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Prenylated Flavonoids and Phenolic Compounds from the Rhizomes of Marine Phanerogam *Cymodocea nodosa*

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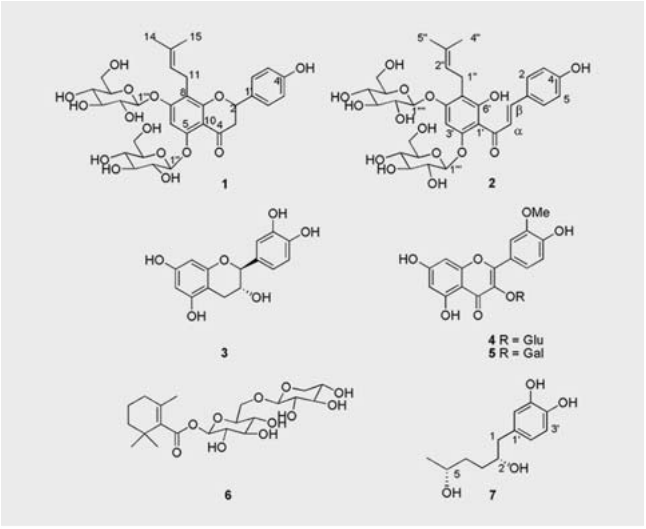
ABSTRACT

Chemical investigation of the rhizomes of the marine phanerogam *Cymodocea nodosa* resulted in the isolation of two new prenylated flavon-di-*O*-glycosides, cymodioside A (**1**) and B (**2**), along with known phenolic compounds **3**–**7**, some of which never reported from seagrasses to date. The structures of compounds **1** and **2** were established by extensive nuclear magnetic resonance analysis. In addition, the absolute configuration of 4-(2,5-dihydroxyhexyl) benzene-1,2-diol (**7**), which was not previously reported in the literature, has been now determined.

Introduction

Marine phanerogams are flowering plants that inhabit the oceans worldwide and play a significant role in coastal ecosystems [1]. Because of their great ecological importance, a number of studies have been dedicated to seagrasses and their ecology [2–4]. On the other side, the chemistry of these plants has not been equally well investigated even though the studies conducted to date report the presence of a wide array of natural products including phenols, flavonoids, sterols, and few terpenoids [5, 6]. *Cymodocea nodosa* (Ucria) (Ascherson) is a seagrass distributed across the Mediterranean Sea and adjacent eastern Atlantic coasts. It belongs to one of the four families of higher plants exclusively marine (Cymodoceaceae, Hydrocharitaceae, Posidoniaceae, and Zosteraceae) encompassing a total of 15 species in five genera [7]. *C. nodosa* holds a significant relevance in various ecosystems as evidenced by numerous ecological investigations [8, 9] whereas a limited number of studies concern with the chemical aspects. To date, the compounds characterized from this species include caffeic acid, quercetin 3-*O*-*β*-glucopyranoside and isorhamnetin 3-*O*-*β*-glucopyranoside [10], sterols and 3-keto steroids [11, 12], and a sulfated polysaccharide [13], all of which are isolated from the aerial parts. Quite recently, four diarylheptanoids, a meroterpenoid, and brominated briarane diterpenes were reported from the whole plant [14, 15], and lastly, chicoric acid was found in high concentration in leaves and rhizomes [16].

In the course of our ongoing studies on marine organisms including seagrasses [17, 18], we have analyzed the chemical content of the rhizomes of *C. nodosa*. In particular, our attention has been focused towards the *n*-butanol-soluble portion of the acetone extract containing polyhydroxy aromatic components. This study led us to isolate several phenolic metabolites including two new prenylated flavonoids, cymodioside A (**1**) and B (**2**), and a series of known metabolites (compounds **3**–**7**), some of them never reported from *C. nodosa* (▶ **Fig. 1**). The structure elucidation of **1** and **2** and the assignment of the absolute configuration of 4-(2,5-dihydroxyhexyl) benzene-1,2-diol (**7**) that was not previously determined are here described.



▶ **Fig. 1** Structures of the isolated compounds from *C. nodosa*.

Results and Discussion

Fresh plants of *C. nodosa* collected in the Gulf of Pozzuoli (Naples) in October 2014 were carefully separated into leaves and rhizomes. Rhizomes were exhaustively extracted with acetone, and after removal of the organic solvent, the aqueous residue was subsequently partitioned with diethyl ether and *n*-butanol. The TLC chromatographic screening of both extracts revealed a complex metabolite pattern in the *n*-butanol part. Several brownish and yellowish spots were observed by developing TLC plates with cerium sulphate. A portion of this extract (1 g) was fractionated on a Sephadex LH-20 column to give 10 fractions (Fr. I–X), all of which were analyzed by NMR. The proton spectra revealed the presence of phenolic components in almost all the fractions that were subsequently submitted to further purification steps including SPE (solid phase extraction) reversed-phase chromatography and HPLC (see "Material and Methods" for details). Two new minor compounds, named cymodiosides A and B, were isolated from two distinct fractions and characterized as 8-prenylnaringenin 5,7-*O*-*β*-diglucopyranoside (**1**) and 5′-prenylchalco-naringenin 2′,4′-*O*-*β*-diglucopyranoside (**2**), respectively. In addition, main (−)-catechin (**3**) [19–21], which is a quite rarely occurring metabolite, isorhamnetin-3-*O*-glucopyranoside (**4**) [22,23] and isorhamnetin-3-*O*-galactopyranoside (**5**) [24,25] (1 : 1 mixture), jasminoside M (**6**) [26], and 4-(2,5-dihydroxyhexyl) benzene-1,2-diol (**7**) [27] (▶ **Fig. 1**) were also isolated and identified by comparing their spectroscopic data with those reported in the literature.

