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Unusual Chlorinated Homo-Diterpenes from the South African Nudibranch *Chromodoris hamiltoni*

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Abstract: Extracts of the South African nudibranch *Chromodoris hamiltoni* were found to contain four unusual chlorinated homo-diterpenes, hamiltonins A – D (**3a–6**), and a new sesterterpene hamiltonin E (**7**), in addition to the known toxins latrunculins A (**1**) and B (**2**).

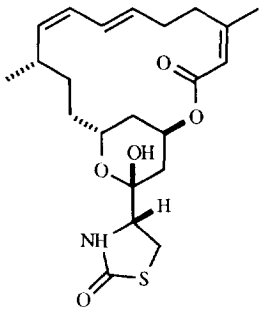
As part of our on-going investigation of the secondary metabolites of South African marine invertebrates, seven specimens of the brilliantly colored nudibranch *Chromodoris hamiltoni* were collected by hand using scuba (-30 m) from Aliwal Shoal, near Durban, South Africa. These organisms attracted our interest because *Chromodoris* species have been shown to sequester compounds possessing new structures and in vitro toxicity towards human pathogens.[1] In addition, these vividly pigmented animals were eye-catching and emitted a pleasing floral fragrance.

The specimens of *C. hamiltoni* were stored in acetone for approximately one month prior to decanting the solvent and repeated extraction with acetone. The extracts were pooled and chromatographed on silica gel with a solvent gradient from hexane through dichloromethane and ethyl acetate to methanol. The least polar fractions contained compounds which were identified by ^{1}H NMR as mixtures of sterols and sweet-smelling alkyl ketones. A more polar fraction contained a mixture of two compounds that was purified by normal phase HPLC to yield latrunculins A (**1**) and B (**2**)[2] which were identified from low resolution mass spectra and by comparison of the ^{1}H and ^{13}C NMR data with literature values.[3] It is interesting to note that latrunculin A (**1**) has previously been reported from *Chromodoris elisabethina* and *C. lochi*.[4] Detailed studies of the biological effects of latrunculins A and B show that these compounds disrupt normal cell organization and function.[2,3]

Further purification of the more polar *C. hamiltoni* fractions by column chromatography and normal phase HPLC led to the isolation of the chlorinated homo-diterpenes hamiltonin A (**3a**, 3.4 mg, 0.5 mg/animal), hamiltonin B (**4a**, 4.3 mg, 0.6 mg/animal), hamiltonin C (**5**), and hamiltonin D (**6**) and the sesterterpene hamiltonin E (**7**, 0.3 mg, 0.04 mg/animal).

Hamiltonin A (**3a**) was isolated as an optically active, colorless oil. The HRFAB mass spectrum indicated that the molecular formula was $C_{21}H_{31}O_3Cl$, which required six degrees of unsaturation. An absorption in the IR spectrum at 1760 cm^{-1} in conjunction with a ^{13}C NMR signal at δ 176.5 established the presence of a γ–lactone which, in addition to olefinic carbons at 109.6 (C-18), 121.3 (C-12), 129.5 (C-13) and

150.0 (C-4), indicated that hamiltonin A (**3a**) was tricyclic (Table I).



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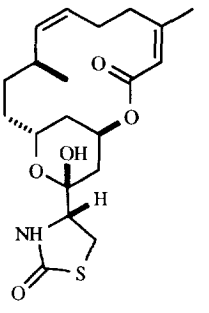

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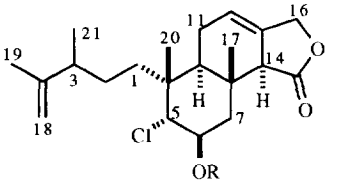

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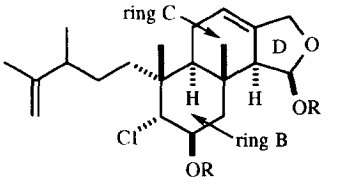
3b R = Ac



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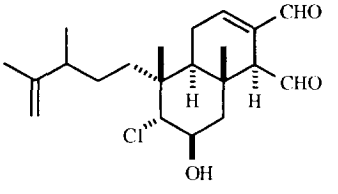

