FULL PAPER

Seco-Tremulanes from Cultures of the Basidiomycete *Flavodon flavus* BCC 17421

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A new *seco*-tremulane, 11,12-epoxy-5,6-secotremula-1,6(13)-dien-5,12-olide (**1**), was isolated together with the known compounds, conocenolides A (**2**) and B (**3**), tremulenediol A (**4**), tremulenolide A (**5**), and two lanostane triterpenoids, trametenolic acid B (**6**), and pinicolic acid A (**7**), from cultures of the basidiomycete *Flavodon flavus* BCC 17421. Interconversion of conocenolides A/B was demonstrated. Compound **1** exhibited weak cytotoxic activities, whereas tremulenediol A showed antiplasmodial activity (IC_{50} 8.6 μg/ml). Pinicolic acid A exhibited activity against herpes simplex virus type-1 (IC_{50} 15 μg/ml) as well as cytotoxic activities.

Keywords: *Flavodon flavus*, Basidiomycete, Tremulane, Antiviral activity, Antimalarial activity, Cytotoxicity

Introduction

Mushrooms have been valued as traditional sources of natural bioactive compounds for many centuries. As a result of research on medicinal mushrooms, many novel biologically active compounds have been reported [1][2]. While some medicinal species have been well investigated, many species remain chemically unexplored or poorly studied. As a part of a continuing research program on the utilization of fungal sources in Thailand, we have been recently focusing on the structural diversity of the secondary metabolites from cell cultures of higher fungi [3][4][5]. In the present study, cultures of the basidiomycete *Flavodon flavus* BCC 17421 have been investigated, because an culture broth extract exhibited antiviral activity against herpes simplex virus type-1 (HSV-1) (IC_{50} 22 μg/ml). To the best of our knowledge, the only report on the chemical constituents from natural fruiting bodies or cell cultures of the genus *Flavodon* is the isolation of a difuranylmethane derivative, flavodonfuran, from the mangrove-derived *Flavodon flavus* PSU-MA201 [6].

Results and Discussion

The fungus *Flavodon flavus* BCC 17421 was fermented in malt extract broth (MEB; total 15 l), and the extracts were fractionated by chromatographic methods. A new *seco*-tremulane, 11,12-epoxy-5,6-secotremula-1,6(13)-dien-5,12-olide (**1**), was isolated together with the known compounds, conocenolides A (**2**) and B (**3**) [7], tremulenediol A (**4**) [8], tremulenolide A (**5**) [8], and two lanostane triterpenoids, trametenolic acid B (**6**) [9] and pinicolic acid A (**7**) [9][10] (*Fig. 1*).

Compound **1** was isolated as viscous oil. The molecular formula of **1** was established as $C_{15}H_{20}O_3$ on the basis of the sodiated *quasi*-molecular ion peak at *m/z* 271.1303 (calc. for $C_{15}H_{20}NaO_3^+$, 271.1305) in the HR-ESI-MS. The ^{1}H- and ^{13}C-NMR spectra showed some resemblance to those of **2** and **3**, which suggested that **1** is a related sesquiterpenoid. Analysis of the ^{1}H- and ^{13}C-NMR, DEPT135, and HMQC spectroscopic data revealed that **1** contained a C=O group (δ(C) 175.6), a tetrasubstitited olefin (δ(C) 139.0 and 131.5), a CH_2=CH group, an acetal CH (δ(C) 108.5, δ(H) 6.09), a quaternary C, two CH, four CH_2, and two Me groups (*Table 1*). The planar structure was elucidated on the basis of the COSY and HMBC correlations (*Fig. 2*). A cyclopentane ring was connected with a CH_2=CH group at C(7) and geminal Me groups at C(9). Besides, C(1) (δ(C) 139.0) was a part of a tetrasubstituted olefin. The location of the olefinic carbons were revealed by the HMBC correlations from H–C(6), H–C(7), H_β–C(8), H_α–C(10), and H_β–C(10) to C(1), and from H–C(7), H_α–C(10), and H_β–C(10) to C(2) (δ(C) 131.5). The other side of the tetrasubstituted olefin, C(2), was a part of a bicyclo-acetal ring. The bicyclo-acetal substructure was also revealed by HMBC correlations (*Fig. 2*), including the key correlations from the acetal proton H–C(12) to C(2), C(3), C(5), and C(11). The linkage to the cyclopentane ring through the tetrasubsituted olefin was revealed by the HMBC correlations from H–C(3), H_α–C(11), and H_β–C(11) to both olefinic carbons C(1) and C(2). The (*E*)-olefinic geometry was assigned on the basis of the NOESY

