

Secondary metabolites from *Opuntia ficus-indica* var. *saboten*

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Abstract

A butanol fraction, from the methanolic extract of *Opuntia ficus-indica* var. *saboten*, on purification either by preparative TLC or reversed phase HPLC, yielded three chemical components: isorhamnetin 3-*O*-(6''-*O*-*E*-feruloyl)neohesperidoside (**1**), (6*R*)-9,10-dihydroxy-4,7-megastigmadien-3-one-9-*O*-β-D-glucopyranoside (**2**) and (6*S*)-9,10-dihydroxy-4,7-megastigmadien-3-one-9-*O*-β-D-glucopyranoside (**3**) along with 15 known compounds. Structures of compounds (**1–3**) were elucidated by aid of spectroscopic analyses. The absolute stereochemistry in compounds **2** and **3** was established with the help of CD data analysis and comparison with the literature data analysis. In a DPPH radical scavenging assay, compound **1** showed moderate inhibitory activity (IC₅₀ = 45.58 μg/ml).

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Keywords: *Opuntia ficus-indica* var. *saboten*; Cactaceae; Isorhamnetin 3-*O*-(6''-*O*-*E*-feruloyl)neohesperidoside; (6*R*)-9,10-Dihydroxy-4,7-megastigmadiene-3-one-9-*O*-β-D-glucopyranoside; (6*S*)-9,10-Dihydroxy-4,7-megastigmadiene-3-one-9-*O*-β-D-glucopyranoside

1. Introduction

Opuntia ficus-indica var. *saboten* Makino (Cactaceae) is widely cultivated in Jeju Island (South Korea) for use in manufacture of health foods such as tea, jam and juice. Its fruits and stems have been traditionally used in oriental folk medicines to treat diabetes, hypertension, asthma, burns, edema and indigestion (Ahn, 1998; Lopez, 1995). It has also been reported that the extracts of fruits or stems exhibit hypoglycemic (Ibanez-Camacho and Roman-Ramos, 1979; Trejo-Gonzalez et al., 1996), anti-ulcer (Galati et al., 2001), and anti-allergic activities (Lee et al., 2000). In addition, Park et al. reported analgesic and anti-inflammatory activity of the fruits and the stem extracts (Park et al., 1998) and isolated β-sitosterol as an active anti-inflammatory principle from the stem extract

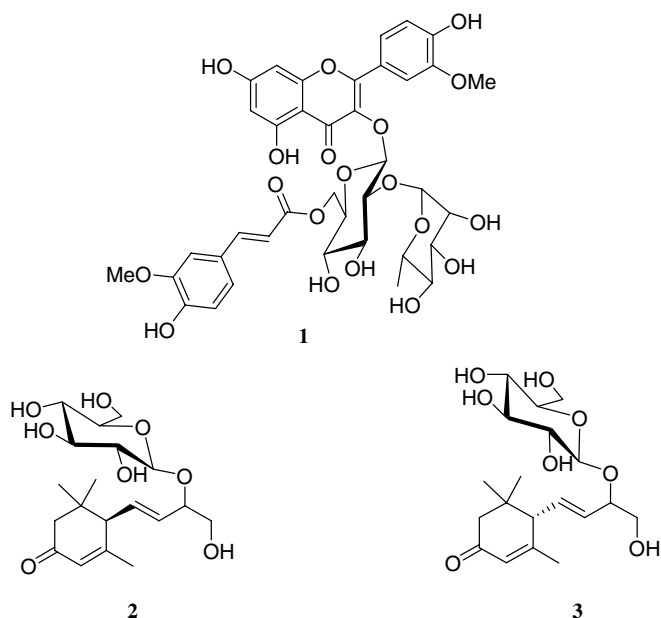
(Park et al., 2001). The high percentage of pectins and fibers can increase fecal mass and intestinal motility, which in turn affect cholesterol and glucose plasma levels (Fernandez et al., 1992). Thus, the medicinal importance of this plant motivated us to investigate its secondary metabolites. Previously, isolation of two alkaloids, indicaxanthin and neobetanin, along with various flavonoids were reported from this plant (Impellizzeri and Piattelli, 1972; Strack et al., 1987; Jeong et al., 1999).