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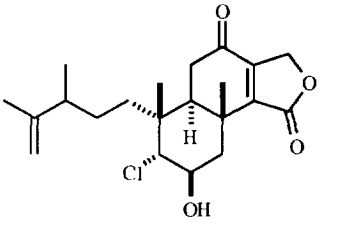

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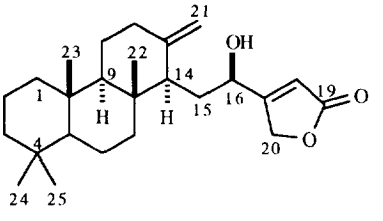

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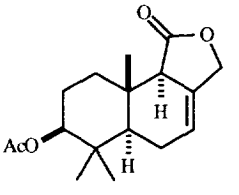

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The ^{1}H NMR spectrum contained four methyl signals at δ 1.01 (Me-21), 1.12 (Me-17), 1.23 (Me-20) and 1.62 (Me-19) as well as signals at 4.64 (H-18') and 4.66 (H-18) which were assigned to an exocyclic methylene (HMQC correlation to a carbon triplet at δ_C = 109.6), indicating that **3a** contained one methyl group more than normal spongian diterpenes. HMBC correlations from the methyl singlet at δ 1.12 (Me-17)

to carbon resonances at 33.8 (C-8), 37.5 (C-7), 43.4 (C-9) and 54.5 (C-14) indicated that Me-17 was located on quaternary carbon C-8 adjacent to two methines (C-9 and C-14) and one methylene (C-7). The COSY spectrum contained correlations from a pair of resonances at δ 2.56 and 2.15 (H-7β and H-7α, respectively, HMQC correlations to δ_C = 37.5) to a multiplet at 4.35 (H-6), which was correlated in turn to a doublet at 4.05 (H-5, HMQC correlation to δ_C = 68.1). Correlations in the HMBC spectrum from the proton resonance at δ 1.23 (Me-20) to carbon signals at 39.0 (C-10), 40.0 (C-1), 43.4 (C-9) and 68.1 (C-5) led to the location of Me-20 on the quaternary carbon C-10 and established the connectivity of the B ring.

Table I. ^1H and ^{13}C NMR Data for Hamiltonin A (**3a**)

Carbon no.	δ_H	(mult.)	δ_C	(mult.)
1	1.26	(m)	40.0	(t)
2	1.41	(m)	27.0	(t)
	1.27	(m)		
3	2.06	(m)	41.7	(d)
4	–		150.0	(s)
5	4.05	(br s)	68.1	(d)
6	4.35	(m)	72.0	(d)
7	2.56	(br d, H_β)	37.5	(t)
	2.15	(m, H_α)		
8	–		33.8	(s)
9	1.85	(dd, J = 12, 5 Hz)	43.4	(d)
10	–		39.0	(s)
11	2.17	(m, H_α)	22.7	(t)
	2.04	(m, H_β)		
12	5.75	(br s)	121.3	(d)
13	–		129.5	(s)
14	2.86	(br s)	54.5	(d)
15	–		176.5	(s)
16	4.66	(H_β)	69.9	(t)
	4.64	(H_α)		
17	1.12	(s)	16.5	(q)
18	4.66	(br s)	109.6	(t)
	4.64	(br s)		
19	1.62	(s)	19.1	(q)
20	1.23	(s)	18.8	(q)
21	1.01	(d, J = 10 Hz)	19.9	(q)

Acetylation of hamiltonin A (**3a**) yielded acetate **3b** as the single product. The ^1H NMR spectrum of **3b** in $CDCl_3$ contained a methyl singlet at δ 2.03 consistent with the presence of one acetate group. The

^{1}H NMR resonance at 4.35 (H-6) in the spectrum of **3a** was shifted downfield to 5.27 while the H-5 signal found at 4.05 in the spectrum of **3a** did not shift appreciably (4.09 in **3b**) leading to the assignment of a chlorine substituent at C-5 and a hydroxy group at the C-6 position of hamiltonin A (**3a**).

