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New Spongiane Diterpenes from the East African Nudibranch *Chromodoris hamiltoni* †

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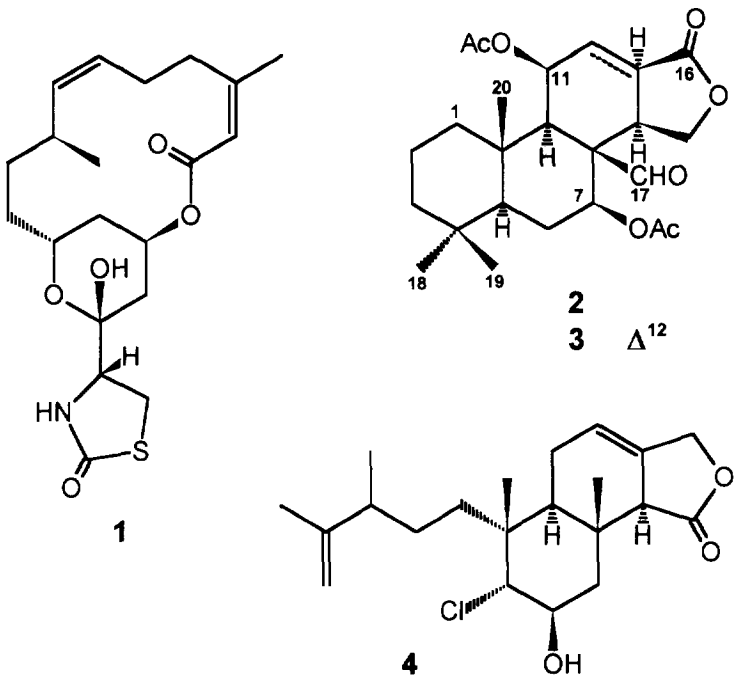
Abstract: The known ichthyotoxin latrunculin B (**1**) and two new spongiane diterpene lactones, 7β,11β-diacetoxy-16-oxospongian-17-al (**2**) and 7β,11β-diacetoxy-16-oxospongi-12-en-17-al (**3**) were isolated from specimens of the nudibranch *Chromodoris hamiltoni* collected off Mozambique.

Dorid nudibranchs, or sea slugs, of the genus *Chromodoris* are brightly colored and have few predators.[1] The vividly colored *C. hamiltoni*, a common species found on subtidal tropical reefs along the east African coast,[2] is no exception and is known to sequester noxious metabolites from the sponges on which it feeds and to concentrate these chemicals in its mantle tissue.[3] "Defense chemicals" so acquired, are exuded from glands in the skin when the nudibranch is molested. Therefore skin extracts from dorid nudibranchs are potentially rich sources of new bioactive secondary metabolites[4] and in continuation of our search for bioactive compounds from Southern African nudibranchs we have examined specimens of *C. hamiltoni* collected from Malangaan reef off southern Mozambique. Although the natural product constituents of *C. hamiltoni* from the Kwazulu-Natal coast of South Africa (500kms south of Malangaan) have been examined before,[3] extracts from nudibranchs that sequester secondary metabolites often vary significantly when collected from different localities.[5] Changes in a nudibranch species' secondary metabolite content reflect biogeographical variation in the nudibranch's dietary organisms and our study has shown that *C. hamiltoni's* acquisition of toxic natural products from sponges is consistent with this observation.

Fifteen small specimens of *C. hamiltoni* were steeped in acetone for four months, the acetone decanted and the nudibranchs extracted repeatedly with further acetone. Concentration under reduced pressure of the combined acetone extracts followed by partitioning between ethyl acetate and water gave a crude ethyl acetate partition fraction which was purified by HPLC (6:4 and 3:7 EtOAc/hexane). The initial HPLC separation yielded a compound whose NMR and HREIMS data were in accordance with those of latrunculin B (**1**, 4.2mg, 0.28mg/animal) a potent ichthyotoxin found in latrunculid sponges.[6] Interestingly, **1** and its homolog latrunculin A, were also identified in the acetone extract of the earlier collection of *C. hamiltoni* from the Natal/Kwazulu coast.[3]

† Dedicated to Professor Emeritus Douglas Rivett on the occasion of his 75th birthday.



