Studies on the Constituents of *Luffa operculata* COGN. I.[1] Isolation and Structures of Luperosides A—H, Dammarane-Type Triterpene Glycosides in the Herb

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Eight dammarane-type triterpene glycosides named luperosides A—H were isolated from the dried herb of *Luffa operculata* COGN. (Cucurbitaceae). Their structures were determined on the basis of chemical and spectral evidence as follows. A: a 3,20-bis-*O*-*β*-D-glucopyranoside of (20*S*)-dammar-24-ene-3*β*,7*β*,20-triol (Ag). B: a 20-*O*-*β*-gentiobioside of Ag. C: a 3-*O*-*β*-neohesperidoside-20-*O*-*β*-D-glucopyranoside of Ag. D: a 3-*O*-*β*-D-glucopyranoside-20-*O*-*β*-gentiobioside of Ag. E: a 3-*O*-*β*-neohesperidoside-20-*O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranoside of Ag. F: a 3-*O*-*β*-neohesperidoside-20-*O*-*β*-gentiobioside of Ag. G: a 3-*O*-*β*-neohesperidoside-20-*O*-*β*-gentiobioside of (20*S*)-dammar-23-ene-3*β*,7*β*,20,25-tetraol. H: 3-*O*-*β*-neohesperidoside-20-*O*-*β*-gentiobiosides of (20*S*, 24*S* and *R*)-dammar-25-ene-3*β*,7*β*,20,24-tetraols.

Keywords *Luffa operculata*; Cucurbitaceae; luperoside; triterpene glycoside; dammarane; (20*S*)-dammar-24-ene-3*β*,7*β*,20-triol; (20*S*)-dammar-23-ene-3*β*,7*β*,20,25-tetraol; (20*S*)-dammar-25-ene-3*β*,7*β*,20,24-tetraol

Luffa operculata COGN. is a cucurbitaceous vine indigenous to South America. The dried fruit, known as "buchinha-do-norte" or "cabacinha" in Brazil, tastes extremely bitter and has a decongestant action on the mucosa and a strong foaming property. It has been used traditionally as a sternulator, a purgative and an expectorant. It has been also used as a folk medicine for nasal congestion.[2] The chemical constituents of the dried fruit were investigated by several groups and cucurbitacins B and D,[3] and isocucurbitacin B[4] were reported as bitter principles. Djerassi *et al.*[5] isolated gypsogenin as an aglycone of the saponin. There is no published information, however, on chemical constituents of the other parts of the plant.

The dried herb does not taste bitter, and foams vigorously when the powder is shaken in water. The latter property suggests that the powder contains saponins. It was found that it does not contain any bitter principle, but contains considerable amounts of glycosides. Eight dammarane-type triterpene glycosides named luperosides A—H could be isolated from the less polar glycoside fraction (fr. II) together with several kinds of pentacyclic triterpene glycosides from the more polar fraction (fr. I). In the present paper, we report on the isolation and structure elucidation of the luperosides.

The procedures for extraction and fractionation are described in the experimental section. The less polar glycoside fraction (fr. II) showed several spots on thin layer chromatography (TLC) which stained violet on heating the plate after spraying sulfuric acid. A combination of chromatography on normal-phase silica gel and reversed-phase material (LiChroprep RP-18) resulted in the isolation of eight glycosides which were assigned the names luperosides A—H (I—VIII) in the order of increasing polarity.

Luperoside F (VI), the major constituent, is a colorless crystalline compound which exhibited in the fast atom bombardment mass spectrum (FAB-MS) an intense $[M+Na]^+$ ion at m/z 1115, indicating a molecular weight of 1092. The results of elemental analysis were consistent with $C_{54}H_{92}O_{22}\cdot 1/5H_2O$. The proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra showed the signals of four anomeric protons at δ 6.54 (br s), δ 5.09 (d, $J=7$ Hz), δ 5.04 (d, $J=8$ Hz) and δ 4.95 (d, $J=7$ Hz) and anomeric carbons (δ 98.7, 101.7 and 105.4 × 2).

The component monosaccharides were proved to be D-glucose and L-rhamnose by gas-liquid chromatography

TABLE I. ^{13}C-NMR Chemical Shifts of the Aglycone Moieties of Luperosides and Related Compounds[a]

