Pergamon

0031-9422(95)00069-0

Phytochemistry, Vol. 39, No. 3, pp. 727–729, 1995

0031-9422/95 $9.50 + 0.00

FLAVONE *C*-GLYCOSIDES FROM *BRYONIA ALBA* AND *B. DIOICA*

MIROSŁAWA KRAUZE-BARANOWSKA and WOJCIECH CISOWSKI*

Department of Pharmacognosy, Medical University, ul. Gen. J. Hallera 107, 80-416 Gdańsk, Poland

(*Received in revised form* 18 *July* 1994)

Key Word Index—*Bryonia alba*; *Bryonia dioica*; Cucurbitaceae; flavone *C*-glycosides; apigenin 6-*C*-glucoside-7-*O*-(6″-caffeoyl)glucoside).

Abstract—Lutonarin was isolated from *Bryonia alba* and *B. dioica*. Five further *C*-glycosides: vitexin, isovitexin, isoorientin, saponarin were isolated from *B. dioica* together with saponarin caffeic ester, a new natural product.

INTRODUCTION

The roots of *Bryonia alba* L. and *Bryonia dioica* Jacq. are well known in homeopathy for their antirheumatic, antiphlogistic and expectorant activity [1, 2]. As yet, the flavonoid constituents of these species have not been completely determined [3–6]. Earlier phytochemical investigations of *B. dioica* revealed the presence of saponarin and vicenin-2 [5] and we have previously reported three flavones from *B. alba*: saponarin, isovitexin and vitexin [6]. In continuation of our studies on the genus *Bryonia* we have now isolated lutonarin (**5**) from *B. alba* and *B. dioica*, and five other flavone *C*-glycosides (**1–4**, **6**) from *B. dioica*, including one new compound, saponarin caffeic ester (**6**).

RESULTS AND DISCUSSION

The flavonoids were isolated from methanolic extracts of the aerial parts of *B. alba* and *B. dioica* by means of preparative CC on polyamide, cellulose, and Sephadex LH-20 (**1–3**, **6**) or preparative TLC (**5**). Compounds were identified using co-chromatography with authentic samples (PC, TLC, HPLC), acid hydrolysis, alkaline hydrolysis and spectroscopic methods: UV, IR, ^{1}H NMR, ^{13}C NMR, 2D NMR (^{1}H–^{13}C COSY), LSI-MS, FD-MS. The structural investigations of **1–4** (vitexin, isovitexin, isoorientin, saponarin) from *B. dioica* gave results identical with those reported by us in the literature [6, 7].

Acid hydrolysis of **5** gave two *C*-glycosylflavones: isoorientin and orientin (Wessely–Moser isomerization) [8, 9] together with glucose (co-PC). The UV spectrum of **5** gave diagnostic shifts characteristic of a luteolin derivative [8–10] and the FD-MS spectrum indicated a molecular ion at m/z 610 $[M]^+$. The ^{1}H NMR spectrum of **5** exhibited two doublets of anomeric protons: at δ5.00 (J = 6.82 Hz) corresponding to H-1″ of glucose bound as an *O*-glycoside and at δ4.66 (J = 9.8 Hz) corresponding to H-1‴ of a carbon bound glucose [8, 9]. In the ^{13}C NMR a signal at δ110.5 was assigned to the C-6 position substituted with a sugar residue [9]. On the basis of this data **5** is identified as luteolin 6-*C*-glucoside-7-*O*-glucoside (lutonarin). This is the first report of lutonarin from either *B. alba* or *B. dioica*.

Alkaline hydrolysis of **6** gave caffeic acid and saponarin (apigenin 6-*C*-glucoside-7-*O*-glucoside) (co-PC with standards). Acid hydrolysis of the alkaline hydrolysis products afforded glucose and two *C*-glycosyflavones: isovitexin and vitexin (Wessely–Moser isomerization). The IR spectrum of **6** showed absorptions for a γ-pyrone carbonyl group (1655 cm^{-1}) and for an ester function (1695 cm^{-1}) [9, 11, 12]. The UV spectrum of **6** with diagnostic shift reagents revealed the presence of free hydroxyl groups at C-4′, C-5 and the presence of a substituted hydroxyl in position C-7 [8, 9]. The LSI-MS of **6** exhibited a molecular ion peak at m/z 756 $[M - H]^-$ in accordance with the structure of a caffeoyl ester of an apigenin diglucoside. The fragment ions at m/z 593 and 431 indicated a successive loss of caffeoyl and glucosyl moieties. In addition the fragment ion at m/z 324 suggested that caffeic acid was attached to the *O*-glucose at position C-7. The ^{1}H NMR spectrum exhibited two doublets at δ6.3 and 7.48 with large coupling constants (15.9 Hz) which assigned a *trans* configuration to the caffeic acid [13–15]. The anomeric proton (H-1″) of the *O*-glucose appeared as a doublet at δ5.01 (J = 7.1 Hz). The signal at δ4.66 (J = 9.8 Hz) was assigned to the H-1‴ of the glucose, confirming the linkage between the sugar residue and the aglycone as *C*-glycosidic in character. In the ^{13}C NMR spectrum a signal due to C-6″ of the *O*-glucose at C-7 shifted downfield by 2.3 ppm while C-5″ had shifted upfield by 4.6 ppm in comparison with the corresponding signals for saponarin (^{1}H–^{13}C COSY) [6]. These shifts are expected from the substituent effect of C-6″ glucose acylation [15, 16]. These results show that the caffeoyl moiety is connected to a hydroxyl group at C-6″ of the *O*-glycosidic bound glucose. From these data

*Author to whom correspondence should be addressed.

