Acta Poloniae Pharmaceutica – Drug Research, Vol. 59 No. 3 pp. 227–229, 2002

ISSN 0001-6837
Polish Pharmaceutical Society

NATURAL DRUGS

FLAVONOID COMPOUNDS IN THE FLOWERS OF *ABUTILON INDICUM* (L.) SWEET (*MALVACEAE*)

IRENA MATŁAWSKA and MARIA SIKORSKA

Department of Pharmacognosy, Karol Marcinkowski University of Medical Sciences, 10 Sieroca Str., 61–771 Poznań, Poland

Abstract: Seven flavonoid compounds: luteolin, chrysoeriol, luteolin 7-*O*-β-glucopyranoside, chrysoeriol 7-*O*-β-glucopyranoside, apigenin 7-*O*-β-glucopyranoside, quercetin 3-*O*-β-glucopyranoside, quercetin 3-*O*-α-rhamnopyranosyl (1→6)-β-glucopyranoside, were isolated and identified from the flowers of *Abutilon indicum* (L.) Sweet (*Malvaceae*).

Keywords: *Abutilon indicum* (L.) Sweet (*Malvaceae*), flowers, flavonoids, luteolin, chrysoeriol, apigenin and quercetin glycosides.

The Abutilon L. genus of the *Malvaceae* family comprises about 150 species, one of them is *Abutilon indicum* (L) Sweet. These plants occur wild in tropic and subtropics regions of the world, whereas in Poland some of them are cultivated for their ornamental values. Earlier phytochemical studies detected linoleic, oleic, stearic, palmitic, myristic, lauric, capric and caprylic acids in the fixed oil obtained from the petrol extract of *Abutilon indicum* roots. This oil is reported to have analgesic activity comparable with that of acetylsalicylic acid and it is devoided of CNS depressant activity (1). The root extracts, obtained with different solvents, exhibited antibacterial and antifungal activity (2). A mixture of the extracts prepared from the fresh *Abutilon indicum* leaves and *Allium cepa* bulbs is used as a remedy against liver discorders (3), whereas the recent studies revealed that the whole plant has an immunostimulating effect. Moreover, in different parts of this plant were also identified vitamin E, gallic acid, terpenes (2) and sesquiterpene lactones (4).

The aim of our work was the isolation and identification of the flavonoids present in the flowers of *Abutilon indicum*, since according to our best knowledge only two derivatives of gossypetin, i.e. 8–glucoside and 7–glucoside, had been isolated before (5).

EXPERIMENTAL

Plant material

The flowers of *Abutilon indicum* were collected in the garden of the Department of Medical Plants (K. Marcinkowski University of Medical Sciences, Poznań). A voucher specimen is deposited in the Department of Pharmacognosy K. Marcinkowski University of Medical Sciences in Poznań.

Extraction and isolation

The air–dried flowers (20.0 g) were extracted with hot MeOH and the extract was concentrated, treated with hot H_2O and filtered. The H_2O sol. was extracted with Et_2O followed by EtOAc. The Et_2O extract (0.5 g) was separated by preparative paper chromatography (Whatman No. 3, S_1) to yield flavonoid compounds A and B, whereas the EtOAc extract (1.5 g) was subjected to CC on a cellulose column using solvent systems S_4 and S_5. The last yielded flavonoid compounds C, D, E, F and G. The crude compounds were further purified by CC on Sephadex LH – 20 using S_6.

Identification

Chromatography, solvent systems:

PC, Whatman No. 1 or 3: S_1: iso–PrOH–HCOOH–H_2O (2:5:5); S_2: n–BuOH–HOAc–H_2O (3:1:1); S_3: HOAc–H_2O (15:85)

The spots of flavonoids were detected under $UV_{365\ nm}$ before and after spraying with 1% KOH.

CC, Cellulose Whatman CF11: S_4: EtOAc–MeOH–H_2O (100:5:5); S_5: H_2O–MeOH–EtOAc (100:14:10)

CC, Sephadex LH–20 (Pharmacia, Uppsala): S_6–MeOH

TLC, Silica gel (Merck): S_7: n–PrOH–EtOAc–H_2O (7:2:1)

Sugars were visualized by spraying with aniline phthalate and heating at 105°C.

