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Pericarp Saponins of *Akebia quinata* Decne. II.[1] Arjunolic and Norarjunolic Acids, and Their Glycosides

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The structures of two free triterpenoids, Y and Z, and two saponins, P_H and P_{J1}, isolated from the fresh pericarps of *Akebia quinata* Decne. (Lardizabalaceae) were investigated. Y, mp 317—320°, $[\alpha]_D$+62°, was identified with arjunolic acid (I), while Z, mp 303—305°, $[\alpha]_D$+75°, was assigned the structure 20 (29)-dehydro-30-norarjunolic acid (XII) and conventionally named norarjunolic acid. P_H, mp 207—210° (decomp.), $[\alpha]_D$+3°, was defined as 28-O-α-L-rha·pyr-(1→4)-β-D-glc·pyr-(1→6)-β-D-glucopyranoside (XIX) of XII, and P_{J1} was characterized as 28-O-β-D-xyl·pyr-(1→3)-α-L-rha·pyr-(1→4)-β-D-glc·pyr-(1→6)-β-D-glucopyranoside (XXIV′) of I.

XXIV′ is the first arjunolic acid oligoglycoside, and XII and XIX are second to eupteleogenin and eupteleoside, respectively, as a naturally occurring noroleanane derivative and its glycoside.

In the preceding paper[1] it was reported that two free triterpenoids, Y and Z, and twelve saponins, P_A—P_H, P_{J1-3}, and P_K, were obtained from the pericarps of *Akebia quinata* Decne., and that ten of the saponins were found to be the glycosides of hederagenin and oleanolic acid.

This paper concerns characterizations of Y and Z and structure elucidations of the remaining two saponins, P_H and P_{J1}.

Y (I), mp 317—320°, $[\alpha]_D$ +62°, $C_{30}H_{48}O_5$ (M^+, *m/e* 488) shows on the infrared (IR) spectrum the hydroxyl and carboxyl absorptions, and the methylester (II), mp 221—223°, $[\alpha]_D$ +58°, $C_{31}H_{50}O_5$ (M^+, *m/e* 502), exhibits on the nuclear magnetic resonance (NMR) spectrum six tertiary methyl and one methoxycarbonyl signals. I acetate (III), mp 135—140°, $[\alpha]_D$ +37°, and its methylester (IV), mp 106—109°, $[\alpha]_D$+34°, show three acetoxyl signals on their NMR spectra. On the mass spectra of I and III the relatively intense peaks at *m/e* 248 and 203 are commonly observed, while on those of II and IV the peak at *m/e* 248 is replaced by that of *m/e* 262. These are characteristic ion peaks[3] due to the retro-Diels-Alder type fragmentation of an olean-12-en- or urs-12-en-28-oic acid derivative and its methylester bearing no substituent on the C, D, and E rings. The NMR spectra of III and IV show equally an AB quartet (2H, J=12 Hz) at 3.55 and 3.86 ppm, which is also observed on the spectra of hederagenin diacetate and asiatic acid triacetate[4] and assigned to their 23-methylene protons. When I was treated with acetone and anhydrous cupric sulfate in the same manner as in the case of methyl asiatate[5] yielding the 3,23-O-isopropylidene derivative, a monoacetonide (V), mp 190—192°, $[\alpha]_D$ +51°, $C_{33}H_{52}O_5$ (M^+, *m/e* 528), was provided, and it was further acetylated to give a monoacetonide monoacetate (VI), mp 201—205°, $[\alpha]_D$ +23°, $C_{35}H_{54}O_6$ (M^+, 570).

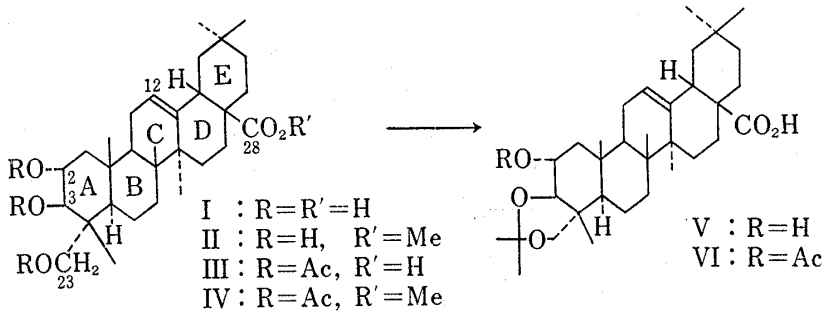
All the above data suggest that I is an olean- or urs-12-en-28-oic acid bearing, alike hederagenin and asiatic acid, both the 3β- and 23-hydroxyl groups and that the third hydroxyl group is located on the A or B ring.

V, presumably the 3,23-O-isopropylidene derivative of I, shows on the NMR spectrum a one-proton doublet (J=10 Hz) at 3.32 ppm, two-proton singlet at 3.48 and a one-proton mul-

1) Part I: R. Higuchi and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo),24, 1021 (1976).
2) Location: *3-1-1 Maedashi, Higashi-ku Fukuoka, 812, Japan.*
3) H. Budzikiewicz, J.M. Wilson, and C. Djerassi, *J. Amer. Chem. Soc.*, **85**, 3688 (1963).
4) J.C. Mani, *Ann. Chim.*, **10**, 533 (1965).
5) H.T. Cheung and M.C. Feng, *J. Chem. Soc.*, **1968**, 1047.

tiplet ($w_{h/2}$=18 Hz) at 3.5—3.9. The first one is assignable to the α (axial) proton at C_3, the second to the 23-methylene protons, and the last one is ascribed to a proton *geminal* to the third hydroxyl group. On the NMR spectrum of VI the above multiplet is replaced by that at 4.8—5.1 ppm due to the proton of a methine carrying an acetoxyl group. The coupling patterns of the doublet and the multiplet on the spectrum of V indicate that the third hydroxyl group is *vicinal* and *trans* to the 3β-hydroxyl group, that is, located at C_2 in α configuration.

Accordingly I is presumed to be 2α,3β,23-trihydroxyolean-12-en-28-oic acid (2α-hydroxyhederagenin=arjunolic acid[6]) or its ursane analog (asiatic acid[7]).



Formulae 1

The melting point and the optical rotation of I are similar to those (mp 315—320°,[8] $[\alpha]_D$ +63.5°) reported for arjunolic acid and different from those (mp 300—305°, $[\alpha]_D$ +51°) of its isomer, asiatic acid. However, since the authentic samples for direct comparisons were not available, in order to confirm the structure, the synthesis of I from hederagenin was carried out according to the scheme shown in Chart 1.

