[Chem. Pharm. Bull. 24(5)1021—1032(1976)]

UDC 547.913.02.05 : 581.192

Pericarp Saponins of *Akebia quinata* Decne. I. Glycosides of Hederagenin and Oleanolic Acid

Ryuichi Higuchi and Toshio Kawasaki

Faculty of Pharmaceutical Sciences, Kyushu University[1)]

(Received August 29, 1975)

Two free triterpenoids and twelve triterpenoid saponins were isolated from the fresh pericarps of *Akebia quinata* Decne. (Lardizabalaceae). Ten saponins, P_{A-G}, P_{J_2}, P_{J_3} and P_K, of the above twelve were characterized as follows: P_A (I), hederagenin (hederag.) 3-O-α-L-ara·pyranoside; P_F (II), hederag. 3-O-β-D-glc·pyr-(1→2)-α-L-ara·pyranoside; P_B (III), oleanolic acid (ol. acid) 3-O-α-L-rha·pyr-(1→2)-α-L-ara·pyranoside; P_C (VI), hederag. 3-O-β-D-xyl·pyr-(1→3)-α-L-ara·pyranoside; P_D (IX), hederag. 3-O-α-L-rha·pyr-(1→2)-α-L-ara·pyranoside; P_E (XI), ol.acid 3-O-β-D-glc·pyr-(1→2)-α-L-ara·pyranoside; P_G (XIII), hederag. 3-O-β-D-xyl·pyr-(1→3)-α-L-rha·pyr-(1→2)-α-L-ara·pyranoside; P_{J_2} (XV′) 3-O-α-L-ara·pyranosyl-hederag. 28-O-α-L-rha·pyr-(1→4)-β-D-glc·pyr-(1→6)-β-D-glc·pyranoside; P_{J_3} (XIX′), 3-O-α-L-rha·pyr-(1→2)-α-L-ara·pyranosyl-ol.acid 28-O-α-L-rha·pyr-(1→4)-β-D-glc·pyr-(1→6)-β-D-glc·pyranoside; P_K (XXI), 3-O-α-L-rha·pyr-(1→2)-α-L-ara·pyranosyl-hederag. 28-O-α-L-rha·pyr-(1→4)-β-D-glc·pyr-(1→6)-β-D-glc·pyranoside.

VI, XI and XV′ are new members of the group of hederagenin and oleanolic acid oligoglycosides. It is noted that they all have the common unit, 3-O-α-L-ara·pyranoside, and that XV′, XIX′ and XX, having the same trisaccharide combined with the 28-carboxyl group of the aglycone, are corresponding to I, III and IX, respectively.

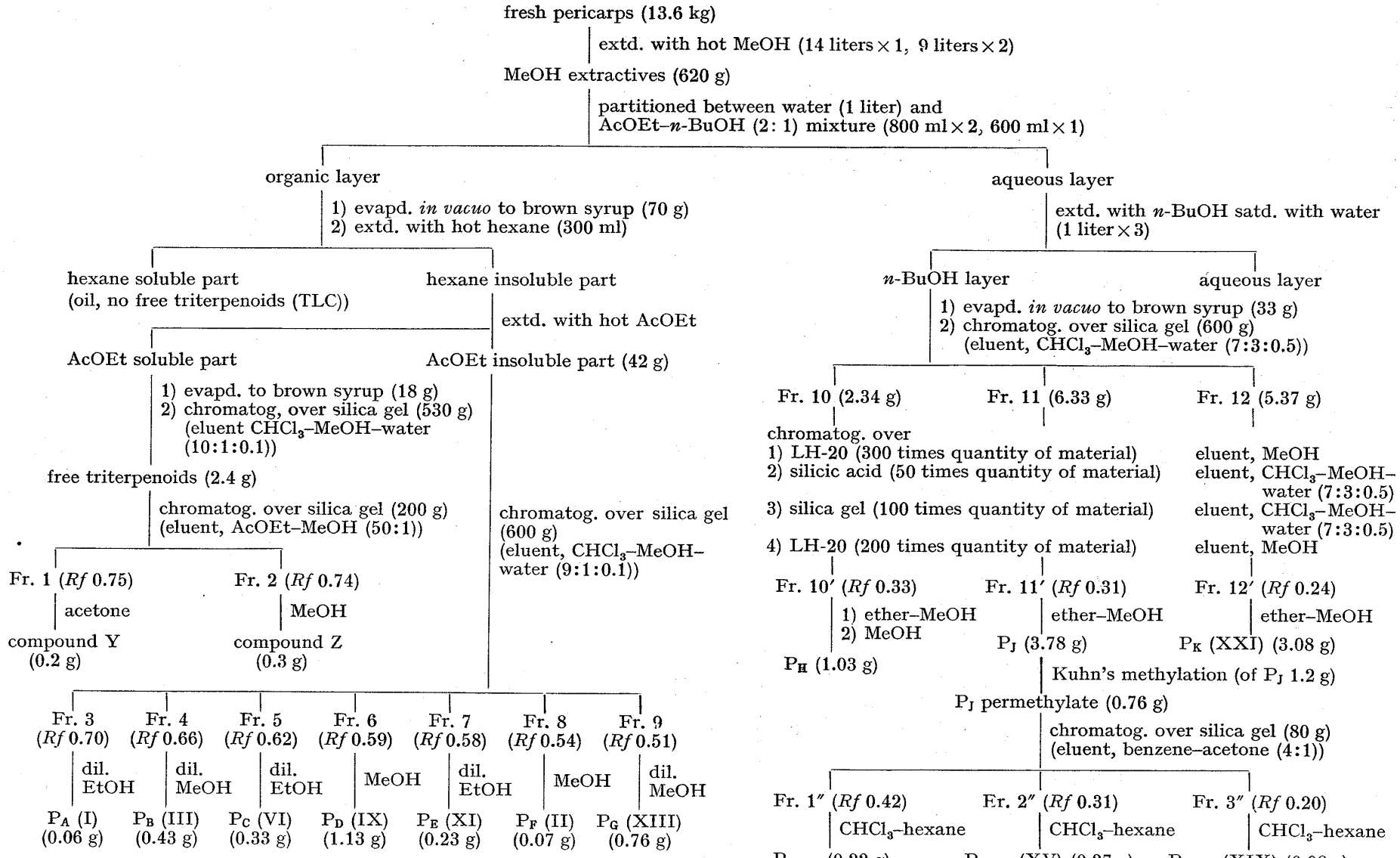
Previously[2)] it was reported that the seeds of *Akebia quinata* Decne. (Lardizabalaceae) contained seven kinds of hederagenin 3-O-glycosides and 3,28-O-bisglycosides, seed saponins A-G. In continuation of the study, the triterpenoid constituents of the fresh pericarps of the same plant were surveyed and two free triterpenoids, Y and Z, and twelve saponins, pericarp saponins A-H, J_{1-2}, K (P_A-P_H, P_{J_1}-P_{J_3}, P_K), were obtained.[3)]

This paper describes the isolation of these compounds and the characterization of ten of them, P_A-P_G, P_{J_2}, P_{J_3}, P_K, as the glycosides of hederagenin and oleanolic acid.

The methanol extractives were fractionated as shown in Chart 1, under monitoring by thin-layer chromatography (TLC), with the aid of partitioning between water and the ethyl acetate–*n*-butanol (2: 1, v/v) mixture[4)] followed by treatment with some solvents and subsequent chromatography. Except P_J, all the compounds thus obtained were considered to be homogeneous on the basis of their behaviors on TLC and of the hydrolysis products. P_J exhibited single spot on TLC using two different solvent systems, but was hydrolyzed with acid to give hederagenin, oleanolic acid and the triterpenoid Y, and with alkali to provide P_A and P_B together with Y. Therefore it is apparent that P_J is a mixture of at least three glycosides P_{J1-3}. Attempted resolution of P_J or its acetate into the components by means of further chromatography was in failure, but the permethylate of P_J prepared by the Kuhn method[5)] was successfully separated over a silica gel column into three kinds of homogeneous methylates, P_{J1-M}-P_{J3-M}.

1) Location: *3-1-1 Maedashi, Higashi-ku, 812, Fukuoka.*
2) *a*) R. Higuchi, K. Miyahara, and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **20**, 1935 (1972); *b*) R. Higuchi and T. Kawasaki, *ibid.*, **20**, 2143 (1972).
3) P_{J_1}-P_{J_3} were obtained as their permethylates.
4) This method was successfully applied also to the separation of furostanol 3,26-O-bisglycosides from spirostanol 3-O-glycosides (T. Nohara, K. Miyahara, and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **23**, 872 (1975)).
5) R. Kuhn, I. Löw, and H. Trischmann, *Chem. Ber.*, **88**, 1492, 1690 (1955).

