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STEROL LIPIDS IN RICE BRAN

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ABSTRACT

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Sterol lipids were isolated from rice bran and systematically analyzed for their chemical compositions. Six classes of sterol lipids—free 4-demethylsterol, free 4-monomethylsterol, free 4,4-dimethylsterol, sterolester, sterylglycoside, and acylsterylglycoside—were found. The principal fatty acid components of sterolester were linoleic and oleic acid in decreasing order, whereas those of acylsterylglycoside were linoleic, palmitic, and oleic acid. The principal 4-demethylsterol components of free 4-demethylsterol, sterolester, sterylglycoside, and acylsterylglycoside were β-sitosterol, campesterol, and stigmasterol. The principal 4-monomethylsterol components of free 4-monomethylsterol and sterolester were gramisterol and citrostadienol. The principal 4,4-dimethylsterol components of free 4,4-dimethylsterol and sterolester were 24-methylenecycloartanol and cycloartenol. The component sugar was only D-glucose both in sterylglycoside and acylsterylglycoside. Possible metabolic relations of sterol lipids were suggested on the basis of their chemical compositions.

Several sterol lipids are known to be present in rice grain (1). However, general and systematic studies on sterol lipids in the rice grain have not been carried out in detail. The present paper describes isolation, identification, chemical composition, and possible metabolic relations of sterol lipids—free 4-demethylsterol (free S), free 4-monomethylsterol (free MMS), free 4,4-dimethylsterol (free DMS), sterolester (SE), sterylglycoside (SG), and acylsterylglycoside (ASG)—in rice bran.

MATERIALS AND METHODS

Extraction and Fractionation of Total Lipids

Rice bran was prepared from Shin-ei (yield: 20.3%), a variety of rice which had been harvested at Hokkaido Prefecture in 1973 and 1974, steamed at 100° C for 3 min, and extracted four times with 3 vol of chloroform-methanol (2:1) and twice with water-saturated butanol, respectively. The extracts were combined, washed with water (2), dehydrated with sodium sulfate, and evaporated to obtain total lipids. Yield was approximately 10.9% for the rice bran. The lipids were successively subjected to silicic acid column chromatography with chloroform,

acetone, and methanol (3). Neutral lipid fraction was eluted with chloroform, glycolipid fraction with acetone, and phospholipid fraction with methanol, respectively.

Fractionation of Sterol Lipids

The neutral lipid fraction was applied to a silicic acid column and eluted stepwise with hexane-benzene and hexane-diethyl ether (4). SE was eluted with hexane-benzene (85:15); free S, free MMS, and free DMS with hexane-diethyl ether (85:15). SE was subjected to preparative silica gel G thin-layer chromatography with hexane-benzene (1:1); free S, free MMS, and free DMS were pretreated with methanolic 1*N* KOH followed by preparative silica gel G thin-layer chromatography with hexane-diethyl ether (80:30). Purified SE, free S, free MMS, and free DMS were thus isolated.

The glycolipid fraction was subjected to silicic acid column chromatography by a stepwide elution with chloroform-acetone (5). The eluates with chloroform-acetone, 9:1 and 7:3, contained ASG and SG, respectively. Each glycoside was run through another silicic acid column followed by preparative silica gel G thin-layer chromatography with chloroform-methanol (95:12) repeatedly. Purified ASG and SG were thus isolated.

Infrared Spectrometry of Sterol Lipids

Infrared spectra were taken on an infrared spectrophotometer (IR-G type, Nippon Bunko kogyo Co. Ltd., Tokyo), using 300 mg KBr pellets for each $2 \sim 3$ mg sterol lipids.

Degradation of Sterol Lipids

The experimental procedures for degradation of SE, ASG, and SG were identical to those reported previously (6).

