Arch. Pharm. Res. 6(1), 75~77 (1983)

Studies on the Anti-inflammatory Activity of *Aralia continentalis*(Ⅱ)

Isolation of Two Phenolic Acids from the Hydrolysate of Butanol Fraction

Byung Hoon Han, Myung Hwan Park, Yong Nam Han and Josefina B. Manalo*

*Natural Products Research Institute, Seoul National University, Seoul 110, Korea and *National Institute of Science and Technology, 727 Pedro Gil, Metro Manila, Philippines*

(Received 22 April 1983)

Abstract □ Two phenolic acids were isolated from the hydrolyzed butanol fraction of *Aralia continentalis* K. in crystalline form. They were identified by chemical tests and by analysis of spectra of UV, IR and NMR as ferulic acid and caffeic acid.

Keyworlds □ *Aralia continentalis* K., Ferulic acid, Caffeic acid, Albumin stabilizing activity, Phenolic acids.

As described in the preceding paper[1], the butanol fraction of A. continentalis also showed the protein stabilizing activity of 9.3% of total methanol extract and the specific activity was about 188 units per gram of butanol fraction.

When chromatographed on TLC by developing solvents for glycosides, butanol fraction showed some spots positive with ferric chloride solution. In order to investigate genuine aglycones, butanol fraction was hydrolyzed with sodium hydroxide, and ether-soluble portion of the hydrolysate yielded two phenolic acids in crystalline states. They were identfied by chemical and spectrometric methods to be ferulic acid and caffeic acid. These acids showed the protein stabilizing activity.

EXPERIMENTAL METHODS

Hydrolysis of Butanol Fraction

One hundred thirty five grams of the concentrated butanol fraction prepared as described in the preceding paper[1], were dissolved in a small amount of methanol, hydrolyzed using 10% sodium hydroxide for 4.0 hours on a boiling water bath, and methanol was removed in vacuo. The hydrolysate cooled was acidified with 10% HCl and extracted with ether. The ether extract was washed with water, dried with anhydrous sodium sulfate and concentrated to dryness.

Isolation of Phenolic Acids

Phenolic compounds in the ether extract were detected on TLC plates by using the solvent system of hexane-ethylacetate-acetic acid(10:10:1), and by using the following visualizing agents: UV light, 10%H_2SO_4, Pauli's reagent and $FeCl_3$ solution.

Separation and isolation of phenolic compounds were done by column chromatography using silica gel 60 as adsorbent and the solvent system mentioned above as elution solvent.Two phenolic compounds isolated were recrystallized with ethylacetate, respectively.

Acetylation of Phenolic Compounds

Acetates of two compounds were prepared by dissolving them in acetic anhydride-pyridine mixture (1:1) and by standing over night. Heated on a water bath for 3 hours, and dried

using a stream of nitrogen. The two acetates were crystallized with ethylacetate, respectively.

Instruments

All melting points were taken on a Mitamura heat block apparatus and given uncorrected values. A recording spectrometer, Gilford type 2600 was used for the measurements of UV-visible absorption spectra. IR spectra were determined in KBr pellets on Perkin-Elmer type 283 B spectrophotometer. PMR spectra were obtained in $CDCl_3$ or DMSO-d_6 solution using TMS as internal standard on Perkin-Elmer NMR spectrometer (90MHz) and recorded by δppm.

Protein Stabilizing Activity

Protein stabilizing activity of two phenolic acids were determined as described in the preceding paper[1].

RESULTS AND DISCUSSION

Characterization of Compounds I and II

Some physico-chemical properties of compounds I and II are summarized in Table I. Both I and II characteristically exhibited bathochromic shifts in their UV spectra in the presence of alkali, suggesting to have a phenolic group.

Table I: Physico-chemical properties of compound I and II.

	I	II
R*f* value	0.25	0.19
mp (free)	162~164°	198~200°
(acetate)	182~184°	188~190°
$FeCl_3$	brown	blue
Pauly	purple	brown
UV (λmax, nm)		
MeOH	292.5	296.5
	312.0	326.0
NaOH	304.0	304.0
	340.0	352.0

IR spectrum of I showed absorption bands at 3410cm^{-1} (hydroxyl group); 3010, 1600 and 1510cm^{-1} (aromatic ring); 1690cm^{-1} (C=O in aromatic acid); 1270 and 1200cm^{-1} (methoxy); 940 and 845cm^{-1} (unsymmetric trisubstituted benzene ring). PMR spectrum of acetate of I gave eleven hydrogens $\delta_{ppm}^{DMSO\text{-}d_6}$; 2.22 (3H, s, CH_3COO-); 3.8 (3H, s, -OCH_3); 6.55 and 7.55 (2×1H, d, $>C=C<^{Hb}_{COOH}$ (Ha) Jab=17); 7.45, 7.24 and 7.06 (3H, C-2, C-5 and C-6 of benzene ring). These properties of I are identical with those of ferulic acid.

IR spectrum of II showed absorption bands at 3420 cm^{-1}(hydroxyl group); 3020, 1615, 1600, and 1520 cm^{-1} (aromatic ring); 1640 cm^{-1} (conjugated carbonyl group). In PMR spectrum acetate of II displayed eleven hydrogens $\delta_{ppm}^{DMSO\text{-}d_6}$; 2.28 (2×3H, s, acetyl-); 6.36 and 7.61 (2×1H, d, $>C=C<^{Hb}_{COOH}$ (Ha) Jab=17.5); 7.18 to 7.48 (3 hydrogens of benzene ring).

These spectral data of II are identical with those of caffeic acid.

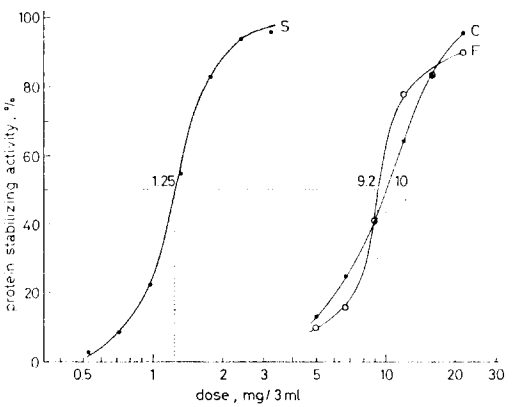

Fig. 1: Protein stabilizing potency of phenolic acids. C; caffeic acid, F; ferulic acid, S; salicylc acid

Portein Stabilizing Activity of Ferulic Acid and Caffeic Acid

The protein stabilizing activities of ferulic acid and caffeic acid against heat denaturation of albumin were determined. Dose-response curves were drawn by plotting values for the inhibition rates of different amounts of two phenolic acids together with salicylic acid as a reference.

The curves (Fig. 1) show that the protein stabilizing activities of two phenolic acids are approximately 1/8 times that of salicylic acid, when 50% inhibition concentrations are compared.

LITERATURE CITED

1) Han, B.H., Han, Y.N., Han, K.A., Park, M.H. and Lee, E.O. *Arch. Pharm. Res.* 6, 17(1983).