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Seed Saponins of *Akebia quinata* Decne. II.[1] Hederagenin 3,28-O-Bisglycosides

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The more polar four triterpenoid saponins, D—G, of the seven, A—G, so far isolated in pure state from the seeds of *Akebia quinata* Decne. were characterized as follows: saponin D (mp>225° (decomp.), $[\alpha]_D$ +15°), 3-O-α-L-arabinopyranosyl hederagenin 28-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (IV); E (mp 210—214° (decomp.), $[\alpha]_D$ +6°), 3-O-β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl hederagenin 28-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (XI); F (mp 211—214° (decomp.), $[\alpha]_D$ −4°), 3-O-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl hederagenin 28-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (XIV); G (mp>218° (decomp.), $[\alpha]_D$ −19°), 3-O-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-α-L-arabinopyranosyl hederagenin 28-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (XVI). They are new hederagenin 3,28-O-bisglycosides and related to each other and to saponins A,B, and C.

As described in the preceding paper[1] seven saponins, A—G, were isolated from the seeds of *Akebia quinata* and three, A, B and C, of them were identified as hederagenin 3-O-glycosides, I, II and III, respectively.

This paper deals with a study on the more polar four saponins, D—G, which has led to characterization of them as hederagenin 3,28-O-bisglycosides[3] related to each other and to A, B and C.

Saponin D (IV), colorless needles, mp>225° (decomp.), $[\alpha]_D$ +15° (MeOH), was hydrolyzed with 2N sulfuric acid in 30% ethanol to yield hedreagenin, arabinose and glucose, and with 1% potassium hydroxide in 30% ethanol to give a compound (V). V gave, on acid hydrolysis, hederagenin and arabinose, and shows on thin-layer chromatogram (TLC) the same *Rf* value and color as those of saponin A (I). The methylester acetate of V, mp 235°, $[\alpha]_D$ +72° ($CHCl_3$), was identical with the corresponding derivative of I.

When the permethylate (VI) of IV prepared by the Kuhn method,[4] mp 109—110°, $[\alpha]_D$ +23° ($CHCl_3$), was subjected to methanolysis, three kinds of methylated monosaccaride along with an aglycon (VII) were provided. The sugars were identified on TLC and gas-liquid chromatogram (GLC) as methyl pyranosides of 2,3,4-tri-O-methyl-arabinose, 2,3,4,6-tetra-O-methyl-, and 2,3,4-tri-O-methyl-glucoses, and VII was converted with diazomethane to 23-O-methyl hederagenin methylester, the aglycon of I permethylate. The lithium aluminum hydride reduction of VI yielded two products, a syrup (VIII) and a white powder (IX), mp 95—97°, $[\alpha]_D$ +59° ($CHCl_3$), which was proved to be the 28-carbinol corresponding to the permethylate of I by direct comparison with the synthetic sample. VIII shows on a mass spectrum (MS) the peaks due to molecular ion (*m/e* 442) and to a terminal permethylated hexose residue (*m/e* 219).[5] The nuclear magnetic rasonance (NMR) spectra of VIII and its acetate, a syrup, $[\alpha]_D$

1) Part I: R. Higuchi, K. Miyahara, and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **20**, 1935 (1972).
2) Location: *1276 Katakasu, Fukuoka.*
3) Tschesche and Wulff have proposed to call the bis- and monoglycosides bis- and monodesmosidic, respectively. *a*) G. Wulff, *Deut. Apoth.-Ztg.*, **108**, 797 (1968); R. Tschesche, *Kagaku No Ryoiki*, **25**, 571 (1971); *b*) H.D. Woitke, J.P. Kayser, and K. Hiller, *Pharmazie*, **25**, 133, 213 (1970).
4) R. Kuhn, *Angew. Chem.*, **67**, 32 (1955).
5) *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, 221.

$-12°$ ($CHCl_3$), exhibit one anomeric proton signal at 4.27 ppm as a doublet ($J=6.5$ Hz) in the former and two acetoxyl in the latter. These data indicate that VIII derived from the 1—6 linked linear glucooligosaccaride moiety at the 28-carboxyl group of VI is 2,3,4,6-tetra-O-methyl-glucopyranosyl-(1→6)-2,3,4-tri-O-methyl-sorbitol, and that IV is, if glucose is assumed to be of the most common D-series, 28-O-D-glucopyranosyl-(1→6)-D-glucopyranoside of I.

