MEDICINAL FOODSTUFFS. XIII.[1] SAPONIN CONSTITUENTS WITH ADJUVANT ACTIVITY FROM HYACINTH BEAN, THE SEEDS OF *DOLICHOS LABLAB* L. (2) : STRUCTURES OF LABLABOSIDES D, E, AND F

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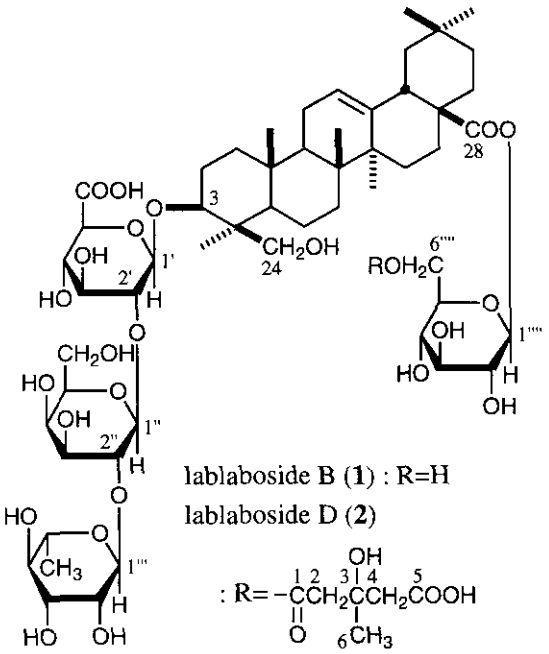
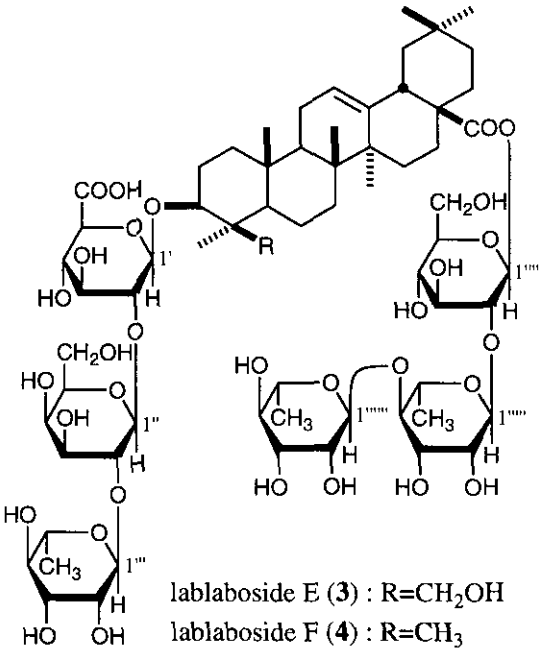
Abstract — Following the characterization of lablabosides A, B, and C, new oleanane-type triterpene bisdesmosides, lablabosides D, E, and F, were isolated from the glycosidic fraction with adjuvant activity obtained from the seeds of *Dolichos lablab* L. (Leguminosae). Their chemical structures were elucidated on the basis of chemical and physicochemical evidence as follows : 3-*O*-[α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→2)-β-D-glucopyranosiduronic acid]-28-*O*-[6-*O*-(3-hydroxy-3-methylglutaroyl)-β-D-glucopyranosyl] 24-*epi*-hederagenin (lablaboside D), 3-*O*-[α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→2)-β-D-glucopyranosiduronic acid]-28-*O*-[α-L-rhamnopyranosyl (1→4)-α-L-rhamnopyranosyl (1→2)-β-D-glucopyranosyl] 24-*epi*-hederagenin (lablaboside E), 3-*O*-[α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→2)-β-D-glucopyranosiduronic acid]-28-*O*-[α-L-rhamnopyranosyl (1→4)-α-L-rhamnopyranosyl (1→2)-β-D-glucopyranosyl] oleanolic acid (lablaboside F).

In the course of our characterization studies on bioactive saponin constituents in medicinal foodstuffs[1,2] and natural medicines,[3] we have recently found that the glycosidic fraction from the white seeds of *Dolichos lablab* L. ("白扁豆" in Chinese) showed adjuvant activity. Thus far, we have isolated six new oleanane-type triterpene bisdesmosides called lablabosides A, B (**1**), C, D (**2**), E (**3**), and F (**4**) from the glycosidic fraction. In the preceding paper,[1] we reported the structures of lablabosides A, B (**1**), and C. As a continuing study, we describe here the structure elucidation of the remaining three new oleanane-type triterpene bisdesmosides, lablabosides D (**2**), E (**3**), and F (**4**).

