Novel Spiciferone Derivatives from the Fungus *Drechslera hawaiiensis* Isolated from the Marine Sponge *Callyspongia aerizusa*

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Sponge-Associated Fungi, *Drechslera hawaiiensis,* Spiciferone

From the marine sponge *Callyspongia aerizusa* collected from the Sea of Bali, Indonesia, fungal isolates of *Drechslera hawaiiensis* were obtained. Culture filtrates of the fungus yielded four spiciferone derivatives which include spiciferone A (**1**) and B (**2**), and two other novel derivatives including spiciferol A (**3**) which is an alcohol congener of spiciferone A (**1**) and compound **4** which is an monocyclic spiciferone congener featuring a butoxyl side chain. The structures of the novel compounds were established on the basis of NMR spectroscopic (^{1}H, ^{13}C, COSY) and mass spectrometric (EIMS) data.

In our search for bioactive secondary metabolites from sponge-associated fungi, a series of phytotoxic spiciferones were obtained from the fungus *Drechslera hawaiiensis* that had been isolated from the marine sponge *Callyspongia aerizusa*, collected at Bali, Indonesia. Spiciferones are azaphilone chromophore natural products which were first reported from the fungus *Cochliobolus spicifer* Nelson (D-5), a pathogen responsible for the leaf spot disease in Gramineae (Nakajima *et al.,* 1989; Nakajima *et al.,* 1991; Nakajima *et al.,* 1992). Spiciferones are fungal metabolites containing a bicyclic unit composed of a fully substituted γ-pyrone and a cyclohexadienone moiety, with a quarternary carbon bearing ethyl and methyl substituents as well as ketonic carbonyl group. In addition to the known spiciferone derivatives (**1–2**), we isolated two novel congeners, spiciferol A (**3**) and a monocyclic butoxyl derivative (**4**), for which we shall propose the name butoxyl spiciferin. One- and two-dimensional homonuclear correlation spectroscopy (^{1}H COSY) afforded unambiguous confirmation of the signal assignments, substituent position, and total structures of the novel compounds (Table I).

Materials and Methods

The fungus *Drechslera hawaiiensis* was isolated from fresh samples of the marine sponge *Callyspongia aerizusa.* Sponge samples were collected by scuba from the Sea of Bali along the shores of Mengangan Island, Indonesia, in September of 1997. Under sterile conditions, tissue samples were taken from the inside of the sponge and were inoculated on malt agar slants, containing malt extract (15 g/l), agar (15 g/l), and seasalt (24.4 g/l). Cultures were incubated at 27 °C. From the growing cultures, pure strains of *Drechslera hawaiiensis* were isolated by reinoculation on malt agar plates. The fungal strain was identified by the Centraalbureau voor Schimmelcultures at Baarn, Netherlands. A voucher strain (no. P22–96) is deposited at the Alfred-Wegener-Institut für Polar und Meeresforschung, Bremerhaven. The sponge was identified by Dr. R. van Soest, Dept. of Coelenterates and Porifera, University of Amsterdam, Netherlands.

Prior to extraction, the fungus *Drechslera hawaiiensis* was grown in liquid malt-broth medium which containing 25 g malt extract in 1 liter of artificial seawater. After 41 days of incubation in 60

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Erlenmeyer flasks, each containing 250 ml malt-broth medium at 27 °C, the mycelia and culture filtrate were collected and extracted with MeOH and EtOAc. The total extract was evaporated under reduced pressure, taken to dryness (3 g) and chromatographed over silica gel employing hexane:EtOAc (70:30) as solvent system. Ten fractions were obtained, the lipophilic fractions 1–5 contained fatty acids and sterols, the semi-polar fractions 6–8 yielded the known spiciferone congeners **1** (6.1 mg), and **2** (4.7 mg). The new derivatives **3** (4.7 mg) and **4** (2.0 mg) were isolated from the polar fractions 9 and 10. Purification of the spiciferones was accomplished by semi-preparative HPLC (Merck, Munich, Germany) on a Eurospher C18 column using the following linear gradient: 0 min, 10% MeOH; 30 min, 90% MeOH; 35–40 min, 100% MeOH.

^{1}H NMR and ^{13}C NMR spectra (chemical shifts in ppm) were recorded in DMSO-d_6 on Bruker ARX 400 NMR and AVANCE DMX 600 NMR spectrometers. Mass spectra (EIMS) were measured on a Finnigan MAT 8430 mass spectrometer. Optical rotations were determined on a Perkin Elmer-241 MC Polarimeter. For HPLC analysis, samples were injected into a HPLC system (Gynkotek) coupled to a photodiode-array detector. Routine detection was at 254 nm. The separation column (125 × 4 mm, i.d.) was prefilled with Eurospher C-18. UV spectra were recorded in MeOH. Solvents were distilled prior to use and spectral grade solvents were used for spectroscopic measurements. TLC was performed on precoated TLC plates with Si gel 60 F254 (Merck, Darmstadt, Germany).

