[Chem. Pharm. Bull. 19(6)1144—1149(1971)]

UDC 547.918.02 : 581.192 : 582.942

Studies on Resin Glycosides. I. Reinvestigation of the Components of Pharbitin, a Resin Glycoside of the Seeds of *Pharbitis Nil* Choisy[1]

Toshio Kawasaki, Hikaru Okabe and Iwao Nakatsuka

Faculty of Pharmaceutical Sciences, Kyushu University[2]

(Received November 16, 1970)

Alkaline hydrolysis of pharbitin, a purgative resin glycoside of the seeds of *Pharbitis Nil* Choisy (Convolvulaceae) gave a glycosidic acid "pharbitic acid" together with (+)-α-methylbutyric and nilic ((-)-α-methyl-β-hydroxybutyric) acids as reported by Asahina, and in addition, valeric and tiglic acids. Tetrahydroxydecanoic acid which was reported by Kromer to exist in the organic acid fraction was not obtained and it is presumed to be a mixture of (-)-α-methyl-β-hydroxybutyric acid and (-)-α-methyl-β-O-(α-methyl-β-hydroxybutyroyl)-butyric acid, an artefact formed from the former. On acid hydrolysis of "pharbitic acid," ipurolic (3,11-dihydroxytetradecanoic) acid was yielded accompanied by a very small amount of convolvulinolic (11-hydroxytetradecanoic) acid, and as the sugar portion, besides D-glucose and L-rhamnose, D-quinovose was provided.

It is well known that a characteristic group of complex glycosides, so-called resin glycosides, is widely distributed in the Convolvulaceae plants as the constituents of their resinous purgative principles.[3,4] Chemical investigations on the "resin glycosides" were initiated as early as in the last century and several papers have appeared also quite recently.[3-8] They occur in different species of the family (for instance, *Pharbitis Nil* Choisy, *Ipomoea purga* Hayne, *I. orizabensis* Ledanois, *I. operculata* Martin and *I. Turpethum* Brown) and in various parts of the plants (underground parts, stems, seeds) and vary in the components, but it is known that they have a common feature in their general compositions. When subjected to alkaline hydrolysis, some organic acids and a glycosidic acid are provided and the latter is cleaved with acid to yield a few kinds of sugars and a hydroxyfatty acid, and hence they have been regarded as a complex glycosides in which a number of units of a hydroxyfatty acid oligoside are combined with each other and some of the free hydroxyl groups are esterified with several kinds of organic acids. In spite that the resin glycosides have been known and studied for a long time, and in contrast to the recent remarkable advances in the chemistry of glycolipids mainly of animal origin,[9] no detailed investigations on their structures have appeared, and even any of the component glycosidic acids has not been fully characterized except for muricatin B [11-hydroxyhexadecanoic (jalapinolic[10]) acid 4-O-L-rhamnopyranosyl-L-rhamnopyranoside] from *Ipomoea muricata* seeds.[7]

1) A part of this work was presented at the 90th Annual Meeting of Pharmaceutical Society of Japan, Sapporo, July, 1970.
2) Location: *Katakasu, Fukuoka.*
3) C.R. Smith, Jr., L.H. Niece, H.F. Zobel and I.A. Wolff, *Phytochemistry*, **3**, 289 (1964).
4) H. Auterhoff and H. Demleitner, *Arzneimittel-Forsch.*, **5**, 402 (1955).
5) E. Graf, E. Dahlke and H. W. Voigtländer, *Arch. Pharm.*, **298**, 6 (1965).
6) G. Legler, *Phytochemistry*, **4**, 29 (1965).
7) S.N. Khanna and P.C. Gupta, *Phytochemistry*, **6**, 735 (1967).
8) L.S. Rae, C.K. Heung and K.H. Sik, *Daehan Hwahak Hwoejee*, **13**, 96 (1969) [*Chem. Abstr.*, **72**, 79354 (1970)].
9) Several glycolipids of which structures seem to be somewhat similar to those of resin glycosides have recently been discovered also in some bacterial metabolites: F.G. Jarvis and M.J. Johnson, *J. Am. Chem. Soc.*, **71**, 4124 (1946); R.U. Lemieux, J.A. Thorn, C. Brice and R.H. Haskins, *Can. J. Chem.*, **29**, 409 (1951); R.U. Lemieux, *ibid.*, 415(1951).
10) Y. Asahina and T. Yaoi, *Yakugaku Zasshi*, **45**, 786 (1925).