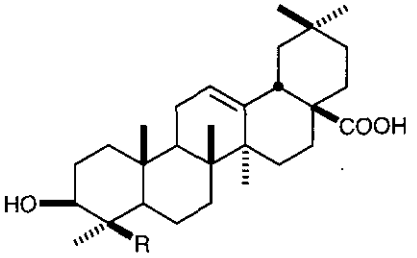
Lablaboside D Lablaboside D (**2**) was isolated as colorless fine crystals of mp 193.4-195.0 °C from aqueous methanol. The IR spectrum of **2** showed absorption bands at 1741, 1736, 1731, and 1724 cm^{-1} ascribable to ester and carboxyl groups and strong broad absorption bands at 3419 and 1076 cm^{-1} suggestive of an oligoglycosidic structure. In the positive-ion FAB-MS of **2**, a quasimolecular ion peak was observed at *m/z* 1285 $(M+Na)^+$, while the negative-ion FAB-MS showed a quasimolecular ion peak at *m/z* 1261 $(M-H)^-$. Furthermore, fragment ion peaks at *m/z* 1115 $(M-C_6H_{11}O_4)^-$, *m/z* 955 $(M-C_{12}H_{19}O_9)^-$, and *m/z* 953 $(M-C_{12}H_{21}O_9)^-$, which were prepared by cleavage of the glycosidic linkage at 2''-, 28-, and 2'-positions, respectively, were observed in the negative-ion FAB-MS of **2**. High-resolution MS analysis of the quasimolecular ion peak $(M+Na)^+$ revealed the molecular formula of **4** to be $C_{60}H_{94}O_{28}$. Methanolysis of **2** with 9% hydrogen chloride in dry methanol liberated 24-*epi*-hederagenin (**5**)[4] and methyl glycosides of glucuronic acid, galactose, rhamnose, and glucose in



lablaboside B (**1**) : R=H
lablaboside D (**2**)



lablaboside E (**3**) : R=CH_2OH
lablaboside F (**4**) : R=CH_3



24-*epi*-hederagenin (**5**) : R=CH_2OH
oleanolic acid (**6**) : R=CH_3

Figure 1

a 1 : 1 : 1 : 1 ratio.[5] Alkaline hydrolysis of **2** with 1% sodium hydroxide furnished lablaboside B (**1**)[1] and 3-hydroxy-3-methylglutaric acid (meglutol). The organic acid was derived to the *p*-nitrobenzyl ester,[6] which was identified by HPLC analysis. The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra of **2**, which were assigned by means of various NMR experiments,[7] showed signals assignable to a 24-*epi*-hederagenin moiety [δ 3.16 (m, 18-H), 3.22, 4.23 (both d, *J*=11.3 Hz, 24-H_2), 3.38 (dd, *J*=4.6, 12.2 Hz, 3-H), 5.37 (br s, 12-H)], a β-D-glucuronic acid moiety [δ 4.96 (d, *J*=7.6 Hz, 1'-H)], a β-D-galactopyranosyl moiety [δ 5.72 (d, *J*=7.6 Hz, 1"-H)], a α-L-rhamnopyranosyl moiety [δ 1.75 (d, *J*=6.4 Hz, 6'''-H_3), 6.22 (br s, 1'''-H)], a β-D-glucopyranosyl moiety [δ 4.76, 4.92 (both dd-like, 6''''-H_2), 6.22 (d, *J*=7.9 Hz, 1''''-H)], and a 3-hydroxy-3-methylglutaroyl group [δ 3.03, 3.15 (both d, *J*=14.1 Hz, 2-H_2), 3.10, 3.17 (both d, *J*=15.3 Hz, 4-H_2), 1.73 (s, 6-H_3)]. The glycosidic structure of **2**, including the position of a 3-hydroxy-3-methylglutaroyl group, was clarified by a heteronuclear multiple bond correlation (HMBC) experiment, which showed long-range correlations between the following protons and carbons : 1'''-H and 2"-C, 1"-H and 2'-C, 1'-H and 3-C, 1''''-H and 28-C, 6''''-H_2 and 1-C of the 3-hydroxy-3-methylglutaroyl

