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Constituents of the Halophyte *Salicornia herbacea*

Yeon Sil Lee, Hye Seung Lee, Kuk Hyun Shin, Bak-Kwang Kim[1], and Sanghyun Lee[1]

Seokwon Life Science Research Institute, World Sea Green Co. Ltd., Paju 413-832, Korea and [1]College of Pharmacy, Seoul National University, Seoul 151-742, Korea

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Four compounds were isolated from *Salicornia herbacea* by repeated column chromatography. Their structures were identified as β-sitosterol (**1**), stigmasterol (**2**), uracil (**3**), and isorhamnetin-3-*O*-β-D-glucopyranoside (**4**) by spectral analysis and comparison with the published data.

Key words: *Salicornia herbacea*, Chenopodiaceae, Uracil, Isorhamnetin-3-*O*-β-D-glucopyranoside

INTRODUCTION

Salicornia herbacea L. (Chenopodiaceae) is one of the halophytes that can grow in salt marshes, or salt fields along the seashores in Korea (Kim and Song, 1983; Lee, 1997). It has been used as a fork medicine as well as a seasoned vegetable by some people living in coastal area. This plant has previously been shown to stimulate cytokine production, nitric oxide release and expression of surface molecules (Im *et al.*, 2003). But there are a few references for the isolation of constituents from this plant.

In a searching of naturally occurring bioactive compounds from *S. herbacea*, constituents were isolated by repeated column chromatography. The present study was reported the isolation and elucidation of constituents from the halophyte *S. herbacea* collected from Korea.

MATERIALS AND METHODS

Instruments and reagents

MS spectra were measured with a Jeol JMS-AX505WA mass spectrometer. IR spectra were recorded with a Jasco FT/IR-300E instrument on KBr disc. ^{1}H- and ^{13}C-NMR spectra were recorded with a Bruker AVANCE 400 NMR spectrometer in $CDCl_3$ or DMSO using TMS as an internal standard. All other chemicals and reagents were analytical grade.

Correspondence to: Sanghyun Lee, College of Pharmacy, Seoul National University, Shillim-dong, Gwanak-gu, Seoul 151-742, Korea
Tel: 82-2-880-9178, Fax: 82-2-878-1652
E-mail: jnp@korea.com

Plant material

The plant material of *Salicornia herbacea* L. was collected at the south seashore of Mokpo, Oct. 2003, Korea. The material was botanically identified by Prof. Jong-Ahm Shin, Yosu National University, Korea. A voucher specimen (No. WSG 2003-02) was deposited at the Herbarium of Seokwon Life Science Research Institute, World Sea Green Co. Ltd., Korea.

Extraction and isolation

The air-dried powdered whole plant (4 kg) of *S. herbacea* was extracted with MeOH under reflux. After removal of the solvent *in vacuo*, the residue (430 g) was suspended in water and then extracted with *n*-hexane (166 g), CH_2Cl_2 (4 g), EtOAc (24 g), and *n*-BuOH (50 g) fraction after evaporation. A portion of the *n*-hexane fraction (20 g) was chromatographed on a silica gel by a gradient elution with *n*-hexane and EtOAc to afford compounds **1** (95:5, 83 mg) and **2** (95:5, 39 mg). A portion of the EtOAc fraction (20 g) was chromatographed on a silica gel by a gradient elution with CH_2Cl_2 and MeOH to afford compounds **3** (85:15, 5 mg) and **4** (90:10, 506 mg).

Compound **3**; EI-MS (rel. int. %): *m/z* 112 $[M]^+$ (100), 97 (0.5), 83 (0.7), 69 (50.0), 68 (17.2), 57 (2.0); IR ν_{max} (KBr) cm^{-1}: 3433 (-OH), 1418, 1235; ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 11.01 (1H, s, -OH), 10.81 (1H, s, -OH), 7.38 (1H, d, $J = 7.6$ Hz, H-6), 5.44 (1H, d, $J = 7.6$ Hz, H-5); ^{13}C-NMR (100 MHz, DMSO-d_6) δ_c (ppm): 164.3 (C-4), 151.5 (C-2), 142.2 (C-6), 100.2 (C-5).

