Published in final edited form as:

J Nat Prod. 2016 March 25; 79(3): 578–583. doi:10.1021/acs.jnatprod.5b01012.

Carolignans from the Aerial Parts of *Euphorbia sikkimensis* and Their Anti-HIV Activity

Cheng Jiang[†], **Pan Luo**[†], **Yu Zhao**[‡], **Jialing Hong**[†], **Susan L. Morris-Natschke**[‡], **Jun Xu**[†], **Chin-Ho Chen**[§], **Kuo-Hsiung Lee**[*,‡,⊥], and **Qiong Gu**[*,†,‡]

†

‡

§

⊥

Abstract

Seven new carolignans, including two pairs of enantiomers (±)-*erythro*-7′-methylcarolignan E (**1a/1b**) and (±)-*threo*-7′-methylcarolignan E (**2a/2b**), (+)-*threo*-carolignan E (**3a**), (+)-*erythro*-carolignan E (**4a**), and (−)-*erythro*-carolignan Z (**5**), together with four known lignans (**3b, 4b, 6,** and **7**) and six polyphenols (**8–13**) were isolated from the aerial parts of *Euphorbia sikkimensis*. The structures of the new compounds were elucidated by spectroscopic analysis, and their absolute configurations were determined by electronic circular dichroism calculations. Seven of the isolates were examined for anti-HIV effects, and compounds **1a** and **1b** showed moderate anti-HIV activity with EC_{50} values of 6.3 and 5.3 μM.

The genus *Euphorbia* belongs to the family Euphorbiaceae, which is characterized by production of a white, milky latex that is somewhat toxic. The family contains about 300 genera and 7000 species. *Euphorbia* is one of the largest genera in the Euphorbiaceae family, with about 1600 species. Numerous chemical studies on the *Euphorbia* genus have

[*]**Corresponding Authors** Tel: 919-962-0066. Fax: 919-966-3893. khlee@unc.edu (K.-H. Lee). Tel/Fax: +86-20-39943077. guqiong@mail.sysu.edu.cn (Q. Gu)..

The authors declare no competing financial interest.

ASSOCIATED CONTENT

Supporting Information The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b01012.

UV, IR, MS, and 1D and 2D NMR for compounds **1a, 1b, 2a, 2b, 3a, 4a,** and **5** (PDF)

DEDICATION

Dedicated to Professors John Blunt and Murray Munro, of the University of Canterbury, for their pioneering work on bioactive marine natural products.

Special Issue: Special Issue in Honor of John Blunt and Murray Munro

been performed.[1,2] *Euphorbia sikkimensis* Boiss. has been used to treat poisoning, malaria, rheumatism, and jaundice.[3] In 2013, new and known diterpenes, triterpenoids, tocopherol derivatives, and other compounds were isolated.[4,5] Two diterpenoids showed antiproliferative effects against the A549 cancer cell line, while (−)-bornyl ferulate exhibited antiangiogenic activity in a zebrafish model.[5]

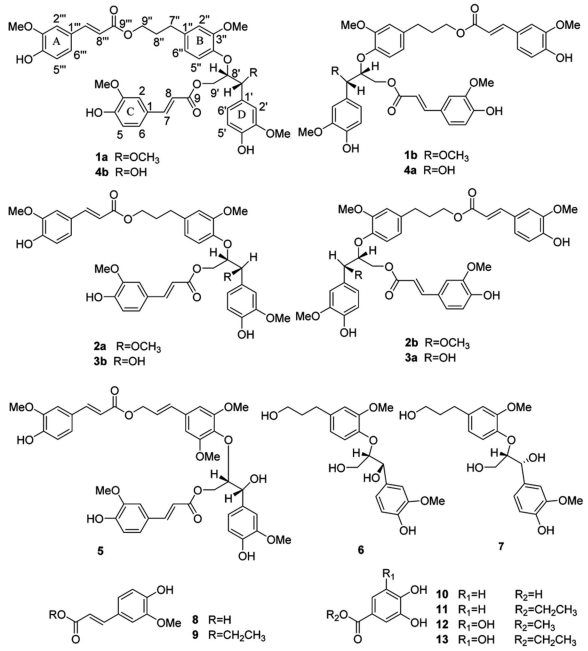
In a continuing search for anti-HIV natural products from herbal medicinal plants, anti-HIV screening showed that an EtOAc-soluble fraction from an ethanol extract of the aerial parts of *E. sikkimensis* had an EC_{50} value of 2.5 μg/mL and an SI (selective index) value of more than 8.3. Prior literature reported that diterpenoids from *Euphorbia* showed potent anti-HIV activity at the nanomolar level,[6,7] whereas one of these diterpenoids, a phorbol ester, also showed tumor-promoting activity.[8] These results encouraged us to investigate the anti-HIV-active constituents of the EtOAc-soluble fraction, which led to the isolation and characterization of seven new lignans (**1–7**) and six polyphenols (**8–13**). Compounds **1a** and **1b** showed moderate anti-HIV activity with EC_{50} values of 6.3 and 5.3 μM, respectively. Herein are reported details of the isolation, structural elucidation, and anti-HIV activity of these compounds.

The known compounds were identified as (−)-(7′*R*,8′*S*)-*erythro*-carolignan E (**3b**),[9,10] (−)-(7′*S*,8′*S*)-*threo*-carolignan E (**4b**),[9,10] *threo*-guaiacylglycerol-*β*-*O*-4′-dihydroconiferyl alcohol (**6**),[11,12] *erythro*-guaiacylglycerol-*β*-*O*-4′-dihydroconiferyl alcohol (**7**),[11,12] (*E*)-ferulic acid (**8**),[13,14] ethyl ferulate (**9**),[14] protocatechuic acid (**10**),[15,16] vanillic acid (**11**),[17,18] methyl gallate (**12**),[19] and ethyl gallate (**13**),[19] based on spectroscopic data analysis and comparison with reported literature values.

