Pergamon

PII: S0031-9422(98)00048-X

Phytochemistry, Vol. 48, No. 3, pp. 573–575, 1998

Printed in Great Britain
0031–9422/98 $19.00+0.00

FLAVONOIDS FROM *PHLOMIS NISSOLII*

FRANZ BUCAR,* STEFAN NINOV,† ILIANA IONKOVA,† THEODOR KARTNIG, MANFRED SCHUBERT-ZSILAVECZ,‡ IVAN ASENOV† and BELMA KONUKLUGIL§

Institute of Pharmacognosy, University of Graz, Universitätsplatz 4/I, A-8010 Graz, Austria; † Department of Pharmacognosy, Faculty of Pharmacy, University of Sofia, Dunav Str. 2, Sofia 1000, Bulgaria; ‡ Institute of Pharmaceutical Chemistry, University of Frankfurt am Main, Marie-Curie-Str. 9, D-60439 Frankfurt am Main, Germany; § Department of Pharmacognosy, Faculty of Pharmacy, University of Ankara, 06100 Tandogan, Ankara, Turkey

(*Received* 27 *October* 1997)

Key Word Index—*Phlomis nissolii*; Lamiaceae; luteolin 7-*O*-(6″-*O*-β-D-apiofuranosyl)-β-D-glucopyranoside; chrysoeriol 7-*O*(6″-*O*-β-D-apiofuranosyl)-β-D-glucopyranoside; structural elucidation.

Abstract—Two new flavone glycosides, luteolin and chrysoeriol 7-(6″-β-D-apiofuranosyl)-β-D-glucopyranosides have been isolated from the aerial parts of *Phlomis nissolii*. The known compounds apigenin, eriodictyol, luteolin as well as their 7-glucosides and luteolin 7-rutinoside were also identified in this plant.

INTRODUCTION

Several *Phlomis* species are used in herbal medicine, e.g. for diseases of the respiratory tract or externally for treatment of wounds [1–3]. Some *Phlomis* species have been studied for their flavonoid constituents [4–6] but *P. nissolii* L., an endemic plant of Turkey, has not been investigated previously. Preliminary TLC investigations revealed that flavonoids are major components in this plant and so a more detailed study was carried out. In this paper we report on the isolation of two new flavone glycosides (**1** and **2**) as well as seven known flavone and flavanone aglycones and glycosides from the aerial parts of *P. nissolii*.

RESULTS AND DISCUSSION

Compounds **1** and **2** were obtained as yellow powders from an 80% methanolic aerial parts extract of *P. nissolii*. Using common shift reagents [7] UV spectral data suggested that **1** was a flavone with a substituted hydroxyl group at C-7 and adjacent hydroxyl groups in ring B. The 2H AX and 3H ABX system in the ^{1}H NMR spectra of **1** confirmed the 3′,4′-substitution pattern of ring B. Two anomeric protons at 5.02 ppm (*d*, J = 6.9 Hz) and 4.97 ppm (*d*, J = 2.4 Hz) indicated the presence of two sugar moieties which, according to UV and ^{1}H NMR spectral data, were attached to the hydroxyl group at C-7. The inner saccharide unit could be identified by ^{1}H and ^{13}C NMR data as β-D-glucopyranose [8, 9]. The ^{13}C and ^{1}H signals of the second monosaccharide moiety were in accordance with literature values for a β-D-apiofuranosyl residue [10–12]. ^{13}C NMR data (Table 1) showed the signal for C-6″ (glucose) at 68.7 ppm which indicated the attachment of the apiose to C-6 of the glucosyl residue. In addition, for comparative purposes NMR spectra of apiin (apigenin 7-*O*-(2″-*O*-β-D-apiofuranosyl)-β-D-glucopyranoside) were recorded. Apiin showed a ^{13}C NMR signal for C-6″ at 62.5 ppm. The ^{1}H NMR data of apiin indicated a downfield shift of H-2″ signal (3.65 ppm) of 0.17 ppm compared to H-2″ of **1** (3.48 ppm), but on the other hand H-6″$_A$ of **1** (4.05 ppm) was shifted downfield by 0.11 ppm compared to apiin (3.92 ppm) indicating the attachment of the apiose to the terminal hydroxyl group of the glucosyl moiety. Signal assignment was done by ^{1}H^{1}H COSY and HMQC spectra. Cross peaks of a HMBC experiment (optimized for J = 7 Hz) confirmed the interglycosidic linkage of **1**. Hence **1** was identified as luteolin 7-*O*-(6″-*O*-β-D-apiofuranosyl)-β-D-glucopyranoside. Compound **2** showed similar spectral data to **1** except in the UV spectra, which showed the absence of two free adjacent hydroxyl groups in ring B but indicated a hydroxyl at C-4′. Two λ_{max} at 269 nm and 251 nm in band 2 were indicative of two substitutents in ring B. ^{1}H NMR spectra showed in

