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ISOSCUTELLAREIN-7-*O*-[ALLOSYL (1→2) GLUCOSIDE] FROM *SIDERITIS LEUCANTHA*

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ABSTRACT.—In continuation with our work on the flavonoids of *Sideritis* species, we have now isolated and identified from *Sideritis leucantha* extracts, the novel flavonoid compound, isoscutellarein-7-*O*-[allosyl (1→2) glucoside].

Sideritis species are used in Spanish folk medicine as anti-inflammatory drugs (1), and a flavonoid compound has been isolated as responsible for this activity (2). The aim of our work is to elucidate the structures of the flavonoid compounds present in extracts from this genus.

As part of our work on the flavonoids present in the *n*-BuOH extract of *Sideritis leucantha* Cav. (3-6), we have now isolated a small quantity of a flavonoid diglycoside (**1**) by chromatographic procedures. This compound (Figure 1) shows a dark brown color after spraying chromatograms with Naturstoffreagenz-A and exposure to uv light (360 nm), suggesting a flavone with a single free hydroxyl on B-ring and substitution at C-6 or C-8 (7).

Its uv spectrum in MeOH indicates a flavone structure; uv λ max nm, 366sh,

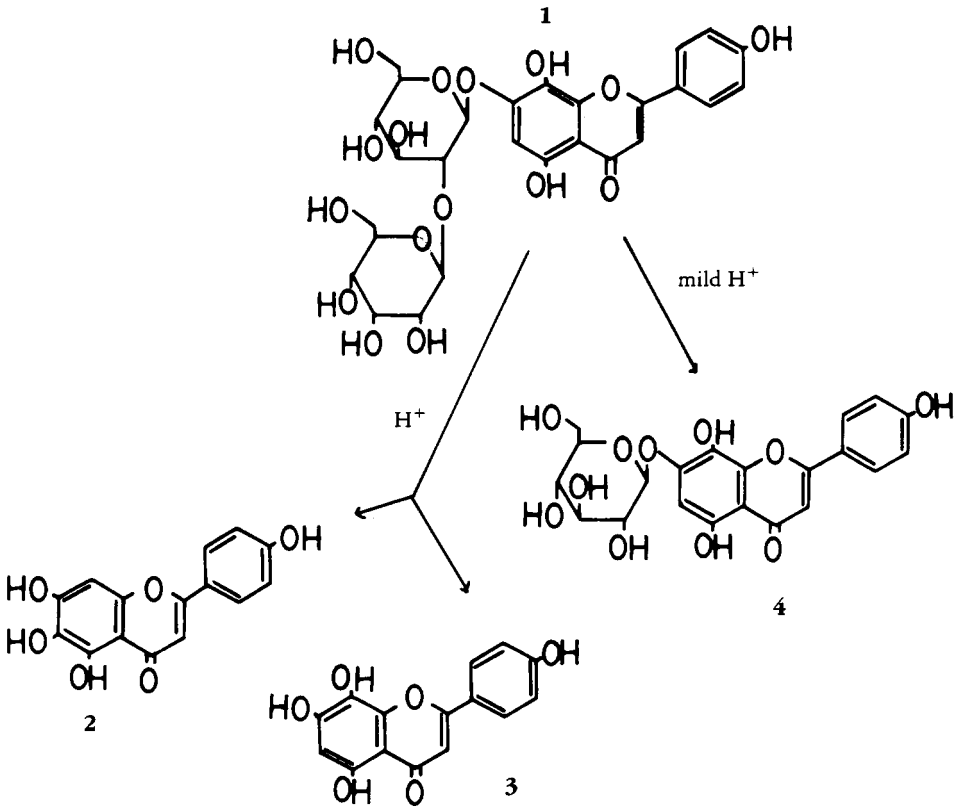

FIGURE 1. Hydrolysis of the glycoside **1**. Mild acidic hydrolysis yields the monoglycoside **4**. Conventional acidic hydrolysis yields the two Wessely-Moser isomers **2** and **3**.

