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Phytochemical Constituents of *Suaeda japonica* Makino

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ABSTRACT: Four compounds were isolated from *Suaeda japonica* by repeated column chromatography. Their structures were identified as 2'-hydroxy-6,7-methylenedioxyisoflavone (1), loliolide (2), dehydrovomifoliol (3), and uridine (4) by spectral analysis and comparison with the published data. All compounds were isolated for the first time from this plant.

Keywords: *Suaeda japonica*, Chenopodiaceae, dehydrovomifoliol, 2'-hydroxy-6,7-methylenedioxyisoflavone, loliolide, uridine

Chenopodiaceae (*Suaeda japonica*) has hard salt component tolerance suggesting nearby salt marsh vegetation communities. They live in stocks in a seaside tidal flat. Genera ca. 100, species ca. 1500 (27 genera, 168 species in the flora): worldwide, especially in desert and semi-desert regions, often in alkaline or saline habitats. *Suaeda japonica* belonging to the family Chenopodiaceae is a halophyte and grows naturally on the beach of Korea and Japan (Breckle, 1990; Choo, 1995; Kim *et al.*, 2002).

S. japonica studies on its secondary metabolites have little been done until now. In this paper, we describe the isolation of compounds and their subsequent structural determination by spectroscopic analysis.

MATERIALS AND METHODS

Plant material

Suaeda japonica Makino was collected from Yeong-Gwang, Chollado in Feb. 2003, and authenticated by Prof. Y. Seo, Division of Ocean Science, Korea Maritime University, Korea. The voucher specimen was deposited at the Herbarium of Division of Ocean Science, Korea Maritime University, Korea.

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Instruments and reagents

MS spectrum was measured with a Jeol JMS-600 mass spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded with a Varian 300 NMR spectrometer in DMSO or CD_3OD using TMS as an internal standard. Chemical shifts were reported in parts per million (δ), and coupling constants (*J*) were expressed in hertz. TLC analysis was performed on Kieselgel 60 F_{254} (Merck) TLC aluminum sheets, with compounds visualized by spraying with 5% H_2SO_4 followed by charring at 100°C. Silica gel (Merck, 200-400 mesh ASTM) was used for column chromatography. YMC column was used for HPLC analysis (Dionex P580). All other chemicals and reagents were analytical grade.

Extraction and isolation

The shade-dried samples (3 kg) of *S. japonica* were chopped into small pieces and repeatedly extracted for 3 days with CH_2Cl_2, MeOH and CH_2Cl_2-MeOH (1:1), successively. The combined crude extracts (235 g) were partitioned between CH_2Cl_2 and water. The organic layer was repartitioned with 85% MeOH and *n*-hexane. The aqueous fraction was also further fractionated with *n*-BuOH and H_2O, successively, to produce the *n*-hexane (15.6 g), 85% MeOH (6.3 g), *n*-BuOH (10.0 g), and H_2O fraction. The 85% aqueous MeOH fraction (6.0 g) was fractionated into three fractions, using silica gel column chromatography (CH_2Cl_2-MeOH = 100:1 → 1:1). The second fraction (3.2 g) was further purified by column chromatography over a silica gel eluting with *n*-hexane-EtOAc (4:1→1:1) solvent system to afford five sub-fractions. The third sub-fraction was recrystallization from *n*-hexane-EtOAc (5:1) to yield compound **1** (9.0 mg). The fourth sub-fraction was subjected to reversed-phase C_{18} column chromatography that produced seven sub-fractions. The second sub-fraction was purified using HPLC (YMC ODS column, 40% MeOH) to yield compounds **2** (3.9 mg) and **3** (3.6 mg). The *n*-BuOH fraction (10.0 g) was chromatographed on a reversed-phase C_{18}

column with a MeOH-H_2O (20% → 100% MeOH). Fractions were combined based on their TLC profiles to yield sub-fraction designated as C1~C6. The sub-fraction C3 (4 g) was rechromatographed on a silica gel eluting with a CH_2Cl_2-MeOH (30:1 → 1:1) solvent system to afford six sub-fractions. The third sub-fraction was purified using HPLC (YMC polyamine column, 90% acetonitrile) to yield compound **4** (3.0 mg).

