Studies on Kochiae Fructus. II.[1] On the Saponin Constituents from the Fruit of Chinese *Kochia scoparia* (Chenopodiaceae): Chemical Structures of Kochianosides I, II, III, and IV

Masayuki YOSHIKAWA,*,[a] Yue DAI,[b] Hiromi SHIMADA,[a] Toshio MORIKAWA,[a] Narumi MATSUMURA,[a] Satoshi YOSHIZUMI,[a] Hisashi MATSUDA,[a] Hideaki MATSUDA,[c] and Michinori KUBO[c]

Kyoto Pharmaceutical University,[a] 5, Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607, Japan, China Pharmaceutical University,[b] 24, Tong Xiang, Narying, Jiangsu, China, and Faculty of Pharmaceutical Sciences, Kinki University,[c] 3–4–1, Kowakae, Higashiosaka, Osaka 577, Japan. Received December 27, 1996; accepted March 1, 1997

Four new saponins called kochianosides I, II, III, and IV were isolated from the glycosidic fraction of the fruit of Chinese *Kochia scoparia*, which has exhibited an antipruritogenic effect, together with eight known saponins. The structures of kochianosides I, II, III, and IV were characterized on the basis of physicochemical evidence as 22α-hydroxyoleanolic acid 3-*O*-β-D-glucopyranosiduronic acid, morolic acid 3-*O*-[β-D-glucopyranosyl(1→2)][β-D-xylopyranosyl(1→3)]-β-D-glucopyranosiduronic acid, 3β,12α-dihydroxyolean-28,13β-olide 3-*O*-β-D-xylopyranosyl(1→3)-β-D-glucopyranosiduronic acid, and betulinic acid 3-*O*-β-D-xylopyranosyl(1→3)-β-D-glucopyranosiduronic acid, respectively.

Key words kochianoside; *Kochia scoparia*; antipruritogenic effect; 22α-hydroxyoleanolic acid; saponin; Chenopodiaceae

A Chinese natural medicine, Kochiae Fructus (地膚子), prepared from the fruit of *Kochia scoparia* SCHRAD. (Chenopodiaceae), has been used for the treatment of pain in micturition, rubella, eczema, and cutaneous pruritus in traditional Chinese preparations. In regard to the chemical constituents of *Kochia scoparia*, phytoecdysteroids, saponins, and alkaloids were isolated from the seed, fruit, and aerial part of this plant.[2]

Recently, we found that the methanol extract and glycosidic fraction of Kochiae Fructus have inhibitory effects on the cutaneous pruritus induced by Compound 48/80 or serotonin in mice.[1] As a continuation of our studies on antipruritogenic principles in Kochiae Fructus, we have isolated four new saponins, called kochianosides I (**1**), II (**2**), III (**3**), and IV (**4**), from the fruit of Chinese *Kochia scoparia*, together with momordins I (**11**),[3] Ic (**5**),[4] IIc (**8**),[4] the 2′-*O*-glucopyranosides (**7**,[5] **10**[5]) and 6′-methyl ester (**6**,[6] **9**[3]) of momordins Ic and IIc, and

