Phenolic acids from the flowers of *Nymphaea alba* L.

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Abstract

Nymphaea alba flowers contain a variety of phenolic acids. Crystalline ellagic and gallic acids were isolated as well as their methyl and ethyl esters. Their identification was made on the basis of the results of spectral analyses ^1H NMR, ^{13}C NMR, MS, UV). Small amounts of p-hydroxybenzoic, p-cumaric, vanillic and ferulic acids were isolated. The presence of bound forms of acids in the material was demonstrated.

Key words: *Nymphaea alba* L., *Nymphaeaceae*, phenolic acids, methyl and ethyl gallate

INTRODUCTION

The structure of flavonoids isolated from *Nymphaea alba* flowers was described on a previous paper (Jambor and Skrzypczak 1991). A phenolic acid fraction was obtained as a by-product. The characterisation of that fraction is presented in this paper.

MATERIAL AND METHODS

The ether fraction obtained as described in a previous paper (Jambor and Skrzypczak 1991) was used to isolate 2.6 g of a mixture of phenolic acid compounds (fraction PA). It was initially analysed by two-dimensional thin-layer chromatography (2D-TLC). The fractions were applied to cellulose-coated plates (Merck) and developed in solvent systems A: benzene (C_6H_6)-acetic acid (AcOH)-water (125:72:3) in direction I and B: 50% AcOH

— direction II (Fig. 1). Homogenous compounds and degradation products were chromatographed on thin layers of cellulose (TLC).

Using column and preparative chromatography (Herborne 1973) the following amounts of crystalline compounds were obtained from 2 g of fraction PA: K1 — 0.114 g, K2 — 0.193 g, K3 — 0.014 g and K4 — 0.278 g. The method of Świątek (1977) and Świątek and Dąbrowicz (1984) was applied to this fraction to obtained more phenolic acids (K5-K8) in the form of amorphous, white powders, in amounts of about 1 mg each. Fractions of phenolic acids occurring in water lily flowers in bound form were also isolated from these flowers.

Fig. 1. 2D-TLC of phenolic fraction. Direction I: C_6H_6 -AcOH- H_2O (125:72:3), II: 50% AcOH, K₁ — ellagic acid, K₂ — gallic acid, K₃ — methylgallate, K₄ — ethylgallate, reagent — $FeCl_3$

IDENTIFICATION OF THE ACIDS

The uncorrected melting points were determined for the compounds crystallised from methanol (MeOH) or ethyl ether (Et_2O). The R_f values for the three mobile phases, A, B, C — water are given below. The chromatographs were examined under UV_{365} before and after treatment with ammonium vapors. The plates were also examined under visible light after

spraying them with a 1% solution of ferric chloride and diazotized sulfanilic acid in a 10% aqueous solution of sodium carbonate. The spectra of the compounds were recorded and their hydrolysis was carried out as described (Jambor and Skrzypczak 1991). ^{13}C NMR was carried out at 22.5 MHz.

Compound K1 — a light yellow, crystalline powder, m.p. exceeding 350°C (MeOH), 360°C according to Nawwar et al. (1982) and Nawwar and Souleman (1984); $\text{UV}\lambda_{\text{max}}^{\text{MeOH}}$ nm, 255, 367, + NaOMe 288; R_f A — 0.05, B — 0.20, C — 0.00, blue spots under UV, greenish navy blue in visible light after spraying with a solution of FeCl_3 .

Compound K2 — cream-colored needles which darken on contact with air, m.p. 239°C (MeOH), 240°C according to Karrer et al. (1977) $238\text{--}240^\circ\text{C}$, according to Tomczyk and Kohlmünzer (1979); $\text{UV}\lambda_{\text{max}}^{\text{MeOH}}$ nm 218, 274, + NaOMe immediate decomposition (Nawwar et al. 1982); R_f A — 0.12, B — 0.05, C — 0.77 violet-blue — UV, greyish navy blue — FeCl_3 .