Cymodioside A (**1**) was obtained as a pale-yellow oil. The molecular formula $C_{32}H_{40}O_{15}$ was deduced by the sodium adduct ion at *m/z* 687.2260 in the HR-ESI-MS spectrum (positive mode) accounting for 13 indices of hydrogen deficiency. Analysis of the ^{1}H NMR spectrum in CD_3OD (▶ **Table 1**) immediately revealed the presence of a glycosyl 4′,5,7-trihydroxy flavanone framework (naringenin) bearing a prenyl moiety. In particular, the spectrum contained multiplets due to an ABX system at *δ* 3.06 (1H, dd, *J* = 17.0, 12.7 Hz, H-3a), 2.82 (1H, dd, *J* = 17.0, 2.9 Hz, H-3b), and 5.38 (1H, dd, *J* = 12.7, 2.9 Hz, H-2), two sets of aromatic doublets at *δ* 7.35 (2H, d, *J* = 8.5 Hz, H-2′,6′) and 6.85 (2H, d, *J* = 8.5 Hz, H-3′,5′) attributed to a *p*-disubstituted benzene ring, a singlet at *δ* 6.78 (1H, s) assigned to an isolated proton on an aromatic penta-substituted ring, and a series of signals at *δ* 3.29 (1H, m, H-11a), 3.45 (1H, m, H-11b), 5.20 (1H, app t, *J* = 7.2 Hz, H-12), 1.64 (3H, s, H-14), and 1.61 (3H, s, H-15) that were attributed to the protons of a prenyl chain. The structure of **1** included two sugar moieties as suggested by analysis of ESI-MS and ESI-MS/MS spectra (see **Fig. S44**, Supporting Information) showing a typical fragmenta-

▶ **Table 1** NMR data[a] in ppm for compound **1** in CD_3OD.

C	δ_H, m (*J* in Hz)	δ_C, m	HMBC[b]
2	5.38, dd (12.7, 2.9)	80.3, CH	C-1′
3	3.06, dd (17.0, 12.7) 2.82, dd (17.0, 2.9)	46.4, CH_2	C-2, C-4
4		194.0, C	
5		159.6, C	
6	6.78, s	98.3, CH	C-4, C-5, C-7, C-8, C-10
7		162.5, C	
8		114.5, C	
9		162.8, C	
10		108.5, C	
11	3.45, m 3.29, m	23.0, CH_2	C-7, C-8, C-9, C-12, C-13
12	5.20, app t (7.2)	123.5, CH	C-13
13		132.0, C	
14	1.64, s	25.9, CH_3	C-12, C-13, C-15
15	1.61, s	18.1, CH_3	C-12, C-13, C-14
1′		131.1, C	
2′, 6′	7.35, d (8.5)	129.0, CH	C-2,
3′, 5′	6.85, d (8.5)	116.3, CH	C-1′, C-2′, C-4′
4′		159.0, C	
1″	4.94, d (7.7)	104.4, CH	C-5
2″	3.57, m	74.73, CH	
3″	3.51, m	77.18, CH	
4″	3.33, m	71.76, CH	
5″	3.69, m	78.26, CH	
6″	3.96, m 3.69, m	62.95, CH_2	
1‴	5.14, d (7.5)	100.7, CH	C-7
2‴	3.56, m	74.85, CH	
3‴	3.51, m	77.18, CH	
4‴	3.33, m	71.86, CH	
5‴	3.69, m	78.32, CH	
6‴	3.96, m 3.69, m	62.95, CH_2	

[a] Assignments aided by HSQC and HMBC (*J* = 7 Hz) experiments; [b] HMBC correlations are from protons stated to the indicated carbons.

tion pattern with *m/z* 501 (M – $C_6H_{12}O_6$)$^-$, and *m/z* 339 (M – 2 × $C_6H_{12}O_6$)$^-$ ion peaks as well as ^{1}H and ^{13}C NMR spectra displaying two anomeric glycosyl signals (▶ **Table 1**). Comparison of NMR data of **1** with the literature suggested that both sugar moieties were glucopyranose. Analysis of the coupling constant values of the two anomeric protons at *δ* 4.94 (H-1″, $J_{H\text{-}H}$ = 7.7 Hz) and 5.14 (H-1‴, $J_{H\text{-}H}$ = 7.5 Hz) indicated that the two sugars had the *β*-configuration. The prenyl chain was located at C-8 of naringenin skeleton, whereas the glucosyl units were positioned at 5-OH and 7-OH groups according to the following evidence. Diagnostic cross-peaks were observed in the HMBC experiment between C-5 (*δ* 159.6) and both H-1″ (*δ* 4.94) and H-6 (*δ* 6.78), as well as between C-7 (*δ* 162.5) and H-1‴ (*δ* 5.14), H-6, and H_2-11 (*δ* 3.45 and 3.29). Furthermore, significant NOESY correlations were observed between H-6 and both H-1″ and H-1‴ anomeric protons, confirming the proposed assignment. Thus, the structure of cymodioside A (**1**) was determined as 8-prenylnaringenin-5,7-*O*-*β*-diglucopyranoside. Compound **1** was closely related to evodioside B, which has been isolated from the two terrestrial plants *Evodia rutaecarpa* and *Evodia daniellii* [28, 29]. NMR spectra of **1** were also recorded in DMSO-d_6 (see "Materials and Methods") and compared with those reported in the literature for evodioside B [28, 29] supporting the structure assignment.