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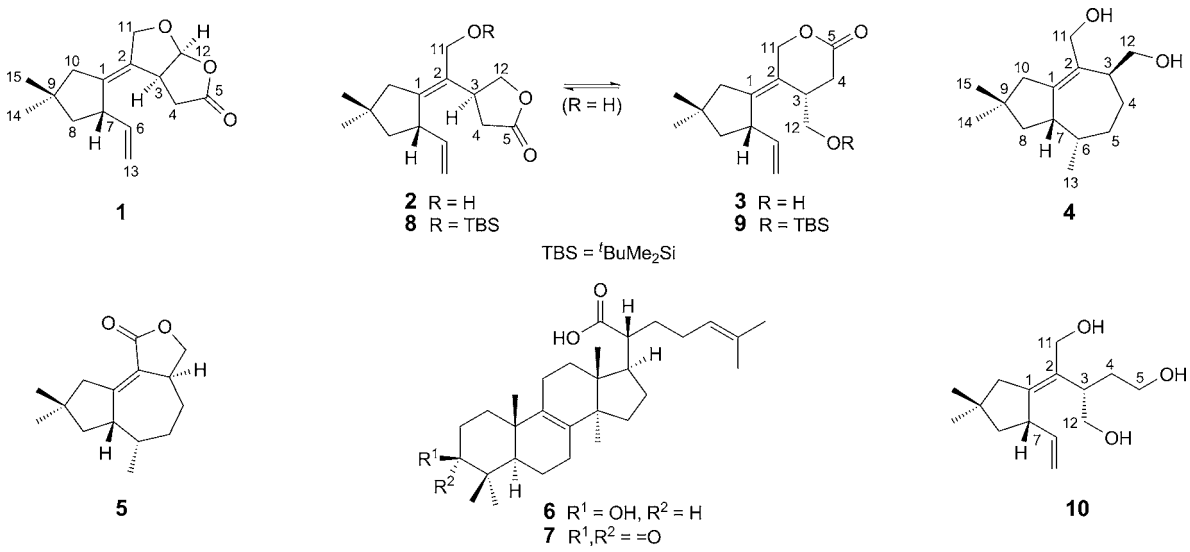

Fig. 1. Structures of compounds **1** – **10**

correlations H_α–C(10)/H_α–C(11) and H_β–C(10)/H_β–C(11). The *cis*-junction of the bicycle-acetal ring was evident from the intense NOESY correlation H–C(3)/H–C(12). Consequently, compound **1** was identified as 11,12-epoxy-5,6-secotremula-1,6(13)-dien-5,12-olide. At this stage, the relative configuration at C(7) and the absolute configuration were not determined. However, they could be correlated with those of the co-metabolite, tremulenediol A (**4**), whose absolute configuration had been unambiguously determined by enantioselective syntheses [11]. The optical rotation value of the isolated natural product **4** from BCC 17421, $[\alpha]_D^{26}$ = +44 (*c* = 0.16, MeOH), was comparable to the literature values for the synthetic **4**, $[\alpha]_D^{24}$ = +40.0 (*c* = 0.24, MeOH) [11], and natural product from *Phellinus tremulae*, $[\alpha]_D^{24}$ = +41.3 (*c* = 0.24, MeOH) [8]. Compound **1** is the 10-deoxy derivative of 11,12-epoxy-10α-hydroxy-5,6-secotremula-1,6(13)-dien-5,12-olide, which was previously isolated, together with **2**, **3**, and related derivatives, from cultures of *Conocybe siliginea* by *Liu* and coworkers [12].

