[Chem. Pharm. Bull. 19(11)2394—2403(1971)]

UDC 547.918.02.05 : 581.192 : 582.942

Studies on Resin Glycosides. II.[1] Unhomogeneity of "Pharbitic Acid" and Isolation and Partial Structures of Pharbitic Acids C and D, the Major Constituents of "Pharbitic Acid"[2]

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(Received June 23, 1971)

"Pharbitic acid" (I), the component glycosidic hydroxyfatty acid of pharbitin, was found to be a mixture of two minor and two major constituents, pharbitic acids A,B,C (II) and D (III). II and III were isolated by silica gel chromatography of their *p*-phenyl phenacyl esters and subsequent saponification. II, mp 120—129°, $[\alpha]_D$ $-51.4°$, $C_{44}H_{78}O_{26}\cdot 10H_2O$, is composed of ipurolic acid, two moles each of D-glucose and L-rhamnose and one mole of D-quinovose, and III, mp 140—145°, $[\alpha]_D$ $-63.8°$, $C_{50}H_{88}O_{30}\cdot 2H_2O$, has another mole of L-rhamnose. Oxidation of I with $Na_2Cr_2O_7$ and treatment with AcONa in boiling Ac_2O both followed by acid hydrolysis provided respectively 10-hydroxytridecan-2-one (VI) and 11-hydroxytetradec-2-enoic acid (VII) as the sole ether soluble product indicating that II and III are 11-O-oligosides of ipurolic acid. On oxidation with $NaIO_4$ and subsequent acid hydrolysis, III provided D-quinovose, while II yielded none, and partial hydrolyses of I, II and III yielded equally monoglucoside (XI), $[\alpha]_D$ $-16.2°$, $C_{21}H_{40}O_9\cdot H_2O$, diglucoside (XII), mp 114—116°, $[\alpha]_D$ $-23.6°$, $C_{27}H_{50}O_{14}\cdot 1/2\ H_2O$, and rhamnodiglucoside (XIII), $[\alpha]_D$ $-25.7°$, $C_{33}H_{60}O_{18}\cdot H_2O$.

On the basis of the above results together with those of gas chromatographic analyses of aglycones and component monosaccharides of the permethylates of II, III, XII and XIII, II and III are assigned the structures either one of IIa—c and IIIa—c, respectively.

"Pharbitic acid" (I) is the glycosidic hydroxyfatty acid obtained and named by Asahina[4] on alkaline hydrolysis of pharbitin, a resin glycoside of the seeds of *Pharbitis nil* Choisy (Convolvulaceae), and it was reported to be composed of ipurolic acid (3, 11-dihydroxytetradecanoic acid), D-glucose and L-rhamnose.[4a]

Our recent reinvestigation[1] has shown that it contains in its sugar moiety D-quinovose in addition to the afore-mentioned two monosaccharides, and this paper deals with unhomogeneity of I, isolation of two major constituents and partial elucidation of their structures.

I obtained according to the Asahina method[4a] was a hygroscopic amorphous powder and when examined by thin-layer chromatography (TLC) on silica gel using BuOH–AcOH–H_2O (4:1:5, top layer) (solvent system 1) showed the presence of two minor (*Rf*: 0.46 and 0.20) and two major constituents (*Rf*: 0.17 and 0.16), which were tentatively named pharbitic acids A,[5] B, C (II) and D (III), respectively, in order of decreasing *Rf* values (Fig. 1).

Attempted separation of these constituents either in a free state or in the form of methyl and phenacyl esters by several methods (column chromatographies on silica gel and cellulose powder, gel permeation and preparative thin-layer chromatography) was not successful, but chromatography of their *p*-phenyl phenacyl esters on silica gel using $CHCl_3$–MeOH–H_2O (7:3:1, bottom layer) (solvent system 2) was found to show satisfactory resolution. *p*-

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[1] Part I: T. Kawasaki, H. Okabe, and I. Nakatsuka, *Chem. Pharm. Bull.* (Tokyo), **19**, 1144 (1971).

[2] A part of this work was presented at the 87th Annual Meeting of the Pharmaceutical Soceity of Japan, Kyoto, April 1967 and at the 89th Annual Meeting, Nagoya, April 1969.

[3] Location: *Katakasu, Fukuoka.*

[4] *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).

[5] Though this substance did not seem to be an acid because it was unchanged on treatment with diazomethane, phenacyl bromide and *p*-phenyl phenacyl bromide.

Phenyl phenacyl esters (IV) and (V) respectively of II and III were obtained by this method in thin-layer chromatographically pure state. Pharbitic acid A and *p*-phenyl phenacyl ester of pharbitic acid B, though detected on a chromatogram, could not be isolated in homogeneous state. IV was recrystallized from isopropanol furnishing fine plates, mp 127—130°, $[\alpha]_D$ $-54.7°$, which was saponified and the product was dissolved in ethanol and precipitated with ethyl acetate to regenerate II as a hygroscopic white powder, mp 120—129°, $[\alpha]_D$ $-54.1°$, molecular weight 990 (titration) showing single spot on TLC. V was recrystallized in the same manner to afford fine plates of mp 135—137°, $[\alpha]_D$ $-63.2°$, which gave, on saponification, thin-layer chromatographically pure III, which was recrystallized from isopropanol to furnish fine plates, mp 140—145°, $[\alpha]_D$ $-63.8°$, molecular weight 1253 (titration).

On complete hydrolysis, II and III gave equally ipurolic acid (Table I), D-glucose, D-quinovose and L-rhamnose (Table II) and the total sugar amounts in the hydrolysates and abovementioned molecular weights indicated that II and III are pentaglycoside and hexaglycoside, respectively. The molar ratios of D-glucose to methylpentose were 2: 3 in II and 2: 4 in III. Separative determination of the two kinds of methylpentose could not be achieved but a visual comparison of intensities and areas of the corresponding two spots on a paper chromatogram suggested the molar ratios of D-quinovose to L-rhamnose were 1: 2 in II and 1: 3 in III.

