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FLAVONOIDS FROM *PHLOMIS LYCHNITYS*

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Abstract—The new naturally occurring acylated flavone glucoside chrysoeriol-7-β-D-(3″-*E*-*p*-coumaroyl)glucoside and other flavonoids have been isolated from the aerial parts of *Phlomis lychnitys*, and identified by spectral techniques. Relationships between flavonoid pattern, phylogeny and geography in *Phlomis* are discussed.

The flavonoids from several *Phlomis* species have been studied previously. Thus, *Phlomis tuberosa* [1–4], *P. agraria* [1], *P. spectabilis* [5], *P. aurea* [6] and *P. floccosa* [6] which grow in different countries and continents have been analysed so far.

In the present work, the flavonoids of *P. lychnitys*, a small perennial plant which grows in the Mediterranean area of Spain, have been studied, and luteolin, apigenin and chrysoeriol and their 7-*O*-glucosides and 7-*p*-coumaroylglucosides, have been isolated and identified. The structure of the new naturally occurring chrysoeriol 7-β-D-(3″-*E*-*p*-coumaroyl)glucoside has been completely characterized. This new glycoside showed a UV spectrum in methanol characterized by a principal maximum at 318 nm that overlapped the two typical bands in the UV spectra of flavones (band I and band II), band I appearing as an inflection at 345 nm and band II as a maximum at 270 nm. These UV values were similar to those reported for acylated derivatives of flavonoids in which the acyl group was *p*-coumaric acid [5–9]. The IR spectrum was characterized by a strong carbonyl ester band at 1730 cm^{-1}, in addition to another carbonyl band (flavone) at 1653 cm^{-1}. Acidic hydrolysis of the original glycoside yielded chrysoeriol, glucose and *E*-*p*-hydroxycinnamic acid, identified by TLC comparisons with authentic samples. The EIMS of the permethylated glycoside was in accordance with an acylated flavone monoglucoside. Upon alkaline hydrolysis, *p*-coumarate and chrysoeriol-7-β-D-glucoside were obtained. The structure of the monoglucoside was proved by classical UV and EIMS techniques [10, 11]. The complete structure of the naturally occurring glycoside was clear from the ^1H NMR spectrum. The olefinic and aromatic resonances of the acid are split into two sets of signals that correspond to *cis* and *trans* isomers around the double bond. Only data from the *trans* major component, identified by the large olefinic *J* value, are reported here. One of the sugar ring proton signals (H-3″) appears as a triplet at an unusually high δ value, and it must correspond to the point of linkage of the acid to the ring. The NMR spectrum is nearly first order, so that the spectral parameters were readily obtainable. The $J_{1'',2''}$ value is compatible with a β-linked glucose. The rest of the measured *J* values are similar to those measured for glucose in water [12].

It is noteworthy from a phytochemical point of view that species from the genus *Phlomis* contain large amounts of *p*-coumaroylglucosides of flavonoids. The previous reports on this genus are as follows: *Phlomis spectabilis* [5], an alpine perennial herb growing in India (2400–2600 m), contained exclusively flavonols; *P. tuberosa* and *P. agraria* [1–4], which grow in continental habitats in Russia, contained flavones (luteolin and apigenin) and their glucosides and glucuronides; and *P. aurea* and *P. floccosa* [6], two Mediterranean species growing in Egypt, the first in the rocky places in Sinai and the second in the north coastal area, contained 7-*O*-glucosides and 7-*O*-*p*-coumaroylglucosides of luteolin, apigenin and chrysoeriol, and the first also of naringenin. *P. lychnitys* showed also chrysoeriol glucosides and a flavonoid pattern quite similar to that observed from *P. floccosa*, suggesting a close relationship between these two coastal Mediterranean species. These results indicate that there are relationships between the flavonoid pattern, phylogeny and geography. Thus, Mediterranean species contain methylated flavones (chrysoeriol) are perhaps the most evolved species, the continental species contained only hydroxylated flavones and lacked chrysoeriol and flavonols, and the Indian species studied contained exclusively flavonols. This latter species could therefore be the most primitive of the *Phlomis* species studied so far [13].

EXPERIMENTAL

Plant material. Phlomis lychnitys L. aerial parts were collected at flowering near Santomera (Murcia) and a voucher specimen was deposited on file in the herbarium of the Faculty of Sciences at Murcia (accession No. 12.259).