In this paper, we describe the isolation of isorhamnetin-3-*O*-(6''-*O*-*E*-feruloyl)neohesperidoside (**1**), (6*R*)-9,10-dihydroxy-4,7-megastigmadien-3-one-9-*O*-β-D-glucopyranoside (**2**) and (6*S*)-9,10-Dihydroxy-4,7-megastigmadien-3-one-9-*O*-β-D-glucopyranoside (**3**), along with 15 known constituents. 9,10-Dihydroxy-4,7-megastigmadien-3-one-9-*O*-β-D-glucopyranoside has previously been reported from the leaves of *Pterospermum semisagittatum* as a mixture with 9,10-dihydroxy-4,7-megastigmadien-3-one-10-*O*-β-D-glucopyranoside. They could only isolate 9,10-dihy-

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droxy-4,7-megastigmadien-3-one-9-*O*- β -D-glucopyranoside in its acetylated form; however, the absolute configurations of this compound have not been determined (Khan et al., 2005).

Herein, we wish to describe the isolation of **2** and **3** in pure form for the first time and determination of the absolute configuration at C-6 position of these compounds.



2. Results and discussion

The ^1H NMR spectrum of **1** indicated a flavonol glycoside moiety, as it displayed two methine signals at δ 6.20 (d, $J = 2.1$ Hz) and 6.03 (d, $J = 1.8$ Hz) for aromatic ring A, and three resonances at δ 7.87 (d, $J = 2.0$ Hz), 7.50 (dd, $J = 8.5$ and 2.0 Hz) and 6.88 (d, $J = 8.5$ Hz) for the ring B of the flavonol skeleton. In addition, the signals at δ 6.99 (d, $J = 1.7$ Hz), 6.85 (dd, $J = 8.1$ and 1.7 Hz) and 6.78 (d, $J = 8.2$ Hz), corresponding to three aromatic protons of an ABX spin system, and the resonances at δ 7.33 (d, $J = 15.9$ Hz) and 6.05 (d, $J = 15.7$ Hz), corresponding to the *trans* olefinic protons, indicated the presence of a *trans*-feruloyl group in **1**. Two methoxy signals observed in the same spectrum at δ 3.98 and 3.88 were attributed to flavonol and feruloyl moieties, respectively. The observation of a methoxy group (δ 3.98) revealed that the flavonol aglycone could be isorhamnetin, which was confirmed by a ^{13}C NMR analysis with the only significant differences being the downfield chemical shift of about 4.7 ppm for C-3' and an upfield shift of 3.0 ppm for C-2' (Markham et al., 1978). The effect of glycosylation of the 3-OH on the C-3 signal is a 2.1 ppm upfield shift and a downfield shift of 9.2 ppm for C-2 linked to the flavonol moiety (Markham et al., 1978). The attachment of a disaccharide at C-3 was

supported by the signals for two anomeric protons at δ 5.80 (d, $J = 7.3$ Hz, for glucose) and 5.20 (d, $J = 1.1$ Hz, for rhamnose) and other sugar proton resonances in the ^1H NMR spectrum of **1**. The downfield shift of about 5.5 ppm for C-2 of glucose and an upfield shift of about 3.5 ppm for C-1 of glucose, pointed to the interglycosidic linkage at C-2 of glucose (Markham et al., 1978). The chemical shift of anomeric protons for both the sugar units also supported this interpretation, i.e. with the glucose being the internal sugar and the rhamnose the terminal one. Thus, it was ultimately confirmed by a HMBC experiment as the glucose anomeric proton at δ 5.80 (C-1'') showed a long range interaction with the carbon at δ 134.2 (C-3) and the rhamnose anomeric proton at δ 5.20 (C-1''') gave an interaction with the glucose carbon signal at δ 80.1 (C-2''). The esterification site of ferulic acid was at C-6 of the glucose moiety, as the proton signals at δ 4.36 and 4.29 (H-6'') were correlated with the signal at δ 168.7 for the carbonyl carbon of ferulic acid. Additionally, the downfield chemical shift of the glucose H-6 by 0.55 ppm was further evidence to deduce the connectivity of feruloyl unit at C-6 of glucose. On the basis of all these data, the compound **1** was characterized as isorhamnetin 3-*O*-(6''-*O*-*E*-feruloyl)neohesperidoside which is a new natural product.

