Medicinal Foodstuffs. XXVII.[1] Saponin Constituents of Gotu Kola (2): Structures of New Ursane- and Oleanane-Type Triterpene Oligoglycosides, Centellasaponins B, C, and D, from *Centella asiatica* Cultivated in Sri Lanka

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Ursane- and oleanane-type triterpene oligoglycosides, centellasaponins B, C, and D, were isolated from the aerial parts of *Centella asiatica* (L.) URBAN cultivated in Sri Lanka together with madecassoside, asiaticoside, asiaticoside B, and sceffoleoside A. The chemical structures of centellasaponins B, C, and D were determined on the basis of chemical and physicochemical evidence to be madecassic acid 28-*O*-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside, madasiatic acid 28-*O*-α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside, and 3β,6β,23-trihydroxyolean-12-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside, respectively.

Key words centellasaponin; *Centella asiatica*; medicinal foodstuff; gotu kola; Umbelliferae

In the course of our characterization studies on bioactive constituents in medicinal foodstuffs,[1,2] we have recently found that the methanolic extract from the aerial parts of *Centella asiatica* (L.) URBAN cultivated in Vietnam shows an inhibitory effect against rat lens aldose reductase. Through bioassay-guided separation, two flavonoids were isolated as the inhibitors on aldose reductase. In addition, a new olean-13-ene triterpene, centellasapogenol A, and its oligoglycoside, centellasaponin A (**8**), were isolated together with three known saponins, madecassoside (**4**, principal saponin), asiaticoside (**5**), and sceffoleoside A (**7**).[1]

The Umbelliferae *C. asiatica*, which has many common names including gotu kola, hydrocotyle, Indian pennywort, marsh penny, thick-leaved pennywort, and white rot, has been widely cultivated as a vegetable or spice in China, Southeast Asia, India, Sri Lanka, Africa, and Oceanic countries. In Sri Lankan and Indian Ayurvedic traditional medicine, the aerial parts of *C. asiatica* have been used for skin diseases, syphilis, rheumatism, mental illness, epilepsy, hysteria, dehydration, and in the treatment of leprosy. This natural medicine is used to prompt bladder activity and for physical and mental exhaustion, diarrhea, eye diseases, inflammation, asthma, and high blood pressure in Southeast Asia. To characterize the difference in the traditional effects between the two regions, we have surveyed the chemical constituents of *C. asiatica* in Southeast Asia countries and the India–Sri Lanka area. As a part of this study, we have isolated two new ursane-type triterpene oligoglycosides, centellasaponins B (**1**) and C (**2**), and an oleanane-type triterpene oligoglycoside, centellasaponin D (**3**), together with madecassoside (**4**), asiaticoside (**5**), asiaticoside B (**6**), and sceffoleoside A (**7**) from the aerial parts of *C. asiatica* cultivated in Sri Lanka. In this paper, we present the isolation of the saponin constituents and the structure elucidations of centellasaponins B (**1**), C (**2**), and D (**3**).

The methanolic extract from the aerial parts of *C. asiatica* cultivated in Sri Lanka was partitioned in an ethyl acetate (AcOEt)–water mixture to give an AcOEt-soluble fraction and a H_2O-soluble fraction. The H_2O-soluble fraction was subjected to Diaion HP-20 (H_2O→MeOH) and reversed-phase silica gel (MeOH–H_2O) column chromatography and finally HPLC (YMC-Pack ODS-A, MeOH–H_2O) to give centellasaponins B (**1**, 0.0063%), C (**2**, 0.0084%), and D (**3**, 0.0036%) together with madecassoside[3] (**4**, =asiaticoside A[4] 0.50%), asiaticoside[4,5] (**5**, 0.99%), asiaticoside B[4] (**6**, 0.033%), and sceffoleoside A[6] (**7**, 0.0068%) (Chart 1).

