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Phenolic Constituents from the Aerial Parts of *Artemisia stolonifera*

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Two acetophenone glycosides, 2,4-dihydroxy-6-methoxy acetophenone 4-O-β-D-glucopyranoside(I), 2,4,6-trihydroxy acetophenone 2-O-β-D-glucopyranoside(II), together with coniferin(III) were isolated from the aerial parts of *Artemisia stolonifera* (Max.) Kom. The structures of the compounds were elucidated on the basis of spectroscopic evidence. Compound II was also confirmed as a new phenolic glycoside from natural sources. In addition, compound I induced cytostatic activity of macrophages, while compounds II and III did not.

Keyword : *Artemisia stolonifera*, Compositae, Phenolic glycoside, 2,4,6-Trihydroxy acetophenone 2-O-β-D-glucopyranoside, 2,4-Dihydroxy-6-methoxy acetophenone 4-O-β-D-Glucopyranoside, Immunomodulating activity

INTRODUCTION

Artemisia stolonifera (Compositae) grows as a perennial herb in mountainous area of Korea where about 30 *Artemisia species* are widely distributed (Lee, 1989). *Artemisia stolonifera* herbs have been used as diuretics, antipyretics and for treating eye diseases (Song, 1990). *Artemisia species* have been extensively investigated for their chemical constituents (Atta-ur-Rahman, 1990, Kelsey *et al.*, 1979). However, there are no reports on *Artemisia stolonifera* except for a herniarin isolated by Konovalova (Konovalova *et al.*, 1976). As part of our systematic survey on the genus Artemisia in Korea (Jang *et al.*, 1993, Jang *et al.*, 1993, Koo et al., 1994), we report herein isolation and structural determination of two acetophenone compounds (I, II) and coniferin (III) from Artemisia stolonifera, and biological activities of the compounds.

MATERIALS AND METHODS

General experimental procedures

Melting points were taken with a Gallenkamp melting point apparatus (uncorr.). ^{1}H-and ^{13}C-NMR spectra were recorded on a Varian Gemini 300 spectrometer. The UV spectra were recorded on Shimadzu UV 240 UV-Visible recording spectrophotometer. The IR spectra were measured on Shimadzu IR-435 Infrared spectrophotometer (KBr disc). All other chemical and solvents were analytical grade and used without further purification.

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Plant material

Aerial parts of *Artemisia stolonifera* (Compositae) were collected August of 1994 at Odaesan, Kangwon-do, Korea. A voucher speciman is deposited in the herbarium of college of Pharmacy, SungKyunKwan University.

Extraction and Isolation

The aerial parts of *A. stolonifera* (2.0 kg) were extracted in 20 liters of 95 % EtOH three times at room temperature. The concentrated EtOH extract (100 g) was partitioned successively with n-hexane, EtOAc and n-BuOH. The EtOAc extract (15 g) was subjected to a column chromatography over SiO_2 eluting with n-hexane-EtOAc-MeOH (5 : 5 : 1). The eluates were fractionated by TLC to yield fractions designated as E1-E5. Fraction E4 was further chromatographed repeatedly by LPLC on Lobar A column using EtOAc-MeOH-H_2O=100 : 7 : 3.5 and $CHCl_3$-MeOH=5 : 1, which afforded 12mg of compound I and 10 mg of compound II as colorless needles. The n-BuOH extract (15 g) was chromatographed repeatedly on SiO_2 column eluted with a gradient of $CHCl_3$-MeOH to give B1-B4 fractions. Fraction B4 was purified by LPLC on Lobar A column eluting with $CHCl_3$-MeOH (5 : 1), which yielded 15 mg of III as colorless crystals.

Compound I: mp 156-158°C ; IR ν_{max}^{KBr} cm^{-1} 3400

Table I. ^{13}C-NMR spectral data of compounds I, II and III

C	I	II	III
1	106.3	106.8	131.0
2	165.3*	162.6	110.0
3	96.1	95.5	149.0
4	163.6*	167.7*	146.0
5	92.0	98.2	115.3
6	162.6	166.3*	119.0
7	—	—	129.0
8	—	—	128.4
9	—	—	61.6*
OCH_3	56.1	—	55.6
C=O	203.0	204.9	—
CH_3	32.7	33.5	—
glc.C-1	99.6	102.0	100.0
C-2	73.1	74.7	73.2
C-3	77.2	78.5	77.0
C-4	69.8	71.1	69.7
C-5	76.5	78.4	76.8
C-6	60.7	62.4	60.7*

