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Cytotoxic Constituents from *Solidago virga-aurea* var. *gigantea* MIQ

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Activity-guided fractionation of the whole plant of *Solidago virga-aurea* var. *gigantea* M_{IQ}. (Compositae) has led to the isolation of three cytotoxic compounds, erythrodiol-3-acetate (**1**), α-tocopherol-quinone (**2**), and trans-phytol (**3**) from the hexane soluble fraction. It is the first report of those compounds from the genus.

Key words: *Solidago virga-aurea* var. *gigantea* M_{IQ}, Compositae, Cytotoxic compounds, Erythrodiol-3-acetate, α-Tocopherolquinone, *Trans*-phytol

INTRODUCTION

Solidago virga-aurea var. *gigantea* M_{IQ}. (Compositae), a subsp. of *Solidago virga-aurea* var. *asiatica* Nakai, is a perennial herb growing inherently in the Ulreung-island of *Korea*. The whole plant (root and aerial parts) has been used as a stomachic and diuretic in Korean folk medicine and the young aerial part, as food (Lee, 1979). A variety of *Solidago* species have been reported to possess antibacterial, anti-inflammatory, spasmolytic, and carminative properties. Even now the original species of this plant, *S. virga-aurea* L., under the trade name of Urol mono®, and *S. virga-aurea* var. *asiatica* Nakai named as Zhi-Huang-Hua have been employed as herbal medicines for the treatments of goitre, bronchitis, old ulcers, laryngitis, jaundice, and urological diseases (Britton *et al.*, 1970; Mitsuhashi, 1988). Earlier investigation on the chemical constituents from various *Solidago* species revealed oleanan-type saponins (solidago saponins) (Inose *et al.*, 1991, 1992; Reznicek *et al.*, 1992; Bader *et al.*, 1995; Miyase *et al.*, 1994), diterpenes (Lu *et al.*, 1995), acetylenic compounds (Matsunaga *et al.*, 1990) and aromatic esters (Mann, 1981). However, no chemical or biological studies of this plant are available so far.

In the course of searching for anti-tumor agents from medicinal herbs, the MeOH extract of the whole plant of *Solidago virga-aurea* var. *gigantea* M_{IQ} was found to be active against a human cancer cell line (SK-MEL-2). Subsequent cytotoxicity-directed fractionation led to the isolation of three active compounds, erythrodiol-3-acetate (**1**), α-tocopherol-quinone (**2**), *trans*-phytol (**3**) together with 2-methoxybenzyl-2,6-dimethoxybenzoate (**4**) (Fig. 1) from the hexane-soluble fraction. These compounds were evaluated for their cyctotoxicity against five human cancer cell lines (Table I).

MATERIALS AND METHODS

Instruments and reagents

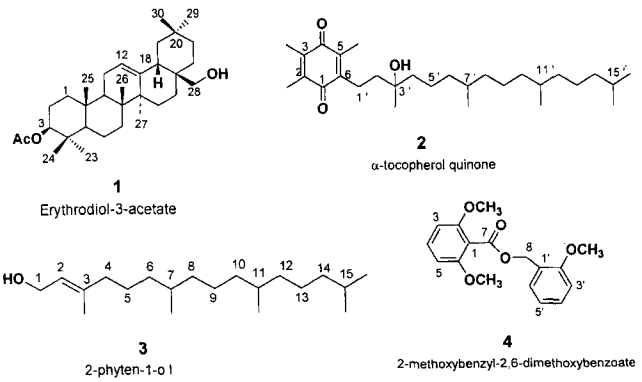

Fig. 1. Structure of the compounds from *Solidago virga-aurea* var. *gigantea* M_{IQ}.

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Table I. Cytotoxic activity of compounds 1-4 [a-b]

Compounds	ED_{50} (μM) of the separated compounds on cancer cells				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	18.2	19.3	19.4	10.3	11.2
2	8.7	22.0	8.2	5.9	6.4
3	16.2	16.2	21.4	21.39	37.8
4	57.6	37.7	50.1	54.8	35.8
Doxorubicin	0.8	0.9	1.4	1.43	2.4

[a] A549 (Non-small lung caner cell); SK-OV-3 (Ovarian cancer cell); SK-MEL-2 (Skin cancer cell); XF498 (CNS cancer cell); HCT15 (Colorectal cancer cell).
[b] Doxorubicin was used as a reference.

