ORGANIC LETTERS

2011
Vol. 13, No. 18
4886–4889

Sterostreins A–E, New Terpenoids from Cultures of the Basidiomycete *Stereum ostrea* BCC 22955

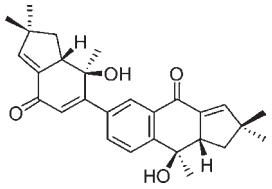
Masahiko Isaka,* Urarat Srisanoh, Wilunda Choowong, and Thitiya Boonpratuang

National Center for Genetic Engineering and Biotechnology (BIOTEC), 113 Thailand Science Park, Phaholyothin Road, Klong Luang, Pathumthani 12120, Thailand

isaka@biotec.or.th

Received July 22, 2011

ABSTRACT



sterostrein A

Sterostreins A–E (1, 2, 3a/3b, 4, and 5), five novel terpenoids, were isolated from cultures of the mushroom fungus *Stereum ostrea* BCC 22955. Sterostrein A (1) exhibited antimalarial activity (IC_{50} 2.3 μg/mL) and cytotoxicity (IC_{50} 5.3–38 μg/mL).

Mushroom fungi belonging to the genus *Stereum* have been the source of bioactive compounds; in particular, sesquiterpenes are one of the most common constituents of fruiting bodies or cell cultures. Examples include hirsutanes,[1−3] sterpuranes,[4] cadinanes,[5,6] and stereumanes.[7] As part of our research program on the utilization of fungal sources in Thailand, we investigated secondary metabolites of *Stereum ostrea* BCC 22955 as the ^{1}H NMR spectrum of an extract from a culture of this fungus showed unique resonance patterns. There has been a few reports of chemical studies on this species; isolation of a drimane-type sesquiterpene (methoxylaricinolic acid),[8] β-lactones (ostalactones A and B),[9] and L-pipecolic acid and *trans*-5-hydroxy-L-pipecolic acid.[10] Scale-up fermentation of strain BCC 22955 and chemical studies resulted in the isolation of three dimeric sesquiterpenes, sterostreins A (**1**), B (**2**) and C (**3a**/**3b**), and two illudalanes, sterostreins D (**4**) and E (**5**).

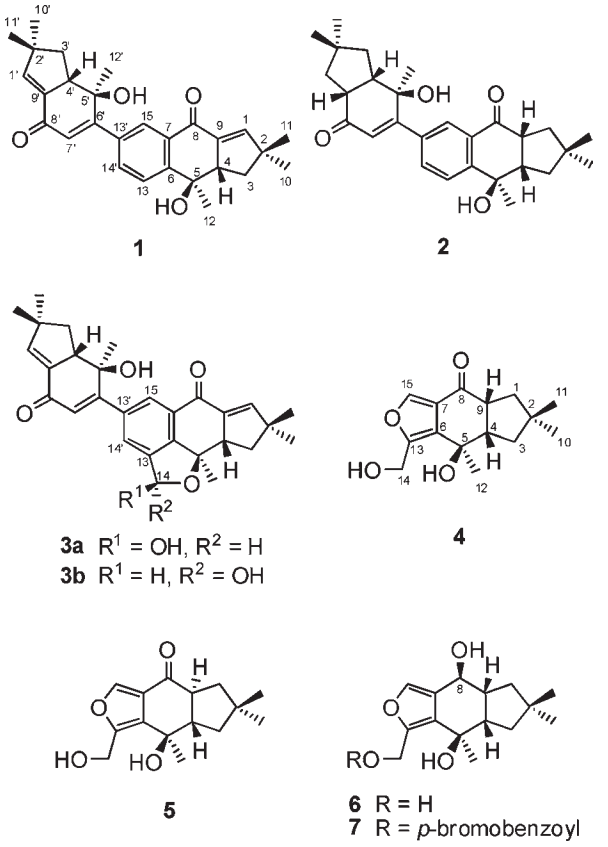
Stereum ostrea BCC 22955[11] was fermented in 20 × 1 L Erlenmeyer flasks containing 250 mL of malt extract broth (MEB) at 25 °C for 130 days under static conditions. The cultures were filtered to separate culture broth (filtrate) and mycelia (residue). The EtOAc extract from culture broth (2.25 g) was subjected to column chromatography on silica gel ($MeOH/CH_2Cl_2$) and the fractions were purified by preparative HPLC using a reversed phase column ($MeCN/H_2O$) to furnish **4** (19.2 mg) and **5** (29.0 mg). The mycelial extract (283 mg) was also fractionated by column chromatography on silica gel and preparative HPLC to obtain **1** (1.9 mg), **2** (3.0 mg), **3a**/**3b** (2.1 mg), and **5** (2.0 mg).