When IV was incubated with almond emulsin, glucose and a prosapogenin (X), colorless needles, mp 211—214° (decomp.), $[\alpha]_D$ +34° (MeOH), were provided, and the latter was further cleaved with acid to give hederagenin, glucose and arabinose. This and the NMR data of VIII imply the existence of β-D-glucopyranosyl residue in Cl conformation[1] at terminal. The NMR spectrum of X in C_5D_5N solution shows two doublets at 4.75 ppm ($J=7$ Hz) and 6.00 ppm ($J=7$ Hz) which could be attributed to the anomeric protons of α-L-arabinopyranosyl (Cl conformation)[1] and β-D-glucopyranosyl (Cl conformation) units. The β-configulation of the two D-glucose residues was also suggested by the molecular rotation differrences[6] between IV and X (−127°), and X and I (−35°).

Consequently, saponin D is defined as 3-O-α-L-arabinopyranosyl hederagenin 28-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside and represented by the formula IV.

Saponin E (XI), white powder, mp 210—214° (decomp.), $[\alpha]_D$ +6° (MeOH), was hydrolyzed in the same manner as in IV with acid to give hederagenin, arabinose, xylose and glucose, and with alkali to yield saponin B (II) which was identified by direct comparison of its methylester peracetate, mp 140—143°, $[\alpha]_D$ +34° ($CHCl_3$), with the authentic specimen. The permethylate (XII), white powder, mp 112—113°, $[\alpha]_D$ +7° ($CHCl_3$), was methanolyzed to give the same aglycon VII as that from VI and four kinds of methylated sugar. The latters were identified on TLC and GLC as methyl pyranosides of 2,3,4-tri-O-methyl-xylose, 3,4-di-O-methyl-arabinose, 2,3,4-tri-O-methyl- and 2,3,4,6-tetra-O-methyl-glucoses. The lithium aluminum hydride reduction of XII yielded a syrup together with colorless plates, mp 220—221°, which are identical with the 28-carbinol derived from II permethylate. The syrup shows the same *Rf* value on TLC and MS as those of VIII. Accordingly, assuming glucose as of D-series, the sugar moiety attached to the 28-carboxy group of XI is regarded as a disaccaride, D-glucopyranosyl-(1→6)-D-glucopyranose. The mode of linkage of the two glucose units is regarded, in both cases, as β on the basis of the NMR spectrum of XII showing an anomeric proton signal of esterglycosidic glucose[7] at 5.40 ppm as a doublet ($J=7$ Hz) and of the fact that XI was cleaved with emulsin to give glucose and a prosapogenin (XIII), mp 205—210° (decomp.), $[\alpha]_D$ +25° (MeOH), consisting of hederagenin, arabinose, xylose, and glucose. The configurations are also supported by the molecular rotation differrences between II and XIII (−42°), and XIII and XI (−169°).

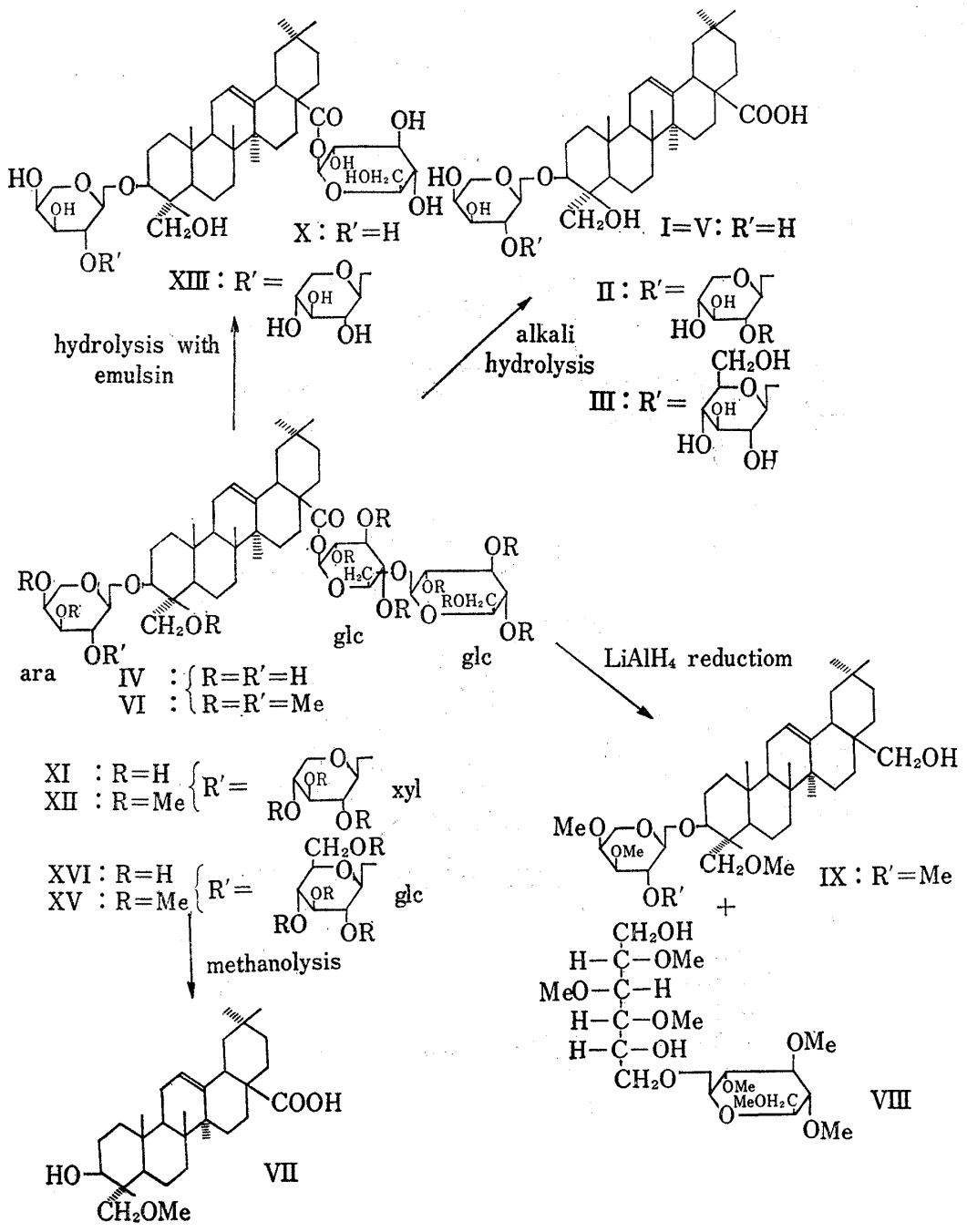
Saponin E is thus considered to be 3-O-β-D-xylopyranosyl-(1→2)-α-L-arabinopyranosyl hederagenin 28-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (XI).