The original report of conocenolides A (**2**) and B (**3**) [7] describes that they were isolated from cultures of *Conocybe siliginea* as an inseparable mixture in the ratio of approximately 2:1. In this study, we also obtained a *ca.* 2.5:1 mixture of **2**/**3** by silica gel column chromatography. Separation of this mixture by preparative reversed-phase HPLC (MeCN/H_2O 10:90) was successfully achieved with clear peak separation in the chromatogram (UV 210 nm; t_R 21.0 min (minor) and 23.6 min (major)) and corresponding fractions were collected and concentrated. However, ^{1}H-NMR spectra of both fractions showed the presence of the mixture of **2**/**3**. These results suggested the slow interconversion of **2** and **3** (intramolecular translactonization) which was accelerated during the removal of the aqueous solvent by evaporation on a warm water bath (35 – 50 °C). In order to confirm this unique reaction, we planned to protect the OH group to block the interconversion and separate the γ- and δ-lactone isomers. Silylation of a mixture of **2**/**3** (TBS-Cl, imidazole, DMF) gave a mixture of silyl ethers **8** and **9** (*ca.* 2.4:1), which were successfully separated by preparative HPLC to furnish pure compounds. Desilylation of the γ-lactone silyl ether **8** under the common mild conditions (TBAF, THF) gave a mixture of **2**/**3** (*ca.* 3:1) as identified by ^{1}H-NMR spectroscopic analysis of the crude reaction product. Similarly, desilylation of the δ-lactone silyl ether **9** also gave a mixture of **2**/**3** (*ca.* 1:1). These results unambiguously confirmed the slow equilibrium of **2**/**3** in solution.

Table 1. ^{1}H- and ^{13}C-NMR Data (400 and 100 MHz resp.; in $CDCl_3$) of Compound **1**. δ in ppm, *J* in Hz

Position	δ(H)	δ(C)	HMBC
1		139.0	
2		131.5	
3	3.60 – 3.62 (*m*)	41.1	1, 2, 4, 5, 11
4	2.48 (*ddd*, *J* = 19.0, 4.3, 0.6, H_α), 2.89 (*dd*, *J* = 19.0, 10.8, H_β)	36.5	2, 3, 5, 12
5		175.6	
6	5.63 – 5.67 (*m*)	142.6	1, 7
7	3.30 – 3.32 (*m*)	47.9	1, 2, 6, 8, 13
8	1.39 (*dd*, *J* = 12.8, 9.4), 1.77 (*dd*, *J* = 12.8, 8.1)	48.6	1, 6, 7, 9, 10, 14, 15
9		38.3	
10	2.03 (*d*, *J* = 16.2, H_α), 2.01 (*d*, *J* = 16.2, H_β)	47.4	1, 2, 9, 14
11	4.36 (*d*, *J* = 12.3,H_α), 4.42 (*d*, *J* = 12.3, H_β)	69.5	1, 2, 3, 12
12	6.09 (*dd*, *J* = 5.3, 0.6)	108.5	2, 3, 5, 11
13	5.05 (*d*, *J* = 17.0), 4.98 (*d*, *J* = 9.8)	114.7	6, 7
14	1.09 (*s*)	28.8	8, 9, 10, 15
15	0.89 (*s*)	27.4	8, 9, 10, 14

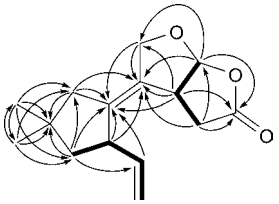

Fig. 2. $^1H,^1H$-COSY and selected HMBC (H→C) correlations for **1**

Biogenetically, compound **1** should be closely related to **2/3**. We could assume that **1** is produced by oxidation of the C(12) $HOCH_2$ group of **2** to CHO group followed by acetal formation. Most likely, these *seco*-tremulanes should be biosynthesized *via* a tremulane intermediate **4**. The relative configurations of **1** and **2/3** were chemically correlated. Thus, treatment of **1** with $LiAlH_4$ gave a triol derivative **10**. Similarly, $LiAlH_4$ reduction of **2/3** gave the same compound (**10**) as a single major reaction product. These results are consistent with the proposed relative configurations of the *seco*-tremulanes.

