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Strobilols A–D: Four cadinane-type sesquiterpenes from the edible mushroom *Strobilurus ohshimae*

Fuminori Hiramatsu, Tetsuya Murayama, Takuya Koseki, Yoshihito Shiono *

Department of Bioresource Engineering, Faculty of Agriculture, Yamagata University, Tsuruoka, Yamagata 997-8555, Japan

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Abstract

Four sesquiterpenoids – strobilols A (**1**), B (**2**), C (**3**), and D (**4**) – were isolated from the organic extracts of fruiting bodies of the edible mushroom *Strobilurus ohshimae*. Their structures were determined by spectroscopic methods. Compound **1** exhibited moderate activity against the brine shrimp *Artemia salina*. This paper is the first report on isolation of cadinane-type sesquiterpenoids from *S. ohshimae*.

Keywords: Cadinane-type sesquiterpenoid; Strobilols; *Strobilurus ohshimae*; *Artemia salina*

1. Introduction

In Asia, mushrooms have long been used as traditional foods and medicines. Edible mushrooms contain an abundance of resources that possess a multitude of biological activities. The natural products extracted from edible mushrooms exhibit lower toxicity and fewer side effects than chemical drugs; therefore, mushrooms represent a potential valuable resource for natural drugs (Chihara, 1992). During our search for naturally occurring, biologically active compounds from Japanese wild edible mushrooms, we reported on the isolation and structural elucidation of lanostane triterpenoids from *Stropharia aeruginosa* (Strophariaceae) (Shiono et al., 2005), as well as eburicoic acid and *N*-phenethylhexadecanamide from *Laetiporus sulphureus* var. *miniatus* (Polyporaceae) (Shiono et al., 2004). Some of these compounds exhibited moderate plant growth inhibitory activity. In a continuation of our investigations of the phytochemical constituents of Japanese wild edible mushrooms, *Strobilurus ohshimae* (Sugiedatake in Japanese) was collected in Yamagata Prefecture, located in the Northeast part of Honshu Island. This edible mushroom, belonging to the family Tricholomataceae, is distributed in the cedar forests of Japan. Since, to the best of our knowledge, there have been no chemical studies on *S. ohshimae*, we were encouraged to study its chemical constituents. We report here the isolation and structure determination of four new cadinane-type derivatives – strobilols A (**1**), B (**2**), C (**3**), and D (**4**) – from the fruiting bodies of *S. ohshimae*.

2. Results and discussion

The fruiting bodies of *S. ohshimae* (190 g fresh wt) were extracted with MeOH. The solvent was removed and the resulting residue was partitioned between water and EtOAc. The EtOAc fraction was successively subjected to silica gel chromatography and ODS column chromatography to yield four new compounds: strobilols A (**1**), B (**2**), C (**3**), and D (**4**) (Fig. 1).

Strobilol A (**1**) was isolated as a yellow oil. Its molecular formula was determined as $C_{15}H_{22}O_5$ by HR-FABMS, and this indicated the presence of five degrees of unsaturation in the molecule. The ^{13}C NMR (Table 1) and DEPT

* Corresponding author. Tel./fax: +81 235 28 2873.
E-mail address: yshiono@tds1.tr.yamagata-u.ac.jp (Y. Shiono).

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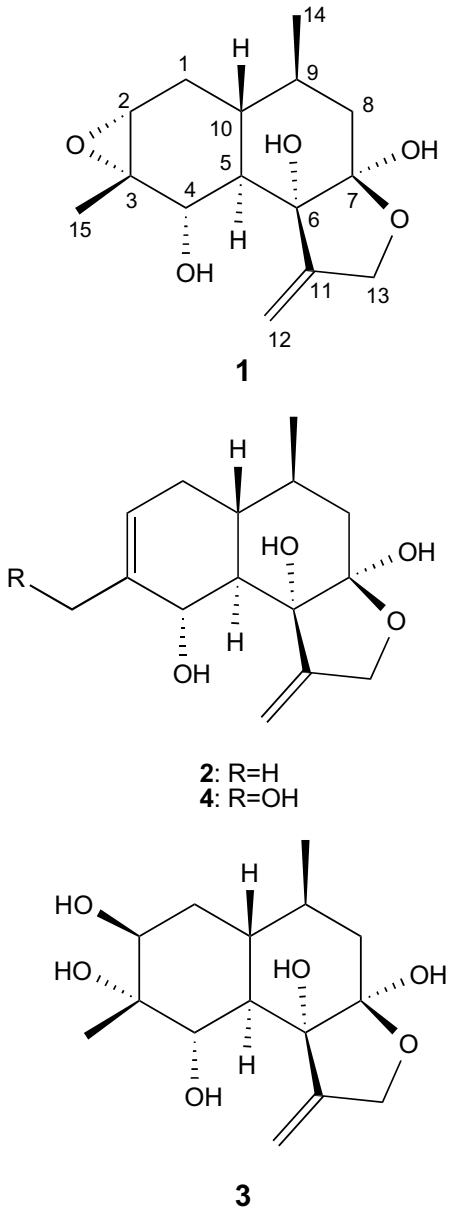