The HRFABMS (positive mode) of **2** gave a molecular ion peak at m/z 386 corresponding to the molecular formula $\text{C}_{19}\text{H}_{30}\text{O}_8$. The ^1H NMR spectrum of **2** had similar peak patterns to those of 9,10-dihydroxy-4,7-megastigmadiene-3-one from Fijian *Aglaia* species (Greger et al., 2001), except that it contained a glucose located on C-9. In the ^1H NMR spectrum, the signals at δ 5.90 (dd, $J = 14.7$ and 10.1 Hz) and 5.64 (dd, $J = 15.4$ and 7.0 Hz) corresponded to the *trans* olefinic protons and the broad singlet at δ 5.89 (H-4), assignable to the vinyl proton indicated the presence of a substituted double bond. The signal of the glucose anomeric proton appeared at δ 4.32 (d, $J = 7.4$ Hz) with a coupling constant characteristic of a β -configuration. In the HMBC spectrum of **2**, the signals at δ 1.04 (H-12) and 1.00 (H-11) showed correlations with the carbon resonance at δ 37.1 (C-1), and the signals at δ 2.06 and 2.50 (H-2) correlated with the carbonyl resonance at δ 202.0 (C-3) and 37.1 (C-1). Therefore, the positions of these two methyl groups were assigned to C-1 and the carbonyl group was proposed as residing at C-3. The signal at δ 4.32 (H-1') of the anomeric proton correlated with the resonance at δ 79.6 (C-9), indicating that the glucose was connected through C-9. The absolute stereochemistry at C-9 was not, however, determined. The absolute stereochemistry at C-6 could be deduced by comparing the data reported for similar type of compounds. In the literature data, the *R* configuration at C-6 was established on the basis of CD data [318 ($\Delta\epsilon -13.3$), 256 (+181.0) nm] exhibiting a positive maximum at 256 nm (Pabst et al., 1992; Ito et al., 2001). Thus the compound **2** has been characterized as (6*R*)-9,10-dihydroxy-4,7-megastigmadiene-3-one-9-*O*- β -D-glucopyranoside and named as opuntiside A.

Compound **3** was a diastereoisomer of **2** with virtually identical NMR and MS data. The CD data [303 ($\Delta\epsilon$ +13.6), 251 (−178.6 nm)] showed a negative maximum at 251 nm which was opposite to the CD data of **2** (Pabst et al., 1992; Ito et al., 2001), indicating that the C-6 of **3** had an absolute *S*-configuration. Therefore, compound **3** has been established as (6*S*)-9,10-dihydroxy-4,7-megastigmadiene-3-one-9-*O*- β -D-glucopyranoside and named as opuntiside B.

The 15 known compounds were isolated and identified as quercetin (**4**), kaempferol (**5**), quercetin 3-*O*-methyl ether (**6**), 2,3-dihydrokaempferol (**7**) (Lee et al., 2003), isorhamnetin 3-*O*-glucoside (**8**) (Karl et al., 1981), 2,3-dihydroquercetin (**9**) (Nonaka et al., 1987), coumaric acid (**10**), kaempferol 7-*O*-glucoside (**11**) (Lee et al., 1998), ferulic acid (**12**), isorhamnetin 3-*O*-neohesperidoside (**13**) (Norbaek and Kondo, 1999), isorhamnetin 3-*O*-rutinosyl-4'-*O*- β -D-glucoside (**14**) (Aquino et al., 1987), isorhamnetin 3-*O*-(2,6-dirhamnosyl)glucoside (**15**) (Liu and Lu, 1998), zataroside-A (**16**) (Ali et al., 1999), *n*-butyl- β -D-fructopyranoside (**17**) (Xu et al., 2005), 4-*O*-glucosyl-mapic acid (**18**) (Materska and Perucka, 2005), the structure of which were determined by spectroscopic analyses.

All isolates were assessed for antioxidant activity in the DPPH radical (Lee et al., 2002) and superoxide anion radical scavenging effect in the xanthine/xanthine oxidase system (Toda et al., 1991), and their cytotoxic activity against five human cancer cell lines: lung cancer (A549), epidermoid carcinoma (KB), colon adenocarcinoma (HCT-15), and malignant melanoma (SK-MEL2) cancer cells. Among the tested compounds, compound **1** showed moderate radical scavenging activity (IC_{50} = 45.58 μ g/ml) against DPPH radical but was inactive for anticancer (data not shown).