The isolated compounds **1** – **7** were subjected to several biological activity tests in our research unit: anti-HSV-1 activity, antiplasmodial activity against *Plasmodium falciparum* K1, and cytotoxicity to cancer cell lines (KB, MCF-7, and NCI-H187) and nonmalignant Vero cells (*Table 2*). Only pinicolic acid A (**7**), a lanostane triterpenoid, showed activity against HSV-1 (IC_{50} 15 μg/ml), which suggested that this compound should be the antiviral principle of the fungus BCC 17421 cell culture. Compound **1** exhibited weak cytotoxicity against all tested cell lines. On the other hand, tremulenediol A (**4**) showed antimalarial activity (IC_{50} 8.6 μg/ml) and its cytotoxic activities were relatively weaker. No notable biological activity of **4** has previously been reported. This study demonstrates that basidiomycete *Flavodon flavus* is a rich source of biologically active terpenoid metabolites.

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Experimental Part

General

Column chromatography (CC): silica gel *60H* (SiO_2; *Merck*, Darmstadt, Germany) and *Sephadex LH-20* (*GE Healthcare*, Uppsala, Sweden). Prep. HPLC: *Waters* 600 controller, *Waters 2996* photodiode array detector (*Waters*, Milford, MA, USA). Optical rotations: *JASCO P-1030* digital polarimeter (*Jasco*, Tokyo, Japan). UV Spectra: *Analytik Jena SPEKOL 1200* spectrophotometer (*Analytik Jena*, Nonthaburi, Thailand); λ_{max} (log ε) in nm. IR Spectra: *Bruker ALPHA* spectrometer (*Bruker*, Billerica, MA, USA); $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker DRX400* and *AV500D* spectrometers; δ in ppm rel. to the solvent signals, *J* in Hz. HR-ESI-MS: *Bruker micrOTOF* mass spectrometer; in *m/z*.

Fungal Material

The mushroom used in this study was collected on a bamboo wood in Khao Yai National Park, Nakhon Rachasima Province, Thailand. The living culture was deposited in the *BIOTEC Culture Collection* as BCC 17421 on April 20, 2005. On the basis of the morphological both macro- and microscopic characteristics, this fungus was identified as *Flavodon flavus* of the family *Meruliaceae* by one of the authors (*R.C.*), who is an expert mycologist.

Fermentation, Extraction, and Isolation

The fungus BCC 17421 was maintained on potato dextrose agar at 25 °C. The agar was cut into small plugs and inoculated into 6 × 250-ml *Erlenmeyer* flasks containing 25 ml of potato dextrose broth (PDB; potato starch 4.0 g/l, dextrose 20.0 g/l). After incubation at 25 °C for 7 d on a rotary shaker (200 rpm), each primary culture was transferred into a 1000-ml *Erlenmeyer* flask containing 250 ml of the same liquid medium (PDB), and incubated at 25 °C for 7 days on a rotary shaker (200 rpm). The secondary cultures were pooled and each 25 ml portion was transferred into 60 × 1000-ml *Erlenmeyer* flasks containing 250 ml of malt extract broth (MEB; malt extract 6.0 g/l, yeast extract 1.2 g/l, maltose 1.8 g/l, dextrose 6.0 g/l), and the final fermentation was carried out at 25 °C for 42 days under static conditions. The cultures were filtered to separate broth and mycelia (residue). The broth was extracted with AcOEt (2 × 9 l) and concentrated under reduced pressure to obtain a brown gum (extract A, 1.32 g). The wet mycelia were macerated in MeOH (1 l, r.t., 2 days) and filtered. Hexane (800 ml) and H_2O (50 ml) were added to the filtrate, and the layers were separated. The H_2O/MeOH (bottom) layer was partially concentrated by evaporation, and the residue was diluted with AcOEt (1 l) and washed with H_2O (50 ml). The AcOEt layer was concentrated under reduced pressure to obtain a brown gum (extract B, 604 mg). The hexane (upper) layer was concentrated under reduced pressure to obtain a yellow gum (extract C, 544 mg). Extract A was passed through a column on *Sephadex LH-20* (3.8 × 50 cm) eluted with MeOH to afford three pooled fractions, *Frs. A1 – A3*. *Fr. A2* (752 mg) was repetitively subjected to CC (SiO_2; MeOH/CH_2Cl_2, step gradient elution from 0:100 to 20:80) to furnish **1** (84 mg), **2/3** (194 mg), **4** (4.7 mg), **5** (4.1 mg), and **6** (14.6 mg). Extract B was fractionated by CC on *Sephadex LH-20* (3.8 × 50 cm, MeOH) to afford three pooled fractions, *Fr. B2* (454 mg) was purified by repetitive CC (SiO_2; MeOH/CH_2Cl_2, step gradient elution from 0:100 to 20:80) to afford **1** (1.1 mg), **2/3** (1.3 mg), **6** (120 mg), and

