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Flavone-coumarin hybrids from *Gnidia socotrana*

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Dedicated to Professor Günter Adam on the occasion of his 70th birthday

Abstract

Phytochemical investigation of leaves and twigs of *Gnidia socotrana* (Balf. f.) Gilg (Thymelaeaceae), a plant occurring endemically on Socotra Island (Yemen), afforded six novel natural products: two compounds consisting of a flavone and a coumarin moiety connected by a C–C linkage, 7,7′-dihydroxy-3,8′-biscoumarin and three substances with the rare spiro-bis-γ-lactone structure. The structures were elucidated on the basis of their spectroscopic data.

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Keywords: *Gnidia socotrana*; Thymelaeaceae; Biscoumarins; Flavone-coumarin hybrids; Spiro-bis-γ-lactones

1. Introduction

The genus *Gnidia* consisting of approximately 140 species represents the largest genus of the family Thymeleaeceae. *Gnidia* species are distributed mainly in the tropical and southern Africa, although some species are found in Madagascar, western India and Arabia (Mabberley, 1990). *Gnidia* species are known to contain toxic diterpene esters of the daphnane type exhibiting antineoplastic activity (Borris and Cordell, 1984; Kupchan et al., 1975a, 1976a,b). Furthermore, phytochemical investigations revealed the occurrence of coumarins (Kupchan et al., 1975b), lignans (Bryan and Shen, 1978), flavonoids and benzophenone glycosides (Ferrari et al., 2000).

Members of the genus are used in African traditional medicine for abdominal pain, sore throat, as a purgative, as laxative and in the treatment of wounds, burns and snakebites. Furthermore, applications as molluscicidal agent, arrow and fish poison are described (Borris and Cordell, 1984).

Within the scope of our work on isolation and identification of constituents of Yemenian plants we investigated leaves and twigs of *Gnidia socotrana*. *Gnidia socotrana* characterized by its remarkable spathaceous bracts and densely haired receptacle is endemically to Socotra Island (Gilg, 1894). Our phytochemical studies of this species afforded 6 new compounds: a biscoumarin, two novel umbelliferylflavonoids and three substances with the rare spiro-bis-γ-lactone structure.

2. Results and discussion

The EI–MS of **1** displayed a molecular ion at m/z 322 (base peak) and key fragments originated by successive CO eliminations typical for coumarins. The molecular formula $C_{18}H_{10}O_6$ determined by EI–HRMS was in accordance with a biscoumarin bearing two hydroxy groups. The assumptions were supported by the NMR results. The ^{1}H NMR spectrum showed a pair of doublets ($J=9.5$) at δ 7.717 and 6.281 which could be assigned to H-4′ and H-3′ of a coumarin nucleus, a singlet at δ 8.048 attributable to H-4 of a 3-substituted coumarin, a pair of doublets and an *ortho* and *meta* coupled aromatic three spin system. The substitution pattern of both parts were established on the base of the observed NOESY correlations between H-4 and H-5 as well as between H-4′ and H-5′. The assignment of all carbon atoms was done by HSQC and HMBC experiments. HMBC correlations between H-4 and C-8′ as well as a $^4J_{CH}$ correlation between H-6′ and C-3 indicated the connection of C-3 and C-8 of the two umbelliferyl units. Thus, compound **1** was characterized as 7,7′-dihydroxy-3,8′-biscoumarin. A similar biscoumarin linked between C-3 and C-8′ was previously isolated from *Ipomopsis aggregata* (Polemoniaceae). This compound named ipomopsin contains two additionally methoxy groups at position 6 and 6′ (Arisawa et al., 1984).

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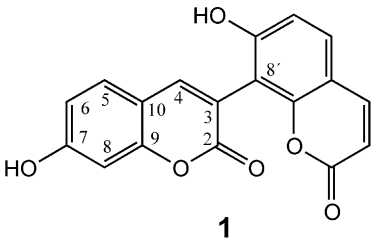

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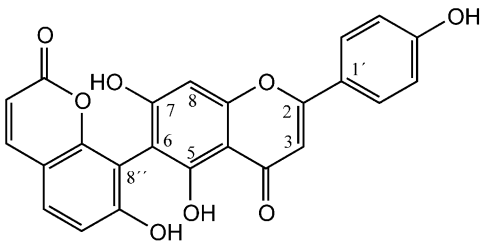