*Author to whom correspondence should be addressed. Fax: +43-316-380 9860; e-mail franz.bucar@kfunigraz.ac.at

Table 1. ^{13}C NMR (CD_3OD) spectra of compounds **1** and **2**

C	1	2
2	166.9	167.1
3	104.2	104.0
4	184.1	184.0
5	163.0	163.0
6	101.1	101.0
7	164.7	164.7
8	96.2	96.2
9	158.9	158.9
10	107.1	107.3
1′	123.5	[a]
2′	114.2	110.6
3′	147.1	155.1
4′	151.3	150.3
5′	116.8	117.3
6′	120.6	122.3
1″	101.6	101.5
2″	74.7	74.7
3″	77.8	77.8
4″	71.5	71.5
5″	77.2	77.2
6″	68.7	68.7
1‴	111.0	111.0
2‴	78.2	78.2
3‴	80.5	80.5
4‴	75.1	75.1
5‴	65.8	65.8
3′-OCH_3	—	56.6

[a] Not detected.

comparison with **1** an additional singlet for 3H at 3.94 ppm for a OCH_3 group at C-3′. Compound **2** therefore was characterized as chrysoeriol 7-*O*-(6″-*O*-β-D-apiofuranosyl)-β-D-glucopyranoside. Both compounds were hydrolysed with β-glucuronidase giving luteolin and chrysoeriol, respectively, as well as glucose and apiose (the latter only in small amounts) which were identified by GC-MS as their TMSi derivatives [13]. The finding of the 6″-apiofuranosyl glucosides of luteolin and chrysoeriol is new since in neither the luteolin 7-apiosylglucoside nor the chrysoeriol 7-apiosylglucoside previously reported from *Apium graveolens* [14], *Petroselinum sativum* [15] and *Luffa echinata* [16] were the interglycosidic linkages determined. However, chrysoeriol 7-(2″-β-D-apiofuranosyl)-β-D-glucopyranoside has been characterized from *Dahlbergia volubilis* [17].

Using TLC and HPLC co-chromatography with authentic markers **3** was identified as apigenin, **4** as eriodictyol and **5** as luteolin. Compounds **6**, **7** and **8** were additionally hydrolysed by 2 N HCl followed by GC-MS of the TMSi-saccharides as well as TLC co-chromatography of the aglycones and sugars. Hence, **6**, **7** and **8** were identified as the 7-*O*-β-D-glucosides of apigenin (**6**), eriodictyol (**7**) and luteolin (**8**). Compound **9** proved to be luteolin 7-*O*-rutinoside by comparison of ^1H and ^{13}C NMR data with literature values [8, 9]. The occurrence of a chrysoeriol glycoside in this Mediterranean species is in accordance with previous investigations [4].

EXPERIMENTAL

Plant material

Phlomis nissolii was collected at the flowering stage in Turkey at Icel (Mt Gulnar, 900 m) and was identified by Professor M. Koyuncu (Department of Pharmaceutical Botany, University of Ankara). A voucher specimen (AEF 18711) has been deposited at the Herbarium of the University of Ankara.

