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Complete isolation and characterization of silybins and isosilybins from milk thistle (*Silybum marianum*) †

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Complete separation, isolation, and structural characterization of four diastereoisomeric flavonolignans, silybins A (**1**) and B (**2**), and isosilybins A (**3**) and B (**4**) from the seeds of milk thistle (*Silybum marianum*) were achieved for the first time using a preparative reversed-phase HPLC method. In addition, three other flavonolignans, silychristin (**5**) isosilychristin (**6**) and silydianin (**7**), and a flavonoid, taxifolin (**8**) were isolated. Structures, including absolute stereochemistries of **1**–**4**, were confirmed using 2D NMR and CD spectroscopy.

Introduction

It has long been recognized that fruits and/or seeds of the plant milk thistle [*Silybum marianum* (L.) Gaertn. (Asteraceae)] have hepatoprotective activity.[1] Previously, numerous studies have been conducted on the isolation of the hepatoprotective principles from this plant.[2–4] The presence of flavonoid-type compounds in the fruits of *S. marianum* was recognized in the early 1950's.[5] By the 1960's, two phenolic compounds, namely, *Silybum* substances E_5 and E_6, were isolated and identified as flavones.[6] Subsequently, a hepatoprotective principle was isolated, identified, and named as silymarin or silybin,[7–9] and it turned out to be the same compound as *Silybum* substance E_6.[10–12] The structure of silybin was proposed as a coupling product of a flavonoid, taxifolin and a lignan, coniferyl alcohol.[11] Later, it was reported that silymarin was a mixture of several flavonolignans.[2] Together with silybin, two additional flavonolignans, silychristin and silydianin, have been identified as the three major compounds from *S. marianum*, and the structures of these compounds were determined by several spectrometric and spectroscopic methods including ^{1}H and ^{13}C NMR, and X-ray crystallography.[2,10,11,13–18] Other flavonolignans including isosilychristin, silymonin, silandrin, silyhermin, and neosilyhermins A and B have been isolated also as minor constituents of the same and/or a different variety of *S. marianum*.[19–24] The structure of silybin was determined by spectroscopic methods,[2,10,11,18] degradation[25] and synthetic approaches.[26–28] During the isolation and structure determination of silybin, its regioisomer isosilybin was isolated and purified.[2,11,28–31] Furthermore, it was recognized that silybin itself exists as two diastereomeric forms, namely, silybins A and B, which differ in stereochemistries at positions C-α and C-β in the lignan moiety.[25] Similarly, isosilybin exists as the same type of two different stereoisomeric forms, isosilybins A and B. The relative stereochemistry of silybin was reported by X-ray crystallography, however, it was determined using a 1 : 1 diastereomeric mixture.[32] Several analytical methods have been developed for the analysis of these flavonolignans, especially silybins A and B, and isosilybins A and B by HPLC, from milk thistle plant extracts,[33–39] plasma samples for pharmacokinetic studies,[40–43] or different milk thistle species and different cultivation conditions.[44–46] Recently, Křen *et al.* reported obtaining pure silybins A and B by glycosylation of the silybin mixtures followed by isolation *via* HPLC and acid hydrolysis.[47,48] However, thus far, it seems apparent that the isolation of diastereomeric silybin and/or isosilybin isomers (silybins A and B, and isosilybins A and B) as pure forms from natural sources in substantial yields has not been achieved. Thus, to date it has not been possible to conduct chemical and/or biological studies using stereochemically and/or regioselectively pure forms of silybins A and B, and/or isosilybins A and B. Herein, we report the first complete isolation of silybin and isosilybin stereoisomers, silybins A and B, and isosilybins A and B (**1**–**4**, Scheme 1) from a crude MeOH extract of milk thistle seeds using preparative HPLC. Three other major flavonolignans, silychristin, isosilychristin, and silydianin (**5**–**7**), and a flavonoid, taxifolin (**8**) were isolated as well. Since this is the first description of silybins and isosilybins in stereochemically pure forms from a natural source, complete structure elucidation data are discussed including absolute stereochemistries and conformational analysis *via* CD.

Results and discussion

The compounds were isolated by repetitive precipitation and column chromatographies, and preparative HPLC was used for the final purification.

