Resin Glycosides. VII.[1] Reinvestigation of the Component Organic and Glycosidic Acids of Pharbitin, the Crude Ether-Insoluble Resin Glycoside ("Convolvulin") of Pharbitidis Semen (Seeds of *Pharbitis nil*)

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Alkaline hydrolysis of the ether-insoluble resin glycoside ("convolvulin") fraction, pharbitin, of the seeds of *Pharbitis nil* (Convolvulaceae) provided three organic acids, *S*-2-methylbutyric acid, (2*R*,3*R*)-nilic acid and tiglic acid, and a new glycosidic acid named pharbitic acid B along with the known compounds pharbitic acids C and D. Pharbitic acid B was characterized as (3*S*,11*S*)-3,11-dihydroxyhexadecanoic acid 11-*O*-α-L-rhamnopyranosyl-(1→3)-*O*-β-D-quinovopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-[*O*-α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside. The structures of the sugar moieties of pharbitic acids C and D were revised.

Keywords resin glycoside; pharbitin; Pharbitidis Semen; *Pharbitis nil*; glycosidic acid; pharbitic acid B, C, D; (3*S*,11*S*)-ipurolic acid oligoside; (3*S*,11*S*)-3,11-dihydroxyhexadecanoic acid oligoside; hydroxyfatty acid FD-MS

Pharbitidis Semen, the seeds of *Pharbitis nil* CHOISY (Convolvulaceae), is a purgative crude drug and its resin glycoside is known to be a typical Mayer's "convolvulin," an ether-insoluble resin glycoside.[2,3] The first systematic chemical investigation on the resin glycoside of this plant was conducted by Kromer,[4] who obtained angelic or tiglic acid and a tetrahydroxydecanoic acid along with a glycosidic acid by alkaline hydrolysis of the resin, and D-glucose and two crystalline fatty acids (mp 68.5 °C and 99 °C) by acidic hydrolysis of the glycosidic acid. Later, Asahina and his co-workers[5] conducted a more detailed investigation and reported that alkaline hydrolysis of the crude glycoside named pharbitin gave, together with (+)-2-methylbutyric and tiglic acids, an organic acid named nilic acid (2-methyl-3-hydroxybutyric acid) and a glycosidic acid named pharbitic acid which was composed of ipurolic acid (3,11-dihydroxytetradecanoic acid), D-glucose and L-rhamnose.

In 1970, Okabe and *et al.*[6] isolated two glycosidic acids named pharbitic acids C and D along with valeric, tiglic, nilic and (+)-2-methylbutyric acids from the alkaline hydrolysate of pharbitin. The glycosidic acids were respectively characterized as ipurolic acid 11-*O*-penta- (**6′**) and 11-*O*-hexaglycoside (**7′**), as shown in Fig. 1.

Prior to study of the genuine resin glycosides of this plant, a reinvestigation of the chemical components of pharbitin was carried out. This paper deals with the reexamination of the structures of pharbitic acids C and D, and characterization of a new glycosidic acid named pharbitic acid B.

The crushed seeds of *Pharbitis nil* were percolated with MeOH and the extractive was partitioned between *n*-BuOH and water. The *n*-BuOH extractive was defatted with ether and the residue was chromatographed over highly porous polymer gel (MCI gel CHP 20P) to afford a crude resin glycoside, pharbitin, as a pale yellow powder, mp 124—140 °C, in the yield of 3.6% based on the crude drug. Alkaline hydrolysis of pharbitin with 1% aqueous K_2CO_3 yielded organic acid and glycosidic acid (pharbitic acid) fractions. Gas chromatography (GC) of the methyl ester of the former revealed the presence of 2-methylbutyric (**1**), tiglic (**2**) and nilic (**3**) acids in the ratio of about 17 : 1 : 11.

The organic acid fraction was acylated with *p*-bromophenacyl bromide followed by chromatographic separation to afford *p*-bromophenacyl 2-methylbutyrate (**1a**) as needles, mp 52—53 °C, $[\alpha]_D$ +11.9°, and *p*-bromophenacyl nilate (**3a**) as needles, mp 98—99 °C, $[\alpha]_D$ −2.2°. The absolute configurations of **1** and **3** were defined as 2*S* and 2*R*,3*R*, respectively, by comparison of the specific rotations of their *p*-bromophenacyl esters with those of the compounds obtained from *Ipomoea muricata*.[7]

Acid hydrolysis of pharbitic acid yielded aglycone and monosaccharide fractions. Methylation of the former with diazomethane yielded two hydroxyfatty acid methyl esters, **4**, colorless needles, mp 69—70 °C, $[\alpha]_D$ +1.2° and **5**, colorless needles, mp 72.5—74.0 °C, $[\alpha]_D$ +0.2°.

Compound **4** showed signals due to two methines adjacent to secondary hydroxy groups (δ 4.44 and 3.83), an ester group (δ 3.65) and a primary methyl group (δ 0.95) in the ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum. The field desorption mass spectrum (FD-MS) of **4** exhibited the $(M+H)^+$ ion peak at *m/z* 275 and fragment peaks at *m/z*

