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Studies on Higher Alcohols V: Thin-Layer Chromatographic Separation of Monoenoic, Dienoic and Trienoic Fatty Alcohols

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1. INTRODUCTION

The separation of saturated higher alcohols from unsaturated alcohols after isolating these alcohols from the unsaponified portion of lipids by thin-layer chromatography (TLC) was previously discussed.[1] The purpose of this paper is to report further trial to separate unsaturated alcohols based on the difference in double bonds. It is important to establish methods of separation of alcohols since the methods established will be utilized for many purposes; for example, when we need to identify or to determine chemical structures of alcohols in lipids, the method will be utilized for fractioning samples. Isolation techniques based on the difference in the number of double bonds have been demonstrated with unsaturated fatty acids. For example, the silver acetate-addition product p. 207 method [2] and TLC method using $AgNO_3$ - impregnated plate [3] are known to be practical. It was expected that these methods were also applicable for unsaturated higher alcohols and similar results would be obtained, but so far it seems no paper has been published on this approach. This study was started by preparing known alcohols having 1, 2 and 3 double bonds, then isolation techniques of these alcohols were developed. The technique developed was applied for the isolation of alcohol-containing natural products and alcohol mixtures obtained by reduction of fatty acid esters, and it was found that the separation of mono-, di- and tri- unsaturated alcohols was possible as shown below.

2. EXPERIMENTAL

2-1 Samples and their preparation.

Alcohol acetates used for this study are as follows:

(1) Stearyl alcohol acetate

(2) Oleyl alcohol acetate

(3) Linoleyl alcohol acetate

(4) Linolenyl alcohol acetate

(5) Docosenyl alcohol acetate

(6) Sperm whale alcohol acetate

(7) Finwhale alcohol acetate

(8) Cow milk alcohol acetate

(9) Shark liver alcohol acetate

Commercial products [(1) - (5)] were purified by the combination of the following techniques, repeating recrystalization, treating with urea and column chromatography. Then alcohols were obtained by reduction. The purity of the alcohols obtained was found to be 95-98% by gas chromatography (GLC). Sample (6) was treated as described previously[1]. Sample (7): After saponification of finwhale oil using KOH-CH_3OH solution, fatty acids were obtained by routine systems and then methyl esterification was performed. The methyl esters produced were treated once with urea. The fraction which did not react with urea was reduced and alcohol mixtures were obtained. Sample (8): Commercial milk cream was heated at 80°C and treated with acetone. The acetone soluble portion was condensed, extracted with ethyl ether and as a result fat was obtained. The treatments thereafter are the same as (7). Sample (9) was prepared from commercial shark liver. The liver was macerated, extracted with acetone and

fat was obtained from the acetone soluble fraction. Thereafter, the sample was treated the same as sample (7) and a mixture of crude alcohols was obtained. Iodine values of samples (6), (7), (8) and (9) are 64.3, 125.2, 102.0 and 150.6 respectively.

2-2 Reduction of fatty acid methyl ester.

The method described by Nystrom et al. [4] was employed; in ethyl ether, fatty acid methyl ester was reduced by the reaction with lithium aluminum hydride. The completion of the reduction of fatty acid to alcohol was confirmed by TLC. For example, with silica gel plate using n-hexane : ethyl ether (7:3 v/v) as a developing solvent, Rf values of spots are around 0.3 for the alcohols obtained and around 0.8 for fatty acid methyl esters, which spots should be seen if the reduction is not completed. The separation is thus quite distinctive.

2-3 TLC and GLC

The methods described in the previous paper [1] were used. Silver nitrate-silica gel TLC was performed by the method of Privett et al.[3]

3. RESULTS and DISCUSSION

3-1 Separation of oleyl, linoleyl and linolenyl alcohols.

3-1-1 TLC of silver acetate addition compounds.

To investigate the most suitable experimental conditions of TLC, acetoxymercurimethoxy-complexes were prepared[5] with samples (2) - (5). Brilliant violet spots are obtained from these complexes when diphenyl-carbazone is sprayed as a chromogenic reagent. The intensity of the color

gradually fades away and accordingly the size of the spot becomes smaller and reaches to a pin point. It might be one of recommendable methods to consider the pin point as the centre of a spot obtained when Rf value is measured. When the spot disappears, it will be revisualized by the second spray using water or dilute $AgNO_3$ aq. solution. Of developing solvent systems examined, diisobutyl ketone : acetic acid (40:10 $^{v}/v$) and n-propanol : acetic acid : pyridine (150:1:1 $^{v}/v$) were found to be good solvent systems. Results of TLC using these two systems are shown in Table 1.

Table-1 TLC of the acetoxymercurimethoxy-complexes of the alcohol acetates.

