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TERPENOID METABOLITES FROM SKIN EXTRACTS OF FOUR SRI LANKAN NUDIBRANCHS IN THE GENUS *CHROMODORIS*

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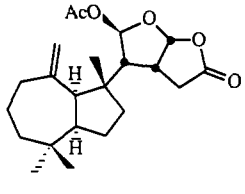
ABSTRACT.—The skin extracts of four species of Sri Lankan dorid nudibranchs belonging to the genus *Chromodoris* have been found to contain diterpenoids. Extracts of *Chromodoris gleneii* contained dendrillolide A [**1**], 12-desacetoxyshahamin C [**2**], and shahamin K [**3**]; extracts of *Chromodoris geminus* contained 12β,15α,16α-triacetoxyspongian [**4**], 6α,15α,16α-triacetoxyspongian [**5**], and 6α,12β,15α,16α-tetraacetoxyspongian [**6**]; extracts of *Chromodoris annulata* contained shahamin F [**7**]; and extracts of *Chromodoris inopinata* contained aplyroseol 2 [**8**], the γ-lactone **9**, and spongian-16-one [**10**]. Compounds **3**, **5**, and **6** are new diterpenoid metabolites. The remaining diterpenoids **1**, **2**, **4**, and **7–10** have been previously isolated from marine sponges or other nudibranchs.

Dorid nudibranchs are shell-less marine molluscs that appear to be extremely vulnerable to predation. It is now well documented that many nudibranchs employ secondary metabolites acquired from sponges in their diets as defensive allomones to thwart predation (1). As part of our ongoing studies of the chemistry of nudibranch skin extracts (2), we have examined four *Chromodoris* species Chromdorididae, (Bergh, 1891) collected in the coastal waters of Sri Lanka. We now report that: extracts of *Chromodoris gleniei* (Kelaart, 1858) contained dendrillolide A [**1**], 12-desacetoxyshahamin C [**2**], and shahamin K [**3**]; extracts of *Chromodoris geminus* (Rudman, 1987) contained 12β,15α,16α-triacetoxyspongian [**4**], 6α,15α,16α-triacetoxyspongian [**5**], and 6α,12β,15α,16α-tetraacetoxyspongian [**6**]; extracts of *Chromodoris annulata* (Eliot, 1904) contained shahamin F [**7**]; and extracts of *Chromodoris inopinata* (Bergh, 1905)



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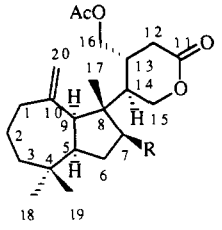
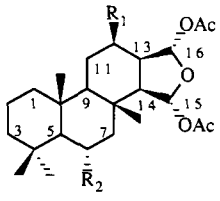
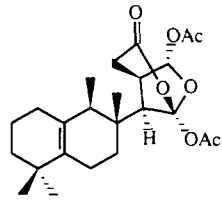


2 R=H
3 R=OAc



4 R_1=OAc, R_2=H
5 R_1=H, R_2=OAc
6 R_1=OAc, R_2=OAc



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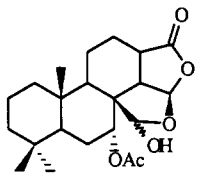

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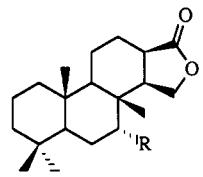

9 R=OAc
10 R=H

contained aplyroseol 2 [**8**], the γ-lactone **9**, and spongian-16-one [**10**]. Metabolites **3**, **5**, and **6** are new diterpenoids.

Specimens of *C. gleniei* (12 animals) were collected on nearshore reefs off Mt. Lavenia, Sri Lanka, in December 1987. Standard extraction and fractionation procedures (see Experimental) gave pure samples of dendrillolide A [**1**] (70 mg), 12-desacetoxyshahamin C [**2**] (7.5 mg), and shahamin K [**3**] (3 mg). The known metabolites dendrillolide A [**1**] and 12-desacetoxyshahamin C [**2**] were identified by comparing their spectral data to literature values (3).

