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Seed Saponins of *Akebia quinata* Decne I. Hederagenin 3-O-Glycosides

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Seven triterpenoid saponins were isolated from the seeds of *Akebia quinata* Decne. (Lardizabalaceae). The less polar three of them, tentatively named saponins A (mp 228—229° (decomp.), $[\alpha]_D$ +49°), B (mp 249.5—250.5° (decomp.), $[\alpha]_D$ +37°) and C (mp 248—250° (decomp.), $[\alpha]_D$ +40°), were characterized as hederagenin 3-O-glycosides; α-L-arabinopyranoside (IX), β-D-xylopyranosyl-(1-2)-α-L-arabinopyranoside (VII) and β-D-glucopyranosyl-(1-2)-α-L-arabinopyranoside (I), respectively. Saponin B is the first one ever reported.

Akebia quinata Decne. (Lardizabalaceae) is a plant widely distributed in Japan and its air-dried voluble stems, called *Akebia Caulis* (Mokutsu, in Japanese), have been known[2] as a japanese folk medicine, for example as antiphlogistic, diuretic and analgesic.

The stems have been reported[2] to contain hederagenin, oleanolic acid and inorganic potassium salts and the recent isolation by Takezaki and his coworkers[3] of an anti-inflammatory oleanolic acid glycoside attracts a considerable attention. Meanwhile, as for the ingredients of the seeds, little has been known except fatty oil,[2] but the existence of triterpenoid compounds seems very likely as suggested by the occurrence[4] of mubenins A, B, and C (oleanolic acid diglycoside, tetraglycoside and hederagenin tetraglycoside, respectively) in the seeds of a related plant *Stauntonia hexaphylla* Decne. of the same family.

A study on the constituents of the seeds of *Akebia quinata* hoping the isolation of physiologically active triterpenoid compounds has been conducted in this laboratory, and we wish to report in this and the succeeding papers the structures of seven triterpenoid saponins (hederagenin glycosides) so far isolated in a pure state.

The procedure of isolation of the glycosides is shown in Chart 1. Among seven saponins thus obtained, tentatively named saponins A—G in the order of increasing polarity, A, B, and C which have been found to be hederagenin 3-O-glycosides are dealt in this communication.

Saponin C (I) was obtained in a pure state as colorless needles, mp 248—250° (decomp.), $[\alpha]_D$+40° (pyridine). I shows an carbonyl absorption on the infrared (IR) spectrum and gave on hydrolysis with 2N sulfuric acid in 50% ethanol for 2 hr hederagenin, arabinose and glucose. A mild acid hydrolysis gave only one prosapogenin (III) together with hederagenin and I. Its permethylate (II), mp 104—105°, $[\alpha]_D$+39° ($CHCl_3$), prepared by the Hakomori method[5] exhibits on a mass spectrum (MS) the peaks of molecular ion at *m/e* 878 and those originated from the aglycone moiety and a terminal permethylated hexose residue[6] at *m/e* 482 and 219, respectively. These data together with those of elemental analyses of I and II suggest that II is represented by the formula $C_{49}H_{82}O_{13}$, and that I is a glucosyl arabinoside

1) Location: *1276 Katakasu, Fukuoka.*
2) K. Akamatsu, "Wakanyaku," Isiyaku Pub. Co, Tokyo, 1970, p. 435, 436.
3) T. Takezaki, F. Kitame, S. Nakamura, M. Sawai, and M. Taruya, Abstracts of Papers, The 90th Annual Meeting of the Pharmaceutical Society of Japan, Sapporo, June, 1970, p. II-212.
4) T. Takemoto and K. Kometani, *Ann.*, **685**, 237 (1965).
5) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).
6) *a*) H. Budzikiewicz, C. Djerassi, and D.H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. II, Holden-Day, Inc., San Francisco, 1964, pp. 203—227; *b*) T. Kawasaki, T. Komori, Y. Ida, Y. Inatsu, K. Miyahara, and T. Nohara, International Conference on Mass Spectroscopy, Kyoto, September, 1969, Preprints, p. 221.