RESULTS AND DISCUSSION

Compound **1** (**1a**/**1b**) was obtained as a white, amorphous solid. A molecular formula of $C_{41}H_{44}O_{13}$ was deduced from a quasimolecular ion peak at *m/z* 743.27039 (calcd 743.27091) observed in the negative HRESIMS. The IR spectrum showed absorption bands for OH (3407 cm^{-1}), carbonyl (1704 cm^{-1}), and aromatic groups (1599, 1514, and 1460 cm^{-1}). The ^1H NMR spectrum showed two pairs of doublets at δ_H 7.59 (1H, d, J = 15.9 Hz, H-7‴) and 6.27 (1H, d, J = 15.9 Hz, H-8‴); 7.48 (1H, d, J = 15.9 Hz, H-7) and 6.23 (1H, d, J = 15.9 Hz, H-8), together with six aromatic protons (ring A: δ_H 7.00 (1H, d, J = 1.8 Hz, H-2‴), 6.91 (1H, d, J = 8.1 Hz, H-5‴), 7.05 (1H, dd, J = 1.8, 8.1 Hz, H-6‴); ring C: δ_H 7.02 (1H, d, J = 1.9 Hz, H-2), 6.85 (1H, d, J = 8.2 Hz, H-5), 7.05 (1H, dd, J = 1.9, 8.2 Hz, H-6)). The ^{13}C NMR spectrum exhibited two conjugated carbonyl carbons at δ_C 167.1 and 167.5 ppm. The above data indicated the presence of two feruloyl moieties. The ^{13}C NMR spectrum (Figure S6, Supporting Information) also displayed two oxymethylene (CH_2O, δ_C 63.9), two methylene (CH_2, δ_C 32.1, 30.6), and two oxymethine (CHO, δ_C 83.5, 82.3) signals, and the COSY correlations (Figure 1) suggested one CH_2O–CH_2–CH_2 fragment and one CH_2O–CHO–CHO group. The ^1H and ^{13}C NMR data (Tables 1 and 2) also indicated two 1,3,4-trisubstituted aromatic units (rings B and D) and five methoxy groups. HMBC correlations (Figure 1) of H-9″ (δ_H 4.15, 2H, t, J = 6.5 Hz) with C-9‴ (δ_C 167.5) and H-9′ (δ_H 4.11, 1H, dd, J = 4.7, 11.6 Hz; 4.31, 1H, dd, J = 2.8, 11.6 Hz) with C-9 (δ_C 167.1) showed that two feruloyl units were connected at C-9 and C-9‴. All of the above data were

closely similar to those of the known compound *erythro*-carolignan E,[9,10] except for an additional methoxy group in the new compound. The position of this methoxy group at C-7′ was confirmed from the HMBC correlation of MeO (δ_H 3.27, s) with C-7′ (δ_C 83.5, d). Its relative configuration was established on the basis of NOESY correlations (Figure 2) and interpretation of ^{1}H–^{1}H coupling constants. A coupling constant of 15.9 Hz between H-8 (8‴) and H-7 (7‴) suggested an *E*-configuration for Δ^7 and $\Delta^{7'''}$. The coupling constant between H-7′ and H-8′ (3.0 Hz) implied an *erythro*-configuration of these two protons.[20–22] Thus, compound **1** (*erythro*-7′-methylcarolignan E) was fully identified.



The specific rotation of **1** approached zero, and no Cotton effect was found in the electronic circular dichroism (ECD) spectrum of **1**, indicating a racemic mixture. Subsequent chiral resolution of **1** afforded the anticipated enantiomers **1a** and **1b**, which showed mirror image-like ECD curves (Figure 3a) and specific rotations (**1a**: $[\alpha]^{20}_D$ −16.2; **1b**: $[\alpha]^{20}_D$ +17.1). In order to define the absolute configuration of the enantiomers **1a** and **1b**, ECD calculations were performed for the two configurations (7′*R*,8′*S*)- and (7′*S*,8′*R*)-**1** using the Gaussian 09 program at the TD-DFT-B3LYP/6-311++G(d,p) level in MeCN. The calculation for the 7′*R*, 8′*S* enantiomer agreed with the experimental ECD data (Figure 3a) of **1a.** Thus, **1a** has a 7′*R*,8′*S*-configuration. The calculated ECD spectrum for the 7′*S*,8′*R*-configuration was in good accordance with the experimental spectrum of **1b** (Figure 3a). Consequently, the absolute configuration of **1b** was unambiguously assigned with the 7′*S*,8′*R*-configuration. Thus, compounds **1a** and **1b** were given the trivial names (−)-(7′*R*,8′*S*)-*erythro*-7′-methylcarolignan E and (+)-(7′*S*,8′*R*)-*erythro*-7′-methylcarolignan E, respectively.

Compound **2** (**2a**/**2b**) was isolated as a white, amorphous solid, having the same molecular formula as that of **1** as deduced from HRESIMS. The NMR data of **2** were almost identical to those of **1**, except for a slight discrepancy in the positions of the oxygenated methines (C-7′ and C-8′). Detailed 2D NMR analysis (Figures S18–20, Supporting Information)

revealed that **2** shares the same planar structure as that of **1**, indicating **2** to be a stereoisomer of **1** with the configuration changed at C-7′ or C-8′. A *threo*-configuration of **2** was further determined by the coupling constants between H-7′ and H-8′ (6.3 Hz).[20,23] Compound **2** also has negligible optical activity and an ECD spectrum devoid of Cotton effects. Chiral isolation of **2** afforded the enantiomers **2a** and **2b**. By comparison of the calculated ECD spectra of 7′*S*,8′*S*-**2** and 7′*R*,8′*R*-**2** with the experimental data of **2a** and **2b** (Figure 3b), the absolute configurations of **2a** and **2b** were assigned as 7′*S*,8′*S* and 7′*R*,8′*R*. Compounds **2a** and **2b** were given the trivial names (−)-(7′*S*,8′*S*)-*threo*-7′-methylcarolignan E and (+)-(7′*R*,8′*R*)-*threo*-7′-methylcarolignan E, respectively.