	II	X	VI	IX	XI[7]	XII[8]	VII	XIII[11]	VIII	XIV[12]
C1	39.4	39.6	39.6	39.4	39.5	39.5	39.6	39.4	39.5	39.3
C2	28.4	26.3	28.1	28.4	28.3	28.3	27.0	26.9	27.0	28.0
C3	78.0	88.7	88.7	78.0	78.0	78.0	88.7	89.3	88.7	78.4[c]
C4	39.5	39.6	39.6	39.5	39.4	39.5	39.6	39.9	39.6	40.2
C5	54.0	54.3	54.2	54.0	54.0	56.5	54.2	56.6	54.2	61.7
C6	29.4	29.1	29.1	29.4	29.4	18.8	29.1	18.6	29.1	67.6
C7	74.8	74.7	74.7	74.8	74.8	35.7	74.8	35.3	74.7	47.3
C8	46.6	46.6	46.6	46.6	46.6	40.7	46.6	40.2	46.6	41.1
C9	51.0	50.9	50.9	51.0	51.0	51.1	50.9	50.3	50.8	49.8
C10	37.0	37.0	37.0	37.0	37.4	37.4	37.0	37.1	37.0	39.3
C11	22.1	22.0	22.1	22.1	22.1	21.9	22.0	30.9	22.1	30.8
C12	26.0	25.8	26.0	25.8	25.8	25.8	25.7	70.6	26.0	78.6[c]
C13	43.6	43.6	43.5	43.6	43.6	42.6	43.4	49.6	43.7	48.8
C14	50.4	50.4	50.4	50.4	50.4	50.6	50.3	51.7	50.4	51.4
C15	35.5	35.4	35.5	35.5	35.5	31.7	35.1	30.7	35.3	30.8
C16	28.1	28.2	27.1	28.2	28.2	28.1	28.1	26.6	28.1	26.6
C17	47.8	49.8	47.7	49.7	49.8	50.3	48.6	52.2 (47.8)	47.9 (52.3)	52.0
C18	10.5	10.4	10.5	10.5	10.5	16.5[b]	10.5	16.2[b]	10.4	17.4[b]
C19	16.8[b]	17.1[b]	17.1[b]	16.7[b]	16.6[b]	16.3[b]	17.1[b]	16.5[b]	17.1[b]	17.4[b]
C20	82.6	74.3	82.6	74.2	74.2	74.0	82.4	83.6 (82.8)	82.7 (83.4)	83.2
C21	21.5	26.3	21.6	26.2	26.2	25.3	22.5	23.4 (21.5)	21.7	22.8
C22	40.4	41.6	40.4	41.6	42.0	41.9	43.5	39.9	36.5 (32.5)	32.3
C23	23.3	23.3	23.4	23.3	22.9	23.3	123.3	123.0 (30.5)	30.4	30.8
C24	126.2	126.1	126.2	126.1	127.7	126.0	142.3	142.3 (76.0)	76.2 (76.1)	75.6
C25	130.2	130.7	130.7	130.7	136.1	130.6	70.0	70.1	149.9	149.7
C26	25.8	25.8	25.9	25.8	21.8	26.1	30.7[c]	30.6	110.3 (110.0)	109.9 (110.2)
C27	18.0	17.7	18.0	17.7	60.9	17.7	30.6[c]	30.7	18.3	18.5
C28	28.1	28.0	28.0	28.6	28.6	28.7	28.0	28.3	27.9	31.8
C29	16.4[b]	16.6[b]	16.7[b]	16.4[b]	16.5[b]	15.8[b]	16.7[b]	16.8[b]	16.6[b]	16.4[b]
C30	16.6[b]	16.7[b]	16.8[b]	16.6[b]	16.7[b]	16.9[b]	16.8[b]	17.4[b]	16.8[b]	17.4[b]

a) Assignments are based on the reports by Iwamoto *et al.*,[7] Asakawa *et al.*,[8] Yoshikawa *et al.*[11] and Yahara *et al.*[12] *b*, *c*) The signals with the same superscripts in each column may be interchanged. The numbers in the parentheses are the chemical shifts of the sub-peaks.

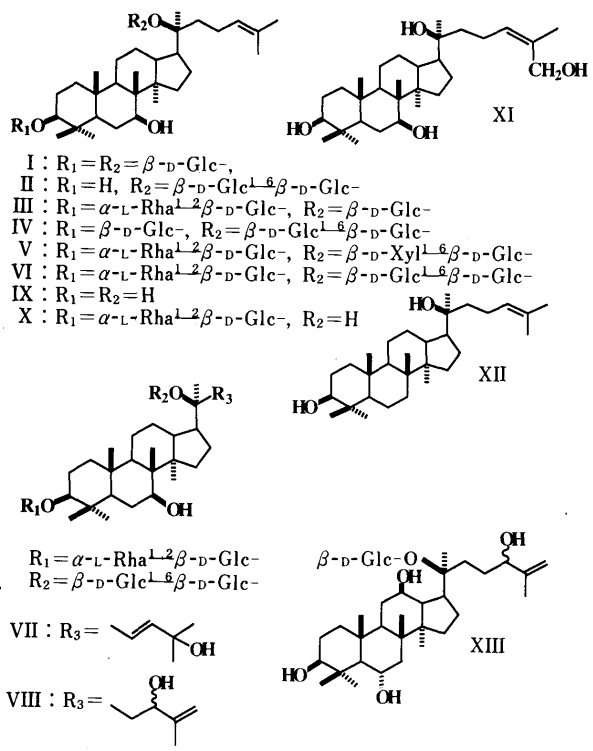
(GLC) of the methanolysate (as trimethylsilyl (TMS) ethers) and hydrolysate (as TMS ethers of methyl 2-(polyhydroxyalkyl)-thiazolidine-4(*R*)-carboxylates[6]) of VI. The NMR data indicate VI to be composed of 1 mol of L-rhamnose, 3 mol of D-glucose and an aglycone $C_{30}H_{52}O_3$.