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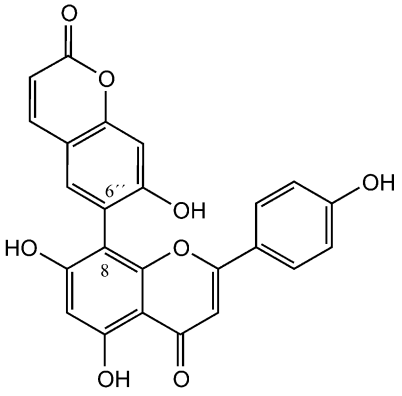

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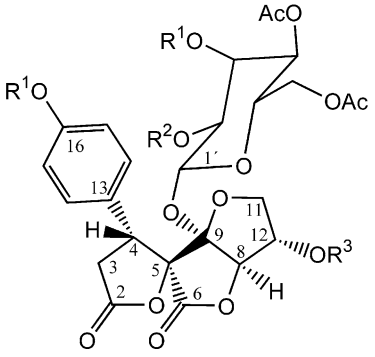
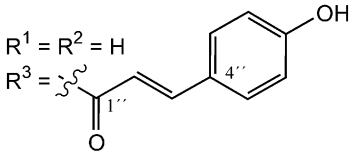


4 $R^1 = R^2 = R^3 = H$

4a $R^1 = R^2 = R^3 = -COCH_3$

5 $R^1 = R^2 = H$, $R^3 =$



6 $R^1 = H$; $R^2 = R^3 = -COCH_3$

The isomeric compounds **2** and **3** gave the molecular ion $[M]^+$ at m/z 430 corresponding to the molecular formula $C_{24}H_{14}O_8$. To the best of our knowledge natural products with this molecular formula are hitherto not described. The ^1H NMR spectrum of **2** measured in CD_3OD (Table 1) displayed signals for 10 aromatic protons: four of them were attributable to a 8-substituted 7-hydroxycoumarin moiety as already found in **1**, the remaining two singlets at δ 6.635 and 6.619 as well as 4 protons belonging to a *para*-substituted phenyl were assigned to a 4′,5,7-trihydroxyflavone (apigenin) substituted at C-6 or C-8. The carbon shifts of the flavone part are in good agreement with a substitution at C-6 (Kitajima et al., 1998). This is further supported by the observed HMBC correlations of H-8 to C-7, C-9, C-6 and C-10 (but not C-5!; Shen et al., 1993). Week HMBC correlations of H-8 to C-8″ and of H-6″ to C-6 indicated the C-C connection of C-6 and C-8″. The observed NOESY correlations, for example between H-3 and H2′/6′ and between H-4″ and H-5″ were consistent with the assumed structure. Compound **2** was optically active ($[\alpha]_D = +56.2$) and displayed a CD spectrum indicating that the free rotation at the biaryl axis is hindered. Thus, compound **2** can be regarded as an atropisomer. According to the spectral data the structure of compound **2** was established as 6-(8″-umbelliferyl)-apigenin.

Compound **3**, similar to **2**, also consists of a coumarin and a flavone part. In the ^1H NMR spectrum of **3** the two doublets of H-5″ and H-6″ found for **2** are replaced by two singlets indicating protons at C-5″ and C-8″ and a substitution at C-6″. Additionally the ^{13}C NMR signal for C-8 arising at δ 94.8 in compound **2** is exchanged by a signal at δ 100.0 attributable to an unsubstituted C-6 of a 5,7-dihydroxyflavon structure (Kuo et al., 2000; Chari et al., 1977). HMBC correlations between H-6 and C-5, C-7 and C-10 support the assignment (Shen et al., 1993). Furthermore, in acetone-d_6 NOE correlations between H-6 and 5-OH are observed. Therefore, the linkage between both moieties is at C-8 (apigenin) and C-6″ (umbelliferon). HMBC correlations from H-5″ to C-8 prove the connective position. Due to the obtained

Table 1
NMR data [δ, multiplicity, (*J*)] of compounds **2** and **3** measured in different solvents