Acid hydrolysis: 1 mg of the compounds, 1% HCl, 105°C, 1 h (total); 0.5% HCl, 100°C, 1/2 h (partial); the hydrolysis was monitored by PC in S_1.

Enzymatic hydrolysis: with β–glucosidase (Koch–Light): 2 mg of the compounds, 1 mg of the enzyme (β–glucosidase) in 1 ml of H_2O at room temp., monitored by PC in S_1.

The EtOAc extracts of the hydrolysates were analyzed for aglycones (co–PC, S_2, S_3) whereas the water residues for sugars (co–TLC, S_7).

Spectral analysis: UV spectra were performed according to the method by Mabry et al. (7), on a Specord UV–VIS spectrometer. ^1H (300 MHz) and ^{13}C (75.5 MHz) NMR spectra were recorded on a Varian Unity – 300 instruments in DMSO – d_6, and in pyridine – d_5 for compound D with TMS as internal standard.

Luteolin [A]
m.p. 330–333°C (2 mg)
PC R_f S_1=0.15; S_2=0.77; S_3=0.08

$UV_{\lambda max}$: MeOH 240, 257, 268, (290), 356; + NaOAc 270, (323), 380; + NaOAc/H_3BO_3 263, (300), 378, 428; + NaOMe 262, (322), 410; + $AlCl_3$ 275, (300), 320, 433; + $AlCl_3$/HCl 266, (278), 291, (365), 380 nm.

Chrysoeriol [B]
m.p. 324–325°C (2 mg)
PC R_f S_1=0.18; S_2=0.80; S_3=0.04

$UV_{\lambda max}$: MeOH 241, (248), 267, 340; + NaOAc 270, (320), 394; + NaOAc/H_3BO_3 267, 345; + NaOMe 261, (273), 328, 403; + $AlCl_3$ 262, 271, 294, (360), 388; + $AlCl_3$/HCl 254, 273, 290, 351, 382 nm.

Luteolin 7–O–β–glucopyranoside [C]
m.p. 260–262°C (6 mg)
PC R_f S_1=0.35; S_2=0.44; S_3=0.15

$UV_{\lambda max}$: MeOH 255, (265), 349; + NaOAc 257, (265), (364), 400; + NaOAc/H_3BO_3 257, 370; + NaOMe 262, (300), 395; + $AlCl_3$ 272, (295), 326, 430; + $AlCl_3$/HCl 271, (291), 360, 388 nm.

^1H NMR DMSO – d_6 δ ppm: 7.41 (1H, d, J=2.0 Hz, H–2'); 7.43 (1H, dd, J=2.0; 8.1 Hz, H–6'); 6.82 (1H, d, J=8.1 Hz, H–5'); 6.78 (1H, d, J=2.0 Hz, H–8); 6.75 (1H, s, J=2.0 Hz, H–3); 6.44 (1H, d, J=2.1 Hz, H–6); 5.09 (1H, d, J=8.0 Hz, H–1'' of glucose); 3.71–3.10 (m, the remaining protons of glucose).

Acid (total) and enzymatic hydrolysis: luteolin and glucose.

Chrysoeriol 7–O–β–glucopyranoside [D]
m.p. 188–192°C (6 mg)
PC R_f S_1=0.41; S_2=0.56; S_3=0.19

$UV_{\lambda max}$: MeOH 250, 270, 348; + NaOAc 252, 270, 351; NaOAc/H_3BO_3 252, 271, 350; + NaOMe 262, 410; +$AlCl_3$ 278, (299), (365), 390; + $AlCl_3$/HCl 277, (297), (350), 390 nm.

^1H NMR Pyridine – d_5 δ ppm; 7.62 (2H, dd, J=2.0; 9.0 Hz, H–2', H–6'); 6.96 (1H, d, J=8.0 Hz, H–5'); 6.87 (1H, d, J=2.0 Hz, H–8); 6.97 (1H, s, H–3); 6.47 (1H, d, J=2.1 Hz, H–6); 5.82 (1H, d, J=8.0 Hz, H–1'' of glucose); 3.85 (3H, s, J=2.0 Hz, CH_3 of C–3'); 3.70–3.10 (m, the remaining protons of glucose).

Acid (total) and enzymatic hydrolysis: chrysoeriol and glucose.