The present studies were commenced in an attempt to elucidate the finer structures of the resin glycosides, complex glycolipids of plant origin, and this paper concerns the chemical reinvestigation of the components of pharbitin, a constituent glycoside of Pharbitidis resin of the seeds of *Pharbitis Nil*, as the first step in the project. A preliminary investigation on this resin glycoside was made by some investigators[11] in the 19th century and systematic examinations were carried out by Kromer[12] and by Asahina and his collaborators.[13] Kromer reported that alkaline hydrolysis of the resin glycoside provided angelic or tiglic and tetrahydroxydecanoic acids together with a glycosidic acid which was acid hydrolyzed to give D-glucose and two crystalline fatty acids of mp 68.5° and 99°. Asahina and his co-workers have made more detailed investigation reporting that the resin glycoside, named pharbitin, yielded, on alkaline hydrolysis, along with a mixture of organic acids, a glycosidic acid "pharbitic acid" which was further cleaved with acid to give ipurolic (3,11-dihydroxytetradecanoic) acid,[14] D-glucose and L-rhamnose. From the organic acid fraction they isolated and charac-

TABLE I. GLC of Component Organic Acids of Pharbitin

Reference acid[a] \ Condition[b]	t_R (min)		
	Free acid	Methyl ester[c]	
	I	II	III
Acetic	1.9		
Propionic	2.4		
Butyric	3.1		
Isobutyric	2.4		
Valeric	4.4	3.3	
Isovaleric	3.4	2.4	
(+)-α-Methylbutyric[d]	3.4	2.4	
Caproic	6.3		
Crotonic	5.8	3.0	
Tiglic	7.3	5.0	
Angelic[e]	7.3	4.5	
Nilic ((−)-α-methyl-β-hydroxybutyric[f])		9.0	0.7
Lauric			1.1
Palmitic			2.8
Stearic			4.8
Convolvulinolic[g]			9.3
Organic acids from pharbitin	3.4	2.4 (Me-A)	0.7 (Me-D)
	4.4	3.2 (Me-B)	
	7.3	5.0 (Me-C)	
		9.0 (Me-D)	

a) Unless otherwise specified all reference organic acids are those in the market.
b) A Shimazu GC-3AF Gas Chromatograph was used. condition I: diethyleneglycol succinate-H_3PO_4(25—1%) on shimalite (60—80 mesh), 1.8 m × 4 mmϕ, temperature; 141°, carrier; N_2, 67 ml/min; II: 3% OV-17 on chromosorb W (AW) DMCS (80—100 mesh), 1.8 m × 4 mmϕ, temperature; 100°, carrier; N_2, 110 ml/min; III: 5% 1,4-butanediol succinate on shimalite W (60—80 mesh), 1.8 × 4 mmϕ, temperature; 185°, carrier; N_2, 46 ml/min
c) Prepared by methylation with diazomethane
d) kindly provided by Dr. A. Yagi
e) kindly provided by Prof. Hata
f) obtained from Prof. Asahina's copper nilate
g) Prof. Asahina's authentic specimen

11) R. Schütze, *Pharmazeutische Centralhalle*, **1887**, 270; K. Hirano, *Tokyo-Teikoku-Daigaku-Kiyo*, **1888**, 209.
12) N. Kromer, *Arch. Pharm.*, **1896**, 459.
13) *a*) Y. Asahina and S. Terada, *Yakugaku Zasshi*, **39**, 821 (1919); *b*) Y. Asahina and T. Shimizu, *ibid.*, **42**, 1 (1922); *c*) Y. Asahina and S. Nakanishi, *ibid.*, **45**, 515 (1925).
14) First isolated from *Ipomoea purpurea* (Convolvulaceae) by Power and Rogerson; F.B. Power and H. Rogerson, *Chem. Zbl.*, **II**, 887 (1908). Its structure was determined by Asahina, *et al*,[13b,c] and confirmed by Kawasaki; T. Kawasaki, *Yakugaku Zasshi*, **79**, 1065 (1951); T. Kawasaki and H. Okabe, *Shoyakugaku Zasshi*, **19**, 4 (1965).

terized (+)-α-methylbutyric acid and α-methyl-β-hydroxybutyric acid, named nilic acid, and the presence of tiglic acid was suspected from the fact that the acid mixture smelled like sweat and decolorized a permanganate solution.

