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43. Nobuo Ikekawa,*1 Shinsaku Natori,*2 Hiroyuki Ageta, Kenji Iwata,*3 and Masami Matsui*1 : Gas Chromatography of Triterpenes. II.*4 Hopane-Zeorinane and Onocerane Groups.

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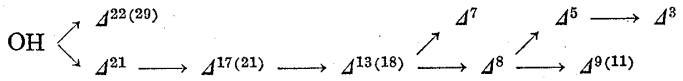
The outline of the correlation between the retention time and the structure of triterpene was described in the previous paper.*4 The gas chromatographic behavior of double bond isomer of triterpenes and the comparison with that of sterols, which have been reported in several reports,[1~3] are discussed in this paper. Generally it is not so easy to separate or identify the mixture of double bond isomers of steroids or triterpenes. However, these isomers can be separable by gas chromatography and some of the previous results on naturally occurring sterols have been corrected by the gas chromatographic evidences.[4]

Availability of this technique to the structural determination of triterpene was already reported from our laboratory.[7,8] Recently, thirteen kinds of hydrocarbons of hopane series, listed in Table I have been either isolated or derived in the studies of fern constituents[5~10] and thus it became possible to investigate the correlation between positions of double bonds and behaviors on the gas chromatogram.

Biogenetically, various double bond isomers of hopane series would be produced by the 1,2-shift of hydrogen and methyl groups in the following sequence :



The separation and identification of these isomers would be very important for the studies on biogenetical point of view and on acid migration reactions of these compunds.

Table I summarized the data of retention time observed, related to cholestane, with each of two kind of column, methyl silicone polymer (G.E. SE-30) and neopentylglycol succinate (NGS) under standard conditions at 225° and 219°, respectively. Some examples of separation from a mixture of several compounds on the two columns are shown in Fig. 1.

Although better resolution was obtained by SE-30 phase, NGS phase gives much better results and the order of retention times on these nonpolar and polar phases are found to be slightly different as mentioned below.

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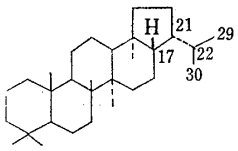
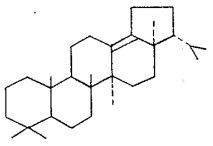
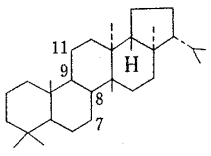
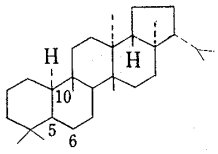
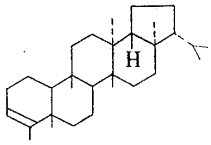
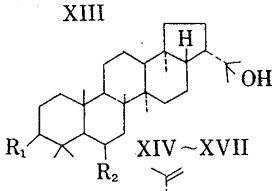
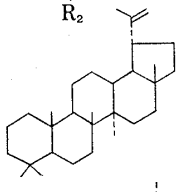
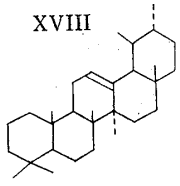
*4 Part I. N. Ikekawa, S. Natori, H. Itokawa, S. Tobinaga, M. Matsui : This Bulletin, **13**, 316 (1965).
1) R. B. Clayton : Nature, **190**, 1071 (1961); Biochemistry, **1**, 357 (1962).
2) K. Tsuda, K. Sakai, N. Ikekawa : This Bulletin, **9**, 835 (1961).
3) B. A. Knights : J. Gas Chromatog., **2**, 160 (1964).
4) For instance : M. J. Thompson, W. E. Robbins, L. Baker : Steroids, **2**, 505 (1963); T. Murakami, H. Itokawa, A. Matsushima, N. Ikekawa : Yakugaku Zasshi, **83**, 427 (1963).
5) H. Ageta, K. Iwata, Y. Otake : This Bulletin, **10**, 637 (1962).
6) *Idem* : *Ibid.*, **11**, 407 (1963).
7) H. Ageta, K. Iwata, K. Yonezawa : *Ibid.*, **11**, 408 (1963).
8) H. Ageta, K. Iwata, S. Natori : Tetrahedron Letters, **1963**, 1447.
9) *Idem* : *Ibid.*, **1964**, 3413.
10) H. Ageta, Y. Otake : Unpublished.