Centellasaponin B (**1**) with positive optical rotation ($[\alpha]_D^{25}$ +13.2°) was obtained as colorless fine crystals with mp 223—224 °C from $CHCl_3$–MeOH. The positive-ion fast atom bombardment (FAB)-MS of **1** showed a quasimolecular ion peak at *m/z* 851 $(M+Na)^+$, while a quasimolecular and fragment ion peaks were observed at *m/z* 827 $(M-H)^-$ and 503 $(M-C_{12}H_{21}O_{10})^-$ in the negative-ion FAB-MS. The molecular formula $C_{42}H_{68}O_{16}$ of **1** was determined from the quasimolecular ion peak $(M+Na)^+$ and by high-resolution MS measurement. The IR spectrum of **1** showed absorption bands at 1736 and 1655 cm^{-1} ascribable to ester carbonyl and olefin functions and strong absorption bands at 3432 and 1073 cm^{-1} suggestive of an oligoglycosidic structure. Alkaline hydrolysis of **1** with 5% aqueous sodium hydroxide (NaOH) furnished madecassic acid (**9**).[7] On acid hydrolysis with 5% aqueous sulfuric acid (H_2SO_4)–1,4-dioxane (1 : 1, v/v), **1** liberated D-glucose, which was identified by GLC analysis of the trimethylsilyl thiazolidine derivative.[8] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra of **1**, which were assigned by various NMR experiments,[9] showed signals assignable to two glucopyranosyl moieties [δ 5.00 (d, J=7.9 Hz, 1″-H), and 6.12 (d, J=8.2 Hz, 1′-H)] together with a madecassic acid moiety [δ 0.85 (d, J=6.9 Hz, 30-H_3), 0.92 (d, J=6.7 Hz, 29-H_3), 1.11, 1.70, 1.71, 1.80 (all s, 27, 26, 24, and 25-H_3), 2.52 (d, J=11.9 Hz, 18-H), 4.02, 4.33 (both d, J=10.3 Hz, 23-H_2), 4.15 (m, 3-H), 4.37 (m, 2-H), 5.02 (br s, 6-H), 5.50 (dd, J=3.4, 3.7 Hz, 12-H)]. As shown in Fig. 1, the oligoglycoside structure and its connectivities to the aglycon in **1** were confirmed by HMBC experiments. That is, long-range correlations were observed between the 1′-proton of the inner glucopyranosyl moiety and the 28-carbon of the madecassic acid moiety, and between the 1″-proton of the