Pharbitin, obtained according to the Asahina's method[13a] was a slightly hygroscopic amorphous white powder with mp 130—135°, $[\alpha]_D$ −43.4°. "Pharbitic acid" prepared by a slightly modified procedure of Asahina's[13a] was a hygroscopic, white to faintly yellow solid of mp 130—140°, $[\alpha]_D$ −65.1°, molecular weight 957 (titration).

A mixture of organic acids obtained together with "pharbitic acid" was examined by gas–liquid chromatography (GLC) in a free state and in the form of methyl ester (Table I). The mixture of free acids provided one major and two minor peaks (Fig. 1) showing identical t_R values with those of (+)-α-methylbutyric or isovaleric, valeric and tiglic or angelic acids, respectively, while the mixture of methyl esters gave the peaks of one predominant (Me-A) and three minor constituents (Me-B, -C and -D) (Fig. 2). They were identified on GLC with authentic samples of the methyl esters of (+)-α-methylbutyric or isovaleric, valeric, tiglic and nilic acids, respectively. Tetrahydroxydecanoic acid (Ba salt, $C_{10}H_{18}O_6Ba+H_2O$) which was reported by Kromer[12] to exist in the organic acid fraction was not detected in our experiment.

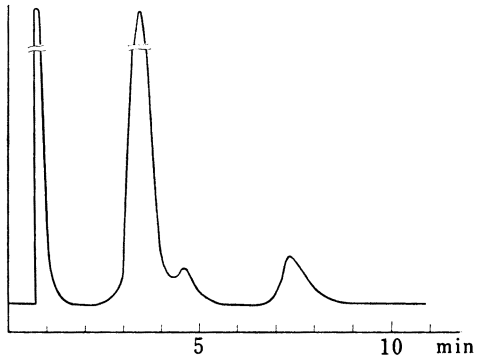


Fig. 1. GLC of Organic Acid Portion from Pharbitin

condition I: See Table I.

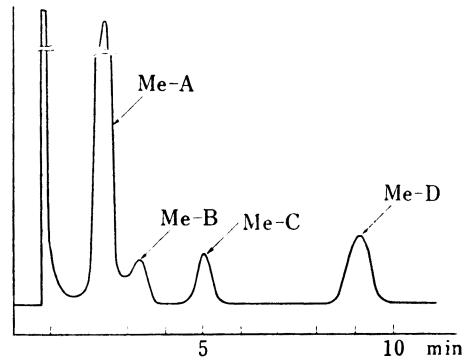
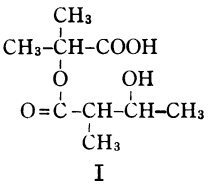


Fig. 2. GLC of Methyl Ester of Organic Acid Portion from Pharbitin

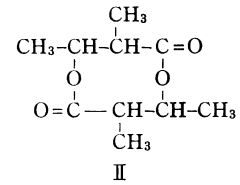
condition II: See Table I.

Repeated distillation of the mixture of methyl esters furnished gas chromatographically pure Me-A, bp 118—120°, $[\alpha]_D$ +18.3°, $C_6H_{12}O_2$. Its nuclear magnetic resonance (NMR) spectrum showed a triplet (3H, 0.90 ppm, J=6.0 cps), a doublet (3H, 1.13 ppm, J=6.0 cps), a multiplet (2H, centered at 1.60 ppm), a multiplet (H, centered at 2.37 ppm) and a singlet (3H, 3.64 ppm). The above data indicate Me-A to be methyl (+)-α-methylbutyrate and not isovalerate. Me-D, obtained by distillation under reduced pressure of the high boiling portion of the mixture of methyl esters, showed bp 80° (45 mmHg), $[\alpha]_D$ −17.5° and analyzed for $C_6H_{12}O_3$. Its infrared (IR) spectrum exhibited an absorption band of hydroxyl group at 3500 cm^{-1} and the NMR spectrum showed a pair of doublets (3H, 1.20 ppm, J=8.0 cps; 3H, 1.23 ppm, J=6.0 cps), a multiplet (H, centered at 2.45 ppm), a singlet (H, 3.15 ppm) due to a hydroxyl proton, a singlet (3H, 3.75 ppm) and a multiplet (H, centered at 3.87 ppm) attributable to the hydrogen on a carbon bearing a hydroxyl group. These data support that Me-D is the methyl ester of nilic acid. Me-B and -C could not be isolated because of their poor contents.

