Flavonoid Glycosides from *Thymus membranaceus*

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Thymus membranaceus is an endemism of southeastern Spain, which belongs to section *Pseudothymbra* of genus *Thymus*. In this work we have achieved an investigation of the flavonoid glycosides present in the aerial parts of this plant. Among the compounds identified are the rare luteolin-7-0-β-D-xyloside, luteolin-7-0-β-D-[rhamnosyl (1→2)glucoside] and luteolin-7-0-β-D-[xylosyl (1→2)glucoside] previously not known to occur in the Labiatae.

Introduction

Infusions and decoctions of thyme aerial parts are used in spanish folk-medicine in whooping cough and as antihelmintic and insects repellent [1]. In the last few years, the spasmolytic activity of some methoxylated flavones isolated from *Thymus* species has been established [2, 3].

Thymus membranaceus Boiss subsp. *membranaceus* is an endemism of southeastern Spain, that has been previously investigated for flavonoid aglycones, and the new flavone thymusin (5,6,4′-trihydroxy-7,8-dimethoxy-flavone) was isolated and identified [4]. In continuation with our work we have now studied the flavonoid glycosides present in the aerial parts of this plant.

Materials and Methods

Plants of *Thymus membranaceus* were collected at flowering in April 1982 near "La Matanza" (Murcia, Spain). A voucher specimen is on file in the herbarium of the Faculty of Sciences at Murcia (Accession number 11,943). The air-dried powdered aerial parts were exhaustively extracted with cold EtOH-H_2O (7:3). The hydroalcoholic extract was concentrated under reduced pressure (40 °C), until only the water remained. The aqueous concentrate was successively extracted with Et_2O and *n*-BuOH. The Et_2O extract contained flavonoid aglycones that were previously studied [4, 5]. The *n*-BuOH extract was paper chromatographed on Whatman N° 3 with 30% HOAc and H_2O, and fractions were visualized under UV_{366}. Further purification was achieved by preparative PC on Whatman N° 1 with *n*-BuOH-HOAc-H_2O (4:1:5, upper phase) or with 30% HOAc. The isolated compounds were purified on sephadex LH-20, eluted with MeOH. UV spectra were recorded in MeOH according to standard procedures [6, 7]. All compounds were cochromatographed with authentic samples on cellulose TLC with 30% HOAc, H_2O and *n*-BuOH-HOAc-H_2O (4:1:5, upper phase). The naturally occurring glycosides were permethylated by means of methyl iodide [8] and the permethylated derivatives purified by TLC on silica gel with EtOAc, $CHCl_3$-Me_2CO (4:1) and $CHCl_3$-EtOAc-Me_2CO (5:1:4 and 5:4:1). The permethylated derivatives were EIMS analysed (70 eV, direct inlet, ion source temperature 240 °C, probe temperature 270–300 °). Sugars and aglycones were analysed after acidic hydrolysis of the naturally occurring glycosides (except for vicenin-2) by chromatographic comparisons (TLC and HPLC) against authentic samples.

Results and Discussion

The *n*-BuOH extract has been analysed for flavonoids and the following flavone glycosides have been isolated and characterized: 6-Hydroxyluteolin-7-0-β-D-glucoside, luteolin-7-0-β-D-glucoside, apigenin-7-0-β-D-glucoside, luteolin-7-0-β-D-glucuronide, luteolin-7-0-β-D-xyloside, apigenin-6,8-di-C-glucoside (vicenin-2), luteolin-7-0-β-D-[rhamnosyl (1→6)glucoside], luteolin-7-0-β-D-[rhamnosyl (1→2)glucoside] (veronicastroside) and luteolin-7-0-β-D-[xylosyl(1→2)glucoside].

6-Hydroxyluteolin-7-0-β-D-glucoside showed the lower Rf values on PC with 30% HOAc and H_2O, similar to those obtained for 8-hydroxyluteolin-8-β-D-glucoside isolated previously from *Sideritis leucantha* [9]. UV: λ_{max} (nm) in MeOH 345, 284 (256); + $AlCl_3$ 425 (343), 302, 275 (246); + $AlCl_3$ + HCl 371, 295, 261. These data are in accordance with those reported for pedalitin (5,6,3′3,4′-tetrahydroxy-7-methoxyflavone [7], suggesting the same substitution pattern and glycosidation on the hydroxyl at C-7. Acidic and enzymic (β-D-glucosidase) hydrolysis yielded 6-hydroxyluetolin and glucose, which

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were identified by chromatographic comparisons against authentic samples. This compound was previously reported from *Thymus loscosii* [10].

