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Studies on the Constituents of *Mallotus japonicus* Muell. Arg. I. Cardiac Glycosides from the Seeds[1]

Hikaru Okabe, Kazuhide Inoue, and Tatsuo Yamauchi

Faculty of Pharmaceutical Sciences, Fukuoka University[2]

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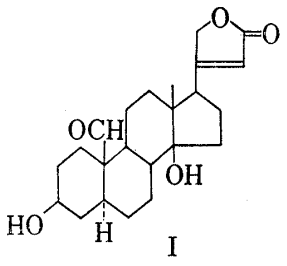
Eight cardiac glycosides, 3-O-α-L-rhamnopyranosides and 3-O-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosides of corotoxigenin, mallogenin, coroglaucigenin and panogenin, were isolated from the seeds of *Mallotus japonicus* Muell. Arg. (Euphorbiaceae).

Mallotus japonicus Muell. Arg. (Japanese name: Akamegashiwa) (Euphorbiaceae) is a deciduous tree widely distributed in Japan and in the southeastern part of Asia, and its bark has been used in Japan for stomach disorders, and in Taiwan as a folk medicine for cancer.

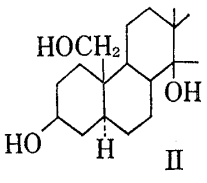
The constituents of this plant were investigated by a number of workers, and was isolated bergenin from its bark[3] and rutin from leaves,[4] but the constituents of the seeds have not

Table I. Cardenolides[a] from the Seeds of *Mallotus philippinensis* and *M. paniculatus*

From *M. philippinensis*	From *M. paniculatus*
Corotoxigenin (I)	Uzarigenin (III)
Coroglaucigenin (II)	11-Oxouzarigenin (IV)
Corotoxigenin rhamnoside	Mallogenin (V)
Coroglaucigenin rhamnoside	Coroglaucigenin
	Mallogenin rhamnoside
	Panogenin (VI) rhamnoside
	Panogenin glucorhamnoside



I



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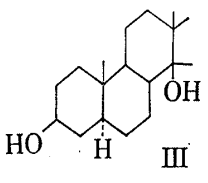

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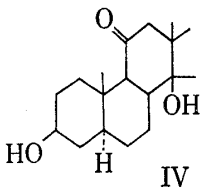

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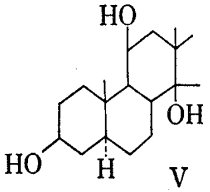
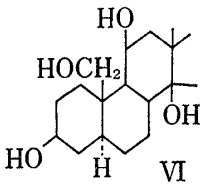


V



VI

a) Sugar moieties of glycosides were placed at C-3 hydroxyl group of aglycones without any experimental proof, and the position and configuration of the glucose linkage to rhamnose of panogenin glucorhamnoside were not determined.

1) This work was reported at the 95th Annual Meeting of the Pharmaceutical Society of Japan, Nishinomiya, April 1975.
2) Location: *Nanakuma, Nishi-ku, Fukuoka.*
3) K. Homma, *J. Agr. Chem. Soc. Japan*, **15**, 394 (1939).
4) K. Shibata and M. Shimokoriyama, *J. Chem. Soc. Japan*, **20**, 36 (1949).

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been reported. Reichstein and his coworkers isolated and characterized several cardenolides shown in Table I from the fermentation products of seeds of *Mallotus philippinensis*[5] and *M. paniculatus*.[6,7]

The seeds of *M. japonicus* were also expected to contain similar cardenolides and/or their glycosides. The authors have examined the constituents of the seeds, and this paper deals with isolation and characterization of eight cardiac glycosides.

The greasy seed powder was washed with *n*-hexane and then percolated with methanol. The methanolic extract was fractionated to the benzene, chloroform, chloroform–ethanol (2: 1) and butanol extracts, the latter three of which were sensitive to the Kedde reaction. The thin-layer chromatograms (TLC) showed eight Kedde positive compounds in all and they were tentatively named MJ-I to VIII in order of increasing polarity.

MJ-I and V stained yellowish brown after spraying 10% sulfuric acid and heating on the TLC plate, II and VI; dark violet, III and VII; dark brown, IV and VIII; green, and these colorations suggested that the paired two compounds have the same aglycone but differ in number of the sugar combined.