Compound K3 — white crystalline powder, m.p. $194\text{--}197^\circ\text{C}$ (MeOH) $198\text{--}200^\circ\text{C}$ according to Karrer et al. (1977), $197\text{--}198^\circ\text{C}$ according to Tomczyk and Kohlmünzer (1979); $\text{UV}\lambda_{\text{max}}^{\text{MeOH}}$ nm 219, 276, + NaOMe 241, 283, 320 inflexion (p), 410p, MeOH/HCl 217, 274; ^1H NMR (DMSO-d_6) 6.94 (2H, s, H-2, H-6), 5.63 (3H, s, wide $3\times\text{OH}$), 3.75 (3H, s, OCH_3); ^1H NMR ($\text{DMSO-d}_6 + \text{D}_2\text{O}$) 6.96 (2H, s, H-2, H-6), 3.75 (3H, s, OCH_3); MS (m/e): 184 (M^+ 56%), 153 (M- OCH_3 , 100%), 125 (M- COOCH_3 , 20%); R_f A — 0.12, B — 0.77, C — 0.36, dark violet — UV, navy blue — FeCl_3 . This compound was subjected to acid hydrolysis and its degradation products were then analysed by TLC.

Compound K4 — long white needles, m.p. $147\text{--}151^\circ\text{C}$ (Et_2O); $\text{UV}\lambda_{\text{max}}^{\text{MeOH}}$ nm 220, 277, + NaOMe 238, 284, 321; ^1H NMR (DMSO-d_6) 6.96 (2H, s, H-2, H-6), 5.97 (3H, s, wide $3\times\text{OH}$) 4.25 (2H, q, $J=7\text{Hz}$, CH_2), 1.28 (3H, t, $J=7\text{Hz}$, CH_3); ^1H NMR ($\text{DMSO-d}_6 + \text{D}_2\text{O}$) 6.99 (2H, s, H-2, H-6), 4.25 (2H, q, $J=7\text{Hz}$, CH_2), 1.28 (3H, t, $J=7\text{Hz}$, CH_3); ^{13}C NMR; C-1 119.9, C-2 108.7, C-3 145.3, C-4 138.4, C-5 145.3, C-6 108.7, C-7 166.0, C-1' 60.1, C-2' 14.2; MS (m/e): 198 (M^+ 45%), 170 (M- C_2H_4 17%), 153 (M- OC_2H_5 100%), 125 (M- COOC_2H_5 17%).

Elementary analysis for the formula: $\text{C}_9\text{H}_{10}\text{O}_5$:

obtained: 54.54% C, 5.09% H

calculated: 54.85% C, 5.03% H

Compound K4 was subjected to acid hydrolysis after which the products were analysed (TLC. m.p.) R_f A — 0.40, B — 0.78, C — 0.46.

Compounds K5-K8. These compounds were obtained in very small amounts, thus only their UV spectra and chromatography with standards were possible to carry out (Herborne 1973). Chromatography on cellulose plates was employed using a number of solvent systems, among which were: D — $\text{C}_6\text{H}_6\text{--AcOH--H}_2\text{O}$ (6:7:3) organic phase; E — sodium formate (HCOONa)-formic acid (HCOOH)- H_2O (10:1:200). The results are presented in Table 1.

Table 1
Results of chromatography and UV analysis of phenolic acids K₅-K₈

Phenolic acid	Rf		Color			UV λ max (nm)	
	D	E	UV	UV/NH ₃	DSA	MeOH+HCl	NaOMe
K ₅ Ferulic	0.83	$\frac{0.71^c}{0.30^t}$	b	b	pv	234, 324	348
K ₆ Vanillic	0.75	0.54	—	—	o	207, 253, 291	299
K ₇ p-Cumaric	0.50	$\frac{0.79^c}{0.41^t}$	—	v	p	228, 310	335
K ₈ p-Hydroxybenzoic	0.41	0.66	—	—	y	208, 255	275

D — C₅H₆-AcOH-H₂O (6:7:3), E — HCOONa-HCOOH-H₂O (10:1:200), c — cis, t — trans, DSA — diazotized sulfanilic acid in 10% Na₂CO₃, b — blue, v — violet, y — yellow, p — pink, o — orange, pv — pink-violet.