Saponin F (XIV), white powder, mp 211—214° (decomp.), $[\alpha]_D$ −4° (MeOH), is composed of hederagenin, arabinose and glucose, and gave saponin C (III) on alkali hydrolysis. XIV permethylate (XV), mp 110°, $[\alpha]_D$ +13° ($CHCl_3$), shows an anomeric proton signal of esterglycosidic glucose at 5.35 ppm as a doublet ($J=7$ Hz) on the NMR spectrum, and its methanolysis yielded VII, as from VI and XII, and methyl pyranosides of 3,4-di-O-methyl-arabinose, 2,3,4-tri-O-methyl- and 2,3,4,6-tetra-O-methyl-glucoses. The reduction products of XV with lithium aluminum hydride were identified as VIII and the 28-carbinol corresponding to III permethylate.

On the basis of the above data and by analogy with the cooccurrence of XI and II, saponin F is regarded as 28-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside of coexisting III, that is 3-O-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl hederagenin 28-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (XIV).

6) W. Klyne, *Biochem. J.*, **47**, xli (1950).
7) N. Kondo, Y. Marumoto, and J. Shoji, *Chem. Pharm. Bull.* (Tokyo), **19**, 1103 (1971).

Saponin G (XVI), white powder, mp>218° (decomp.), $[\alpha]_D$ −19° (MeOH), consists of hederagenin, arabinose, glucose and rhamnose, and its hydrolysis with alkali afforded a compound (XVII), mp 225—227° (decomp.), $[\alpha]_D$ +10° (MeOH). XVII was acid hydrolyzed in the same manner as in XVI to give the same products and in a milder condition to provide saponin C (III). The permethylate (XVIII) of XVII prepared by the Hakomori method,[8] colorless needles, mp 211—211.5°, $[\alpha]_D$ +15° ($CHCl_3$), shows on a MS the molecular ion (*m/e* 1052) and the fragment ions originated from terminal permethylated methylpentose (*m/e* 189)[5b] and hexose (*m/e* 219)[5] residues, and its methanolysis yielded a compound identical with the aglycon of I permethylate and methyl pyranosides of 2,3,4-tri-O-methyl-

