Fucosterol	5,24(28)*E*	1.62	33.62	1.72	36.56
Clerosterol (24*S*/β)	5,25(27)	1.54	33.43	1.64	36.39
5,22,25-Stigmastatrienol (24*S*/β)	5,22*E*,25(27)	1.39	33.05	1.52	36.10
23-Demethylgorgosterol (22*R*,23*R*,24*R*)	5,22:23-Cyclo	1.65	33.69	1.73	36.59
22-Dihydrospinasterol (24*R*/α)	7	1.81	34.03	1.94	37.02
		(1.805)		(1.938)	
22-Dihydrochondrillasterol (24*S* β)	7	1.80	34.01	1.93	37.00
		(1.797)		(1.928)	
Spinasterol (24*S* α)	7,22*E*	1.58	33.53	1.70	36.52
		(1.582)		(1.698)	
Chondrillasterol (24*R*/β)	7,22*E*	1.59	33.55	1.71	36.54
		(1.589)		(1.705)	
7,22,25-Stigmastatrienol (24*R* α)	7,22*E*,25(27)	1.55	33.46	1.77	36.66
		(1.549)		(1.765)	
7,22,25-Stigmastatrienol (24*S*·β)	7,22*E*,25(27)	1.57	33.50	1.80	36.71
		(1.567)		(1.793)	
Avenasterol	7,24(28)*Z*	1.89	34.20	2.15	37.40
28-Isoavenasterol	7,24(28)*E*	1.82	34.06	2.04	37.21
Pepositerol (7,24-stigmastadienol)	7,24(25)	2.01	34.43	2.31	37.68
7,25-Stigmastadienol (24*S*/β)	7,25(27)	1.75	33.91	1.95	37.04
23,24-Dimethyl-7,22-cholestadienol (24*R*/β)	7,22*E*	1.53	33.41	1.62	36.34
Vernosterol	8,14,24(28)*Z*	1.72	33.85	1.94	37.02
8(14)-Stigmastenol (24*R*, α)	8(14)	1.62	33.62	1.67	36.47
		(1.618)		(1.674)	
8(14)-Stigmastenol (24*S*/β)	8(14)	1.61	33.60	1.66	36.45
		(1.610)		(1.664)	
14-Stigmastenol (24*R*/α)	14	1.59	33.56	1.64	36.39
C_{30} sterol					
Gorgosterol (22*R*,23*R*,24*R*)	5,22:23-Cyclo	2.19	34.75	2.32	37.69
Acanthasterol (22*R*,23*R*,24*R*)	7,22:23-Cyclo	2.46	35.19	2.74	38.32