1 2 3 Δ^{12} 4

No evidence of the latter latrunculin was found in the extract of the Mozambique specimens of *C. hamiltoni*. Dissection of five specimens of this nudibranch and examination of the gut contents revealed acanthorhab spicules, typically found in latrunculid sponges, suggesting that these sponges are a common source for the defensive chemicals sequestered by *C. hamiltoni* along the south east coast of Africa.

Further HPLC purification of the ethyl acetate partition fraction yielded two new spongiane diterpenes, 7β, 11β-diacetoxy-16-oxospongian-17-al (**2**, 2.1mg, 0.14mg/animal) and 7β, 11β-diacetoxy-16-oxospongi-12-en -17-al (**3**, 1.9mg, 0.13mg/animal) as colorless oils. A molecular formula of $C_{24}H_{34}O_7$ for **2** ($[\alpha]_D^{21}$ +38°) was deduced from NMR data and confirmed by the HREI mass spectrum (*m/z* 434.2287, Δmmu -9). Of the eight degrees of unsaturation required by the molecular formula, four could be assigned to the carbonyl groups of a lactone functionality, an aldehyde and two acetate moieties, evidenced in the ^{13}C NMR spectrum of **2** by resonances at δ176.3, 201.0, 169.2 and 169.6 respectively. The remaining four degrees of unsaturation therefore implied a tetracyclic substructure for **2**.

The NMR data for **2** (Table 1) were consistent with published data for a basic tetracyclic spongiane skeleton[7] and were supported by characteristic spongiane diterpene fragment ions observed at *m/z* 201, 123 and 109 in the LREI mass spectrum of **2**.[8] Two and three bond HMBC correlations from the aldehyde proton singlet (δ10.19) to carbon resonances at δ 53.8 (C-8) and 52.3 (C-14), together with a long range COSY coupling to H-14 (δ1.93), placed the aldehyde moiety at C-8. A three bond HMBC correlation from the deshielded proton doublet H-7

Table 1. ^{13}C (100 MHz, $CDCl_3$), ^{1}H (400 MHz, $CDCl_3$) and HMBC NMR data for compound 2

Carbon number	δ_C ppm (Mult)	δ_H ppm (Mult, J/Hz)	HMBC to
1	39.1(t)	0.99(td), 1.80(d)	-
2	18.3(t)	1.58(br m)	-
3	41.3(t)	1.19(td), 1.48(d, 12)	C2, C4
4	33.4(s)	-	-
5	53.9(d)	1.06(d, 13)	C22, C20, C6, C4, C10
6	25.2(t)	2.13(br d), 1.79(q, 12)	C10, C8, C7
7	79.9(d)	4.78(dd, 5,12)	C14, C8, Ac CO, C17
8	53.8(s)	-	-
9	61.3(d)	1.24(s)	C22, C10, C14, C5
10	37.7(s)	-	-
11	67.5(d)	5.59(br s)	C13, C8
12	31.5(t)	2.35(dt,3,14) 1.46(t,12)	C13
13	35.3(d)	3.47(td, 4,13)	-
14	52.3(d)	1.93 (br d, 7)	-
15	68.4(t)	4.46(m, 3,10) 4.04(t, 8)	C13, C14,C16
16	176.3(s)	-	-
17	201.0(d)	10.2(s)	C14, C8
18	21.4(q)	0.86(s)	C3, C4, C5, C19, C20
19	33.3(q)	0.92(s)	C3, C4, C5, C18, C20
20	17.9(q)	1.03(s)	C1, C5, C10
$CH_3\underline{CO}$	169.6(s)	-	-
	169.2(s)	-	-
$\underline{CH_3}CO$	21.3(q)	2.05(s)	Ac CO
	21.1(q)	1.99(s)	Ac CO

(δ4.78) to the acetate carbonyl carbon at δ169.6 positioned one acetate functionality at C-7. Although no useful HMBC correlations were observed between proton resonances and the second acetate carbonyl carbon signal (δ169.2), COSY couplings from H-9 (δ1.24) to the deshielded H-11 (δ5.59) proton signal and from the latter proton to the multiplets at δ1.46 and 2.35 (2H-12) unequivocally sited this acetate moiety at C-11. Three bond HMBC correlations from H-11 to C-13 (δ35.3) and C-8, and from H-9 to C-14 (δ52.3) provided conclusive evidence for the connectivity of rings B and C. The five membered lactone ring (ring D) was also delineated from the HMBC data where correlations were observed between the H-15α and H-15β proton triplets (δ4.46 and 4.04) and the ^{13}C resonances at δ176.3 (C-16), 35.3 (C-13) and 52.3(C-14).