Compound **4**; EI-MS (rel. int. %): *m/z* 316 $[M]^+$ (100), 301

(21.9), 287 (9.7), 273 (7.2), 245 (10.7), 217 (6.7), 153 (5.0), 128 (4.6), 121 (1.8), 108 (3.9); FAB-MS: *m/z* 479 $[M+H]^+$; IR ν_{max} (KBr) cm^{-1}: 3383 (OH), 1652 (α,β-unsaturated C=O), 1055 (C-O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 12.61 (1H, s, 5-OH), 7.94 (1H, d, *J* = 2.0 Hz, H-2'), 7.49 (1H, dd, *J* = 8.4, 2.0 Hz, H-6'), 6.91 (1H, d, *J* = 8.4 Hz, H-5'), 6.44 (1H, d, *J* = 2.0 Hz, H-8), 6.20 (1H, d, *J* = 2.0 Hz, H-6), 5.57 (1H, d, *J* = 7.4 Hz, anomeric H-1), 3.83 (3H, s, $-OCH_3$); ^{13}C-NMR (100 MHz, DMSO-d_6) δ_c (ppm): 177.4 (C-4), 164.3 (C-7), 161.2 (C-5), 156.4 (C-2), 156.3 (C-9), 149.4 (C-4'), 146.9 (C-3'), 133.0 (C-3), 122.0 (C-6'), 121.1 (C-1'), 115.2 (C-5'), 113.4 (C-2'), 104.0 (C-10), 100.8 (Glc C-1), 98.7 (C-6), 93.7 (C-8), 77.5 (Glc C-5), 76.4 (Glc C-3), 74.3 (Glc C-2), 69.8 (Glc C-4), 60.6 (Glc C-6), 55.7 ($-OCH_3$).

Acid hydrolysis of 4

Compound **4** (10 mg) was refluxed with 5% H_2SO_4 in MeOH (3 mL) for 4 h. Workup in the usual way, followed by crystallization afforded glucose (co-TLC, *n*-BuOH: $HOAc:H_2O$ = 4:1:5) and an aglycone identified as isorhamnetin (**4a**).

Compound **4a**; EI-MS (rel. int. %): *m/z* 316 $[M]^+$ (100), 301 (28.5), 287 (11.3), 273 (9.8), 245 (17.5), 217 (9.6), 153 (7.8), 128 (6.4), 121 (1.9), 108 (4.9); ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H (ppm): 12.62 (1H, s, 5-OH), 7.93 (1H, d, *J* = 1.9 Hz, H-2'), 7.48 (1H, dd, *J* = 8.3, 1.9 Hz, H-6'), 6.90 (1H, d, *J* = 8.3 Hz, H-5'), 6.45 (1H, d, *J* = 2.0 Hz, H-8), 6.19 (1H, d, *J* = 2.0 Hz, H-6), 3.84 (3H, s, $-OCH_3$); ^{13}C-NMR (100 MHz, DMSO-d_6) δ_c (ppm): 176.4 (C-4), 163.3 (C-7), 161.5 (C-5), 156.3 (C-2), 156.1 (C-9), 149.3 (C-4'), 147.0 (C-3'), 133.5 (C-3), 122.0 (C-6'), 121.2 (C-1'), 115.3 (C-5'), 112.4 (C-2'), 103.9 (C-10), 98.6 (C-6), 93.6 (C-8), 55.6 ($-OCH_3$).

RESULTS AND DISCUSSION

Four compounds were isolated from the *n*-hexane and EtOAc fraction of *S. herbacea* by repeated column chromatography.

Compounds **1** and **2** were elucidated as β-sitosterol and stigmasterol, respectively, by spectral analysis and comparison with the published data (Do *et al.*, 1988).

Compound **3** was obtained as white powder from MeOH. The EI-MS of **3** showed an $[M]^+$ ion at *m/z* 112 as a base peak. In the ^{1}H-NMR spectrum of **3**, the doublets at δ 7.38 (*J* = 7.6 Hz) and 5.44 (*J* = 7.6 Hz) assigned H-6 and -5, respectively. The each singlet at δ 11.01 and 10.81 showed hydroxyl signals. Its ^{13}C-NMR spectrum of **3** showed two C-O signals at δ 164.3 and 151.5. The IR spectrum of **3** showed adsorption bands for hydroxy at 3433 cm^{-1} and C-O at 1418, 1235 cm^{-1}. Accordingly, the

