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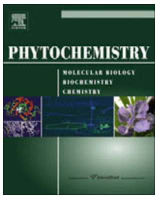

Oxygenated verticillene derivatives from *Bursera suntui* ☆

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Medium polarity fractions of the hexane extracts of the stems of *Bursera suntui* afforded six previously known (**1**–**6**) and four hitherto unknown verticillane derivatives: (1*S*,3*Z*,7*S*,8*S*,11*S*,12*S*)-(+)-7,8-epoxyverticill-3-en-12,20-diol (**7**), (1*S*,3*Z*,7*S*,8*S*,11*S*,12*S*)-(+)-7,8-epoxyverticill-3-en-12,20-diol 20-acetate (**8**), (1*S*,3*Z*,7*S*,11*S*,12*S*)-(+)-verticilla-3,8(19)-dien-7,12,20-triol (**9**), and (1*S*,3*Z*,7*S*,11*S*,12*S*)-(+)-verticilla-3,8(19)-dien-7,12,20-triol 20-acetate (**10**). Acetylation of **9** and **10** yielded (1*S*,3*Z*,7*S*,11*S*,12*S*)-(+)-verticilla-3,8(19)-dien-7,12,20-triol 7,20-diacetate (**11**), while hydrolysis of **8** gave **7**. The structures and stereochemistry of **7**–**11** were established by spectroscopic analyses, particularly by 1D and 2D NMR spectra and HRESIMS. The conformational preferences of **7**–**11** were studied by molecular mechanics modelling employing the Monte Carlo protocol followed by B3LYP/DGDZVP DFT calculation, thus supporting the observed ^{1}H NMR NOESY cross peaks.

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1. Introduction

Verticillane derivatives constitute a relevant group of diterpenes whose hydrocarbon skeleton is thought to be a key intermediate in taxadiene biosynthesis (Jin et al., 2005a,b). These metabolites have been isolated from diverse sources such as the conifer *Sciadopitys verticillata* (Karlsson et al., 1978), the yew trees *Taxus canadensis* (Zamir et al., 1995, 1998), *T. cuspidata* (Renzulli et al., 2004), *T. chinensis* (Fang et al., 1995), *T. chinensis* var. *mairei* (Shi et al., 1998), and *T. mairei* (Shi et al., 2005), as well as the soft corals *Cespitularia hypotentaculata* (Duh et al., 2002) and *C. taeniata* (Shen et al., 2005), antipodal structures have also been found in the liverworts *Jackiella javanica* and *Jungermannia infusca* (Nagashima et al., 1997, 2005). Some verticillene derivatives display relevant biological activity against diverse tumour cell lines; for example, verticillenes isolated from *Taxus sp.* showed significant cytotoxicity against murine leukemia L1210 cells and human oral epidermoid carcinoma KB cells (Kobayashi and Shigemori, 2002); other verticillene derivatives obtained from *Cespitularia sp.* also exhibited important cytotoxicity against mouse lymphocytic leukemia P-388 cells, human colon adenocarcinoma A549 cells (Duh et al., 2002), human colon adenocarcinoma WiDr cells, human medulloblastoma Daoy cells, human oral epidermoid carcinoma KB cells and Hepa59T/VGH cells (Shen et al., 2005). There are also specific biological assays in which these diterpenes show promising activity. For example, verticillene-type derivatives isolated from *T. cuspidata* produced accumulation of vincristine in cells that originated from tissues constitutively expressing the multidrug-resistance gene (MDR1), such as the human ovarian cancer 2780AD cells (Kobayashi et al., 2000; Kobayashi and Shigemori, 2002). In addition, some verticillenes were found to remarkably reduce $CaCl_2$-induced depolymerization of microtubules (Kobayashi and Shigemori, 2002).

In previous work, the isolation and structure elucidation of several low-functionalized verticillene derivatives obtained for the first time from the genus *Bursera* were reported. Thus, the stems of *Bursera suntui* previously afforded three major verticillane diterpenes, (1*S*,3*E*,7*E*,11*S*,12*S*)-(+)-verticilla-3,7-dien-12-ol (**1**), (1*S*,3*E*,7*E*,11*S*,12*S*)-(+)-verticilla-3,7-dien-12,20-diol (**2**) and (1*S*,3*E*,7*E*,11*S*,12*S*)-(+)-verticilla-3,7-dien-12,20-diol 20-acetate (**3**) as well as three hydrocarbon derivatives, (1*S*,3*E*,7*E*,11*R*)-(+)-verticilla-3,7,12(18)-triene (**4**), (1*R*,3*E*,7*E*,11*R*,12*Z*)-(+)-verticilla-3,7,12-triene (**5**) and (1*R*,7*E*,11*Z*)-(−)-verticilla-4(20),7,11-triene (**6**) (Hernández-Hernández et al., 2005). Now, isolation of four new functionalized verticillane derivatives (**7**–**11**) obtained from the hexane extracts of *B. suntui* are described (Fig. 1). Verticillene **7** was chemically correlated with **8** by means of alkaline hydrolysis of the latter, while compound **9** was correlated with **10** via the diacetate **11**. The global minimum energy structures for each