The relative stereochemistry of **2** was determined from a combination of 1D NOE difference experiments and a ROESY experiment. The former experiments confirmed the β-orientation of the C-7 acetate moiety as follows. Irradiation of H-7 resulted in enhancements of the equatorial H-6 proton doublet (δ2.13), the axial H-14α multiplet (δ2.93), the axial H-5α doublet (δ1.06) and the axial H-9α singlet (δ1.24). The β-configuration of the

C-11 acetoxy group followed from a ROESY experiment in which NOE correlations were observed between H-11, H-9, H-1α (δ1.80) and both the C-12 protons. This assignment was confirmed by a series of NOE difference experiments. Strong NOE correlations in the ROESY spectrum of **2** between the aldehyde proton and the angular methyl group, C-20 (δ1.03), and H-6β (δ1.79) confirmed the β-orientation of the aldehyde functionality. Finally, irradiation of H-13 (δ3.47) in an NOE difference experiment resulted in a positive enhancement of H-14, confirming the *cis* fusion of rings C and D as found in previously reported spongiane diterpenes.[8]

HREIMS data (432.2153 Δ mmu +13) yielded a molecular formula of $C_{24}H_{32}O_7$ for the second spongiane diterpene, **3** ($[\alpha]_D^{21}$ +97°), isolated from *C. hamiltoni*. Close similarities in NMR data, and a difference of only two mass units between the molecular ions of **2** and **3**, suggested that the latter was an unsaturated analog of **2**. This assumption was supported by the presence of deshielded olefinic resonances at δ129.6 and 129.9 in the ^{13}C NMR spectrum of **3**. The quaternary character of the latter signal required one carbon of the olefinic moiety to be at a ring junction. Accordingly, the coalesence of the signals at δ4.46 and 4.04 (H-15α and H-15β) in the ^1H NMR spectrum of **2**, to give the overlapping triplets (δ4.23 and 4.29) in the analogous spectrum of **3**, and the downfield shift of H-14 (δ1.93 to 2.93) located the double bond at the junction of rings C and D. The olefinic moiety was unequivocally placed in a Δ^{12} position from COSY coupling between the deshielded vinylic proton H-12 (δ6.65) and the oxymethine proton H-11 (δ5.86). Although HMBC correlations from H-11 to C-12 were not observed, correlations from H-14 (δ2.93) and 2H-15 to C-13 were clearly evident. Finally, a ROESY experiment confirmed that the stereochemistry of the aldehyde and the two acetate functionalities in **3** was consistent with that of **2**.

Although the previous examination of *C. hamiltoni* yielded four new unusual homo-diterpenes, analogous to hamiltonin A (**4**), no spongiane diterpenes were found.[3] Conversely, no evidence of hamiltonin type compounds were evident in the extract of the Mozambique specimens of *C. hamiltoni*. The isolation of spongiane diterpenes from nudibranchs is not unique and both spongiane and rearranged spongiane diterpenes have been previously found in nudibranchs from the genera *Chromodoris*[9,10] and *Ceratasoma*.[11] Spongiane diterpenes have also been reported from the sponge genera *Aplysilla*,[7,10,12] *Dictyodendrilla*,[8] *Dysidea*,[13] *Igernella*[14] and *Spongia*[15] and have been shown to be mildly cytotoxic.[15] Unfortunately, the paucity of material isolated in this study precluded any bioactivity investigations while the dietary sponge source of the two spongiane diterpenes in *C. hamiltoni* also needs to be established.