ESMS of compound **1** showed the pseudomolecular ion peak at *m/z* 481 $[M - 1]^-$, which is consistent with the formula of $C_{25}H_{22}O_{10}$. In the ^{1}H and ^{13}C NMR spectra, an exchangeable proton signal at δ 11.89 (5-OH), a carbonyl carbon signal at δ 197.6 (C-4), and signals at δ 5.06/82.6 (C-2), 4.60/71.4 (C-3), 163.3 (C-5), 5.89/96.1 (C-6), 167.1 (C-7), and 5.84/95.1 (C-8) represented the presence of a dihydroflavonol moiety with C-5 and C-7 positions that were hydroxylated.[49] Two AB systems at δ 5.06 and 4.60 indicated the characteristic axial conformation of H-2 and H-3 from the observation of large coupling constants (J = 11.3 and 11.1 Hz for H-2 and H-3, respectively).[49] NMR signals at δ 7.07/116.5 (C-2′), 6.96/116.3 (C-5′), and 7.01/121.3 (C-6′), and two quaternary carbon NMR signals at

† Electronic supplementary information (ESI) available: HPLC chromatograms of isolates and extracts. See http://www.rsc.org/suppdata/ob/b3/b300099k/

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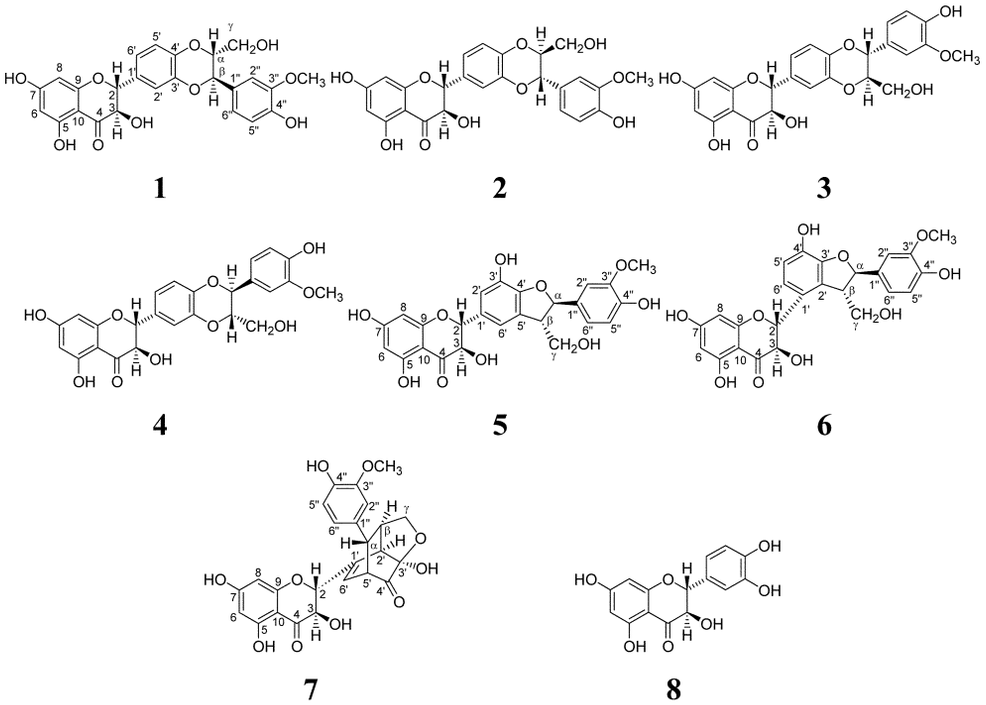

Scheme 1 Structures of isolates.