Correlations in the HMBC spectrum of hamiltonin A (**3a**) from the methyl singlet at δ 1.62 (Me-19) to carbon resonances at 41.7 (C-3), 109.6 (C-18) and 150.0 (C-4) located Me-19 adjacent to an exocyclic methylene which was, in turn, attached to a methine carbon (C-3, HMQC correlation to $\delta_H = 2.06$). The proton multiplet at δ 2.06 (H-3) had a COSY correlation to a resonance at 1.01 (Me-21) in addition to HMBC correlations to carbon signals at 27.0 (C-2) and 40.0 (C-1) establishing the connectivity of the *seco*-A ring.

Ring C connectivity was determined by analysis of COSY correlations from the doublet of doublets at δ 1.85 (H-9, HMQC correlation to $\delta_C = 43.4$) to resonances assigned to a methylene pair at 2.04 (H-11β) and 2.17 (H-11α) which were strongly coupled, in turn, to an olefinic proton resonance at 5.75 (H-12, HMQC correlation to $\delta_C = 121.3$). The overlapping ^{1}H NMR resonances of H-16 (δ 4.64, 4.66) and H-18 (4.64, 4.66) in $CDCl_3$ complicated elucidation of the D ring structure; consequently the carbon and proton NMR resonances of **3a** were measured in benzene-d_6, improving dispersion of the ^{1}H NMR spectrum, so that all signals could be assigned by analysis of COSY, HMQC and HMBC data. The HMBC spectrum in benzene-d_6 contained correlations from a proton resonance at δ 4.01 (H-16, H-16') to carbon signals at 120 (C-12), 131 (C-13) and 175 (C-15) thus the lactone carbonyl was assigned to C-15. The ^{1}H and ^{13}C NMR shifts assigned to the D ring of **3a** in $CDCl_3$ were in close agreement with those reported for the synthetic drimane sesquiterpene **8**.[5] These results established that **3a** possessed the new 3-homo-4,5-*seco*-spongian skeleton.

The relative stereochemistry of hamiltonin A (**3a**) was determined from coupling constants and NOE difference experiments. Upon irradiation of the methyl signal at δ 1.12 (Me-17) an NOE enhancement was observed for the methyl signal at 1.23 (Me-20, 6% enhancement) locating Me-17 in a 1,3-diaxial conformation relative to Me-20. Irradiation of the signal at δ 1.23 (Me-20) caused enhancement of signals at 1.12 (Me-17, 1%) and 4.05 (H-5, 10%), permitting assignment of the C-5 chlorine substituent to the α face of **3a**. From the ^{1}H NMR spectrum it was determined that H-6 (δ 4.35) was coupled to H-5 with a coupling constant of 4 Hz, to H-7β with $J < 1$ Hz, and to H-7α with $J = 4$ Hz. These coupling constants were consistent with H-6 in the equatorial position. Irradiation of the broad singlet at δ 2.86 (H-14) induced NOE enhancements in resonances at 1.85 (H-9, 15%) and 2.15 (H-7α, 8%) consistent with the location of H-9, H-14 and H-7α on the α face of the carbon skeleton. The stereochemistry at C-3 was not determined.

The ^{1}H NMR spectrum (Table II) of hamiltonin B (**4a**), isolated as a transparent oil, closely resembled that of hamiltonin A (**3a**). The mass spectrum established the molecular formula to be $C_{21}H_{33}O_3Cl$. The IR spectrum of metabolite **4a** contained a broad band at 3500–3300 cm^{-1} but lacked a band corresponding to the presence of a carbonyl moiety while the ^{13}C NMR spectrum of **4a** contained a resonance at δ 99.1 (C-15) in place of the signal at 176.5 (C-15) in the spectrum of **3a**. Analysis of the COSY, HMQC and HMBC data established that the 3-homo-4,5-*seco*-A, B and C rings of **4a** were identical to those of **3a**. A broad doublet at δ 4.17 (H-16β) showed HMBC correlations to carbon signals at 62.1 (C-14), 99.1 (C-15), 117.2 (C-12) and 136.0 (C-13) which indicated that the C and D rings of **4a** were functionalized with a $\Delta^{12,13}$ unsaturation and a hemiketal at C-15. Acetylation of **4a** led to the formation of the diacetate **4b** indicating that the 5-chloro-6-hydroxy substitution pattern found in hamiltonin A (**3a**) was also found in **4a**. The ^{1}H and ^{13}C NMR chemical shifts, the magnitude of the coupling constants and results of NOE difference experiments indicated that the

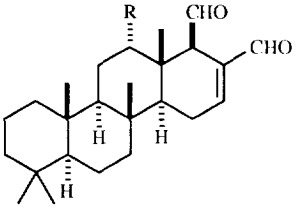
relative stereochemistry of **4a** did not differ from that of hamiltonin A (**3a**). Irradiation of the broad singlet at δ 2.27 (H-14) induced NOE enhancements in resonances at 1.79 (H-9, 8%) and 5.30 (H-15, 4%), consistent with the location of H-9, H-14 and H-15 on the α face of the carbon skeleton.