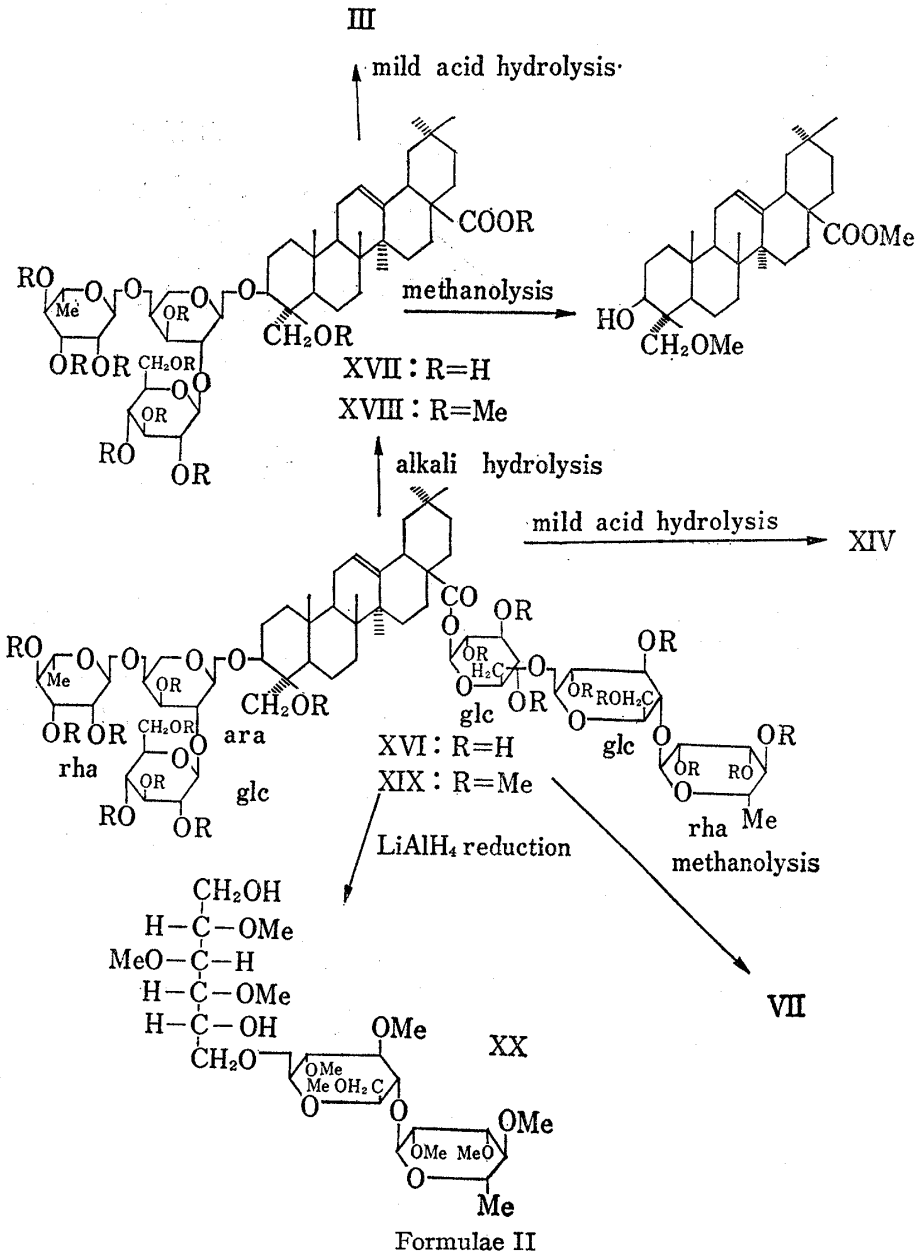


Formulae I

8) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).

rhamnose, 2,3,4,6-tetra-O-methyl-glucose and 3-O-methyl-arabinose. If the rhamnose residue in XVII is assumed to be of L-series, as is usually the case in natural glycosides, the molecular rotation difference (−139°) between XVII and III suggests α-configulation[9] of the L-rhamnose unit. Thus the structure, 3-O-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-α-L-arabinopyranosyl hederagenin, is assigned to XVII.

The permethylate (XIX) of XVI, white powder, mp 113—115°, $[\alpha]_D$ −13° ($CHCl_3$), gave on methanolysis VII and five kinds of methylated sugar. The latters were identified on TLC and GLC as methyl pyranosides of 2,3,4-tri-O-methyl-rhamnose, 3-O-methyl-arabinose, 2,3,4,6-tetra-O-methyl-, 2,3,4-tri-O-methyl- and 2,3,6-tri-O-methyl-glucoses. The lithium aluminum hydride reduction of XIX provided a colorless syrup (XX), $[\alpha]_D$ −34° ($CHCl_3$), (diacetate, a colorless syrup, $[\alpha]_D$ −45° ($CHCl_3$)), together with a powder which should be the 28-carbinol corresponding to XVIII. XX exhibits on a MS the molecular ion (*m/e* 616) and the peak due to a terminal permethylated methylpentose residue (*m/e* 189). These data



Formulae II

9) The small *J* value (1.5 Hz) of the anomeric proton signal of α-L-rhamnose unit at 5.12 ppm on the NMR spectrum of XVIII (*cf.* Experimental) might be due to the 1C conformation of the unit.

along with the finding that XVI gave saponin F (XIV) on mild acid hydrolysis indicate that the oligosaccaride moiety conjugated with the 28-carboxy group in XVI is rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranose. On the NMR spectrum of XX the anomeric proton signals appear at 4.34 ppm (doublet, $J=7$ Hz) and 4.99 ppm (singlet) and they are assigned respectively to those of β-D-glucopyranose (Cl conformation) and of rhamnopyranose assumingly of L-series with α-linkage in 1C conformation.

