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A NEW QUERCETIN-ACYLGLUCURONIDE FROM *SCOLYMUS HISPANICUS*

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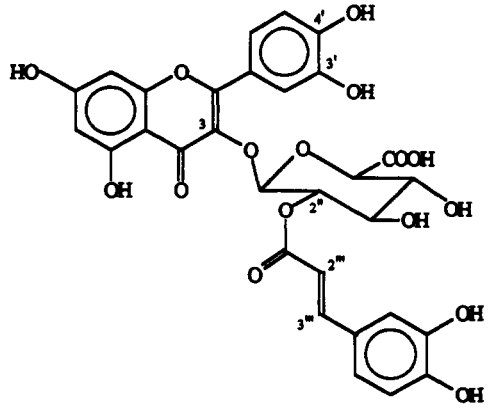
ABSTRACT.—The aerial part of *Scolymus hispanicus* was examined for the presence of phenolic compounds. A new flavonoid, quercetin-3-*O*-(2″-*O*-caffeoyl)-β-D-glucuronopyranoside [**1**], was isolated and identified. In addition, six known flavonoids, kaempferol, kaempferol-3-*O*-β-D-glucuronopyranoside, its 6″-methyl ester, quercetin, its 3-*O*-and 5-*O*-β-D-glucopyranosides, and four known phenolic acids, *p*-coumaric, protocatechuic, chlorogenic, and isochlorogenic, were identified.

The increasing interest in flavonoids as chemotaxonomic markers led us to study the different phenolic compounds present in the tribe Lactuceae of the Asteraceae. During the course of this research, we began a phytochemical study of the genus *Scolymus*, the only genus in the subtribe Scolyminae. The peculiarity of *Scolymus* lies in its chromosome number, 10, which is different from the basic number of all the other genera in the tribe. Its thistle-like habit and the presence of oil ducts make *Scolymus* transitional towards genera of Cynareae and Arctoteae (1).

Of the three species belonging to this genus, we selected *Scolymus hispanicus* L. for our study. This species is found in the Mediterranean region and in Southeastern Europe and has traditionally been used as a choleretic plant (2). Other studies on the isolation and identification of different phenolic compounds of this species have been carried out in the past (2–4).

Six known flavonoid compounds, namely kaempferol, quercetin, kaempferol-3-*O*-β-D-glucuronopyranoside, kaempferol-3-*O*-β-D-glucuronopyranoside-6″-methyl ester, quercetin-3-*O*-β-D-glucopyranoside, and quercetin-5-*O*-β-D-glucopyranoside, and four phenolic acids, *p*-coumaric, procatechuic, isochlor-



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ogenic, and chlorogenic, were isolated and identified on the basis of uv-vis, ^{1}H-nmr, and ^{13}C-nmr spectral analysis, as well as by acid hydrolysis and chromatographic data.

A new natural product, quercetin-3-*O*-(2″-*O*-caffeoyl)-β-D-glucuronopyranoside [**1**], was also isolated and identified. Uv spectroscopy revealed the existence of a 3-*O*-substituted quercetin structure, although its uv spectra showed some particularities due to the fact that the absorbances of the flavonol and caffeic acid moieties were coincident. Generally speaking, the position of flavonoid band II is unaffected by the presence of the acyl group because of caffeic acid has only a low absorbance maximum (243 nm) in this range. However, the position of band I of this glycoside was seriously modified by the caffeoyl group, since it is responsible for a very broad band with a peak that in dicaffeoyl-quinic acid, an ester used as a reference in this study, is placed at 328 nm. The fact that this band was more intense than flavonoid band I led to hypsochromic shifts in the spectra recorded with different reagents in comparison with the analogous spectra of simple flavonol-3-*O*-glycosides (5).

Tlc and hplc behavior of **1** indicated a relatively low polarity, and no sugar was detected by tlc after hydrolysis. However, nmr analysis clearly showed the presence of one glycosidic moiety. Given its anomalous chromatographic properties, we considered the possibility of an acylated-glycosyl substitution. Mild alkaline hydrolysis resulted in the transformation of **1** into a substance with an R_f in the range of the other quercetin glucosides. ^{1}H- and ^{13}C-nmr spectroscopy confirmed the uv spectroscopy data. The presence of a glucuronic moiety was supported by the -CHOH signals between 72.0 and 76.9, an anomeric carbon at 103.5, and a carboxyl group at 172.8 (6). This explained the difficult acid hydrolysis. Comparison of sugar signals in the ^{13}C-nmr spectrum with data of kaempferol-3-*O*-β-D-glucuronopyranoside allowed unequivocal identification of a 2″-*O* substitution on the glucuronic acid residue; the C-2 of the sugar at 74.1 ppm was 1.9 ppm lower compared to that of kaempferol-3-*O*-β-D-glucuronopyranoside, and C-3 at 72.0 ppm is 2.1 ppm higher than that of the same compound. The acyl group was identified as caffeic acid, and the aglycone was confirmed as quercetin by comparison of the nmr signals with those of previous reports (6,7).

The heterosides of quercetin are the most prevalent flavonoids identified in *Scolymus* (2,3). Quercetin-3-*O*-β-D-glucopyranoside, identified here for the first time in *Scolymus*, has been detected in other subtribes (4,8). Therefore, its systematic value is quite limited. Because the phenolic acids also have scattered occurrence in different subtribes of Lactuceae (8,9), we can consider them of little use from a chemotaxonomic point of view. On the other hand, quercetin-5-*O*-β-D-glucopyranoside may be of taxonomic interest. The lack of reports of its occurrence in other Lactuceae species, its relative rarity, and the ease with which it is detected make it useful as a systematic tool. The identification of the new flavonoid, quercetin-3-*O*-(2″-*O*-caffeoyl)-β-D-glucuronopyranoside, is also phytochemically relevant and suggests that this flavonoid may become useful in future chemotaxonomic studies.