Melting points were determined on a Electrothermal IA 9200 melting point appratus and were uncorrected. Optical rotations were measured with a JASCO DIP-1000 instrument. Nuclear magnetic resonance spectra (^{1}H-NMR and ^{13}C-NMR spectra taken at 300 and 125 MHz, respectively) were recorded on a Bruker-DPS-300 and -DPS-500 spectrometer using TMS as the internal standard. The EI/MS (70 eV) spectra were determined using a Jeol JMS-AX 505H. HR-MS (70 eV) were measured with the Jeol JMS-SX 102 spectrometer. UV spectra were obtained using a Jasco 500 series. IR spectra were measured in KBr disk using a Jasco FT/IR-300E. Medium pressure liquid chromatography (MPLC) system was comprised of a metering pump (Eldex B-100-S-4, Eldex Ltd., USA) and UV detector (Hitachi L-4200, Japan) by using columns from Merck Lobar Si 60 (40-63 μm) or Lichroprep RP-18 (40-63 μm). HPLC was conducted on a Jasco PU-980 and PU-986 pump equipped with UV- and DAD detector (Gynkotek UVD-340DS, Germany). TLC work was carried out using plates coated with silica gel 60 F_{254} (Merck Co.). All solvents were routinely distilled prior to use. Silica gel column chromatography was performed on Merck silica gel 60 (70-230 mesh and 230-400 mesh). Other reagents were obtained form commercial suppliers and were used without purification.

Plant materials

Whole plants (the aerial part and roots) of *S. virga-aurea* var. *gigantea* M_{IQ}.(Compositae) was collected at Ulreung-island, Korea in August 1995 and identified taxonomically with respect to morphology. A voucher specimen of the plant (CBNU 9502AC) was deposited at the College of Pharmacy, Chungbuk National University, Korea.

Cytotoxicity assay

The *in vitro* cytotoxic activity of compounds **1-4** was evaluated according to the standard procedures of the NCI (Skehan *et al.*, 1990) on cell line panel consisting of 5 lines, A-549 (human lung carcinoma), SK-OV-3 (human ovarian adenocarcinoma), SK-MEL-2 (melanoma), XF498 (human CNS carcinoma), and HCT15 (human colon adenocarcinoma).

Extraction and isolation

The air-dried fresh whole plants (1.8 kg) were ground and extracted three times with MeOH for 7 hrs. at below 50°C. The resultant extracts were combined and concentrated under reduced pressure to afford 161 g of the residue. This MeOH extract was suspended in 10 volumes of water and then partitioned successively with equal volumes of n-hexane, EtOAc, and n-BuOH, leaving a residual water soluble fraction. Each fraction was evaporated in vaccuo to yield the residues of *n*-hexane fraction (fr.), (6.0 g), EtOAc fr., (13.4 g), and *n*-BuOH fr., (24.3 g), respectively. The *n*-hexane soluble fraction showed the most significant cytotoxicity (ED_{50} 29.3 μg/mL) against the human melanoma cell line (SK-MEL-2).

The *n*-hexane soluble fraction (5.8 g) was chromatographed on a silica gel column (250 g, 70-230 mesh, 8.2 × 60 cm) using stepwise gradient elution with the solvents $CHCl_3$-MeOH (100:1, 50:1, 0:100, v/v) to divide the fraction into seven sub-fractions (Fr.1-Fr.7).

As the sub-fraction, 5 and 6 showed activity on cytotoxicity assay, Fr. 6 (1.9 g) was re-chromatographed on a silica gel column (60 g, 230-400 mesh, 2×60 cm) by elution with $CHCl_3$ - MeOH (50:1, v/v) to give four fractions (Fr. 61 Fr. 64). Fr. 62 (168 mg) was subjected to MPLC on a silica column (Lobar® Si 60, 40-63 μm) by elution with $CHCl_3$-MeOH (150:1, v/v) to give two fractions (Fr. 621 Fr 622). The Fr. 621 was subjected to a reversed-phase HPLC (C_{18}, 1.050 cm) using isocratic elution with 50% MeOH to afford compound **1** (10.8 mg). Fr. 63 (538 mg) was subjected to MPLC on a silica gel column (Lobar® Si 60, 40-63 μm) eluting with $CHCl_3$-MeOH (150:1, v/v) to give two sub-fractions (Fr. 631 and Fr. 632). The Fr. 631 was finally subjected to a reversed-phase HPLC (C_{18},1.0 × 50 cm) using isocratic

elution with 50% MeOH to afford compound **2** (10 mg).

