Antioxidant Compounds from the Root Bark of *Hibiscus syriacus*

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Abstracts

A new lignan named as hibiscuside, (+) pinoresinol 4-O-[-glucopyranosyl (12) -rhamnoside] (1), and a known lignan, syringaresinol (2) were isolated from the root bark of *Hibiscus syriacus* together with two feruloyltyramines (3,4) and three known isoflavonoids (5,6,7). The structures of these compounds have been established on the basis of their NMR, Mass, UV spectra. Among these phenolic compounds, 6-O-acetyl daidzin (5), 6-O- acetyl genistin (6), and 3-hydroxy daidzein (7) with IC50 values of 8.2, 10.6, and 4.1 M, respectively, significantly inhibited lipid peroxidation in rat liver microsomes. Hibiscuside (1), E and Z-N-feruloyl tyramines (3,4) exhibited moderate antioxidant activity.

INTRODUCTION

Hibiscus syriacus L. (Malvaceae) is widely distributed in eastern and southern Asia. The dried flower of *H. syriacus* is used as a folk medicine for the cure of hematochezia, dysentery, obstruction due to wind phlegm, and vomiting of food in orient.1,2 Previous phytochemical investigations performed on this species resulted in the isolation of some fatty acids including malvalic acid, sterculic acid, and lauric acid and the flavonoids, saponarin, apigenin-7-O--glucoside, and taxifolin-3-O--glucopyranoside and cylic peptide.3-7 In the search for effective new antioxidant compounds from methanol extracts of *H. syriacus* that showed moderate activity in a 1,1-diphenyl 2-picrylhydrazyl (DPPH) free-radical assay, seven constituents 1-7 were isolated from methanol extracts of this plant.(Fig.1) These compounds were examined for antioxidant activity against lipid peroxidation in rat liver microsomes.