NII-Electronic Library Service



Rf values of Fr. 1—9 and 10′—12′ and of Fr. 1″—3″ were measured on TLC using solvent b ($CHCl_3$–MeOH–water (25:17:3)) and solvent e (benzene–acetone (4:1)), respectively.

Chart 1

NII-Electronic Library Service

P_A (I), mp 228—230° (decomp.), $[\alpha]_D$ +48°, (peracetate, mp 205—207°, $[\alpha]_D$ +67°; peracetate methylester, mp 238—239°, $[\alpha]_D$ +76°; permethylate, mp 181—182°, $[\alpha]_D$ +44°) and P_F (II), mp 248—250° (decomp.), $[\alpha]_D$ +41°, (peracetate, mp 172—175°, $[\alpha]_D$ +38°), were respectively identified with hederagenin 3-O-α-L-arabinopyranoside (seed saponin A[2a]) and 3-O-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranoside (seed saponin C[2a]) by examination of their acid hydrolysates, by comparisons of their *Rf* values on TLC, infrared (IR) and nuclear magnetic resonance (NMR) spectra with those of the authentic specimens and by a mixed fusion.

P_B (III), mp 222—225° (decomp.), $[\alpha]_D$ +9°, (peracetate, mp 170—172°, $[\alpha]_D$ +22°), shows the carboxyl absorptions on the IR spectrum and its permethylate (IV) prepared by the Hakomori method,[6] mp 210—212°, $[\alpha]_D$ +14°, exhibits on its mass spectrum the molecular ion peak at *m/e* 818 and the fragment peak due to the terminal permethylated methylpentose residue at *m/e* 189.[7a] III was hydrolyzed with 2N sulfuric acid for 2 hr to give oleanolic acid, arabinose and rhamnose, and with 0.4N H_2SO_4 for 1 hr to yield only one prosapogenin (V), mp 255—259° (decomp.), $[\alpha]_D$ +49°, consisting of oleanolic acid and arabinose. When IV was subjected to methanolysis, methyl oleanolate and two kinds of methylated monosaccharides were provided and the latters were identified with methyl pyranosides of 2,3,4-tri-O-methyl-α-L-rhamnose[8a] and 3,4-di-O-methyl-β-L-arabinose[8b] on TLC and gas–liquid chromatography (GLC) and on the basis of the optical rotations of them and of the corresponding free sugars. The molecular rotation differences[9] between III and V and V and oleanolic acid indicate the α-linkage of both the L-rhamnose and L-arabinose units.

Consequently III is defined as oleanolic acid 3-O-α-L-rhamnopyranosyl-(1—2)-α-L-arabinopyranoside.

P_C (VI), mp 236—240° (decomp.), $[\alpha]_D$ +45°, (peracetate, mp 179—182°, $[\alpha]_D$ +35°), was hydrolyzed with acid to yield hederagenin, arabinose and xylose, and in a milder condition to give I along with hederagenin and unchanged VI. VI permethylate (VII), mp 112—115°, $[\alpha]_D$ +28°, showed on its mass spectrum the peaks at *m/e* 834 and 175, which are assignable respectively to the molecular ion and the fragment ion originating from the terminal permethylated pentose residue.[7a] Methanolysis of VII afforded 23-O-methyl-hederagenin methylester (VIII) and two kinds of methylated monosaccharides, which were separated over a silica gel column and identified with methyl pyranosides of 2,3,4-tri-O-methyl-α-D-xylose and 2,4-di-O-methyl-β-L-arabinose by comparisons of their optical rotations, *Rf* values on TLC and retention times on GLC with those of the authentic samples.[8b] The NMR spectrum of VII shows two one-proton doublet (J=6 Hz) at 4.16 and 4.51 ppm, which are ascribable respectively to the anomeric protons of the α-L-arabinopyranose (in Cl conformation)[10] and the β-D-xylopyranose (in Cl conformation) units by comparing with the spectrum of I permethylate. The β-configuration of the D-xylose unit was supported by the molecular rotation difference between VI and I.

Now it follows that VI is represented as hederagenin 3-O-β-D-xylopyranosyl-(1→3)-α-L-arabinopyranoside.

P_D (IX), mp 256—259° (decomp.), $[\alpha]_D$ +15°, (peracetate, mp 146—150°, $[\alpha]_D$ +37°) is composed of hederagenin, arabinose and rhamnose, and was hydrolyzed with acid under a

6) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).
7) *a*) T. Komori, Y. Ida, Y. Muto (née Inatsu), K. Miyahara, T. Nohara, and T. Kawasaki, *Biomedical Mass Spectrometry*, **2**, 65 (1975); *b*) H. Budzikiewicz, C. Djerassi, and D.H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," Vol. II, Holden-Day, Inc., San Francisco, 1964, p. 203.
8) *a*) H. Okabe, N. Koshito, K. Tanaka, and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **19**, 2394 (1971); *b*) M. Nishimura, R. Higuchi, K. Miyahara, and T. Kawasaki, Meeting of Kyushu Branch, Pharmaceutical Society of Japan, Fukuoka, February 1971.
9) W. Klyne, *Biochem. J.*, **47**, xli (1950).
10) K. Miyahara and T. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **17**, 1369 (1969).

NII-Electronic Library Service

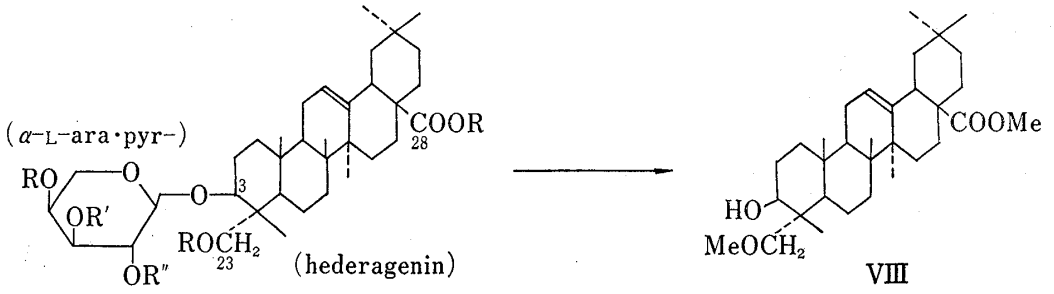
mild condition to give I as the sole prosapogenin. IX permethylate (X), mp 110—112°, $[\alpha]_D$ +37°, exhibits on its mass spectrum the molecular ion at *m/e* 848 and the fragment ion due to the terminal permethylated methylpentose unit at *m/e* 189. On methanolysis X gave VIII and methyl pyranosides of 2,3,4-tri-O-methyl-α-L-rhamnose and 3,4-di-O-methyl-β-L-arabinose. The molecular rotation differrence between IX and I indicates the α-linkage of the rhamnose residue to the arabinose unit of I.

Therefore IX is considered to be hederagenin 3-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.

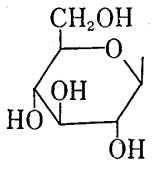
P_E (XI), mp 263—266° (decomp.), $[\alpha]_D$ +22°, (peracetate, mp 183—186°, $[\alpha]_D$ +20°), was hydrolyzed with acid to give oleanolic acid, arabinose and glucose, and in a milder condition to afford V along with XI and oleanolic acid. XI permethylate (XII), mp 144—146°, $[\alpha]_D$ +16°, shows on the mass spectrum the peaks at *m/e* 848 (M^+) and 219 (the terminal permethylated hexose unit[7]), and was methanolyzed to yield methyl oleanolate and methyl pyranosides of 2,3,4,6-tetra-O-methyl-α-D-glucose and 3,4-di-O-methyl-β-L-arabinose. The molecular rotation difference between XI and V indicates the β-linkage of the D-glucose unit to V, and the NMR spectrum of XII also supported the β-configuration.

Accordingly XI is oleanolic acid 3-O-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranoside.

