

The Isolation of Carboxylic Acids from the Flowers of *Delphinium formosum*

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From the flowers of *Delphinium formosum* Boiss. et Huet. (Ranunculaceae) *trans*-aconitic, *p*-hydroxybenzoic, *p*-coumaric, caffeic and chlorogenic acids were isolated and identified. In addition, protocatechuic, vanillic and gentisic acids were identified.

Key Words: Carboxylic acid, isolation, *Delphinium formosum*, chromatography.

Introduction

The genus *Delphinium* is represented by 28 species in Turkey^{1,2}. *Delphinium* species are used in folk medicine as antirheumatics, pediculicides and insecticides for cattle³. Studies have been carried out on alkaloids⁴ and flavonoids⁵ of *Delphinium formosum*, which is endemic to Turkey. But, to our knowledge, there is no report regarding the carboxylic acids of this plant. In the present paper, we report the isolation and identification of organic acids from *Delphinium formosum* Boiss. et Huet.

In previous papers, the isolation of carboxylic acids of *Consolida orientalis*⁶, *Consolida armeniaca*³ and various *Consolida species*⁷ was reported by some of us. The plant acids, especially, gallic, chlorogenic and caffeic acids, are known to have anticarcinogenic activity⁸.

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Experimental

Plant material and chemicals

Delphinium formosum, which has large clusters of flowers, was collected on the plateau of Santa-Gümüşhane in July and August, 1996. The dried flowers were stored in an airy place. Authentic acids were purchased from Aldrich. All the solvents, HCl and NaHCO₃ were obtained from Merck. Silica Gel 60 HF₂₅₄ (Merck) was used as adsorbent. The mobile phase solvent systems were as follows:

I CHCl₃-AcOH (9:1); II EtOAc-Toluene-AcOH (50:40:20); III Benzene-MeOH-AcOH (79:14:7); IV CHCl₃-MeOH-H₂O (65:45:12). The spots were visualized by UV light, diazotized *p*-nitro aniline and FeCl₃. Isolation was succeeded by repetitive preparative thin layer chromatography. NMR spectra were recorded on a Varian Unity spectrometer (¹H, 400 MHz and ¹³C, 100 MHz). IR spectra were measured with a Shimadzu FT-IR 1640 instrument.

Extraction and isolation

To a known amount of dried and thoroughly ground flowers, methanol was added and heated in a water bath overnight with stirring. The solution obtained after filtration was evaporated at reduced pressure. The residual matter was poured into water and extracted with petroleum ether and chloroform to discard organic residues other than acids. Then, the main solution was extracted with ether to obtain the organic acids. The ethereal extract was found to have a great amount of *trans*-acotinic acid and lesser amounts of the others. The flavonoids were removed by extracting the aqueous solution with ethyl acetate. The remaining aqueous solution was extracted with *n*-butanol to separate the chlorogenic acid. To develop the chlorogenic acid on the plate, a number of solvent systems were tried, but only the CHCl₃-MeOH-H₂O (65:45:12) system was able to separate it.

For the isolation of the other acids, HCl 8% was added to a known amount of dried and finely ground flowers and hydrolyzed by heating on a hot plate for 1 h. After filtration, the aqueous acidic solution was extracted with ethyl acetate. The ethyl acetate extract was treated with NaHCO₃, 5%. The organic acids in the aqueous phase was extracted with ethyl acetate again and *p*-hydroxybenzoic, caffeic and *p*-coumaric acids were isolated from this extract.

Results and Discussion

The structures of the carboxylic acids analyzed are shown in the Scheme below.

The isolation of organic acids **1-5** was carried out by means of preparative thin layer chromatography. The identification and structure elucidation of the isolated carboxylic acids were established by direct comparison of their melting points, mixed melting points, IR, ¹H-NMR, ¹³C-NMR spectra and thin layer chromatography with the authentic samples^{9,10} (Tables 1 and 2).

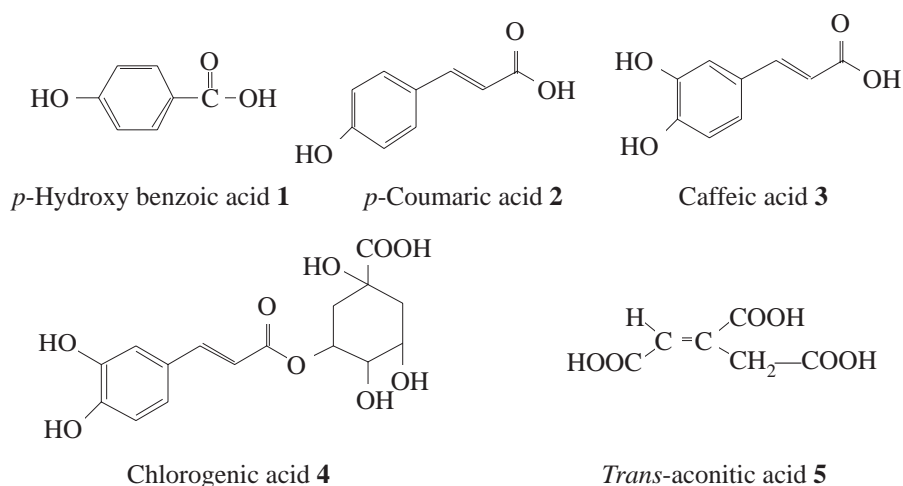


Table 1. Solvent systems, R_f values, melting points, spray reagents and colours for the diagnosis of acids.

