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173. Takao Murakami,*1 Hideji Itokawa,*2 Fumiko Uzuki,*3 and Naotoshi Sawada*1 : Thin-layer Chromatography of Tetra- and Pentacyclic Triterpenes and Related Compounds.

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Thin-layer silica gel chromatography has recently been applied in many laboratories. For the separation of triterpenoids, Tschesche, *et al.*,[1,2] Thomas, *et al.*,[3] and Huneck[4] were successful with thin-layer chromatography (TLC) technic for triterpenoids of *Bredemeyera floribunda*, *Commiphora glandulosa*, and *Sorbus torminalis* respectively. However, a systematic survey has not been reported on TLC of triterpenoids. Recently, Ikan, *et al.*[5] reported the relationships between Rf values and the structures of triterpenoids. They applied alumina G using heptane-benzene-ethanol mixture (50:50:0.5) as developing agent and revealed the following results : the epimers and the ketones show higher Rf values than the corresponding secondary alcohols; the Rf value decreases with the increasing number of hydroxyl groups; among the β-amyrin type compounds, the higher Rf value was obtained according to the position of a double bond in the order of Δ^{18}, Δ^{12}, and Δ^{14}.

In the present paper, we wish to report the new findings about correlations between Rf values and chemical structures of triterpenoids on the basis of TLC of 50 samples on silica gel G[6] (Table I) and alumina G (Table II).

For convenience, the structural formulae of the compounds investigated are given in Chart 1.

In Tables I and II are listed in the approximate order of decreasing Rf value, tetra- and pentacyclic triterpenoids, sterols and some of the unknown compounds which are now under study by us. Eleven and nine kinds of solvent systems were used for silica gel G and alumina G plates respectively.

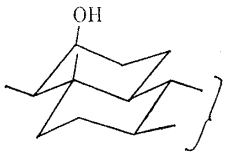
The systematic survey has revealed the following four items are in good agree ment with Ikan's results.

(1) The increasing number of hydroxyl groups gives lower Rf value; *e.g.**4 β-amyrin $(OH)_1$>erythrodiol $(OH)_2$>longispinogenin $(OH)_3$.

(2) The esters have higher Rf values than the free alcohols.

(3) The ketones have higher Rf values than the corresponding secondary alcohols.

(4) The epi-compounds show higher Rf values than normal, presumably because of the increased steric hindrance in epi-form; *e.g.* epi-friedelanol (I)>friedelanol (II).



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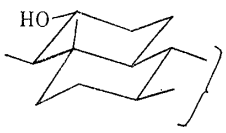

II

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*4 These expressions are used for the comparison of Rf value and the similar ways for the following sentences.

1) R. Tschesche, F. Lampert, G. Snatzke : J. Chromatog., **5**, 217 (1961).

2) R. Tschesche, A. K. Sen Gupta : Chem. Ber., **93**, 1903 (1960).

3) A. F. Thomas, I. M. Muller : Experientia, **16**, 62 (1960).

4) S. Huneck : J. Chromatog., **7**, 561 (1962).

5) R. Ikan, J. Kashman, E. D. Bergmann : *Ibid.*, **14**, 275 (1964).

6) T. Murakami, H. Itokawa, F. Uzuki, N. Sawada : Presented to The Pharmacognostical Society of Japan Annual Meeting (September, 1964).