Gas-Liquid Chromatography

Analyses were performed with a Hitachi Gas Chromatograph (Model 063, Hitachi Seisakusho Co. Ltd., Tokyo) equipped with a hydrogen-flame ionization detector. A glass tube of 0.3×200 cm was used for the column and N_2 as carrier gas. The column was packed with 10% diethyleneglycolsuccinate polyester on Chromosorb W and the column temperature was held at 175° C for analysis of fatty acid methylesters. Methylglycosides were chromatographed after trimethylsilylation (7) through a column packed with 5% SE-30 on Chromosorb W and kept at 170° C. S, MMS, and DMS were chromatographed through a column packed with 1.5% OV-17 on Chromosorb W. The column was operated at 250° C with the carrier gas flow at 50 ml/min (8).

Peaks revealed in the chart were identified by comparing the retention times with those of standards (myristic, palmitic, stearic, oleic, and linoleic acid, methylglucoside, cholesterol, β-sitosterol, and campesterol) and by referring to relative retention times in the literature (8,9).

Gas Chromatography-Mass Spectrometry

The main peak compounds in S, MMS, and DMS were analyzed with a Hitachi Gas Chromatograph-Mass Spectrometer (Model RMU-6MG, Hitachi Seisakusho Co. Ltd., Tokyo). The chromatograph was fitted with a glass

column, 0.3 × 100 cm, packed with Diasolid-ZS. As operating conditions, column temperature of 240°C, ionization voltage of 20 eV, trap current of 80 μA, ion source temperature of 200°C, and accelerated voltage of 3.2 kV were adopted, respectively.

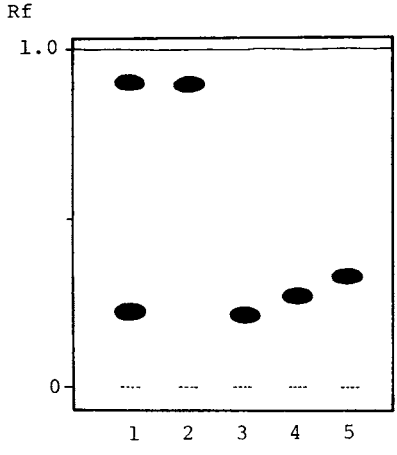

Fig. 1. Thin-layer chromatogram of neutral sterol lipids in rice bran. Developed by hexane-diethyl ether-acetic acid (80:30:10 and detected by 50% sulfuric acid. 1, Mixture of SE and free S, standards; 2, SE from rice bran; 3, free S from rice bran; 4, free MMS from rice bran; 5, free DMS from rice bran.

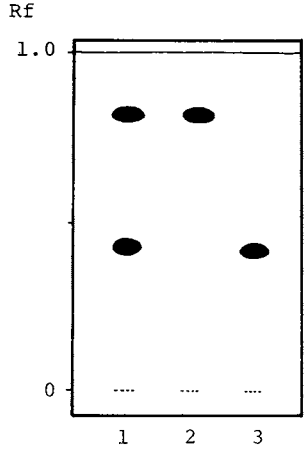

Fig. 2. Thin-layer chromatogram of sterol glycosides in rice bran. Developed by chloroform-methanol (95:12) and detected by 50% sulfuric acid. 1, Mixture of SG and ASG, standards; 2, ASG from rice bran; 3, SG from rice bran.

RESULTS

Thin-Layer Chromatograms of Sterol Lipids

Thin-layer chromatograms of neutral sterol lipids and polar sterol lipids isolated from rice bran are shown in Fig. 1 and Fig. 2. Sterol lipids gave single spots on the plate and R_f values agreed with those of the standards. From the R_f values, stability against alkaline treatment and a color reaction with sulfuric acid differing from that of free S, compounds 4 and 5 in Fig. 1 were tentatively identified as free MMS and free DMS (8,10), respectively. After hydrolysis of SE, three spots, which had the same R_f values as those of free S, free MMS, and free DMS, were detected. Both the sterol glycosides contained only S.

A spot, which was seen between triglyceride and free fatty acid on the thin-layer plate seemed to be ferulic ester (11), but could not be investigated further because only a minor amount was present.