Enzymatic hydrolysis with cellulase yielded an aglycone (IX) and a prosapogenin (X). Elemental analysis of IX suggested $C_{30}H_{52}O_3 \cdot 1/2H_2O$ and the ^{1}H-NMR spectrum showed the signals of five methyl groups on C–C bonded quaternary carbons at δ 0.93, 1.05, 1.16, 1.23 and 1.25, the signal (δ 1.45s) of a methyl group which seems to be adjacent to oxygenated quaternary carbon, and signals (δ 1.66 and 1.70) of two methyl groups attached to the same olefinic carbon. Two hydroxymethine protons appeared at δ 3.46 (dd, J=6, 10 Hz) and δ 4.06 (dd, J=4, 11 Hz), and one olefinic proton signal was observed at δ 5.32 as a triplet (J=7 Hz). The ^{13}C-NMR spectrum showed the signals of four C–C bonded quaternary carbons (δ 37.4, 39.5, 46.6 and 50.4), one hydroxylated quaternary carbon (δ 74.2), two hydroxymethine carbons (δ 74.8 and 78.0) and a pair of carbons (δ 126.1 and 130.7) of a tri-substituted double bond. The degree of unsaturation of the molecular formula and the above-mentioned spectral feature implied IX to be a dammarane-type triterpene having two hydroxymethine groups in the nucleus, and one tertiary hydroxyl group and a tri-substituted double bond in the side chain. The ^{13}C-NMR spectrum of IX is strikingly similar to that of the aglycone (XI) of actinostemmoside B, which was isolated in this laboratory from the herb of *Actinostemma lobatum* MAXIM. (Cucurbitaceae).[7] The differences between the two spectra are that the signals of C_{26} and C_{27} of XI appear at δ 21.8 and 60.9, respectively, while those of IX appear at δ 25.8 and 17.7, respectively. Some minor differences were observed between the chemical shifts of other carbons in the side chains. The chemical shifts of the side chain carbons were almost identical with those of (20*S*)-dammarenediol [(20*S*)-dammar-24-ene-3β,20-diol] (XII) reported by Kasai *et al.*[8] From these data, IX was identified as (20*S*)-dammar-24-ene-3β,7β,20-triol. The coupling constants of the two hydroxymethine protons support the β-orientation of the hydroxyl groups.

The prosapogenin (X) showed in FAB-MS an $[M+Na]^+$ ion at m/z 791, elemental analysis suggested $C_{42}H_{72}O_{12} \cdot 2H_2O$. The same prosapogenin[9] (X′) was obtained by acetic acid hydrolysis of VI. The molecular formula indicated that X has 1 mol each of L-rhamnose and D-glucose as the component monosaccharides. The ^{13}C-NMR signals of C_7 and C_{20} of X appeared at δ 74.7 and 74.3, respectively, and these values are almost the same as those of the aglycone (IX), except that the C_3 signal of X is shifted downfield by 10.7 ppm compared with that of IX, indicating that the biose is linked to the aglycone at C_3.

The permethylate of X gave methyl glycosides of 2,3,4-tri-*O*-methyl-α-L-rhamnopyranose and 3,4,6-tri-*O*-methyl-α- and -β-D-glucopyranoses, thus indicating X to be a 3-*O*-L-rhamnopyranosyl-(1→2)-D-glucopyranoside of (20*S*)-dammar-24-ene-3β,7β,20-triol.

When VI was treated with EtONa according to the method reported by Ogihara *et al.*,[10] four prosapogenins were obtained together with the aglycone (IX) and another prosapogenin (X). The new prosapogenins were proved to be identical with luperosides A (I), B (II), C (III) and D



(IV), respectively, in the order of increasing polarity.

Luperoside A (I) showed in FAB-MS an $[M+Na]^+$ ion at m/z 807, and it gave D-glucose on acid hydrolysis. The ^{1}H-NMR spectrum showed anomeric proton signals at δ 4.93 (d, J=8 Hz) and 5.09 (d, J=8 Hz), and corresponding anomeric carbon signals were observed at δ 106.9 and 98.6, respectively. The carbon signals of C_3 and C_{20} of the aglycone moiety appeared at δ 88.8 and 82.5, respectively. These data clearly indicate that I is (20*S*)-dammar-24-ene-3β,7β,20-triol-3,20-bis-*O*-β-D-glucopyranoside.

Luperoside B (II) gave elemental analysis data consistent with $C_{42}H_{72}O_{13} \cdot 2H_2O$, and gave D-glucose on acid hydrolysis. The NMR spectra showed anomeric proton signals at δ 5.01 (d, J=7 Hz) and 5.02 (d, J=8 Hz), and corresponding carbon signals at δ 98.6 and 105.4, respectively. Among three oxygenated carbons in the aglycone moiety, only one signal due to C_{20} was shifted downfield (Δ8.4 ppm) when compared with that of the aglycone (IX), indicating that II is a monodesmoside in which a glucobiose is linked at C_{20}. The glycosylation shift of one hydroxymethylene carbon (δ 70.6, C_6 of glucose) and the coupling constants of the anomeric protons indicate that the sugar moiety is β-gentiobiose. Thus, II is (20*S*)-dammar-24-ene-3β,7β,20-triol-20-*O*-β-gentiobioside.

Luperoside C (III) showed in FAB-MS an $[M+Na]^+$ ion at m/z 953 and it gave D-glucose and L-rhamnose on acid hydrolysis. The ^{1}H-NMR spectrum showed anomeric proton signals at δ 4.95 (d, J=7 Hz), 5.10 (d, J=8 Hz) and 6.54 (br s), and corresponding carbon signals were seen at δ 105.4, 98.6 and 101.7, respectively. Acetic acid hydrolysis of III gave X′ as a prosapogenin, thus indicating III to be a 3-*O*-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside-20-*O*-β-D-glucopyranoside of IX.

Luperoside D (IV) gave an $[M+Na]^+$ ion at m/z 969 in FAB-MS, and the NMR spectra showed anomeric proton signals at δ 4.95 (d, J=8 Hz), 5.07 (d, J=7 Hz) and 5.09 (d,

$J=8$ Hz), and corresponding carbon signals at δ 106.8, 98.6 and 105.4, respectively. Compound IV gave D-glucose on acid hydrolysis. The ^{13}C-NMR spectrum showed glycosylation shifts at C_3 (δ 88.8) and C_{20} (δ 82.6), indicating IV to be a bisdesmoside. Acetic acid hydrolysis of IV gave a 3-*O*-monoglucoside of (20*S* and 20*R*)-dammar-24-ene-3β,7β,20-triol. Accordingly, IV is (20*S*)-dammar-24-ene-3β,7β,20-triol-3-*O*-β-D-glucopyranoside-20-*O*-β-gentiobioside.

