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Phenolic and Furan Type Compounds Isolated from *Gastrodia elata* and their Anti-Platelet Effects

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Nine phenolic (**1~9**) and two furan type (**10**, **11**) compounds, were isolated from the methanolic extract of the tuber of *Gastrodia elata* Blume (Orchidaceae) in the course of continuing search for platelet anit-aggregating plant components. Compound **1** was identified as 4,4'-dihydroxybenzyl sulfone, a novel compound for the best of our knowledge. Compound **10**, 5-hydroxymethyl-2-furancarboxaldehyde, was isolated for the first time from this plant. Compound **1** (IC_{50}; 83 μM) was about four times more inhibitory to U46619 induced aggregation than ASA (IC_{50}; 340 μM). Compound **9**, 4,4'-dihydroxy-dibenzylether, (IC_{50}; 5 μM, 3 μM and 33 *u*M, respectively) was 10~80 fold more potent than ASA (IC_{50}; 420 μM, 53 μM and 340 μM respectively) to collagen, epinephrine and U46619 induced aggregation, although it is less active than ASA to AA induced aggregation.

Key words: *Gastrodia elata*, 4,4-Dihydroxybenzyl sulfone, 5-Hydroxymethyl-2-furancaboxaldehyde, 4,4-Dihydroxy-dibenzylether

INTRODUCTION

Gastrodia elata Blume (Orchidaseae) has been considered as one of the most important herbal medicines and used for the treatment of headache, migraine, dizziness, epilepsy, rheumatism, neuralgia, paralysis and other neuralgic and nervous disorders in oriental traditional or folk medicine (Bensky and Gamble, 1986; Tang and Eisenbrand, 1992). In our previous screening for anti-platelet and/or anti-thrombotic plant materials, several solvent fractions prepared from MeOH extract of the tubers of *G. elata* showed anti-platelet effects. The fractions also attenuated the thrombotic symptoms in both mouse and rat models of thrombosis (Paik *et al.*, 1995; Yun-Choi *et al.*, 1985; Yun-choi *et al.*, 1986).

The previous phytochemical studies of this plant have revealed the presence of several phenolic compounds, including 4-hydroxybenzaldehyde, 4-hydroxybenzylmethylether, 4-hydroxybenzylalcohol, 4,4'-dihydroxy-dibenzylether, 4,4'-dihydroxy-diphenyl methane, 4,4'-dihydroxybenzyl sulfoxide, 4-[4'-(4"-hydroxybenzyloxy)benzyloxy]benzyl methyl ether, 3-*O*-(4'-hydroxybenzyl)-β-sitosterol, gastrodin, parisin and gastrol (Zhou *et al.*, 1980; Tagauchi *et al.*, 1981; Noda *et al.*, 1995; Lin *et al.*, 1996; Yun-Choi and Pyo, 1997; Yun-Choi *et al.*, 1999; Hayashi *et al.*, 2002), and a furan type compound, cirsiumaldehyde (Yun-Choi *et al.*, 1999). In our continuing search for plant anti-platelet components, the methanolic extracts of the tubers of *G. elata,* was subjected to the activity-guided fractionation and the isolated compounds were evaluated on rat platelet aggregation induced by ADP, collagen, epinephine, arachidonic acid (AA) and U46619, a thromboxane A_2 mimetic agent.

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MATERIALS AND METHODS

Materials

Melting points were determined on a Mitamura-Riken melting point apparatus and were uncorrected. IR spectra were recorded on a Jasco FT/IR-5300 spectrometer. ^{1}H- and ^{13}C-NMR spectra were taken at 300 MHz and 75.5 MHz, respectively on a Varian Gemini-2000 spectrometer with tetramethylsilane as an internal standard. EI-MS were taken on 5989 B GC/MS system (Hewlett Packard, U.S.A.). FABMS were taken on a VG70-VSEQ (VG Analytical,