Table 2. Biological Activities of Compounds **1** – **7**

Compound	Anti-HSV-1	Antiplasmodial	Cytotoxicity (IC_{50} [μg/ml])			
	IC_{50} [μg/ml]	IC_{50} [μg/ml]	KB	MCF-7	NCI-H187	Vero
1	> 50	> 10	27	28	26	27
2/3	> 50	> 10	> 50	> 50	> 50	> 50
4	> 50	8.6	> 50	> 50	18	> 50
5	> 50	> 10	> 50	> 50	> 50	> 50
6	> 50	> 10	18	> 50	> 50	> 50
7	15	> 10	28	22	36	17
Acyclovir[a])	4.5	–	–	–	–	–
Dihydroartemisinin[b])	–	0.00076	–	–	–	–
Doxorubicin[c])	–	–	0.43	7.3	0.097	–
Ellipticine[c])	–	–	2.5	–	2.8	1.3

[a]) Standard compound for the anti-HSV-1 assay. [b]) Standard antimalarial drug. [c]) Standard compounds for the cytotoxicity assays.

7 (8.2 mg). Extract C was fractionated by CC (SiO_2; MeOH/CH_2Cl_2) to obtain **6** (91 mg) and ergosterol (41 mg).

(3*S*,7*S*,12*S*)-11,12-Epoxy-5,6-secotremula-1,6(13)-dien-5,12-olide (= **(3a*S*,4*E*,6a*S*)-4-[(2*S*)-2-Ethenyl-4,4-dimethylcyclopentylidene]tetrahydrofuro[2,3-*b*]furan-2(3*H*)-one**; **1**): Pale brown oil. $[\alpha]_D^{26} = -120$ ($c = 0.21$, MeOH). UV (MeOH): 215 (3.46). IR (ATR): 2953, 1782. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 271.1303 ($[M + Na]^+$, $C_{15}H_{20}NaO_3^+$; calc. 271.1305).

Synthesis of Compounds **8** *and* **9**

A mixture of **2/3** (15 mg, 60 μmol), TBS-Cl (27 mg, 180 μmol), and imidazole (30 mg, 45 μmol) in DMF (0.3 ml) was stirred at room temperature for 18 h. The mixture was diluted with AcOEt (5 ml), and washed with H_2O (2 × 2 ml). The organic layer was concentrated under reduced pressure to leave a pale brown oil (21 mg), which was subjected to prep. HPLC (*CAPCell-Pak*, 20 × 150 mm, 5 μm, *Shiseido, Tokyo,* Japan; MeCN/H_2O = 90:10, flow rate 8 ml/min) to furnish **8** (5.5 mg, t_R 23.5 min) and **9** (2.3 mg, t_R 21 min).