Fr. 5 (1.46 g) was re-chromatographed on a silica gel column (50 g, 230-400 mesh, 2 × 60 cm) using isocratic elution with *n*-hexane-EtOAc (5:1, v/v) to give five sub-fractions (Fr. 51 Fr. 55). Fr. 52 (284 mg) was chromatographed on silica gel (230-400 mesh, 2.822 cm) eluting with 100% $CHCl_3$ to give two fractions (Fr. 521 and Fr. 522). Fr. 522 was finally subjected to a reversed-phase HPLC (C_{18}) using isocratic elution with MeOH to afford compound **3** (100 mg). Fr. 55 was subjected to a MPLC on a Lobar Si column using 85% MeOH as eluent to afford a single peak and followed by re-crystalization from MeOH and $CHCl_3$ to afford compound **4** (328 mg).

Compound 1 (Erythrodiol-3-acetate:12-Oleanene-3β,28β-diol-3-acetate): Colorless needles (MeOH); m.p. 228 - 230°C; $[\alpha]_D^{21.5}$ +47.57 (c 1.0, $CHCl_3$); $C_{32}H_{52}O_3$ (MW 484.761); UV λ_{max} 239.5 nm ($CHCl_3$); IR ν_{max} (KBr) cm^{-1}: 3491, 2945, 1709, 1269; EI-MS *m/z* (rel. int. %), 484 (5), 466 (5), 234 (23), 203 (100); ^{1}H-NMR ($CDCl_3$), δ_H (ppm), 0.86 (s, 3H, CH_3), 0.87 (s, 6H, CH_3), 0.94 (s, 3H, CH_3), 0.96 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 1.16 (s, 3H, CH_3), 2.05 (s, 3H, Ac), 3.21 and 3.55 (ABq, J=11.0 Hz, H-28), 5.19 (m, H, H-12), 4.50 (dd, H, J=8.7, 8.0 Hz, H-3); ^{13}C-NMR ($CDCl_3$), δ_C (ppm), 80.9 (C-3), 122.3 (C-12), 144.2 (C-13), 171.0 (Ac);. The spectral data were identical with those reported in the literature (Bhattacharyya *et al.*, 1986; Nes *et al.*, 1981).

Compound 2 (α-tocopherolquinone: 2,3,5-trimethyl-6(3´-hydroxy)-phytyl-1,4-benzoquinone): Yellow oil; $C_{29}H_{50}O_3$ (MW 446.3759); UV λ_{max} 262 nm (MeOH); IR ν_{max} (Film) cm^{-1}, 3550, 1660; EI-MS *m/z* (rel. int. %), 446 (28), 428 (15), 221 (100), 178 (75); ^{1}H-NMR ($CDCl_3$), δ_H (ppm), 0.81 (3H, J=6.6Hz, Me-7´), 0.83 (6H, d, J=6.6 Hz, 2Me-15´), 0.84 (3H, d, J=6.6Hz, Me-11´), 1.21 (3H, s, Me-3´), 1.98* (6H, s, Me-2,3), 2.01* (3H, s, Me-5), 2.52 (2H, m, H-1´); ^{13}C-NMR ($CDCl_3$) δ_c (ppm), 187.70 (CO, C-4), 187.23 (CO, C-1),144.42 (C-6), 140.52 (C-5), 140.42 (C-3), 140.16 (C-2), 11.96 (Me-3)*, 12.29 (Me-2)*, 12.37 (Me-5)*, 19.69 (Me-7´), 19.74 (Me-11´), 21.30 (C-5´), 21.39 (C-1´), 22.61 (Me-15´), 22.71 (Me-15´), 24.48 (C-9´), 24.78 (C-13´), 26.57 (Me-3´), 27.97 (C-15´), 32.76 (C-7´), 32.79 (C-11´), 37.27 (C-12´), 37.42(C-10´, C-8´), 37.59 (C-6´), 39.36 (C-14´), 40.25 (C-2´), 42.27 (C-4´), 72.68 (C-3´). The spectral data were identical with those reported in the literature (Rasool *et al.*, 1991; Teresa *et al.*, 1987). *Assignments may be interchanged.