The structure of luperoside F (VI), the mother compound of the above-mentioned luperosides, was reconstructed from those of the prosapogenins as (20*S*)-dammar-24-ene-3β,7β,20-triol-3-*O*-β-neohesperidoside-20-*O*-β-gentiobioside. The α-linkage of the L-rhamnopyranosyl group was established from the difference ($\Delta[M]_D$ $-153.6°$) of molecular rotations between VI ($[M]_D$ $-221.7°$) and IV ($[M]_D$ $-68.1°$).

Luperoside E (V) gave elemental analysis data consistent with $C_{53}H_{90}O_{21}\cdot 2H_2O$ and it gave D-glucose, L-rhamnose and D-xylose on acid hydrolysis. The NMR spectra suggested that V is a 3,20-*O*-bisdesmoside of the same aglycone as VI. Acetic acid hydrolysis of V gave a prosapogenin (X′) indicating that a biose composed of 1 mol each of D-glucose and D-xylose is linked to the C_{20}-hydroxyl group. The downfield shift of one of two C_6-carbon signals of glucopyranosyl groups indicates that the xylopyranosyl group is linked to the C_6-hydroxyl group of glucose. Accordingly, V is (20*S*)-dammar-24-ene-3β,7β,20-triol-3-*O*-β-neohesperidoside-20-*O*-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside.

Luperoside G (VII) was obtained as a crystalline powder from benzene–EtOH and the FAB-MS showed an $[M+Na]^+$ ion at *m/z* 1131 and an $[M-H]^-$ ion at *m/z* 1107, indicating the molecular weight to be 1108. Compound VII gave elemental analysis data consistent with $C_{54}H_{92}O_{23}\cdot 2H_2O$, and it gave D-glucose and L-rhamnose on acid hydrolysis. The NMR spectra showed the anomeric proton signals at δ 4.92 (d, $J=7$ Hz), 5.08 (2H, d, $J=8$ Hz) and 6.50 (br s), and the corresponding carbon signals were observed at δ 105.4, 105.2, 98.6 and 101.7. From these data, the sugar moiety of VII was proved to be composed of 1 mol of L-rhamnose and 3 mol of D-glucose. The ^{13}C-NMR signals of the aglycone moiety were compared with those of VI. Compound VII showed the signals of a pair of the di-substituted olefin carbons (δ 123.3 and 142.3) instead of those of tri-substituted olefin carbons. It also showed one more signal of one oxygenated quaternary carbon (δ 70.0). Two signals of methyl carbons linked to the olefinic carbon in VI had disappeared in VII, and instead, they appeared downfield (δ 30.6 and 30.7). The ^{1}H-NMR spectrum showed olefin proton signals at δ 6.28 (ddd, $J=7$, 7, 15 Hz) and δ 6.05 (d, $J=15$ Hz), indicating the presence of a *trans* double bond connected to a methylene carbon and a quaternary carbon. The carbon signals other than those of the side chain have almost the same chemical shifts as those of VI. These NMR data indicate that VII is a 3,20-*O*-bisdesmoside of (20*S*)-dammar-23-ene-3β,7β,20,25-tetraol or of (20*S*)-dammar-22-ene-3β,7β,20,25-tetraol.

The ^{13}C-NMR spectrum of VII was compared with that of gypenoside LXIX (XIII), which was isolated by Takemoto *et al.* from *Gynostemma pentaphyllum* MAKINO.[11] The chemical shifts of signals assignable to C_{23}, C_{24}, C_{25}, C_{26} and C_{27} were identical, although the signals of C_{20}, C_{21} and C_{22} did not have the same chemical shifts, probably due to the absence of the C_{12}-OH group in VII. From comparison of the NMR data with those of gypenoside LXIX, the structure of the aglycone of VII was determined as (20*S*)-dammar-23-ene-3β,7β,20,25-tetraol.

Acetic acid hydrolysis of VII gave a prosapogenin and a sugar. The former gave D-glucose and L-rhamnose, and the latter gave D-glucose only on acid hydrolysis, showing that a glucobiose is linked to the C_{20} and a rhamnoglucose to the C_3 of the aglycone. The NMR chemical shifts of the signals of the sugar moiety were almost identical with those of VI. Thus, the structure of VII was elucidated as (20*S*)-dammar-23-ene-3β,7β,20,25-tetraol-3-*O*-β-neohesperidoside-20-*O*-β-gentiobioside.

Luperoside H (VIII) was isolated as a thin-layer-chromatographically homogeneous amorphous powder. The FAB-MS showed an $[M+Na]^+$ ion at *m/z* 1131 and an $[M-H]^-$ ion at *m/z* 1107, the same mass number as those in the case of VII. Elemental analysis data were consistent with $C_{54}H_{92}O_{23}\cdot 3.5H_2O$. The component monosaccharides were proved to be 1 mol of L-rhamnose and 3 mol of D-glucose.

The ^{1}H-NMR spectrum showed the signals of the exomethylene protons at δ 5.29 (br s) and 4.9[illegible], and a proton signal due to a methyl group linked to an olefinic carbon was observed at δ 1.96 (3H, s).