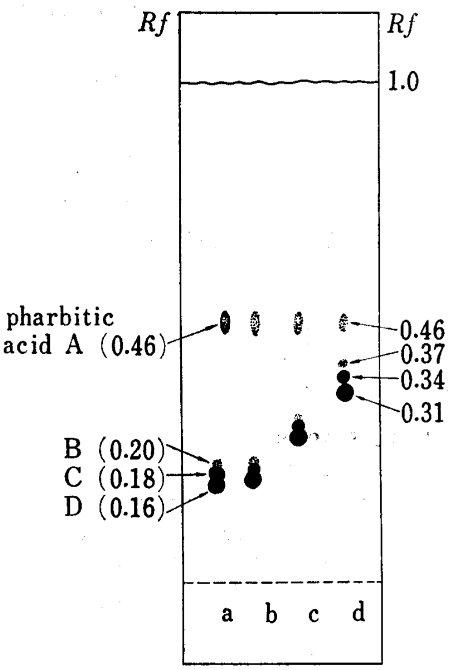

Fig. 1. TLC of "Pharbitic Acid" and Its Derivatives

Kiesel gel G nach Stahl
BuOH-AcOH-H_2O (4:1:5, top layer)
(solvent system 1)
a) "pharbitic acid", b) methyl ester,
c) phenacyl ester, d) *p*-phenyl phenacyl ester

Table I. Retention Times in GLC of Methyl Esters of Aglycones of Pharbitic Acids and Their Degradation Products

Methyl ester of	t_R (min)
Aglycones of II, III, XI, XII and XIII	8.30
Convolvulinolic acid[a]	4.70
Jalapinolic acid[b]	9.80
Ipurolic acid	8.30

condition: Shimazu GC-1B Gas Chromatograph equipped with hydrogen flame ionization detector. 1.5% SE-52 on chromosorb W (60—80 mesh), 2.25m × 4 mmϕ, temperature; 176°, carrier: N_2, 30 ml/min
a) prof. Asahina's authentic specimen[6]
b) prof. Asahina's authentic specimen[7]

Oxidation of I with sodium dichromate followed by acid hydrolysis gave as the sole ether soluble product a neutral substance (VI), $C_{13}H_{26}O_2$, mp 37—38°, $[\alpha]_D$ $+5.2°$. Its infrared (IR) spectrum exhibited the absorptions of hydroxyl and methyl ketonic groups, and the nuclear magnetic resonance (NMR) spectrum showed a singlet (3H) at 2.19 ppm attributable to the

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6) Y. Asahina and M. Akasu, *Yakugaku Zasshi*, **45**, 779 (1925).
7) Y. Asahina and T. Yaoi, *Yakugaku Zasshi*, **45**, 786 (1925).

TABLE II. *Rf* Values in PC of Component Sugars of Pharbitic Acids and Their Degradation Products

	Rf value		R_{Rha} value[b]
Condition[a]	I	II	III
Component sugars of			
II	0.11, 0.30	0.27, 0.45	0.20, 0.66, 1.00
III	0.11, 0.30	0.27, 0.45	0.20, 0.66, 1.00
XI	0.11	0.27	0.20
XII	0.11	0.27	0.20
XIII	0.11, 0.30	0.27, 0.45	0.20, 1.00
D-Glucose	0.11	0.27	0.20
L-Rhamnose	0.30	0.47	1.00
D-Quinovose	0.31	0.45	0.66

[a] I: BuOH–AcOH–H_2O (4:1:5, top layer) (solvent system 1), Toyo-roshi No. 50, ascending
II: BuOH–pyridine–H_2O (6:2:3), top layer+pyridine (1) (solvent system 3) Toyo-roshi No. 50, ascending, triple development
III: BuOH–$CH_3COC_2H_5$ (1:1) saturated with boric acid-borax buffer[8] (solvent system 4), Toyo-roshi No. 2 treated with the same buffer, descending for 68 hr.

[b] relative movement to that (15 cm) of L-rhamnose.

hydrogens of methylketone. Accordingly VI is considered to be 10-hydroxytridecan-2-one, which was yielded by cleavage of glycosidic linkage and ketonic fission of the 11-hydroxy-3-keto-tetradecanoic acid glycosides formed from I. When I was refluxed with sodium acetate in acetic anhydride[4c] and subsequently hydrolyzed with alkali and acid, an acidic oily substance (VII) was provided as the aglycone. VII was methylated with diazomethane and chromatographed on silica gel furnishing a methyl ester, $C_{15}H_{28}O_3$, bp 166—167° (4 mmHg), $[\alpha]_D$ $-11.0°$. Its IR spectrum showed the absorptions of hydroxyl and ester groups and a double bond conjugated with the latter, and on the NMR spectrum a double triplet (H, $J=1.5$, 16.8 Hz) at 5.99 ppm and a double triplet (H, $J=7.5$, 16.8 Hz) at 7.20 ppm were observed and they were assigned respectively to the hydrogen on the α- and β-carbon atoms of an α,β-unsaturated acid methyl ester. Therefore VII is regarded as 11-hydroxytetradec-2-enoic acid, the aglycone of a glycoside formed by dehydration of I.

The above results indicate that the aglycone, ipurolic acid, in I and hence in II and III, is combined with an oligosaccharide only through the hydroxyl group at C_{11}.

On oxidation with excess sodium periodate followed by reduction with sodium borohydride and subsequent acid hydrolysis, III gave D-quinovose, while II provide none in the sugar fraction. This suggests that the hydroxyl group at C_3 of the D-quinovose residue in II is combined with another sugar to form III.

Methylation of II and III by the Hakomori method[9] and subsequent column chromatography of the products on silica gel using benzene–methanol (9: 1) provided fully methylated products VIII and IX, respectively, both in thin-layer chromatographically homogeneous state. VIII and IX were then subjected to methanolysis and the products were examined by gas liquid chromatography (GLC). The aglycones yielded from VIII and IX were in both cases methyl ipurolate 3-methyl ether and methyl 11-hydroxytetradec-2-enoate (Table III, Fig. 2). The monosaccharides obtained from VIII and detected on a chromatogram (condition 1, Table IV, Fig. 2) were methyl pyranosides of 2,3,4-tri-O-methyl ethers of β-D-quinovose, α-D-quinovose or/and α-L-rhamnose and β-L-rhamnose, and methyl pyranosides of 2,3-di-O-methyl-α- and -β-L-rhamnose, 3,4,6-tri-O-methyl-α- and β-D-glucose, 3,4-di-O-methyl-α- and

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8) M.T. Krauss, H. Jäger, O. Schindler, and T. Reichstein, *J. Chromatog.*, 3, 63 (1960).
9) S. Hakomori, *J. Biochem* (Tokyo), 55, 255 (1964).

