Three New Dihydroisocoumarins from the Greek Endemic Species *Scorzonera cretica*[1]

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Extracts of the Greek endemic species *Scorzonera cretica* afforded three new compounds, the dihydroisocoumarin scorzocreticin (**1**) and its glycosides, scorzocreticoside I (**2**) and scorzocreticoside II (**3**), as well as 11 known compounds. The structures of the isolated compounds were elucidated on the basis of spectral data and chemical methods. The absolute configurations of **1**−**3** were established using circular dichroism.

The genus *Scorzonera* (Compositae) includes 28 European species distributed all over the continent, from northern Russia to Spain and Crete.[2] Eleven of the European species are found in Greece, four of which, as well as one subspecies, are endemic.[2] *Scorzonera cretica* Willd. is endemic to Crete and the South Aegean region.[2] It is a plant commonly used in traditional Cretan cuisine as an ingredient in savory meat dishes. No previous chemical work on the species has been recorded, and only a few articles on other members of the genus *Scorzonera* have appeared.[3−6] In this paper we report the isolation and structure elucidation of three new compounds, 6,8-dihydroxy-3-(4-methoxyphenyl)isochroman-1-one (scorzocreticin, **1**), 8-*O*-*β*-D-glucopyranosylscorzocreticin (scorzocreticoside I, **2**), and 8-*O*-[α-L-rhamnopyranosyl(1→6)-*β*-D-glucopyranosyl]scorzocreticin (scorzocreticoside II, **3**), and the identification of 11 known compounds.

Scorzocreticin (**1**) was obtained as a yellowish powder from the MeOH extract of *S. cretica*. Its molecular formula was determined by HRFABMS as $C_{16}H_{14}O_5$, and the UV spectrum was suggestive of a 6,8-dihydroxylated dihydroisocoumarin derivative.[7] The ^{1}H NMR spectrum showed a proton (*δ* 5.42, H-3) coupled with two methylene protons (*δ* 2.94, 3.19, H-4), a methoxy group at 3.77 ppm, and two meta-coupled aromatic protons on a disubstituted A-ring (*δ* 6.18, H-5 and *δ* 6.26, H-7). The sharp ^{1}H NMR signal at 11.04 ppm indicated the presence of one hydrogen-bonded hydroxyl group. Additionally, the presence of a pair of 2H ortho-coupled aromatic protons (*δ* 6.88, H-2′,6′ and *δ* 7.32, H-3′,5′) suggested a disubstituted B-ring. The ^{13}C NMR spectrum showed the presence of one carbonyl group (*δ* 169.9, C-1) characteristic of dihydroisocoumarins.[7,8] Moreover, the ^{13}C NMR and DEPT spectra revealed the presence of 12 aromatic carbons (three quaternary, three quaternary oxygenated, and six protonated), one methylene, one oxygenated methine, and one methoxy group. In the HMBC spectrum, a ^3J correlation between the methylene protons and C-5 (*δ* 106.9) confirmed the dihydroisocoumarin skeleton. The connectivity of the B-ring to the benzopyranone unit was indicated from the HMBC spectrum by the correlations of C-3 (*δ* 80.1) with H-2′ and H-6′. The protons of the methoxy group were correlated with C-4′ (*δ* 159.8). The attachment site of the methoxy group was confirmed by its ^5J correlations with H-3′ and H-5′ observed in the COSY-LR spectrum. The absolute configuration at C-3 was determined by comparison of the CD spectrum with that of mellein (**4**), which has also been used as reference for other dihydroisocoumarins.[8−10] Scorzocreticin (**1**) gave a positive Cotton effect at 233 nm and a negative Cotton effect at 255 nm, as in the case of **4**.[11] This suggested that the two compounds possess the same absolute configuration at C-3, which in the case of **1** is *S*.