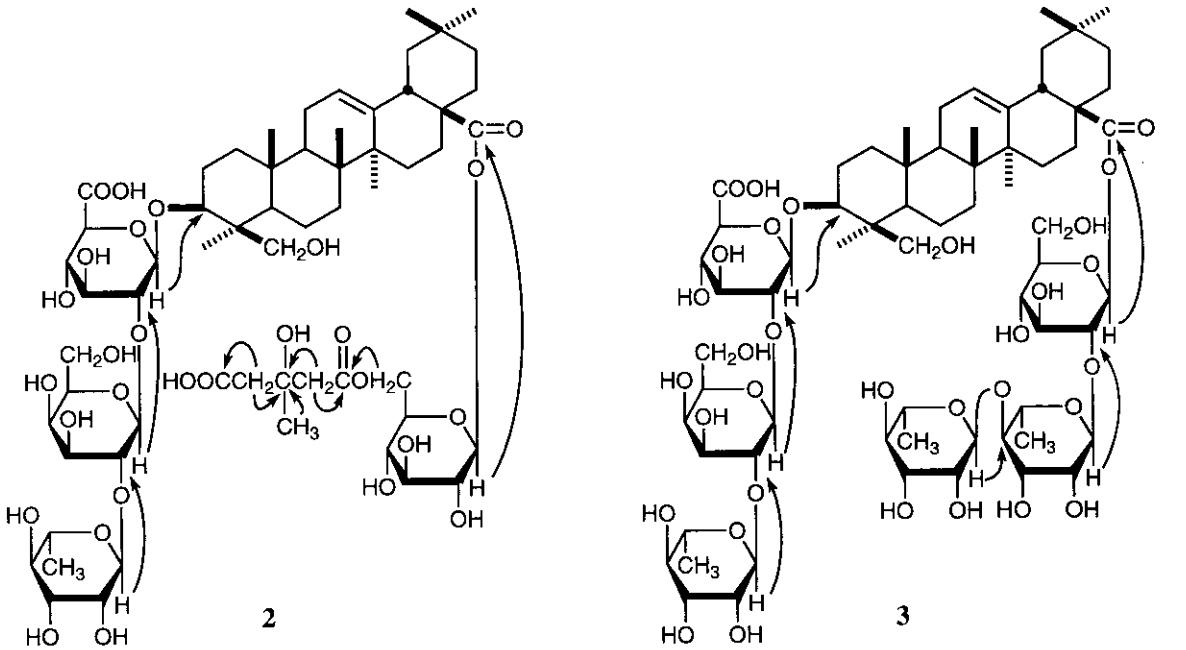


Figure 2. HMBC Correlations of Lablabosides D (**2**) and E (**3**)

(Hmg) group (Figure 2). Furthermore, comparison of the ^{13}C-NMR data for **2** with that for **1** indicated an acetylation shift around the 6""-position of the 28-glucoside moiety (Table 1). On the basis of this evidence, the structure of lablaboside D was determined to be 3-*O*-[α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→2)-β-D-glucopyranosiduronic acid]-28-*O*-[6-*O*-(3-hydroxy-3-methylglutaroyl)-β-D-glucopyranosyl] 24-*epi*-hederagenin (**2**).