Table III. Retention Times in GLC of Methyl Esters of Aglycones of VIII, IX, XIV and XV

Methyl ester of	t_R (min)
Aglycones of VIII, IX, XIV and XV	5.00, 5.80
Palmitic acid	2.40
Ipurolic acid	8.40
3-methyl ether[a]	5.80
11-methyl ether[a]	5.30
3,11-dimethyl ether[a]	3.80
11-Hydroxytetradec-2-enoic acid	5.00

condition: Shimazu GC-3AF, 1.5% silicone DC-QF 1 on chromosorb W (AW) DMCS (60—80 mesh), 1.8 m × 4 mmϕ, temperature; 160°, carrier; N_2; 66 ml/min

a) Synthesized from ipurolic acid (*cf.* experimental).

-β-D-glucose and on another chromatogram (condition 2, Table IV, Fig. 3) the peaks of methyl pyranosides of 2,3,4-tri-O-methyl-α- and -β-D-quinovose and 2,3,4-tri-O-methyl-α-L-rhamnose were separately observed. The sugar portion of the methanolysate of IX gave similar chromatograms (TableI V, Fig. 2 and 3) to those of VIII but the peaks of methyl 2,3,4-tri-O-methyl-α- and -β-D-quinovopyranosides (t_R: 0.8 and 0.6, respectively in condition 1) disappeared and instead, a new peak (t_R: 1.9) was observed. Since the D-quinovose unit in III is linked with another sugar at the hydroxyl group at C_3, the new peak is thought to be due to either methyl 2,4-di-O-methyl -α- or -β-D-quinovopyranosides and that of the anomer seems to be overlapped with the peak of methyl 2,3-di-O-methyl-α-L-rhamnopyranoside.

Consequently, II is considered to be a 11-O-pentaglycoside of ipurolic acid of which sugar moiety consists of two moles each of D-glucose and L-rhamnose and one mole of D-quinovose and is branched at one of the two glucose units having the rhamnose and quinovose residues at terminals, and III is assumed as a homologous hexaglycoside which has another mole of L-rhamnose combined with the hydroxyl group at C_3 of the terminal D-quinovose residue in II.

In order to determine the monosaccharide sequences in II and III, the degradation of their sugar moieties were then carried out. Attempted partial hydrolysis of I by dilute mineral acid, acetic acid[10] and by enzymes (amylase[11], naringinase[12], rhamnosidase[12]) failed to give any satisfactory result, but when I was heated with 80% formic acid and the product was methylated with diazomethane, four substances (X—XIII) were detected on TLC: *Rf*; 1.00, 0.35, 0.25 and 0.12 (Kiesel gel G, solvent system 2) (Fig. 4).

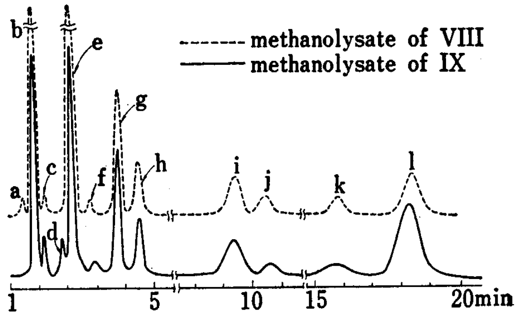

Fig. 2. GLC of Methanolysates of VIII and IX

condition 1: Shimazu GC-1B, 5% 1,4-butanediol succinate on Shimalite (60—80 mesh) 1.5 m × 4 mmϕ, temperature; 197°, carrier gas: N_2, 22 ml/min

a, methyl pyranoside of 2,3,4-tri-O-methyl-β-D-quinovose
b, methyl pyranoside of 2,3,4-tri-O-methyl-α-D-quinovose and/or 2,3,4-tri-O-methyl-α-L-rhamnose
c, methyl pyranoside of 2,3,4-tri-O-methyl-β-L-rhamnose
d, methyl pyranoside of 2,4-di-O-methyl-β-D-quinovose(?)
e, methyl pyranoside of 2,4-di-O-methyl-α-D-quinovose(?) and/or 2,3-di-O-methyl-α-L-rhamnose
f, methyl pyranoside of 2,3-di-O-methyl-β-L-rhamnose(?)
g, methyl pyranoside of 3,4,6-tri-O-methyl-α-D-glucose
h, methyl pyranoside of 3,4,6-tri-O-methyl-β-D-glucose
i, methyl pyranoside of 3,4-di-O-methyl-α-D-glucose
j, methyl pyranoside of 3,4-di-O-methy -β-D-glucose
k, methyl 11-hydroxytetradec-2-enoate
l, methyl ipurolate 3-methyl ether

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[10] S. Shibata, T. Ando, and O. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **14**, 1157 (1966).

[11] Commercial preparation (Takadiastase, Sankyo Co., Ltd.).

[12] Prepared from *Aspergillus niger* kindly provided by Dr. S. Kamiya of Women's Junior Coll. Shizuoka, Japan; S. Kamiya, S. Esaki, and M. Hama, *Agr. Biol. Chem.* (Tokyo), **31**, 133 (1967).

Table IV. Retention Times in GLC of Component Sugars of VIII, IX, XIV and XV

	t_R (min)	
	Condition 1[a]	Condition 2[b]
Component sugars of		
VIII	0.6, 0.8, 1.2	5.2
	2.1, 2.8, 3.7	6.6
	4.5, 9.3, 10.6	7.2
IX	0.8, 1.2, 1.9	7.1
	2.1, 2.8, 3.7	
	4.5, 9.3, 10.6	
XIV	1.9, 3.8, 4.5	
XV	0.8, 1.9, 9.3	7.1
	10.6	
Methyl pyranoside[c] of		
2,3,4,6-tetra-O-methyl-α-D-glucose	1.9	
2,3,4-tri-O-methyl-α-D-glucose	4.4	
2,3,6-tri-O-methyl-α-D-glucose	5.5	
2,3,6-tri-O-methyl-β-D-glucose	4.2	
2,4,6-tri-O-methyl-α-D-glucose	5.6	
2,4,6-tri-O-methyl-β-D-glucose	4.0	
3,4,6-tri-O-methyl-α-D-glucose	3.7	
3,4,6-tri-O-methyl-α- and -β-D-glucoses[d]	3.8, 4.5	
2,3-di-O-methyl-α-D-glucose	15.6	
2,4-di-O-methyl-α-D-glucose	13.7	
2,4-di-O-methyl-β-D-glucose	9.7	
3,4-di-O-methyl-α-D-glucose	9.3	
3,6-di-O-methyl-α-D-glucose	10.6	
3,6-di-O-methyl-α- and -β-D-glucoses[e]	10.6, 13.5	
4,6-di-O-methyl-α-D-glucose	10.7	
2,3,4-tri-O-methyl-α-L-rhamnose	0.8	7.1
2,3,4-tri-O-methyl-β-L-rhamnose	1.2	
2,3-di-O-methyl-α-L-rhamnose	2.1	
2,4-di-O-methyl-α-L-rhamnose	1.7	
3,4-di-O-methyl-α-L-rhamnose	1.5	
2,3,4-tri-O-methyl-α-D-quinovose	0.8	6.6
2,3,4-tri-O-methyl-β-D-quinovose	0.6	5.2