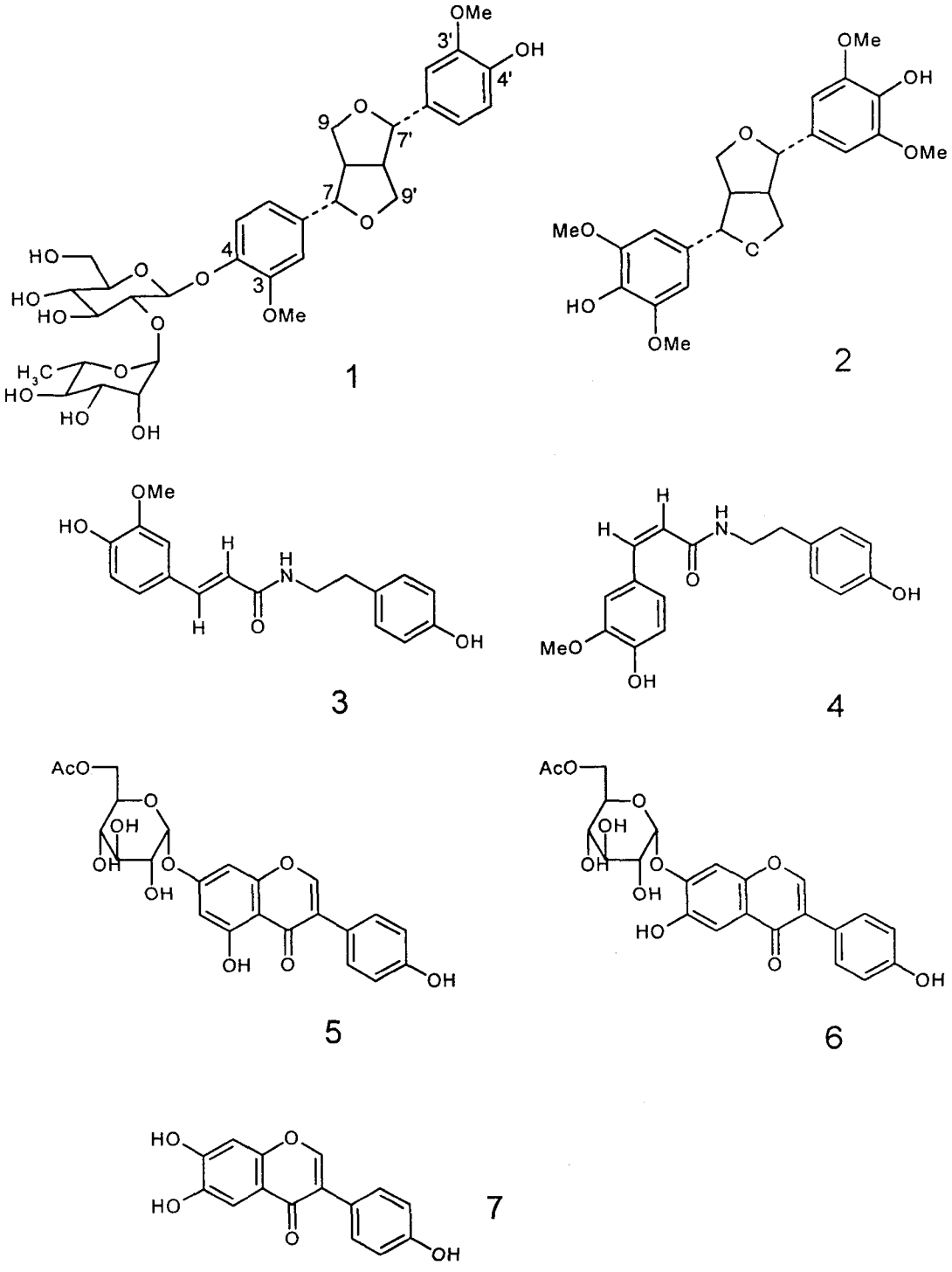

Fig. 1. Structures of Compounds 1-7 from *Hibiscus syriacus*

MATERIALS AND METHODS

General Method

UV spectrum was measured on a Kontron Uvicon 930 spectrometer. 1H and 13C NMR spectra were measured on Bruker DRX 300 and DMX 600 NMR spectrometers, respectively. Two dimensional NMR spectra were recorded on a Bruker AM 600 NMR spectrometer. All two-dimensional homonuclear spectra were recorded in pure-phase absorption mode. FAB mass spectra were recorded on a JMX-HX 110A/HX110A (Jeol, JAPAN) FAB mass spectrometer. Generated solution was mixed with 3-nitrobenzyl alcohol as the matrix on the FAB probe tip. Optial rotation and IR spectrum were obtained on a Schmidt+Haensch Polartronic polarimeter and on Perkin Elmer 16000 FTIR spectrometers, respectively.

Material

Root bark of *Hibiscus syriacus* L. was collected at the vicinity of Taejon, Korea, in Oct. 1995 and identified by Dr. Yoo at Korea Research Institute of Bioscience and Biotechnology (KRIBB). A voucher specimen (Yoo 98-8) has been deposited at the laboratory of Cell Function Regulator Research Group at KRIBB.

Isolation

Dried root bark of *Hibiscus syriacus* (1.6 kg) were cut into small pieces and extracted two times with 100 % MeOH (3 l, 1day). The extracts were concentrated in vacuo to 100 ml, and redissolved in 500 ml distilled water. The aqueous layer was extracted successively with CHCl3 (500 ml 3), EtOAc (500 ml 4), and BuOH (500 ml 3). The extracts obtained from each solvent were combined and evaporated to dryness. The CHCl3 extract was chromatographed on a silica gel (70~230 mesh, Merck) column with hexane-EtOAc (10:1 1:1) mixture. The fractions active against the DPPH assay were rechromatographed on a silica gel column with CHCl3-MeOH mixtures (20:1~5:1), followed on a Sephadex LH 20 (25~100 mmol, Pharmacia Fine Chemicals) column with 80 % MeOH yielding fractions a h (100 ml for each) which were examined for antioxidative activity in the DPPH assay system. The active compounds, 2 (18 mg), 3 (15 mg) and 4 (2 mg), were obtained using preparative RP-TLC (70 % MeOH, Rf 0.65 for 2), preparative TLC (CHCl3-MeOH=10:1, Rf 0.4 for 4) and preparative