The ^{13}C-NMR spectrum of VIII showed olefinic carbon signals at δ 149.9 and 110.0, and signals of oxygenated carbons in the aglycone moiety (determined by ^{1}H–^{1}H COSY and ^{1}H–^{13}C COSY) at δ 88.7, 74.7, 82.8 and 76.2, the first three of which were assigned to C_3, C_7 and C_{20} by comparison with the data of other luperosides. The last one was presumed to be due to a hydroxymethine carbon in the side chain because the carbon signals assigned to the nucleus showed almost the same chemical shifts as those of VI. The location of the new hydroxyl group was postulated at C_{24} and the ^{13}C-NMR spectrum was compared with that of ginsenoside M_{7cd} (XIV), which was isolated by Tanaka *et al.*[12] from the flower buds of *Panax ginseng*, The chemical shifts of C_{23}, C_{24}, C_{25}, C_{26} and C_{27} were almost identical with those of XIV, although the chemical shifts of C_{21} and C_{22} were not the same because of the absence of a C_{12}-hydroxyl group in VIII. Thus, the structure of the aglycone was concluded to be (20*S*)-dammar-25-ene-3β,7β,20,24-tetraol. Each signal peak of C_{17}, C_{20}, C_{21}, C_{23}, C_{24} and C_{26} was accompanied with a small signal (chemical shifts are shown in parentheses in the Table I), which indicates that the aglycone is a mixture of the 24*R*- and 24*S*-isomers, as ginsenoside M_{7cd} was reported to be.

The structure of the sugar moiety was determined in the same way as for VII, and was proved to be the same as that of VII by NMR spectral comparison. Accordingly, the structure of VIII was concluded to be (20*S*, 24*R* and *S*)-dammar-24-ene-3β,7β,20,24-tetraol-3-*O*-β-neohesperidoside-20-*O*-β-gentiobioside.

The more polar glycoside fration (fr. I) foams vigorously when the aqueous solution is shaken. The fraction showed several spots on TLC developed with $CHCl_3$–MeOH–H_2O–AcOH (21 : 15 : 3 : 1), and these spots stained bluish green when the plate was heated after spraying sulfuric acid, suggesting the presence of acidic pentacyclic triterpene saponins. The isolation and structure elucidation of these

saponins are in progress.

Experimental[13]

Plant Material *Luffa operculata* COGN. was cultivated in a suburb of Fukuoka City in 1986. The herb was harvested in September, air-dried and powdered.

Extraction and Fractionation The powder (200 g) of the herb was percolated with MeOH (2 l). The MeOH extract was refluxed with 50% MeOH (1 l) and the insoluble material was separated. The 50% MeOH solution was evaporated to remove MeOH and the remaining aqueous solution was applied to a column of a highly porous polystyrene polymer, Diaion HP-20 (200 ml). After washing of the column with H_2O (1 l), the materials absorbed in the column were eluted with 1 l each of 50% MeOH and MeOH. The eluates were combined and concentrated *in vacuo*. The residue was again suspended in hot MeOH (90 ml). The insoluble powdery materials (fr. I, 3.2 g) was filtered off and the filtrate was concentrated *in vacuo* to give a brown powder (fr. II, 5.8 g). The yield of fr. II from 3.9 kg of the dried herb was 79 g.

Fraction II was chromatographed portionwise on an HP-20 column (20 times the weight of the material) using MeOH–H_2O solvent systems and separated into four fractions (fr. II-1—4).

Fraction II-2 (eluate with 40—50% MeOH) was repeatedly chromatographed on silica gel with AcOEt–MeOH–H_2O (70 : 25 : 5), then chromatographed on LiChroprep RP-18 (15% PrOH) to give VII (301 mg) and VIII (367 mg). The fr. II-3 (eluate by 60% MeOH) was repeatedly chromatographed on silica gel using the solvent systems $CHCl_3$–MeOH–H_2O (100 : 40 : 5) and AcOEt–MeOH–H_2O (80 : 15 : 5), and on LiChroprep RP-18 (20% PrOH) to give III (964 mg), IV (8.4 g), V (342 mg) and VI (12.7 g). The fr. II-4 (eluate with 70—90% MeOH) was chromatographed on silica gel [$CHCl_3$–MeOH (8 : 2)] and then purified by passage through a LiChroprep RP-18 column (20—30% MeOH) to give I (297 mg) and II (81 mg).

I: Colorless needles from MeOH, mp 168—170 °C. $[\alpha]_D^{26}$ +2.4° (c=1.02, MeOH). *Anal.* Calcd for $C_{42}H_{72}O_{13}\cdot 2H_2O$: C, 61.44; H, 9.26. Found: C, 61.53; H, 9.43. FAB-MS m/z: 807 ($[M+Na]^+$), 783 ($[M-H]^-$). ^{1}H-NMR δ: Sugar moiety; 4.93 (d, J=8 Hz, C_3-*O*-Glc-1), 5.09 (d, J=7 Hz, C_{20}-*O*-Glc-1). ^{13}C-NMR δ: Sugar moiety; 106.8 (C_3-*O*-Glc-1), 98.6 (C_{20}-*O*-Glc-1).

II: Colorless needles from MeOH, mp 148—150 °C, $[\alpha]_D^{26}$ −3.3° (c=1.00, MeOH). *Anal.* Calcd for $C_{42}H_{72}O_{13}\cdot 2H_2O$: C, 61.44; H, 9.33. Found: C, 61.41; H, 9.31. FAB-MS m/z: 807 ($[M+Na]^+$), 783 ($[M-H]^-$). ^{1}H-NMR δ: 5.00 [d, J=7 Hz, Glc(in)-1], 5.02 [d, J=8 Hz, Glc(out)-1]. ^{13}C-NMR δ: Sugar moiety; 98.6 [Glc(in)-1], 105.4 [Glc(out)-1], 70.6 [Glc(in)-6].

