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Cyanthiwigin AC and AD, Two Novel Diterpene Skeletons from the Jamaican Sponge *Myrmekioderma styx*

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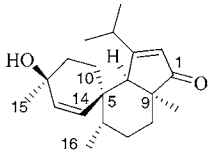
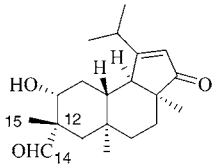
ABSTRACT



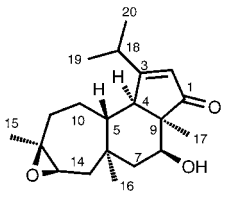
cyanthiwigin AC (2)



cyanthiwigin AD (3)

Three novel diterpenes, called cyanthiwigins AB (1), AC (2), and AD (3), were isolated from the Jamaican sponge *Myrmekioderma styx*. Their structures were elucidated by means of 1D and 2D NMR spectroscopic methods. Cyanthiwigin AC (2) and AD (3) represent novel diterpene-type skeletons.

The marine sponge *Myrmekioderma styx* has been shown to be a rich source of new bioactive diterpenes and sesquiterpenes. The 5,6,7-tricarbocyclic diterpenes, cyanthiwigin C, and two epoxide analogues were isolated from a Venezuelan sponge *Myrmekioderma styx* by Sennett et al. in 1992, along with a linear diterpene, styxenol.[1] Albrizio et al. reported six linear diterpenes from a *M. styx* sample collected from the Caribbean.[2,3] The cyanthiwigin-type diterpenes, cyanthiwigins A−D, were also reported from a Jamaican sponge *Epipolasis reiswigi*,[4] and the antifouling sesquiterpenes, curcuphenol and curcudiol, were reported from *M. styx* by Tsukamoto et al.[5] In the interest of identifying antiinfective and anticancer leads from marine sponges, we examined a deep-reef collection of the Jamaican sponge *M. styx*. Twenty-three new diterpenes called cyanthiwigins E−AA, two novel sesquiterpenes named styxone A and B, and a novel lactone named styxlactone were isolated and reported in our earlier publications.[6,7] In this paper, we report three additional minor diterpenes, called cyanthiwigins AB (**1**), AC (**2**), and AD (**3**) of which cyanthiwigin AC (**2**) and AD (**3**) represent novel



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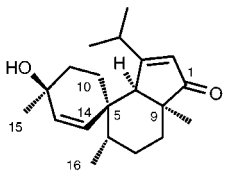

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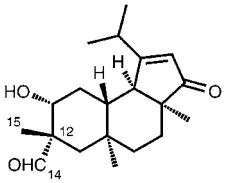

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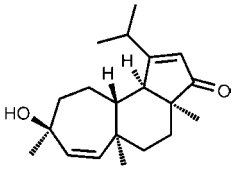

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(1) Sennett, S. H.; Pomponi, S. A.; Wright, A. E. *J. Nat. Prod.* **1992**, *55*, 1421−1429.

(2) Albrizio, S.; Fattorusso, E.; Magno, S.; Mangoni, A.; Pansini, M. *J. Nat. Prod.* **1992**, *55*, 1287−93.

(3) Albrizio, S.; Ciminiello, P.; Fattorusso, E.; Magno, S. *Z. Naturforsch. B Chem. Sci.* **1993**, *48*, 488−492.

(4) Green, D.; Goldberg, I.; Stein, Z.; Ilan, M.; Kashman, Y. *Nat. Prod. Lett.* **1992**, *1*, 193−199.

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Table 1. ^{1}H and ^{13}C NMR Data of Cyanthiwigins AB, AC, and AD (**1**, **2**, and **3**)[a]