330sh, 306, 277, and the single BII signal suggest a mono-substituted B-ring (8). The vis-uv study with the classical shift reagents (8) evidences the presence of free hydroxyls at C-4′ (large bathochromic shift of BI 52 nm with an increase in its intensity in NaOMe relative to MeOH) and C-5 ($AlCl_3$/HCl spectrum) and the substitution of the hydroxyl at C-7 (lack of BII bathochromic shift in NaOAc relative to MeOH, and lack of BIII in NaOMe spectrum) (8,9). The maximum at 277 nm in the MeOH spectrum supports the existence of an additional substituent at C-6 or C-8, and the $AlCl_3$/HCl spectrum showing a BIa shift relative to MeOH of 58 nm confirms the existence of a free hydroxyl at C-8 (10-12). The important alkaline decomposition observed corroborated the assignment of a 5,8-dihydroxyl-system (7). The shape and values of these spectra, which coincided with those of 5,8,4′-trihydroxy-7-methoxyflavone (12), confirm definitely the substitution pattern on the flavone nucleus.

The ms of the permethylated derivative of the naturally occurring glycoside shows a molecular peak at *m/z* 750 (1% rel. int.) and an aglycone ion $A+H^+$ at *m/z* 328 (100%). The difference between these two ions and the presence of an OS^+ ion (disaccharide) at *m/z* 423 (1.1%) suggest that the naturally occurring glycoside is a hexosyl-hexoside (Figure 2) (13,14). The presence of an important $OS\text{-}MeOH^+$ ion, the characteristic fragments of the (1↦2) linkage $A+2H^+$, $OS+H^+$, and $S+2H^+$, and the lack of the typical fragments for (1↦6) linkage (13-14) (Table 1) show clearly that the interglycosidic linkage is (1↦2). This is in agreement with data reported for diglycosides of flavonoids isolated previously from these Labiatae species (7) since all of them are hexosyl (1↦2) hexosides. At last, the RDA fragments of the aglycone obtained by acidic hydrolysis show the presence of two methoxyls and one hydroxyl on A-ring, and one methoxyl on B-ring, confirming the substitution pattern on the flavone nucleus and the glycosidation on the A-ring ($A_1\text{-}HCO^+$ *m/z* 167; B_2^+ *m/z*135; B_1+H^+ *m/z* 133) (Figure 2) (15).

The 7-*O*-glycosidation is confirmed by means of acidic hydrolysis of the permethylated glycoside, and the NaOAc-uv study of the aglycone obtained (8), which shows a bathochromic shift on BII of 8 nm relative to MeOH and evidences the presence of a free hydroxyl at C-7.

The acid hydrolysis of the natural glycoside yielded glucose and allose, as evidenced by chromatographic comparisons against authentic samples, and two flavonoid aglycones (**2** and **3**) (Figure 1) as products of the Wessely-Moser rearrangement. The ms of the major aglycone (**2**) shows a molecular ion at *m/z* 286, in accordance with a tetrahydroxyflavone. The RDA fragments [A_1^+ *m/z* 168 (100); B_2^+ *m/z* 121 (41) and B_1^+ *m/z* 118 (24)] are evidence for three hydroxyls on A-ring and one hydroxyl on B-ring. Its vis-uv spectra confirm that this aglycone is scutellarein (5,6,7,4′-tetrahydroxyflavone), and this was corroborated by chromatographic comparisons against an authentic sample. Its permethylated derivative coincided chromatographically in several systems with 5,6,7,4′-tetramethoxyflavone (16), confirming the substitution pattern. A second aglycone (**3**) is found in trace quantity and is identified by vis-uv procedures and

TABLE 1. Ms Characteristic Fragments for Distinguishing Interglycosidic Linkages in Flavonoid Disaccharides (13). Presence of Fragments for Interglycosidic Linkage (1↦2) in the Diglycoside **1**