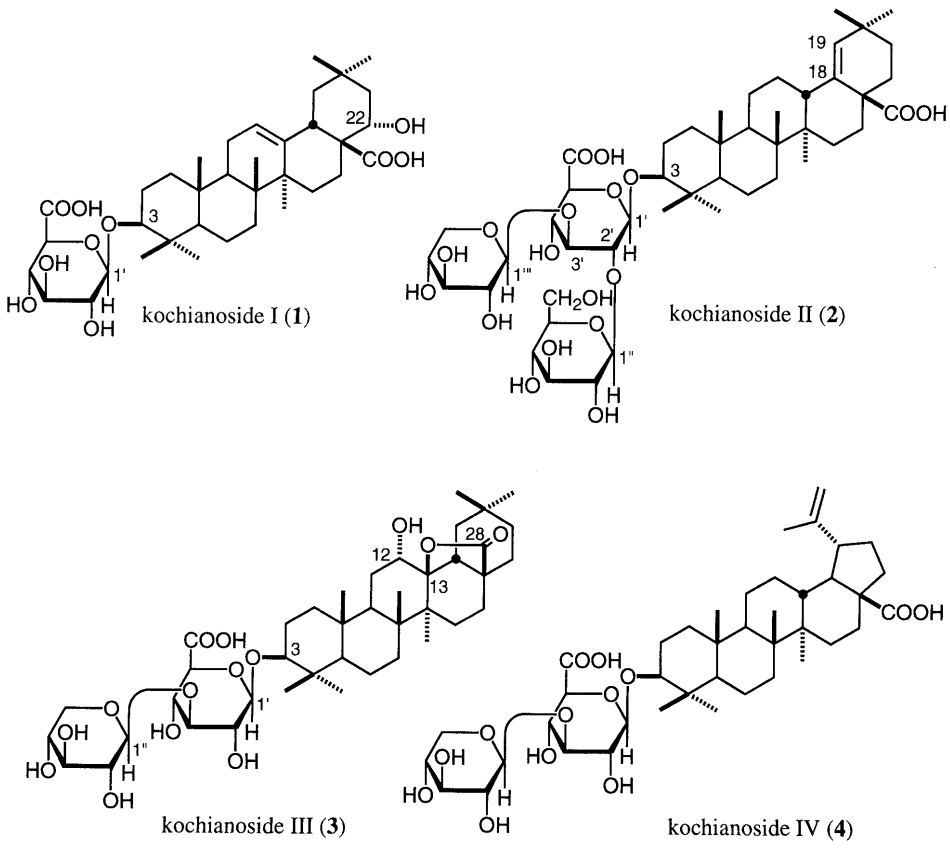

Chart 1

* To whom correspondence should be addressed.

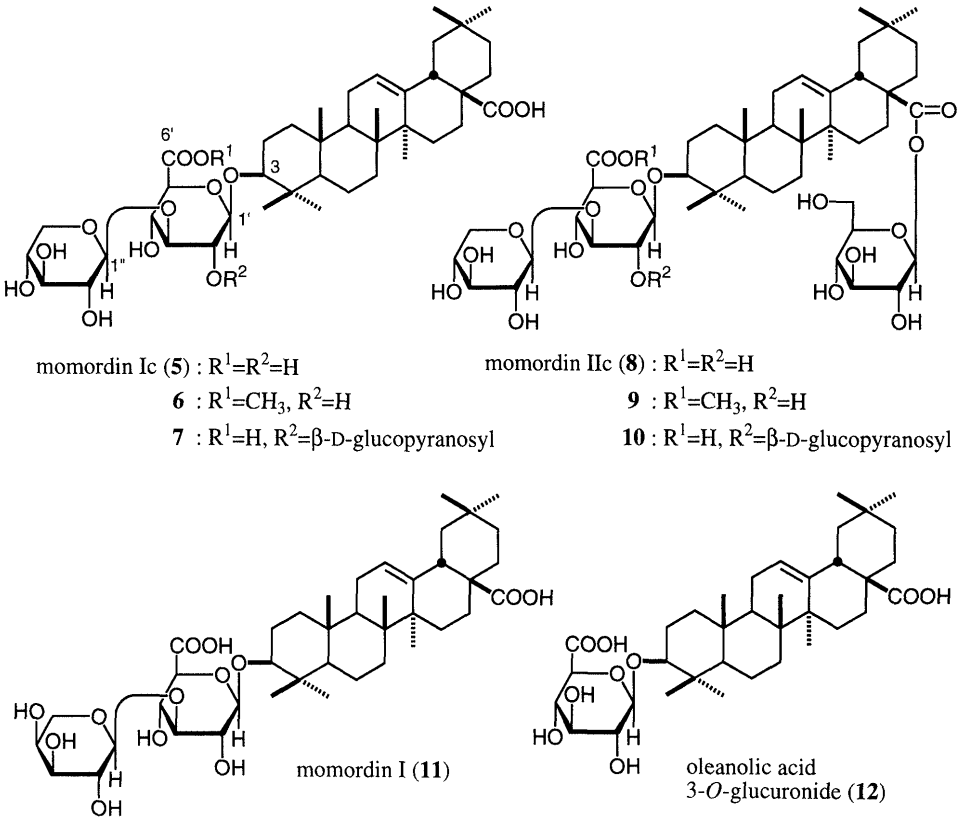

Chart 2

oleanolic acid 3-*O*-glucuronide (**12**).[7] This paper describes the structural elucidation of kochianosides I (**1**), II (**2**), III (**3**), and IV (**4**).

