NOTE

Wax Lipid in Rice Bran

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Wax on the outer cuticular surface of plants is known as one of the functional lipids, which protect plants from hydration and dehydration as well as from damage by microorganisms, insects, and other elements. Lipids in rice grains have been well studied (Fujino 1978),[1] but only a few studies have been done on wax. We describe the isolation and the chemical characterization of wax lipid from rice bran.

About 0.6 kg of rice bran, prepared from 4 kg of nonglutinous brown rice (Tomoyutaka var.) harvested in 1976 at Hokkaido Central Agricultural Experimental Station, Naganuma, Japan, was extracted with chloroform-methanol (2:1) and water-saturated butanol (Acker and Schmitz 1967) to obtain the total lipid (12%). This was chromatographed on a silicic-acid column (Rouser et al 1967) to separate into fractions of neutral lipid, glycolipid, and phospholipid. The ratio of each fraction was approximately 91:4:5. The neutral lipid fraction was further applied to a silicic-acid column (Barron and Hanahan 1958) to isolate the wax fractions of sterylester, longer alkylester, and shorter alkylester. These were then purified by preparative silica gel G thin-layer chromatography (TLC) with a developing solvent of hexane-benzene (2:1) and magnesium oxide TLC (Nicolaides 1970). The wax lipids thus obtained gave a single spot on both TLC plates and were identified by infrared spectrophotometry, which showed that the absorptions were due to the CH_3 group (2960, 2870, 1460, 1380 cm^{-1}), the CH_2 group (2930, 2850, 1460 cm^{-1}), ester carbonyl (1740, 1180 cm^{-1}), and skeletal vibration of CH_2 group (720 cm^{-1}), respectively. These were then analyzed with a gas chromatograph-mass spectrometer. Sterylester and longer alkylester were analyzed using a glass column (0.3 × 100 cm) packed with Diasolid ZT at 300°C. Shorter alkylester was also done as the temperature increased from 160 to 210°C at the rate of 4°C/min. The temperatures of molecular separator and ion source were 300 and 220°C, respectively. Ionizing voltage was 20 eV, and trap current was 70 μA.

Yields of sterylester, longer alkylester, and shorter alkylester were 9.5% of the neutral lipid fraction. The ratio of the three compounds was about 8:1:1.

A gas chromatogram of sterylester and its molecular species obtained by mass spectrometry is shown in Fig. 1 and further explained in Table I. In the major peak 3 (61%), the fragment ions of m/e 281 (linoleic acid) and 283 (oleic acid) due to fatty acid moieties were detected; linoleic acid was predominant, as judged from the relative ionic intensity. The ion of m/e 396 due to sitosterol moiety was also recognized. These data showed that the principal molecular species of sterylester in peak 3 was linoleoyl sitosterol. Similarly, the main molecular species in peaks 1 and 2 were identified as palmitoyl sitosterol, and linoleoyl campesterol, respectively. The main sterol components of sterylester in rice bran reportedly are sitosterol and campesterol (Kuroda et al 1977).

The composition of longer alkylester is shown in Table II. The gas chromatogram gave ten peaks, and the mass spectrum of each peak lipid showed that the main carbon numbers of longer alkylester were C_{44}, C_{46}, C_{54}, and C_{56}. As the major ions, m/e 257 (palmitic acid) and 392 (octacosanol) were detected for C_{44}, 257 (palmitic acid) and 420 (triacontanol) for C_{46}, 341 (behenic acid) and 448 (dotriacontanol) for C_{54}, and 341 (behenic acid) and 476 (tetratriacontanol) for C_{56}. The data and the peak areas calculated from the gas chromatogram showed that the predominant molecular species of longer alkylesters were behenoyl dotriacontanol, palmitoyl octacosanol, behenoyl tetratriacontanol, and palmitoyl