a), *b*) See Fig. 2 and 3.
c) Unless otherwise specified reference compounds were all synthesized (*cf.* experimental).
d) Obtained by hydrolysis of prosapogenin A of dioscin permethylate[13] followed by treatment with methanolic hydrogen chloride.
e) Obtained by hydrolysis of dioscin permethylate[14] followed by treatment with methanolic hydrogen chloride.

The same treatments of II and III gave the identical products. The four products (X—XIII) from I were separated on silica gel column using solvent system 2 and recrystallized. X, colorless needles, mp 65—66°, was identified with methyl ipurolate by direct comparison with an authentic specimen and XI, a hygroscopic powder, $C_{21}H_{40}O_9 \cdot H_2O$, was acid hydrolyzed to give D-glucose and methyl ipurolate suggesting it to be a monoglucoside. XII, colorless needles, mp 114—116°, $[\alpha]_D$ $-23.6°$, $C_{27}H_{50}O_{14} \cdot 1/2\ H_2O$, afforded on hydrolysis D-glucose and methyl ipurolate, and XIII, a colorless powder, $[\alpha]_D$ $-25.9°$, $C_{33}H_{60}O_{18} \cdot H_2O$, gave methyl ipurolate, D-glucose and L-rhamnose (Tables I and II). Fully methylated XII (XIV), prepared by the Hakomori method[9] yielded on methanolysis methyl ipurolate 3-methyl ether accompanied by a small amount of methyl 11-hydroxytetradec-2-enoate and methyl pyranosides of 2,3,4,6-tetra-O-methyl-α-D-glucose and 3,4,6-tri-O-methyl-α- and -β-D-glucose. XIII permethylate (XV) gave the same aglycones as above and methyl pyranosides of 2,3,4-tri-O-

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13) T. Kawasaki and T. Yamauchi, *Chem. Pharm. Bull.* (Tokyo), **16**, 1070 (1968).
14) T. Kawasaki and T. Yamauchi, *Chem. Pharm. Bull.* (Tokyo), **10**, 703 (1962).

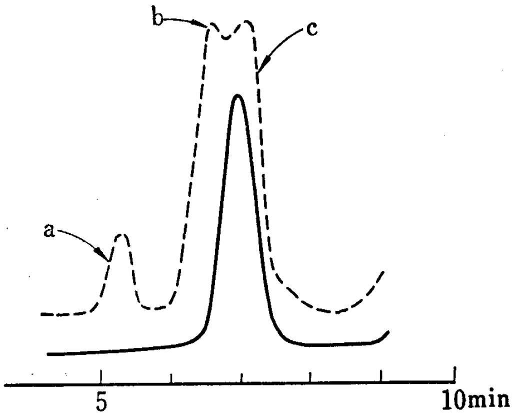


Fig. 3. GLC of Methanolysates of VIII and IX

-------: methanolysate of VIII
———: methanolysate of IX
condition 2: Shimazu GC-3AF, 3% OV-17 on chromosorb W (AW) DMCS (80—100 mesh) 1.7 m × 4 mmϕ. temperature; 138.5°, carrier gas; N_2, 18 ml/min
a, methyl pyranoside of 2,3,4-tri-O-methyl-β-D-quinovose
b, methyl pyranoside of 2,3,4-tri-O-methyl-α-D-quinovose
c, methyl pyranoside of 2,3,4-tri-O-methyl-α-L-rhamnose

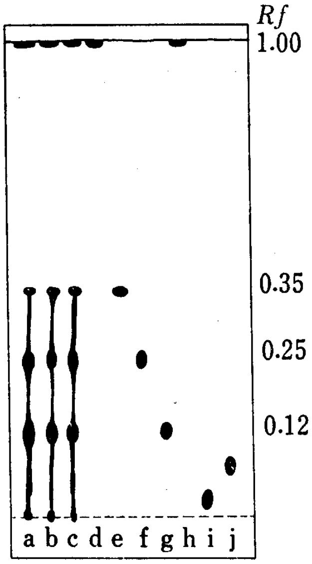


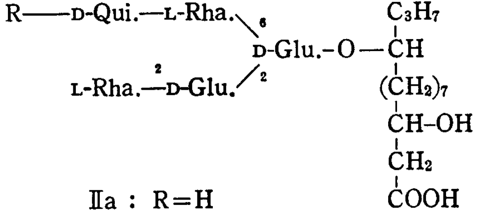
Fig. 4. TLC of Methyl Esters of Partial Hydrolysates of Pharbitic Acids

Kieselgel G nach Stahl
$CHCl_3$-MeOH-H_2O (7:3:1, bottom layer)
(solvent system 2)
a, methyl ester of partial hydrolysate of I
b, methyl ester of partial hydrolysate of II
c, methyl ester of partial hydrolysate of III
d, Fr. A (X); e, Fr. B (XI); f, Fr. C (XII);
g, Fr. D (XIII); h, methyl ipurolate; i, D-glucose; j, L-rhamnose

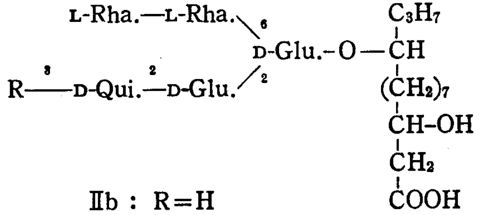
methyl-α-L-rhamnose, 2,3,4,6-tetra-O-methyl-α-D-glucose and 3,4-di-O-methyl-α- and -β-D-glucoses (Tables III and IV).

