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Phytochemical Constituents from *Diodia teres*

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All ten compounds were isolated from the methanolic extract of the whole plants of *Diodia teres* through repeated silica gel and Sephadex LH-20 column chromatography. Their chemical structures were elucidated as three iridoid glycosides, asperuloside, geniposidic acid and asperulosidic acid, a coumarin glycoside, scopolin, and six flavonoids, rutin, kaempferol-3-*O*-rutinoside, quercitrin, astragalin, isoquercitrin and quercetin by spectroscopic analysis.

Key words: *Diodia teres*, Asperuloside, Geniposidic acid, Asperulosidic acid, Scopolin, Flavonoids

INTRODUCTION

Diodia teres Walter (Rubiaceae), as an annual plant native to America is naturalized in Korea and Japan (Lee, 1986; Park, 1995). A literature survey revealed that no phytochemical and pharmacological studies have been carried out on this plant. Therefore, we were interested in the chemical constituents of this plant.

Our research on this source led to the isolation of three iridoid glycosides, asperuloside, geniposidic acid and asperulosidic acid, a coumarin glycoside, scopolin, and six flavonoids, rutin, kaempferol-3-*O*-rutinoside, quercitrin, astragalin, isoquercitrin and quercetin. This paper describes the isolation and structural characterization of these compounds.

MATERIALS AND METHODS

General experimental procedures

^{1}H- and ^{13}C-NMR spectra were determined on a JEOL JMN-EX 400 spectrometer in CD_3OD. IR spectra were obtained on a JASCO FT/IR 410 spectrometer and UV spectra were recorded on Shimadzu UV-1601 UV-Visible spectrophotometer. TLC work was carried out using plates coated with silica gel 60 F_{254} (Merck Co.). Silica gel column chromatography was performed on Merck silica gel 60 (230-400 mesh). Sephadex LH-20 was used for the column chromatography (Pharmacia, 25-100 μm). The column used for LPLC was Lobar-A (Merck Lichroprep Si 60, 240-10 mm). All solvents were routinely distilled prior to use. Other chemicals were commercial grade without purification.

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Plant materials

The whole plants of *D. teres* were collected in October 2000 at Wanju, Chonbuk, Korea. A voucher specimen is deposited at the herbarium of college of pharmacy, Woosuk University, Korea (WSU-00-007).

Extraction and isolation

The air-dried plant materials (700 g) was extracted twice with MeOH under 50°C. The resultant MeOH extract (120 g) was suspended in water, and then fractionated successively with equal volumes of ethyl acetate and *n*-BuOH, leaving residual water soluble fraction. Each fraction was evaporated *in vacuo* to yield the residues of ethyl acetate soluble fraction (25 g) and *n*-BuOH soluble fraction (38 g).

10 g of Ethyl acetate soluble fraction was chromatographed on the Sephadex LH-20 column (MeOH) to give five fractions (E1-E5). Recrystallization of the fraction E2 with MeOH gave **1** (12 mg). Silica gel column chromatography of the fraction E3 with EtOAc-MeOH-H_2O (25:5:1) afforded **2** (7 mg) and **3** (12 mg). Fraction E4 was chromatographed on a silica gel column eluting with EtOAc-MeOH-H_2O (80:10:1) and purified by Lobar-A

column chromatography (MeOH) to afford **4** (6 mg) and **5** (8 mg). Silica gel column chromatography of the fraction E5 with EtOAc-MeOH (30:1) gave **6** (10 mg).

10 g of *n*-BuOH soluble fraction was chromatographed over Sephadex LH-20 column with MeOH to give three fractions (B1-B3). The major fraction B1 was chromatographed on silica gel column using EtOAc-MeOH-H_2O (25:5:1) to yield four fractions (B11-B14). Fraction B11 was purified by Lobar-A column (EtOAc-MeOH-H_2O, 40:5:1) to give **7** (12 mg) and **8** (8 mg). Fraction B13 was chromatographed on reversed-phase silica gel column with MeOH-H_2O (1:1) and purified by Lobar-A column (CH_2Cl_2-MeOH, 1:1) to give **9** (15 mg). Fraction B14 was purified by Lobar-A column (EtOAc-MeOH-H_2O, 25:5:1) to yield **10** (10 mg).

