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This article is dedicated to Professor Satoshi Ōmura in celebration of his 2015 Nobel Prize.

Note

Structures of Aromatic Glycosides from the Seeds of *Cassia auriculata*

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A new benzocoumarin glycoside, cassiaglycoside I (1), a new naphthol glycoside, cassiaglycoside II (2), a new chromon glycoside, cassiaglycoside III (3), a new phenylethyl glycoside, cassiaglycoside IV (4), were isolated from the seeds of *Cassia auriculata*, together with seven known constituents. The chemical structures of four new constituents were characterized on the basis of chemical and physicochemical evidence.

Key words *Cassia auriculata*; Leguminosae; aromatic glycoside; Ayurvedic traditional medicine

Cassia auriculata LINN. belongs to the family Leguminosae and is used for the treatment of diabetes, rheumatism, asthma, and skin diseases in the Ayurvedic traditional medicine. In the course of our studies on the constituents of traditional Asian medicine,^{1–11)} we previously reported the isolation of flavonols and flavanol dimers from the leaves of *C. auriculata*.¹²⁾ In addition, several diterpene glycosides were isolated from the seeds of *C. auriculata*.¹³⁾ As a continuing study, a new benzocoumarin glycoside, cassiaglycoside I (1), a new naphthol glycoside, cassiaglycoside II (2), a new chromon glycoside, cassiaglycoside III (3), and a new phenylethyl glycoside, cassiaglycoside IV (4), were isolated from the seeds of *C. auriculata*, together with seven known constituents. This paper deals with the structure elucidation of four new constituents (1–4).

The MeOH extract (11.0% from the seeds of *C. auriculata*) was partitioned into an *n*-hexane–H₂O (1:1, v/v) mixture to furnish *n*-hexane- and H₂O-layers, respectively. The H₂O layer was subjected to Diaion HP-20 column chromatography to give H₂O- and MeOH-eluted fractions as reported previously.¹³⁾ The MeOH-eluted fraction was furthermore subjected to normal-phase and reversed-phase silica gel column chromatography and repeated HPLC to give cassiaglycoside I (1, 0.00048%), cassiaglycoside II (2, 0.00040%), cassiaglycoside III (3, 0.00040%), and cassiaglycoside IV (4, 0.0014%), together with avaroside I (5, 0.039%),¹²⁾ aloesol (6, 0.00052%),¹⁴⁾ benzyl *O*- α -L-arabinopyranosyl(1 $\rightarrow$ 6)- β -D-glucopyranoside (7, 0.00040%),¹⁵⁾ benzyl *O*- β -D-apiofuranosyl(1 $\rightarrow$ 2)- β -D-glucopyranoside (8, 0.0014%),¹⁶⁾ 2-phenylethyl D-rutinoside (9, 0.00034%),¹⁷⁾ 2-phenylethyl *O*- α -L-arabinopyranosyl(1 $\rightarrow$ 6)- β -D-glucopyranoside (10, 0.0012%),¹⁸⁾ and sayaendoside (11, 0.0048%).¹⁹⁾ (Fig. 1)

Structures of Cassiaglycosides I–IV (1–4)

Cassiaglycoside I (1) was obtained as a yellow powder with a negative optical rotation ($[\alpha]_D^{27}$ –125.7 in MeOH). FAB-MS in