δ 143.3 (C-3′) and 143.7 (C-4′) suggested a double-oxygenated aromatic C-ring. HMBC correlations of proton signals H-2′ and H-6′, and other HMBC correlations (see Experimental section) confirmed the dihydroflavonol moiety as taxifolin. NMR signals from another aromatic group were observed at δ 127.5 (C-1″), 6.99/111.8 (C-2″), 147.6 (C-3″), 147.0 (C-4″), 6.79/115.3 (C-5″), and 6.85/120.5 (C-6″). From the multiplicities and several HMBC correlations, it was postulated that two of the positions were substituted with a methoxy group (δ 3.76/55.7) and a hydroxy group. The position of the methoxy group at C-3″ was determined from HMBC, where a methoxy singlet proton signal at δ 3.76 had a long-range correlation with a carbon signal at δ 147.6 (C-3″). In the ^{1}H NMR spectrum, two oxygenated methine proton signals at δ 4.16 (H-α) and 4.89 (H-β) were observed, and the latter was correlated with three aromatic carbon signals at δ 127.5 (C-1″), 111.8 (C-2″), and 120.5 (C-6″), and this suggested that the aromatic group was connected to the methine carbon C-β. Long range HMBC cross correlations were observed between these methine protons and other methine carbons [δ 4.89 (H-β) and δ 78.1 (C-α), and H-α (δ 4.16) and δ 75.9 (C-β)]. In the ^{1}H NMR spectrum, the coupling constant of H-β (7.9 Hz) suggested that the two methine protons could be in as a *trans* configuration.[50,51] Proton signals of a hydroxymethylene moiety at δ 3.52 and 3.33 (H-γ) were observed, and one of them (δ 3.52) was long-range correlated with a methine carbon signal at δ 75.9 (C-β). These observations indicated the presence of a lignan moiety, and this was confirmed by a TOCSY experiment where proton signals H-2″, H-5″, and H-6″ were correlated with H-α and H-β to become a network. The connectivity between the lignan and flavonoid moieties was verified from the HMBC correlations, wherein the methine protons at δ 4.16 (H-α) and 4.89 (H-β) were three-bond, long-range correlated with oxygenated aromatic carbon signals at δ 143.7 (C-4′) and 143.3 (C-3′), respectively. These correlations strongly suggested the presence of a hydroxymethylene moiety at C-α and a 4-hydroxy-3-methoxyphenyl moiety at C-β, and these HMBC correlations were achieved by the optimization of the coupling constant [$^{2,3}J$ (C,H)] to 2.0 Hz. The absolute stereochemistries of the C-2, C-3, C-α, and C-β positions were determined by CD spectra (Fig. 1) with the comparison of model compounds.[52–55] Upon comparison with the result with the benzodioxane moiety in the model compound, a

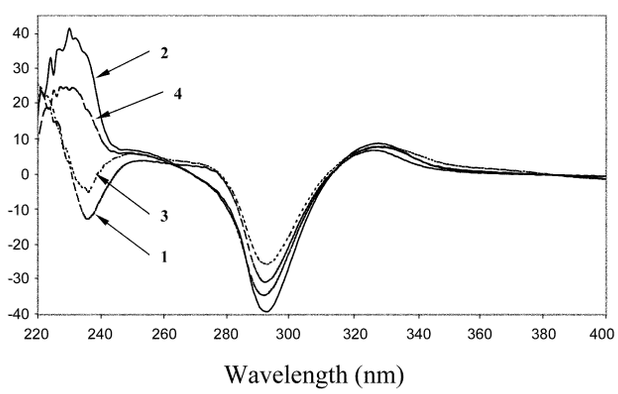

Fig. 1 CD spectra of compounds **1**–**4**.

positive Cotton effect ($\Delta\varepsilon$ +4.9) at the peak of 326 nm and a negative Cotton effect ($\Delta\varepsilon$ −22.4) at the peak of 292 nm for a dihydroflavonol moiety represented the 2*R* and 3*R* configuration while a negative Cotton effect ($\Delta\varepsilon$ −13.9) at the peak of 236 nm for the benzodioxane moiety represented the α*S* and β*S* configuration.[52–55] The energy-minimized structure of compound **1** showed a good agreement with these observations. Therefore, the structure and the absolute stereochemistry of compound **1** were determined as silybin A {(2*R*,3*R*)-3,5,7-trihydroxy-2-[(2*S*,3*S*)-3-(4-hydroxy-3-methoxyphenyl)-2-hydroxymethyl-2,3-dihydrobenzo[1,4]dioxin-6-yl]-chroman-4-one}.

ESMS of the compound **2** showed the pseudomolecular ion peak at *m*/*z* 481 $[M - 1]^-$, which is consistent with the formula of $C_{25}H_{22}O_{10}$ as in compound **1**. Analogous to compound **1**, compound **2** showed similar ^{1}H and ^{13}C NMR signals and 2D NMR correlations. However, unlike compound **1**, a positive Cotton effect ($\Delta\varepsilon$ +8.4) was observed at the peak of 230 nm (Fig. 1). Incorporating these data into the results from the model compounds,[52–55] the stereochemistries of the C-α and C-β positions of compound **2** should be *R* and *R*, respectively. Therefore, the structure of compound **2** was determined as silybin B {(2*R*,3*R*)-3,5,7-trihydroxy-2-[(2*R*,3*R*)-3-(4-hydroxy-3-methoxyphenyl)-2-hydroxymethyl-2,3-dihydrobenzo[1,4]-dioxin-6-yl]-chroman-4-one}.