Cymodioside B (**2**) was isomeric with **1** with the same molecular formula $C_{32}H_{40}O_{15}$ as deduced by the sodium adduct ion at *m/z* 687.2258 in the HR-ESI-MS spectrum. The ^{1}H NMR spectrum of **2** recorded in both CD_3OD (▶ **Table 2**) and DMSO-d_6 ("Materials and Methods") revealed the presence of two phenol rings bearing two glycosyl residues and a prenyl moiety the same as **1**. The main difference resides in the presence in **2** of an olefinic AB system at *δ* 8.08 (1H, d, *J* = 16.0 Hz, H-*α*) and 7.76 (1H, d, *J* = 16.0 Hz, H-*β*) (▶ **Table 2**) attributable to a *trans* double bond replacing the aliphatic CH_2-CH moiety of the flavanone framework in **1**.

These features suggested that cymodioside B (**2**) was the corresponding chalcone form of cymodioside A (**1**). Analysis of 2D NMR experiments confirmed this assumption. Particularly diagnostic were the HMBC spectra revealing correlations between H-*β* and C=O, C-2, and C-6 as well as between H-*α* and both C-1 and C=O. Additional HMBC correlations were observed between H-3′ and C-1′, C-2′, C-4′ and C-5′, and H-1″ with C-4′, C-5′ and C-6′ according to the substitution pattern with the two glucose moieties attached to 2′,4′ positions and the prenyl group linked to C-5′ of the chalcone skeleton. Comparison of spectroscopic data of **2** with those reported in the literature for plant chalcones revealed that it was the di-*O*-glucoside of desmethyl xanthohumol, previously reported from hops of *Humulus lupulus* L [30–32].

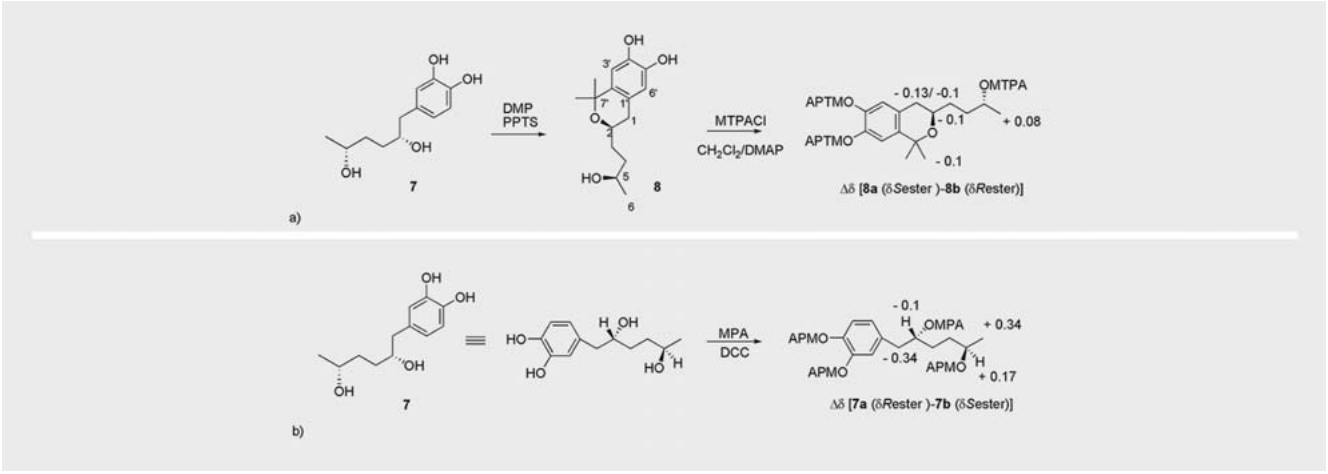
The structural relationship between cymodiosides was definitively confirmed by the chemical interconversion of compounds **1** and **2** that was observed to occur during workup in some experimental conditions including a prolonged storage in organic solvents [32, 33]. Due to this, compound **1** was a scalemic mixture with a predominance of 2*S* stereoisomer [34]. In fact, the CD profile of **1** showed very weak positive and negative Cotton effects in the n → π* (~ 330 nm) and π → π* (~ 290–280 nm) regions, respectively, with absorption magnitudes greatly reduced with respect to optically pure 2*S*-naringin derivatives [34, 35].