the positive-ion mode revealed a quasimolecular ion (M+H)⁺ at *m/z* 699 from which the molecular formula C₃₁H₃₈O₁₈ was determined via high resolution (HR)-MS and ¹³C-NMR data. Acid hydrolysis of 1 liberated D-apiose, D-glucose, and L-rhamnose, which were identified by HPLC analysis using an optical rotation detector. The ¹H- (DMSO-*d*₆) and ¹³C-NMR (Table 1) spectra of 1, which were assigned by various NMR experiments,²⁰⁾ showed signals ascribable to a methyl [δ 2.69 (3H, s, CH₃-5)], an olefinic proton [δ 5.95 (1H, s, H-3)], three aromatic protons [δ 6.54, 6.56 (1H each, both d, *J*=2.2 Hz, H-9, 7), 7.22 (1H, s, H-6)], a β -D-apiofuranosyl moiety [δ 5.44 (1H, d, *J*=2.8 Hz, H-1'')], an α -L-rhamnopyranosyl moiety [δ 4.53 (1H, brs, H-1'''), 1.02 (3H, d, *J*=6.4 Hz, H-6'''), and a β -D-glucopyranosyl moiety [δ 5.48 (1H, d, *J*=7.6 Hz, H-1')]. The proton and carbon signals of 1 in the ¹H- and ¹³C-NMR spectra were superimposable on those of 5,¹²⁾ except for the signals around the 2-position of the glucose, while the proton and carbon signals due to the apiofuranosyl part were very similar to those of 8¹⁶⁾ and 11.¹⁹⁾ In the heteronuclear multiple bond connectivity (HMBC) experiment, long-range correlations were observed between the following protons and carbons: H-3 and C-2, 4, 4a; H-6 and C-4a, 6a, 7, 10a, CH₃-5; H-7 and C-9, 10a; H-9 and C-7, 10, 10a; CH₃-5 and C-4a, 5, 6; OH-8 and C-7, 8; OH-10 and C-10, 10a; H-1' and C-4; H-6' and C-1''; H-1'' and C-2', 4'', H-2'' and C-5'', H-4'' and C-1'', 3'', 5'', H-5'' and C-2'', 4'', H-1''' and C-6' (Fig. 2). Furthermore, in the nuclear Overhauser effect spectroscopy (NOESY) experiment, nuclear Overhauser effect (NOE) correlations were observed between the following proton pairs: H-6 and H-7, CH₃-5; H-1' and H-3 (Fig. 2). Consequently, the chemical structure of cassiaglycoside I (1) was determined to be pannorin 4-*O*- β -D-apiofuranosyl(1 $\rightarrow$ 2)- α -L-rhamnopyranosyl(1 $\rightarrow$ 6)- β -D-glucopyranoside.

Cassiaglycoside II (2) was obtained as an orange powder with negative optical rotation ($[\alpha]_D^{26}$ –98.2 in MeOH). FAB-

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Fig. 1. Structures of Aromatic Compounds Isolated from the Seeds of *C. auriculata*Table 1. ^{13}C -NMR Spectroscopic Data for **1–3**

Position	1	2	3	Position	1	2	3
1		153.5		1'	97.0	101.7	98.1
2	161.1	124.4	164.3	2'	77.4	74.9	75.7
3	90.0	135.5	110.9	3'	76.8	78.2	76.7
4	167.0	120.5	178.2	4'	69.5	71.7	69.8
4a	107.2	138.8	116.1	5'	75.4	78.3	76.9
5	132.1	107.2	141.3	6'	65.7	62.8	60.5
6	124.5	158.0	116.8	1''	108.7	104.2	108.6
6a	138.0			2''	76.1	74.9	76.0
7	100.8	103.9	159.6	3''	78.6	78.0	79.2
8	158.9	157.1	101.4	4''	73.2	71.5	73.9
8a		110.9	158.7	5	62.9	78.8	64.1
9	102.7			6''		62.7	
10	156.5			1'''	100.3		
10a	106.3			2'''	70.2		
10b	153.8			3'''	70.6		
				4'''	71.9		
				5'''	68.3		
				6'''	17.7		

Solvent: DMSO- d_6 for **1** and **3**, CD $_3$ OD for **2**.