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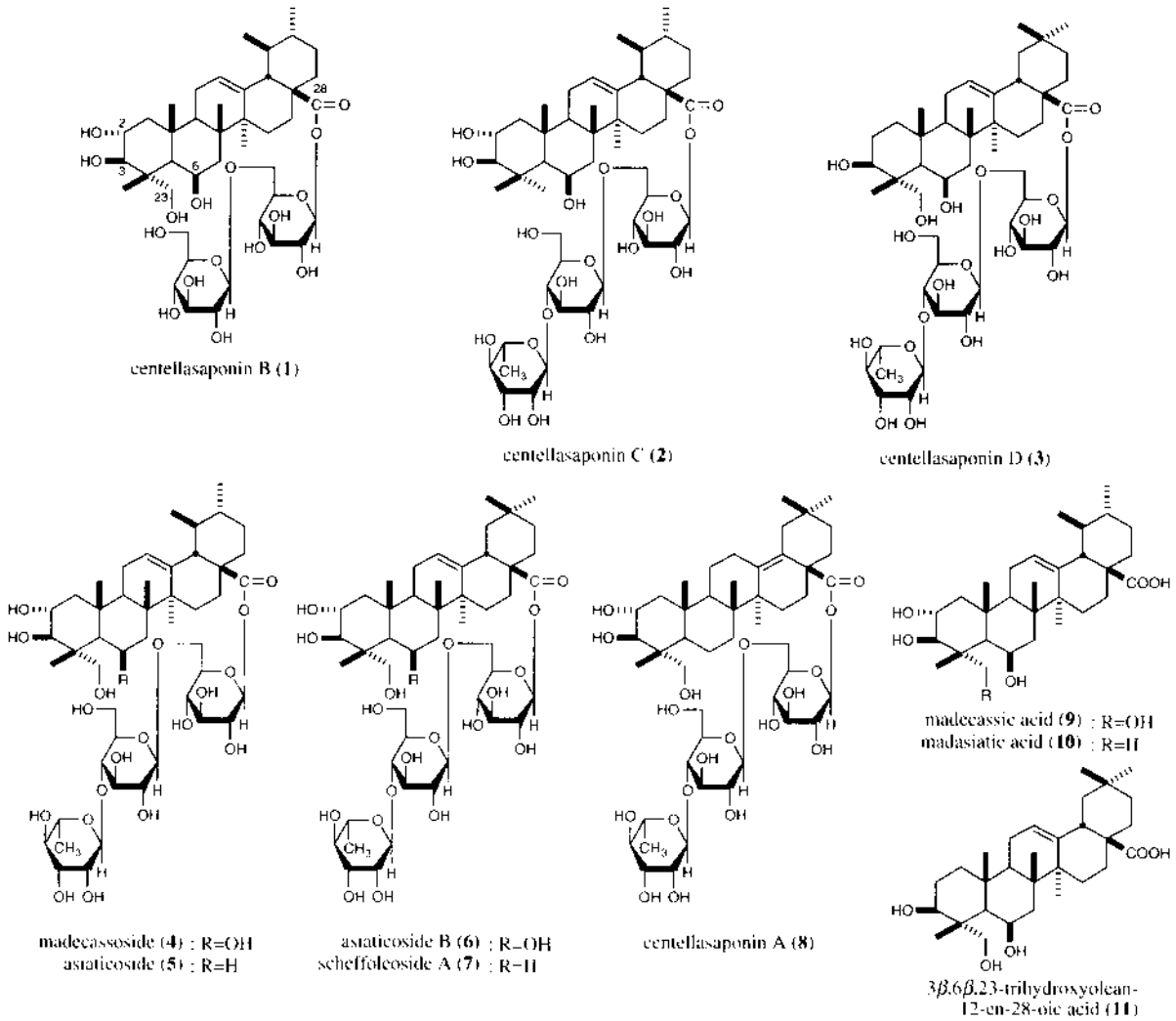

Chart 1

terminal glucopyranosyl moiety and the 6′-carbon of the inner glucopyranosyl moiety. This evidence allowed us to formulate the structure of centellasaponin B as madecassic acid 28-*O*-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranoside (**1**).

Centellasaponin C (**2**) with negative optical rotation ($[\alpha]_D^{25}$ −9.0°) was also isolated as colorless fine crystals with mp 209—210 °C from $CHCl_3$–MeOH. The molecular formula $C_{48}H_{78}O_{19}$ of **2** has been determined from the positive- and negative-ion FAB-MS [*m/z* 981 $(M+Na)^+$, and *m/z* 957 $(M-H)^-$] and by high-resolution MS measurement. Furthermore, fragment ion peaks at *m/z* 811 $(M-C_6H_{11}O_4)^-$ and 487 $(M-C_{18}H_{31}O_{14})^-$, which were thought to be derived by cleavage of the glycosidic linkage at the 4″- and 28-positions, were observed in the negative-ion FAB-MS of **2**. The IR spectrum of **2** showed absorption bands ascribable to hydroxyl, ester carbonyl, and olefin functions at 3431, 1736, and 1655 cm^{-1}. Alkaline hydrolysis of **2** with 5% aqueous NaOH provided madasiatic acid (**10**),[10] while acid hydrolysis of **1** with 5% aqueous H_2SO_4–1,4-dioxane (1 : 1, v/v) liberated D-glucose and L-rhamnose.[8] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra of **2** showed signals assignable to a madasiatic acid moiety [δ 0.90, 0.96 (each d, J=6.4 Hz, 30 and 29-H_3), 1.17, 1.43, 1.67, 1.725, 1.731 (all s, 27, 23, 26, 24, and 25-H_3), 2.53 (d, J=11.6 Hz, 18-H), 3.38 (d, J=10.4 Hz, 3-H), 4.24 (m, 2-H), 4.86 (m, 6-H), 5.52 (dd, J=3.4, 3.7 Hz, 12-H)], two glucopyranosyl moieties [δ 4.94 (d, J=7.9 Hz, 1″-H), and 6.11 (d, J=8.2 Hz, 1′-H)], and a rhamnopyranosyl moiety [δ 1.66 (d, J=6.1 Hz, 6‴-H_3), 5.78 (d, J=1.2 Hz, 1‴-H)]. The proton and carbon signals assignable to the oligoglycoside moiety were superimposable on those of known saponins (**4**—**8**). The oligoglycoside structure and its connectivities to the madasiatic acid moiety in **2** were determined by HMBC experiments, which showed long-range correlations between the 1′-proton and the 28-carbon, between the 1″-proton and the 6′-carbon, and between the 1‴-H and the 4″-carbon (Fig. 1). Consequently, the structure of centellasaponin C was determined to be madasiatic acid 28-*O*-*α*-L-rhamnopyranosyl(1→4)-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranoside (**2**).