▶ **Table 2** NMR data[a] in ppm for compound **2** in CD_3OD.

C	δ_H, m (*J* in Hz)	δ_C, m	HMBC[b]
β	7.76, d (16.0)	144.8, CH	C=O, C-2, C-6
α	8.08, d (16.0)	125.6, CH	C=O, C-1
C=O	–	195.1, C	
1	–	130.4, C	
2	7.65, d (8.5)	132.0, CH	*α*, C-4
3	6.86, d (8.5)	117.1 CH	C-1
4	–	161.8, C	
5	6.86, d (8.5)	117.1 CH	C-1
6	7.65, d (8.5)	132.0, CH	*α*, C-4
1′	–	108.6, C	
2′	–	159.3, C	
3′	6.59, s	94.3, CH	C-1′, C-2′, C-3′, C-4′
4′	–	161.8, C	
5′	–	112.8, C	
6′	–	164.2, C	
1″	3.45, m 3.30, m	22.5, CH_2	C-4′, C-5′, C-6′, C-2″, C-3″
2″	5.25, m	124.0, CH	
3″	–	131.7, C	
4″	1.82, s	25.9, CH_3	C-2″, C-3″
5″	1.67, s	18.0, CH_3	C-2″, C-3″
1‴	5.27, d (7.6)	101.4, CH	C-2′
2‴	3.62, m	74.93, CH	C-1‴
3‴	3.51, m	78.34, CH	
4‴	3.33, m	71.83, CH	
5‴	3.75, m	78.55, CH	
6‴	4.00, m 3.71, m	63.05, CH_2	
1′′′′	5.14, d (7.4)	100.9, CH	C-4′
2′′′′	3.56, m	75.10, CH	C-1′′′′
3′′′′	3.51, m	78.42, CH	
4′′′′	3.33, m	71.90, CH	
5′′′′	3.75, m	78.63, CH	
6′′′′	4.00, m 3.71, m	62.97, CH_2	

[a] Assignments aided by HSQC and HMBC (*J* = 8 Hz) experiments; [b] HMBC correlations are from protons stated to the indicated carbons.

Along with the chemical characterization of the prenylated pair flavanone (**1**) – chalcone (**2**), the absolute configuration of two carbinolic stereogenic centers of 4-(2,5-dihydroxyhexyl) benzene-1,2-diol (**7**) has been also determined. Analysis of NMR spectra of **7** evidenced that carbon and proton resonances were almost identical with those of the compound recently isolated by anaerobic microbial fermentation of green tea [27]. In particular, the multiplicities of H_2-1 and H-3a signals were identical in the two compounds, suggesting that **7** had the same relative configuration at two stereogenic centers as the literature compound



▶ **Fig. 2** **a** Reaction of **7** with DMP to give compound **8**. Reaction of **8** with MTPA chlorides and *Δδ* values calculated for the *tri*-MTPA esters **8a** and **8b. b** Reaction of **7** with R- and S-MPA and *Δδ* values calculated for the *tetra*-MPA esters **7a** and **7b**.

[27]. In that paper, compound **7** was assumed to derive by degradation of co-occurring (−)-*epi*-catechin and, consequently, the 2-(*R*) configuration, the same as (−)-*epi*-catechin, was assigned whereas the configuration at C-5 remained undetermined [27]. Analogous with the literature, in *C. nodosa* compound **7** could derive from co-occurring (−)-catechin (**3**) and thus the 2-(*R*) absolute configuration, the same as (−)-catechin (**3**), should be suggested also in this case. With the aim at establishing the C-5 absolute configuration as well as at securing the C-2 absolute configuration, it was decided to apply the modified Mosher method after protecting phenolic 3′−OH and 4′−OH by formation of the corresponding acetonide. The protection reaction was conducted by using dimethoxypropane (DMP) and catalytic amount of pyridinium *p*-toluenesulfonate, but under the reaction conditions, **7** underwent an intramolecular cyclization following the Oxa-Pictet-Spengler mechanism [36, 37] and gave the main isochromane **8** rather than the expected acetonide derivative. Thus, the modified Mosher method was nevertheless used on derivative **8** to determine the absolute configuration of the unaffected C-5 stereogenic center. The reaction of **8** with *R* and *S* MTPA chlorides gave the corresponding *S* and *R tri*-MTPA ester derivatives **8a** and **8b**. The evaluation of *Δδ* values (δ_S-δ_R) of protons close to H-5 indicated the 5-(*R*) absolute configuration (▶ **Fig. 2a**). The 1D and 2D of cymodioside A (**1**) and B (**2**), compound **7** and its MPA esters **7a**–**7b**, compound **8**, and MTPA esters **8a**–**8b** are available as Supporting Information.