*Within the same column assignments are interchangeable

(OH), 1625 (C=O), 1590 (aromatic C=C), 1070 (glycosidic C-O) ; UV max (MeOH) nm : 282, 320 sh ; ^{1}H-NMR (DMSO-d6, 300 MHz, ppm): δ 2.57(3H, s, $COCH_3$), 3.10~3.75 (sugar H), 3.86 (3H, s, OCH3), 4.99 (1H, d, J=7.4 Hz, anomeric H of glc.), 6.15 (1H, d, J=2.0 Hz, H-3), 6.21 (1H, d, J=2.0 Hz, H-5) ; ^{13}C-NMR (DMSO-d6, 75.5 MHz,ppm): see Table I

Compound II: mp 201-203°C ; IR ν_{max}^{KBr} cm^{-1} 3400 (OH), 1625 (C=O), 1600 (aromatic C=C), 1065 (glycosidic C-O) UV λmax (MeOH) nm: 283, 320sh ^{1}H-NMR (CD_3OD, 300 MHz, ppm): δ 2.68(3H, s, $COCH_3$), 3.25~3.95 (sugar H), 5.02 (1H, d, J=7.4 Hz, anomeric H of glc.), 5.94 (1H, d, J=2.2 Hz, H-5), 6.17 (1H, d, J=2.2 Hz, H-3) ; ^{13}C-NMR (CD_3OD, 75.5 MHz,ppm): see Table I

Compound III: mp 185-188°C ; IR ν_{max}^{KBr} cm^{-1} 3400 (OH), 2850, 1590, 1510 (aromatic C=C), 1070 (glycosidic C-O) ; UV max (MeOH) nm: 260, 293 ; ^{1}H-NMR (DMSO-d6, 300 MHz, ppm): δ 3.10~3.80 (sugar H), 3.78 (3H, s, OCH_3), 4.09 (2H, br.s, H-9), 4.88 (1H, d, J=6.0 Hz, anomeric H of glc.), 6.27 (1H, dt, J=15.9, 4.9 Hz, H-8), 6.47 (1H, d, J=15.9 Hz, H-7) ; ^{13}C-NMR (DMSO-d6, 75.5 MHz, ppm): see Table I

Acetylation of Compound II: Compound II (6.5 mg) was acetylated with Ac_2O/pyridine (1 : 1) at room temperature overnight. The usual workup gave an acetate. (compound II_a, 5.2 mg), IR ν_{max}^{KBr} cm^{-1} 1755(C=O), 1605 (aromatic C=C), 1215, 1060 (glycosidic C-O) ; UV λmax (MeOH) nm: 290 sh, 296 ; ^{1}H-NMR ($CDCl_3$, 300 MHz, ppm): δ 2.02, 2.04(×2), 2.10, 222, 2.29 (each 3H, s, OAc×6), 2.44 (3H, s, $COCH_3$), 3.89 (1H, m, glc. H-5), 4.19 (1H,dd, J=12.3, 2.4 Hz, glc. H-6), 4.26 (1H, dd, J=12.3, 5.4 Hz, glc. H-6), 5.05 (1H,d, J=7.3 Hz, glc. H-1), 5.14 (1H, m, glc. H-4), 5.27 (2H, m, glc. H-2, 3), 6.71 (1H, d, J=2.3 Hz, H-5), 6.82 (1H, d, J=2.3 Hz, H-3)

Table II. *In vitro* augmentation of cytostatic activity of macrophages by compounds

Treatment[a]	Concentration (μg/ml)	% Cytostasis of target cell (B16)
None		28.79±1.26
Compound I	100	72.09±2.03*
	10	70.73±3.35*
	1	60.77±2.44*
Compound II	100	39.15±4.22
	10	33.24±3.12
	1	30.32±2.45
Compound III	100	35.32±3.98
	10	33.34±4.55
	1	27.89±1.67
LPS[b] (1 μg/ml)+ IFN[c]-γ (100 U/ml)		85.89±4.74

[a]Peritoneal macrophages were co-cultured for 12 hours in medium alone or in medium supplemented with compounds. Macrophages were co-cultured for 48 hours with targets at in initial effector/target cell ratio of 10 : 1. Effects on target cell viability are expressed as net percent [^{3}H]-thymidine incorporated. The results are mean±S.D. of quintuplicates from representative experiment.
[b]LPS; Lipopolysaccharide
[c]IFN; Interferon
* Significantly differently from control (no treatment); P<0.001

Immunomodulatory activity

To examine the activity of compounds on murine macrophages which are involved in the defense against tumors and virus infection, macrophages were treated with various doses of compounds. The ability of macrophages to resist infection with herpes simplex virus type-1 and inhibit growth of tumor cells was assessed as described previously(Pyo *et. al.*, 1993).