6 is identified as apigenin 6-*C*-β-D-glucoside-7-*O*-(6″-caffeoyl-β-D-glucopyranoside). To our knowledge, this compound has never been described in the plant kingdom.

EXPERIMENTAL

Plant material. Flowering plants of *Bryonia alba* L. and *B. dioica* Jacq. were collected from the Medicinal Plants Garden of Medical University in Gdańsk (Poland). Voucher specimen of these plants have been deposited in our laboratory.

Extraction and isolation. The fresh, undried aerial parts of *B. alba* (2 kg) were extracted (× 3) with MeOH. The combined concd extracts were cooled in the refrigerator to give a ppt. of **4** and **5**, which were subjected to prep. TLC on cellulose using BAW (*n*-BuOH–HOAc–H_2O, 4:1:5) as eluent in a 'sandwich' chamber modificated by Rumiński [17]. After crystallization, **5** (30 mg) was obtained in pure form. Compound **4** was isolated and identified as described in our previous paper [6].

Dried and pulverized herb of *B. dioica* (400 g) was defatted with petrol followed by $CHCl_3$ in a Soxhlet. Flavonoids were extracted (× 3) with MeOH. The combined MeOH extracts were concd, suspended in H_2O and successively extracted with Et_2O–EtOAc mixts with increasing proportions of EtOAc. During extraction **4** and **5** were pptd and sepd as described for *B. alba* above. After crystallization **4** (3.5 g) and **5** (35 mg) were obtained in pure form. The Et_2O–EtOAc extracts were combined and after concn fractionated over a polyamide column by successive elution with 30% MeOH, 60% MeOH, 80% MeOH. Frs were rechromatographed on a cellulose column with 30% MeOH—**1** (4 mg), **3** (35 mg), H_2O—**2** (30 mg) and EtOAc–MeOH–M_2CO–H_2O (100:15:5:10) **6** (60 mg) which was purified by Sephadex LH-20 eluted with MeOH.

Identification of flavonoids. UV data were recorded according to standard procedure [8], ^1H and ^{13}C NMR spectra were recorded on a Bruker MSL 300 instrument at 300 MHz and 75.5 MHz respectively. FD-MS ((8 + 3) KeV, J_E = 20 mA) was recorded on Varian MAT 711. Negative LSI-MS was obtained with an AMD-604 Intectra spectrometer: caesium ions with energy 6 keV, thioglycerol as a matrix. HPLC analysis was carried out as reported previously [18]. Sugar analyses were carried out after acid hydrolysis [9] by PC using *n*-BuOH–pyridine–H_2O (3:3:1) and (6:4:7) and aniline phthalate spray reagent. Caffeic acid was identified after alkaline hydrolysis (4%KOH, 4 hr) on PC with HCOONa–85% HCOOH–H_2O (10:1:200) by spraying diazosulphanilic acid soln and 25% NH_3.

Luteolin 6-C-glucoside-7-O-glucoside (**5**). Mp 239–242° (aq. MeOH). UV λ_{max}^{MeOH} nm: + $AlCl_3$ 258, 272, 350; 277, 300sh, 340, 426; + $AlCl_3$/HCl 246sh, 280, 295sh, 354, 387); + NaOMe; + NaOAc 268, 305sh, 402, 268, 402; + H_3BO_3 266, 392. FD-MS *m/z* (rel. int.): 610 $[M]^+$ (100), 611 $[M + H]^+$ (68). ^1H NMR (DMSO-d_6): δ13.58 (1H, *s*, C-5–OH), 7.47 (2H, *dd*, *J* = 2.8 Hz/8.5 Hz, H-2′,6′), 6.92 (1H, *d*, *J* = 8.5 Hz, H-5′), 6.83 (1H, *s*, H-8), 6.70 (1H, *s*, H-3), 5.00 (1H, *d*, *J* = 6.8 Hz, H-1″ *O*-glucose), 4.66 (1H, *d*, *J* = 9.8 Hz, H-1‴ C-glucose). ^{13}C NMR (DMSO-d_6) δ: 181.8 (C-4), 164.2 (C-2), 162.3 (C-7), 159.2 (C-5), 156.3 (C-9), 149.9 (C-4′), 145.7 (C-3′), 121.1 (C-1′), 119.1 (C-6′), 115.9 (C-5′), 113.4 (C-2′), 110.5 (C-6), 104.8 (C-10), 103.1 (C-3), 101.1 (C-1″), 93.5 (C-8), 80.9 (C-5″), 78.9 (C-3‴), 77.2 (C-5″), 75.6 (C-3″), 73.8 (C-1‴), 73.0 (C-2″), 72.6 (C-2‴), 70.8 (C-4‴), 69.5 (C-4″), 60.7 (C-6‴), 60.3 (C-6″).