Luteolin-7-0-β-D-glucoside, luteolin-7-0-β-D-glucuronide, apigenin-7-0-β-D-glucoside and vicenin-2 were characterized by chromatographic comparisons against authentic samples and by UV and EIMS standard procedures [11–13]. Vicenin-2 is an useful flavonoid compound for chemotaxonomic purposes, and has been reported previously from section *Pseudothymbra* of genus *Thymus* [14].

Luteolin-7-0-β-D-xyloside exhibited R_f values as for a monoglycoside, and the UV study confirmed a 7-substituted luteolin. After acidic hydrolysis, luteolin and xylose were identified. EIMS of permethylated derivative *m/z* (rel. int.): 502 (29, M^+), 328 (100, A+H), 175 (7), 143 (59). These results supported the existence of a monopentosyl-luteolin, and confirmed the structure of the naturally occurring glycoside.

Luteolin-7-0-β-D-[rhamnosyl(1→6)glucoside] and luteolin-7-0-β-D-[rhamnosyl(1→2)glucoside] exhibited the same UV values that luteolin-7-0-β-D-glucoside, and upon acidic hydrolysis rendered luteolin, rhamnose and glucose. These two compounds were rather difficult to separate by the classical PC and TLC methods, but they were clearly differentiated as permethylated derivatives. EIMS of permethylated luteolin-7-0-β-D-[rhamnosyl (1→6)glucoside] *m/z* (rel. int.): 720 (1, M^+), 575 (5, S+60), 516 (23, S+H), 393 (6, OS), 329 (44, A+2H), 328 (100, A+H), 189 (26), 157 (5). EIMS of permethylated luteolin-7-0-β-D-[rhamnosyl-(1→2)glucoside] *m/z* (rel. int.): 720 (4, M^+), 517 (45, S+2H), 393 (30, OS), 361 (100, OS-MeOH), 329 (65, A+2H), 328 (54, A+H), 189 (74), 157 (19). The EIMS data evidenced that rhamnose was the terminal sugar, and that they were flavonoid disaccharides (rhamnosyl-glucosides), and the interglycosidic linkage was evidenced by the presence of the characteristic fragments for (1→6) or (1−→2) linkage [12, 15]. Thus, the EIMS of permethylated luteolin-7-0-β-D-[rhamnosyl(1→6)glucoside] showed S+60 and S+H ions and an important A+H peak, meanwhile the EIMS of permethylated luteolin-7-0-β-D-[rhamnosyl(1→2)glucoside] showed S+2H and OS-MeOH ions and an important A+2H peak. These structures were confirmed by chromatographic comparisons of the naturally occurring glycosides.

Luteolin-7-0-β-D-[xylosyl(1→2)glucoside] exhibited the same UV data as luteolin-7-0-β-D-glucoside. Acidic hydrolysis yielded luteolin, xylose and glucose. EIMS of the permethylated derivative *m/z* (rel. int.): 706 (3, M^+), 503 (44, S-12), 379 (11, OS), 347 (53, OS-MeOH), 329 (65, A+2H), 328 (100, A+H), 175 (5), 143 (59). These data were in accordance with a pentosyl(1→2)glucoside, as evidenced the characteristic fragments S-12, OS, OS-MeOH and an important A+2H [15].

Luteolin-7-0-β-D-glucuronide and luteolin-7-0-β-D-[rhamnosyl(1→6)glucoside] were previously unknown to occur in *Thymus* species, and this is the first report on luteolin-7-0-β-D-xyloside, luteolin-7-0-β-D-[rhamnosyl(1→2)glucoside] and luteolin-7-0-β-D-[xylosyl(1→2)glucoside] (luteolin-7-0-β-D-sambubioside) within the Labiatae. This is also the first time that a flavone neohesperidoside or sambubioside have been found within this family.

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