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Abietane diterpenoid dimers from the roots of *Salvia prionitis*

Jun Xu, Jun Chang, Ming Zhao, Jin-Sheng Zhang *

State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Shanghai 201203, People's Republic of China

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Abstract

Three abietane diterpenoid dimers, bisprioterones A–C (**1**–**3**), were isolated from roots of the Chinese folk medicinal plant *Salvia prionitis* Hance (Labiatae). Compounds **1**–**3** possessed two different abietane diterpenoid skeleta, which were linked via either a C–C single bond (**1** and **2**) or an ether bridge (**3**). Their structures were elucidated by analysis of 1D and 2D NMR spectroscopic data. The structure of **1** was further confirmed by a single-crystal X-ray diffraction determination.
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Keywords: *Salvia prionitis*; Labiatae; Abietane diterpenoid dimer; Bisprioterones A–C

1. Introduction

Various species of the genus *Salvia* (Labiatae), especially *Salvia mittiorrhiza* (Danshen), are very important Chinese traditional medicines as antiphlogistic, antibacterial and cardiovascular agents. Most biologically active components reported from plants of the genus *Salvia* were abietane diterpenoid quinones bearing either *para*-quinone or *ortho*-quinone moieties (Xu, 1990). *Salvia prionitis* Hance, a medicinal herb distributed in the southern provinces of the People's Republic of China, is used in Chinese folk medicine for treatment of tonsillitis, pharyngitis, and bacillary dysentery (Luo, 1994). More than 40 compounds, i.e., tashinone II-A, tashinone I, and cryptotanshinone, have previously been isolated from this plant (Yang et al., 1988; Lin et al., 1989, 1990, 1995; Huang et al., 1990; Li et al., 2000, 2001; Chen et al., 2002). In a continuing study on its constituents, three abietane diterpenoid dimers, bisprioterones A–C (**1**–**3**), were obtained from *S. prionitis*. In this paper, we report on the isolation and structural elucidation of bisprioterones A–C (see Fig. 1).

2. Results and discussion

Compound **1** was obtained as white needles, whose molecular formula was determined to be $C_{40}H_{48}O_4$ from the HR-ESI-MS ($[M + Na]^+$, m/z found 615.3483, calcd. 615.3450). The ^1H NMR spectrum (Table 1) indicated the presence of five aromatic protons at δ 6.94 (1H, *d*, $J = 8.4$ Hz), δ 6.98 (1H, overlapping), δ 6.99 (1H, overlapping), δ 7.03 (1H, *s*) and δ 7.41 (1H, *d*, $J = 8.4$ Hz), and two aromatic angular methyls at δ 2.20 (3H, *s*) and δ 2.22 (3H, *s*). Two isopropyl groups were observed as the signals at δ 1.86 (1H, septet, $J = 6.6$ Hz), and δ 0.77, δ 0.80 (each 3H, *d*, $J = 6.6$ Hz); δ 3.28 (1H, septet, $J = 6.9$ Hz) and δ 1.21, δ 1.24 (each 3H, *d*, $J = 6.9$ Hz). The ^{13}C NMR and DEPT spectroscopic data revealed the presence of 40 carbons in the molecule, corresponding to 16 quaternary carbons, 12 methines, two methylenes and 10 methyls. Further analysis of the COSY, HMQC and HMBC (Fig. 2) spectra suggested two 4,5-*seco* abietane diterpenoid quinone moieties in the molecule. In one 4,5-*seco* abietane diterpenoid moiety, the side-chain $C(1)H_2$–$C(2)H_2$–$C(3)H{=}C(4)(CH_3)_2$, assigned to C-10, was established unambiguously by analysis of its COSY and HMBC spectra. In the other 4,5-*seco* rearranged abietane diterpenoid moiety, the side-chain $C(1')H_2$–$C(2')H_2$–$C(3')H{=}C(4')(CH_3)_2$ and the rearranged

* Corresponding author. Tel.: +86 21 50805892; fax: +86 21 50807088.
E-mail address: jszhang@mail.shcnc.ac.cn (J.-S. Zhang).