Rutin (1)

Yellow powder (MeOH), mp 109-111°C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 12.56 (1H, s, 5-OH), 10.81, 9.67, 9.18 (each 1H, 7, 3', 4'-OH), 7.54 (2H, dd, *J* = 8.5, 2.0 Hz, H-2', 6'), 6.85 (1H, d, *J* = 8.8 Hz, H-5'), 6.41 (1H, d, *J* = 1.8 Hz, H-8), 6.18 (1H, d, *J* = 1.8 Hz, H-6), 5.30 (1H, d, *J* = 7.0 Hz, Glc H-1), 4.40 (1H, brs, Rha H-1), 3.80-3.04 (sugar H), 1.00 (3H, d, *J* = 6.0, Rha CH_3). ^{13}C-NMR (100 MHz, DMSO-d_6) see the Table I.

Table I. ^{13}C-NMR spectral data of compounds **1-6** from *D. teres*

Carbon	1[a]	2[a]	3[b]	4[b]	5[b]	6[a]
2	156.5	156.5	158.1	158.5	157.4	146.8
3	133.4	133.3	136.0	135.4	134.5	135.7
4	177.4	177.5	179.3	179.5	178.4	175.6
5	161.3	160.9	159.1	163.1	162.1	160.6
6	98.6	98.8	99.5	99.9	99.1	98.2
7	164.3	164.1	165.8	166.0	165.5	163.8
8	93.7	94.0	94.3	94.7	93.8	93.5
9	156.7	156.9	163.0	159.1	158.2	156.2
10	104.0	104.2	105.5	105.8	104.5	103.0
1'	121.3	121.2	122.5	122.8	122.1	122.0
2'	115.4	131.1	116.2	132.3	115.1	115.2
3'	144.8	115.3	146.2	116.1	149.0	145.0
4'	148.5	159.8	149.5	161.6	145.0	147.5
5'	116.4	115.3	116.7	116.1	116.6	115.5
6'	121.6	131.0	122.7	132.3	122.1	120.1
1"	101.3	101.4	103.4	104.1	103.5	
2"	74.3	74.3	71.8	75.7	74.8	
3"	76.5	76.4	72.0	78.4	77.2	
4"	70.1	70.7	73.1	71.4	70.4	
5"	76.0	75.8	71.7	78.0	77.5	
6"	67.1	67.0	17.5	62.6	61.6	
1'''	100.8	101.0				
2'''	70.5	70.4				
3'''	70.7	70.0				
4'''	71.9	71.8				
5'''	68.3	68.5				
6'''	17.9	17.9				

[a]Recorded at 100 MHz in DMSO-d_6.
[b]Recorded at 100 MHz in CD_3OD.

Kaempferol-3-*O*-rutinoside (2)

Yellow powder (MeOH), mp 183-184°C; ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.96 (2H, d, *J* = 8.8 Hz, H-2', 6'), 6.78 (2H, d, *J* = 8.8 Hz, H-3', 5'), 6.23 (1H, d, *J* = 1.8 Hz, H-6), 5.00 (1H, d, *J* = 7.0 Hz, glc.H-1), 4.41 (1H, br s, rha.H-1). ^{13}C-NMR (100 MHz, DMSO-d_6) see the Table I.

Quercitrin (3)

Yellow powder (MeOH), mp 181-182°C; ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.57 (1H, d, *J* = 1.9 Hz, H-2'), 7.53 (1H, dd, *J* = 8.2, 1.9 Hz, H-6'), 6.77 (1H, d, *J* = 8.2 Hz, H-5'), 6.29 (1H, d, *J* = 1.8 Hz, H-8), 6.10 (1H, d, *J* = 1.8 Hz, H-6), 5.01 (1H, d, *J* = 2.0 Hz, H-1"), 4.22-3.14 (sugar H), 1.02 (3H, d, *J* = 6.0 Hz, H-6"). ^{13}C-NMR (100 MHz, CD_3OD) see the Table I.