Apigenin 7–O–β–glucopyranoside [E]
m.p. 180–182°C (8 mg)
PC R_f S_1=0.52; S_2=0.60; S_3=0.21

$UV_{\lambda max}$: MeOH 268, 331; + NaOAc 254, 265, 355, 385; NaOAc/H_3BO_3 265, 338; + NaOMe 244, 267, (300), 384; +$AlCl_3$ 274, 300, 345, 384; + $AlCl_3$/HCl 275, 295, 340, 380 nm.

^1H NMR DMSO – d_6 δ ppm: 7,98 (2H, d, J=8.8 Hz, H–2', H–6'); 6.98 (2H, d, J=8.8 Hz, H–3', H–5'); 6,82 (1H, d, J=2.0 Hz, H–8); 6.85 (1H, s, H–3), 6.46 (1H, d, J=2.1 Hz, H–6); 5.06 (1H, d, J=7.1 Hz, H–1'' of glucose); 3.64–3.28 (m, the remaining protons of glucose).

Acid (total) and enzymatic hydrolysis: apigenin and glucose.

Quercetin 3–O–β–glucopyranoside [F]
m.p. 217–220°C (10 mg)
PC R_f S_1=0.62; S_2=0.49; S_3=0.38

$UV_{\lambda max}$: MeOH 53, 267, (286), 350; + NaOAc 276, 324, 388; + NaOAc/H_3BO_3 266, 297, 370; + NaOMe 25, 326, 406; + $AlCl_3$ 278, 300, 438; + $AlCl_3$/HCl 274, 296, 360, 401 nm.

^1H NMR DMSO – d_6 δ ppm: 7.55 (2H, dd, J=1.9; 8.5 Hz, H–2', H–6'); 6.90 (1H, d, J=8.5, H–5'); 6.42 (1H, d, J=2.0 Hz, H–8); 6.20 (1H, d, J=2.1 Hz, H–6); 5.33 (1H, d, J=7.5 Hz, H–1'' of glucose); 3.70–3.10 (m, the remaining protons of glucose).

Acid (total) and enzymatic hydrolysis: quercetin and glucose.

Quercetin 3–O–α–rhamnopyranosyl (1→6)–β–glucopyranoside [G]
m.p. 187–191°C (15 mg)
PC R_f S_1=0.69; S_2=0.44; S_3=0.49

$UV_{\lambda max}$: MeOH 260, 268, 362; + NaOAc 275, 384; + NaOAc/H_3BO_3 253, 384; + NaOMe 275, 418; + $AlCl_3$ 275, (310), 440; + $AlCl_3$/HCl 272, (310), 372, 410 nm.

^1H NMR DMSO – d_6 δ ppm: 7.56 (2H, dd, J=2.2; 10.0 Hz, H–2', H–6'); 6.86 (1H, d, J=8.5, H–5'); 6.41 (1H, d, J=1.9 Hz, H–8); 6.22 (1H, d,

J=2.0 Hz, H–6); 5.34 (1H, d, J=7.6 Hz, H–1'' of glucose); 4.39 (1H, d, J=1.2 Hz, H–1''' rhamnose); 1.00 (3H, d, J=6.2 Hz, CH_3 of rhamnose); 3.38–3.05 (m, the remaining protons of glucose and rhamnose).

^{13}C NMR δ ppm: 177.2 (C–4); 163.9 (C–7); 161.1 (C–5); 156.4 (C–2); 156.2 (C–9); 148.2 (C–4'); 144.6 (C–3'); 133.1 (C–3); 121.4 (C–1'); 121.0 (C–6'); 116 (C–5'); 115.1 (C–2'); 103.8 (C–10); 98.5 (C–6); 93.4 (C–8); 100.6 (C–1''); 101.0 (C–1'''); 76.3 (C–5''); 75.7 (C–3''); 73.9 (C–2''); 71.7 (C–4'''); 70.4 (C–4''); 70.2 (C–2'''); 69.8 (C–3'''); 68.1 (C–5'''); 66.8 (C–6''); 17.6 (C–6''').

Acid total hydrolysis: quercetin, glucose and rhamnose.

Acid partial hydrolysis: 3–O–glucoside as a secondary heteroside.

