Furodysin Lactone and Pyrodysinoic Acid, New Sesquiterpenes from a Philippines Dysidea Species

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Furodysin Lactone and Pyrodysinoic Acid, New Sesquiterpenes from a Philippines *Dysidea* Species

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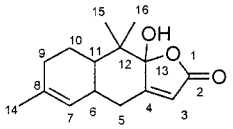
Chemical investigation of a *Dysidea* sponge, collected at Siquijor, Philippines, has afforded two new sesquiterpenoid metabolites, which we have termed furodysin lactone (**1**) and pyrodysinoic acid (**2**). The known 3,6,11-trihydroxy-9,11-secoergostane-7,24(28)-dien-9-one (**3**) was also encountered. Structure elucidation of the isolated metabolites involved high-field 2-D NMR spectroscopy including ^{1}H−^{1}H COSY, HSQC, and HMBC.

The marine sponge *Dysidea* sp. (order Dendroceratida, family Dysidae) is a prolific producer of structurally diverse secondary metabolites including bromophenols,[1] sesquiterpenes,[2] sesterpenes,[3] sterols,[4] and polychlorinated compounds including dysidenin,[5] dysidamides,[6] dysideathiazoles,[7] and chlorinated diketopiperazines.[8]

We recently reported the discovery of the novel polychlorinated dysideaprolines and barbaleucamides from a *Dysidea* sp. collected at Bararin Island, Philippines.[9] This prompted us to chemically investigate a *Dysidea* sp. collected from Siquijor, Philippines. Silica gel chromatography and semipreparative reversed-phase HPLC of the MeOH extract of this sponge afforded the new metabolites furodysin lactone (**1**) and its *N*-acetic acid analogue, pyrodysinoic acid (**2**). The known 3,6,11-trihydroxy-9,11-secoergostane-7,24(28)-dien-9-one (**3**) was also encountered.



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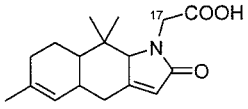

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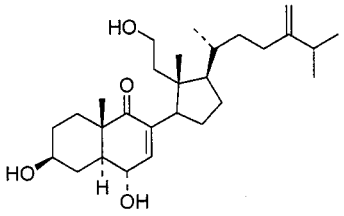

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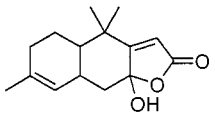

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HRESIMS of **1** gave a pseudomolecular ion $[M + H]^+$ at *m*/*z* 249.1496, corresponding to a molecular formula of $C_{15}H_{21}O_3$, and six degrees of unsaturation. An IR band at 1758 cm^{-1}, ^{1}H NMR signals at δ 5.85 (H-3) and 7.10 (−O*H*), and ^{13}C NMR signals at δ_C 170.7 (C-2), 113.3 (C-3), 168.4 (C-4), and 107.5 (C-13) were suggestive of a 3,4-disubstituted-4-hydroxybutenolide moiety. This moiety is encountered in several *Dysidea*-derived sesquiterpenes[10] including the well-known furodysinin lactone (**4**).[11] Indeed, the NMR data (Table 1) of **1**, superficially at least, suggested that **1** may be this compound. Further examination by 2-D NMR including ^{1}H−^{1}H COSY, TOCSY, HMQC, and HMBC (Table 1), however, highlighted inconsistencies with such an assignment and offered support for the structure now given. For example, ^{1}H−^{1}H COSY demonstrated the presence of strong allylic coupling between H-3 and H-5b ($J_{H-3-H-5b}$ = 2 Hz). Indeed, COSY and TOCSY were able to reveal two spin systems, H-3-H-5a,b-H-6 and H-5a,b-H-6-H-7-H-14-H-9-H-10-H-11. This not only indicated that the *gem*-dimethyl group had a different structural relationship to H-3 in **1** than in **4** but supported the placement of the olefinic methyl group at C-8. The *gem*-dimethyl was placed at C-12 on the basis of HMBC correlations between H-15/16 with C-11, C-12, and C-13. If this geminal system were part of a furodysinin skeleton, those correlations would have been replaced by correlations between the olefinic carbon (δ 168.4) and the *gem*-dimethyl groups.[12] In addition, HMBC correlations (typically weak across an oxygen bond) between O−H and C-4 (^3J), C-12 (^3J), and C-13 (^2J) confirm the hydroxyl location to be on C-13, one bond away from C-4 and C-12. Finally, selective inversion of H-5a (δ 2.17) produced a nuclear Overhauser enhancement of H-5b, H-6, H-7, and H-3, confirming the two olefinic protons to be on the same side of the molecule as the methylene at C-5. The *cis* ring junction is confirmed with the inversion of H-15 producing a nuclear Overhauser enhancement of H-11 and H-6, while inversion of H-16 produced a nuclear Overhauser enhancement of O−H and H-5b (Figure 1). We have termed this new compound furodysin lactone (**1**).