Astragalin (4)

Yellow powder (MeOH), mp 184-185°C; ^{1}H-NMR (400 MHz, CD_3OD) δ: 8.05 (2H, d, *J* = 8.7 Hz, H-2', 6'), 6.87 (2H, d, *J* = 8.7 Hz, H-8), 6.38 (1H, d, *J* = 2.0 Hz, H-8), 6.20 (1H, d, *J* = 2.0 Hz, H-6), 5.26 (1H, d, *J* = 7.0 Hz, anomeric H), 3.70-3.09 (sugar H). ^{13}C-NMR (100 MHz, CD_3OD) see the Table I.

Isoquercitrin (5)

Yellow powder (MeOH), mp 234-235°C; ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.61 (1H, d, *J* = 2.0 Hz, H-2'), 7.49 (1H, dd, *J* = 8.2, 2.0 Hz, H-6'), 6.77 (1H, d, *J* = 8.2 Hz, H-5'), 6.29 (1H, d, *J* = 1.9 Hz, H-8), 6.10 (1H, d, *J* = 1.9 Hz, H-6), 5.17 (1H, d, *J* = 7.2 Hz, H-1"). ^{13}C-NMR (100 MHz, CD_3OD) see the Table I.

Quercetin (6)

Yellow powder (MeOH), mp 316-317°C; ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.62 (1H, d, *J* = 2.2 Hz, H-2'), 7.53 (1H, dd, *J* = 8.4, 2.2 Hz, H-6'), 6.78 (1H, d, *J* = 8.4 Hz, H-5'), 6.28 (1H, d, *J* = 1.8 Hz, H-8), 6.07 (1H, d, *J* = 1.8 Hz, H-6). ^{13}C-NMR (100 MHz, DMSO-d_6) see the Table I.

Scopolin (7)

Colorless prisms (MeOH), mp 213-214°C; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.97 (1H, d, *J* = 9.5 Hz, H-4), 7.29 (1H, s, H-5), 7.15 (1H, s, H-8), 6.32 (1H, d, *J* = 9.5 Hz, H-3), 5.35 (1H, d, *J* = 4.2 Hz, H-1'), 3.82 (3H, s, $OC\underline{H}_3$), 3.15-3.73 (sugar H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 160.6 (C-2), 149.7 (C-7), 148.8 (C-9), 145.9 (C-6), 144.2 (C-4), 113.2 (C-3), 112.3 (C-10), 109.7 (C-5), 103.0 (C-8), 99.7 (C-1'), 77.0 (C-5'), 76.5 (C-3'), 73.1 (C-2'), 69.5 (C-4'), 60.7 (C-6'), 56.1 ($O\underline{C}H_3$).

Asperuloside (8)

Amorphous powder, mp 128-130°C, UV λ_{max} (MeOH) nm: 230, IR ν_{max} (KBr) cm^{-1}: 1750, 1735, 1655, ^{1}H-NMR (400

Table II. ^{13}C-NMR (DEPT) spectral data of compounds **8-10** from *D. teres*

Carbon	8	9	10
1	99.9	97.9 CH	101.1
3	150.3	150.5 CH	154.5
4	106.2	116.4 C	109.6
5	37.4	37.3 CH	42.7
6	86.3	39.9 CH_2	75.5
7	128.9	128.4 CH	131.8
8	144.3	144.9 C	145.9
9	45.2	47.2 CH	46.4
10	64.4	61.6 CH_2	63.8
11	172.3	174.5 C	172.1
1'	93.3	100.2 CH	100.5
2'	73.8	74.9 CH	74.9
3'	78.4	78.3 CH	78.5
4'	71.6	71.5 CH	71.5
5'	77.9	77.8 CH	77.8
6'	61.9	62.6 CH_2	62.9
<u>C</u>OCH$_3$	172.6		172.6
CO<u>C</u>H$_3$	20.6		20.8

Recorded at 100 MHz in CD_3OD.