MS in the positive-ion mode revealed a quasimolecular ion $(\text{M}+\text{Na})^+$ at m/z 579 from which the molecular formula $\text{C}_{25}\text{H}_{32}\text{O}_{14}$ was determined *via* HR-MS and ^{13}C -NMR data. Acid hydrolysis of **2** liberated D-glucose, which was identified by HPLC analysis using an optical rotation detector. The ^1H - (CD $_3$ OD) and ^{13}C -NMR (Table 1) spectra of **2**, which were assigned by various NMR experiments,²⁰ showed signals ascribable to a methyl [δ 2.32 (3H, s, CH $_3$ -3)], an acetyl group [δ 2.62 (3H, s, C(=O)CH $_3$)], three aromatic protons [δ 7.04 (1H, d, J =2.8 Hz, H-5), 7.05 (1H, s, H-4), 7.14 (1H, d, J =2.8 Hz, H-7)], and two β -D-glucopyranosyl moiety [δ 5.07 (1H, d, J =7.6 Hz, H-1'), 5.15 (1H, d, J =8.2 Hz, H-1'')]. The proton

and carbon signals of **2** in the ^1H - and ^{13}C -NMR spectra were similar to those of 6-hydroxymusizin,²¹⁾ except for the signals around the 6 and 8-positions. The positions of two glucopyranoses were confirmed by HMBC spectroscopy analysis. Namely, long-range correlations were observed between the following proton and carbon pairs: H-1' and C-6; H-1'' and C-8. Furthermore, in the NOESY experiment, NOE correlations were observed between the following proton pairs: H-1' and H-5, 7; H-1'' and H-7 (Fig. 2). Consequently, the chemical structure of cassiaglycoside II (**2**) was determined to be 6-hydroxymusizin 6,8-di- O - β -D-glucopyranoside.

Cassiaglycoside III (**3**) was obtained as a pale yellow

Fig. 2. Important 2D-NMR Correlations of 1–4

powder with negative optical rotation ($[\alpha]_D^{27} -117.7$ in MeOH). FAB-MS in the positive-ion mode revealed a quasimolecular ion $(M+Na)^+$ at m/z 507 from which the molecular formula $C_{22}H_{28}O_{12}$ was determined *via* HR-MS and ^{13}C -NMR data. Acid hydrolysis of **3** liberated D-apiose and D-glucose, which were identified by HPLC analysis using an optical rotation detector. The 1H - (DMSO- d_6) and ^{13}C -NMR (Table 1) spectra of **3**, which were assigned by various NMR experiments,²⁰ showed signals ascribable to a 7-hydroxy-2,5-dimethylchromone [two methyl [δ 2.31 (3H, s, CH_3 -2), 2.70 (3H, s, CH_3 -5)], three aromatic protons [δ 6.06 (1H, s, H-3), 6.82, 6.97 (1H each, both brs, H-6, 8)], a β -D-apiofuranosyl moiety, and a β -D-glucopyranosyl moiety. The proton and carbon signals of **3** in the 1H - and ^{13}C -NMR spectra were similar to those of 7-hydroxy-2,5-dimethylchromone,²² except for the signals around the 7-position, while the proton and carbon signals due to the glycosyl part were very similar to those of **8**¹⁶ and **11**.¹⁹ The positions of two sugars were confirmed by HMBC spectroscopy analysis. Namely, long-range correlations were observed between the following proton and carbon pairs: H-1' and C-7; H-1'' and C-2'. Furthermore, in the NOESY experiment, NOE correlations were observed between the following proton pairs: H-1' and H-6, 8 (Fig. 2). Consequently, the chemical structure of cassiaglycoside III (**3**) was determined to be 7-hydroxy-2,5-dimethylchromone 7-O- β -D-apiofuranosyl(1 $\rightarrow$ 2)- β -D-glucopyranoside.