RESULTS AND DISCUSSION

The concentrated methanol extract prepared from the air–dried flowers of *Abutilon indicum* was extracted successively with ethyl ether and ethyl acetate. Two flavon aglycones (A and B) were isolated from the ethereal fraction by preparative paper chromatography and characterized as luteolin and chrysoeriol by a direct comparison with the authentic samples as well as by the spectral analysis in UV (6). Three flavone glycosides (C, D, E) and two flavonol glycosides (F and G) were obtained from the ethyl acetate fraction by column chromatography.

Compounds C, D, E, F were identified by acid and enzymatic hydrolysis, and their structures were confirmed by the spectral analysis (UV, ^1H NMR). They turned to be luteolin 7–*O*–β–glucopyranoside, chrysoeriol 7–*O*–β–glucopyranoside, apigenin 7–*O*–β–glucopyranoside and quercetin 3–*O*–β–glucopyranoside, respectively.

Acid hydrolysis of G gave quercetin, glucose and rhamnose. In the ^1H NMR spectra in DMSO – d_6, the signals for aglycone were the same as for 3–*O*–substituted quercetin. Moreover, two signals were observed in the region characteristic of anomeric sugar protons. A doublet at δ 5.33 ppm (J=7.5 Hz) was assigned to β–glucopyranose linked to quercetin at C–3, while a signal at δ 4.39 ppm (J=1.2 Hz) corresponded to the anomeric proton of a terminal α–rhamnopyranose (7). The linkage of rhamnose to C–6'' of the glucose moiety was determined on the basis of the ^{13}C NMR spectra. A direct comparison of the ^{13}C NMR spectrum of G with the spectrum of quercetin 3–*O*–glucoside revealed an upfield shift of δ 4.5 ppm for C–6'' of glucose and a downfield shift of δ 1.2 ppm for C–5'' of glucose, resulting from the presence of the rhamnose moiety. These shifts correspond to those reported for a quercetin rhamnopyranosyl (1→6) glucopyranoside (8), thus confirm the sugar linkage in compound G.

Flavonol derivatives of kaempferol and quercetin predominate in the species from the *Malvaceae* family (9). Flavones are only represented by the apigenin glycosides in the flowers of *Althaea rosea* L. (10), hypolaetin derivatives detected in *Althaea officinalis* L. (11) and *Malva silvestris* L. (12, 13) as well as apigenin, luteoline and chrysoeriol glucosides in *Kitaibelia vitifolia* Willd. (14, 15). In our work we did not confirm the presence of gossypetin derivatives in *Abutilon indicum*.

REFERENCES

1. Bagi M.K., Kalyani G.A., Deenis T.J., Akshaya K.K., Kakrani H.K.: Fitoterapia 3, 168 (1985).
2. Mehta B.K., Neogi R., Kotra S., Mall O.P.: Fitoterapia 48, 273 (1997).
3. Parchezhian E., Ansari S.H.: Pharmazie 55, 9 (2000).
4. Sharma P.V., Ahmad Z.A.: Phytochemistry 12, 3525 (1989).
5. Subramanian S.S., Nair A.G.R.: Phytochemistry 11, 1518 (1972).
6. Mabry T.J., Markham K.R., Thomas M.B.: The Systematic Identification of Flavonoids. Springer Verlag, Berlin, Haidelberg, New York 1970.
7. Harborne J.B.: The Flavonoids Advances in Research Since 1986. Chapman and Hall, London 1994.
8. Markham K.R., Ternai B., Stanley R., Geiger H., Mabry T.J.: Tetrahedron 34, 1389 (1978).
9. Hegnauer R.: Chemotaksonomie der Pflanzen. Birkhäuser Verlag Basel. Stuttgart 1973.
10. Gudej J.: Acta Polon. Pharm. 42, 192 (1985).
11. Gudej J.: Acta Polon. Pharm. 44, 369 (1987).
12. Nawwar M.A.M., Dein A., Sherbeiny A., Ansari M.A., Sissi H.I.: Phytochemistry 16, 145 (1977).
13. Billeter M., Meier B., Sticher O.: Phytochemistry 30, 987 (1991).
14. Matławska I.: Herba Polonica 36, 71 (1990).
15. Matławska I.: Acta Polon. Pharm. 58, 127 (2001).

Received: 23.01.2002