TABLE I *(continued)*

Acetate	*Position of double bond**	*OV-1*		*OV-17*	
		RRT	*MU*	*RRT*	*MU*
II. 4α-Methylsterol					
4α-Monomethylsterol (4α-methylcholestane group)					
4α-Methylcholestanol		1.19	32.46	1.12	34.95
Lophenol	7	1.28	32.75	1.33	35.60
24-Methyllophenol (24,*R*,*S*)	7	1.66	33.72	1.73	36.59
24-Ethyllophenol (24*R*/α)	7	2.06 (2.062)	34.53	2.16 (2.156)	37.41
24-Ethyllophenol (24*S*/β)	7	2.05 (2.051)	34.51	2.15 (2.145)	37.39
24-Methyl-23-dehydrolophenol	7,23*E*	1.63	33.64	1.79	36.72
Gramisterol (24-methylenelophenol)	7,24(28)	1.63	33.64	1.79	36.72
24-Methyl-24(25)-dehydrolophenol	7,24(25)	1.87	34.16	2.17	37.44
24-Ethyl-23-dehydrolophenol	7,23*E*	1.97	34.35	2.16	37.42
24-Ethyl-24(25)-dehydrolophenol	7,24(25)	2.28	34.91	2.60	38.12
Citrostadienol	7,24(28)*Z*	2.16	34.70	2.41	37.84
28-Isocitrostadienol	7,24(28)*E*	2.08	34.55	2.29	37.65
24-Ethyl-25-dehydrolophenol (24*S*/β)	7,25(27)	1.99	34.39	2.19	37.47
4α-Methyl-8-cholestenol	8	1.21	32.53	1.18	35.15
4α-Methyl-8(14)-cholestenol	8(14)	1.15	32.35	1.14	35.02
4α-Methyl-8(14)-ergostenol (24*R*,*S*)	8(14)	1.48	33.28	1.49	36.03
4α-Methyl-8(14)-stigmastenol (24*R*,*S*)	8(14)	1.83	34.08	1.86	36.86
4α,14α-Dimethylsterol (31-norlanostane group)					
31-Norcycloartanol	9:19-Cyclo	1.33	32.89	1.31	35.54
31-Norcycloartenol	9:19-Cyclo,24(25)	1.45	33.21	1.58	36.26
24(28)-Dihydrocycloeucalenol (24*R*,*S*)	9:19-Cyclo	1.71	33.83	1.71	36.54
24-Methyl-31-nor-23-dehydrocycloartanol	9:19-Cyclo,23*E*	1.67	33.74	1.77	36.67
Cycloeucalenol	9:19-Cyclo,24(28)	1.69	33.78	1.77	36.67
24-Methyl-31-norcycloartenol	9:19-Cyclo,24(25)	1.94	34.30	2.14	37.39
31-Norcyclolaudenol (24*S*/β)	9:19-Cyclo,25(27)	1.64	33.67	1.72	36.56
31-Nor-7-lanostenol	7	1.32	32.86	1.33	35.60
24-Methyl-31-nor-7-lanostenol (24*R*,*S*)	7	1.70	33.81	1.74	36.62
31-Nor-8-lanostenol	8	1.18	32.43	1.10	34.87
31-Norlanosterol	8,24(25)	1.29	32.78	1.33	35.60
24(28)-Dihydroobtusifoliol (24*R*,*S*)	8	1.51	33.36	1.44	35.90
Obtusifoliol	8,24(28)	1.48	33.28	1.49	36.03
24-Methyl-31-norlanosterol	8,24(25)	1.72	33.85	1.79	36.72
24-Ethyl-31-nor-8-lanostenol (24*S*/β)	8	1.86	34.14	1.78	36.69
24-Ethyl-31-nor-8,25-lanostadienol (24*S*/β)	8,25(27)	1.79	33.99	1.80	36.74
31-Nor-9(11)-lanostenol	9(11)	1.28	32.75	1.26	35.39
24-Methyl-31-nor-9(11)-lanostenol (24*R*,*S*)	9(11)	1.65	33.69	1.64	36.39
24-Methylene-31-nor-9(11)-lanostenol	9(11),24(28)	1.63	33.64	1.69	36.50
III. Triterpene alcohol					
Tetracyclic triterpene alcohol					
4,4-Dimethylsterol (32-norlanostane group)					
4,4-Dimethylcholestanol	–	1.43	33.16	1.33	35.60
Lanostane group					
Lanostanol	–	1.76	33.93	1.71	36.54
24-Methyllanostanol (24*R*,*S*)	–	2.25	34.85	2.23	37.53
Cycloartanol	9:19-Cyclo	1.59	33.56	1.54	36.15
Cycloartenol	9:19-Cyclo,24(25)	1.74	33.89	1.86	36.86
24-Methylcycloartanol (24*R*,*S*)	9:19-Cyclo	2.04	34.49	2.01	37.15

(Continued on p. 70)