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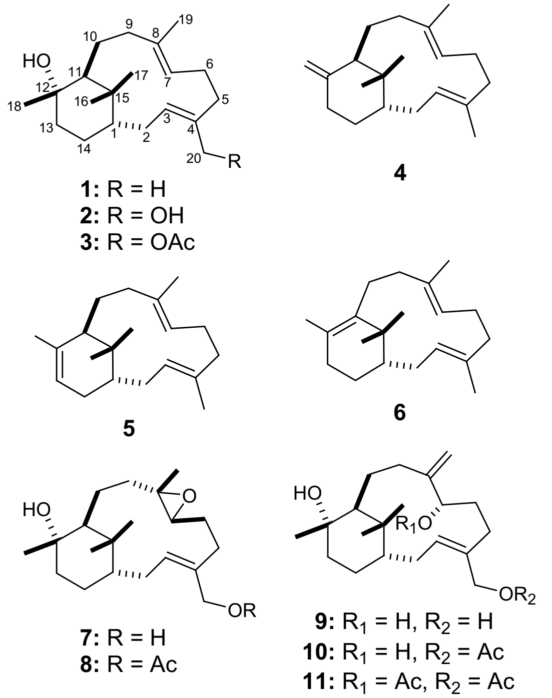


Fig. 1. Molecular structures.

verticillane derivative (**7**–**11**) were obtained by a Monte Carlo search (Chang et al., 1989) using molecular mechanics (Halgren, 1996a,b,c,d; Halgren and Nachbar, 1996) followed by density functional theory (DFT) (Andzelm and Wimmer, 1992; Godbout et al., 1992) at the B3LYP/DGDZVP level of theory. Comparison of ^{1}H NMR NOESY correlations and molecular models confirmed the stereochemistry and conformation of verticillane derivatives obtained from *B. suntui*.

2. Results and discussion

Hexane extraction of the fresh stems of *B. suntui* yielded a viscous oil which was chromatographed on silica gel. Fast elution with hexane, hexane–chloroform (1:1), chloroform, and EtOAc, respectively, afforded four main fractions. The EtOAc fraction was subjected to successive flash chromatographic purifications over silica gel to give pure verticillane derivatives **7**–**10**.

The IR spectrum of (1*S*,3*Z*,7*S*,8*S*,11*S*,12*S*)-(+)-7,8-epoxyverticill-3-en-12,20-diol (**7**) showed the presence of a hydroxyl group at 3602 and 3457 cm^{-1}, its HRESIMS peak at *m*/*z* 345.2408 indicated the molecular formula $C_{20}H_{34}O_3$ + Na, indicating that **7** possessed four degrees of unsaturation, while the optical rotation value $[\alpha]_D$ +69 was in agreement with those displayed by most compounds belonging to the (+)-verticillol series. The ^{1}H NMR spectrum (Table 1) exhibited a signal for a vinylic proton at δ 6.10 (*dd*, *J* = 13.4, 3.7 Hz), a hydroxymethylene group at δ 4.44 (*br d*, *J* = 11.6 Hz) and δ 3.95 (*dd*, *J* = 11.6, 5.7 Hz); the latter was also coupled to a hydroxyl group, a signal for a methine group bearing an oxirane ring at δ 2.98 (*d*, *J* = 9.0 Hz), and four tertiary methyl groups at δ 1.23 (geminal to oxirane ring), δ 1.27 (geminal to a hydroxyl group), δ 0.80 and δ 0.91 (*gem*-dimethyl group). The ^{13}C NMR spectrum (Table 2) showed the presence of a trisubstituted C=C double bond at δ 136.2 (C) and δ 131.9 (CH), four signals for oxygen-bearing carbon atoms at δ 75.3 (C), δ 66.0 (CH), δ 62.0 (C) and δ 58.4 (CH_2), as well as four methyl groups, seven methylenes, two methines and one quaternary carbon. The HMBC spectrum was very informative and the structure was confirmed to be a verticillane-type compound with two hydroxyl groups at C-12 and C-20, and a double bond at C-3 from the correlations observed between: H-2 and C-1, C-3, C-4 and C-14; H-3 and C-20; H-5 and C-3, C-4, C-7 and C-20; H-6 and C-4, C-7 and C-8; H-9 and C-8 and C-11; H-10 and C-9 and C-12; H-11 and C-9, C-10, C-12, C-15 and C-16; H-18 and C-11, C-12 and C-13; H-19 and C-7, C-8 and C-9, and between H-20 and C-3, C-4 and C-5. The NOESY correlations between H-1 and H-17 (*d* = 2.50 Å), H-2β and H-17 (*d* = 1.83 Å), H-2β and H-20a (*d* = 2.08 Å), H-3 and H-11 (*d* = 2.61 Å), H-3 and H-13α (*d* = 2.04 Å), H-7 and H-11 (*d* = 2.49 Å), H-16 and H-17 (*d* = 1.84 Å), H-16 and H-18 (*d* = 1.58 Å), and H-17 and H-19 (*d* = 1.98 Å), together with coupling constant values (Table 1), were in full agreement with the stereochemistry and conformation observed in the molecular model of **7** obtained by geometry optimization at the B3LYP/DGDZVP level of theory using a DFT calculation (Andzelm and Wimmer, 1992; Godbout et al., 1992) (Fig. 2) from which the distances (*d*) were measured.