Results and Discussion

The fungus *Drechslera hawaiiensis* was isolated from the Indo-Pacific sponge *Callyspongia aerizusa*. For extraction of the metabolites the fungus was grown in liquid malt-broth medium (25 g malt extract per l seawater) at 27 °C for 41 days. The secondary metabolites were obtained from the EtOAc-MeOH extract of the mycelia and its culture broth. Isolation of the spiciferones was achieved by normal phase chromatography on SiO gel employing mixtures of hexane and EtOAc as solvent system. The semi-polar fractions afforded the known compounds, **1** and **2**, while the non-polar fractions yielded the novel derivatives, **3** and **4**. All compounds were readily identified by their spectroscopic data. Through-bond homonuclear (^{1}H-^{1}H COSY) correlations were used to establish assignments and atom connectivities. Chemical shifts were compared with literature data for compounds containing similar structural subunits.

Spiciferone A (**1**) was obtained as a white powder residue; $[\alpha]_D$ −20.6 ° (*c* 0.15, EtOH), (lit. $[\alpha]_D$ −44 ° (*c* 1.0, $CHCl_3$) [Nakajima *et al*, 1989]); UV max (EtOH) nm 202 (ε 9,800), 240 (ε 5,450), 261 sh (ε 4,800), 318 (ε 2,450); EIMS (70eV) *m/z* $[M]^+$ 232 (52), 217 (100), 204 (28), 189 (15), 175 (8). The isolated spiciferone A (**1**) showed a molecular ion peak $[M]^+$ at *m/z* 232 in the EIMS which is compatible with the molecular composition of $C_{14}H_{16}O_3$. Its NMR spectral data are identical to those already published in the literature (Nakajima *et al.*, 1989).

Spiciferone B (**2**) was obtained as a white powder residue; $[\alpha]_D$ −21.8 ° (*c* 0.06, EtOH), (lit. $[\alpha]_D$ −34 ° (*c* 0.695, EtOH) [Nakajima *et al*, 1989]); UV max (EtOH) nm 204 (ε 10,210), 238 (ε 4,600), 262 sh (ε 4,000), 324 (ε 2,800); EIMS (70eV) *m/z* $[M]^+$ 248 (22), 233 (100), 220 (20), 205 (10), 145 (8). The isolated spiciferone B (**2**) showed a molecular ion peak $[M]^+$ at *m/z* 248 in the EIMS which is compatible with the molecular composition of $C_{14}H_{16}O_4$. Its NMR spectral data are also identical to those already published by the group of Nakajima (1991).

The new spiciferol A (**3**) was obtained as a white powder residue; $[\alpha]_D$ +18.1 ° (*c* 0.27, EtOH); UV max (EtOH) nm 202 (ε 12,200), 239 (ε 9,200), 260 sh (ε 6,600), 325 (ε 1,200); EIMS (70eV) *m/z* $[M]^+$ 234 (12), 233 (22), 221 (20), 205 (100), 178 (22), 145 (38), 123 (10), 97 (25), 83 (16), 69 (20), 57 (42). Spiciferol A (**3**) showed a molecular ion peak $[M]^+$ at *m/z* 234 in the EIMS which is compatible with the molecular composition of $C_{14}H_{18}O_3$. The ^{13}C and ^{1}H NMR spectra are similar but not identical to those of **1** (Table I). The major difference of derivative **3** with that of **1** is the reduction of the ketone group at C-8 which was indicated in the ^{13}C NMR spectrum by a resonance at δ72.1 which corresponds to the loss of the quarternary carbonyl signal at δ200.1 (Nakijima *et al.*, 1989). 1D and 2D COSY ^{1}H NMR spectra of **3** identified fragments belonging to the cyclohexadienol system. The shift of H-10 indicates the conjugation of

Table I. NMR data of **3** and **4** in DMSO-d_6.

		3	**4**
No.	δC	δH	δH
2	160.5 s		
3	119.4 s		
4	174.5 s		
5	115.1 s		6.25 s
6	168.0 s		
7	43.0 s		
8_{axial}	72.1 d	4.16 bt (3.5 Hz)	
9	117.4 d	5.80 dd(9.7 Hz, 3.5 Hz)	2.55 ddd (6.3 Hz, 6.3 Hz, 16.9 Hz) 2.45 ddd (6.4 Hz, 6.4 Hz, 16.9 Hz)
10	129.1 d	6.40 dd (9.7 Hz, 1.5 Hz)	3.57 q (~5.9 Hz)
11	22.0 t	1.81 dq (7.5 Hz, 13.7 Hz) 1.60 dq (7.5 Hz, 13.7 Hz)	1.84 q (7.5 Hz)
12	8.5 q	0.83 t (7.5 Hz)	0.72 t (7.5 Hz)
13	19.3 q	1.25 s	1.28 s
14	17.4 q	2.29 d (0.6 Hz)[a]	2.22 s[a]
15	9.5 q	1.82 d (0.6 Hz)[a]	1.81 s[a]
OH-8		5.07 bd(6.0 Hz)	
OH-10			4.47 t (5.3 Hz)