RESULTS AND DISCUSSION

Compound I was obtained as colorless needles. Its ir spectrum showed presence of hydroxyl (3400 cm^{-1}), carbonyl (1625) and aromatic C=C (1590) functional groups. The uv spectrum of compound I exhibited absorption maxima at 282 nm and 320 (sh) nm, suggesting it contains a benzoyl group (Silverstein *et al.*, 1991; Nagarajan *et al.*, 1977; Yenesew *et al.*, 1994). The ^{1}H-NMR spectrum of compound I showed presence of two meta coupled aromatic protons at 6.15 (d, J=2.0 Hz) and 6.21 (d, J=2.0 Hz), an acetyl methyl group at 2.57 (s), a methoxy group at 3.86 (s), and sugar protons at 3.10-3.75 ppm. From acid hydrolysis of compound I, glucose was identified by TLC compared with an authentic sample. The coupling constant (J=7.4 Hz) of a signal at 4.99 ppm (d)

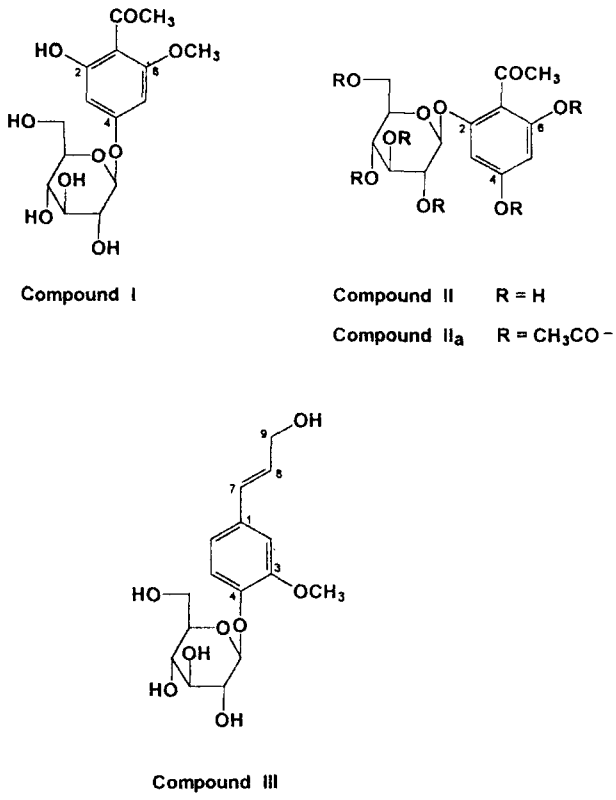

Fig. 1. Structures of Compounds I, II, II_a and III.

corresponded to the β-configuration for the glucose moiety (Friebolin *et al.*, 1993). In the ^{13}C-NMR spectrum of compound I, signals at 203.0 and 32.7 ppm indicated an acetophenone moiety, and 165.3, 163.6, 162.6 ppm indicated oxygen bearing aromatic carbons. From these data, compound I was identified as 2,4-dihydroxy-6-methoxy acetophenone 4-O-β-D-glucopyranoside. Compound I was previously isolated in an acetate form from *A. santolinifolia* (Jakupovic *et al.*, 1991), but the free form has not been reported yet.

Compound II was also obtained as colorless crystals. The ^{1}H-and ^{13}C-NMR spectra of II were similar to those of compound I, but a methoxy group was missing and the ^{1}H-NMR spectrum of the acetate of II showed four aliphatic and two aromatic acetyl signals. This suggested the presence of two free hydroxyl groups at C-4 and C-6. The location of a glucose molecule was confirmed by a detailed analysis of the NOEDS spectrum. Irradiation of the anomeric proton of glucose at 5.02 ppm increased the intensity of the aromatic proton at 6.17 ppm (10%), but the aromatic proton at 5.94 ppm was not affected. If the glucose moiety was located at C-4, both of the protons must be affected. Thus, our results indicated that compound II is 2,4,6-trihydroxy acetophenone 2-O-β-D-glucopyranoside. Although compound II has been reported as a synthetic intermediate (Diedrich *et al.*, 1962), we isolated compound II from a natural source for the first time.

Compound III was identified as coniferin (Falshaw *et al.*, 1969; Podimuang *et al.*, 1971) on the basis of spectral data (^{1}H, ^{13}C-NMR, NOEDS and ^{1}H-^{1}H COSY).

To investigate whether the isolated compounds (I-III) could render macrophages cytostatic or antiviral, thioglycolate-elicited macrophages from CD-1 mice were incubated for 12 hours at various concentrations in the presence of these compounds. As shown in Table II, compound I showed significant in vitro enhancement of tumoricidal activity of macrophages, whereas compounds II and III did not. None of the tested compounds enhanced antiviral activity of macrophages (data not shown). Preliminary experiments indicated that, under similar conditions and concentrations used, the isolated compounds have no direct toxic effect on macrophages or tumor cells. These findings suggest that compound I could be used as an immunomodulator. Further experiments are needed to elucidate the mechanisms of action induced by compound I.

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