ESMS of compound **3** showed a pseudomolecular ion peak at *m*/*z* 481 $[M - 1]^-$, which is consistent with the formula of $C_{25}H_{22}O_{10}$ as observed in both compounds **1** and **2**. The ^1H and ^{13}C NMR spectra are similar to those of compounds **1** and **2**. However, in the HMBC spectrum, a methine proton signal at δ 4.15 (H-β), adjacent to a hydroxymethylene moiety, correlated with a carbon signal at δ 143.2 (C-3′), while the other methine proton at δ 4.90 (H-α), adjacent to an aromatic group, correlated with a carbon signal at δ 143.6 (C-4′). Opposite to the previous cases for compounds **1** and **2**, these observations suggest connectivities of the hydroxymethylene moiety to the C-β position and the 4-hydroxy-3-methoxyphenyl moiety to the C-α position. The *trans* configuration of H-α and H-β was identified by coupling constant ($J = 7.8$ Hz). In the CD spectrum (Fig. 1), a negative Cotton effect was observed ($\Delta\varepsilon$ −4.2) at 236 nm, which represented the configuration of C-α and C-β positions as *R* and *R*, respectively. This observation indicated that compound **3** is a regioisomer of compound **1**. Therefore, the structure of compound **3** was determined as isosilybin A {(2*R*,3*R*)-3,5,7-trihydroxy-2-[(2*R*,3*R*)-2-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-2,3-dihydrobenzo[1,4]dioxin-6-yl]-chroman-4-one}.

As with the compounds described previously, compound **4** showed the same pseudomolecular ion peak at *m*/*z* 481 $[M - 1]^-$ in the ESMS spectrum, which is consistent with the formula of $C_{25}H_{22}O_{10}$. While similar chemical shifts in the ^1H and ^{13}C NMR spectra were observed, two HMBC correlations were different compared to those seen in compounds **1** and **2**. As described above in **3**, a methine signal at δ 4.90 (H-α), adjacent to a hydroxymethylene moiety correlated with a quaternary aromatic carbon signal at δ 143.6 (C-4′). Another methine proton signal at δ 4.15 (H-β), adjacent to the 4-hydroxy-3-methoxyphenyl moiety, correlated with another quaternary aromatic carbon signal at δ 143.2 (C-3′). These observations suggested that the hydroxymethylene and 4-hydroxy-3-methoxyphenyl moieties were connected in the same fashion as described in compound **3**. However, a positive Cotton effect ($\Delta\varepsilon$ +6.4) at 231 nm in the CD spectrum (Fig. 1) indicated that the configurations of the C-α and C-β positions were opposite to those of compound **3**, but the same as silybin B (**2**), which gave the configurations as *S* and *S*, respectively. Accordingly, compound **4**, the diastereoisomer of compound **3**, has the structure of isosilybin B {(2*R*,3*R*)-3,5,7-trihydroxy-2-[(2*S*,3*S*)-2-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-2,3-dihydrobenzo-[1,4]dioxin-6-yl]-chroman-4-one}.

Structures and stereochemistries of silychristin (**5**), isosilychristin (**6**), silydianin (**7**), and taxifolin (**8**) were determined by the comparison with published data.[11,13,14,20,21,56–59]

Experimental

General experimental procedures

Melting points were determined with a Bristoline hot-stage instrument and are uncorrected. Optical rotations were measured with a Rudolph Autopol IV polarimeter at 25 °C and are given in units of 10^{-1} deg cm^2 g^{-1}. UV spectra were recorded on Varian Cary 3G spectrometer and CD spectra were recorded on a Aviv Circular Dichroism Stopped Flow Model 202. IR spectra were recorded on a Nicolet Avatar 360 FT-IR (KBr) spectrometer. NMR spectra were recorded in DMSO-d_6 using a Bruker AMX 500 and a Bruker DRX 500 spectrometer (500 MHz for both) with TMS as an internal standard. Electrospray mass spectrometry (ESMS) was performed on a Finnigan LCQ mass spectrometer. Analytical HPLC was performed using Varian Prostar 210 pumps attached to a YMC ODS-A (S 5 μm 120 Å, 4.6 × 150 mm) column and a MetaChem 0396MG guard column. The Star Chromatography Workstation V.5.51 was used as a controlling system. Signals were monitored at UV 280 nm using a Varian ProStar 330 PDA detector. The flow rate for analytical HPLC was 1 mL min^{-1}. Preparative HPLC was carried out using the same system as analytical HPLC except a YMC ODS-A (S 5 μm 120 Å, 25 × 250 mm) semi-preparative column was utilized. The flow rate for preparative HPLC was 7 mL min^{-1}. Molecules were modeled using SYBYL (Tripos Inc., St. Louis, MO) and electrostatic charges of each compound were calculated with the Gasteiger–Hückle method. Each compound was energy minimized using a SYBYL force field with a conjugate gradient of 0.001 kcal mol^{-1} as the termination criteria.