III: Colorless needles from MeOH, mp 238—240 °C, $[\alpha]_D^{26}$ −30.5° (c=1.07, pyridine). *Anal.* Calcd for $C_{48}H_{82}O_{17}\cdot 2H_2O$: C, 59.61; H, 8.96. Found: C, 59.34; H, 8.83. FAB-MS m/z: 953 ($[M+Na]^+$). ^{1}H-NMR (pyridine-d_5–D_2O) δ: Sugar moiety; 6.54 (br s, Rha-1), 5.10 (d, J=8 Hz, C_{20}-*O*-Glc-1), 4.95 (d, J=7 Hz, C_3-*O*-Glc-1). ^{13}C-NMR (pyridine-d_5–D_2O) δ: Sugar moiety; 105.4 (C_3-*O*-Glc-1), 101.7 (Rha-1), 98.6 (C_{20}-*O*-Glc-1), 79.8 (C_3-*O*-Glc-2), aglycone moiety; the chemical shifts of the signals are almost the same as those of I.

IV: Colorless needles from EtOH–H_2O, mp 241—242 °C, $[\alpha]_D^{23}$ −7.2° (c=1.05, MeOH). *Anal.* Calcd for $C_{48}H_{82}O_{18}\cdot 2H_2O$: C, 58.64; H, 8.82. Found: C, 58.54; H, 8.69. FAB-MS m/z: 969 ($[M+Na]^+$). ^{1}H-NMR δ: Sugar moiety; 5.09 [d, J=8 Hz, C_{20}-*O*-Glc(out)-1], 5.07 [d, J=7 Hz, C_{20}-*O*-Glc(in)-1], 4.95 [d, J=8 Hz, C_3-*O*-Glc-1]. ^{13}C-NMR δ: Sugar moiety; 106.8 (C_3-*O*-Glc-1), 105.4 [C_{20}-*O*-Glc(out)-1], 98.6 [C_{20}-*O*-Glc(in)-1], 70.6 [C_{20}-*O*-Glc(in)-6], aglycone moiety; the chemical shifts of the signals are almost the same as those of I.

V: Colorless needles from EtOH–H_2O, mp 191—193 °C, $[\alpha]_D^{26}$ −23.9° (c=1.04, MeOH). *Anal.* Calcd for $C_{53}H_{90}O_{21}\cdot 2H_2O$: C, 57.91; H, 8.62. Found: C, 57.79; H, 8.89. FAB-MS m/z: 1085 ($[M+Na]^+$), 1061 ($[M-H]^-$). ^{1}H-NMR δ: Sugar moiety; 6.53 (br s, Rha-1), 5.04 (d, J=8 Hz, C_{20}-*O*-Glc-1), 4.96 (d, J=8 Hz, C_3-*O*-Glc-1*), 4.94 (d, J=8 Hz, Xyl-1*). ^{13}C-NMR δ: Sugar moiety; 105.8 (Xyl-1**), 105.4 (C_3-*O*-Glc-1**), 101.7 (Rha-1), 98.6 (C_{20}-*O*-Glc-1), 79.8 (C_3-*O*-Glc-2), 70.3 (C_{20}-*O*-Glc-6), aglycone moiety; almost identical with I. *, ** The assignments might be reversed in each case.

VI: Colorless needles from EtOH–H_2O, mp 232—234 °C, $[\alpha]_D^{26}$ −20.3° (c=1.01, MeOH). *Anal.* Calcd for $C_{54}H_{92}O_{22}\cdot 1/5H_2O$: C, 57.43; H, 8.57. Found: C, 57.69; H, 8.69. FAB-MS m/z: 1115 ($[M+Na]^+$). ^{1}H-NMR δ: Sugar moiety; 6.54 (br s, Rha-1), 5.09 [d, J=7 Hz, C_{20}-*O*-Glc(out)-1], 5.04 [d, J=8 Hz, C_{20}-*O*-Glc(in)-1], 4.95 (d, J=7 Hz, C_{20}-*O*-Glc-1). ^{13}C-NMR δ: Sugar moiety; 105.4 [C_{20}-*O*-Glc(out)-1, C_3-*O*-Glc-1], 101.7 (Rha-1), 98.7 [C_{20}-*O*-Glc(in)-1], 79.8 (C_3-*O*-Glc-2), 70.6 [C_{20}-*O*-Glc(in)-6], aglycone moiety; almost identical with I.

VII: Crystalline powder from benzene–EtOH, mp 194—195 °C, $[\alpha]_D^{27}$ −19.1° (c=0.99, MeOH). *Anal.* Calcd for $C_{54}H_{92}O_{23}\cdot 2H_2O$: C, 56.63; H, 8.45. Found: C, 56.70; H, 8.55. FAB-MS m/z: 1131 ($[M+Na]^+$), 1107 ($[M-H]^-$). ^{1}H-NMR δ: Aglycone moiety; ≻CH$_3$; 1.56 (C_{21}), 1.55 (C_{26} or C_{27}), 1.54 (C_{27} or C_{26}), 1.26 (C_{18}), 1.23 (C_{28}), 1.20, 1.10, 0.85. Olefinic H; 6.05 (d, J=15 Hz, C_{24}-H), 6.28 (ddd, J=7, 7, 15 Hz, C_{23}-H). C_{22}-H_2; 2.83 (2H, br d, J=7 Hz). C_3-H; 3.35 (dd, J=4, 12 Hz), sugar moiety; 6.50 (br s, Rha-1), 5.08 [2H, d, J=8 Hz, C_{20}-*O*-Glc(in)-1, C_{20}-*O*-Glc(out)-1], 4.92 (d, J=7 Hz, C_3-*O*-Glc-1). ^{13}C-NMR δ: Sugar moiety; 105.4 (C_3-*O*-Glc-1), 105.2 [C_{20}-*O*-Glc(out)-1], 101.7 (Rha-1), 98.6 [C_{20}-*O*-Glc(in)-1], 79.8 (C_3-*O*-Glc-2), 70.4 [C_{20}-*O*-Glc(in)-6].