TABLE I. Relative Retention Times of Hopane-Zeorinane Group

		1.5% SE-30[a]	1% NGS[b]	position of double bond	
I	Hopane (Zeorinane)	2.68	4.05		
II	Hopane-b (Diploptene)	2.78	5.00	$\Delta^{22(29)}$	
III	Hopene-a	2.87	4.80	Δ^{21}	
IV	Hopene-I	1.73	2.10	$\Delta^{17(21)}$	I~IV
V	Hopene-II	2.01	2.70	$\Delta^{13(18)}$	 V
VI	Fern-7-ene	2.34	3.10	Δ^{7}	
VII	Fern-8-ene (Isofernene)	1.96	2.66	Δ^{8}	
VIII	Fern-9(11)-ene	2.08	2.96	$\Delta^{9(11)}$	
IX	Ferna-7,9(11)-diene	1.99	2.60	$\Delta^{7,9(11)}$	 VI~IX
X	Adian-5-ene	2.38	3.30	Δ^{5}	
XI	Adian-5(10)-ene	2.22	3.00	$\Delta^{5(10)}$	
XII	Adiana-1(10),5-diene	2.39	3.74	$\Delta^{1(10),5}$	 X~XII
XIII	Filic-3-ene	3.06	4.98	Δ^{3}	 XIII
XIV	Hydroxyhopane (Diplopterol)	2.80	$R_2=R_2=H$		 XIV~XVII
XV	Hydroxyhopanone	5.07	$R_1=O$, $R_2=H$		
XVI	Zeorin	4.96	$R_1=H$, $R_2=\cdots OH$		
XVII	Zeorinone	4.42	$R_1=H$, $R_2=O$		
XVIII	α-Lupene	1.76	2.60		 XVIII
XIX	Urs-12-ene	1.74	2.13		 XIX

a) Column, 1.5% SE-30 on Gas Chrom P, 80~100 mesh, 150 cm.×4 mm.i.d.; column temp., 225°; carrier gas, N_2, 80 ml./min.; retention time of cholestane, 7.4 min.
b) Column, 1% NGS on Gas Chrom P, 80~100 mesh, 150 cm.×4 mm.i.d.; column temp., 219°; carrier gas, N_2, 80 ml./min.; retention time of cholestane, 3.0 min.