P_G (XIII), mp 218—220° (decomp.), $[\alpha]_D$ +11°, (peracetate, mp 155—158°, $[\alpha]_D$ +26°), consisting of hederagenin, arabinose, xylose and rhamnose, was hydrolyzed with acid under a mild condition to provide two prosapogenins identical with I and IX. XIII permethylate (XIV), mp 118—120°, $[\alpha]_D$ +8°, showing on the mass spectrum the molecular ion peak (*m/e* 1008) and the peak of the terminal permethylated pentose residue (*m/e* 175), gave on methanolysis VIII and methyl pyranosides of 2,4-di-O-methyl-α-L-rhamnose, 2,3,4-tri-O-methyl-α-D-xylose and 3,4-di-O-methyl-β-L-arabinose. The mode of linkage of the terminal D-xylose unit to the L-rhamnose residue of IX was regarded as β on the basis of the molecular rotation

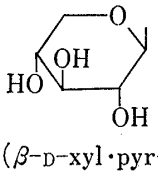


I : R=R'=R''=H
II : R=R'=H, R''=



(β-D-glc·pyr-)

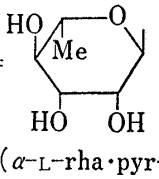
VI : R=R''=H, R'=



(β-D-xyl·pyr-)

VII : R=R''=Me,
R'=2,3,4-tri-O-Me-β-D-xyl·pyr-

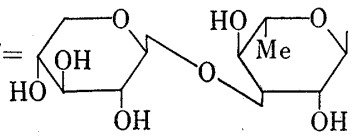
IX : R=R'=H, R''=



(α-L-rha·pyr-)

X : R=R'=Me,
R''=2,3,4-tri-O-Me-α-L-rha·pyr-

XIII : R=R'=H, R''=

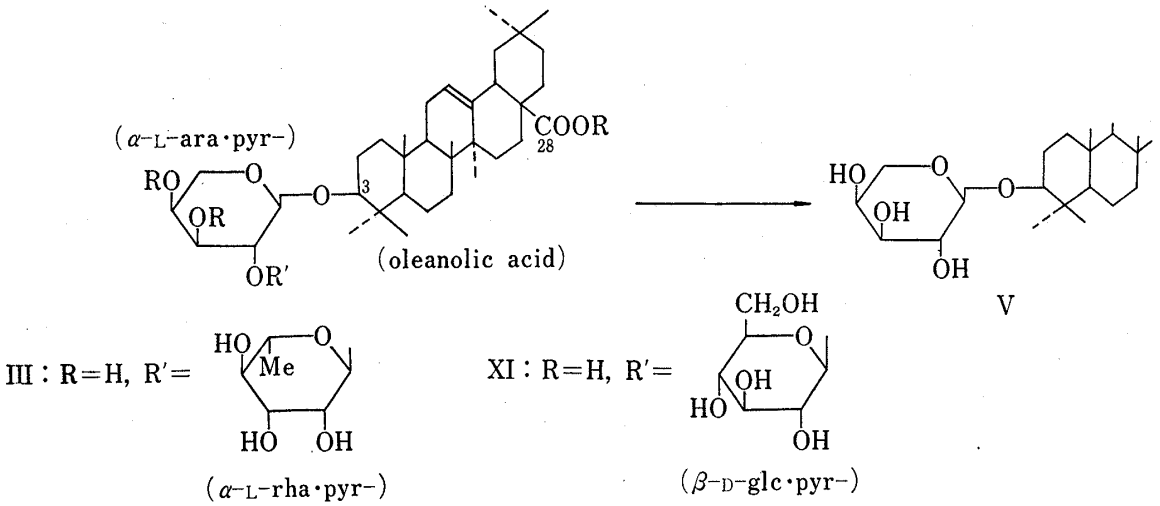


(β-D-xyl·pyr-(1→3)-α-L-rha·pyr-)

XIV : R=R'=Me,
R''=2,3,4-tri-O-Me-β-D-xyl·pyr-
(1→3)-2,4-di-O-Me-α-L-rha·pyr-

Chart 2

NII-Electronic Library Service



IV : R=Me,
R'=2,3,4-tri-O-Me-α-L-rha·pyr-

XII : R=Me,
R'=2,3,4,6-tetra-O-Me-β-D-glc·pyr-

Chart 3

difference between XIII and IX and the NMR spectrum of XIV in comparison with that of X.

In consequence XIII is defined as hederagenin 3-O-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.

P_{J2-M} (XV), mp 109—111°, $[\alpha]_D$ −7°, was methanolyzed to yield 23-O-methyl-hederagenin (XVI) and methyl pyranosides of 2,3,4-tri-O-methyl-arabinose, 2,3,4-tri-O-methyl-rhamnose, 2,3,4- and 2,3,6-tri-O-methyl-glucoses. Reduction of XV with lithium aluminum hydride yielded a colorless syrup (XVII), $[\alpha]_D$ −32°, and colorless prisms (XVIII), mp 111—113°, $[\alpha]_D$ +58°. XVII shows on the mass spectrum the molecular ion at *m/e* 616 and the fragment ion due to the terminal permethylated methylpentose residue at *m/e* 189. On methanolysis XVII gave methyl pyranosides of 2,3,6-tri-O-methyl-α-D-glucose and 2,3,4-tri-O-methyl-rhamnose and a syrup, which was identical with 2,3,4-tri-O-methyl-D-sorbitol. From these data XVII was presumed to be the nonamethyl ether of rhamnopyranosyl-(1→4)-D-glucopyranosyl-(1→6)-D-sorbitol and was identified with the authentic sample of 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 obtained by lithium aluminum hydride reduction of the permethylate of seed saponin G.[2b] Accordingly XV should have the trisaccharide corresponding to XVII combined with the 28-carboxyl group of XVI. Since P_J, a mixture of P_{J1}-P_{J3}, was hydrolyzed with alkali to give I together with III and Y, P_{J2} is regarded as a 28-O-glycoside of I and hence another reduction product (XVIII) of XV is considered to be the 28-ol corresponding to I permethylate. XVIII was actually identified as such by examination of the methanolysis products and by direct comparisons with the authentic sample.[2a] The mode of linkage of the D-glucose unit to the 28-carboxyl group was regarded as β on the basis of the NMR spectrum of XV in comparison with that of the permethylate of seed saponin G.[2b]

Consequently XV is defined as the tridecamethyl ether of 3-O-α-L-arabinopyranosyl-hederagenin 28-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (XV'), and the originally existing saponin P_{J2} is presumed to be XV'.

P_{J3-M} (XIX), mp 108—110°, $[\alpha]_D$ −16°, gave on methanolysis oleanolic acid and methyl pyranosides of 2,3,4-tri-O-methyl-rhamnose, 3,4-di-O-methyl-arabinose, 2,3,4- and 2,3,6-tri-O-methyl-glucoses. Lithium aluminum hydride reduction of XIX provided XVII as from XV and colorless prisms (XX), mp 120—122°, $[\alpha]_D$ +21°. Taking into account the fact that P_J was hydrolyzed with alkali to give III together with I and Y, XX was presumed to be the 28-ol corresponding to III permethylate (IV) and the presumption was evidenced by comparisons with the authentic sample prepared by lithium aluminumhydride reduction of IV. The

NII-Electronic Library Service

NMR spectrum of XIX shows a one-proton doublet (J=8 Hz) at 5.35 ppm. By comparisons with the spectra of XV and IV, it is ascribable to the anomeric proton of the D-glucopyranose residue (in Cl conformation) combined in β configuration with the 28-carboxyl group of the aglycone.

Now it follows that XIX is the tetradecamethyl ether of 3-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-oleanolic acid 28-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (XIX'). The originally existing saponin P_{J3} is supposed to be XIX'.

P_K (XXI), mp 212—215° (decomp.), $[\alpha]_D$ −18°, (peracetate, mp 148—150°, $[\alpha]_D$ +3°), is composed of hederagenin, L-arabinose, D-glucose and L-rhamnose, and was hydrolyzed with alkali to yield IX. XXI permethylate (XXII), mp 119—122°, $[\alpha]_D$ −8°, was methanolyzed to give XVI and the same four methylated monosaccharides as from XIX. Lithium aluminum hydride reduction of XXII provided XVII, as from XIX and XV, and colorless needles