	1		**2**		**3**	
	^{1}H (mult, *J* in Hz)	^{13}C (mult)	^{1}H (mult, *J* in Hz)	^{13}C (mult)	^{1}H (mult, *J* in Hz)	^{13}C (mult)
1		216.4 (s)		213.8 (s)		211.1 (s)
2	5.79 (s)	124.4 (d)	5.98 (s)	125.6 (d)	5.76 (s)	123.0(d)
3		195.6 (s)		188.2 (s)		191.9 (s)
4α	2.51 (d, 10.4)	57.8 (d)	2.70 (s)	58.6 (d)	2.42 (d, 10.8)	54.1 (d)
5*β*	1.05 (m)	59.5 (d)		41.9 (s)	0.88 (m)	52.4 (d)
6		38.6 (s)	1.42 (m)	30.5 (d)		34.2 (s)
7α	1.63 (dd, 4.5, 12.2)	49.1 (t)	1.50 (m)	25.6 (t)	0.83 (m)	38.2 (t)
7*β*	1.23 (m)		1.15 (m)		1.29 (m)	
8α	3.75 (ddd, 4.5, 9.6, 12.4)	72.2 (d)	2.16 (ddd, 1.5, 9.5, 14.3)	26.1 (t)	1.39 (dt, 5.5, 14.4)	26.6 (t)
8*β*			1.63 (m)		2.14 (m)	
9		53.8 (s)		51.2 (s)		51.5 (s)
10α	1.29 (m)	27.0 (t)	1.72 (2H, m)	26.8 (t)	1.77 (q, 12.0)	34.5 (t)
10*β*	1.89 (m)				2.11 (m)	
11α	1.50 (m)	35.0 (t)	1.85 (2H, m)	34.7 (t)		76.1 (d)
11*β*	1.94 (m)				3.43 (m)	
12		59.7 (s)		69.2 (s)		49.8 (s)
13α	2.69 (t, 7.2)	60.3 (d)	5.59 (d, 8.0)	133.8 (d)	1.81 (d, 14.2)	50.6 (t)
					1.02 (d, 14.2)	
14α	1.98 (m)	44.6 (t)	4.97 (d, 8.0)	136.0 (d)	10.04 (s)	208.3 (d)
14*β*	1.25 (m)					
15	1.27 (3H, s)	22.5 (q)	1.31 (3H, s)	28.7 (q)	1.06 (3H, s)	23.0 (q)
16	1.03 (3H, s)	16.5 (q)	0.71 (3H, d, 5.4)	16.6 (q)	0.74 (3H, s)	17.7 (q)
17	1.32 (3H, s)	28.1 (q)	1.04 (3H, s)	30.2 (q)	1.01 (3H, s)	30.2 (q)
18	2.84 (m)	33.5 (d)	2.81 (m)	32.6 (d)	2.80 (m)	32.9 (d)
19	1.23 (3H, d, 6.8)	19.8 (q)	1.19 (3H, d, 6.0)	20.2 (q)	1.24 (3H, d, 6.8)	20.3 (q)
20	1.16 (3H, d, 6.8)	21.7 (q)	1.12 (3H, d, 6.0)	22.9 (q)	1.15 (3H, d, 6.8)	21.8 (q)

[a] ^{1}H NMR at 400 MHz, ^{13}C NMR at 100 MHz, in acetone-d_6, multiplicities inferred from DEPT and HMQC experiments.

diterpene-type skeletons. The sponge *M. styx* was collected in July 2000 off the coast of Rio Beuno, Jamaica, using closed circuit rebreathers, at the depth of −50 to −60 m. The freeze-dried sponge was extracted with methanol, and the acetone-soluble part of the extract was subjected to silica gel vacuum-liquid chromatography followed by column chromatography, preparative thin-layer chromatography, and reverse-phase HPLC to yield compounds **1**−**3**.