The fruit of Chinese *Kochia scoparia* was extracted with methanol and the extract was partitioned into a mixture of ethyl acetate and water. The water-soluble portion was further extracted with 1-butanol. The 1-butanol-soluble portion (so-called glycosidic fraction), which was found to exhibit a potent antipruritogenic effect,[1] was subjected to ordinary-phase and reversed-phase silica-gel column chromatography, and finally HPLC, to give **1** (0.00055%), **2** (0.00020%), **3** (0.00024%), **4** (0.00034%), **5** (0.45%), **6** (0.0071%), **7** (0.17%), **8** (0.14%), **9** (0.0016%), **10** (0.024%), **11** (0.0021%), and **12** (0.00063%).

Kochianoside I (**1**) was isolated as colorless fine crystals with a mp of 172—175 °C from a mixture of chloroform and methanol. The IR spectrum of **1** showed absorption bands at 3432, 1726, 1701, 1665, and 1081 cm^{-1}, ascribable to hydroxyl, carboxyl, and olefin groups. The molecular formula $C_{36}H_{56}O_{10}$ was determined by its positive and negative-mode FAB-MS and by high-resolution MS measurement. Thus, in the positive-mode FAB-MS of **1**, a quasimolecular ion peak was observed at *m/z* 671 $(M+Na)^+$, while the negative-mode FAB-MS of **1** showed a quasimolecular ion peak at *m/z* 647 $(M-H)^-$. Furthermore, a fragment ion peak was observed at *m/z* 471 $(M-C_6H_9O_6)^-$ in the negative-mode FAB-MS of **1**. The ^{1}H-NMR (pyridine-d_5) spectrum[8] of **1** showed signals assignable to a *β*-D-glucopyranosiduronic acid moiety [*δ* 5.01 (d, *J*=7.7 Hz, 1′-H)] and a sapogenol moiety [*δ* 1.02 (s, 29-H_3), 1.12 (s, 30-H_3), 3.34 (m, 18-H), 3.40 (dd, *J*=4.6, 11.9 Hz, 3-H), 4.53 (dd, *J*=4.6, 10.7 Hz, 22-H), and 5.46 (br s, 12-H)]. The ^{13}C-NMR (Table 1) spectrum[8] of **1** was superimposable on that of oleanolic acid 3-*O*-*β*-D-glucuronide (**12**), except for some signals due to the 22-hydroxy group of **1**. In the heteronuclear multiple bond correlations (HMBC) experiment of **1**, long-range correlations were observed between the 1′-proton and the 3-carbon, between the 29,30-methyl protons and the 19,20,21-carbons, and between the 21-methylene protons and the 22-carbon. The stereostructure of the 22-carbon was characterized by considering the coupling pattern (dd, *J*=4.5, 10.7 Hz) of the 22-axial proton and by the ^{1}H-NMR nuclear Overhauser and exchange spectroscopy (NOESY) experiment of **1**, which showed nuclear Overhauser effect (NOE) correlations between the 30-methyl and the 22-proton, between the 18-proton and the 22-proton, and between the 30-methyl and the 18-proton. Finally, comparison of the ^{1}H-NMR and ^{13}C-NMR data for **1** with those for 22*β*-hydroxyoleanene and 22*α*-hydroxyhederagenin derivatives[9] led us to formulate the structure of kochianoside I (**1**), as shown.