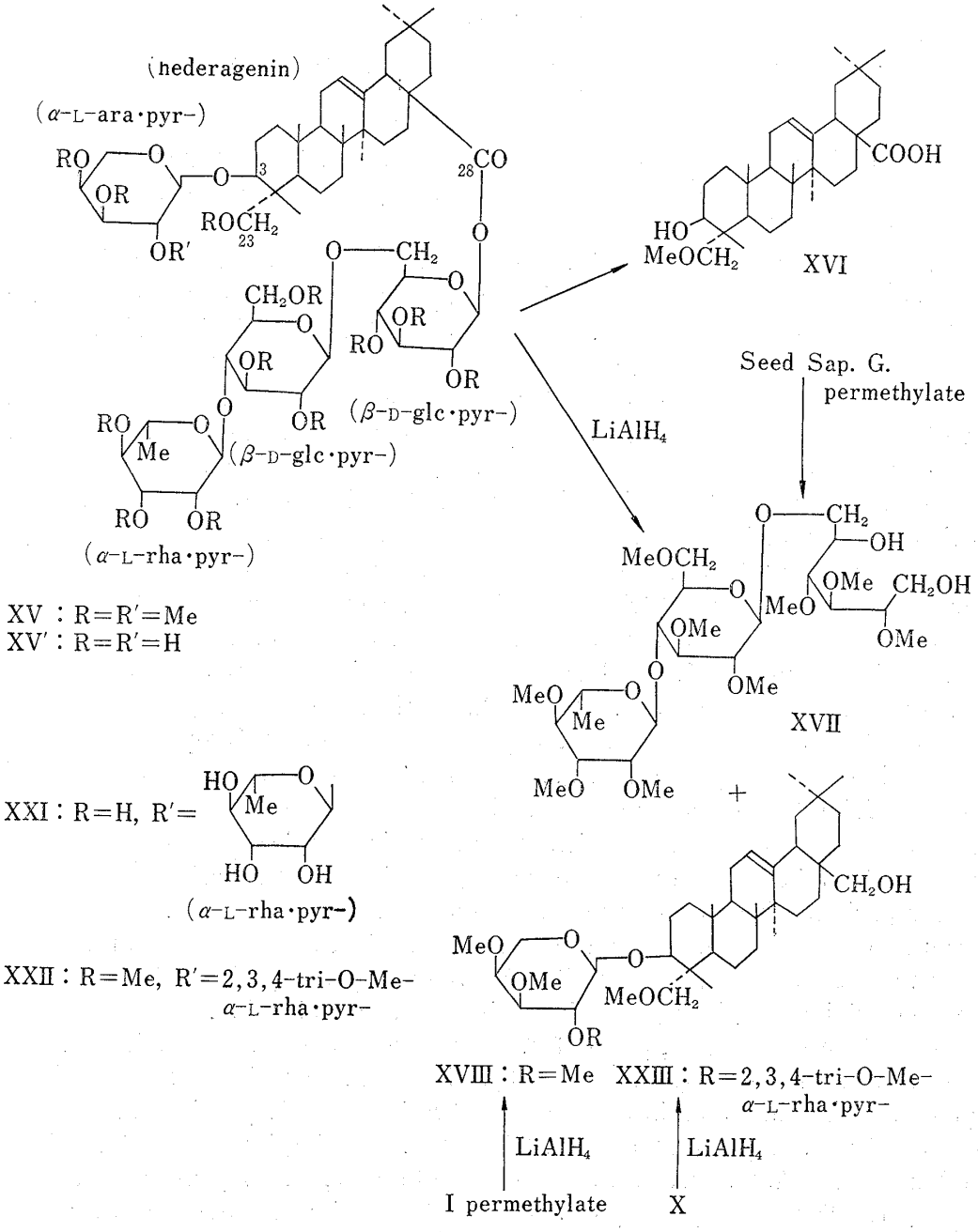


Chart 4

NII-Electronic Library Service

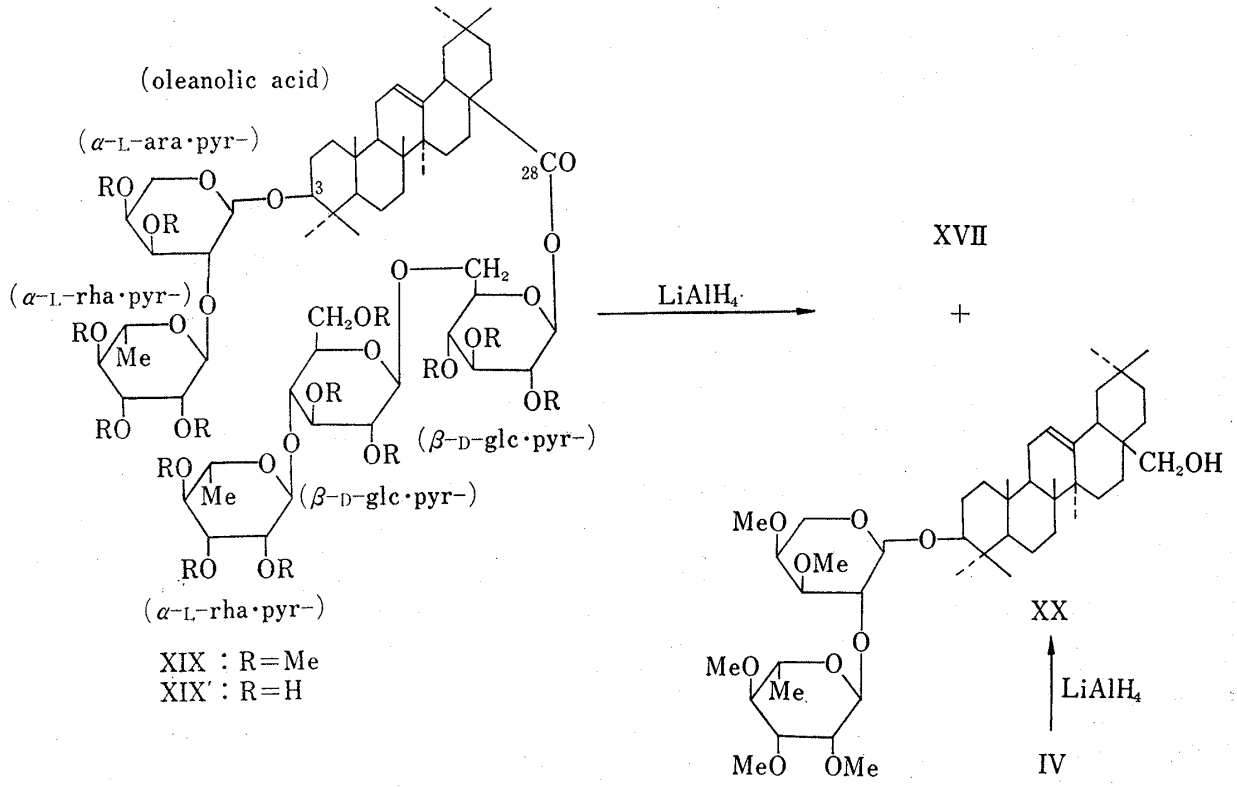


Chart 5

(XXIII), mp 124—127°, $[\alpha]_D$ +36°, which was identified with the 28-ol corresponding to and prepared from X. The NMR spectrum of XXII shows a one-proton doublet (J=8 Hz) at 5.38 ppm, and, in analogy to the cases of XV and XIX, it is assigned to the anomeric proton of the β-D-glucopyranose (in Cl conformation) linked to the 28-carboxyl group of the aglycone.

Therefore XXI is 3-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-hederagenin 28-O-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside.

P_A (I), P_F (II), P_B (III), P_D (IX), P_G (XIII), P_K (XXI), and P_{J3} (XIX′) are respectively identical with akebia seed saponin A[2a] (=saponin A of *Caulophyllum robostrum*,[11] leontoside A,[12] scabioside A,[13] koelreuteria saponin A,[14] akeboside St_b[15]), and C[2a] (=saponin B of *Caulophyllum robostrum*,[11] akeboside St_d[15]), β-hederin,[16] kalopanax saponin A[17] (=α-hederin,[16] sapindoside A,[18] akeboside St_c[15]), sapindoside B,[18] kalopanax saponin B[19] (=hederasaponin C,[16] akeboside St_h[15]), and hederasaponin B,[16] but P_C (VI), P_E (XI), and P_{J2} (XV′) are new members of the group of hederagenin and oleanolic acid oligoglycosides.

It is of interest to see that they all have the common unit, hederagenin or oleanolic acid 3-O-α-L-arabinopyranoside, and that the 3,28-O-bisglycosides, P_{J2} (XV′), P_{J3} (XIX′) and

11) T. Murakami, M. Nagasawa, S. Urayama, and N. Satake, *Yakugaku Zasshi*, **88**, 321 (1968).
12) L.G. Mzhelskaya and N.K. Abubakirov, *Khim. Prir. Soedin*, **3**, 101 (1967) [*C.A.*, **67**, 100383 (1967)].
13) V.G. Bukharov, V.V. Karlin, and T.N. Sidorovich, *Khim. Prir. Soedin*, **6**, 69 (1970) [*C.A.*, **73**, 99162 (1970)].
14) V. Ya. Chirva, P.K. Kintya, and V.A. Sosnovskii, *Khim. Prir. Soedin*, **6**, 328 (1970) [*C.A.*, **73**, 110063 (1970)].
15) Y. Kumekawa, H. Itokawa, and M. Fujita, *Chem. Pharm. Bull.* (Tokyo), **22**, 2294 (1974).
16) R. Tschesche, W. Schmidt, and G. Wulff, *Z. Naturforsch*, **20b**, 708 (1965) [*C.A.*, **63**, 18178 (1965)].
17) A. Ya. Khorlin, A.G. Venyaminova, and N.K. Kochetkov, *Dokl. Akad. Nauk SSSR*, **155**, 619 (1964) [*C.A.*, **60**, 15964 (1964)].
18) V. Ya. Chirva, P.K. Kintya, V.A. Sosnovskii, P.E. Krievenshuk, and N.V. Zykova, *Khim. Prir. Soedin*, **6**, 218 (1970) [*C.A.*, **73**, 77544 (1970)].
19) A. Ya. Khorlin, A.G. Venyaminova, and N.K. Kochetkov, *Izv. Akad. Nauk SSSR, Ser. Khim.*, **1966**, 1588 [*C.A.*, **66**, 65803 (1967)].