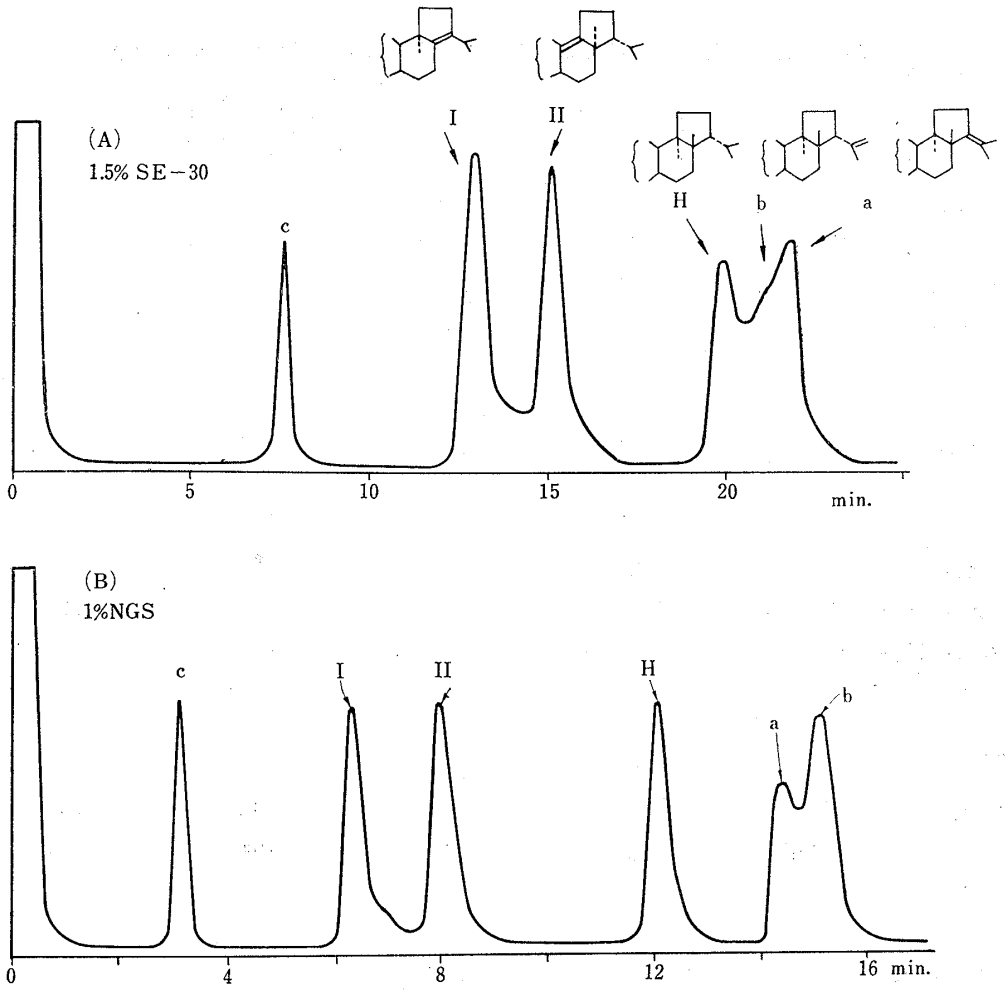


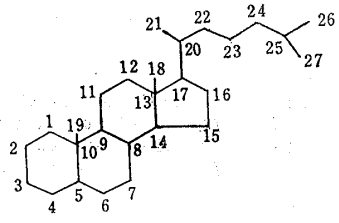
Fig. 1. Separation of a Mixture of Hopane Series

I : Hopene-I II : Hopene-II H : Hopane
a : Hopene-a b : Hopene-b c : Cholestane (Standard)
(A) 1.5% SE-30 on Chromosorp W, Column Temp. 230°, N_2, 85 ml./min.
(B) 1% NGS on Chromosorb W, Column Temp. 219°, N_2, 85 ml./min.

SE-30 $\Delta^{17(21)} < \Delta^{8} < \Delta^{7,9(11)} < \Delta^{13(18)} < \Delta^{9(11)} < \Delta^{5(10)} < \Delta^{5} < \Delta^{1(10),5} < \Delta^{7} < \Delta^{0} < \Delta^{22(29)} < \Delta^{21} < \Delta^{3}$

NGS $\Delta^{17(21)} < \Delta^{7,9(11)} < \Delta^{8} < \Delta^{13(18)} < \Delta^{9(11)} < \Delta^{5(10)} < \Delta^{5} < \Delta^{7} < \Delta^{1(10),5} < \Delta^{0} < \Delta^{21} < \Delta^{3} < \Delta^{22(29)}$

The compounds having double bond in the side chain and in C_3–C_4 show longer retention times than that of hopane, the corresponding saturated hydrocarbon; on the other hand, the compounds having double bond in the ring systems show shorter retention times on both phases. Magnitude of the effect of a double bond depends upon its position in the molecule, but generally tetrasubstituted double bonds in the skeleton, such as $\Delta^{17(21)}$, Δ^{8}, and $\Delta^{13(18)}$, have been found to shorten the retention time greater than the trisubstituted double bond on both phases. It is also interesting to note that the decreasing effect of double bond on the retention time at C_7 and C_9 was observed to be additive in the case of ferna-7,9(11)-diene, although the additivity of the increasing effect of double bonds was already observed in the case of sterols.[1,2]



In the case of sterol, the correlation between structure and retention time was investigated by Tsuda, *et al.*,[2] using SE-30, and by Clayton,[1] using polyester phase. Those results are summarized that the double bonds of

Δ^9, Δ^{16}, and Δ^{22} decrease the retention time of saturated parent compound and the double bonds of Δ^5, Δ^6, Δ^7, Δ^8, $\Delta^{8(14)}$, Δ^{14}, Δ^{24}, and $\Delta^{28(28)}$ increase, but the effect of Δ^5, Δ^8, and $\Delta^{8(14)}$ are very little.