	2				**3**		
	Pyridine-d_5		CD_3OD		Pyridine-d_5		Acetone-d_6
C	^{13}C (125.7 MHz)	^{1}H (499.83 MHz)	^{13}C (75.5 MHz)	^{1}H (499.83 MHz)	^{13}C (125.7 MHz)	^{1}H (499.83 MHz)	^{1}H (300.24 MHz)
2	164.3		166.0		164.6		
3	103.9	6.890 *s*	103.8	6.635 *s*	103.6	6.953 *s*	6.439 *s*
4	182.8		183.8		183.1		
5	161.9		161.0		162.4		
6	104.9		104.6		100.0	6.888 *s*	6.671 *s*
7	164.5		164.4		164.2		
8	94.6	6.890 *s*	94.8	6.619 *s*	104.9		
9	158.0		159.0		156.0		
10	105.0		105.2		104.9		
1′	122.4		123.2		122.5		
2′/6′	128.8	7.917 *d* (8.8)	129.3	7.885 *d* (8.5)	128.8	7.910 *d* (8.8)	7.643 *d* (8.9)
3′/5′	116.8	7.213 *d* (8.8)	116.9	6.947 *d* (8.5)	116.9	7.169 *d* (8.8)	6.884 *d* (8.9)
6′	162.6		162.6		162.7		
2″	161.7		164.0		161.2		
3″	111.7	6.234 *d* (9.4)	111.8	6.183 *d* (9.3)	112.1	6.250 *d* (9.3)	6.232 *d* (9.5)
4″	144.5	7.667 *d* (9.4)	146.4	7.917 *d* (9.3)	144.2	7.760 *d* (9.3)	7.940 *d* (9.5)
5″	128.9	7.454 *d* (8.5)	129.7	7.508 *d* (8.2)	133.3	7.850 *s*	7.643 *s*
6″	113.9	7.258 *d* (8.5)	114.2	6.939 *d* (8.2)	118.9		
7″	161.4		161.3		162.0		
8″	109.8		109.4		103.5	7.21 *s*[a]	6.994 *s*
9″	155.4		155.2		156.2		
10″	112.2		113.3		111.9		
5-OH		14.260 *s*				13.904 *s*	13.171 *s*

[a] Chemical shift of HSQC correlation peak (hidden behind solvent signal).

data compound **3** was elucidated as 8-(6″-umbelliferyl)-apigenin. Contrary to **2** compound **3** did not exhibit optical activity.

To our knowledge natural compounds consisting of a flavonoid and a coumarin moiety were not detected before. Thus, the flavone-coumarin hybrid compounds **2** and **3** represent a new structural type of natural products. However, biscoumarins and flavonoid dimers are frequently described in Thymelaeaceae (Hegnauer, 1990).

Compound **4** gave no molecular ion in its EIMS. According to the $[M-H]^-$ ion at *m*/*z* 567 obtained by ESI–MS the molecular formula of **4** was determined as $C_{25}H_{28}O_{15}$ by HR–ESI–FTICRMS. Careful examination of all spectral data suggested that compound **4** is similar to viburnolide A (also referred to as dilaspirolactone) a spiro-bis-γ-lactone glucoside isolated from *Viburnum* species (Caprifoliaceae; Machida and Kikuchi, 1994; Iwagawa and Hase, 1984). In comparison to viburnolide A compound **4** contains two additionally acetyl groups. COSY and HMBC experiments localized the acetyl functions at C-4 and C-6 of the glucosyl moiety. The stereochemistry of **4** is assumed to be identical to that observed in viburnolide A based on the following evidence: peracetylation of **4** yielded the product **4a**. All spectral data of **4a** including $[\alpha]_D$ were in agreement with those reported for the peracetylated viburnolide A (Machida and Kikuchi, 1994). For example, the ^{1}H NMR signal of H-8 in all compounds appeared as a singlet indicating that the dihedral angle between H-8 and H-12 is nearly 90°. The stereochemistry of viburnolide A has been elucidated as 4*R*, 5*S*, 8*R*, 9*R* and 12*S* (Machida and Kikuchi, 1994). Consequently, **4** was identified as 4′,6′-diacetylviburnolide A.

The molecular formula of compound **5** was deduced as $C_{34}H_{34}O_{17}$ from high-resolution ESI–MS in agreement with its $[M-H]^-$ ion at *m*/*z* 713. ^{1}H and ^{13}C NMR spectra of **5** indicated the same basic structure as in **4**. Additional resonances were assigned to a coumaroyl residue. In the ESI–CID–MS of the $[M-H]^-$ ion ions at *m*/*z* 163 and *m*/*z* 145 appear representing coumaric acid derived fragments. HMBC correlation between H-12 and C-1″ demonstrated the coumaroyl substitution at C-12. Therefore, the structure of **5** was elucidated as 4′, 6′-diacetyl-12-cumaroyl-viburnolide A. The structure of **5** was confirmed by COSY, TOCSY, HSQC, HMBC and NOESY measurements.