Compound **3** (**3a**/**3b**), obtained as a white, amorphous solid, gave a molecular formula $C_{40}H_{42}O_{13}$ determined from the HRESIMS ion at *m/z* 729.2542 $[M - H]^-$(calcd $C_{40}H_{41}O_{13}$). Its NMR data (Tables 1 and 2) were almost identical to those of *threo*-carolignan E.[9,10] Compound **3** showed a negligible specific rotation and CD spectrum, indicating it to be a racemic mixture. Subsequent chiral separation of **3** afforded a pair of enantiomer **3a** and **3b**, which had opposite ECD curves (Figure 3c) and optical rotations (**3a**: $[\alpha]^{20}_D$ +30.4; **3b**: $[\alpha]^{20}_D$ −29.8). The absolute configurations of **3a** and **3b** were determined using the same methods as described in **1a** and **1b**. Thus, **3a** was defined as (+)-(7′*R*,8′*R*)-*threo*-carolignan E, and **3b** was identified as the known compound (−)-(7′*S*,8′*S*)-*threo*-carolignan E.[10]

Compound **4** (**4a**/**4b**), a white, amorphous solid, showed the same molecular formula as that of **3**. The two compounds had similar NMR data, except for the position of the C-7′ and C-8′ oxygenated methines. Detailed 2D NMR analysis confirmed that **4** and **3** shared the same planar structure, indicating that **4** is a stereoisomer of **3** with a different orientation of the substituents at C-7′ or C-8′. The coupling constant $J_{7',8'}$ was 3.3 Hz, so therefore the relative configuration at C-7′ and C-8′ was *erythro*. Compound **4** also showed a negligible optical activity and an ECD spectrum devoid of Cotton effects. Chiral isolation of **4** afforded the enantiomers **4a** and **4b**. By comparison of the calculated ECD spectra of the 7′*R*,8′*S* and 7′*S*,8′*R* configurations of **4** with the experimental data of **4a** and **4b** (Figure 3d), the absolute configurations of **4a** and **4b** were assigned as 7′*S*,8′*R* and 7′*R*,8′*S*, respectively. Thus, **4a** was identified as (+)-(7′*S*,8′*R*)-*erythro*-carolignan E, and **4b** was elucidated as the known compound (−)-(7′*R*,8′*S*)-*erythro*-carolignan E.[10]

Compound **5**, a white, amorphous solid, gave the molecular formula $C_{41}H_{42}O_{14}$ as determined by the HRESIMS ion at *m/z* 757.24921 $[M - H]^-$(calcd 757.25018). The NMR data of **5** were similar to those of **1** with an additional methoxy group in the B-ring and two olefinic carbons (δ_C 134.0, 123.8) rather than two methylene signals. The B-ring proton signals at δ_H 6.60 (2H, d, J = 1.8 Hz) implied a symmetrical 1,3,4,5-tetrasubstituted aromatic ring, consistent with the location of the additional methoxy group in **5** at C-5″. This assignment was confirmed by the HMBC correlations (Figure 4) of the methoxy protons (δ_H 3.90) with C-6″ (δ_C 103.9). The location of the double bond was determined by HMBC correlation from the olefinic protons (δ_H 6.30, H-8″) with C-1″ (δ_C 130.9). The overall structure was further established by analysis of its 2D NMR spectra (Figures S46–49, Supporting Information). An *erythro*-configuration of **5** was further determined by the

coupling constants between H-7′ and H-8′ (3.1 Hz).[20–22] The experimental ECD spectrum of **5** showed an ECD curve with Cotton effects around 324 (−), 290 (−), and 242 (+) nm (Figure 3e). The absolute configuration of **5** was assigned as *R*,*S* through comparison with the calculated ECD spectra of (7′*R*,8′*S*)-**5** (Figure 3e). Thus, **5** was elucidated as (−)-7′*R*,8′*S*-*erythro*-carolignan Z.

Seven compounds (**1a, 1b, 2a, 2b, 5, 8,** and **9**) were tested for in vitro inhibitory effects against HIV-1 replication in MT4 cell lines, with AZT used as the positive control. One pair of enantiomers, **1a** and **1b**, showed more potent anti-HIV activity.

EXPERIMENTAL SECTION

General Experimental Procedures

Optical rotations were measured on a PerkinElmer 341 automatic polarimeter. UV spectra were recorded using a Shimadzu UV-2450 spectrophotometer. CD spectra were obtained on an Applied Photophysics Chirascan spectrometer. IR spectra were determined on a Bruker Tensor 37 infrared spectrophotometer. The ^{1}H (400 MHz), ^{13}C (100 MHz), and 2D NMR spectra were obtained on a Bruker AM-400 with tetramethylsilane as an internal reference at 25 °C. Chemical shifts (δ) are expressed in ppm with reference to the solvent signals. HRESIMS were acquired on a Shimadzu LCMS-IT-TOF instrument, and the ESIMS data were measured on an Agilent 1200 series LC-MS/MS system. Macroporous resin D101 (Sinopharm Chemical Reagent Co. Ltd., Shanghai, People's Republic of China), RP-C18 silica gel (Fuji, 40–75 μm), MCI gel CHP20P (75–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan), silica gel (200–300 mesh, Marine Chemical Ltd., Qingdao, People's Republic of China), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Sweden) were used for column chromatography. Analytical and semipreparative HPLC separation were carried out on an LC-20AT Shimadzu liquid chromatography system with a Zorbax SB-C18 column (250 × 9.4 mm, 5 μm) or an Agilent SB-C18 column connected with an SPD-M20A diode array detector. Semipreparative chiral HPLC separation was carried out on an LC-20AT Shimadzu liquid chromatography system with a Phenomenex Lux cellulose-2 chiral-phase column (250 × 10 mm, 5 μm). Thin-layer chromatography (TLC) analysis was carried out on silica gel plates (Marine Chemical Ltd.). Fractions were monitored by TLC and visualized by heating plates sprayed with 5% H_2SO_4 in EtOH. All solvents were of analytical grade (Guangzhou Chemical Reagents Company Ltd., Guangzhou, People's Republic of China).

Plant Material

The aerial parts of *Euphorbia sikkimensis* were collected at Longli County of the Qiannan Buyi National Minority Miao National Minority Autonomous Region, Guizhou Province, People's Republic of China, in July 2014. The sample was identified by Dr. Qingwen Sun from Guiyang College of Traditional Chinese Medicine, and a voucher specimen (GZQSY356) has been deposited at the School of Pharmaceutical Science, Sun Yat-sen University.