Extraction and isolation

Dried aerial parts (740 g) were extracted with MeOH (80%) in a steam bath. MeOH was evaporated and the aq. residue extracted successively with Et_2O (3 l), $CHCl_3$ (5.5 l), EtOAc (4.5 l) and *n*-BuOH (7 l). The Et_2O fr. was chromatographed on Sephadex LH20/Me_2CO, Me_2CO–MeOH and MeOH, respectively. 34 frs (50 ml each) were collected. Frs 12–16 yielded **3** (20 mg), frs 17–19 **4** (4 mg). The EtOAc fraction (6.8 g) was separated on Sephadex LH20/MeOH–EtOAc (1:1), 43 frs (30 ml each) were collected. Frs 24–27 contained a mixture of **5**–**8**. Further separation of frs 24–27 was done by HPLC on a LiChrospher 100 RP-18 column (10 μm, 250 × 10 mm, Merck) with THF–H_2O (27:73) at 2.0 ml min^{-1} yielding **5** (5 mg), **6** (4 mg) and a mixture of **7** and **8** which was separated with THF–H_2O (22:78) at 2.0 ml min^{-1} yielding 8 mg **7** and 60 mg **8**. The *n*-BuOH fr. (25.2 g) was dissolved in H_2O and chromatographed on polyamide (Woelm) with a H_2O–MeOH gradient (0–80% MeOH). Frs 124–133 (60% MeOH) contained **1**, **2** and **9**. This mixture was further separated on Sephadex LH20/MeOH–EtOAc (1:1), 25 frs (20 ml each) were collected. Frs 17–18 were separated by semiprep. HPLC on LiChrospher 100 RP-18 (10 μm, 250 × 10 mm) with AcCN–H_2O (23:77) at 2.0 ml min^{-1} to give **2** and **9**. HPLC of frs 19–20 using the same conditions yielded **2** and a mixture of **1** and **9** which was separated on Sephadex LH20/MeOH giving 5 mg of pure **1**, 4 mg of **2** and 3 mg of **9**. TLC monitoring of frs and hydrolysis products was done on precoated silica gel 60 F254 aluminium sheets (Merck) with toluene–HCO_2Et–HCO_2H (5:4:1) (aglycones), EtOAc–MeCOEt–HCO_2H–H_2O (5:3:1:1) (glycosides) and $CHCl_3$–MeOH–H_2O (64:36:8) (monosaccharides). The best separation of **1**, **2** and **9** was achieved on polyamide-6 precoated plastic sheets (Macherey-Nagel) with MeOH–HOAc–H_2O (18:1:1), R_f = 0.53 (**1**), 0.73 (**2**), 0.78 (**9**). Enzymic hydrolysis of **1** and **2** was carried out as in [18] using β-glucuronidase from *Helix pomatia*, except that the hydrolysis solution was left for 12 h at room temp. A reference of apiose was obtained by hydrolysis of apiin. For GC-MS of TMSi-sugars a fused silica capillary with dimethylpolysiloxane (HP-5MS) was used.

^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Plus 400 at 399.98 MHz and 100.57 MHz, respectively.

Luteolin 7-O-(6″-O-β-D-apiofuranosyl)-β-D-glucopyranoside (**1**). Yellow amorphous powder, mp uncorr. 226–230. UV λ_{max} (MeOH) nm: 349, 269 sh, 225; (+NaOMe) 395, 299 sh, 267; (+$AlCl_3$) 420, 330 sh, 293 sh, 270; (+$AlCl_3$/HCl) 390, 362 sh, 293 sh, 270; (+NaOAc) 405, 262; (+NaOAc/H_3BO_3) 374, 259. ^{1}H NMR (CD_3OD) δ: 7.42 (*dd*, J = 8, 2 Hz, H-6′), 7.41 (*d*, J = 2 Hz, H-2′), 6.91 (*d*, J = 8 Hz, H-5′), 6.76 (*d*, J = 2.1 Hz, H-8), 6.60 (*s*, H-3), 6.54 (*d*, J = 2.1 Hz, H-6), 5.02 (*d*, 6.9 Hz, H-1″), 4.96 (*d*, J = 2.4 Hz, H-1‴), 4.05 (*dd*, J = 11, 1.5 Hz, H-6_A), 3.99 (*d*, J = 9.7 Hz, H-$4'''_A$), 3.91 (*d*, J = 2.4 Hz, H-2‴), 3.75 (*d*, J = 9.7 Hz, H-$4'''_B$), 3.68 (*m*, H-5″), 3.61 (*dd*, J = 11, 6.9 Hz, H-$6''_B$), 3.57 (*s*, H-$5'''_{A,B}$), 3.47 (*m*, H-2″, H-3″), 3.36 (*t*, J = 9.1 Hz, H-4″).