TABLE 1. ^{1}H- (400 MHz) and ^{13}C- (75 MHz) nmr Data for 12-Desacetoxyshahamin C [**2**] and Shahamin K [**3**] (recorded in $CDCl_3$).

Carbon	Compound					
	2		**3**			
	^{1}H[a]	^{13}C[b]	^{1}H[a]	COSY	nOe[c]	^{13}C[b]
C-1	2.36, m	37.1	2.39, dd (5.1, 12.7) 1.95, dd (12.6, 12.7)			36.7
C-2		28.8				28.8
C-3		37.7				37.8
C-4		36.2				36.0[d]
C-5	1.92, m	54.4	1.82, m	H-9, H-6		48.7
C-6		25.9	2.17, m	H-5, H-6′, H-7	H-6′, H-7	32.5
C-6′			1.80, m	H-7, H-6		
C-7		37.6	4.98, dd (9, 5)	H-6, H-6′, H-9	H-6, H-15′, H-16	78.5
C-8		48.5				49.7[d]
C-9	2.73, d (8.7)	54.8	2.80, bd (7.7)	H-5, H-20*Z*	H-5 or H-14, H-13, H-20*Z*	54.2
C-10		153.7				153.0
C-11		170.8				172.6
C-12	2.55, m	32.2	2.55, dd	H-13		32.2
C-12′	2.55, m		2.56, dd	H-13		
C-13	2.48, m	32.0	2.49, m	H-12, H-12′	H-7, H-16, H-16′, H-20*Z*	31.5
C-14		44.1	1.80, m	H-15, H-15′		44.6
C-15	4.21, dd (10.0, 11.8)	68.2	4.20, dd (6.0, 12.0)	H-14		67.9
C-15′	4.32, dd (6.1, 11.8)		4.28, dd (9.8, 12.)	H-14		
C-16	4.19, dd (4.3, 11.2)	67.5	4.21, dd (4.4, 11.4)	H-13, H-16′		67.0
C-16′	3.84, dd (7.8, 11.2)		3.89, dd (7.5, 11.2)	H-13, H-16	H-16	
C-17	0.92, s	21.3	0.93, s			21.2
C-18	0.95, s	25.6	0.95, s			25.3
C-19	1.00, s	34.4	1.01, s			34.4
C-20*Z*	4.63, d (2.0)	115.0	4.69, bd (1.7)	20*E*		116.3
C-20*E*	4.86, d (2.0)		4.94, bd (1.7)	20*Z*		
OAc	2.08, s	172.9; 20.8		2.06, s		20.8; 172.6
OAc				2.07, s		16.1; 170.7

[a]Referenced to internal TMS.
[b]Referenced to solvent.
[c]Proton in carbon atom column irradiated.
[d]May be interchanged.

Shahamin K [**3**] was obtained as a clear oil that showed a parent ion in the hreims at *m/z* 420.2517 appropriate for a molecular formula of $C_{24}H_{36}O_6$ ($\Delta M = +0.5$ mmu), which differed from that of 12-desacetoxyshahamin C [**2**] by the addition of $C_2H_2O_2$. The ^{1}H- and ^{13}C-nmr data (Table 1) for shahamin K [**3**] were very similar to those for 12-desacetoxyshahamin C [**2**] (3), indicating that the two metabolites were closely related. Differences in th ^{1}H-nmr data of shahamin K included the presence of not one but two acetate methyl resonances [δ 2.06 (s), 2.07 (s)] and one additional deshielded methine resonance (δ 4.98). This ^{1}H-nmr evidence suggested that the extra atoms in **3** could be assigned to a secondary acetate (Table 1) and, therefore, that shahamin K [**3**] was an acetoxy derivative of 12-desacetoxyshahamin C [**2**].

