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Novel sesquiterpenes and a lactone from the Jamaican sponge *Myrmekioderma styx*

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Abstract—Two novel sesquiterpenes, styxone A (**1**) and styxone B (**2**), and a novel lactone, styxlactone (**3**), were isolated from the Jamaican sponge *Myrmekioderma styx*. Their structures were elucidated by detailed ^{1}H, ^{13}C and 2D NMR data and the absolute stereochemistry of styxone A and B was determined by CD spectra. Styxone A represents a novel sesquiterpene skeleton. (*S*)-(+)-Curcuphenol (**4**), (*S*)-(+)-curcudiol (**5**), and abolene (**6**) were also isolated. An activity enhancement by cyanthiwigin B (**7**) to curcuphenol was observed in the antimicrobial assays when the two compounds were administered together.

In the interest of identifying antiinfective compounds from marine invertebrates,[1,2] we studied the Jamaican marine sponge *Myrmekioderma styx*. A number of bioactive linear[3] and tricyclic[4,5] diterpenes have been identified from *M. styx*, and in our previous paper, the isolation of 27 tricyclic diterpenes (cyanthiwigins A-AA) and their cytotoxic and antiinfective activities were reported.[6] In this paper, we report two novel sesquiterpenes, styxone A (**1**) and B (**2**), and a new lactone, styxlactone (**3**), from *M. styx*, along with the previously characterized compounds (*S*)-(+)-curcuphenol (**4**), (*S*)-(+)-curcudiol (**5**) and abolene (**6**).

The sponge *M. styx* was collected in July 2000 off the coast of Rio Beuno, Jamaica. The freeze-dried sponge was extracted with methanol. 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**–**6**.

Styxone A (**1**) was obtained as a colorless oil. It's molecular formula $C_{15}H_{22}O$ (5 degrees of unsaturation) was determined by HRESIMS 219.1744 [M+H] (calcd 219.1749) and 241.1566 [M+Na] (calcd 241.1568). The ^{1}H and ^{13}C NMR spectra suggested a sesquiterpene structure. A carbonyl signal at δ 195.2 ppm indicated an α,β-unsaturated ketone. Two trisubstituted double bonds were observed at δ 141.1 (s, C-2), 133.9 (d, C-3), 163.7 (s, C-10), and 129.5 (d, C-11) ppm; thus, compound **1** is bicyclic. The remaining signals in the ^{13}C and DEPT NMR spectra were identified as four methyls, four methylenes and two quaternary carbons. The methyl signal at δ 1.86 ppm (H-12) indicated it was allylic. The long range heteronuclear correlations of the methyl protons (δ 1.86) with C-2 (δ 141.2), C-3 (δ 133.9) and C-1 (δ 195.2) located this methyl group at C-2 and linked the double bond between C-2 and C-3 with the ketone. The C-1–5,10,11 seven-membered ring system was established by the following HMBC correlations: H-3 (δ 6.23) with C-1, C-2, C-4 (δ 41.5) and C-5 (δ 39.7); H-4 (δ 2.58, 1.96) with C-2, C-3, C-5 and C-10 (δ 163.7); H-11 (δ 5.88) with C-2, C-10, and C-5. The C-5–10 six-membered ring system was determined by the following HMBC correlations: H-9 (δ 2.19) with C-10, C-11 (δ 129.5), and C-8 (δ 33.7); H-9 (δ 1.82) with C-5, C-8 and C-7 (δ 34.9); and H-6 (δ 1.61, 1.42) with C-5 and C-7. Two geminal methyl groups at δ 32.2 (C-14) and 24.9 (C-15) were located at C-8 on the basis that both H-14 (δ 0.99) and H-15 (δ 0.81) showed HMBC correlations with C-8. The 13-methyl group was assigned at C-5 by the HMBC correlations of H-13 (δ 1.07) with C-5, C-6, C-4, and C-10. Thus, the structure of compound **1** was established as a novel sesquiterpene skeleton. The absolute configuration of 5*S* was deter-

Keywords: Myrmekioderma styx; sesquiterpene; styxones A and B; styxlactone; activity enhancement.