3. Experimental section

3.1. General experimental procedures

Optical rotations were determined on an Autopol III Automatic polarimeter (Rudolph Research Flanders, NJ). Circular dichroism (CD) spectra were measured on a JASCO J-715 spectropolarimeter. 1H and ^{13}C NMR spectra were recorded as CD_3OD solution on Bruker 300 and Varian 500 MHz spectrometer for 1H , and 75.43 and 125 MHz for ^{13}C , respectively. 1H - 1H COSY, HMBC and HMQC spectra were obtained with the usual pulse sequences and data processing was performed with standard software. Chemical shifts are given in ppm referred to the residual CH_3OH signal set at δ 3.33 and 4.88 or CD_3OD middle band set at δ 49.0. TLC and column chromatography were carried out on pre-coated silica gel F_{254} plates (Merck, art. 5715), RP-18 F_{254S} plates (Merck, art. 15423), silica gel 60 (230–400 mesh, Merck), Sephadex LH-20 (Bead size 25–100 μ m, Sigma) and LiChroprep RP-18 (40–63 μ m, Merck). Preparative HPLC was performed on a Waters pump model 510 with a photodiode

array detector (Waters model 996) using a LichroCART RP-18 (10 mm \times 250 mm, 7 μ m column).

3.2. Plant material

The leaves of *Opuntia ficus-indica* var. *saboten* were purchased from a local, Korean herbal drug market in January 2001. Voucher specimens (901-15) have been deposited in the laboratory of Korea Institute of Science and Technology (KIST).

3.3. Extraction and isolation

The dry powered leaves (1.2 kg) were extracted two times with $EtOH-H_2O$ (1:1, 8.4 l) at 80 °C. The organic solvent was evaporated and water residue was extracted with *n*-BuOH (2.5 l). The BuOH extracts were concentrated in vacuo to yield a residue (50.2 g), which was dissolved in water and washed with CH_2Cl_2 (400 \times 4 ml) to give 35.9 g of water-soluble fraction. An aliquot of the water-soluble fraction (14.5 g) was subjected to Sephadex LH-20 CC eluting with MeOH to give eight fractions (1–8). Fraction 8 (372.2 mg) was subjected to RP-18 chromatography eluting with $MeOH-H_2O$ (6:4) to yield three sub-fractions (8.1–8.3), and fraction 7 (980.5 mg) was subjected to column chromatography under same conditions to give three sub-fractions (7.1–7.3). Fraction 7.1 (450.0 mg) was mixed with fraction 8.2 (308.3 mg) and purified by silica gel CC eluting with a mixture of 5% MeOH in CH_2Cl_2 to afford compounds **4** (122.5 mg) and **5** (88.3 mg). The fraction 5.4 (82.3 mg) was further purified by preparative normal phase silica TLC (MC:MeOH, 9:1) to afford **6** (22.5 mg). Fraction 4 (2.25 g) was subjected to silica gel column chromatography by eluting with a gradient solvent system (0.5% MeOH in CH_2Cl_2 to 20% MeOH) to give **7** (15.5 mg). Fraction 4.12 (188.0 mg) was further purified by RP-18 column chromatography using 30% MeOH as eluting solvent to give compounds **8** (65.7 mg), **9** (6.4 mg) and **10** (6.2 mg). Fraction 4.14 were separated by HPLC using a gradient mobile phase system (13% to 43% MeOH) to afford **11** (12.3 mg), and fraction 4.14.2 was purified by preparative RP-18 TLC ($CH_3CN:H_2O$, 3:7) to yield **1** (12.3 mg). The fraction 4.6 (56.5 mg) was subjected to preparative RP-18 TLC ($CH_3CN:H_2O$, 25:75) to yield **12** (16.5 mg). Fraction 2 (1.514 g) was purified using a RP-18 column eluted with $MeOH-H_2O$ (35:65) to yield an additional amount of **9**, **10** and **12** along with **13** (4.8 mg). Fraction 1 (5.346 g) was subjected to RP-18 column chromatography eluted with a gradient mobile phase system ($MeOH:H_2O$, 25:75 to 65:35) to give **14** (12.2 mg) and **15** (69.5 mg). Fraction 1.2 (180.0 mg) was purified by preparative HPLC (LiChrosorb 250-10, 7 μ m, RP-18, Merck) eluted with $MeOH-H_2O$ (1:4), 1.8 ml/min, to yield **16** (6.2 mg) and **17** (7.2 mg). Fractions 1.3 (140.9 mg) and 1.4 (265.1 mg) were mixed to load on to an RP-18 column eluted with $MeOH-H_2O$ (35:65) and then by preparative HPLC to yield **3** (R_t 15.7 min, 9.3 mg) and **2** (R_t 20.3 min, 21.8 mg) by eluting

with 21% aq. MeOH at a flow rate of 2.2 ml/min. Fraction 1.5 (180.5 mg) could also be purified by HPLC eluting in an isocratic mobile phase system MeOH-H₂O (22:78), 2.2 ml/min to give **18** (R_t 19.0 min, 7.1 mg).