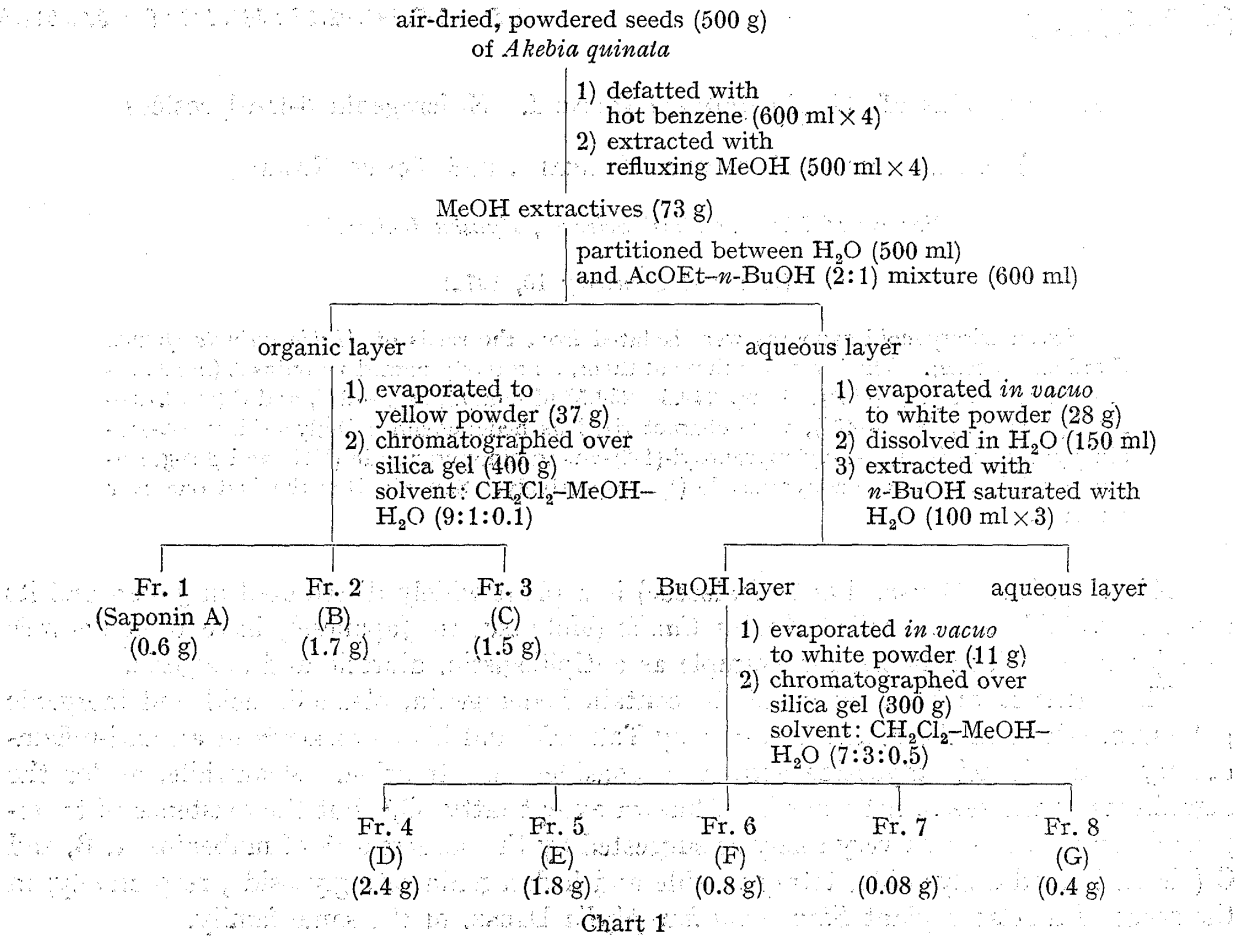


Chart 1

of hederagenin. The methanolysis of II provided an aglycone (IV), mp 190—192.5°, $C_{32}H_{52}O_4$, and two kinds of methylated monosaccaride. IV was acetylated to give an acetate (V), mp 213—213.5°, $C_{34}H_{54}O_5$. The nuclear magnetic resonance (NMR) spectrum of V shows the signals of one acetoxy, one methylester and one methyl ether groups, and a quartet (2H, J=18 Hz, 12 Hz) at 2.96 ppm and a triplet (1H, J=8 Hz, 8 Hz) at 4.90. The former is ascribable to the methylene protons of 23-CH_2OCH_3 group of a hederagenin derivative and the latter assigned to the proton at C-3 bearing β(equatorial)-acetoxy group. Therefore V and IV are regarded respectively as 3-O-acetyl-23-O-methyl hederagenin methylester and the corresponding 3-hydroxy compound, and I is considered to have the glucosyl-arabinose moiety combined with the 3β-hydroxy group of hederagenin. Two methylated sugars in the methanolysate were separated over silica gel column and the one, colorless