[Chem. Pharm. Bull. 13(3) 316~319 (1965)]

UDC 547.597 : 543.544.25

42. **Nobuo Ikekawa,**[*1] **Shinsaku Natori,**[*2] **Hideji Itokawa,**[*3] **Seishô Tobinaga,**[*4] **and Masami Matsui**[*1] : Gas Chromatography of Triterpenes. I. Ursanane, Oleanane, and Lupane Groups.

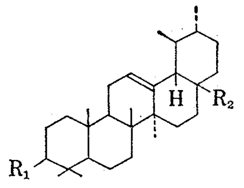
(*Institute of Physical and Chemical Research,*[*1] *National Institute of Hygienic Sciences,*[*2] *Tokyo College of Science,*[*3] *and Showa College of Pharmacy*[*4])

Gas chromatographic techniques recently developed for steroids[1)] are now widely used in the studies of natural products. Correlations between structures and retention times in the experiments of various steroids are considered to be very useful for the work with the complex mixtures of natural origin. However, only a few systematic surveys have been reported on gas chromatography of triterpenes.[2),3)]

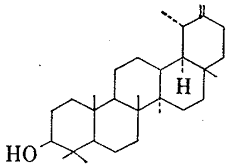
Eglinton, *et al.*[4)] reported the gas chromatography of some triterpene hydrocarbons using 4% Apiezon L, but longer retention times were necessary in obtaining a limited success. According to the method, developed by Horning, *et al.*[5)] for steroid separation, it was considered that triterpenes could be separated under suitable conditions using relatively thin film column packing prepared with highly thermostable stationary phase.

Table I. Relative Retention Times of Pentacyclic Triterpenes

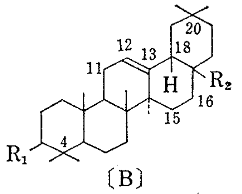
			1.3% SE-30
[A] Ursane group (α-Amyrin group)			
I	Urs-12-ene	$R_1=H$, $R_2=CH_3$	1.74
II	α-Amyrin	$R_1=OH$, $R_2=CH_3$	3.65
	acetate	$R_1=OAc$, $R_2=CH_3$	4.74
III	Methyl ursolate	$R_1=OH$, $R_2=COOCH_3$	5.25
	acetate	$R_1=OAc$, $R_2=COOCH_3$	6.80
IV	Taraxasterol		5.75
[B] Oleanane group (β-Amyrin group)			
V	β-Amyrin	$R_1=OH$, $R_2=CH_3$	3.23
VI	Methyl desoxyoleanolate	$R_1=H$, $R_2=COOCH_3$	2.66
VII	Methyl oleanolate	$R_1=OH$, $R_2=COOCH_3$	4.8
	acetate	$R_1=OAc$, $R_2=COOCH_3$	7.35
VIII	Methyl oleanonate	$R_1==O$, $R_2=COOCH_3$	4.4



[A]



IV



[B]

[*1] Komagome, Bunkyo-ku, Tokyo (池川信夫, 松居正巳).

[*2] Tamagawayoga, Setagaya-ku, Tokyo (名取信策).

[*3] Funagawara-machi, Shinjuku-ku, Tokyo (糸川秀治).

[*4] Tsurumaki-cho, Setagaya-ku, Tokyo (飛永精照).

1) E.C. Horning, W.J.A. VandenHeuvel, B.G. Creech : "Methods of Biochemical Analysis," XI, p. 69, Interscience Publ., 1963.

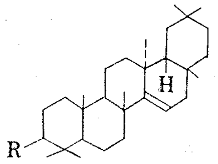
2) P. Capella, E. Fedeli, M. Cirimele : Chem. & Ind., **1963**, 1590.

3) M. Shimizu, F. Uchimaru, G. Ohta : This Bulletin, 12, 74 (1964).

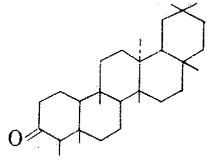
4) G. Eglinton, R.J. Hamilton, R. Hodges, R.A. Raphael : Chem. & Ind., **1959**, 955.

5) W.J.A. VandenHeuvel, C.C. Sweeley, E.C. Horning : J. Am. Chem. Soc., 82, 3481 (1960).