TABLE I *(continued)*

Acetate	*Position of double bond**	*OV-1*		*OV-17*	
		RRT	*MU*	*RRT*	*MU*
Cyclosadol(24-methyl-23-dehydrocycloartanol)	9:19-Cyclo,23*E*	2.00	34.41	2.08	37.28
24-Methylenecycloartanol	9:19-Cyclo,24(28)	2.01	34.43	2.07	37.26
Cyclobranol (24-methylcycloartenol)	9:19-Cyclo,24(25)	2.33	34.98	2.50	37.98
Cycloläudenol (24*S*/*β*)	9:19-Cyclo,25(27)	1.95	34.32	2.03	37.19
24-Dihydrocimicifugenol	7,9:19-Cyclo	1.22	32.56	1.13	34.99
Cimicifugenol (7-dehydrocycloartenol)	7,9-Cyclo,24(25)	1.34	32.91	1.37	35.71
7-Lanostenol	7	1.58	33.54	1.57	36.23
24-Methyl-7-lanostenol (24*R*,*S*)	7	2.04	34.49	2.05	37.23
24-Dihydroagnosterol	7,9(11)	1.32	32.86	1.28	35.45
Agnosterol	7,9(11),24(25)	1.43	33.16	1.55	36.17
24-Dihydrolanosterol	8	1.39	33.05	1.30	35.51
Lanosterol	8,24(25)	1.52	33.39	1.57	36.23
24-Methyl-24-dihydrolanosterol (24*R*,*S*)	8	1.78	33.97	1.70	36.52
24-Methylene-24-dihydrolanosterol	8,24(28)	1.75	33.91	1.76	36.65
24-Dihydroparkeol	9(11)	1.54	33.43	1.49	36.03
Parkeol	9(11),24(25)	1.69	33.78	1.79	36.72
24-Methyl-24-dihydroparkeol (24*R*,*S*)	9(11)	1.97	34.35	1.94	37.02
24-Methylene-24-dihydroparkeol	9(11),24(28)	1.93	34.28	2.00	37.13
24,24-Dimethyl-9(11)-lanostenol	9(11)	2.54	35.31	2.39	37.81
24,25-Dimethyl-9(11)-lanostenol (24*R*,*S*)	9(11)	2.35	35.01	2.40	37.82
24,25-Dimethyl-9(11),23-lanostadienol**	9(11),23ξ	2.33	34.98	2.37	37.78
24,24-Dimethyl-9(11),25-lanostadienol	9(11),25(27)	2.37	35.05	2.49	37.96
Euphane-tirucallane group					
24-Dihydrobutyrospermol (20*R*)	7	1.44	33.18	1.40	35.80
Butyrospermol (20*R*)	7,24(25)	1.58	33.54	1.70	36.52
24-Dihydroeuphol (20*R*)	8	1.20	32.50	1.07	34.78
Euphol (20*R*)	8,24(25)	1.32	32.86	1.30	35.51
7-Tirucallenol (20*S*)	7	1.60	33.58	1.59	36.28
7,24-Tirucalladienol (20*S*)	7,24(25)	1.76	33.93	1.92	36.98
24-Dihydrotirucallol (20*S*)	8	1.34	32.91	1.22	35.27
Tirucallol (20*S*)	8,24(25)	1.46	33.23	1.47	35.97
Dammarane group					
Dammaradienol	20(21),24(25)	1.50	33.34	1.64	36.39
24-Methylenedammarenol	20(21),24(28)	1.70	33.81	1.78	36.69
Isoeuphenol (20*R*)	13(17)	0.91	31.79	0.93	34.25
Isotirucallenol (20*S*)	13(17)	1.12	32.24	1.01	34.56
Cucurbitane group					
10α-5-Cucurbitaenol	5	1.21	32.53	1.19	35.18
10α-5,24-Cucurbitadienol	5,24(25)	1.32	32.86	1.43	35.87
Pentacyclic triterpene alcohol					
α-Amyrin (12-ursenol)	12	1.64	33.67	1.84	36.82
Taraxasterol (20[30]-taraxastenol)	20(30)	2.06	34.52	2.50	37.98
ψ-Taraxasterol (20-taraxastenol)	20(21)	2.01	34.43	2.40	37.82
Bauerenol (7-bauerenenol)	7	1.98	34.37	2.26	37.59
Epibauerenol (7-baueren-3α-ol)	7	1.64	33.64	1.94	37.02
Isobauerenol (8-bauerenenol)	8	1.73	33.87	1.83	36.84
β-Amyrin (12-oleanenol)	12	1.52	33.39	1.65	36.41
Germanicol (18-oleanenol)	18	1.54	33.43	1.65	36.41
Glutinol (5-glutinenol)	5	1.59	33.56	1.91	36.96
Taraxerol (14-taraxerenol)	14	1.45	33.21	1.57	36.23
Friedelinol (5β-methyl friedelan-3α-ol)	–	2.10	34.59	2.66	38.21
Epifriedelinol (5β-methyl friedelanol)	–	2.09	34.57	2.55	38.04