Plant material

Seeds of *Silybum marianum* were purchased from Frontier Natural Products (Norway, Iowa) in 2001. A voucher specimen has been deposited in the Herbarium of the University of North Carolina, Chapel Hill, under the accession number NCU542201.

Extraction and isolation

Ground milk thistle seeds (2 kg) were extracted with hot MeOH using a Soxhlet (×2). The MeOH extract was concentrated under vacuum and dissolved in 2 L of MeOH–H_2O (9 : 1) and partitioned with hexane (3 × 800 mL). The aqueous MeOH extract was partitioned successively with EtOAc, $CHCl_3$, and *n*-BuOH. The EtOAc extract showed that the silybin band as monitored by TLC co-chromatographed with a reference standard of silybin (Silibinin, Sigma Chemical Co.), and thus, this extract was chromatographed over Si gel using $CHCl_3$–MeOH (100 : 0⟶90 : 10, gradient mixtures) as eluents. A precipitate (>600 mg) was formed in combined fractions 246–300. Low pressure reversed-phase (C_{18}) Si gel column chromatography of this precipitate (600 mg) using MeOH–H_2O (1 : 1⟶4 : 1, gradient mixtures) as eluents resulted in precipitates forming in two combined fractions 64–164 and 191–310. A preparative HPLC of the precipitate from fractions 64–164, using gradient mixtures of MeOH–H_2O (50 : 50⟶65 : 35 for 60 min, then 65 : 35⟶100 : 0 for 20 min) as eluents, enabled the isolation of isosilybins A (**3**, 16.3 mg) and B (**4**, 24.0 mg), and a preparative HPLC of the precipitate from fractions 191–310 using the same mobile phase afforded silybins A (**1**, 5.3 mg) and B (**2**, 5.2 mg). The purity was confirmed by analytical HPLC using MeOH–H_2O (30 : 70⟶40 : 60 for 50 min, gradient mixtures, then hold for 15 min) as the mobile phase (t_R for silybin A, silybin B, isosilybin A, and isosilybin B were 35.6, 37.6, 41.8, and 42.9 min, respectively). Combined fractions 333–384 of Si gel column chromatography showed four major peaks in the analytical HPLC chromatogram. These were isolated by preparative HPLC using gradient mixtures of MeOH–H_2O (30 : 70⟶40 : 60 for 60 min, then 40 : 60⟶100 : 0 for 5 min, then 100% MeOH for 45 min) as eluents. Analytical HPLC of each isolate using gradient mixtures of MeOH–H_2O (30 : 70⟶45 : 55 for 20 min, then holding 100% MeOH in 5 min) yielded pure silychristin (**5**, 16.4 mg), isosilychristin (**6**, 11.1 mg), silydianin (**7**, 8 mg), and taxifolin (**8**, 21.9 mg). The purity of these isolates were confirmed also by analytical HPLC using the same conditions described above (t_R for silychristin, isosilychristin, silydianin, and taxifolin were 23.2, 19.9, 25.3, and 12.8 min, respectively).

Silybin A (1). Mp 158–160 °C; $[\alpha]_D$ +6.1 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 288 (5.1), 230 (5.2), 217 (5.2) nm; CD (MeOH) λ_{ext} ($\Delta\varepsilon$) 326 (+4.9), 292 (−22.4), 236 (−9.3) nm; IR (KBr) 3456, 3191, 3086, 1638, 1592, 1510, 1467, 1435, 1365, 1279, 1237, 1188, 1165, 1082 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HMBC H-2/C-3, C-1′, C-6′, H-6/C-8, C-10, H-8/C-6, C-10, H-2′/C-2, C-4′, C-6′, H-5′/C-1′, C-3′, H-6′/C-2′, C-4′, C-5′, H-α/C-4′, H-β/C-3′, C-α, C-1″, C-2″, C-6″, H-2″/C-β, C-1″, C-4″, C-6″, H-5″/C-1″, C-3″, H-6″/C-2″, C-4″;

ESMS (negative-ion mode) *m*/*z* 481 [M − 1]$^{-1}$; MS-MS 463, 453, 301, 257.

