Natural Product Sciences
7(2) : 49~52 (2001)

Flavonoids from the Leaves of *Glycine max* Showing Anti-lipid Peroxidative Effect

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Abstract – Anti-lipid peroxidative activity and phytochemical study on the leaves of *Glycine max* Meer. were investigated. The methanol extract of the leaves of *G. max* reduced the level of lipid peroxides induced by bromobenzene *in vitro*. From the leaves of this plant, apigenin, genistein 7-*O*-β-D-glucopyranoside, kaempferol 3-*O*-β-D-glucopyranoside, and kaempferol 3-*O*-sophoroside were isolated and characterized by spectral data.

Key words – *Glycine max*, Leguminosae, lipid peroxide, flavonoid, genistein 7-*O*-glucoside.

Introduction

Glycine max Meer. (Leguminosae) is a perennial herbaceous plant (Lee, 1993). The leaves of this plant have been used in Korean folk medicine as the detoxifying agent for snake poison (Shanghai Science and Technological Publisher, 1985) and particularly as food in southern province. Triterpenoids and flavonoids (Curl *et al.*, 1988; Kitagawa *et al.*, 1988a; Kitagawa *et al.*, 1988b; Ohta *et al.*, 1979; Kudou *et al.*, 1991) have been isolated from the seed of *G. max*. As a part of our continuing studies to find the anti-lipid peroxidative compounds from natural resources (Park *et al.*, 1995), we found that the title plant inhibited the formation of lipid peroxide, and four flavonoids were isolated from the leaves of *G. max*.

Experimental

Plant material and apparatus – The leaves of *G. max* were collected in Sunchon, Jeonnam, Korea on October 14, 1998. The voucher specimen (No. 0359) is deposited in the Department of Oriental Medicine Resources, Sunchon National University.

Animals – Male Sprague-Dawley rats were used for the study. Animals were fed with commercial standard rat diet and water *ad lib.*, and maintained at 20±2°C and with the illumination of a 12 hrs light/dark cycle. The bromobenzene (460 mg/kg) (Zampaglione *et al.*, 1973) was i.p. injected four times with 12 hrs interval for final two days of the fourth weeks. The animals were starved overnight before being sacrificed in order to reduce the variation of hepatic metabolism. Animals were sacrificed by exsanguination from the abdominal aorta under anesthesia with CO_2 gas. The liver was exhaustively perfused with ice-cold normal saline through the portal vein until uniformly pale and immediately removed and weighed.

Determination of lipid peroxide – The content of lipid peroxide was determined by the method of Ohkawa *et al.* (1979) and represented as the content of malondialdehyde (*n* mol) per g of tissue. 0.4 ml of 10% liver homogenate in 0.9% NaCl was added to 1.5 ml of 8.1% sodium dodecyl sulfate, 1.5 ml of 20% acetate buffer (pH 3.5) and 1.5 ml of 0.8% thiobarbituric acid (TBA) solution. The mixture was heated at 95°C for 1 hr. After cooling, 5.0 ml of *n*-butanol-pyridine (15:1) was added for extraction, and the absorbance of the *n*-butanol-pyridine layer at 532 nm was measured for the determination of TBA reactive substance. The statistical differences between the experimental group were determined by using Duncan's multiple range test.

Extraction and isolation – The dried and powdered aerial parts (2.6 kg) of *G. max* was refluxed with MeOH. This extract (400 g) has been partitioned with

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organic solvents of the different polarities to afford dichloromethane (217 g), ethyl acetate (9 g), *n*-butanol (75 g) and aqueous (80 g) fractions, respectively. The ethyl acetate and *n*-butanol fractions were subjected to column chromatography using silica gel with $CHCl_3$-MeOH-H_2O (7:3:1, lower layer; 65:35:10, lower layer) as solvents to give E-1~E-10 and B-1~B-20 subfractions (volume of each tube: 30 ml), respectively. We isolated pure compound **1** (88 mg) from subfr. E-2 (tubes no. 13-15) in ethyl acetate fraction, and compound **2** (43 mg) from subfr. B-2 (tubes no. 36-38), and compound **3** (88 mg) from B-4 (tubes no. 78-81), and compound 4 (116 mg) from B-5 (tubes no. 115-145), respectively. The IR spectra were determined in KBr pellets on a Hitachi 2703 spectrophotometer and the NMR spectra were recorded with a Bruker AM-200 spectrometer.