Lablaboside E Lablaboside E (**3**) was also isolated as colorless fine crystals of mp 202.8-204.5 °C from aqueous methanol and its IR spectrum showed absorption band at 3419, 2936, 1736, 1716, 1638, and 1075 cm^{-1} ascribable to hydroxyl, ester, and carboxyl groups. The molecular formula $C_{66}H_{106}O_{32}$ was determined from the positive-ion and negative-ion FAB-MS and by high-resolution MS measurement. Namely, a quasimolecular ion peak was observed at *m/z* 1433 $(M+Na)^+$ in the positive-ion FAB-MS of **3**, while the negative-ion FAB-MS showed a quasimolecular ion peak at *m/z* 1409 $(M-H)^-$. In addition, fragment ion peaks at *m/z* 1263 $(M-C_6H_{11}O_4)^-$, *m/z* 1101 $(M-C_{12}H_{21}O_9)^-$, and *m/z* 955 $(M-C_{18}H_{31}O_{13})^-$, which were derived by cleavage of the glycosidic linkage at the 2"- (or 4""'-), 2'-, and 28-positions, respectively, were observed in the negative-ion FAB-MS. Methanolysis of **3** liberated **5** together with the methyl glycosides of glucuronic acid, galactose, rhamnose, and glucose in a 1 : 1 : 3 : 1 ratio.[5] In order to confirm the absolute configuration of the component monosaccharides, **3** was subjected to acid hydrolysis with 5% aqueous sulfuric acid-dioxane (1 : 1, v/v) to furnish D-glucuronic acid, D-galactose, L-rhamnose, and D-glucose, which were identified by gas-liquid chromatography (GLC) analysis of the trimethylsilyl thiazolidine derivatives.[8] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[8] of **3** showed signals due to a 24-*epi*-hederagenin moiety [δ 3.10 (m, 18-H), 3.09, 4.17 (both d, *J*=11.3 Hz, 24-H$_2$), 3.32 (dd, *J*=4.6, 11.6 Hz, 3-H), 5.42 (br s, 12-H)], a β-D-glucuronic acid moiety [δ 4.93 (d, *J*=7.7 Hz, 1'-H)], a β-D-galactopyranosyl moiety [δ 5.70 (d, *J*=7.7 Hz, 1"-H)], three α-L-rhamnopyranosyl moieties [δ 1.62 (d, *J*=6.1 Hz, 6""'-H$_3$), 1.74 (d, *J*=6.1 Hz, 6"'-H$_3$), 1.75 (d, *J*=5.8 Hz, 6""-H$_3$), 6.18 (br s, 1"'-H, 1""'-H), 6.62 (br s, 1""-H)], and a β-D-glucopyranosyl moiety [δ

Table 1. ^{13}C-NMR Data of Lablabosides B (**1**), D (**2**), E (**3**), and F (**4**)

	1[a]	**2**[a]	**3**[a]	**4**[b]		**1**[a]	**2**[a]	**3**[a]	**4**[b]
C-1	38.6	38.6	38.5	38.9	Gal-1"	101.8	101.8	101.8	102.9
2	26.7	26.6	26.6	26.6	2"	77.7	77.7	78.0	77.0
3	91.3	91.3	91.3	90.0	3"	76.6	76.6	76.5	76.1
4	43.9	43.9	43.8	39.7	4"	71.3	71.2	71.2	70.5
5	56.2	56.2	56.1	55.9	5"	76.4	76.4	76.4	76.3
6	18.6	18.6	18.6	18.7	6"	61.8	61.7	61.8	62.0
7	33.3	33.3	33.8	33.6	Rha-1"'	102.3	102.3	102.4	102.2
8	39.9	39.9	39.9	40.0	2"'	72.4	72.4	72.4	72.4
9	47.9	47.9	47.9	48.1	3"'	72.8	72.7	72.8	72.7
10	36.6	36.6	36.6	37.1	4"'	74.4	74.4	74.4	74.4
11	24.0	24.0	24.0	23.4	5"'	69.4	69.4	69.4	69.5
12	122.8	122.8	122.5	122.7	6"'	18.9	18.9	18.9	18.9
13	144.1	144.1	144.3	144.3	Glc-1""	95.8	95.5	94.8	94.9
14	42.2	42.2	42.5	42.5	2""	74.2	73.9	74.9	74.9
15	28.3	28.2	29.0	28.9	3""	78.9	78.6	80.0	79.9
16	23.5	23.4	23.3	23.9	4""	71.3	71.2	71.5	71.5
17	47.1	47.1	47.2	47.2	5""	79.2	76.0	79.0	79.0
18	41.8	41.7	42.1	42.2	6""	62.4	64.2	62.1	62.1
19	46.3	46.3	46.3	46.4	Rha-1""'			101.0	100.9
20	30.8	30.8	30.7	30.7	2""'			72.8	72.8
21	34.1	34.1	34.1	34.1	3""'			73.2	73.2
22	32.6	32.6	32.2	32.1	4""'			80.3	80.1
23	23.0	23.0	22.7	28.7	5""'			67.9	67.9
24	63.6	63.6	63.5	16.9	6""'			19.0	19.0
25	15.7	15.7	15.7	15.6	Rha-1"""			103.7	103.6
26	17.4	17.3	17.4	17.6	2"""			72.2	72.2
27	26.0	26.0	25.9	26.0	3"""			73.1	73.0
28	176.4	176.3	176.3	176.3	4"""			74.1	74.1
29	33.1	33.1	33.0	33.1	5"""			70.4	70.3
30	23.7	23.6	23.7	23.7	6"""			18.4	18.5
GlcA-1'	105.4	105.4	105.4	105.3	Hmg-1		171.6		
2'	77.0	76.9	76.9	79.5	2		46.6		
3'	78.5	78.4	78.4	78.7	3		70.0		
4'	73.8	73.8	73.8	73.4	4		46.3		
5'	77.6	77.6	77.6	77.4	5		174.6		
6'	172.3	172.3	172.3	172.6	6		28.2		