However, with the aim at definitively proving the absolute configuration of C-2, the CDA (chiral derivatization agents) approach proposed by Riguera's group for secondary 1,*n*-diols was followed by using MPA as chiral derivatizing reagent [38–40]. The reaction of **7** with *R* and *S* MPA reagents gave the corresponding *R* and *S tetra*-MPA ester derivatives **7a** and **7b**. It was assumed that MPA substituents esterifying the *ortho*-hydroxyl groups in the aromatic ring did not alter for each derivative the representative conformation as it relates to NMR [38–40]. According to the CDA method [38–40], comparison of ^{1}H-NMR spectra of **7a** and **7b** allowed to calculate the signs of *Δδ* values (δ_R-δ_S) of C-1/C-6 fragment (▶ **Fig. 2b**). The sign distribution of *Δδ* was homogeneous and consistent with the *anti*-1,4 disposition [39], implying the assignment of the (*R*) absolute configuration at both C-2 and C-5 stereogenic centers, in agreement with the already obtained results.

In conclusion, this study added new insights to the chemistry of marine plants by characterization of two new phenolic metabolites, cymodioside A (**1**) and cymodioside B (**2**), as well as the isolation of known compounds **3**–**7**. In particular, (−)-catechin (**3**), jasminoside M (**6**), and 4-(2,5-dihydroxyhexyl) benzene-1,2-diol (**7**) have never been described from seagrasses. Phenolic compounds are widely distributed in marine plants and a number of biological functions have been attributed to them [5, 6]. They include flavones and flavonols [10, 17, 41–44] whereas flavanone components have not been reported till now. Thus, this study represents the first finding of flavanones as well as of prenylated flavonoids in seagrasses.

Materials and Methods

General experimental procedures

Optical rotations were obtained with a Jasco P2000 digital polarimeter (Jasco). UV spectra were acquired on a Jasco V-650 spectrophotometer. ECD (electronic circular dichroism) curves were acquired on a Jasco J-815 spectropolarimeter. IR (infrared) were recorded on a Jasco FTIR 4100. NMR experiments were recorded at the Istituto di Chimica Biomolecolare (ICB) NMR Service Centre. Chemical shifts values are reported in ppm and referenced to the internal signals of residual protons (CD_3OD, ^{1}H *δ* 3.34, ^{13}C 49.0 ppm; DMSO-*d*6, ^{1}H *δ* 2.50, ^{13}C 40.0 ppm; $CDCl_3$, ^{1}H *δ* 7.26, ^{13}C 77.0 ppm). The 1D and 2D NMR spectra were acquired on a Bruker Avance-400 operating at 400 MHz using an inverse probe fitted with a gradient along the z-axis and a Bruker DRX-600 operating at 600 MHz using an inverse TCI CryoProbe fitted with a gradient along the z-axis. ESI-MS and ESI-MS/MS spectra were measured in positive and negative mode, respectively, on a Micromass Q-TOF Micro spectrometer coupled with an HPLC Waters Alliance

2695. HR-ESI-MS spectra were recorded on a Thermo Q-Exactive spectrometer coupled with a UHPLC Agilent Infinity 1290 and on a Shimadzu IT-TOF spectrometer equipped with an ESI interface. HPLC separation was performed on a Shimadzu HPLC system using a Shimadzu liquid chromatograph LC-10AD equipped with an UV SPD-10A wavelength detector with a reversed-phase (RP) column (10 × 250 mm, Aventis-Supelco). Silica gel chromatography was performed using precoated KieselGel 60 F254 plates (TLC) and Kieselgel 60 powder (70–230 mesh) from Merck. The spots on TLC were visualized under UV light (254 nm) and then sprayed with 10% H_2SO_4 in water followed by heating. Sephadex LH-20 was from Amersham Pharmacia Biotech. RP-18 cartridge (SPE) were purchased from Macherey-Nagel.

Biological material

The seagrass *C. nodosa* was collected in the Gulf of Pozzuoli (Naples, Italy) by scuba diving in October 2014. The plant has been identified by one of us (G.V.). Fresh rhizomes were accurately cleaned from the leaves and immediately submitted to the extraction procedure. A frozen voucher specimen of rhizomes is stored at ICB (code CNL).