NII-Electronic Library Service

P_K (XXI), are corresponding to the 3-O-glycosides, P_A (I), P_B (III) and P_D (IX), respectively, and have the same trisaccharide, 4-O-α-L-rhamnopyranosyl-β-gentiobiose, combined with the 28-carboxyl group of the aglycone.

Experimental

All melting points were determined on a micro melting point apparatus (an air-bath type) and are uncorrected. Optical rotations were measured at 15—25° with a JASCO DIP-SL automatic polarimeter. IR spectra were obtained with a JASCO IR-G spectrometer and NMR spectra were taken at 100 MHz with a JEOL-C-100H spectrometer in $CDCl_3$ solution and chemical shifts are given in δ (ppm) scale with tetramethyl silane as the internal standard. Mass spectra were recorded on a JMS-O1SG mass spectrometer with an accelerating potential of 4.5—4.8 kV, an ionizing potential of 30 or 35 eV. GLC was run on a Yanagimoto GSG 550-F with flame ionization detector using glass column (1.2 m × 4 mmϕ) packed with 5% 1,4-butanediol succinate on Shimalite W (60—80 mesh); column temperature 152—155°, H_2 60 ml/min, N_2 80 ml/min. The authentic samples of methylated sugars for comparisons were prepared in this laboratory.[8] PPC was conducted on Tôyô Roshi No. 50 by double ascending method using the upper layer of *n*-BuOH–pyridine–water (6: 2: 3)+pyridine (1) as the solvent and aniline hydrogen phthalate as the spray reagent. TLC was performed on Kieselgel G (Merck) by using the following solvent systems: a) $CHCl_3$–MeOH–water (7: 3: 1) (bottom layer), b) $CHCl_3$–MeOH–water (25: 17: 3), c) AcOEt–MeOH (50: 1), d) hexane–AcOEt (1: 3), e) benzene–acetone (4: 1). Detection was made by spraying anisaldehyde reagent[20] followed by heating. Column chromatography was carried out with Kieselgel (0.05—0.5 mm) (Merck), silicic acid (100 mesh) (Mallinckrodt) and Sephadex LH-20 (25—100 μ) (Pharmacia Fine Chemicals). The ratios of solvents and reagents in mixture are given in v/v. Methylation of the glycosides was conducted by the Hakomori method[6] unless otherwise indicated.

Isolation of Triterpenoid Constituents——The fresh pericarps collected in the suburbs of Fukuoka city during October were extracted and the extractives were fractionated as shown in Chart 1. Each fraction was examined by TLC using solvent b. P_J (530 mg), showing single spot on TLC using two kinds of solvent systems (a, b), was heated with 2N H_2SO_4 (25 ml) for 2 hr. The precipitates (200 mg) formed on cooling were collected and chromatographed over silica gel (eluent, $CHCl_3$–MeOH–water (10: 1: 0.5)) to give three compounds: colorless needles (28 mg) (from MeOH), mp 300—302°, identified with oleanolic acid;[21] colorless prisms (30 mg) (from EtOH), mp 322—325°, identified with hederagenin;[22] colorless needles (50 mg) (from acetone), mp 315—317° (methylester, mp 220—222°), identified with Compound Y by direct comparisons (TLC, IR, NMR, mixed fusion). P_J (150 mg) was heated with 1% KOH in 30% EtOH (10 ml) for 1 hr. The hydrolysate was diluted with water, neutralized with dil.HCl and extracted with AcOEt–*n*-BuOH (2: 1). The organic layer was washed with water, evaporated *in vacuo* and the residue was examined by TLC (solvent a): *Rf* 0.53 (Compound Y), 0.42 (P_A), 0.35 (P_B). The peracetate of P_J prepared on heating with Ac_2O–pyridine (1: 1) showed single spot on TLC using two kinds of solvent systems (d, e), while the permethylate prepared by the Kuhn method[5] showed three spots (solv. d, e). The permethylate was fractionated as shown in Chart 1.

P_A (I)——Colorless needles, mp 228—230° (decomp.), $[\alpha]_D$ +48° (c=1.16, MeOH). I (30 mg) was hydrolyzed on boiling with 2N H_2SO_4 in 50% EtOH (2 ml) for 2 hr. After cooling the reaction mixture was diluted with water and the precipitates were collected by filtration. The precipitates were crystallized from EtOH to give colorless prisms, mp 325—327° (acetate, colorless needles, mp 162—163°, $[\alpha]_D$+79° (c=2.30, $CHCl_3$), which were identified with hederagenin by direct comparisons (IR, NMR, mixed fusion). The filtrate of the hydrolysate was passed through a column of Amberlite A-400, evaporated *in vacuo* and the residue was examined by PPC to show the presence of arabinose. I was acetylated on boiling with Ac_2O–pyridine (1: 1) to give the peracetate as colorless needles (from MeOH), mp 205—207°, $[\alpha]_D$ +67° (c=0.99, $CHCl_3$), which was further methylated in $CHCl_3$ with CH_2N_2–ether to provide the peracetate methylester as colorless needles (from MeOH), mp 238—239°, $[\alpha]_D$ +76° (c=1.00, $CHCl_3$). Methylation of I gave the permethylate as colorless needles (from MeOH), mp 181—182°, $[\alpha]_D$ +44° (c=1.25, $CHCl_3$). NMR: 4.16 (1H, doublet, J=6 Hz, C_1–H of arabinose residue). I and its peracetate methylester were identified with seed saponin A and its peracetate methylester,[2a] respectively, by direct comparisons.

P_F (II)——Colorless needles, mp 248—250° (decomp.), $[\alpha]_D$ +41° (c=1.48, pyridine). Hydrolyzed with acid in the same way as in I to give hederagenin, arabinose and glucose. II and its peracetate, mp 172—175°, $[\alpha]_D$ +38° (c=0.77, $CHCl_3$), were respectively identified with seed saponin C[2a] and its peracetate, mp 172—175°, by direct comparisons.

20) E. Stahl, "Dünnschicht Chromatographie," Springer-Verlag, Berlin, 1962, p. 498.
21) The authentic sample of oleanolic acid, mp 301—302°, 300—302° (taken in this laboratory), was kindly provided by Prof. T. Takemoto of Tohoku University.
22) The melting point (315—317°) of hederagenin obtained from seed saponins and reported in the previous paper[2a] was raised up to 326—329°.

NII-Electronic Library Service

P_B (III)——Colorless needles, mp 222—225° (decomp.), $[\alpha]_D$ +9° (c=1.83, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1690 (COOH). *Anal.* Calcd. for $C_{41}H_{66}O_{11}\cdot 2H_2O$: C, 63.87; H, 9.15. Found: C, 64.30; H, 9.04. Acetylation of III as I provided the peracetate as colorless needles (from MeOH), mp 170—172°, $[\alpha]_D$ +22° (c=2.96, $CHCl_3$). *Anal.* Calcd. for $C_{51}H_{76}O_{16}$: C, 64.81; H, 8.11. Found: C, 64.90; H, 8.06.

Permethylate (IV) of III——III (80 mg) was methylated and the product was passed through a silica gel column (eluent, hexane–AcOEt (1: 1)) and crystallized from hexane to give IV as colorless plates (47 mg), mp 210—212°, $[\alpha]_D$ +14° (c=0.90, $CHCl_3$). IR: no OH. Mass Spectrum *m/e*: 818 ($C_{47}H_{78}O_{11}^+$, M^+), 189 ($C_{10}H_{17}O_4^+$, terminal permethylated methylpentose residue[7a]). NMR: 4.42 (1H, doublet, J=6 Hz, C_1–H of arabinose residue), 5.14 (1H, doublet, J=1.5 Hz, C_1–H of rhamnose residue). *Anal.* Calcd. for $C_{47}H_{78}O_{11}$: C, 68.91; H, 9.60. Found: C, 68.55; H, 9.56.

Hydrolysis of III with Acid——1) III (40 mg) was hydrolyzed and worked up in the same way as in I to give an aglycone together with arabinose and rhamnose (identified by PPC and TLC (solvent b)). The aglycone was crystallized from EtOH to provide colorless needles (10 mg), mp 300—302°, (acetate, mp 260—262°; acetate methylester, mp 217—218°), which were identified with oleanolic acid (TLC, IR, mixed fusion). 2) III (440 mg) was hydrolyzed on boiling with 0.4N H_2SO_4 in 50% EtOH (20 ml) for 1 hr. The hydrolysate was neutralized with dil.KOH, concentrated *in vacuo*, diluted with water and extracted with AcOEt–*n*-BuOH (2: 1). The organic layer, containing oleanolic acid (*Rf* 0.83), III (*Rf* 0.38) and a new compound (*Rf* 0.57) as evidenced on TLC (solv. a), was evaporated and the residue was chromatographed over silica gel (eluent, $CHCl_3$–MeOH–water (10: 1: 0.1)).

