On the Occurrence of Caffeoyltartronic Acid and Other Phenolics in *Chondrilla juncea*

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Caffeoyltartronic acid and other eleven phenolic compounds were identified in the MeOH extract of *Chondrilla juncea:* the flavonoids luteolin, luteolin-7-glucoside, luteolin-7-galactosylglucuronide and quercetin-3-galactoside; the phenolic acids protocatechuic, caffeic, chlorogenic, isochlorogenic and isoferulic and the coumarins cichoriin and aesculetin. The taxonomic implications of these compounds have been discussed.

Introduction

The genus *Chondrilla* (Asteraceae) belongs to the subtribe Crepidinae of the tribe Lactuceae. This genus includes four European species, but *Chondrilla juncea* L. is the only one that grows in the Iberian Peninsula [1]. The chemistry of this plant has not been studied before. This report describes the phenolic compounds from *C. juncea* and discusses their chemotaxonomic value within the family Asteraceae.

Results

Twelve phenolic compounds were detected in the MeOH extract of aerial parts of *C. juncea*. Two of them were identified by spectroscopic methods (^{1}H, ^{13}C NMR and UV) as 2-O-caffeoyltartronic acid and luteolin-7-O-galactosylglucuronide.

Compound **1** was characterized as 2-O-caffeoyltartronic acid according to the following results: UV spectra showed that the addition of NaOH caused a strong bathochromic shift of λ_{max}^{MeOH} from 325 to 373 nm, which is typical of caffeoyl ester. The change from blue to greenish-blue observed when the ester was analyzed by TLC under UV (350 nm) with treatment of NH_3 vapour suggested the compound to be a caffeic acid ester [2]. ^{13}C NMR (D_2O) tartronic moiety: δ (ppm) 169.4 (C-1′ and C-3′), 75.5 (C-2′); caffeoyl moiety: 127.9 (C-1), 123.7 (C-2), 147.9 (C-3), 145.1 (C-4), 117.0 (C-5), 116.1 (C-6), 147.5 (C-7), 114.9 (C-8), 174.5 (C-9). The presence of the signal corresponding to C-7 at 147.5, *ca.* 2 ppm at a lower field than expected when we compare it with other caffeoate derivatives, is due to the strong electronegative effect of the two carboxylic groups in the tartronic moiety. ^{1}H NMR (D_2O) δ (ppm) 7.62 (d, H-7, J = 16 Hz), 7.14 (d, H-2, J = 2 Hz), 7.05 (dd, H-6, J = 8, J' = 2 Hz), 6.86 (d, H-5, J = 8 Hz), 6.39 (d, H-8, J = 16 Hz), 5.48 (s, H-2′). These data constitute the first report of a ^{13}C NMR spectrum for the compound, whereas the ^{1}H NMR spectrum coincides with that of caffeoyltartronic acid, which has been localized so far in *Vigna radiata* (Fabaceae) [3].

Compound **2** was identified as luteolin-7-O-galactosylglucuronide on the basis of its R_f value, partial hydrolysis and UV spectra. It gave an UV spectrum nearly equal to that of the luteolin [4] except for the fact that the lack of any shift in band II with NaOAc indicated a 7-O-substituted structure [5]. Its chromatographic behaviour suggested the presence of two sugars linked to the aglycone. Acid hydrolysis gave luteolin, galactose and glucuronic acid as products whereas enzymatic hydrolysis with galactosidase gave luteolin-7-O-glucuronide [6] and galactose.

In addition to the above compounds ten phenolics were detected by HPLC-DAD. These substances were: three flavonoids: luteolin (**3**), luteolin-7-glucoside (**4**) and quercetin-3-galactoside (**5**); five phenolic acids: protocatechuic (**6**), caffeic (**7**), chlorogenic (**8**), isochlorogenic (**9**) and isoferulic (**10**); and two coumarins: cichoriin (**11**) and aesculetin (**12**). They were identified by comparing their R_f values obtained by co-TLC with authentic samples and by their HPLC retention times and UV spectra of the HPLC-DAD peaks with those of the

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reference compounds (Table I). The position of the glycosyl moiety was established on the basis of the UV bands of absorption.

Table I. TLC and spectral data of the identified compounds. A: cellulose layer, BuOH:AcOH:H_2O (4:1:5) upper phase; B: cellulose layer, $CHCl_3$:AcOH:H_2O (25:23:2.5), C: silica gel layer, EtOAc:AcOH:H_2O (100:11:27). R_t data of HPLC-DAD.

