Notes

Three Unprecedented Chloro Steroids from the Maui Sponge *Strongylacidon* sp.: Kiheisterones C, D, and E†

John R. Carney and Paul J. Scheuer*

Department of Chemistry, University of Hawaii at Manoa, 2545 The Mall, Honolulu, Hawaii 96822

Michelle Kelly-Borges

Division of Biomedical Marine Research, Harbor Branch Oceanographic Institution, Inc., 5600 Old Dixie Highway, Fort Pierce, Florida 34946

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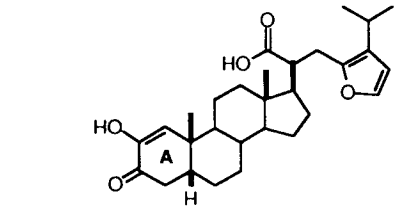
Marine organisms, particularly sponges, have been a prolific source of unique steroids[1] and also of halogenated metabolites—isoprenoid, acetate, or shikimate-derived. Yet, amazingly, not a single halogenated marine steroid has so far been reported. We now describe the first three halo steroids, which we isolated from the sponge *Strongylacidon* sp., whose major cytotoxic constituents are kiheisterones A and B (**1**, **2**).[2]

The sponge, collected off Maui, was extracted with 2-propanol/CH_2Cl_2 (1:1). The residue was partitioned between EtOAc/heptane/MeOH/H_2O (7:4:4:3). The residue from the upper phase was partitioned between heptane/CH_2Cl_2/CH_3CN (50:15:35). The polar residue of that system was subjected to high speed countercurrent chromatography (HSCCC) with EtOAc/heptane/MeOH/H_2O (7:4:4:3) as solvents. Reversed-phase HPLC (MeOH/H_2O/TFA, 82:18:0.1) of the fraction containing the chloro steroids gave kiheisterones C (**3**), D (**4**), and E (**5**).



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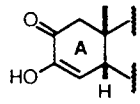

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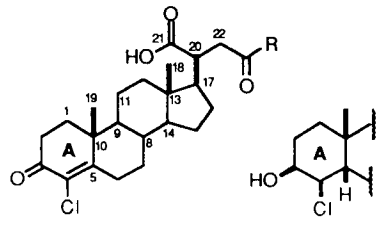

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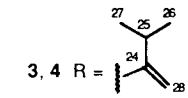
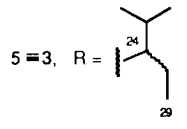


3, 4 R =



5 = 3, R =

A molecular formula of $C_{28}H_{39}ClO_4$, established by HREIMS, indicated nine degrees of unsaturation for **3**. DEPT and HMQC NMR experiments established C–H connectivities and revealed 38 protons attached to 28 carbons; a carboxyl group (δ 179.8; ν 1740 cm^{-1}) accounted for the exchangeable proton. NMR, UV, and IR data suggested two enones. One was a vinyl ketone [δ 200.2 (C-23), 154.9 (C-24), 121.6 (C-28); 5.71, 1 H, d, J = 1.1 Hz (H_a-28), 5.96, 1 H, s (H_b-28); λ_{max} 224 nm, log ϵ 4.0; ν 1710 cm^{-1}], and the other was an α-chloro enone [δ 190.8 (C-3), 127.2 (C-4), 164.8 (C-5); λ_{max} 257 nm, log ϵ 3.9;[3] ν 1680 cm^{-1} [4]]. These functions accounted for five degrees of unsaturation, leaving four for the sterone nucleus.

HMBC and ^1H–^1H COSY experiments were used to determine the C–C connectivities (Table I). A methyl singlet at δ 0.86 (H_3-18) showed HMBC correlations to carbon signals at 55.3 (C-14), 42.2 (C-13), 36.8 (C-12), and 52.1 (C-17). A proton resonating at δ 1.60, attached to C-17, correlated to carbons at δ 27.7 (C-16), 42.1 (C-20), and the carboxyl carbon (C-21), as well as to C-12, C-13, and C-18. COSY data revealed that H-17 was coupled to H-20 (δ 2.76), which was in turn coupled to protons at δ 2.85 and 3.08 (H_2-22). HMBC crosspeaks were observed for H_2-22 to the carbonyl (δ 200.2, C-23) and α-carbon (154.9, C-24) of the vinyl ketone. COSY data also indicated that two methyl doublets at δ 0.99 (H_3-26, H_3-27) were coupled to a one proton septet (δ 2.85), attached to a carbon at 27.8 (C-25). HMBC correlations of H_3-26 and H_3-27 to C-24 and C-25 and of the vinyl protons to C-23, C-24, and C-25 established the structure of the side chain.