	Solvent systems*			
Alcohol acetates	(a)	R_f	(b)	R_f
Stearyl	0.8-0.85		0.8 -0.85	
Complexes of :				
Oleyl, Docosenyl	0.6-0.65		0.65-0.7	
Linoleyl	0.4-0.45		0.45-0.5	
Linolenyl	0 -0.1		0 -0.1	

* (a) Diisobutyl ketone : acetic acid (40 : 10vol/vol)
(b) *n*-Propanol : acetic acid : pyridine (150 : 1 : 1 vol/vol/vol)

As shown in the Table, when more double bonds are present, lower Rf values are obtained. However, if the number of double bonds is the same between two compounds, the separation of these two compounds is rather difficult even if carbon chain lengths are different. For example, Rf values of complexes of oleyl- and docosenyl - alcohol acetates are identical. Stearyl alcohol always gives higher Rf values and consequently the separation from unsaturated alcohols is very clear. The spots of saturated alcohols are visualized by spraying a fluorescein sodium solution. Thus, the separation of alcohol acetates by this acetoxymercurimethoxy-complex

is excellent, but the following are some disadvantages which should be considered. To obtain objective alcohols, silver must be removed and in having this removing procedure, sample loss might be incurred. From these aspects the following method seems to be more advantageous.

3-1-2 TLC by using $AgNO_3$ - impregnated silica gel

The influence of the amount of silver nitrate was first investigated. Silver nitrate-impregnated plates were made up at different concentrations; amounts of silver nitrate added were 5%, 10%, 15% and 20% of silica gel. p. 208 Results obtained with a 5% plate were unsatisfactory, but all others were excellent and the difference found between 3 plates was very little. Therefore, 10% was chosen as a standard concentration and used for further study.

Fig.-1 Thin-layer chromatogram of the alcohol acetates on silica gel impregnated with silver nitrate (1).

Fig. 1 shows the chromatogram obtained by using 10% $AgNO_3$ - impregnated plate and petroleum ether : ethyl ether (80:20 $^{v}/v$) as a developing solvent. In accordance with the increase of the number of double bonds (acetates of oleyl alcohol, linoleyl alcohol and linolenyl alcohol) Rf

values become smaller, and the separations of these three compounds were distinctive. Docosenyl alcohol acetate and oleyl alcohol acetate showed the same Rf values. Stearyl alcohol acetate gave a higher Rf value and the separation from oleyl alcohol acetate was satisfactory. The separation of alcohol-acetates and fatty acid methyl esters was also investigated; samples (1) - (4) (alcohol acetates) and methyl esters of oleic - linolic - and linolenic-acids were spotted on the same plate and developed. Results are shown in Table 2. As shown very little difference was found between the two groups.

Table-2 Relation between of R_f value of the fatty alcohol acetates and fatty acid methyl esters on $AgNO_3$-TLC.

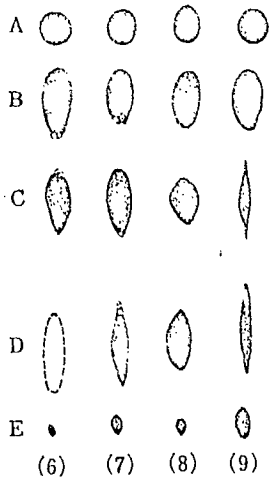
	Alcohol acetates	Acid methyl esters
18 : 1	0.55—0.58	0.60—0.65
18 : 2	0.35—0.40	0.40—0.45
18 : 3	0.10—0.15	0.15—0.20

Solvent system :
petroleum ether : ethyl ether (80 : 20 vol/vol)

3-2 TLC of sperm whale alcohol acetate and some others

The conditions described in 3-1-2 was applied for sperm whale alcohol acetates and some other alcohol acetates. Fig. 2 shows the chromatogram obtained with these samples. The separation was satisfactory with Rf values of 0.55 - 0.58, 0.35 - 0.40 and 0.10 - 0.15 for monoenoic, dienoic and trienoic unsaturated alcohol acetates and 0.60 - 0.65 for saturated alcohol acetate. As previously demonstrated[1], when conc. H_2SO_4 is used as a chromogenic reagent 3 - 5γ of alcohol is detectable. In Fig. 2, the spot for trienoic alcohol acetate in sample (6) is invisible but the spot of monoenoic alcohol acetate is very dense. Thus semi-quantitative

determination of these compounds is not impossible.