High-resolution mass measurements for compound **2** of *m*/*z* 290.1756 $[M + H]^+$ indicated a molecular formula of $C_{17}H_{24}NO_3$. Several features in the ^{1}H and ^{13}C NMR spectra of **2** suggested that it was closely related to furodysin lactone (**1**). 2-D NMR spectroscopy including ^{1}H−^{1}H-COSY, HMQC, and HMBC (Table 2) supported the furodysin skeleton represented by C-2 through C-13. The fact that H-13 is a singlet is key evidence for the furodysin skeleton vs the furodysinin skeleton (in which this proton would have been coupled to the neighboring methylene group). On the basis of the molecular formula, this left only a $C_2H_3NO_2$ unit to be accounted for. It most logically followed that this could be incorporated as now given for **2**. C-13 is protonated and its chemical shift (δ_C 67.0) is considerably upfield relative to the corresponding C-13 in compound **1**. An isolated CH_2 group (δ 3.80, 4.27) is also required, and significantly, both of these methylene protons showed a long-range correlation in the HMBC spectrum to C-2 and one (H-17b) showed a long-range correlation to

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Table 1. NMR Data for Furodysin Lactone (**1**) in DMSO-d_6 at 25 °C

H/C	δH	mult (*J* Hz)	$\delta^{13}C^a$	$^1H-^1H$ COSY	HMBC
2			170.7		H-3
3	5.85	d (2.0)	113.3	H-5b	H-5b, H-5a
4			168.4		H-3, O-H, H-5b, H-5a
5a	2.67	dd (3.9, 13.3)	29.1	H-5b, H-6	H-3, H-11
5b	2.17	dt (2.0, 13.0)		H-3, H-5a, H-6	
6	2.42	m	33.6	H-7, H-5b, H-5a, H-14, H-11	H-7, H-5a, H-5b, H-10, H-11
7	5.35	br dd (1.6, 5.1)	124.3	H-6, H-9, H-14	H-5b, H-14, H-9
8			134.3		H-9, H-14, H-10
9	1.89	m	31.4	H-7, H-10, H-14	H-7, H-14
10	1.71	m	19.5	H-9, H-11	H-9
11	1.40	br dt (5.0, 10.0)	44.6	H-6, H-10	H-7, H-5a, H-9, H-10, H-16, H-15
12			41.9		O-H, H-16, H-15, H-11
13			107.5		H-3, O-H, H-5a, H-11, H-16, H-15
14	1.60	s	22.8	H-7, H-6, H-9	H-7
15	0.80	s	23.1	H-16 weak	H-16
16	1.10	s	19.9	H-15 weak	H-15
OH	7.10	s			

[a] Deduced from HSQC and gHMBC.

Table 2. NMR Data for Pyrodysinoic Acid (**2**) in DMSO-d_6 at 25 °C

H/C	δH (*J* in Hz)	$\delta^{13}C$	$^1H-^1H$ COSY	HMBC[a]
2		172.5		H-17a, H-17b, H-3
3	5.77, m (1.7)	117.7	H-5a	H-5a, H-5b, H-13
4		161.7		H-3, H-13, H-5a, H-5b
5a	2.13 ddd (14.1, 2.2, 0.5)	31.0	H-5b	
5b	2.70 dd (4.9, 14.1)		H-5a	
6	2.35, m	32.6	H-11, H-7, H-5ab	H-5ab
7	5.40, br dd (1.6, 5.4)	124.9	H-14, H-6	H-14
8		133.3		H-14
9ab	1.98, m (7.0)	31.2	H-10b	H-14
10a	1.56, m	17.6	H-11, H-10b	
10b	1.76, m		H-10a, H-9ab	
11	1.37, m	45.8	H-10a, H-5a	H-5b, H-15, H-16
12		39.7		H-13, H-15, H-16
13	3.97, s	67.0		H-17b, H-3, H-5b, H-15, H-16
14	1.60, br s	22.7		
15	0.70, s	20.3		H-16
16	1.17, s	26.7		H-15
17a	3.80, d (18.0)	44.0	H-17b	
17b	4.27, d (18.0)		H-17a	
18		170.8		H17a, H-17b

[a] Proton showing long-range correlation to indicated carbon.