ODS-HPLC (Phenomenex 10 250 mm, 80 % MeOH, for 3) from b and c fractions, respectively. EtOAc extract was subjected to silica gel column chromatography. Six fractions were obtained. Further Sephadex LH 20 column chromatography (MeOH 100 %) of Fr. 2 afforded compound 7 (2.1 mg), and Fr. 4 afforded compound 5 (5.1 mg) and 6 (2.0 mg), respectively. The BuOH extract was chromatographed on a HP-20 column with 80 % MeOH (800 ml), 60 % MeOH (800 ml), and 40 % MeOH (1 l), successively. The 80 % MeOH extracts demonstrated antioxidant activity. Further purification was carried out by ODS-HPLC column (Phenomenex 10 250 mm, 30 % MeOH, flow rate 3 ml/min). This step afforded the new compound (Rt 21.20 min, 7 mg).

Hibiscuside (1). Amorphous compound: []20D + 8.5 (c 0.01, 80% MeOH); UV max nm (80 % MeOH): 279 (0.78), 229 (1.79), 215 sh. (1.58); IR max cm-1: 3400, 1610, 1595, 1520; FABMS (negative ion mode): m/z 665.47 [M-H], FABMS (positive ion mode): m/z 667.22 [M+H]+.

RESULTS AND DISCUSSION

Negative FABMS spectrometry of compound 1 showed [M-H] at m/z 665.47. The 1H NMR spectrum of 1 was in agreement with a 2,6-diaryl tetrahydrofurofuran ring system. The 1H NMR and 13C NMR spectra showed signals for two methoxyl groups, six aromatic protons in two aryl groups as well as for eight protons in the furofuran ring. The 1H signals for H-8 and H-8 which appear at 3.04 and the axial and equatorial 1H signals for H-9 and H-9 at 4.13 and 3.75 suggest that the 2,6-diaryltetrahydro furofuran ring is symetric with both aryl group. Slightly different aryl groups were revealed by doublets at 4.68 (H-7) and 4.62 (H-7). The 1H-NMR and 13C-NMR spectra of compound 1 showed that both possessed a diequatorial stereochemistry at H-7 and H-7 since the fusion of tetrahydrofuranes to obtain the bicyclic is always cis and axial position of one aryl group is able to affect the chemical shifts of arylic proton for the other side due to the shielding cone of axial aryl group.8 The 1H NMR spectrum also supported the presence of two 1,3,4-trisubstituted rings. In HMBC spectrum, it was evident that the two methoxyl groups are placed at each 3 and 3 position of the two aromatic rings. 1H-NMR and DQF-COSY spectra data implied that compound 1 contained a diglycoside moiety possessing a -glucose and a -rhamnose

moiety. In the 1H and 13C NMR spectra, a doublet (3H, J=6.2 Hz) at 1.11 and a peak at 18.1 for a methyl group and a broad singlet at 5.22 for an anomeric proton suggested the existence of a -rhamnose. In the HMBC spectrum, a correlation peak of the H-1 signal of the glucose moiety at 4.99 and the C-4 signal of the phenyl group at 145.5 suggested that the glucose moiety is connected to a 4-hydroxy group at the phenyl group. Correlation peaks in the HMBC spectrum of the H-2 signal for glucose at 3.54, the C-1 signal for glucose at 98.2 and C-1 signal for rhamnose at 100.1 suggested that these two sugar moieties were connected by 12 linkage between -glucose and -rhamnose, consistent with a neohesperidoside structure. Thus, the structure of 1 was established as (+)-pinoresinol 4-O-[-glucopyranosyl (12)--rhamnoside], and named hibiscuside.

Compound 2 was identified as syringaresinol by 1H, 13C NMR, and FABMS data. Compound 3 was identified as E-N-feruloyl tyramine and compound 4 is Z-E-feruloyl tyramine as compared with authentic literature data.11 Compound 5, 6, and 7 were identified as 6-O-acetyldaidzin,12 6-O-acetylgenistin,13 and 3-hydroxy daidzein by 1H NMR and mass spectra as well as UV spectra with some diagnostic reagents.18 Compounds 2-7 were reported for the first time from *Hibiscus syriacus*.