The IR spectrum of (1*S*,3*Z*,7*S*,8*S*,11*S*,12*S*)-(+)-7,8-epoxyverticill-3-en-12,20-diol 20-acetate (**8**) displayed bands at 3599, 3475 and 1729 cm^{-1} indicating the presence of hydroxyl groups and carbonyl groups. The molecular formula of **8** was established as $C_{22}H_{36}O_4$ + Na by HRESIMS (*m*/*z* 387.2511) and the optical activity data showed an expected dextrorotatory value of $[\alpha]_D$ +60. The ^{1}H NMR spectrum (Table 1) of **8** showed a signal pattern very similar to that of **7** with the vinylic proton at δ 6.22 (*dd*, *J* = 13.8, 3.6 Hz). However, the methylene group at C-20 appeared shifted downfield to δ 4.79 (*d*, *J* = 12.1 Hz) and δ 4.50 (*d*, *J* = 12.1 Hz), and a new signal for an acetyl group was observed at δ 2.06. The ^{13}C NMR spectrum (Table 2) together with the COSY, NOESY, HSQC, and HMBC correlations of this new substance reinforced the structure, stereochemistry, and conformation (Fig. 2). In addition, verticillane **8** was hydrolyzed with potassium hydroxide in methanol to yield **7**, which was identical to the natural product.

B. suntui also afforded two new verticillane derivatives with exocyclic methylene groups (**9** and **10**). The IR spectrum of (1*S*,3*Z*,7*S*,11*S*,12*S*)-(+)-verticilla-3,8(19)-dien-7,12,20-triol (**9**) showed the presence of a hydroxyl group (3598 and 3417 cm^{-1}), while its molecular formula was established as $C_{20}H_{34}O_3$ + Na by HRESIMS (*m*/*z* 345.2409). The compound also had an optical rotation $[\alpha]_D$ +29. The ^{1}H NMR spectrum (Table 1) exhibited three signals for vinylic protons: one at δ 5.98 (*dd*, *J* = 12.5, 4.4 Hz) for a trisubstituted C=C double bond, and two at δ 5.17 (*t*, *J* = 1.8), and δ 4.87 (*br s*) which confirmed the presence of an exocyclic methylene, a resonance for a proton geminal to a hydroxyl group at δ 4.71 (*br d*, *J* = 7.5 Hz), an AB system for a methylene group bearing a hydroxyl group at δ 4.43 (*d*, *J* = 11.6 Hz) and δ 3.96 (*d*, *J* = 11.6 Hz), and three tertiary methyl groups at δ 1.15 (geminal to hydroxyl group), δ 0.83 and δ 0.90 (*gem*-dimethyl group). The ^{13}C NMR spectrum (Table 2) confirmed the presence of the exocyclic methylene at δ 155.9 (C) and δ 109.5 (CH_2), three signals for oxygen-bearing carbon atoms at δ 74.1 (C), δ 73.5 (CH), and δ 59.4 (CH_2), as well as three methyl groups, seven methylenes, two methines and one quaternary carbon. The HMBC spectrum showed correlations of: H-3 with C-5 and C-20; H-7 with C-5, C-6, C-8 and C-19; H-18 with C-11, C-12 and C-13; H-19 with C-7, C-8 and C-9, and of H-20 with C-4 and C-5. The NOESY correlations between H-1 and H-16 (*d* = 2.13 Å), H-1 and H-17 (*d* = 2.53 Å), H-2β and H-17 (*d* = 1.82 Å), H-2β and H-20a (*d* = 2.08 Å), H-3 and H-11 (*d* = 2.57 Å), H-3 and H-13α (*d* = 2.05 Å), H-7 and H-11 (*d* = 2.41 Å), H9b and H-19a (*d* = 2.62 Å), H-16 and H-17 (*d* = 1.86 Å), and H-16 and H-18 (*d* = 1.51 Å), together with coupling constant values, were in full agreement with the stereochemistry and molecular conformation obtained by DFT calculations at the B3LYP/DGDZVP level of theory of **9** (Fig. 2).