We suspected that the α-chloro enone moiety was in the A ring by analogy with the monoenolized diketones of **1** and **2**, and this was confirmed by NMR data. The C-19 protons (δ 1.22) showed HMBC correlations to C-1 (δ 34.4), C-5 (164.8), C-9 (53.9), and C-10 (41.4). The chemical shifts of C-1, C-9, and C-10 were in the typical range, but C-5 was upfield by 5 ppm, compared with these carbons in 4-en-3-one steroids.[5] Substitution of a proton with chlorine in an alkene is known to cause an upfield shift of approximately 6 ppm of the adjacent alkene carbon.[6] The protons attached to C-1 (δ 1.68, 2.01) exhibited COSY correlations to a methylene group at δ 2.55, attached to a carbon at 34.0 (C-2), as well as HMBC correlations to C-2 and an enone carbonyl carbon signal at 190.8 (C-3). Two protons resonating at δ 2.17 and 3.23, attached to a carbon at δ 28.6 (C-6), were correlated in the HMBC experiment to a quaternary carbon at δ 127.2 (C-4) and to C-5, C-7, C-8, and C-10. Additional NMR data were entirely consistent with the remaining conventional steroid portion and thus secured the structure of **3**.

^1H and ^{13}C NMR data revealed that **4** shared B, C, and D rings and side chain with **3** but differed in ring A. Its molecular formula of $C_{28}H_{43}ClO_4$ (HREIMS) implied seven instead of nine degrees of unsaturation. The UV (λ_{max} 220 nm, log ϵ 4.1) and ^{13}C NMR spectra (δ 70.1, C-3; 66.7,

† Contribution No. 955 from Harbor Branch Oceanographic Institution.

(1) Kerr, R. G.; Baker, B. J. *Nat. Prod. Rep.* **1991**, *8*, 465–497.
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(5) (a) Schun, Y.; Cordell, G. A. *J. Nat. Prod.* **1987**, *50*, 195–198. (b) Ksebati, M. B.; Schmitz, F. J. *J. Org. Chem.* **1988**, *53*, 3926–3929.
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Table I. NMR Data for 3 (in $CDCl_3$)

no.	^{13}C	^1H (J, Hz)	HMBC	COSY
1	34.4 (t)[a]	ax 1.68, 1 H, td (13.0, 6.5)	H_2-2, H_3-19	H-1eq, H_2-2
		eq 2.01, 1 H, dt (13.3, 4.2)		H-1ax, H_2-2
2	34.0 (t)	2.55, 2 H, m	H-1eq	
3	190.8 (s)		H-1eq, H_2-2	
4	127.2 (s)		H_2-2, H_2-6	
5	164.8 (s)		H-1eq, H_2-6, H-7eq, H_3-19	
6	28.9 (t)	ax 2.17, 1 H, td (14.5, 5.1)		H-6eq, H_2-7
		eq 3.23, 1 H, ddd (14.7, 3.6, 2.4)		H-6ax, H_2-7
7	31.0 (t)	ax 1.05, 1 H, m	H_2-6	
		eq 1.90, 1 H, m		
8	35.1 (d)	1.57, 1 H, m	H_2-6	
9	53.9 (d)	0.95, 1 H, td (11.1, 3.9)	H-11ax, H_3-19	H-11ax
10	41.4 (s)		H-1eq, H_2-2, H_2-6, H_3-19	
11	20.9 (t)	ax 1.40, 1 H, qd (13.1, 3.8)		
		eq 1.55, 1 H, m		H-11ax
12	36.8 (t)	ax 1.08, 1 H, m	H-11ax, H-17, H_3-18	H-11ax
		eq 1.89, 1 H, m		
13	42.2 (s)		H-17, H_3-18	
14	55.3 (d)	1.02, 1 H, m	H_3-18	
15	23.4 (t)	1.17, 1 H, m	H-16a	
		1.66, 1 H, m		
16	27.7 (t)	a 1.31, 1 H, m	H-17	
		b 1.91, 1 H, m		
17	52.1 (d)	1.60, 1 H, q (10.7)	H_3-18, H-20, H_2-22	H-20
18	11.5 (q)	0.86, 3 H, s	H-17	
19	17.7 (q)	1.22, 3 H, s	H-1eq	
20	42.1 (d)	2.76, 1 H, td (11.0, 3.4)	H-17, H_2-22	
21	179.8 (s)		H-17, H-20, H_2-22	
22	40.9 (t)	a 2.85, 1 H, dd (17.6, 3.5)	H-20	
		b 3.08, 1 H, dd (17.6, 10.8)		H-20
23	200.2 (s)		H-20, H_2-22, H_2-28	
24	154.9 (s)		H-22a, H_3-26, H_3-27, H_2-28	
25	27.8 (d)	2.85, 1 H, septet (6.9)	H_3-26, H_3-27, H_2-28	H_3-26, H_3-27
26	21.7 (q)	0.99, 3 H, d (6.9)	H-25, H-27	H-25
27	21.8 (q)	0.99, 3 H, d (6.9)	H-25, H-26	H-25
28	121.6 (t)	5.71, 1 H, d (1.1)	H-22a	
		5.96, 1 H, s		

[a] Multiplicities were determined by DEPT and HMQC spectra.