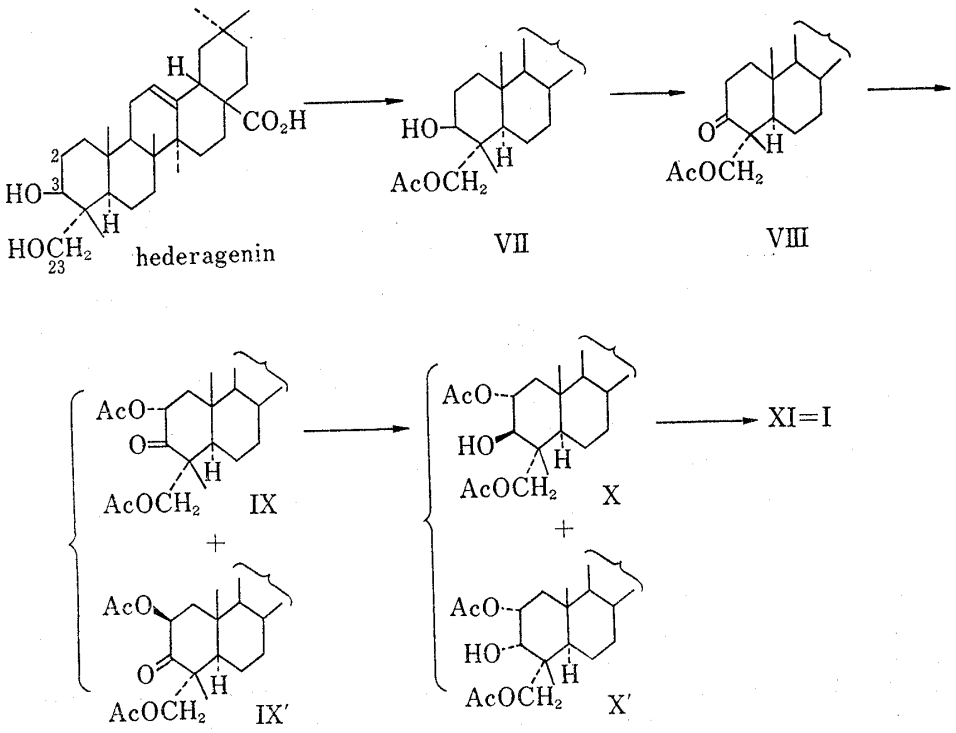


Chart 1

6) F.E. King, T.J. King, and J.M. Ross, *J. Chem. Soc.*, **1954**, 3995.
7) J. Polonsky, *Bull. Soc. Chim. France*, **1952**, 649, 1015; *idem*, *ibid.*, **1953**, 173.
8) According to King, *et al.*[6] this melting point was raised upto 337—340° by repeated recrystallization of a large amount of sample.

Acetylation of hederagenin in a mild condition gave the 23-monoacetate (VII) as the major product, which was oxidized to provide the corresponding 3-one (VIII). VIII was treated in an acetic acid–acetic anhydride mixture with lead tetraacetate and boron trifluoride according to the Henbest method[9] which is employed for introduction of 2α-acetoxyl group onto a 3-oxo-5α-steroid. Of the two epimeric 2-acetoxy-3-ones (IX, IX′) afforded, the 2α-epimer (IX) was isolated by means of chromatography and reduced with sodium borohydride to give a mixture of 2α-acetoxy-3β-ol (X) and its 3α-epimer (X′). The mixture was separated over silica gel and X was saponified to give 2α-hydroxyhederagenin (XI), mp 317—320°, $[\alpha]_D$ +60°, which was identified with I.

Consequently I is defined as 2α,3β,23-trihydroxyolean-12-en-28-oic acid, that is arjunolic acid.

Z(XII), mp 303—305°, $[\alpha]_D$ +75°, $C_{29}H_{44}O_5$ (M^+ 472), shows the hydroxyl and carboxyl absorptions on the IR spectrum, and its methylester (XIII), mp 211—212°, $[\alpha]_D$ +106°, $C_{30}H_{46}O_5$, exhibits on the NMR spectrum one methoxycarbonyl signal. Usual acetylation of XII gave its acetate (XIV), mp 140—145°, $[\alpha]_D$ +44°, $C_{35}H_{50}O_8$, which shows three acetoxyl signals on the NMR spectrum and was treated with diazomethane to give the methylester (XV), mp 102—105°, $[\alpha]_D$ +41°, $C_{36}H_{52}O_8$. On the mass spectra of XII and XIV the fragment peaks at *m/e* 232 and 187 were observed, while on those of XIII and XV the former is replaced by the *m/e* 246 peak. The mass numbers of these peaks are respectively sixteen mass units less than those of the peaks due to the retro-Diels-Alder type fragmentation of I and II. All the NMR spectra of XIII—XV show four tertiary methyl signals and a two-proton singlet at 4.62 ppm ascribable to the exo-methylene protons.

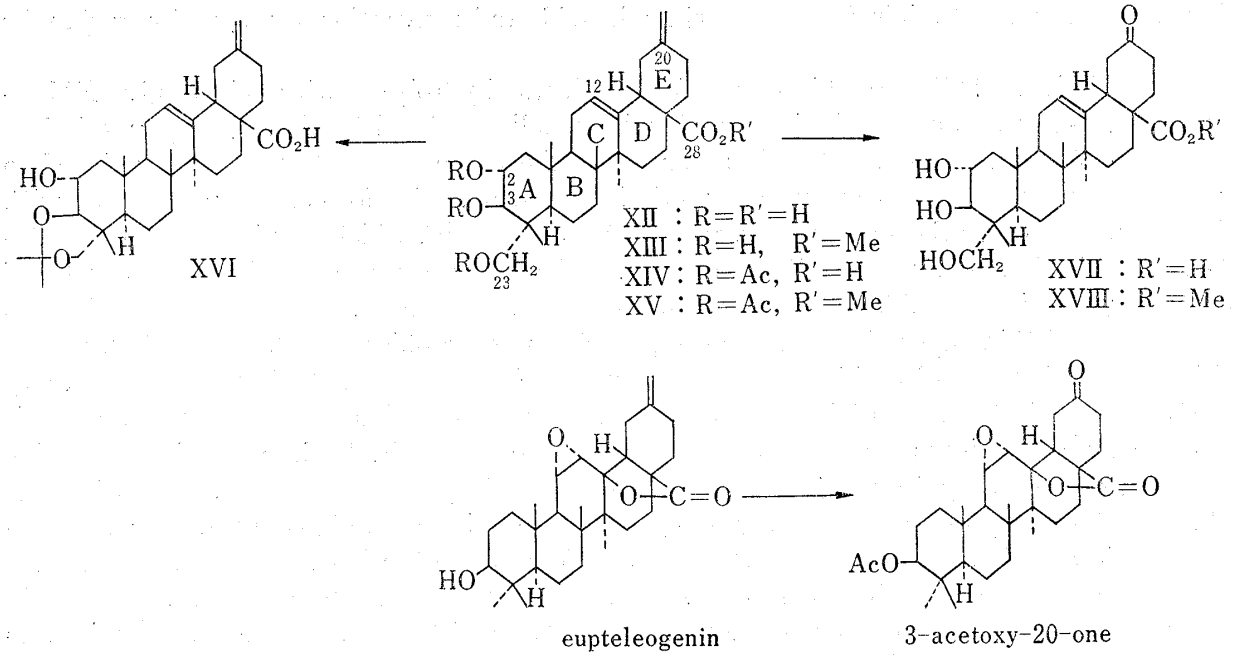
Therefore XII is presumed to be 30-norolean-12,20(29)-dien- or 30-norurs-12,19(29)-dien-28-oic acid having three hydroxyl groups attached to the A and/or B ring.