The fourth compound, (1*S*,3*Z*,7*S*,11*S*,12*S*)-(+)-verticilla-3,8(19)-dien-7,12,20-triol 20-acetate (**10**), showed in its IR spectrum bands

Table 1
^1H NMR spectroscopic data of verticillane derivatives **7**–**11** ($CDCl_3$, 300 MHz)

Proton	**7**	**8**	**9**	**10**	**11**
H-1	1.50 (*m*)	1.53 (*m*)	1.52 (*m*)	1.54 (*m*)	1.53 (*m*)
H-2α	1.95 (*m*)	1.97 (*m*)	2.03 (*m*)	2.04 (*m*)	2.03 (*m*)
H-2β	2.86 (*ddd*, 14.5, 13.4, 5.9)	2.87 (*td*, 13.8, 6.1)	2.86 (*ddd*, 14.3, 12.7, 6.4)	2.88 (*td*, 13.4, 6.1)	2.90 (*ddd*, 14.0, 13.4, 6.3)
H-3	6.10 (*dd*, 13.4, 3.7)	6.22 (*dd*, 13.8, 3.6)	5.98 (*dd*, 12.5, 4.4)	6.11(*dd*, 12.7, 3.7)	6.18 (*dd*, 12.9, 3.6)
H-5α	2.20 (*td*, 14.1, 2.6)	2.27 (*td*, 13.8, 2.8)	2.19 (*br t*, 13.8)	2.22 (*br t*, 12.7)	1.90 (*m*)
H-5β	2.73 (*br d*, 14.1)	2.52 (*br d*, 13.8)	2.55 (*dd*, 13.8, 7.0)	2.36 (*m*)	2.26 (*m*)
H-6α	1.68 (*m*)	1.68 (*m*)	1.52 (*m*)	1.51 (*m*)	1.66 (*m*)
H-6β	1.96 (*m*)	1.96 (*m*)	1.78 (*m*)	1.76 (*m*)	1.89 (*m*)
H-7	2.98 (*d*, 9.0)	2.99 (*d*, 9.4)	4.71 (*br d*, 7.5)	4.72 (*br d*, 6.6)	5.85 (*br d*, 6.6)
H-9α	1.71 (*m*)	1.73 (*m*)	1.91 (*m*)	1.91 (*m*)	1.97 (*m*)
H-9β	1.98 (*m*)	1.97 (*m*)	2.32 (*ddd*, 13.8, 6.8, 2.4)	2.33 (*ddd*, 14.1, 6.6, 2.2)	2.29 (*td*, 6.6, 2.2)
H-10α	1.52 (*m*)	1.55 (*m*)	1.37 (*m*)	1.37 (*m*)	1.76 (*m*)
H-10β	1.52 (*m*)	1.55 (*m*)	1.58 (*m*)	1.59 (*m*)	1.50 (*dtd*, 14.3, 7.1, 2.5)
H-11	2.14 (*dd*, 9.0, 1.3)	2.11 (*m*)	2.40 (*dd*, 6.0, 2.4)	2.37 (*m*)	2.18 (*dd*, 6.3, 2.7)
H-13α	1.94 (*m*)	1.92 (*m*)	1.85 (*m*)	1.86 (*m*)	1.85 (*m*)
H-13β	1.69 (*m*)	1.70 (*m*)	1.72 (*m*)	1.72 (*m*)	1.80 (*m*)
H-14α	1.62 (*m*)	1.63 (*m*)	1.64 (*m*)	1.65 (*m*)	1.66 (*m*)
H-14β	1.94 (*m*)	1.97 (*m*)	1.96 (*m*)	1.97 (*m*)	1.99 (*m*)
Me-16	0.80 (*s*)	0.80 (*s*)	0.83 (*s*)	0.83 (*s*)	0.85 (*s*)
Me-17	0.91 (*s*)	0.93 (*s*)	0.90 (*s*)	0.90 (*s*)	0.91 (*s*)
Me-18	1.27 (*s*)	1.27 (*s*)	1.15 (*s*)	1.15 (*s*)	1.07 (*s*)
Me-19	1.23 (*s*)	1.26 (*s*)	–	–	–
H-19a	–	–	5.17 (*t*, 1.8)	5.17 (*t*, 1.8)	5.00 (*t*,1.7)
H-19b	–	–	4.87 (*br s*)	4.87 (*br s*)	4.91 (*br s*)
H-20a	4.44 (*br d*, 11.6)	4.79 (*d*, 12.1)	4.43 (*d*, 11.6)	4.79 (*d*, 12.1)	4.77 (*d*, 11.8)
H-20b	3.95 (*dd*, 11.6, 5.7)	4.50 (*d*, 12.1)	3.96 (*d*, 11.6)	4.52 (*d*, 12.1)	4.55 (*d*, 11.8)
AcO	–	2.06 (*s*)	–	2.06 (*s*)	2.06 (*s*)
AcO	–	–	–	–	2.13 (*s*)