Silybin B (2). Mp 157–159 °C; [*α*]$_D$ +0.6 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log *ε*) 288 (5.2), 230 (5.3), 217 (5.2) nm; CD (MeOH) λ_{ext} (Δ*ε*) 328 (+2.1), 292 (−9.5), 230 (+10.1) nm; IR (KBr) 3565, 3422, 1639, 1595, 1510, 1470, 1435, 1361, 1277, 1182, 1162, 1086 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HMBC H-2/C-3, C-9, C-1′, C-6′, H-3/C-2, H-6/C-5, C-7, C-8, C-10, H-8/C-6, C-7, C-9, C-10, H-2′/C-2, C-3′, C-4′, C-6′, H-5′/C-1′, C-3′, H-6′/C-2, C-2′, C-5′, H-α/C-4′, C-β, H-β/C-3′, C-α, C-1″, C-2″, C-6″, H-γ/C-β, H-2″/C-β, C-3″, C-4″, C-6″, H-5″/C-1″, C-3″, H-6″/C-β, C-2″, C-4″, 5-OH/C-6, C-10, OCH$_3$/C-3″; ESMS (negative-ion mode) *m*/*z* 481 [M − 1]$^{-1}$; MS-MS 463, 453, 301, 283, 257.

Isosilybin A (3). Mp 158–160 °C; [*α*]$_D$ +30.4 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log *ε*) 288 (5.1), 230 (5.3), 212 (5.4) nm; CD (MeOH) λ_{ext} (Δ*ε*) 330 (+2.9), 293 (−9.3), 236 (−1.9) nm; IR (KBr) 3424, 2939, 1640, 1595, 1510, 1468, 1365, 1271, 1163, 1085, 1027 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HMBC H-2/C-1′, C-2′, C-6′, H-6/C-10, H-8/C-10, H-2′/C-2, C-4′, C-6′, H-5′/C-1′, C-3′, H-6′/C-2′, C-5′, H-α/C-4′, C-β, C-1″, C-2′, C-6″, H-β/C-3″, H-2″/C-α, C-3″, C-6″, H-5″/C-1″, C-3″, H-6″/C-2″, C-4″, OCH$_3$/C-3″; ESMS (negative-ion mode) *m*/*z* 481 [M − 1]$^{-1}$; MS-MS 453, 437, 283, 257.

Isosilybin B (4). Mp 194–196 °C; [*α*]$_D$ −17.6 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log *ε*) 288 (5.1), 230 (5.3), 212 (5.4) nm; CD (MeOH) λ_{ext} (Δ*ε*) 328 (+2.8), 292 (−12.6), 229 (+9.0) nm; IR (KBr) 3423, 2940, 2888, 1640, 1595, 1510, 1466, 1444, 1275, 1163, 1086, 1030 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HMBC H-2/C-3, C-4, C-9, C-1′, C-2′, C-6′, H-3/C-2, C-1′, H-6/C-5, C-7, C-8, C-10, H-8/C-6, C-7, C-9, C-10, H-2′/C-2, C-1′, C-3′, C-4′, C-6′, H-5′/C-1′, C-3′, C-4′, H-6′/C-2, C-2′, C-4′, C-5′, H-α/C-4′, C-β, C-γ, C-1″, C-2″, C-6″, H-β/C-3″, C-γ, C-1″, H-γ/C-β, H-2″/C-α, C-1′, C-3″, C-4″, C-6″, H-5″/C-1″, C-3″, C-4″, H-6″/C-α, C-2″, C-4″, OCH$_3$/C-3″; ESMS (negative-ion mode) *m*/*z* 481 [M − 1]$^{-1}$; MS-MS 453, 301, 283, 257.

Silychristin {(2*R*,3*R*)-3,5,7-trihydroxy-2-[(2*R*,3*S*)-7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-2,3-dihydrobenzofuran-5-yl]-chroman-4-one} (5). Physical data were comparable with literature values;[2,14,15,20,24] CD (MeOH) λ_{ext} (Δ*ε*) 328 (+5.6), 298 (−5.2), 224 (+18.5) nm; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HMBC H-2/C-3, C-1′, C-2′, H-3/C-2, C-1′, H-6/C-5, C-7, C-8, C-10, H-8/C-6, C-7, C-9, C-10, H-2′/C-2, C-4′, C-6′, H-6′/C-2, C-2′, C-β, H-α/C-5′, C-β, C-γ, C-1″, C-2″, C-4″, C-6″, H-β/C-5′, C-α, C-γ, C-1″, C-4″, H-γ/C-5′, C-α, H-2″/C-3′, C-α, C-1″, C-6″, H-5″/C-1″, H-6″/C-α, C-2″, OCH$_3$/C-3″; ESMS (negative-ion mode) *m*/*z* 481 [M − 1]$^-$; MS-MS 463, 433, 355, 325, 179.