Consequently the structure 3-O-β-D-glucopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→4)]-α-L-arabinopyranosyl hederagenin 28-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (XVI) is postulated for saponin G.

There have recently been known several hederagenin 3,28-O-bisglycosides,[3b,10] but the four saponins, IV, XI, XIV and XVI here reported are somewhat different in their sugar moieties from those so far recorded. Coexistence of the glycosides closely related to each other are noted.

Experimental[11]

Saponin D (IV)——IV was obtained on recrystallization from AcOEt–BuOH as colorless needles, mp >225° (decomp.), $[\alpha]_D$ +15° ($c=1.64$, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1755, 1740, 1725 (COOR). *Anal.* Calcd. for $C_{47}H_{76}O_{18}\cdot 4H_2O$: C, 56.38; H, 8.46. Found: C, 55.66; H, 8.15. IV (50 mg) was hydrolyzed on refluxing with 2N H_2SO_4 in 30% EtOH (2 ml) for 2 hr. The reaction mixture was diluted with H_2O, the aglycon precipitated was collected by filtration and the filtrate was neutralized with Amberlite A-400 and evaporated *in vacuo*. The aglycon was recrystallized from EtOH to give colorless prisms (23 mg), mp 315—317° (acetate, mp 162—163°). It was identified as hederagenin by direct comparison (mixed melting point, IR, TLC) with authentic sample.[1] The sugar portion was examined by paper chromatography (PPC) and arabinose and glucose were detected. IV (130 mg) was refluxed with 1% KOH in 30% EtOH (8 ml) for 1 hr and the reaction mixture was evaporated *in vacuo* to a residue (V) (77 mg), which showed on TLC one spot identical with that of I run in pallarel and gave on acid hydrolysis hederagenin and arabinose (TLC and PPC). Acetylation of V with Ac_2O–pyridine followed by methylation with CH_2N_2 and recrystallization of the product from MeOH gave a methylester acetate as colorless needles, mp 235°, $[\alpha]_D$ +72° ($c=1.0$, $CHCl_3$). *Anal.* Calcd. for $C_{44}H_{66}O_{12}$: C, 67.15; H, 8.45. Found: C, 66.54; H, 8.40. It was identified by direct comparison with the methylester acetate, mp 238°, $[\alpha]_D$ +76° ($c=1.0$, $CHCl_3$), derived from I.

Permethylate (VI) of IV——IV (900 mg) was methylated in dimethylformamide (9 ml) with Ag_2O (2 g) and CH_3I (10 ml) for 150 hr according to the Kuhn method.[4] The precipitates were filtered off, the filtrate was diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, dried and evaporated. The residue was then passed through a silica gel column by using hexane–AcOEt (1:1) to give VI (157 mg) as white powder, mp 109—110°, $[\alpha]_D$ +23° ($c=3.3$, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1755, 1725 (COOR), none of OH. NMR: 5.40 ppm (1H, d, $J=7$ Hz, anomeric proton of esterglycosidic glucose[7]).

Methanolysis of VI——VI (65 mg) was refluxed with 10% HCl–MeOH (5 ml) for 3 hr, the reaction mixture was neutralized with Ag_2CO_3, filtered, evaporated and the residue was recrystallized from MeOH to give an aglycon (VII) (10 mg) as colorless needles, mp 203—206°, IR ν_{max}^{KBr} cm^{-1}: 3500, 3200 (OH), 1725 (COOH). The mother liquor of recrystallization was examined by TLC and GLC and three methylated sugars were detected and identified as methyl pyranosides of 2,3,4,6-tetra-O-methyl- and 2,3,4-tri-O-methyl-glucoses and 2,3,4-tri-O-methyl-arabinose by comparison with the synthetic samples[12] including 2,3,6-, 2,4,6- and 3,4,6-tri-O-methyl-α-D-glucopyranosides. VII was methylated with CH_2N_2 to yield colorless needles (AcOEt), mp 190—192°, which was identified (mixed mp, TLC) with the aglycon[1] of I permethylate.