Infrared Spectra of Sterol Lipids

Infrared spectra of sterol lipids from rice bran are shown in Fig. 3 and Fig. 4. Absorptions at 2940, 2860, 1467, 1445, 1385, and 1370 cm^{-1} based on methylene and methyl groups in sterol and fatty acid structure were indicated in the spectra of all the sterol lipids. An absorption for hydroxyl group was seen at 3440 cm^{-1} in the spectra of free S, free MMS, free DMS, SG, and ASG, but not in that of SE. A strong absorption at 1730 cm^{-1} present in the spectra of SE and ASG was attributed to ester bond. A broad absorption in the region of 1125 to 1000 cm^{-1}, characteristic for sugar, was recognized in the spectra of SG and ASG. The

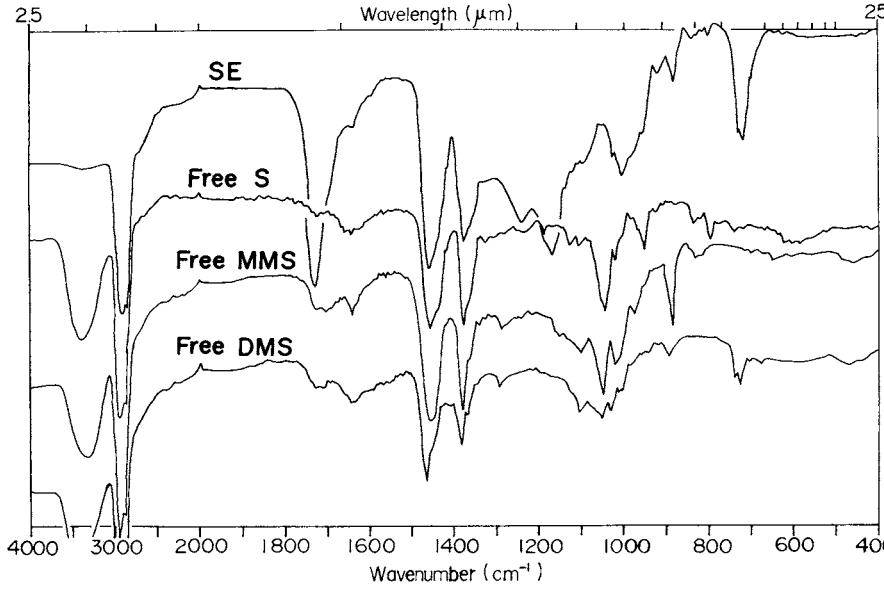

Fig. 3. Infrared spectra of neutral sterol lipids in rice bran.

infrared spectra of free S, SG, and ASG were in agreement with those from pea seed (6).

Fatty Acid Composition of Sterol Lipids

Fatty acid composition, analyzed by gas-liquid chromatography, of SE and ASG obtained from rice bran is shown in Table I. Seven fatty acids were found in both SE and ASG. The principal component fatty acids in decreasing order were linoleic and oleic acids in SE, whereas linoleic, palmitic, and oleic acids were in ASG.

TABLE I
Fatty Acid Composition of Sterol Lipids in Rice Bran

Fatty Acid[a]	SE %	ASG %
14:0	trace	1.4
16:0	7.4	29.9
16:1	0.5	0.6
18:0	0.9	1.2
18:1	30.4	22.7
18:2	58.3	42.5
18:3	2.5	1.7

[a]In (m:n), m and n indicate carbon number and double-bond number, respectively.