Acids, m.p (°C)	Solvent systems ^a and R_f values				Spray reagents and colours		
	I	II	III	IV	Diazotised <i>p</i> nitroaniline	FeCl ₃	UV (366 nm)
<i>p</i> -Hydroxy benzoic acid 213-214	0.55	0.87	0.67	0.95	yellow	yellow	—
<i>p</i> -Coumaric acid 210-213	0.51	0.88	0.58	0.90	dark brown	orange	—
Caffeic acid 223-225	0.22	0.83	0.49	0.80	pink-brown	bluish-grey	blue
Chlorogenic acid 208-211	—	0.10	0.14	0.25	pink-orange	dark blue	blue
<i>Trans</i> -aconitic acid 198-199	0.18	0.75	0.29	0.60	—	pink	—

^a I: CHCl₃-AcOH(9:1); II: EtOAc-Toluene-AcOH (50:40:20); III : Benzene-MeOH-AcOH (79:14:7); IV: CHCl₃-MeOH-H₂O (65:45:12)

From the ethereal solution of the aqueous methanol extract of the flowers of *Delphinium formosum*, *trans*-aconitic acid was isolated. The soluble part in *n*-butanol of the same extract was found to contain chlorogenic acid in a substantial amount. Chlorogenic acid was also detected in the ethyl acetate extract, but its amount was too low to isolate; however, it was found to be present in high yield in *n*-butanol extract. Chlorogenic acid, since it is more polar and bulky in comparison with the other acids, could be separated in the solvent system IV CHCl₃-MeOH-H₂O (65:45:12). The chromatographic studies showed that the amounts of other free acids are too low for separation and isolation, and they are bound.

The ethyl acetate extract obtained by hydrolysis with HCl 8% contained *p*-hydroxy, *p*-coumaric and caffeic acids in high percentages.

The spectroscopic data of the carboxylic acids detected and isolated from the source plant are given in Table 2. All the proton and carbon NMR peak assignments for the isolated compounds were done by comparing the literature values, and they were found to be consistent with the literature data. Furthermore, they were assayed for thin layer chromatographic behaviour with various solvent systems and visualising reagents and the results were the same as those of the pure authentic samples. The proton and carbon NMR data of chlorogenic acid are seen in Table 2.

Table 2. Spectroscopic data for the carboxylic acids isolated from *Delphinium formosum* Boiss. et Huet.

Acids	¹ H-NMR(DMSO-d ₆) σ ppm	¹³ C-NMR(DMSO-d ₆) σ ppm	IR (KBr); $\sqrt{\text{cm}^{-1}}$
<i>p</i>-Hydroxy benzoic acid 1	12.04 (s, broad, 1H), 9.14 (s, broad, 1H), 6.97(dd, J=1.5, 7.7 Hz, 2H), 6.65 (dd, 2H, J=1.5, 7.7 Hz)	179.8, 158.4, 145.2, 130.2, 120.6, 116.1, 113.6	3388, 2821, 2547, 1674, 1608, 1595, 1508, 1448, 1421, 1317, 1292, 1244, 1, 101, 929, 854, 619
<i>p</i>-Coumaric acid 2	12.09 (s, broad, 1H), 9.18 (s, broad, 1H), 7.34 (d, 1H, J=15.8 Hz), 7.16 (t, 1H, J=7.7Hz), 6.97 (d, 1H, J=7.7Hz), 6.95 (d, 1H, J=2.2Hz), 6.74 (dd, 1H, J=2.2, 7.7 Hz), 6.45 (d, 1H, J=15.8 Hz).	170.6, 160.1, 146.5, 137.2, 131.3, 120.8, 119.4, 118.2, 115.3	3384, 2910, 1672, 1627, 1602, 1512, 1450, 1245, 977, 833, 516
Caffeic acid 3	7.42 (d, 1H, J=15.8 Hz), 7.02 (d, 1H, J=2.1 Hz), 6.95 (dd, 1H, J=2.1, 8.1 Hz), 6.75 (d, 1H, J=8.1 Hz), 6.17 (d, 1H, J=15.8 Hz).	168.1, 148.2, 145.4, 144.4, 125.6, 121.1, 115.7, 115.2, 114.6	3433, 3234, 2910, 1645, 1620, 1448, 1278, 1217, 1120, 974, 576
Chlorogenic acid 4	12.10 (s, broad, 1H, COOH), 9.53 (s, broad, 1H), 9.12 (s, broad, 1H), 7.40 (d, J=15.0 Hz, 1H), 7.02 (d, J=1.6 Hz, 1H), 6.94 (dd, 8.0, 1.6, 1H), 6.75 (d, J=8.0, 1H), 6.17 (d, J=15.0, 1H), 5.19 (m, 1H), 4.85 (d, J=3.6, 1H), 4.72 (br, 1H), 3.76-3.53 (m, 4H), 2.56-1.80 (m, 4H)	176.0 (COOH), 166.2 (OCOCH=CH-), 149.3, 145.5 (CH=CH), 144.3, 125.9, 121.2, 116.1 (CH=CH) 115.1, 114.7, 79.1, 73.3, 70.8, 67.3, 38.7, 37.1	3421, 2929, 1697, 1635, 1456, 1398, 1278, 1182, 812
<i>Trans</i>-aconitic acid 5	11.10 (m, 3H, COOH), 6.98 (s, 1H, CH=C), 3.96 (s, 2H, CH ₂)	175.1, 170.2, 143.3, 131.2, 36.2	2950 (broad), 1697, 1417, 1290, 1222, 900, 852, 646, 615

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