Furthermore, LC–MS measurements indicated the presence of tri- and tetraacetylated viburnolides in the ethyl acetate extract (R_t 13.78 min: $[M-H]^-$ ion *m*/*z* 609, R_t 15.72 min: $[M-H]^-$ ion *m*/*z* 651). However, these compounds were not very stable and tended to loose acetyl groups. In spite of that we were able to isolate

and characterize the tetraacetate **6**. The molecular ion at m/z 652 obtained by EI–MS corresponded to the molecular formula $C_{29}H_{32}O_{17}$. The ^1H and ^{13}C NMR spectra of **6** were similar to those of **4** but displayed signals for two additional acetyl functions. Most of the carbon and proton signals of **6** were assigned by comparison with the NMR data of the compounds **4** and **5**. In compound **6** the signals of C-8 and C-11 are clearly shifted to higher field indicating acetyl substitution at C-12. The second additional acetyl function is assumed to be localized at C-2′ since H-2′ is significantly downfield shifted.

To our knowledge this is the first report on compounds with the rare spiro-bis-γ-lactone structure in the family of Thymeleaeceae. Similar compounds, e.g. conocarpin, leucodrin and related substances, are typical constituents of Proteaceae (Kruger and Perold, 1970; Perold et al., 1972; Murray and Bradshaw, 1966). Murray and Bradshaw (1966) discussed a possible role of these substances as carbohydrate storage compounds or as products of a detoxification mechanism that removes phenolic compounds. On the other hand these compounds could represent ascorbigens which are formed by the coupling of ascorbic acid and *p*-coumaric acid or 3-hydroxy-3-*p*-hydroxyphenylpropionic acid (Glennie and Perold, 1980). The synthesis of leucodrin and the aglycon of viburnolide A starting from ascorbic acid was reported previously (Poss and Belter, 1987, 1988).

3. Experimental

3.1. General methods

CC: silica gel (Merck, 40–63 μm) and Sephadex LH 20 (Fluka). Mp: uncorr., $[\alpha]_D$: JASCO DIP 1000 Polarimeter, solvent MeOH. [θ]: JASCO J 710. UV: Kontron Uvicon 940, solvent MeOH. NMR, 1D: Varian Unity 500 spectrometer, 499.83 MHz (^1H) and 125.7 MHz (^{13}C) or Varian Gemini spectrometer 300, 300.24 MHz (^1H) and 75.5 MHz (^{13}C), 2D: (GHMBC, GHSQC, NOESY, ^1H–^1H COSY) Varian Unity 500 spectrometer, 499.83 MHz. Chemical shifts were referenced to internal TMS ($\delta = 0$, ^1H) and pyridine-d_5 ($\delta = 123.5$, ^{13}C) or CD_3OD ($\delta = 49.0$, ^{13}C), respectively. HR–EIMS (resolution 7000) and EIMS (DIS): 70 eV, AMD 402, AMD Intectra. positive and negative ion ESI–MS: TSQ 7000 (Finnigan, electrospray voltage 4.5 kV), heated capillary temperature 220 °C; sheath gas: nitrogen) coupled with a Micro-Tech Ultra-Plus MicroLC system, equipped with a RP18-column (4 μm, 1×100 mm, Ultrasep), HPLC conditions: gradient system from H_2O:CH_3CN 4:1 (each of them contained 0.2% HOAc) to 1:9 within 15 min, then hold on this value for 10 min; flow rate 70 μl min^{-1}. All mass spectra are averaged and background substracted. The high resolution ESI mass spectra were obtained from a Bruker BioApex 70e Fourier transform ion cyclotron resonance mass spectrometer (Bruker Daltonics, Billerica, USA) equipped with an Infinity™ cell, a 7.0 Tesla supraconducting magnet (Bruker, Karlsruhe, Germany), an RF-only hexapole ion guide and an external electrospray ion source (Apollo™ API source). Nitrogen was used as drying gas at 150 °C.