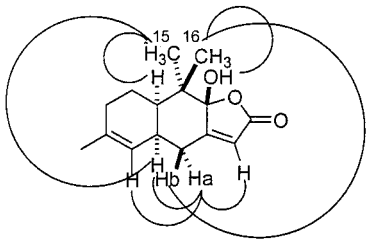

Figure 1. Selected NOE correlations in **1**.

C-13 (Table 2). We have termed this new compound pyrodysinoic acid (**2**).

The known 3,6,11-trihydroxy-9,11-secoergostane-7,24(28)-dien-9-one (**3**)[13] originally reported from *Spongia officinalis* and *Sinularia hirta* was also encountered. *Dysidea* sp. are a well-known source of cytotoxic 9,11-seco-sterols,[14] but surprisingly this was the first time this particular compound has been reported from this species.

Experimental Section

General Experimental Procedures. ^1H and ^{13}C NMR spectra were recorded in DMSO-d_6 at 25 °C on a Varian spectrometer operating at 500 and 125 MHz, respectively, using residual solvent signals as an internal reference. All 2-D NMR experiments were performed on the same spectrometer and in the same solvent. HSQC experiments were optimized for $^1J_{CH} = 150$ Hz, and HMBC experiments were optimized for $^nJ_{CH} = 7$ Hz, respectively. Selective 1-D transient NOE experiments were performed on a Varian spectrometer operating at 600 MHz using the pulse sequence of Stott et al.[15] A mixing time of 2 s and a recycle time of 14.3 s, which was equal to ~3 × T1 of the slowest relaxing proton (4.5 s, H-3), were used. HRESIMS were recorded in positive mode on a Mariner Electrospray-Time-of-Flight Biospectrometry workstation (PerSeptive Biosystems).

Animal Material. The *Dysidea* sp. (NCI 815) was supplied by Prof. D. J. Faulkner, Scripps Oceanographic Institute, La Jolla, CA. It was collected at Siquijor, Phillipines, at an unspecified depth. On collection, it was described as a gray-blue encrusting sponge, 1−2 mm thick. It was described by Prof. John Hooper, Queensland Centre for Biodiversity, Queensland Museum, Australia, as follows: "Growth form. Encrusting, 1−2 mm thick, slightly foliose-lobate. Colour. Grey-blue alive, chocolate brown in EtOH, beige fibres protruding through the surface. Oscules. Not visible. Texture. Very soft, compressible, floppy, fibrous, mucusy. Surface ornamentation. Regularly conulose, small conules interconnected by ridges. Ectosomal skeleton. Membraneous, pushed up into conules by choanosomal fibres. Choanosomal skeleton. Irregular reticulation of fibres partially cored by detritus and foreign spicule material, sometimes obscured by dark mesohyl collagen. Primary fibres mainly near the surface, 70% packed with debris, occasionally less, fibre pith obscured. Debris mostly calcitic or siliceous sand grains, with no foreign sponge spicules seen. Secondary fibres mostly clear of debris, sparse, irregularly reticulate. Choano-

cyte chambers not seen due to poor preservation. This species has been found previously from the southern Philippines and therefore is probably fairly common." It has been registered in the Queensland Museum as QM G318546.

Extraction and Isolation. A fresh freeze-dried collection of *Dysidea* sp. (70 g) was suspended in 400 mL of CH_2Cl_2 overnight and the solvent put aside. The residue was then resuspended sequentially three times in 800 mL of fresh MeOH for 6, 12, and 6 h. The MeOH extracts were combined and the solvent was evaporated in vacuo to give 14 g of a dark green oil. This oil was subjected to reversed-phase (100 g) vacuum liquid column chromatography using a MeCN−0.01%TFA gradient in water−0.01%TFA (200 mL each of 0%, 20%, 40%, 60%, 80%, 100%, then 3 times with 200 mL of MeOH). After evaporation, the 40% fraction was subjected to semipreparative reversed-phase HPLC (YMC ODS SH-434-10 S-10 120A AQ, 2.0 × 25.0 cm) using the following elution profile: 45 min linear gradient from at 10% MeCN−0.01%TFA to 100% MeCN−0.01%TFA then 15 min isocratic at 100% MeCN−0.01%TFA. The flow rate was 10 mL/min. Detection was at 210 nm. The fractions collected between 39 and 42 min were dried and then resubjected to semipreparative reversed-phase HPLC (Alltech Alltima C18, 1.0 × 25.0 cm, 5 μM), using the same elution profile at a flow rate of 3 mL/min. Detection was at 210 nm. The fraction collected at 39 min afforded pure furodysin lactone (**1**) (2.5 mg) as a pale yellow oil.