(1) Yun, B.-S.; Lee, I.-K.; Cho, Y.; Cho, S.-M.; Yoo, I.-D. *J. Nat. Prod.* **2002**, *65*, 786–788.
(2) Yoo, N.-H.; Kim, J.-P.; Yun, B.-S.; Ryoo, I.-J.; Lee, I.-K.; Yoon, E.-S.; Koshino, H.; Yoo, I.-D. *J. Antibiot.* **2006**, *59*, 110–113.
(3) Liermann, J. C.; Schüffler, A.; Wollinsky, B.; Birnbacher, J.; Kolshorn, H.; Anke, T.; Opatz, T. *J. Org. Chem.* **2010**, *75*, 2955–2961.
(4) Xie, J.-L.; Li, L.-P.; Dai, Z.-Q. *J. Org. Chem.* **1992**, *57*, 2313–2316.
(5) Li, G.-H.; Duan, M.; Yu, Z.-F.; Li., L.; Dong, J.-Y.; Wang, X.-B.; Guo, J.-W.; Huang, R.; Wang, M.; Zhang, K.-Q. *Phytochemistry* **2008**, *69*, 1439–1445.
(6) Li, G.-H.; Li, L.; Duan, M.; Zhang, K.-Q. *Chem. Biodiv.* **2006**, *3*, 210–216.
(7) Li, G.; Liu, F.; Shen, L.; Zhu, H.; Zhang, K. *J. Nat. Prod.* **2011**, *74*, 296–299.
(8) Kim, Y.-H.; Yun, B.-S.; Ryoo, I.-J.; Kim, J.-P.; Koshio, H.; Yoo, I.-D. *J. Antibiot.* **2006**, *59*, 432–434.
(9) Kim, J. P. Korean Patent, 2010, KR 2010063970.
(10) Hatanaka, S. *Sci. Papers Coll. Gen. Educ., Univ. Tokyo* **1972**, *22*, 117–120.

(11) The fungus *Stereum ostrea* was collected from bark of a dead hardwood tree in Khao Yai National Park, Nakhon Nayok Province, Thailand, and the living culture was deposited in the BIOTEC Culture Collection as BCC 22955 on August 21, 2006, by one of the authors (T.B.).

(12) Sterostrein A (**1**): colorless gum; $[\alpha]^{27}_D$ −163 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 209 (4.10), 283 (4.16) nm; IR (neat) ν_{max} 3344, 1659, 1651, 1622 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table 1; HRESI-MS *m*/*z* 455.2194 $[M + Na]^+$ (calcd for $C_{28}H_{32}O_4Na$, 455.2193).

10.1021/ol2019778
Published on Web 08/17/2011



Sterostrein A (**1**) was isolated as a colorless gum.[12] The molecular formula was determined by HRESI-MS as $C_{28}H_{32}O_4$. The ^{1}H and ^{13}C NMR, DEPT135, and HMQC data for **1** supported the presence of eight sp^2 quaternary carbons, six sp^2 methine carbones, four sp^3 quaternary carbons, three methines, two methines, two methylenes, and six methyl groups (Table 1). The planar structure was elucidated by interpretation of COSY and HMBC data. Thus, HMBC correlations from geminal dimethyl groups (H_3-10 and H_3-11) to an olefinic methine (C-1), C-2, and C-3, and the correlations from the olefinic proton H-1 to C-2, C-3, C-4, and C-9 revealed the five-membered ring. The tricyclic linkages were established by the HMBC correlations: from H-1 and H-15 to the doubly conjugated ketone carbonyl (C-8, δ_C 184.7), from H-4 and H-13 to the tertiary alcohol carbon (C-5, δ_C 73.9), and from H_3-12 to C-4 and C-6. The bicyclic unit was also deduced from the HMBC correlations: from the geminal dimethyl group (H_3-10′ and H_3-11′) to C-1′, C-2′, and C-3′, from H-1′ to C-2′, C-3′, C-4′, C-8′, and C-9′, from H_3-12′ to C-4′, C-5′, and C-6′, and from H-7′ to C-5′, C-6′, and C-9′. The connection of the two units at C-13′−C-6′ was also accomplished by the key HMBC correlations from H-15 and H-14′ to C-6′ and from H-7′ to C-13′ (Figure 1). The relative configurations at C-4/C-5 and C-4′/C-5′ were addressed on the basis of NOESY correlations (Figure 1). The NOESY correlation of H_α-3/H-4 was relatively much weaker than that of H_β-3/H-4, while an intense cross-peak of H_α-3/H_3-12 was observed. Similar NOESY correlations for the bicyclic unit indicated the *anti*-relation of H-4′ and CH_3-12′. NOESY correlations from H_3-12′ to H-15 and H-14′ and from H-7′ to H-15 and H-14′ suggested that the rotation of the C-13′−C-6′ axis was not restricted in $CDCl_3$.