3.2. Plant material

Leaves and twigs of *Gnidia socotrana* (Balf. f.) Gilg were collected in February 1999 on Socotra Island, Hajhir (Haggier) Mountains, Yemen. The species was identified by Professor Abdul Karim Nasher, Department of Biology, Sanáa University. A voucher specimen is deposited at the herbarium of the Dhamar University, Yemen.

3.3. Extraction and purification

The dried and powdered plant material (258 g) was extracted exhaustively with 95% EtOH. After evaporation of the solvent the resulting crude extract (53 g) was dissolved in water, successively partitioned with *n*-heptane (4.8 g), EtOAc (5.2 g) and *n*-BuOH (17.9 g) and evaporated to dryness.

The *n*-BuOH extract contained mainly flavonoid glycosides (determined by LC–MS) and was not further processed.

Of the EtOAc extract, 2.5 g were subjected to CC on Sephadex LH 20 eluting with MeOH to give 6 fractions which were combined according to TLC monitoring.

Further separation of fraction 1 by repeated CC on silica gel using $CHCl_3$:MeOH gradient systems gave compound **4** (25.5 mg). Compound **6** (13.5 mg) crystallized from the fraction eluted from the silica gel column previous to **4**.

Re-chromatography of fractions 3 and 5 by CC on silica gel ($CHCl_3$:MeOH) followed by prep. HPLC resulted in the isolation of the compounds **5** (8 mg) and **1** (2.3 mg), and **2** (4.5 mg) and **3** (12.5 mg), respectively.

3.4. 7,7′-Dihydroxy-3,8′-biscoumarin (*1*)

Amorphous solid. UV λ_{max}: 327 nm. ^1H NMR (pyridine-d_5) δ (*J*): 8.048 (1H, *s*, H-4), 7.717 (1H, *d*, 9.5 Hz, H-4′), 7.485 (1H, *d*, 8.6 Hz, H-5′), 7.456 (1H, *d*, 8.5 Hz, H-5), 7.195 (1H, *d*, 8.6 Hz, H-6′), 7.040 (1H, *dd*, 8.5/2.3 Hz, H-6), 6.965 (1H, *d*, 2.3 Hz, H-8), 6.281 (1H, *d*, 9.5 Hz, H-3′). ^{13}C NMR (pyridine-d_5) δ: 162.9 (C-7), 161.1 (C-7′), 161.0 (C-2′), 160.4 (C-2), 156.5 (C-8a), 154.7 (C-8a′), 145.3 (C-4), 144.4 (C-4′), 129.9 (C-5), 129.4 (C-5′), 116.6 (C-3), 114.0 (C-6), 113.6 (C-6′), 112.3 (C-4a), 112.1 (C-3′), 112.1 (C-4a′), 112.0 (C-8′), 103.1 (C-8). EIMS (rel. int.): 322.0461 $[M]^+$ (100, calc. for

$C_{18}H_{10}O_6$ 322.0472), 305 (8), 294 (45), 266 (15), 249 (4), 237 (18), 221 (5), 210 (6), 181 (4), 165 (2).

3.5. 6-(8″-Umbelliferyl)-apigenin (**2**)

Yellow crystals, mp >360 °C. $[\alpha]_D^{25}$ +56.2 (*c* 0.10). CD: $[\theta]_{231}$ +16 905, $[\theta]_{246}$ +4 572, $[\theta]_{265}$ −22 870, $[\theta]_{283}$ +18 192, $[\theta]_{319}$ +38 323, $[\theta]_{356}$ −16 600. UV λ_{max} nm (log ε): 210 (4.64), 274 (4.33), 330 (4.52). ^1H and ^{13}C NMR: Table 1. EIMS (rel. int.): 430 $[M]^+$ (43), 413 $[M-OH]^+$ (100), 385 (15), 360 (11), 310 (2), 294 (4), 270 (2), 268 (4), 242 (5), 121 (13). HR–ESI–MS: *m*/*z* 453.05784 $[M+Na]^+$ (calc. for $C_{24}H_{14}O_8Na$ 453.05864)

3.6. 8-(6″-Umbelliferyl)-apigenin (**3**)

Yellow crystals, mp 270–275 °C. UV λ_{max} nm (log ε): 216 (4.44), 271 (4.23), 330 (4.33). ^1H and ^{13}C NMR: Table 1. EIMS (rel. int): 430 $[M]^+$ (100), 413 $[M-OH]^+$ (37), 310 (6), 294 (9), 270 (8), 268 (10), 226 (18), 198 (13), 121 (82). HR–ESI–MS: *m*/*z* 453.05786 $[M+Na]^+$ (calc. for $C_{24}H_{14}O_8Na$ 453.05864).