It should be mentioned that all the isomers of sterol have the same ring system, but in the case of hopane series the isomers have some different ring systems as shown in Table I, because the isomerisation of triterpene is accompanied with concerted 1,2-shift(s) of methyl and hydrogen groups. However, from these data, it might be concluded that retention time might be affected by polarity, molecular volumn or shape of the compound, and usually double bond may give effects on the increase of polarity, but that a decrease effect on retention time may not be anomalous. The polarity of double bonds at the inside of ring, especially the tetrasubstituted one, may be shielded by some steric effect, while the molecular shape would be changed to the curtailment of solute-solvent interaction. On the other hand, double bond in the outside of ring, *i.e.* the side chain, increases polarity of a molecule, namely increases the retention time, except Δ^{22} of sterol. By the same way, a compound having exo double bond show longer retention time than that of endo double bond.*[4]

Although Clayton has tried to explain the reason of the effect of Δ^9, Δ^{16}, and Δ^{22} double bond of sterol, it may be rather difficult to find a reasonable interpretation for either the decrease or increase effect on the retention time of each double bonds. Lipsky[11] described that an additional double bond in A or B ring of steroids increases the retention time, relative to the saturated compound, and double bond in C or D ring decreases, but such a simple conclusion could not be elucidated from our results.

The relationships above mentioned between position of double bonds and retention times may be applicable to the other pentacyclic triterpenes such as oleanane, ursane and lupane groups and also may be helpful for the structural prediction.

Hydroxyhopane Series

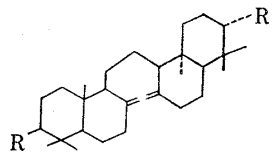
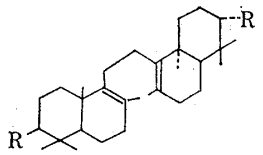
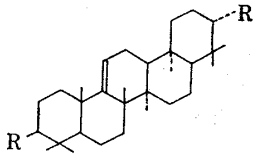
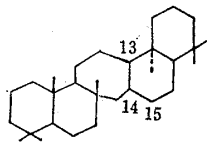
In Table I, the relative retention times of 22-hydroxyhopane derivatives are included. These values were not in agreement with what would be expected from their molecular structures. Since the relative retention time of hydroxyhopane was found to be very close to those of hopene-a and hopene-b, the dehydration might occur during the chromatography. The eluted product showed the same retention time in the second gas chromatographic determination and the same Rf value by thin-layer chromatography[12] as that of hopene-a. These evidences suggested the elimination of hydroxyl group from 22 position to form Δ^{21} compound in the flash heater zone as well as in the column, After repeated experiments with different SE-30 column, it was found that the main peak was sometimes accompanied with a small peak showing the relative retention time of 4.3, which would be the peak of unchanged substance. This fact suggested that some parts of the compound have not been dehydrated in the column. However, trimethylsilyl derivative of hydroxyhopane gave the relative retention time of 5.35, which may not show any elimination. Similarly the relative retention times of hydroxyhopanone, zeorin and zeorinone in Table I may be found actually that of dehydration products.

Onocerane Group

The relative retention times of four series of onocerane group and their acetates are summarized in Table II.

11) S. R. Lipsky, R. A. Landowne : Anal. Chem., 33, 818 (1961).
12) The Rf values of hopene-a, hopene-b and hydroxyhopane were 0.74, 0.74 and 0.43, respectively, in a solvent system of benzene-acetone (27:1).

Table II. Relative Retention Times of Onocerane Group

			1.5% SE-30[a]	1% NGS[b]	
XX	Onocerin	R=OH	4.76		
XXI	α-Onocerin	R=OH	4.76		
XXII	α-Onocerin diacetate	R=OAc	7.83		
XXIII	α-Onoceradiene	R=H	1.34	1.78	XX~XXIII
XXIV	β-Onocerin	R=OH	6.20		
XXV	β-Onocerin diacetate	R=OAc	9.77		
XXVI	β-Onoceradiene	R=H	1.68	1.89	XXIV~XXVI
XXVII	γ-Onocerin	R=OH	8.0		
XXVIII	γ-Onocerin diacetate	R=OAc	14.5		
XXIX	γ-Onocerene	R=H	2.16	2.90	XXVII~XXIX
XXX	Serratene (Δ^{14})		2.51	4.00	
XXXI	Isoserratene (Δ^{13})		2.01	2.78	XXX, XXXI[10,15]

a) Column, 1.5% SE-30 on Anakrom A, 80~100 mesh, 150 cm.×4 mm.i.d.; column temp., 230°; carrier gas, N_2, 90 ml./min., retention time of cholestane, 4.4 min.
b) Column, 1% NGS on Gas Chrom P, 80~100 mesh, 150 cm.×4 mm.i.d.; column temp., 220°; carrier gas, N_2, 85 ml./min., retention time of cholestane, 2.8 min.