The NMR spectra of XIV and XV show the same AB quartet (2H, J=12 Hz, at 3.55 and 3.86 ppm) as that observed on the spectra of III and IV and assigned to the 23-methylene protons. Similarly to I, XII gave a monoacetonide (XVI), mp 202—205°, $[\alpha]_D$ +64°, $C_{32}H_{48}O_5$, (acetate, mp 155—158°, $[\alpha]_D$ +36°) which exhibits on the NMR spectrum a one-proton doublet (J=10 Hz) at 3.30 ppm and a one-proton multiplet ($w_{h/2}$=18 Hz) at 3.5—3.9. Accordingly, in analogy to the case of I, XII is regarded as a 2α,3β,23-triol.

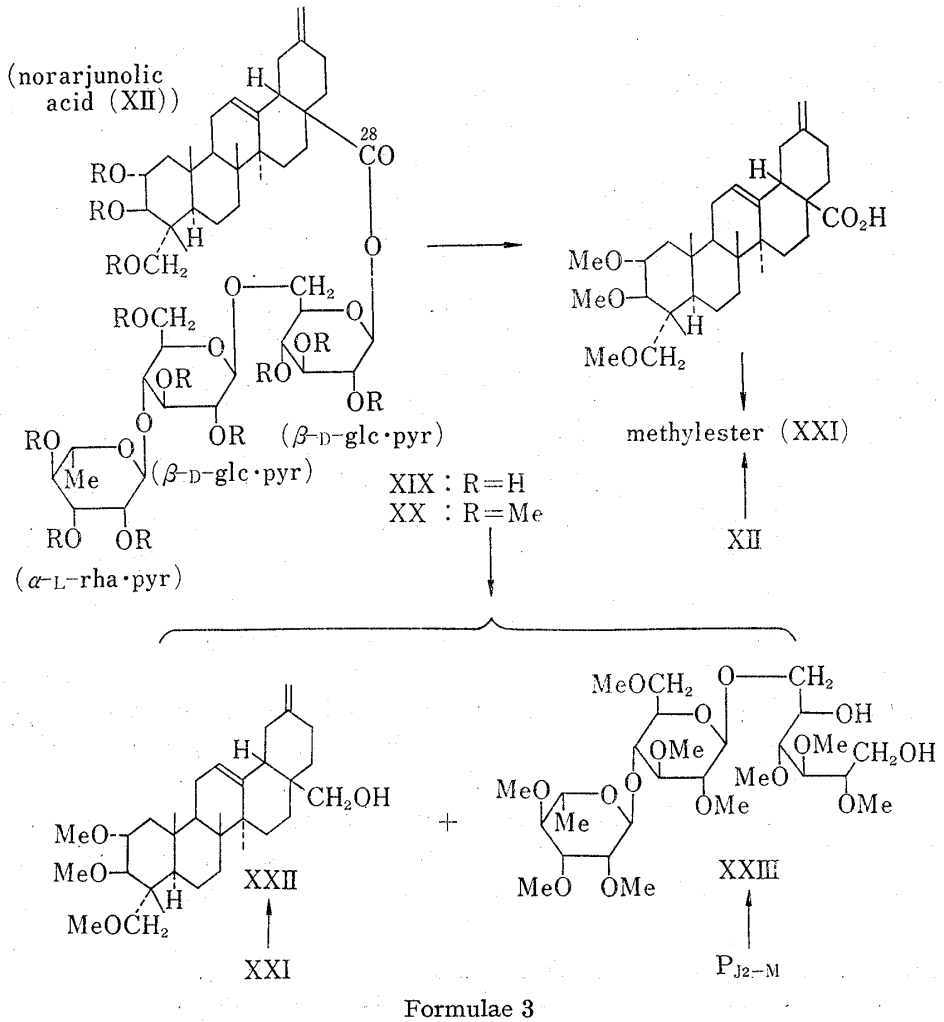
When XII was oxidized with ozone, a ketone (XVII), mp 290—295°. $[\alpha]_D$ +31°, $C_{28}H_{42}O_6$, having one carbon less than the parent compound, was afforded. XVII shows on the IR spectrum the absorption of a six-membered ring ketone and on the mass spectrum were observed the peaks at *m/e* 234 and 189, of which mass numbers are two mass unit more than those provided by the retro-Diels-Alder type fragmentation of XII. XVII methylester (XVIII), mp 145—147°, $[\alpha]_D$ +54°, shows on the circular dichroism (CD) spectrum a negative Cotton curve due to the six-membered ring ketone (n-π^*), which is in good agreement with that[10] of the 20-one[11] derived from eupteleogenin[12] (30-nor-20(29)-dehydro-11α,12α-epoxyoleanolic lactone).

Therefore XVII is considered to be 20-one and hence XII to have the exo-methylene group at C_{20}.

9) H.B. Henbest, *J. Chem. Soc.*, **1961**, 4472.
10) T. Murata, dissertation (1970).
11) The ketone derived from eupteleogenin is assigned[12] the structure 20-one on the basis of its negative Cotton curve on the ORD spectrum which is in agreement with that of 5β-3-keto-steroid. 5β-4-Keto-steroid shows a positive curve[13] and 5β-2-keto-steroid is known[14] to have a more negative Cotton effect than that of 5β-3-keto-steroid.
12) T. Murata, S. Imai, and M. Imanishi, *Tetrahedron Letters*, **1965**, 3215; M. Nishikawa, K. Kamiya, T. Murata, Y. Tomiie, and I. Nitta, *ibid.*, **1965**, 3223; T. Murata, S. Imai, M. Imanishi, and M. Goto, *Yakugaku Zasshi*, **90**, 744 (1970).
13) C. Djerassi and W. Klyne, *J. Chem. Soc.*, **1963**, 2390.
14) K. Takeda and H. Minato, *Steroids*, **1**, 345 (1963).



Formulae 2



Formulae 3

In consequence, XII is represented as 2α,3β,23-trihydroxy-30-norolean-12,20(29)-dien-28-oic acid, that is, 20(29)-dehydro-30-norarjunolic acid, and conventionally named norarjunolic acid.

P_H (XIX), mp 207—210° (decomp.), $[\alpha]_D$ +3°, was hydrolyzed with acid to give XII, L-rhamnose, D-glucose and gentiobiose, while with alkali also to yield XII. The permethylate (XX), mp 95—98°, $[\alpha]_D$ −3°, of XIX prepared by the Kuhn method[15] was methanolyzed to provide the aglycone and a mixture of methylated sugars. The former was methylated with diazomethane to give the methylester, mp 85—87°, $[\alpha]_D$ +79°, which was identified with 2,3,-23-trimethyl ether methylester (XXI) of XII. The sugar mixture was found to consist of methyl pyranosides of 2,3,4-tri-O-methyl-rhamnose, 2,3,6- and 2,3,4-tri-O-methyl-glucoses with the aid of thin-layer (TLC) and gas-liquid chromatographies (GLC). Reduction of XX with lithium aluminum hydride gave colorless prisms (XXII), mp 118—120°, $[\alpha]_D$ +90°, $C_{32}H_{52}O_4$, and a colorless syrup (XXIII), $[\alpha]_D$ −34°. By examination of the methanolysis products and by comparisons with the authentic sample[1] obtained by lithium aluminum hydride reduction of the permethylate (P_{J2-M}) of pericarp saponin J_2 (P_{J2}), XXIII was identified as 2,3,4-tri-O-methyl-α-L-rhamnopyranosyl-(1→4)-2,3,6-tri-O-methyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-O-methyl-D-sorbitol. XXII was considered to be the 28-ol corresponding to XXI and evidenced as such by direct comparisons with the sample prepared from XXI. The NMR spectrum of XX shows a one-proton doublet (J=7 Hz) at 5.34 ppm ascribable, in analogy to the case of P_{J2-M},[1] to the anomeric proton of the β-D-glucopyranose residue (in Cl conformation) conjugated with the 28-carboxyl group of the aglycone.

