NATURAL DRUGS

FLAVONOID COMPOUNDS IN THE FLOWERS OF
URENA LOBATA L. (MALVACEAE)

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Abstract: The following flavonoid compounds have been isolated and identified from flowers of Urena lobata L.: kaempferol 3-O-(6-O-trans-p-coumaroyl)β-glucoside (tilisroside), dihydrokaempferol 4’-O-β-glucopyranoside, kaempferol and quercetin 3-O-β-glucosides and 3-O-β-rutinosides also evident 4’-O-β-glucopyranosides. Their structures were established by conventional (acid, enzymatic and alkaline hydrolysis) and spectral analysis (UV, 1H NMR, 13C NMR). Chromatographical investigations indicated the presence of quercetin, kaempferol and kaempferol 7-O-glucoside.

Keywords: Urena lobata L., Malvaceae, flavonoid: kaempferol, quercetin, luteolin, dihydrokaempferol glycosides, tilisroside.

Urena lobata L. (Malvaceae) occurs wild in nature in China and is cultivated in tropic and subtropic countries for the fibre used in production of ropes and sacks. Because of the big flowers it is sometimes grown as a decorative plant. Some species from Malvaceae family are used in medicine for the treatment of cough and inflammation of mucous membranes as well as for treating haemorrhoids (1). The aim of this work was the isolation and identification of flavonoids present in the flowers of Urena lobata L.

EXPERIMENTAL

Material and methods

Urena lobata L. flowers were collected from the plants cultivated in the garden of the Department of Medical Plants at the University of Medical Sciences, Poznań, from the seed obtained from the Botanical Garden of Kisantu. A voucher specimen has been deposited in the author’s laboratory.

UV spectra were run in MeOH on a UV-VIS spectrophotometer. 1H NMR and 13C NMR spectra (Jeol FX 90Q) were recorded in DMSO-d6 at 90.0 and 22.5 MHz respectively, chemical shifts are given in δ (ppm) downfield of TMS (for I, V, VI compounds). 1H NMR spectrum of compound V was also registered in DMSO-d6, 2H O and pyridine-d5 (py-d5).

PC was carried out on a Whatman No 1 paper by using the following solvent systems:
S-1. n-BuOH–HOAc–H2O (4:1:2); upper phase
S-2. HOAc–H2O (15:85)
S-3. EtOAc–HCOOH–H2O (10:2:3)
S-4. iso-ProOH–HCOOH–H2O (2:5:5)
S-5. EtOAc–Pyridine–H2O (12:5:4)

TLC was run on Kieselgel G (Merck) by using the solvent system:
S-6. CHCl3–MeOH–H2O (6:4:2)

The spots of flavonoids were detected under UV light (366 nm) before and after spraying with KOH, AlCl3, and NA–reagent.

Sugars obtained on hydrolysis were analysed by co-TLC (solvent system S–6) and co-PC (solvent system S–5). The spots of sugars were visualized by spraying with aniline phthalate and heating at 105°C. Violet spots of p-coumaric trans-, cis–acid were observed after spraying with a mixture of diazotized sulphanilic acid and 20% Na2CO3.

Extraction and isolation

Air-dried flowers of Urena lobata L. (250 g) were extracted with MeOH and 50% MeOH. The combined extracts were evaporated to dryness and partitioned between H2O and Et2O. The ethereal extract was chromatographed by CC on a silica gel using CHCl3–MeOH (4 : 1) as eluent to yield crude compounds I (30 mg), II (10 mg), III (2 mg) and IV (trace amounts). The aqueous portion was chromatographed on cellulose powder (Whatman CF 11) with EtOAc–MeOH–H2O (100:5:5) as eluent to yield crude compound V (25 mg). Subsequently eluted compounds VI–X were rechromatographed by preparative PC (Whatman No 3, solvent system S–2). The crude compounds I–X were further purified by CC on Sephadex LH 20 using MeOH as eluent.
Identification

Flavonoids were identified according to standard methods and spectral analysis (UV, \(^1\)H and \(^{13}\)C NMR) (2). Total acid hydrolysis was carried out with 2 N HCl, mild acid hydrolysis with 0.1 N HCl at 90°C, enzymatic hydrolysis with \(\beta\)-glucosidase (Koch-Light); EtOAc extract of hydrolysates were analysed for aglycones and \(\text{H}_2\text{O}\) residues for sugars.

Alkaline hydrolysis of compound I: 0.5% NaOH, 30 min at room temp. The solution was acidified (5% HCl) and extracted with Et\(_2\)O, p-coumaric acid was identified by PC in the solvent system S–2.

**Compound I.** Kaempferol 3–O–(6-O-trans-p-coumaryl)–\(\beta\)-glucopyranoside (trans tiliroside). Yellow powder Rf: S–1 0.9; S–2 0.28. UV \(\lambda_{max}\) MeOH 269,301,319,359sh; +NaOAc 276,312,371sh; +NaOAc+H\(_2\)BO\(_3\) 267,315,359sh; +AlCl\(_3\) 275,308,397; +AlCl\(_3\)+HCl 274,306,397sh nm.

\(^1\)H NMR (DM SO–d\(_6\)) 8 ppm: 1.03 and 6.91 (each 2H, J=9.0 Hz, H–2’,6’) and H 3’,5’, respectively; 7.36 and 6.75 (each 2H, J=9.0 Hz, H–2”,6” and H–3”,5”), respectively; 7.35 and 6.27 (each 1H, d, J=16.0 Hz, H–7” and H–8” respectively); 6.40 and 6.17 (1H, d, J=2.0 Hz, H–8 and H–6 respectively); 5.41 (1H, d, J=9.0 Hz, H” of sugar).