Cyanthiwigin AB (**1**, 0.7 mg) was obtained as a white crystalline material, and the molecular formula $C_{20}H_{30}O_3$ was determined by HRESIMS 341.2090 [M + Na] (calcd 341.2093). The ^{1}H NMR spectrum (Table 1) showed characteristic signals for a cyanthiwigin-type diterpene[6] including a singlet olefinic proton at δ 5.79 (H-2), three singlet methyl protons at δ 1.27, 1.03, and 1.32 (H-15, H-16, and H-17, respectively), two doublet methyl protons at δ 1.23 and 1.16 (H-19 and H-20), and a doublet methine proton at δ 2.51 (H-4). An oxygenated methine proton was observed at δ 3.75 (H-8), suggesting hydroxyl functionality. Twenty carbon signals appeared in the ^{13}C NMR spectrum, including a carbonyl carbon signal at δ 216.4 (C-1), and olefinic carbon signals at δ 195.6 (C-3) and 124.4 (C-2) indicated an α,*β*-unsaturated ketone in the five-membered ring.[6] The signal at δ 72.2 (C-8) indicated a hydroxymethine carbon, and the signals at δ 59.7 and 60.3 suggested an epoxy group. The HMBC experiment showed a correlation between δ 1.32 (H-17) and δ 72.2 (C-8), indicating the hydroxyl group at C-8. The relative configuration of the C-8-hydroxyl was determined as *β* in configuration by the NOE correlation between H-8 and H-17. The long-range heteronuclear correlation of H-15 (δ 1.27) to C-12 (δ 59.7) and C-13 (δ 60.3) indicated that the epoxy group was between C-12 and C-13. The comparable chemical shifts of H-13 (δ 2.69), C-13, and C-12 with the data of cyanthiwigins H, K, and J[6] suggested that the epoxy moiety was *β* in configuration, and this was confirmed by an NOE correlation between H-13 and H-16. Cyanthiwigin AC (**2**, 0.3 mg) was obtained as a white powder and its molecular formula $C_{20}H_{30}O_2$ was determined by HRESIMS 325.2167 [M + Na] (calcd 325.2144). The ^{1}H and ^{13}C NMR data (Table 1) of **2** showed signals for an isopropyl (C-18−C-20) group, α,*β*-unsaturated five-membered ring (C-1−C-4, C-9), and a double bond (C-13−C-14), a pattern with similarity to cyanthiwigin U (**4**).[6] However, some significant differences were observed including H-4 (δ 2.70), which appeared as a singlet indicating a quaternary C-5, and H-16 (δ 0.71), which appeared as a doublet indicating a tertiary C-6. These data suggested that **2** did not have a seven-membered ring as in cyanthiwigin U (**4**). In the COSY spectrum, the correlations of H-16 (d 0.71)

(5) Tsukamoto, S.; Kato, H.; Hirota, H.; Fusetani, N. *Biofouling* **1997**, *11*, 283−291.
(6) Peng, J.; Walsh, K.; Weedman, V.; Braude, I. A.; Kelly, M.; Hamann, M. T. *Tetrahedron* **2002**, *58*, 7809−7819.
(7) Peng, J.; Franzblau, S. G.; Zhang, F.; Hamann, M. T. *Tetrahedron Lett.* **2002**, *43*, 9699−9702.

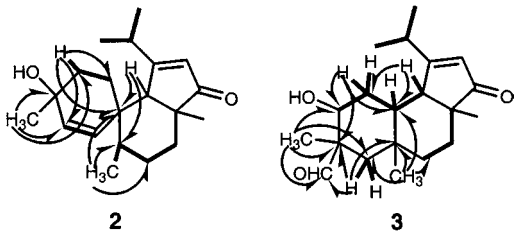

Figure 1. Bold bonds indicate COSY correlations, and arrows indicate HMBC.

with H-6 (d 1.42); H-6 with H-7b (d 1.15); H-7b with H-7a (d 1.50) and H-8b (d 1.63); and H-8α (δ 2.16) with H-8β and H-7α, indicated a $CH_3-CH-CH_2-CH_2$ ($C_{16}-C_6-C_7-C_8$) spin system. The long-range heteronuclear correlations (Figure 1) of H-4 (δ 2.70) to C-5, C-6, and C-9 (δ 51.2), and H-17 (δ 1.04) to C-4, C-8, and C-9, connected C-4, -5, -9 with the substructure to form the C-4−C-9 six-membered ring. The correlations of H-13 (δ 5.59) with H-14 (δ 4.97) and H-10 (δ 1.72) with H-11 (δ 1.85) in the COSY spectrum indicated a 1,2-substituted ethylene and a 1,2-substituted ethane spin system. The long-range heteronuclear correlations of H-15 (δ 1.31) to C-12 (δ 69.2), C-13 (δ 133.8), and C-11 (δ 34.7) connected the 1,2-substituted ethylene and the 1,2-substituted ethane through the oxygenated carbon (C-12). The long-range heteronuclear correlations of H-4 to C-10 (δ 26.8) and H-11 (δ 1.85) to C-5 (δ 41.9) connected C-10 to C-5. Finally the olefinic methine (C-14) must link to the quaternary carbon (C-5) to form a six-membered spiro-ring (C-5, C-10−C-14), and thus the structure of cyanthiwigin AC was elucidated and represents a novel diterpene ring system.