a) : 125 MHz, b) : 68 MHz in pyridine-d_5. Hmg : 3-hydroxy-3-methylglutaroyl

6.10 (d, J=8.2 Hz, 1""-H)]. The carbon signals due to the 3-*O*-glycosidic moiety in the ^{13}C-NMR spectrum of **3** were superimposable on those of **1**, **2**, and dehydrosoyasaponin I.[9] The oligoglycosidic structures bonding to the 3- and 28-positions of the 24-*epi*-hederagenin moiety were determined by the HMBC experiment of **3**, in which long-range correlations

were observed between the following protons and carbons shown in Figure 2 : 1'''-H and 2''-C, 1''-H and 2'-C, 1'-H and 3-C, 1''''''-H and 4''''-C, 1''''-H and 2'''-C, 1'''-H and 28-C. The above evidence led us to formulate the structure of lablaboside E as 3-*O*-[α-L-rhamnopyra-nosyl (1→2)-β-D-galactopyranosyl (1→2)-β-D-glucopyranosiduronic acid]-28-*O*-[α-L-rhamnopyranosyl (1→4)-α-L-rhamno-pyranosyl (1→2)-β-D-glucopyranosyl] 24-*epi*-hederagenin (**3**).

Lablaboside F Lablaboside F (**4**), obtained as colorless fine crystals of mp 200.6-202.0 °C from aqueous methanol, showed absorption bands ascribable to hydroxyl, ester, and carboxyl groups. Here again, the molecular formula $C_{66}H_{106}O_{31}$ of **4** was determined from the quasimolecular ion peaks observed in the negative-ion and positive-ion FAB-MS [*m/z* 1393 $(M-H)^-$, *m/z* 1417 $(M+Na)^+$] and by high-resolution MS measurement. Furthermore, fragment ion peaks at *m/z* 1247 $(M-C_6H_{11}O_4)^-$, *m/z* 1085 $(M-C_{12}H_{21}O_9)^-$, and *m/z* 939 $(M-C_{18}H_{31}O_{13})^-$ were observed in the negative-ion FAB-MS of **4**. Methanolysis of **4** liberated oleanolic acid (**6**)[10] and methyl glycosides of glucuronic acid, galactose, rhamnose, and glucose in a 1 : 1 : 3 : 1 ratio,[5] while acid hydrolysis of **4** furnished D-glucuronic acid, D-galactose, L-rhamnose, and D-glucose, which were identified by the GLC analysis of their trimethylsilyl thiazolidine derivatives.[8] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra[7] of **4** showed signals due to an oleanolic acid moiety [δ 3.10 (m, 18-H), 3.27 (m, 3-H), 5.43 (br s, 12-H)], a β-D-glucuronic acid moiety [δ 5.01 (d, *J*=7.3 Hz, 1'-H)], a β-D-galactopyranosyl moiety [δ 5.62 (d, *J*=7.2 Hz, 1''-H)], three α-L-rhamnopyranosyl moieties [δ 1.62 (d, *J*=6.3 Hz, 6''''-H_3), 1.73 (d, *J*=5.9 Hz, 6'''-H_3), 1.74 (d, *J*=5.6 Hz, 6''''''-H_3), 6.20, 6.22, 6.61 (each br s, 1'''''', 1''', 1''''-H)], and a β-D-glucopyranosyl moiety [δ 6.11 (d, *J*=8.2 Hz, 1''''-H)]. The carbon signals of the 3-*O*-triglycosidic structure in the ^{13}C-NMR spectrum of **4** were superimposable on those of kaikasaponin III[11] and lablaboside A,[1] whereas the carbon signals of the 28-*O*-triglycosidic structure were very similar to those of **3**. Finally, the oligoglycosidic structures of the 3- and 28-positions in **4** were also clarified by HMBC experiment, which showed long-range correlations between the following protons and carbons : 1'''-H and 2''-C, 1''-H and 2'-C, 1'-H and 3-C, 1''''''-H and 4''''-C, 1''''-H and 2'''-C, 1'''-H and 28-C. Consequently, the structure of lablaboside F was determined as 3-*O*-[α-L-rhamnopyranosyl (1→2)-β-D-galactopyranosyl (1→2)-β-D-glucopyranosiduronic acid]-28-*O*-[α-L-rhamnopyranosyl (1→4)-α-L-rhamnopyranosyl (1→2)-β-D-glucopyranosyl] oleanolic acid (**4**).