Cassiaglycoside IV (**4**) was obtained as a colorless amorphous powder with negative optical rotation ($[\alpha]_D^{26} -74.9$ in MeOH). In the positive- and negative-ion FAB-MS of **4**, quasimolecular ion peaks were observed at m/z 585 $(M+Na)^+$ and m/z 561 $(M-H)^-$, respectively. HR-MS analysis of the quasimolecular ion peak in the positive-ion FAB-MS indicated the molecular formula of **4** to be $C_{25}H_{34}O_{14}$. Acid hydrolysis of **4** liberated D-apiose, D-glucose, and L-rhamnose, which were identified by HPLC analysis using an optical rotation detector. The 1H - and ^{13}C -NMR (CD_3OD , Table 2) spectra²⁰ showed the signals caused by a phenylethyl group, a β -D-apiofuranosyl moiety, a β -D-glucopyranosyl moiety, and an α -L-rhamnopyranosyl moiety. The proton and carbon signals of phenylethyl group of **4** in the 1H - and ^{13}C -NMR spectra were similar to those of **9–11**,^{17–19} while the proton and carbon signals due to the glycosyl part were very similar to those of **1**. The positions of three sugars were confirmed by HMBC spectroscopy analysis. Namely, long-range correla-

Table 2. ^{13}C -NMR Spectroscopic Data for **4**

Position	4	Position	4
1	71.7	1'''	110.5
2	37.4	2'''	77.9
1'	140.1	3'''	80.7
2', 6'	129.4	4'''	75.3
3', 5'	130.0	5'''	66.2
4'	127.3	1''''	102.2
1''	103.3	2''''	72.2
2''	78.5	3''''	72.4
3''	78.6	4''''	74.0
4''	71.7	5''''	69.8
5''	76.7	6''''	18.1
6''	68.0		

Solvent: CD_3OD .

tions were observed between the following proton and carbon pairs: H-1 and C-1'', H-1'' and C-1; H-1''' and C-2''; H-6'' and C-1''''; H-1'''' and C-6''. Consequently, the chemical structure of cassiaglycoside IV (**4**) was determined to be 2-phenylethyl O- β -D-apiofuranosyl(1 $\rightarrow$ 2)- α -L-rhamnopyranosyl(1 $\rightarrow$ 6)- β -D-glucopyranoside.

Experimental

General Experimental Procedures The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter ($l=5$ cm); IR spectra, Shimadzu FTIR-8100 spectrometer; UV spectra, Shimadzu UV-1600 spectrometer; electron ionization (EI)-MS and HR-EI-MS, JEOL JMS-GCMATE mass spectrometer; FAB-MS and HR-FAB-MS, a JEOL JMS-SX 102A mass spectrometer; 1H -NMR spectra, JNM-LA500 (500 MHz) and JEOL JNM-ECA600 (600 MHz) spectrometers; ^{13}C -NMR spectra, JNM-LA500 (125 MHz) and JEOL JNM-ECA600 (150 MHz) spectrometers; HPLC detector, Shimadzu RID-6A refractive index detector and SPD-10A UV-VIS detectors; and HPLC column, Cosmosil 5C₁₈-MS-II (Nacalai Tesque Inc. (Kyoto, Japan), 250 $\times$ 4.6 mm i.d.) and (250 $\times$ 20 mm i.d.) columns were used for analytical and preparative purposes, respectively. The following experimental materials were used for chromatography: normal-phase silica gel column chromatography (CC), Silica gel BW-200 (Fuji Silysia Chemical, Ltd. (Japan), 150–350 mesh); reversed-phase silica gel column chromatog-

raphy, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100–200 mesh); TLC, precoated TLC plates with Silica gel 60F₂₅₄ (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reversed phase); reversed-phase HPTLC, precoated TLC plates with Silica gel RP-18 WF_{254S} (Merck, 0.25 mm); and detection was achieved by spraying with 1% Ce(SO₄)₂–10% aqueous H₂SO₄ followed by heating.

Plant Material The seeds of *C. auriculata* were purchased from N.T.H. Co., Ltd. A voucher of the plant is on file in our laboratory [CAS-1].