Extraction and isolation

Fresh rhizomes (1 kg wet, 300 g after extraction) were cut and exhaustively extracted with acetone (0.6 L × 4) by using ultrasounds. After filtration and evaporation *in vacuo* of the organic solvent, the aqueous residue was partitioned first with diethyl ether (500 mL × 4) and subsequently with *n*-BuOH (500 mL × 4) to obtain two extracts of 0.5 g and 1.5 g, respectively. Chromatographic screening of both extracts revealed a more complex metabolite pattern in the *n*-BuOH extract, a portion of which (1.0 g) was fractionated by Sephadex LH-20 (column diameter: 3 cm diameter, 120 cm height, 300 g Sephadex) and eluted in isocratic mode with $CHCl_3$/MeOH 1:1. The collected eluates (52 tubes, each with a volume of 20 mL) were combined to obtain 10 fractions (I–X). NMR analysis of all fractions revealed that some of them contained mainly phenolic and several UV-absorbing compounds. Fraction IV (50.2 mg) was subjected to C18 cartridge (SPE, Macherey-Nagel) and eluted with a gradient of MeOH in H_2O (0–100%) to give eight fractions [IV(1) to IV(8)]. Subfraction IV(5), 10.0 mg, eluted with MeOH/H_2O 3:7, was further purified by HPLC (Supelco, Ascentis C18 column, 1.0 × 25 cm) with a 30-min eluent gradient starting from 45 to 60% MeOH in H_2O (flow rate 0.8 mL/min) to yield pure compounds **1** (3.7 mg) and **6** (0.8 mg). Fraction V (95.1 mg) was further fractionated on C18 cartridge (SPE, Macherey-Nagel) with a gradient of MeOH in H_2O (0–100%) to give eight fractions [V(1) to V(8)]. Fractions V(1) (8.0 mg) and V(2) (4.1 mg), eluted with MeOH/H_2O 1:9, contained pure compound **7** (12.2 mg), whereas fraction V(5) (14.3 mg), eluted with MeOH/H_2O 6:4, was a mixture containing compounds **1**, **4**, and **5**. Half of fraction VI (50.1 mg) was additionally purified on C18 cartridge (SPE, Macherey-Nagel) with a gradient of MeOH in H_2O (0–100%) to give subfraction VI(1) eluted with MeOH/H_2O 1:9 containing compound **7** (7.3 mg) and subfraction VI(4) (MeOH/H_2O 3:7) containing compounds **4** and **5** (mixture 1:1, 17.0 mg). Fraction VII (60.5 mg) was subjected to C18 cartridge (SPE, Macherey-Nagel) with a gradient of MeOH in H_2O (0–100%) to yield a subfraction VII(4) (2.1 mg) eluted with MeOH/H_2O 4:6 containing compound **2**. Finally, fraction X (48.4 mg) from the first purification column was further submitted to a Sephadex LH-20 column (column diameter: 1 cm diameter, 60 cm height, 20 g Sephadex) eluted with $CHCl_3$/MeOH 1:1 to give (−)-catechin (**3**, 20.7 mg).

Cymodioside A (**1**): pale-yellow oil; R_f 0.38 RP-18 F_{254} MeOH/H_2O (6:4); $[\alpha]_D^{25}$ − 1.7 (c 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 348 (7.67), 283 (8.36), 225 (9.11); CD (MeOH) $\Delta\varepsilon_{342}$ + 1,70, $\Delta\varepsilon_{288}$ − 1.48, $\Delta\varepsilon_{237}$ − 0.03; IR (KBr) ν_{max} 3405, 2924, 1645, 1597, 1076 cm^{-1}; ^1H and ^{13}C NMR (MeOD) see ▶ **Table 1**; ^1H NMR (DMSO-d_6, 400 MHz) δ 7.30 (1H, d, J = 8.5 Hz, H-2′ and H-6′), 6.78 (2H, d, J = 8.5 Hz, H-3′ and H-5′), 6.61, (1H, s, H-6), 5.38 (1H, dd, J = 13.0, 2.7 Hz, H-2), 5.16 (1H, m, H-12), 3.35 (1H, m overlapped, H-11a), 3.11 (1H, m, overlapped, H-11b), 3.03 (1H, dd, J = 13.0, 17.4 Hz, H-3ax), 2.70 (1H, dd, J = 2.7, 17.4 Hz, H-3eq), 1.57 (3H, s, H_3-15), 1.55 (3H, s, H_3-14), 5.12 (1H, overlap, H-1″), 3.12–3.49 (8H, overlap, H-2″, H-2‴, H-3″, H-3‴, H-4″, H-4‴, H-5″, H-5‴), 3.45 (2H, overlap, H-6″a and H-6‴a), 3.74 (2H, overlap, H-6″b and H-6‴b); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 190.7 (C, C-4), 162.6 (C, C-9), 160.5 (C, C-7), 157.7 (C, C-4′ and C-5), 131.2 (C, C-1′), 130.5 (C, C-13), 127.9 (CH, C-2′ and C-6′), 122.7 (CH, C-12), 116.6 (CH, C-3′ and C-5′), 110.1 (C, C-8), 106.2 (C, C-10), 103.0 (CH, C-1″), 99.0 (CH, C-1‴), 96.5 (CH, C-6), 77.8 (CH, C-2), 77.2–69.9 (CH, C-2″-C-5″ and C-2‴-C-5‴), 60.9 (CH_2, C-6″ and C-6‴), 44.6 (CH_2, C-3), 25.5 (CH_3, C-14), 21.2 (CH_2, C-11), 17.7 (CH_3, C-15); ESI-MS (pos. ion mode) m/z 687 $[M + Na]^+$; ESI-MS (neg. ion mode) m/z 663 $[M - H]^-$; HR-ESI-MS m/z 687.2260 $[M + Na]^+$ (calcd for $C_{32}H_{40}O_{15}Na$ 687.2265).