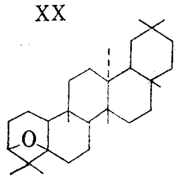
IX	Oleanoyl chloride	$R_1=OH$, $R_2=COCl$	7.40
X	Oleanolactone	$R_1=OH$, R_2-C_{13}=-COO- (non Δ^{12})	3.4
XI	Erythrodiol	$R_1=OH$, $R_2=CH_2OH$	7.32
	diacetate	$R_1=OAc$, $R_2=CH_2OAc$	10.52
XII	Methyl glycyrrhate	$R_1=OH$, $R_2=CH_3$ $C_{11}=O$, $C_{20}\begin{smallmatrix}-CH_3\\ \diagdown COOCH_3\end{smallmatrix}$	9.51
XIII	Hederagenin methyl ester	$R_1=OH$, $R_2=COOCH_3$ $C_4\begin{smallmatrix}-CH_3\\ \diagdown CH_2OH\end{smallmatrix}$	8.55
	trimethylsilyl ether		7.37
XIV	Hederatriol diacetate	$R_1=OAc$, $R_2=CH_2OH$ $C_4\begin{smallmatrix}-CH_3\\ \diagdown CH_2OAc\end{smallmatrix}$	9.9
XV	Primulagenin	$R_1=OH$, $R_2=CH_2OH$ $C_{16}\cdots OH$	
	trimethylsilyl ether		6.57
	triacetate		14.8
XVI	Longispinogenin	$R_1=OH$, $R_2=CH_2OH$ C_{16}-OH	
	trimethylsilyl ether		5.3
	triacetate		13.1
XVII	Sojasapogenol A	$R_1=OH$, $R_2=CH_3$ $C_{21}\sim OH$, $C_{22}\sim OH$ $C_4\begin{smallmatrix}-CH_3\\ \diagdown CH_2OH\end{smallmatrix}$	
	trimethylsilyl ether		6.57
[C]	Modified Oleanane group		
XVIII	Taraxerol (Skimmiol)	$R=OH$	3.14
XIX	Taraxerone (Skimmione)	$R==O$	4.08
XX	Friedelin		5.07
XXI	Dendropanoxide		2.57
[D]	Lupane group		
XXII	α-Lupene	$R_1=H$, $R_2=CH_3$	1.76
XXIII	Betulin	$R_1=OH$, $R_2=CH_2OH$	8.93
	trimethylsilyl ether		5.36
	diacetate		11.9



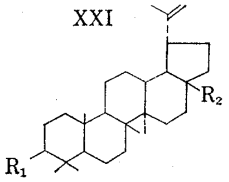
XVIII, XIX



XX



XXI



[D]

The feasibility of the technique for separation of triterpenes has been investigated in order to make use for the studies of natural products and this paper is concerning with pentacyclic triterpenes of oleanane, ursane, and lupane groups. For the separation of relatively large molecule and highly substituted comounds, methyl silicone gum, SE-30, is considered to be the most suitable liquid phase. The retention times, relative

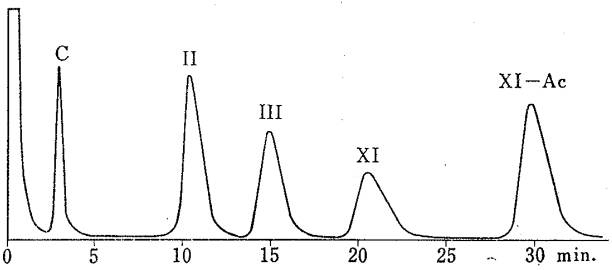


Fig. 1. Separation of a Mixture of Cholestane (C), α-Amyrin (II), Methyl Ursolate (III), Erythrodiol (XI) and Erythrodiol Acetate (XI-Ac)

Column, 1.5% SE-30 on Gas Chrom P, 240°. (For conditions see experimental part.)

to cholestane, of four kinds of ursane, eighteen kinds of oleanane and two kinds of lupane series of compounds using 1.5% SE-30 are summarized in Table I and an example of the separation of some mixtures is shown in Fig. 1.

The precise correlation between structure and retention time was not obtained in this study because of the limited samples having the same ring system. However, the effect of substituents on the retention time may be as same as the case of steroids. An introduction of additional substituents to the skeleton causes relatively a larger increase in retention time, and consequently an extent of substitution may be recognized from chromatographic behaviors. The relation between numbers of substituent, such as hydroxyl, ketone, and methoxycarbonyl, etc., and relative retention time (R. R. T.) to cholestane can be shown below.

	R. R. T.		R. R. T.
no substituent	1.4~3	one substituent	3~6
two substituents	4.5~9	three substituents	8.5~10
diacetoxy compounds	10~12	triacetoxy compounds	12~15

The compounds with other ring systems, which will be discussed in the following papers, also show the same range listed above for the number of substituents.

Acetylation of hydroxy group increases the retention time greatly and an increased value of relative retention time is found to be 1.1~1.8 for monoacetate and about two times of the value for diacetate. Separation factors of hydroxy and acetoxy compounds were found to be 1.3~1.5 for both cases of monohydroxy-monoacetate and dihydroxy-diacetate.

A series of compounds having methyl, hydroxymethyl, and carboxyl groups at C-17 of ursane, oleanane and also lupane groups have been isolated from natural resources. It is interesting to note that some correlations were observed among those series of compounds; namely, the separation factor of methyl and carbomethoxy compounds, for example α-amyrin and methyl ursolate, is found to be about 1.5, and that of methyl and hydroxymethyl compounds, for example, β-amyrin and erythrodiol, is shown to be about 2.2.