Compound	R_fA	R_fB	R_fC	R_t [min]	nm (MeOH)
1	73	37	25	17.43	325, 238, 217
2	34	13	6	24.85	343, 267, 254
3	85	86	92	42.49	348, 266, 253
4	48	52	26	28.71	348, 255
5	63	38	29	29.36	357, 266, 255
6	79	55	81	6.31	294, 260, 216
7	79	88	91	11.64	321, 217
8	61	31	12	11.89	328, 218
9	77	52	49	29.04	327, 219
10	82	96	81	24.98	323, 293
11	53	55	14	8.11	342, 252, 230
12	76	65	87	11.20	350, 256, 231

Discussion

Two remarkable facts revealed by this study are the occurrence of 2-O-caffeoyltartronic acid and the presence of the rare flavonoid luteolin-7-O-galactosylglucuronide. The former is significant because this is not only the first time that a tartronic acid ester derivative has been identified in the tribe Lactuceae, but also in the whole Compositae. Hydroxycinnamic esters are in nature in the form of caffeoates, *p*-coumaroates, feruloates and sinapoates of mono-, di-, or trisaccharides and quinic acid, being restricted, mainly though not entirely, to sympetalous plants [7]. The hydroxycinnamic esters of aliphatic hydroxy acids (tartaric, malic, isocitric, tartronic, ...), constitute a group which seems to be present in a highly sporadic manner. Various caffeoyl-tartaric derivatives were localized in wines and in edible members of the tribe Lactuceae *(Cichorium, Lactuca)*, but in recent years these esters of hydroxy acids has been reported in several taxa with markedly different phylogeny. Many hydroxycinnamoyl derivatives of tartaric acid are present in Lamiaceae [8], Asteraceae [9], Vitaceae [10], Chenopodiaceae [11] and even in Equisetaceae [12]; malic conjugates can be found in Brassicaceae [13] and Urticaceae [14] (both families belonging to the subclass Dilleniidae); isocitric derivatives in Amaranthaceae [15], and finally, caffeoyl-tartronic acid, which has been localized only in Fabaceae [3] and now in Asteraceae. The presence of the luteolin-7-O-galactosylglucuronide, on the other hand, is significant because we have very recently described it as a new compound in *Andryala ragusina,* another species of subtribe Crepidinae [6].

Like other genera of the same subtribe, *C. juncea* contains the widespread luteolin and its 7-glucoside, although apigenin and its derivatives are absent, in contrast to the Crepidinae species studied so far. The presence of quercetin-3-galactoside is noteworthy because this flavonol has only been detected before in one member of this subtribe, *Sonchus asper* subsp. *asper* [16], whereas it is common in species of Leontodontinae [17], another subtribe of Lactuceae. With respect to the coumarin content, the presence of cichoriin and aesculetin serves to strengthen the links between *Chondrilla* and other genera of the Crepidinae (*Crepis* [18], *Launaea* [19], *Hieracium* [20] and *Sonchus* [16]) and distinguishes this subtribe from Leontodontinae where they are absent [17].

Phenolic acids are not of high value chemotaxonomically because of their wide distribution in these species [16–20].

Materials and Methods

Plant material and extraction

Leaves and stems of *C. juncea* was collected at the flowering stage in Cillas (Guadalajara, Spain) in August 1989 and a voucher specimen was deposited in the Herbarium of the Faculty of Pharmacy (Valencia, Spain). Air-dried and powdered plant (300 g) was extracted in a soxhlet with CH_2Cl_2 and MeOH. MeOH extract (26.6 g) was concentrated, dissolved in water and fractionated with Et_2O and BuOH to yield Et_2O (4.4 g) and BuOH (6.2 g) extracts. A small amount of fresh plant material was extracted with hot EtOH proving the presence of free flavonoid aglycones.