The chloroform, chloroform–ethanol (2: 1) and butanol extracts were respectively passed through polyamide column. The fractions positive to the Kedde reaction were further chromatographed over neutral alumina repeatedly, and eight cardiac glycosides were obtained in crystalline form.

MJ-I: Colorless needles, $C_{29}H_{42}O_9 \cdot H_2O$, mp 188—192°, $[\alpha]_D$ −18.5°, λ_{max} 218 nm.

MJ-II: Colorless needles, $C_{29}H_{44}O_9 \cdot 1/2H_2O$, mp 275—278°, λ_{max} 220 nm.

MJ-III: Colorless prisms, $C_{29}H_{44}O_9 \cdot H_2O$, mp 235—238°, $[\alpha]_D$ −37.05°, λ_{max} 220 nm.

MJ-IV: Colorless needles, $C_{29}H_{44}O_{10} \cdot 1/2H_2O$, mp 281—283°, $[\alpha]_D$ −30.52°, λ_{max} 220 nm.

MJ-V: Colorless plates, $C_{35}H_{52}O_{14}$, mp 197—200°, $[\alpha]_D$ −24.11°, λ_{max} 218 nm.

MJ-VI: Colorless needles, $C_{35}H_{54}O_{14} \cdot H_2O$, mp 225—228°, $[\alpha]_D$ −27.00°, λ_{max} 220 nm.

MJ-VII: Colorless needles, $C_{35}H_{54}O_{14} \cdot 2H_2O$, mp 190—195°, $[\alpha]_D$ −31.50°, λ_{max} 220 nm.

MJ-VIII: Colorless needles, $C_{35}H_{54}O_{15} \cdot H_2O$, mp 238—240°, $[\alpha]_D$ −33.20°, λ_{max} 220 nm.

The former four glycosides (MJ-I to IV) gave L-rhamnose on acid hydrolysis, and the molecular formulae indicated that they are all mono-rhamnosides and this conclusion was confirmed by the nuclear magnetic resonance (NMR) spectra in which each compound depicted one singlet due to the anomeric proton of the rhamnosyl group. MJ-I is rather unstable and its NMR spectrum exhibited a singlet due to an aldehydic proton together with a singlet due to the angular methyl suggesting that one of two angular methyl groups on a steroidal framework is oxidized to an aldehyde, and when treated with $NaBH_4$ in 80% aqueous ethanol, MJ-I gave MJ-III which showed no aldehydic proton signal.

The chemical correlation of MJ-I to MJ-III, and their physical constants strongly suggested that MJ-I and III are L-rhamnosides of corotoxigenin and coroglaucigenin, respectively.

MJ-II and IV were found to be identical with malloside (mallogenin rhamnoside) and panoside (panogenin rhamnoside) by comparison of physical properties and infrared (IR) spectra with those reported by Reichstein, *et al.*[6]

The acetate of MJ-II showed on NMR spectrum three singlets due to the acetyl methyl protons. On the other hand, the acetates of MJ-III and IV both exhibited four singlets due to acetyl methyl protons and an AB quartet at 4—5 ppm which was not discernible in the spectra of the parent compounds, and was assigned to the signal due to the acetoxymethylene protons on C_{19}. These NMR data have led us to the conclusion that the sugar moiety of each rhamnoside is linked to the C_3 hydroxyl group of the aglycone.

5) K.D. Roberts, Ek. Weiss, and T. Reichstein, *Helv. Chim. Acta*, **46**, 2886 (1963).
6) K.D. Roberts, Ek. Weiss, and T. Reichstein, *Helv. Chim. Acta*, **49**, 316 (1966).
7) K.D. Roberts, Ek. Weiss, and T. Reichstein, *Helv. Chim. Acta*, **50**, 1645 (1967).