Onocerin and α-onocerin, which have different melting points and different specific rotations,[13] show the same retention time on SE-30 and also on QF-1 phase. Their trimethylsilyl derivatives gave also the same retention time.[14]

It is interesting to add that the order of the retention time of the isomers of onocerin, their acetate and onocerane, respectively, was the same order of α, β, γ, and serratene[10,15] series in each case.

Experimental

Samples——The samples used in these studies were either isolated or derived in the studies on fern constituents.[5~10]

Apparatus and Method——A Shimadzu Seisakusho Model GC-1B Gas Chromatograph attached with a hydrogen flame detector (dual column and differential flame) was used in this series of studies. A stainless steel column of 150 cm. × 4 mm. i. d. was packed with 1.5% SE-30, 1% NGS, 1% GF-1 on Gas Chrom P,

13) H. Schulze: Z. physiol. Chem., **238**, 35 (1936); J. Zimmermann: Helv. Chem. Acta, **21**, 853 (1938).
14) The conditions for the trimethylsilyl derivatives were as follows: Column, 2% QF-1 on Gas Chrom P, 80~100 mesh, 150 cm. × 4 mm. i. d.; column temp., 210°; carrier gas, N_2, 90 ml./min. The relative retention time was 4.68 (cholestane, 3.7 min.).
15) Y. Tsuda, T. Sano, K. Kawaguchi, Y. Inubushi: Tetrahedon Letters, **1964**, 1279; Y. Inubushi, T. Sano, Y. Tsuda: *Ibid.*, **1964**, 1303.

80~100 mesh. The solid support was used after a washing with acid, silanized with dimethyldichlorosilane in toluene and then the packing was prepared by the solution coating technique. The standard operating conditions were listed in Tables I and II. The temperature of the detector and sample heater were adjusted at 240° and 270°, respectively. A sample of 0.5% solution of either hexane or acetone was injected with a Hamilton microsyring.

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Summary

Gas chromatographic behaviors of seventeen kinds of hopane-zeorinane group and of twelve kinds of onocerane group were investigated using NGS and SE-30 phases. The relationships between the position of double bond and the effect to the retention time were discussed.

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44. Yoshinobu Hirasaka and Kenji Umemoto: Structure of D-Glucosaccharodilactone.

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D-Glucosaccharodilactone (Ⅳ) was first isolated in crystalline state (m.p. 134~135°) by Rehorst and Scholz[1] in 1936. Later in 1944, Smith[2] obtained the similar substance (m.p. 133°) by lactonization of D-glucosaccharo-3,6-lactone (Ⅲ) and presumed that this dilactone possessed the 1,5-3,6-dilactone structure since it was different from that derived from D-glucosaccharo-1,4-lactone (Ⅱ)[*2] and indicated rapid mutarotation was shown in aqueous solution in contrast to D-mannosaccharo-1,4-3,6-dilactone (Ⅹ). However, the authors have recently isolated a crystalline dilactone from either Ⅱ or Ⅲ respectively, and found that two dilactones were completely identical in mixed melting point, infrared spectrum and optical rotation, and therefore the presumption proposed by Smith has become doubtful. The structure of Ⅳ has not so far been substantiated certainly as such.

The present investigation was undertaken to elucidate the structure of this dilactone using its acetyl derivative, since methylation of this dilactone gave an unsatisfactory result in affording principally 2,5-di-O-methyl-Δ^4-D-glucosaccharo-3,6-lactone 1-methyl ester (Ⅸ) instead of the desired 2,5-di-O-methyl-D-glucosaccharo-dilactone.

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*1 Ukima-cho, Kita-ku, Tokyo (平坂義信, 梅本賢次).

*2 Smith assumed in his report that the syrupy dilactone derived from Ⅱ would possess the 1,4-3,6-dilactone structure.

1) K. Rehorst, H. Scholz: Ber., 69, 524 (1936).
2) F. Smith: J. Chem. Soc., 1944, 633.