Compound 1 (apigenin) IRv_{max}^{KBr} cm^{-1}: 3330, 1650, 1610, 1580; ^{1}H-NMR (DMSO-d_6, 200 MHz) δ: 7.92 (2H, d, J=8.6 Hz, H-2' & 6'), 6.92 (2H, d, J=8.6 Hz, H-3' & 5'), 6.78 (1H, s, H-3), 6.47 (1H, d, J=1.7 Hz, H-8), 6.18 (1H, d, J=1.7 Hz, H-6).

Compound 2 (genistein 7-*O*-β-D-glucopyranoside) IRv_{max}^{KBr} cm^{-1}: 3368, 1654, 1622, 1579, 1182, 1045; ^{1}H-NMR (DMSO-d_6, 200 MHz) δ: 8.39 (1H, s, H-2), 7.39 (2H, d, J=8.6 Hz, H-2' & 6'), 6.82 (2H, d, J=8.6 Hz, H-3' & 5'), 6.71 (1H, d, J=2.4 Hz, H-8), 6.46 (1H, d, J=2.4 Hz, H-6), 5.06 (1H, d, J=7.0 Hz, anomeric H).

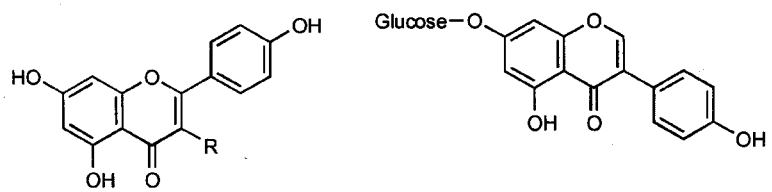
Compound 3 (kaempferol 3-*O*-β-D-glucoside) IRv_{max}^{KBr} cm^{-1}: 3284, 1653, 1605, 1505, 1358, 1288, 1177, 1077; ^{1}H-NMR (DMSO-d_6, 200 MHz) δ: 8.06 (2H, d, J=8.7 Hz, H-2' & 6'), 6.89 (2H, d, J=8.7 Hz, H-3' & 5'), 6.43 (1H, d, J=1.9 Hz, H-8), 6.19 (1H, d, J=1.9 Hz, H-6), 5.46 (1H, d, J=7.0 Hz, anomeric H).

Compound 4 (kaempferol 3-*O*-sophoroside) IRv_{max}^{KBr} cm^{-1}: 3376, 1651, 1607, 1586, 1500, 1358, 1286, 1179, 1069; ^{1}H-NMR (DMSO-d_6, 200 MHz) δ: 8.08 (2H, d, J=9.0 Hz, H-2' & 6'), 6.92 (2H, d, J=9.0 Hz, H-3' & 5'), 6.43 (1H, d, J=2.5 Hz, H-8), 6.20 (1H, d, J=2.5 Hz, H-6), 5.72 (1H, d, J=7.5 Hz, anomeric H), 4.64 (1H, d, J=7.5 Hz, anomeric H).

Results and Discussion

Bromobenzene, a xenobiotic liver toxin, is converted to bromobenzene 3,4-oxide by mixed function oxidase system in the liver. The electrophilic bromobenzene 3,4-oxide acts as a liver toxin. *G. max* has been used for the treatment of stomachache, vomiting and diarrhea (Hsu, 1986).

In the present study the effects of *G. max* on anti-lipid peroxidation and phytochemical study were investigated. When 1 mg/ml of the methanol extract from the leaves of this plant was added, lipid peroxide formation in the bromobenzene-treated rat liver was decreased by 43% *in vitro*. The methanol extract of the leaves of *G. max* was fractionated with dichloromethane, ethyl acetate, *n*-butanol and water successively. Column chromatography of ethyl acetate soluble fraction afforded four compounds. Compound **1** was identified as a well known compound, apigenin by comparison of reported NMR data (Park *et al.*, 2000). Compounds **2-4** showed absorption bands for glycosidic linkage (1000-1100 cm^{-1}) in their IR spectra. The ^{1}H-NMR spectra of compounds **2**, **3** and **4** showed anomeric proton signal at δ5.06 (J=7.0 Hz), and 5.46 (J=7.0 Hz), and 5.72 (J=7.5 Hz), 4.64 (J=7.5 Hz), respectively. The sugar moiety