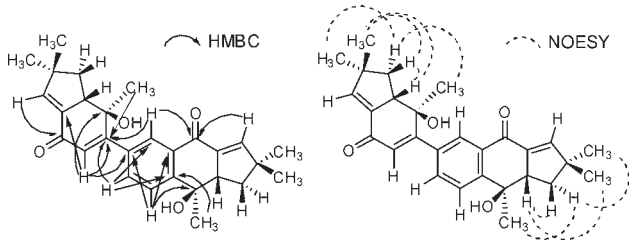


Figure 1. Selected HMBC and NOESY correlations for **1**.

The molecular formula of sterostrein B (**2**) was established by HRESIMS as $C_{28}H_{36}O_4$.[13] Detailed analysis of the NMR spectroscopic data (^{1}H, ^{13}C, DEPT135, COSY, HMQC, and HMBC) revealed that it was a tetrahydro derivative of **1**. The *cis* ring junction of the tricyclic unit was addressed by the NOESY correlations. Thus, intense NOESY correlation of H-4 and H-9 was observed, and both these protons exhibited cross-peaks to H_3-11. The relative configuration of the bicyclic unit (C-9′, C-4′, and C-5′ positions) proved to be the same sense as the tricyclic unit on the basis of the similar NOESY correlations.

Sterostrein C was isolated as an inseparable mixture of a pair of isomers **3a**/**3b**.[14] The ^{1}H and ^{13}C NMR spectra showed two sets of resonances of dimeric sesquiterpene similar to **1** in a ratio of ca. 2:1. The significant differences of the NMR data were the presence of a hemiacetal carbon, **3a** (δ_C 101.1, δ_H 6.64) and **3b** (δ_C 101.4, δ_H 6.50), and the absence of H-13. The location of the hemiacetal functionality was supported by the NOESY correlation from H-14 to H-14′ both for **3a** and **3b** and the downfield shift of C-5 (**3a** δ_C 83.9; **3b** δ_C 84.9) when compared with **1** (δ_C 73.9). Intense NOESY correlations of H_α-3/H_3-12 were observed for both **3a** and **3b**, which indicated that these isomers possess the same relative configuration of C-4/C-5. Since the significant ^{1}H and ^{13}C NMR chemical shift differences between **3a** and **3b** were found around the hemiacetal moiety (Table 1), they were assigned as C-14 emimers, which could interconvert. The NOESY correlation from H-14 to H_3-12 was observed for **3a** but not for **3b**. These data indicated that **3a** should be the 14β−OH isomer.

Sterostrein D (**4**) was assigned the molecular formula of $C_{15}H_{20}O_4$ by HRESI-MS.[15] The illudalane skeleton was

(13) Sterostrein B (**2**): pale yellow gum; $[\alpha]^{27}_D$ −90 (*c* 0.135, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.27), 248 (4.23) nm; IR (neat) ν_{max} 3343, 1668, 1620 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table 1; HRESI-MS *m*/*z* 459.2508 $[M + Na]^+$ (calcd for $C_{28}H_{36}O_4Na$, 459.2506).

(14) Sterostrein C (**3**): pale yellow powder; $[\alpha]^{27}_D$ −139 (*c* 0.115, MeOH); UV (MeOH) λ_{max} (log ε) 209 sh (4.24), 283 (4.22) nm; IR (neat) ν_{max} 3343, 1660, 1621 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) data, see Table 1; HRESI-MS *m*/*z* 461.2323 $[M + H]^+$ (calcd for $C_{29}H_{33}O_5$, 461.2320).