Extraction and Isolation

The air-dried and powdered aerial parts (20 kg) of *E. sikkimensis* were extracted with 95% EtOH (3 × 40 L) at room temperature for 48 h. The solvent was concentrated under reduced pressure to give a crude extract (2.562 kg). The 95% EtOH extract was then suspended in H_2O (2 L) and successively partitioned with petroleum ether (3 × 8 L), EtOAc (3 × 8 L), and *n*-BuOH (3 × 8 L) to yield three corresponding portions. The EtOAc-soluble extract (334 g) was chromatographed on D101 macroporous resin eluting with a step gradient of EtOH–H_2O (2:8, 5:5, 7:3, 10:0) to afford four fractions (A–D). Fraction A was separated using MCI gel CHP20P eluting with an increasing gradient of MeOH–H_2O from 30% to 100% and then further purified by silica gel columns to give compounds **8** (4 mg), **10** (6 mg), **11** (20 mg), **12** (2.0 g), and **13** (5.2 g). Fraction B was subjected to an RP-18 column with MeOH–H_2O (30–100%) as eluent to afford four subfractions (B1–B4). Fraction B1 (16.4 g) was chromatographed on a silica gel column using CH_2Cl_2–MeOH (1:0, 100:1, 15:1) to yield six subfractions (B1a–B1f). Fraction B1c was submitted to separation over a Sephadex LH-20 column eluting with CH_2Cl_2–MeOH (1:1) to give four subfractions (B1c1–B1c4). Fraction B1c2 was further purified by semipreparative HPLC eluting with MeOH–H_2O (60:40) to produce compounds **3** (10 mg) and **4** (6.2 mg). Fractions B1d and B1e were combined and further subjected to MPLC using CH_2Cl_2–EtOAc (50:1) and further purified with semipreparative HPLC eluting with MeCN–H_2O (55:45) to give compounds **1** (8.2 mg) and **2** (15.8 mg). Compounds **5** (10.2 mg), **6** (1.0 mg), and **7** (0.8 mg) were isolated from fraction B1f by semipreparative HPLC with MeCN–H_2O (50:50). Fraction C was chromatographed over a silica gel-containing column eluting with CH_2Cl_2–MeOH (200:1, 20:1) to obtain compound **9** (4 mg). Compounds **1–4** were further separated by semipreparative chiral HPLC (CH_3OH–H_2O, 9:1, 3 mL/min) to give **1a** (3.5 mg, t_R 30.4 min), **1b** (3.4 mg, t_R 33.2 min), **2a** (7.3 mg, t_R 31.2 min), **2b** (5.7 mg, t_R 34.6 min), **3a** (2.9 mg, t_R 28.2 min), **3b** (5.5 mg, t_R 25.7 min), **4a** (2.1 mg, t_R, 29.4 min), and **4b** (2.5 mg, t_R, 26.8 min), respectively.

*(−)-(7′R,8′S)-erythro-7′-Methylcarolignan E (**1a**):* white, amorphous solid; $[\alpha]^{20}_D$ −16.2 (*c* 0.1, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 231 (4.58), 288 (3.87), 327 (4.68) nm; ECD (MeCN) λ_{max} ($\Delta\varepsilon$) 232 (1.03), 211 (−3.72) nm; IR ν_{max} 3407, 2962, 2930, 2853, 1704, 1633, 1599, 1514, 1460, 1428, 1262, 1096, 1027, 803 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Tables 1 and 2; HRESIMS *m/z* 743.27039 $[M - H]^-$ (calcd for $C_{41}H_{43}O_{13}$, 743.27091).

*(+)-(7′S,8′R)-erythro-7′-Methylcarolignan E (**1b**):* white, amorphous solid; $[\alpha]^{20}_D$ +17.1 (*c* 0.1, $CHCl_3$); ECD (MeCN) λ_{max} ($\Delta\varepsilon$) 232 (−1.03), 211 (3.80) nm; UV, IR, NMR, and HRESIMS were the same as those of **1a**.

*(−)-(7′S,8′S)-threo-7′-Methylcarolignan E (**2a**):* white, amorphous solid; $[\alpha]^{20}_D$ −35.1 (*c* 0.2, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 232 (4.36), 288 (2.92), 326 (4.64) nm; ECD (MeCN) λ_{max} ($\Delta\varepsilon$) 325 (−1.78), 295 (−1.80), 240 (−1.14) nm; IR ν_{max} 3402, 2961, 2919, 2850, 1704, 1633, 1597, 1514, 1464, 1428, 1263, 1097, 1028, 803 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Tables 1 and 2; HRESIMS *m/z* 743.26971 $[M - H]^-$ (calcd for $C_{41}H_{43}O_{13}$, 743.27091).

*(+)-(7′R,8′R)-threo-7′-Methylcarolignan E (**2b**):* white, amorphous solid; $[\alpha]^{20}_{D}$ +32.6 (*c* 0.2, $CHCl_3$); ECD (MeCN) λ_{max} (**Δ**ε) 325 (1.73), 294 (1.76), 240 (1.17) nm; UV, IR, NMR, and HRESIMS were the same as those of **2a**.

*(+)-(7′R,8′R)-threo-Carolignan E (**3a**):* white, amorphous solid; $[\alpha]^{20}_{D}$ +30.4 (*c* 0.1, $CHCl_3$); ECD (MeCN) λ_{max} (**Δ**ε) 333 (+1.08), 298 (+1.34), 240 (+1.58) nm; UV (MeOH) λ_{max} (log ε) 230 (4.51), 284 (2.75), 320 (4.83) nm; IR ν_{max} 3413, 2940, 2913, 2852, 1704, 1631, 1597, 1513, 1461, 1430, 1172, 1097, 1031, 801 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Tables 1 and 2; HRESIMS *m/z* 729.2542 $[M - H]^-$ (calcd for $C_{40}H_{41}O_{13}$, 729.2553).