Analysis of the COSY spectrum showed that the new acetoxy substituent in **3** was situated at C-7. Starting with the easily identifiable H-20*Z* (δ 4.69) and H-20*E* (δ 4.94) olefinic methylene proton resonances it was possible to identify correlations that led in sequence to the assignment of resonances to H-9 (δ 2.80), H-5 (δ 1.82), H-6 (δ 2.17), H-6′ (δ 1.80), and finally to the new deshielded methine at H-7 (δ 4.98) (Table 1). A series of double resonance experiments confirmed these COSY assignments. In particular, irradiation of H-6 (δ 2.17) converted the H-7 resonance (δ 4.98) into a sharp doublet with $J = 9$ Hz, in agreement with the placement of the acetoxy functionality at C-7. Difference nOe experiments demonstrated that the H-7 methine was cis to the δ-lactone substituent at C-8 (Table 1). Thus, irradiation of H-7 (δ 4.98) induced nOe's in H-15′ (δ 4.28), H-16 (δ 4.21), H-6 (δ 2.17), and in the complex multiplet at δ 1.8 (H-5, H-14 and H-6′).

A collection of *C. geminus* (12 individuals) was made in the coastal waters of Sri Lanka in January 1988. Standard extraction and fractionation procedures gave pure samples of 12β,15α,16α-triacetoxyspongian [**4**] (23 mg), 6α,15α,16α-triacetoxyspongian [**5**] (10 mg), and 6α,12β,15α,16α-tetracetoxyspongian [**6**] (34 mg). The known metabolite 12β,15α,16α-triacetoxyspongian [**4**] was identified by comparing its spectral data (Table 2) to the literature values (4). COSY and nOe experiments carried out on **4** supported the identification.

6α,12β,15α,16α-Tetraacetoxyspongian [**6**] was isolated as an optically active colorless oil. The ^{13}C-nmr spectrum of **6** contained 28 well dispersed resonances, and an APT experiment indicated that there were 42 hydrogen atoms attached to the carbon atoms (Experimental). Closer inspection of the ^{1}H- and ^{13}C-nmr data for **6** revealed that it was a tetraacetate [^{1}H δ 1.98 (s), 2.04 (s), 2.05 (s), 2.06; ^{13}C δ 168.9 (C), 169.0 (C), 169.3 (C), 169.4 (C)]. Compound **6** failed to show a parent ion in either the hreims or cims but it did give an intense fragment ion at *m/z* 402.2405 ($C_{24}H_{34}O_5$ ΔM −0.1 mmu), which in light of the nmr data could be attributed to the loss of two equivalents of HOAc.

The similarity of the nmr data for **6** and the companion metabolite **4** (Table 2 and Experimental) suggested that **6** was an acetoxy derivative of 12β,15α,16α-triacetoxyspongian [**4**]. A deshielded ^{1}H resonance at δ 5.23 (dt, $J = 3.5$, 11.1) and a deshielded ^{13}C resonance at δ 69.3 (CH) in the nmr spectra of **6** were assigned to a secondary carbon attached to the new acetoxy substituent. COSY data revealed that the acetoxy methine at δ 5.23 (H-6) was coupled to a pair of mutually coupled methylene protons at δ 2.16 (H-7_{eq}) and 1.21 (H-7_{ax}) and to a methine proton at δ 1.2 (H)5). The absence of additional COSY correlations into the resonances assigned to H-5, H-7_{ax}, and H-7_{eq} in either C_6H_6-d_6 (Experimental) or $CDCl_3$ (Table 2) indicated that the new acetoxy methine resonance (H-6 δ 5.23) was part of an isolated five-proton spin system. The only way to incorporate this spin system into a 12β,15α,16α-triacetoxyspongian framework was to place the fourth acetoxy substituent at C-6. Irradiation of the H-15 resonance at δ 6.05 induced an nOe in the H-7 resonance at δ 2.16, confirming the

TABLE 2. ^{1}H- (400 MHz) and ^{13}C- (75 MHz) nmr Data for 12-β,15α,16α-Triacetoxyspongian [**4**], 6α,15α,16α-Triacetoxyspongian [**5**], and 6α,12β,15α,16α-Tetracetoxyspongian [**6**] (recorded in $CDCl_3$).