Characteristic fragments	(1↦2) Linkage (rel. int. %)	Characteristic fragments	(1↦6) Linkage (rel. int. %)
$S+2H^+$	(1) +	$S+H^+$	—
$A+2H^+$	(71) +	$A+H^+$ PROMINENT .	—
$OS+H^+$	(1) +	OS^+ PROMINENT . .	—
$OS\text{-}MeOH^+$	(18) +	S+59	—

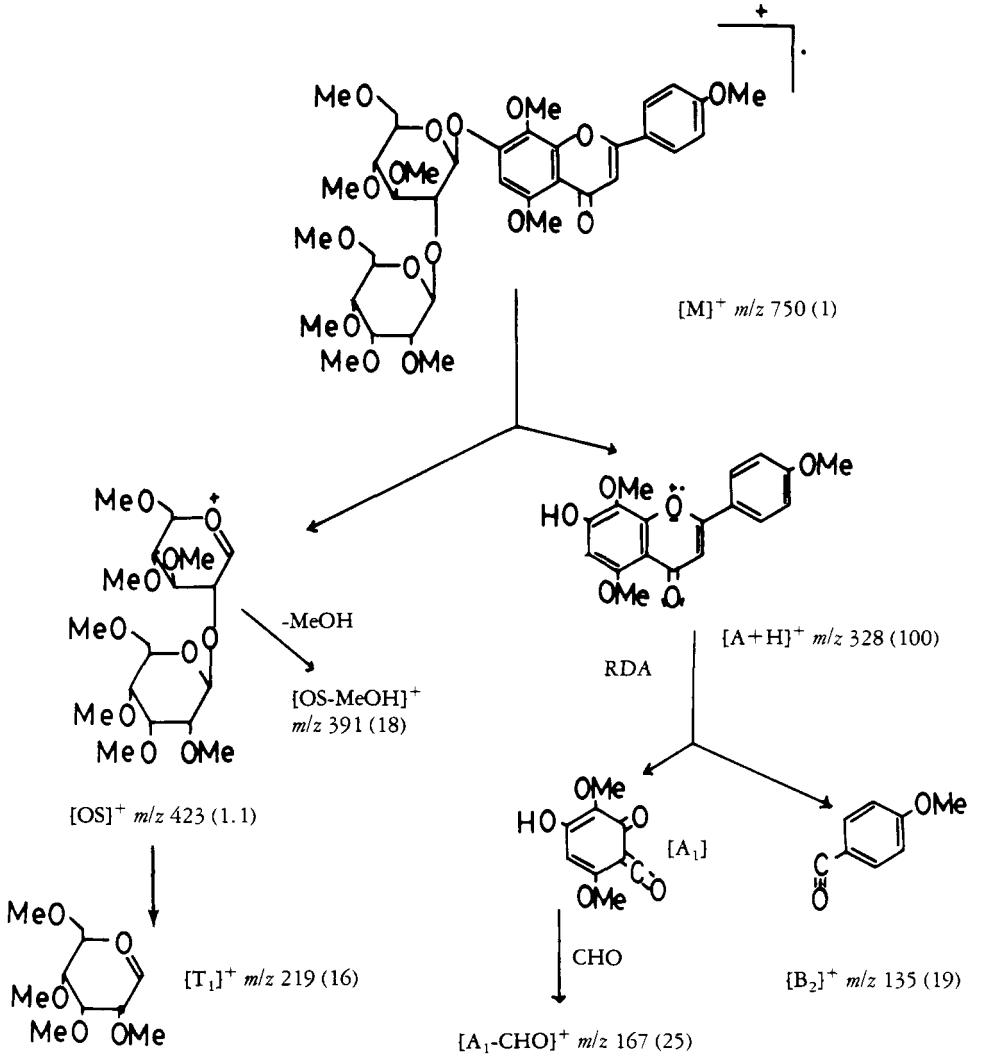

FIGURE 2. Eims fragmentation of the permethylated derivative of the natural glycoside **1**.

chromatographic comparisons against an authentic marker as isoscutellarein (5,7,8,4′-tetrahydroxyflavone). Its permethylated derivative coincided chromatographically in several systems with 5,7,8,4′-tetramethoxyflavone (16). This is in accordance with the well-known Wessely-Moser rearrangement that transforms 8-hydroxyflavones almost completely into 6-hydroxyflavones (17).