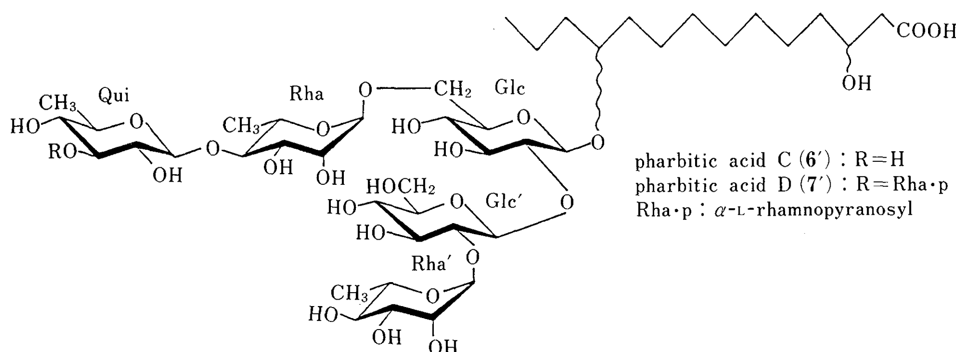

Fig. 1

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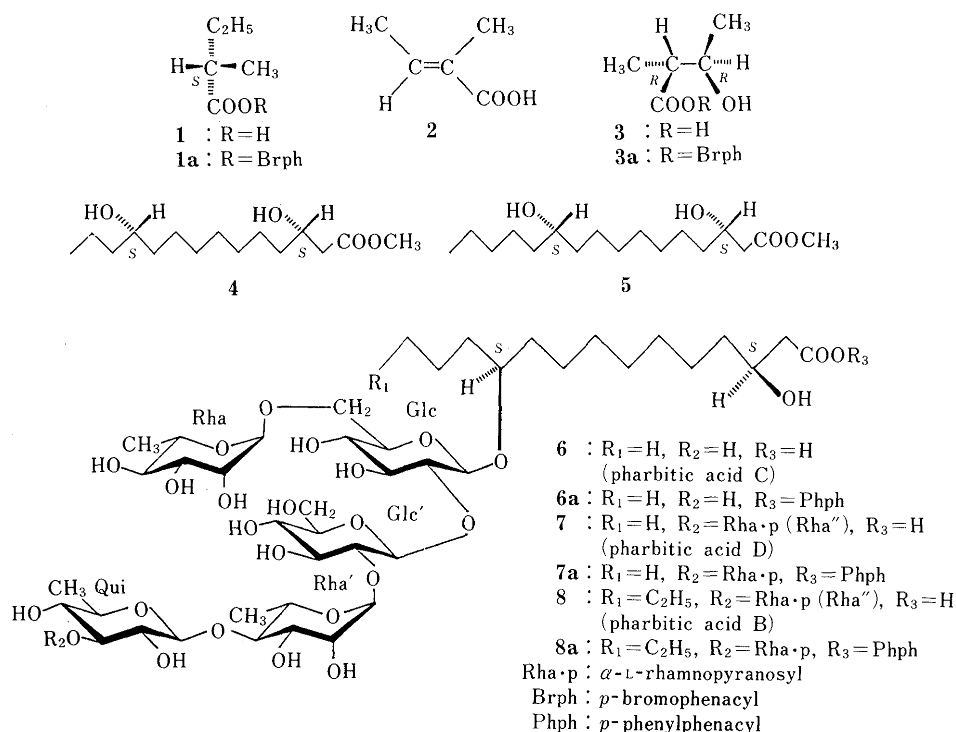

Fig. 2

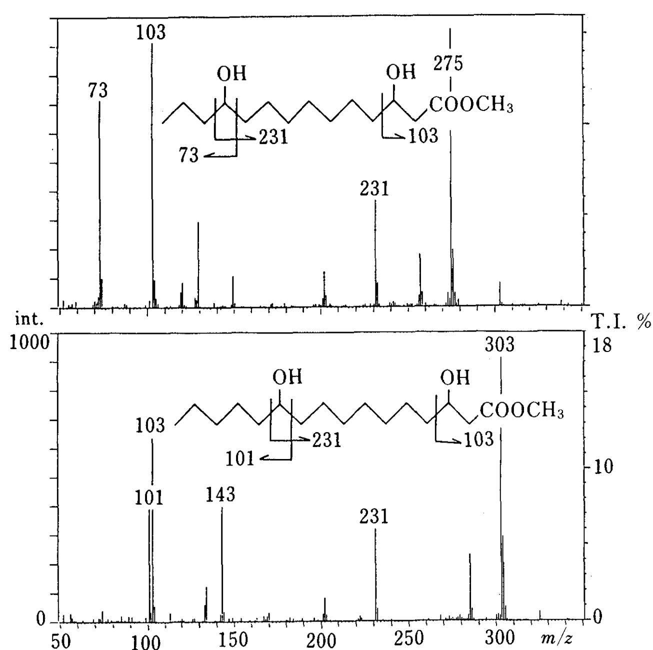

Fig. 3. FD-MS of **4** and **5**

231 $[M-CH_3(CH_2)_2]^+$, 103 $[CH(OH)CH_2COOCH_3]^+$ and 73 $[CH_3(CH_2)_2CHOH]^+$ (Fig. 3). These data indicated that **4** is the methyl ester of dihydroxytetradecanoic acid, in which the hydroxy groups are located at C_3 and C_{11}. The compound was identical with an authentic sample of methyl ipurolate.[6] Further, the configurations at C_3 and C_{11} were determined to be *S* by Mosher's method.[8]

Compound **5** showed a quite similar ^{1}H-NMR spectrum to that of **4**, suggesting **5** to be a new dihydroxyfatty acid. The FD-MS showed the $(M+H)^+$ ion peak at *m*/*z* 303 and fragment peaks at *m*/*z* 231, 103 and 101 $[CH_3(CH_2)_4CHOH]^+$ (Fig. 2). These data indicated **5** to be the methyl ester of 3,11-dihydroxyhexadecanoic acid. The configurations at C_3 and C_{11} were defined as *S* in the same manner as in the case of **4**.[8]

In order to determine the component monosaccharides and their absolute configurations, the sugar fraction was converted into trimethylsilyl (TMS) ethers of diastereomeric thiazolidine derivatives and then analyzed by GC according to Hara *et al.*[9] D-Glucose, L-rhamnose and D-quinovose were detected.

Pharbitic acid was derived into *p*-phenylphenacyl ester and chromatography afforded three glycosidic acid esters, **6a**, a white powder, mp 128—131 °C (dec.), $[\alpha]_D$ −50.6°, **7a**, a white powder, mp 136—140 °C (dec.), $[\alpha]_D$ −63.7°, and **8a**, a white powder, mp 138—142 °C (dec.), $[\alpha]_D$ −57.1°. Compounds **6a** and **7a** gave, on alkaline hydrolysis, free glycosidic acids, **6**, a white powder, mp 130—134 °C (dec.), $[\alpha]_D$ −50.5° and **7**, a white powder, mp 141—146 °C (dec.), $[\alpha]_D$ −66.2°, respectively. The ^{13}C-NMR spectra of **6** and **7** were superimposable on those of pharbitic acids C and D,[6] respectively.