Carbon	Compound						
	4		**5**		**6**		
	^{1}H[a]	^{13}C[b]	^{1}H[a]	COSY	^{1}H[a]	COSY	^{13}C[b]
C-1		40.0					
C-2		18.3					
C-3		42.2					
C-4		33.2					
C-5		56.6	1.2, m		1.2, m		
C-6		18.0	5.23, dt (3.6, 11.1)	H-5, H-7_{eq}, H-7_{ax}	5.23, dt (3.5, 11.1)	H-7_{ax}, H-5	69.3[c]
C-7_{eq}		41.8	2.14, dd (3.5, 12.3)	H-6, H-7_{ax}	2.16, dd (3.5, 12.3)		
C-7_{ax}			1.2, m	H-6, H-7_{eq}	1.21, m	H-6, H-7_{eq}	
C-8		34.6					
C-9	0.80, m	55.0				0.80, m	
C-10		37.4					
C-11_{ax}	1.47, q (12.4)	23.3			1.49, q (12.4)	H-9, H-11_{eq}, H-12	
C-11_{eq}	1.80, bdd (5.9, 12.8)				1.84, bdd (5.8, 12.7)	H-9, H-11_{ax} H-12	
C-12_{ax}	5.07, m	71.2			5.07, m	H-11_{ax}, H-11_{eq}, H-13	70.7[c]
C-13	3.09, q (7.5)	41.9	2.58, q (7.4)	H-16	3.10, q (7.3)	H-12, H-14, H-16	
C-14	1.99, d (7.5)	59.5	1.9		2.03, d (7.3)	H-13	
C-15	6.06, s	99.3	6.08, s		6.05, s		99.7[d]
C-16	6.36, d (7.6)	98.5	6.10, d (7.3)	H-13	6.36, d (7.5)	H-13	98.5[d]
C-17	0.99, s	17.0	1.2, s[c]		1.12, s[c]		
C-18	0.85, s	33.2	1.08, s[c]		0.98, s[c]		
C-19	0.80, s	21.3	0.96, s[c]		1.03, s[c]		
C-20	0.86, s	16.5	0.89, s[c]		0.87, s[c]		
OAc	1.98, s; 2.05, s; 2.07, s	169.7; 169.9; 170.1	2.10, s; 2.05, s; 2.04, s		2.06, s; 2.05, s; 2.04, s; 1.98, s		

[a]Referenced to internal TMS.
[b]Referenced to solvent.
[c,d]Values in the same column with the same superscript may be interchanged.

placement of this five-proton spin system on ring B. Irradiation of the H-6 methine resonance at δ 5.23 induced nOe's in three methyl singlets at δ 0.87, 1.03, and 1.12 (Me-17, Me-20, and Me-19). These nOe results were consistent with the equatorial orientation of the C-6 acetoxy group that was indicated by the coupling pattern observed for the H-6 resonance (dt, $J = 3.5$, 11.1).

6α,15α,16α-Triacetoxyspongian [**5**] was isolated as an optically active colorless glass that gave an $[M - HOAc]^+$ fragment ion at *m/z* 404.2537 ($C_{24}H_{36}O_5$ ΔM −2.5 mmu) as the highest mass peak in the mass spectrum. The ^{1}H- and ^{13}C-nmr data for **5** (Table 2 and Experimental) indicated that the molecule was simply the 12-desacetoxy derivative of 6α,12β,15α,16α-tetraacetoxysapongian [**6**]. COSY and nOe experiments confirmed this assignment (Table 2).

A collection of *C. annulata* (6 individuals) was made in the coastal waters of Sri Lanka in December 1988. Extracts from *C. annulata* yielded a single known metabolite, shahamin F [**7**] (5 mg), that was identified by comparing its spectral data to the literature values (5).

A single specimen of *C. inopinata* was obtained from a tropical fish and invertebrate exporting company in January 1990. The nudibranch had been collected on the west coast of Sri Lanka. Fractionation of the *C. inopinata* extract yielded pure samples of the diterpenoids aplyroseol 2 [**8**] (4 mg) (6,7), the γ-lactone **9** (2 mg) (8,9) and spongian-

16-one [**10**] (2 mg) (10). All three known terpenoids were identified by comparison of their spectral data to the literature values.