*(−)-(7′S,8′S)-threo-Carolignan E (**3b**):* white, amorphous solid; $[\alpha]^{20}_{D}$ −29.8 (*c* 0.1, $CHCl_3$); ECD (MeCN) λ_{max} (**Δ**ε) 334 (−1.08), 298 (−1.32), 240 (−1.54) nm; UV, IR, NMR, and HRESIMS were the same as those of **3a**.

*(+)-(7′S,8′R)-erythro-Carolignan E (**4a**):* white, amorphous solid; $[\alpha]^{20}_{D}$ +16.6 (*c* 0.1, $CHCl_3$); ECD (MeCN) λ_{max} (**Δ**ε) 234 (+3.18), 222 (−1.22) nm; UV (MeOH) λ_{max} (log ε) 232 (4.36), 288 (2.92), 326 (4.64) nm; IR λ_{max} 3512, 2941, 2917, 2850, 1700, 1631, 1592, 1513, 1464, 1430, 1260, 1090, 1030, 802 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Tables 1 and 2; HRESIMS *m/z* 729.2542 $[M - H]^-$ (calcd for $C_{40}H_{41}O_{13}$, 729.2553).

*(−)-(7′R,8′S)-erythro-Carolignan E (**4b**):* white, amorphous solid; $[\alpha]^{20}_{D}$ −16.4 (*c* 0.1, $CHCl_3$); ECD (MeCN) λ_{max} (**Δ**ε) 234 (−3.20), 222 (+1.21) nm; UV, IR, NMR, and HRESIMS were the same as those of **4a**.

*(−)-(7′R,8′S)-erythro-Carolignan Z (**5**):* white, amorphous solid; $[\alpha]^{23}_{D}$ −34.1 (*c* 0.24, $CHCl_3$); ECD (MeCN) λ_{max} (**Δ**ε) 324 (−2.54), 290 (−2.78), 242 (+1.28) nm; UV (MeOH) λ_{max} (log ε) 219 (6.76), 286 (3.40), 326 (4.31) nm; IR λ_{max} 3427, 2963, 2930, 2853, 1704, 1632, 1588, 1514, 1463, 1427, 1262, 1152, 1097, 1022, 801 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) data, see Tables 1 and 2; HRESIMS *m/z* 757.24921 $[M - H]^-$ (calcd for $C_{41}H_{41}O_{14}$, 757.25018).

Multicycle Viral Replication in MT4 Cell Assay

The anti-HIV-1 assay was performed as previously described.[24] HIV-1 NL4-3 Nanolucsec at an infecting dose of 50 $TCID_{50}$/well was used to infect MT4 cells (1×10^5 cells/mL) in the presence of compounds at various concentrations in 96-well plates. On day 3 postinfection, supernatant samples were harvested and assayed for luciferase activity using the Promega Nano-Glo luciferase assay system. The antiviral potency is defined as the drug concentration that reduces the luciferase activity by 50% (EC_{50}).

Cytotoxicity Assay

A CytoTox-Glo cytotoxicity assay (Promega) was used to determine the cytotoxicity of the isolates. MT4 cells were cultured in the presence of various concentrations of the compounds for 3 days. Cytotoxicity of the compounds was determined by following the

protocol provided by the manufacturer. The 50% cytotoxic concentration (CC_{50}) was defined as the concentration that caused a 50% reduction of cell viability.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

This study was supported in part by the National High-Tech R&D Program of China (863 Program) (2012AA020307), the Guangdong Innovative Research Team Program (No. 2009010058), and the National Natural Science Foundation of China (No. 81173470). Partial support was also received from the National Institute of Allergy and Infectious Diseases (NIAID) Grant AI33066 (K.H.L.).

REFERENCES

1. Vasas A, Hohmann J. Chem. Rev. 2014; 114:8579–8612. [PubMed: 25036812]
2. Shi QW, Su XH, Kiyota H. Chem. Rev. 2008; 108:4295–4327. [PubMed: 18817355]
3. Fan JW, Yu L, Ma LZ, Guo N, Gao QS, Zhao QM, Zeng FL, Ge F, Wang QK, Deng XM, Zeng L. Chin. Agric. Sci. Bull. 2009; 25:1–7.
4. Yang DS, Zhang YL, Peng WB, Wang LY, Li ZL, Wang X, Liu KC, Yang YP, Li HL, Li XL. J. Nat. Prod. 2013; 76:265–269. [PubMed: 23327832]
5. Yang DS, Peng WB, Li ZL, Wang X, Wei JG, Liu KC, Yang YP, Li XL. Nat. Prod. Bioprospect. 2013; 3:112–116.
6. Vasas AL, Gyuris A, Minarovics J, Hohmann J. Planta Med. 2007; 73:947–947.
7. Pan LL, Fang PL, Zhang XJ, Ni W, Li L, Yang LM, Chen CX, Zheng YT, Li CT, Hao XJ, Liu HY. J. Nat. Prod. 2011; 74:1508–1512. [PubMed: 21534540]
8. Kujubu DA, Fletcher BS, Varnum BC, Lim RW, Herschman HR. J. Biol. Chem. 1991; 266:12866–12872. [PubMed: 1712772]
9. Paula VF, Barbosa LCA, Howarth OW, Demuner AJ, Cass QB, Vieira IJC. Tetrahedron. 1995; 51:12453–12462.
10. Seca AML, Silva AMS, Silvestre AJD, Cavaleiro JAS, Domingues FMJ, Neto CP. Phytochemistry. 2001; 56:759–767. [PubMed: 11314965]
11. Matsuda N, Kikuchi M. Chem. Pharm. Bull. 1996; 44:1676–1679.
12. Kim KH, Kim HK, Choi SU, Moon E, Kim SY, Lee KR. J. Nat. Prod. 2011; 74:2187–2192. [PubMed: 21936523]
13. Guo J, Zhang J, Wang W, Liu T, Xin Z. Eur. Food Res. Technol. 2015; 241:37–47.
14. Yoshioka T, Inokuchi T, Fujioka S, Kimura YZ. Naturforsch., C: J. Biosci. 2004; 59:509–514.
15. Wang SM, Kadota S, Liu ZQ, Liu SY. Nat. Prod. Res. Dev. 2005; 17:131–137.
16. Wu JF, Chen SB, Wu LJ, Chen SL, Tu PF. Chin. J. Chin. Mater. Med. 2007; 32:819–821.
17. Zhang DW, Dai SJ, Liu WH, Li GH. Zhongguo Tianran Yaowu. 2010; 8:196–198.
18. Huang SC, Yen GC, Chang LW, Yen WJ, Duh PD. J. Agric. Food Chem. 2003; 51:2380–2383. [PubMed: 12670184]
19. Ekaprasada MT, Nurdin H, Ibrahim S. Indon. J. Chem. 2009; 9:457–460.
20. Cutillo F, Abrosca B, Greca MD, Fiorentino A, Zarrelli AJ. Agric. Food Chem. 2003; 51:6165–6172.
21. Besombes S, Robert D, Utille JP, Taravel FR, Mazeau KJ. Agric. Food Chem. 2003; 51:34–42.
22. Xiong L, Zhu C, Li Y, Tian Y, Lin S, Yuan S, Hu J, Hou Q, Chen N, Yang Y, Shi JJ. Nat. Prod. 2011; 74:1188–1200.
23. Kijima K, Oha H, Ide T, Ogimi, Hirata CE, Takushi A, Takeda Y. Phytochemistry. 1998; 48:669–676.