Extraction and isolation. Air-dried and powdered aerial parts (*ca* 200 g) were extracted with *n*-hexane, $CHCl_3$ and MeOH in succession. The MeOH extract was concd under red. pres. and column chromatographed on silica gel G-60 with EtOAc–MeOH (10:1). Fractions with flavonoid colour under UV light were collected, concd and purified by prep. TLC on silica gel with EtOAc–MeOH (10:1).

Identification of the new flavonoid. UV λ_{max}^{MeOH} nm: 345i, 318

(1.0), 302 sh, 290 sh, 270 (0.76), 258 sh, 240 sh; + NaOMe: 380 (increased), 309 sh, 259; + $AlCl_3$: 385, 359, 316 sh, 298, 278, 262 sh, 238 sh; + $AlCl_3$ + HCl: 380 sh, 355 sh, 316 sh, 299, 278, 262 sh, 238 sh; + NaOAc: 404, 317, 300 sh, 267; + NaOAc + H_3BO_3: the same shape and values as in MeOH. IR ν_{max}^{Nujol} cm^{-1}: 2984 (Me), 2939, 1730 (ester C=O), 1653 (flavone C=O), 1602, 1450, 1370, 1291, 1174, 1127, 1074, 1038, 1017, 816, 800, 746, 704. EIMS, permethylated derivative obtained by Brimacombe's method [14] (MeI, NaH), *m/z* (rel. int.) (70 eV, ion source temp. 240°, probe temp. 300°): 692 $[M]^+$ (0.5), 532 $[M - acyl]^+$ (30), 328 $[A + H]^+$ (100), 161 $[acyl]^+$ (81). ^1H NMR (360 MHz, DMSO-d_6): δ3.466 (1H, *t*, *J* = 9.0 Hz, H-4″), 3.506 (1H, *dd*, *J* = 9.4 Hz, H-2″), 3.530 (1H, *dd*, *J* = 11.3 Hz, H-6″$_B$), 3.624 (1H, *m*, *J* = 1.5 Hz, H-5″), 3.727 (1H, *dd*, *J* = 5.4 Hz, H-6″$_A$), 3.852 (3H, *s*, OMe), 5.065 (1H, *t*, *J* = 9.4, H-3″), 5.254 (1H, *d*, *J* = 7.8 Hz, H-1″), 5.925 (1H, *s*, H-3), 6.424 (1H, *d*, *J* = 15.7, Ph-C=CH-CO-), 6.457 (1H, *d*, *J* = 2.0 Hz, H-6), 6.794 (1H, *dd*, *J* = 8.6 Hz, H-5′), 6.813 (2H, *d*, *J* = 9.0 Hz, H-2‴, H-6‴), 6.865 (1H, *d*, H-8), 7.483 (1H, *dd*, *J* = 1.0 Hz, H-2′), 7.554 (1H, *dd*, *J* = 2.0 Hz, H-6′), 7.564 (2H, *d*, *J* = 9.0 Hz, H-3‴, H-5‴), 7.585 (1H, *d*, *J* = 15.7 Hz, Ph-CH=C-CO-). Signals from the OH protons were not seen due to rapid exchange with the water protons. Sequential double resonance starting with the H-1″ proton of glucose was employed to identify the rest of glucose protons. The assignment of the remaining signals was done on the basis of both signal multiplicities and reference spectra [15].

Acidic hydrolysis. The naturally occurring glucoside was hydrolysed by means of aq. 2 N HCl (1 hr, 90°) yielding *p*-coumaric acid, chrysoeriol and glucose that were identified by chromatographic comparisons with authentic samples.

Alkaline hydrolysis. The original glycoside was treated with aq. 2 N NaOH, and after 48 hr (room temp.) *p*-coumarate and a flavone monoglucoside were obtained.

Monoglucoside identification. UV λ_{max}^{MeOH} nm: 343, 268, 253 sh; + NaOMe: 388 (increased), 296 sh, 266; + $AlCl_3$, 390 sh, 362, 303 sh, 274; + $AlCl_3$ + HCl: 380 sh, 355, 303 sh, 275, 260 sh; + NaOAc: 405, 353 sh, 262 sh; + NaOAc + H_3BO_3: 347, 268. EIMS of the permethylated derivative (70 eV, ion source temperature 240°, probe temp. 300°): 546 $[M]^+$ (43), 328 $[A + H]^+$ (100), 218 $[T_1]^+$ (52), 187 $[T_2]^+$ (73), 155 $[T_3]^+$ (41).

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