Centellasaponin D (**3**) with negative optical rotation ($[\alpha]_D^{25}$ −12.4°) was obtained as colorless fine crystals with mp 202—203 °C from $CHCl_3$–MeOH. The positive- and negative-ion FAB-MS of **3** showed a quasimolecular and fragment ion peaks at *m/z* 981 $(M+Na)^+$, 957 $(M-H)^-$, 811 $(M-C_6H_{11}O_4)^-$, and 487 $(M-C_{18}H_{31}O_{14})^-$. The high-resolution MS analysis revealed the molecular formula of **3** to be $C_{48}H_{78}O_{19}$, and the IR spectrum showed absorption bands

Table 1. ^{13}C-NMR Data for Centellasaponins B (**1**), C (**2**), and D (**3**)

	1	**2**	**3**		**1**	**2**	**3**
C-1	50.5	50.5	41.3	Glc-1′	95.8	95.8	95.8
C-2	69.1	68.8	28.0	2′	73.9	73.8	73.9
C-3	78.5	84.2	73.6	3′	78.4	78.6	78.5
C-4	44.5	40.7	44.0	4′	71.4	71.2	71.1
C-5	48.7	56.5	49.6	5′	77.9	77.9	77.9
C-6	67.7	67.7	67.7	6′	69.7	69.6	69.4
C-7	41.4	41.6	41.2				
C-8	39.7	39.7	39.4	Glc-1″	105.3	104.9	104.8
C-9	48.8	48.8	48.8	2″	75.3	75.3	75.3
C-10	38.1	38.3	37.0	3″	78.6	76.6	76.5
C-11	24.0	24.0	24.0	4″	71.7	78.5	78.5
C-12	126.5	126.5	123.3	5″	78.4	77.1	77.1
C-13	137.9	137.8	143.5	6″	62.8	61.5	61.4
C-14	43.2	43.2	42.8				
C-15	28.8	28.8	28.4	Rha-1‴		102.7	102.7
C-16	24.8	24.8	23.6	2‴		72.5	72.5
C-17	48.6	48.5	47.1	3‴		72.7	72.7
C-18	53.4	53.4	41.8	4‴		74.0	74.0
C-19	39.5	39.5	46.4	5‴		70.3	70.3
C-20	39.1	39.2	30.8	6‴		18.5	18.5
C-21	30.9	30.9	34.1				
C-22	36.9	36.9	32.6				
C-23	66.5	29.3	67.7				
C-24	15.9	18.8	14.7				
C-25	19.4	19.3	17.6				
C-26	19.3	19.3	18.9				
C-27	23.8	23.8	26.1				
C-28	176.3	176.3	176.5				
C-29	17.3	17.4	33.1				
C-30	21.2	21.3	23.7				

Measured in pyridine-d_5 at 125 MHz. Glc: β-D-glucopyranosyl; Rha: α-L-rhamnopyranosyl.