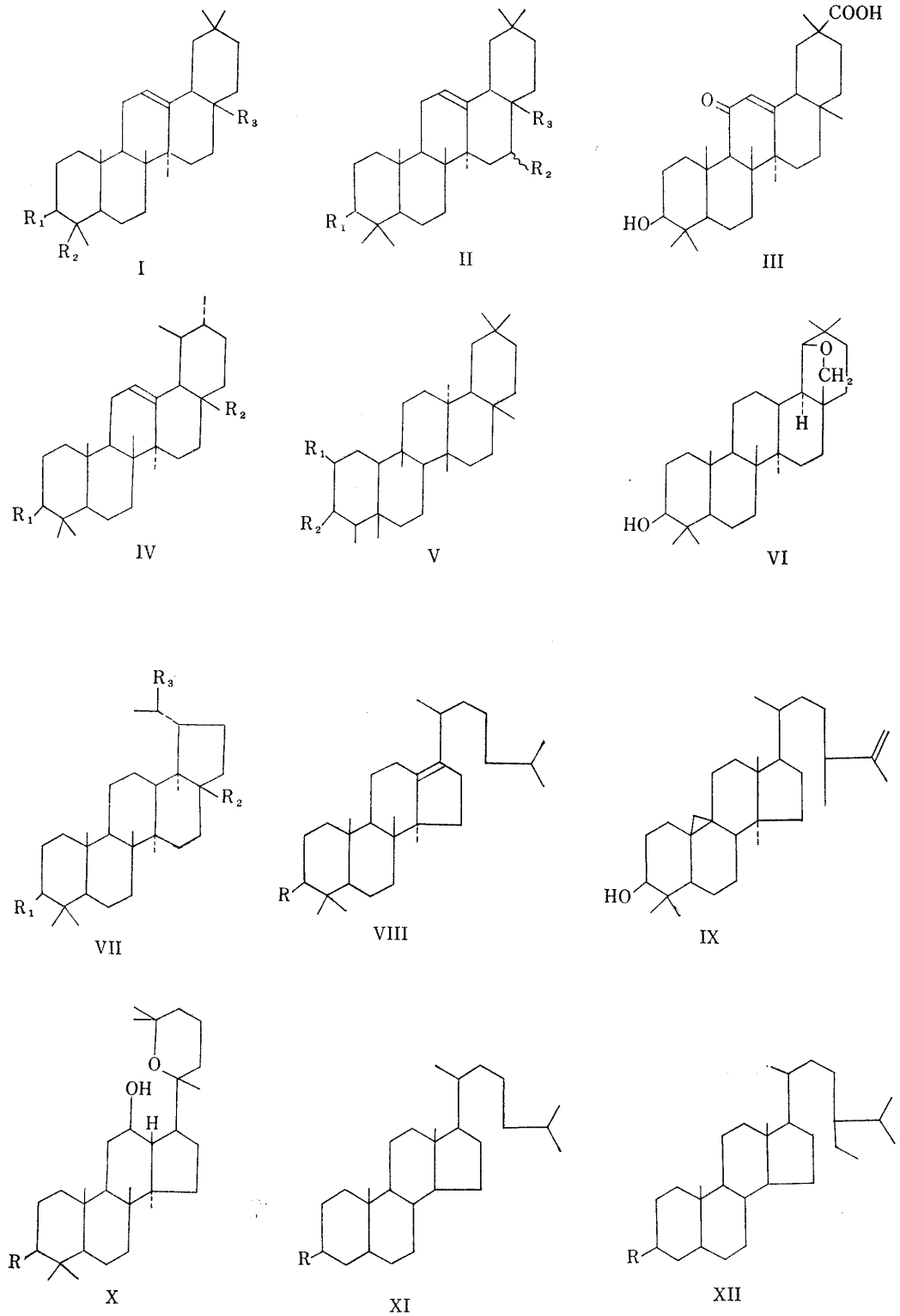


Chart 1.

Savard,[7] Barton[8] have already discussed concerning this subject on the paper chromatography of steroids.

7) K. Savard : J. Biol. Chem., **202**, 457 (1953).
8) D. H. R. Barton : J. Chem. Soc., **1953**, 1036.