ANALYSIS OF CONJUGATED ACIDS

A fraction of phenolic acids was obtained from *Nymphaea alba* flowers after hydrolysis under both acidic and alkaline conditions (Świątek 1977, Komorowski and Świątek 1982, Świątek and Dombrowicz 1984). Chromatography was carried out in two directions on paper (Whatman No. 1) and on cellulose plates using system D (as described above) for direction I, system E — in direction II. The results were compared with standards. Staining was carried out as given in Table 1.

RESULTS AND DISCUSSION

A PA fraction was obtained from fresh *Nymphaea alba* L. flowers (Jambor and Skrzypczak 1991). This fraction was then further separated using chromatographic techniques obtaining a total of eight crystalline phenolic compounds. They were identified on the basis of spectral analyses.

Compounds K1 and K2 were identified as ellagic and gallic acids. The next phenolic compounds (K3 and K4) were found to be the methyl and ethyl esters of gallic acid. Small amounts of other phenolic acids were then obtained (K5-K8): ferulic, vanillic, p-cumaric and p-hydroxybenzoic acids (Table 1). Phenolic acids frequently occur in plant material, eg, in esters and glycosides. The presence of bound acids was demonstrated in this study using well-known analytical methods (Świątek 1977, Komorowski and Świątek 1982, Świątek et al. 1984).

The obtained results indicate that the following acids are found, probably in bound form, in water lily flowers: gentisic, protocatechuic, caffeic and syringic.

Ellagic and gallic acids are components of hydrolysing tannins, the so-called elago- and gallotannins which belong to one of the four chemical classes of tannins (Hegnauer 1986a). During recent years this group of compounds has become the focus of rising interest due to the variety of chemical structures found in it, its role in the biochemical taxonomy of plants and their wide range of biological effects.

Water lilies contain hydrolysing tannins. Their presence in roots, rhizomes and seeds was reported many years ago (according to Hegnauer 1969). There were, however, no data on the occurrence of free phenolic acids. There is only a passing mention of the presence of gallic acid in the flowers of an Indian species of *Nymphaea alba* L. having been determined (Joshi et al. 1974).

Our studies resulted in the isolation of crystalline phenolic compounds from flowers, including ellagic and gallic acids and the methyl and ethyl esters of the latter. The dominant compound in the material is ethyl gallate. Smaller amounts of its methyl derivative were found. These compounds and the remaining acids that were found to occur had not yet been described from the species *Nymphaea alba* L.

A chemical characteristic of the order *Nymphaeales*, which includes the family *Nymphaeaceae*, is the occurrence of significant amounts of gallic- and ellagotannins (Hegnauer 1986b). It would be premature to draw conclusions on the taxonomic significance of phenolic acids while there is a lack of broader-ranged studies of this group of compounds in the particular taxonomic groups of the mentioned order.

It should be emphasised that the metabolites that have been discovered are polyphenols. This group of compounds is considered to be intermediated in the pathway leading to the formation of the major resistance substances of plants (Beart et al. 1985). These compounds also draw attention because of their strong antioxidant properties (Su et al. 1988). Ellagic and gallic acids have been shown to be effective agents in combating the larvae of moths feeding on tobacco (Locke et al. 1986). It has also been found that the acids referred to above along with their tannic structures, gallo- and ellagotannins, are inhibitors of enzymes (Kakiuchi et al. 1985). The presence of ellagic acid and methylgallate, which are considered to possess tranquillising properties, support the suggestions of using water lily flowers in place of its rhizomes and roots in phytotherapy.

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Kwasy fenolowe kwiatów Nymphaea alba L.

Streszczenie

Jako produkt uboczny przy izolacji flawonoidów otrzymano frakcję fenolokwasów, z której wydzielono osiem krystalicznych związków. Obok kwasów: elagowego, galusowego, p-hydroksybenzoesowego, p-kumarynowego, wanilinowego i ferulowego, wyodrębniono galusan metylu i etylu. Ten ostatni ester jest dominującym związkiem fenolowym badanego surowca. Analizy chromatograficzne wskazują, że kwiaty grzybieni białych zawierają w postaciach związanych dalsze kwasy, jak gentyzynowy, protokatechowy, kawowy i syryngowy.