24. Dang Z, Zhu L, Lai W, Bogerd H, Lee KH, Huang L, Chen CH. ACS Med. Chem. Lett. 2016 in press.

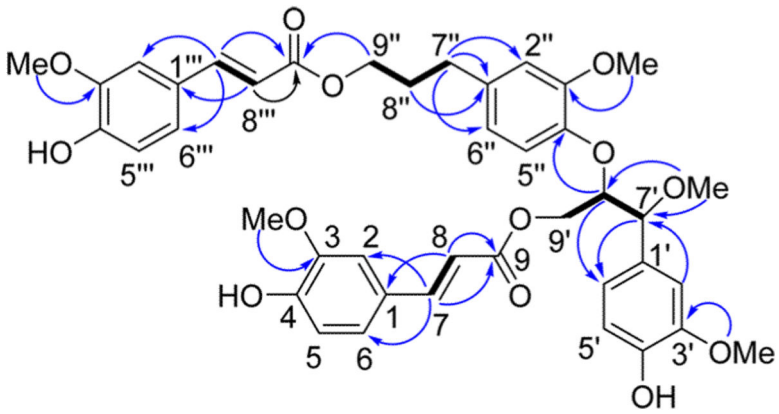


Figure 1.
Key ^{1}H–^{1}H COSY (—) and HMBC (→) correlations of **1**.

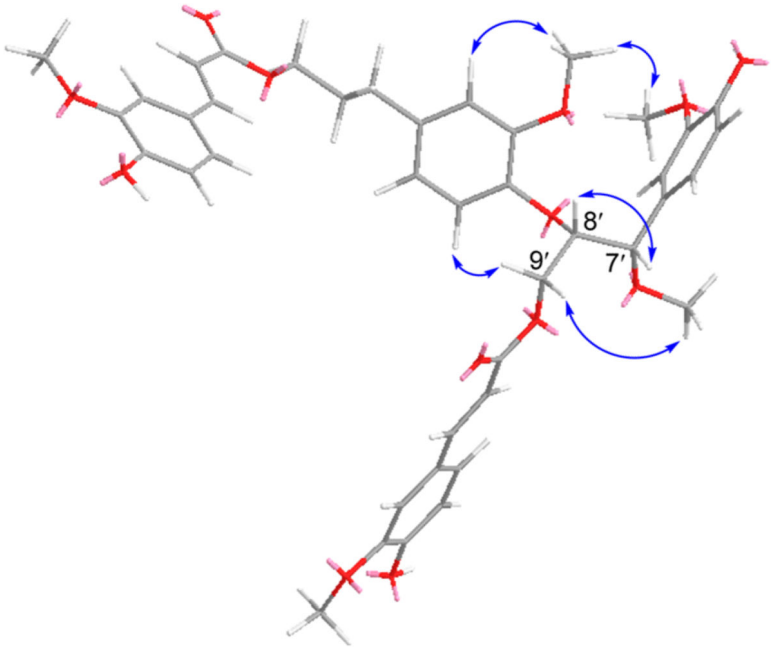


Figure 2.
Key NOE correlations (↔) of **1**.