syrup, $[\alpha]_D$+125° ($CHCl_3$), and the other, colorless syrup, $[\alpha]_D$+180° ($CHCl_3$), were identified by their optical rotations and on thin-layer (TLC) and gas liquid chromatograms (GLC) as methyl 2,3,4,6-tetra-O-methyl-α-D-glucopyranoside and methyl 3,4-di-O-methyl-β-L-arabinopyranoside (VI), respectively. The NMR spectrum of II shows two anomeric proton signals at 4.33 ppm (doublet, J=7 Hz) and 4.59 (doublet, J=8 Hz), and the coupling constants indicate that the protons at C-1 and C-2 in both sugar units are oriented nearly in *trans*-diaxial, and hence that D-glucopyranose is β-linked in Cl (N) conformation and L-arabinopyranose α-linked in Cl (N).[7]

Consequently saponin C (I) is hederagenin 3-O-β-D-glucopyranosyl-(1-2)-α-L-arabinopyranoside and represented by the formula I.

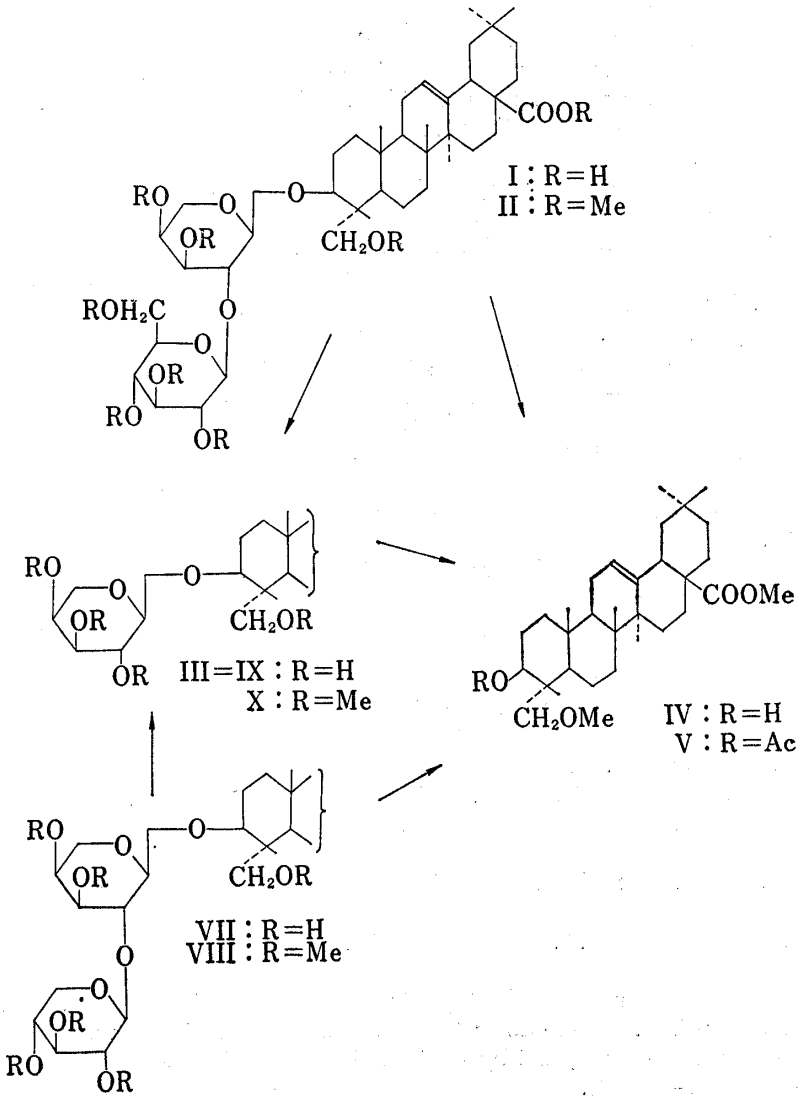
Saponin B (VII), colorless needles, mp 249.5—250.5° (decomp.), $[\alpha]_D$+37° (MeOH), was hydrolyzed in the same manner as in I to give hederagenin, arabinose and xylose, and in