3.7. 4′,6′-Diacetyl-viburnolide A (**4**)

Amorphous solid. $[\alpha]_D^{27}$ −18.2 (c 0.10). UV λ_{max} nm (log ε): 227 (3.54), 2.77 (2.74), 2.84 *sh*. ^1H NMR (CD_3OD) δ (*J*): 7.238 (2H, *d*, 8.5 Hz, H-14/18), 6.790 (2H, *d*, 8.5 Hz, H-15/17), 4.954 (1H, *d*, 8.0 Hz, H-1′), 4.917 (1H, *dd*, 10.2/9.4 Hz, H-4′), 4.617 (1H, *dd* 12.9/8.8, H-4), 4.331–4.418 (2H, *m*, H-11a/H-12), 4.155 (2H *d*, 3.0, H-6′), 4.067 (1H, *dd*, 8.8/2.7 Hz, H-11b), 3.987 (1H, *s*, H-8), 3.713 (1H, *dt*, 10.2/ Hz, H-5′), 3.654 (1H, *t*, 10.4 Hz, H-3′), 3.524 (1H, *dd*, 9.4/8.0 Hz, H-2′), 3.150 (1H, *dd*, 17.3/12.9 Hz, H-3a), 2.842 (1H, *dd*, 17.3/8.8 Hz, H-3b), 2.090 (3H, *s*, Me-CO-CH-4′), 2.042 (3H, *s*, Me-CO-CH-6′). ^{13}C NMR (CD_3OD) δ: 175.6 (C-2), 172.3 (CO-C-6′), 171.8 (C-6), 171.6 (CO-C-4′), 159.1 (C-16), 131.1 (C-14/C-18), 123.9 (C-13), 116.7 (C-15/C17), 109.1 (C-9), 97.2 (C-1′), 90.8 (C-5), 89.0 (C-8), 77.8 (C-11), 75.2 (C-3′), 74.7 (C-2′), 74.6 (C-12), 73.8 (C-5′), 71.3 (C-4′), 62.7 (C-6′), 45.4 (C-4), 34.2 (C-3), 20.9 (Me-CO-C-4′), 20.8 (Me-CO-C-6′). R_t (LC–MS): 12.10 min, negative ion ESI–MS: *m*/*z* 567 $[M-H]^-$; ESI–CIDMS (30 eV) of *m*/*z* 567 $[M-H]^-$ (*m*/*z*, rel int.): 525 (10), 483 (4), 450 (9), 439 (18), 421 (44), 321 (14), 261 (67), 259 (44), 221 (100), 203 (21), 145 (6). HR–ESI–MS *m*/*z*: 567.13557 $[M-H]^-$ (calc. for $C_{25}H_{27}O_{15}$ 567.13445). Acetylation of **4** yielded the peracetyleted vinburnolide A (**4a**): EIMS (rel. int.): 736 $[M]^+$ (0.5), 694 (13), 389 (29), 346 (8), 331 (40), 169 (100), 109 (39).

3.8. 4′-6′-Diacetyl-12-coumaroyl-viburnolide A (**5**)