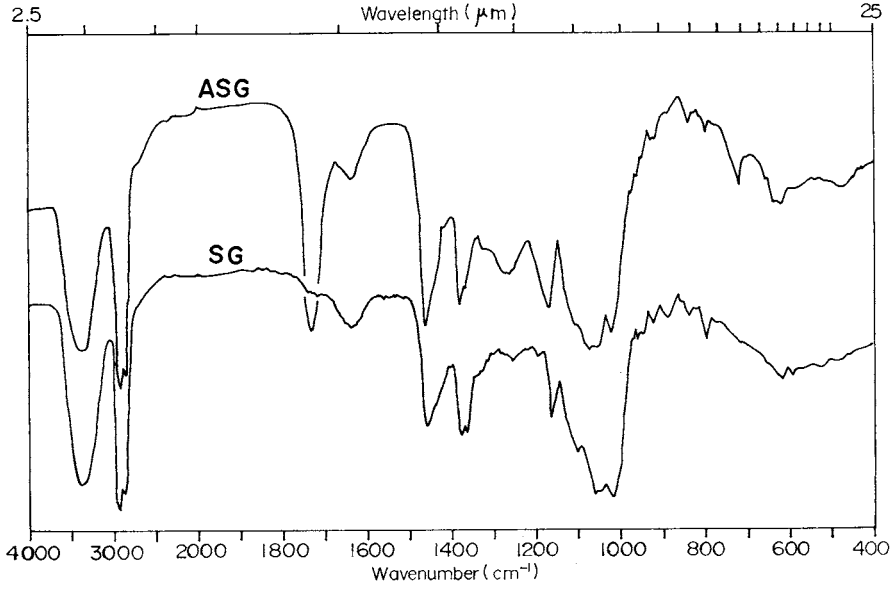

Fig. 4. Infrared spectra of sterol glycosides in rice bran.

S Composition of Sterol Lipids

Table II shows S compositions analyzed by gas-liquid chromatography of free S, SE, SG, and ASG from rice bran. Seven components S in SE, six in free S, and four both in SG and ASG were found, respectively. In each case, the predominant components were β-sitosterol, campesterol, and stigmasterol, among which β-sitosterol was preponderant. Brassicasterol (8) was not detected. These results reflected the general pattern of free S in plants (12). β-Sitosterol was confirmed by gas chromatography-mass spectrometry as shown in Fig. 5, where m/e 414 for the molecular ion, m/e 329 [M−(67+18)] and m/e 303 [M−

TABLE II
S Composition of Sterol Lipids in Rice Bran

S	SE %	Free S %	ASG %	SG %
Cholesterol	1.3	1.8	...	...
Campesterol	20.2	16.2	8.0	9.3
Stigmasterol	9.2	18.8	11.3	11.8
β-Sitosterol	51.6	57.2	80.2	77.2
Δ^5-Avenasterol	11.4	6.0	...	...
Δ^7-Stigmasterol	3.6	trace	0.5	1.0
Δ^7-Avenasterol	2.7	...	...	...

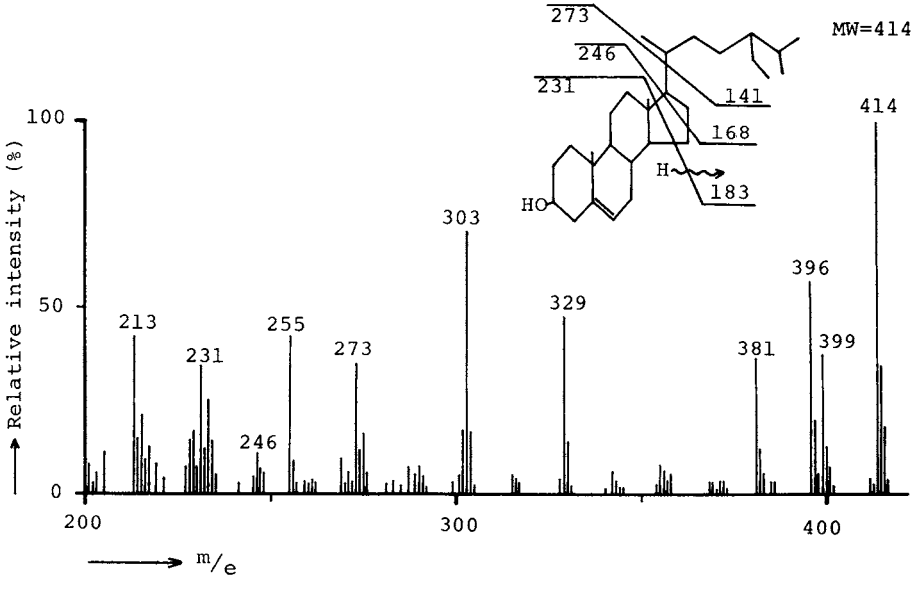

Fig. 5. Mass spectrum of β-sitosterol in rice bran.