$LiAlH_4$ Reduction of VI——VI (40 mg) in anhydrous tetrahydrofuran (4 ml) and $LiAlH_4$ (18 mg) were refluxed for 3 hr. Excess reagent was decomposed with H_2O, the reaction mixture was evaporated *in vacuo* and extracted with $CHCl_3$. The extracts were dried, evaporated and the residue was separated by chromatography on silica gel using hexane–AcOEt (1:1) into two fractions, a colorless syrup (VIII), Mass Spectrum *m/e*:

10) V.G. Bukharov and V.V. Karlin, *Khim. Prir. Soedin.*, **6**, 64 (1970) [*C. A.*, **73**, 56350 (1970)]; V. Ya, Chirva, P.K. Kintya, and V.A. Sosnovskii, *ibid.*, **6**, 431 (1970) [*C.A.*, **74**, 13384 (1971)]; M.M. Mukhamedziev, P.K. Alimbaera, T.T. Gorovits, and N.K. Abubakirov, *ibid.*, **7**, 153 (1971) [*C.A.*, **75**, 49494 (1971)].

11) For general methods, except for the mass spectra of VIII and XX (accelerating potential of 6.1 KV, ionizing potential of 30 eV, source temperature of 100°), refer to the preceding paper.[1]

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

442 (M^+, $C_{19}H_{38}O_{11}^+$), 219[5] ($C_{10}H_{19}O_5^+$); NMR: 4.27 ppm (1H, d, J=6.5 Hz, anomeric proton), and a white powder (IX), mp 95—97°, $[\alpha]_D$ +59° (c=0.54, $CHCl_3$). VIII was acetylated with Ac_2O-pyridine to give an acetate, a syrup, $[\alpha]_D$ −12° (c=1.4, $CHCl_3$), NMR (benzene): 1.74 ppm (3H, s, -OCOC<u>H</u>$_3$), 1.81 (3H, s, -OCOC<u>H</u>$_3$). IX was identified (mixed mp, IR, TLC) with a product from I permethylate on $LiAlH_4$ reduction in the same manner as in VI.

Hydrolysis of IV with Emulsin——IV (570 mg) in H_2O (25 ml) was incubated with almond emulsin[13] (80 mg) at 37° for 2 days and the hydrolysate was shaken with n-BuOH saturated with H_2O. The aqueous layer shows only one spot of glucose on PPC and the organic layer was concentrated and chromatographed over silica gel (CH_2Cl_2–MeOH–H_2O (7:3:1, bottom layer)) to provide a prosapogenin (X) as colorless needles (dil. MeOH), mp 211—214° (decomp.), $[\alpha]_D$ +34° (c=3.64, MeOH), $\Delta[M]_D$ (IV-X) −127°, (X-I) −35° ($[M]_D$ of methyl D-glucopyranoside: β, −62°; α, +276°). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1755, 1725 (COOR). NMR (C_5D_5N): 4.75 ppm (1H, d, J=7 Hz, anomeric proton of arabinose), 6.00 (1H, d, J=7 Hz, anomeric proton of esterglycosidic glucose). The acid hydrolysate of X was shown by PPC and TLC to contain hederagenin, glucose and arabinose.

Saponin E (XI)——White powder (precipitated from MeOH with ether), mp 210—214° (decomp.), $[\alpha]_D$ +6° (c=1.8, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1755, 1740, 1725 (COOR). *Anal.* Calcd. for $C_{52}H_{84}O_{22}\cdot 4H_2O$: C, 55.02; H, 8.18. Found: C, 55.43; H, 8.02. Acid hydrolysis and examination of the product were carried out in the same way as in IV, and hederagenin, arabinose, xylose and glucose were identified. XI (180 mg) was cleaved as IV with alkali, the product was successively acetylated (Ac_2O–pyridine), methylated (CH_2N_2) and chromatographed over silica gel (hexane–AcOEt (3:1)) to give colorless needles (Me_2CO) (80 mg), mp 140–143°, $[\alpha]_D$ +34° (c=1.0, $CHCl_3$). *Anal.* Calcd. for $C_{53}H_{78}O_{13}$: C, 63.45; H, 7.84. Found: C, 63.55; H, 7.99. It was identified by direct comparison with the methylester peracetate of II.

Permethylate (XII) of XI——XI (500 mg) was methylated, worked up, and the product was purified in the same way as in IV to give XII (180 mg) as a white powder (precipitated from $CHCl_3$ with hexane), mp 112—113°, $[\alpha]_D$ +7° (c=3.8, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1755, 1740 (COOR), none of OH. NMR: 5.40 ppm (1H, d, J=7 Hz, anomeric proton of esterglycosidic glucose). *Anal.* Calcd. for $C_{65}H_{110}O_{22}$: C, 62.78; H, 8.92. Found: C, 62.23; H, 8.82.