UK) mass spectrometer in *m*-nitrobenzylalcohol (NBA) matrix in the positive ion mode and the elemental analysis was performed with a GmbH Vario EL Elemental Analysensystem by Seoul Branch Analytical Lab., Korea Basic Science Institute. Platelet count was determined on an Excell 18 Hematology Analyzer (MWI, Inc., Dallas, U.S.A.). Platelet aggregation was measured on a Platelet Aggregometer (490X, Chrono-Log Corp., U.S.A.). Collagen and ADP (adenosine 5'-diphosphate dicyclohexylammonium salts) were purchased from Chroro-Log., Co. (U.S.A). Sodium arachidonate (AA) and 9,11-dideoxy-11α,9α-epoxymethanoprostagrandin F2α (U46619, TX_2 mimetic) were from Sigma Chemical Co. (U.S.A.). The rats (Sprague-Dawley) were bred at the Animal Station of Natural Products Research Institute, Seoul National University. They were maintained and cared in accordance with the Guide for the Care and Use of Laboratory Animals by Seoul National University.

Plant materials

Steamed and dried tubers and fresh tubers of *G. elata* were purchased from a crude drug market and Korean Agricultural Development Farm respectively in Seoul. They were identified by Prof. Hyung Joon Chi and the voucher specimens were deposited at the Herbarium, Natural Products Research Institute, Seoul National University.

Extraction and isolation

The steamed and dried tubers of *G. elata* (SDG, 6 kg) were refluxed with MeOH three times for six hours each. The concentrated MeOH extract was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer (64 g), after concentration, was further partitioned between *n*-hexane and 90% MeOH to provide hexane fraction (28 g) and MeOH fr. (36 g), respectively. The aqueous layer was extracted with EtOAc and then with BuOH to obtain EtOAc fr. (40 g) and BuOH fr. (95 g) respectively. Fresh tubers of *G. elata* (FG, 20 kg) were sliced and percolated in MeOH for several weeks at room temperature. The fractionation of the MeOH extract of FG was proceeded with the same method as described above for that of SDG. The MeOH extract from FG yielded hexane (18 g), MeOH (35 g), EtOAc (33 g) and BuOH (112 g) fraction, respectively. The MeOH fr. (30 g) from SDG was chromatographed on a silica gel (2 kg) column eluted with $CHCl_3$ containing increasing proportions of MeOH to afford compounds **1** (15 mg), **2** (33 mg), and **3** (29 mg). The EtOAc fr. (25 g) from SDG was also subjected to a silica gel (2.5 kg) column eluting with $CHCl_3$-MeOH (90:1) or *n*-hexane-EtOAc (1:1) to afford compounds **4** (25 mg), **5** (22 mg) and **6** (34 mg). The BuOH fr. (20 g) from SDG was applied to a silica gel (1.5 kg) column and eluted with $CHCl_3$-MeOH-H_2O (90:9:1) yielding compound **7** (1.2 g). The MeOH fr. (25 g) from FG, was chromatographed on a silica gel (2 kg) column eluting with $CHCl_3$-MeOH (100:1), afforded compound **8** (10 mg), **9** (30 mg), **10** (50 mg), **2** (6 g), **3** (50 mg) and **4** (80 mg). The EtOAc fr. (25 g) from FG was also subjected to a silica gel (2.5 kg) column and eluted with n-hexane-EtOAc (1:1) to afford compound **11** (42 mg).

4,4'-Dihydroxybenzyl sulfone (1)

Pale yellow needle from EtOAc-ether; mp, 223-226°C; Anal. Calcd. for $C_{14}H_{14}O_4S_1 1/4H_2O$: C, 59.52; H, 5.08; S, 11.35, found: C, 59.22; H, 4.73; S, 10.85; IR ν_{max} cm^{-1} (KBr); 3387 (br, OH), 1613, 1512 (aromatic C=C), 1250 (aromatic C-O); Positive HR-FABMS *m/z*: 279.0718 $[M+H]^+$; for $C_{14}H_{15}O_4S_1$; EI-MS m/z: 278 $[M]^+$, 246, 214, 107; ^{1}H-NMR (Me_2CO-d_6): δ 8.52 (OH), 7.24, 6.84 (4H each, d, *J*=8.7 Hz, H-2, 2', 3, 3'), 4.18 (4H, s, H-α, α'); ^{13}C-NMR (Me_2CO-d_6): δ 159.06 (C-4,4'), 133.43 (C-2,2'), 119.81 (C-1,1'), 116.41 (C-3,3), 57.87 (C-α, α').