7) K. Miyahara and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **17**, 1369 (1969); J. Sakakibara, Y. Hotta, and M. Yasue, *Yakugaku Zasshi*, **91**, 1318 (1971).

a milder condition, III and unchanged VII. Its permethylate (VIII), mp 187—188°, $[\alpha]_D$+25° ($CHCl_3$), exhibits on a MS the peaks at *m/e* 834 (molecular ion), 482 and 175 (fragments originated from the aglycone moiety and a terminal permethylated pentose residue,[6b] respectively). The elemental analyses of VII and VIII and the above data indicate VIII to have molecular formula $C_{47}H_{78}O_{12}$ and VII to consist of one mole each of hederagenin, arabinose and xylose. Methanolysis of VIII afforded an aglycone and two methylated pentoses, the former being nothing but IV and one of the latters being identical with VI. The other methylated pentose was proved to be methyl 2,3,4-tri-O-methyl-β-D-xylopyranoside on the basis of its optical rotation ($[\alpha]_D$−70° ($CHCl_3$)) and by comparison with the authentic sample on TLC and GLC. The NMR spectrum of VIII shows two anomeric proton signals at 4.42 and 4.60 ppm both as doublet with J=6 Hz implying existence of the β-D-xylopyranosyl residue in Cl (N) conformation as well as of the α-L-arabinopyranosyl unit (Cl (N) conformation).

Therefore saponin B (VII) is hederagenin 3-O-β-D-xylopyranosyl-(1-2)-α-L-arabinopyranoside.

Saponin A (IX), colorless needles, mp 228—229° (decomp.), $[\alpha]_D$+49° (MeOH), was acid hydrolyzed to yield hederagenin and arabinose, and behaved in the same manner on TLC as the prosapogenin derived from both I and VII suggesting that it is hederagenin 3-O-α-L-arabinopyranoside. The structure was verified by MS and NMR spectra of its per-



methylate (X), colorless prisms, mp 182°, $[\alpha]_D$+44° ($CHCl_3$), $C_{40}H_{66}O_8$, and by characterization of the methanolysis products as IV and methyl 2,3,4-tri-O-methyl-β-L-arabinopyranoside.

I[8] and IX have the same structures, respectively, as those proposed by Murakami and his coworkers[9] for saponins B and A of *Caulophyllum robustrum* Maxim. (Berberidaceae), and IX was also isolated from *Leontice eversmanni* Bunge. (Berberidaceae) (named leontoside A[11]), from *Patrinia scabiosuefolia* Link. (Valerianaceae) (scabioside A[12]) and from *Koelreuteria paniculata* Laxm. (Sapindaceae) (koelreuteria saponin A[13]), but VII is, to the authors' knowledge, the first one ever reported.