C-4) suggested that the α-chloro enone of **3** was reduced to a 4,3-chlorohydrin; HMQC and ^1H–^1H COSY experiments confirmed this (supplementary material, Table I). The chemical shifts of C-9 (δ 41.4), C-18 (11.7), and C-19 (23.9) implied that the A/B rings were cis fused[7] and the coupling patterns and constants for H-4 (δ 4.47, dd, J = 11.8, 2.7 Hz) and H-3 (4.05, bs) placed the hydroxyl and chloro groups in axial and equatorial positions, respectively. Correlations in a ROESY experiment of H_3-19 (δ 1.00) to H-5 (1.87), and H-4 to H-9 (1.21), confirmed relative stereochemistry for kiheisterone D (**4**).

A molecular formula for kiheisterone E (**5**) of $C_{29}H_{43}ClO_4$ by HRFABMS suggested eight degrees of unsaturation. From the ^1H and ^{13}C NMR data, including ^1H–^1H COSY, HMQC, and HMBC (supplementary material, Table II), it was apparent that 24-methylene in **3** was replaced by 24-ethyl. Major ^1H and ^{13}C signals of the side chain were accompanied by adjacent smaller signals, indicating that partial epimerization had occurred at C-24,[8] presumably due to TFA in the HPLC solvent system.

The only known naturally occurring halogenated steroids are chlorinated withanolides isolated from *Solanaceae*. These *trans*-6,5-chlorohydrins are accompanied by their putative biosynthetic precursor, 5,6-epoxides.[9-14] 4-Chloro 4-en-3-one hormone analogs have been synthesized by epoxidation of 4-en-3-ones, followed by chloride opening of the epoxide with concomitant dehydration.[3] One can envisage parallel biosynthesis for **3** and **5**.[15] The existence in many organisms of reductases capable of converting 4-en-3-one-containing steroids to the corresponding 3β-hydroxy-5β(H) compounds is well documented,[16] and kiheisterone D (**4**) with its *cis*-chlorohydrin may have arisen from enzymatic reduction of **3**.

Experimental Section

Taxonomy. The sample was collected at −10–20 m in Maalaea Bay near Kihei, Maui, on June 4, 1991. The sponge formed a small, circular, thickly encrusting patch, with short, broad mammilate to conulose papillae on a smooth surface. The sponge was bright orange-red in life, and is dull grey in ethanol preservative. The skeleton is a plumo-reticulation of strongyles in fibers, and the microscleres are unguiferate to anchorate isochelae. Rhaphides are also present. The sponge is an undescribed species of *Strongylacidon* (*Porifera, Demospongiae, Poecilosclerida, Desmacidonidae*). The sample differs from other Hawaiian or Pacific *Strongylacidon*-like species, such as *Xytopsiphum kaneohe* de Laubenfels, X. (=*Strongylacidon*) *meganese* de Laubenfels and *Xytopsues* (=*Strongylacidon*) *zukerani* de Laubenfels, in details of spicule complement and dimensions and coloration. A voucher specimen has been deposited at the Harbor Branch Oceanographic Museum, Fort Pierce, Florida (Catalog No. 003:00826).

Isolation. The freeze-dried sponge (400 g) was extracted with 3 L of 2-propanol/CH_2Cl_2 (1:1). The extract was concentrated in vacuo yielding 6.44 g, of which a 3.05 g portion was partitioned between 900 mL of EtOAc/heptane/MeOH/H_2O (7:4:4:3). The residue from the upper phase (1.56 g) was partitioned between 400 mL of heptane/CH_2Cl_2/CH_3CN (50:15:35). The lower layer residue (0.839 g) was subjected to high-speed countercurrent chromatography (HSCCC) with a P. C. Inc. multilayer coil separator–extractor equipped with a no. 10 column (380 mL volume, flow rate of 2 mL/min), collecting fractions of 20 mL. Reversed-phase HPLC (MeOH/H_2O/TFA, 82:18:0.1, YMC 5 μ ODS 10 × 250 mm column, flow rate 2 mL/min, UV detection

(7) Blunt, J. W.; Stothers, J. B. *Org. Magn. Reson.* **1977**, *9*, 439–464.