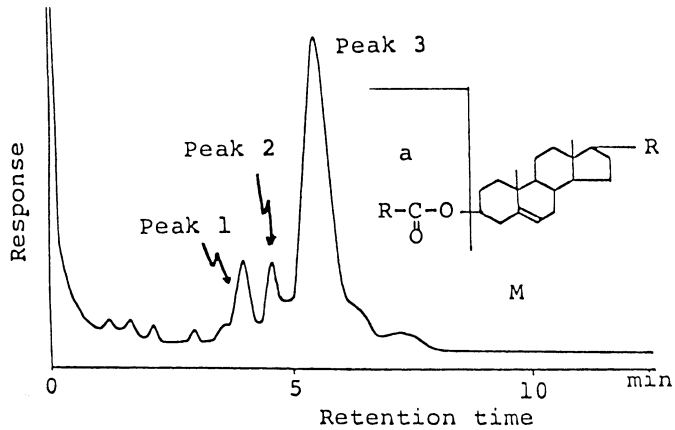

Fig. 1. Gas chromatogram of sterylester in rice bran.

TABLE I
Molecular Species of Sterylester in Rice Bran

	Peak 1		Peak 2		Peak 3	
	(m/e)[a]	(%)	(m/e)[a]	(%)	(m/e)[a]	(%)
Fragment ions						
M	652	2	662	5	676	3
M − (a+H)	396	100	382	100	396	100
a + 2H	257 (16:0)	12	283 (18:1)	15	283 (18:1)	11
			281 (18:2)	34	281 (18:2)	17
Molecular species (fatty acid–sterol[b]	16:0–29:1		18:2–28:1 18:1–28:1		18:2–29:1 18:1–29:1	

[a] m/e = Ratio of mass/electron.
[b] Carbon number and number of double bond.

TABLE II
Composition of Longer Alkylester in Rice Bran

Carbon Number	Major Molecular Species[a]			Percent Esters
38	16:0–22:0			1.1
40	16:0–24:0	18:0–22:0		2.1
42	16:0–26:0	22:0–20:0		7.9
44	16:0–28:0	22:0–22:0		16.4
46	16:0–30:0	22:0–24:0		12.5
48	16:0–32:0	22:0–26:0		10.3
50	22:0–28:0	24:0–26:0		6.2
52	18:1–34:0	22:0–30:0	24:0–28:0	8.9
54	22:0–32:0	24:0–30:0		16.6
56	22:0–34:0	24:0–32:0		13.6
58	22:0–36:0	24:0–34:0		4.4

[a] Number of carbon atoms in acid and alcohol moiety - number of double bonds present.

[1] B. O. Juliano. Rice lipids. Presented at 61st AACC Annual Meeting, New Orleans, LA, October 1976.

TABLE III
Composition of Shorter Alkylester in Rice Bran

Carbon Number	Molecular Species[a]	Percent Esters
15	14:0–1:0	2.2
17	16:0–1:0	29.8
18	16:0–2:0	5.5
19	18:0–1:0	0.8
19	18:1–1:0	43.4
19	18:2–1:0	1.5
20	18:1–2:0	16.8

[a]Number of carbon atoms in acid and alcohol moiety - number of double bonds present.

triacontanol, in decreasing order. The component alcohols of longer alkylester in rice bran were reported to be tetracosanol, hexacosanol, octacosanol (Cousins et al 1953), octacosanol, triacontanol, tetratriacontanol, and hexatriacontanol (Takagi 1978). Results of our study agreed closely with the latter.

Shorter alkylester was composed mainly of methyl oleate, methyl palmitate, and ethyl oleate (Table III). In the mass spectrometric analysis of shorter alkylester, the characteristic ions of m/e 59 (CH_3OCO), 74 ($CH_3OC(OH)CH_2$), 87 ($CH_3OC(OH)CHCH_2$), 196 (M-74), and 239 (M-31) were detected for methyl palmitate and 222 (M-74) and 265 (M-31) for methyl oleate, and those of m/e 73 (C_2H_5OCO), 88 ($C_2H_5OC(OH)CH_2$), 101 ($C_2H_5OC(OH)CHCH_2$), 222(M-45), and 265 (M-45) for ethyl oleate. The shorter alkylester should not be artifactual because the composition of fatty acids of shorter alkylester was different from that of free fatty acid in rice bran (Sakata et al 1973) and because ethanol was not employed in the experimental procedure.

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