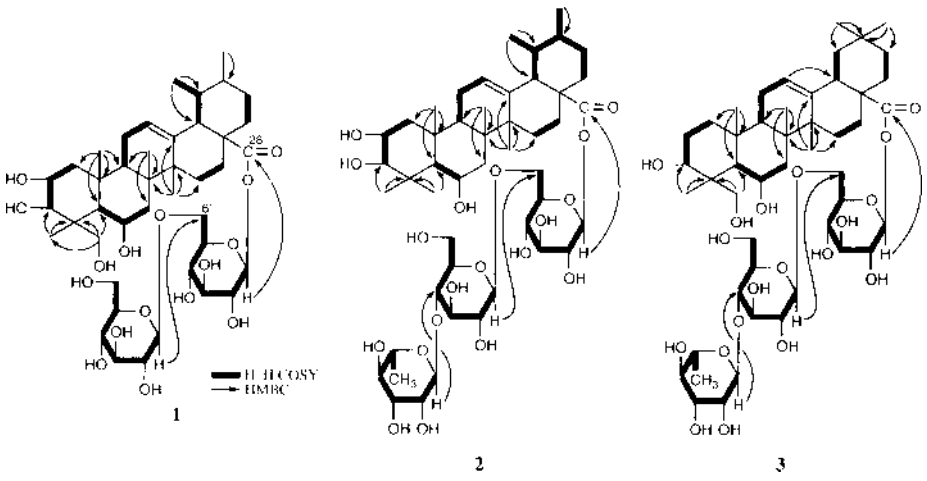

Fig. 1

ascribable to hydroxyl (3432 cm^{-1}), ester carbonyl (1739 cm^{-1}), and olefin (1655 cm^{-1}) functions. Alkaline hydrolysis of **3** liberated 3β,6β,23-trihydroxyolean-12-en-28-oic acid (**11**),[11] while acid hydrolysis of **3** furnished D-glucose and L-rhamnose.[8] The ^{1}H-NMR (pyridine-d_5) and ^{13}C-NMR (Table 1) spectra of **3** showed signals assignable to an aglycon moiety [δ 0.88, 0.90, 1.21, 1.68, 1.69, 1.71 (all s, 30, 29, 27, 26, 25, and 24-H$_3$), 3.21 (dd, J=4.6, 13.7 Hz, 18-H), 4.00, 4.34 (both d, J=10.3 Hz, 23-H$_2$), 4.20 (m, 3-H), 4.99 (br s, 6-H), 5.52 (dd, J=3.4, 3.6 Hz, 12-H)], two glucopyranosyl moieties [δ 4.94 (d, J=7.9 Hz, 1″-H), 6.15 (d, J=8.2 Hz, 1′-H)], and a rhamnopyranosyl moiety [δ 1.66 (d, J=6.1 Hz, 6‴-H$_3$), 5.77 (br s, 1‴-H)]. The proton and carbon signals due to the oligoglycoside moiety in the ^{1}H- and ^{13}C-NMR spectra of **3** were similar to those of **2** and known saponins (**4**—**8**). In the HMBC experiments on **3**, long-range correlations were observed between the following protons and carbons: 1′-H and 28-C; 1″-H and 6′-C; 1‴-H and 4″-C (Fig. 1). These findings led us to elucidate the structures of centellasaponin D to be 3β,6β,23-trihydroxyolean-12-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl(1→4)-β-D-glucopyranosyl(1→6)-β-D-glucopyranoside (**3**).

Experimental

The following instruments were used to obtain physical data: melting points, Yanagimoto micro hot-stage apparatus (uncorrected); specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); ^{1}H-NMR spectra, JNM-LA500 (500 MHz) spectrometers; ^{13}C-NMR spectra, JNM-LA500 (125 MHz) spectrometers with tetramethylsilane as an internal standard; MS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer; HPLC detector, Shimadzu RID-6A refractive index detector.