To confirm the structures of **6** and **7**, MS and ^{1}H-, ^{13}C-, chemical shift correlation spectroscopy (COSY), ^{1}H–^{13}C-COSY and nuclear Overhauser effect spectroscopy (NOESY) spectra were recorded. In the negative ion fast atom bombardment mass spectrum (FAB-MS), **6** showed the $(M-H)^-$ ion peak at *m*/*z* 1021 and fragment peaks at *m*/*z* 875 $[1021-146(\text{6-deoxyhexose unit})]^-$, 729 $[875-146]^-$, and 567 $[729-162(\text{hexose unit})]^-$. The ^{1}H-NMR spectrum exhibited the signals of five anomeric protons (δ 6.36, 5.94, 5.40, 5.27 and 4.92) and three secondary methyls due to 6-deoxyhexose (δ 1.92, 1.65 and 1.55) as well as an nonequivalent 2-methylene (δ 2.87 and 2.91) and a primary methyl (δ 0.97) ascribable to an ipurolic acid moiety. All the ^{1}H- and ^{13}C-signals due to the sugar moiety

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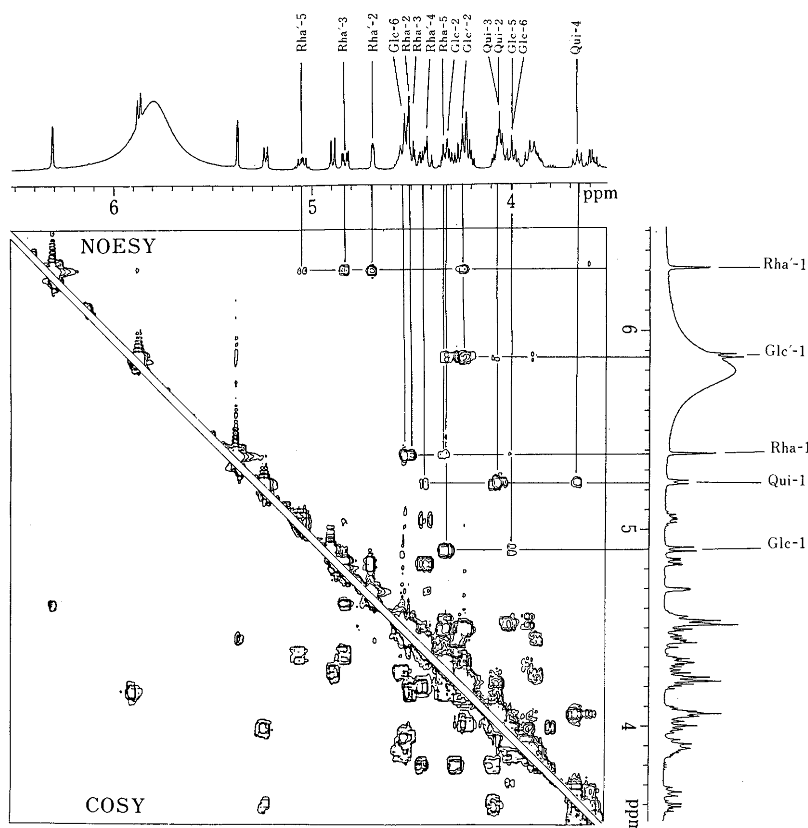

Fig. 4. COSY and NOESY Spectra of **6** (in Pyridine-d_5, 400 MHz)

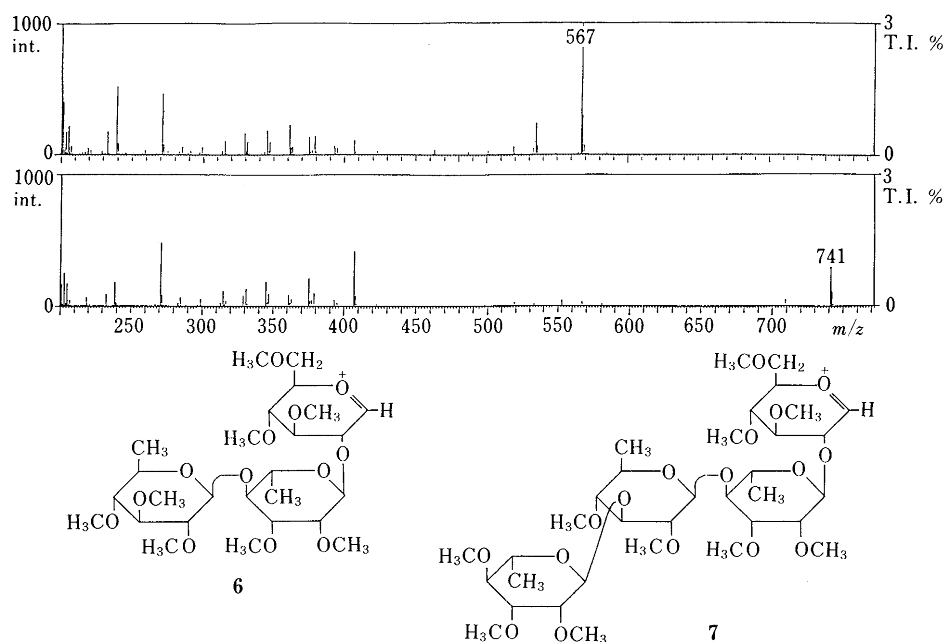

Fig. 5. EI-MS of the Permethylates of **6** and **7**

were assigned with the aid of COSY and ^{1}H–^{13}C-COSY spectra (Tables I and II). The component hydroxyfatty acid and the monosaccharides as well as the sites of glycosidic linkages derived from the above data were consistent with those expected from the structure (**6′**) proposed by Okabe *et al.*[6] (Fig. 1).