MHz, CD_3OD) δ: 7.21 (1H, d, J = 2.0 Hz, H-3), 5.86 (1H, m, H-7), 5.63 (1H, s, H-1), 5.47 (1H, d, J = 6.8 Hz, H-6), 4.58 (2H, s, H-10), 3.55 (1H, m, H-9), 3.21 (1H, m, H-5), 1.98 (3H, s, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$) see the Table II.

Geniposidic acid (9)

Amorphous powder, UV λ_{max} (MeOH) nm: 235, IR ν_{max} (KBr) cm^{-1}: 1740, 1725, 1650, ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.25 (1H, s, H-3), 5.68 (1H, s, H-7), 5.00 (1H, d, J = 7.2 Hz, H-1), 4.63 (1H, d, J = 8.0 Hz, H-1'), 4.21, 4.08 (each 1H, d, J = 14.4 Hz, H-10), 3.74 (2H, m, H-6'), 3.30 (1H, m, H-5'), 3.22-3.19 (3H, H-4',3',2'), 3.13 (1H, m, H-5), 2.75 (1H, dd, J = 16.0, 8.4 Hz, H-6α), 2.58 (1H, m, H-9), 2.00 (1H, dd, J = 16.0, 1.9 Hz, H-6β). ^{13}C-NMR (100 MHz, CD_3OD) see the Table II.

Asperulosidic acid (10)

Amorphous powder, mp 126-127°C, UV λ_{max} (MeOH) nm: 230, IR ν_{max} (KBr) cm^{-1}: 1730, 1640, ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.51 (1H, s, H-3), 5.92 (1H, s, H-7), 4.94 (1H, d, J = 8.8 Hz, H-1), 4.63 (1H, d, J = 8.0 Hz, H-6), 2.93 (1H, t, J = 6.0 Hz, H-9), 2.53 (1H, t, J = 8.4 Hz, H-5), 2.00 (3H, s, CH_3). ^{13}C-NMR (100 MHz, CD_3OD) see the Table II.

RESULTS AND DISCUSSION

In the course of phytochemical study of the MeOH extract from the whole plants of *D. teres*, ten compounds were isolated by the chromatographic separation of each fraction. They were identified as three iridoid glycosides, a coumarin glycoside, and six flavonoids by spectroscopic analysis.