Isosilychristin {(2*R*,3*R*)-3,5,7-trihydroxy-2-[(2*R*,3*S*)-7-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-2,3-dihydrobenzofuran-4-yl]-chroman-4-one} (6). Mp 154–156 °C (lit. 155–157 °C[21]); [*α*]$_D$ +176.9 (*c* 0.06, MeOH) (lit. +245[21]); UV (MeOH) λ_{max} (log *ε*) 289 (5.2), 231 (5.3) nm; CD (MeOH) λ_{ext} (Δ*ε*) 326 (+1.4), 298 (−2.3), 226 (+44.3) nm; IR (KBr) 3403, 2941, 1640, 1515, 1495, 1361, 1271, 1162, 1084, 1031 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HMBC H-2/C-3, H-3/C-2, H-6/C-8, C-10, H-8/C-6, C-10, H-5′/C-2′, C-3′, H-6′/C-2, C-2′, C-3′, C-4′, C-5′, H-α/C-β, C-γ, H-γ/C-α, H-2″/C-α, C-1″, C-3″, C-4″, C-6″, H-5″/C-1″, C-4″, H-6″/C-α, C-2″; ESMS (negative-ion mode) *m*/*z* 481 [M − 1]$^-$; MS-MS 463, 453, 445, 179.

Silydianin {(3*R*,6*R*,7*R*,10*R*)-3-hydroxy-10-(4-hydroxy-3-methoxyphenyl)-8-[(2*R*,3*R*)-3,5,7-trihydroxy-4-oxochroman-2-yl]-4-oxatricyclo[4.3.1.0]dec-8-en-2-one} (7). Mp 185–187 °C (lit. 190–192 °C[13]); [*α*]$_D$ +256.4 (*c* 0.004, MeOH) (lit. +175[13]);

Table 1 ^{1}H NMR data for compounds **1**–**7** (500 MHz, DMSO-d_6)

Position	1	2	3	4	5	6	7
2	5.06 d (11.3)	5.06 d (11.3)	5.08 d (11.2)	5.06 d (11.2)	4.99 d (11.4)	5.16 d (11.7)	4.84 d (10.4)
3	4.60 d (11.1)	4.59 dd (11.0, 2.9)	4.57 d (11.2)	4.59 d (11.3)	4.51 dd (11.3, 4.8)	4.63 dd (11.6, 5.5)	4.42 dd (10.4, 5.2)
6	5.89 d (1.8)	5.90 d (2.1)	5.89 d (1.6)	5.90 d (2.1)	5.90 d (1.9)	5.92 d (1.9)	5.90 d (1.4)
8	5.84 d (1.8)	5.86 d (2.0)	5.85 d (1.6)	5.86 d (2.1)	5.85 d (2.0)	5.87 d (2.0)	5.88 d (1.8)
2′	7.07 d (1.4)	7.07 d (1.8)	7.08 d (1.3)	7.07 d (1.8)	6.83 br s		3.43 m
5′	6.96 d (8.3)	6.96 d (8.3)	6.92 d (8.2)	6.96 d (8.3)		6.74 d (8.1)	3.17 dd (6.9, 2.8)
6′	7.01 d (1.6)	7.07 d (1.7)	6.99 br s	7.00 d (1.8)	6.87 br s	6.95 d (8.4)	6.01 d (6.6)
α	4.16 m	4.15 m	4.90 d (7.8)	4.90 d (7.9)	5.46 d (6.9)	5.57 m	3.29 m
β	4.89 d (7.9)	4.90 d (7.9)	4.15 m	4.15 m	3.47 dd (12.5, 6.4)	3.67 dd (13.2, 6.3)	2.71 m
γ	3.52 d (10.6)	3.52 d (10.5)	3.52 d (10.6)	3.52 d (10.5)	3.72 m	3.76 m	4.11 dd (7.9, 3.0)
	3.33 d (7.6)	3.33 dd (12.0, 4.5)	3.33 dd (12.3, 4.5)	3.33 dd (12.2, 4.4)	3.63 dd (10.6, 6.8)	3.44 m	
2″	6.99 dd (6.1, 1.5)	7.06 d (8.2, 1.5)	6.98 dd (8.1, 1.4)	7.01 dd (8.2, 1.9)	6.96 d (1.7)	6.86 d (1.8)	6.74 d (1.3)
5″	6.79 d (8.1)	6.79 d (8.0)	6.79 d (8.0)	6.79 d (8.1)	6.76 d (8.1)	6.70 d (8.1)	6.62 d (8.1)
6″	6.85 dd (8.1, 1.4)	6.84 dd (8.4, 1.7)	6.84 dd (9.2, 1.5)	6.85 dd (8.2, 1.8)	6.78 dd (8.9, 2.2)	6.76 dd (8.1, 1.8)	6.54 dd (8.1, 1.3)
5-OH	11.89 s	11.88 s	11.83 s	11.89 s	11.92 s	11.93 s	10.57 s
OCH$_3$	3.76 s	3.77 s	3.77 s	3.77 s	3.76 s	3.69 s	3.72 s