Fig. 1. Structures of strobilols A (**1**), B (**2**), C (**3**) and D (**4**).

spectra of **1** revealed two methyls (δ 20.0 and 20.3), one sp^2 methylene (δ_C 110.7), three sp^3 methylenes (δ 31.3, 41.9, and 69.4), five sp^3 methines (δ_C 37.9, 40.3, 45.7, 62.7, and 73.9), three sp^3 quaternary carbons (δ_C 63.7, 80.4, and 106.6), and one sp^2 quaternary carbon (δ_C150.8). The ^{1}H NMR spectrum (Table 1) of **1** contained one singlet [1.38 (3H, *s*, H_3-15)], one secondary methyl [δ_H 0.87 (3H, *d*, $J = 6.4$ Hz, H-14)], an *exo*-methylene [δ_H 5.14 (1H, *t*, $J = 2.2$ Hz, H-12) and 5.52 (1H, *t*, $J = 2.2$ Hz, H-12)], one isolated methylene bearing an oxygen [δ_H 4.14 (1 H, *dt*, $J = 12.6$, 2.2 Hz, H-13) and 4.52 (1H, *dt*, $J = 12.6$, 2.2 Hz, H-13)], and two oxygenated methines [δ_H 3.07 (1H, *d*, $J = 4.9$ Hz, H-2) and 4.60 (1H, *d*, $J = 10.8$ Hz, H-4)]. The ^{1}H–^{1}H COSY of **1** established the presence of partial segments (*a* and *b*) as shown in Fig. 2. The doublet methyl signal (H-14) was coupled to a multiplet (H-9)

Table 1
^{1}H and ^{13}C NMR spectroscopic data for strobilol A (**1**)

No.	δ_C (Mult)	δ_H (Mult. *J* Hz)	HMBC
1	31.3 *t*	α 1.41 (1H, *dd*, 15.9, 9.8)	2, 3, 5, 9, 10
		β 2.22 (1H, *ddd*, 15.7, 7.6, 4.9)	2, 3, 5, 9, 10
2	62.7 *d*	3.07 (1H, *d*, 4.9)	1, 3, 4, 10, 15
3	63.7 *s*		
4	73.9 *d*	4.60 (1H, *d*, 10.8)	2, 5, 6, 10
5	45.7 *d*	1.92 (1H, *dd*, 12.5, 10.8)	3, 4, 6, 10, 11
6	80.4 *s*		
7	106.6 *s*		
8	41.9 *t*	1.26 (1H, *t*, 13.6)	7, 9, 10
		1.66 (1H, *dd*, 12.5, 3.5)	6, 7, 9, 10
9	37.9 *d*	1.35 (1H, *m*)	1, 8, 10
10	40.3 *d*	1.13 (1H, *m*)	4, 5
11	150.8 *s*		
12	110.7 *t*	5.14 (1H, *t*, 2.2)	6, 11, 13
		5.52 (1H, *t*, 2.2)	6, 11, 13
13	69.4 *t*	4.14 (1H, *dt*, 12.6, 2.2)	7, 11, 12
		4.52 (1H, *dt*, 12.6, 2.2)	7, 9, 11, 12
14	20.0[a] *q*	0.87 (3H, *d*, 6.4)	8, 9, 10
15	20.3[a] *q*	1.38 (3H, *s*)	2, 3, 4

Measured in CD_3OD, with values in parentheses being coupling constants in Hz.
[a] Interchangeable.