TABLE I *(continued)*

Acetate	*Position of double bond**	*OV-1*		*OV-17*	
		RRT	*MU*	*RRT*	*MU*
Multiflorenol (7-multiflorenenol)	7	1.86	34.14	2.13	37.39
Isomultiflorenol (8-multiflorenenol)	8	1.61	33.60	1.73	36.59
Lupeol (20[29]-lupenol)	20(29)	1.66	33.72	1.93	37.00
Epilupeol (20[29]-lupen-3α-ol)	20(29)	1.35	32.94	1.63	36.36
Fernenol (9[11]-fernenol, 21*S*)	9(11)	1.98	34.37	2.28	37.63
Epifernenol (9[11]-fernen-3α-ol)	9(11)	1.58	33.54	1.94	37.02
Trematol (21-epi-9[11]-fernenol, 21*R*)	9(11)	2.27	34.89	2.79	38.39
Moretenol (21-epi-22[29]-hopenol)	22(29)	1.95	34.32	2.26	37.59
Simiarenol (5-adianenol)	5	1.92	34.26	2.40	37.82
Isoarborinol (9[11]-arborinenol)	9(11)	2.15	34.68	2.57	38.08

* Cyclopropyl group is also included and denoted by Cyclo.

** From *Quercus myrsinaefolia* (W. H. Hui and M. M. Li, *J. Chem. Soc., Perkin Trans. 1*, (1977) 897, most probably has a 23*E*-configuration.

which are of higher plant origin or are derived therefrom. It is worth mentioning here that there are differences in the retention data of 24-alkylsterols epimeric at C-24 as reported recently[27–29]. The 24α-epimers of 24-methyl- and 24-ethylsterols with a saturated side chain showed slightly larger RRT and MU values than those of their 24β-counterparts, whereas the opposite elution order was observed for the 24-ethyl-Δ^{22}- and 24-ethyl-$\Delta^{22,25}$-sterols[27]. The use of more highly polar phases and longer capillary columns had resulted in GLC being a diagnostic tool in the differentiation and characterization of the epimeric 24-alkylsterols[28,29]. Chromatography of a 1:1 mixture of the C-24 epimeric pair of 24-alkylsterols with a saturated or a Δ^{22}-monounsaturated side chain afforded a single peak on OV-1 and OV-17 glass capillary columns in this study, instead of separated peaks, and hence several C-24 epimeric mixtures of 24-alkylsterols, which were derived from the corresponding dehydro-sterols by hydrogenation, are considered to be eluted at the midpoint of the peaks of the epimers of each epimeric pair.

The separation factors related to the presence of double bonds, steric effects and alkyl substituents, which were calculated from the RRT data given in Table I, are shown in Tables II–IV. As found previously[5], there is almost no interdependence of the individual separation characteristics related to the skeleton and to the side chain, and therefore, the two sets of features are presented separately in these tables.

Skeletal double bond and steric separation factors are shown in Table II. Since most of the compounds analyzed are of higher plant origin and such plants contain Δ^5, Δ^7 and 9β:19-cyclopropyl compounds as the major sterol constituents[37–39], these compounds were taken, for convenience, as the references in calculating the skeletal double bond separation factors. The separation factors related to the skeletal double bond of cholestane and of 4α-methylcholestane were almost identical, but markedly different from those of 31-norlanostane and lanostane which possess quite similar separation factors. This indicates that the skeletal double bond separation factor is affected solely by the angular methyl group at C-14α, whereas almost no influence is exerted by the methyl group at C-4. Several stationary phases have been shown to distinguish saturated 4-desmethylsterols from the corresponding Δ^5-sterols in packed

TABLE II

SKELETAL DOUBLE BOND AND STERIC SEPARATION FACTORS

Separation factors in this and in the subsequent tables refer to mean values when two or more examples are available.