Scorzocreticoside I (**2**) was isolated as a yellowish powder, and its molecular formula was determined by HRFABMS as $C_{22}H_{24}O_{10}$. Its UV spectrum indicated a different substitution pattern in the aromatic rings compared with that of **1**. The data observed in the ^{1}H and ^{13}C NMR spectra showed a strong similarity with **1** but also indicated the presence of a sugar moiety. The anomeric proton at *δ* 4.81 (d, $J = 7$ Hz) and the splitting pattern of the other sugar protons gave evidence of a *β*-glucose unit. The attachment site of glucose was indicated by the ^3J correlation between the anomeric proton and C-8 (*δ* 163.2) in the HMBC spectrum. The carbon at position C-8 was identified by its ^2J correlation with only one aromatic proton [H-7 (*δ* 6.79)], while C-6 (*δ* 165.8) showed two ^2J correlations with both H-5 and H-7. Furthermore, the disappearance of the hydrogen-bonded hydroxyl signal indicated the absence of a free hydroxyl group on C-8. Acid hydrolysis of **2** afforded **1** and glucose. The specific rotation of glucose revealed that it was a D sugar. Consequently, compound **2** was assigned as 8-*O*-*β*-D-glucopyranosylscorzocreticin. The CD spectrum of **2** was similar to that of **1**, which indicated that the absolute configuration at C-3 is identical to **1**.

The third new dihydroisocoumarin, scorzocreticoside II (**3**), was isolated as a yellowish powder, and its molecular formula was determined by HRFABMS as $C_{28}H_{34}O_{14}$. The UV spectrum was similar to that of **2**. The ^{1}H NMR spectrum of **3** was comparable to that of **2**, but the presence of two anomeric protons at *δ* 4.84 (H-1″ of glucose) and *δ* 4.75 (Ç-1‴ of rhamnose) was indicative of a disaccharide moiety in **3**.[12,13] The attachment site of glucose to the aglycon was determined in a similar way to **2**. The numerous signals between 3.37 and 4.02 ppm corresponded to the protons of the two sugars in **3**. The ^{13}C NMR spectrum confirmed the presence of an additional sugar in comparison to **2** (26 peaks in **3** compared to 20 peaks in **2**). The characteristic downfield shift of C-6″ of glucose (*δ* 67.6) indicated that a rhamnose unit was attached to this position. This was confirmed by the ^3J correlation of H-1‴ with C-6″ in the HMBC spectrum. Acid hydrolysis of **3** afforded **1**, glucose, and rhamnose. The specific rotation of glucose revealed that it was a D sugar, and that of

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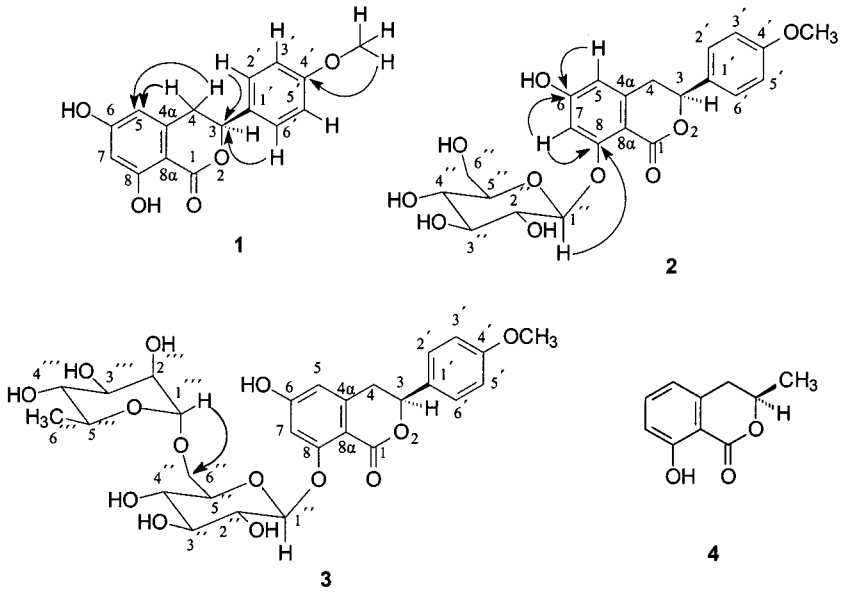

Figure 1. Structures of **1**−**4** and selected HMBC correlations.