Accordingly the sugar moieties of XII and XIII are D-glucopyranosyl-(1-2)-D-glucopyranose and D-glucopyranosyl-(1-2)-[L-rhamnopyranosyl-(1-6)]-D-glucopyranose, respectively.

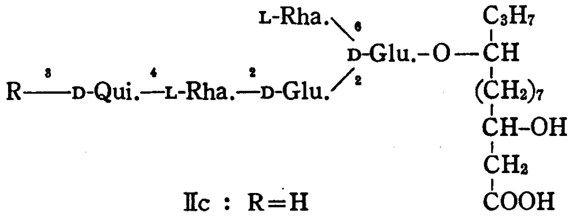
On the basis of all the above results, the structures of II and III are considered respectively to be one of the following three IIa, IIb, IIc and IIIa, IIIb, IIIc.



IIa : R=H
IIIa : R=L-Rha.



IIb : R=H
IIIb : R=L-Rha.



IIc : R=H
IIIc : R=L-Rha.

Experimental[15]

Isolation of *p*-Phenyl Phenacyl Esters (IV and V) of Pharbitic Acids C and D——"Pharbitic acid" (I) (10 g) obtained by the Asahina method[4a] (TLC: Fig. 1) was dissolved in H_2O (40 ml) and EtOH (160 ml), neutralized with 2N NaOH (7 ml) and *p*-phenyl phenacyl bromide (5 g) was added. The mixture was refluxed for 90 min and then evaporated *in vacuo* to give a syrup which was puffed to a faintly yellowish powder (15.8 g) (TLC: Fig. 1). The powder (2 g) was treated with boiling $CHCl_3$ (30 ml) and the insoluble portion (1.8 g) was chromatographed on silica gel (500 g) using the bottom layer of $CHCl_3$–MeOH–H_2O (7:3:1) (solvent system 2) as a solvent. A typical run was as follows: Fr. 1 (~900 ml), none; Fr. 2 (~4180 ml), 107 mg, *Rf* (solvent system 1) 0.46, 0.37; Fr. 3 (~5630 ml), 79 mg, *Rf* 0.37, 0.34; Fr. 4 (~7230 ml), 336 mg, *Rf* 0.34; Fr. 5 (~8730 ml), 500 mg, *Rf* 0.31. Fraction 4 (1 g) was recrystallized four times from iso PrOH to yield IV as fine plates (87 mg): mp 127—130°, $[\alpha]_D^{15°}$ $-54.7°$ ($c=1.30$, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3600—3000 (OH), 1745 (-$COOCH_2$-) and 1695 (aromatic ketone). *Anal.* Calcd. for $C_{58}H_{88}O_{27}\cdot 2H_2O$: C, 55.58; H, 7.35. Found: C, 55.69; H, 7.49. Fraction 5 was recrystallized three times from iso PrOH to yield chromatographically pure V as fine plates (203 mg): mp 135—137°, $[\alpha]_D^{22°}$ $-63.2°$ ($c=0.76$, MeOH), IR: almost same as that of IV. *Anal.* Calcd. for $C_{64}H_{98}O_{31}\cdot 3H_2O$: C, 54.46; H, 7.37. Found: C, 54.30; H, 7.70.

Pharbitic Acid C (II)——IV (1 g) in EtOH (10 ml) and 1% NaOH (10 ml) was refluxed for 30 min and the mixture was passed through a column of Amberlite IRC-50 (H^+) (50 ml). An yellowish eluate was concentrated under reduced pressure to half volume and repeatedly extracted with ether (30 ml in total). The acidic layer was evaporated *in vacuo* to a syrup, which was dissolved in EtOH (20 ml) and treated with active charcoal. Filtrate was evaporated *in vacuo* to a syrup, to which AcOEt was added to precipitate II as a hygroscopic powder (625 mg): single spot (*Rf* 0.18, solvent system 1) on TLC, mp 120—129°, $[\alpha]_D^{8°}$ $-54.1°$ ($c=1.48$, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3500—3200 (OH), 1720 (COOH). *Anal.* Calcd. for $C_{44}H_{78}O_{26}\cdot 10H_2O$: C, 43.92; H, 8.15. Found: C, 43.98; H, 7.59. Molecular weight, 990 (titration with 0.005N NaOH, indicator, phenolphthalein).

Pharbitic Acid D (III)——V (5 g) in EtOH (25 ml) and 1% NaOH (25 ml) was refluxed for 30 min. The reaction mixture was diluted with H_2O (100 ml), concentrated to 100 ml, passed through a column of Amberlite IRC-50 (H^+) (100 ml) and an yellowish eluate was extracted repeatedly with ether (200 ml). The aqueous layer was evaporated *in vacuo* to give a faintly yellow syrup, which was dissolved in MeOH, treated with charcoal and the filtrate was evaporated *in vacuo* to provide a colorless syrup (3.5 g). Three recrystallization from iso PrOH gave III as fine plates (617 mg): single spot (*Rf* 0.16, solvent system 1) on TLC, mp 140—145°, $[\alpha]_D^{15°}$ $-63.8°$ ($c=0.99$, MeOH), IR ν_{max}^{KBr} cm^{-1}: 3500—3200 (OH), 1724 (COOH). *Anal.* Calcd. for $C_{50}H_{88}O_{30}\cdot 2H_2O$: C, 49.83; H, 7.64. Found: C, 49.53; H, 7.86. Molecular weight, 1253 (titration as in II).

Identification of Component Sugars and Aglycones of II and III——II and III (60 mg each) were respectively dissolved in 2N HCl (5 ml) and heated in a boiling water bath for 2 hr,[16] diluted with H_2O (10 ml) and extracted with $CHCl_3$ (30 ml). $CHCl_3$ solution was washed with H_2O, dried over Na_2SO_4 and evaporated to dryness (yield; *ca.* 10 mg in each case) and the residue was methylated with CH_2N_2 and submitted to GLC. The results are summarized in Table I. Aqueous layer was neutralized with Ag_2O and the filtrate was treated with H_2S. The precipitates were filtered off and the filtrate was evaporated *in vacuo* to yield a syrup, which was examined by PC. The results are shown in Table II.