(93+18)] for the Δ^5-sterol (13), and m/e 273 [M−141], m/e 255 [M−(141+18)], m/e 246 [M−(141+27)], m/e 231 [M−(141+42)], and m/e 213 [M−(141+42+18)] for the saturated C_{10} side chain were recognized. Campesterol was also characterized by gas chromatography-mass spectrometry, where m/e 400 for the molecular ion, m/e 315 [M−(67+18)] and m/e 289 [M−(93+18)] for the Δ^5-sterol, m/e 273 [M−127], m/e 255 [M−(127+18)], m/e 246 [M−(127+27)], m/e 231 [M−(127+42)], and m/e 213 [M−(127+42+18)] for the saturated C_9 side chain were observed.

MMS Composition of Sterol Lipids

Table III shows MMS composition analyzed by gas-liquid chromatography of SE and free MMS from rice bran. Five MMS were found in both, though two minor components could not be identified. Predominant components were gramisterol and citrostadienol, particularly the former, which comprised over one-half of MMS. These two MMS have usually been detected in large quantities

TABLE III
MMS Composition of Sterol Lipids in Rice Bran

MMS	SE %	Free MMS %
Obtusifoliol	9.5	7.7
?	1.5	1.4
Gramisterol	55.4	60.5
?	5.7	6.6
Citrostadienol	27.9	23.6

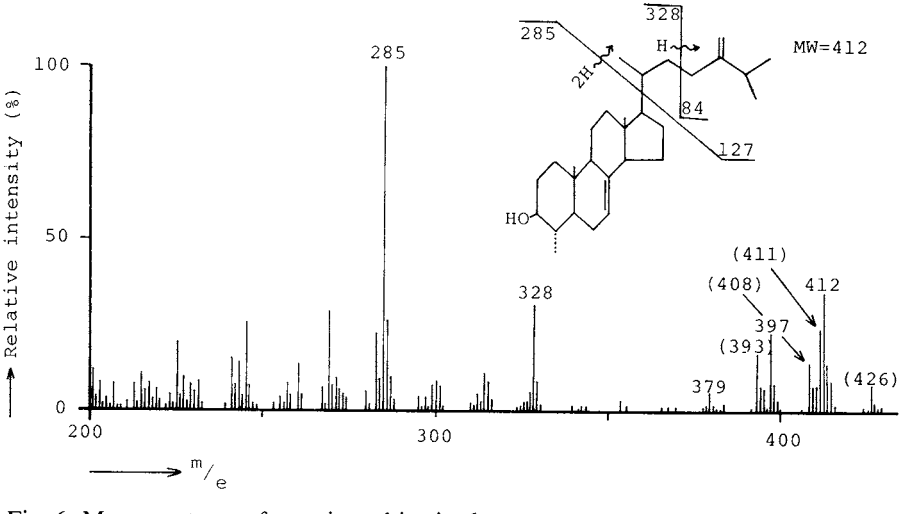

Fig. 6. Mass spectrum of gramisterol in rice bran.

in vegetable oils (9,14). Gramisterol was characterized by gas chromatography-mass spectrometry as shown in Fig. 6, where m/e 412, m/e 397 [M−15], and m/e 379 [M− (15+18)] for mol wt, m/e 328 [M−84] and m/e 285 [M−127] for $\Delta^{7,24}$ $^{(28)}$-structure (13) were observed, respectively. Ions seen in the chart at m/e 426, 411, 408, and 393 may indicate that cycloeucalenol (mol wt = 426) was present in a small amount, but was inseparable from gramisterol (9).