Experimental

All melting points were determined on a micro melting point apparatus (an air-bath type) and are uncorrected. Optical rotations were measured at 20—25° with a JASCO DIP-SL automatic polarimeter. IR spectra were obtained with a JASCO IR-G spectrometer. NMR spectra were taken at 60 MHz on a JEOL-C-60H spectrometer in $CDCl_3$ solution unless otherwise specified and chemical shifts are given in δ scale with tetramethylsilane as internal standard (s, singlet: d, doublet: t, triplet: q, quartet). Mass spectra were recorded on a JMS-01SG mass spectrometer with an accelerating potential of 6.2 kV, an ionizing potential of 75 eV and a source temperatue of 160°. GLC was run on a Yanagimoto GSG 550-F with flame ionization detector using glass column (1.2 m×4 mm ϕ) packed with 5% 1,4-butanediol succinate on Shimalite W (60—80 mesh). PPC was conducted on Toyo Roshi No. 50 in double ascending method using upper layer of *n*-BuOH–pyridine–H_2O (6:2:3)+pyridine (1) as a solvent and aniline hydrogen phthalate as a spray reagent. TLC was performed on Kieselgel G (E. Merck, A.G.) using solvent systems, $CHCl_3$–MeOH–H_2O (7:3:1) (bottom layer) (for hederagenin and saponins A-C), $CHCl_3$–MeOH–H_2O (25:17:3) (for saponins D—G), AcOEt–MeOH (50:1) (for methylated sugars), and visualized with *p*-anisaldehyde reagent.[14] Column chromatography was performed with Kieselgel (0.05—0.2 mm) (E. Merck, A.G.).

Isolation of Saponins A—G——The seeds collected in the suburb of Fukuoka city during October were treated as shown in Chart 1.

Saponin C (I)——Fr. 3 in Chart 1 was recrystallized from MeOH to give I as colorless needles, mp 248—250° (decomp.), $[\alpha]_D$ +40° (c=1.66, pyridine), +30° (c=0.5, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1690 COOH). *Anal.* Calcd. for $C_{41}H_{66}O_{13}\cdot 3H_2O$: C, 59.98; H, 8.84. Found: C, 59.65; H, 8.62. I (50 mg) was refluxed with 2 N H_2SO_4 in 50% EtOH (2 ml) for 2 hr and then diluted with H_2O. The precipitates (32 mg) were collected by filtration, dried and recrystallized from EtOH to give colorless prisms, mp 315—317°, $[\alpha]_D$ +81° (c=1.5, pyridine). *Anal.* Calcd. for $C_{30}H_{48}O_4$: C, 76.22; H, 10.24. Found: C, 75.57; H, 10.22. (acetate, mp 162—163°, $[\alpha]_D$ +79°, (c=2.3, $CHCl_3$); methylester acetate, mp 192—193°, $[\alpha]_D$ +74°, (c=2.4, $CHCl_3$); methylester, mp 236—237°, $[\alpha]_D$ +70° (c=1.0, $CHCl_3$)). The aglycone and its acetate were identified with authentic samples[15] of hederagenin and the acetate, respectively, by mixed melting point determination and TLC. The filtrate was neutralized with Amberlite A-400, concentrated and examined by paper chromatography (PPC), and arabinose and glucose were detected. When I was heated with 0.1 N H_2SO_4 in 50% EtOH for 2 hr, the hydrolysate was shown by TLC to contain hederagenin, a prosapogenin (III) and unchanged I.

Permethylate (II) of I——I (400 mg) was methylated by the Hakomori method.[5] The reaction mixture was diluted with H_2O, extracted with $CHCl_3$ and the $CHCl_3$ layer was washed, dried and evaporated. The residue was placed on a silica gel column and eluted with *n*-hexane–AcOEt (1:1) mixture to give II