Determination of the Total Sugar Amounts and the Molar Ratios of D-Glucose to Methylpentose in the Complete Hydrolysates of II and III——Yield of total sugar and molar ratio of D-glucose to methylpentose in each hydrolysate of II and III were determined in the same manner as reported by Kawasaki, *et al.*[17] Total sugar yield: II, Found (as glucose); 67.8%, Calcd. for $C_{44}H_{78}O_{26}\cdot 10H_2O$; 70.8%. III, Found (as glucose): 83.0%. Calcd. for $C_{50}H_{88}O_{30}\cdot 2H_2O$; 84.4%. The molar ratios of D-glucose to methylpentose in II; 1:1.5, in III; 1:1.9.

10-Hydroxytridecan-2-one (VI)——I (2.1 g) was heated in 10% $Na_2Cr_2O_7$ solution (11 ml) at 90° for 5 hr. Excess dichromate was decomposed with 10% $NaHSO_3$ (25 ml) and the reaction mixture was evaporated *in vacuo* to dryness. The MeOH soluble portion was heated at 90° for 1 hr with 2N HCl (20 ml) and extracted with ether. The ether solution was washed with H_2O, dried and evaporated to yield an oily substance, which was recrystallized from hexane to give VI as a white crystalline powder (66 mg): mp 37—38°, $[\alpha]_D^{25°}$

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[15] 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 in $CDCl_3$ solution and chemical shifts are given in δ scale with tetramethylsilane as internal reference (s, singlet; d, doublet; t, triplet). TLC was conducted on Kiesel gel G nach Stahl. In column chromatography, "Kanto" silica gel (100—200 mesh) was employed.

[16] Preliminary experiment have shown that II and III were completely hydrolyzed by this condition and the loss of sugars was negligible.

[17] T. Kawasaki and T. Yamauchi, *Chem. Pharm. Bull.* (Tokyo), **11**, 1221 (1963).

+5.2° (c=1.32, $CHCl_3$), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH), 1709 (-$COCH_3$). NMR (ppm): 2.19 (3H, s). *Anal.* Calcd. for $C_{13}H_{26}O_2$: C, 72.89; H, 12.14. Found: C, 72.62; H, 12.17.

Methyl 11-Hydroxytetradec-2-enoate (VII Methyl Ester)——A mixture of I (5.0 g) and AcONa (10 g) in Ac_2O (100 ml) was refluxed for 5 hr and poured into ice water to yield brownish yellow precipitates (6.3 g), which were collected and saponified with 5% alcoholic KOH (25 ml). The reaction mixture was neutralized with HCl, evaporated *in vacuo* and a dark brown residue was further hydrolyzed with 2N HCl (25 ml) at 90° for 1 hr. The mixture was extracted repeatedly with ether (100 ml) and ether layers were combined, washed with H_2O, dried and evaporated to yield VII as a dark brown oil (1 g). It was methylated with CH_2N_2 and chromatographed on silica gel (180 g) using hexane–AcOEt (5:1) to provide a colorless oil (330 mg), which was vacuum-distilled to give an oil (196 mg): bp 166—167°/4 mmHg, $[\alpha]_D^{23°}$ −11.0° (c=0.71, $CHCl_3$), IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1710 (-CH=CH-COO-), 1655 (-CH=CH-), 3610 (OH). *Anal.* Calcd. for $C_{15}H_{28}O_3$: C, 70.31; H, 10.97. Found: C, 69.86; H, 11.05. NMR (ppm): 5.99 (H, double t, J=1.5, 16.8 Hz), 7.20 (H, double t, J=7.5, 16.8 Hz).

Periodate Oxidation of II and III——II and III (100 mg each) were respectively added to 2.7% sodium periodate (7 ml) and stirred at 0° for 2 hr in the dark. Excess $NaIO_4$ was quenched with glycerine (3 drops), EtOH (50 ml) added and the mixture was evaporated *in vacuo* to yield a white residue, which was reduced in MeOH with $NaBH_4$ (10 mg) under stirring for 10 min at room temperature. Excess $NaBH_4$ was decomposed with AcOH, the mixture was evaporated *in vacuo* and the residue was hydrolyzed with 2N HCl (2 ml) in a boiling water bath for 1 hr. The sugar portion obtained in an usual manner was examined by PC. No sugar was detected in the product from II, whereas D-quinovose (*Rf*, 0.31, 0.45, R_{Rha}, 0.66 by solvent systems 1,3 and 4, respectively) was found in that from III.

Permethylates (VIII and IX) of II and III——II (150 mg) and III (200 mg) were respectively methylated by the Hakomori method[9] and the products were chromatographed on silica gel using 5% MeOH in benzene to give VIII (69 mg) and IX (100 mg) both as a resinous mass: no hydroxyl absorption on IR spectrum in either case, *Rf* (Kiesel gel G, 10% MeOH in benzene), 0.41 (VIII) and 0.34 (IX).

Indentification of the Components of VIII and IX——VIII and IX (20 mg each) were respectively heated with 2N HCl in MeOH on a boiling water bath for 2 hr and the reaction mixtures were worked up as usaul and the methanolysates were examined by GLC. The results are shown in Tables III, IV and Fig. 2 and 3.

Syntheses of Reference Compounds[18]——Methyl Ipurolate 3- and 11-Monomethyl Ethers: Methyl ipurolate (1.1 g) in CH_3I (10 ml) was refluxed with Ag_2O (1 g) for 24 hr, precipitates were removed and the filtrate was evaporated to dryness. The residue was again methylated and worked up as above to give an oil. It was chromatographed on silica gel (200 g) using hexane–AcOEt (5:1) to afford 3-methyl ether (oil, 69.4 mg) and 11-Methyl ether (oil, 225 mg). 3-Methyl ether: bp 140—145°/3 mmHg. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3623 (OH), 1733 (-$COOCH_3$), 1092 (-OCH_3). Mass Spectrum *m/e*: 73 ($C_3H_7\text{-}\overset{+}{C}H\text{-}OH$), 117 ($CH_3O\text{-}\overset{+}{C}H\text{-}CH_2COOCH_3$). 11-methyl ether: bp 130—135°/3 mmHg, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3571 (OH), 1731 (-$COOCH_3$), 1087 (-OCH_3). Mass Spectrum *m/e*: 87 ($C_3H_7\text{-}\overset{+}{C}H\text{-}OCH_3$), 103 ($HO\text{-}\overset{+}{C}H\text{-}CH_2COOCH_3$).