When the mixture of free organic acids was steam-distilled and the non-volatile portion, mainly consisting of nilic acid, was left stand at room temperature for two years and subsequently treated with diazomethane, a new compound tentatively named Me-E was detected



I



II

on GLC accompanying Me-D. Rohrbeck[15] suggested the formation of an esteranhydride (I or II) by a long storage of α-methyl-β-hydroxybutyric (nilic) acid, and Kromer[16] also reported a probable co-existence of esteranhydride(s) (I or II, or both) in α-methyl-β-hydroxybutyric acid which was stored for a long period after obtaining from Jalapin, a resin glycoside of Jalap resin from *Ipomoea purga* HAYNE. Either investigator, however, was not successful in isolation and identification of these artefacts. Since Me-E seemed most likely to be II or the methyl ester of I, the isolation and characterization of this compound was then carried out and gas chromatographically pure Me-E, bp 160—180° (bath) (8 mmHg), $[\alpha]_D$ −29.7°, $C_{11}H_{20}O_5$, was successfully obtained by column chromatography on silica gel followed by distillation under reduced pressure. It showed a similar IR spectrum to that of Me-D and the mass spectrum[17] exhibited the peaks of *m/e* 232 $[M]^+$, 216 $[M-CH_4]^+$, 144 $[CH_3CH(OH)CH(CH_3)COOCH=CH_2]^+$, 115 $[CH_3{}^+CH(CH_3)COOCH_3]$, 114 $[CH_3CH=C(CH_3)COOCH_3]^+$ and 100 $[CH_3CH=CHOOCH_3]^+$. On its NMR spectrum, four pairs of doublets (12H, 1.0—1.5 ppm) due to four secondary methyl groups, a multiplet (3H, centered at 2.50 ppm), a singlet (3H, 3.17 ppm), a multiplet (H, 3.86 ppm), a multiplet (H, 5.15 ppm) attributable to the hydrogen to the carbon bearing the ester oxygen were observed. Alkaline hydrolysis of Me-E followed by methylaton of the product with diazomethane gave a methyl ester which showed on GLC a peak with the same t_R value as that of Me-D.

On the basis of these experimental results, the structure of Me-E is defined as the methyl ester of (−)-α-methyl-β-O-(α-methyl-β-hydroxybutyroyl)-butyric acid (I). Taking his procedure of isolation and Rohrbeck's suggestion into account, Kromer's tetrahydroxydecanoic acid[12] is presumed to be a mixture of nilic acid and the esteranhydride I.

Acid hydrolysis of "pharbitic acid" gave an aglycone and sugars, the former of which was methylated with diazomethane and crystallized from hexane to provide a methyl ester, mp 69—69.5°, $C_{15}H_{30}O_4$. On saponification, the ester yielded an acid, mp 97—99°, $C_{14}H_{28}O_4$, which was identified as ipurolic acid by direct comparison with an authentic specimen.[13a] A gas chromatogram of the mother liquor of crystallization of the aglycone methyl ester exhibited a large peak of methyl ipurolate accompanied by a very small one whose t_R value was identical with that of methyl convolvulinolate (11-hydroxytetradecanoate[18]). The Kromer's fatty acid with mp 68.5° is assumed to be ipurolic acid contaminated with convolvulinolic acid.