DMS Composition of Sterol Lipids

Table IV shows DMS composition analyzed by gas-liquid chromatography of SE and free DMS. Five components were found in both sterols. Predominant components were 24-methylenecycloartanol and cycloartenol; the former was close to 70% of DMS. These two DMS have generally been found in large amounts in vegetable oils (9,14). 24-Methylenecycloartanol was confirmed by gas chromatography-mass spectrometry as shown in Fig. 7, where m/e 440, m/e 425 [M−15], and m/e 407 [M− (15+18)] for mol wt, m/e 300 [M−140], m/e 285 [M− (140+15)], and M/e 175 [M− (140+125)] for 9:19-cyclopropane ring (13),

TABLE IV
DMS Composition of Sterol Lipids in Rice Bran

DMS	SE %	Free DMS %
Cycloartanol	2.5	3.9
Cycloartenol	26.6	19.6
24-Methylenecycloartanol	67.9	67.0
?	2.1	6.6
Cyclobranol	0.9	2.9

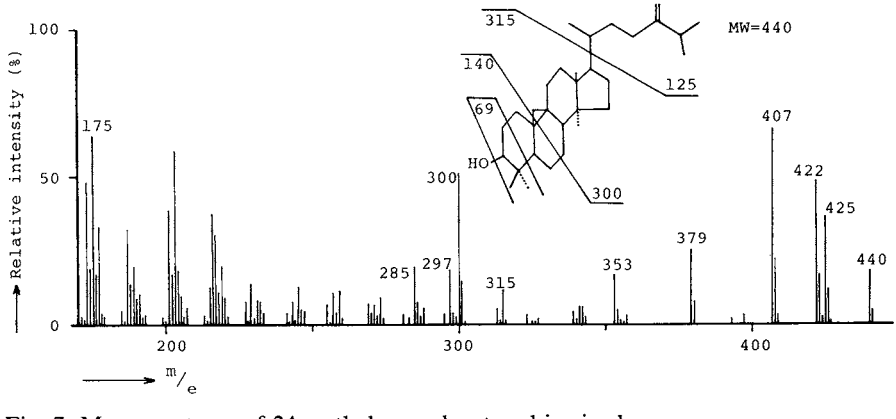

Fig. 7. Mass spectrum of 24-methylenecycloartanol in rice bran.

m/e 353 [M− (69+18)] for 4,4-dimethyl group (13), m/e 315 [M−125] and m/e 297 [M− (125+18)] for side chain, and m/e 379 [M− (43+18)] for this sterol (13) were found, respectively.

Sugar Composition of Sterol Lipids

Component sugars analyzed by gas-liquid chromatography of sterol glycosides obtained from rice bran are shown in Fig. 8. Only D-glucose was detected both in SG and ASG. It has been reported that the component sugar of free and esterified sterylglycosides is generally glucose (6, 15−18).

DISCUSSION

Sterol lipids in rice bran were fractionated into six classes and analyzed for the components S, MMS, DMS, fatty acid, and sugar. The compositions of S, MMS, and DMS from SE were, respectively, like those from free S, free MMS, and free DMS (Tables II, III, and IV). The results indicate that SE is metabolically closely related to free S, free MMS, and free DMS. The compositions of sugar and S in SG were similar to those in ASG (Fig. 8, Table II). The results demonstrate that SG is metabolically closely related to ASG.

Neutral sterol lipids (SE and free sterols) contained not only S but also MMS and DMS, whereas polar sterol lipids (ASG and SG) contained only S. The sterol pattern of neutral sterol lipids was different from that of polar sterol lipids (Table II). In addition, the fatty acid composition of SE was quite different from that of ASG (Table I). The results suggest that neutral sterol lipids and polar sterol lipids might be considerably different from each other metabolically as well as in constitutional structure.