R

1 H
3 O-glucose
4 O-sophorose

2

Fig. 1. Flavonoids isolated from the leaves of *Glycine max*. 1: apigenin, 2: genistein 7-*O*-β-D-glucoside, 3: kaempferol 3-*O*-β-D-glucoside, 4: kaempferol 3-*O*-sophoroside.

Table 1. Effect of methanol extract from the leaves of *Glycine max* on the hepatic lipid peroxidation in bromobenzene-treated rats *in vitro*

Group	Conc. (mg/ml)	content: MDA n mole/g of tissue	% of control
Control		58.5±9.68[a]	
Extract	1	33.3±4.01[b]	56.9

The values are mean±S.D. of three replications.
Means sharing the same superscript letter are not significantly different from control ($p<0.05$).

Table 2. ^{13}C-NMR data for compounds isolated from the leaves of *Glycine max*

Carbon No.	1	2	3	4
2	163.7	154.6	156.3	156.5
3	102.8	122.6	132.9	133.0
4	181.7	180.5	177.6	77.7
5	161.2	161.7	161.3	161.4
6	98.8	99.6	98.7	98.2
7	164.1	163.0	164.2	164.6
8	94.0	94.5	93.7	93.6
9	157.3	157.2	155.9	156.5
10	103.9	106.1	104.0	104.4
1'	121.1	121.0	121.0	121.0
2'	128.5	130.2	131.1	131.2
3'	116.0	115.1	115.3	115.1
4'	161.4	157.5	160.0	160.2
5'	116.0	115.0	115.3	115.1
6'	128.5	130.1	131.1	131.2
1"		99.9	101.0	98.2
2"		73.1	74.4	82.0
3"		76.4	76.5	77.2
4"		69.6	69.8	70.3
5"		77.2	77.5	75.9
6"		60.6	60.7	61.0
1"'				103.6
2"'				74.6
3"'				76.8
4"'				70.0
5"'				75.5
6"'				61.0

of compounds **2-4** were determined to be β-D-glucopyranoses by the J values of the anomeric proton signals and the ^{13}C-NMR data. Confirmation of the isoflavone skeleton was provided by the ^{1}H-NMR spectrum of compound **2** which contained the characteristic singlet at δ8.39 due to the C-2 proton. Two protons resonate at δ6.46 and δ6.71 show meta coupling (J=2.4 Hz) whilst the aromatic signals at δ6.82 and δ7.39 represent a typical ortho coupled doublet (J=8.6 Hz). These data indicated that compound **2** were genistein glucoside. The location of sugar was substantiated by inspection of the ^{13}C-NMR spectrum of compound **2** compared with its aglycone (Agrawal, 1989). Thus the structure of compound **2** was elucidated as genistein 7-*O*-β-D-glucopyranoside. The ^{1}H-NMR spectra of compounds **3** and **4** showed ortho-coupled doublets ascribable to H-2', 6' and H-3', 5' of B-ring in the flavonoid skeleton and two meta-coupled doublets ascribable to H-8 and H-6 of A-ring in the flavonoid skeleton. These data indicated that compounds **3** and **4** were kaempferol glycosides. The ^{13}C-NMR data of compounds **3** and **4** supported the attachment of sugar moiety to the C-3 position of flavonoid glycosides. The sugar moieties of compounds **3** and **4** were determined to be β-D-glucose and sophorose, respectively, by the J values of the anomeric proton signals and the ^{13}C-NMR data. Compounds **3** and **4** were, therefore, identified as kaempferol 3-*O*-β-D-glucoside and kaempferol 3-*O*-sophoroside, respectively. The ^{1}H- and ^{13}C-NMR data of compounds **3** and **4** were in agreement with those of reference (Markham *et al.*, 1978). Investigation of anti-lipid peroxidative compounds from *G. max* is now in progress.

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(Accepted May 28, 2001)