Compound 3 (*trans*-phytol; (2*E*)-3,7,11,15-Tetramethyl-2-hexadecen-1-ol) **:** Colorless oil, $C_{20}H_{40}O$ (MW 269.535), UV λ_{max} 240 nm ($CHCl_3$); IR ν_{max} (Film) cm^{-1}: 3354 (OH), 1662 (C=C); EI-MS *m/z* (rel. int.): 296 $[M]^+$(7), 278 $[M-H_2O]^+$ (5), 71 (100); ^{1}H-NMR ($CDCl_3$), δ_H (ppm), 0.8, 1.0 (m, 4 CH_3), 1.0, 1.6 (CH_2, CH), 1.67 (s, 3H, 3-CH_3), 4.15 (d, 2H, J = 6.96, H-1), 5.41 (m, H, H-2). ^{13}C-NMR ($CDCl_3$), δ_C (ppm), 59.8 (C-1), 123.5 (C-2), 140.6 (C-3). The spectral data were in agreement with previously reported ones (Aoki *et al.*, 1982; Goodman *et al.*, 1973).

Compound 4 (2-Methoxybenzyl-2,6-dimethoxybenzoate): Colorless needles (MeOH); m.p. 104 - 106°C ; UV λ_{max} 276.0 and 239.5 nm ($CHCl_3$); IR ν_{max}(KBr) cm^{-1}: 3006, 1596, 1500, 1721; EI-MS *m/z* (rel. int. %): 302 (33), 165 (100), 121 (48). HRMS *m/z*: 302.1151 $[M]^+$ for $C_{17}H_{18}O_5$ requires 302.1154; ^{1}H-NMR ($CDCl_3$), δ_H (ppm), 3.81 (s, 6H, 2,6-OCH_3), 3.86 (s, 3H, 2'-OCH_3), 5.46 (s, 2H, H-8), 6.55 (d, 2H, J=8.4 Hz, H-3, H-5), 6.89 (d, H, J=8.2, H-3'), 6.95 (t, H, J=7.5 Hz, H-5'), 7.28 (t, H, J=8.4 Hz, H-4), 7.29 (td, H, J=8.0, 1.6 Hz, H-4'), 7.48 (d, H, J=7.5 Hz, H-6'). ^{13}C-NMR ($CDCl_3$), δ_C (ppm), 55.3 (2'-OCH_3), 55.9 (2,6-OCH_3), 62.2 (C-8), 103.9 (C-3), 110.2 (C-3'), 120.2 (C-5'), 124.4 (C-1'), 129.0 (C-4'), 129.1 (C-6'), 130.9 (C-4), 157.4 (C-2, 6), 157.1 (C-2'), 166.5 (C-7), 124.4 (C-1). The spectral data were identical with those reported in the literature (Mann *et al.*, 1981).

RESULTS AND DISCUSSION

The hexane-soluble fraction was separated by using a variety of chromatographies to give compounds **1-4.** The structure of **1-4** were identified on the basis of their spectrosopic properties and by comparison of their physical and spectral data with published values.

Compound **1** was obtained as white needles by a variety of chromatographies followed by re-crystallization (in EtOAc). Mass spectrum showed that its molecular ion peak at *m/z* 484 and the most intense peak at *m/z* 203, which is characteristic of the triterpene of erythrodiol with methylol group (Inose *et al.*, 1992). Some fragment ions from rDA cleavage was observed at *m/z* 249, 234, and 189 indicating that it is the ester of 3-α- or 3-β-with acyl group. We were able to identify and assign the peaks characterizing the 32 carbon atoms of the ^{13}C NMR spectrum and from the C12 and C13 carbon shift values the compound was assigned as oleanene type triterpene. The signals due to the remaining carbon atoms of the compound was assigned by comparison with those reported for β-amyrin. Thus the structure of **1** was determined to be 12-Oleanene-3β,28β-diol-3-acetate(erythrodiol-3-acetate) by comparison with those reported in the literature (Bhattacharyya *et al.*, 1986; Nes *et al.*, 1981). Erythrodiols are mainly widely distributed in several species of plants, but this is the first report from the genus.