(8) The ratio of diastereomers was approximately 2:1. Diastereomeric side chain signals were only observed in the NMR spectra of **5**, implying that epimerization had occurred at C-24 and not at the C-20 methine common to **3**, **4**, and **5**. Examination of the ^1H NMR spectrum of **3** after it had been stirred overnight in the HPLC solvent revealed no diastereotopic signals.

(9) Nittala, S. S.; Velde, V. V.; Frolow, F.; Lavie, D. *Phytochemistry* **1981**, *20*, 2547–2552.

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(13) Tschesche, R.; Annen, K.; Welzel, P. *Chem. Ber.* **1971**, *104*, 3556–3566.

(14) Tschesche, R.; Baumgarth, M.; Welzel, P. *Tetrahedron* **1968**, *24*, 5169–5179.

(15) Biogenetic pathways involving a haloperoxidase-generated chloronium ion are also plausible, but while bromoperoxidase activity has been detected in several marine sponges and algae, we are not aware of chloroperoxidase activity being adequately characterized in any marine organism.

(16) See Dorfman, R. I.; Ungar, F. *Metabolism of Steroid Hormones*; Academic Press: New York, 1965.

at 254 nm) of fraction 3 (214.0 mg) gave **3** (6.0 mg), **4** (5.7 mg), and **5** (4.9 mg). Fraction 2 (154.4 mg) contained primarily **1** and **2**.

Kiheisterone C (3): $[\alpha]_D$ +71° (c 0.55, $CHCl_3$); HREIMS (M^+) 474.2538, $C_{28}H_{39}ClO_4$ (Δ −0.1 mmu), (M^+ − HCl) 438.2769, $C_{28}H_{38}O_4$ (Δ 0.0 mmu), (M^+ − $C_7H_{11}O$) 363.1722, $C_{21}H_{28}ClO_3$ (Δ 0.5 mmu); FABMS (MH^+) 475; HRFABMS 475.2608, $C_{28}H_{40}ClO_4$ (Δ 0.7 mmu); UV (MeOH) λ_{max} 224 (log ε 4.0), 257 nm (log ε 3.9); IR ($CHCl_3$) ν 1740, 1710, 1680, 1580 cm^{-1}; ^{13}C and ^1H NMR, see Table I.

Kiheisterone D (4): $[\alpha]_D$ +46° (c 0.57, $CHCl_3$); HREIMS (M^+) 478.2862, $C_{28}H_{43}ClO_4$ (Δ 1.3 mmu), (M^+ − H_2O − HCl) 424.2978, $C_{28}H_{40}O_3$ (Δ 0.0 mmu); UV (MeOH) λ_{max} 220 nm (log ε 4.1); ^{13}C NMR ($CDCl_3$) δ 11.7 (C-18), 20.9 (C-11), 21.7 (C-27), 21.8 (C-26), 22.4 (C-6), 23.4 (C-15), 23.9 (C-19), 25.6 (C-7), 26.4 (C-2), 27.7 (C-16), 27.8 (C-25), 29.1 (C-1), 35.6 (C-8), 37.4 (C-12), 38.5 (C-10), 41.0 (C-22), 41.4 (C-9), 42.2 (C-20), 42.5 (C-13), 44.5 (C-5), 52.4 (C-17), 56.3 (C-14), 66.7 (C-4), 70.1 (C-3), 121.5 (C-28), 154.9 (C-24), 179.8 (C-21), 200.2 (C-23); ^1H NMR ($CDCl_3$) δ 0.79 (s, 3 H, H-18), 0.99 (d, 3 H, J = 6.8 Hz, H-26), 0.99 (d, 3 H, J = 6.8 Hz, H-27); 1.00 (s, 3 H, H-19), 1.03 (m, 1 H, H-14), 1.04 (m, 1 H, H_{ax}-12), 1.16 (m, 1 H, H_a-15), 1.20 (m, 1 H, H_a-11), 1.21 (m, 1 H, H-9), 1.29 (m, 1 H, H_a-16), 1.39 (m, 1 H, H_b-11), 1.46 (m, 1 H, H-8), 1.47 (m, 2 H, H-7), 1.47 (m, 1 H, H_a-1), 1.59 (q, 1 H, J = 10.5 Hz, H-17), 1.59 (m, 1 H, H_a-2), 1.60 (m, 1 H, H_b-1), 1.64 (m, 1 H, H_b-15), 1.70 (m, 1 H, H_a-6), 1.76 (m, 1 H, H_b-2), 1.84 (m, 1 H, H_{eq}-12), 1.87 (m, 1 H, H-5), 1.88 (m, 1 H, H_b-16), 1.89 (m, 1 H, H_b-6), 2.76 (td, 1 H, J = 10.7, 3.3 Hz, H-20), 2.85 (septet, 1 H, J = 6.9 Hz, H-25), 2.86 (dd, 1 H, J = 17.7, 3.5 Hz, H_a-22), 3.07 (dd, 1 H, J = 17.7, 10.7 Hz, H_b-22), 4.05 (bs, 1 H, H-3), 4.47 (dd, 1 H, J = 11.8, 2.7 Hz, H-4), 5.70 (d, 1 H, J = 1.1 Hz, H_a-28), 5.95 (s, 1 H, H_b-28).