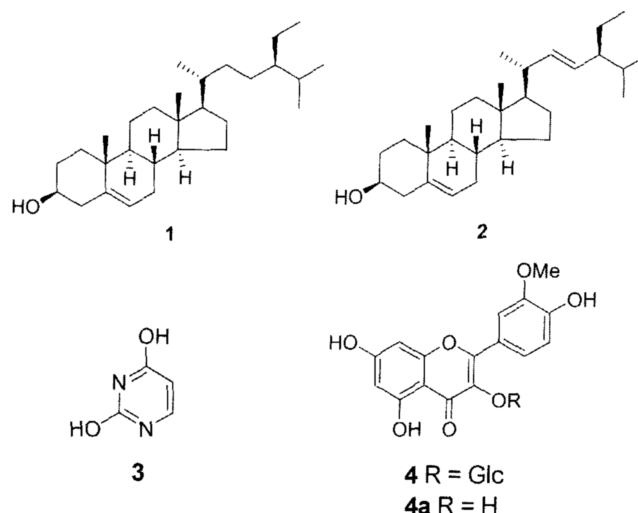

Fig. 1. Structures of compounds **1**-**4**

structure of **3** was elucidated as uracil by comparing its spectral data in the literature (Ko *et al.*, 1992). Uracil (**3**) has previously been isolated from *Angelica gigas* (Lee *et al.*, 2002), *Euodia daniellii* (Yoo *et al.*, 2002), *Ganoderma capens* (Yu and Zhai, 1979), *Nothapodytes foetida* (Wu *et al.*, 1995) and *Peucedanum japonicum* (Hisamoto *et al.*, 2003).

Compound **4** was obtained as yellow crystals from MeOH. It responded positively to the Shinoda and the Molisch test. In the EI-MS of **4**, the aglycone peak showed at *m/z* 316. The characteristic fragment ion peaks at *m/z* 153 and 121 showed the *retro* Diels Alder fragmentation of flavonoids (Markham, 1982). The aglycone of **4** was identified as isorhamnetin (**4a**) by chemical reaction (acid hydrolysis). The FAB-MS of **4** showed $[M+H]^+$ peak at *m/z* 479 corresponding to the molecular formula $C_{22}H_{22}O_{12}$. In the ^{1}H-NMR spectrum of **4**, the typical flavonoid signals were observed. Two *meta*-coupled signals at δ 6.20 (d, *J* = 2.0 Hz, H-6) and 6.44 (d, *J* = 2.0 Hz, H-8), and three ABX type signals at δ 7.94 (d, *J* = 2.0 Hz, H-2'), 7.49 (dd, *J* = 8.4, 2.0 Hz, H-6'), and 6.91 (d, *J* = 8.4 Hz, H-5') due to (B) ring were observed. The singlets of aromatic 5-OH at δ 12.61 and of $-OCH_3$ signal at δ 3.83 were observed. The position of $-OCH_3$ was at C-3' of (B) ring by HMBC analysis. Glucose position was deduced at C-3 of aglycone by the HMBC analysis. Its ^{13}C-NMR spectrum of **4** showed C=O at δ 177.4, $-OCH_3$ at δ 55.7 and carbons of glucose. The carbon signal at δ 100.8 showed anomeric C-1. The IR spectrum of **4** showed absorption bands for hydroxyl at 3383 cm^{-1}. Accordingly, the structure of **4** was elucidated as isorhamnetin-3-*O*-β-D-glucopyranoside by comparing its spectral data in the literature (Kang *et al.*, 1983). Isorhamnetin-3-*O*-β-D-glucopyranoside (**4**) has previously been isolated from *Astragalus tribuloides* (El-Sebakhy *et al.*, 2000), *Brassica rapa* (Kim *et al.*, 1998), *Calotropis*

gigantea (Sen *et al.*, 1992), *Diospyros kaki* (Chen *et al.*, 2002), *Pyrus communis* (Rychlinska and Gudej, 2002), *Syzygium aromaticum* (Son *et al.*, 1998), *Typha latifolia* (Kang *et al.*, 1983) and *Warburgia stuhlmannii* (Manguro *et al.*, 2003).

To the best of our knowledge, this is the first report on the isolation of β-sitosterol (**1**), stigmasterol (**2**), uracil (**3**) and isorhamnetin-3-*O*-β-D-glucopyranoside (**4**) from *S. herbacea*.