Compound **2** was obtained as a yellow oil. HR-MS spectrum showed a parent ion peak at *m/z* 446.3759 that is consistent with the molecular formula $C_{29}H_{50}O_3$ (calcd. 446.3759). The IR spectrum showed bands corres-

ponding to a hydroxyl group (3550 cm^{-1}) and of an α,β -unsaturated ketone (1660 cm^{-1}). The UV absorption spectrum shows λ_{max} 262 nm, which is typical for a quinonic group. The ^1H NMR ($CDCl_3$) spectrum showed characteristic features of tetra-alkyl benzoquinones (Mahmood *et al.*, 1984). It displayed three methyl singlet on a quinonic ring (δ 2.01, 3H, s; 1.98, 6H, s). Further signals were observed for a methyl germinal to a hydroxyl group (1.21, 3H, s) and four secondary methyl groups (0.81, 3H, d, J=6.6 Hz; 0.83, 6H, d, J=6.6 Hz; 0.84, 3H, d, J=6.6 Hz). The ^{13}C NMR spectrum clearly showed a totally substituted quinonic ring together with an aliphatic chain of the phytol type with a hydroxyl group at C-3´.The EI-MS spectrum showed prominent fragment ions at *m/z* 221, 178, and 203 which arise by characteristic process involving the cleavage of the bond on either side of C-3´ with formation of six- or five-member rings clearly showed the structure. Other physical and spectroscopic properties agreed with those described in the literature of α-tocopherolquinone (2,3,5-trimethyl-6(3´-hydroxy)-phytyl-1,4-benzoquinone) (Rasool *et al.*, 1991; Teresa *et al.*, 1987).

Compound **3** was obtained as an oily liquid through a variety of chromatographic separations. The EI-MS spectrum showed the molecular ion at *m/z* 296, and fragments at *m/z* 278 $[M-H_2O]^+$, 71 (100, $[M - C_{16}H_{33}]^+$). ^1H-NMR ($CDCl_3$) showed four methyl protons at δ 0.8-1.67, a methylene protons bearing oxygen at δ 4.15 (d, J=6.96, H-1) and an olefinic proton at δ 5.41 (m, H-2). ^{13}C-NMR ($CDCl_3$) showed 59.8 (C-1), 123.5 (C-2) and 140.6 (C-3). Thus, the compound was determined as *trans*-phytol, (2*E*)-3,7,11,15-tetramethyl-2-hexadecen-1-ol, which was in good agreement with previously reported one (Aoki *et al.*, 1982; Goodman *et al.*, 1973).

Compound **4**, a crystal which is detected as a red spot with the spray of 5% phosphomolybdic acid, was obtained in a large quantity (300 mg). The HR-MS assigned the molecular formula $C_{17}H_{18}O_5$ ($[M]^+$ peak at *m/z* MW 302.1151; calcd. 302.1154). The IR spectrum indicated the presence of an ester group through peaks at 1720 and 1250 cm^{-1}. The ^1H NMR ($CDCl_3$) exhibited singlets at δ 3.81, δ 3.86, and δ 5.46 that assigned to three aromatic methoxyl-and a methylene group adjacent to ester oxygen. The other signals observed were doublets at δ 6.55 (J=8.4 Hz, H-3, H-5), δ 6.89 (J=8.2, H-3'), and triplets at δ 6.95 (J=7.5 Hz, H-5'), δ 7.28 (J=8.4 Hz, H-4). The ^{13}C-NMR ($CDCl_3$) showed 17 signals assigned as three methoxyl groups (δ 55.3, $\times OCH_3$, δ 55.9 $\times$ 2 OCH_3), a carbon bearing oxygen, 12 aromatic carbons and a carbonyl carbon (δ 166.5). Position of the three methoxyl groups (-2, -2´ and -6) and other protons and carbons were determined from the ^1H-^1H- and ^{13}C-^1H COSY spectra. Thus, the structure of **4** was elucidated as 2-methoxybenzyl-2,6-dimethoxy benzoate, which was in agreement with the previously reported one (Mann *et al.*, 1981).

Cytotoxicity measured according to SRB method of these compounds, **1-4** against the five human cancer cell lines (A549, SK-OV-3, SK-Mel-2, XF-498 and HCT15) are shown in Table I. Compound **1-3** showed mild cytotoxicities against the cancer cell lines tested. This is the first report on the chemical constitutes of cytotoxic principles from the genus *Solidago* and the plant, *S. virga-aurea* var. *gigantea* M_{IQ}.

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