Consequently XIX is defined as norarjunolic acid 28-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside.

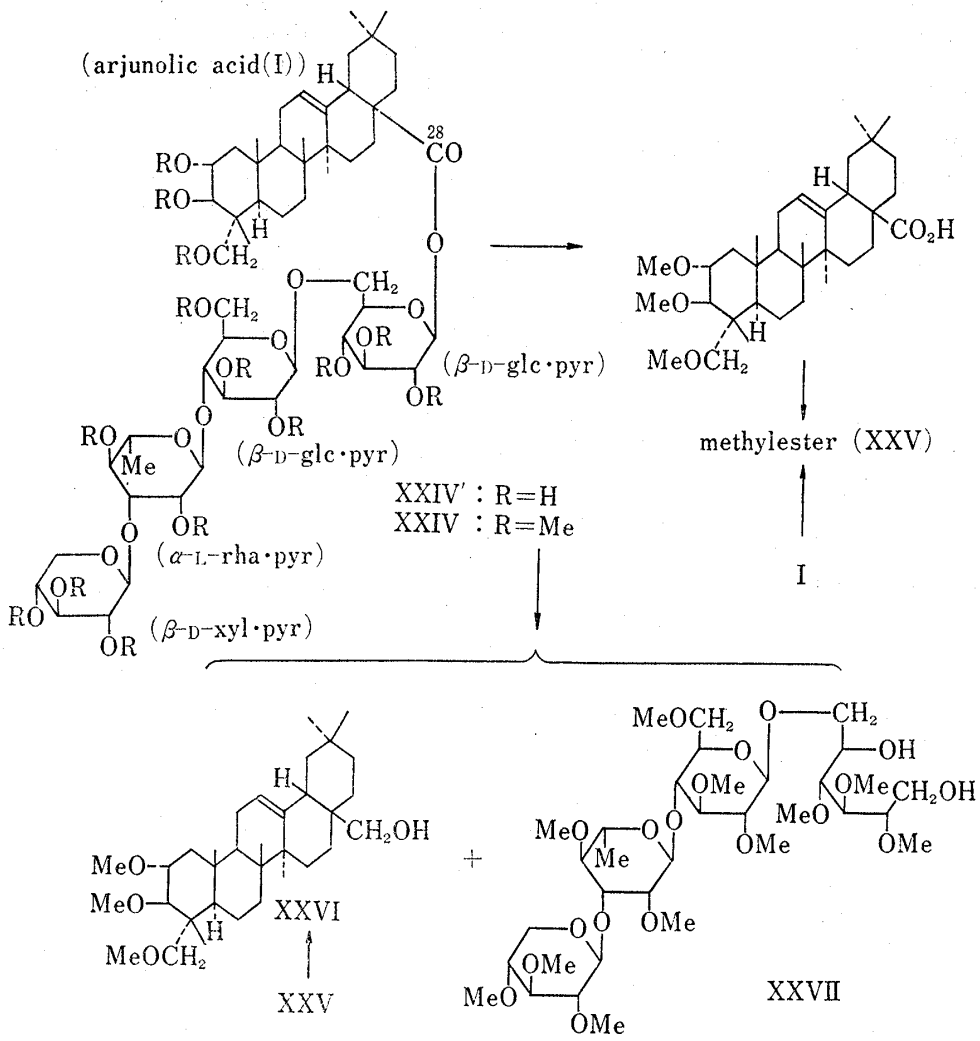
Permethylate (P_{J1-M}) (XXIV), mp 110—113°, $[\alpha]_D$ −19°, of pericarp saponin J_1 (P_{J1}) was methanolyzed to yield the aglycone and a mixture of methylated sugars consisting of methyl pyranosides of 2,3,4-tri-O-methyl-xylose, 2,4-di-O-methyl-rhamnose, 2,3,4- and 2,3,6-tri-O-methyl-glucoses. The aglycone was treated with diazomethane and the resulting methylester, mp 84—86°, $[\alpha]_D$ +50°, $C_{34}H_{56}O_5$, was identified with trimethyl ether methylester (XXV) of I. Lithium aluminum hydride reduction of XXIV gave a white powder (XXVI), mp 88—90°, $[\alpha]_D$ +46°, $C_{33}H_{56}O_4$, and a colorless syrup (XXVII), $[\alpha]_D$ −56°. XXVI was identified with the 28-ol corresponding to and prepared from XXV. XXVII shows on the mass spectrum the molecular ion peak at *m/e* 776 and the peak due to the terminal permethylated pentose residue[16] at 175. Hydrolysis of XXVII with acid in a mild condition yielded three kinds of homogeneous colorless syrups, one of which was identified as 2,3,4-tri-O-methyl-xylose on TLC. The other two syrups were treated with hydrogen chloride in methanol, and one yielded methyl 2,4-di-O-methyl-rhamnopyranoside, while another provided a mixture of 2,3,6-tri-O-methyl-glucopyranoside and 2,3,4-tri-O-methyl-sorbitol as identified on TLC and GLC.

All the above data indicate that XXIV is the permethylate of a glycoside of I, where the tetrasaccharide, xylopyranosyl-(1→3)-rhamnopyranosyl-(1→4)-glucopyranosyl-(1→6)-glucopyranose, is combined with the 28-carboxyl group of the aglycone.

In consideration of the fact that P_{J1} is coexistent with XIX, pericarp saponin J_2, J_3 and K (P_{J2-3}, P_K)[1] all having the same trisaccharide, α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranose, combined with the 28-carboxyl group of their aglycones, and on the basis of the NMR spectrum of XXVII and XXIV, the component monosaccharides of XXIV could be safely regarded as β-D-xylose, α-L-rhamnose and β-D-glucose.

Accordingly XXIV is considered to be the tetradecamethyl ether of arjunolic acid 28-O-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-gluco-

15) R. Kuhn, I. Löw, and H. Trischmann, *Chem. Ber.*, **88**, 1492, 1960 (1955).
16) T. Komori, Y. Ida, Y. Muto (née Inatsu), K. Miyahara, T. Nohara, and T. Kawasaki, *Biomedical Mass Spectrometry*, **2**, 65 (1975).



Formulae 4

pyranoside (XXIV′) and the originally existing saponin P_{J1} is presumed to be XXIV′.

XXIV′ is the first arjunolic acid oligoglycoside, and XII and XIX are second to eupteleogenin and eupteleoside,[11] respectively, as a naturally occurring noroleanane derivative and its glycoside.