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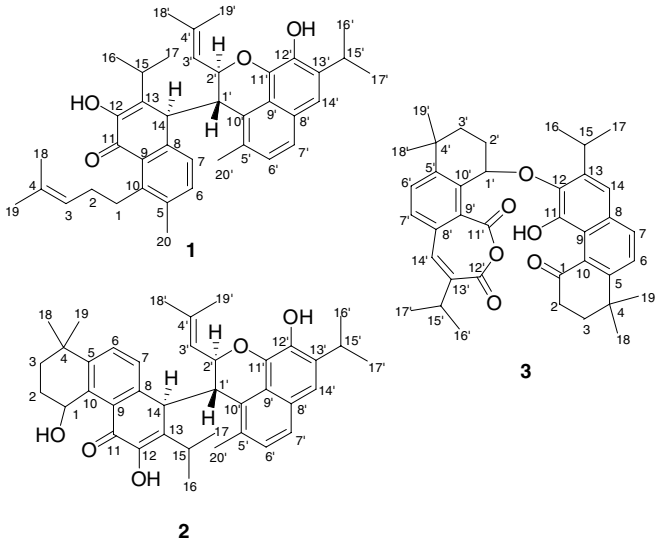

Fig. 1. Structures of compounds **1**–**3**.

linkage site were established through long-range HMBC correlations between H-2′ (δ_H 5.07) and C-11′ (δ_C 133.2). Finally, the two abietane diterpenoids was linked by a C–C single bond between C-14 and C-1′ according to correlations between H-1′ (δ_H 3.35) and C-8 (δ_C 144.7), H-14 (δ_H 3.94) and C-2′ (δ_C 73.3) in the HMBC spectrum. The X-ray diffraction analysis of compound **1** (Fig. 3) verified the above deductions, and the relative configurations at C-14, C-1′, and C-2′ were thus confirmed. The H-1′ and H-14 could be assigned with β-configuration, while the H-2′ was in an α-configuration. The upfield shifts of H-15 ($\Delta\delta_H$ −0.97), H_3-16 ($\Delta\delta_H$ −0.64) and H_3-17 ($\Delta\delta_H$ −0.70) compared with those of **2** (Table 1) were considered to be caused by shielding effects due to the aromatic ring (C-5′–C-14′). The non-observed coupling between the two neighboring protons H-1′ and H-2′ may be caused by the effect of the dihedral angle of H-1′ and H-2′, which was approximately 90° according to the perspective ORTEP drawing for **1** (Fig. 3). Consequently, the structure of **1** was established as a 4,5-*seco* arbietane diterpenoid dimer, and given the trivial name bisprioterone A.

Compound **2** was obtained as orange cuboid crystals, whose molecular formula was determined to be $C_{39}H_{46}O_5$ from the HR-ESI-MS ($[M + Na]^+$, *m*/*z* found 617.3260, calcd. 617.3243), suggesting **2** was another abietane diterpenoid dimer. According to the NMR spectroscopic data, one part of the structure was deduced to have the same skeleton as 1R-hydroxymitirone (Michavila et al., 1986). The remaining part of the NMR spectrum revealed similarities between compounds **1** and **2**, including the same moiety (C-1′–C-20′), the same linkage site of two parts, similar coupling constants for H-14 and H-1′, and absence of coupling between H-1′ and H-2′. The last two points suggested similar relative spatial locations of the three consecutive protons in **1** and **2**, this being one of the bases for the later computer modeling. The obvious upfield shift of the aromatic proton at C-7 (H-7, δ_H 5.44) in compound **2** instead of that of H-15 in compound **1**, suggested a reverse configuration at the linkage site of this moiety, C-14, in compound **2**. The results of computer modeling (Fig. 4) verified our deductions and supplied a possible conformation. Attempts to obtain suitable crystals for X-ray analysis to determine the relative configuration at C-1 were unsuccessful. Hence, the structure was elucidated as **2**, and given the trivial name bisprioterone B.

Compound **3** was obtained as an orange powder, whose molecular formula was $C_{38}H_{42}O_6$ according to the HR-EI-MS at *m*/*z* 594.2993 (M^+, calcd. 594.2981). The NMR spectroscopic data suggested two abietane moieties in the molecule. In one moiety, two resonances for an anhydride moiety were observed at δ_C 170.1 and δ_C 166.8, which together with the HMBC spectroscopic data (Fig. 2) suggested the presence of a seven-membered-ring anhydride in the molecule. Several compounds with similar structures have been isolated from plants of the same genus (Chang and Cheng et al., 1990; Chang and Choang et al., 1990). The ^1H and ^{13}C NMR spectroscopic data (Table 1) of the other moiety were the same as those of arucadiol (Michavila et al., 1986), except for absence of the OH-12 signal. Thus, an ether bridge was deduced to be located between C-12 and the oxygenated carbon C-1′. The structure of **3** was thus established and given the trivial name bisprioterone C.