5-Hydroxymethyl-2-furancarboxaldehyde (10)

Yellow needle from *n*-hexane-ether; mp, 34-35; IR ν_{max} cm^{-1} (KBr); 3397 (br, OH), 1674 (C=O), 1524; ^{1}H-NMR ($CDCl_3$): δ 9.52 (1H, s, CHO), 7.19 (1H, d, *J*=3.6 Hz, H-3), 6.48 (1H, d, *J*=3.6 Hz, H-4), 4.67 (2H, s, H-α); ^{13}C-NMR: δ 177.9 (CHO), 161.2 (C-5), 152.3 (C-2), 123.4 (C-3), 110.0 (C-4), 57.2 (C-α).

Platelet aggregation

Blood, collected from rat heart using a syringe containing 0.1 volume of 2.2% sodium citrate, was centrifuged at 200 g for 10 min to obtain the supernatant platelet rich plasma (PRP). Platelet poor plasma (PPP) was obtained from the residue by centrifugation at 1500×g for 10 min. PRP was diluted with saline to adjust the number of platelets (400~450×10^6 platelets/mL) with the aid of platelet counter. The degree of platelet aggregation was monitored by the turbidimetric method using optical aggregometer (McNicol, 1996). The reduction in turbidity of PRP was measured as the degree of aggregation assuming that PPP represented 100% light transmission and PRP represented 0% transmission. The test sample or vehicle was added to the adjusted PRP after 3 min pre-incubation and an aggregation inducing agent [(ADP (2~5 μM) or collagen (2~5 μg/mL)] was added at 1 min after the sample addition. Epinephrine-induced aggregation was measured by the previously described method (Yun-Choi, 2000) in the presence of threshold concentration of collagen. Briefly, sample was added to PRP 30 sec before the addition of the threshold concentration of collagen (0.8~1.0 μg/mL), at which only platelet shape change was induced without aggregation. Epinephrine (1~4 μM) was added 30 sec after the addition of the threshold concentration of collagen. AA (10~40 μM) and U46619 (1~5 μM) induced platelet

aggregation were also measured in the presence of the threshold concentration of collagen. The minimum inducer concentration that elicited maximal aggregation was employed as the control for each PRP.

Statistical analysis

The concentrations of the compounds causing 50% inhibitory effects (IC_{50}) were determined from the Regression Wizard from the SigmaPlot equation library.

RESULT AND DISCUSSION

Nine phenolic and two furan type compounds (Fig. 1) were isolated from the MeOH fr., EtOAc fr. and BuOH fr. prepared from the MeOH extract of SDG and FG, which showed inhibitory effects on rat platelet aggregation. Nine of them were identified as the previously reported compounds from this plant; 4-hydroxybenzylmethylether (**2**), 4-hydroxybenzylalcohol (**3**), 4-hydroxybenzaldehyde (**4**), 4,4'-dihydroxybenzyl sulfoxide (**5**), gastrodin (**6**), 4-[4'-(4"-hydroxybenzyloxy)benzyloxy]benzyl methyl ether (**7**), 4,4'-dihydroxy-diphenyl methane (**8**), 4,4-dihydroxy-dibenzylether (**9**) and cirsiumaldehyde (**11**) (Taguchi *et al.*, 1981; Noda *et al.*, 1995; Yun-Choi and Pyo, 1997; Yun-Choi *et al.*, 1997). The structure of the remaining compounds **1** and **10** were determined based upon various spectral data (IR, MS. ^{1}H-, ^{13}C-NMR).