The last two glycosides isolated, kaempferol-3-*O*- β-D-glucuronopyranoside and its 6″-methyl ester are also reported here in *Scolymus* for the first time. Although several glucuronides and 6″-methyl esters are found in different Lactuceae species (4,8), the fact that they are detected is always noteworthy because this particular glycosylation often constitutes a useful variation of the general patterns.

Other flavonoids not isolated in our survey have been reported by other authors in different organs of *S. hispanicus* of

uncertain origins, such as *C*-glycosyl flavones, some kaempferol glycosides, and isorhamnetin-3-galactoside (2,3). These discrepancies may be attributed to geobotanical and methodological differences.

EXPERIMENTAL

PLANT MATERIAL AND EXTRACTION.—The aerial parts of *S. hispanicus* were collected in Corbera (Valencia, Spain) in June 1990, and a voucher specimen was deposited in the Herbarium of the Faculty of Pharmacy (Valencia, Spain). Air-dried and powdered plant material (240.1 g) was extracted in a Soxhlet extractor with hexane, CH_2Cl_2, and MeOH. MeOH extract (30.2 g) was evaporated under reduced pressure, dissolved in H_2O, and fractionated with Et_2O and *n*-BuOH to yield Et_2O (3.4 g) and *n*-BuOH (6.4 g) extracts.

SEPARATION AND IDENTIFICATION.—The Et_2O extract was chromatographed on a Sephadex LH-20 column with MeOH to obtain nine fractions. By hplc-diode array detector (dad) and tlc analysis, *p*-coumaric and protocatechuic acids were identified from fraction 4 and isochlorogenic acid from fraction 6. Quercetin (10 mg) was obtained directly from fraction 9 and kaempferol (9 mg) was isolated from fraction 8 after further purification on a Si gel cc with CH_2Cl_2-MeOH (9:1). *n*-BuOH extract was chromatographed on a Sephadex LH-20 column with MeOH to obtain seven fractions. Chlorogenic acid was identified from fraction 3 by hplc-dad and tlc. Fractions 4 and 5 were rechromatographed on a Si gel column with EtOAc-MeOH-H_2O (105:5:5, 95:5:5, 65:35:5, and 50:45:5) to give nine fractions. Quercetin-5-*O*-β-D-glucopyranoside (2 mg), quercetin-3-*O*-β-D-glucopyranoside (4 mg), and kaempferol-3-*O*-β-D-glucuronopyranoside (16 mg) were obtained from fractions 2, 5, and 9, respectively. Kaempferol-3-*O*-β-D-glucuronopyranoside-6″-methyl ester (3 mg) and quercitin-3-*O*-(2″-*O*-caffeoyl)-β-D-glucuronopyranoside (15 mg) were isolated from fractions 6 and 7 by further purification in the same conditions.

Phenolic compounds were detected by tlc (Si gel or cellulose) and hplc-dad under conditions previously reported (8,10). Identification was carried out by comparison of uv, ^{1}H-nmr, and ^{13}C-nmr spectra with those of authentic samples and data found in the literature (2,11). Substances suspected to be glycosides were subjected to acid hydrolysis. This was carried out directly on Si gel plates with 2 M HCl 0.1 ml (2 h at 100°); sugars were detected by tlc on Si gel with EtOAc-HOAc-MeOH-H_2O (65:20:15:15), after spraying with 0.5% thymol in H_2SO_4-EtOH (5:95) and heating at 120° 5 min. Alkaline hydrolysis was performed according to Markham (12). Uv spectra were interpreted according to well established criteria (5,12,13).

Quercetin-3-O-(2″-O-caffeoyl)-β-D-glucuronopyranoside [**1**].—Uv λ max (MeOH) nm 254, 264 sh, 295 sh, 345; (+ $AlCl_3$) 265, 310 sh, 372; (+$AlCl_3$/HCl) 266, 298 sh, 345; (+NaOAc) 265, 295 sh, 349; (+NaOMe) 214, 270, 335 sh, 397; ^{13}C nmr (50 MHz, DMSO-d_6) δ (aglycone moiety) 157.0 (C-2), 134.3 (C-3), 177.3 (C-4), 161.3 (C-5), 99.9 (C-6), 166.3 (C-7), 94.3 (C-8), 157.4 (C-9), 103.0 (C-10), 120.6 (C-1′), 115.5 (C-2′), 145.1 (C-3′), 148.9 (C-4′), 118.1 (C-5′), 120.8 (C-6′), (sugar moiety) 103.5 (C-1″), 74.1 (C-2″), 72.0 (C-3″), 74.3 (C-4″), 76.9 (C-5″), 172.8 (C-6″), (acyl moiety) 167.4 (C-1‴), 115.0 (C-2‴), 145.6 (C-3‴), 125.4 (C-4‴), 113.8 (C-5‴), 145.9 (C-6‴), 149.1 (C-7‴), 115.8 (C-8‴), 121.5 (C-9‴).

AUTHENTIC SAMPLES.—Quercetin (Merck, Darmstadt, Germany); kaempferol, quercetin-3-*O*-β-D-glucopyranoside, and isochlorogenic acid (Apin Chemicals, Abingdon, U.K.); *p*-coumaric, protocatechuic, and chlorogenic acids (Sigma Chemical Co., St. Louis).

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