Chrysoeriol 7-O-(6″-O-β-D-apiofuranosyl)-β-D-glucopyranoside (**2**). Yellow amorphous powder, mp uncorr. 168–173. UV λ_{max} (MeOH) nm: 345, 269, 251; (+NaOMe) 399, 299 sh, 264, 247 sh; (+$AlCl_3$) 390, 355 sh, 297, 269, 263 sh; (+$AlCl_3$/HCl) 379 sh, 356, 292 sh, 274 sh, 265; (+NaOAc) 408, 350 sh, 275 sh, 259; (+NaOAc/H_3BO_3) 345, 268, 251. ^{1}H NMR (CD_3OD) δ: 7.54 (*dd*, J = 8.5, 2.2 Hz, H-6′), 7.47 (*d*, J = 2.2 Hz, H-2′), 6.90 (*d*, J = 8.5 Hz, H-5′), 6.78 (*d*, J = 2.2 Hz, H-8), 6.65 (*s*, H-3), 6.53 (*d*, J = 2.2 Hz, H-6), 5.04 (*d*, J = 7.6 Hz, H-1″), 4.96 (*d*, J = 2.5 Hz, H-1‴), 4.04 (*dd*, J = 11, 1.7 Hz, H-$6''_A$), 3.99 (*d*, J = 9.7 Hz, H-$4'''_A$), 3.94 (*s*, $OC\underline{H}_3$-3′), 3.88 (*d*, J = 2.5 Hz, H-2‴), 3.74 (*d*, J = 9.7 Hz, H-$4'''_B$), 3.69 (*ddd*, J = 9.5, 6.5, 1.6 Hz, H-5″), 3.62 (*dd*, J = 11, 6.5, H-$6''_B$), 3.55 (*s*, H-$5'''_{A,B}$), 3.47 (*m*, H-2″, H-3″), 3.36 (*t*, J = 9.5 Hz, H-4″).

Acknowledgement—Financial support by the CEEPUS program of the EU is gratefully acknowledged.

REFERENCES

1. Hoppe, H. A., *Drogenkunde*. Walter de Gruyter, Berlin, New York, 1975, p. 825.
2. Akopov, E. I., Styptic plants, 2nd edn, Tashkent, Pub: *Medicine*, UZSSR, 1981, p. 136.
3. Tammaro, F. and Xepapadakis, G., *Journal of Ethnopharmacology*, 1986, **16**, 167.
4. Tomas, F., Nieto, J. L., Barberan, F. A. T. and Ferreres, F., *Phytochemistry*, 1986, **25**, 1253.
5. Kumar, R., Bhan, S., Kalla, A. K. and Dhar, K. L., *Phytochemistry*, 1985, **24**, 1124.
6. El-Negoumy, S. I., Abdalla, M. F. and Saleh, N. A. M., *Phytochemistry*, 1986, **25**, 772.
7. Mabry, T. J., Markham, K. R. and Thomas, M. B., *The Systematic Identification of Flavonoids*. Springer, Berlin, 1970.
8. Markham, K. R. and Geiger, H., In *Flavonoids. Advances in Research since 1986* Ch. 10, (ed. J. B. Harborne). Chapman & Hall, London, 1994.
9. Agrawal, P. D. (ed.), *Carbon-13 NMR of Flavonoids*, Elsevier, Amsterdam, 1989.
10. Snyder, J. R. and Serriani, A. A., *Carbohydrate Research*, 1987, **166**, 85.
11. Gross, G. A., Sticher, O. and Anklin, C., *Helvetica Chimica Acta*, 1987, **70**, 91.
12. Ishii, H., Tori, K., Tozyo, T. and Yoshimura, Y., *Journal of the Chemical Society, Perkin Transactions I*, 1984, 661.
13. Jurenitsch, J., Kopp, B., Gabler-Kolacsek, I. and Kubelka, W., *Journal of Chromatography*, 1981, **210**, 337.
14. Galensa, R. and Herrmann, K., *Zeitschrift fur Lebensmittel-Untersuchung und -Forschung*, 1979, **169**, 170.
15. Eriashvili, V. M. and Chumburidze, V. I., *Soobshcheniya Akademii Nauk Gruzinskoi SSR*, **63**, 101; cit. CA 1971, **75**, 154995, 1971.
16. Seshradi, T. R. and Vydeeswaran, S., *Phytochemistry*, 1971, **10**, 667.
17. Biswass, K. M., Ali, M. E. and Haque, M. E., *Indian Journal of Chemistry, Section B*, 1977, **15b**, 396.
18. Markham, K. R., *Techniques of Flavonoid Identification*. Academic Press, London, 1982.