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

Isolation of Centellasaponins B (1), C (2), and D (3) and Known Saponins (4—7) from the Aerial Parts of *C. asiatica* L. Cultivated in Sri Lanka The dried aerial parts of *C. asiatica* L. (5.0 kg, purchased in Colombo, Sri Lanka) were finely cut and extracted three times with MeOH under reflux. Evaporation of the solvent under reduced pressure provided the MeOH extract (1296 g, 25.9%) and this extract (520 g) was partitioned in an AcOEt–H_2O (1 : 1) mixture. Removal of the solvent under reduced pressure from the AcOEt- and H_2O-soluble fractions yielded 96 g and 424 g of residue, respectively. The H_2O-soluble fraction (424 g) was subjected to Diaion HP-20 column chromatography [Nippon Rensou, Co., Ltd. (4.0 kg), $H_2O \rightarrow$MeOH] to give three fractions [fr. 1 (364.1 g), fr. 2 (5.8 g), fr 3. (54.1 g)]. Fraction 3 (30.6 g) was subjected to reversed-phase silica gel column chromatography [600 g, MeOH–H_2O (40 : 60 → 50 : 50 → 60 : 40 → 70 : 30 → 80 : 20 → 90 : 10) → MeOH] to furnish seven fractions [Fr. 3-1 (6.3 g), fr. 3-2 (3.9 g), fr. 3-3 (16.6 g), fr. 3-4 (0.5 g), fr. 3-5 (0.9 g), fr. 3-6 (1.4 g), fr. 3-7 (1.0 g)]. Fraction 3-2 (354 mg) was purified by HPLC [YMC-Pack ODS-A (250×20 mm i.d., YMC Co., Ltd.), MeOH–H_2O (50 : 50)] to give madecassoside (**4**, 71 mg, 0.068%) and asiaticoside B (**6**, 34 mg, 0.033%). Fraction 3-3 (5.4 g) was purified by HPLC [YMC-Pack ODS-A, MeOH–H_2O (55 : 45)] to give centellasaponin B (**1**, 23 mg, 0.0063%), **4** (1.63 g, 0.44%), asiaticoside (**5**, 3.65 g, 0.99%), and sceffoleoside A (**7**, 25 mg, 0.0068%). Fraction 3-4 (440 mg) was purified by HPLC [YMC-Pack ODS-A, MeOH–H_2O (70 : 30)] to give centellasaponins C (**2**, 96 mg, 0.0084%), and D (**3**, 41 mg, 0.0036%). The known compounds (**4**—**7**) were identified by comparison of their physical data ($[\alpha]_D$, IR, ^{1}H-, ^{13}C-NMR) with reported values.[3—6]

Centellasaponin B (**1**): Colorless fine crystals from $CHCl_3$–MeOH, mp 223—224 °C, $[\alpha]_D^{25}$ +13.2° (c=0.3, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{42}H_{68}O_{16}Na$ $(M+Na)^+$: 851.4406. Found: 851.4417. IR (KBr): 3432, 2928, 1736, 1655, 1073 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.85 (3H, d, J=6.9 Hz, 30-H_3), 0.92 (3H, d, J=6.7 Hz, 29-H_3), 1.11, 1.70, 1.71, 1.80 (3H each, all s, 27, 26, 24, and 25-H_3), 2.52 (1H, d, J=11.9 Hz, 18-H), 4.02, 4.33 (1H each, both d, J=10.3 Hz, 23-H_2), 4.15 (1H, m, 3-H), 4.37 (1H, m, 2-H), 5.00 (1H, d, J=7.9 Hz, 1″-H), 5.02 (1H, br s, 6-H), 5.50 (1H, dd, J=3.4, 3.7 Hz, 12-H), 6.12 (1H, d, J=8.2 Hz, 1′-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Positive-ion FAB-MS: m/z 851 $(M+Na)^+$. Negative-ion FAB-MS: m/z 827 $(M-H)^-$, 503 $(M-C_{12}H_{21}O_{10})^-$.