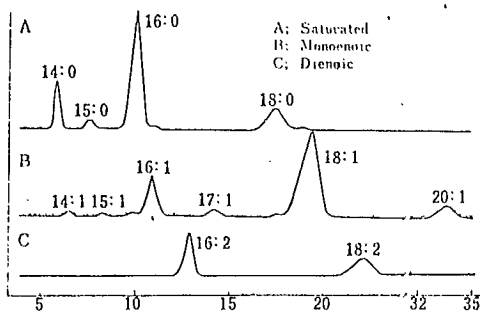
(6) Sperm whale alcohol acetate
(7) Alcohol acetates prepared from fin whale
(8) Alcohol acetates prepared from cow milk fat
(9) Alcohol acetates prepared from shark liver oil

Solvent system;
petroleum ether : ethyl ether (80 : 20 vol/vol)
A; Saturated,
B; Monoenoic,
C; Dienoic,
D; Trienoic,
E; Polyenoic

Fi.-2 Thin-layer chromatogram of the alcohol acetates on silica gel impregnated with silver nitrate(2).

3-3 Discussion on GLC

After TLC separation, samples were collected from the plate and analysed by GLC for confirmation. The procedures used are as follows: after developing the plate, fluorescein sodium solution was sprayed, each band obtained was scraped off, extracted with ethyl ether, ether was evaporated under nitrogen stream, column chromatographed (diameter 1 cm, height 2-3 cm) to remove fluorescein sodium using petroleum ether (40-60°C) as an eluent, the eluate was concentrated and examined the purity by TLC, which showed a single spot at the expected Rf position in each case. Scraped off the silica gel from each position and analysed by GLC.



GLC conditions : App. YANAGIMOTO GCG-2 , 3m column packed with Diasolid S (80-100mesh) containing poly diethylene glycol succinate , column temp. 220°C; flow rate of helium gas 60ml/min , bridge current 150mA; rhart speed 10mm/min.

Fig.-3 Gas-liquid chromatograms of the saturated, monoenoic and dienoic alcohol acetates of sperm whale obtained by $AgNO_3$-TLC.

Fig. 3 shows the chromatograms obtained with sperm whale alcohol acetates; A is from the fraction for saturated compounds on TLC, B is from monoenoic and C is from dienoic fractions. A very small amount of $C_{18=1}$ was found in the saturated compounds fraction, and similarly, $C_{16=0}$ was found in the monoenoic compounds fraction. However, it was understood that our main objective was essentially achieved. Although the contents of $C_{16=2}$ and $C_{18=2}$ in sperm whale alcohol are very small (1.0% and 0.6% respectively), peaks for these two compounds are very clear (Fig. 3, C), thus identification of these compounds by this technique is proved to be satisfactory. It is obvious from the results of this study that the separation technique based on the double bond difference by TLC is useful to improve the accuracy of GLC analysis. Table 3 shows the results obtained with shark liver fatty alcohols by the same technique described above. From the results, it was interpreted that unsaturated alcohols (mono-, di- and tri-) were obtained in high purity.

Table-3 The composition of shark liver fatty alcohols obtained by $AgNO_3$-TLC.

Fatty alcohol	Spot of Fig.-2 (9)				
	A	B	C	D	E
	%	%	%	%	%
14 : 0	16.3	0.2	—	—	—
16 : 0	69.6	0.6	—	—	—
18 : 0	13.5	—	—	—	—
16 : 1	—	16.7	—	—	—
18 : 1	0.4	42.3	—	—	—
20 : 1	—	18.5	—	—	—
22 : 1	0.1	19.1	—	—	—
24 : 1	—	2.0	—	—	—
18 : 2	—	—	100	—	—
18 : 3	—	—	—	100	0.1
18 : 4	—	—	—	—	3.6
20 : 4	—	—	—	—	6.0
20 : 5	—	—	—	—	27.2
22 : 5	—	—	—	—	14.5
22 : 6	—	—	—	—	48.4

3-4 Discussion

As shown above, two TLC methods, acetoxymercurimethoxy-complex method and silver nitrate-impregnated silica gel method, are found to be useful for practical isolation and analysis of higher alcohols. The silver nitrate method has some advantage over the other in obtaining objective unsaturated alcohols after TLC. However, in performing TLC, the following consideration must be paid to maintain a clear separation between saturated and monoenoic compounds after a continuous use of the same developing solvent. When fractions of large amounts of samples are necessary and more than 10 plates are prepared, the frequent renewal of the solvent is suggested and occasional GLC check is recommended to avoid getting an unexpected contamination. This sort of consideration was not necessary with di- and trienoic compounds. The reason for this difference is not clear, but it is likely due to the gradual change in the solvent system . Recently, Arvidson[6] reported that calcium sulfate used as a binder in his TLC caused some influence to Rf

values of lecithins he studied. The study is also based on the double bond difference. The authors did not try other adsorbents since results obtained with silica gel G ~~was~~ were satisfactory. It is possible, however, to find better conditions to separate complexes composed of double bonds and silver ion (utilization of π-complex). The spots obtained near the origin of Fig. 3 (This must be Fig. 2) are of polyenoic compounds which have more than 4 double bonds. Further attempt to separate polyenoic compounds was not made, but will be made some time in the future.

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