Cymodioside B (**2**): yellow oil; R_f 0.20 RP-18 F_{254} MeOH/H_2O (6:4); $[\alpha]_D^{25}$ − 3.2 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 369 (9.25), 285 (8.69); IR (KBr) ν_{max} 3369, 2923, 1598, 1076, 839 cm^{-1}; ^1H NMR (DMSO-d_6, 400 MHz) δ 8.00 (1H, d, J = 15.5 Hz, H-β), 7.68 (1H, d, J = 15.5 Hz, H-α), 7.63 (2H, d, J = 8.6 Hz, H-2 and H-6), 6.78 (2H, d, J = 8.6 Hz, H-3 and H-5), 6.43, (1H, s, H-3′), 5.16 (1H, m, overlap,H-2″), 3.34 (1H, m overlapped, H-1″a), 3.12 (1H, m, overlapped, H-1″b), 1.70 (3H, s, H_3-5″), 1.58 (3H, s, H_3-4″), 5.12 (1H, overlap, H-1‴), 4.97 (1H, d, J = 7.3 Hz, H-1′′′′), 3.12–3.49 (8H, overlap, H-2‴, H-2′′′′, H-3‴, H-3′′′′, H-4‴, H-4′′′′, H-5‴, H-5′′′′), 3.45 (2H, m, H-6‴a and H-6′′′′a), 3.74 (2H, m, H-6‴b and H-6′′′′b); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 192.9 (C, C=O), 162.7 (C, C-6′), 160.4 (C, C-4, C-4′), 157.7 (C, C-2′), 144.0 (CH, C-β), 131.2 CH, C-2 and C-6), 130.2 (C, C-1 and C-3″), 122.8 (CH, C-α and C-2″), 115.2 (CH, C-3 and C-5), 110.3 (C, C-5′), 106.3 (C, C-1′), 99.9 (CH, C-1‴), 99.5 (CH, C-1′′′′), 93.2 (CH, C-3′), 77.3–70.0 (CH, C-2‴-C-5‴ and C-2′′′′-C-5′′′′), 61.0–60.9 (CH_2, C-6‴ and C-6′′′′), 25.6 (CH_3, C-4″), 21.3 (CH_2, C-1″), 17.8 (CH_3, C-5″); ESI-MS (pos. ion mode) m/z 687 $[M + Na]^+$; HR-ESI-MS m/z 687.2258 $[M + Na]^+$ (calcd for $C_{32}H_{40}O_{15}Na$ 687.2265).

4-(2,5-dihydroxyhexyl)-benzene-1,2-diol (**7**): white powder, $[\alpha]_D^{25}$ − 7.5 (c 0.4, MeOH); $[\alpha]_D^{25}$ lit. − 4.1 (c 0.1, MeOH) [27]; ^1H NMR (CD_3OD, 400 MHz) δ 6.69 (1H, d, J = 8.0 Hz, H-3′), 6.68, (1H, d, J = 1.0 Hz, H-6′), 6.55, (1H, dd, J = 8.0, 1.0 Hz, H-2′), 3.72, (2H, m, H-2 and H-5), 2.65 (1H, dd, J = 13.6, 6.9 Hz, H-1a), 2.58 (1H, dd, J = 13.6, 6.4 Hz, H-1b), 1.65 (3H, m, H-3a and H_2-4), 1.38 (1H, bdd, J = 18.6, 8.4, H-3b), 1.15 (3H, d, J = 6.4 Hz, H_3-6); ^{13}C

NMR (MeOD, 400 MHz, indirect detection from 2D heteronuclear experiments) δ 145.3 (C, C-5′), 144.2 (C, C-4′), 132.0 (C, C-1′), 121.6 (CH, C-2′), 117.3 (CH, C-6′), 116.0 (CH, C-3′), 74.0 (CH, C-2), 68.4 (CH, C-5), 44.3 (CH_2, C-1), 36.0 (CH_2, C-4), 33.4 (CH_2, C-3), 23.2 (CH_3, C-6); HR-ESI-MS *m/z* 249.1102 $[M + Na]^+$ (calcd for $C_{11}H_{18}NaO_4$ 249.1103).

Reaction of compound 7 with DMP

An aliquot of compound **7** (8.0 mg) was allowed to react with DMP (1 mL) and a catalytic amount of pyridinium *p*-toluenesulfonate at 70 °C for 45 min. The reaction was then quenched with H_2O (5 mL) and extracted with Et_2O (5 mL × 3). All the organic layers were combined and evaporated, and the crude reaction mixture (11 mg) was purified on a silica-gel pipette Pasteur packed in $CHCl_3$ and eluted with a gradient of MeOH in $CHCl_3$ (1–5%). Fractions (50 tubes) of 2 mL were collected and checked by TLC chromatography ($CHCl_3$/MeOH, 9:1). Fractions (34–49) eluted with 5% MeOH were combined to yield compound **8** (4.6 mg).

3R-(3R-hydroxybutyl)-1,1-dimethylisochroman-6,7-diol (**8**): ^1H NMR values in ppm ($CDCl_3$, 400 MHz) δ 6.59 (1H, s, H-3′), 6.53 (1H, s, H-6′), 3.91 (1H, m, H-5), 3.79 (1H, m, H-2), 2.63 (1H, dd, *J* = 15.5, 11.3 Hz, H-1ax), 2.42 (1H, dd, *J* = 15.5, 2.5 Hz, H-1eq), 1.72 (2H, m, H_2-3), 1.70 (1H, m, H-4a), 1.58 (1H, m, H-4b), 1.46 (6H, s, H_3-8′ and H_3-9′), 1.22 (3H, d, *J* = 6.3 Hz, H_3-6); ^{13}C NMR ($CDCl_3$, indirect detection from HSCQ and HMBC) δ 142.4 (C × 2, C-4′ and C-5′), 135.4 (C, C-2′), 125.4 (C, C-1′), 114.9 (CH, C-6′), 111.9 (CH, C-3′), 75.5 (C, C-7′), 69.1 (CH, C-2), 67.5 (CH, C-5), 35.2 (CH_2, C-4), 34.6 (CH_2, C-1), 31.9 (CH_2, C-3), 31.0 (CH_3, C-8′), 28.6 (CH_3, C-9′) 23.0 (CH_3, C-6); ESI-MS (pos. ion mode) *m/z* 289 $[M + Na]^+$. HR-ESI-MS *m/z* 289.1409 $[M + Na]^+$ (calcd for $C_{15}H_{22}O_4Na$ 289.1416).