Prosapogenin (V) of III——The fraction containing only the compound of *Rf* 0.57 in the above chromatography was crystallized from MeOH to give V as colorless needles (50 mg), mp 255—259° (decomp.), $[\alpha]_D$ +49° (c=0.78, MeOH). $\Delta[M]_D$: III—V, −226°; V-oleanolic acid, −51°. $[M]_D$ of Me L-rhamnopyranoside[23]: α, −109°; β, +169°, $[M]_D$ of Me L-arabinopyranoside[24]: α, +28°; β, +403°. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1690 (COOH). *Anal.* Calcd. for $C_{35}H_{56}O_7\cdot 2H_2O$: C, 67.27; H, 9.68. Found: C, 67.10; H, 9.52. Hydrolysis of V as I gave oleanolic acid (TLC) and arabinose (PPC, TLC). Methylation of V (15 mg) yielded the permethylate as colorless prisms (8 mg) (from hexane), mp 180—182°, $[\alpha]_D$ +46° (c=0.68, $CHCl_3$). IR: no OH. NMR: 4.24 (1H, doublet, J=6 Hz, C_1–H of arabinose residue). Acetylation of V afforded the peracetate as colorless prisms (from MeOH), mp 155—160°, $[\alpha]_D$ +42° (c=0.51, $CHCl_3$). NMR: 4.40 (1H, doublet, J=6 Hz, C_1–H of arabinose residue).

Methanolysis of IV——IV (200 mg) was boiled with 8% HCl in MeOH (6 ml) for 2 hr. The reaction mixture was neutralized with Ag_2CO_3, the precipitates were filtered off and the filtrate was evaporated. The residue was crystallized from MeOH to give colorless needles (40 mg), mp 197—198°, which were identified with an authentic sample of methyl oleanolate[25] by comparisons of their *Rf* values on TLC and IR spectra and by a mixed fusion. The mother liquor of crystallization was evaporated and the residue, showing two spots on TLC and two peaks on GLC, was chromatographed over silica gel (eluent, AcOEt–MeOH (25: 1)) to give two kinds of colorless syrups. One (20 mg), *Rf* 0.61 (solv. c), t_R 2.3 min, $[\alpha]_D$ −60° (c=1.85, EtOH) (Me 2,3,4-tri-O-Me-α-L-rhamnopyranoside, lit.[8a] $[\alpha]_D$ −45° (MeOH)), was boiled with 1N HCl for 2 hr, and the hydrolysate was neutralized with Amberlite A-400, concentrated *in vacuo* and passed through a Sephadex LH-20 column (eluent, MeOH) to give a colorless syrup, $[\alpha]_D$ +24° (c=1.30, water) (2,3,4-tri-O-Me-L-rhamnose, lit.[26] $[\alpha]_D$ +26° (water)). Another syrup (22 mg), *Rf* 0.25. t_R 9.6 min, $[\alpha]_D$ +196° (c=2.10, $CHCl_3$) (Me 3,4-di-O-Me-β-L-arabinopyranoside, lit.[27] $[\alpha]_D$ +211° ($CHCl_3$); 2,4-di-O-Me-, *Rf* 0.25, t_R 10.6 min; 2,3-di-O-Me-, *Rf* 0.29, t_R 8.8 min), was hydrolyzed with acid and worked up as above to yield a colorless syrup, $[\alpha]_D$ +120° (c=1.90, water) (3,4-di-O-Me-L-arabinose, lit.[27] $[\alpha]_D$ +116° (water)).

P_C (VI)——Colorless needles, mp 236—240° (decomp.), $[\alpha]_D$ +45° (c=2.42, MeOH). $\Delta[M]_D$: VI—I, +34°. $[M]_D$ of Me D-xylopyranoside[24]: α, +253°; β, −108°. JR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1690 (COOH). *Anal.* Calcd. for $C_{40}H_{64}O_{12}\cdot H_2O$: C, 63.63; H, 8.81. Found; C, 63.18; H, 8.83. Peracetate, colorless needles (from MeOH), mp 179—182°, $[\alpha]_D$ +35° (c=3.18, $CHCl_3$). *Anal.* Calcd. for $C_{55}H_{80}O_{20}$: C, 62.65; H, 7.60. Found: C, 61.75; H, 7.80.

Hydrolysis of VI with Acid——VI (50 mg) was hydrolyzed with 2N H_2SO_4 in 50% EtOH in the same way as in III to give hederagenin (24 mg) and a sugar mixture, which was shown to consist of xylose and arabinose by TLC and PPC. VI was hydrolyzed with 0.4N H_2SO_4 in 50% EtOH and the hydrolysate was treated in the same way as in III. The product was chromatographed over silica gel (eluent, $CHCl_3$–MeOH–water (9: 1: 0.1)) to provide hederagenin, unchanged VI and a prosapogenin, colorless needles (from dil. EtOH), mp 223—226° (decomp.), $[\alpha]_D$ +52° (c=1.85, MeOH), which was identified with I by direct comparisons.

Permethylate (VII) of VI——VI (200 mg) was methylated and the product was precipitated from hexane–$CHCl_3$ to give VII as a white powder (150 mg), mp 112—115°, $[\alpha]_D$ +28° (c=3.66, $CHCl_3$). IR: no OH.

23) E. Fischer, M. Bergmann, and A. Rabe, *Chem. Ber.*, **53**, 2364 (1920).
24) C.S. Hudson, *J. Am. Chem. Soc.*, **47**, 265 (1925).
25) Prepared from oleanolic acid by methylation with CH_2N_2–ether, mp 198—200°.
26) T. Takemoto and K. Kometani, *Ann.*, **685**, 237, (1965).
27) J. Honeyman, *J. Chem. Soc.*, **1946**, 990.

NII-Electronic Library Service

Mass Spectrum *m/e*: 834 ($C_{47}H_{78}O_{12}^+$, M+), 175 ($C_8H_{15}O_4^+$, terminal permethylated pentose residue[7a]). NMR: 4.16 (1H, doublet, J=6 Hz, C_1–H of arabinose residue), 4.51 (1H, doublet, J=6 Hz, C_1–H of xylose residue). *Anal.* Calcd. for $C_{47}H_{78}O_{12}$: C, 67.67; H, 9.42. Found: C, 67.77; H, 9.41.

Methanolysis of VII——VII (150 mg) was boiled with 8% HCl in MeOH (7 ml) for 2 hr and the reaction mixture was worked up in the same way as in IV. The aglycone was crystallized from AcOEt to give colorless prisms (50 mg), mp 189—190°, which were identified with 23-O-methyl-hederagenin methylester[2a] (VIII) by direct comparisons. The sugar portion was chromatographed over silica gel (eluent, hexane–AcOEt (2: 1)) to give two kinds of colorless syrups: *Rf* 0.57 (solv. c), t_R 2.9 min, $[\alpha]_D$ +85° (c=0.64, MeOH) (12 mg) (Me 2,3,4-tri-O-Me-α-D-xylopyranoside, lit.[28] $[\alpha]_D$ +86° (MeOH)) and *Rf* 0.25, t_R 10.5 min, $[\alpha]_D$ +205° (c=1.27, $CHCl_3$) (20 mg) (Me 2,4-di-O-Me-β-L-arabinopyranoside, lit.[29] $[\alpha]_D$ +220° ($CHCl_3$); 3,4,-di-O-Me-, *Rf* 0.25, t_R 9.5 min; 2,3-di-O-Me-, *Rf* 0.29, t_R 8.6 min).

PD (IX)——Colorless needles, mp 256—259° (decomp.), $[\alpha]_D$ +15° (c=1.35, EtOH). $\Delta[M]_D$: IX-I, −160°. $[M]_D$ of Me L-rhamnopyranoside: α, −109°; β, +169°. IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1700 (COOH). *Anal.* Calcd. for $C_{41}H_{66}O_{12}\cdot 2H_2O$: C, 62.57; H, 8.97. Found: C, 63.00; H, 8.84. Peracetate, colorless needles (from hexane), mp 146—150°, $[\alpha]_D$ +37° (c=1.10, $CHCl_3$). *Anal.* Calcd. for $C_{53}H_{78}O_{18}$: C, 63.45; H, 7.84. Found: C, 63.01; H, 8.09.

Hydrolysis of IX with Acid——IX (50 mg) was hydrolyzed as VI with 2N H_2SO_4 in 50% EtOH to give hederagenin (23 mg), rhamnose and arabinose, while with 0.4N H_2SO_4 in 50% EtOH to provide I along with hederagenin and IX.