Centellasaponin C (**2**): Colorless fine crystals from $CHCl_3$–MeOH, mp 209—210 °C, $[\alpha]_D^{25}$ −9.0° (c=0.6, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{48}H_{78}O_{19}Na$ $(M+Na)^+$: 981.5035. Found: 981.5049. IR (KBr): 3431, 2926, 1736, 1655, 1067 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.90, 0.96 (3H each, both d, J=6.4 Hz, 30 and 29-H_3), 1.13 (1H, br s, 5-H), 1.17, 1.43, 1.67, 1.725, 1.731 (3H each, all s, 27, 23, 26, 24, and 25-H_3), 1.66 (3H, d, J=6.1 Hz, 6‴-H_3), 2.53 (1H, d, J=11.6 Hz, 18-H), 3.38 (1H, d, J=10.4 Hz, 3-H), 4.24 (1H, m, 2-H), 4.86 (1H, m, 6-H), 4.94 (1H, d, J=7.9 Hz, 1″-H), 5.52 (1H, dd, J=3.4, 3.7 Hz, 12-H), 5.78 (1H, d, J=1.2 Hz, 1‴-H), 6.11 (1H, d, J=8.2 Hz, 1′-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Positive-ion FAB-MS: m/z 981 $(M+Na)^+$. Negative-ion FAB-MS: m/z 957 $(M-H)^-$, 811 $(M-C_6H_{11}O_4)^-$, 487 $(M-C_{18}H_{31}O_{14})^-$.

Centellasaponin D (**3**): Colorless fine crystals from $CHCl_3$–MeOH, mp 202—203 °C, $[\alpha]_D^{25}$ −12.4° (c=0.3, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{48}H_{78}O_{19}Na$ $(M+Na)^+$: 981.5035. Found: 981.5040. IR (KBr): 3432, 2932, 1739, 1655, 1062 cm^{-1}. ^{1}H-NMR (500 MHz, pyridine-d_5) δ: 0.88, 0.90, 1.21, 1.68, 1.69, 1.71 (3H each, all s, 30, 29, 27, 26, 25, and 24-H_3), 1.66 (1H, d, J=6.1 Hz, 6‴-H_3), 3.21 (1H, dd, J=4.6, 13.7 Hz, 18-H), 4.00, 4.34 (1H each, both d, J=10.3 Hz, 23-H_2), 4.20 (1H, m, 3-H), 4.94 (1H, d, J=7.9 Hz, 1″-H), 4.99 (1H, br s, 6-H), 5.52 (1H, dd, J=3.4, 3.6 Hz, 12-H), 5.77 (1H, br s, 1‴-H), 6.15 (1H, d, J=8.2 Hz, 1′-H). ^{13}C-NMR (125 MHz, pyridine-d_5) δ_C: given in Table 1. Positive-ion FAB-MS: m/z 981 $(M+Na)^+$. Negative-ion FAB-MS: m/z 957 $(M-H)^-$, 811 $(M-C_6H_{11}O_4)^-$, 487 $(M-C_{18}H_{31}O_{14})^-$.

Alkaline Hydrolysis of Centellasaponins B (1), C (2), and D (3) A solution of **1**—**3** (10 mg each) in 5% aqueous NaOH (1.5 ml) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Dowex HCR-W2 (H^+ form) and the resin was removed by filtration. Evaporation of the solvent from the filtrate under reduced pressure yielded a product that was subjected to ordinary-phase silica gel column chromatography [1.0 g, $CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower layer) → MeOH] to give madecassic acid (**9**, 5 mg, 85% from **1**), madasiatic acid (**10**, 5 mg, 92% from **2**), and 3β,6β,23-trihydroxyolean-12-en-28-oic acid (**11**, 4 mg, 78% from **3**), respectively.