Apigenin 6-C-glucoside-7-O-(6″-caffeoyl-β-D-glucoside) (**6**). Mp 251–254° (aq. MeOH). IR cm^{-1}: 3300 (OH), 1695 (C=O ester), 1655 (C=O γ-pyrone), 1610 (–CH=CH aromatic), 1580, 1515, 900–670. UV λ_{max}^{MeOH} nm: + $AlCl_3$ 272, 334; 281, 302, 341, 375; + $AlCl_3$/HCl 281, 301, 339, 375; + NaOMe 275, 313sh, 392; + NaOAc 276, 315sh, 342, 401sh; + H_3BO_3 274, 348. Negative ion LSI-MS *m/z* (rel. int.): 755 $[M - H]^-$ (80), 593 $[M - H -$ caffeoyl] (42), 431 $[M - H -$ caffeoyl − glucosyl] (85), 324 [caffeoyl + glucosyl] (5). ^1H NMR (DMSO-d_6) δ: 7.3 (2H, *d*, *J* = 8.7 Hz, H-2′6′), 7.48 (1H, *d*, *J* = 15.9 Hz, H-7 caff), 6.91–6.78 (6H, *m*, H-3′5′, 3, 8, H-2.5 caff), 6.55 (1H, *d*, *J* = 7.9 Hz, H-6 caff), 6.30 (1H, *d*, *J* = 15.9 Hz, H-8 caff), 5.01 (1H, *d*, *J* = 7.1 Hz, H-1″ *O*-glucose), 4.66 (1H, *d*, *J* = 9.8 Hz, H-1‴ *C*-glucose). ^{13}C NMR (DMSO-d_6): δ181.8 (C-4), 164.0 (C-2), 162.0 (C-7), 161.2 (C-4′), 159.2 (C-5), 156.2 (C-9), 128.3 (C-2′, 6′), 120.6 (C-1′), 115.8 (C-3′, 5′), 110.4 (C-6), 104.8 (C-3), 103.0 (C-3), 93.4 (C-8), 101.2 (C-1″), 80.7 (C-5‴), 78.7 (C-3‴), 75.4 (C-3″), 73.6 (C-2″, 5″), 72.5 (C-1‴), 70.7 (C-2‴), 69.6 (C-4‴), 69.3 (C-4″), 63.3 (C-6″), 61.0 (C-6‴), caffeoyl moiety 166.3 (C-9), 148.1 (C-3), 145.3 (C-7), 145.2 (C-4), 125.0 (C-1), 120.5 (C-6), 115.4 (C-5), 115.1 (C-2), 113.3 (C-8).

REFERENCES

1. Charrette, G. (1987) in *Homeopatische Arzneimittellehre fur die Praxis*, p. 115. Hippokrates, Stuttgart.
2. Zepernick, B., Langhammer, L. and Lüdcke, J. B. P. (1984) in *Lexikon der Offizinallen Arzneipflanzen*, p. 87. Gruyter, Berlin, New York.
3. Imperato, F. (1980) *Experientia* **36**, 1136.
4. Leiba, S. Delavean, P. and Paris, R. R. (1968) *Plant Med. Phytother.* **2**, 81.
5. Proliac, A., Chaboud, A. and Raynaud, J. (1989) *Pharm. Acta Helv.* **64**, 207.
6. Krauze, M. and Cisowski, W. (1992) *Pol. J. Chem.* **66**, 951.
7. Krauze-Baranowska, M. and Cisowski, W. (1995) *Acta Pol. Pharm.* in press.
8. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids.* Springer, Berlin.
9. Markham, K. R. (1982) *Techniques of Flavonoid Identification.* Academic Press, London.
10. Sherwood, R. T., Shamma, M., Moniol, J. L. and Kroschewsky, J. R. (1973) *Phytochemistry* **12**, 2775.

11. Cisowski, W. and Lamer-Zarawska, E. (1976) *Herba Pol.* **22**, 185.
12. Dembińska-Migas, W. (1988) *Herba Pol.* **32**, 191.
13. Clifford, M. N., Kellard, B. and Ah-sing, E. (1989) *Phytochemistry* **28**, 943.
14. Otsuka, H., Kubo, N., Yamasaki, K. and Padolina, W. G. (1992) *Phytochemistry* **31**, 943.
15. Hasler, A., Gross, G. A. Meier, B. and Sticher, D. (1992) *Phytochemistry* **31**, 1931.
16. Nasr, C., Haag-Berrurier, M., Lobstein-Guth, A. and Anton, R. (1986) *Phytochemistry* **25**, 770.
17. Rumiński, J. K. (1988) *Chem. Analit.* **33**, 479.
18. Krauze-Baranowska, M. and Cisowski, W. (1994) *J. Chromatogr.* **A675**, 240.