(15) Sterostrein D (**4**): colorless gum; $[\alpha]^{28}_D$ −41 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 207 (4.10), 264 sh (3.35) nm; IR (neat) ν_{max} 3342, 2955, 1763, 1687, 756 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Supporting Information; HRESI-MS *m*/*z* 287.1256 $[M + Na]^+$ (calcd for $C_{15}H_{20}O_4Na$, 287.1254).

Table 1. NMR Data of Sterostreins A (**1**), B (**2**), and C (**3a**/**3b**) in $CDCl_3$

	1		**2**		**3a/3b** (ca. 2:1)	
no.	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)
1	151.3	6.81, d (2.6)	45.2	α 1.48, dd (13.2, 7.5) *β* 1.93, ddd (13.2, 9.3, 1.3)	152.9[e]/152.8[e]	6.87, d (2.5)/6.87, d (2.5)
2	45.59[a]		39.2		46.3[f]/46.2[f]	
3	39.7	α 1.97, dd (13.2, 8.3) *β* 2.19, dd (13.2, 8.1)	44.1	α 0.94, t (12.4) *β* 1.61, m	39.4/39.4	α 1.98, m/1.99, m *β* 2.09, m/2.04, m
4	54.5	3.56, dt (2.6, 8.1)	50.4	2.94, m	54.9/54.1	3.56, dt (2.6, 8.1)/3.51, dt (2.5, 8.2)
5	73.9		70.9		83.9/84.9	
6	152.6		145.4		152.8/153.1	
7	132.1		132.6		129.1/129.0	
8	184.7		201.9		184.1/184.0	
9	137.4		47.9	3.35, q (8.6)	137.9[h]/138.2[h]	
10	28.5[b]	1.27, s	29.4	0.91, s	28.3/28.3	1.28,[j] s/1.27,[j] s
11	27.6[c]	1.17, s	28.4	1.04, s	27.2[g]/27.1[g]	1.12, s/1.12, s
12	25.9	1.32, s	25.9	1.69, s	22.3/24.6	1.38, s/1.56, s
13	124.4	7.82, d (8.1)	124.8	7.49, d (8.0)	138.3[h]/138.2[h]	
14					101.1/101.4	6.64, m/6.50, br d (4.2)
14-OH						3.32, m/3.49, br d (4.2)
15	127.6	8.10, d (1.8)	126.9	7.94, d (1.8)	126.8/126.6	7.92,[k] br s/7.90,[k] br s
1′	148.9	6.62, d (2.6)	42.6	α 2.21, dd (13.6, 1.7) *β* 1.68, dd (13.6, 8.6)	149.1/149.1	6.63, d (2.4)/6.63, d (2.4)
2′	45.56[a]		37.4		45.6/45.6	
3′	39.4	α 1.88, dd (13.2, 8.4) *β* 2.02, dd (13.2, 8.3)	44.4	α 1.22, t (12.9) *β* 1.58, m	39.5[i]/39.4[i]	α 1.89, dd (13.2, 8.5)/ 1.89, dd (13.2, 8.5) *β* 2.02, m/2.02, m
4′	54.9	3.67, dt (2.6, 8.3)	53.2	2.69, dt (12.9, 6.5)	55.0/54.8	3.70, dt (2.4, 8.1)/ 3.68, dt (2.4, 8.1)
5′	75.1		71.2		75.1	
6′	167.2		158.3		167.0/167.0	
7′	129.1	6.06, s	127.9	5.86, s	129.6/129.6	6.04, s/6.02, s
8′	185.9		201.0		185.7/185.6	
9′	137.1		47.4	2.97, m	137.0/137.0	
10′	28.6[b]	1.25, s	31.40[d]	0.99, s	28.3/28.3	1.28,[j] s/1.27,[j] s
11′	27.7[c]	1.16, s	31.37[d]	1.05, s	27.6/27.6	1.18, s/1.18, s
12′	24.0	1.42. s	26.8	1.18, s	24.1/24.0	1.42,[l] s/1.40,[l] s
13′	136.2		138.8		138.6/139.1	
14′	134.1	7.71, dd (8.1, 1.8)	132.3	7.66, dd (8.0, 1.8)	128.4/128.1	7.70, br s/7.67, br s