TABLE I. Rf Values of Triterpenes and

No.	Compound	Structure	Substituents	A	B	C
1	β-Amyrin benzoate	I	R_1=OBz, R_2=R_3=CH_3	0.28	0.96	
2	β-Amyrin acetate	I	R_1=OAc, R_2=R_3=CH_3	0.14	0.84	
3	Isotirucallenyl acetate	VIII	R=OAc,	0.14	0.82	
4	Stigmasteryl acetate	XII	R=OAc, $\Delta^{22\sim23}$, $\Delta^{5\sim6}$	0.11	0.80	
5	Cholesteryl acetate	XI	R=OAc, $\Delta^{5\sim6}$	0.13	0.76	
6	Friedelanyl acetate	V	R_1=H, R_2=OAc	0.11	0.72	
7	epi-Friedelanyl acetate	V	R_1=H, R_2=OAc(β)	0.12	0.71	
8	3-Acetyl-28-methyl oleanolic acid	I	R_1=OAc, R_2=CH_3, R_3=$COOCH_3$	0.01	0.58	
9	3-Acetyl-28-chloro oleanolic acid	I	R_1=OAc, R_2=CH_3, R_3=COCl	0.01	0.57	
10	Betulin diacetate	VII	R_1=OAc, R_2=CH_2OAc, R_3= =CH_2		0.57	
11	Betulin dibenzoate	VII	R_1=OBz, R_2=CH_2OBz, R_3= =CH_2		0.56	
12	Erythrodiol diacetate	I	R_1=OAc, R_2=CH_3, R_3=CH_2OAc		0.56	
13	Friedelin	V	R_1=H, R_2= =O	0.05	0.54	
14	3,23,28-Trimethyl hederagenin	I	R_1=OCH_3, R_2=CH_2OCH_3, R_3=$COOCH_3$		0.35	0.84
15	Longispinogenin triacetate	II	R_1=OAc, R_2=OAc(β), R_3=CH_2OAc		0.23	0.82
16	epi-Friedelanol	V	R_1=H, R_2=OH(β)		0.50	0.76
17	Primulagenin diacetate	II	R_1=OAc, R_2=OH(α), R_3=CH_2OAc		0.18	0.68
18	Cyclolaudenol	IX			0.33	0.66
19	Isotirucallenol	VIII	R=OH		0.31	0.64
20	α-Amyrin	IV	R_1=OH, R_2=CH_3		0.31	0.64
21	β-Amyrin	I	R_1=OH, R_2=R_3=CH_3		0.31	0.64
22	Friedelanol	V	R_1=H, R_2=OH(α)		0.26	0.60
23	Desoxyoleanolic acid	I	R_1=H, R_2=CH_3, R_3=COOH		0.15	0.59
24	Methyl oleanolate	I	R_1=OH, R_2=CH_3,R_3=$COOCH_3$			0.60
25	Allobetulin	VI				0.55
26	3-Acetyl panaxadiol	X	R=OAc			0.53
27	Cerin	V	R_1=OH, R_2= =O			0.40
28	Acetyl oleanolate	I	R_1=OAc, R_2=CH_3, R_3=COOH			0.40
29	Acetyl ursolate	IV	R_1=OAc, R_2=COOH			0.44
30	Dihydrobetulin	VII	R_1=OH, R_2=CH_2OH, R_3=CH_3			0.44
31	Betulin	VII	R_1=OH, R_2=CH_2OH, R_3= =CH_2			0.43
32	Uvaol	IV	R_1=OH, R_2=CH_2OH			0.40
33	Erythrodiol	I	R_1=OH, R_2=CH_3, R_3=CH_2OH			0.40
34	Oleanonic acid	I	R_1= =O, R_2=CH_3, R_3=COOH			0.37
35	Oleanolic acid	I	R_1=OH, R_2=CH_3, R_3=COOH			0.09
36	Ursolic acid	IV	R_1=OH, R_2=COOH			0.09
37	Hederagenin methyl ester	I	R_1=OH, R_2=CH_2OH, R_3=$COOCH_3$			0.10
38	Longispinogenin	II	R_1=OH, R_2=OH(β), R_3=CH_2OH			0.09
39	Panaxadiol	X	R=OH			0.09
40	Camellia sapogenol I		4-OH			
41	Genin diacetate[a]		2-COOH, 2-OAc, -CO-O-			
42	Hederagenin	I	R_1=OH, R_2=CH_2OH, R_3=COOH			
43	Camellia sapogenol II					
44	Glycyrrhetic acid	III				
45	Genin[a]		2-COOH, 2-OH, -CO-O-			
46	Camellia sapogenol III					
47	Cholesterol	XI	R=OH, $\Delta^{5\sim6}$			
48	Cholestanol	XI	R=OH			
49	Stigmasterol	XII	R=OH, $\Delta^{5\sim6}$, $\Delta^{22\sim23}$			
50	Stigmastanol	XII	R=OH			

a) Sapogenin from *Polygala tenuifolia*

Solvent :