Table II. ^{1}H and ^{13}C NMR Data for Hamiltonin B (**4a**)

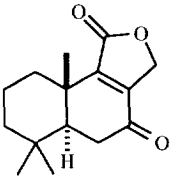
Carbon no.	δ_H	(mult.)	δ_C	(mult.)
1	1.27		39.8	(t)
2	1.38		27.0	(t)
	1.27	(m)		
3	2.03	(m)	41.6	(d)
4	–		149.9	(s)
5	4.02	(d, J = 3 Hz)	68.7	(d)
6	4.32	(m)	72.1	(d)
7	2.15	(dd, J = 16, 4 Hz, H_α)	38.6	(t)
	1.86	(br d, J = 16 Hz, H_β)		
8	–		32.5	(s)
9	1.79	(dd, J = 12, 5 Hz)	43.6	(d)
10	–		38.4	(s)
11	2.12	(br s, H_α)	23.0	(t)
	1.93	(br t, J = 15 Hz, H_β)		
12	5.52	(br s)	117.2	(d)
13	–		136.0	(s)
14	2.27	(br s)	62.1	(d)
15	5.30	(t, J = 5 Hz)	99.1	(d)
16	4.45	(br d, J = 11 Hz, H_α)	68.8	(t)
	4.17	(br d, J = 11 Hz, H_β)		
17	1.05	(br s)	16.4	(q)
18	4.65	(br s)	109.4	(t)
	4.66	(br s)		
19	1.62	(s)	18.7	(q)
20	1.23	(s)	19.3	(q)
21	0.99	(d, J = 7 Hz)	19.8	(q)
OH	2.70	(d, J = 5 Hz)		

Hamiltonins C (**5**) and D (**6**) were isolated in sub-milligram amounts. Like **4a**, both appeared from ^{1}H NMR data to be related to hamiltonin A (**3a**). While the small quantities of **5** and **6** precluded the possibility of obtaining ^{13}C NMR spectra, excellent COSY and good HMQC and HMBC spectra were acquired. Analysis of the data indicated that **5** and **6** differed from **3a** only in D ring structure. The ^{1}H NMR spectrum of hamiltonin C (**5**) contained aldehyde signals at δ 9.52 (d, 1H, J = 5 Hz, H-15) and 9.46 (s, 1H,

H-16). Bands at 1730 and 1685 cm^{-1} in the IR spectrum of **5** were diagnostic of saturated and α,β-unsaturated aldehydes, respectively. The COSY spectrum of **5** contained correlations from a resonance at δ 1.71 (H-9) to multiplets at 2.39 (H-11) and 2.49 (H-11') which were correlated, in turn, to an olefinic proton at 7.13 (H-12). The resonance at δ 7.13 (H-12) exhibited allylic coupling to a signal at 2.83 (H-14) which was coupled in turn to the doublet at 9.52 (H-15) permitting location of the saturated aldehyde functionality at C-14 while the α,β-unsaturated aldehyde was placed at C-13 by process of elimination. The chemical shifts for protons and protonated carbons of **5** were in excellent agreement with those reported for **9**, a secondary metabolite possessing the same 1,2-dialdehyde moiety as **5**, which was isolated from the Mediterranean nudibranch *Hypselodoris orsini*.[6]