Methanolysis of XII——XII (20 mg) was methanolyzed as VI to give VII (mixed mp, TLC) and methyl pyranosides of 2,3,4-tri-O-methyl-xylose, 3,4-di-O-methyl-arabinose, 2,3,4-tri-O-methyl- and 2,3,4,6-tetra-O-methyl-glucoses (identified on TLC and GLC by comparing with the synthetic samples[11] including three di-O-methyl-β-L-arabinopyranosides and four tri-O-methyl-α-D-glucopyranosides).

$LiAlH_4$ Reduction of XII——XII (120 mg) was reduced with $LiAlH_4$ (50 mg) and the products were separated in the same way as in VI to give a syrup (30 mg) and colorless plates (MeOH) (10 mg), mp 220—221°, $[\alpha]_D$ +34° (c=1.7, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), none of C=O. *Anal.* Calcd. for $C_{46}H_{78}O_{11}$: C, 68.45; H, 9.74. Found: C, 68.13; H, 9.26. The syrup was identical with VIII on TLC and MS, and the crystals were identified (TLC, IR, mixed mp) with one of the $LiAlH_4$ reduction products of II permethylate.

Hydrolysis of XI with Emulsin——The procedure is same as for VI. XI (560 mg) gave glucose (PPC) and a white powder (precipitated from MeOH with ether) (XIII) (390 mg), mp 205—210° (decomp.), $[\alpha]_D$ +25° (c=4.55, MeOH), $\Delta[M]_D$ (XIII—II) −42°, (XI—XIII) −169° ($[M]_D$ of methyl D-glucopyranoside: β, −62°; α, +276°). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1755, 1740, 1725 (COOR). *Anal.* Calcd. for $C_{46}H_{74}O_{17}\cdot 3H_2O$: C, 57.96; H, 8.46. Found: C, 58.19; H, 8.32. XIII was acid hydrolyzed as IV to yield hederagenin (TLC and mixed mp) arabinose, xylose and glucose (PPC).

Saponin F (XIV)——White powder (precipitated from MeOH with ether), mp 211—214° (decomp.), $[\alpha]_D$ −4° (c=2.65, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1755, 1740, 1725 (COOR). *Anal.* Calcd. for $C_{53}H_{86}O_{23}\cdot 5H_2O$: C, 53.88; H, 8.19. Found: C, 53.41; H, 8.01. Hydrolyses conducted in the same way as for IV yielded with acid hederagenin, arabinose and glucose, and with alkali a compound, colorless needles (MeOH), mp 245—246° (decomp.), $[\alpha]_D$ +38° (c=0.72, pyridine), which is identical with III (mixed mp, IR, TLC).

Permethylate (XV) of XIV——XIV (440 mg) was methylated and worked up as IV to give XV (157 mg) as a white powder (precipitated from $CHCl_3$ with hexane), mp 110°, $[\alpha]_D$ +13° (c=2.77, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1755, 1740 (COOR), none of OH. NMR: 5.35 ppm (1H, d, J=7 Hz, anomeric proton of esterglycosidic glucose).

Methanolysis of XV——The products obtained and characterized in the same way as in VI and XII were VII and methyl pyranosides of 2,3,4,6-tetra-O-methyl- and 2,3,4-tri-O-methyl-glucoses and 3,4-di-O-methyl-arabinose.

$LiAlH_4$ Reduction of XV——The reduction of XV (55 mg) as VI with $LiAlH_4$ (30 mg) afforded a colorless syrup (14 mg) and a white powder (precipitated from MeOH) (27 mg), mp 105—107°, $[\alpha]_D$ +31° (c=0.5, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), none of C=O. The former was identical with VIII on TLC and the latter was identified by direct comparison with the corresponding reduction product of III permethylate.

13) Sigma Chemical Company.

Saponin G (XVI)——A white powder (precipitated from MeOH with ether), mp >218° (decomp.), $[\alpha]_D$ −19° (c=4.4, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1755, 1740, 1725 (COOR). *Anal.* Calcd. for $C_{65}H_{106}O_{31}\cdot 7H_2O$: C, 51.40; H, 7.72. Found: C, 51.71; H, 8.01. In a similar manner to IV, XVI was cleaved with acid and alkali respectively to give hederagenin, arabinose, glucose and rhamnose and to yield colorless needles (dil. MeOH) (XVII), mp 225—227° (decomp.), $[\alpha]_D$ +10° (c=3.05, MeOH), $\Delta[M]_D$ (XVII-III) −139° ($[M]_D$ of methyl L-rhamnoside: α, −111°; β, +170°). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1690 (COOR). *Anal.* Calcd. for $C_{47}H_{76}O_{17}\cdot 3H_2O$: C, 57.88; H, 8.43. Found: C, 58.37; H, 8.55. On refluxing XVI with 0.1N H_2SO_4 in 50% EtOH for 2 hr, a mixture of unchanged XVI, XIV, XVII, III and hederagenin (TLC) was provided.