Table 2
^{13}C NMR spectroscopic data of verticillane derivatives **7**–**11** ($CDCl_3$, 300 MHz)

Carbon	**7**	**8**[a]	**9**	**10**[b]	**11**[c]
1	43.7	43.6	43.1	43.1	43.2
2	33.2	33.4	33.0	33.3	33.7
3	131.9	134.5	130.8	133.5	133.8
4	136.2	131.6	137.1	132.6	131.9
5	33.4	34.0	32.6	33.3	32.2
6	26.4	26.5	33.7	33.7	31.8
7	66.0	65.9	73.5	73.4	75.9
8	62.0	62.1	155.9	155.9	148.9
9	39.6	39.6	34.6	34.6	34.4
10	21.1	21.1	26.5	26.5	25.9
11	45.3	45.3	47.8	48.0	49.7
12	75.3	75.3	74.1	74.1	72.7
13	41.3	41.2	39.1	39.2	40.9
14	28.0	27.9	27.8	27.7	28.3
15	37.5	37.5	37.8	37.8	38.4
16	24.9	24.8	24.7	24.5	25.3
17	28.9	28.9	27.3	27.3	27.4
18	25.0	25.0	25.0	25.0	22.7
19	16.5	16.6	109.5	109.5	111.5
20	58.4	60.3	59.4	61.3	61.4

[a] AcO: δ 171.1, 20.9.
[b] AcO: δ 171.2, 21.0.
[c] AcO's: δ 172.3, 171.2, 21.1, 21.0.

for a hydroxyl group (3598 and 3416 cm^{-1}) and a carbonyl group (1732 cm^{-1}). The molecular formula of **10** was established as $C_{22}H_{36}O_4$ + Na by HRESIMS (*m*/*z* 387.2507), and it had an optical rotation of $[\alpha]_D$ +29. The spectroscopic data of **10** were analogous to those of **9** with the exception of the signal for one acetyl group at δ 2.06. HMBC correlations of H-3 with C-2, C-5, and C-20, of H-7 with C-6, C-8, and C-19, of H-18 with C-12, and of H-20 with C-21, together with the NOESY spectrum, allowed the structure determination of **10**.