9 R = OAc
11 R = H



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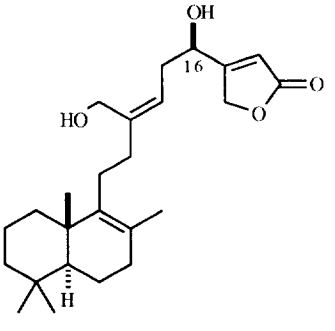
The COSY spectrum of hamiltonin D (**6**) contained correlations from a resonance at δ 2.39 (H-9) to a pair of methylene proton resonances at 2.53 (H-11β) and 2.65 (H-11α), forming an isolated spin system. The downfield shift of the H-11 proton resonances compared with corresponding signals in the spectra of hamiltonins A–C (**3a**–**5**) in conjunction with absorptions in the IR spectrum of **6** at 1760 and 1685 cm^{-1} indicated an α,β-unsaturated carbonyl was likely situated at C-12 and conjugated to an α,β-unsaturated γ-lactone located at C-15. The UV absorptions at 206 and 238 nm were in reasonable agreement with absorptions at 217 and 242 nm in the UV spectrum of the terrestrial plant metabolite **10**.[7] The ^{13}C NMR shifts of C-13 and C-14 (δ 150, 153) and the IR spectrum of **6** were also in excellent agreement with those reported for **10**, supporting the assignment of the unsaturated carbonyl to C-15 rather than C-16.[6] The ^{1}H and ^{13}C NMR shifts for the A–C ring portions of **5** and **6** were in good agreement with the data for hamiltonin A (**3a**) and consequently both were assigned the same stereochemistry.

The final compound isolated was hamiltonin E (**7**), a colorless oil, present as a minor metabolite in the extract of *C. hamiltoni*. The molecular formula, $C_{25}H_{38}O_3$, required seven degrees of unsaturation. The connectivity of the A and B rings was determined by analysis of the HMQC, HMBC and COSY spectra of **7**. The ^{1}H and ^{13}C NMR chemical shift data for this moiety were in excellent agreement with the values reported for the A and B rings of 1,2-deacetoxyscalaradial (**11**) from *Cacospongia mollior*.[8] The HMBC spectrum of **7** contained correlations from the methyl singlets at δ 0.66 (Me-22) and 0.77 (Me-23) to a carbon resonance at 60 (C-9, HMQC correlation to δ_H = 1.01). The proton resonance at δ 1.01 (H-9), in turn, had COSY correlations to a pair of methylene proton resonances at 1.30 (H-11) and 1.70 (H-11') which were further coupled to methylene signals at 1.90 (H-12) and 2.38 (H-12'). The proton resonance at δ 1.90 (H-12) showed further coupling to a signal assigned to an exocyclic methylene proton at 4.90 (H-21). The carbon resonance

at δ 107 had correlations in the HMQC spectrum to protons at 4.90 (H-21) and 4.63 (H-21') and the latter proton signal was coupled to a proton multiplet at 1.56 (H-14). The proton resonance at δ 1.56 (H-14) showed an HMBC correlation to a carbon signal at 15 (C-22), establishing the connectivity of the C ring, and was also coupled to a pair of methylene proton signals at 1.86 (H-15) and 1.80 (H-15'). The COSY spectrum showed couplings from the H-15 and H-15' resonances to a multiplet at δ 4.68 (H-16, HMQC correlation to δ_C = 69) which, in turn, was coupled to an exchangeable proton at 1.99 and exhibited allylic coupling to an olefinic proton at 5.89 (H-18). The only remaining, unassigned proton signals, at δ 4.85 (dd, 1H, J = 1, 18 Hz, H-20') and 4.91 (dd, 1 H, J = 1, 18 Hz, H-20), exhibited geminal coupling to one another and allylic coupling to the proton resonance at 5.89 (H-18) leading to the conclusion that **7** contained an α,β-unsaturated γ-lactone (IR 1740 cm^{-1}). The IR, ^1H and ^{13}C NMR data for the γ-lactone of **7** were comparable to the assigned values for luffarin-I (**12**), a sponge metabolite that contained the same moiety.[9]

The absolute stereochemistry at C-16 of hamiltonin E (**7**) was studied by measuring the CD spectrum and comparing it with that of luffarin-I (**12**) and (4*S*)- and (4*R*)-manoalide diols (**13a** and **13b**, respectively),[9,10] all of which contained a secondary alcohol adjacent to a γ-lactone. The CD spectrum of hamiltonin E (**7**) contained a minimum at 218 nm (Θ -2 300) which compared favorably to the corresponding data for luffarin-I (**12**, (16*R*), $(\Theta)_{218}$ -1 500) and (4*R*)-manoalide diol (**13b**) which had a negative CD minimum at 217 nm.[9,10] Conversely, synthetic (4*S*)-manoalide diol (**13a**) had a positive CD maximum at 217 nm and thus the data for **7** was consistent with the assignment of 16*R* stereochemistry to hamiltonin E (**7**).[9,10] The relative stereochemistry for rings A and B was determined by comparing ^1H and ^{13}C NMR shifts with published values.[10]