A. Hexane
B. Benzene
C. Chloroform
D. Chloroform-Methanol (40:1)
E. Hexane-Ethylacetate (7:3)
F. Benzene-Ethylacetate (7:3)
G. Isopropyl ether-Acetone (19:1)
H. Benzene-Ethylacetate (3:7)
I. Chloroform-Methanol (20:1)
J. Ethylacetate
K. Ether

Related Compounds (Adsorbent : Silica gel G)

D	E	F	G	H	I	J	K	H_2SO_4	$SbCl_3$	$SbCl_5$	STA
								DuRO	PaPuPi	RPu	PaBr
								〃	PuPi	PuPi	GryRPu
								LBr	PaYO	DaYO	Y
								DuO	DaBrPu	DaYBr	GryRPu
								DeBlPu	DePuR	〃	LRO
								LBr	Pu	PaPu	RY
								〃	〃	〃	〃
								DuRO	DePuR	DePuR	〃
								〃	RPu	〃	LYO
								DaBr	LBr	DaYO	GrnY
								〃	PaBr	PaBr	Pi
								DaPaBr	〃	Pu	RY
								PaYW	GryRPu	PaYW	LYO
								DePuR	DuO	Pu	LRO
	0.95							DaPaBr	GryRPu	DePuR	GrnY
0.90	0.95	0.83	0.94					PaBr	Pu	Pu	PaPuPi
0.95	0.92	0.88	0.93					GryRPu	〃	PaBr	DaYO
0.80	0.92	0.81	0.92					DuRO	DuO	DaYO	YW
0.80	0.91	0.81	0.92					YBr	〃	〃	〃
0.78	0.91	0.80	0.92					DuRO	LBr	Pu	PaYO
0.77	0.91	0.80	0.92					RPu	DuRO	〃	PaYW
0.60	0.88	0.79	0.90					PaBr	Pu	PaBr	PaPuPi
0.54	0.91	0.80	0.91					〃	〃	Pu	
0.74	0.88	0.63	0.88					DuRO	DuO	DePuR	LYO
0.70	0.87	0.65	0.87					YBr	Y	PaYO	GrnY
0.95	0.78	0.51	0.81					DuRO	DuO	RPu	LYO
0.69	0.81	0.64	0.82					PaBr			
0.58	0.79	0.50	0.87					GryRPu	PuR	Pu	DePuR
0.53	0.80	0.51	0.87					RPu	DePuR	PaBr	LYO
0.47	0.78	0.57	0.81	0.93		0.92	0.89	GryRPu	PaBr	〃	GrnY
0.46	0.77	0.55	0.80	0.93		0.91	0.87	YBr	PaYW	PaYO	〃
0.49	0.75	0.56	0.80	0.92		0.92	0.88	DeBlGrn	DeBlGrn	BlPu	BlPu
0.51	0.75	0.56	0.80	0.92	0.85	0.92	0.88	DaPaBr	PaBr	PaBr	PaBr
0.46	0.70	0.51	0.80	0.91	0.82	0.88	0.87	GryRPu	LBr	DaYO	DuO
0.26	0.51	0.32	0.78	0.88	0.52	0.84	0.80	〃	Pu	Pu	RPu
0.24	0.50	0.31	0.77	0.89	0.56	0.82	0.79	RPu	DePuR	PaBr	〃
0.22	0.43	0.24	0.52	0.74	0.58	0.76	0.56	DePuR	DuO	Pu	Pi
0.24	0.42	0.28	0.63	0.84	0.64	0.87	0.76	RPu	DaYBr	〃	DaBrPu
0.33	0.32	0.23	0.50	0.74	0.81	0.77	0.67	DuRO	DuO	〃	PaBr
0.14	0.17	0.15	0.45	0.72	0.42	0.78	0.63	RPu	Pu	DaBrPu	RPu
0.07	0.13	0.08	0.35 t	0.31 t	0.13 t	0.36 t	0.25 t	Gry	BlPu	YBr	GrnY
0.06	0.10	0.06	0.33	0.53	0.16	0.62	0.50	RPu	Pu	PaBr	RPu
0.07		0.04	0.24	0.61	0.31	0.73	0.50	DeYGrn	LYO	DaPaBr	PaBr
0.05				0.27	0.15	0.45	0.24	LYO	PaPuPi	LBr	PaYW
			0.07	0.18 t	0.02	0.08	0.09	Gry	BlPu	DaYO	GrnY
			0.07	0.22	0.08	0.39	0.16	RPu	DuO	DaBrPu	LBr
0.61	0.80	0.60	0.80					Pu	DePuR	DaYBr	PaPuPi
0.60	0.78	0.59	0.78					PaBr	Pi	DaYO	PaYW
0.61	0.80	0.60	0.80					DeBlPu	RPu	PaBr	PaPuPi
0.60	0.78	0.59	0.78					PaBr	Pi	〃	PaYW