[a] Long range couplings were observed in the COSY between Me-14 and Me-15.

the double bond with the γ-pyrone system being maintained and additionally the shift of H-8 argues against alternative structures in which either the C-8/C-9/C-10 or C-7/C-8/C-9/C-10 units are inverted. From the ^{1}H NMR data, the relative stereochemistry of the hydroxyl group at C-8 is pseudoequatorial as H-8_{ax} has a coupling with H-9 of 3.5 Hz (dihedral angle *ca.* 100 °) and an allylic coupling with H-10 of 1.5 Hz. Its relative stereochemistry was also determined by comparison with the dihydrospiciferol derivatives which were previously obtained by hydrogenation and subsequent reduction of spiciferone A (Nakijima *et al.*, 1993). Both of the dihydrospiciferol congeners, pseudo-equatorially (**5**) and -axially (**6**) oriented OH, were found to be dextro-rotatory [+78 ° and +9 °, respectively]. Comparison of the UV spectra of **3** with those of compounds **5** and **6** (Fig. 1) indicates that **3** posseses an additional double bond in the cyclohexane ring from the weak band at *ca.* 305 nm which is not perceptible in the latter. Hence compound **3** is the alcohol congener of spiciferone A.

Butoxyl-spiciferin (**4**) was obtained as a white powder residue; $[\alpha]_D$ −34.85 ° (*c* 0.16, EtOH); UV max (EtOH) nm 202 (ε 10,000), 218 sh (ε 5,200), 252 (ε 3,400); EIMS (70eV) *m/z* $[M]^+$ 252 (12), 248 (22), 233 (20), 180 (22), 165 (100), 151 (38), 125

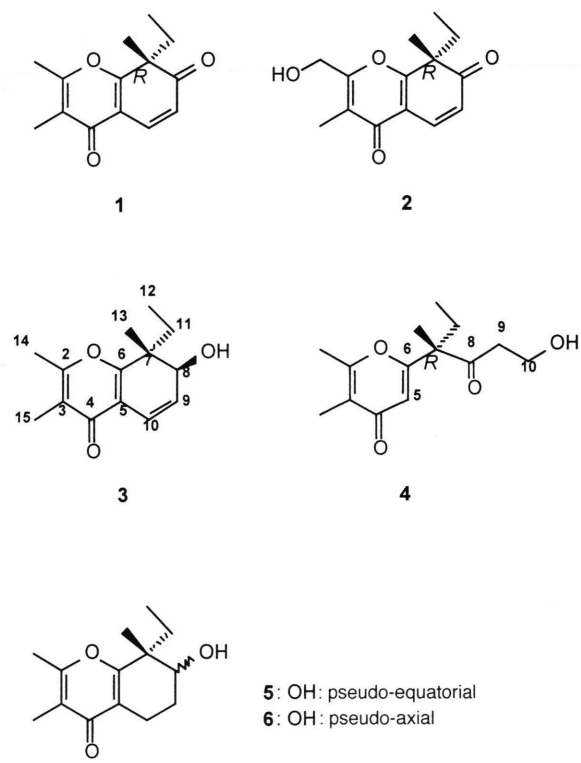

Fig. 1. Spiciferone derivatives.

(10), 97 (25), 83 (16), 70 (20), 55 (42). Compound **4** showed the molecular ion peak $[M]^+$ at *m/z* 252 in the EIMS which is compatible with the molecular composition of $C_{14}H_{20}O_4$. Unlike compounds **1–3** there was no perceptible band at *ca.* 305 nm in the UV spectra which indicates a total loss of conjugation or olefinicity in the hexane ring. The ^1H NMR spectra, however, are still comparable with those of the spiciferones (Table I). The loss of aromaticitiy in the second ring system was indicated by the absence of the doublet pair of signals in the olefinic region which was replaced by new methylene signals at *ca.* 2.40 to 4.0 ppm with coupling constants from which the presence of a butoxyl fragment (CH_2CH_2OH) was identified in the 2D COSY ^1H NMR spectrum. The OH proton was observed at 4.47 ppm as a triplet signal of 5.3 Hz coupling with a methylene proton at 3.57 ppm. The singlet signal at 6.25 ppm corresponding to H-5 also confirms the opening of the hexanone ring. An alternative structure in which the carbonyl group and C-7 unit are interchanged is ruled out by the chemical shift of the associated methylene group C-9. Compound **4** is thus, the 6-(1-ethyl, 1-methyl, 4-hydroxy, 2-butoxyl) congener of spiciferone A.

Biosynthesis of the spiciferones as fungal metabolites has been proposed to arise from a 10-membered monocyclic precursor *via* retro-aldol condensation (Nakajima *et al.*, 1993 and 1994). The novel derivatives **3** and **4** can be considered as possible intermediates in new reactions during polyketide biosynthesis.

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