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11. Masao Shimizu, Fumihiko Uchimaru, and Genkichi Ohta : Studies on the Constituents of Rice-Bran Oil. VI.*2 Detection of Cycloartanol by Gas Chromatography.

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The isolation of cycloartenol (VI) and 24-methylenecycloartanol (VII) from rice-bran oil was previously reported.[1~5] Both are triterpenoid alcohols containing a cyclopropane ring and the structure of the latter compound which is the fifth compound of this type was elucidated by the authors. Recently Djerassi[6] isolated four compounds of this group from Spanish Moss having hydroxyl, methoxy and carbonyl groups in the side chains.

Generally these substances occur together with other phytosteroids or triterpenoids and their separation is very difficult since they occur in small quantities. Also in our experiments, purification was extremely difficult although good results were obtained when these substances were purified as their ferulates.

Recently new methods for the detection of naturally occuring substances such as thin layer chromatography or gas chromatography have been rapidly developed. This paper concerns the re-examination of purity of these triterpenoid alcohols and also the survey of the crude fractions from rice-bran oil using above mentioned modern techniques.

Thin layer chromatography using two kinds of solvents was first attempted and proved to be unsuccessful in this case. Both VI and VII showed single spot of the same Rf value. Gas chromatography of these triterpenoids was next attempted according to the general procedure for steroids.[7]

Table I. Relative Retention Time

Substance	m.p. (°C)	Relative R_t
Cholestane (I)	73~ 78	1.00
Cholesterol (II)	147~148	1.74
Lanosterol[a] (III)	135~138	(2.43) 2.64
α-Amyrin[b] (IV)	178~181	2.85
Cycloartanol (V)	99~101	2.83
Cycloartenol (VI)	97~100/114~115	3.06
24-Methylenecycloartanol (VII)	121.5~122.5	3.53
24-Methylcycloartanol (VIII) (24 α and β mixture)	136	3.56
Mixture-1[c] (Alcohol-B)	114.5~115.5	
Mixture-2[c]		
Mixture-3[c]		

a) commercial sample
b) We are indebted to Dr. S. Natori, National Institute of Hygienic Sciences, for his kind supply of this sample.
c) see Chart 1.

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*2 Part V : This Bulletin, 8, 108 (1960).

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2) G. Ohta, M. Shimizu : *Ibid.*, **5**, 40 (1957).
3) G. Ohta : *Ibid.*, **8**, 5 (1960).
4) *Idem* : *Ibid.*, **8**, 9 (1960).
5) M. Shimizu, G. Ohta : *Ibid.*, **8**, 108 (1960).
6) R. McCrindle, C. Djerassi : Chem. & Ind. (London), **1961**, 1311; *Idem* : J. Chem. Soc., **1962**, 4034.
7) *e.g.* E.C. Horning, W.J.A. VandenHeuvel, B.G. Creech : "Methods of Biochemical Analysis," Interscience Publishers, New York-London, **11**, 69 (1963).

Sample——The samples listed in Table I were used in this study. Among them VI and VII were isolated from rice-bran oil, and V and VIII were derived from the reduction of VI and VII respectively.

Apparatus——A packed column was used in conjunction with a Barber-Colman Model 10 chromatographic unit containing an argon ionization detector. The U-shaped column, 6 feet×6 mm. (int. diam.), was packed with 1% SE-30 silicon polymer on Anakrom ABS (70~80 mesh). Column temperature was 240°, cell temp. 190°, flash heater temp. 280°, and argon flow rate was 110 ml./min.

The relative retention times in Table I were calculated from the retention time of cholestane, 4.59~4.63 min., which was a reference compound. Samples were 4~5 μl. of a 0.05% solution of compound in Me_2CO.

Eight compounds gave a single peak without decomposition except III, which had a relative retention time as shown in Table I. Lanosterol gave a small peak (2.43) before the main peak (2.64) which probably corresponds to dihydrolanosterol present in lanosterol.

Naturally occurring VI and VII had somewhat different retention times and also it seemed possible to separate VI and its dihydroderivative (V). But the separation of VII and VIII was impossible under these conditions as their retention times were almost identical. To examine the crude fraction during the course of purification, a mixture of V, VI, VII, and cholestane