The fractions collected at 34, 35, 36, 47, and 48 min were evaporated and then resubjected to the exact same chromatographic procedure. The fraction collected at 33 min was once more subjected to the exact same chromatographic procedure. The fraction collected at 33 min afforded pure pyrodysinoic acid (**2**) (2.7 mg) as a pale yellow oil.

The fraction eluting from the vacuum liquid column chromatography at 80% MeCN−0.01%TFA was subjected to semipreparative reversed-phase HPLC (YMC ODS SH-434-10 S-10 120A AQ, 2.0 × 25.0 cm) using the following elution profile: 45 min linear gradient from 10% MeCN−0.01%TFA to 100% MeCN−0.01%TFA, and then 15 min isocratic at 100% MeCN−0.01%TFA. The flow rate was 10 mL/min. Detection was at 210 nm. The fractions collected between 29 and 47 min were evaporated, and then resubjected to semipreparative reversed-phase HPLC, using the same column, flow rate, and detection with the following elution profile: 80 min convex gradient from 10% MeCN−0.01%TFA to 100% MeCN−0.01%TFA, and then 10 min isocratic at 100% MeCN−0.01%TFA. The fractions collected between 47 and 49 min were evaporated and then resubjected to semipreparative reversed-phase HPLC (Alltech Alltima C18, 1.0 × 25.0 cm, 5 μM), using an isocratic elution at 60% MeCN−0.01%TFA and at a flow rate of 3 mL/min. Detection was at 210 nm. The fraction collected at 25 min afforded pure 3,6,11-trihydroxy-9,11-secoergostane-7,24(28)-dien-9-one (**3**) (5.1 mg) as a white crystalline solid.

Furodysin lactone (1): pale yellow oil, $[\alpha]^{23}_D$ +100° (*c* 0.011, MeOH); UV (MeOH) λ_{max} (log ϵ) 216 (3.79) nm; IR (film) ν_{max} 3520, 2965, 2927, 2855, 1758, 1710, 1440, 1387, 1264, 1176, 1142, 1035, 938 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 1; HRESIMS found *m*/*z* 249.1496 [M + H]$^+$, calcd for $C_{15}H_{21}O_3$, 249.1491.

Pyrodysinoic acid (2): pale yellow oil, $[\alpha]^{23}_D$ +9.1° (*c* 0.066, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (1.46) nm; IR (film) ν_{max} 3524, 2965, 2927, 2855, 1723, 1666, 1461, 1379, 1281, 1200, 1125, 967 cm^{-1}; ^{1}H NMR, see Table 2; ^{13}C NMR, see Table 2; HRESIMS found *m*/*z* 290.1756 [M + H]$^+$, calcd for $C_{17}H_{24}NO_3$, 290.1756.

3,6,11-Trihydroxy-9,11-secoergostane-7,24(28)-dien-9-one (3): white amorphous powder, IR (film) ν_{max} 3305, 2952, 2925,2875, 1660,1439, 1365, 1194, 1042, 1003, 879 cm^{-1}; ^{1}H and ^{13}C NMR data consistent with that presented by Migliuolo et al.[12] and corroborated by ^{1}H−^{1}H-COSY, HMQC, and HMBC spectroscopy (see Supporting Information); HRESIMS found *m*/*z* 290.1756 [M + H]$^+$, calcd for $C_{17}H_{24}NO_3$, 290.1756.

Acknowledgment. We are greatly indebted to Prof. D. J. Faulkner, Scripps Oceanographic Institute, La Jolla, CA, who provided the sponge material and the original descriptions of the sponge, and to Prof J. A. Hooper, Queensland Centre for Biodiversity, Queensland Museum, Australia, for his expert taxonomic descriptions. Mass spectrometric analyses were performed by David Masters-Moore and James Doom of the Pharmacia Mass Spectroscopic Facilities.

Supporting Information Available: NMR data for **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) (a) Sharma, G. M.; Vig, B. *Tetrahedron Lett.* **1972**, 1715−1719. (b) Fu, X.; Schmitz, F. J. *J. Nat. Prod.* **1996**, *59*, 1102−1103. (c) Handayani, D.; Edrada, R. A.; Proksch, P.; Wray, V.; Witte, L.; Van Soest, R. W. M.; Kunzman, A.; Soedarsono. *J. Nat. Prod.* **1997**, *60*, 1313−1316.