Spray reagents :
H_2SO_4 : 10% aq. sol.
$SbCl_3$: 20% Chloroform sol.
$SbCl_5$: 25% Chloroform sol.
STA : Silicotungustic acid 40% ethanol sol.
t : tailing

Color :
Br-Brown Bl-Blue Du-Dull
Da-Dark De-Deep Grn-Green
Gry-Gray L-Light O-Orange
Pa-Pale Pi-Pink Pu-Purple
R-Red W-White Y-Yellow
(New Color Index, published by Japanese Color Research Laboratory)

TABLE II. Rf Values of Triterpenes and Related Compounds
(Adsorbent : Alumina G)

No.	Compound	A	B	C	E	F	G	H	L	M
1	β-Amyrin benzoate	0.22	0.98							
2	β-Amyrin acetate	0.14	0.96							
3	Isotirucallenyl acetate	0.11	0.96							
4	Stigmasteryl acetate	0.10	0.94							
5	Cholesteryl acetate	0.14	0.94							
6	Friedelanyl acetate	0.12	0.93							
7	epi-Friedelanyl acetate	0.12	0.92							
8	3-Acetyl-28-methyl oleanolic acid	0.07	0.89							
9	Betulin diacetate		0.89							
10	Betulin dibenzoate		0.89							
11	Erythrodiol diacetate		0.89							
12	3-Acetyl-28-chloro oleanolic acid		0.88							
13	3,23,28-Trimethyl hederagenin		0.84	0.89						
14	Friedelin		0.82	0.87						
15	Longispinogenin triacetate		0.69	0.89						
16	epi-Friedelanol		0.53	0.65	0.96	0.92	0.95		0.63	
17	Primulagenin diacetate		0.42	0.64	0.96	0.96	0.93		0.70	
18	3-Acetyl panaxadiol		0.31	0.65	0.95	0.95	0.90		0.71	
19	Cyclolaudenol		0.30	0.47	0.94	0.86	0.88		0.44	
20	Isotirucallenol		0.30	0.47	0.93	0.85	0.88		0.44	
21	α-Amyrin		0.30	0.47	0.93	0.85	0.88		0.42	
22	β-Amyrin		0.30	0.47	0.93	0.85	0.86		0.42	
23	Methyl oleanolate		0.28	0.45	0.91	0.84	0.79		0.36	
24	Friedelanol		0.26	0.39	0.93	0.83	0.75		0.32	
25	Allobetulin		0.26	0.46	0.90	0.82	0.77		0.33	
26	Erythrodiol		0.17	0.27	0.78	0.72	0.66	0.91	0.14	0.42
27	Uvaol		0.17	0.26	0.77	0.72	0.64	0.91	0.14	
28	Betulin		0.14	0.26	0.77	0.72	0.62	0.89	0.13	
29	Cerin		0.14	0.14	0.88	0.64	0.33	0.82	0.21	
30	Dihydrobetulin		0.14	0.26	0.81	0.73	0.66	0.92	0.14	
31	Panaxadiol			0.21	0.54	0.47	0.33	0.84		0.39
32	Longispinogenin				0.33	0.33	0.27	0.69		0.07
33	Hederagenin methyl ester				0.16	0.13	0.09	0.34		
34	Camellia sapogenol I									
35	Oleanonic acid									
36	Acetyl oleanolate									
37	Acetyl ursolate									
38	Oleanolic acid									
39	Ursolic acid									
40	Desoxyoleanolic acid									
41	Genin diacetate[a)]									
42	Camellia sapogenol II									
43	Hederagenin									
44	Glycyrrhetic acid									
45	Genin[a)]									
46	Camellia sapogenol III									
47	Cholesterol		0.16	0.26	0.75	0.62	0.61		0.18	
48	Cholestanol		0.16	0.26	0.75	0.62	0.61		0.18	
49	Stigmasterol		0.16	0.26	0.74	0.62	0.61		0.18	
50	Stigmastanol		0.16	0.26	0.74	0.62	0.61		0.18	

