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FLAVONOID GLYCOSIDES AND A BITTER PRINCIPLE FROM *LOMATOGONIUM CARINTHIACUM*

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Abstract—Three flavone glycosides, including the novel 6-hydroxyluteolin 7-*O*-gentiobioside, and a bitter secoiridoid glycoside, swertiamarin, have been identified in the aerial parts of *Lomatogonium carinthiacum*. Four minor xanthone glycosides were also detected. Separations were made with droplet counter-current chromatography (DCCC) in combination with column chromatography. Structure elucidation was achieved by UV, ^{1}H and ^{13}C NMR, FDMS and D/CIMS spectra.

INTRODUCTION

Lomatogonium carinthiacum (Wulfen) Rchb., Syn: *Pleurogyne carinthiaca* (Wulfen) G. Don, (Gentianaceae) is an annual plant which is used in the Mongolian system of medicine against liver and bile diseases [1]. In previous studies [1–4], the occurrence of erythrocentaurin, xanthones and flavonoids such as luteolin, luteolin 7-*O*-glucoside, swertisin and 6-hydroxyluteolin 7-*O*-glucoside in the aerial parts has been reported. The present paper reports the results of a reinvestigation of *L. carinthiacum* collected in Switzerland where this plant is very rare. This study was undertaken in the course of our systematic screening of plants for xanthones with possible monoamine oxidase (MAO) inhibition activity [5, 6]. Xanthone glycosides could only be detected in trace amounts, but it was possible to isolate the previously undescribed 6-hydroxyluteolin 7-*O*-gentiobioside (**1**) along with known flavone glycosides (**2**, **3**) and swertiamarin (**4**).

RESULTS AND DISCUSSION

The crude methanol extract (6.4 g) from aerial parts of *L. carinthiacum* was submitted to droplet counter-current chromatography (DCCC) [7] with chloroform–methanol–propanol–water (5:6:1:4) in the descending mode and collected in several fractions. Glycosides **1** and **2** were obtained in a pure form after filtration over Sephadex LH-20 with methanol. The fraction containing compound **3** was purified by DCCC with chloroform–methanol–*iso*-propanol–water (4:6:1:4) in the descending mode. The bitter glycoside **4** was obtained after chromatography on silica gel with dichloromethane–methanol (82.5:17.5), followed by filtration over Sephadex LH-20 with methanol. Finally, a fraction from the initial DCCC separation afforded, after column chromatography on Sephadex and polyamide, a mixture of four xanthone glycosides which were determined by an HPLC-UV method.

The UV spectrum of compound **1** was characteristic of a 5,3′,4′-trihydroxyflavone with a hydroxyl or methoxyl group at position 6 [8, 9] and was identical to that of glycoside **2**. Acidic hydrolysis of **1** and **2** afforded the same aglycone 6-hydroxyluteolin [10, 11] and glucose. Comparison of the UV spectra of **1** and **2** before and after hydrolysis indicates that the site of glycosylation in both is at position 7. Furthermore, the UV spectra of **1** and **2** correspond in all respects with those of 6-hydroxyluteolin 7-*O*-glucoside, previously isolated from *L. carinthiacum* [4], and of a 6-hydroxyluteolin 7-*O*-diglucoside reported by Harborne and Williams [11] in the leaves of *Globularia cordifolia* (Globulariaceae). The underivatized glycosides **1** and **2** were submitted to field desorption MS (FDMS) [12] and to desorption/chemical ionization MS (D/CIMS) [13]. The FDMS spectrum of **1** shows signals at *m/z* 665, 649 (base peak) and 627 corresponding to the quasi-molecular ions $[M+K]^+$, $[M+Na]^+$ and $[M+H]^+$, respectively. A weak signal at *m/z* 487, $[M+Na-162]^+$, is due to the loss of a glucosyl unit. Signals at *m/z* 347 (4%), 325 (26%) and 302 (83%) correspond to the fragments produced by the loss of a diglucosyl unit, respectively $[M+K-324]^+$, $[M+Na-324]^+$ and $[M-324]^+$. In the D/CIMS spectrum of **1**, the base peak appears at *m/z* 303 corresponding to the loss of the diglucosyl sugar moiety $[M+H-324]^+$. For the quasi-molecular ion, only a weak peak could be observed at *m/z* 627 $[M+H]^+$. The structure of **1** was confirmed by ^{1}H and ^{13}C NMR [14–16]. The two glucopyranosyl units forming the sugar moiety attached at C-7 are linked in position $1 \rightarrow 6$. The C-6‴ signal (terminal glucose) appears at δ61.3 (*t*), whereas the chemical shift of C-6″ (inner glucose moiety) is at δ68.8 (*t*). Similar shifts have been observed in other gentiobiosides [17]. Thus, the structure of **1** is 6-hydroxyluteolin 7-*O*-gentiobioside which is described for the first time. Several 6-hydroxyluteolin 7-*O*-diglucosides have already been reported [11, 18], but in these compounds, the interglycosidic linkage was not determined.

