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 isolerulic and the coumarins cichorin 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 (1H, 13C 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 \( \lambda_{\text{max}} \) 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 NH3 vapour suggested the compound to be a caffeic acid ester [2]. 13C NMR (D2O) tartronic moiety: 3 (ppm) 169.4 (C-1'), 75.5 (C-2'); caffeoyl moiety: 127.9 (C-1), 116.1 (C-6), 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. 1H NMR (D2O) \( \delta \) (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 13C NMR spectrum for the compound, whereas the 1H NMR spectrum coincides with that of caffeoyltartronic acid, which has been localized so far in Vigna radiata (Fabaceae) [3].

Compounds 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 isoflurulic (10); and two coumarins: cichorin (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...
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:H₂O (4:1:5) upper phase; B: cellulose layer, CHCl₃:AcOH:H₂O (25:23:2.5), C: silica gel layer, EtOAc:AcOH:H₂O (100:11:25). Rₜ data of HPLC-DAD.

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Discussion

Two remarkable facts revealed by this study are the occurrence of 2-O-cafeoyltartaric 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 tartaric 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, ferulooates and sinapooates 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, trartronic, ...), constitute 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 chemotaxononomically 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 material (300 g) was extracted in a soxhlet with CH₂Cl₂ and MeOH. MeOH extract (26.6 g) was concentrated, dissolved in water and fractionated with Et₂O and BuOH to yield Et₂O (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 Et₂O and BuOH extracts. Fraction 8 of the BuOH extract was rechromatographed on a Lobar Licha-
spher RP-8 column with MeOH:H₂O (4:6) and compound 1 was obtained directly from the first fraction. Further purification of the last one by preparative TLC (cellulose, CHCl₃:AcOH:H₂O, 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 x 0.7 mm) of Lichrospher RP-18 (5 µm). The following conditions were used: eluents: H₂O + 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 ¹³C NMR spectra on Varian Gemini 200 spectrometer (200 MHz) in D₂O.

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:H₂O, 65:20:15:15) spraying with 0.5% thymol in H₂SO₄: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].

Acknowledgements

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