Compound **8**. Colorless oil. IR (ATR): 1781, 1464, 1254, 1171, 1057, 836, 776. ^{1}H-NMR (400 MHz, $CDCl_3$): 5.69 – 5.71 (*m*, H–C(6)); 4.98 – 5.00 (*m*, H_a–C(13)); 4.95 – 4.97 (*m*, H_b–C(13)); 4.30 – 4.33 (*m*, CH_2(12)); 4.26 (*d*, *J* = 11.9, H_a–C(11)); 4.13 (*d*, *J* = 11.9, H_b–C(11)); 3.76 – 3.78 (*m*, H–C(3)); 3.32 – 3.34 (*m*, H–C(7)); 2.56 (*dd*, *J* = 17.4, 10.2, H_a–C(4)); 2.50 (*dd*, *J* = 17.4, 9.2, H_b–C(4)); 2.20 (*d*, *J* = 15.4, H_a–C(10)); 2.09 (*d*, *J* = 15.4, H_b–C(10)); 1.79 (*dd*, *J* = 12.8, 8.8, H_a–C(8)); 1.34 (*dd*, *J* = 12.8, 8.5, H_b–C(8)); 1.08 (*s*, Me(15)); 0.88 (*s*, 12 H, Me(14), TBS); 0.07 (*s*, 6 H, TBS). ^{13}C-NMR (100 MHz, $CDCl_3$): 177.7 (C(5)); 145.9 (C(1)); 142.2 (CH(6)); 128.6 (C(2)); 113.4 (CH_2(13)); 71.3 (CH_2(12)); 61.2 (CH_2(11)); 48.1 (CH_2(8)); 46.0 (CH(7), CH_2(10)); 37.8 (C(9)); 37.2 (CH(3)); 32.6 (CH_2(4)); 28.7 (Me(14)); 27.5 (Me(15)); 25.8 (Me, TBS); 18.1 (C, TBS); −5.5 (Me, TBS). HR-ESI-MS: 387.2332 ($[M + Na]^+$, $C_{21}H_{36}NaO_3Si^+$; calc. 387.2326).

Compound **9**. Colorless oil. IR (ATR): 1754, 1464, 1253, 1100, 836, 777. ^{1}H-NMR (400 MHz, $CDCl_3$): 5.62 – 5.64 (*m*, H–C(6)); 5.06 (*dd*, *J* = 10.0, 1.5, H_a–C(13)); 5.01 – 5.03 (*m*, H_b–C(13)); 4.76 (*d*, *J* = 13.4, H_a–C(11)); 4.68 (*d*, *J* = 13.4, H_b–C(11)); 3.57 – 3.60 (*m*, CH_2(12)); 3.37 – 3.39 (*m*, H–C(7)); 3.27 – 3.29 (*m*, H–C(3)); 2.79 (*dd*, *J* = 15.8, 5.5, H_a–C(4)); 2.48 – 2.50 (*m*, *J* = 15.8, 6.5, H_b–C(4)); 2.11 – 2.13 (*m*, CH_2(10)); 1.78 (*ddd*, *J* = 12.6, 8.3, 1.5, H_a–C(8)); 1.39 (*dd*, *J* = 12.6, 9.0, H_b–C(8)); 1.09 (*s*, Me(15)); 0.92 (*s*, Me(14)); 0.88 (*s*, 9 H, TBS); 0.04 (*s*, 6 H, TBS). ^{13}C-NMR (100 MHz, $CDCl_3$): 172.8 (C(5)); 142.6 (C(1)); 140.3 (CH(6)); 124.6 (C(2)); 114.2 (CH_2(13)); 69.4 (CH_2(11)); 64.1 (CH_2(12)); 48.7 (CH_2(8)); 46.3 (CH(7)); 46.2 (CH_2(10)); 37.9 (C(9)); 35.7 (CH(3)); 32.3 (CH_2(4)); 28.7 (Me(14)); 27.5 (Me(15)); 25.9 (Me, TBS); 18.3 (C, TBS); −5.5 (Me, TBS). HR-ESI-MS: 387.2327 ($[M + Na]^+$, $C_{21}H_{36}NaO_3Si^+$; calc. 387.2326).