Solvents :
L. Heptane-Benzene-Ethanol (50:50:0.5)
M. Heptane-Benzene-Ethanol (50:50:1)

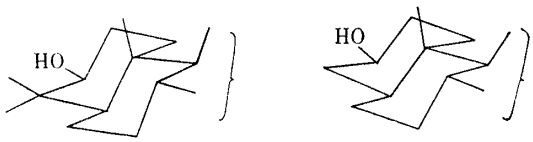
They found the higher Rf values of axial isomers than the corresponding equatorial OH compounds: 3-a (OH)>3-e (OH) (A/B *trans*); 3-a (OH)>3-e (OH) (A/B *cis*); 6-a (OH)>6-e (OH); 7-a (OH)>7-e (OH); 11-a (OH)>11-e (OH).

(5) The esters have higher Rf values than the corresponding free carboxylic acids.

(6) Comparing to a hydroxyl group, the ethereal function shows approximately a half efficacy to Rf value; *e.g.* β-amyrin $(OH)_1$>allobetulin $(OH)_1$, one ether linkage>betulin $(OH)_2$, erythrodiol $(OH)_2$.

(7) The carboxyl group corresponds to about one and a half hydroxyl functions in Rf value; *e.g.* β-amyrin $(OH)_1$>3-desoxyoleanolic acid $(COOH)_1$>erythrodiol $(OH)_2$.

(8) Sterically hindered OH group at C_3 by geminal dimethyl group of C_4 in triterpene (Ⅲ) shows less effect for lowering Rf value comparing to the ordinary steroidal OH function at C_3 (Ⅳ). Therefore, the Rf values of sterols are lower than the corresponding triterpenes.



III IV

(9) The increasing number of carboxyl function make Rf value decreased as expected (in the case of using silica gel G); *e.g.* oleanolic acid, ursolic acid >a genin from *Polygala tenuifolia*[9] having two carboxyl functions. In the case of using alumina G as an adsorbent on the contrary, none of carboxylic triterpenes (compounds No. 35~46, except 42, 46) moves from starting point by any solvent system illustrated in Fig. 1. However, if one applied a large

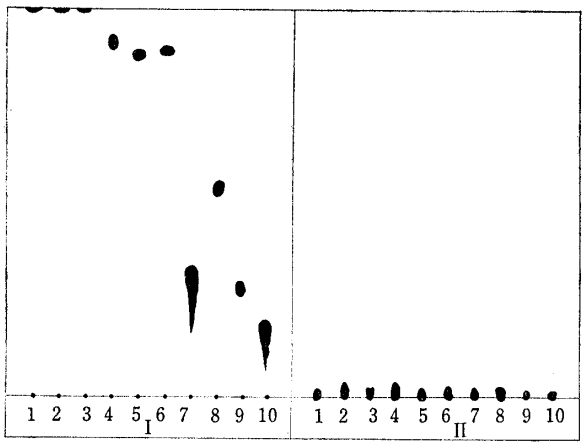


Fig. 1. Comparison of Silica Gel G (I) and Alumina G (Ⅱ) in the Case of Triterpenes Possessing Carboxyl Function by Solvent H