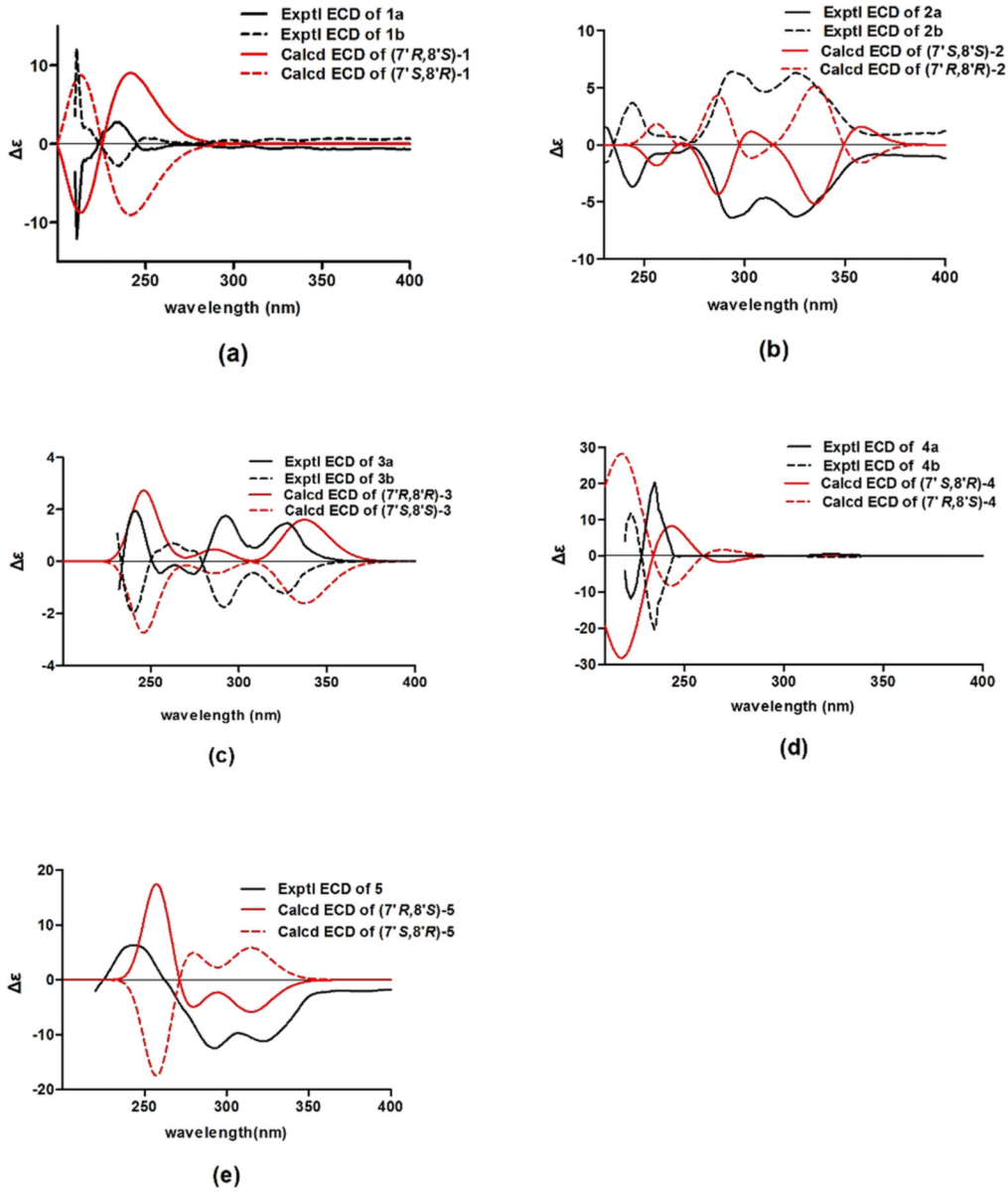


Figure 3.

(a) Experimental ECD spectra of (−)- and (+)-*erythro*-7′-methylcarolignan E (**1a/1b**) in MeCN and calculated ECD spectra of (*S*′,*R*′)-**1** and (*R*′,*S*′)-**1**. (b) Experimental ECD spectra of (−)- and (+)-*threo*-7′-methylcarolignan E (**2a/2b**) in MeCN and calculated ECD spectra of (*R*′,*R*′)-**2** and (*S*′,*S*′)-**2**. (c) Experimental ECD spectra of (+)- and (−)-*threo*-carolignan E (**3a/3b**) in MeCN and calculated ECD spectra of (*R*′,*R*′)-**3** and (*S*′,*S*′)-**3**. (d) Experimental ECD spectra of (+)- and (−)-*erythro*-carolignan E (**4a/4b**) in MeCN and calculated ECD spectra of (*S*′,*R*′)-**4** and (*R*′,*S*′)-**4**. (e) Experimental ECD spectra of (−)-*erythro*-carolignan Z (**5**) in MeCN and calculated ECD spectra of (*R*′,*S*′)-**5** and (*S*′,*R*′)-**5**. The calculated ECD spectra were computed at the B3LYP/6-311++G(d,p) level.

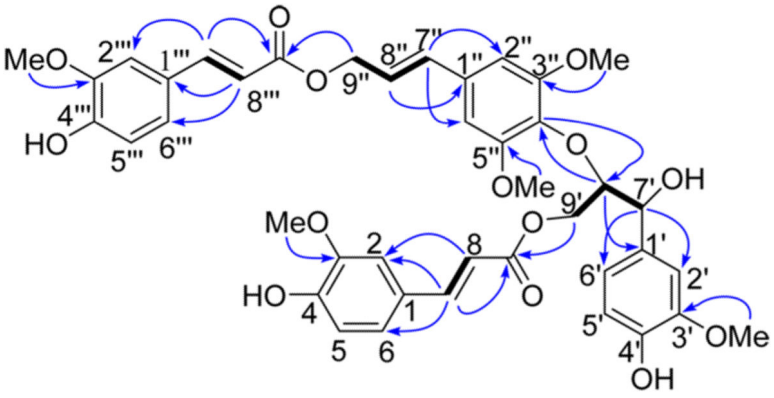


Figure 4.
Key ^{1}H–^{1}H COSY (—) and HMBC (→) correlations of **5**.

Table 1

^{1}H NMR Spectroscopic Data (400 MHz, in $CDCl_3$) for Compounds 1–5 (δ_H in ppm, *J* in Hz)