The sugar portion of the hydrolysate was examined by paper chromatography (PC) with two solvent systems (Table II) and in either case none but two spots identical with those of D-glucose and L-rhamnose was detected. However, since it has recently been reported that D-quinovose occurs sometimes as one of the component sugars of the resin glycosides,[3,6] and D-quinovose could not be differentiated from L-rhamnose on PC in the conditions employed, a possibility of its existence is not excluded. The hydrolysate was then examined by PC according to the Krauss method[19] (Table II). The chromatogram revealed besides two spots of glucose (relative movement (*R*Rha) to rhamnose, 0.2) and rhamnose, an additional one

15) H. Rohrbeck, *Liebig's Ann.*, **188**, 229 (1877).
16) N. Kromer, *Arch. Pharm.*, **239**, 373 (1902).
17) H. Budzikiewicz, C. Djerassi and D. H.Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. II, HOLDEN-DAY, Inc., San Francisco, Calif., 1964, p.167.
18) T. Kawasaki, *Yakugaku Zasshi*, **70**, 35 (1950).
19) M.T. Krauss, H. Jäger, O. Schindler and T. Reichstein, *J. Chromatog.*, 3, 63 (1960).

TABLE II. PC of Component Sugars of "Pharbitic Acid"

Solvent system[a] / Reference sugar	*Rf* value I	*Rf* value II	*R*Rha value[b] III
D-Glucose	0.11	0.27	0.20
L-Rhamnose	0.31	0.47	1.00
D-Fucose[c]			0.38
D-Quinovose[d]	0.31	0.47	0.66
Sugar portion of "pharbitic acid"	0.11	0.27	0.20
	0.31	0.45	0.66
			1.00

a) solvent system I: BuOH–AcOH–H_2O (4:1:5), top layer, Toyo-roshi No. 50, ascending. II: BuOH–pyridine–H_2O (6:2:3), top layer+pyridine(1), Toyo-roshi No. 50, ascending, triple development. III: BuOH–$CH_3COC_2H_5$ (1:1) saturated with boric acid–borax buffer,[19] Toyo-roshi No. 2 treated with boric acid–borax buffer,[19] descending for 68 hr
b) relative movement to that (15 cm) of L-rhamnose
c) synthesized according to the method of Schmidt[20]
d) synthesized according to the methods of Karrer[21] and Wood[22]

(*R*Rha, 0.66) which was identical with that of synthetic D-quinovose. Attempted isolation of quinovose was in failure, but since the occurrence of natural L-quinovose has not been reported, it might safely be regarded as of D-series.

Consequently "pharbitic acid" is composed of ipurolic acid, D-glucose, L-rhamnose and D-quinovose, and pharbitin is a complex glycoside in which the "pharbitic acid" units are combined with each other and some of the free hydroxyl groups are esterified with (+)-α-methylbutyric, valeric, tiglic and nilic acid.

Experimental[23]

Pharbitin——Powdered seeds of *Pharbitis Nil* CHOISY ("shirokengo-shi", collected in Nara pref.)(1 kg) were defatted with hot benzene (1.5 liters × 2) and extracted with boillng 95% EtOH for 5 hr(1.5 liters × 3). The ethanolic solution was evaporated *in vacuo* to give a brown resin, which was washed with H_2O (1 liter × 2) and insoluble substance was dissolved in 95% EtOH. $(AcO)_2Pb$ was added to the solution until no more precipitation ocuurred. Yellow precipitates were filtered off and the filtrate was treated with H_2S. Precipitates being filtered off, the filtrate was evaporated *in vacuo* at 60—80° to give a brownish yellow resin, which was dissolved in a small amount of 95% EtOH and AcOEt was added to afford pharbitin as a slightly hygroscopic amorphous white powder (30 g), mp 130—140°, $[\alpha]_D^{15°}$ −43.4° (*c*=1.54, MeOH)(lit. [13a]; mp 145—150°, $[\alpha]_D$ −43.5°). IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 1745 (–COO–).

Alkaline Hydrolysis of Pharbitin——Pharbitin (40 g) was dissolved in 10% $Ba(OH)_2$ (400 ml) and heated at 90° for 30 min. Precipitates being filtered off, the reddish brown filtrate was passed through a column of Amberlite-120 (H^+) (25cm × 4cm ϕ) and the column was washed with H_2O until the eluate became neutral. The acidic eluate was shaken with ether (100 ml × 5) and the ether layer was evaporated at 40—60° to give a brownish oil (3.5 g). The aqueous layer was evaporated *in vacuo* to yield a resin.