Table II. Relative Retention Times of Trimethysilyl (TMSi) Derivatives of some Triterpenes

	2% CNSi	1.5% QF-1	1% NGS	1.5% SE-30 (not TMSi Deriv.)
α-Amyrin TMSi	3.77	3.65	3.96	3.65
β-Amyrin TMSi	3.34	3.12	3.42	3.26
Taraxerol TMSi	3.15	3.12	3.33	3.14
Taraxasterol TMSi	13.0	7.52	—	5.75
Cholestane	1 (5.7 min.)	1 (1.7 min.)	1 (5.5 min.)	1 (2.8 min.)
Column Temp.	225°	220°	220°	240°

Sample heater temp. and detector temp. were 270° and 240°, respectively, and carrier gas(N_2) flow rate was 90 ml./min. in each case. Columns were 1.5 m.×4 mm. i.d. for QF-1, NGS and SE-30 and 2.25 m.×4 mm. i.d. for CNSi packing.

Tri- and tetra-hydroxy compounds gave a large tailing peak or, otherwise, could not be eluted from the column. However, trimethylsilyl derivatives of these compounds show a sharp peak and short retention time. Therefore, no difficulty is found on the gas chromatographic separation for polyhydroxy triterpene, such as sojasapagenol A, primulagenin and longispinogenin.

α-Amyrin and taraxastrol, in which the positions of double bond and the configuration at C-18 are different, exhibited much different retention time and consequently could be separated completely. However, the position isomers of methyl group show relatively close retention time. A mixture of α-amyrin, β-amyrin, and taraxerol could not be separated on the SE-30 phase. Trimethylsilyl derivatives of α-amyrin and β-amyrin could be separated on QE-1 or NGS phase but β-amyrin and taraxerol showed the same retention time on these phases. Nitrile silicone, XE-60, phase was found to be most suitable for the separation of these three compounds as shown in Fig. 2 and Table II.

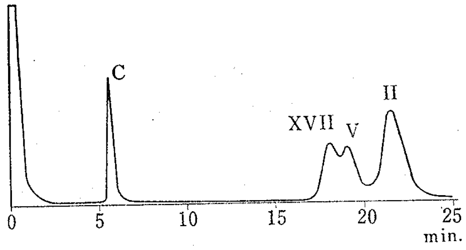


Fig. 2. Separation of a Mixture of Cholestane (C), Taraxerol (XVII) β-Amyrin (V) and α-Amyrin (II) Trimethylsilyl Derivatives

Column, 2% CNSi on Gas Chrom P, 225°. (For condition see Table II).

Experimental

Samples——The samples used in this studies were either isolated or derived from the natural resources in our laboratories.

Apparatus and Methods——A Shimadzu Model GC-1B Gas Chromatograph attached with a hydrogen flame detector (dual column and differential flame type) was used in this studies. A stainless steel column of 150 cm. (U-shape, 75 cm. × 2) × 4 mm. i. d. was packed with 1.5% SE-30 (G. E. methyl silicone), 1% NGS (neopentyl glycol succinate), and 1% QF-1 (FS-1265) (D. C. fluorinated alkylsilicone), and 225 cm. × 4 mm. i. d. column for CNSi (G. E. XE-60, nitrile silicone). The solid support, Gas Chrom P, was used after being washed with acid and silanized with dimethyldichlorosilane in toluene. Solution coating technique was used for the preparation of the packing and the conditioning was carried out by heating for 48 hr. with a slow nitrogen stream at 260° for SE-30 and 220° for other packings.

The standard operating conditions for the determination of relative retention times in Table I was as follows; column packing, 1.5% SE-30 on Gas Chrom P, 80～100 mesh; column temp. 240°; sample heater temp. 270°; detector temp. 240°; carrier gas, N_2, 90 ml./min.; retention time of cholestane, 2.8 min. A sample, 2～3 μl. of 0.5% acetone solution, was injected with 10 μl. Hamilton syring.

The trimethylsilyl derivatives were prepared by the following method. The sample, 2 mg., in a microtest tube with stopper was treated with 0.2 ml. of dry pyridine, 0.1 ml. of hexamethyldisilazane and 0.1 ml. of trimethylchlorosilane. After a vigorous shaking for 3 min. and then standing for about 5 min. at room temperature, 1～3 μl. of the reaction mixture was injected directly into the chromatograph.

The authors are deeply indebted to Professor E. Ochiai for his encouragement. They are also indebted to Dr. O. Tanaka, Tokyo University, for his valuable discussion and to Dr. Arthur Martellock, General Electric Co., for a sample of nitrile silicone polymer. Part of expenses of this work was financed Grant-in-Aid for Scientific Research from the Ministry of Education and from the Hoansha Foundation, to which authors' thanks are due.

Summary

The feasibility of gas chromatographic techniques for the separation of triterpenes has been investigated. The relative retention times of ursane, oleanane and lupane groups were examined using SE-30 phase and some correlations between structures and retention times were discussed.

(Received Octobet 27. 1964)