Permethylate (X) of IX——IX (700 mg) was methylated to give X as colorless needles (500 mg) (from hexane), mp 110—112°, $[\alpha]_D$ +37° (c=3.75, $CHCl_3$). IR: no OH. Mass Spectrum *m/e*: 848 ($C_{48}H_{80}O_{12}^+$, M+), 189 ($C_9H_{17}O_4^+$, terminal permethylated methylpentose residue). NMR: 4.27 (1H, doublet, J=6 Hz, C_1–H of arabinose residue), 5.14 (1H, doublet, J=1.5 Hz, C_1–H of rhamnose residue). *Anal.* Calcd. for $C_{48}H_{80}O_{12}$: C, 67.89; H, 9.50. Found: C, 67.56; H, 9.60.

Methanolysis of X——X (500 mg) was boiled with 8% HCl in MeOH (25 ml) for 2 hr. The reaction mixture was treated as in IV. The aglycone was crystallized from AcOEt to give colorless prisms (140 mg), mp 192—193°, identical (TLC, IR, NMR, mixed fusion) with VIII. The sugar portion, showing two spots on TLC (solv. c) and two peaks on GLC, was subjected to silica gel chromatography (eluent, AcOEt–MeOH (25: 1)) to give two kinds of colorless syrups: *Rf* 0.61, t_R 2.3 min, $[\alpha]_D$ −59° (c=4.91, EtOH) (40 mg) (Me 2,3,4-tri-O-Me-α-L-rhamnopyranoside) and *Rf* 0.25, t_R 9.6 min, $[\alpha]_D$ +226° (c=1.40, $CHCl_3$) (50 mg) (Me 3,4-di-O-Me-β-L-arabinopyranoside).

PE (XI)——Colorless needles, mp 263—266° (decomp.), $[\alpha]_D$ +22° (c=1.33, MeOH). $\Delta[M]_D$: XI-V, −126°. $[M]_D$ of Me D-glucopyranoside[30]: α, +307°; β, −62°. IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1695 (COOH). *Anal.* Calcd. for $C_{41}H_{66}O_{12}\cdot 2H_2O$: C, 61.17; H, 9.02. Found: C, 60.86; H, 8.85. Peracetate, colorless needles (from MeOH), mp 183—186°, $[\alpha]_D$ +20° (c=3.26, $CHCl_3$). *Anal.* Calcd. for $C_{53}H_{78}O_{18}$: C, 63.45; H, 7.84. Found: C, 63.30; H, 7.73.

Hydrolysis of XI with Acid——Hydrolysis and identification of the products were conducted in the same way as in III. 1) With 2N H_2SO_4 in 50% EtOH, oleanolic acid, arabinose and glucose were provided. 2) With 0.4N H_2SO_4 in 50% EtOH, oleanolic acid, XI and V were obtained.

Permethylate (XII) of XI——XI (160 mg) was methylated to give XII as colorless needles (80 mg) (from hexane), mp 144—146°, $[\alpha]_D$ +16° (c=2.90, $CHCl_3$). IR: no OH. Mass Spectrum *m/e*: 848 ($C_{48}H_{80}O_{12}^+$, M+), 219 ($C_{10}H_{19}O_5^+$, terminal permethylated hexose residue[7]). NMR: 4.52 (1H, doublet, J=6 Hz, C_1–H of arabinose residue), 4.58 (1H, doublet, J=7 Hz, C_1–H of glucose residue). *Anal.* Calcd. for $C_{48}H_{80}O_{12}$: C, 67.89; H, 9.50. Found: C, 67.23; H, 9.43.

Methanolysis of XII——Conducted in the same way as in IV to give methyl oleanolate and a sugar mixture. The latter was chromatographed over silica gel (eluent, AcOEt–MeOH (50: 1)) to give two kinds of colorless syrups: *Rf* 0.44 (solv. c), t_R 6.7 min, $[\alpha]_D$ +128° (c=0.80, $CHCl_3$) (Me 2,3,4,6-tetra-O-Me-α-D-glucopyranoside, lit.[8a] $[\alpha]_D$ +139° ($CHCl_3$)) and *Rf* 0.25, t_R 9.6 min, $[\alpha]_D$ +208° (c=0.60, $CHCl_3$) (Me 3,4-di-O-Me-β-L-arabinopyranoside).

PG (XIII)——Colorless needles, mp 218—220° (decomp.), $[\alpha]_D$ +11° (c=5.43, MeOH). $\Delta[M]_D$: XIII-IX, −39°. $[M]_D$ of Me D-xylopyranoside: α, +253°; β, −108°. IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1690 (COOH). *Anal.* Calcd. for $C_{47}H_{74}O_{16}\cdot 4H_2O$: C, 58.36; H, 8.55. Found: C, 58.63; H, 8.43. Peracetate, a white powder (from hexane–$CHCl_3$), mp 155—158° $[\alpha]_D$ +26° (c=1.80, $CHCl_3$).

Hydrolysis of XIII with Acid——Carried out in the same way as in III. With 2N H_2SO_4 in 50% EtOH, hederagenin, rhamnose, xylose and arabinose were yielded, while with 0.4N H_2SO_4 in 50% EtOH, two prosapogenins were provided along with hederagenin and XIII as detected on TLC. The prosapogenins were isolated by chromatography of the hydrolysate over silica gel (eluent, $CHCl_3$–MeOH–water (8: 2: 0.2)) as

28) A.E. Carruthers and E.L. Hirsh, *J. Chem. Soc.*, **121**, 2299 (1922).
29) P. Kovac and R. Palovcik, *Carbohydrate Research*, **36**, 379 (1974).
30) E. Pacsu, "Method in Carbohydrate Chemistry," Vol. II, ed. by R.L. Whistler, M.L. Wolfrom, Academic Press Inc., New York and London, 1963, p. 356.

NII-Electronic Library Service

colorless needles (from dil.EtOH), mp 228—229° (decomp.) and colorless needles (from MeOH), mp 256—259° (decomp.), which were identified with I and IX, respectively, by direct comparisons.

Permethylate (XIV) of XIII——XIII (700 mg) was methylated and the product was passed through a silica gel column (eluent, hexane–AcOEt (3: 2)) and crystallized from hexane to give XIV as colorless needles (300 mg), mp 118—120°, $[\alpha]_D$ +8° (c=3.50, $CHCl_3$). IR: no OH. Mass Spectrum m/e: 1008 ($C_{55}H_{92}O_{16}^+$, M^+), 175 ($C_8H_{15}O_4^+$, terminal permethylated pentose residue). NMR: 4.30 (1H, doublet, J=6 Hz, C_1–H of arabinose residue), 4.54 (1H, doublet, J=7 Hz, C_1–H of xylose residue), 5.15 (1H, doublet, J=1.5 Hz, C_1–H of rhamnose residue). *Anal.* Calcd. for $C_{55}H_{92}O_{16}$: C, 65.44; H, 9.19. Found: C, 65.31; H, 9.00.

Methanolysis of XIV——XIV (250 mg) was methanolyzed and worked up in the same way as in VII. The aglycone (120 mg) was identified with VIII. The sugar portion was chromatographed over silica gel (eluent, hexane–AcOEt (2: 1) and AcOEt–MeOH (25: 1)) to give three kinds of colorless syrups: *Rf* 0.57 (solv. c), t_R 2.6 min, $[\alpha]_D$ +86° (c=0.59, MeOH) (6 mg) (Me 2,3,4-tri-O-Me-α-D-xylopyranoside); *Rf* 0.52, t_R 5.6 min, $[\alpha]_D$ −63° (c=1.33, MeOH) (13 mg) (Me 2,4-di-O-Me-α-L-rhamnopyranoside, lit.[8a] $[\alpha]_D$ −67° (MeOH); 3,4-di-O-Me-, *Rf* 0.54, t_R 4.9 min; 2,3-di-O-Me-, *Rf* 0.47, t_R 6.9 min); *Rf* 0.25, t_R 9.4 min, $[\alpha]_D$ +205° (c=2.36, MeOH) (23 mg) (Me 3,4-di-O-Me-β-L-arabinopyranoside; 2,4-di-O-Me-, *Rf* 0.25, t_R 10.4 min; 2,3-di-O-Me-, *Rf* 0.29, t_R 8.6 min).

$P_{J2\text{-}M}$ (XV)——A white powder, mp 109—111°, $[\alpha]_D$ −7° (c=3.92, $CHCl_3$). IR: no OH. NMR: 5.35 (1H, doublet, J=8 Hz, C_1–H of esterglycosidic glucose residue). *Anal.* Calcd. for $C_{66}H_{112}O_{22}$: C, 63.02; H, 8.98. Found: C, 62.58; H, 8.98.

Methanolysis of XV——XV (80 mg) was methanolyzed and worked up in the same way as in VII to give the aglycone as colorless needles (10 mg) (from MeOH), mp 217—219°, and a sugar mixture. The former was identified with 23-O-methyl-hederagenin (XVI) by direct comparisons with an authentic sample[2b], and the latter was shown to consist of methyl pyranosides of 2,3,4-tri-O-methyl-rhamnose 2,3,4-tri-O-methyl-arabinose, 2,3,4- and 2,3,6-tri-O-methyl-glucoses by TLC and GLC.