Extraction and Isolation The seeds of *C. auriculata* (3.93 kg) were crushed and extracted three times with MeOH under reflux for 3 h. Evaporation of the solvent under reduced pressure provided the MeOH extract (430.6 g, 10.96%). A part of the extract (399.0 g) was partitioned into an *n*-hexane–H₂O (1:1, v/v) mixture to furnish *n*-hexane- (46.4 g, 1.27%) and H₂O- (350.8 g, 9.63%) layers, respectively. A part of the H₂O layer (322.7 g) was subjected to Diaion HP-20 column chromatography (CC) (3.0 kg, H₂O→MeOH) to give H₂O- (248.2 g) and MeOH- (63.2 g) eluted fractions as reported previously. The MeOH-eluted fraction (60.0 g) was subjected to ordinary-phase silica gel CC [1.5 kg, CHCl₃→CHCl₃–MeOH (20:1, v/v)→CHCl₃–MeOH–H₂O (10:3:1→7:3:1→6:4:1, v/v/v, low layer)→MeOH] to give 9 fractions {Fr. 1–Fr. 4, Fr. 5 (10.4 g), Fr. 6 (5.7 g), Fr. 7 (16.7 g), Fr. 8 (16.3 g), Fr. 9}. Fraction 5 (10.4 g) was subjected to reversed-phase silica gel CC [300 g, MeOH–H₂O (20:80→30:70→40:60→50:50→60:40→70:30→80:20, v/v)→MeOH] to give 13 fractions [Fr. 5-1, Fr. 5-2 (522 mg), Fr. 5-3 (282 mg), Fr. 5-4 (783 mg), Fr. 5-5 (1991 mg), Frs. 5-6–5-13]. Fraction 5-2 (522 mg) was purified by HPLC [MeOH–H₂O (20:80, v/v), CH₃CN–MeOH–H₂O (8:8:84, v/v/v)] to give **7** (9.7 mg, 0.00031%) and **8** (22 mg, 0.00070%). Fraction 5-3 (282 mg) was purified by HPLC [MeOH–H₂O (25:75, v/v), CH₃CN–MeOH–H₂O (9:9:82, v/v/v)] to give **8** (17 mg, 0.00052%), **10** (14 mg, 0.00045%), and **11** (41 mg, 0.0013%). Fraction 5-4 (783 mg) was purified by HPLC [MeOH–H₂O (35:65 and 30:70, v/v)] to give **3** (13 mg, 0.00040%), **8** (1.3 mg, 0.00004%), **9** (11 mg, 0.00034%), **10** (17 mg, 0.00053%), and **11** (91 mg, 0.0029%). Fraction 5-5 (1991 mg) was purified by HPLC [MeOH–H₂O (45:55, 40:60, v/v) and CH₃CN–MeOH–H₂O (19:8:73, v/v/v)] to give **6** (17 mg, 0.00052%) and **11** (18 mg, 0.00058%). Fraction 6 (5.7 g) was subjected to reversed-phase silica gel CC [200 g, MeOH–H₂O (10:90→20:80→30:70→40:60→50:50→60:40→70:30→80:20, v/v)→MeOH] to give 8 fractions [Fr. 6-1, Fr. 6-2 (300 mg), Fr. 6-3, Fr. 6-4 (436 mg), Frs. 6-5–6-8]. Fraction 6-2 (300 mg) was purified by HPLC [MeOH–H₂O (25:75, v/v) and CH₃CN–MeOH–H₂O (8:8:84, v/v/v)] to give **7** (2.9 mg, 0.00009%), **8** (5.5 mg, 0.00017%), and **10** (7.9 mg, 0.00025%). Fraction 6-4 (436 mg) was purified by HPLC [MeOH–H₂O (35:65, v/v) and CH₃CN–MeOH–H₂O (13:8:79, v/v/v)] to give **5** (98 mg, 0.0031%). Fraction 7 (16.7 g) was subjected to reversed-phase silica gel CC [480 g, MeOH–H₂O (10:90→20:80→30:70→40:60→50:50→60:40→70:30, v/v)→MeOH] to give 12 fractions [Frs. 7-1–7-4, Fr. 7-5 (428 mg), Fr. 7-6 (**5**, 1074 mg, 0.034%), Frs. 7-7–7-12]. Fraction 7-5 (428 mg) was purified by HPLC [MeOH–H₂O (30:70, v/v) and CH₃CN–MeOH–H₂O (11:9:80)] to give **2** (13 mg, 0.00040%), **4** (46 mg, 0.0014%), and **5** (77 mg, 0.0024%). Fraction 8 (16.3 g) was subjected to reversed-phase silica gel CC [480 g, MeOH–H₂O (20:80→30:70→40:60→50:50, v/v)→MeOH] to give 12 frac-