Enzymic hydrolysis to avoid the Wessely-Moser rearrangement yields exclusively isoscutellarein (**3**), and, thus, this is the naturally occurring aglycone.

The sugar sequence is evidenced by means of analysis of the sugar moiety present in the monoglycoside (**4**) obtained by mild acidic hydrolysis (Figure 1), which shows an Rf value on Whatman No 1 with 30% HOAc of 0.58 (the diglycoside occurs at Rf 0.82), this being glucose, the sugar directly linked to the aglycone, so allose is the terminal sugar (6).

The data presented here establish that the compound **1** is 5,7,8,4′-tetrahydroxyflavone-7-*O*-[allosyl(1→2)glucoside]. This is a novel, natural flavone glycoside. An acetylated derivative of this compound was isolated previously from *Veronica filiformis* in trace quantity (18), and this was the first time that allose was found as part of a

flavonoid diglycoside. In the last few years, three allosyl (1→2) glucosides of flavones have been isolated from *Sideritis* species (6,19,20).

EXPERIMENTAL

PLANT MATERIAL.—The material used was collected in southeastern Spain, near Santomera (Murcia), and a voucher specimen was deposited in the Department of Botany, Faculty of Sciences, Murcia University. The plant material was air-dried before extraction. Dried aerial parts (1 kg) were homogeneously powdered.

GENERAL EXPERIMENTAL PROCEDURES.—Spectra were recorded with the following instruments: uv, Pye Unicam SP8-100; ms, Hewlett & Packard 5993 GC-MS; adsorbants used for tlc were from Macherey-Nagel (polyamide DC-6) and from Carlo Erba (silica gel). Naturstoffreagenz-A (β-aminoethyl ester of diphenyl boric acid) and allose were from Fluka. Rapidase C-40 was from Societé Rapidase, Seclin, France.

EXTRACTION AND SEPARATION OF FLAVONOID (**1**).—The vegetal material was exhaustively extracted with cold EtOH-H_2O (7:3) until all pigments and other colored materials were removed. This hydroalcoholic extract was concentrated under reduced pressure until only the H_2O remained. The aqueous layer was extracted with Et_2O followed by EtOAc and finally *n*-BuOH. From the *n*-BuOH extract, three new flavonoid compounds were previously isolated (5,6), and in continuation with this work, a small quantity of a new glycoside has now been isolated by means of pc with Whatmann No 3 and 30% HOAc (Rf 0.65), and on Whatmann No 1 with *n*-BuOH-HOAc-H_2O (4:1:5, upper phase) (Rf 0.60). Final purification was carried out by tlc on polyamide with $CHCl_3$-iPrOH-MeCOEt-HOAc (10:3:3:4) (Rf 0.61). This compound is unstable in solution and on storage and oxidizes rapidly to black polymers; all operations were carried out as quickly as possible.