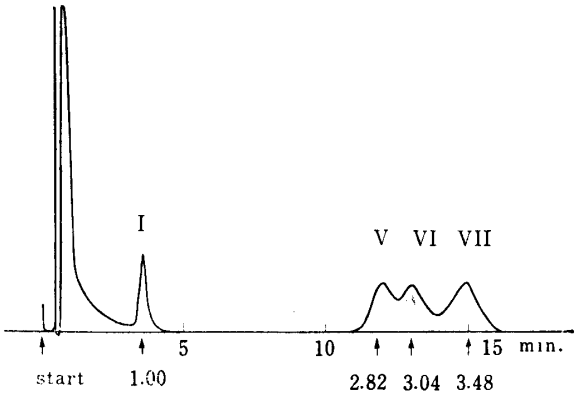
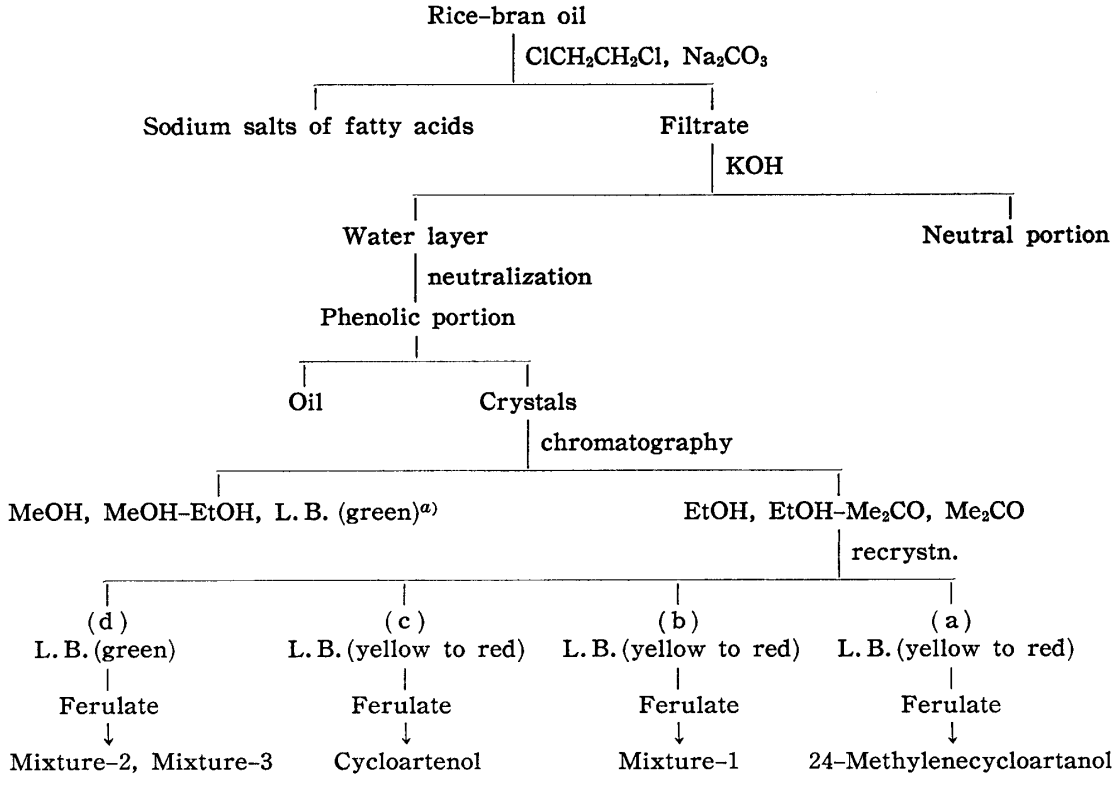


Fig. 1. Separation of a Mixture of Three Terpenoids (V, VI and VII)



a) The Liebermann-Burchard Reaction.

Chart 1.

was injected. As shown in Fig. 1, these compounds were clearly separated on the gas chromatogram, although both reversed-phase chromatography and thin layer chromatography were unsuccessful for this purpose. The retention times of each compound were almost the same as compared to those of the pure substances.

Mixture-1 in Table I (originally designated as Alcohol-B), having a constant melting point of 114.5~115.5° (yellow to red color in the Liebermann-Burchard reaction), was proved to be a mixture of cycloartenol (VI) and 24-methylenecycloartanol (VII) as already described in Part V of this series. The gas chromatogram of this substance shows a small shoulder of relative retention time 2.82 along with two more intense peaks of 3.02 and 3.43 which correspond to VI and VII respectively. This small shoulder seemed to be due to cycloartanol (V) and when small quantity of V was added to this substance only this peak became more intense. From this result and also from the chemical evidence during ozonolysis (see Part V) the natural occurrence of cyclroartanol in minor amounts was ascertained.

Furthermore, Mixture-2 and Mixture-3 in Table I were examined by gas chromatography. These mixtures are cruder fractions as illustrated in Chart 1 and give a green color in the Liebermann-Burchard reaction. Mixture-2 gave two peaks and a shoulder just the same as Mixture-1. Mixture-3 showed an intense peak of relative retention time 2.22 besides the peaks above-mentioned. This peak seemed to arise from a phytosteroid but detailed examination of this substance has not yet been made.

A typical tetracyclic triterpenoid, lanosterol, originating from animal sources always occurs with dihydrolanosterol. This was also indicated in a gas chromatogram of commercial lanosterol just described above. It is interesting that in plants cycloartenol also occurs together with cycloartanol.

The authors wish to express their deep gratitude to Dr. Nobuo Ikekawa, Institute of Physical and Chemical Research and Mr. Yoshihiro Sato, Institute of Applied Microbiology, University of Tokyo, for their valuable suggestions about gas chromatography. They are also indebted to Dr. Junzo Shinoda, Chairman of Board of Directors, Dr. Takeo Ishiguro, President of this Company for their kind encouragement.

Summary

Gas chromatographic properties of several triterpenoid alcohols from rice-bran oil were demonstrated. These alcohols are pure enough from their gas chromatograms and further natural occurence of cycloartanol was ascertained by gas chromatography.

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