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MJ-V has an aldehyde group, and it was converted to MJ-VII when treated with $NaBH_4$. MJ-V, VI, VII and VIII gave L-rhamnose and D-glucose on acid hydrolysis. Molecular formulae and NMR spectra indicated that their sugar moieties are composed of one mole each of L-rhamnose and D-glucose. On treatment with β-glucosidase, they gave MJ-I, II, III and IV, respectively. However, on incubation with the crude hesperidinase,[8] MJ-VI, VII and VIII were hydrolyzed almost quantitatively into aglycones, which were proved to be mallogenin, coroglaucigenin and panogenin, respectively, by comparison of their melting points and IR spectra with those reported.[5-7]

Glucorhamnosides were methylated by the Hakomori method[9] and methylated products were subjected to methanolysis to give in each instance methyl pyranosides of 2,3,4,6-tetra-O-methyl-α-D-glucose and 2,3-di-O-methyl-α-L-rhamnose, which were identified on the basis of thin-layer and gas-liquid cochromatographies (GLC) using authentic specimens.

Configurations of the sugar linkages were indicated α-form for L-rhamnose by differences of molecular rotations between those of rhamnosides and aglycones (Table II) and β-form for D-glucose by molecular rotation differences (Table II) and by NMR spectra which depicted the signal of the anomeric proton of the D-glucopyranosyl group as a doublet with a coupling constant of *ca.* 6 Hz.

Table II. Molecular Rotation Differences

	$[\alpha]_D$	$[M]_D$	$\Delta[M]_D$
MJ-V	−24.1	−168.8	−70.2
MJ-I	−18.5	−98.6	−264.5
Corotoxigenin[a]	+43.0	+167.0	
MJ-VI	−27.0	−188.5	−20.2
MJ-II (malloside)[a]	−31.4	−168.3	−283.0
Mallogenin[a]	+29.4	+114.7	
MJ-VII	−31.5	−219.9	−21.3
MJ-III	−37.1	−198.6	−293.0
Coroglaucigenin[a]	+24.2	+94.4	
MJ-VIII	−33.2	−237.0	−68.5
MJ-IV	−29.0	−168.5	−273.2
Panogenin[a]	+25.8	+104.7	
Methyl pyranoside of α-D-glucose		+309	
β-D-glucose		−66	
α-L-rhamnose		−111	
β-L-rhamnose		+170	

a) data after Reichstein, *et al.*[5-7]

Above-mentioned evidence indicates that MJ-I, II, III and IV are 3-O-α-L-rhamnopyranosides of corotoxigenin, mallogenin, coroglaucigenin and panogenin, respectively, and MJ-V, VI, VII and VIII are the corresponding β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosides.

As already mentioned in the introduction, Reichstein and his collaborators isolated cardenolide aglycones and their rhamnosides from the fermentation products of the seeds of two species of *Mallotus*. Glucopanoside is the only one glucorhamnoside that they isolated and its yield is very low. On the other hand, in this study without fermentation process, glucorhamnosides were isolated as the major constituents with small amounts of rhamnosides and no aglycone. Considering from our results, it is most probable that the genuine cardiac

8) H. Kohda and O. Tanaka, *Yakugaku Zasshi*, **95**, 246 (1975).

9) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 255 (1964). When cardiac glycosides are methylated by the Hakomori or Kuhn methods, they usually provide a complex mixture of methylated products. This result is probably due to isomerization and decomposition of the alkali labile butenolide ring. The authors confirmed preliminarily using the known cardiac glycosides that the sugar moiety is fully methylated intact.

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glycosides in *M. philippinensis* and *paniculatus* are also β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosides.

Experimental[10]

Extraction and Fractionation——The seeds (23 kg) of *Mallotus japonicus* collected in the suburbs of Fukuoka City, Fukuoka Pref. in September, 1974 were crushed and the tarry seed mass was washed with *n*-hexane to give an oily hexane extract (10 kg). The defatted seed powder was percolated with MeOH (total volume 260 liters). The MeOH solution was concentrated to 4 liters, water (4 liters) was added and extracted successively with benzene (24 liters), $CHCl_3$ (16 liters) and then with $CHCl_3$–EtOH (2: 1) (20 liters). The solvents were evaporated to give the benzene extract (480 g), $CHCl_3$ extract (7.7 g) and $CHCl_3$–EtOH (2: 1) extract (16 g). The aqueous MeOH layer after extraction was concentrated to a half volume and extracted with BuOH (20 liters) to give a resinous extract (72 g). $CHCl_3$, $CHCl_3$–EtOH (2: 1) and BuOH extracts were chromatographed on polyamide (10× weight of sample) and fractions positive to the Kedde reaction were repeatedly chromatographed over neutral alumina (grade III for rhamnosides and IV for glucorhamnosides; eluting solvents: solvent A for rhamnosides and B for glucorhamnosides) until thin-layer chromatographically homogeneous compounds were separated. Homogeneity was checked by TLC (developing solvents: solvent B for rhamnosides and C for glucorhamnosides; detection of spots: Kedde reagent and 10% H_2SO_4).