Compounds compared		*Stationary phase*	
		OV-1	*OV-17*
Stanol Δ^{7}	Cholestane	1.03	1.01
Δ^{7}, Δ^{5}	Cholestane	1.13	1.19
Δ^{8}, Δ^{5}	Cholestane	1.07	1.06
$\Delta^{8(14)}$, Δ^{5}	Cholestane	1.01	1.02
Δ^{14}, Δ^{5}	Cholestane	0.99	1.01
$\Delta^{5,7}$, Δ^{5}	Cholestane	1.13	1.17
$\Delta^{5,8}$, Δ^{5}	Cholestane	1.06	1.07
Stanol Δ^{7}	Cholestane	0.91	0.86
	4α-Methylcholestane	0.93	0.84
	Lanostane	1.11	1.09
Δ^{5}, Δ^{7}	Cholestane	0.95	0.90
	4α-Methylcholestane	0.95	0.89
	31-Norlanostane	0.89	0.83
	Lanostane	0.88	0.83
	Euphane-tirucallane	0.83	0.77
	Bauerenane	0.87	0.82
	Multiflorenane	0.87	0.81
$\Delta^{9(11)}$, Δ^{7}	Cholestane	0.89	0.86
	4α-Methylcholestane	0.89	0.86
$\Delta^{9(11)}$, Δ^{7}	31-Norlanostane	0.97	0.95
	Lanostane	0.97	0.95
Δ^{14}, Δ^{7}	Cholestane	0.85	0.88
$\Delta^{7,9(11)}$, Δ^{7}	Lanostane	0.84	0.81
$\Delta^{8,14}$, Δ^{7}	Cholestane	0.91	0.90
Stanol 9:19-cyclo	Lanostane	1.11	1.11
Δ^{7}, 9:19-cyclo	31-Norlanostane	1.00	1.02
	Lanostane	1.00	1.03
Δ^{8}, 9:19-cyclo	31-Norlanostane	0.89	0.84
	Lanostane	0.87	0.85
$\Delta^{9(11)}$ 9:19-cyclo	31-Norlanostane	0.96	0.96
	Lanostane	0.97	0.97
$\Delta^{7,9(11)}$, 9:19-cyclo	Lanostane	0.83	0.83
Δ^{7}, 9:19-Cyclo, 9:19-cyclo	Lanostane	0.77	0.74
Δ^{18}, Δ^{12}	Oleanane	1.01	1.00
$\Delta^{20(30)}$, $\Delta^{20(21)}$	Taraxastane	1.02	1.04
Stan-3α-ol stanol	Cholestane	0.89	0.87
	Lupane	0.81	0.84
	Fernane ($\Delta^{9(11)}$)	0.80	0.85
	Bauerenane (Δ^{7})	0.83	0.87
	Friedelane	1.00	1.04
5β-Stanol/stanol	Cholestane	0.84	0.80
5β-Stan-3α-ol stanol	Cholestane	0.86	0.82
Euphane/lanostane	Δ^{8}	0.87	0.83
	Δ^{7}	0.91	0.88
Tirucallane/lanostane	Δ^{8}	0.96	0.94
	Δ^{7}	1.01	1.01
Ursane, oleanane	Δ^{12}	1.08	1.12
Bauerenane, multiflorenane	Δ^{8}	1.07	1.06
	Δ^{7}	1.06	1.06
20*R*(α-H) 20*S*(β-H)	Cholestane	1.10	1.12
	Euphane-tirucallane	0.90	0.88
	Dammarane($\Delta^{13(17)}$)	0.81	0.92
21*R*(α-H) 21*S*(β-H)	Fernane	1.15	1.22

columns[4,5,15,16], but none gave complete separations of these sterols[5]. The OV-1 glass capillary column used in this study, as well as in other studies[40,41], afforded almost complete separations. Δ^7-Lanostane was eluted simultaneously or slightly after its 9β:19-cyclopropyl isomer, but the co-existence of the Δ^7-bond and the cyclopropyl group in the lanostane skeleton greatly reduced its retention time.

