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LDL-Antioxidant pterocarpans from roots of Glycine max (L.) Merr.

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Objectives

The oxidative modification of low-density lipoprotein (LDL) plays a considerable role in early atherosclerosis process. When LDL is oxidized, it is modified inseveral ways through the reaction with reactive oxygen species (ROS), and the oxidized LDLwithin arterial walls promotes several steps in atherosclerosis, including endothelial cell damage foam cell accumulation and growth, and synthesis of auto-antibodies. Moreover, oxidized LDL promotes monocytes to cause expression of adhesion molecules on the cell surface. Monocyte-derived macrophages recognize oxidized LDL through the scavenger receptor, resulting inthe massive accumulation of lipids.

In this study, we isolated ten flavonoids from roots of *G. max* and identified their structures through spectroscopic methods. Isolated compounds were also evaluated for their inhibitory activity on copper-induced LDL oxidation through four methods: thiobarbituric acid reactive substances (TBARS) assay, measurement of the formation of conjugated diene, relative electrophoretic mobility (REM), and fragmentation of apoB-100.

Materials and Methods

○ Material

The roots of *G. max* (Taekwangkong) were collected on ten days after R8 at Moonsan, Jinju, Korea at the end of September 2003. The fresh roots of *G. max* were then dried.

○ Methods

All purification were monitored by TLC (E. Merck Co., Darmstadt, Germany), using commercially available glass-backed plates. Visualized under UV at 254 and 366 nm or sprayed with *p*-anisaldehyde solution. Column chromatography was carried out using 230-400 mesh silica gel (kieselgel 60, Merck, Germany). Melting points were measured on a Thomas Scientific capillary melting point apparatus (Electrothermal 9300, UK) and are uncorrected. IR spectrawere recorded on a Bruker IFS66 (Bruker, karlsruhe, Germany) infrared Fourier transform spectrophotometer (KBr) and UV spectra were measured on a Beckman DU650 spectrophotometer (Beckman Coulter, Fullerton, CA, USA). ^{1}H- and

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^{13}C-NMR along with 2D-NMR data were obtained on a Bruker AM 500 (^{1}H-NMR at 500 MHz, ^{13}C-NMR at 125 MHz) spectrometer (Bruker, karlsruhe, Germany) in $CDCl_3$, acetone-d_6, DMSO-d_6, and CD_3OD (Sigma Chemical Co, St. Louis, MO, USA). EIMS was obtained on a JEOLJMS-700 mass spectrometer (JEOL, Tokyo, Japan).All the reagent grade chemicals were purchased from sigma (Sigma Chemical Co, St. Louis, MO, USA).

Results and Discussion

The methanolic roots extract of *Glycine max* (L.) Merr. was chromatographed, which yielded ten flavonoids , including three isoflavones **1-3**, five pterocarpans **4-8**, one flavonol **9**, and one anthocyanidin **10**. All isolated compounds were examined in regard to LDL-antioxidant activities using four different assay systems on the basis of Cu^{2+}-mediated oxidation. Among them, seven compounds showed potent LDL-antioxidant activities in the thiobarbituric acid-reactive substances (TBARS) assay, the lag time of conjugated diene formation, relative electrophoretic mobility (REM), and fragmentation of apoB-100 on copper-mediated LDL oxidation. Three pterocarpans **4, 6**, and **7**, never reported as LDL-antioxidant, showed potent activities with IC_{50} values of 19.8 μM, 0.9 μM, 45.0 μM respectively, in comparison with probucol (IC_{50} = 5.6 μM) as positive control. Interestingly, coumestrol **6** (IC_{50} = 0.9 μM) showed twenty times more activity in the TBARS assay than genistein (IC_{50} = 30.1 μM) and daidzein (IC_{50} = 21.6 μM), are representative antioxidants in soybean. Moreover, coumestrol **6** had an extended lag time of 190 min at 3.0 μM in measuring conjugated diene formation, while both genistein (120 min) and daidzein (93 min) lag times were extended to less than 120 min at the same concentration. Also, methanolic extracts of *G. max* (L.) Merr. showed an inhibitory effect on the NO production and iNOS expression in RAW264.7 cell.