tions [Frs. 8-1–8-7, Fr. 8-8 (1175 mg), Frs. 8-9–8-12]. Fraction 8-8 (1175 mg) was purified by HPLC [MeOH–H₂O (35:65, v/v) and CH₃CN–MeOH–H₂O (11:9:80)] to give **1** (15 mg, 0.00048%).

Cassia glycoside I (**1**)

A yellow powder; $[\alpha]_D^{27}$ –125.7 ($c=0.73$, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 324 (3.76), 289 (4.34), 234 (4.64) nm; IR (KBr) $\nu_{\max}$ 3420, 2936, 1686, 1638, 1458, 1084 cm^{–1}; ¹H-NMR (DMSO-*d*₆, 600 MHz) δ : 1.02 (3H, d, $J=6.4$ Hz, H-6''), 2.69 (3H, s, CH₃-5), 3.47 (1H, m, H-6'a), 3.66 (1H, dd, $J=7.6$, 8.6 Hz, H-2'), 3.82 (1H, brd like, $J=ca.$ 11 Hz, H-6'b), 4.53 (1H, brs, H-1''), 5.44 (1H, d, $J=2.8$ Hz, H-1'), 5.48 (1H, d, $J=7.6$ Hz, H-1'), 5.95 (1H, s, H-3), 6.54, 6.56 (1H each, both d, $J=2.2$ Hz, H-9, 7), 7.22 (1H, s, H-6), 10.00 (1H, brs, OH-10), 10.03 (1H, brs, OH-8); ¹³C-NMR data see Table 1; positive-ion FAB-MS m/z 699 [M+H]⁺; HR-FAB-MS: m/z 699.2142 (Calcd for C₃₁H₃₈O₁₈ [M+H]⁺, 699.2135).

Cassia glycoside II (**2**)

An orange powder; $[\alpha]_D^{26}$ –98.2 ($c=0.62$, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 339 (2.98), 262 (4.19), 236 (4.54) nm; IR (KBr) $\nu_{\max}$ 3420, 2943, 1630, 1584, 1075 cm^{–1}; ¹H-NMR (CD₃OD, 500 MHz) δ : 2.32 (3H, s, CH₃-3), 2.62 (3H, s, C(=O)CH₃), 5.07 (1H, d, $J=7.6$ Hz, H-1'), 5.15 (1H, d, $J=8.2$ Hz, H-1''), 7.04 (1H, d, $J=2.8$ Hz, H-5), 7.05 (1H, s, H-4), 7.14 (1H, d, $J=2.8$ Hz, H-7); ¹³C-NMR data see Table 1; positive-ion FAB-MS m/z 579 [M+Na]⁺; HR-FAB-MS: m/z 579.1692 (Calcd for C₂₅H₃₂O₁₄Na [M+Na]⁺, 579.1690).

Cassia glycoside III (**3**)

A pale yellow powder; $[\alpha]_D^{27}$ –117.7 ($c=0.17$, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 277 (3.99), 242 (4.21), 224 (4.30) nm; IR (KBr) $\nu_{\max}$ 3405, 2926, 1655, 1609, 1509, 1084 cm^{–1}; ¹H-NMR (DMSO-*d*₆, 500 MHz) δ : 2.31 (3H, s, CH₃-2), 2.70 (3H, s, CH₃-5), 3.52 (1H, m, H-2'), 5.14 (1H, d, $J=7.4$ Hz, H-1'), 5.35 (1H, brs, H-1''), 6.06 (1H, s, H-3), 6.82, 6.97 (1H each, both brs, H-6, 8); ¹³C-NMR data see Table 1; positive-ion FAB-MS m/z 507 [M+Na]⁺; HR-FAB-MS: m/z 507.1484 (Calcd for C₂₂H₂₈O₁₂Na [M+Na]⁺, 507.1478).