Table 2 ^{13}C NMR data for compounds **1**–**7** (125 MHz, DMSO-d_6)

Position	1	2	3	4	5	6	7
2	82.6	82.5	82.5	82.5	83.2	79.9	80.1
3	71.4	71.5	71.5	71.4	71.7	71.5	69.3
4	197.6	197.7	197.7	197.6	197.5	197.9	194.9
5	163.3	163.3	163.3	163.3	163.3	163.4	161.8
6	96.1	96.1	96.1	96.0	96.1	96.2	94.7
7	167.1	166.9	166.9	166.9	167.2	167.1	165.4
8	95.1	95.1	95.1	95.0	95.0	95.2	93.5
9	162.5	162.5	162.5	162.4	162.5	162.6	160.5
10	100.4	100.4	100.4	100.4	100.3	100.5	98.7
1′	130.1	130.1	130.1	130.1	129.9	124.5	138.0
2′	116.5	116.6	116.6	116.6	115.6	128.9	47.1
3′	143.3	143.2	143.2	143.2	146.3	141.6	95.2
4′	143.7	143.6	143.6	143.6	140.7	145.9	200.4
5′	116.3	116.3	116.3	116.6	129.0	116.1	51.8
6′	121.3	121.1	121.1	121.1	115.3	119.5	122.5
α	78.1	78.2	75.9	75.8	87.0	86.4	44.5
β	75.9	75.9	78.2	78.1	53.3	52.0	42.4
γ	60.2	60.2	60.2	60.1	62.9	63.5	71.2
1″	127.5	127.5	127.5	127.5	132.4	132.9	131.4
2″	111.8	111.7	111.7	111.6	110.4	110.3	110.9
3″	147.6	147.7	147.7	147.6	147.5	147.5	145.6
4″	147.0	147.0	147.0	147.0	147.0	146.3	143.5
5″	115.3	115.3	115.3	115.3	115.3	115.2	113.4
6″	120.5	120.5	120.5	120.5	118.7	118.5	118.8
OCH_3	55.7	55.7	55.7	55.7	55.6	55.6	53.9

UV (MeOH) λ_{max} (log ε) 289 (5.3), 231 (5.4, sh), 216 (5.4) nm; CD (MeOH) λ_{ext} ($\Delta\varepsilon$) 345 (+3.9), 295 (+2.1), 282 (−5.6) nm; IR (KBr) 3460, 2958, 1745, 1641, 1516, 1468, 1275, 1155, 1080, 1033 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HMBC H-2/C-3, C-4, C-9, C-1′, C-2′, H-3/C-2, C-4, C-1′, H-6/C-5, C-7, H-8/C-9, C-10, H-2′/C-β, H-5′/C-1′, C-3′, C-4′, C-6′, C-β, C-1″, H-6′/C-2′, C-4′, C-5′, H-α/C-6′, C-γ, C-1″, C-2″, C-6″, H-β/C-3′, H-γ/C-2′, C-3′, C-α, H-2″/C-α, C-1″, C-3″, C-4″, C-6″, H-5″/C-1″, C-3″, C-4″, H-6″/C-α, C-2″, C-4″, 5-OH/C-5, C-10, OCH_3/C-3″; ESMS (negative-ion mode) *m*/*z* 481 [M − 1]$^-$; MS-MS 453, 301, 178, 151, 125.

Taxifolin {2-(3,4-dihydroxyphenyl)-(2*R*,3*R*)-3,5,7-trihydroxychroman-4-one} (8). Mp 132–134 °C; $[\alpha]_D$ +19.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 290 (5.0), 230 (5.0) nm; CD (MeOH) λ_{ext} ($\Delta\varepsilon$) 326 (+1.1), 292 (−4.4), 232 (+4.5) nm; IR (KBr) 3416, 3262, 1640, 1616, 1507, 1475, 1371, 1266, 1166, 1138, 1083 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.91 (1H, s, 5-OH), 6.88 (1H, s, H-2′), 6.75 (1H, s, H-5′), 6.75 (1H, s, H-6′), 5.90 (1H, br s, H-6), 5.86 (1H, br s, H-8), 4.97 (1H, d, J = 11.2, H-2), 4.49 (1H, d, J = 11.0, H-3); ^{13}C NMR data were consistent with literature values;[59] ESMS (negative-ion mode) *m*/*z* 303 [M − 1]$^{-1}$; MS-MS 285, 241, 217, 175, 151, 125.

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