We are currently engaged in examination of the adjuvant effect of lablabosides, which will be reported in our forthcoming paper.

EXPERIMENTAL

The following instruments were used to obtain physical data : melting points, Yanagimoto micro-melting point apparatus MP-500D (values are uncorrected); specific rotations, Horiba SEPA-300 digital polarimeter (*l*=5 cm); UV spectra, Shimadzu UV-1200 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrophotometer; FAB-MS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JEOL EX-270 (270 MHz) spectrometer and LNM-LA500 (500 MHz) spectrometer; ^{13}C-NMR spectra, JEOL EX-270 (68 MHz) spectrometer and LNM-LA500 (125 MHz) spectrometer with tetramethylsilane as an internal standard.

The following experimental conditions were used for chromatography : ordinary-phase silica gel column chromatography, Silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150-350 mesh); reversed-phase silica gel column chromatography, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100-200 mesh); TLC, pre-coated TLC plate with Silica gel $60F_{254}$ (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 $60F_{254}$ (Merck, 0.25 mm) (reversed phase); reversed-phase HPTLC, pre-coated TLC plate with Silica gel RP-18 $60WF_{254S}$ (Merck, 0.25 mm); detection was done by spraying 1% $Ce(SO_4)_2$-10% aqueous H_2SO_4 and heating.

Isolation of Lablabosides D (2), E (3), and F (4) from the White Seeds of *Dolichos lablab* L.

Lablabosides D (**2**), E (**3**), and F (**4**) were isolated as described earlier.[1]

Lablaboside D (**2**) : Colorless fine crystals from MeOH-H_2O, mp 193.4-195.0 °C, $[\alpha]_D^{26}$ -15.1° (*c*=2.5, MeOH). High-resolution positive-ion FAB-MS : Calcd for $C_{60}H_{94}O_{28}Na$ $(M+Na)^+$: 1285.5829; Found : 1285.5818. IR (KBr) : 3419, 2946, 1741, 1736, 1731, 1724, 1638, 1076 cm^{-1}. ^{1}H-NMR (pyridine-d_5, 500 MHz) δ : 0.68, 0.88, 0.89, 1.00, 1.25, 1.41, 1.73 (3H each, all s, 25, 29, 30, 26, 27, 23, Hmg-6-H_3), 1.75 (3H, d, *J*=6.4 Hz, 6'''-H_3), 3.03, 3.15 (1H each, both d, *J*=14.1 Hz, Hmg-2-H_2), 3.10, 3.17 (1H each, both d, *J*=15.3 Hz, Hmg-4-H_2), 3.16 (1H, m, 18-H), 3.22, 4.23 (1H each, both d, *J*=11.3 Hz, 24-H_2), 3.38 (1H, dd, *J*=4.6, 12.2 Hz, 3-H), 4.76, 4.92 (1H each, both dd-like, 6''''-H_2), 4.96 (1H, d, *J*=7.6 Hz, 1'-H), 5.37 (1H, br s, 12-H), 5.72 (1H, d, *J*=7.6 Hz, 1''-H), 6.22 (1H, d, *J*=7.9 Hz, 1''''-H), 6.22 (1H, br s, 1'''-H). ^{13}C-NMR (pyridine-d_5, 125 MHz) δc : given in Table 1. Negative-ion FAB-MS (*m/z*) : 1261 $(M-H)^-$, 1115 $(M-C_6H_{11}O_4)^-$, 955 $(M-C_{12}H_{19}O_9)^-$, 953 $(M-C_{12}H_{21}O_9)^-$. Positive-mode FAB-MS (*m/z*) : 1285 $(M+Na)^+$.