However, the NOESY spectrum of **6** showed four cross peaks between 2-H of glucose (Glc) and 1-H of the second glucose (Glc′), 6-H of Glc and 1-H of rhamnose (Rha), 2-H of Glc′ and 1-H of the second rhamnose (Rha′), and 4-H of Rha′ and 1-H of quinovose (Qui), but no peak between 4-H of Rha and 1-H of Qui required for the structure **6′** was observed (Fig. 4). Therefore, the arrangement of monosaccharides in the sugar moiety of **6** should be revised as shown in Fig. 2. This revision was supported by the strong peak at *m/z* 567.3022 ($C_{26}H_{47}O_{13}^{+}$) that was

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TABLE I. ^{1}H-NMR Spectral Data for **6**, **7** and **8** (in Pyridine-d_5, 400 MHz)

	6	7	8
Glc-1	4.92 (d, 7.6)	4.91 (d, 8.0)	4.91 (d, 7.0)
Glc-2	4.37 (dd, 7.6, 9.0)	4.36[a]	4.31[a]
Glc-3	4.57 (dd, 9.0, 9.0)	4.53 (dd, 8.5, 8.5)	4.52[a]
Glc-4	3.94 (dd, 9.0, 9.0)	3.92 (dd, 8.5, 8.5)	3.90 (dd, 8.5, 8.5)
Glc-5	4.01[a]	4.02[a]	3.99[a]
Glc-6	4.02[a]	4.05[a]	4.03[a]
	4.55[a]	4.54[a]	4.53[a]
Glc'-1	5.94 (d, 7.0)	5.91 (d, 7.0)	5.85 (d, 7.6)
Glc'-2	4.25[a]	4.24[a]	4.22[a]
Glc'-3	4.24 (dd, 9.0, 9.0)	4.22 (dd, 9.0, 9.0)	4.19 (dd, 9.0, 9.0)
Glc'-4	4.10 (dd, 9.0, 9.0)	4.08 (dd, 9.0, 9.0)	4.07 (dd, 9.0, 8.5)
Glc'-5	3.88 (ddd, 3.0, 6.0, 9.0)	3.88[a]	3.85 (ddd, 3.0, 6.5, 8.5)
Glc'-6	4.29 (dd, 6.0, 11.0)	4.27[a]	4.26 (dd, 6.5, 11.5)
	4.46[a]	4.46 (dd, 3.5, 11.0)	4.43 (dd, 3.0, 11.5)
Rha-1	5.40 (d, 1.5)	5.42 (d, 1.0)	5.42 (d, 1.2)
Rha-2	4.54[a]	4.52[a]	4.50
Rha-3	4.53[a]	4.52[a]	4.49[a]
Rha-4	4.26 (dd, 9.0, 9.0)	4.25[a]	4.20[a]
Rha-5	4.36 (dq, 9.0, 6.1)	4.34[a]	4.28[a]
Rha-6	1.65 (d, 6.1)	1.66 (d, 6.4)	1.65 (d, 6.1)
Rha'-1	6.36 (d, 1.5)	6.33 (d, 1.6)	6.29 (d, 1.2)
Rha'-2	4.72 (dd, 1.5, 3.3)	4.69 (dd, 1.6, 3.1)	4.67 (dd, 1.2, 3.1)
Rha'-3	4.81 (dd, 3.3, 9.6)	4.79[a]	4.76 (dd, 3.1, 9.4)
Rha'-4	4.46 (dq, 9.6, 9.6)	4.40 (dd, 9.5, 9.5)	4.36 (dd, 9.4, 9.4)
Rha'-5	5.09 (dq, 9.6, 6.1)	5.05 (dq, 9.5, 6.7)	5.02 (dq, 9.4, 6.1)
Rha'-6	1.92 (d, 6.1)	1.89 (d, 6.7)	1.86 (d, 6.1)
Rha"-1		6.17 (d, 1.6)	6.17 (d, 1.2)
Rha"-2		4.78 (dd, 1.6, 3.1)	4.75[a]
Rha"-3		4.60 (dd, 3.1, 9.4)	4.56 (dd, 3.4, 9.1)
Rha"-4		4.32 (dd, 9.4, 9.4)	4.28[a]
Rha"-5		4.93 (dd, 9.4, 6.4)	4.89 (dd, 9.1, 6.1)
Rha"-6		1.66 (d, 6.4)	1.65 (d, 6.1)
Qui-1	5.27 (d, 7.3)	5.14 (d, 8.0)	5.12 (d, 7.9)
Qui-2	4.09[a]	4.03 (dd, 8.0, 9.0)	4.02 (dd, 7.9, 9.0)
Qui-3	4.09[a]	4.19 (dd, 9.0, 9.0)	4.16 (dd, 9.0, 9.0)
Qui-4	3.69 (dd, 9.0, 9.0)	3.56 (dd, 9.0, 9.0)	3.54 (dd, 9.0, 9.0)
Qui-5	3.59 (dq, 9.0, 6.1)	3.55[a]	3.53[a]
Qui-6	1.55 (d, 6.1)	1.52 (d, 5.5)	1.51 (d, 6.7)
Ag-2	2.87 (dd, 15.0, 5.5)	2.87 (dd, 15.0, 5.0)	2.86 (dd, 15.0, 5.0)
	2.91 (dd, 15.0, 8.0)	2.91 (dd, 15.0, 8.0)	2.90 (dd, 15.0, 8.0)
Ag-3	4.45[a]	4.54[a]	4.53[a]
Ag-11	3.90[a]	3.90[a]	3.87[a]
Ag-14	0.97 (t, 7.0)	0.96 (t, 7.0)	
Ag-16			0.87 (t, 7.4)

a) Signals are overlapping. δ in ppm from TMS (coupling constants (J) in Hz are given in parentheses). Glc, glucopyranosyl; Rha, rhamnopyranosyl; Qui, quinovopyranosyl; Ag, aglycone. All assignments are based on the COSY and NOESY spectral data.

assignable to a trisaccharide unit composed of 2 mol of 6-deoxyhexose and 1 mol of hexose in the high resolution MS of the permethylate of **6** (Fig. 5). The coupling constants (J_{H-H} and J_{C-H}) of the signals due to the sugar moiety indicated that the modes of glycosidic linkages of the rhamnopyranose units are α in $^1C_4$ conformation and those of the glucopyranose and quinovopyranose units are β in $^4C_1$ conformation (Tables I and II).