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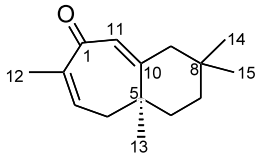
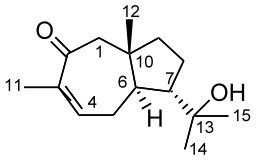


styxone A (**1**)



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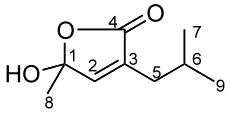
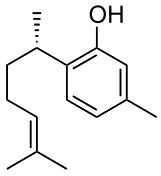
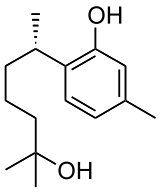


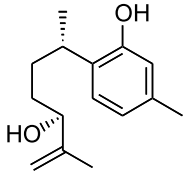
styxlactone (**3**)



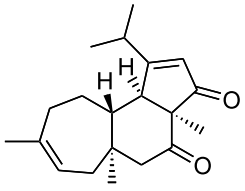
curcuphenol **(4)**



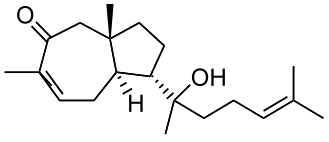
curcudiol **(5)**



abolene **(6)**



cyanthiwigin B (**7**)



polasol A (**8**)

mined by the positive Cotton effect at 284 nm in the CD spectrum. Styxone A has the same six- and seven-membered ring system as well as the same 5*S* configuration as the cyanthiwigin diterpenes (**7**) isolated from this sponge previously,[6] suggesting a similar biosynthetic pathway.

Styxone B (**2**) was obtained as an oil and a molecular formula of $C_{15}H_{24}O_2$ was determined by HRESIMS 259.1666 [M+Na] (calcd 259.1674), 495.3417 [2M+Na] (calcd 495.3450), suggesting the presence of four degrees of unsaturation. The IR absorption at 3436 and 1697 cm^{-1} suggested a hydroxyl and carbonyl group. The ^{13}C and DEPT NMR spectra showed fifteen carbon signals including one carbonyl carbon at δ 201.5 (C-2), two olefinic carbons at δ 137.0 (s, C-3) and 139.5 (d, C-4), and one oxygenated quaternary carbon at δ 72.6, which requires that **2** must have two rings. The ^1H NMR spectrum presented four methyl singlets at δ 1.77, 1.18, 1.16, and 0.89, and one olefinic proton at δ 6.32. The ^1H–^1H COSY NMR spectrum provided the partial structure: $=CHCH_2CHCHCH_2CH_2$, C-4–C-9. The following long-range couplings were observed in the HMBC spectrum: H-1β (δ 2.62) to C-2 (δ 201.5), C-3 (δ 137.0), C-6 (δ 48.8), C-9 (δ 42.1), and C-10 (δ 41.7); H-1α (δ 2.48) to C-2, C-9, C-10, and C-12 (18.4); H-4 (δ 6.32) to C-2, C-3, C-5 (δ 34.3), C-6, and C-11 (δ 21.7); H-11 (δ 1.77) to C-2, C-3, and C-4 (δ 139.5); H-12 (δ 0.89) to C-1, C-6, C-9 and C-10. These data established the bicyclic ring skeleton. An isopropanol group connecting to C-7 was deduced by the following HMBC correlations: H-14 (δ 1.18, 3H, s) to C-13 (δ 72.6), C-7, and C-15 (δ 25.9); H-15 (δ 1.16, 3H, s) to C-13, C-7 and C-14 (δ 29.6). Thus, the planar structure of **2** was determined as a new carotane (daucane) sesquiterpene. Carotane sesquiterpenes were reported from terrestrial plants in the genera *Ferula*, *Rosa* and *Trichoderma*.[7,8] The relative stereochemistry was determined by the NOESY experiment. The NOEs between H-1α/H-6, H-12/H-5β,1β,7, and H-14/H-5α,6 suggested a trans A/B ring, an α configuration for H-6, and a β configuration for H-7. Styxone B is a structural moiety of polasol A (**8**), a diterpene which is structurally equivalent to an isopentene connected to C-15 of **2**, isolated from the marine sponge *Epipolsis* sp.[9] The absolute configuration of 6*R*, 7*S*, and 10*S* was determined by the negative Cotton effect at 245 nm in the CD spectrum, which is same as polasol A (**8**).[9]