Cassia glycoside IV (**4**)

A colorless amorphous powder; $[\alpha]_D^{26}$ –74.9 ($c=0.66$, MeOH); IR (KBr) $\nu_{\max}$ 3440, 2934, 1069 cm^{–1}; ¹H-NMR (CD₃OD, 500 MHz) δ : 1.24 (3H, d, $J=6.1$ Hz, H₃-6''), 2.93 (2H, dd like, $J=7.3$, 7.3 Hz, H₂-2), 3.34 (1H, m, H-2''), 3.60 (1H, m, H-6'a), 3.96 (1H, brd like, $J=ca.$ 11 Hz, H-6'b), 3.74, 4.02 (1H each, both m, H₂-1), 4.34 (1H, d, $J=8.0$ Hz, H-1''), 4.74 (1H, brs, H-1''), 5.37 (1H, d, $J=1.6$ Hz, H-1''), 7.16 (1H, m, H-4'), 7.25 (4H, m, H-2', 3', 5', 6'); ¹³C-NMR data see Table 2; positive-ion FAB-MS m/z 585 [M+Na]⁺; negative-ion FAB-MS m/z 561 [M–H][–]; HR-FAB-MS: m/z 585.2164 (Calcd for C₂₅H₃₄O₁₄Na [M+Na]⁺, 585.2159).

Acid Hydrolyses of 1–4 A solution of **1–4** (each 1.0 mg) in 1 M HCl–1,4-dioxane (1:1, v/v, 1.0 mL) was heated under reflux for 3 h, respectively. After cooling, the reaction mixture was extracted with EtOAc. The aqueous layer was subjected to HPLC analysis under the following conditions, respectively: HPLC column, Kaseisorb LC NH₂-60-5, 4.6 mm i.d.×250 mm (Tokyo Kasei Co., Ltd., Tokyo, Japan); detection, optical rotation [Shodex OR-2 (Showa Denko Co., Ltd., Tokyo, Japan); mobile phase, CH₃CN–H₂O (85:15, v/v); flow rate 0.8 mL/min]. Identifications of D-apiose for **1**, **3**, and **4**, D-glucose for **1–4**, and L-rhamnose for **1** and **4**, present in the aqueous layer were carried out by comparison of their retention times and

optical rotations with those of authentic samples [retention time (t_R): D-apiose, 6.3 min (positive optical rotation), D-glucose, 11.5 min (positive optical rotation), L-rhamnose, 7.4 min (negative optical rotation)].

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Conflict of Interest The authors declare no conflict of interest.