MJ-I: Yield, 0.0012% on fresh seed basis. Colorless needles from MeOH–H_2O, mp 188—192°, $[\alpha]_D^{24°}$ −18.5° (c=1.50, MeOH). *Anal.* Calcd. for $C_{29}H_{42}O_9 \cdot H_2O$: C, 63.04; H, 7.97. Found: C, 63.47; H, 7.95. IR ν_{max}^{KBr} cm^{-1}: 3400—3600, 1780, 1735, 1712. NMR ($CDCl_3$–CD_3OD): 0.80 (3H, s, >C–CH_3), 1.21 (3H, d, J=6.0 Hz, >CH–CH_3 of rhamnosyl group), 4.63 (1H, s, anomeric H of rhamnosyl group), 4.62, 4.86 (2H, AB quartet, J_{AB}=18.0 Hz, =C–CH_2–OCO–), 5.62 (1H, s, >C=CH–COO–), 9.52 (1H, s, –CHO). UV λ_{max}^{MeOH} nm (log ε): 218 (4.20).

MJ-II: 0.0004%. Colorless needles from MeOH, mp 275—278°. *Anal.* Calcd. for $C_{29}H_{44}O_9 \cdot 1/2H_2O$: C, 63.85; H, 8.25. Found: C, 63.30; H, 8.12. IR ν_{max} cm^{-1}: 3420, 1780, 1755, 1745, 1625; identical with the spectrum of malloside reported by Reichstein, *et al.* UV λ_{max}^{MeOH} nm (log ε): 220 (4.25). Acetate of MJ-II: Colorless needles from EtOH, mp 220—230°. NMR ($CDCl_3$): 2.12, 2.02, 1.96 (3H, each, s, –$OCOCH_3$).

MJ-III: 0.0016%. Colorless prisms from MeOH, mp 235—238°, $[\alpha]_D^{24°}$ −37.05° (c=2.00, MeOH). *Anal.* Calcd. for $C_{29}H_{44}O_9 \cdot H_2O$: C, 62.81; H, 8.30. Found: C, 62.68; H, 8.30. IR ν_{max} cm^{-1}: 3200—3600, 1820, 1725, 1650, 1612. NMR ($CDCl_3$–CD_3OD): 0.89 (3H, s, >C–CH_3), 1.23 (3H, d, J=5.4 Hz, >CH–CH_3 of rhamnosyl group), 4.72 (1H, s, anomeric H of rhamnosyl group), 4.68, 4.95 (2H, AB quartet, J_{AB}=18.0 Hz, =C–CH_2–OCO–), 5.69 (1H, s, >C=CH–COO–). UV λ_{max}^{MeOH} nm (log ε): 220 (4.02). Acetate of MJ-III: Amorphous powder. NMR ($CDCl_3$): 1.96, 2.02, 2.05, 2.11 (3H each, s, –$OCOCH_3$), 4.10, 4.34 (2H, AB quartet, J_{AB}=11.9 Hz, –CH_2OAc).