The molecular formula of styxlactone (**3**) was determined to be $C_9H_{14}O_3$ by HRESIMS 193.0847 [M+Na] (calcd 193.0841) and 363.1789 [2M+Na] (calcd 363.1784), which indicated three degrees of unsaturation. The IR spectrum suggested a carbonyl group resonating at 1700 cm^{-1}. One double bond and one carboxyl group were observed at δ 133.9 (s, C-3), 149.3 (d, C-2), and 171.5 (C-4) in the ^{13}C NMR spectrum, indicating a single ring in the structure. The ^1H NMR spectrum contained three methyl groups at δ 1.60 (s, H-8), 0.90 (d, H-7), and 0.91 (d, H-9), one olefinic proton at δ 7.03 (s, H-2), one methylene group at δ 2.09 (H-5), one methine proton at δ 1.88 (H-6), and one exchangeable proton at δ 6.18 (OH). The chemical shift of C-1 at δ 104.5 indicated a connection to a hydroxyl and a carboxyl group. The following HMBC correla-

tions established the structure: H-2 to C-1 (δ 104.5), C-8 (δ 24.8), C-3 (δ 133.9), C-4 (δ 171.5), and C-5 (δ 33.9); H-8 to C-1 and C-2 (δ 149.3); the hydroxyl proton to C-1, C-2, and C-8 (δ 24.8); H-5 to C-2, C-3, C-4, C-6 (δ 27.1), and C-7,9 (δ 22.0); H-6 to C-3, C-5, and C-7,9; H-7 and H-9 to C-6 and C-5. The very low yield of this compound precluded further determination of the absolute stereochemistry.

(*S*)-(+)-Curcuphenol (**4**) and (*S*)-(+)-curcudiol (**5**) as two major sesquiterpenes were also isolated together with a minor one, abolene (**6**). Curcuphenol exhibited modest antifungal activity with an IC_{50} of 15 μg/mL against both *Candida albicans* and *Cryptococcus neoformans*, and antibacterial activities against *Staphylococcus aureus* and methicillin-resistant *S. aureus* with IC_{50} values of 20 and 15 μg/mL, respectively. Cyanthiwigin B (**7**), the major diterpene isolated from this sponge,[6] may inhibit the efflux pump system, which promotes the excretion of small molecules out of the cell. The antimicrobial activities of a mixture of the inactive cyanthiwigin B and curcuphenol were assayed, and cyanthiwigin B clearly enhanced the antimicrobial activities of curcuphenol (Table 2). An activity enhancement by cyanthiwigin B (**7**) to curcuphenol was also observed in the antituberculosis assays when the two compounds were administered together (Table 2). Antimicrobial activity was not observed (IC_{50} >15 μg/mL) for styxone A (**1**) and styxone B (**2**) in these assays.

Styxone A (**1**): Colorless oil; HRESIMS 219.1744 [M+H] (calcd for $C_{15}H_{23}O$ 219.1749), 241.1566 [M+Na] (calcd 241.1568), 257.1301 [M+K] (calcd 257.1308), 459.3228 [2M+Na] (calcd 459.3239); $[\alpha]_D$ +164° (*c* 0.09, MeOH); UV λ_{max} (nm) 252 (ε=6808); IR (film) *v* 2952, 2925, 1706, 1617, 14554, 1371, 1251, 1105, 894 cm^{-1}; CD $[\theta]_{284}$ +21786, $[\theta]_{342}$ −3640 (*c* 1.03×E-4, MeOH); ^1H and ^{13}C NMR data see Table 1.