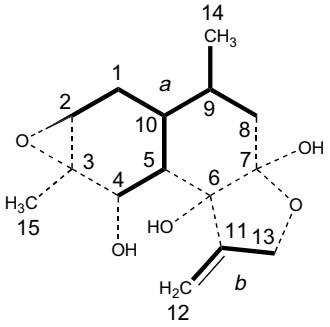

Fig. 2. Important ^{1}H–^{1}H COSY (bold lines) correlations observed for **1**.

and long-range correlations was observed between: H-14 and C-8 and C-10; H-8 and C-6 and C-10; H-4 and C-6; H-2 and C-4, and H-1 and C-3. These results indicated that segment *a* was comprised of a decalin moiety. In the HMBC spectrum of **1**, correlations were noted between H-5 and C-11, and H-13 and C-7, which indicated that a five-membered ether ring was positioned at C-6 and C-7. Furthermore, the HMBC correlations of H-12 to C-6, C-11, and C-13 established an *exo*-methylene group to be located at C-11. The presence of an epoxide ring at C-2 and C-3 was deduced from the chemical shifts of the NMR spectroscopic data for these positions and the molecular formula. The relative stereochemistry of **1** was also established by NOE difference experiments (see Fig. 3). NOE correlations from H-2 to H-4 and H-15, and from H-14 to H-1 β, indicated that they are oriented on the same side, while correlation from H-9 to H-5 revealed that these protons are α-oriented. These NOE data confirmed the *trans*-junction of the decalin ring system. The *trans*-juncture of the decalin ring was also supported by the coupling

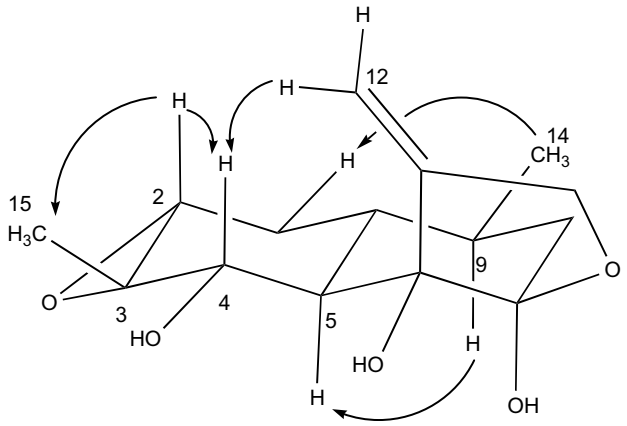

Fig. 3. Selected NOE correlations for **1**.

constant of H-4 ($J_{4,5} = 10.8$ Hz) and H-5 ($J_{5,10} = 12.5$ Hz). Irradiation of H-12 caused NOE on H-4, thus requiring a *cis*-juncture between the five- and six-membered rings with α-diols at C-6 and C-7, leading to determination of structure **1** (see Fig. 1).

Strobilol B (**2**) was isolated as a yellow oil with a molecular formula of $C_{15}H_{22}O_4$, as determined by HR-FABMS. The molecular formula indicated five degrees of unsaturation in the molecule. The appearance of resonances for four sp^2 carbons in the ^{13}C NMR spectrum indicated that two degrees of unsaturation were attributed to the presence of two double bonds and that the remaining degrees could be satisfied by the assignment of three rings. Since the physico-chemical properties of **2** were similar to those of **1**, the structural assignment was initiated by comparison of the NMR spectra of **2** with those of **1**. The ^1H and ^{13}C NMR spectroscopic data for **2** are listed in Table 2. The ^{13}C NMR spectrum of **2** established a similar spectroscopic pattern to that of **1**, with the exception of differences in the chemical shift values observed for C-2 and C-3. The carbon signals for C-2 at δ 124.3 and for C-3 at δ 133.8 resonated at a lower field than those of **1**. In the ^1H NMR spectrum of **2**, one proton signal at δ 3.07 due to an epoxide ring was absent and an olefinic methine proton at δ 5.54 was instead observed. In the HMBC spectrum, the methine signal at δ_C 5.54 exhibited HMBC correlations with C-4, C-10, and C-15, and the methyl resonance at δ_C 1.77 (H-15) exhibited HMBC correlations with C-2 and C-4; this suggested the presence of a Δ^2 double bond. The relative stereochemistry of **2** was deduced from NOE experiments. Thus, the structure of strobilol B was elucidated to be **2** (Fig. 1).