Kochianoside II (**2**) was also isolated as colorless fine crystals of mp 203—204 °C. The IR spectrum of **2** showed absorption bands at 1736, 1701, and 1655 cm^{-1} due to carboxyl and olefin groups, and strong absorption bands at 3436 and 1078 cm^{-1}, suggestive of the oligoglycosidic structure. The positive and negative-mode FAB-MS of **2** showed quasimolecular ion peaks at *m/z* 949 $(M+Na)^+$ and *m/z* 925 $(M-H)^-$, respectively, and the molecular formula $C_{47}H_{74}O_{18}$ was determined by high-resolution MS analysis of the quasimolecular ion peak $(M+Na)^+$. Furthermore, fragment ion peaks at *m/z* 793 $(M-C_5H_9O_4)^-$, *m/z* 763 $(M-C_6H_{11}O_5)^-$, and *m/z* 631

Table 1. ^{13}C-NMR Data of Kochianosides I (**1**), II (**2**), III (**3**), and IV (**4**) (125 MHz, Pyridine-d_5)

	1	2	3	4
C-1	38.7	39.0	39.0	39.0
C-2	26.6	26.8	26.7	26.8
C-3	89.1	89.6	89.4	89.4
C-4	39.6	39.7	39.6	39.7
C-5	55.8	55.9	55.8	55.9
C-6	18.5	18.4	18.0	18.5
C-7	33.1	35.0	34.5	34.9
C-8	40.0	41.0	42.8[a]	41.2
C-9	48.0	51.4	45.0	50.9
C-10	37.0	37.0	36.5	37.2
C-11	23.8	21.3	29.3	21.2
C-12	122.9	26.5	75.8	26.1
C-13	144.2	41.6	91.4	38.7
C-14	42.6	43.0	42.7[a]	42.9
C-15	28.0	30.0	28.3	30.3
C-16	17.0	34.4	21.8	32.9
C-17	53.1	48.6	45.0	56.7
C-18	43.5	139.0	51.8	49.9
C-19	46.1	132.0	39.7	47.8
C-20	31.5	32.4	31.8	151.3
C-21	43.3	34.2	34.6	31.3
C-22	71.5	34.2	28.4	37.6
C-23	28.3	27.8	28.0	28.0
C-24	17.0	16.5	16.7	16.8
C-25	15.4	16.8	16.6	16.3
C-26	17.4	16.3	18.8	16.4
C-27	26.8	15.3	19.0	14.9
C-28	179.4	179.1	179.6	178.8
C-29	33.4	30.8	33.3	109.9
C-30	25.2	29.3	23.9	19.5
GlcA-1′	107.2	105.5	106.9	106.8
GlcA-2′	75.6	79.3	74.6	74.6
GlcA-3′	78.2	85.4	86.6	86.6
GlcA-4′	73.4	71.5	71.4	71.4
GlcA-5′	77.8	77.4	77.5	77.5
GlcA-6′	172.7	171.9	172.0	172.0
Glc-1″		103.8		
Glc-2″		76.5		
Glc-3″		78.5		
Glc-4″		72.4		
Glc-5″		78.0		
Glc-6″		63.3		
Xyl-1‴		105.0	106.2	106.2
Xyl-2‴		75.3	75.2	75.3
Xyl-3‴		78.6	78.1	78.1
Xyl-4‴		70.9	71.0	71.0
Xyl-5‴		67.4	67.4	67.4

a) Assignment may be interchangeable.

$(M-C_{11}H_{19}O_9)^-$ were observed in the negative-mode FAB-MS. The ^{1}H-NMR (pyridine-d_5) spectrum[8] of **2** showed signals due to a trisaccharide moiety [δ 5.01 (d, J=7.9 Hz, 1′-H), 5.75 (d, J=7.6 Hz, 1″-H), and 5.43 (d, J=7.9 Hz, 1‴-H)] and a sapogenol moiety [δ 1.07 (s, 30-H_3), 1.13 (s, 29-H_3), 2.68 (dd-like, 13-H), 3.30 (dd, J=4.2, 11.9 Hz, 3-H), and 5.30 (br s, 19-H)]. The carbon signals of the trisaccharide moiety in the ^{13}C-NMR (Table 1) spectrum[8] of **2** were superimposable on those of **7** and **10**, whereas the carbon signals due to the sapogenol moiety of **2** were very similar to those of morolic acid derivatives.[10] The HMBC experiment of **2** showed long-range correlations between the following protons and carbons: [1‴-H and 3′-C, 1″-H and 2′-C, 1′-H and 3-C, 30-H_3 and 19, 20-C, 29-H_3 and 19, 20-C, 19-H and 18, 20-C]. On the basis of the above mentioned evidence, the structure of kochianoside II (**2**) was characterized as shown.