Desilylation of **8** *and* **9**

To a stirred solution of **8** (2.0 mg) in THF (0.1 ml) at 0 °C was added a solution of tetrabutylammonium fluoride (100 μl, 1M in THF) dropwise, and the mixture was stirred at 0 °C for 30 min, then at room temperature for 1 h. The reaction was quenched by addition of saturated aqueous NH_4Cl (1 ml) at 0 °C. The mixture was diluted with AcOEt, the organic layer was separated, and the aqueous layer was extracted with AcOEt. The organic layer was concentrated *in vacuo* to obtain a pale yellow gum. The ^{1}H-NMR ($CDCl_3$, 400 MHz) spectrum of this crude reaction product indicated the presence of a mixture of **2/3** (*ca*. 3:1). Using the similar procedures, compound **9** was desilylated. The ^{1}H-NMR ($CDCl_3$, 400 MHz) spectrum of the crude reaction products indicated the presence of a mixture of **2/3** (*ca*. 1:1).

*$LiAlH_4$ Reduction of **2/3***

To a solution of **2/3** (15 mg) in THF (0.1 ml) was added a small portion of $LiAlH_4$ (*ca.* 10 mg), and the mixture was stirred at room temperature for 1 h. The reaction was quenched by slow addition of H_2O (100 µl), and extracted with AcOEt. The mixture was dried by addition of $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure to leave a pale yellow gum, which was purified by CC (SiO_2; MeOH/CH_2Cl_2) to furnish **10** (4.4 mg). By a similar procedure, compound **1** (10 mg) was reacted with $LiAlH_4$ to give **10** (2.8 mg).

Compound **10**. Colorless oil. $[\alpha]_D^{20}$ = +25 (*c* = 0.09, MeOH). IR (ATR): 3315, 1013, 990, 908. ^{1}H-NMR (500 MHz, $CDCl_3$): 5.73 (*ddd*, *J* = 17.2, 9.8, 8.8, H–C(6)); 5.03 (*ddd*, *J* = 17.2, 1.6, 0.7, H_a–C(13)); 4.96 (*dd*, *J* = 9.9, 1.6, H_b–C(13)); 4.16 (*d*, *J* = 11.4, H_a–C(11)); 3.98 (*d*, *J* = 11.4, H_b–C(11)); 3.57 – 3.64 (CH_2(5,12)); 3.40 – 3.42 (*m*, H–C(7)); 3.00 – 3.02 (*m*, H–C(3)); 2.38 (*dd*, *J* = 14.9, 2.2, H_a–C(10)); 2.18 (br. *d*, *J* = 14.9, H_b–C(10)); 1.79 (*ddd*, *J* = 12.8, 8.5, 2.2, H_a–C(8)); 1.66 – 1.70 (*m*, CH_2(4)); 1.35 (*dd*, *J* = 12.8, 8.5, H_b–C(8)); 1.09 (*s*, Me(15)); 0.90 (*s*, Me(14)). ^{13}C-NMR (125 MHz, $CDCl_3$): 148.7 (C(1)); 142.9 (CH(6)); 133.1 (C(2)); 113.4 (CH_2(13)); 65.3 (CH_2(12)); 61.4 (CH_2(5)); 51.3 (CH_2(11)); 48.2 (CH_2(8)); 46.1 (CH_2(10)); 45.6 (CH(7)); 40.2 (CH(3)); 37.7 (C(9)); 32.4 (CH_2(4)); 28.6 (Me(14)); 27.2 (Me(15)). HR-ESI-MS: 255.1948 ([*M* + H]$^+$, $C_{15}H_{27}O_3^+$; calc. 255.1955).

Biological Assays

Assay for activity against *Plasmodium falciparum* (K1, multidrug resistant strain) was performed using the microculture radioisotope technique [13]. IC_{50} represents the concentration that causes 50% reduction in parasite growth as indicated by the *in vitro* uptake of [^{3}H]-hypoxanthine by *P. falciparum*. Antiviral activity against herpes simplex virus type 1 and cytotoxicity to Vero cells (African green monkey kidney fibroblasts) were performed using the green fluorescent protein microplate assay (GFPMA) [14]. Test materials were assayed in triplicate in 96-well plate for anti-HSV-1 activity, and in quadruplicate in 384-well plate for cytotoxicity to host cells (Vero). Cytotoxic activities against human cancer cell lines (KB, MCF-7, and NCI-H187) were evaluated using the resazurin microplate assay [15]. The assays were performed in triplicate in 384-well plate.

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