The results reported in this manuscript extend the range of association of dorid nudibranchs in the genus *Chromodoris* and spongian-derived diterpenoids (4,11). Six of the diterpenoids, dendrillolide A [**1**] (3), 12-desacetoxyshahamin C [**3**] (3), shahamin F [**7**] (5), aplyroseol 2 [**8**] (6,7), the γ-lactone **9** (8,9), and spongian-16-one [**10**] (10) have been previously isolated from sponges, and it is most likely that the remainder of the compounds have also been sequestered by the nudibranchs from their sponge diets for use as defensive allomones.

EXPERIMENTAL

STANDARD EXTRACTION AND PURIFICATION PROCEDURE.—Voucher specimens of the nudibranchs have been deposited in the invertebrate collection, Department of Zoology, UBC.

Live animals were immediately immersed in MeOH-CH_2Cl_2 (1:1) and stored in a freezer. At the time of workup, the organic solvents were decanted from thawed samples and evaporated in vacuo to give an oily residue that was partitioned between H_2O and CH_2Cl_2. Fractionation of the CH_2Cl_2-soluble materials by sequential application of Si gel flash chromatography (EtOAc/hexane gradients) and normal phase hplc (EtOAc/hexane mixtures) yielded pure metabolites.

Shahamin K [**3**].—Colorless oil; $[\alpha]_D$ +84.0° ($c = 0.10$, CH_2Cl_2); ir (film) 2952, 2931, 2867, 1745 (sh), 1740, 1366, 1243, 1135, 1110, 615 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 1; lreims *m/z* (rel. int.) 420 (18), 378 (2), 360 (8), 300 (8), 191 (2), 177 (3), 171 (6), 166 (3), 164 (5), 161 (7), 159 (9), 158 (3), 150 (23), 137 (31), 136 (88), 121 (39); hreims $[M]^+$ 420.2517 ($C_{24}H_{36}O_6$ ΔM +0.5 mmu); dcims $[M+NH_4]^+$ 438.

6α,15α,16α-Triacetoxyspongian [**5**].—Colorless glass; $[\alpha]_D$ +3° ($c = 0.3$, $CHCl_3$); ir (film) 2931, 1734, 1464, 1366, 1244 cm^{-1}; ^1H nmr see Table 2; ^{13}C nmr (C_6H_6-d_6) δ 17.7 (q), 18.0 (q), 18.4 (q), 19.0 (t), 21.2 (q), 21.4 (q), 22.0 (q), 22.7 (t), 23.3 (t), 34.0 (s), 36.4 (s), 37.0 (q), 39.9 (t), 40.1 (s), 40.4 (t), 44.2 (t), 50.2 (d), 56.4 (d), 59.5 (d), 59.8 (d), 70.1 (d), 100.6 (d), 102.4 (d), 169.4 (s), 169.9 (s), 170.3 (s); lreims *m/z* (rel. int.) 404 (0.2), 344 (43), 284 (33), 269 (100); hreims *m/z* $[M-HOAc]^+$ 404.2537 ($C_{24}H_{36}O_5$ ΔM −2.5 mmu).

6α,12β,15α,16α-Tetracetoxyspongian [**6**].—Colorless glass; $[\alpha]_D$ +40° ($c = 0.7$, $CHCl_3$); ir (film) 2930, 1732, 1463, 1367, 1243 cm^{-1}; ^1H nmr see Table 2; ^{13}C nmr (C_6H_6-d_6) δ 17.8 (q), 17.8 (q), 18.4 (q), 20.4 (q), 20.7 (q), 20.8 (q), 21.4 (q), 22.1 (q), 23.7 (t), 33.4 (s), 35.6 (s), 36.4 (q), 39.4 (s), 39.8 (t), 42.1 (d), 43.6 (t), 49.0 (t), 53.9 (d), 58.7 (d), 59.4 (d), 69.4 (d), 70.7 (d), 96.8 (d), 99.7 (d), 168.9 (s), 169.0 (s), 169.3 (s), 169.3 (s); lreims *m/z* (rel. int.) 402 (69), 342 (51), 282 (91); hreims *m/z* $[M-(2XHOAc)]^+$ 402.2405 ($C_{24}H_{34}O_5$ ΔM −0.1 mmu).

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