Methyl Ipurolate 3,11-Dimethyl Ether: Methyl ipurolate (700 mg) was methylated four times in the same way as above. The product was vacuum-distilled to give the dimethyl ether as a colorless oil (212 mg): bp 125—128°/3 mmHg. Mass Spectrum *m/e*: 87 ($C_3H_7\text{-}\overset{+}{C}H\text{-}OCH_3$), 117 ($CH_3O\text{-}\overset{+}{C}H\text{-}CH_2COOCH_3$).

Methyl 2,3,4,6-Tetra-O-methyl-α-D-glucopyranoside: Prepared by the Hakomori's methylation[9] of methyl α-D-glucopyranoside and purified by distillation; $[\alpha]_D^{23°}$ +139.1° (lit.[19] $[\alpha]_D$ +144°).

Methyl 2,3,4-Tri-O-methyl-α-D-glucopyranoside: Methyl 6-O-trityl-α-D-glucopyranoside prepared according to Helferich, *et al.*[20] was methylated and detritylated; bp 111°/1.5 mmHg, $[\alpha]_D^{16°}$ +157.3° (c=1.11, MeOH).

Methyl 2,3,6-Tri-O-methyl-α- and -β-D-glucopyranosides: Methanolysis of methyl hepta-O-methyllactoside prepared according to Hirst[21] and subsequent chromatography on silica gel provided 2,3,6-tri-O-methyl ethers along with methyl 2,3,4,6-tetra-O-methyl-α-D-galactopyranoside. α-Anomer; bp 104—105°/1.5 mmHg, $[\alpha]_D^{16°}$ +143.6° (c=1.00, MeOH) (lit.[22] $[\alpha]_D$ +149°). β-Anomer; bp 123—124°/2 mmHg, $[\alpha]_D^{21°}$ −47.9° (c=1.08, $CHCl_3$) (lit.[23] $[\alpha]_D$ −48°).

Methyl 2,4,6-Tri-O-methyl-α- and -β-D-glucopyranosides: 1,2,5,6-Di-O-isopropylidene glucofuranose prepared according to Schmidt[24] was converted to 3-O-benzyl ether (bp 180—185°/3 mmHg, $[\alpha]_D^{21°}$ −29.7° (c=4.00, MeOH)). It was subjected to methanolysis and subsequent methylation by the Hakomori method[9] gave methyl 2,4,6-tri-O-methyl-3-O-benzyl-α-D-glucopyranoside (bp 100°/1 mmHg, $[\alpha]_D^{21°}$ +88.8° (c=1.90,

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[18] Analytical data were in good agreement with those calculated for all compounds.

[19] T. Purdie and J.C. Irvine, *J. Chem. Soc.*, **85**, 1049 (1904).

[20] B. Helferich, L. Moog, and A. Junger, *Ber.*, **58**, 872 (1925).

[21] E.L. Hirst and E. Percival, "Methods in Carbohydrate Chemistry," Vol. II, ed. by R.L. Whistler and M.L. Wolfrom, Academic Press Inc., New York and London, 1963, p. 145.

[22] K. Freudenberg and E. Plankenhorn, *Ber.*, **73B**, 621 (1940).

[23] J.C. Irvine and I.M.A. Black, *J. Chem. Soc.*, **1926**, 862.

[24] O.T. Schmidt, "Methods in Carbohydrate Chemistry," Vol. II, 1963, p. 318.

$CHCl_3$)) and its β-anomer (bp 80°/1 mmHg, $[\alpha]_{\mathrm{D}}^{21^\circ}$ -0.8° ($c=1.68$, $CHCl_3$)). Benzyl groups were removed by catalytic hydrogenation over Pd-black; α-anomer: bp 116—117°/2.5 mmHg, $[\alpha]_{\mathrm{D}}^{16^\circ}$ $+133.7^\circ$ ($c=1.33$, $CHCl_3$); β-anomer: bp 120°/2 mmHg, $[\alpha]_{\mathrm{D}}^{16^\circ}$ -29.6° ($c=1.00$, $CHCl_3$) (lit.25) $[\alpha]_{\mathrm{D}}$ -27.4°).

Methyl 3,4,6-Tri-O-methyl-α-D-glucopyranoside: Prepared according to Sundberg, *et al.*26); bp 116°/2.5 mmHg, $[\alpha]_{\mathrm{D}}^{16^\circ}$ $+154.0^\circ$ ($c=1.00$, $CHCl_3$).

Methyl 2,3-Di-O-methyl-α-D-glucopyranoside: Methyl 4,6-O-benzylidene-α-D-glucopyranoside was methylated and then methanolyzed; mp 82—84°, $[\alpha]_{\mathrm{D}}^{28^\circ}$ $+150.6^\circ$ ($c=0.64$, MeOH) (lit.27) mp 83—85°, $[\alpha]_{\mathrm{D}}$ $+150.6^\circ$).

Methyl 2,4-Di-O-methyl-α- and -β-D-glucopyranosides: Synthesized according to Adams, *et al.*28); α-anomer: mp 75—76°, $[\alpha]_{\mathrm{D}}^{16^\circ}$ $+181.2^\circ$ ($c=1.00$, acetone) (lit.28) mp 79—81°, $[\alpha]_{\mathrm{D}}+186^\circ$). β-anomer: $[\alpha]_{\mathrm{D}}^{16^\circ}-19.6^\circ$ ($c=1.35$, acetone) (lit.28) $[\alpha]_{\mathrm{D}}$ -18.6°).

Methyl 3,4-Di-O-methyl-α-D-glucopyranoside: Methyl 2-O-benzyl-3-O-methyl-α-D-glucopyranoside26) was tritylated and subsequently methylated. Detritylation followed by debenzylation gave 3,4-di-O-methyl ether: bp 107°/0.35 mmHg, $[\alpha]_{\mathrm{D}}^{16^\circ}$ $+143.7^\circ$ ($c=1.07$, $CHCl_3$).

Methyl 3,6--DiO-methyl-α-D-glucopyranoside: Methyl 2-O-benzyl-3-O-methyl-6-O-trityl-α-D-glucopyranoside, an intermediate in the synthesis of 3,4-di-O-methyl ether, was subjected successively to benzylation, detritylation, methylation and debenzylation; bp 119°/0.64 mmHg, $[\alpha]_{\mathrm{D}}^{16^\circ}$ $+141.6^\circ$ ($c=1.22$, EtOH).

Methyl 4,6-Di-O-methyl-α-D-glucopyranoside: Synthesized according to Bell, *et al.*29); bp 114°/0.48 mmHg, $[\alpha]_{\mathrm{D}}^{16^\circ}$ $+148.8^\circ$ ($c=0.88$, $CHCl_3$) (lit.29) $[\alpha]_{\mathrm{D}}$ $+157^\circ$).