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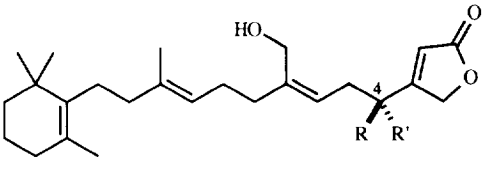

13a R = OH, R' = H
13b R = H, R' = OH

Relatively few chlorinated metabolites have been reported from sponges or nudibranchs.[11] In a number of instances it was proposed that halogenation resulted from reaction of polyene precursors with "chloronium ions".[12] Since halonium ion initiated cyclization inevitably produces cyclic products in which the halogen is equatorial, the diaxial conformation of the 5α-chloro,6β-hydroxyl substituents suggests that the biosynthesis of the hamiltonins A–D (**3a–6**) may proceed via chloride attack on a 5β,6β-epoxide intermediate. The feasibility of such a reaction has been demonstrated by Minale and co-workers.[13]

Two of the *Chromodoris hamiltoni* specimens were dissected and the internal organs dissolved in bleach. Inspection of the gut contents revealed spicules typical of sponges of the genus *Latrunculia*. Hamiltonins A and B showed no significant activity in antimicrobial and cytotoxicity (HCT-116) bioassays.

EXPERIMENTAL SECTION

Infrared spectra were recorded on a Perkin-Elmer 1600 series FTIR spectrophotometer. A Perkin-Elmer Lambda 3B UV/vis spectrometer was used to measure UV spectra. NMR experiments were recorded on Brüker WP-200 SY and Varian 500 MHz spectrometers. Unless otherwise specified, all NMR data were acquired on samples in $CDCl_3$ and chemical shifts were reported relative to the residual solvent peaks. Optical rotations were measured on a Rudolf Research Autopol III polarimeter using a 10 cm cell and the CD spectrum was acquired using a Cary 300 spectrometer. Low resolution mass spectra were recorded on a Hewlett-Packard 5988A spectrometer while high resolution spectra were obtained from the University of British Columbia and the regional facility at UC Riverside.

Collection and Extraction of *Chromodoris hamiltoni* : Seven specimens of *Chromodoris hamiltoni* were collected by hand using scuba (-30 m) from Aliwal Shoal, Republic of South Africa. The nudibranchs were stored in acetone (50 mL) for one month. The extract was decanted and the specimens were re extracted with acetone. The two extracts were pooled and concentrated to yield a fragrant residue (57 mg). The metabolites were separated by normal phase short column chromatography (TLC-grade silica gel 5–40 μ mesh) using a solvent gradient of hexane through dichloromethane through ethyl acetate through methanol. The natural products were purified by short column chromatography or by normal phase HPLC. The acetone extract yielded latrunculin A (**1**, 5.3 mg, 0.7 mg/animal), latrunculin B (**2**, 3.4 mg, 0.5 mg/animal), hamiltonin A (**3a**, 3.4 mg, 0.5 mg/animal), hamiltonin B (**4a**, 4.3 mg, 0.6 mg/animal), hamiltonin C (**5**,), hamiltonin D (**6**) and hamiltonin E (**7**, 0.3 mg, 0.04 mg/animal).