The inhibitory effects against lipid peroxidation in rat liver microsomes were measured according to method of Hageboom17. The inhibition was shown in a concentration-dependant manner. As shown in Table 2, the isoflavonoids, 6-O-acetyl daidzin (5), 6-O-acetyl genistin (6), and 3-hydroxy daidzein (7), showed significant inhibitory activities with IC_{50} values of 8.2, 10.6, and 4.1 M, respectively. IC_{50} values of these isoflavonoids were slightly weaker than those of vitamin E, quercetin, and catechin with IC_{50} values of 3.4, 2.1, and 3.1 mM, which are known to be strong antioxidants.14-16 The new compound, hibiscuside, showed moderate inhibitory activity against lipid peroxidation in rat liver microsomes with an IC_{50} value of 27.0 M. The IC_{50} value of hibiscuside is weaker than that of pinoresinol with an IC50 value of 18.9 M. E and Z-N-feruloyl tyramines exhibited moderate antioxidant activity with IC_{50} values of 47.3 M. However, Z-N-feruloyl tyramine and syringaresinol did not exhibit any activity even at a concentration of 50 M.

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Table 1. Proton and carbon chemical shift assignments and long-range connectivities observed in the HMBC spectrum of (+) pinoresinol 4-O-[?-glucopyranosyl (1?2) ?-rhamnoside] (1) (600MHz, DMSO-d_6).

Position	^{1}H(mult, J=Hz)	^{13}C	^{1}H → ^{13}C[a]
1		135.5	
2	6.92 (d,1.6)	110	C-3,4,6,7
3		115.2	
4		145.5	
5	7.02 (d,8.4)	149.4	C-1,3,4
6	6.82 (dd,1.6,8.4)	117.9	C-2,4,7
7	4.68 (d,4.6)	85.2	C-1,2,6,8
8	3.04 (m)	53.9	-[b]
9 ax.	3.75 (m)	71.2	C-7′,8
eq.	4.13 (m)		
OCH_3	3.77 (s)	55.5	C-4
1′		132.5	
2′	6.87 (d,1.6)	110.5	C-3′,4′,6′,7′
3′		115.3	
4′		146	
5′	6.71 (d,9.1)	147.7	C-1′,3′,4′
6′	6.74 (dd,1.6,9.1)	118.8	C-2′,4′,7′
7′	4.62 (d,4.8)	85.4	C-1′,2′,6′,8′
8′	3.04 (m)	53.8	-
9′ax.	3.75 (m)	71.1	C-7,8′
9′eq.	4.13 (m)		
OCH_3	3.76 (s)	55.7	C-4′
1″	4.99 (d,7.8)	98.2	C-4
2″	3.54 (d,7.9)	75.8	C-1″,3″,1‴
3″	3.44 (m)	77.9	-
4″	3.18 (m)	72.4	C-5″
5″	3.29 (m)	76.9	-
6″	3.63 (m),3.43 (m)	60.6	-
1‴	5.22 (br s)	100.1	C-2‴,5‴
2‴	3.68 (dd,3.2,1.5)	70.7	-
3‴	3.37 (dd,9,3.5)	70.5	-
4‴	3.19 (m)	69.9	-
5‴	3.92 (m)	68.4	-
6‴	1.11 (d,6)	18.1	-

[a]Long-range couplings were based on a HMBC spectrum and are specified from the proton indicated to the specified carbon

[b]Not observed

Table 2. Inhibition activity(IC_{50}) of the compounds 1-7 from *Hibiscus syriacus* against lipid peroxidtion in rat liver microsomes.

compound	1	2	3	4	5	6	7	vitamine E	quercetin	catechin
[a]IC_{50}(μM)	27.0	>50	47.3	>50	8.2	10.6	4.1	3.4	2.1	3.1

[a]Average of IC_{50} values is from three independent experimenst