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TWO FLAVONE GLYCOSIDES FROM *SIDERITIS LEUCANTHA*

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Key Word Index—*Sideritis leucantha*, Labiatae; 5,8,3′,4′-tetrahydroxy-6,7-dimethoxyflavone 8-β-D-glucoside; 8-hydroxyluteolin 7-(2″-*O*-allosylglucoside).

Abstract—8-Hydroxyluteolin occurs in *Sideritis leucantha* as the 7-(2″-allosylglucoside) and the 8-glucoside of its 6,7-dimethyl ether has also been characterized from the same plant.

INTRODUCTION

In continuation of our previous work on the flavonoids of Mediterranean Labiatae, in particular on *Sideritis leucantha* Cav. [1–4], we have now isolated and identified two new flavonoid glycosides, 5,8,3′,4′-tetrahydroxy-6,7-dimethoxyflavone 8-β-D-glucoside and 8-hydroxyluteolin 7-(2″-*O*-allosylglucoside); this is also the first report of 5,8,3′,4′-tetrahydroxy-6,7-dimethoxyflavone in nature. Allose is a rare natural sugar and has recently been identifed in disaccharide combination in the flavonoids of *Veronica filiformis* [5], *Sideritis grandiflora* [6] and *S. hirsuta* [7].

RESULTS

The UV values of the first glycoside (**1**) in presence of classical reagents [8–12], revealed free hydroxyls in 5,3′ and 4′ positions, as well as the presence of three supplementary substituents on the A ring (6,7 and 8 positions) by comparison with the data obtained for 5,3′,4′-trihydroxy-6,7,8-trimethoxyflavone [1]. Moreover, the mass spectrum of the permethylated derivative showed that the natural glycoside was a monohexoside ($[M]^+$ at *m/z* 606) [13, 14], and the RDA fragments confirmed the substitution pattern on the flavone nucleus, and suggested that the sugar was linked to a hydroxyl on A ring.

Its enzymic hydrolysis yielded an aglycone (**3**) and glucose. The UV study of this aglycone confirmed the presence of a methoxyl group at C-7 [9], and a free hydroxyl at C-8 position (BIa $\lambda_{max}^{AlCl_3+HCl}$ at 430 nm) [11, 12], and an important alkaline decomposition (not observed in the glycoside) supported a 5,8-dihydroxy system. Further, the MS-RDA fragmentation of **3** revealed the presence of two hydroxyls on B ring and two hydroxyls and two methoxyls on A ring, and an $[M]^+ < [M - Me]^+$ coinciding with results for other flavones with the 5,8-dihydroxy-6,7-dimethoxy system [15, 16].

Acidic hydrolysis yielded the two Wessely-Moser isomers which showed by cellulose TLC with HOAc 30%, R_f values of 0.22 (**3**) and 0.14 (**4**). The comparative study of these two isomers confirmed that the natural aglycone (**3**), was 5,8,3′,4′-tetrahydroxy-6,7-dimethoxyflavone, on the basis of chromatographic (8-hydroxyflavones showed higher R_f values than 6-hydroxyflavones) [17] and UV behaviour (6-hydroxyflavones showed higher BII UV λ_{max}^{MeOH} than 8-hydroxyflavones) [10, 17], and so, 5,8,3′,4′-tetrahydroxy-6,7-dimethoxyflavone 8-β-D-glucoside is the naturally occurring glucoside.

The UV study of the second glycoside (**2**) indicated the existence of free hydroxyls in 5,8,3′ and 4′ positions, as well as a substituted hydroxyl on C-7, suggesting glycosidation at this position. Enzymic hydrolysis yielded 8-hydroxyluteolin, identified by chromatographic and UV comparisons with an authentic sample.

The mass spectrum of the permethylated derivative of the glycoside, showed that the natural compound was a hexosyl (1 → 2) hexoside ($[M]^+$ *m/z* 780, $[OS]^+$ *m/z* 423, $[OS - MeOH]^+$ *m/z* 391, and T_1, T_2 and T_3 fragments) [13], and the RDA fragmentation confirmed the substitution pattern on the flavone nucleus. Acid hydrolysis of the PM glycoside yielded an aglycone which showed free hydroxyl at C-7 (UV study with NaOAc), corroborating 7-*O*-glycosidation, and this aglycone by complete methylation yielded isosinensetin (5,7,8,3′,4′-pentamethoxyflavone).