Compound **1** was obtained as pale yellow needles upon recrystalization from EtOAc-ether. The IR spectrum exhibited the presence of hydroxyl (3387 cm^{-1}), aromatic C=C (1613, 1512 cm^{-1}), and aromatic C-O (1250 cm^{-1}). The molecular formula of **1** was determined as $C_{14}H_{14}O_4S_1$ from HR-FABMS (*m/z*: 279.0718 $[M+H]^+$). The EI Mass spectrum of **1** showed the molecular ion peak at *m/z* 278, a peak due to fragmentation of SO_2 moiety at *m/z* 214, and a base peak at *m/z* 107 corresponding to 4-hydroxybenzyl fragment. The ^{1}H-NMR spectrum showed signals ascribable to 1,4-disubstituted aromatic protons at δ 7.24 and 6.84 (4H each, d, *J*=8.7 Hz) and a peak at 8.52 due to the phenolic hydroxyl protons. The singlet signal at δ 4.18 (4H) indicated two methylene protons of benzyl sulfone group. The ^{13}C-NMR (with DEPT) showed signals for quaternary aromatic carbons at δ 159.06 and 119.81, two protonated aromatic carbon signals at δ 133.43 and 116.41 and an sulfoxygenated methylene carbon signal at δ 57.87. On the basis of above spectral data, compound **1** was determined as 4,4'-dihydroxybenzyl sulfone, which is a novel compound for the best of our knowledge.

Compound **10** was obtained as a yellow needles from *n*-hexane-ether. The IR spectrum revealed the presence of the hydroxyl group at 3397 cm^{-1} and a carbonyl group

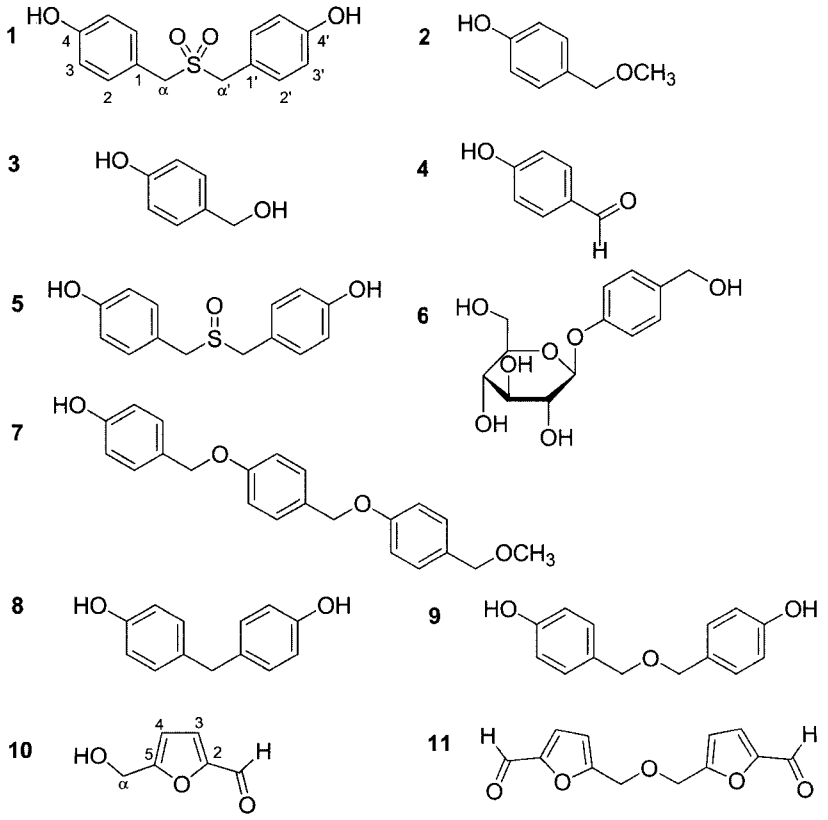

Fig. 1. Compounds isolated from *Gastrodia elata*

at 1674 cm^{-1}. The presence of two aromatic doublets in ^{1}H-NMR, at δ 7.19 and 6.48 with *J*=3.6 Hz suggested 2,5-disubstituted furan ring in the structure (Silverstein *et al.*, 1991). One aldehyde peak at δ 9.52 (s) and one hydroxylated methylenic singlet at δ 4.67 were also observed. The ^{13}C-NMR showed only 6 peaks, one at δ 177.9 due to the carboxylic carbon, four aromatic carbon peaks at δ 161.2, 152.3, 123.4, 110.0 and one hydroxylated methylenic carbon at 57.2. On the basis of above spectral data and by comparison with the literature values (Shen and Mu, 1990), compound **10** was identified as 5-hydroxymethyl-2-furancarboxadehyde. This compound has been isolated from *Cirsium chlorolepis* (Shen and Mu, 1990) but was isolated for the first time from this plant.