Kochianoside III (**3**), obtained as colorless fine crystals of mp 224—225 °C, showed absorption bands at 3456, 1736, 1719, and 1040 cm^{-1} due to hydroxyl, carboxyl, and lactone groups in the IR spectrum, and the molecular formula $C_{41}H_{64}O_{14}$ was determined from its positive and negative-mode FAB-MS and by high-resolution MS measurement. Thus, in the positive-mode FAB-MS of **3**, a quasimolecular ion peak was observed at m/z 803 $(M+Na)^+$, while the negative-mode FAB-MS of **3** showed a quasimolecular ion peak at m/z 779 $(M-H)^-$ in addition to fragment ion peaks at m/z 647 $(M-C_5H_9O_4)^-$ and m/z 471 $(M-C_{11}H_{17}O_{10})^-$. The ^{1}H-NMR (pyridine-d_5) spectrum[8] of **3** showed signals assignable to the disaccharide moiety [δ 4.95 (d, J=7.9 Hz, 1′-H) and 5.30 (d, J=7.6 Hz, 1″-H)] and the sapogenol moiety [δ 2.28 (m, 18-H), 3.29 (dd, J=3.9, 11.6 Hz, 3-H), and 4.12 (m, 12-H)]. The carbon signals of the disaccharide moiety in the ^{13}C-NMR (Table 1) spectrum[8] of **3** closely resembled those of **5** and **8**, whereas the carbon signals due to the sapogenol moiety were very similar to those of a 3β,12α-dihydroxyolean-28,13β-olide derivative.[11] In the HMBC experiment of **3**, long-range correlations were observed between the following protons and carbons: [1″-H and 3′-C, 1′-H and 3-C, 12-H and 13-C, 18-H and 13, 17-C]. Consequently, the structure of kochianoside III (**3**) was determined as shown.

Kochianoside IV (**4**) was also obtained as colorless fine crystals of mp 212—214 °C and its IR spectrum showed absorption bands due to hydroxyl, carboxyl, and olefin groups. Here again, the molecular formula $C_{41}H_{64}O_{13}$ of **4** was clarified from quasimolecular ion peaks observed in the positive and negative-mode FAB-MS and by high-resolution MS measurement. Namely, quasimolecular ion peaks were observed at m/z 787 $(M+Na)^+$ in the positive-mode FAB-MS of **4**, while the negative-mode FAB-MS of **4** showed a quasimolecular ion peak at m/z 763 $(M-H)^-$ and a fragmentation peak at m/z 631 $(M-C_5H_9O_4)^-$. The ^{1}H-NMR (pyridine-d_5) spectrum[8] of **4** showed signals assignable to the disaccharide moiety [δ 5.00 (d, J=7.3 Hz, 1′-H) and 5.30 (d, J=7.3 Hz, 1″-H)] and the sapogenol moiety [δ 1.78 (m, 18-H), 2.72 (dt-like, 13-H), 3.39 (dd, J=4.9, 11.6 Hz, 3-H), 3.50 (m, 19-H), and 4.74, 4.92 (both br s, 30-H_2)]. The carbon signals of the disaccharide moiety in the ^{13}C-NMR (Table 1) spectrum[8] of **4** were superimposable on those of **3**, **5**, and **8**, whereas the carbon signals of the sapogenol moiety were very similar to those of betulinic acid derivatives.[12] Finally, the HMBC experiment of **4** showed long-range correlations between the following protons and carbons [1″-H and 3′-C, 1′-H and 3-C, 18-H and 13, 17, 19-C, 29-H_3, 30-H_2 and 19-C]. Based on this evidence, the structure of kochianoside IV (**4**) was clarified as shown.