Methyl 2,3,4-Tri-O-methyl-α-and -β-L-rhamnopyranosides: Prepared from L-rhamnose by the Haworth's methylation followed by chromatography on silica gel: α-anomer; bp 98—100°/24 mmHg, $[\alpha]_{\mathrm{D}}^{26^\circ}$ -44.9° ($c=1.11$, MeOH). β-anomer: mp 45—46°, $[\alpha]_{\mathrm{D}}^{26^\circ}$ $+104.8^\circ$ ($c=1.24$, MeOH).

Methyl 2,3-Di-O-methyl-α-L-rhamnopyranoside: Synthesized according to Brown, *et al.*30): bp 80—85°/1 mmHg, $[\alpha]_{\mathrm{D}}^{21^\circ}$ -37.6° ($c=1.07$, MeOH) (lit.30) $[\alpha]_{\mathrm{D}}$ -37.6°).

Methyl 2,4-Di-O-methyl-α-L-rhamnopyranoside and Methyl 3,4-Di-O-methyl-α-L-rhamnopyranoside: Methyl 4-O-methyl-α-L-rhamnopyranoside prepared according to Butler, *et al.*31) was partially methylated by Purdie method32) and the products were separated on silica gel. 2,4-Di-O-methyl ether: bp 90—92°/1 mmHg, $[\alpha]_{\mathrm{D}}^{25^\circ}$ -66.6° ($c=3.10$, MeOH), hydrolysate (2,4-Di-O-methyl-L-rhamnose); *Rf* (solvent system 1), 0.74; tetrazolium chloride test, negative. 3,4-Di-O-methyl ether: bp 79—80°/1 mmHg, $[\alpha]_{\mathrm{D}}^{25^\circ}$ -99.0° ($c=2.05$, MeOH), hydrolysate (3,4-di-O-methyl-L-rhamnose); *Rf*, 0.77; tetrazolium chloride test, pink.

Methyl 2,3,4-Tri-O-methyl-α- and -β-D-quinovopyranosides: A mixture of methyl -α- and β-D-quinovopyranosides was methylated by the Hakomori method9) and the products were separated on silica gel. α-Anomer: bp 137—140°/13 mmHg, $[\alpha]_{\mathrm{D}}^{12^\circ}$ $+168.0^\circ$ ($c=1.05$, MeOH), β-anomer: mp 58—58.5°, $[\alpha]_{\mathrm{D}}^{12^\circ}$ -37.7° ($c=1.04$, MeOH).

Partial Hydrolyses of I, II and III——I (7 g) in 80% formic acid33) (70 ml) was heated at 90° for 90 min and the reaction mixture was evaporated *in vacuo* giving a resinous residue, which was dissolved in MeOH (50 ml) and methylated with CH_2N_2 in ether. The solvent being removed *in vacuo*, the residue was dissolved in H_2O saturated with BuOH (100 ml) and repeatedly extracted with BuOH saturated with H_2O. The BuOH layer was evaporated *in vacuo* to give a brown mass (4 g) (TLC; *Rf*, 1.00, 0.35, 0.25 and 0.12) (Fig. 4). II (26.7 mg) and III (47.5 mg) were respectively hydrolyzed with 80% formic acid (0.5 ml) and treated in the same way as I. Thin-layer chromatograms (Fig. 4) of the products were identical with that in the case of I. The products (4 g) from I were separated on silica gel (250 g) column using solvent system 2 giving four fractions (Fr.A—D). Fr. A (150 mg) was recrystallized twice from hexane to give X as colorless needles. Its melting point, alone (65—66°) and on admixture, and IR spectrum identical with those of an authentic sample of methyl ipurolate. Fr. B (150 mg) was placed on a silica gel (10 g) column and eluted first with $CHCl_3$–MeOH (3:1) (50 ml) and then with solvent system 2. The latter eluate was evaporated *in vacuo* to give XI as a hygroscopic powder (80 mg): $[\alpha]_{\mathrm{D}}^{24^\circ}$ -16.2° ($c=0.89$, MeOH). *Anal.* Calcd. for $C_{21}H_{40}O_9\cdot H_2O$: C, 55.50; H, 9.03. Found: C, 55.26; H, 8.87. Fr. C (520 mg) was recrystallized from MeOH to give XII as fine needles: mp 114—116°, $[\alpha]_{\mathrm{D}}^{24^\circ}$ -23.6° ($c=1.62$, MeOH). *Anal.* Calcd. for $C_{27}H_{50}O_{14}\cdot 1/2H_2O$: C, 53.37; H, 8.32. Found: C, 53.20; H, 8.33. Fr. D (420 mg) could not be crystallized and puffed *in vacuo* to give XIII as a white powder: $[\alpha]_{\mathrm{D}}^{24^\circ}$ -25.7° ($c=1.19$, MeOH). *Anal.* Calcd. for $C_{33}H_{60}O_{18}\cdot H_2O$: C, 51.96; H, 8.13. Found: C, 51.86; H, 8.03.

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32) E.L. Hirst and E. Percival, "Methods in Carbohydrate Chemistry," Vol. II, 1963, p. 146.
33) Commercial formic acid (purity>89%) (80 ml) was diluted with water to 100 ml.

Identification of Components of XI, XII and XIII——XI, XII and XIII (10 mg each) were respectively hydrolyzed with 2N HCl (1 ml) in a boiling water bath. The precipitates formed on cooling were collected, washed with water, treated with CH_2N_2 and examined by GLC (Table I), and the aqueous layers were worked up in an usual manner and checked by PC (Table II).

Permethylates (XIV and XV) of XII and XIII and Their Methanolyses——XII (70 mg) was methylated by the Hakomori method[9] and the product was chromatographed on silica gel (7 g) using hexane–AcOEt (1:1) to give XIV as a resinous mass (25 mg); *Rf* (Kiesel gel G, hexane–AcOEt, 2:1), 0.68. In the same way XIII (90 mg) gave XV (50 mg): *Rf* (Kiesel gel G. hexane–AcOEt, 1:1), 0.61. XIV and XV (10 mg each) were respectively refluxed with 4N HCl in MeOH (0.5 ml) for 2 hr and the products were examined by GLC. The results are shown in Tables III and IV.