Acid Hydrolysis of Centellasaponins B (1), C (2), and D (3) A solution of **1**—**3** (4 mg each) in 5% aqueous H_2SO_4–1,4-dioxane (0.5 ml, 1 : 1, v/v) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and the residue was removed by filtration. After removal of the solvent from the filtrate *in vacuo*, the residue was transferred to a Sep-Pak C18 cartridge with H_2O and MeOH. The H_2O eluate was concentrated and the residue was treated with L-cysteine methyl ester hydrochloride (4 mg) in pyridine (0.5 ml) at 60 °C for 1 h. After reaction, the solution was treated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (0.2 ml) at 60 °C for 1 h. The supernatant was then subjected to GLC analysis to identify the derivatives of D-glucose (i) from **1**—**3**; and L-rhamnose (ii) from **2** and **3**. GLC conditions: column, Supeluco STB™-1, 30 m×0.25 mm (i.d.) capillary column; injector temperature, 230 °C , detector temperature, 230 °C; column temperature, 230 °C; He flow rate, 15 ml/min; t_R, i 24.2 min, ii 15.5 min.

References and Notes

1) Part XXVI: Matsuda H., Morikawa T., Ueda H., Yoshikawa M., *Heterocycles*, **55**, 1499—1504 (2001).
2) *a*) Murakami T., Kishi A., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull*., **48**, 994—1000 (2000); *b*) Yoshikawa M., Uemura T., Shimoda H., Kishi A., Kawahara Y., Matsuda H., *ibid*., **48**, 1039—1044 (2000); *c*) Murakami T., Kohno K., Kishi A., Matsuda H., Yoshikawa M., *ibid*., **48**, 1673—1680 (2000); *d*) Matsuda H., Murakami T., Nishida N., Kageura T., Yoshikawa M., *ibid*., **48**, 1429—1435 (2000); *e*) Murakami T., Emoto A., Matsuda H., Yoshikawa M., *ibid*., **49**, 54—63 (2001); *f*) Murakami T., Kohno K., Matsuda H., Yoshikawa M., *ibid*., **49**, 73—77 (2001); *g*) Murakami T., Hirano K., Yoshikawa M., *ibid*., **49**, 776—779 (2001); *h*) Murakami T, Kishi A., Matsuda H., Hattori M., Yoshikawa M., *ibid*., **49**, 845—848 (2001).
3) Pihas H., Bondiou J. C., *Bull. Soc. Chim. Fr.*, **6**, 1888—1890 (1967).
4) Sahu N. P., Roy S. K., Mahato S. B., *Phytochemistry*, **28**, 2852—2854 (1989).
5) Mahato S. B., Sahu N. P., Luger P., Muller E., *J. Chem. Soc*., *Perkin Trans. II*, **1987**, 1509—1515.
6) Maeda C., Ohtani K., Kasai R., Yamasaki K., Duc N. M., Nham N. T., Cu N. K. Q., *Phytochemistry*, **37**, 1131—1137 (1994).
7) Pinhas H., Billet D., Heitz S., Chaigneau M., *Bull. Soc. Chim. Fr*., **6**, 1890—1895 (1967).
8) Hara S., Okabe H., Mihashi K., *Chem. Pharm. Bull*., **34**, 1843—1845 (1986).
9) The ^{1}H- and ^{13}C-NMR spectra of **1**, **2**, and **3** were assigned on the basis of homo- and heterocorrelation spectroscopy (^{1}H–^{1}H, ^{13}C–^{1}H COSY), homo- and heteronuclear Hartmann-Hahn spectroscopy (^{1}H–^{1}H, ^{13}C–^{1}H HOHAHA), and heteronuclear multiple-bond correlation (HMBC) experiments.
10) Pinhas H., *Bull. Soc. Chim. Fr*., **10**, 3592—3595 (1969).
11) Khan I. A., Sticher O., Rali T., *J. Nat. Prod*., **56**, 2163—2165 (1993).