Accordingly, the structure of pharbitic acid C (**6**) was defined as (3*S*,11*S*)-ipurolic acid 11-*O*-β-D-quinovopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-[*O*-α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside (Fig. 2).

Pharbitic acid D (**7**) had been reported to have the structure **7'** (Fig. 1) in which an additional rhamnopyranose is located at 3-OH of the terminal quinovose of **6'**.[6] Taking the above revision into account, it was considered that the structure might also be revised to **7** in Fig. 2.

The negative ion FAB-MS and ^{1}H- and ^{13}C-NMR spectra of **7** showed no contradiction with the proposed structure. On the other hand, there was no NOE relation between 4-H of Rha and 1-H of Qui as would be expected for the structure **7'**, but an interaction between 4-H of Rha' and 1-H of Qui was observed. In addition, the permethylate of **7** exhibited a peak at m/z 741.3906 ($C_{34}H_{61}O_{17}$) which was ascribable to a tetrasaccharide unit composed of three 6-deoxyhexose residues and one hexose (Fig. 5). The modes of the glycosidic linkages were determined as α in $^1C_4$ conformation for all rhamnopyranose units and β in $^4C_1$ conformation for glucopyranose and quinovopyranose units from the J values of all methine protons due to the sugar moiety (Table I) and the J_{C-H} values of the anomeric carbon signals.

Consequently, pharbitic acid D (**7**) was concluded to be (3*S*,11*S*)-ipurolic acid 11-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-quinovopyranosy-(1→4)-*O*-α-L-rhamnopyranosyl-

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Table II. ^{13}C-NMR Spectral Data for **6**, **7** and **8** (in Pyridine-d_5, 100 MHz)

C atom	6	7	8
Glc-1	102.7 (154.1)	102.7 (153.8)	102.7
Glc-2	79.3	79.2	79.2
Glc-3	79.3	79.2	79.2
Glc-4	71.9	71.9	71.9
Glc-5	76.2	76.2	76.3
Glc-6	67.9	68.1	68.3
Glc'-1	101.8 (157.2)	101.8 (163.3)	101.9
Glc'-2	78.5	78.6	78.7
Glc'-3	79.4	79.4	79.5
Glc'-4	72.4	72.4	72.4
Glc'-5	77.8	77.8	77.8
Glc'-6	63.1	63.1	63.1
Rha-1	102.2 (167.9)	102.4 (167.9)	102.4
Rha-2	72.2	72.2	72.2
Rha-3	72.7	72.7	72.7
Rha-4	74.1	74.0	74.1
Rha-5	69.6	69.6	69.7
Rha-6	18.5	18.7	18.7
Rha'-1	101.6 (174.0)	101.7 (175.5)	101.7
Rha'-2	72.0	72.0	72.0
Rha'-3	72.4	72.4	72.5
Rha'-4	84.1	84.1	84.2
Rha'-5	67.8	67.9	67.9
Rha'-6	18.7	18.5	18.5
Rha"-1		102.7 (169.4)	102.7
Rha"-2		72.6	72.6
Rha"-3		72.5	72.6
Rha"-4		74.0	74.0
Rha"-5		69.9	69.9
Rha"-6		18.6	18.6
Qui-1	105.6 (157.2)	105.4 (158.6)	105.4
Qui-2	76.3	76.5	76.5
Qui-3	78.1	83.6	83.7
Qui-4	76.6	74.8	74.9
Qui-5	73.0	72.9	72.9
Qui-6	18.9	18.8	18.8
Ag-1	175.3	175.3	175.3
Ag-2	43.9	43.9	43.9
Ag-3	68.5	68.5	68.6
Ag-11	81.1	81.1	81.1
Ag-14	14.6	14.6	
Ag-16			14.3

δ in ppm from TMS (coupling constants (J_{C-H}) for anomeric carbons are given in parentheses). Glc, glucopyranosyl; Rha, rhamnopyranosyl; Qui, quinovopyranosyl; Ag, aglycone. All assignments are based on the ^{1}H–^{13}C-COSY spectral data.

(1→2)-*O*-β-D-glucopyranosyl-(1→2)-[*O*-α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside (Fig. 2).

Compound **8a** gave, on alkaline hydrolysis, a new glycosidic acid named pharbitic acid B (**8**), a white powder, mp 136—141 °C (dec.), $[\alpha]_D$ −58.5°, $C_{52}H_{92}O_{30}$. The negative ion FAB-MS of **8** showed the $(M-H)^-$ ion peak at *m/z* 1195 and fragment peaks at *m/z* 1049, 903, 757 and 595 which were assignable to $[M-H-146$(6-deoxyhexose unit)$]^-$, $[1049-146]^-$, $[903-146]^-$ and $[757-162$(hexose unit)$]^-$ ion peaks, respectively. The fact that all the peaks are 28 mass units larger than those of **7** suggested that the aglycone of **8** is **5** and the sugar moiety is that of **7**. This suggestion was confirmed by analyses of the ^{1}H- and ^{13}C-NMR spectra of **8** with the aid of COSY, NOESY and long-range ^{13}C–^{1}H-COSY spectra (Tables I and II).

Accordingly, pharbitic acid B (**8**) was characterized as (3*S*,11*S*)-3,11-dihydroxyhexadecanoic acid 11-*O*-α-L-rhamnopyranosyl-(1→3)-β-D-quinovopyranosyl-(1→4)-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-β-D-glucopyranosyl-(1→2)-[*O*-α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside (Fig. 2).

Experimental

General Procedures All instruments and materials used were the same as those cited in the preceding report[10] unless otherwise specified.