8) Hederacoside A,[10] leontoside B[11] and scabioside C[12] are also known to be 3-O-glucosyl arabinosides of hederagenin but they are different from I in the structures of the sugar moieties.
9) T. Murakami, M. Nagasawa, S. Urayama, and N. Satake, *Yakugaku Zasshi*, **88**, 321 (1968).
10) J.J. Scheidegger and E. Cherbuliez, *Helv. Chim. Acta*, **38**, 547 (1955).
11) L.G. Mzhelskaya and N.K. Abubakirov, *Khim. Prir. Soedin*, **3**, 101—105 (1967) [*C.A.*, **67**, 100383 (1967)].
12) V.G. Bukharov, V.V. Karlin, and T.N. Sidorovich, *Khim. Prir. Soedin*, **6**, 69—74 (1969) [*C.A.*, **73**, 99162 (1970)].
13) V. Ya. Chirva, P.K. Kintya, and V.A. Sosnovskii, *Khim. Prir. Soedin*, **6**, 328—331 (1970) [*C.A.*, **73**, 110063 (1970)].
14) E. Stahl, "Dünnschicht Chromatographie," Springer-Verlag, Berlin, 1962, p. 498.
15) Kindly provided by Prof. T. Takemoto of Tohoku University (hederagenin, mp >300°, 315—317°*; acetate, mp 162—165°, 162—165°*) and by Prof. T. Murakami of Science University of Tokyo (hederagenin, mp 332—335°, 316—319°*).
* taken in this laboratory

(250 mg) as white powder (precipitated from *n*-hexane), mp 104—105°, $[\alpha]_D$ +39° (c=1.65, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1730 (COOR), none of OH. Mass Spectrum *m/e*: 878 (M^+, $C_{49}H_{82}O_{13}^+$), 482 ($C_{32}H_{50}O_3^+$), 219 ($C_{10}H_{19}O_5^+$).[6a] NMR: 4.33 ppm (1H, d, J=7 Hz, anomeric proton of arabinose), 4.59 (1H, d, J=8 Hz, anomeric proton of glucose). *Anal.* Calcd. for $C_{49}H_{82}O_{13}$: C, 66.98; H, 9.40. Found: C, 66.75; H, 9.40.

Methanolysis of II——II (120 mg) was refluxed with 2 N HCl in MeOH (4 ml) for 2 hr, the mixture was treated with Ag_2CO_3 and filtered. The filtrate was evaporated and the residue was crystallized from AcOEt to provide an aglycone (IV) as colorless needles, mp 190—192.5°, $[\alpha]_D$ +102° (c=0.7, $CHCl_3$) (23-O-methyl hederagenin methylester, mp 182—184°[9]). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1720 (COOR). *Anal.* Calcd. for $C_{32}H_{52}O_4$: C, 76.75; H, 10.47. Found: C, 76.59; H, 10.48. IV was acetylated with Ac_2O–pyridine to give an acetate (V) as colorless needles (MeOH), mp 213—213.5°. NMR: 2.02 ppm (3H, s, -OCOC<u>H</u>$_3$), 2.96 (2H, q, J=18 Hz, 12 Hz, -C<u>H</u>$_2$-OCH_3), 3.24 (3H, s, -CH_2-OC<u>H</u>$_3$), 3.61 (3H, s, -COOC<u>H</u>$_3$), 4.90 (1H, t, J=8 Hz, 8 Hz, -CH_2-C<u>H</u>-$OCOCH_3$ (eq)). *Anal.* Calcd. for $C_{34}H_{54}O_5$: C, 75.23; H, 10.03. Found: C, 75.24; H, 9.94. The mother liquor of recrystallization of methanolysate, showing the existence of two methylated sugars on GLC and TLC, was placed on a silica gel column and eluted with AcOEt to give two substances, colorless syrup, $[\alpha]_D$ +125° (c=0.8, $CHCl_3$), and colorless syrup, $[\alpha]_D$ +180° (c=0.69, $CHCl_3$), which were identified as methyl pyranosides of 2,3,4,6-tetra-O-methyl-α-D-glucose and 3,4-di-O-methyl-β-L-arabinose (VI), respectively, by comparison on GLC and TLC with the synthetic samples[16,17] of them and of methyl 2,3- and 2,4-di-O-methyl-β-L-arabinopyranosides.