MJ-IV: 0.0012%. Colorless needles from MeOH, mp 281—283°, $[\alpha]_D^{24°}$ −30.52° (c=1.15, MeOH). *Anal.* Calcd. for $C_{29}H_{44}O_{10} \cdot 1/2H_2O$: C, 61.96; H, 8.00. Found: C, 62.03; H, 8.00. IR ν_{max} cm^{-1}: 3200—3600, 1785, 1750, 1720, 1664, 1630; identical with the spectrum of Reichstein's panoside. NMR ($CDCl_3$–CD_3OD): 1.09 (3H, s, >C–CH_3), 1.23 (3H, d, J=5.8 Hz, >CH–CH_3 of rhamnosyl group), 4.66 (1H, s, anomeric H of rhamnosyl group), 4.63, 4.91 (2H, AB quartet, J_{AB}=18.0 Hz, =C–CH_2–O–CO–), 5.63 (1H, s, =CH–COO–). UV λ_{max}^{MeOH} nm (log ε): 220 (4.17). Acetate of MJ-IV: Amorphous powder. NMR ($CDCl_3$): 1.95, 2.01, 2.09, 2.12 (3H each, s, –$OCOCH_3$), 4.11, 4.46 (2H, AB quartet, J_{AB}=11.8 Hz, –CH_2OAc).

MJ-V: 0.0163%. Colorless plates from MeOH–H_2O, mp 197—200°, $[\alpha]_D^{24°}$ −24.11° (c=1.00, MeOH). *Anal.* Calcd. for $C_{35}H_{52}O_{14}$: C, 60.34; H, 7.47. Found: C, 60.52; H, 7.40. IR ν_{max} cm^{-1}: 3200—3600, 1740, 1710, 1650, 1630. NMR ($CDCl_3$–CD_3OD): 0.80 (3H, s, >C–CH_3), 1.28 (3H, d, J=5.4 Hz, >CH–CH_3 of rhamnosyl group), 4.34[11] (1H, d, J=6.3 Hz, anomeric H of glucosyl group), 4.61 (1H, s, anomeric H of rhamnosyl

10) Melting points: Yanaco micro melting point apparatus, uncorrected. UV: Shimadzu multiconvertible spectrophotometer Double-40. NMR: JEOL JNM-MH-100 NMR spectrometer, 100 MHz, internal reference; Me_4Si, δ-scale (s: singlet, d: doublet). IR: Hitachi grating infrared spectrophotometer Model EPI-G3, KBr pellets. Specific Rotations: Yanaco digital automatic polarimeter OR-50D. GLC: Shimadzu gas chromatograph GC-3BF, column: 5% 1,4-butanediol succinate on shimalite W (60—80 mesh), 1.7 m×3 mmϕ, conditions: carrier gas N_2; 0.6 kg/cm², H_2; 0.6 kg/cm², column temperature; 184°. TLC: Kieselgel G nach Stahl (E. Merck). Paper Chromatography: Toyo filter paper No. 50, ascending. Column Chromatography: Kieselgel (70—200 mesh) (E. Merck), Aluminum oxide (M. Woelm), Polyamide C-200 (Wako Pure Chemical Industries, Ltd.). Solvent systems: solvent A; $CHCl_3$–MeOH–H_2O (7: 2: 1, bottom layer), B; $CHCl_3$–MeOH–H_2O (7: 3: 1, bottom layer), C; solvent B (30)+MeOH (3), D; BuOH–AcOH–H_2O (4: 1: 5, top layer), E; hexane–AcOEt (1: 2).

11) This signal was overlapped by the signal of hydroxyl proton, therefore, the spectrum was taken after adding CF_3COOH (up to 5%) to the solution (K. Miyahara and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **22**, 1407 (1974)).

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group), 4.63, 4.86 (2H, AB quartet, J_{AB}=18.0 Hz, =C–CH_2–O–CO–), 5.62 (1H, s, =CH–COO–), 9.52 (1H, s, –CHO). UV λ_{max}^{MeOH} nm (log ε): 218 (4.25).

MJ-VI: 0.006%. Colorless needles from MeOH, mp 225—228°, $[\alpha]_D^{24°}$ −27.00° (c=1.07, MeOH). *Anal.* Calcd. for $C_{35}H_{54}O_{14}\cdot H_2O$: C, 58.80; H, 7.82. Found: C, 58.94; H, 7.79. IR ν_{max} cm^{-1}: 3300—3600, 1815, 1725, 1699, 1650, 1622. NMR ($CDCl_3$–CD_3OD): 1.01 (3H, s, >C–CH_3), 1.09 (3H, s, >C–CH_3), 1.30 (3H, d, J=5.3 Hz, >CH–CH_3 of rhamnosyl group), 4.42[11] (1H, d, J=6.2 Hz, anomeric H of glucosyl group), 4.73 (1H, s, anomeric H of rhamnosyl group), 4.69, 4.92 (2H, AB quartet, J_{AB}=17.1 Hz, =C–CH_2–OCO–), 5.71 (1H, s, =CH–COO–). UV λ_{max}^{MeOH} nm (log ε): 220 (4.19).