Lablaboside E (**3**) : Colorless fine crystals from MeOH-H_2O, mp 202.8-204.5 °C, $[\alpha]_D^{25}$ -20.3° (*c*=2.8, MeOH). High-resolution positive-ion FAB-MS : Calcd for $C_{66}H_{106}O_{32}Na$ $(M+Na)^+$: 1433.6565; Found : 1433.6547. IR (KBr) : 3419, 2936, 1736, 1716, 1638, 1075 cm^{-1}. ^{1}H-NMR (pyridine-d_5, 500 MHz) δ : 0.65, 0.79, 0.88, 0.98, 1.30, 1.43 (3H each, all s, 25, 30, 29, 26, 23, 27-H_3), 1.62 (3H, d, *J*=6.1 Hz, 6''''''-H_3), 1.74 (3H, d, *J*=6.1 Hz, 6'''-H_3), 1.75 (3H, d, *J*=5.8 Hz, 6''''-H_3), 3.09, 4.17 (1H each, both d, *J*=11.3 Hz, 24-H_2), 3.10 (1H, m, 18-H), 3.32 (1H, dd, *J*=4.6, 11.6 Hz, 3-H), 4.93 (1H, d, *J*=7.7 Hz, 1'-H), 5.42 (1H, br s, 12-H), 5.70 (1H, d, *J*=7.7 Hz, 1''-H), 6.10 (1H, d, *J*=8.2 Hz, 1''''-H), 6.18 (1H, br s, 1''''''-H), 6.18 (1H, br s, 1'''-H), 6.62 (1H, br s, 1''''-H). ^{13}C-NMR (pyridine-d_5, 125 MHz) δc : given in Table 1. Negative-ion FAB-MS (*m/z*) : 1409 $(M-H)^-$, 1263 $(M-C_6H_{11}O_4)^-$, 1101 $(M-C_{12}H_{21}O_9)^-$, 955 $(M-C_{18}H_{31}O_{13})^-$. Positive-ion FAB-MS (*m/z*) : 1433 $(M+Na)^+$.

Lablaboside F (**4**) : Colorless fine crystals from MeOH-H_2O, mp 200.6-202.0 °C, $[\alpha]_D^{24}$ -46.1° (*c*=1.3, MeOH). High-resolution positive-ion FAB-MS : Calcd for $C_{66}H_{106}O_{31}Na$ $(M+Na)^+$: 1417.6616; Found : 1417.6635. IR (KBr) : 3423, 2940, 1736, 1716, 1638, 1078 cm^{-1}. ^{1}H-NMR (pyridine-d_5, 270 MHz) δ : 0.80, 0.83, 1.04, 1.08, 1.27, 1.40, 1.82 (3H each, all s, 30, 25, 26, 24, 23, 27, 29-H_3), 1.62 (3H, d, *J*=6.3 Hz, 6''''''-H_3), 1.73 (3H, d, *J*=5.9 Hz, 6'''-H_3), 1.74 (3H, d, *J*=5.6 Hz, 6''''-H_3), 3.10 (1H, m, 18-H), 3.27 (1H, m, 3-H), 5.01 (1H, d, *J*=7.3 Hz, 1'-H), 5.43 (1H, br s, 12-H), 5.62 (1H, d, *J*=7.2 Hz, 1''-H), 6.11 (1H, d, *J*=8.2 Hz, 1''''-H), 6.20 (1H, br s, 1''''''-H), 6.22 (1H, br s, 1'''-H) , 6.61 (1H, br s, 1''''-H). ^{13}C-NMR (pyridine-d_5, 68 MHz) δc : given in Table 1. Negative-ion FAB-MS (*m/z*) : 1393 $(M-H)^-$, 1247 $(M-C_6H_{11}O_4)^-$, 1085 $(M-C_{12}H_{21}O_9)^-$, 939 $(M-C_{18}H_{31}O_{13})^-$. Positive-mode FAB-MS (*m/z*) : 1417 $(M+Na)^+$.