Styxone B (**2**): Colorless oil; HRESIMS 259.1666 [M+Na] (calcd 259.1674), 495.3417 [2M+Na] (calcd 495.3450); $[\alpha]_D$ +48° (*c* 0.10, MeOH); UV λ_{max} (nm) 238 (ε=4289); IR (film) *v* 3436, 2962, 2929, 1697, 1644, 1454, 1378, 1168, 752 cm^{-1}; CD $[\theta]_{259}$ −2664, $[\theta]_{320}$ +790 (*c* 1.06×E-4, MeOH); ^1H and ^{13}C NMR data see Table 1.

Table 1. ^1H and ^{13}C NMR data of **1**–**3**

	1		**2**		**3**	
	^1H (mult, *J* in Hz)	^{13}C (mult)	^1H (mult, *J* in Hz)	^{13}C (mult)	^1H (mult, *J* in Hz)	^{13}C (mult)
1		195.2 (s)	2.62 (d, 15.2) 2.48 (d, 15.2)	58.3 (t)		104.5 (s)
2		141.1 (s)		201.5 (s)	7.03 (s)	149.3 (d)
3	6.23 (m)	133.9 (d)		137.0 (s)		133.9 (s)
4	2.58 (dd, 2.0, 14.5) 1.96 (dd, 9.2, 14.5)	41.5 (t)	6.32 (d, 7.2)	139.5 (d)		171.5 (s)
5		39.7 (s)	2.92 (m) 2.18 (m)	34.3 (t)	2.09 (2H, d, 7.2)	33.9 (t)
6	1.59 (m) 1.42 (m)	38.9 (t)	2.01 (m)	48.8 (d)	1.88 (m)	27.1 (d)
7	1.30 (m) 1.52 (m)	34.9 (t)	1.82 (m)	55.2 (d)	0.90 (3H, d, 6.6)	22.0 (q)
8		33.7 (s)	1.82 (m) 1.54 (m)	26.0 (t)	1.60 (3H, s)	24.8 (q)
9	2.19 (d, 14.3) 1.82 (dd, 2.6, 14.3)	49.3 (t)	1.42 (2H, m)	42.1 (t)	0.90 (3H, d, 6.6)	22.0 (q)
10		163.7 (s)		41.7 (s)		
11	5.88 (s)	129.5 (d)	1.77 (3H, s)	21.7 (q)		
12	1.86 (3H, s)	19.47 (q)	0.89 (3H, s)	18.7 (q)		
13	1.07 (3H, s)	20.9 (q)		72.6 (s)		
14	0.99 (3H, s)	32.2 (q)	1.18 (3H, s)	29.6 (q)		
15	0.81 (3H, s)	24.9 (q)	1.16 (3H, s)	25.9 (q)		

Table 2. Antimicrobial activity

	Anti-TB IC_{50} (μM)	Antifungal IC_{50} (μg/mL)		Antibacterial IC_{50} (μg/mL)	
		C. albicans	*C. neoformans*	*S. aureus*	MRS[a]
Curcuphenol	126.7	15	15	20	15
Cyanthiwigin B	NA[b]	NA	NA	NA	NA
Curcuphenol + cyanthiwigin B[c]	53.7	10	8.5	7.5	8.0

[a] MRS = methicillin-resistant *S. aureus*.
[b] NA = not active.
[c] Curcuphenol and cyanthiwigin B mixed 1:1. IC_{50} calculated based on the mass of curcuphenol.

Styxlactone (**3**): Colorless oil; HRESIMS 193.0847 [M+Na] (calcd 193.0841), 363.1789 [2M+Na] (calcd 363.1784). $[\alpha]_D$ +10° (*c* 0.10, MeOH); UV λ_{max} (nm) 235 (ε=2769); IR (film) ν 3401, 2958, 1745, 1458, 1375, 1178, 1043, 929 cm^{-1}; CD $[\theta]_{244}$ +1824, $[\theta]_{273}$ −1743 (*c* 1.30×E-4, MeOH); ^1H and ^{13}C NMR data see Table 1.

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