MJ-VII: 0.0202%. Colorless needles from MeOH, mp 190—195°, $[\alpha]_D^{24°}$ −31.5° (c=2.19, MeOH). *Anal.* Calcd. for $C_{35}H_{54}O_{14}\cdot 2H_2O$: C, 57.22; H, 7.90. Found: C, 57.18; H, 7.64. IR ν_{max} cm^{-1}: 3200—3600, 1775, 1735, 1620. NMR ($CDCl_3$–CD_3OD): 0.89 (3H, s, >C–CH_3), 1.28 (3H, d, J=5.7 Hz, >CH–CH_3 of rhamnosyl group), 4.32[11] (1H, d, J=6.0 Hz, anomeric H of glucosyl group), 4.64 (1H, s, anomeric H of rhamnosyl group), 4.63, 4.87 (2H, AB quartet, J_{AB}=18.0 Hz, =C–CH_2O–), 5.60 (1H, s, =CH–COO–). UV λ_{max}^{MeOH} nm (log ε): 220 (4.17).

MJ-VIII: 0.0072%. Colorless fine needles from MeOH, mp 238—240°, $[\alpha]_D^{24°}$ −33.20° (c=1.18, MeOH). *Anal.* Calcd. for $C_{35}H_{54}O_{15}\cdot H_2O$: C, 57.37; H, 7.65. Found: C, 57.44; H, 7.59. IR ν_{max} cm^{-1}: 3100—3600, 1750, 1719, 1660, 1630; identical with the spectrum of Reichstein's glucopanoside. NMR ($CDCl_3$–CD_3OD): 1.09 (3H, s, >C–CH_3), 1.29 (3H, d, J=5.0 Hz, >CH–CH_3 of rhamnosyl group), 4.40[11] (1H, d, J=7.1 Hz, anomeric H of rhamnosyl group), 4.66, 4.89 (2H, AB quartet, J_{AB}=18.0 Hz, =C–CH_2OCO–), 5.69 (1H, s, =CH–COO–). UV λ_{max}^{MeOH} nm (log ε): 220 (4.18).

Identification of Sugars——A glycosides (20 mg) was suspended in 1N aqueous HCl (1 ml) and heated in a boiling water bath for 1 hr. The reaction mixture was worked up as usual manner and the sugar mixture was subjected to paper chromatography (developing solvent: solvent D). MJ-I, II, III, and IV gave one spot (*Rf*, 0.39) while MJ-V, VI, VII, and VIII gave two spots (*Rf*, 0.39, 0.19) (L-rhamnose, 0.39; D-glucose, 0.19). A glucorhamnoside (300 mg) was dissolved in 2N methanolic HCl (5 ml) and refluxed for 1 hr. The reaction mixture was treated in a usual manner to give a resinous residue (300 mg), which was chromatographed over Kieselgel (10 g) using solvent B as an eluting solvent to give methyl glycosides I and II. Two methyl glycosides were respectively hydrolyzed with 1N aqueous HCl (2 ml) and the resulting sugars were converted to the corresponding phenylosazones.

Phenylosazone from Methyl Glycosides I: Brownish yellow powder from EtOH, mp 181° (decomp.), $[\alpha]_D^{20°}$ +65.4° (c=0.60, MeOH), IR: identical with the spectrum of the authentic sample of 6-deoxy-L-arabino-hexose phenylosazone.

Phenylosazone from Methyl Glycoside II: Brownish yellow powder from EtOH, mp 206—207° (decomp.), $[\alpha]_D^{20°}$ +75.0° (c=0.60, pyridine). IR: identical with the spectrum of the authentic sample of D-arabino-hexose phenylosazone.