3. Conclusions

Hongencaotone (Li et al., 2001), which was also isolated from the roots of *Salvia prionitis*, was the first abietane diterpenoid dimer discovered from the genus *Salvia*. Our findings on the discovery of the three abietane diterpenoid dimers bisprioterones A–C (**1**–**3**) could be regarded as some further insight into the diversity of natural products in the genus *Salvia*. Unfortunately, these diterpenoid dimers including hongencaotone, though with unique structural frameworks, exhibit no acute cytostatic, antiphlogistic, or antibacterial activities. In contrast to their monomers, the disappearance of some functional groups during the process of dimerization might account for the decrease of the bioactivities.

4. Experimental

4.1. General

Melting points were determined on a Fisher–Johns melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin–Elmer 241 automatic digital polarimeter, whereas UV spectral data were obtained on a Shimadzu UV-260 instrument. IR spectral data were acquired on a Perkin–Elmer 599B instrument with KBr disks, whereas ^1H NMR and ^{13}C NMR spectra were

Table 1
NMR spectroscopic data for compounds **1**–**3**[a]

Site	**1**		**2**		**3**	
	^1H[b]	^{13}C[c]	^1H[b]	^{13}C[c]	^1H[b]	^{13}C[c]
1	2.88 *m*, 3.12 *m*	30.2 *t*	5.47 br *t*	63.5 *d*		205.0 *s*
2	1.91 *m*, 2.07 *m*	28.9 *t*	1.86 *m*, 2.05 *m*	27.1 *t*	2.93 *t* (6.8)	36.6 *t*
3	5.22 *t* (6.6)	124.6 *d*	2.02 *m*	32.2 *t*	2.07 *t* (6.8)	35.4 *t*
4		131.7 *s*		34.1 *s*		36.2 *s*
5		143.0 *s*		145.1 *s*		157.7 *s*
6	6.99 overlapping	132.8 *d*	6.43 *d* (8.3)	129.4 *d*	7.43 *d* (8.7)	123.0 *d*
7	6.98 overlapping	126.5 *d*	5.44 *d* (8.3)	128.1 *d*	7.95 *d* (8.7)	137.5 *d*
8		144.7 *s*		143.6 *s*		132.3 *s*
9		128.6 *s*		128.0 *s*		121.4 *s*
10		136.1 *s*		139.0 *s*		127.7 *s*
11		183.2 *s*		184.3 *s*		145.3 *s*
12		145.3 *s*		146.6 *s*		137.7 *s*
13		133.2 *s*		134.1 *s*		141.8 *s*
14	3.94 *d* (3.3)	49.1 *d*	3.97 *d* (3.5)	48.6 *d*	7.22 *s*	117.2 *d*
15	1.86 septet (6.6)	32.3 *d*	2.83 septet (6.9)	32.9 *d*	2.98 septet (6.8)	27.5 *d*
16	0.77 *d* (6.6)	19.7 *q*	1.41 *d* (6.9)	19.8 *q*	1.15 *d* (6.8)	23.0 *q*
17	0.80 *d* (6.6)	20.8 *q*	1.50 *d* (6.9)	20.0 *q*	1.17 *d* (6.8)	22.7 *q*
18	1.61 *s*	25.8 *q*	0.87 *s*	30.5 *q*	1.43 *s*	29.7 *q*
19	1.67 *s*	17.7 *q*	1.09 *s*	31.4 *q*	1.43 *s*	29.6 *q*
20	2.20 *s*	19.7 *q*				
1′	3.35 *d* (3.3)	50.3 *d*	3.68 *d* (3.4)	44.3 *d*	5.16 *dd* (11.6, 5.2)	77.9 *d*
2′	5.07 *d* (8.4)	73.3 *d*	5.10 *d* (9.4)	69.3 *d*	1.59 *m*, 2.38 *m*	26.4 *t*
3′	4.81 *d* (8.4)	123.2 *d*	4.88 *d* (9.3)	122.3 *d*	1.86 *m*	37.3 *t*
4′		136.1 *s*		137.7 *s*		34.5 *s*
5′		132.0 *s*		130.4 *s*		141.8 *s*
6′	6.94 *d* (8.4)	126.6 *d*	7.23 *d* (8.4)	126.6 *d*	7.38 *d* (8.0)	130.0 *d*
7′	7.41 *d* (8.4)	126.2 *d*	7.60 *d* (8.4)	126.5 *d*	7.87 *d* (8.0)	130.9 *d*
8′		126.0 *s*		125.6 *s*		147.4 *s*
9′		119.9 *s*		120.8 *s*		121.5 *s*
10′		123.6 *s*		124.7 *s*		133.8 *s*
11′		133.2 *s*		132.5 *s*		170.1 *s*
12′		139.2 *s*		138.7 *s*		166.8 *s*
13′		136.0 *s*		136.2 *s*		145.3 *s*
14′	7.03 *s*	115.4 *d*	7.09 *s*	114.9 *d*	7.48 *s*	127.3 *d*
15′	3.28 septet (6.9)	27.6 *d*	3.15 septet (6.8)	27.2 *d*	3.19 septet (6.7)	33.1 *d*
16′	1.24 *d* (6.9)	22.1 *q*	1.25 *d* (6.8)	21.5 *q*	1.41 *d* (6.7)	22.2 *q*
17′	1.21 *d* (6.9)	23.0 *q*	1.17 *d* (6.8)	23.3 *q*	1.43 *d* (6.7)	22.2 *q*
18′	1.37 *s*	25.5 *q*	1.50 *s*	25.6 *q*	1.44 *s*	30.8 *q*
19′	1.33 *s*	17.9 *q*	1.71 *s*	18.7 *q*	1.15 *s*	31.6 *q*
20′	2.22 *s*	18.7 *q*	2.68 *s*	18.9 *q*		
OH-11					10.65 *s*	
OH-12	6.71 *s*		7.28 *s*			
OH-12′	5.48 *s*		4.92 *s*			