Hamiltonin A (3a): oil; $[\alpha]_D$ -60° (*c* 0.027, CH_2Cl_2); UV (methanol) 218 nm (ε 3100); IR (film) 3500–3400, 2930, 1760 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) see Table I; (C_6D_6) δ: 1.06 (d, *J* = 7 Hz, Me-21), 1.08 (s, Me-20), 1.12 (m, H-1), 1.13 (s, Me-17), 1.24 (m, H-2), 1.27 (m, H-1'), 1.46 (m, H-2'), 1.60 (m, H-9), 1.60 (m, H-11), 1.62 (br s, Me-19), 1.81 (m, H-11'), 2.03 (dd, *J* = 4, 16 Hz, H-7α), 2.09 (m, H-3), 2.19 (br s, H-14), 2.66 (br d, *J* = 16 Hz, H-7β), 3.66 (br s, H-5), 3.92 (t, *J* = 3 Hz, H-6), 4.01 (br s, H-16, H-16'), 4.81 (br s, H-18, H-18'), 5.00 (br s, H-12); ^{13}C NMR (125 MHz, $CDCl_3$) see Table I; (C_6D_6) [15] δ: 16 (q, C-17), 18. (q, C-19), 18 (q, C-20), 20 (q, C-21), 22 (t, C-11), 27 (t, C-2), 33 (s, C-8), 37 (t, C-7), 39 (s, C-10), 40 (t, C-1), 42 (d, C-3), 43 (d, C-9), 54 (d, C-14), 68 (d, C-5), 69 (t, C-16), 72 (d, C-6), 109 (t, C-18), 120 (d, C-12), 131 (s, C-13), 150 (s, C-4), 175 (s, C-15); HRCIMS obsd. *m/z* 384.2325, $C_{21}H_{35}NO_3{}^{35}Cl$ $(M+NH_4)^+$ requires 384.2306.

Acetylation of hamiltonin A (3a): Hamiltonin A (**3a**, 0.5 mg) was dissolved in dry pyridine (0.5 mL) and freshly distilled acetic anhydride (0.5 mL). The reaction was stirred overnight and excess reagent removed under vacuum. One product was observed in the TLC and ^1H NMR spectrum. **Hamiltonin A acetate (3b):** ^1H NMR (500 MHz, $CDCl_3$) δ: 0.99 (d, *J* = 7 Hz, Me-21), 1.13 (s, Me-17), 1.61 (s, Me-19), 2.03 (s, OAc),

2.88 (br s, H-14), 4.09 (dd, J = 2, 2 Hz, H-5), 4.66 (m, H-16, H-16', H-18, H-18'), 5.27 (m, H-6), 5.77 (m, H-12); LRCIMS obsd. m/z $(M+NH_4)^+$ 426 (100%), 428 (49%).

Hamiltonin B (4a): oil; $[\alpha]_D$ -8° (c 0.29, CH_2Cl_2); IR (film) 3500–3300, 2930 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) see Table II; ^{13}C NMR (50 MHz, $CDCl_3$) see Table II; HRCIMS obsd. m/z 351.2069, $C_{21}H_{32}O_2{}^{35}Cl$ $(M-OH)^+$ requires 351.2091.

Acetylation of hamiltonin B (4a): Hamiltonin B (**4a**) was acetylated in the same way as described for hamiltonin A (**3a**). **Hamiltonin B diacetate (4b):** ^1H NMR (500 MHz, $CDCl_3$) δ: 0.99 (d, J = 7 Hz, Me-21), 1.02 (s, Me-17), 1.61 (s, Me-19), 2.04 (s, OAc), 2.07 (S, OAc), 4.08 (d, J = 2 Hz, H-5), 4.24 (d, J = 12 Hz, H-16'), 4.40 (br d, J = 12 Hz, H-16), 4.64 (br s, H-18'), 4.66 (br s, H-18), 5.22 (m, H-6), 5.60 (m, H-12), 6.16 (br d, J = 3 Hz, H-15).

Hamiltonin C (5): oil; UV (methanol) 215 nm; IR (film) 3400–3300, 2930, 1730, 1685, 1650 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ: 1.00 (d, J = 7 Hz, Me-21), 1.19 (s, Me-17), 1.25 (H-2), 1.27 (s, Me-20), 1.62 (br s, Me-19), 1.71 (m, H-9), 1.86 (br d, J = 15 Hz, H-7β), 2.03 (m, H-3), 2.30 (dd, J = 3, 15 Hz, H-7α), 2.39 (m, H-11), 2.49 (m, H-11'), 2.83 (m, H-14), 4.01 (d, J = 3 Hz, H-5), 4.35 (m, H-6), 4.65 (br s, H-18), 4.67 (br s, H-18'), 7.13 (m, H-12), 9.46 (s, H-16), 9.52 (d, J = 5 Hz, H-15); ^{13}C NMR (125 MHz, $CDCl_3$)[15] δ: 18 (q, C-17), 19 (q, C-19), 20 (q, C-20), 20 (q, C-21), 27 (t, C-2), 39 (t, C-7), 40 (t, C-1), 42 (d, C-3), 68 (d, C-5), 72 (d, C-6), 111 (t, C-18), 150 (s, C-4); LRCIMS obsd. m/z $(M+H)^+$ 367 (21%), 369 (12%).