Methylation of the Glucorhamnosides and Identification of Component Methylsugars——A glucorhamnoside (10 mg) was dissolved in DMSO (1 ml) and 0.4 ml of dimethylsulfinyl carbanion solution prepared by warming NaH (200 mg) in DMSO (4 ml) was added. After stirring the mixture for 10 min at room temperature, MeI (1 ml) was added and stirred for another 30 min. The reaction mixture was poured into water and extracted with $CHCl_3$ (5 ml). After washing with water and drying over Na_2SO_4, the solvent was evaporated. The residue was dissolved in 2N methanolic HCl (1 ml) and refluxed for 30 min. HCl was removed by adding Ag_2O and the neutral residue was subjected to TLC (developing solvent: solvent E). By spraying 10% H_2SO_4 and heating, an intense black spot (*Rf*, 0.48) and a yellow spot (*Rf*, 0.33) were detected (methyl 2,3,4,6-tetra-O-methyl-α-D-glucopyranoside: 0.48, methyl 2,3,4-tri-O-methyl-α-L-rhamnopyranoside: 0.76, 2,3-di-O-methyl-α-L-rham.: 0.33, 2,4-di-O-methyl-α-L-rham.: 0.46, 3,4-di-O-methyl-α-L-rham.: 0.56). Two compounds were separated by column chromatography on Kieselgel (2 g) using $CHCl_3$ as eluting solvent and they were subjected to GLC. Two compounds had the same t_R value (3.1 min), but were clearly distinguished from other possible methyl glycosides of partially methylated sugars (methyl 2,3,4,6-tetra-O-methyl-α-D-glucopyranoside: 3.1, methyl 2,3,4-tri-O-methyl-α-L-rhamnopyranoside: 1.2, 2.3,-di-O-methyl-α-L-rham.: 3.1, 2.4-di-O-methyl-α-L-rham.: 2.6,3,4-di-O-methyl-α-L-rham.: 2.3).

Enzymic Hydrolysis of Glucorhamnosides——(A) β-Glucosidase: A thin-layer chromatographically homogeneous glucorhamnoside (100 mg) and β-glucosidase (200 mg) were dissolved in water (5 ml) (MJ-V and VI were dissolved in 5 ml of 20% aqueous EtOH) and shaken overnight at 38°. Water was evaporated to dryness and flashed repeatedly with EtOH to prepare a dry powder, which was chromatographed on neutral alumina (grade III, 15 g) using solvent A as developer. MJ-V, VI, VII, and VIII gave the corresponding desglucosides and they were identical with MJ-I, II, III, and IV respectively in all respects.

(B) Crude Hesperidinase: MJ-VI (31 mg) was dissolved in 2 ml of 20% aqueous EtOH. The crude hesperidinase (24 mg) was added and shaken for 2 days at 38°. Precipitates were filtered and recrystallized from MeOH to give colorless prisms, mp 280—290° (decomp.). Its IR spectrum was identical with that of mallogenin reported by Reichstein, *et al.* The same work on MJ-VII and VIII gave the corresponding aglycones. The aglycone of VII: colorless prisms from acetone, mp 247—250°, IR ν_{max} cm^{-1}: 3500, 3420, 3250,

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1785, 1750, 1742, 1720, 1710, 1670, 1630. The aglycone of VIII: colorless fine needles from EtOH, mp 250—260° (decomp.), IR: superimposable on the spectrum of Reichstein's panogenin.

$NaBH_4$ Reduction of MJ-I and V——MJ-I (160 mg) was dissolved in 80% aqueous EtOH (10 ml) and $NaBH_4$ (56 mg) was added. After the mixture was stirred overnight in a refrigerator, the solution was neutralized with AcOH and evaporated *in vacuo* to dryness. The residue was chromatographed on neutral alumina (grade III, 20 g) using solvent D as an eluting solvent and the thin-layer chromatographically homogeneous fractions were combined and crystallized from MeOH to give colorless prisms (25 mg): mp 230—236°, its IR spectrum was identical with that of MJ-III. Similar treatment of MJ-V (306 mg) gave colorless prisms (75 mg), mp 192—195°, its IR spectrum was quite identical with that of MJ-VII.

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