To determine the relative stereochemistry of cyanthiwigin AC (**2**), a combination of NOESY experiments and molecular modeling analysis was utilized. In the NOESY spectrum, the strong correlation of H-4 (δ 2.70) to H-17 (δ 1.04) indicated a *cis* junction of the five- and six-membered ring; the correlations of H-4 to both H-10 (δ 1.72) and H-11 (δ 1.85) and H-14 (δ 4.97) to H-18 (δ 2.81) indicated that C-10 was α in orientation and C-14 was β in configuration. Because the two six-membered ring systems could exist in several different conformations, a conformational analysis was essential for the determination of the configurations of both the 15- and 16-methyl groups. The conformation with minimized energy by molecular modeling was shown in Figure 2.[9] H-14 showed NOE correlations with both H-16 (δ 0.71) and H-6 (δ 1.42), and H-16 correlated with H-10 (δ 1.72), requiring an α, equatorial 16-CH_3. H-15 (δ 1.31) showed NOE correlations with both H-10 and H-11, indicating an axial 15-methyl group, which is α in configuration on the basis of the molecular model. The calculated distances between the NOE correlated protons are shown in Figure 2 and are highly consistent with the experimentally determined NOESY data.

(8) α]$_D$ −23 (*c* 0.10, MeOH); UV λ_{max} (nm) 240 (ε = 7963); IR (film) ν cm^{-1} 3415, 2966, 2925, 2873, 1724, 1681, 1457, 1378, 1299, 1103, 1027, 869; CD [θ]$_{220}$ +5130, [θ]$_{316}$ −5170 (*c* 7.86 × 10^{-5}, MeOH).

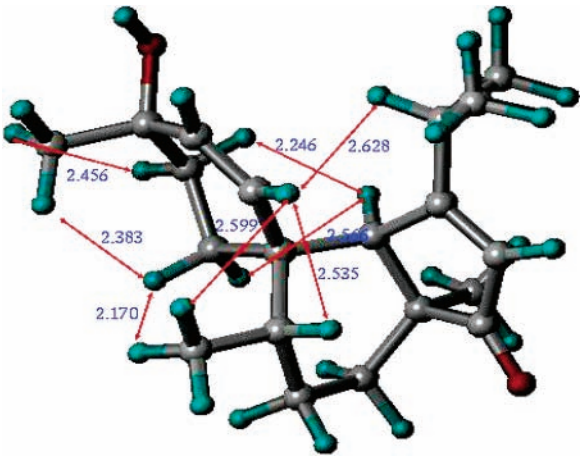

Figure 2. The conformation of **2** with minimized energy. The arrows show the NOE correlations, and the data are the distances in Å between correlated protons.

Cyanthiwigin AD[10] (**3**, 0.4 mg) was obtained as a white crystalline material, and its molecular formula was determined to be $C_{20}H_{30}O_3$ by HRESIMS 341.2084 [M + Na] (calcd 341.2093). The ^{1}H NMR of **3** showed some similarity

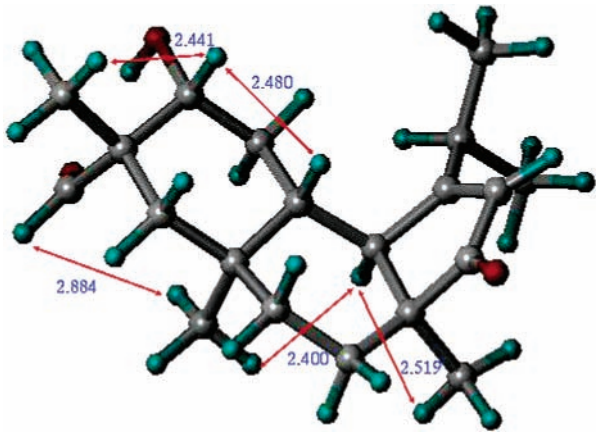

Figure 3. The conformation of **3** with minimized energy. The arrows show the NOE correlations, and the data are the distances in Å between correlated protons.

(9) Molecular modeling was performed using SYBYL 6.8 (Tripos Associates, St. Louis, MO). Initial conformations of the molecule were obtained by 10 rounds of dynamics simulations, in which the molecule was heated to 500 K within 500 fs and then allowed to cool to 200 K within 5000 fs. Ten lowest energy conformations were selected and refined by molecular mechanics minimization using Powell's gradient algorithm method with the MMFF94 force field, a constant-dependent dielectric of 2.00, and partial atomic charges, until a root-mean-square deviation 0.001 kcal/mol·Å was achieved. Finally, from these refined conformations, the conformer with the lowest energy was selected as the global minimum, representing the most likely molecular configuration.