Experimental[17]

Y(I)——Colorless needles, mp 317—320°, $[\alpha]_D$+62° (c=1.07, EtOH). IR ν_{max} cm^{-1}: 3350 (OH), 1690 (COOH). Mass Spectrum *m/e*: 488 ($C_{30}H_{48}O_5^+$, M$^+$), 248, 203. *Anal.* Calcd. for $C_{30}H_{48}O_5$: C, 73.73; H, 9.90. Found: C, 73.67; H, 9.78.

I Methylester (II)——I was methylated in $CHCl_3$-MeOH with diazomethane in ether to give II as colorless needles (from MeOH), mp 221—223°, $[\alpha]_D$+58° (c=1.12). IR ν_{max} cm^{-1}: 3350 (OH), 1730 (COOR). NMR: 0.72, 0.80, 0.92, 0.93, 1.00, and 1.14 (each 3H, s, Me-■ ×6), 3.60 (3H, s, -COOMe), 5.29 (1H, m, >C=CH-). Mass Spectrum *m/e*: 502 ($C_{31}H_{50}O_5^+$, M$^+$), 262, 203. *Anal.* Calcd. for $C_{31}H_{50}O_5$: C, 74.06; H, 10.03. Found: C, 73.57; H, 10.04.

I Triacetate (III)——I was acetylated with Ac_2O–pyridine (1:1) at room temperature overnight to yield III as a white powder (precipitated from hexane), mp 135—140°, $[\alpha]_D$+37° (c=2.68). IR ν_{max} cm^{-1}: 1745 (AcO), 1690 (COOH). NMR: 1.98, 2.02, and 2.09 (each 3H, s, AcO×3), 3.55 and 3.86 (2H, q, J=12 Hz, -CH$_2$-OAc).[4] Mass Spectrum *m/e*: 614 ($C_{36}H_{54}O_8^+$, M$^+$), 248, 203. *Anal.* Calcd. for $C_{36}H_{54}O_8$: C, 70.33; H, 8.55. Found: C, 70.16; H, 8.82.

17) For general method, refer to the preceding paper.[1] Unless otherwise specified, optical rotations were measured in $CHCl_3$ solution and IR spectra were taken in nujol mulling.

III Methylester (IV)——III was methylated with diazomethane to give IV as a white powder (precipitated from hexane), mp 106—109°, $[\alpha]_D$+34° (c=2.65). IR ν_{max} cm^{-1}: 1745 (AcO), 1730 (COOR). NMR: 1.98, 2.01 and 2.08 (each 3H, s, AcO×3), 3.62 (3H, s, -COOMe), 3.55 and 3.86 (2H, q, J=12 Hz, -C$\underline{H}_2$-OAc). Mass Spectrum m/e: 628 ($C_{37}H_{56}O_8^+$, M$^+$), 262, 203. *Anal.* Calcd. for $C_{37}H_{56}O_8$: C, 70.67; H, 8.98. Found: C, 70.61; H, 9.00.

I Monoacetonide (V)——I (700 mg) and $CuSO_4$ (2 g) in anhydrous acetone (50 ml) were stirred at room temperature for 24 hr,[5] and the precipitates were filtered off. To the filtrate a small amount of aqueous $NaHCO_3$ solution was added, the mixture was concentrated *in vacuo*, diluted with water and extracted with $CHCl_3$. The extract was washed with water, dried over Na_2SO_4 and evaporated. The residue (syrup, 500 mg) was passed through a silica gel column (eluent, hexane–AcOEt (3:2)) and crystallized from hexane–acetone to give V as colorless needles (200 mg), mp 190—192°, $[\alpha]_D$+51° (c=1.88). NMR: 1.46 (6H, s, >C-Me$_2$), 3.32 (1H, d, J=10 Hz, >C$\underline{H}$-O-C-Me$_2$), 3.48 (2H, s, -C$\underline{H}_2$-O-C-Me$_2$), 3.5—3.9 (1H, m, $w_{h/2}$=18 Hz, >C$\underline{H}$-OH). Mass Spectrum m/e: 528 ($C_{33}H_{52}O_5^+$, M$^+$). *Anal.* Calcd. for $C_{33}H_{52}O_5$: C, 74.96; H, 9.91. Found: C, 75.34; H, 10.03.

I Monoacetonide Monoacetate (VI)——V was acetylated in a usual manner to give VI as colorless prisms (from MeOH), mp 201—205°, $[\alpha]_D$+23° (c=1.54). NMR: 2.02 (3H, s, AcO), 4.8—5.1 (1H, m, >C$\underline{H}$-OAc). Mass Spectrum m/e: 570 ($C_{35}H_{54}O_6^+$, M$^+$). *Anal.* Calcd. for $C_{35}H_{54}O_6$: C, 73.63; H, 9.54. Found: C, 73.87; H, 9.49.

Synthesis of I from Hederagenin (Chart 1)——1) 23-Monoacetate (VII): To the solution of hederagenin (500 mg) in pyridine (5 ml) was added Ac_2O (5 ml) under stirring at room temperature. The mixture was left standing for 3 min and then poured into ice-water. The precipitates (Rf 0.10 (hederagenin), 0.67, 0.40 (main) on TLC (solvent, hexane–AcOEt (3:2)) were collected and chromatographed over silica gel (eluent, hexane–AcOEt (3:2)) to give the main product, which was crystallized from hexane–AcOEt to provide VII as colorless needles (100 mg), mp 196—198°, $[\alpha]_D$+57° (c=0.70). IR ν_{max} cm^{-1}: 3450 (OH), 1720 (AcO), 1690 (COOH). NMR: 2.12 (3H, s, AcO), 3.2—3.6 (1H, m, >C$\underline{H}$-OH), 3.88 and 4.25 (2H, q, J=11 Hz, -C$\underline{H}_2$-OAc). Mass Spectrum m/e: 514 ($C_{32}H_{50}O_5^+$, M$^+$). *Anal.* Calcd. for $C_{32}H_{50}O_5$: C, 74.67; H, 9.79. Found: C, 74.59; H, 9.74.

2) 23-Acetoxy-3-one (VIII): VII (3.0 g) in dimethylformamide (DMF) (25 ml) was oxidized with CrO_3 (1.2 g) and conc. H_2SO_4 (0.5 ml) in DMF (30 ml) under stirring and ice-cooling. The mixture was left standing for 1 hr and poured into water. The precipitates were collected, passed through a silica gel column (eluent, hexane–AcOEt (3:2)) and crystallized from hexane to give VIII as colorless needles (1.7 g), mp 122—125°, $[\alpha]_D$+81° (c=1.22). IR ν_{max} cm^{-1}: 1740 (AcO), 1710 (six-membered ring ketone), 1690 (COOH). NMR: 2.02 (3H, s, AcO), 4.06 (2H, s, -C$\underline{H}_2$-OAc). CD (c=0.11, MeOH, 17°) [θ] (nm): −601 (300) (negative maximum). Mass Spectrum m/e: 512 ($C_{32}H_{48}O_5^+$, M$^+$). *Anal.* Calcd. for $C_{32}H_{48}O_5$: C, 74.96; H, 9.44. Found: C, 74.64; H, 9.62.

