Phenolic Compounds from *Lactuca viminea* L.

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The aerial part of *Lactuca viminea* L. was examined for the presence of phenolic compounds. Six different flavonoids were isolated and identified by UV spectra, TLC, HPLC-DAD, ^{1}H NMR and ^{13}C NMR. They were identified as apigenin, luteolin, quercetin, luteolin-7-O-glucoside, quercetin-3-O-glucoside and luteolin-7-O-glucuronide-6″-methyl ester. Three phenolic acids were also detected: caffeic, ferulic and chlorogenic acid.

Introduction

Species of the genus *Lactuca* (Asteraceae) have been used for a long time in folk medicine as sedative plants [1]. Moreover, analgesic, antipyretic and antiinflammatory properties have been attributed to other species of this genus *L. chinensis* [2]. Previous phytochemical studies paid considerable attention to sesquiterpene lactones [3], which are the compounds most frequently isolated in this genus and seem to be typical of *Lactuca*. In spite of different terpenes having been described, flavonoids and their derivatives are now considered more important because of their possible use as chemotaxonomic markers. The aim of the present work was to isolate and characterize flavonoids and other phenolic compounds present in *Lactuca viminea* L., the chemical characteristics of which have never been studied previously. The results will also be used to determine the role of these products in the chemotaxonomy of this genus and the whole Lactuceae tribe. Further studies of this kind will be carried out with different *Lactuca* species, *L. tenerrima, L. virosa* and *L. muralis*.

Results

Phenolic compounds were obtained as explained in Materials and Methods. The main tool for determining their structure was UV spectral analysis. Peak wavelengths of the spectral measurements with the usual shifts [4, 5] are shown in Table I. R_f values in different TLC conditions and t_r on HPLC-DAD are given in Table II.

Compounds **2, 3,** and **4** were identified as apigenin, luteolin and quercetin respectively. This conclusion was reached on the basis of the coincidence between data from UV spectra, TLC and HPLC-DAD and the authentic samples.

Compound **5** gave a UV spectrum nearly equal to that of luteolin except for the fact that the lack of any shift in band II with NaOAc indicates a 7-O-substituted structure. Substitution at C-7 was

Table I. UV spectral data of the isolated compounds. Maxima in nm, dec. = decomposition, values in brackets correspond to shoulders.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
MeOH	326 237 215	334 (295) 266	348 (290) 266 253 (242)	371 (300) (269) 255	349 (266) 254	356 (296) (268) 256	348 (267) 255	328 218
NaOMe		388 321 274	406 (330) 270	447 281 dec.	398 261	410 (355) 272	402 (305) 268	
$AlCl_3$		(382) 347 (300) 273	422 (330) (298) 271	448 330 (302) 270	421 (328) (300) 272	426 (315) 213	426 325 (296) 272	
$AlCl_3$/ HCl		(380) 338 (298) 275	385 356 (296) 274 (265)	424 358 (301) 266	387 358 (300) 272	399 (365) (300) 268	386 357 (292) 271	
NaOAc		373 (301) 272	395 (327) 268	393 328 272 (258)	351 260	401 (317) 272	404 260	

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Table II. TLC data of the isolated compounds. A: silica gel layer, $CHCl_3$:MeOH (9:1); B: cellulose layer, $CHCl_3$:AcOH:H_2O (50:45:5); C: cellulose layer BuOH:AcOH:H_2O (4:1:5) u.p. t_r data of HPLC-DAD.