S is known to be formed from squalene via DMS and MMS in many plants. In general, the main components of S, MMS, and DMS in rice bran were similar to

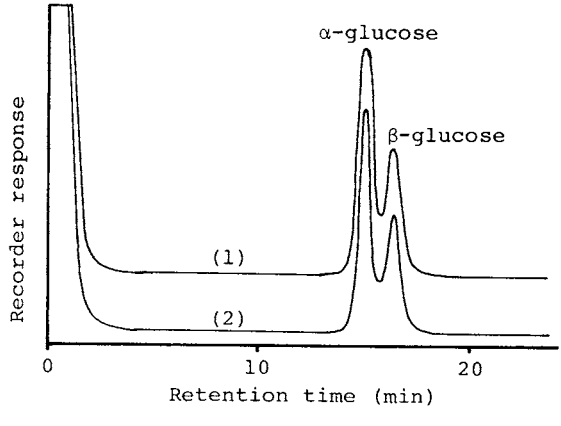

Fig. 8. Gas-liquid chromatogram of trimethylsilyl ether derivatives of methylglycosides obtained from SG (1) and ASG (2) in rice bran.

those of higher plants (8,9). The data suggest that the biosynthetic pathways of DMS, MMS, and S in rice bran are similar to those of higher plants.

Literature Cited

1. JULIANO, B. O. The rice caryopsis and its composition. In Rice: Chemistry and technology, ed. by D. F. Houston, Vol. IV, p. 16. Amer. Ass. Cereal Chem.: St. Paul, Minn. (1972).
2. FOLCH, J., LEES, M., and STANLEY, G. H. S. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497 (1957).
3. ROUSER, G., KRITCHEVSKY, G., SIMON, G., and NELSON, G. J. Quantitative analysis of brain and spinach leaf lipids employing silicic acid column chromatography and acetone for elution of glycolipids. Lipids 2: 37 (1968).
4. BARRON, E. J., and HANAHAN, D. J. Observations on the silicic acid chromatography of the neutral lipids of rat liver, beef liver and yeast. J. Biol. Chem. 231: 493 (1958).
5. VORBECK, M. L., and MARINETTI, G. V. Separation of glycosyl diglycerides from phosphatides using silicic acid column chromatography. J. Lipid Res. 6: 3 (1965).
6. MIYAZAWA, T., ITO, S., and FUJINO, Y. Sterol lipids isolated from pea seeds. Cereal Chem. 51: 623 (1974).
7. CARTER, H. E., and GAVER, R. C. Improved reagent for trimethylsilylation of sphingolipid bases. J. Lipid Res. 8: 391 (1967).
8. ITO, T., TAMURA, T., and MATSUMOTO, T. Sterol composition of 19 vegetable oils. J. Amer. Oil Chem. Soc. 50: 122 (1973).
9. ITO, T., TAMURA, T., and MATSUMOTO, T. Methylsterol compositions of 19 vegetable oils. J. Amer. Oil Chem. Soc. 50: 300 (1973).
10. JEONG, T. M., TAMURA, T., ITO, T., and MATSUMOTO, T. Cycloeucalenol in rice bran oil (in Japanese). Yukagaku 22: 153 (1973).
11. ENDO, T., UENO, K., and INABA, Y. Studies on the ferulates contained in rice bran oil (in Japanese). Yukagaku 17: 344 (1968).
12. NODA, M. Lipids of seeds (in Japanese). Yukagaku 20: 708 (1971).
13. TAMURA, T., and IIDA, T. Isolation and identification of natural sterols (in Japanese). Yukagaku 22: 581 (1973).
14. JEONG, T. M., ITO, T., TAMURA, T., and MATSUMOTO, T. Analysis of methylsterol fractions from twenty vegetable oils. Lipids 10: 634 (1975).
15. KIRIBUCHI, T., CHEN, C., and FUNAHASHI, S. Systematic analysis of sterols in soybeans and other oilseeds. Agr. Biol. Chem. 29: 265 (1965).
16. KIRIBUCHI, T., MIZUNAGA, T., and FUNAHASHI, S. Separation of soybean sterols by florisil chromatography and characterization of acylated steryl glucoside. Agr. Biol. Chem. 30: 770 (1966).
17. LEPAGE, M. Isolation and characterization of an esterified form of steryl glucoside. J. Lipid Res. 5: 587 (1964).
18. ITO, S., and FUJINO, Y. Steryl glucoside in alfalfa leaves (in Japanese). Nippon Nogeikagaku kaishi 47: 229 (1973).

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