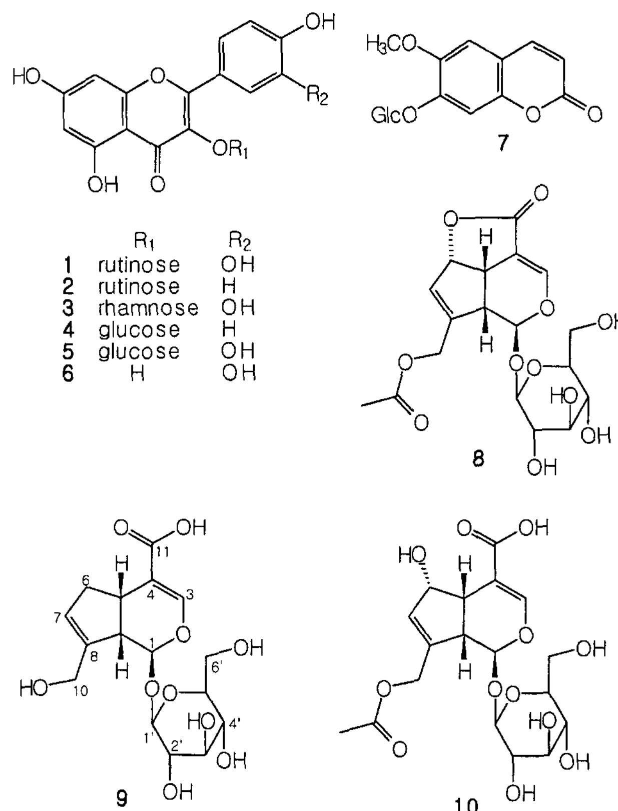

Fig. 1. The structures of compounds **1-10**

Compounds **1**-**6** were identified as rutin (**1**), kaempferol-3-*O*-rutinoside (**2**), quercitrin (**3**), astragalin (**4**), isoquercitrin (**5**) and quercetin (**6**). Compound **7** was elucidated as scopolin by comparing physicochemical and spectral data with that of published literatures (Hasan *et al.*, 1995; Chaurasia *et al.*, 1987; Harborne *et al.*, 1994; Agrawal *et al.*, 1989; Do *et al.*, 1992; Kim *et al.*, 1999; Hwang *et al.*, 1994; Kim *et al.*, 1992).

Compounds **8-10** have similar patterns in their NMR spectra. Compound **8** was obtained as an amorphous powder from MeOH. The absorption band of UV spectrum at 230 nm and IR spectrum of at 1735 and 1655 cm^{-1} indicated the probable presence of a carbonyl conjugated enol ether group. The ^{1}H-NMR spectrum of **8** showed a doublet at δ 7.21 which was assigned to the enol ether proton at C-3, and the multiplet at δ 3.21 and 3.55 were assigned to the protons at C-5 and C-9. In the ^{13}C-NMR spectrum, 18 carbon signals were observed, which included two carbonyl groups at δ 172.6 and 172.3, four olefinic carbons at δ 153.0, 144.3, 128.9 and 106.2, six sugar carbons at δ 93.3, 78.4, 77.9, 73.8, 71.6 and 61.9, and a methyl group at δ 20.6. From these results, compound **8** was indicated to be an iridoid glycoside. The structure of **8** was determined to be asperuloside on the

basis of the above evidences, together with a comparison of the above data with those published in the literature (Bailleul *et al.*, 1977; El-Naggar *et al.*, 1980).

Compound **10** was obtained as an amorphous powder from MeOH. The absorption band of UV and IR spectra were similar to those of compound **1**. The ^{1}H-NMR spectrum of **10** showed a doublet at δ 7.51 which was assigned to the enol ether proton at C-3 and the triplet at δ 2.53 (1H, t, J = 8.4 Hz) and 2.93 (1H, t, J = 6.0 Hz) were assigned to the protons at C-5 and C-9. The ^{13}C-NMR spectrum of **10** was similar to that of **8**, suggesting it has the similar carbon skeleton. The main difference was C_3-C_4-C_5-C_6-C_7 chemical shift. In the ^{13}C-NMR spectrum, 18 carbon signals were observed, which included two carbonyl groups at δ 172.6 and 172.1, four olefinic carbons at δ 154.5, 145.9, 131.8 and 109.6, six sugar carbons at δ 100.5, 78.5, 77.8, 74.9, 71.5 and 62.9, and a methyl group at δ 20.8. From these results, compound **10** was indicated to be a iridoid glycoside. The structure of **10** was determined to be asperulosidic acid on the basis of the above evidences, together with a comparison of the above data with those published in the literature (Chaudhuri *et al.*, 1977; El-Naggar *et al.*, 1980).

Compound **9** was obtained as an amorphous powder from MeOH. The absorption band of UV and IR spectra were similar to those of compound **1**. The ^{1}H-NMR spectrum of **9** showed a doublet at δ 7.25 which was assigned to the enol ether proton at C-3 and the multiplet at δ 3.13 and 2.58 was assigned to the protons at C-5 and C-9. In the ^{13}C- and DEPT NMR spectrum, 16 carbon signals were observed, which included a carbonyl group at δ 174.5, four olefinic carbons at δ 150.5, 144.9, 128.4 and 116.4, six sugar carbons at δ 100.2, 78.3, 77.8, 74.9, 71.5 and 62.6. The above results suggested the structure of **9** was a iridoid glycoside. Finally, the structure of **9** was determined to be geniposidic acid on the basis of the above evidences, together with a comparison of the above data with those published in the literature (Chaudhuri *et al.*, 1977; El-Naggar *et al.*, 1980).

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