20-Isocholesterol (20*S*, β-H) was eluted before its 20*R* epimer, cholesterol (20α-H), as observed previously[42,43]. This is most probably due to the conformational difference in the side chain, since rotation of C-20 about the 17(20)-bond can place the side chain in significantly different positions[42–44]. Thus the most stable conformer at the 17(20)-bond should be the non-eclipsed one with the 20-H (the smallest group on C-20) close to C-18, and therefore presumably the preferred conformer for cholesterol is a "right-handed" structure (C-22, *trans*-oriented to C-13). When the configuration at C-20 is inverted as in 20-isocholesterol, the preferred conformation should be that with a "left-handed" side chain (C-22, *cis*-oriented to C-13). Based on these considerations, we can postulate conformation at C-20 of euphane-tirucallane and $\Delta^{13(17)}$-dammarane triterpenes from their retention behaviour. The 20*S*(β-H) epimers of these triterpenes were eluted after their 20*R*(α-H) counterparts, and therefore the former should have a "right-handed" side chain conformation and the latter a "left-handed" conformation.

Table III shows the separation factors associated with the side chain double bonds of sterols and tetracyclic triterpenes. The configurational isomerism at C-24 of 24-alkylsterols exerted almost no influence on the side chain double bond separation factors, while the separation factors related to the 22*E*,25(27)-double bonds of 24-ethylsterol differed appreciably according to the nature of the isomerism. The presence of

TABLE III

SIDE CHAIN DOUBLE BOND SEPARATION FACTORS

Relative to the RRT of the steryl acetates with the corresponding saturated side chain.

Position of double bond	*Alkyl or alkylene substituent*	*Stationary phase*	
		OV-1	*OV-17*
22*Z*	–	0.88	0.90
22*E*	–	0.92	0.93
	24-Methyl	0.86	0.88
	24-Ethyl	0.86	0.88
23*E*	24-Methyl	0.98	1.04
	24-Ethyl	0.96	1.00
24(25)	–	1.09	1.21
	24-Methyl	1.14	1.25
	24-Ethyl	1.11	1.21
24(28)	24-Methylene	0.98	1.03
	24*Z*-Ethylidene	1.04	1.12
	24*E*-Ethylidene	1.01	1.06
25(27)	24-Methyl	0.96	1.01
	24-Ethyl	0.97	1.02
	24,24-Dimethyl	0.93	1.04
22*E*,25(27)	24α-Ethyl	0.86	0.91
	24β-Ethyl	0.87	0.93

TABLE IV

ALKYL SUBSTITUENT SEPARATION FACTORS

Alkyl substituent	*Position of double bond**	*Stationary phase*	
		OV-1	*OV-17*
4α-Methyl	–	1.16	1.10
	7	1.14	1.12
	8	1.13	1.11
	8 (14)	1.14	1.11
4α,14α-Dimethyl/14α-methyl	9:19-Cyclo	1.14	1.10
4,4-Dimethyl/4α-methyl	–	1.20	1.19
4,4,14α-Trimethyl/4α,14α-dimethyl	9:19-Cyclo	1.19	1.18
	7	1.20	1.19
	8	1.18	1.18
	9 (11)	1.19	1.18
4,4-Dimethyl	–	1.39	1.30
4α,14α-Dimethyl/4α-methyl	7	1.03	1.01
	8	0.98	0.93
4,4,14α-Trimethyl/4,4-dimethyl	–	1.23	1.29
24-Methyl	–	1.29	1.31
	22*E*	1.22	1.23
	24 (25)	1.35	1.35
23,24-Dimethyl	22*E*	1.49	1.47
24,24-Dimethyl	–	1.65	1.60
24,25-Dimethyl	–	1.53	1.61
23,24-Dimethyl/24-methyl	22*E*	1.22	1.20
24,24-Dimethyl/24-methyl	–	1.29	1.23
24,25-Dimethyl/24-methyl	–	1.19	1.24
24-Ethyl	–	1.60	1.63
	22*E*	1.52	1.54
	24 (25)	1.65	1.63
24-Ethyl/24-methyl	–	1.24	1.24
	22*E*	1.25	1.25
	24 (25)	1.22	1.20
	25 (27)	1.25	1.25