Experimental Section

The ^1H (400MHz) and ^{13}C (100MHz) NMR spectra were recorded on a Bruker AMX400 spectrometer. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. Low resolution mass spectra were recorded on a Hewlett-Packard 5988A spectrometer and high resolution spectra were obtained by Dr P. Boshoff of the Mass Spectrometry Unit at the Cape Technikon, Cape Town. High-performance liquid chromatographic separations were performed on a Whatman Magnum 9 Partisil column.

Collection and Extraction of *Chromodoris hamiltoni*: Fifteen specimens of *Chromodoris hamiltoni* were collected by hand using SCUBA (-27m) from Malangaan Reef off southern Mozambique in September, 1995. The nudibranchs were stored in acetone (100ml) for four months. The extract was decanted and the specimens were re-extracted with acetone. The acetone extracts were pooled, concentrated and partitioned between ethyl acetate and water. The three metabolites, separated as colorless oils from the concentrated ethyl acetate partition layer by normal phase HPLC, were latrunculin B (**1**, 4.2mg, 0.28mg/animal), 7β, 11β-diacetoxy-16-oxospongian-17-al (**2**, 2.1mg, 0.14mg/animal) and 7β, 11β-diacetoxy-16-oxospongi-12-en-17-al (**3**, 1.9mg, 0.13mg/animal).

7β, 11β-Diacetoxy-16-oxospongian-17-al (2): oil; $[\alpha]_D^{21}$ = +38° (*c*, 0.19, $CHCl_3$); ^1H and ^{13}C NMR spectra see Table 1; EIMS (70eV), *m/z* (int, %), 314 (6), 201 (5), 123 (9), 109 (19), 91(12), 69(15), 55 (12), 43 (100); HREIMS obsd. *m/z* 434.2287, $C_{24}H_{34}O_7$ (M^+) requires 434.2296.

7β, 11β-Diacetoxy-16-oxospongi-12-en-17-al (3): oil; $[\alpha]_D^{21}$ = +97° (*c*, 0.18, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 10.02 (s, 1H, H-17), 6.65 (t, 1H, $J_{11,12}$ = 4Hz, H-12), 5.86 (m, 1H, H-11), 4.87 (dd, 1H, $J_{7,8}$ = 12Hz, $J_{6,7}$ = 5Hz), 4.29 (m, 1H, H-15a), 4.23 (m, 1H, H-15b), 2.93 (m, 1H, H-14), 2.11 (dd, 1H, $J_{6,7}$ = 5Hz, $J_{5,6}$ = 16Hz, H-6α), 2.07 (s, 3H, OAc), 2.02 (s, 3H, OAc), 1.91 (m, 1H, H-6β), 1.72 (m, 1H, H-1α), 1.70 (brs, 1H, H-9), 1.55 (m, 2H, 2H-2), 1.49 (dd, 1H, J=3Hz, 12Hz, H-3α), 1.19 (td, 1H, J= 4Hz, 13Hz, H-3β), 1.14 (s, 3H, 3H-20), 1.09 (t, 1H, $J_{1,2}$=12, H-1β), 1.08 (d, 1H, $J_{5,6}$= 12Hz, H-5), 0.94 (s, 3H, 3H-19), 0.89 (s, 3H, 3H-18); ^{13}C NMR ($CDCl_3$) δ 199.2 (s, C-17), 169.76 (s, OAc), 169.2 (s, OAc), 168.3 (s, C-16), 129.9 (s, C-13), 129.6 (d, C-12), 80.5 (d, C-7), 67.9 (t, C-15), 65.2 (d, C-11), 58.3 (d, C-9), 53.7 (d, C-5), 53.2 (s, C-8), 47.6 (d, C-14), 41.1 (t, C-3), 39.1 (t, C-1), 37.9 (s, C-10), 33.5 (q, C-19), 33.3 (s, C-4), 25.5 (t, C-6), 21.8 (q, C-18), 21.2 (q, OAc), 21.1 q, OAc), 18.5 (q, C-20), 18.2 (t, C-2); EIMS (70eV), *m/z* (int, %), 205(5), 161(9), 133(9), 123(13), 109(25), 105(12), 95(11), 91(17), 81(13), 69(18), 43(100); HREIMS obsd. *m/z* 432.2153, $C_{24}H_{32}O_7$ (M^+) requires 432.2140.

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