Separation and identification

Both extracts were subjected to gel filtration with Sephadex LH-20 and eluted with MeOH to yield nine and eight fractions, respectively, of the Et_2O and BuOH extracts. Fraction 8 of the BuOH extract was rechromatographed on a Lobar Lichro-

spher RP-8 column with MeOH:H_2O (4:6) and compound **1** was obtained directly from the first fraction. Further purification of the last one by preparative TLC (cellulose, $CHCl_3$:AcOH:H_2O, 50:45:5) yielded compound **2.**

TLC was performed using silica gel 60 G and microcrystalline cellulose (Merck). Compounds were visualized by 365 nm UV light and aminoethyl ester of diphenylboric acid 1% in MeOH (Neu's reagent). HPLC-DAD analysis was performed using a Merck-Hitachi HPLC system (L-6200 pump) equipped with a L-3000 photodiode array detector and a prepacked 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 rates was 1 ml/min, column pressure 60–80 bar, and the UV detector was set at 255 nm.

Identification was carried out by UV spectra on a Perkin-Elmer Lambda 3 UV/Vis spectrophotometer and by ^1H and ^{13}C NMR spectra on Varian Gemini 200 spectrometer (200 MHz) in D_2O.

Acid hydrolysis was carried out with 2 N HCl for 2 h. Aglycones were identified by co-chromatography. TLC of the sugars was performed with silica gel (EtOAc:AcOH:MeOH:H_2O, 65:20:15:15) spraying with 0.5% thymol in H_2SO_4:EtOH (5:95).

Enzymatic hydrolysis was carried out with 1 mg of compound **2** in 2 ml of pH 5 buffer (an aqueous 0.5 M NaOAc solution adjusted to pH 5 with AcOH) and 1 mg of β-galactosidase (Sigma). The reaction was maintained for 12 h at 37 °C [5].

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[1] T. Tutin, V. H. Heywood, N. Burges, D. Moose, D. Valentine, S. Walters, and D. Weebs, Flora Europea, **Vol. IV,** p. 343, Richardson, Cambridge 1976.
[2] D. Strack, P. Leicht, M. Bokern, V. Wray, and L. Grotjahn, Phytochemistry **26,** 2919 (1987).
[3] D. Strack, F. Hartfeld, F. A. Austenfeld, L. Grotjahn, and V. Wray, Phytochemistry **24,** 147 (1985).
[4] M. C. Terencio, M. J. Sanz, M. L. Fonseca, S. Máñez, and J. L. Ríos, Z. Naturforsch. **47c,** 17 (1992).
[5] K. R. Markham, Techniques of Flavonoid Identification, pp. 36–61, Academic Press, London 1982.
[6] M. C. Recio, M. C. Terencio, J. A. Arenas, R. M. Giner, M. J. Sanz, S. Máñez, and J. L. Ríos, submitted for publication in Pharmazie.
[7] P. Molgaard and H. Ravn, Phytochemistry **27,** 2411 (1988).
[8] W. Sumaryono, P. Proksch, V. Wray, L. Witte, and T. Hartmann, Planta Med. **57,** 176 (1991).
[9] H. Soycke, G. Al-Hassan, and K. Gorler, Planta Med. **54,** 175 (1988).
[10] V. Chenier, J. Rigaud, and M. Moutouned, Phytochemistry **29,** 1751 (1990).
[11] D. Strack, J. Heilemann, B. Boehnert, L. Grotjahn, and V. Wray, Phytochemistry **26,** 107 (1987).
[12] C. Weiduer, M. Veit, and F. C. Czygan, Planta Med. **57** (Suppl. 2), A 37 (1991).
[13] J. K. Nielsen, O. Olsen, L. H. Pedersen, and H. Sorensen, Phytochemistry **23,** 1741 (1984).
[14] J. Budzianowski, Phytochemistry **29,** 3299 (1990).
[15] F. Strack, P. Leicht, M. Bokern, V. Wray, and L. Grotjahn, Phytochemistry **26,** 2919 (1987).
[16] R. M. Giner, A. Ubeda, M. J. Just, A. Serrano, S. Máñez, and J. L. Ríos, submitted for publication in Biochem. Syst. Ecol.
[17] J. L. Ríos, R. M. Giner, M. J. Cuéllar, M. C. Recio, and A. Serrano, Planta Med. **58** (Suppl.), A 701 (1992).
[18] S. Máñez, R. M. Giner, M. C. Recio, M. C. Terencio, and J. L. Ríos, Planta Med. **58** (Suppl.), A 698 (1992).
[19] R. M. Giner, J. Díaz, S. Máñez, M. C. Recio, and J. L. Ríos, Biochem. Syst. Ecol. **20,** 187 (1992).
[20] R. M. Giner, M. C. Recio, A. M. Villalba, S. Máñez, and J. L. Ríos, Pharmazie **47,** 308 (1992).