Strobilol C (**3**) had the molecular formula, $C_{15}H_{24}O_6$, as determined by HR-FABMS. The ^1H and ^{13}C NMR spectra (Table 3) for **3** corresponded well with those of **1**, with the exception of the presence of an oxymethine signal [δ_H 3.60; δ_C 75.6]. These data suggest that **3** possesses a hydroxyl group at C-2. The structure of **3** was further supported by HMBC correlations (Table 3). The β-configuration of a hydroxyl group at C-2 was determined from the coupling constants of H-2 ($J_{1,2 \text{ and } 2,3} = 2.5$ Hz). The relative configurations of C-3, C-4, C-5, C-6, C-7, C-9, and C-10 were deduced to be the same as those of **1** and **2** at all chiral centers. This was concluded on the basis of the similarity of various spectroscopic parameters, particularly ^{13}C NMR chemical shifts, with the corresponding values for **1** and **2**.

The molecular formula of strobilol D (**4**), $C_{15}H_{22}O_5$, was determined from its HR-FABMS data, corresponding to one oxygen atom more than that of **2**. An examination of the ^1H and ^{13}C NMR spectra established the absence of a methyl group at C-3, and instead the presence of an oxymethylene group. In the HMBC spectrum, correlations

Table 2
^1H and ^{13}C NMR spectroscopic data for strobilol B (**2**)

No.	δ_C (Mult)	δ_H (Mult. *J* Hz)	HMBC
1	30.4 *t*	1.65 (1H, *m*)	2, 3, 5, 10
		2.27 (1H, *m*)	
2	124.3 *d*	5.54 (1H, *m*)	1, 4, 10, 15
3	133.8 *s*		
4	71.5 *d*	4.90 (1H, *d*, 9.2)	2, 5, 12
5	49.3 *d*	1.88 (1H, *dd*, 12.4, 9.2)	4, 6, 11
6	77.9 *s*		
7	104.3 *s*		
8	40.2 *t*	1.30 (1H, *m*)	
		1.86 (1H, *dd*, 13.9, 3.8)	7, 9, 10, 14
9	35.4 *d*	1.50 (1H, *m*)	1, 8, 10
10	39.3 *d*	1.33 (1H, *m*)	4, 5
11	148.0 *s*		
12	109.5 *t*	5.15 (1H, *t*, 2.0)	6, 11, 13
		5.34 (1H, *t*, 2.0)	6, 11, 13
13	68.0 *t*	4.18 (1H, *dt*, 12.9, 2.0)	7, 11, 12
		4.63 (1H, *dt*, 12.9, 2.0)	7, 11, 12
14	18.7[a] *q*	0.88 (3H, *d*, 6.4)	8, 9, 10
15	18.8[a] *q*	1.77 (3H, *br.* s)	2, 3, 4

Measured in $CDCl_3$, with values in parentheses being coupling constants in Hz.
[a] Interchangeable.

Table 3
^1H and ^{13}C NMR spectroscopic data for strobilol C (**3**)

No.	δ_C (Mult)	δ_H (Mult. *J* Hz)	HMBC
1	34.8 *t*	1.55 (1H, *dd*, 8.3, 2.5)	2, 3, 5, 10
		1.75–1.84[a]	
2	75.6 *d*	3.60 (1H, *t*, 2.5)	4
3	75.3 *s*		
4	74.1 *d*	4.33 (1H, *d*, 11.3)	5
5	47.1 *d*	2.03 (1H, *t*, 10.0)	4, 6, 9, 10, 11
6	80.0 *s*		
7	106.8 *s*		
8	42.3 *t*	1.33 (1H, *t*, 12.8)	9
		1.75–1.84[a]	
9	36.4 *d*	1.40 (1H, *m*)	7, 10
10	38.5 *d*	1.15 (1H, *m*)	4, 5
11	150.9 *s*		
12	110.6 *t*	5.14 (1H, *t*, 1.9)	6, 13
		5.53 (1H, *t*, 1.9)	11
13	69.4 *t*	4.18 (1H, *dt*, 12.6, 1.9)	11
		4.42 (1H, *dt*, 12.6, 1.9)	7, 11
14	20.6 *q*	0.89 (3H, *d*, 6.2)	8, 9, 10
15	24.4 *q*	1.29 (3H, *s*)	2, 3, 4

Measured in CD_3OD, with values in parentheses being coupling constants in Hz.
[a] Overlaping signals.