VIII: Amorphous powder, mp 188—193 °C, $[\alpha]_D^{26}$ −19.9° (c=0.98, MeOH). *Anal.* Calcd for $C_{54}H_{92}O_{23}\cdot 3.5H_2O$: C, 55.32; H, 8.51. Found: C, 55.38; H, 8.44. FAB-MS m/z: 1131 ($[M+Na]^+$), 1107 ($[M-H]^-$). ^{1}H-NMR δ: Aglycone moiety; ≻CH$_3$; 0.85, 1.12, 1.13, 1.19, 1.23 (C_{18}, C_{28}), 1.53 (C_{21}), 1.95, 1.96 (C_{27}). C_3-H; 3.35 (dd, J=4, 11 Hz), C_{26}-H_2; 4.98 (br s), 5.29 (br s), sugar moiety; *ca.* 4.95 (overlapped with other signals, C_3-*O*-Glc-1), 5.02, 5.03 [d, J=8 Hz, C_{20}-*O*-Glc(out)-1], 5.05, 5.07 [d, J=7 Hz, C_{20}-*O*-Glc(in)-1], 6.49 (br s, Rha-1).
^{13}C-NMR δ: Sugar moiety; 105.34 (C_3-*O*-Glc-1), 105.25 [C_{20}-*O*-Glc(out)-1], 101.7 (Rha-1), 98.5 [C_{20}-*O*-Glc(in)-1], 79.8 (C_3-*O*-Glc-2), 70.5 [C_{20}-*O*-Glc(in)-6].

Determination of Component Monosaccharides Each compounds (5 mg) was dissolved in 1 N HCl–MeOH (0.5 ml) and heated at 100 °C in a sealed tube for 30 min. The acid was neutralized with Ag_2CO_3 and the solution was filtered. The filtrate was bubbled through with H_2S and the solvent was evaporated off. The residue was dissolved in MeOH and Ag_2S was removed by filtration. One half of the filtrate was set aside and the MeOH was evaporated off. The residue was trimethylsilylated with trimethylimidazole and analyzed by GLC [column, G-SCOT OV-17 (0.3 mmϕ × 50 m); column bath temperature, 130 °C and 150 °C; injection port temperature, 250 °C; carrier gas, He 0.75 ml/min; split ratio, 1/70; make-up gas, He at 50 ml/min]. The results are summarized in Table II.

The other half of the methanolysate was hydrolyzed by heating in 1 N HCl (0.5 ml) at 100 °C for 1 h. The reaction solution was treated in a usual

Table II. GLC of TMS Ethers of Methanolysates of Luperosides[a]

Column bath temp.	t_R (min) 130 °C	150 °C
Methanolysate of		
I		21.2, 21.7
II		21.3, 21.8
III	13.1	21.3, 21.8
IV		21.3, 21.7
V	13.3, 18.6 18.7	21.5, 21.8
VI	13.1	21.3, 21.6
VII	13.1	21.3, 21.7
VIII	13.1	21.3, 21.8
L-Rhamnose	13.1	
D-Xylose	18.6, 18.7	
D-Glucose		21.3, 21.8

a) Conditions are given in the experimental section.

Table III. GLC of TMS Ethers of Thiazolidine Derivatives of the Hydrolysates of Luperosides[a]

	t_R (min)
Hydrolysate of V	11.4, 12.4, 17.0
VI	12.4, 17.0
VII	12.4, 17.1
VIII	12.4, 17.0
Xylose	11.4 (D-), 12.2 (L-)
Rhamnose	12.4 (L-), 12.8 (D-)
Glucose	17.0 (D-), 18.0 (L-)

a) Conditions are given in the experimental section.

manner to give a hydrolysate. The hydrolysate was converted into the thiazolidine derivative according to the method reported by Hara *et al.*[6] The GLC conditions were the same as for TMS ethers of methyl glycosides except for the column bath temperature (210 °C) and injection port temperature (280 °C). The results are shown in Table III.

Enzymatic Hydrolysis of VI Compound VI (1.0 g) was suspended in 20% EtOH (500 ml) and the suspension was shaken at 37 °C for 64 h after addition of cellulase (Sigma, type II) (1.8 g). The precipitates were centrifuged and recrystallized from MeOH to give an aglycone (IX, 16 mg). The supernatant was concentrated to dryness and the residue was subjected to column chromatography on HP-20 (30 ml). The column was eluted with H_2O and 30% MeOH. The product obtained from the MeOH eluate was crystallized from EtOH–H_2O after purification by silica gel chromatography [$CHCl_3$–MeOH–H_2O (85 : 15 : 1)] to give 152 mg of a prosapogenin (X).

IX: Colorless prisms from MeOH, mp 89—91 °C, $[\alpha]_D^{26}$ +10.8° (c=1.09, pyridine). *Anal.* Calcd for $C_{30}H_{52}O_3 \cdot 1/2H_2O$: C, 76.71; H, 11.37. Found: C, 76.34; H, 11.31. FAB-MS m/z: 459 ($[M+Na]^+$). ^{1}H-NMR δ: >CH$_3$; 1.70 (C_{26}), 1.65 (C_{27}), 1.45 (C_{21}), 1.25 (C_{18}), 1.20 (C_{28}), 1.16, 1.05, 0.93. C_3-H; 3.46 (dd, J=6, 10 Hz). C_7-H; 4.06 (dd, J=4, 11 Hz). C_{24}-H; 5.34 (t, J=7 Hz).