Methanolysis of Lablaboside D (2), E (3), and F (4)

A solution of lablabosides (**2**, **3**, **4**, 1.0 mg each) in 9% HCl-dry MeOH (0.5 mL) was heated under reflux for 2 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 and the insoluble portion was removed by filtration. The sapogenol constituent of each product, which was obtained from the filtrate by removal of the solvent under reduced pressure, was shown to be identical with an authentic samples [24-*epi*-hederagenin (**5**)[4] from **2**, **3**; oleanolic acid (**6**) from **4**;] by TLC [$CHCl_3$-MeOH (10 : 1), benzene-acetone (3 : 1), *n*-hexane-AcOEt (1 : 2)] and HPLC [YMC-Pack ODS-A (YMC Co., Ltd., 250 x 4.6 mm i.d.), MeOH-1% aq. AcOH (85 : 15, v/v)] comparisons. The sugar composition of the product was analyzed by GLC. After removal of the solvent *in vacuo* from the filtrate, each residue was dissolved in pyridine (0.01 mL) and the solution was treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, 0.02 mL) for 1 h. The reaction solution was then subjected to GLC analysis to identify the trimethylsilyl (TMS) derivatives of methyl glycoside [methyl glucuronide (i),

methyl galactoside (**ii**), methyl rhamnoside (**iii**), and methyl glucoside (**iv**), from **2**, **3**, and **4**; GLC conditions : CBR-M25-025, 0.25 mm (i.d.) x 25 m capillary column, column temperature 140-280 °C, He flow rate 15 ml/min, t_R : **i** (18.4, 18.6 min), **ii** (18.9, 19.4 min), **iii** (11.5, 13.9 min), **iv** (17.8, 18.2, 19.2 min)].

Alkaline Hydrolysis of Lablaboside D (2)

A solution of lablaboside D (**2**, 2 mg) in 10% aqueous KOH-50% aqueous dioxane (1 : 1, v/v, 0.5 mL) was stirred at 37 °C for 15 min. After removal of the solvent from a part (0.1 mL) of the reaction mixture under reduced pressure, the residue was dissolved in $(CH_2)_2Cl_2$ (5 mL) and the solution was treated with *p*-nitrobenzyl-*N,N'*-diisopropylisourea (10 mg), then the whole was stirred at 80 °C for 1.5 h. The reaction solution was subjected to HPLC analysis to identify the *p*-nitrobenzyl ester of 3-hydroxy-3-methylglutaric acid (**a**), HPLC conditions : column, YMC-Pack ODS (250 x 4.6 mm i.d.); solvent, MeOH-H_2O (60 : 40, v/v); flow rate, 0.9 mL/min; t_R, **a** : 7.1 min.

The rest of the reaction mixture was neutralized with Dowex HCR Wx2 (H^+ form) and the resin was removed by filtration. Evaporation of the solvent from the filtrate under reduced pressure yielded a crude product (20 mg), which was subjected to ordinary-phase silica gel column chromatography [600 mg, $CHCl_3$-MeOH-H_2O (7 : 3 : 1, lower layer)] to give lablaboside B (**1**, 1.5 mg), which was identified an authentic sample by TLC and ^1H- and ^{13}C-NMR with an authentic sample.[1]

Acid Hydrolysis of Lablaboside E (3) and F (4)

A solution of lablabosides (**3**, **4**, 2.0 mg each) in 5% aqueous H_2SO_4-1,4-dioxane (1 : 1, v/v, 1.0 mL) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and the resin was removed by filtration. After removal of the solvent *in vacuo* from the filtrate, the residue was subjected to Sep-Pak C_{18} cartridge with H_2O and MeOH. The H_2O eluate was concentrated and the residue was treated with L-cysteine methyl ester hydrochloride (3.0 mg) in pyridine (0.5 mL) at 60 °C for 1 h. After reaction, the solution was treated with BSTFA (0.02 mL) at 60 °C for 1 h. The supernatant was then subjected to GLC analysis to identify the derivatives of D-glucuronide (i), D-galactoside (ii), L-rhamnoside (iii), and D-glucoside (iv), from **3** and **4**; GLC conditions : Supelco^{TM}-1, 0.25 mm (i.d.) x 30 m capillary column, column temperature 230 °C, He flow rate 15 mL/min, t_R : i (26.4 min), ii (13.8 min), iii (15.5 min), iv (24.2 min).

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