3.4. Isorhamnetin 3-O-(6''-O-E-feruloyl)neohesperidoside (**1**)

Yellow amorphous powder; UV λ_{\max} (MeOH): 332, 300 (sh), 267(sh), 252 nm; IR ν_{\max} cm⁻¹: 3401, 2925, 1655, 1605, 1514, 1453, 1430 1357, 1284, 1205, 1179, 1126, 1055, 1031, 811; $[\alpha]_{\text{D}}^{28}$: -99.83 (*c* 0.46, MeOH); HRFABMS (positive ion mode): *m/z* 823.2086 (C₃₈H₄₀O₁₉Na, calcd. 823.2061); ¹H NMR (CD₃OD, 300 MHz): δ 7.87 (1H, d, *J* = 2.0 Hz, H-2'), 7.50 (1H, dd, *J* = 8.5 and 2.0 Hz, H-6'), 7.33 (1H, d, 15.9 Hz, H-3'''), 6.99 (1H, d, *J* = 1.7 Hz, H-5'''), 6.88 (1H, d, *J* = 8.5 Hz, H-5'), 6.85 (1H, dd, *J* = 8.1 & 1.7 Hz, H-8'''), 6.78 (1H, d, *J* = 8.2 Hz, H-9'''), 6.20 (1H, d, *J* = 2.1 Hz, H-8), 6.05 (1H, d, *J* = 15.7 Hz, H-2'''), 6.03 (1H, d, *J* = 1.8 Hz, H-6), 5.80 (1H, d, *J* = 7.3 Hz, H-1''), 5.20 (1H, d, *J* = 1.1 Hz, H-1'''), 4.36 (1H, dd, *J* = 11.9 and 6.1 Hz, H_a-6''), 4.29 (1H, dd, *J* = 12.0 and 2.8 Hz, H_b-6''), 4.03 (1H, m, H-5'''), 4.01 (1H, dd, *J* = 3.0 and 1.6 Hz, H-2'''), 3.98 (3H, s, OMe), 3.88 (3H, s, OMe), 3.77 (1H, dd, *J* = 9.6 and 3.4 Hz, H-3'''), 3.68 (1H, dd, *J* = 8.3 and 6.3 Hz, H-2''), 3.63 (1H, t, *J* = 9.1 Hz, H-3''), 3.57–3.50 (1H, m, H-5''), 3.30–3.36 (2H, m, H-4'' and 4'''), 0.90 (3H, d, *J* = 6.0 Hz, H-6'''), ¹³C NMR (CD₃OD, 75 MHz): δ 168.7 (C, C-1'''), 179.2 (C, C-4), 165.6 (C, C-7), 163.0 (C, C-5), 158.7 (C, C-2), 158.3 (C, C-9), 150.6 (2C, C-4', 7'''), 148.4 (2C, C-3', 6'''), 146.9 (CH, C-3'''), 134.2 (C, C-3), 127.6 (C, C-4'''), 124.3 (CH, C-6'), 123.7 (CH, C-9'''), 123.4 (C, C-1'), 116.4 (CH, C-8'''), 116.0 (CH, C-5'), 114.8 (CH, C-2'''), 114.6 (CH, C-2'), 111.4 (CH, C-5'''), 105.8 (C, C-10), 102.8 (CH, C-1'''), 100.2 (CH, C-1''), 99.8 (CH, C-6), 94.7 (CH, C-8), 80.1 (CH, C-2''), 78.8 (CH, C-3''), 75.7 (CH, C-5''), 74.0 (CH, C-4'''), 72.4 (3CH, C-4'', 2'', 3''), 70.0 (CH, C-5'''), 63.9 (CH₂, C-6''), 57.0 (CH₃, OMe), 56.5 (CH₃, OMe), 17.5 (CH₃, C-6''').