Amorphous solid. $[\alpha]_D^{24}$ +41.9 (*c* 0.13). UV λ_{max} nm (log ε): 228 (4.18), 316 (4.10), 2.85 *sh*. ^1H NMR (CD_3OD) δ (*J*): 7.665 (1H, *d*, 16.0 Hz, H-3″), 7.490 (2H, *d*, 8.6 Hz, H-5″/9″), 7.252 (2H, *d*, 8.7 Hz, H-14/18), 6.807 (2H, *d*, 8.6 Hz, H-6″/8″), 6.792 (2H, *d*, 8.7 Hz, H-15/17), 6.387 (1H, *d*, 16.0 Hz, H-2″), 5.240 (1H, *dd*, 6.6/4.3 Hz, H-12), 4.933 (1H, *d*, 8.0 Hz, H-1′), 4.949 (1H, *t*, 9.6 Hz, H-4′), 4.650 (1H, *dd*, 13.0/8.8 Hz, H-4), 4.601 (1H, *dd*, 10.5/6.6 Hz, H-11a), 4.266 (1H, *dd*, 10.5/4.3 Hz, H-11b), 4.199 (1H, *s*, H-8), 4.191 (1H, *dd*, 12.6/3.8 Hz, H-6′a), 4.119 (1H, *dd*, 12.6/2.2 Hz, H-6′b), 3.707 (1H, *m*, H-5′), 3.645 (1H, *t*, 9.3 Hz, H-3′), 3.548 (1H, *dd*, 9.3/8.0 Hz, H-2′), 3.171 (1H, *dd*, 17.4/13.0 Hz, H-3a), 2.857 (1H, *dd*, 17.4/8.8 Hz, H-3b), 2.084 (3H, *s*, Me-CO-CH-4′), 2.054 (3H, *s*, Me-CO-CH-6′). ^{13}C NMR (CD_3OD) δ: 175.5 (C-2), 172.4 (CO-C-6′), 171.6 (C-6), 171.6 (CO-C-4′), 167.8 (C-1″), 161.4 (C-7″), 159.1 (C-16), 147.8 (C-3″), 131.4 (C5″/9″), 131.0 (C14/18), 126.9 (C-4″), 123.8 (C-13), 116.7 (C-6″/8″), 116.7 (C-15/17), 113.8 (C-2″), 109.1 (C-9), 97.3 (C-1′), 90.7 (C-5), 87.1 (C-8), 76.2 (C-12), 76.0 (C-11), 75.6 (C-3′), 74.6 (C-2′), 73.6 (C-5′), 71.2 (C-4′), 62.7 (C-6′), 45.3 (C-4), 34.3 (C-3), 20.9 (Me-CO-C-4′), 20.8 (Me-CO-C-6′. R_t (LC–MS): 16.03 min, negative ion ESI–MS: *m*/*z* 713 $[M-H]^-$; ESI–CIDMS (40 eV) of *m*/*z* 713 $[M-H]^-$ (*m*/*z*, rel. int.): 321 (7), 285 (3), 221 (8), 203(5), 163 (11), 145 (100). HR–ESI–MS *m*/*z*: 713.17176 $[M-H]^-$ (calc. for $C_{34}H_{33}O_{17}$ 713.17178).

3.9. Tetraacetylviburnolide A (**6**)

Colourless crystals, mp. 219–221 °C. $[\alpha]_D^{25}$ −27.5 (*c* 0.10). UV λ_{max} nm (log ε): 2.28 (3.80), 2.76 (3.08), 2.84 sh. ^1H NMR (CD_3OD) δ (*J*): 7.217 (2H, *d*, 8.8 Hz, H-14/18), 6.784 (2H, *d*, 8.8, H-15/17), 5.179–4.991 (4H), 4.678–4.5713 (2H, H-4, H-11a), 4.184 (2H, *bdd*, H-6′), 3.999 (1H, *dd*, 10.4/4.7, H-11b), 3.994 (1H, *s*, H-8), 3.840 (1H, *t*, 9.3, H-3′), 3.293 (1H, *dt*, 9.9/3.0, H-5′), 3.164 (1H, *dd*, 12.9/17.3, H-3a), 2.860 (1H, *dd*, 17.3/8.8, H-3b), 2.153 (3H, *s*, Me-CO), 2.089 (3H, *s*, Me-CO), 2.059 (6H, *s*, 2×Me-CO). ^{13}C NMR (CD_3OD) δ: 175.4 (C-2), 172.3, 171.4, 171.3, 171.2, 171.0 (C-6, 5×Me-CO), 159.2 (C-16), 131.0 (C-14/18), 123.5 (C-13), 116.8 (C-15/17), 109.1 (C-9), 95.2 (C-1′), 90.5 (C-5), 86.9 (C-8), 76.2 (CH), 75.0 (C-11), 74.4, 73.9, 73.6, 70.9 (4×CH), 62.3 (C-6′), 45.3 (C-4), 34.1 (C-3), 21.4, 20.8, 10.7, 20.6 (4×Me-CO). EIMS (rel. int.): 652. 1644 $[M]^+$ (7, calc. for $C_{29}H_{32}O_{17}$ 652.1639), 610 (1), 347 (30), 289 (83), 229 (59), 187 (25), 169 (100), 147 (43), 127 (66), 120 (27), 109 (63). R_t (LC–MS): 15.72 min, negative ion ESI–MS: *m*/*z* 651 $[M-H]^-$.

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