Table 4
^{1}H and ^{13}C NMR spectroscopic data for strobilol D (**4**)

No.	δ_C (Mult)	δ_H (Mult. *J* Hz)	HMBC
1	31.9 *t*	1.77 (1H, *m*)	2, 3
		2.36 (1H, *dt*, 17.7, 6.5)	2, 3, 5, 10
2	126.5 *d*	5.81 (1H, *d*, 6.5)	3, 4, 10, 15
3	140.7 *s*		
4	69.5 *d*	5.08 (1H, *d*, 9.0)	2, 5, 12
5	51.5 *d*	1.89 (1H, *dd*, 12.5, 9.0)	1, 4, 7, 9, 10, 11
6	80.1 *s*		
7	106.7 *s*		
8	42.9 *t*	1.34 (1H, *m*)	7, 9, 10, 14
		1.74 (1H, *m*)	7, 9, 10, 14
9	37.4 *d*	1.45 (1H, *m*)	7, 10
10	41.7 *d*	0.91 (1H, *m*)	1
11	150.4 *s*		
12	110.9 *t*	5.15 (1H, *t*, 2.8)	6, 13
		5.39 (1H, *t*, 2.8)	6, 13
13	69.7 *t*	4.16 (1H, *dt*, 12.3, 2.8)	11, 12
		4.59 (1H, *dt*, 12.3, 2.8)	11, 12
14	20.0 *q*	0.89 (3H, *d*, 6.0)	8, 9,10
15	64.4 *t*	4.02 (1H, *d*, 12.2)	2, 3, 4
		4.22 (1H, *d*, 12.2)	2, 3, 4

Measured in $CDCl_3$, with values in parentheses being coupling constants in Hz.

from oxymethylene protons at δ_H 4.02 and 4.22 to C-2, C-3, and C-4 confirmed the placement of the hydroxymethyl group. The structure of **4** was further supported by HMBC correlations (Table 4). The relative stereochemistry of **4** was confirmed by NOE experiments.

The determination of the absolute stereochemistry of **3** was examined by a modification of Mosher's method. However, the esterification of **3** with (+)/(−)-MTPA-Cl did not take place at the two secondary hydroxyl groups. The unfavorable steric hindrance of the methyl (C-15) and methylene (C-12) groups could result in a decrease in the reactivity of the secondary hydroxyl groups. This is the first evidence of the isolation of four new cadinane-type derivatives – strobilols A (**1**), B (**2**), C (**3**) and D (**4**) – from *S. ohshimae*.

We studied the biological activity of **1**, **2**, **3**, and **4** using an antimicrobial activity assay. At a concentration of 100 μg/disk, compounds **1**–**4** were inactive against *Candida albicans* ATCC 2019, *Staphylococcus aureus* NBRC 13276, and *Pseudomonas aeruginosa* ATCC 15442. The compounds isolated from *S. ohshimae* were tested in a brine shrimp (*A. salina*) bioassay (Meyer et al., 1982). Of these isolates, compound **1** exhibited moderate activity ($LD_{50} = 100$ μg/ml), while compounds **2**, **3**, and **4** were inactive ($LD_{50} > 100$ μg/ml).

3. Concluding remarks

In conclusion, *S. ohshimae* is a wild mushroom collected from Yamagata Prefecture, Japan. The isolation of cadinane-type sesquiterpenoids – strobilols A–D – is the first example from the fruiting bodies of *S. ohshimae*. Although *S. ohshimae* has not been previously investigated, a study carried out by Anke et al. previously identified the structures, strobilurins A and B, from liquid cultures of *S. tenacellus*, a mushroom belonging to the same family (Tricholomataceae) growing on pine cones (Anke et al., 1977; Schramm et al., 1978). At present, synthetic analogs of strobilurins are used worldwide as agricultural fungicides for crop protection (Sauter et al., 1999). Therefore, we are commencing research to investigate further the bioactivity properties of the metabolites derived from liquid cultures of *S. ohshimae*.

4. Experimental

4.1. General procedures

Optical rotation values were measured with a Horiba SEPA-300 polarimeter, and IR and UV spectra were respectively recorded with Jasco J-20A, Shimadzu UV mini-1240, and Jasco J-20A spectrophotometers. Mass spectra were obtained with a Jeol JMS-700 instrument, and ^{1}H and ^{13}C NMR spectra were acquired with a Jeol EX-400 spectrometer. Chemical shifts are given on a δ (ppm) scale with TMS as an internal standard. Column chromatography was conducted on silica gel 60 (Kanto Chemical Co., Inc., Japan) and ODS (Fuji Silysia, Japan). TLC analysis was carried out by using precoated silica gel plates (Merck), and the spots were detected by spraying with 10% vanillin in H_2SO_4 and then heating.