Table 1. ^{13}C ($CDCl_3$/TMS for **1**, CD_3OD/TMS for **2** and **3**, 50 MHz, δ ppm) and ^{1}H ($CDCl_3$/TMS for **1**, CD_3OD/TMS for **2** and **3**, 400 MHz, δ ppm, J in Hz) NMR Data for **1** ($CDCl_3$), **2**, and **3** (CD_3OD)

	1		**2**		**3**	
position	δ_C	δ_H (J Hz)	δ_C	δ_H (J Hz)	δ_C	δ_H (J Hz)
aglycon						
1	169.9		166.4		166.4	
3	80.1	5.42 dd (13, 2)	80.8	5.38 dd (13, 2)	80.8	5.37 dd (13, 2)
4	35.1	2.94 dd (16, 2)	37.2	2.96 dd (16, 2)	37.2	2.96 dd (16, 2)
		3.19 dd (16, 13)		3.19 dd (16, 13)		3.20 dd (16, 13)
4α	141.4		145.5		145.5	
5	106.9	6.18 d (2)	110.4	6.46 d (2)	110.5	6.47 d (2)
6	164.3		165.8		165.9	
7	101.6	6.26 d (2)	105.6	6.79 d (2)	105.3	6.70 d (2)
8	164.1		163.2		163.0	
8α	102.4		107.0		106.9	
1′	130.2		131.9		131.9	
2′	127.6	7.32 d (9)	129.0	7.38 d (9)	129.0	7.39 d (9)
3′	114.0	6.88 d (9)	115.0	6.93 d (9)	115.0	6.94 d (9)
4′	159.8		161.5		161.4	
5′	114.0	6.88 d (9)	115.0	6.93 d (9)	115.0	6.94 d (9)
6′	127.6	7.32 d (9)	129.0	7.38 d (9)	129.0	7.39 d (9)
8-OH		11.04 s				
4′-OCH_3	55.4	3.77 s	55.8	3.79 s	55.8	3.79 s
glucose						
1″			105.1	4.81 d (7)	104.6	4.84 d (7)
2″			74.9	3.51 dd (9.5, 7)	74.9	3.49 dd (9.5, 7)
3″			77.1	3.52 t (9.5)	77.4	3.50 t (9.5)
4″			71.2	3.44 t (9.5)	71.1	3.42 t (9.5)
5″			78.7	3.48 m	77.1	3.59 m
6″			62.5	3.75 dd (11, 5) 3.95 dd (11, 2)	67.6	3.69 dd (11, 5) 4.02 dd (11, 2)
rhamnose						
1‴					102.3	4.75 d (2)
2‴					72.1	3.89 m
3‴					72.3	3.71 m
4‴					74.1	3.37 d (9.5)
5‴					69.8	3.69 m
6‴					17.9	1.22 d (6)

rhamnose revealed that it was an L sugar. Consequently, compound **3** was assigned as 8-*O*-[α-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranosyl]scorzocreticin. The absolute configuration of **3** at C-3 was *S* also, which was proven by the similarity of the CD spectrum of **3** to that of **1**.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 341 polarimeter. UV spectra were recorded in spectroscopic grade MeOH on a Shimadzu-160A spectrophotometer. A JASCO J-715 spectropolarimeter was used for the circular dichroism spectra. IR spectra were taken on a Perkin-Elmer Paragon 500 instrument. ^{1}H NMR spectra were measured on a Bruker DRX-400 (400 MHz) spectrometer and ^{13}C NMR on a Bruker AC-200 (50 MHz) spectrometer. Chemical shifts are given in δ values with TMS as an internal standard. Coupling constants (J) are given in Hz. The signals in the ^{1}H and ^{13}C NMR spectra were assigned unambiguously using 2D NMR techniques (COSY, COSY-LR, HMQC, and HMBC). These 2D experiments were

performed using standard Bruker microprograms. EIMS and HRFABMS were obtained on an HP-6890 and an AEI MS-902 mass spectrometer, respectively. Column chromatography was conducted using Si flash gel 60 Merck (40−63 μm), with an overpressure of 300 mbar. Medium-pressure liquid chromatography (MPLC) was performed with a Büchi model 688 apparatus on columns containing RP-18 Si gel 60 Merck (20−40 μm).