Acid hydrolysis of the natural glycoside had clearly caused the well known Wessely-Moser rearrangement to take place so that the original compound yielded 6-hydroxyluteolin as a major product, and 8-hydroxyluteolin. Moreover, glucose and allose were identified as products of hydrolysis by chromatographic comparisons against authentic markers [5]. Information about the sequence of sugar was gained by means of analysis of the sugar moiety present in the monoglycoside obtained by mild acidic hydrolysis [18]. This sugar was identified as glucose, and so the terminal sugar would be allose and the complete structure of the naturally occurring glycoside is 8-hydroxyluteolin 7-(2″-*O*-allosylglucoside).

EXPERIMENTAL

Plant material. Plants of *Sideritis leucantha* Cav were collected near Santomera (Mucia, Spain) and a voucher specimen was deposited in the herbarium of the Botany Department of the Facultad de Ciencias Biológicas at Murcia

Extraction. The air-dried powdered whole plant (*ca* 1 kg), was exhaustively extracted at room temp. with EtOH–H_2O (7 : 3), and this extract concd under red pres. and subjected to extraction first with Et_2O and secondly with *n*-BuOH

Glycoside **1**. Isolation and identification. from the *n*-BuOH

extract it was isolated by means of PC with 30% HOAc (R_f 0.50) and *n*-BuOH–HOAc–H_2O (4.1:5 upper phase) (R_f 0.53), and after that by TLC on cellulose with 15% HOAc (R_f 0.28) and on polyamide DC-6 (Macherey-Nagel) H_2O–MeOH–MeCOEt–2,4-pentanedione (6:3.2:1) (R_f 0.54). Permethylated derivative: TLC on silica gel, EtOAc (R_f 0.46), $CHCl_3$–EtOAc–Me_2CO (5:4·1) (R_f 0.55), $CHCl_3$–EtOAc–Me_2CO (5:1·4) (R_f 0.81). UV λ_{max}^{MeOH} nm: 346, 277, 258 sh; + NaOMe, 410, 270; + $AlCl_3$, 434, 348 sh, 308 sh, 277, + $AlCl_3$–HCl, 420 sh, 362, 306 sh, 288, 264 sh; + NaOAc, 407, 330 sh, 271; + NaOAc–H_3BO_3, 376, 267 EIMS (probe) 70 eV, *m/z* (rel. int.): *PM*, 606 $[M]^+$ (10), 389 (24), 388 $[A+H]^+$ (100), 387 (8), 374 (15), 373 $[A+H-Me]^+$ (71), 225 $[A_1-H]^+$ (10), 218 (31), 197 $[A_1-CHO]^+$ (29), 188 (7), 187 (62), 183 (9), 182 (14), 179 (7), 168 (7), 165 $[B_2]^+$ (22), 162 $[B_1]^+$ (17), 155 (65), 101 (375).

Enzymic hydrolysis. This was carried out with β-D-glucosidase (Sigma), 1 hr, 30°, acetate buffer 0.1 N pH 4.6. Yielded an aglycone (**3**) which on TLC cellulose 30% HOAc (R_f 0.28) and 60% HOAc (R_f 0.71). UV λ_{max}^{MeOH} nm: 341, 298 sh, 285, 254; + NaOMe, 385, 272 (dec.), + $AlCl_3$, 432, 314, 275; + $AlCl_3$–HCl, 430 sh, 362, 328 sh, 308, 289, 260; + NaOAc, 395, 336, 275; + NaOAc–H_3BO_3, 366, 264. EIMS (probe) 70 eV, *m/z* (rel. int.). 346 $[M]^+$ (32), 345 (4), 332 (9), 331 $[M-Me]^+$ (41), 313 (14), 213 $[A_1+H]^+$ (7), 197 $[A_1-Me]^+$ (100), 185 (23), 183 $[A_1-CHO]^+$ (27), 169 $[A_1-MeCO]^+$ (68), 137 $[B_2]^+$ (55), 134 $[B_1]^+$ (89), 109 $[B_2-CO]^+$ (41).