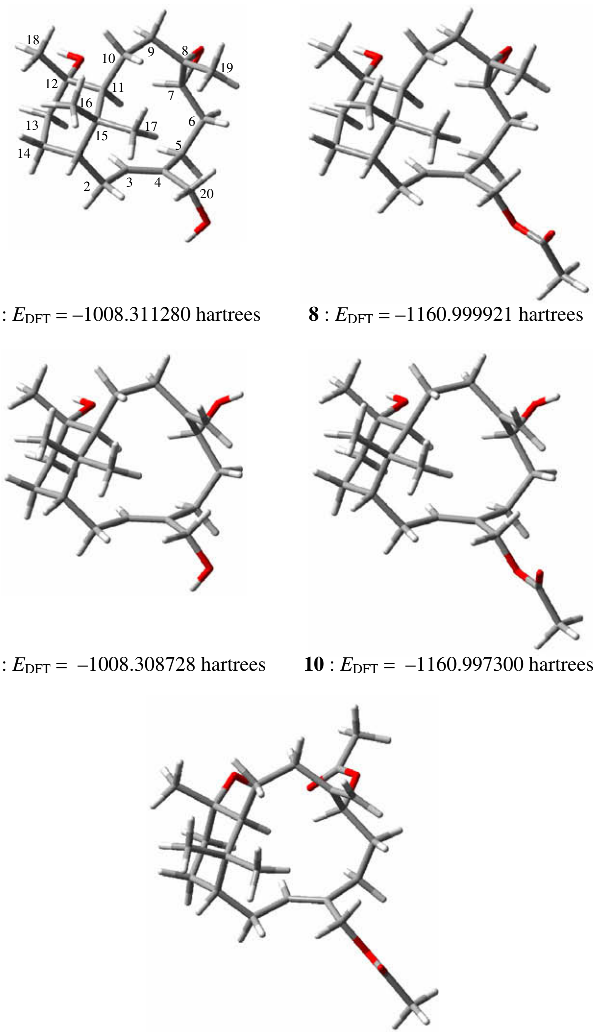
In order to correlate **9** with **10**, both substances were subjected to acetylation under standard reaction conditions yielding (1*S*,3*Z*,7*S*,11*S*,12*S*)-(−)-verticilla-3,8(19)-dien-7,12,20-triol 7,20-diacetate (**11**). The IR spectrum of **11** showed the presence of a hydroxyl group (3507 cm^{-1}) and carbonyl signals (1726 cm^{-1}). The molecular formula of **11** was established as $C_{24}H_{38}O_5$ + Na by HRESIMS (*m*/*z* 429.2625). The spectroscopic data of derivative **11** were analogous to those of **10** with the exception of a shift for the signal due to the hydrogen geminal to the hydroxyl group at C-7 from δ 4.72 in **10** to 5.85 in **11**, and a new acetyl group signal at δ 2.13. HMBC correlations of H-3 with C-2, C-5 and C-20, of H-7 with C-5, C-6, C-8, C-19 and C-23 (carbonyl carbon), of H-18 with C-12, and of H-20 with C-21, together with the NOESY spectrum were in full agreement with structure **11**. Correlation of epoxide **7** with allylic alcohol **9** was not straightforward because the hydroxyl group at C-12 was very sensitive to dehydration under acid treatments. Therefore, this task was not undertaken.

The absolute configuration of verticillol **1**, also isolated from *B. suntui*, was securely assigned as 1*S*,3*E*,7*E*,11*S*,12*S* on the basis of a vibrational circular dichroism study (Cerda-García-Rojas et al., 2007). Therefore, the absolute configuration of the new verticillane derivatives **7**–**11** can be established on biogenetic grounds, taking into account that for the natural product **1**.

The structural novelty of compounds **7** and **8** mainly resides in that both are the first verticillenes with an oxirane ring isolated from a natural source. Previously, several epoxyverticillanes were obtained by oxidation of verticillenes with peroxyacids, such as *m*-chloroperoxybenzoic acid (Nagashima et al., 2005) and monoperphthalic acid (Karlsson et al., 1978), or by treatment with H_2O_2 and SeO_2 in *t*-BuOH (Cerda-García-Rojas et al., 2007). Finally, allylic alcohols **9**–**11** are relevant because they closely resemble some structural features of the cytotoxic verticillenes cespitularins C and E isolated from the soft coral *Cespitularia hypotentaculata* (Duh et al., 2002).

3. Conclusion

Four new functionalized verticillane derivatives (**7**–**10**) were obtained from the hexane extracts of *B. suntui*. This is the first occurrence of epoxyverticillene derivatives (**7** and **8**) as natural



7 : $E_{DFT} = -1008.311280$ hartrees

8 : $E_{DFT} = -1160.999921$ hartrees

9 : $E_{DFT} = -1008.308728$ hartrees

10 : $E_{DFT} = -1160.997300$ hartrees

11 : $E_{DFT} = -1313.687419$ hartrees

Fig. 2. Global minimum energy conformers for verticillane derivatives **7**–**11**.

products. Additionally, the new group of verticillenes **7**–**11** can be employed for the preparation of functionalized derivatives which resemble substances with proven antitumour activity.

4. Experimental

4.1. General remarks

Optical rotations were measured in $CHCl_3$ at 25 °C in a Perkin–Elmer 341 polarimeter. IR spectra were obtained in $CDCl_3$ on a BUCK Scientific 500 spectrophotometer. 1D and 2D NMR spectra were measured from $CDCl_3$ solutions containing TMS as the internal standard at 300 MHz for ^1H and 75.4 MHz for ^{13}C on a Varian Mercury 300 spectrometer. Low-resolution mass spectra were recorded at 70 eV and chemical ionization was done using acetonitrile (Lawrence and Brenna, 2006) on a Varian Saturn 2000 spectrometer, while the high-resolution mass spectra were measured on an Agilent LCTOF spectrometer at the UCR Mass Spectrometry Facility, University of California, Riverside. Column chromatography (CC) was carried out on either Merck silica gel 60 (230–400 mesh ASTM) or Merck aluminum oxide 90 active neutral (70–230 mesh ATSM). TLC was developed on Merck silica gel 60 F_{254} plates.