Compound **1**: EI-MS (70 eV, rel. int., %): *m/z* 282 $[M]^+$ (76.5), 265 (18.7), 225 (1.3), 164 (100), 145 (5.7), 136 (9.7), 118 (9.5), 89 (3.9); ^{1}H-NMR (300 MHz, DMSO-d_6) δ_H (ppm): 9.35 (2'-OH), 8.26 (1H, s, H-2), 7.36 (1H, s, H-5), 7.28 (1H, s, H-8), 7.21 (1H, d, J = 8.4 Hz, H-6'), 7.17(1H, d, J = 8.4 Hz, H-3'), 6.86 (1H, dd, J = 8.4, 1.2 Hz, H-4'), 6.82 (1H, dd, J = 8.4, 1.2 Hz, H-5'), 6.21 (2H, s, -OCH_2O-); ^{13}C-NMR (75 MHz, DMSO-d_6) δ_C (ppm): 174.2 (C-4), 155.4 (C-2'), 154.5 (C-7), 153.0(C-2), 152.5 (C-9), 146.1 (C-6), 131.7 (C-4'), 129.3 (C-6'), 121.5 (C-3), 119.2 (C-1'), 118.7 (C-5'), 118.6 (C-10), 115.8 (C-5), 102.9 (C-3'), 101.3(-OCH_2O-), 98.3 (C-8).

Compound **2**: EI-MS (70 eV, rel. int., %): *m/z* 196 $[M]^+$ (35.9), 178 (96.1), 163 (31.9), 153 (22.6), 140 (55.8), 135 (35.8), 125 (11.9), 111 (100), 95 (25.9), 85 (24.6); ^{1}H-NMR (300 MHz, CD_3OD) δ_H (ppm): 5.73 (1H, s, H-3), 4.20 (1H, m, H-6), 2.41 (1H, dt, J = 13.7, 2.7 Hz, H-7β), 1.97 (1H, dt, J = 14.7, 2.4 Hz, H-5β), 1.76 (3H, s, H-10), 1.73 (1H, dd, J = 13.7, 3.6 Hz, H-7α), 1.52 (1H, dd, J = 14.7, 3.9 Hz, H-5α), 1.46 (3H, s, H-8), 1.27 (3H, s, H-9); ^{13}C-NMR (75 MHz, CD_3OD) δ_C (ppm): 185.4 (C-3a), 174.6 (C-2), 113.2 (C-3), 88.9 (C-7a), 67.2 (C-6), 48.0 (C-5), 46.4 (C-7), 37.2 (C-4), 31.15 (C-8), 27.5 (C-10), 27.0 (C-9).

Compound **3**: EI-MS (70 eV, rel. int., %): *m/z* 222 $[M]^+$ (2.7), 196 (3.8), 183 (26.3), 166 (31.8), 148 (26.8), 124 (100), 96 (25.9), 69 (13.9); ^{1}H-NMR (300 MHz, CD_3OD) δ_H (ppm): 6.95 (1H, d, J = 16.2 Hz, H-7), 6.41 (1H, d, J = 16.2 Hz, H-8), 5.91 (1H, t, J = 1.2 Hz, H-4), 2.58 (1H, br d, J = 17.1 Hz, H-2β), 2.29 (3H, s, H-10), 2.24 (1H, dd, J = 17.1, 1.2 Hz, H-2α), 1.89 (3H, s, H-13), 1.06 (3H, s, H-12), 1.01 (3H, s, H-11); ^{13}C-NMR (75 MHz, CD_3OD) δ_C (ppm): 200.4 (C-3), 200.1 (C-9), 164.5 (C-5), 148.2 (C-7), 131.6 (C-8), 127.9 (C-4), 79.9 (C-6), 50.5 (C-2), 42.7 (C-1), 27.7 (C-10), 24.8 (C-12), 23.6 (C-11), 19.2 (C-13).

Compound **4**: EI-MS (70 eV, rel. int., %): *m/z* 244 $[M]^+$ (6.6), 226 (29.6), 209 (5.0), 171 (18.1), 141 (24.8), 133 (71.2), 113 (100), 98 (17.9), 73 (76.9); ^{1}H-NMR (300 MHz, CD_3OD) δ_H (ppm): 7.98 (1H, d, J = 8.1 Hz, H-6), 5.88 (1H, d, J = 4.5 Hz, anomeric H), 5.68 (1H, d, J = 8.1 Hz, H-5); ^{13}C-NMR (75 MHz, CD_3OD) δ_C (ppm): 165.9 (C-4), 152.3 (C-3), 142.6 (C-6), 102.5 (C-5), 90.6 (C-1'), 86.3 (C-4'), 75.7 (C-2'), 71.3 (C-3'), 62.2 (C-5').

RESULTS AND DISCUSSION

A portion of the 85% MeOH and *n*-BuOH fractions from *S. japonica* was chromatographed on a silica gel to afford compounds **1-4**.