position	1a/1b[a]	2a/2b[a]	3a	4a	5
2	7.02 d (1.9)	7.00 d (1.9)	7.01 d (1.9)	6.91 d (1.8)	7.00 d (2.0)
5	6.85 d (8.2)	6.89 d (8.2)	6.88 d (8.3)	6.87 d (8.3)	6.73 d (8.3)
6	7.05 d (1.9, 8.2)	7.06 d (1.9, 8.2)	7.05 d (1.9, 8.3)	7.00 m	7.05 d (2.0, 8.3)
7	7.48 d (15.9)	7.48 d (15.9)	7.59 d (15.9)	7.59 d (15.9)	7.64 d (15.9)
8	6.23 d (15.9)	6.26 d (15.9)	6.24 d (15.9)	6.21 d (15.9)	6.21 d (15.9)
2′	6.93 m	6.89 d (1.8)	7.04 m	7.01 m	6.98 m
5′	6.90 d (8.2)	6.87 d (8.1)	6.87 d (8.1)	6.89 d (8.1)	6.96 d (8.2)
6′	6.86 m	6.85 dd (1.8, 8.1)	6.75 dd (1.8, 8.1)	6.84 dd (2.0, 8.0)	6.87 d (1.7, 8.2)
7′	4.52 d (3.0)	4.25 t (6.3)	4.91 d (8.1)	4.90 d (3.3)	4.88 d (3.1)
8′	4.48 m	4.43 m	4.20 m	4.44 m	4.55 m
9′	4.11 dd (4.7, 11.6)	4.13 m	4.13 dd (4.8, 12.1)	4.26 m	4.83 d (6.4)
	4.31 dd (2.8, 11.6)	4.44 m	4.34 dd (3.4, 12.1)	4.48 m	
2″	6.68 d (1.8)	6.63 d (1.8)	6.72 d (1.8)	6.75 d (1.8)	6.60 d (1.8)
5″	6.90 m	6.84 d (8.0)	6.91 d (8.0)	6.95 d (8.0)	
6″	6.80 dd (1.8, 8.1)	6.81 dd (1.8, 8.0)	6.85 dd (1.8, 8.0)	6.81 dd (1.8, 8.0)	6.60 d (1.8)
7″	2.62 t (6.5)	2.58 m	2.68 t (7.9)	2.68 t (6.5)	6.63 d (15.7)
8″	1.96 m	1.91 m	2.00 m	1.99 m	6.30 d (15.7)
9″	4.15 t (6.5)	4.13 t (6.4)	4.20 t (6.0)	4.19 t (6.5)	4.25 d (3.7)
					4.44 m
2‴	7.00 d (1.8)	7.00 d (1.8)	7.01 d (1.8)	7.01 m	6.93 d (1.7)
5‴	6.91 d (8.1)	6.94 d (8.1)	6.91 d (8.1)	6.95 d (7.9)	6.75 d (8.2)
6‴	7.05 dd (1.8, 8.1)	7.02 dd (1.8, 8.1)	7.01 dd (1.8, 8.1)	7.06 dd (1.8, 8.3)	7.08 dd (1.7, 8.2)
7‴	7.59 d (15.9)	7.59 d (15.9)	7.51 d (15.9)	7.49 d (15.9)	7.69 d (15.9)
8‴	6.27 d (15.9)	6.28 d (15.9)	6.28 d (15.9)	6.28 d (15.9)	6.23 d (15.9)
OCH_3-3	3.90 s	3.86 s	3.92 s	3.90 s	3.85 s
OCH_3-3′	3.82 s	3.78 s	3.88 s	3.86 s	3.87 s
OCH_3-3″	3.72 s	3.72 s	3.84 s	3.86 s	3.90 s
OCH_3-3‴	3.90 s	3.86 s	3.92 s	3.90 s	3.85 s
OCH_3-5″					3.90 s
OCH_3-7′	3.27 s	3.25 s	3.25 s	3.25 s	

[a] **1a/1b** and **2a/2b** showed the same ^{1}H NMR data.

Table 2

^{13}C NMR (100 MHz) Data (δ) of Compounds 1–5 (δ_C in ppm)

position	1a/1b[a]	2a/2b[a]	3a	4a	5
1	127.1	127.1	127.1	127.0	127.1
2	109.3	109.3	109.6	109.5	109.5
3	146.9	146.9	147.0	147.0	147.0
4	148.2	148.1	148.3	148.2	148.3
5	114.3	114.3	114.6	114.3	115.0
6	123.3	123.3	123.3	123.3	123.3
7	145.3	145.2	145.6	145.4	145.0
8	115.6	115.5	115.6	115.6	115.3
9	167.1	167.3	167.0	167.3	167.2
1′	130.0	130.3	131.4	131.2	130.8
2′	109.9	110.0	109.5	109.0	108.6
3′	146.9	146.9	146.9	146.8	146.8
4′	145.8	145.6	145.8	145.3	145.6
5′	114.9	114.9	114.9	114.9	114.3
6′	120.6	120.6	120.9	121.0	119.0
7′	83.5	82.8	74.6	72.3	71.9
8′	82.3	82.6	86.6	84.7	83.7
9′	63.9	63.8	63.2	62.8	62.7
1″	136.0	136.2	137.6	137.6	130.9
2″	112.6	112.6	112.5	112.6	103.9
3″	150.9	151.0	151.0	151.6	153.7
4″	146.7	146.8	146.3	145.3	134.1
5″	118.9	119.2	120.6	119.5	153.7
6″	121.0	121.2	121.2	121.3	103.9
7″	32.1	32.0	32.2	32.2	134.0
8″	30.6	30.6	30.62	30.6	123.8
9″	63.9	63.9	63.8	63.9	65.0
1‴	127.1	127.1	127.1	127.1	127.2
2‴	109.5	109.5	109.6	109.6	109.4
3‴	146.9	146.9	147.0	147.0	147.0
4‴	148.1	148.1	148.2	148.2	148.1
5‴	114.9	114.9	114.9	115.0	114.9
6‴	123.4	123.5	123.4	123.4	123.3
7‴	145.1	145.2	145.2	145.2	145.0
8‴	115.2	115.6	114.9	115.2	115.6
9‴	167.5	167.6	167.5	167.5	167.2
OCH_3-3	56.2	56.2	56.2	56.2	56.2
OCH_3-3′	56.2	56.1	56.1	56.2	56.2

position	1a/1b[a]	2a/2b[a]	3a	4a	5
OCH_3-3″	56.0	55.9	56.0	56.1	56.4
OCH_3-3‴	56.2	56.2	56.2	56.2	56.2
OCH_3-5″					56.4
OCH_3-7′	57.4	57.4			

[a] **1a/1b** and **2a/2b** showed the same ^{13}C NMR data.

Table 3

Anti-HIV Data of the Isolates from *E. sikkimensis*

compound	EC_{50} (μM)	number of tests	CC_{50} (μM)	SI (CC_{50}/EC_{50})[b]
1a	6.3 ± 2.1	3	21 ± 1.1	3.3
1b	5.3 ± 1.2	3	17 ± 1.2	3.2
2a [a]	>10	1	>10	
2b [a]	>10	1	>10	
5 [a]	>10	1	>10	
8 [a]	>10	1	>10	
9 [a]	>10	1	>10	
AZT	0.004 ± 0.0012	3	>0.1	>25

[a] The highest concentration tested was 10 μM for all the samples in the first test; 0.1 μg/mL for the control AZT. Testing concentration ranges were adjusted in subsequent tests based on potency.

[b] SI is the selective index, SI = CC_{50}/EC_{50}.