Acid Hydrolysis of XVII——Acid hydrolysis of XVII with 2N H_2SO_4 gave hederagenin, arabinose, glucose and rhamnose (TLC and PPC), while partial hydrolysis with 0.1N H_2SO_4 afforded unchanged XVII, III, I and hederagenin (TLC).

Permethylate (XVIII) of XVII——Methylation of XVII (100 mg) as I by the Hakomori method[8] and purification of the product over silica gel (solvent, AcOEt) gave XVIII as colorless needles (MeOH), mp 211—211.5°, $[\alpha]_D$ +15° (c=1.9, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1720 (COOR), none of OH. NMR: 5.12 ppm (1H, d, J=1.5 Hz, anomeric proton of rhamnose). Mass Spectrum m/e: 1052 (M^+, $C_{57}H_{96}O_{17}^+$), 219 ($C_{10}H_{19}O_5^+$),[5] 189 ($C_9H_{17}O_4^+$).[5b] *Anal.* Calcd. for $C_{57}H_{96}O_{17}$: C, 64.66; H, 9.23. Found: C, 64.99; H, 9.19.

Methanolysis of XVIII——XVIII was methanolyzed as VI to give colorless needles (AcOEt), mp 192°, (acetate mp 213°), identical with the aglycon of I permethylate, and methyl pyranosides of 2,3,4-tri-O-methyl-rhamnose, 3-or 4-O-methyl-arabinose, and 2,3,4,6-tetra-O-methyl-glucose (PPC and GLC). The mixture of methylated sugars was acetylated with Ac_2O–pyridine and the product was shown to contain methyl 2,4-di-O-acetyl-3-O-methyl-arabinopyranoside and not 2,3-di-O-acetyl-4-O-methyl derivative by comparison on GLC with synthetic samples.

Permethylate (XIX) of XVI——Prepared by the Kuhn method.[4] A white powder (precipitated from $CHCl_3$ with hexane), mp 113—115°, $[\alpha]_D$ −13° (c=1.7, $CHCl_3$). IR ν_{max}^{Nujol} cm^{-1}: 1755, 1740 (COOR), none of OH. NMR: 4.99 ppm (1H, s, anomeric proton of rhamnose), 5.15 (1H, s, anomeric proton of rhamnose), 5.41 (1H, d, J=7 Hz, anomeric proton of esterglycosidic glucose). *Anal.* Calcd. for $C_{83}H_{142}O_{31}$: C, 60.70; H, 8.78. Found: C, 60.93; H, 8.74.

Methanolysis of XIX——Cleavage and identification of the products were carried out in the same way as in VI. The aglycon was VII and the sugar portion consisted of the three from XVIII and methyl 2,3,4-tri-O-methyl- and 2,3,6-tri-O-methyl-glucopyranosides.

$LiAlH_4$ Reduction of XIX——XIX was reduced and worked up as VI and the crude product was extracted successively with ether and $CHCl_3$. The ether extractive was a white powder (precipitated from MeOH), mp 118—120°, $[\alpha]_D$ +16° (c=0.5, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3500 (OH), none of C=O. The $CHCl_3$ extractive was placed on a silica gel column and eluted with AcOEt–MeOH (50:1) to give a colorless syrup (XX), $[\alpha]_D$ −34° (c=1.25, $CHCl_3$).[14] MS: m/e 616 (M^+, $C_{27}H_{52}O_{15}^+$), 189 ($C_9H_{17}O_4^+$). NMR: 1.30 ppm (3H, d, J=7 Hz, 6-CH_3 of rhamnose), 4.34 (1H, d, J=7 Hz, anomeric proton of glucose), 4.99 (1H, s, anomeric proton of rhamnose). XX was acetylated to yield an acetate as a colorless syrup, $[\alpha]_D$ −45° (c=0.85, $CHCl_3$). NMR (benzene): 1.48 ppm (3H, d, J=7 Hz, 6-CH_3 of rhamnose), 1.74 (3H, s, -OCOCH_3), 1.82 (3H, s, -OCOCH_3), 5.22 (1H, d, J=1.5 Hz, anomeric proton of rhamnose).

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14) 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, a colorless syrup, $[\alpha]_D^{20}$ −33° (c=4.2, $CHCl_3$), (A. Ya. Khorlin, A.G. Venyaminova, and N.K. Kochetkov, *Bull. Acad. Sci. USSR.*, **9**, 1530 (1966) [*C.A.*, **66**, 65803 (1967)]).