4.2. Molecular modelling

Minimum energy structures were generated using the MMFF94 (Halgren, 1996a,b,c,d; Halgren and Nachbar, 1996) force-field calculations employing the Monte Carlo protocol (Chang et al., 1989) in the Spartan'04 program. Gaussian 03W was used to optimize the minimum energy structures for **7**–**11** by DFT (Andzelm and Wimmer, 1992; Godbout et al., 1992) at the B3LYP/DGDZVP level of theory at 298 K and 1 atm.

4.3. Plant material

Specimens of *Bursera suntui* Toledo were collected at 72 km on Iguala-Chilpancingo state road No. 95, near Zumpango del Río, State of Guerrero, Mexico, in April 2003. A voucher specimen (B-291) was deposited at the Herbarium of the Instituto de Ecología, A.C., Pátzcuaro, Michoacán, Mexico, where Prof. Jerzy Rzedowski kindly identified the plants.

4.4. Extraction and isolation

The stems of *B. suntui* (12 kg) were extracted with hexane (10 L) at room temperature during 4 weeks to yield, after solvent removal, a slightly greenish viscous oil (26 g) which was subjected to silica gel column chromatography (CC). Fast elution with hexane, hexane–$CHCl_3$ (1:1, v/v), $CHCl_3$, and EtOAc, respectively, afforded four main fractions. The first three fractions gave previously reported verticillene derivatives **1**-**6** (Hernández-Hernández et al., 2005). The EtOAc fraction (4.0 g) was then subjected to flash chromatography over silica gel (15 g). Elution with hexane–EtOAc, (4:1, v/v) afforded fractions containing **8** (28 mg), elution with hexane–EtOAc, 1:1, gave a mixture of verticillane derivatives **7** and **10** (783 mg) and elution with EtOAc, afforded fractions enriched in **9**. Rechromatography of an aliquot of the mixture of **7** and **10** (317 mg) over $AgNO_3$–impregnated silica gel (7.4 g) (Jin et al., 2005a) eluting with hexane–EtOAc 4:1 yielded pure **7** (18 mg), a mixture of **7** and **10** (228 mg), and pure **10** (28 mg). Further chromatography of an aliquot of the enriched fractions of **9** (56 mg) over silica gel (3 g) eluting with EtOAc:MeOH 19:1 yielded pure **9** (15 mg).

4.4.1. *(1S,3Z,7S,8S,11S,12S)-(+)-7,8-Epoxyverticill-3-en-12,20-diol (**7**)*

Colorless oil, $[\alpha]_{589}$ +69, $[\alpha]_{578}$ +72, $[\alpha]_{546}$ +82, $[\alpha]_{436}$ +150 (*c* 1.21, $CHCl_3$); IR ($CDCl_3$) ν_{max} cm^{-1}: 3602, 3457, 1219; for ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75.4 MHz) spectroscopic data, see Tables 1 and 2; EIMS *m*/*z* (rel. int.): 304 $[M-H_2O]^+$ (2), 289 (6), 271 (17), 253 (9), 231 (13), 213 (29), 187 (22), 161 (37), 147 (37), 133 (66), 121 (80), 107 (76), 67 (38), 43 (100); HRESIMS *m*/*z* 345.2408 (calc. for $C_{20}H_{34}O_3$ + Na, 345.2406).

4.4.2. *(1S,3Z,7S,8S,11S,12S)-(+)-7,8-Epoxyverticill-3-en-12,20-diol 20-acetate (**8**)*

Colorless oil, $[\alpha]_{589}$ +60, $[\alpha]_{578}$ +63, $[\alpha]_{546}$ +73, $[\alpha]_{436}$ +134 (*c* 3.77, $CHCl_3$); IR ($CDCl_3$) ν_{max} cm^{-1}: 3599, 3475, 1729, 1242; for ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75.4 MHz) spectroscopic data see Tables 1 and 2; CIMS (CH_3CN) *m*/*z* (rel. int.) 418 $[M + H_2C{=}C{=}N^+{=}CH_2]^+$ (3), 305 (5), 287 (46), 269 (100), 244 (1), 229 (6), 213 (11), 199 (12), 177 (6), 159 (8), 123 (6), 67 (10); HRESIMS *m*/*z* 387.2507 (calc. for $C_{22}H_{36}O_4$ + Na, 387.2511).