3) 2α, 23-Diacetoxy-3-one (IX): VIII (700 mg) was added to the solution of BF_3-etherate (1 ml) and Pb(OAc)$_4$ (1 g) in AcOH–Ac_2O (10:1) (20 ml) and stirred for 2 hr at room temperature.[9] The reaction mixture was poured into aqueous $NaHCO_3$ solution and extracted with $CHCl_3$. The extract was washed with water, dried, evaporated and the residue was chromatographed over silica gel (eluent, hexane–AcOEt (1:1)) to give two fractions. The more polar fraction was crystallized from MeOH to yield IX as colorless needles (300 mg), mp 127—130°, $[\alpha]_D$+56° (c=3.04). IR ν_{max} cm^{-1}: 1740 and 1730 (AcO), 1690 (COOH). NMR: 2.01 and 2.13 (each 3H, s, AcO×2), 4.15 and 4.27 (2H, q, J=12 Hz, -C$\underline{H}_2$-OAc), 5.58 (1H, q, J=13, 6 Hz, >C$\underline{H}$-OAc). CD (c=0.15, MeOH, 17°) [θ] (nm): −564 (275) (negative maximum).[18] Mass Spectrum m/e: 570 ($C_{34}H_{50}O_7^+$, M$^+$). *Anal.* Calcd. for $C_{34}H_{50}O_7$: C, 71.55; H, 8.83. Found: C, 71.34; H, 8.80. Another fraction gave the 2β-epimer (IX′) as colorless needles (100 mg) (from MeOH), mp 117—120°, $[\alpha]_D$ +117° (c=2.02). NMR: 5.67 (1H, q, J=12, 8 Hz, >C$\underline{H}$-OAc). CD (c=0.15, MeOH, 17°) [θ] (nm): +5392 (286) (positive maximum).[18]

4) 2α, 23-Diacetoxy-3β-ol (X): IX (500 mg) in MeOH (20 ml) was reduced with $NaBH_4$ (50 mg). The crude product was chromatographed over silica gel (eluent, hexane–AcOEt (1:1)) to give two compounds. The more polar compound was precipitated from hexane–AcOEt to give X as a white powder (250 mg), mp 144—146°, $[\alpha]_D$+36° (c=1.72). IR ν_{max} cm^{-1}: 3450 (OH), 1740 and 1730 (AcO), 1690 (COOH). NMR: 2.08 (6H, s, AcO×2), 3.49 (1H, d, J=10 Hz, >C$\underline{H}$-OH),[20] 3.90 and 4.09 (2H, q, J=12 Hz, -C$\underline{H}_2$-OAc), 5.00 (1H, m, >C$\underline{H}$-OAc). Mass Spectrum m/e: 572 ($C_{34}H_{52}O_7^+$, M$^+$). *Anal.* Calcd. for $C_{34}H_{52}O_7$: C, 71.29; H, 9.15. Found: C, 71.02; H, 9.07. Another compound was precipitated from hexane–AcOEt to give the 3α-epimer (X′) as a white powder (50 mg), mp 135—140°, $[\alpha]_D$+54° (c=1.27). NMR: 3.69 (1H, d, J=3 Hz, >C$\underline{H}$-OH).

5) 2α-Hydroxyhederagenin (XI=I): X (200 mg) was boiled with Na_2CO_3 (250 mg) in 90% MeOH (50 ml) for 1 hr. The hydrolysate was concentrated *in vacuo*, diluted with water and extracted with AcOEt–*n*-BuOH

18) A weak negative Cotton curve on the CD spectrum of IX is quite similar to that of VIII, while IX′ has a strong positive Cotton effect. Therefore IX is regarded to have the 2α (equatorial) acetoxyl group and IX′ is assigned the 2β-epimeric structure.[19]

19) G.R. Chaudhry, T.G. Halsall, and E.R.H. Jones, *J. Chem. Soc.*, **1961**, 2725.

20) The J value (10 Hz) of the one-proton doublet due to C_3-H indicates that the protons at C_3 and C_2 have *trans* diaxial orientation and the α and β configurations, respectively.

(2:1). The organic layer was washed with water, evaporated and the residue was crystallized from acetone to give **XI** as colorless needles (50 mg), mp 318—320°, $[\alpha]_D$+60° (c=1.19, EtOH). Mass Spectrum m/e: 488 ($C_{30}H_{48}O_5^+$, M^+), 248, 203. *Anal.* Calcd. for $C_{30}H_{48}O_5$: C, 73.73; H, 9.90. Found: C, 73.55; H, 9.44. Identical with I on TLC (solv. a),[1] IR and mixed fusion.

Z (XII)——Colorless needles, mp 303—305°, $[\alpha]_D$+75° (c=0.66, EtOH). IR ν_{max} cm^{-1}: 3350 (OH), 1690 (COOH). Mass Spectrum m/e: 472 ($C_{29}H_{44}O_5^+$, M^+), 232, 187. *Anal.* Calcd. for $C_{29}H_{44}O_5$: C, 73.69; H, 9.38. Found: C, 73.87; H, 9.49.

XII Methylester (XIII)——XII was methylated with diazomethane to give XIII as colorless needles (from MeOH), mp 211—212°, $[\alpha]_D$+106° (c=1.51). IR ν_{max} cm^{-1}: 3350 (OH), 1725 (COOR). NMR: 0.72, 0.84, 1.02, and 1.17 (each 3H, s, Me-▪×4), 3.60 (3H, s, -COOMe), 4.62 (2H, s, >C=C$\underline{H}_2$), 5.35 (1H, m, >C=C$\underline{H}$-). Mass Spectrum m/e: 486 ($C_{30}H_{46}O_5^+$, M^+), 246, 187. *Anal.* Calcd. for $C_{30}H_{46}O_5$: C, 74.03; H, 9.53. Found: C, 74.01; H, 9.61.

XII Triacetate (XIV)——XII was acetylated as I to give XIV as a white powder (precipitated from hexane), mp 140—145°, $[\alpha]_D$+44° (c=2.35). IR ν_{max} cm^{-1}: 1740 (AcO), 1690 (COOH). NMR: 0.76, 0.89, 1.10 and 1.16 (each 3H, s, Me-▪×4), 1.99, 2.02 and 2.09 (each 3H, s, AcO×3), 3.55 and 3.86 (2H, q, J=12 Hz, -C$\underline{H}_2$OAc), 4.62 (2H, s, >C=C$\underline{H}_2$). Mass Spectrum m/e: 598 ($C_{35}H_{50}O_8^+$, M^+), 232, 187. *Anal.* Calcd. for $C_{35}H_{50}O_8$: C, 70.20; H, 8.42. Found: C, 70.16; H, 8.36.

XIV Methylester (XV)——XIV was methylated with diazomethane to give XV as a white powder (precipitated from hexane), mp 102—105°, $[\alpha]_D$+41° (c=2.65). IR ν_{max} cm^{-1}: 1745 (AcO), 1730 (COOR). NMR: 0.72, 0.88, 1.09, and 1.14 (each 3H, s, Me-▪×4), 3.60 (3H, s, -COOMe), 4.62 (2H, s, >C=C$\underline{H}_2$). Mass Spectrum m/e: 618 ($C_{36}H_{52}O_8^+$, M^+), 246, 187. *Anal.* Calcd. for $C_{36}H_{52}O_8$: C, 70.55; H, 8.55. Found: C, 70.24; H, 8.39.