[a] Assignments were made by COSY, HMBC and HMQC data.
[b] 500 MHz, $CDCl_3$; chemical shifts in ppm relative to TMS; coupling constant (*J*) in Hz.
[c] 125 MHz, $CDCl_3$; multiplicity was established from DEPT data.

recorded at 500 and 125 MHz, respectively, with TMS as an internal standard. EI-MS data were obtained on a MAT-711 mass spectrometer, and ESI-MS data were measured on a Micromass Quattro mass spectrometer. Computer modeling was carried out on a Pentium III workstation by the use of Chem3D of the CS Chemoffice Ultra® version 7.0 program package.

4.2. Plant material

The roots of *Salvia prionitis* were collected in July 2003 at Shangrao, Jiangxi Province of People's Republic of China. The plant was authenticated by Prof. Xiu-lan Huang of Shanghai Institute of Materia Medica. A voucher specimen has been deposited in the Herbarium of Shanghai Institute of Materia Medica (No. SIMMP95068).

4.3. Extraction and isolation

Dried roots of *Salvia prionitis* (10 kg) were extracted with EtOH–H_2O (95:5, 60 L) for 6 days at room temperature, the solvent removed in vacuo to yield a gummy residue (340 g), which was subjected to silica gel CC (15 × 100 cm) and eluted with a PE–EtOAc gradient at a flow rate of 15 mL/min to obtain fraction 1 [5.0 L, PE–EtOAc (60:1)], fraction 2 [5.0 L, PE–EtOAc (40:1)],

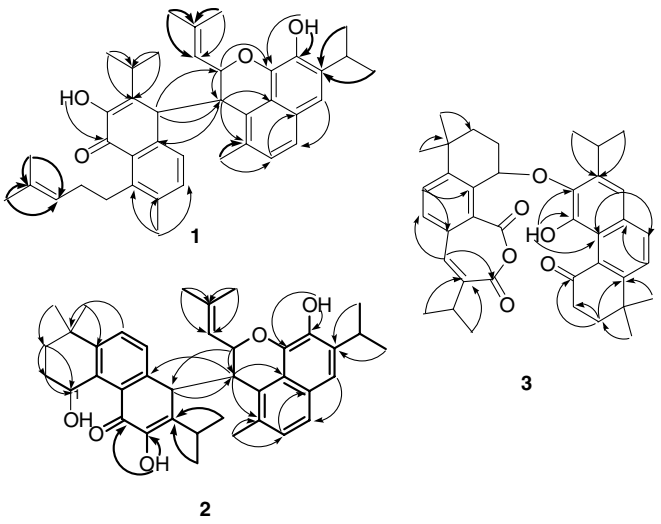

Fig. 2. Selected HMBC correlations of compounds **1**–**3** (H → C).