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REFERENCES

Chen, G., Lu, H., Wang, C., Yamashita, K., Manabe, M., Meng, Z., Xu, S., and Kodama, H., Effect of five flavonoid compounds isolated from leaves of *Diospyros kaki* on stimulus-induced superoxide generation and tyrosyl phosphorylation of proteins in human neutrophils. *Clin. Chim. Acta*, 326, 169-175 (2002).

Do, J. C., Son, K. H., and Kang, S. S., Studies on the constituents of the roots of *Rubus parvifolius* (I). Isolation of (-)-epicatechin. *Kor. J. Pharmacogn.*, 19, 170-173 (1988).

El-Sebakhy, N. A., Asaad, A. M., Abdallah, R. M., Toaima, S. M., Verotta, L., and Orsini F., Constituents of Egyptian *Astragalus tribuloides* Del. *Nat. Prod. Sci.*, 6, 11-15 (2000).

Hisamoto, M., Kikuzaki, H., Ohigashi, H., and Nakatani, N., Antioxidant compounds from the leaves of *Peucedanum japonicum* Thunb. *J. Agric. Food Chem.*, 51, 5255-5261 (2003).

Im, S.-A., Kim, G.-W., and Lee, C.-K., Immunomodulatory activity of *Salicornia herbacea* L. components. *Nat. Prod. Sci.*, 9, 273-277 (2003).

Kang, S. S., Choi, J. S., Woo, W. S., and Chi, H.-J., Isorhamnetin glycosides from the leaves of *Typha latifolia*. *Kor. J. Pharmacogn.*, 14, 137-139 (1983).

Kim, C. S. and Song, T. G., Ecological studies on the halophyte communities at western and southern coasts in Korea. *Kor. J. Ecol.*, 6, 167-176 (1983).

Kim, H. K., Kang, B. J., Park, K. J., Ko, B. S., and Whang W. K., Anti-herpes simplex virus type 1 (HSV-1) effect of isorhamnetin 3-*O*-β-D-glucopyranoside isolated from *Brassica rapa*. *Yakhak Hoeji*, 42, 607-612 (1998).

Ko, S. H., Do, S. H., Kwon, Y. S., and Kim, C. M., A study on the chemical components from the roots of *Anthriscus sylvestris*. *Kor. J. Pharmacogn.*, 23, 225-228 (1992).

Lee, S., Kang, S. S., and Shin, K. H., Coumarins and a pyrimidine from *Angelica gigas* roots. *Nat. Prod. Sci.*, 8, 58-61 (2002).

Lee, Y. N., Flora of Korea, Kyohak Publishing Co., Seoul, p. 144 (1997).

Manguro, L. O. A., Ugi, I., Hermann, R., and Lemmen, P., Flavonol and drimane-type sesquiterpene glycosides of *Warburgia stuhlmannii* leaves. *Phytochemistry*, 63, 497-502 (2003).

Markham, K. R., Techniques of flavonoid identification, Academic press, London, pp. 87-90 (1982).

Rychlinska, I. and Gudej, J., Flavonoid compounds from *Pyrus communis* L. flowers. *Acta Pol. Pharm.*, 59, 53-56 (2002).

Sen, S., Sahu, N. P., and Mahato, S. B., Flavonol glycosides from *Calotropis gigantea*. *Phytochemistry*, 31, 2919-2921 (1992).

Son, K. H., Kwon, S. Y., Kim, H. P., Chang, H. W., and Kang, S. S., Constituents from *Syzygium aromaticum* Merr. et Perry. *Nat. Prod. Sci.*, 4, 263-267 (1998).

Wu, T. S., Leu, Y. L., Hsu, H. C., Ou, L. F., Chen, C. C., Chen, C. F., Ou, J. C., and Wu, Y. C., Constituents and cytotoxic principles of *Nothapodytes foetida*. *Phytochemistry*, 39, 383-385 (1995).

Yoo, S. W., Kim, J. S., Kang, S. S., Son, K. H., Chang, H. W., Kim, H. P., Bae, K. H., and Lee, C.-O., Constituents of the fruits and leaves of *Euodia daniellii*. *Arch. Pharm. Res.*, 25, 824-830 (2002).

Yu, J. and Zhai, Y., Studies on the constituents of *Ganoderma capens*. *Acta Pharmaceut. Sin.*, 14, 374-378 (1979).