4.2. Mushroom material

The fruiting bodies of *S. ohshimae* were collected at the foot of Mt. Gassan, Yamagata Prefecture, Japan, in autumn 2005 and identified by one of authors (F.H.). A voucher specimen YUSO-1 has been deposited at our laboratory of the Faculty of Agriculture, Yamagata University, Yamagata, Japan.

4.3. Extraction and isolation

Fresh fruiting bodies of *S. ohshimae* (190 g) were extracted with MeOH (2 L × 2). The MeOH extract was combined and concentrated under reduced pressure to yield a yellow residue (1.1 g). The latter was next partitioned between EtOAc and H_2O with the organic layer evaporated in vacuo to afford the EtOAc extract (300 mg). The latter was subject to silica gel CC using first a gradient of *n*-hexane–EtOAc (100:0–0:100) and then a gradient of EtOAc–MeOH (100:0–0:100) as eluting solvent systems to give fractions 1 through 13 (Fr. 1–13). Purification of compounds in the eluates was monitored by the characteristic coloration with vanillin–sulfuric acid reagent. Fraction 8 (34.0 mg, *n*-hexane–EtOAc, 30:70) was further subjected to silica gel CC using a gradient of $CHCl_3$–EtOAc (100:0–0:100) to yield strobilol B (**2**) (9.7 mg). Fraction 9 (*n*-hexane–EtOAc, 20:80) was separated by ODS using a gradient of H_2O–MeOH

(100:0–0:100) to afford fraction of 50–70% MeOH eluates. These fractions were subjected to silica gel with CC $CHCl_3$–MeOH (20:1) to obtain strobilol A (**1**) (5.8 mg). Fractions 12 and 13 (EtOAc–MeOH, 50:50 and 0:100) were also combined and further purified by silica gel CC using a gradient of $CHCl_3$–EtOAc (100:0–0:100) to afford 90–100% EtOAc eluates. These fractions were combined and applied to an ODS column, eluted with H_2O–MeOH (10% stepwise gradient) to yield strobilols C (**3**, 6.4 mg) and D (**4**, 4.9 mg).

4.4. Strobilol A (*1*)

Oil; $[\alpha]_D^{20}$ +3.1 (*c* 0.13, MeOH); IR (KBr) ν_{max} cm^{-1}; 3421, 2927, 1029; HRFABMS *m/z* [M−H$^-$]: 281.1392, calcd. for $C_{15}H_{21}O_5$, 281.1389; FABMS *m/z* 281 [M−H$^-$]. For ^1H and ^{13}C NMR spectra, see Table 1.

4.5. Strobilol B (*2*)

Oil; $[\alpha]_D^{20}$ −18.4 (*c* 0.53, MeOH); IR (KBr) ν_{max} cm^{-1}; 3399, 2933, 1031; HRFABMS *m/z* [M−H$^-$]: 265.1442, calcd. for $C_{15}H_{21}O_4$, 265.1440; FABMS *m/z* 265 [M−H$^-$]. For ^1H and ^{13}C NMR spectra, see Table 2.

4.6. Strobilol C (*3*)

Oil; $[\alpha]_D^{20}$ +14.6 (*c* 0.46, MeOH); IR (KBr) ν_{max} cm^{-1}; 3399, 2933, 1031; HRFABMS *m/z* [M+Na$^+$]: 323.1471, calcd. for $C_{15}H_{24}O_6Na$, 323.1471; FABMS *m/z* 301 [M+H$^+$]. For ^1H and ^{13}C NMR spectra, see Table 3.

4.7. Strobilol D (*4*)

Oil; $[\alpha]_D^{20}$ −2.8 (*c* 0.25, MeOH); IR (KBr) ν_{max} 3455, 2955, 1024 cm^{-1}; HRFABMS *m/z* [M−H$^-$]: 281.1395, calcd. for $C_{15}H_{21}O_5$, 281.1389; FABMS *m/z* 281 [M−H$^-$]. For ^1H and ^{13}C NMR spectra, see Table 4.

4.8. Brine shrimp toxicity bioassay

The brine shrimp toxicity was performed using a slight modification of the original method (Meyer et al., 1982). Approximately 20 hatched brine shrimp larvae (*A. salina*) in artificial seawater (3.0 mL) were added to each well containing different concentrations of sample in MeOH (20 μL). Samples and controls were run in duplicate. After 24 h at 25 °C, the number of live, immobile and dead brine shrimp larvae were counted using a magnifying glass.

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