fraction 3 [6.0 L, PE–EtOAc (30:1)], fraction 4 [6.0 L, PE–EtOAc (20:1)], fraction 5 [6.0 L, PE–EtOAc (10:1)], fraction 6 [7.0 L, PE–EtOAc (5:1)], fraction 7 [7.0 L, PE–EtOAc (2:1)], and fraction 8 [7.0 L, PE–EtOAc (1:1)]. Fraction 4 (17 g) was subjected to additional silica gel CC (8×50 cm) using PE–EtOAc (30:1) as eluent to obtain 11 fractions 4A–4K (eluent volume: 500 mL/fraction). Fraction 4B (1.5 g) was next applied to Sephadex LH-20 CC [eluted with CH_2Cl_2–MeOH (1:1)] and then preparative TLC on silica gel (solvent system: PE/EtOAc, 15:1) to give bisprioterone A (**1**, 16 mg) and bisprioterone C (**3**, 9 mg). Fraction 4J (1.3 g) was carried out for similar procedure to give bisprioterone B (**2**, 13 mg).

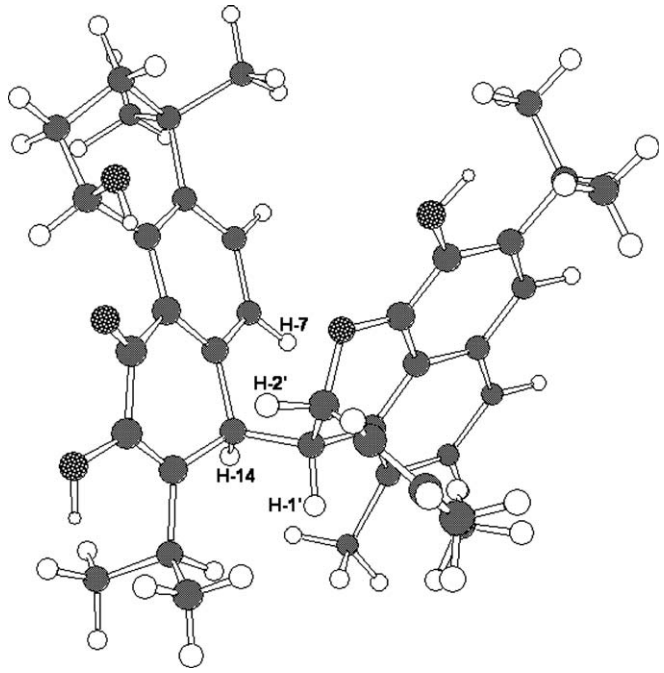

Fig. 4. Stereoviews of compound **2** generated from computer modeling.

4.4. Characterization

4.4.1. Bisprioterone A (*1*)

White needles (methanol); m.p. 153–155 °C; $[\alpha]_D^{20}$ +123° ($CHCl_3$; *c* 0.16); UV (MeOH) λ_{max} nm (log ε): 246 (4.22); IR (KBr) ν_{max} cm^{-1}: 3535, 3379; 2962, 2924, 1666, 1623, 1589, 1429, 1375, 1215, 1172, 1024, 866, 469; For ^1H and ^{13}C NMR spectra, see Table 1; EI-MS *m*/*z* (rel. int.): 592