Hamiltonin D (6): oil; UV (methanol) 206, 238 nm; IR (film) 3550–3400, 2925, 1760, 1685 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ: 1.00 (d, J = 7 Hz, Me-21), 1.22 (s, Me-17), 1.29 (s, Me-20), 1.62 (br s, Me-19), 1.95 (d, J = 4 Hz, OH), 2.05 (m, H-3), 2.19 (dd, J = 5, 15 Hz, H-7'), 2.39 (dd, J = 3, 14 Hz, H-9), 2.53 (dd, J = 14, 17 Hz, H-11β), 2.65 (dd, J = 3, 17 Hz, H-11α), 2.75 (br d, J = 15 Hz, H-7), 4.06 (d, J = 3 Hz, H-5), 4.40 (m, H-6), 4.65 (br s, H-18), 4.67 (br s, H-17'), 4.85 (s, H-16, H-16'); ^{13}C NMR (125 MHz, $CDCl_3$)[15] δ: 19 (q, C-19), 19 (q, C-20), 20 (q, C-21), 27 (t, C-2), 39 (d, C-10), 40 (t, C-1), 42 (d, C-3), 45 (d, C-9), 68 (t, C-16), 68 (d, C-5), 111 (t, C-18), 150 (s, C-4), 150 (s, C-14[14]), 153 (s, C-13[14]); HREIMS obsd. m/z 398.2091 $C_{21}H_{33}NO_4{}^{35}Cl$ $(M+NH_4)^+$ requires 398.2098.

Hamiltonin E (7): oil; UV (methanol) 203, 263 nm; IR (film) 3500–3400, 2830, 1780, 1740 cm^{-1}; CD (MeOH) $(\Theta)_{202}$ 9 200, $(\Theta)_{218}$ -2 300, $(\Theta)_{244}$ -2 300; ^1H NMR (500 MHz, $CDCl_3$) δ: 0.66 (s, Me-22), 0.77 (s, Me-23), 0.77 (s, Me-25), 0.80 (H-1), 0.82 (H-5), 0.83 (s, Me-24), 1.01 (m, H-7), 1.01 (m, H-9), 1.10 (m, H-3), 1.30 (m, H-11), 1.34 (m, H-3'), 1.35 (H-6), 1.37 (m, H-2), 1.56 (H-2'), 1.56 (H-6'), 1.56 (m, H-14), 1.62 (H-1'), 1.70 (H-11'), 1.74 (m, H-7'), 1.80 (m, H-15'), 1.86 (m, H-15), 1.90 (H-12), 1.99 (d, J = 4 Hz, OH), 2.38 (ddd, J = 3, 4, 13 Hz, H-12'), 4.63 (br s, H-21'), 4.68 (m, H-16), 4.85 (dd, J = 1, 18 Hz, H-20'), 4.90 (br s, H-21), 4.91 (dd, J = 1, 18 Hz, H-20), 5.89 (br d, J = 1 Hz, H-18); ^{13}C NMR (125 MHz, $CDCl_3$)[15] δ: 15 (q, C-22), 16 (q, C-23), 18 (t, C-6), 19 (t, C-2), 21 (q, C-25), 23 (t, C-11), 30 (t, C-15), 33 (q, C-24), 38 (s, C-10), 38 (t, C-12), 40 (t, C-1), 40 (t, C-7), 42 (t, C-3), 52 (d, C-5), 53 (d, C-14), 60 (d, C-9), 69 (d, C-16), 70 (t, C-20), 107 (t, C-21), 116 (d, C-18); HREIMS obsd. m/z 386.2837, $C_{25}H_{38}O_3$ $(M)^+$ requires 386.2821.

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14. Values may be interchanged.
15. ^{13}C NMR shifts were determined from HMQC and HMBC spectra.

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