The inhibitory effects of **1-11** on rat platelet aggregation were examined and compared with those of acetylsalicylic acid (ASA) (Table I). All of the tested compounds showed dose-dependent inhibitory activities to collagen, epinephrine, AA and U46619 induced platelet aggregation. In platelets, AA is metabolized to cyclic endoperoxides, prostaglandin G (PGG) and H (PGH), which is subsequently converted to TXA_2, a most potent platelet aggregation inducing agent (Sinakos, 1967). However, rat platelets were observed not to aggregate in response to epinephrine, AA or U46619, a PGH_2/TXA_2 receptor agonist, in the concentration dependent manner. The aggregations with epinephrine, AA or U46619 were observed in the presence of threshold concentration of collagen. All of the tested compounds, including ASA, showed only very mild effects to ADP induced aggregation. Compound **1** (IC_{50}; 83 μM) was about four times more inhibitory to U46619 induced aggregation than ASA (IC_{50}; 340 μM), while it is less active than ASA to all the other agonists. Only very mild and less inhibitory effects were observed with the simple phenolic compounds **2**, **3** and **4** than with ASA to all the aggregation inducing agents. Compound **7** was about 2 fold more inhibitive than ASA to collagen induced aggregation, but less active to the other stimulators. Compound **8** showed approximately equivalent effects as ASA to epinephrine, AA and U46619 induced aggregation. Compound **9** (IC_{50}; 5 μM, 3 μM and 33 μM respectively) was 10~80 fold more potent than ASA (IC_{50}; 420 μM, 53 μM and 340 μM respectively) to collagen, epinephrine and U46619 induced aggregation, although it is less inhibitive than ASA to AA induced aggregation. The remaining compounds, the sulfoxide **5**, the glycoside **6**, and the furano compounds, **10** and **11**, were rather inert to all the stimulators tested. The result is indicative that compound **1**, although it is not very potent, might selectively acting on thromboxane receptor and could serve as a lead compound for the development of selective TXA_2/PGH_2 receptor antagonists. Compound **9**, the most potent inhibitor of platelet aggregation among all the tested compounds, also appear to show inhibitory effects with other mechanisms than ASA which is known to inhibit both AA and epinephrine induced platelet aggregation by blocking cyclooxygenase in the arachidonic acid cascade. Since compound **9** showed only very mild effect on AA induced platelet aggregation, while it exhibited strong effect on both collagen and epinephrine induced platelet aggregation, it is unreasonable for compound **9** acting on cyclooxygenase as ASA, although the precise mechanism should further be clarified.

Table I. Platelet anti-aggregating activities of compounds isolated from *Gastrodia elata*

Compounds	IC_{50} (μM)			
	Collagen[a]	Epinephrine[b,e]	AA[c,e]	U46619[d,e]
ASA[f]	420	53	66	340
1	>1000	560	>1000	83
2	620	741	275	660
3	680	794	661	740
4	650	524	513	620
5	>1000	560	>1000	>1000
6	>1000	>1000	>1000	>1000
7	191	174	381	>1000
8	>1000	60	30	417
9	5	3	398	33
10	>1000	>1000	>1000	>1000
11	>1000	>1000	>1000	>1000

[a]collagen 2~5 μg/mL, [b]epinephrine 1~4 μM, [c]sodium arachidonate 10~40 μM, [d]U46619 1~5 μM, [e]with the threshold concentration of collagen (collagen 0.8~1.0 μg/mL), [f]ASA; acetylsalicylic acid.

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