(10) α]$_D$ +30 (*c* 0.12, MeOH); UV λ_{max} (nm) 240 (ε = 3708); IR (film) ν cm^{-1} 3438, 2964, 2927, 2869, 1702, 1598, 1456, 1382, 1263, 1191, 1078, 1045, 1004, 734; CD [θ]$_{237}$ +6908, [θ]$_{277}$ −1820, [θ]$_{330}$ −2210 (*c* 9.43 × 10^{-5}, MeOH).

with those of the previously characterized cyanthiwigin diterpenes,[6] including five methyl signals at δ 0.74 (s), 1.01 (s), 1.06 (s), 1.15 (d), and 1.24 (d); one α,β-unsaturated ketone proton at δ 5.76 (s); and an oxygenated methine proton at δ 3.43 (m) suggesting a hydroxyl moiety. A singlet methine proton at δ 10.04 was assigned to the carbon signal at δ 208.3 (C-14) on the basis of the HMQC experiment indicating an aldehyde group, which is significantly different from the previously reported cyanthiwigins. Detailed analysis of the 2D NMR data revealed that cyanthiwigin AC has the same five- and six-membered ring system as the other cyanthiwigins but the seven-membered ring was rearranged to form a new six-membered ring based on the following HMBC correlations (see Figure 1): H-4 (δ 2.42) to C-5 (δ 52.4), C-6 (δ 34.2), and C-10 (δ 34.5); H-13α (δ 1.81) to C-5 (δ 52.4), C-6 (δ 34.2), C-11 (δ 76.1), and C-12 (δ 49.8); H-15 (δ 1.06) to C-11 (δ 76.1), C-12 (δ 49.8), and C-13 (δ 50.6) and finally H-10α (1.77) to C-5 (δ 52.4), C-11 (δ 76.1), and C-12 (δ 49.8). Both H-15 and H-13α have long heteronuclear correlations with the aldehyde carbon (δ 208.3) indicating the aldehyde group was vicinal to C-12. The aldehyde proton H-14 (δ 10.04) showed NOE correlations with H-16 (δ 0.74) in the NOESY experiment, indicating its α orientation. The NOE correlations of H-11 (δ 3.43) to H-5 (δ 0.88) and H-15 (δ 1.06) suggested the α orientation of the hydroxyl group. The calculated minimum energy conformation of **3**, shown in Figure 3, furnishes calculated distances between protons, coinciding well with experimentally determined NOE data.

Cyanthiwigin AC (**2**) and AD (**3**) present novel diterpene skeletons. A putative biosynthetic pathway is shown in Figure 4.

Previous studies had shown the anticancer, antituberculosis, and anti-HIV activities of cyanthiwigins.[1,4,6] Because only very small quantities of cyanthiwigin AB, AC, and AD were obtained from this sponge, it was not possible to screen for bioactivity of these three diterpenes. It seems likely that total synthesis will be required to access sufficient quantities of these novel diterpenes for pharmaceutical evaluation.

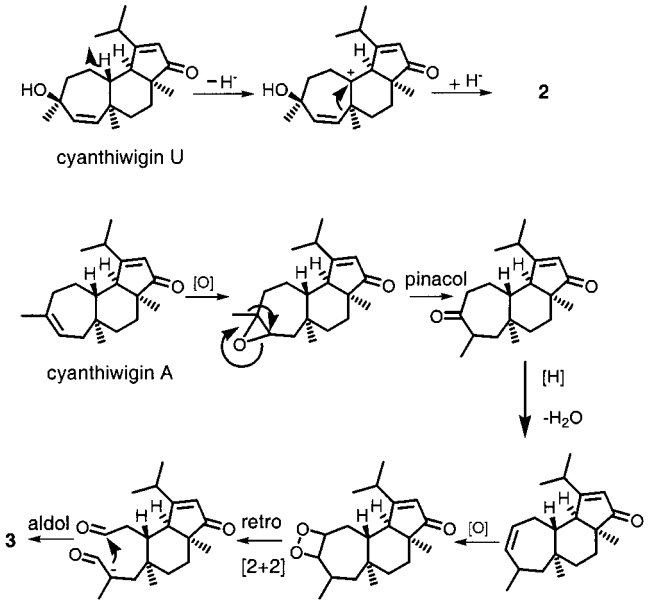

Figure 4. Plausible biosynthetic pathway of cyanthiwigin AC (**2**) and AD (**3**).

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