Experimental

The instruments used to obtain physical data and experimental conditions for chromatography were the same as described in our previous paper.[13]

Extraction and Isolation The fruit of *Kochia scoparia* (10 kg, purchased from Tochimototenkaido Co., Ltd., Osaka) was powdered and extracted three times with methanol under reflux. Evaporation of the solvent from the extract solution under reduced pressure gave the methanol extract (1060 g). This extract (850 g) was partitioned into an AcOEt–H_2O (1 : 1) mixture. The aqueous layer was further extracted with 1-butanol. Removal of the solvent *in vacuo* from the 1-butanol-soluble portion gave the 1-butanol extract (359 g). The 1-butanol extract (242 g) was subjected to ordinary-phase silica-gel column chromatography [3.4 kg, $CHCl_3$–MeOH–H_2O = 7 : 3 : 1 (lower layer)→6 : 4 : 1] to give seven fractions [fraction 1 (65 g), fraction 2 (19 g), fraction 3 (25 g), fraction 4 (41 g), fraction 5 (36 g), fraction 6 (16 g), fraction 7 (10 g)]. Fraction 3 (25 g) was separated by reversed-phase silica-gel column chromatography (150 g, MeOH–H_2O) and then HPLC (YMC-pack ODS, 80% MeOH–1% AcOH, 75% MeOH–1% AcOH) to give kochianosides I (**1**, 30 mg), III (**3**, 13 mg), IV (**4**, 18 mg), momordin Ic (**5**, 7.7 g), the 6′-methyl esters (**6**: 100 mg, **9**: 87 mg) of momordins Ic and IIc and oleanolic acid 3-*O*-glucuronide (**12**, 12 mg). Fraction 4 (3.0 g) was subjected to HPLC (YMC-pack ODS, 80% MeOH–1% AcOH, 75% MeOH–1% AcOH) to furnish momordins Ic (**5**, 672 mg) and IIc (**8**, 359 mg) and the 2′-*O*-glucopyranoside (**7**, 456 mg) of momordin Ic. Fraction 5 (3.0 g) was purified by HPLC (YMC-pack ODS, 85% MeOH–1% AcOH) to provide **5** (412 mg), **6** (7 mg), **7** (205 mg), **8** (187 mg), the 2′-*O*-glucopyranoside (**10**, 102 mg) of momordin IIc, and momordin I (**11**, 9 mg). HPLC separation (YMC-pack ODS, 80% MeOH–1% AcOH, 70% MeOH–1% AcOH) of fraction 6 (5 g) furnished **5** (348 mg), **7** (125 mg), **8** (77 mg), and **10** (24 mg). Eight known triterpene glycosides (**5**—**10**) were identified by comparison of their physicochemical data with the reported values.[3-7]

Kochianoside I (**1**): Colorless fine crystals from $CHCl_3$–MeOH, mp 172—175 °C, $[\alpha]_D^{24}$ +84.4° (c = 0.1, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{36}H_{56}NaO_{10}$ $(M+Na)^+$: 671.3772. Found: 671.3754. IR (KBr, cm^{-1}): 3432, 2932, 2876, 1726, 1701, 1665, 1081. ^{1}H-NMR (pyridine-d_5) δ: 0.82, 0.98, 1.02, 1.02, 1.12, 1.31, 1.36 (3H each, all s, 25, 24, 26, 29, 30, 23, 27-H_3), 3.34 (1H, m, 18-H), 3.40 (1H, dd, J = 4.6, 11.9 Hz, 3-H), 4.53 (1H, dd, J = 4.6, 10.7 Hz, 22-H), 5.01 (1H, d, J = 7.7 Hz, 1′-H), 5.46 (1H, br s, 12-H). ^{13}C-NMR (pyridine-d_5) δ_C: given in Table 1. Negative-mode FAB-MS (m/z): 647 $(M-H)^-$, 471 $(M-C_6H_9O_6)^-$. Positive-mode FAB-MS (m/z): 671 $(M+Na)^+$.