* Cyclopropyl group is also included.

the 23*E*-double bond resulted in shorter retention times of sterols on the OV-1 column, whereas on the OV-17 column the retention times were increased or unaffected. The other separation characteristics listed are in reasonable agreement with those previously reported[4,5]. The alkyl substituent at C-24 affected the separation characteristics of the $\Delta^{22}E$, $\Delta^{23}E$, $\Delta^{24(25)}$ and $\Delta^{25(27)}$ sterols.

The separation factors for various alkyl substituents of sterol and lanostane triterpenes are listed in Table IV. Of all the methyl substituents examined, that at C-24 gave the largest retention time increment, whereas the smallest effect on the retention time was observed, as for Δ^7 and Δ^8 sterols, on introduction of the 14α-methyl group. In Δ^8 sterols, the 14α-methyl group actually resulted in a decrease in retention time, as observed previously[5]. The effect of this group was, however, exceptionally large for compounds with saturated skeletons. The increase of retention time attributed to an alkyl substituent was affected by the presence of an adjacent double bond.

most probably due to the interaction between the double bond and the alkyl group. For example, introduction of a $\Delta^{22}E$-bond decreased the retention time increment due to a methyl or ethyl substituent at C-24. Gas chromatography can be used to distinguish between 4-desmethyl-, 4-monomethyl- and 4,4'-dimethylsterols[5,8–10].

The RRT and MU values of the large number of sterols and triterpene alcohols given in Table I are very useful for the identification of these compounds. Several sets of "critical pairs" of sterols were encountered on both the column systems, but these can be distinguished by argentation thin-layer chromatography or by some spectroscopic techniques, or may be differentiated in GLC on other column systems. Using the RRT data, additional separation factors can be calculated for other structural features, and the probable RRT of unknown or undetected sterols and triterpene alcohols can be predicted.

ACKNOWLEDGEMENTS

The authors are grateful to Professor A. Kanazawa for samples of desmosterol, 27-nor-5,22-, 27-nor-7,22-, 5,25- and 7,22-ergostadienol, 23,24-dimethyl-5,22-cholestadienol, 23-demethylgorgosterol, 23,24-dimethyl-7,22-cholestadienol, gorgosterol and acanthasterol; to Dr. M. Hayakawa for epicholestanol, coprostanol, epicoprostanol, 24-methyl- and 24-ethylcoprostanol and 24-ethyl-22-coprostenol; to Dr. H. Yokokawa for 5,7-ergostadienol and 5,8,22-ergostatrienol; to Professor W. R. Nes for 20-isocholesterol; to Dr. M. J. Thompson for 24-dehydropollinastanol; to Dr. S. C. Pakrashi for 5,22,25-stigmastatrienol; to Professor L. Cattel for 28-isoavenasterol; to Dr. J. P. Ward for vernosterol; to Professor T. Ohmoto for bauerenol, epibauerenol, glutinol, friedelinol, fernenol, epifernenol and isoarborinol; to Dr. W. H. Hui for 24,25-dimethyl-9(11),23- and 24,24-dimethyl-9(11),25-lanostadienol; to Dr. L. Ogunkoya for trematol and simiarenol; to Dr. G. Kusano for cimicifugenol; to Dr. K. Stransky for epilupeol; to Professor S. K. Talapatra for isobauerenol; to Professor M. U. S. Sultanbawa for epifriedelinol; to Professor P. Sengupta for multiflorenol and to Professor D. J. Slatkin for moretenol. Our thanks are also due to Professor W. R. Nes for valuable advice.

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