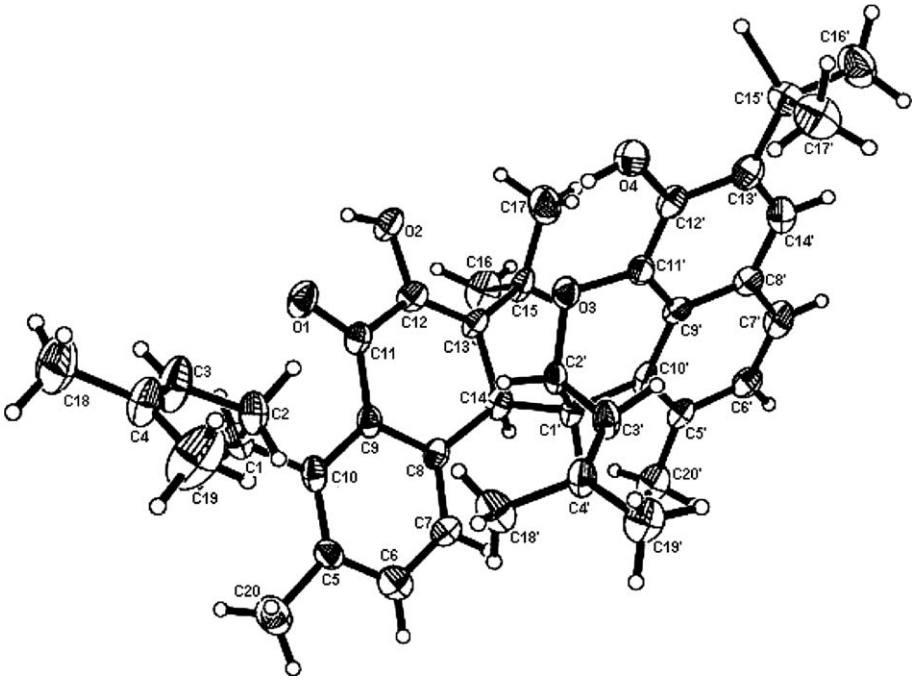

Fig. 3. Perspective ORTEP drawing for compound **1**.

$[M]^+$ (0.5), 296 (52), 295 (100), 229 (24), 227 (86); HR-ESI-MS *m/z*: 615.3483 (calcd. for $C_{40}H_{48}O_4Na$, 615.3450).

4.4.2. X-ray crystallography data of 1

$C_{40}H_{26}O_4$, mol. wt. = 592.78, triclinic space group $P\bar{1}$, $a = 8.0397(17)$ Å, $b = 14.194(3)$ Å, $c = 16.628(4)$ Å, $\alpha = 68.714(5)°$, $\beta = 85.535(6)°$, $\gamma = 74.031(4)°$, $V = 1699.4(6)$ Å^3, $Z = 2$, $d = 1.158$ g/cm^3. $F(000) = 640$, $\mu = 0.073$ mm^{-1}. A single crystal of dimensions $0.489 \times 0.201 \times 0.053$ mm was used for X-ray measurements. The intensity data of all unique reflections within the θ range 2.54–26.00° were collected at 293 K in a SMART APEX CCD diffractometer, using graphite monochromated Mo Kα ($\lambda = 0.71073$ Å) radiation. A total of 6576 independent reflections were measured, and 1931 were considered to be observed ($|F|^2 \geqslant 2\sigma|F|^2$). Hydrogen atoms were fixed at calculated positions. The structure was solved by the direct method SHELX-97 and expanded using difference Fourier techniques, refined by the program and full-matrix least-squares calculations. CCDC 263818 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road; Cambridge CB2 1EZ, UK; fax: +44 1223 336 033; email: deposit@ccdc.cam.ac.uk).

4.4.3. Bisprioterone B (2)

Orange cuboid crystals; m.p. 184–186 °C; $[\alpha]_D^{20}$ +5° ($CHCl_3$; *c* 0.39); UV (MeOH) λ_{max} nm (log ε): 249 (4.20); IR (KBr) ν_{max} cm^{-1}: 3396, 2960, 2931, 2869, 1668, 1608, 1583, 1431, 1263, 1174, 1147, 1043, 586; For ^{1}H and ^{13}C NMR spectra, see Table 1; EI-MS *m/z* (rel. int.): 594 $[M]^+$ (0.5), 297 (34), 294 (50), 282 (32), 227 (100); HR-ESI-MS *m/z*: 617.3260 (calcd. for $C_{39}H_{46}O_5Na$, 617.3243).

4.4.4. Bisprioterone C (3)

Orange powder; $[\alpha]_D^{20}$ −21° ($CHCl_3$; *c* 0.12); UV (MeOH) λ_{max} nm (log ε): 216 (3.85); IR (KBr) ν_{max} cm^{-1}: 3433, 2962, 2929, 2870, 1761, 1639, 1496, 1460, 1412, 1298, 1198, 1147, 1072, 1005, 604; For ^{1}H and ^{13}C NMR spectra, see Table 1; EI-MS *m/z* (rel. int.): 594 $[M]^+$ (2), 297 (100), 253 (38); HR-EI-MS *m/z*: 594.2993 (calcd. for $C_{38}H_{42}O_6$, 594.2981).

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