[a–i] The assignment of carbons can be interchanged. [j–l] The assignment of protons can be interchanged.

established by interpretation of the 1D and 2D NMR spectroscopic data. The presence of a furan ring attached with a hydroxymethyl group was deduced on the basis of the HMBC correlations from an sp^2 methine at δ_H 7.85 (H-15) to the aromatic quaternary carbons C-6, C-7, and C-13, and from H_2-14 to C-6 and C-13. The relative configuration was deduced from the NOESY data. Intense cross-peak between H-4 and H-9 and a weak correlation from H-4 to H_β-1 revealed the *cis* ring junction.

Sterostrein E (**5**)[16] was identified as the C-9 epimer of **4**. The NOESY correlations H-4/H_β-1, H-9/H_α-3, and H-9/H_3-12 established the *trans* ring junction and relative configuration of the C-9, C-4, and C-5 positions. In an initial effort to determine the absolute configurations of **4** and **5**, we prepared their 14-*O*-*p*-bromobenzoate derivatives (*p*-bromobenzoyl chloride, pyridine, rt), wherein we observed the epimerization of **5** at C-9 to give a mixture of isomeric acylated products. This result further confirmed that **4** and **5** possess the same relative configuration of C-4/C-5.

The absolute configuration of the key illudalane monomer **4** was determined by application of the modified Mosher's method.[17] $LiAlH_4$ reduction (THF, 0 °C) of sterostrein D (**4**) gave the 8*β*-hydroxy derivative **6** as the major reaction product, which was transformed to a *p*-bromobenzoate **7**. The configuration at C-8 was addressed on the basis of the NOESY correlations for both **6** and **7**. Intense NOESY correlation from H-8 to H_α-1 indicated the α-orientation of H-8. The cross-peak from H-8 to H-9 was relatively much weaker, and the correlation between H-8 and H_β-1 was not observed. (*S*)- and (*R*)-MTPA ester derivatives, **8a** and **8b**, were synthesized by reactions of **7**

(16) Sterostrein E (**5**): colorless gum; $[\alpha]^{27}_D$ −22 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 207 (4.21), 269 (3.43) nm; IR (neat) ν_{max} 3343, 2953, 1761, 1690, 755 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) data, see Supporting Information; HRESI-MS *m*/*z* 287.1259 $[M + Na]^+$ (calcd for $C_{15}H_{20}O_4Na$, 287.1254).

(17) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.

with (−)-(*R*)-MTPA-Cl and (+)-(*S*)-MTPA-Cl, respectively. The Δδ-values indicated the 8*S*-configuration (Figure 2).

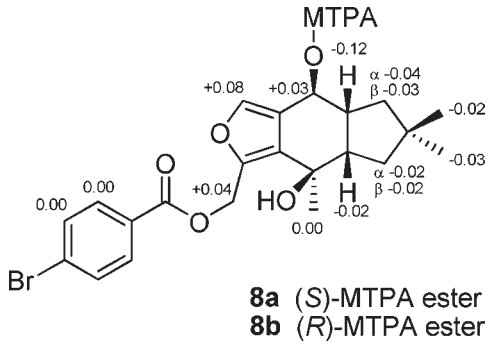


Figure 2. Δδ-Values ($\delta_S - \delta_R$) of the Mosher esters **8a** and **8b**.

A plausible biogenetic pathway for the dimeric compounds **1** and **3a**/**3b** is proposed in Scheme 1. Illudalane **9** (a dehydro analogue of **4**/**5**)[18] may be converted to a norilludalane **10**,[18] which could undergo Diels–Alder reaction with **9** to give a cycloadduct **11**. Subsequent dehydrative aromatization and oxidation of the C-15 primary alcohol of **12** to aldehyde will give sterostrein C (**3a**/**3b**). Sterostrein A (**1**) may be produced by further oxidation to carboxylic acid and decarboxylation.