Compound	R_fA	R_fB	R_fC	t_r [min]
1	21	92	88	19.2
2	62	83	88	47.9
3	39	63	85	42.4
4	28	34	65	39.5
5	16	62	49	37.8
6	4	43	52	30.0
7	2	33	44	27.8
8	0	57	62	11.3
9	12	59	86	11.9

determined by NMR according to the following results: ^1H NMR of **5** aglycone moiety: δ 6.77 (s, H-3), 6.48 (d, J = 2 Hz, H-6), 6.84 (d, J = 2 Hz, H-8), 7.44 (s, H-2′), 6.94 (d, J = 8 Hz, H-5′), 7.46 (dd, J = 8 Hz, J' = 2.0 Hz, H-6′). ^{13}C NMR of aglycone moiety: δ 121.6 (C-2), 103.4 (C-3), 182.1 (C-4), 161.4 (C-5), 99.2 (C-6), 162.6 (C-7), 94.7 (C-8), 157.2 (C-9), 105.7 (C-10), 121.6 (C-1′), 113.8 (C-2′), 146.0 (C-3′), 150.2 (C-4′), 116.2 (C-5′), 118.5 (C-6′); sugar moiety: 99.6 (C-1″), 71.6 (C-2″), 73.0 (C-3″), 75.4 (C-4″), 75.6 (C-5″), 169.5 (C-6″); and 51.9 ($-OCH_3$). These spectral data proved the presence of a methyl group esterifying a glucuronic acid, therefore, compound **5** was identified as luteolin-7-O-glucuronide-6″-methyl ester, which has never been described before in this genus.

Compounds **6** and **7** were identified as quercetin-3-O-glucoside (isoquercitroside) and luteolin-7-O-glucoside respectively. Structural determination was performed by UV spectroscopy [4] and acid hydrolysis, and glucose was identified in both compounds. Compounds **1** and **8** were identified as ferulic and chlorogenic acids on the basis of their UV spectra [6], R_f value (TLC) and t_r value (HPLC-DAD). Comparison of chromatographic data with authentic samples confirmed these results. Finally, the presence of caffeic acid, **9,** was also detected, although its isolation was not performed.

Discussion

Apigenin and luteolin are the flavonoid aglycones most frequently isolated in the *Lactuca* genus. Apigenin was found in *L. indica* [7] and luteolin in *L. chinensis* [2] and *L. indica* [7]. Because they are so abundant in species of this genus and in Compositae, we believe that they may be of no taxonomical value. The presence of free quercetin has not been reported before in *Lactuca,* although it was often present in glycosidic form. These three aglycones were also detected in fresh plant samples, therefore their isolation was not due to possible hydrolytic processes during desiccation, storage or extraction.

Of the flavonoid heterosides isolated in this work, luteolin-7-O-glucoside is quite common in the genus and was to be expected that it would appear in *L. viminea* as it did in *L. chinensis* [2], *L. indica* [8], *L. repens* [9] and *L. tamagawaensis* [10]. On the other hand, several quercetin heterosides have been described in other *Lactuca* species, like quercetin-3-O-malonylglucoside in *L. sativa* [11], quercetin-3-O-galactoside in *L. indica* [8] and rutin from *L. sativa* [3]. However, isoquercitroside, which has just been identified in *L. viminea,* was only described in *L. sativa* [11]. The presence of luteolin-7-O-glucuronide-6″-methyl ester in *L. viminea* is also noteworthy. This compound has not been described before in species of *Lactuca* or other species of the Asteraceae family, although glucuronic derivatives have been isolated in species of *Lactuca,* like quercetin-3-β-D-glucuronide and luteolin-7-β-D-glucuronide in *L. sativa* [11].

Finally, caffeic and chlorogenic acids, present in *L. viminea,* have been detected previously in *L. sativa* [3] and *L. sonchifolia* [12]. Although isoferulic acid was described in *L. pontica* [13], the occurrence of ferulic acid in species of this genus has not been described before.

Materials and Methods

Plant material and extraction

The aerial part of *L. viminea* was collected in Eslida (Castellón, Spain) in October 1989 and a voucher specimen was deposited in the Herbarium of the Faculty of Pharmacy (Valencia, Spain). Air dried and powdered plant (636.7 g) was extracted in a soxhlet with solvents of increasing polarity (Hexane, CH_2Cl_2 and MeOH). MeOH extract (52.6 g) was evaporated under reduced pressure, dissolved in water and fractionated with Et_2O, EtOAc and BuOH to yield Et_2O (6.6 g), EtOAc (3.0 g) and BuOH (10.1 g) extracts. Little scale extraction of fresh plant was carried out with hot EtOH to inhibit enzymatic hydrolysis, and EtOH extract was then treated with Et_2O to separate free flavonoid aglycones.