X: Colorless needles from MeOH, mp 161—163 °C, $[\alpha]_D^{23}$ −20.3° (c=1.12, MeOH). *Anal.* Calcd for $C_{42}H_{72}O_{12} \cdot 2H_2O$: C, 62.66; H, 9.26. Found: C, 62.74; H, 8.77. FAB-MS m/z: 791 ($[M+Na]^+$). ^{1}H-NMR δ: Anomeric H; 4.89 (d, J=7 Hz, Glc-1), 6.42 (br s, Rha-1). ^{13}C-NMR δ: Sugar moiety; 105.4 (Glc-1), 101.7 (Rha-1), 79.6 (Glc-2).

Acetic Acid Hydrolysis of VI Compound VI (200 mg) was suspended in 10% AcOH (20 ml) and heated at 80 °C until the solution became clear (*ca.* 3 h). After evaporation of the solvent, the residue was dissolved in H_2O and passed through a column of HP-20 (40 ml). The MeOH eluate was chromatographed on silica gel [$CHCl_3$–MeOH (9 : 1)] to give a prosapogenin (X′, 65 mg).

X′: Colorless crystalline powder from EtOH–H_2O. $[\alpha]_D^{25}$ −23.2° (c=1.07, MeOH). FAB-MS m/z: 791 ($[M+Na]^+$), 767 ($[M-H]^-$).

Methylation of X and Methanolysis of the Methylation Product Compound X (30 mg) was fully methylated according to Hakomori's method. The product was purified by silica gel column chromatography [AcOEt–hexane (2 : 1)] to give a permethylate (23 mg). The permethylate was methanolysed by refluxing it in 1 N HCl–MeOH for 5 h. The reaction mixture was treated in the usual manner and the methanolysate was checked by TLC [benzene–acetone (2 : 1), (6 : 1)]. The methanolysate showed spots which had the same *Rf* values as those of methyl 2,3,4-tri-*O*-methyl α-L-rhamnopyranoside and methyl 3,4,6-tri-*O*-methyl α-, and β-D-glucopyranosides (by co-chromatography with authentic samples).

Alkaline Degradation of VI Compound VI (960 mg) was dissolved in 2 N EtONa (70 ml) and heated at 50 °C for 24 h. After neutralization with acetic acid, the solvent was evaporated off. The residue was dissolved in H_2O and passed through an HP-20 column. The MeOH eluate after elution with H_2O and 30% MeOH was chromatographed on silica gel [$CHCl_3$–MeOH (85 : 15), $CHCl_3$–MeOH–H_2O (10 : 4 : 0.5)] and LiChroprep RP-18 (60% MeOH) to give an aglycone (X, 15 mg), IX (38 mg), I (26 mg), II (30 mg), III (63 mg) and IV (42 mg).

Acetic Acid Hydrolysis of III and V The procedure was the same as for acetic acid hydrolysis of VI. Compound III (160 mg) gave a prosapogenin (53 mg), which was identical (NMR spectral comparison) with X′.

Compound V (78 mg) was hydrolyzed in the same way to give X′ (24 mg).

Acetic Acid Hydrolysis of IV Compound IV (490 mg) was likewise hydrolyzed with acetic acid to give a prosapogenin (149 mg): a crystalline powder from EtOH–H_2O. FAB-MS m/z: 645 ($[M+Na]^+$). ^{1}H-NMR δ: Anomeric H, 4.93 (d, J=8 Hz). ^{13}C-NMR δ: Sugar moiety; 106.9 (C_1), 75.8 (C_2), 78.6 (C_3), 72.0 (C_4), 78.3 (C_5), 63.1 (C_6), aglycone moiety; chemical shifts of carbon signals were identical with those of X′.

Determination of Component Monosaccharides of the C_3- and C_{20}-Linked Sugar Moieties of VII and VIII Each of VII and VIII (10 mg) was suspended in 10% AcOH (1 ml) and heated at 80 °C for 2 h. After evaporation of the solvent, the residue was subjected to LiChroprep RP-18 column chromatography. The H_2O eluate gave a sugar which was linked to C_{20}, and the MeOH eluate gave a prosapogenin. Both eluates were respectively methanolyzed and checked by GLC under the same conditions as above. The H_2O eluate gave methyl glucosides (t_R 21.3 and 21.7) and the MeOH eluate gave methyl glucosides (t_R 21.3 and 21.7) and a methyl rhamnoside (t_R 13.1).

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13) The instruments and materials used in this work were as follows: JASCO DIP-360 digital polarimeter, JEOL JNM GX-400 spectrometer (100 MHz for ^{13}C-NMR spectra and 400 MHz for ^{1}H-NMR spectra), JEOL JMS DX-300 mass spectrometer, Shimadzu gas chromatograph GC-8A, Kieselgel 60 (63—210 μm, E. Merck), LiChroprep RP-18 (40—63 μm, E. Merck), precoated Kieselgel 60 F_{254} plates, precoated HPTLC RP-18 F_{254} plates (E. Merck).

NMR spectra were obtained in pyridine-d_5 solutions unless otherwise specified. Chemical shifts are expressed on the δ scale with tetramethyl silane as an internal standard. The FAB-MS were measured in glycerol and/or thioglycerol matrix containing NaI.