Total acid hydrolysis (1 hr) gave kaempferol, trans- and cis- p-coumaric acid (co-PC S–2), glucose (co-PC S–5).

Alkaline hydrolysis: kaempferol 3-glucoside, trans- and cis- p-coumaric acid (co-PC S–5).

**Compound V.** Dihydroykaempferol 4’–O–\(\beta\)-glucopyranoside crystallised from H\(_2\)O as long white needles, colour reaction: FeCl\(_3\) –mauve; PC RF S–1 0.63, S–2 0.82

UV \(\lambda_{max}\) MeOH 295,334sh; +NaOAc 332; +NaOAc 331; +NaOAc+H\(_2\)BO\(_3\) 297,333; +AlCl\(_3\) 320, 390; +AlCl\(_3\)+HCl 320, 390 nm.

\(^1\)H NMR (DM SO–d\(_6\)) 8 ppm: 7.5 (2H, dd, J=16.0 Hz, H–2’, H–6’); 6.94 (2H, dd, J=16.0 Hz, H–3’,5’); 6.10 (2H, d, J=7.0 Hz, H–6,8); 5.19 (1H, dd, J=20.0 Hz, H–2); 4.70 (1H, dd, J=20.0 Hz, H–3). The signal for the anomic proton of glucose was overlapped by water resonance which appeared at 4.00–2.85 \(^1\)H NMR (py–d\(_5\)) 5.65 (1H, d, J=8.0 Hz, H” of glucose).

**Compound VI.** Luteoline 4’–O–\(\beta\)-glucopyranoside, pale yellow needles.

UV \(\lambda_{max}\) MeOH 267,294,312sh; +NaOAc 275,389 (without increase of intensity); +NaOAc 276,304sh, 372; +NaOAc+H\(_2\)BO\(_3\) 267,339; +AlCl\(_3\) 276,301sh, 349,384sh; +AlCl\(_3\)+HCl 275,300,345,384sh nm.

\(^1\)H NMR (DM SO–d\(_6\)) 8 ppm: 7.48 (2H, d, J=4.0 Hz, H–2’, H–6’); 7.27 (1H, d, J=8.0 Hz, H–5’); 6.80 (1H, s, J=2.0 Hz, H–3’); 6.49 (1H, d, J=2.0 Hz, H–8’); 6.19 (1H, d, J=2.0 Hz, H–6); 5.08 (1H, d, J=8.0 Hz, H” of glucose).

Total hydrolysis and enzymatic hydrolysis (\(\beta\)-glucosidase): luteoline, glucose.

**Compound II, VII, VIII, IX, X.** Kaempferol and quercetin 3–O–glucosides and 3–O–rutinosides, respectively, and kaempferol 7–O–glucoside were identified by their spectroscopic data (UV), co-PC with standards, acid hydrolys.

**RESULTS AND DISCUSSION**

The flavonoid fraction of Urena lobata L. flowers contained ten flavonoid compounds which were isolated by chromatographic methods.

Separation of the ethereal and aqueous portions of the methanolic extract from the flowers yielded I–IV and V–X, respectively. Flavonoid compounds were identified by conventional and spectral analysis (2) as kaempferol 3–O–\(\beta\)-glucoside [II] quercetin 3–O–\(\beta\)-glucoside [VIII], kaempferol 3–O–rutinoside [VIII] and quercetin 3–O–rutinoside [IX]. Kaempferol 7–O–glucoside [XI], kaempferol [III] and quercetin [IV] were identified by comparison with authentic samples and literature data.

The \(^1\)H NMR spectrum (DM SO–d\(_6\)) of I displayed signals corresponding to the trans configuration of p-coumaryl moiety typical for tiliroside.

The structure of I was confirmed by \(^{13}\)C NMR. Tiliroside was found for the first time in Tilia argentea (3), then in species belonging to different families like Quercus ilex L. and Q. cerris L. (Fagaceae) (4,5), Althaea rosea (L.) Cav. var. nigra (6), Kitaibelia vitifolia Willd. (7), Althaea officinalis L. (8,9) (Malvaceae) and Lamium album L. (Labiatae) (10).

The comparative chromatography, UV spectral analysis and results of acid and enzymatic hydrolysates indicated that V was dihydrokaempferol glucoside with free hydroxyls at C–5 and C–7. In the \(^1\)H NMR spectrum recorded in DM SO–d\(_6\), the signal for the anomic proton of glucose was overlapped by water resonance which appeared at 4.00–2.85 ppm. This proton was observable when pyridine–d\(_5\) was used as solvent.

The \(^{13}\)C NMR spectrum of V showed a downfield shift of C–4’ (4.9 ppm) and upfield shift (1.14 ppm) of C–3’ and C–5’ signals expected from the substituent effects of C–4’ glycosylation. Thus compound V was identified as dihydrokaempferol 4’–O–\(\beta\)-glucoside. It was described for the first time in Petunia hybrida (11) and also in flowers of Althaea officinalis L. (8,9) and Malva sylvestris L. (12).

The UV spectra of compound VI were consistent with the structure of 4’-substituted luteoline.
In the 'H NMR spectrum the aglycone signals matched well with those for luteoline. The presence of the signal for the anomeric proton with the coupling constant of 8.0 Hz confirmed the β-conformation of glucose. Luteoline 4′-O-β-glucoside is a rarely occurring compound (13,14,15,16). Among species of Malvaceae family it has previously been isolated from the flowers of Althaea rosea (L.) Cav. var. nigra (6).

REFERENCES


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