Extraction and Preparation of Pharbitin The crushed seeds of *P. nil* (2.4 kg) imported from China by Kanebo Co., Ltd., were percolated with MeOH (5 l) at room temperature and the extract was evaporated under reduced pressure to afford a brown syrup (357 g). This was suspended in water (4 l) and then extracted with *n*-BuOH (3 l × 3). Concentration of the water layer furnished a brown syrup (149.1 g). The *n*-BuOH extractive (184.9 g) was defatted with Et_2O (3 × 250 ml) to afford a brown powder (166.5 g), which was chromatographed on an highly porous polymer gel (MCI gel CHP 20P, Mitsubishi Chemical Industries Ltd.) column (70% MeOH→90% MeOH→acetone) to provide three fractions, a brown powder (38.4 g), a pale yellow powder (86.2 g, pharbitin), mp 124—140 °C (dec.) and a brown syrup (38.3 g).

Alkaline Hydrolysis of Pharbitin Pharbitin (1.23 g) in 1% aqueous K_2CO_3 (30 ml) was heated at 95 °C for 2 h. The mixture was adjusted to pH 4 with 1 N HCl and extracted with Et_2O (20 ml). The organic phase was washed with H_2O and dried over $MgSO_4$. Evaporation of the Et_2O gave an oil (organic acid fraction, 210 mg), which was methylated with CH_2N_2 then analyzed by GC (column, 2.0 m × 3.2 mm glass column packed with Unisole F-200; carrier N_2, 1.5 kg (117 ml/min); column temperature, 40 °C for 5 min then elevated by 10 °C/min), t_R (min): 3.65 (methyl 2-methylbutyrate), 8.71 (methyl tiglate), 13.63 (methyl nilate) in the ratio of *ca.* 17 : 1 : 11.

The H_2O layer was chromatographed over MCI gel CHP 20P (H_2O→acetone). The acetone fraction gave a white powder (920 mg) (pharbitic acid).

Identification of Organic Acids The organic acid fraction (2.88 g) in dry acetone (50 ml) was neutralized with triethylamine. *p*-Bromophenacyl bromide (600 mg) was added and the mixture was left to stand at room temperature for 1 h. The resulting precipitate was filtered off and the filtrate was concentrated. The residue was fractionated between H_2O (20 ml) and Et_2O (20 ml). The Et_2O fraction was chromatographed over silica gel (*n*-hexane–benzene (1 : 1)→benzene–AcOEt (4 : 1)) to afford *p*-bromophenacyl 2-methylbutyrate (**1a**, 149 mg) and *p*-bromophenacyl nilate (**3a**, 55 mg).

1a: Colorless needles (*n*-hexane–acetone (3 : 1)), mp 52—53 °C, $[\alpha]_D^{19}$ +11.9° (c=3.3, $CHCl_3$). IR (KBr) cm^{-1}: 1740, 1690 (C=O), 1590 (arom.). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 0.98 (3H, t, J=7.3 Hz, 4-H_3), 1.24 (3H, d, J=7.0 Hz, 2-C$\underline{H}_3$), 1.56, 1.79 (each 1H, ddq, J=13.7, 7.0, 7.3 Hz, 3-H_2), 2.56 (1H, ddq, J=7.0, 7.0, 7.0 Hz, 2-H), 5.29 (2H, s, O–C$\underline{H}_2$–CO), 7.63 (2H, ddd, J=8.6, 2.1, 2.1 Hz, arom. H), 7.78 (2H, ddd, J=8.6, 2.1, 2.1 Hz, arom. H). This compound was identical with an authentic sample of *p*-bromophenacyl *S*-2-methylbutyrate obtained from the root of *Ipomoea muricata*.[7]

3a: Colorless needles (*n*-hexane–acetone, 3 : 1), mp 98—99 °C, $[\alpha]_D^{20}$ −2.2° (c=1.2, $CHCl_3$). IR (KBr) cm^{-1}: 3500 (OH), 1730 (C=O), 1690 (C=O), 1590 (arom.). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 1.25 (3H, d, J=7.0 Hz, 2-C$\underline{H}_3$), 1.30 (3H, d, J=6.4 Hz, 4-H_3), 2.63 (1H, dq, J=7.3, 7.0 Hz, 2-H), 3.51 (1H, d, J=4.9 Hz, 3-OH), 3.97 (1H, ddq, J=7.3, 6.4, 4.9 Hz, 3-H), 5.33, 5.44 (each 1H, d, J=16.5 Hz, O–C$\underline{H}_2$–CO), 7.65 (2H, ddd, J=8.5, 2.1, 2.1 Hz, arom. H), 7.79 (2H, ddd, J=8.5, 2.1, 2.1 Hz, arom. H). This compound was identical with an authentic sample of *p*-bromophenacyl (2*R*,3*R*)-nilate obtained from the root of *I. muricata*.[7]

Acid Hydrolysis of Pharbitic Acid Pharbitic acid (4.63 g) in 5% H_2SO_4 (50 ml) was heated at 95 °C on a water bath for 3 h. The mixture was extracted with Et_2O (20 ml × 3) and the combined Et_2O extractive was dried over $MgSO_4$ and concentrated *in vacuo* to give a residue. Treatment of the residue with CH_2N_2 in Et_2O followed by evaporation yielded a white powder (1.216 g), which was chromatographed over silica gel (*n*-hexane–AcOEt (4 : 1)→AcOEt) to afford **4** (211 mg) and **5** (49 mg).