XII Monoacetonide (XVI)——XII (500 mg) was treated with $CuSO_4$ in anhydrous acetone and worked up in the same way as in I to yield XVI as colorless plates (from MeOH) (140 mg), mp 202—205°, $[\alpha]_D$+64° (c=1.49). NMR: 1.46 (6H, s, >C-Me_2), 3.30 (1H, d, J=10 Hz, >C$\underline{H}$-O-C-Me_2), 3.48 (2H, s, -C$\underline{H}_2$-O-C-Me_2), 3.5—3.9 (1H, m, $w_{h/2}$=18 Hz, >C$\underline{H}$-OH). Mass Spectrum m/e: 512 ($C_{32}H_{48}O_5^+$, M^+), 232, 187. *Anal.* Calcd. for $C_{32}H_{48}O_5$: C, 74.96; H, 9.44. Found: C, 74.54, H, 9.27. XVI was acetylated to give the monoacetate as colorless prisms (from MeOH), mp 155—158°, $[\alpha]_D$+36° (c=3.44). NMR: 2.02 (3H, s, AcO), 4.7—5.1 (1H, m, >C$\underline{H}$-OAc).

Oxidation of XII with Ozone——XII (60 mg) in AcOH (10 ml) was treated with ozone (0.3 g/h) for 30 min, diluted with water and extracted with AcOEt–*n*-BuOH (3:1). The organic layer was washed with water, evaporated *in vacuo* and the residue was crystallized from EtOH to give colorless plates (XVII) (20 mg), mp 290—295°, $[\alpha]_D$+31° (c=0.72, MeOH). IR ν_{max} cm^{-1}: 3400 (OH), 1710 (six-membered ring ketone), 1690 (COOH). Mass Spectrum m/e: 474 ($C_{28}H_{42}O_6^+$, M^+), 234, 189. *Anal.* Calcd. for $C_{28}H_{42}O_6$: C, 70.85; H, 8.92. Found; C, 71.09; H, 8.93.

XVII Methylester (XVIII)——XVII was methylated with diazomethane to yield XVIII as colorless needles (from ether), mp 145—147°, $[\alpha]_D$+54° (c=2.63, MeOH). IR ν_{max} cm^{-1}: 3400 (OH), 1720 (COOR), 1710 (C=O). ORD (c=0.25, dioxane, 26°) [ϕ] (nm): +937 (300), −586 (308), −1230 (315), +586 (350). CD (c=0.25, dioxane, 26°) [θ] (nm): −3929 (291) (negative maximum) (3-acetoxy-20-one derived from eupteleogenin, ORD[11] (c=0.13, dioxane, 11°) [ϕ] (nm): +792 (300), −293 (309), −478 (320), +478 (356). CD[10] (c=0.107, dioxane, 25°) [θ] (nm): −3690 (293) (negative maximum)). Mass Spectrum m/e: 488 ($C_{29}H_{44}O_6^+$, M^+), 248, 189. *Anal.* Calcd. for $C_{29}H_{44}O_6$: C, 71.28; H, 9.08. Found: C, 71.08; H, 9.03.

P_H (XIX)——Colorless needles, mp 207—210° (decomp.), $[\alpha]_D$+3° (c=2.72, MeOH). IR ν_{max} cm^{-1}: 3350 (OH), 1735 (COOR). *Anal.* Calcd. for $C_{47}H_{74}O_{19}\cdot 5H_2O$: C, 54.64; H, 8.19. Found: C, 54.80; H, 7.96.

Hydrolysis of XIX——1) With Acid: XIX (300 mg) in 2 N H_2SO_4 (15 ml) was boiled for 2 hr and the reaction mixture was diluted with water. The precipitates were collected by filtration, washed with water and dried, while the filtrate was passed through an Amberlite A-400 column and evaporated *in vacuo* to a syrup. The precipitates were crystallized from MeOH to give colorless needles (60 mg), mp 302—305°, which were methylated with diazomethane to provide colorless needles (from MeOH), mp 210—211°. They were identified with XII and XIII, respectively, by comparisons of *Rf* values on TLC (solv. a), IR spectra and by a mixed fusion. The syrup obtained above showed three spots on paper partition chromatogram (PPC) and was chromatographed over silica gel (eluent, $CHCl_3$–MeOH–water (7:3:0.5)) to give D-glucose, a colorless syrup, $[\alpha]_D$+52° (24 hr) (c=5.03, water), L-rhamnose, colorless needles (from MeOH), mp 68—70°, $[\alpha]_D$−3° (5 min)→+6° (24 hr) (c=4.52, water) (lit.[21] $[\alpha]_D$−7.7°→+8.9° (water)), and gentiobiose, a white powder, $[\alpha]_D$+11° (5 min)→+8° (3 hr) (c=2.75, water) (lit.[22] $[\alpha]_D$+21.4°→+8.7° (water)), which were respectively identified with the authentic samples on PPC.

2) With Alkali: XIX (50 mg) was boiled with 1% KOH in 30% EtOH (4 ml) for 1 hr. The mixture was diluted with water, neutralized with dil. HCl and extracted with AcOEt–*n*-BuOH (2:1). The extract was washed with water and evaporated *in vacuo*. The residue revealed one spot on TLC (solv. a) identical with that of XII.

21) C.S. Hudson and E. Yanovsky, *J. Amer. Chem. Soc.*, **39**, 1032 (1917).
22) F.J. Bates, "Polarimetry, Saccharimetry and the Sugars," National Bureau of Standards, Washington, 1942, p. 722.

XIX Permethylate (XX)——XIX (600 mg) was methylated by the Kuhn method[12] (DMF 10 ml, Ag_2O 2 g, CH_3I 5 ml). The crude product was passed through a silica gel column (eluent, AcOEt–hexane (3:2)) and precipitated from $CHCl_3$–hexane to give XX as a white powder (200 mg), mp 95—98°, $[\alpha]_D$ −3° (c=5.23). IR: no OH. NMR: 5.34 (1H, d, J=7 Hz, C_1-H of ester-glycosidic glucose unit), 4.95 (1H, s, C_1-H of rhamnose unit). *Anal.* Calcd. for $C_{59}H_{98}O_{19}$: C, 63.76; H, 8.89. Found: C, 63.52; H, 8.91.