4.4.3. *(1S,3Z,7S,11S,12S)-(+)-Verticilla-3,8(19)-dien-7,12,20-triol (**9**)*

Colorless oil, $[\alpha]_{589}$ +29, $[\alpha]_{578}$ +31, $[\alpha]_{546}$ +35, $[\alpha]_{436}$ +62, $[\alpha]_{365}$ +104 (*c* 1.05, $CHCl_3$); IR ($CDCl_3$) ν_{max} cm^{-1}: 3598, 3417, 1218; for ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75.4 MHz) spectroscopic data see Tables 1 and 2; EIMS *m*/*z* (rel. int.): 304 $[M-H_2O]^+$ (4), 289 (4), 271 (6), 253 (36), 225 (16), 187 (41), 159 (68), 147

(61), 135 (88), 121 (90), 107 (100), 91 (80), 77 (48), 55 (59), 43 (96); HRESIMS *m/z* 345.2409 (calc. for $C_{20}H_{34}O_3$ + Na, 345.2406).

4.4.4. (1S,3Z,7S,11S,12S)-(+)-Verticilla-3,8(19)-dien-7,12,20-triol 20-acetate (**10**)

Colorless oil, $[\alpha]_{589}$ +29, $[\alpha]_{578}$ +30, $[\alpha]_{546}$ +34, $[\alpha]_{436}$ +62 (*c* 1.09, $CHCl_3$); IR ($CDCl_3$) ν_{max} cm^{-1}: 3598, 3416, 1732, 1240; for ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75.4 MHz) spectroscopic data see Tables 1 and 2; EIMS *m/z* (rel. int.) 346 $[M–H_2O]^+$ (4), 331 (5), 313 (5), 286 (18), 271 (39), 253 (34), 225 (17), 201 (19), 187 (34), 173 (39), 159 (54), 147 (46), 135 (37), 121 (58), 107 (65), 43 (100); HRESIMS *m/z* 387.2507 (calc. for $C_{22}H_{36}O_4$ + Na, 387.2511).

4.4.5. (1S,3Z,7S,11S,12S)-(−)-Verticilla-3,8(19)-dien-7,12,20-triol 7,20-diacetate (**11**)

A solution of **3** (7.6 mg) in pyridine (1.0 mL) was treated with Ac_2O (2.0 mL). The reaction mixture was heated on a steam bath for 14 h, poured over ice-H_2O, and extracted with EtOAc. The organic layer was washed with 10% HCl, H_2O, aqueous $NaHCO_3$, and H_2O, dried, filtered, and evaporated to give a brown residue, which was subjected to chromatography using aluminium oxide. Fractions eluted with hexane–EtOAc, (49:1, v/v) yielded pure **5** (7.7 mg, 81%). Similarly, a solution of **4** (29.4 mg) was heated for 12 h, to yield after chromatography, pure **5** (27.0 mg, 83%). Colourless oil, $[\alpha]_{589}$ −8, $[\alpha]_{578}$ −9, $[\alpha]_{546}$ −11, $[\alpha]_{436}$ −15, $[\alpha]_{365}$ −16 (*c* 0.7, $CHCl_3$); IR ($CDCl_3$) ν_{max} cm^{-1}: 3507, 1726, 1257; for ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75.4 MHz) spectroscopic data see Tables 1 and 2; EIMS *m/z* (rel. int.) 345 $[M–H–AcOH]^+$ (2), 328 (5), 313 (8), 286 (12), 271 (26), 253 (61), 225 (31), 213 (48), 185 (55), 171 (48), 145 (73), 133 (78), 119 (85), 107 (83), 91 (100), 79 (80); HRESIMS *m/z* 429.2625 (calc. for $C_{24}H_{38}O_5$ + Na, 429.2617).

4.4.6. Hydrolysis of **8**

A solution of **8** (115 mg) in MeOH (2.0 mL) was treated with KOH (107 mg) in H_2O (0.5 mL). The reaction mixture was heated until reflux began, this being maintained for 2 h. The mixture was then poured over ice-H_2O (50 mL) and extracted once with EtOAc (200 mL). The organic layer was then washed with H_2O, dried, filtered and evaporated to dryness, giving a pale yellow oil (69 mg), which was subjected to silica gel CC (5 g). Fractions eluted with hexane–EtOAc, 7:3, yielded pure **7** (65 mg, 64%) identical to the natural product.

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