1. Desoxyoleanolic acid
2. Acetyl oleanolate
3. Acetyl ursolate
4. Oleanonic acid
5. Oleanolic acid
6. Ursolic acid
7. Genin diacetate
8. Hederagenin
9. Glycyrrhetic acid
10. Genin

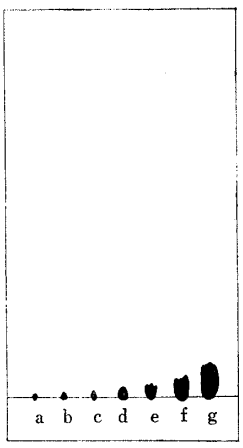


Fig. 2. Correlation of Rf Value and Concentration of Oleanolic Acid

Solvent: Ethyl acetate
Adsorbent: Alumina G

a: 0.5γ c: 2γ e: 8γ g: 24γ
b: 1γ d: 4γ f: 16γ

quantity of sample on a thin-layer plate, one may get a tailing figure as shown in Fig. 2. Therefore, it is required to spot as small amount of sample as possible.

On the other hand, these fiindings make us possible to determine the existence of a carboxyl function in a compound. For instance, hederagenin

9) M. Fujita, H. Itokawa: This Bulletin, **9**, 1006 (1961).

has Rf value of 0.53 with solvent system H on silica gel G, but zero on alumina G by the same solvent mixture.

(10) Double bond usually has only a little effect on Rf value. A double bond in triterpene appears to make Rf value slightly lower than the corresponding saturated one; *e.g.* dihydrobetulin>betulin. On the other hand, some sterols having double bond at $C_{5\sim6}$ show higher Rf values on silica gel G; *e.g.* cholesterol>cholestanol, but on alumina G plate, no difference was observed; *e.g.* stigmasterol=stigmastanol. It might be pertinent to say that the positions of double bond have substantial effect for Rf value in both sterols and triterpenes. The differences of fundamental skeleton among triterpenes, such as oleanane, ursane and lupane etc. will not have any remarkable effect on Rf value.

The relative Rf values of free alcohols and free carboxylic acids would not be affected whatever solvent systems had been applied. However, in the case of some esters, the relative Rf values might change depending upon the solvent systems used. For instance, primulagenin diacetate shows higher Rf value than epi-friedelanol unexpectedly by solvent D and F, but normally lower by other solvent systems. Also abnormally reversed relative Rf values were observed by solvent D in the following pairs : 3-acetyl panaxadiol>epi-friedelanol; acetyl oleanolate>acetyl ursolate.

Experimental

For the preparation of five thin-layer plates (20×20 cm.) a mixture of 30 g. of silica gel G (Meck) or alumina G (Merck) and 70 ml. of distilled water was used.

The well-shaken mixture was applied to thickness of 0.25 mm. After standing 5 min. at room temperature, the plates were dried for 40 min. at 110° and then kept in a desiccator.

The base line was fixed at a distance of 2 cm. from the rim of the plate and the compounds were applied in methanol or ethyl acetate solution by using a micro-pipet. The distance between samples on the same plate was about 1 cm.

The development of the chromatogram with the solvent mixture cited above was carried out in one dimension at 18~25°. Within 30~40 min., the solvent reached a distance of 15 cm. After drying at room temperature for 10 min., the compounds were detected by spraying with 4 kinds of reagents illustrated in Table I, and heating at 110° for 10 min. therefore.

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Summary

It was discussed that the relations between Rf values and the structures of 50 samples of triterpenoids and related compounds on silica gel G and alumina G, using 11 kinds of solvent systems. Four kinds of reagents were used for detection.

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