Pharbitic Acid: The water soluble resin was dissolved in a smal amount of 95% EtOH and precipitated with AcOEt to give "pharbitic acid" as hygroscopic, white to faintly yellowish powder (30 g), mp 130—140°, $[\alpha]_D^{15}$ −65.1° (*c*=2.50, MeOH), molecular weight 957 (titration with 0.005 N NaOH, indicator; phenolphthalein) (lit., [13a] mp 155—162°, $[\alpha]_D$ −47.6°, molecular weight 911), IR ν_{max}^{KBr} cm^{-1}: 3600—3200 (OH), 1712 (COOH).

Examination of Ether-Soluble Fraction: The ether-soluble fraction was examined by GLC in a free state and in the form of methyl ester. The results are summerized in Table I.

20) O. T. Schmidt, "Methods in Carbohydrate Chemistry," Vol. I, ed. by R. L. Whistler and M. L. Wolfrom, Academic Press, New York, N.Y., 1962, p. 191.
21) P. Karrer and A. Boettcher, *Helv. Chim. Acta*, **36**, 570 (1953).
22) H.B. Wood, H.W. Oiehl and H.G. Heetcher, *J. Am. Chem. Soc.*, 79, 3862 (1957).
23) Melting points were taken on a Kofler block. Melting and boiling points are uncorrected. NMR spectra were determined on a JEOL-JNM-C-60H spectrometer at 60Mcps in deuteriochloroform solution and chemical shifts are given in δ scale with tetramethylsilane as internal reference (s, singlet; d, doublet; t, triplet; m, multiplet). TLC was conducted on a Kiesel gel G nach Stahl. In column chromatography, "Kanto" silica gel (100—200 mesh) was employed.

Me-A (Methyl (+)-α-Methylbutyrate)——The ether-soluble fraction (2.5 g) was dissolved in ether and treated with diazomethane. After removal of the solvent, the residue was distilled to give a colorless oil (2.0 g), bp 150° (bath), which was redistilled giving Me-A as a colorless oil, bp 118—120°, $[\alpha]_D^{14°}$ +18.3° (c=11.1, $CHCl_3$). *Anal.* Calcd. for $C_6H_{12}O_2$: C, 62.07; H, 10.34. Found: C, 61.80; H, 10.29. NMR (δ): 0.90 (3H, t, J=6.0 cps, CH_3–CH_2–), 1.13 (3H, d, J=6.0 cps, CH_3–CH–), 1.30—1.90 (2H, m, CH_3 –CH_2–CH–), 2.30 (H, m, –CH_2–CH(CH_3)–COO–), 3.64 (3H, s, –$COOCH_3$).

Me-D (Methyl Nilate ((−)-α-Methyl-β-hydroxybutyrate))——The residue of the first distillation of Me-A was distilled under reduced pressure to give Me-D as a colorless oil (100 mg), bp 80° (45 mmHg), $[\alpha]_D^{18°}$ −17.5° (c=2.37, MeOH). IR ν_{max}^{Liquid} cm^{-1}: 3500 (OH), 1740 ($COOCH_3$). *Anal.* Calcd. for $C_6H_{12}O_3$: C, 54.54; H, 9.09. Found: C, 54.75; H, 8.99. NMR (δ): 1.14 (3H, d, J=8.0 cps, CH_3–CH(OH)–), 1.23 (3H, d, J=6.0 cps, –CH(OH)–CH(CH_3)–COO–), 2.45 (H, m, –CH(OH)–CH(CH_3)–COO–), 3.15 (H, s, –OH), 3.75 (3H, s, –$COOCH_3$), 3.87 (H, m, –CH–OH).