Acid hydrolysis. This was achieved by heating with aq. 2 N HCl for 2 hr (80°) yielding two aglycones (**3** and **4**). Aglycone **3** coincident with the aglycone obtained by enzymic hydrolysis. Aglycone **4**, on cellulose TLC (30% HOAc, R_f 0.11 and 60% HOAc, R_f 0.58). UV λ_{max}^{MeOH} nm: 343, 288, 255 sh; + NaOMe, 393 (dec.), + $AlCl_3$, 425, 372 sh, 310, 271 sh; + $AlCl_3$–HCl, 365, 304.

Glycoside **2**. Isolation and identification: from the *n*-BuOH extract it was isolated by PC on Whatmann n°3 with 30% HOAc (R_f 0.63) and with *n*-BuOH–HOAc–H_2O (4:1:5 upper phase) (R_f 0.44) and posteriorly by preparative TLC on polyamide DC-6 (Macherey-Nagel) with $CHCl_3$–*iso*-PrOH–MeCOEt–HOAc (10·3·3:4) (R_f 0.44). The last purification was carried out on polyamide SC-6 (Macherey-Nagel) column with $CHCl_3$–MeOH–MeCOEt–H_2O (11:8:4:2). UV λ_{max}^{MeOH} nm: 340, 302, 271, 255 sh; + NaOMe, 398, 266 (dec.); + $AlCl_3$, 434, 352 sh, 317, 274, + $AlCl_3$–HCl, 412 sh, 353, 328 sh, 317 sh, 275, 263 sh; + NaOAc, 398 sh, 340, 298 sh, 262 (dec); + NaOAc–H_3BO_3, 362, 298 sh, 266.

Permethylated derivative. Permethylation was carried out first by CH_2N_2 and completed by Brimacombe's method [19] to avoid alkaline decomposition, and purified by TLC on silica gel with EtOAc (R_f 0.08), $CHCl_3$–EtOAc–Me_2CO (5·4 1) (R_f 0.09), $CHCl_3$–EtOAc–Me_2CO (5:1:4) (R_f 0.35), C_6H_6–$C_4H_8O_2$–HOAc (90:25:4) (R_f 0.16) and $CHCl_3$–Me_2CO (4:1) (R_f 0.18). EIMS (probe) 70 eV, *m/z* (rel. int.): PM, 780 $[M]^+$ (0.5), 546 $[S+H]^+$ (1), 545 $[S]^+$ (1), 423 $[OS]^+$ (2), 391 $[OS-MeOH]^+$ (32), 358 $[A+H]^+$ (100), 357 $[A]^+$ (32), 343 $[A+H-Me]^+$ (50), 328 $[A+H-30]^+$ (30), 313 $[A+H-MeCO]^+$ (11), 219 $[T_1]^+$ (105), 187 $[T_2]^+$ (1472), 167 $[A_1-CHO]^+$ (235), 165 $[B_2]^+$ (109), 155 $[T_3]^+$ (691).

Enzymic hydrolysis. This was achieved by means of a crude enzymic preparation, Rapidase C-40 (Societé Rapidase. Seclin. France) in acetate buffer 0.1 N pH 4.6, 48 hr, 30°.

Acid hydrolysis of the PM glycoside. This was carried out with aq. 2 N HCl, 2 hr, 70°, yielding an aglycone. UV λ_{max}^{MeOH} nm: 336, 292 sh, 271, 248 sh; + NaOAc, 365, 281. This aglycone was further methylated and coincided with isosinensetin by co-chromatography with an authentic sample.

Acid hydrolysis of the natural glycoside. This yielded 6-hydroxyluteolin and 8-hydroxyluteolin, and allose and glucose that showed R_f values of 0.40 and 0.32 respectively on Whatmann n°1 PC with PhOH (5)

Mild acid hydrolysis. Aq. 0.1 N HCl, 90°, 10 min [18], yielded a monoglycoside which showed PC Whatmann n°1 (30% HOAc) R_f of 0.43 against 0.55 for the diglycoside. Acid hydrolysis of this monoglycoside rendered glucose (PC with PhOH) and a mixture of 6-hydroxyluteolin and 8-hydroxyluteolin.

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