3.5. (6*R*)-9,10-Dihydroxy-4,7-megastigmadien-3-one-9-O- β -D-glucopyranoside (**2**)

Yellowish amorphous powder; UV λ_{\max} (MeOH): 244 nm; IR ν_{\max} cm⁻¹: 3401, 2930, 1650, 1376, 1077, 1040, 900; $[\alpha]_{\text{D}}^{20}$: +67.23 (*c* 0.89, MeOH); CD [MeOH, nm ($\Delta\epsilon$): 318 (-13.3), 256 (+181.0)]; HRFABMS (positive ion mode): *m/z* 409.1831 (C₁₉H₃₀O₈Na, calcd. 409.1838); ¹H NMR (CD₃OD, 300 MHz): δ 5.90 (1H, dd, *J* = 14.7 and 10.1 Hz, H-7), 5.89 (1H, br s, H-4), 5.64 (1H, dd, *J* = 15.4 and 7.0 Hz, H-8), 4.40 (1H, m, H-9), 4.32 (1H, d, *J* = 7.4 Hz, H-1'), 3.89 (1H, dd, 12.0 and 2.0 Hz, H_a-6'), 3.68–3.56 (3H, m, H_b-6' and H-10), 3.32–3.12 (4H, m, H-2', 3', 4' & 5'), 2.73 (1H, d, *J* = 9.4 Hz, H-6), 2.50 and 2.06 (1H each, d, *J* = 16.7 Hz, H-2), 1.98 (3H, d, *J* = 1.1 Hz, H-13), 1.04 (3H, s, H-12), 1.00 (3H, s, H-11);

¹³C NMR (CD₃OD, 75 MHz): δ 202.0 (C, C-3), 165.6 (C, C-5), 133.5 (CH, C-7), 132.1 (CH, C-8), 126.3 (CH, C-4), 101.4 (CH, C-1'), 79.6 (CH, C-9), 78.1 (CH, C-3' and C-5'), 74.9 (CH, C-2'), 71.7 (CH, C-4'), 66.1 (CH₂, C-10), 62.8 (CH₂, C-6'), 57.1 (CH, C-6), 48.1 (CH₂, C-2), 37.1 (C, C-1), 28.1 (CH₃, C-12), 27.5 (CH₃, C-11), 23.9 (CH₃, C-13).

3.6. (6*S*)-9,10-Dihydroxy-4,7-megastigmadien-3-one-9-O- β -D-glucopyranoside (**3**)

Yellowish amorphous powder; UV λ_{\max} (MeOH): 244 nm; IR ν_{\max} cm⁻¹: 3401, 2928, 1652, 1376, 1077, 1038, 898; $[\alpha]_{\text{D}}^{20}$: -111.48 (*c* 0.27, MeOH); CD [MeOH, nm ($\Delta\epsilon$): 303 (+13.6), 251 (-178.6)]; HRFABMS (positive ion mode): *m/z* 409.1821 (C₁₉H₃₀O₈Na, calcd. 409.1838); ¹H NMR (CD₃OD, 300 MHz): δ 5.89 (1H, br s, H-4), 5.88 (1H, dd, *J* = 15.2 and 9.4 Hz, H-7), 5.64 (1H, dd, *J* = 15.4 and 7.6 Hz, H-8), 4.42 (1H, m, H-9), 4.34 (1H, d, *J* = 7.3 Hz, H-1'), 3.89 (1H, dd, 11.8 and 2.0 Hz, H_a-6'), 3.68–3.56 (3H, m, H_b-6' and H-10), 3.32–3.12 (4H, m, H-2', 3', 4' and 5'), 2.74 (1H, d, *J* = 9.4 Hz, H-6), 2.46 and 2.07 (1H each, d, *J* = 16.8 Hz, H-2), 1.96 (3H, d, *J* = 1.1 Hz, H-13), 1.05 (3H, s, H-12), 1.03 (3H, s, H-11); ¹³C NMR (CD₃OD, 75 MHz): δ 201.9 (C, C-3), 165.3 (C, C-5), 133.7 (CH, C-7), 132.2 (CH, C-8), 126.3 (CH, C-4), 101.0 (CH, C-1'), 79.5 (CH, C-9), 78.1 (CH, C-3'), 78.0 (CH, C-5'), 74.9 (CH, C-2'), 71.7 (CH, C-4'), 66.0 (CH₂, C-10), 62.7 (CH₂, C-6'), 57.0 (CH, C-6), 48.0 (CH₂, C-2), 37.0 (C, C-1), 28.0 (CH₃, C-12), 27.6 (CH₃, C-11), 23.7 (CH₃, C-13).

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