Methanolysis of XX——XX (50 mg) was boiled with 8% HCl in MeOH (3 ml) for 2 hr. The hydrolysate was neutralized with Ag_2CO_3, the precipitates were filtered off and the filtrate was evaporated. The residue was chromatographed over silica gel (eluent, AcOEt–MeOH (50:1)) to give the aglycone and a sugar mixture. The former was methylated with diazomethane to give a white powder (from dil. MeOH) (12 mg), mp 85—87°, $[\alpha]_D$ +79° (c=1.93). IR ν_{max} cm^{-1}: 1730 (COOR), no OH. NMR: 0.64, 0.68, 0.93, and 1.14 (each 3H, s, Me-▪ ×4), 3.32, 3.42, and 3.54 (each 3H, s, MeO- ×3), 3.60 (3H, s, -$COOCH_3$), 4.60 (2H, s, >C=C$\underline{H}_2$), 5.33 (1H, m, >C=C$\underline{H}$-). Mass Spectrum m/e: 528 ($C_{33}H_{52}O_5^+$, M+), 246, 187. *Anal.* Calcd. for $C_{33}H_{52}O_5$: C, 74.96; H, 9.91. Found: C, 74.64; H, 10.00. Identified (*Rf* values on TLC (solvent, hexane–AcOEt (4:1)), IR, NMR and mass spectra) with the trimethyl ether methylester (XXI), mp 86—87°, $[\alpha]_D$ +80° (c=1.03), prepared from XII by the Hakomori's methylation.[23] The sugar mixture obtained from the methanolysate was examined by TLC (solv. c)[1] (and GLC) to show three spots (and peaks) identical with those of methyl pyranosides of 2,3,4-tri-O-methyl-α-L-rhamnose, 2,3,6- and 2,3,4-tri-O-methyl-α-D-glucoses.

Reduction of XX with $LiAlH_4$——XX (50 mg) in tetrahydrofuran (5 ml) was treated with $LiAlH_4$ (30 mg). The reaction mixture was extracted with ether and then with $CHCl_3$. The ether extract was washed with water, dried, evaporated and the residue was crystallized from hexane to give colorless prisms (XXII), mp 118—120°, $[\alpha]_D$ +90° (c=1.09). Mass Spectrum m/e: 500 ($C_{32}H_{52}O^+_4$, M+). *Anal.* Calcd. for $C_{32}H_{52}O_4$: C, 76.57; H, 10.47. Found: C, 76.29; H, 10.38. Identical (*Rf* values on TLC (solv. c), IR and NMR spectra and mixed fusion) with the 28-ol, mp 120—123°, $[\alpha]_D$ +92° (c=2.72), prepared by $LiAlH_4$ reduction of XXI. The $CHCl_3$ extract of the reduction product was washed with water, evaporated and the residue was passed through a Sephadex LH-20 column (eluent, MeOH) to give a colorless syrup (XXIII), $[\alpha]_D$ −34° (c=5.52). NMR: 1.30 (3H, d, J=7 Hz, 6-Me of rhamnose unit), 4.32 (1H, d, J=7 Hz, C_1-H of glucose unit), 4.95 (1H, d, J=1.5 Hz, C_1-H of rhamnose unit). Mass Spectrum m/e: 616 ($C_{27}H_{52}O_{15}^+$, M+), 189 ($C_9H_{17}O_4^+$, terminal permethylated methylpentose residue). XXIII (50 mg) was methanolyzed with 8% HCl in MeOH (3 ml) in the same way as in XX and the product was chromatographed over silica gel (eluent, AcOEt–MeOH (50:1)) to give three kinds of colorless syrups. Two of them were identified (TLC, GLC, optical rotations) with methyl pyranosides[1] of 2,3,4-tri-O-methyl-α-L-rhamnose and 2,3,6-tri-O-methyl-α-D-glucose. The third one was acetylated with Ac_2O–pyridine (1:1) and the crude acetate was passed through a silica gel column (eluent, hexane–AcOEt (3:1)) to give a colorless syrup, identical (optical rotations, TLC, NMR and mass spectra) with 2,3,4-tri-O-methyl-D-sorbitol triacetate.[1] XXIII was identified by direct comparisons with the product[1] obtained by $LiAlH_4$ reduction of P_{J2} permethylate (P_{J2-M}).

P_{JI-M} (XXIV)——A white powder, mp 110—113°, $[\alpha]_D$ −19° (c=3.31). IR ν_{max} cm^{-1}: 1750 (COOR), no OH. NMR: 5.37 (1H, d, J=7 Hz, C_1-H of ester-glycosidic glucose unit). *Anal.* Calcd. for $C_{67}H_{114}O_{23}$: C, 62.49; H, 8.92. Found: C, 62.76; H, 8.62.

Methanolysis of XXIV——XXIV (80 mg) was methanolyzed and worked up in the same way as in XX. The product was separated over silica gel (eluent, hexane–AcOEt (1:1)) into the aglycone and a mixture of methylated sugars. The former was methylated with diazomethane and precipitated from hexane to yield a white powder (10 mg), mp 84—86°, $[\alpha]_D$ +50° (c=1.00), which was identified (*Rf* values on TLC (solvent, hexane–AcOEt (4:1)), IR and NMR spectra, mixed fusion) with the trimethyl ether methylester (XXV) of I prepared by the Hakomori's methylation of I. The sugar mixture was examined by TLC (solv. c) and GLC, and the spots and peaks identical with those of methyl pyranosides of 2,3,4-tri-O-methyl-α- and β-D-xylose, 2,4-di-O-methyl-α-L-rhamnose, 2,3,6- and 2,3,4-tri-O-methyl-α-D-glucoses were detected.

Reduction of XXIV with $LiAlH_4$——XXIV (180 mg) was reduced with $LiAlH_4$ and worked up in the same way as in XX. The ether-soluble product was precipitated from hexane to give a white powder (XXVI) (20 mg), mp 88—90°, $[\alpha]_D$ +46° (c=1.18). Mass Spectrum m/e: 516 ($C_{33}H_{56}O_4^+$, M+). *Anal.* Calcd. for $C_{33}H_{56}O_4$: C, 76.69; H, 10.92. Found: C, 76.73; H, 10.81. Identified by direct comparisons with the $LiAlH_4$ reduction product, mp 88—90°, $[\alpha]_D$ +46° (c=1.50), of XXV. The $CHCl_3$-soluble product was passed through a Sephadex LH-20 column (eluent, MeOH) to give a colorless syrup (XXVII) (70 mg), $[\alpha]_D$ −56° (c=4.61). Mass Spectrum m/e: 776 ($C_{34}H_{64}O_{19}^+$, M+), 175 ($C_8H_{15}O_4^+$, terminal permethylated pentose residue).[13] NMR: 1.30 (3H, d, J=6 Hz, 6-Me of rhamnose unit), 4.32 (1H, d, J=7 Hz, C_1-H of glucose unit), 4.50 (1H, d, J=7 Hz, C_1-H of xylose unit), 4.84 (1H, d, J=1.5 Hz, C_1-H of rhamnose unit). XXVII was hydrolyzed with 0.5N HCl and the product was chromatographed over silica gel (eluent, AcOEt and AcOEt–MeOH (10:1)) to give two kinds of homogeneous (TLC) colorless syrups together with unchanged XXVII and a colorless syrup identical on TLC (solv. c) with 2,3,4-tri-O-methyl-D-xylose. Two syrups were respectively treated with 8% HCl in MeOH for 1 hr. One yielded methyl 2,4-di-O-methyl-rhamnopyranoside, while the other provided a mixture

23) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 203 (1964).

of methyl 2,3,6-tri-O-methyl-glucopyranoside and 2,3,4-tri-O-methyl-sorbitol[1] as identified by comparisons with the authentic samples on TLC (solv. c) and GLC.

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