4: Colorless needles (*n*-hexane–AcOEt), mp 69—70 °C, $[\alpha]_D^{26}$ +1.2° (c=5.0, EtOH). IR (KBr) cm^{-1}: 3350 (OH), 3250 (OH), 1730 (C=O). EI-MS *m/z*: 256 $[M-H_2O]^+$, 103 $[CH(OH)CH_2COOCH_3]^+$. FD-MS *m/z*: 275 $[M+H]^+$, 257 $[M+H-H_2O]$, 231 $[M-CH_3(CH_2)_2]^+$, 103 $[CH(OH)CH_2COOCH_3]^+$, 73 $[CH_3(CH_2)_2CHOH]^+$. ^{1}H-NMR (pyridine-d_5, 400 MHz) δ: 4.44 (1H, m, 3-H), 3.83 (1H, m, 11-H), 3.65 (3H, s, COOC$\underline{H}_3$), 2.76 (1H, dd, J=8.2, 15.0 Hz, 2-H_a), 2.70 (1H, dd, J=4.6, 15.0 Hz, 2-H_b), 0.95 (3H, t, J=7.3 Hz, 14-H_3). ^{1}H-NMR ($CDCl_3$,

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400 MHz) δ: 4.00 (1H, m, 3-H), 3.60 (1H, m, 11-H), 3.72 (3H, s, COOC$\underline{H}_3$), 2.52 (1H, dd, J=3.4, 16.5 Hz, 2-H_a), 2.41 (1H, dd, J=9.2, 16.5 Hz, 2-H_b), 0.93 (3H, t, J=7.0 Hz, 14-H_3). ^{13}C-NMR (pyridine-d_5, 100 MHz) δ: 14.5 (C_{14}), 19.5, 26.2, 26.4, 30.0, 30.1, 30.2, 38.1, 38.5, 40.7, 43.5 (C_2), 51.3 (COO$\underline{C}H_3$), 68.2 (C_3), 70.6 (C_{11}), 172.8 (C_1). This compound was identical with an authentic sample of methyl ipurolate[6] on the basis of mixed melting point determination, and ^{1}H-, ^{13}C-NMR and FD-MS comparisons.

5: Colorless needles (*n*-hexane–AcOEt), mp 72.5—74.0 °C, $[\alpha]_D^{17}$ +0.2° (c=7.5, EtOH). IR (KBr) cm^{-1}: 3325 (OH), 3240 (OH), 1725 (C=O). EI-MS *m/z*: 284 $[M-H_2O]^+$, 103 $[M-CH(OH)CH_2COOCH_3]^+$. FD-MS *m/z*: 303 $[M+H]^+$, 285 $[M+H-H_2O]^+$, 231 $[M-CH_3(CH_2)_4]^+$, 103 $[CH(OH)CH_2COOCH_3]^+$, 101 $[CH_3(CH_2)_4CHOH]^+$. ^{1}H-NMR (pyridine-d_5, 400 MHz) δ: 4.45 (1H, m, 3-H), 3.84 (1H, m, 11-H), 3.64 (3H, s, $COOCH_3$), 2.76 (1H, dd, J=8.2, 15.0 Hz, 2-H_a), 2.70 (1H, dd, J=4.6, 15.0 Hz, 2-H_b), 0.87 (3H, t, J=7.0 Hz, 16-H_3). ^{13}C-NMR (pyridine-d_5, 100 MHz): 14.3 (C_{16}), 23.0, 26.1, 26.2, 26.4, 30.0, 30.1, 30.2, 32.4, 38.1, 38.5, 38.5, 43.5 (C_2), 51.3 (COO$\underline{C}H_3$), 68.2 (C_3), 71.0 (C_{11}), 172.8 (C_1). ^{13}C-NMR ($CDCl_3$, 22.5 MHz) δ: 14.0 (C_{16}), 22.7, 25.4, 25.5, 25.6, 29.5, 29.6, 31.9, 36.5, 37.4, 41.2 (C_2), 51.7 (COO$\underline{C}H_3$), 68.0 (C_3), 72.0 (C_{11}), 173.5 (C_1). *Anal.* Calcd for $C_{17}H_{34}O_4$: C, 67.50; H, 11.33. Found: C, 67.43; H, 11.29.

A small portion of the aqueous layer was neutralized with 3% KOH and the mixture was evaporated. Desalting of the residue by chromatography on an LH-20 column (MeOH) followed by evaporation afforded a syrup (5 mg). The syrup (1 mg) was subjected to GC-analysis as the TMS ether of the thiazolidine derivatives according to Hara *et al.*[9] GC (Hitachi G-3000 gas chromatograph equipped with 30 : 1 splitter and flame ionizing detector; column, fused silica capillary column Bonded MPS-50 (Quadrex), 50 m × 0.25 μ, 0.25 μ film thickness; carrier gas, He (30 ml/min); column temperature, 220 °C), t_R (min), 18.83 (D-quinovose), 19.43 (L-rhamnose), 27.10 (D-glucose) in the ratio of *ca.* 1 : 4 : 4.

Isolation of Pharbitic Acids C (6), D (7) and B (8) from Pharbitic Acid Triethylamine (8 ml) was added to a solution of pharbitic acid (4.6 g) in dioxane (50 ml) and H_2O (10 ml) and the mixture was heated at 90 °C for 1 h, then the solvent was removed. A mixture of the residue and *p*-phenylphenacyl bromide (1.7 g) in dimethylformamide (DMF) (60 ml) was heated at 90 °C for 1 h. The mixture was concentrated to dryness and the residue was chromatographed over silica gel ($CHCl_3$–MeOH–H_2O, 7 : 3 : 0.5→6 : 4 : 1) to afford a solid (3.12 g). This (2 g) was subjected to preparative high performance liquid chromatography (HPLC) (Chemcopak Nucleosil 5C8 (2.0 cm i.d. × 25 cm), MeOH–H_2O, 3 : 1), to furnish fr. 1 (1.19 g) and fr. 2 (0.17 g). Low pressure preparative HPLC (Kusano CIG prepacked Si gel, 2.2 cm i.d. × 30 cm, $CHCl_3$–MeOH–H_2O, 7 : 3 : 0.5) of fr. 1 (400 mg) afforded **6a** (103 mg), a white powder, mp 128—131 °C (dec.), $[\alpha]_D^{17}$ −50.6° (c=1.13, MeOH) and **7a** (235 mg), a white powder, mp 136—140 °C (dec.), $[\alpha]_D^{17}$ −63.7° (c=1.05, MeOH). Fraction 2 gave **8a** (0.17 g), a white powder, mp 138—142 °C (dec.), $[\alpha]_D^{17}$ −57.1° (c=1.94, MeOH).