Kiheisterone E (5): FABMS m/z 491 (MH^+); HRFABMS 491.2939, $C_{29}H_{44}ClO_4$ (Δ −1.1 mmu); HREIMS (M^+ − HCl) 454.3087, $C_{29}H_{42}O_4$ (Δ −0.4 mmu), (M^+ − HCl − H_2O) 436.2976, $C_{29}H_{40}O_3$ (Δ 0.1 mmu); UV (MeOH) λ_{max} 258 nm (log ε 4.0); ^{13}C NMR (CD_3OD) δ 11.9 (C-18), 12.1 (C-29), 17.9 (C-19), 20.1 (C-26), 21.3 (C-27), 22.0 (C-11), 22.9 (C-28), 24.4 (C-15), 28.7 (C-16), 30.0 (C-6), 30.8 (C-25), 32.8 (C-7), 34.9 (C-2), 35.5 (C-1), 36.4 (C-8), 38.2 (C-12), 42.7 (C-10), 43.3 (C-13), 43.4 (C-20), 47.9 (C-22), 53.5 (C-17), 55.5 (C-9), 56.8 (C-14), 61.4 (C-24), 127.8 (C-4), 167.5 (C-5), 179.4 (C-21), 193.0 (C-3), 215.7 (C-23); ^1H NMR (CD_3OD) δ 0.78 (m, 3 H, H-29), 0.87 (d, 3 H, J = 7.0 Hz, H-26), 0.88 (d, 3 H, J = 7.0 Hz, H-27), 0.88 (s, 3 H, H-18), 1.00 (m, 1 H, H-9), 1.02 (m, 1 H, H_a-7), 1.04 (m, 1 H, H-14), 1.05 (m, 1 H, H_a-12), 1.08 (m, 1 H, H_a-8), 1.22 (qd, 1 H, J = 12.1, 5.4 Hz, H_a-15), 1.26 (s, 3 H, H-19), 1.29 (m, 1 H, H_a-16), 1.44 (qd, 1 H, J = 12.7, 3.9 Hz, H_{ax}-11), 1.52 (m, 2 H, H-28), 1.54 (m, 1 H, H-17), 1.56 (m, 1 H, H_{eq}-11), 1.65 (qd, 1 H, J = 11.5, 3.1 Hz, H_b-15), 1.73 (td, 1 H, J = 13.6, 4.5 Hz, H_{ax}-1), 1.84 (octet, 1 H, J = 7.0 Hz, H-25), 1.91 (m, 1 H, H_b-8), 1.92 (m, 1 H, H_b-7), 1.92 (m, 1 H, H_b-12), 1.93 (m, 1 H, H_b-16), 2.06 (ddd, 1 H, J = 13.6, 4.7, 3.6 Hz, H_{eq}-1), 2.22 (td, 1 H, J = 7.3, 5.1 Hz, H-24), 2.27 (td, 1 H, J = 14.1, 5.5 Hz, H_{ax}-6), 2.49 (ddd, 1 H, J = 16.9, 4.5, 3.6 Hz, H_a-2), 2.60 (m, 1 H, H_b-2), 2.61 (m, 1 H, H-20), 2.63 (m, 1 H, H_a-22), 2.81 (dd, 1 H, J = 17.6, 10.1 Hz, H_b-22), 3.22 (ddd, 1 H, J = 14.1, 3.6, 2.7 Hz, H_{eq}-6).

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Supplementary Material Available: Tables of ^1H and ^{13}C NMR assignments for **4** and **5** including two-dimensional spectral correlations, ^1H, ^{13}C, HMQC, and ^1H–^1H COSY spectra for **3**–**5**, HMBC spectra for **3** and **5**, and ROESY spectra for **4** (23 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.