Me-E (Methyl (−)-α-Methyl-β-O-(α-methyl-β-hydroxybutyroyl)Butyrate)——The ether-soluble fraction (6 g) was dissolved in H_2O (50 ml) and steam-distilled. The non-volatile portion was saturated with NaCl, extracted with ether (50 ml×3) and the solvent was evaporated to give a slightly yellowish oil (300 mg). GLC (as methyl ester): Me-A, -B, -C (all in trace), Me-D (main). The oil was stored in a cork-stoppered flask at room temperature for two years. The aged oil was dissolved in ether and methylated with diazomethane. The product showed in GLC two peaks of Me-D (t_R 0.7) and a new compound Me-E (t_R 3.2). It was subjected to column chromatography on silica gel (5 g) using dichloromethane as solvent to give two fractions. Fr. 1 (trace) was identified as a mixture of Me-A, -B, and -C by GLC. Fr. 2 (250 mg) which showed on GLC two peaks of Me-D and -E was distilled under reduced pressure. The first distillate (120 mg), bp 120—150° (bath) (8 mmHg) was identified with Me-D, and second (50 mg), bp 160—180° (bath) (8 mmHg), was gas chromatographically homogeneous Me-E, $[\alpha]_D^{15°}$ −29.8° (c=1.46, MeOH). *Anal.* Calcd. for $C_{11}H_{20}O_5$: C, 56.89; H, 8.62. Found: C, 56.58; H, 8.50. IR ν_{max}^{Liquid} cm^{-1}: 3400 (OH), 1735 ($COOCH_3$). NMR (δ): 1.00—1.50 (12H, d, four pairs, –CH–CH_3×4), 2.00—3.00 (3H, m, –OH, –CH–COO–×2), 3.67 (3H, s, –$COOCH_3$), 3.50—4.20 (H, m, –CH–OH), 5.15 (H, m, –COOCH–). Mass Spectrum (on JEOL-JMS-OISG spectrometer) m/e: 232, 216, 144, 155, 114, 100.

Alkaline Hydrolysis of Me-E: A solution of Me-E (5 mg) in 10% Ba $(OH)_2$ (1 ml) was heated at 95° for 30 min, diluted with H_2O and treated with IR-120 (H^+) (2 ml). The acidic solution was extracted with ether (10 ml×3). Ether being evaporated at 40—60°, the residue was methylated with diazomethane and examined by GLC to show one peak identical with that of Me-D.

Acid Hydrolysis of "Pharbitic Acid"——A solution of "pharbitic acid" (24 g) in 5% H_2SO_4 (200 ml) was heated in a boiling water bath for 30 min, cooled and extracted with ether (100 ml×3). Aqueous layer was again heated and extracted with ether as above. The procedure was repeated another five times. The ethereal layers were combined, washed with H_2O, dried over Na_2SO_4 and evaporated to give an yellowish oil (5 g). The aqueous layer was neutralized with dilute NaOH solution and evaporated under reduced pressure to give a syrup.

Aglycone (Ipurolic Acid): The oil was dissolved in ether, methylated with diazomethane and the product was recrystallized from hexane four times and finally from ether giving colorless silky needles, mp 69—69.5° (lit.[13a] mp 68—69°). *Anal.* Calcd. for $C_{15}H_{30}O_4$: C, 65.69; H, 10.94. Found: C, 65.66; H, 11.03. IR ν_{max}^{Nujol} cm^{-1}: 3330 (OH), 1738 ($COOCH_3$). The mother liquor of first crystallization was concentrated and subjected to GLC giving two peaks at t_R 4.70 (trace) and 8.30 (major) (methyl convolvulinolate;[24] 4.70, methyl ipurolate[13a]; 8.30, methyl jalapinolate[10]; 9.80. Shimazu GC-1B with hydrogen flame ionization detector, 1.5% SE-52 on chromosorb W (60—80 mesh) 2.25 m×4 mmϕ, tempeature; 176°, carrier; N_2, 30 ml/min). The methyl ester (1 g) was hydrolyzed and a resulting free acid was recrystallized from ether to provide colorless needles, mp 97—99°. It was identified as ipurolic acid by direct comparison (IR, TLC, GLC as methyl ester) with Asahina's authentic sample (mp 101°).[13a]

Examination of Sugar Fraction: The syrup obtained from the aqueous layer of the hydrolysate was dissolved in MeOH and examined by PC. The results are summerized in Table II.

Acknowledgement The authors are grateful to Prof. K. Hata of Osaka College of Pharmacy and Dr. A. Yagi of this university for the authentic samples. A part of the expenses for this work was defrayed by a Grant-in-Aid for Scientific Research from the Ministry of Education, which is gratefully acknowledged.

24) Y. Asahina and M. Akasu, *Yakugaku Zasshi*, **45**, 779 (1925).