All the spectral data (UV, ^{1}H NMR) of **2** correspond to 6-hydroxyluteolin 7-*O*-glucoside [4]. Further evidence for

this structure is given by the FDMS of **2**. Quasi-molecular ions are observed at m/z 487 $[M+Na]^+$ (base peak) and 465 $[M+H]^+$. Signals at m/z 325 $[M+Na-162]^+$ and 302 $[M-162]^+$ correspond to the loss of one glucosyl unit. Compound **3** was identified as isoorientin by comparison with an authentic sample [19].

Compound **4** was of bitter taste and identified as the secoiridoid glucoside swertiamarin [20]. Of particular interest was the D/CIMS (reactant gas NH_3) of this rather unstable glucoside. At m/z 392 and 375 appear the quasi-molecular ions $[M+NH_4]^+$ and $[M+H]^+$, respectively. Other fragments can be observed at m/z 212, 198 and 177 (base peak). Swertiamarin was not mentioned by Sorig *et al.* [1] in their study of *L. carinthiacum*, but these authors reported the occurrence of erythrocentaurin (MW 176). This compound could not be found during the present investigation and it was most likely an artifact (derived from swertiamarin) formed during the extraction procedure.

Xanthone glycosides, characteristic constituents of most gentianaceous plants, were also detected in *L. carinthiacum*. However, isolation and structure elucidation could not be achieved, due to the scarcity of plant material. A mixture of these xanthones (**5–8**) was analysed by HPLC-UV. The spectra of **5–8** are typical of xanthones and are given in the Experimental.

The reinvestigation of the polar compounds of *L. carinthiacum* afforded the new flavone glycoside 6-hydroxyluteolin 7-*O*-gentiobioside **1**. Although numerous flavonoids have been identified in the Gentianaceae, 6-hydroxyflavones have only been reported from the genus *Lomatogonium*. This could be of taxonomic significance since 6-hydroxyflavones are supposed to be phyletic markers and present only in highly evolved families, e.g. from the Tubiflorae group [11]. *L. carinthiacum*, first described as *Swertia carinthiaca*, is botanically close to *Swertia* and considered to be evolved from this genus [21]. This can be underlined by the fact that swertiamarin (very common in *Swertia* species) is the major bitter principle of *L. carinthiacum*, whereas gentiopicrin (characteristic of *Gentiana* species) could not be detected.

EXPERIMENTAL

Plant material. L. carinthiacum was collected in August 1981 at Avers, GR, Switzerland, alt. 2200 m, by E. Anchisi, Alpine Botanical Garden of Champex, VS, Switzerland. Voucher specimens are deposited in the Herbarium of the Musée Botanique de l'Univ. de Lausanne (Pharma. 81–01).