Compound **1** was obtained as yellow crystals. The EI-MS showed a molecular ion peak at *m/z* 282 $[M]^+$. In the ^{1}H-NMR spectrum, the typical singlet signal of H-2 of the isoflavone nucleus was shown at δ 8.26, and the position of a 2'-hydroxyl proton was determined at δ 9.35. In the aromatic proton region, two doublets at δ 7.21 (8.4 Hz, H-6') and 7.17 (8.4 Hz, H-3') and two double doublets at δ 6.86 (8.4, 1.2 Hz, H-4') and 6.82 (8.4, 1.2 Hz, H-5') were observed. The ^{1}H-NMR signal at δ 6.21 (s) and ^{13}C-NMR signal at δ 101.3 revealed the presence of a methylenedioxy group between C-6 and -7 in **1** by analysis of H-5 (s) and -8 (s) (Chihi *et al.*, 1986; Ferreira & Dias, 2000; Wong *et al.*, 1987). Accordingly, the structure of **1** was elucidated as 2'-hydroxy-6,7-methylenedioxyisoflavone by comparing its spectral data in the literature (Arakawa *et al.*, 1982; Chihi *et al.*,1986; Ferreira & Dias, 2000; Wong *et al.*,1987).

Compound **2** was obtained as white powders. The EI-MS showed a molecular ion peak at *m/z* 196 $[M]^+$. In the ^{1}H-NMR spectrum, two germinal methyl protons at δ 1.46 (s, H-8) and 1.27 (s, H-9), and one olefinic proton at δ 5.73 (s, H-3) were observed. α,β-unsaturated-γ-lactone group at δ 185.4 (C-3a), 174.6 (C-2) and 113.2 (C-3), and oxygenated carbon signals at δ 88.9 (C-7a) and 67.2 (C-6) were observed in the ^{13}C-NMR spectrum. Accordingly, the structure of **2** was elucidated as loliolide by comparing its spectral data in the literature (Chung *et al.*, 2002; Mori, 1974; Kim *et al.*, 2004b; Park *et al.*, 2004; Valdes III, 1986).

Compound **3** was obtained as light yellow oil. The EI-MS showed a molecular ion peak at *m/z* 222 $[M]^+$. In the ^{1}H-NMR spectrum, it revealed signals assignable to a vinyl proton at δ 5.91 (t, 1.2 Hz, H-4), and two trans olefinic protons at δ 6.95 (d, 16.2 Hz, H-7) and 6.41 (d, 16.2 Hz, H-8), in addition to two *gem*-dimethyl protons at δ 1.06 (s, H-12) and 1.01 (s, H-11), a methyl proton at δ 1.89 (H-13) connected to a double bond, and a methyl group at proton δ 2.29 adjacent to a carbonyl group. Geminally coupled signals at δ 2.58 (br d, 17.1 Hz, H-2β) and 2.24 (dd, 17.1, 1.2 Hz, H-2α) suggested that the carbonyl group was connected to this methylene and that *gem*-dimethyl was substituted to C-1. Two carbonyl signals at δ 200.4 (C-3) and 200.1 (C-9), four olefinic carbon signals at δ 164.5 (C-5), 148.2 (C-7), 131.6 (C-8) and 127.9 (C-4), and an oxygenated carbon signal at δ 79.9 (C-6) were observed in the ^{13}C-NMR spectrum. Thus, compound **3** was predicted to be a 3-oxo-6-hydroxy-ionone. Accordingly, the structure of **3** was elucidated as dehydrovomifoliol by comparing its spectral data in the literature

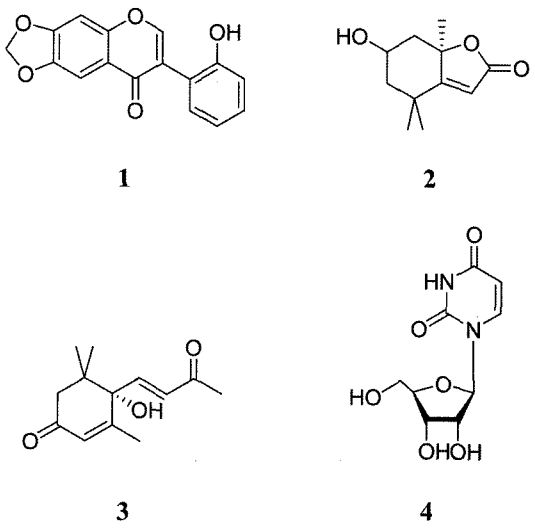

Fig. 1. Structures of compounds **1-4**.

(Chung *et al.*, 2002; Mori, 1974; Kato *et al.*, 1977; Kim *et al.*, 2004a; Kim *et al.*, 2004b).

Compound **4** was obtained as white powders. The EI-MS showed a molecular ion peak at *m/z* 244 $[M]^+$. The typical signals of uracil derivatives were shown in the ^1H- and ^{13}C-NMR spectra. Accordingly, the structure of **4** was elucidated as uridine by comparing its spectral data in the literature (Ham *et al.*, 1999; Pouchert & Behnke, 1993).

To the best of our knowledge, 2'-hydroxy-6,7-methylenedioxyisoflavone (**1**), loliolide (**2**), dehydrovomifoliol (**3**), and uridine (**4**) were isolated for the first time from this plant.

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