Separation and identification

Et_2O extract (6.6 g) was chromatographed on a silica gel 60 (Merck) column with CH_2Cl_2 : MeOH (9:1), and this mixture was progressively enriched in MeOH. Five fractions were obtained (A_1-A_5). Fraction A_2 was rechromatographed on a silica gel 60 (Merck) column with a gradient of CH_2Cl_2 : MeOH (95:5 to 80:20) to obtain seven fractions (B_1-B_7). Compound **1** (5 mg) was obtained from fraction B_2 by PTLC on cellulose with $CHCl_3$: AcOH : H_2O (50:45:5). Compound **2** (4 mg) was isolated from fraction B_4 by PTLC on silica gel with CH_2Cl_2 : MeOH (9:1). Two other compounds **3** (2 mg) and **4** (5 mg) were obtained from B_7 in the same way as **1**. EtOAc extract (1.5 g) was chromatographed on a Sephadex LH-20 column with MeOH : H_2O (8:2). Six fractions were obtained (C_1-C_6). Compound **5** (11 mg) was isolated by precipitation from fraction C_1, and purified by washing with MeOH. Compounds **6** (4 mg) and **7** (3 mg) were obtained from fraction C_4 by PTLC on cellulose with BuOH : AcOH : H_2O (4:1:5), upper phase (u.p.). Finally a portion of BuOH extract (200 mg) was chromatographed on a Lobar Lichroprep RP-8 column with MeOH : H_2O (6:4). Compound **8** was obtained directly.

Phenolic compounds were detected by TLC and HPLC-Diode Array Detector (HPLC-DAD). TLC was performed using silica gel 60 G and microcrystalline cellulose (Merck) on aluminium sheets. 365 nm UV light and aminoethyl ester of diphenylboric acid 1% in MeOH (Neu's reagent) were used as detectors. R_f values were compared with authentic samples and literature data [14]. HPLC-DAD was carried out on a Merck-Hitachi HPLC system (L-6200 pump) equipped with a L-3000 Photodiode Array detector and a pre-packed analytical column (12.5 × 0.7 mm) of Lichrospher RP-18 (5 μm). The following conditions were used: Eluents: H_2O + TFA 0.05% (A), MeOH + TFA 0.05% (B). Elution profile: 0 min, 90% A; 0–5 min, 80% A; 5–45 min, 50% A; 45–55 min, 20% A; 55–59 min, 80% A. Flow rate was 1 ml/min and column pressure 60–80 bar, UV detector was set at 255 nm. Data were compared with authentic samples. Identification was carried out by UV spectra on a Perkin Elmer Lambda 3 UV/VIS spectrophotometer by ^1H NMR and ^{13}C NMR spectra on Varian Gemini 200 spectrometer (200 MHz) in deuterated DMSO. Substances which were suspected to be glycosides were subjected to acid hydrolysis: a solution of the glycoside (1 mg) in 5 ml of 2 N HCl–MeOH (1:1) was kept for 60 min at 100 °C. The aglycones were separated by repeated extraction with EtOAc after removing MeOH. TLC of sugars was performed with silica gel layer and EtOAc : AcOH : MeOH : H_2O (65:20:15:15). Spots were detected by spraying with 0.5% thymol in H_2SO_4–EtOH (5:95) and heating at 120 °C for 15 min.

Authentic samples

Luteolin and apigenin (C. Roth, Karlsruhe); quercetin (Merck, Darmstadt); quercetin-3-O-glucoside and luteolin-7-O-glucoside (Apin Chemicals, Abingdon, U.K.); ferulic, chlorogenic and caffeic acid (Sigma Chemical Co., St. Louis, U.S.A.).

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