Although sesquiterpenes are one of the most common constituents of Basidiomycete (mushroom fungi), illudalanes are relatively rare.[19] Sterostreins D (**4**) and E (**5**) are novel furan-containing illudalanes. Chemical skeleton of the dimeric compounds **1**, **2** and **3a**/**3b** is hitherto unknown.[20] Agrocybone, a bis-sesquiterpene recently isolated from a baidiomycete *Agrocybe salicacola*, is proposed as a plausible Diels–Alder adduct of an illudane

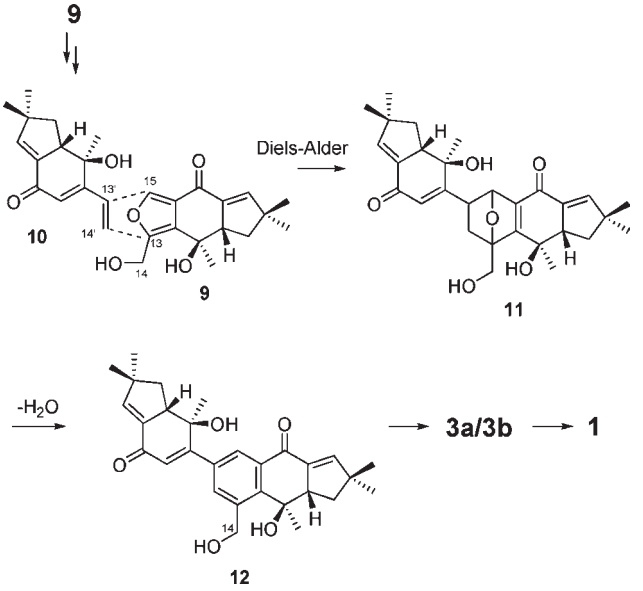
(18) Compounds **9** and **10** have not been previously reported. In our further study on this fungus (BCC 22955), we isolated **9** and a hydrated analogue of **10** (possessing a 2-hydroxyethyl group instead of the C-13′/C-14′ vinyl group) from a new fermentation batch.

(19) (a) Clericuzio, M.; Pan, F.; Han, F.; Pang, Z.; Sterner, O. *Tetrahedron Lett*. **1997**, *38*, 8237–8240. (b) Pettit, G. R.; Meng, Y.; Pettit, R. K.; Herald, D. L.; Cichacz, Z. A.; Doubek, D. L.; Richert, L. *J. Nat. Prod*. **2010**, *73*, 388–392. (c) Suzuki, S.; Murayama, T.; Shiono, Y. *Phytochemistry* **2005**, *66*, 2329–2333. (d) Fushimi, K.; Horikawa, M.; Suzuki, K.; Sekiya, A.; Kanno, S.; Shimura, S.; Kawagishi, H. *Tetrahedron* **2010**, *66*, 9332–9335.

(20) A recent review of natural disesquiterpenoids: Zhan, Z.-J.; Ying, Y.-M.; Ma, L.-F.; Shan, W.-G. *Nat. Prod. Rep*. **2011**, *28*, 594–629.

(21) Zhu, Y.-C.; Wang, G.; Yang, X.-L.; Luo, D.-Q.; Zhu, Q.-C.; Liu, J.-K. *Tetrahedron Lett*. **2010**, *51*, 3443–3445.

Scheme 1. Plausible Biogenetic Pathway for **1** and **3a/3b**



(dienophile) and an illudalane (diene),[21] although its structure totally differs from **1**, **2**, and **3a**/**3b**.

As a part of our search for novel drug leads from Thai fungal sources, compounds **1**, **4**, and **5** were subjected to our biological assay protocols. Compounds **2** and **3a/3b** were not tested due to the sample shortage. Sterostrein A (**1**) exhibited cytotoxicity against cancer cell lines (KB, MCF-7, and NCI-H187) and nonmalignant Vero cells with IC_{50} values of 38, 7.2, 5.3, and 12 μg/mL, respectively. This compound also displayed activity against the malarial parasite *Plasmodium falciparum* K1 with an IC_{50} 2.3 μg/mL, while it was inactive against *Mycobacterium tuberculosis* H37Ra at a concentration of 50 μg/mL. Monomeric illudalanes **4** and **5** were inactive in these assays except that **4** showed weak cytotoxicity to KB cells (IC_{50} 26 μg/mL).

Acknowledgment. Financial support from the Bioresources Research Network, National Center for Genetic Engineering and Biotechnology (BIOTEC), is gratefully acknowledged.

Supporting Information Available. Experimental section and NMR spectra of the new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.