References and Notes

- 1) Nakashima S., Oda Y., Nakamura S., Liu J., Onishi K., Kawabata M., Miki H., Himuro Y., Yoshikawa M., Matsuda H., *Bioorg. Med. Chem. Lett.*, **25**, 2702–2706 (2015).
- 2) Ohta T., Nakamura S., Nakashima S., Matsumoto T., Ogawa K., Fujimoto K., Fukaya M., Yoshikawa M., Matsuda H., *Tetrahedron*, **71**, 846–851 (2015).
- 3) Liu J., Nakamura S., Xu B., Matsumoto T., Ohta T., Fujimoto K., Ogawa K., Fukaya M., Miyake S., Yoshikawa M., Matsuda H., *J. Nat. Med.*, **69**, 135–141 (2015).
- 4) Ogawa K., Nakamura S., Sugimoto S., Tsukioka J., Hinomaru F., Nakashima S., Matsumoto T., Ohta T., Fujimoto K., Yoshikawa M., Matsuda H., *Phytochem. Lett.*, **12**, 98–104 (2015).
- 5) Matsumoto T., Nakamura S., Ohta T., Fujimoto K., Yoshikawa M., Ogawa K., Matsuda H., *Org. Lett.*, **16**, 3076–3078 (2014).
- 6) Matsumoto T., Nakamura S., Nakashima S., Fujimoto K., Yoshikawa M., Ohta T., Ogawa K., Matsuda H., *J. Nat. Prod.*, **77**, 990–999 (2014).
- 7) Yamaguchi I., Matsuda H., Zhang H., Hamao M., Yamashita C., Kogami Y., Kon'I H., Murata M., Nakamura S., Yoshikawa M., *J. Nat. Med.*, **68**, 74–82 (2014).
- 8) Matsumoto T., Nakamura S., Nakashima S., Yoshikawa M., Fujimoto K., Ohta T., Morita A., Yasui R., Kashiwazaki E., Matsuda H., *Bioorg. Med. Chem. Lett.*, **23**, 5178–5181 (2013).
- 9) Mourad A. A., Nakamura S., Ueno T., Minami T., Yagi T., Yasue H., Komatsu R., Yoshikawa M., Taye A. M., El-Moselhy M. A., Khalifa M. M., Matsuda H., *Bioorg. Med. Chem. Lett.*, **23**, 4813–4816 (2013).
- 10) Nakamura S., Nakashima S., Oda Y., Yokota N., Fujimoto K., Matsumoto T., Ohta T., Ogawa K., Maeda S., Nishida S., Matsuda H., Yoshikawa M., *Bioorg. Med. Chem.*, **21**, 1043–1049 (2013).
- 11) Nakamura S., Nakashima S., Tanabe G., Oda Y., Yokota N., Fujimoto K., Matsumoto T., Sakuma R., Ohta T., Ogawa K., Nishida S., Miki H., Matsuda H., Muraoka O., Yoshikawa M., *Bioorg. Med. Chem.*, **21**, 779–787 (2013).
- 12) Nakamura S., Xu F., Ninomiya K., Nakashima S., Oda Y., Morikawa T., Muraoka O., Yoshikawa M., Matsuda H., *Chem. Pharm. Bull.*, **62**, 1026–1031 (2014).
- 13) Zhang Y., Nakamura S., Nakashima S., Wang T., Yoshikawa M., Matsuda H., *Tetrahedron*, **71**, 6727–6732 (2015).
- 14) Kashiwada Y., Nonaka G., Nishioka I., *Chem. Pharm. Bull.*, **32**, 3493–3500 (1984).
- 15) Cui B., Nakamura M., Kinjo J., Nohara T., *Chem. Pharm. Bull.*, **41**, 178–182 (1993).
- 16) Miyaichi Y., Tomimori T., *Natural Medicines*, **49**, 350–353 (1995).
- 17) Umehara K., Hattori I., Miyase T., Ueno A., Hara S., Kageyama C., *Chem. Pharm. Bull.*, **36**, 5004–5008 (1988).
- 18) Yoshikawa M., Shimada H., Horikawa S., Murakami T., Shimoda H., Yamahara J., Matsuda H., *Chem. Pharm. Bull.*, **45**, 1498–1503 (1997).
- 19) Murakami T., Kohno K., Ninomiya K., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **49**, 1003–1008 (2001).
- 20) The ^1H - and ^{13}C -NMR spectra of **1–4** were assigned with the aid of distortionless enhancement by polarization transfer (DEPT), DQF COSY, heteronuclear multiple quantum coherence spectroscopy (HMQC), and HMBC experiments.
- 21) Horikawa M., Noguchi T., Takaoka S., Kawase M., Sato M., Tsunoda T., *Tetrahedron*, **60**, 1229–1234 (2004).
- 22) Hu L., Chen N.-N., Hu Q., Yang C., Yang Q.-S., Wang F.-F., *Molecules*, **19**, 11453–11464 (2014).