6a (105 mg) was heated with 1% aqueous KOH (3 ml) for 2 h. After cooling, the mixture was acidified (pH 4) with AcOH, then extracted with $CHCl_3$. The aqueous layer was placed on an MCI gel column and eluted successively with H_2O and 40% acetone to give **6** (64 mg).

6: A white powder, mp 130—134 °C (dec.), $[\alpha]_D^{24}$ −50.5° (c=1.3, MeOH). IR (KBr) cm^{-1}: 3400 (OH), 1710 (C=O). Negative ion FAB-MS *m/z*: 1021 $[M-H]^-$, 875 [1021 − 146(6-deoxyhexose unit)]$^-$, 729 [875 − 146(6-deoxyhexose unit)]$^-$, 567 [729 − 162(hexose unit)]$^-$. ^{1}H- and ^{13}C-NMR (pyridine-d_5) δ: see Tables I and II.

Similar alkaline hydrolysis of **7a** (205 mg) and **8a** (90 mg) gave **7** (151 mg) and **8** (77 mg), respectively.

7: A white powder, mp 141—146 °C (dec.), $[\alpha]_D^{24}$ −66.2° (c=1.2, MeOH). IR (KBr) cm^{-1}: 3400 (OH), 1710 (C=O). Negative FAB-MS *m/z*: 1167 $[M-H]^-$, 1021 [1167 − 146(6-deoxyhexose)]$^-$, 875 [1021 − 146(6-deoxyhexose unit)]$^-$, 729 [875 − 146(6-deoxyhexose unit)]$^-$, 567 [729 − 162 (hexose)]$^-$. ^{1}H- and ^{13}C-NMR (pyridine-d_5) δ: see Tables I and II.

8: A white powder, mp 136—141 °C (dec.), $[\alpha]_D^{24}$ −58.5° (c=1.0, MeOH). IR (KBr) cm^{-1}: 3400 (OH), 1710 (C=O). Negative ion FAB-MS *m/z*: 1195 $[M-H]^-$, 1049 [1195 − 146(6-deoxyhexose unit)]$^-$, 903 [1049 − 146(6-deoxyhexose)]$^-$, 757 [903 − 146(6-deoxyhexose unit)]$^-$, 595 [757 − 162(hexose unit)]$^-$. ^{1}H- and ^{13}C-NMR (pyridine-d_5) δ: see Tables I and II. *Anal.* Calcd for $C_{52}H_{92}O_{30} \cdot 1/2H_2O$: C, 51.78; H, 7.77. Found: C, 51.84; H, 7.93.

Permethylation of Pharbitic Acids C (6) and D (7) NaH (50 mg) and CH_3I (2 ml) were added to a solution of **6** (20 mg) in DMF (2 ml) were added under stirring. The mixture was stirred overnight at room temperature. The solvent was removed under an N_2 stream and then H_2O (2 ml) was added. The mixture was extracted with Et_2O. The extractive was purified by silica gel chromatography (*n*-hexane→*n*-hexane–AcOEt (10 : 1)→benzene–AcOEt (3 : 1)→AcOEt) to furnish a colorless syrup (16 mg). ^{1}H-NMR (pyridine-d_5, 400 MHz) δ: 1.01 (3H, t, J=7.0 Hz, 14-H_3), 1.23 (3H, d, J=6.1 Hz), 1.41 (3H, d, J=6.1 Hz), 1.70 (3H, d, J=6.1 Hz), 2.60 (1H, dd, J=5.2, 15.3 Hz, 2-H), 2.70 (1H, dd, J=7.6, 15.3 Hz, 2-H), 4.87 (1H, d, J=7.5 Hz), 5.00 (1H, d, J=7.6 Hz), 5.16 (1H, d, J=7.9 Hz), 5.20 (1H, br s), 5.73 (1H, br s), 3.37, 3.43, 3.50, 3.51, 3.54, 3.56, 3.57, 3.58, 3.59, 3.63, 3.65, 3.66, 3.71, 3.84, 3.94 (3H each, s).

Compound **7** (10 mg) was subjected to permethylation as above to yield a colorless syrup (8 mg). ^{1}H-NMR (pyridine-d_5, 400 MHz) δ: 1.02 (3H, t, J=7.1 Hz, 14-H_3), 1.23 (3H, d, J=6.1 Hz), 1.43 (3H, d, J=6.1 Hz), 1.46 (3H, d, J=6.4 Hz), 1.71 (3H, d, J=6.1 Hz), 2.60 (1H, dd, J=3.9, 15.0 Hz, 2-H), 2.70 (1H, dd, J=7.3, 15.0 Hz, 2-H), 4.62 (1H, d, J=7.6 Hz), 5.00 (1H, d, J=7.6 Hz), 5.16 (1H, d, J=7.6 Hz), 5.17 (1H, br s), 5.72 (1H, d, J=1.0 Hz), 5.75 (1H, d, J=1.2 Hz), 3.38, 3.43, 3.50, 3.52, 3.54, 3.56, 3.56, 3.57, 3.57, 3.59, 3.59, 3.61, 3.61 3.69, 3.72, 3.88, 3.96 (3H each, s).

Acidic Hydrolysis of Pharbitic Acid B (8) A solution of **8** (93 mg) in 3% H_2SO_4 (5 ml) was heated on a boiling water bath. After cooling, the mixture was extracted with Et_2O (3 × 4 ml). The combined Et_2O layer was washed with H_2O and dried over $MgSO_4$. It was treated with CH_2N_2 and the solvent was removed. The residue was crystallized from *n*-hexane to furnish **5**, colorless needles, mp 72.5—74 °C. FD-MS *m/z*: 303 $[M+1]^+$, 285 $[M+1-H_2O]^+$, 231 $[CH(OH)(CH_2)_7CH(OH)CH_2COOCH_3]^+$, 103 $[CH(OH)CH_2COOCH_3]^+$, 101 $[CH_3(CH_2)_4CH(OH)]^+$.

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