STRUCTURE ELUCIDATION.—Spectrophotometric vis-uv studies with the classical shift reagents (8-11), ms of the permethylated derivative of glycoside **1**, and ms of the aglycone **2** obtained by acidic hydrolysis aided the characterization. Natural glycoside (**1**) uv λ max (MeOH) nm: 366sh (0.35), 330sh (0.86), 306 (1.00), 277 (0.83); NaOMe: 382, 272, 250 (decomp.); $AlCl_3$: 424, 350, 323, 281; $AlCl_3$/HCl: 424, 348, 320, 281; NaOAc: 392, 335, 276 (decomp.). Acidic hydrolysis was carried out by means of 2 N aqueous HCl, 80°, 30 min. The hydrolysate was extracted with Et_2O; this extract was developed on pc (Whatmann No 1) with 60% HOAc, and two flavone aglycones (Wessely-Moser rearrangement) were obtained (Figure 1). Aglycone **2** (Rf 0.44); uv λ max MeOH nm: 336, 284; $AlCl_3$: 375, 304, 292sh, 262; $AlCl_3$/HCl: 364, 302, 292sh, 262sh; NaOAc: (decomp.). Ms of aglycone **2** (direct inlet 70 eV): M^+ 286 (83), M-H_2O^+ 268 (12), M-CHO^+ 257 (10), A_1^+ 168 (100), B_2^+ 121 (41), B_1+H^+ 119 (32), B_1^+ 118 (24). The permethylated derivative of this aglycone obtained by CH_2N_2 methylation coincided chromatographically with 5,6,7,4′-tetramethoxyflavone in several systems (16). Aglycone **3** (Rf 0.53); uv λ max MeOH nm: 364sh, 328sh, 305, 281; $AlCl_3$: 432, 360sh, 326, 299, 250sh; $AlCl_3$/HCl: 404, 347, 316, 286, 262sh; NaOMe: (decomp.). Its permethylated derivative obtained by CH_2N_2 methylation coincided chromatographically with 5,7,8,4′-tetramethoxyflavone in several systems. The aqueous phase was neutralized by means of IRA-410 and concentrated under reduced pressure (60°). Sugars were analyzed in this extract.

SUGAR IDENTIFICATION.—Sugars were identified by chromatographic comparisons against authentic markers. Allose shows Rf values very similar to those obtained for glucose in the usual systems in which hexoses are analyzed. Nevertheless, allose (Rf 0.44) clearly separates from glucose (Rf 0.33) on pc with phenol saturated with H_2O (18). The presence of allose and glucose was confirmed by gc on silicon column (180°) of their tetramethylsilyl derivatives (19), where β-D-allopyranose (Rt 1880) is clearly distinguished from β-D-glucopyranose (Rt 2024).

PERMETHYLATION OF GLYCOSIDE **1**.—To avoid alkaline decomposition of the diglycoside, a first CH_2N_2 methylation was carried out to block the free phenolic hydroxyls responsible for the high instability in alkaline media, and secondly, the permethylation was achieved in the usual way (21) with CH_3I and sodium hydride in DMF. The permethylated derivative showed bright blue fluorescence under uv light (360 nm). This derivative was purified by tlc on silica gel with $CHCl_3$-EtOAc-Me_2CO (5:4:1) (Rf 0.10), $CHCl_3$-Me_2CO (4:1) (Rf 0.27), $CHCl_3$-EtOAc-Me_2CO (5:1:4) (Rf 0.44). Ms of the permethylated derivative: M^+ 750 (1), S+2H^+ 517 (1), S+H^+ 516 (0.6), S^+ 515 (2), OS+H^+ 424 (1), OS^+ 423 (2), OS-MeOH^+ 391 (18), A+2H^+ 329 (71), A+H^+ 328 (100), A^+ 327 (18), A+H-Me^+ 313 (6), A+H-CHO^+ 299 (17), T_1^+ 219 (16), T_2^+ 187 (154), A_1-CHO^+ 167 (25), T_3^+ 155 (46), 143 (229), B_2^+ 135 (19), B_1+H^+ 133 (18), 127 (44), 111 (159), 101 (153).

ENZYMIC HYDROLYSIS.—A crude pectinase enzyme complex, Rapidase C-40, in acetate buffer 0.1

N pH 4.6, 48 h, 30°, was used. The aglycone obtained was extracted with Et_2O and purified in the same way as the acidic hydrolysis products.

MILD ACIDIC HYDROLYSIS.—This was achieved by 0.1 N aqueous HCl, 100°, 10 min (22) and rendered the monoglycoside **4** (Figure 1).

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