$LiAlH_4$ Reduction of XV——XV (50 mg) in anhydrous tetrahydrofuran (5 ml) was treated with $LiAlH_4$ (30 mg). The reaction mixture was extracted with ether and then with $CHCl_3$. The both extracts were respectively washed with water, dried over Na_2SO_4 and evaporated to dryness. The residue from the ether extracts was crystallized from hexane to give a product (XVIII) as colorless prisms, mp 111—113°, $[\alpha]_D$ +58° (c=0.93, $CHCl_3$). *Anal.* Calcd. for $C_{39}H_{66}O_7$: C, 72.40; H, 10.28. Found: C, 72.28; H, 10.25. The residue from the $CHCl_3$ extracts was placed on a Sephadex LH-20 column and eluted with MeOH to give another product (XVII) as a colorless syrup, $[\alpha]_D$ −32° (c=2.61, $CHCl_3$). Mass Spectrum m/e: 616 ($C_{27}H_{52}O_{15}^+$, M^+), 189 ($C_9H_{17}O_4^+$, terminal permethylated methylpentose residue). XVIII (40 mg) was methanolyzed with refluxing 8% HCl in MeOH (2 ml) for 2 hr to yield methyl 2,3,4-tri-O-methyl-arabinopyranoside (TLC, GLC) and the aglycone (12 mg), colorless prisms (from hexane), mp 205—208°, $[\alpha]_D$ +60° (c=1.00, $CHCl_3$), which was identified with the $LiAlH_4$ reduction product of VIII by direct comparisons. XVIII showed the same melting point (also on admixture), *Rf* values on TLC, IR and NMR spectra as those of the 28-ol corresponding to and prepared from I permethylate. XVII was methanolyzed as XVIII and the product was chromatographed over silica gel (eluent, AcOEt–MeOH (50: 1)) to give three compounds: a colorless syrup, *Rf* 0.87 (solv. a), t_R 2.3 min (Me 2,3,4-tri-O-Me-rhamnopyranoside); a colorless syrup, *Rf* 0.76, t_R 20.9 min, $[\alpha]_D$ +142° (c=1.12, MeOH) (Me 2,3,6-tri-O-Me-α-D-glucopyranoside); a colorless syrup, *Rf* 0.36 (acetate, a colorless syrup, $[\alpha]_D$ +17° (c=1.60, $CHCl_3$)), which was identical in all respects (TLC, Mass and NMR spectra (acetate)) with 2,3,4-tri-O-methyl-D-sorbitol prepared by acid hydrolysis of 2,3,4,6-tetra-O-methyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-O-methyl-D-sorbitol obtained from seed saponin D permethylate.[2b] XVII was identified with 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 by direct comparisons with the authentic sample prepared from seed saponin G permethylate.

$P_{J3\text{-}M}$ (XIX)——A white powder, mp 108—110°, $[\alpha]_D$ −16° (c=4.20, $CHCl_3$). IR: no OH. NMR: 5.35 (1H, doublet, J=8 Hz, C_1–H of esterglycosidic glucose residue). *Anal.* Calcd. for $C_{73}H_{124}O_{25}$: C, 62.54; H, 8.92. Found: C, 62.24; H, 8.72.

Methanolysis of XIX——XIX (40 mg) was methanolyzed and worked up in the same way as in VII to give oleanolic acid (12 mg) and a mixture of methyl pyranosides of 2,3,4-tri-O-methyl-rhamnose, 3,4-di-O-methyl-arabinose, 2,3,4- and 2,3,6-tri O-methyl-glucoses as identified by TLC and GLC.

$LiAlH_4$ Reduction of XIX——Carried out in the same way as in XV to provide a colorless syrup, $[\alpha]_D$ −37° (c=2.70, $CHCl_3$), and colorless prisms (XX) (from hexane), mp 120—122°, $[\alpha]_D$ +21° (c=1.42, $CHCl_3$). The syrup was identified with XVII obtained from XV by comparisons of their *Rf* values on TLC, NMR and mass spectra. XX was methanolyzed to yield methyl pyranosides of 2,3,4-tri-O-methyl-rhamnose and 3,4-di-O-methyl arabinose together with colorless needles (from MeOH), mp 229—230°, $[\alpha]_D$ +88° (c=0.72, $CHCl_3$), which were identified with erythrodiol by direct comparisons with the authentic sample prepared from methyl oleanolate. XX was identical in every respect (TLC, IR, NMR, mixed fusion) with the 28-ol corresponding to and prepared from IV.

P_K (XXI)——A white powder, mp 212—215° (decomp.), $[\alpha]_D$ − 18° (c=2.76, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3350 (OH), 1740 (COOR). *Anal.* Calcd. for $C_{59}H_{96}O_{26}\cdot 3H_2O$: C, 55.56; H, 8.06. Found: C, 55.12; H, 8.20. Peracetate, a white powder (from $CHCl_3$–hexane), mp 148—150°, $[\alpha]_D$ +3° (c=1.88, $CHCl_3$). IR: no OH.

NII-Electronic Library Service

Hydrolysis of XXI——1) XXI (400 mg) in 2N H_2SO_4 (20 ml) was heated on a water-bath for 2 hr and worked up as usual. The aglycone (160 mg) was identified with hederagenin and the sugar portion was found to consist of arabinose, rhamnose, glucose and gentiobiose (trace) (TLC and PPC). The sugar mixture was chromatographed first over silica gel (eluent, $CHCl_3$–MeOH–water (7: 3: 0.5)) and then over Sephadex LH-20 (eluent, MeOH) to give three kinds of sugars: a colorless syrup (80 mg), *Rf* 0.33 (solv. b), $[\alpha]_D$ +10° →+7° (24 hr) (c=7.19, water) (L-rhamnose); colorless needles (20 mg) (from MeOH), *Rf* 0.25, mp 143—145°, $[\alpha]_D$ +125°→+101° (24 hr) (c=1.58, water) (L-arabinose); a colorless syrup (50 mg), *Rf* 0.21, $[\alpha]_D$ +44° (24 hr) (c=4.64, water) (D-glucose).

2) XXI (300 mg) was heated on a water-bath with 1% KOH in 30% EtOH (20 ml) for 1 hr. The reaction mixture was diluted with water, neutralized with dil.HCl and extracted with AcOEt-*n*-BuOH (2: 1). The organic layer was washed with water and evaporated *in vacuo* to dryness. The residue was passed through a Sephadex LH-20 column (eluent, MeOH) and crystallized from MeOH to afford colorless needles (40 mg), mp 256—259° (decomp.), $[\alpha]_D$ +17° (c=1.66, EtOH), which were identified with IX by direct comparisons.

Permethylate (XXII) of XXI——XXI (1.2 g) was methylated by the Kuhn method and the product was purified by passing through a silica gel column (eluent, benzene–acetone (4: 1)) and subsequent crystallization from hexane to give XXII as a white crystalline powder (430 mg), mp 119—122°, $[\alpha]_D$ −8° (c=1.56, $CHCl_3$). IR: no OH. NMR: 5.38 (1H, doublet, J=8 Hz, C_1–H of esterglycosidic glucose residue). *Anal.* Calcd. for $C_{74}H_{126}O_{26}$: C, 61.99; H, 9.00. Found: C, 61.50; H, 8.74.

Methanolysis of XXII——XXII (50 mg) was methanolyzed and worked up in the same way as in XV. The aglycone was crystallized from MeOH to provide colorless needles (12 mg), mp 215—217°, which were methylated with CH_2N_2 to yield colorless needles, mp 187—189°. They were identified with XVI and VIII, respectively. The sugar portion was examined by TLC and GLC to show the presence of the same four methylated sugars as from XIX.

$LiAlH_4$ Reduction of XXII——Conducted in the same way as in XV to yield XVII and colorless needles (XXIII) (from hexane), mp 124—127°, $[\alpha]_D$ +36° (c=2.09, $CHCl_3$). *Anal.* Calcd. for $C_{47}H_{80}O_{11}$: C, 68.74; H, 9.82. Found: C, 68.81; H, 9.81. XXIII was identified (TLC, IR, mixed fusion) with the 28-ol, mp 124—126°, $[\alpha]_D$ +38° (c=3.43, $CHCl_3$), corresponding to and prepared from X by $LiAlH_4$ reduction.

Acknowledgement The authors are grateful to Professor T. Takemoto of Tohoku University for the authentic sample of oleanolic acid, to Professors J. Shoji of Showa University and H. Itokawa of Tokyo College of Pharmacy for valuable discussions. Thanks are also due to Miss M. Kawamura for the mass spectra, to Mr. Y. Tanaka for the NMR spectra, and to the members of the Central Analysis Room of this University for microanalysis.

NII-Electronic Library Service