Kochianoside II (**2**): Colorless fine crystals from $CHCl_3$–MeOH, mp 203—204 °C, $[\alpha]_D^{23}$ −9.6° (c = 0.1, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{47}H_{74}NaO_{18}$ $(M+Na)^+$: 949.4773. Found: 949.4789. IR (KBr, cm^{-1}): 3436, 2940, 2891, 1736, 1701, 1655, 1078. ^{1}H-NMR (pyridine-d_5) δ: 0.71, 0.98, 0.98, 1.05, 1.07, 1.13, 1.23 (3H each, all s, 25, 26, 27, 24, 30, 29, 23-H_3), 2.68 (1H, dd-like, 13-H), 3.30 (1H, dd, J = 4.2, 11.9 Hz, 3-H), 5.01 (1H, d, J = 7.9 Hz, 1′-H), 5.30 (1H, br s, 19-H), 5.43 (1H, d, J = 7.9 Hz, 1‴-H), 5.75 (1H, d, J = 7.6 Hz, 1″-H). ^{13}C-NMR (pyridine-d_5) δ_C: given in Table 1. Negative-mode FAB-MS (m/z): 925 $(M-H)^-$, 793 $(M-C_5H_9O_4)^-$, 763 $(M-C_6H_{11}O_5)^-$, 631 $(M-C_{11}H_{19}O_9)^-$. Positive-mode FAB-MS (m/z): 949 $(M+Na)^+$.

Kochianoside III (**3**): Colorless fine crystals from $CHCl_3$–MeOH, mp 224—225 °C, $[\alpha]_D^{26}$ +47.9° (c = 0.6, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{41}H_{64}NaO_{14}$ $(M+Na)^+$: 803.4194. Found: 803.4207. IR (KBr, cm^{-1}): 3456, 2932, 2900, 1736, 1719, 1655, 1040. ^{1}H-NMR (pyridine-d_5) δ: 0.80, 0.82, 0.92, 0.98, 1.28, 1.29, 1.62 (3H each, all s, 25, 30, 29, 24, 27, 23, 26-H_3), 2.28 (1H, m, 18-H), 3.29 (1H, dd, J = 3.9, 11.6 Hz, 3-H), 4.12 (1H, m, 12-H), 4.95 (1H, d, J = 7.9 Hz, 1′-H), 5.30 (1H, d, J = 7.6 Hz, 1″-H). ^{13}C-NMR (pyridine-d_5) δ_C: given in Table 1. Negative-mode FAB-MS (m/z): 779 $(M-H)^-$, 647 $(M-C_5H_9O_4)^-$, 471 $(M-C_{11}H_{17}O_{10})^-$. Positive-mode FAB-MS (m/z): 803 $(M+Na)^+$.

Kochianoside IV (**4**): Colorless fine crystals from $CHCl_3$–MeOH, mp 212—214 °C, $[\alpha]_D^{21}$ +15.5° (c = 0.8, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{41}H_{64}NaO_{13}$ $(M+Na)^+$: 787.4245. Found: 787.4219. IR (KBr, cm^{-1}): 3453, 2962, 2898, 1736, 1719, 1655, 1040. ^{1}H-NMR (pyridine-d_5) δ: 0.74, 0.96, 1.02, 1.12, 1.28, 1.78 (3H each, all s, 25, 24, 26, 27, 23, 29-H_3), 1.78 (1H, m, 18-H), 2.72 (1H, dt-like, 13-H), 3.39 (1H, dd, J = 4.9, 11.6 Hz, 3-H), 3.50 (1H, m, 19-H), 4.74, 4.92 (both br s, 30-H_2), 5.00 (1H, d, J = 7.3 Hz, 1′-H), 5.30 (1H, d, J = 7.3 Hz, 1″-H). ^{13}C-NMR (pyridine-d_5) δ_C: given in Table 1. Negative-mode FAB-MS (m/z): 763 $(M-H)^-$, 631 $(M-C_5H_9O_4)^-$. Positive-mode FAB-MS (m/z): 787 $(M+Na)^+$.

References and Notes

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