Plant Material. *Scorzonera cretica* was collected in Agia Galini (Crete, Heraklion region) in May 1999. The plant was in full bloom. A voucher specimen (KL080) has been deposited in the herbarium of the Laboratory of Pharmacognosy, University of Athens, Greece.

Extraction and Isolation. The whole plant (1.5 kg), after being air-dried and pulverized, was extracted with CH_2Cl_2 (3 × 2 L) and then with MeOH (3 × 2 L). The extracts were evaporated to dryness and then subjected to vacuum liquid chromatography over Si gel 60 Merck (40−63 μm) with increasing polarity mixtures of CH_2Cl_2−MeOH. The CH_2Cl_2 fractions were further purified by flash column chromatography over Si gel with a CH_2Cl_2−MeOH gradient. Ten triterpenoids were isolated and identified on the basis of ^{1}H and ^{13}C NMR spectral data, as well as MS and optical rotation data (oleanol acetate,[14] germanicol acetate,[14] lupeol acetate,[15] germanicone,[14,15] lupenone,[15] oleanol,[14] germanicol,[14] lupeol,[15] taraxasterol acetate,[15] and taraxasterol[15]). The fractions eluted with MeOH were purified by MPLC [RP-18 Si gel 60 Merck (20−40 μm), H_2O−MeOH gradient] and afforded 3-*O*-β-D-glucopyranosylsitosterol,[16] the dihydroisocoumarin scorzocreticin (**1**, 20.0 mg), and its two glycosides, scorzocreticoside I (**2**, 35.0 mg) and scorzocreticoside II (**3**, 72.3 mg).

Scorzocreticin (1): yellowish powder; $[\alpha]^{20}_D$ +23.3° (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 219 (4.14), 269 (3.84), 303 (3.50) nm; CD $\Delta\epsilon$ +35 (233 nm), −8 (255 nm) (*c* 5 × 10^{-6}, dioxane); IR ν_{max} 3300, 1665 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1; HRFABMS *m*/*z* [M + 1]$^+$ 287.0915 (calcd for $C_{16}H_{14}O_5$, 287.0919); EIMS *m*/*z* 286 [M$^+$] (100), 268 (69), 240 (78), 225 (26), 197 (32).

Scorzocreticoside I (2): yellowish powder; $[\alpha]^{20}_D$ −17.65° (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ϵ) 225 (4.29), 267 (3.75) nm; CD $\Delta\epsilon$ +24 (233 nm), −4 (255 nm) (*c* 5 × 10^{-6}, MeOH); IR ν_{max} 3314, 1682 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1; HRFABMS *m*/*z* [M + 1]$^+$ 449.1444 (calcd for $C_{22}H_{24}O_{10}$, 449.1448).

Scorzocreticoside II (3): yellowish powder; $[\alpha]^{20}_D$ −28.32° (*c* 0.4, MeOH); UV (MeOH) λ_{max} (log ϵ) 210 (4.14), 267 (3.75) nm; CD $\Delta\epsilon$ +24 (233 nm), −4 (255 nm) (*c* 5 × 10^{-6}, MeOH); IR ν_{max} 3317, 1682 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1; HRFABMS *m*/*z* [M + 1]$^+$ 595.2022 (calcd for $C_{28}H_{34}O_{14}$, 595.2027).

Acid Hydrolysis of 2 and 3. A solution of **2** (15 mg) or **3** (20 mg) in HCl (2 N) was refluxed for 20 min under an Ar atmosphere. On cooling, the reaction mixture was neutralized with Amberlite IR-50 resin. The solvent was removed under reduced pressure, and the residue was chromatographed by MPLC [RP-18 Si gel 60 Merck (20−40 μm), H_2O−MeOH gradient] to afford, in the case of **2**, D-glucose {$[\alpha]^{20}_D$ +52° (*c* 0.1, H_2O)} and **1**, and in the case of **3** D-glucose {$[\alpha]^{20}_D$ +52° (*c* 0.1, H_2O)}, L-rhamnose {$[\alpha]^{20}_D$ +8° (*c* 0.1, H_2O)}, and **1**.

References and Notes

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