Preparation of MTPA esters of compound **8**: (*R*)- and (*S*)-MTPA-Cl (α-Methoxy-α-trifluoromethylphenylacetyl chloride) (10 µL) and a catalytic amount of DMAP (N,N-dimethylaminopyridine) were separately added to two different aliquots of compound **8** (1.0 mg each) in dry CH_2Cl_2 (0.5 mL). The resulting mixtures were allowed to stir at room temperature for 3 h. After evaporation of the solvent, each mixture was purified on a SiO_2 pipette Pasteur eluting with $CHCl_3$ to afford pure (*S*)- and (*R*)-MTPA esters **8a** and **8b**, respectively.

(S)-MTPA ester (**8a**): selected ^1H NMR ($CDCl_3$, 400 MHz) δ 6.94 (1H, s, H-6′), 6.92 (1H, s, H-3′), 5.218 (1H, m, H-5), 3.60 (1H, m, H-2), 2.545 (1H, dd, *J* = 15.5, 11.3 Hz, H-1ax), 2.437 (1H, dd, *J* = 15.5, 2.5 Hz, H-1eq), 1.836 (1H, m, H-4a), 1.672 (1H, m, H-4b), 1.379 (3H, d, *J* = 6.3 Hz, H_3-6); HR-ESI-MS *m/z* 937.2604 $[M + Na]^+$ (calcd for $C_{45}H_{43}O_{10}F_9Na$ 937.2610).

(R)-MTPA ester (**8b**): selected ^1H NMR ($CDCl_3$, 400 MHz) δ 6.98 (1H, s, H-6′), 6.93 (1H, s, H-3′), 5.232 (1H, m, H-5), 3.708 (1H, m, H-2), 2.646 (1H, dd, *J* = 15.5, 11.3 Hz, H-1ax), 2.567 (1H, dd, *J* = 15.5, 2.5 Hz, H-1eq), 1.862 (1H, m, H-4a), 1.738 (1H, m, H-4b), 1.296 (3H, d, *J* = 6.3 Hz, H_3-6); HR-ESI-MS *m/z* 937.2601 $[M + Na]^+$ (calcd for $C_{45}H_{43}O_{10}F_9Na$ 937.2610).

Preparation of MPA esters of compound 7

R-(MPA) and *S*-(MPA) (α-Methoxyphenylacetic acid) (6 mg each), DCC (dicyclohexylcarbodiimide) (4.5 mg), and a catalytic amount of DMAP were added to distinct aliquot of compound **7** (2 mg each) in dry CH_2Cl_2 and left stirring overnight. The reaction mixtures were first purified on distinct Pasteur pipettes eluting with an ethyl acetate gradient in *n*-hexane, and the fractions obtained from *n*-hexane/EtOAc 8:2 were further purified by HPLC on a Kinetex 5 µm Phenyl-Hexyl 100 Å column (Phenomenex, 250 × 4.6 mm), eluting with MeOH/H_2O (7:3 isocratic mode, flow 1 mL/min) to give *R*-(MPA)-(**7a**)(R_t 25 min) and *S*-(MPA)-(**7b**) (R_t 27 min) esters, respectively.

R-*(MPA)-ester* (**7a**): selected ^1H NMR ($CDCl_3$, 400 MHz) δ 6.64 (1H, s, H-3′), 6.62 (1H, br s, H-6′), 6.47 (1H, s, H-2′), 4.89 (1H, m, H-2), 4.80 (1H, m, H-5), 2.37 (2H, app d, *J* = 6.0 Hz, H_2-1); HR-ESI-MS *m/z* 841.3198 $[M + Na]^+$ (calcd for $C_{48}H_{50}O_{12}Na$ 841.3199).

S-*(MPA)-ester* (**7b**): selected ^1H NMR values ($CDCl_3$, 400 MHz) δ 6.75 (1H, s, H-3′), 6.69 (1H, br s, H-6′), 6.55 (1H, s, H-2′), 4.99 (1H, m, H-2), 4.63 (1H, m, H-5), 2.71 (2H, app d, *J* = 6.0 Hz, H_2-1); HR-ESI-MS *m/z* 841.31.95 $[M + Na]^+$ (calcd for $C_{48}H_{50}O_{12}Na$ 841.3199).

Supporting information

The 1D and 2D of cymodioside A (**1**) and B (**2**), compound **7** and its MPA esters **7a–7b**, compound **8**, and MTPA esters **8a–8b** are available as Supporting Information.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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