General techniques. TLC was carried out on pre-coated silica gel 60-F254 aluminium sheets (Merck) with EtOAc–MeCOEt–HOAc–H_2O (50:30:10:10) (system 1a) or $CHCl_3$–Me_2CO–H_2O (20:80:5) (system 1b), on pre-coated polyamide 11 plates (Macherey-Nagel) with MeOH 90% (system 2) and on pre-coated cellulose F254 aluminium sheets (Merck) with HOAc 15% (system 3a) or HOAc 30% (system 3b). Column chromatography (CC) was achieved on polyamide SC 6, particle size less than 0.07 mm (Macherey-Nagel) with MeOH–H_2O in different concns and on Sephadex LH-20 (Pharmacia) using MeOH as eluent. Hydrolyses and recording of the UV spectra with the usual shift reagents were made according to standard procedures [22]. Sugars were analysed by TLC on silica gel with EtOAc–H_2O–MeOH–HOAc (65:15:15:20) and visualized by spraying with *p*-anisidine phthalate. ^1H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 with TMS as internal standard on a Bruker WP-200 instrument at 200 MHz and 50, 29 MHz, respectively. FDMS spectra were produced on a Varian MAT 371 spectrometer equipped with a SS-200 data system. D/CIMS spectra were recorded on a Ribermag R10–10B quadrupole spectrometer with a SIDAR data system.

Isolation and identification. The crude MeOH extract (6.4 g) was submitted to DCCC with $CHCl_3$–MeOH–PrOH–H_2O (5:6:1:4) in the descending mode (286 columns, 40 cm × 3.4 mm, flow 50 ml/hr, instrument DCCC-S, Tokyo Rikakikai, Japan). The eluate was collected in ten fractions. Fraction 2 (166 mg) was purified over Sephadex (MeOH) and polyamide (40% aq. MeOH to 100% MeOH) and yielded 5 mg of a mixture of four xanthone glycosides **5–8**. Fraction 4 (1451 mg) was submitted to preparative liquid chromatography (prep. LC) with axial compression of the column (Chromatospac Prep 10, Jobin-Yvon, France): silica gel G60, 15 μm (Merck), column 40 cm × 4 cm, solvent CH_2Cl_2–MeOH, 82.5:17.5. Pure swertiamarin **4** (777 mg) was obtained after filtration over Sephadex LH-20. Fraction 5 was concd under red. pres. and the ppt. formed was crystallized from MeOH to afford 18 mg of **2**. Fraction 6 (200 mg) was submitted to a second DCCC separation with $CHCl_3$–MeOH–*iso*-PrOH–H_2O (4:6:1:4) in the descending mode (283 columns 40 cm × 2.7 mm, flow 50 ml/hr, instrument DCC Chromatograph 670, Büchi, Switzerland). Pure isoorientin **3** (4.5 mg) was obtained after purification over polyamide with 70% aq. MeOH. Finally, the last fraction (400 mg) from the first DCCC separation afforded, after chromatography on Sephadex LH 20 with MeOH, 23 mg of the new glycoside **1**: yellow powder; mp 215–218°; R_f 0.14 (system 1a), 0.49 (system 2) and 0.22 (system 3b). Acidic hydrolysis afforded glucose and 6-hydroxyluteolin. UV λ_{max} nm: (MeOH) 257 sh, 273, 294 sh, 340; (NaOMe) 250, 393; (NaOAc) 265, 336, 394; ($AlCl_3$) 277, 313, 440; ($AlCl_3$–HCl) 264 sh, 278, 308, 357. The ^1H NMR and the ^{13}C NMR data of **a** 1 and **2** supported a 6-hydroxyluteolin 7-*O*-glycoside structure [14–16]. MS data and the determination of the interglycosidic linkage of **1** are discussed above. R_f values and spectral data of **2–4** were in accordance with published data or with authentic samples. The xanthone glycosides **5–8** were separated by HPLC, coupled with a Photodiode Array Detection System HP 1040 A (Hewlett Packard), on a HP-C_8 column, particle size 5 μm (10 cm × 4.6 mm), with MeOH–H_2O gradient 20:80 to 80:20 in 15 min. UV spectra of the separated compounds could directly be obtained. $\lambda_{max}^{MeOH+H_2O}$ nm: 237, 246, 277, 309, 376 (**5**); 239, 266, 312, 378 (**6**); 234, 259, 302, 374 (**7**); 239, 253, 306, 358 (**8**). Hydrolysis of the xanthone mixture afforded glucose and xylose. The aglycones have not yet been identified.

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