Saponin B (VII)——Fr. 2 in Chart 1 was recrystallized from $CHCl_3$–MeOH–H_2O (7:3:1) mixture (bottom layer) to give VII as colorless needles, mp 249.5—250.5° (decomp.), $[\alpha]_D$ +37° (c=1.05, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1690 (COOH). *Anal.* Calcd. for $C_{40}H_{64}O_{12}\cdot 1\frac{1}{2}H_2O$: C, 62.88; H, 8.84. Found: C, 63.05; H, 8.84. VII was hydrolyzed in the same way as in I to yield hederagenin (identified by direct comparison), arabinose and xylose (identified on PPC). A mild hydrolysis of VII as in I gave a product showing on TLC three spots of hederagenin, III and VII.

Permethylate (VIII) of VII——Methylation of VII in the same manner as in I and chromatography of the product over silica gel with *n*-hexane–AcOEt (4:1) mixture gave VIII as colorless needles (MeOH), mp 187—188°, $[\alpha]_D$ +25° (c=4.9, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1725 (COOR), none of OH. Mass Spectrum *m/e*: 834 (M^+, $C_{47}H_{78}O_{12}^+$), 482 ($C_{32}H_{50}O_3^+$), 175 ($C_8H_{15}O_4^+$).[6b] NMR: 4.42 ppm (1H, d, J=6 Hz, anomeric proton of arabinose), 4.60 (1H, d, J=6 Hz, anomeric proton of xylose). *Anal.* Calcd. for $C_{47}H_{78}O_{12}$: C, 67.67; H, 9.42. Found: C, 67.58; H, 9.42.

Methanolysis of VIII——VIII (60 mg) was methanolyzed with 8% HCl in MeOH (5 ml) for 2 hr and worked up as in II. An aglycone and its acetate were identical in all respects with IV and V, respectively, and the sugar portion which showed two peaks on GLC was separated by silica gel chromatography using AcOEt as solvent into two fractions. One was identified with VI and the other, colorless syrup, $[\alpha]_D$ −70° (c=0.5, $CHCl_3$) was identical on GLC and TLC with an authentic sample of methyl 2,3,4-tri-O-methyl-β-D-xylopyranoside.

Saponin A (IX)——Fr. 1 in Chart 1 was recrystallized from dil. EtOH to give IX as colorless needles, mp 228—229° (decomp.), $[\alpha]_D$ +49° (c=3.65, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1690 (COOH). *Anal.* Calcd. for $C_{35}H_{56}O_8\cdot 2H_2O$: C, 65.59; H, 9.94. Found: C, 66.30; H, 9.23. It showed the same *Rf* value and color on TLC as those of III from I and VII. Acid hydrolysis of IX (100 mg) in the same way as in I gave hederagenin (67 mg) as an aglycone and the sugar portion showed only one spot of arabinose on PPC.

Permethylate (X) of IX——IX (150 mg) was methylated as I and VII and the product was directly recrystallized from MeOH to give X (60 mg) as colorless prisms, mp 182°, $[\alpha]_D$ +44° (c=1.95, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1725 (COOR), none of OH. Mass Spectrum *m/e*: 674 (M^+, $C_{40}H_{66}O_8^+$), 482 ($C_{32}H_{50}O_3^+$), 175 ($C_8H_{15}O_4^+$).[6b] NMR: 4.22 ppm (1H, d, J=5 Hz, anomeric proton of arabinose).

Methanolysis of X——Conducted in the same manner as in VIII to give an aglycone identical with IV and only one methylated sugar identical on TLC and GLC with the authentic sample of methyl 2,3,4-tri-O-methyl-β-L-arabinopyranoside.

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16) H. Okabe, N. Koshito, K. Tanaka, and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **19**, 2394 (1971).
17) M. Nishimura, R. Higuchi, K. Miyahara, and T. Kawasaki, Meeting of Kyushu Branch, Pharmaceutical Society of Japan, Fukuoka, February 1971.