(2) (a) R.; Murphy, P. T.; Wells, R. J. *Tetrahedron Lett.* **1978**, *49*, 4949−4950. (b) Montagnac, A.; Martin, M.-T.; Debitus, C.; Pais, M. *J. Nat. Prod.* **1996**, *59*, 866−868. (c) Paul, V. J.; Seo, Y.; Cho, W. K.; Rho, J.-R.; Shin, J.; Bergquist, P. R. *J. Nat. Prod.* **1997**, *60*, 1115−1120. (d) Sera, Y.; Adachi, K.; Nishida, F.; Shizuri, Y. *J. Nat. Prod.* **1999**, *62*, 395−396.

(3) Rudi, A.; Yosief, T.; Schleyer, M.; Kashman, Y. *Org. Lett.* **1999**, *1*, 471−473.

(4) (a) Gunasekera, S. P.; Schmitz, F. J. *J. Org. Chem.* **1985**, *48*, 885−886. (b) West, R. R.; Cardellina, J. H., II. *J. Org. Chem.* **1989**, *54*, 3234. (c) Leone, P. de. A.; Redburn, J.; Hooper, J. N. A.; Quinn, R. J. *J. Nat. Prod.* **2000**, *63*, 694−697.

(5) (a) Kazlauskus, R.; Liddgard, R. O.; Wells, R. J.; Vetter, W. *Tetrahedron Lett.* **1977**, 3183−3186. (b) Charles, C.; Braekman, J. C.; Daloze, D.; Tursch, B. *Tetrahedron* **1980**, *36*, 2133−2135.

(6) (a) Gebreyesus, T.; Yosief, T.; Carmely, S.; Kashman, Y. *Tetrahedron Lett.* **1988**, *29*, 3863−3864. (b) Carmely, S.; Gebreyesus, T.; Kashman, Y.; Skelton, B. W.; White, A. H.; Yosief, T. *Aust. J. Chem.* **1990**, *43*, 1881−1888. (c) Isaacs, S.; Berman, R.; Kashman, Y.; Gebreyesus, T.; Yosief, T. *J. Nat. Prod.* **1991**, *54*, 83−91.

(7) Unson, M. D.; Rose, C. B.; Faulkner, D. J.; Brinen, L. S.; Steiner, J. R.; Clardy, J. *J. Org. Chem.* **1993**, *58*, 6336−6343.

(8) (a) Kazlauskas, R.; Murphy, P. T.; Walls, R. J. *Tetrahedron Lett.* **1978**, 4945−4948. (b) Fu, X.; Ferreira, M. L. G.; Schmitz, F. J.; Kelly-Borges, M. *J. Nat. Prod.* **1998**, *61*, 1226−1231.

(9) Harrigan, G. G.; Goetz, G.; Luesch, H.; Yang, S.; Likos, J. *J. Nat. Prod.* **2001**, *64*, 1133−1138.

(10) Carte, B.; Hong, S.; Poehland, B.; Sarau, H.; Westley, J. W.; Faulkner, D. J. *Tetrahedron Lett.* **1989**, *30*, 2725−2726.

(11) Furodysinin lactone was first isolated and reported as a levorotary compound by: Grode, S. H.; Cardellina, J. H., II. *J. Nat. Prod.* **1984**, *47*, 76−83. An identical structure with a positive optical rotation has recently been reported by: Reddy, N. S.; Venkatesham, U.; Rao, T. P.; Venkateswarlu, Y. *Indian J. Chem.* **2000**, *39B*, 393−395. The presented ^{13}C NMR data for these compounds are identical. The presented ^{1}H NMR data differ most significantly for H-10 (δ 1.12, 1.70 by Grode and Cardellina, δ 1.83, 2H, by Reddy et al.) and for H-11 (δ 1.38 by Grode and Cardellina, δ 0.95 by Reddy et al.). Reddy et al. suggest that (+)-furodysinin lactone and (−)-furodysinin lactone differ at the C-11 position in that the stereochemistry is 11*R* for the (+)-isomer and 11*S* for the (−)-isomer.

(12) Cameron, G. M.; Stapelton, B. L.; Simonse, S. M.; Brecknell, D. J.; Garson, M. J. *Tetrahedron* **2000**, *56*, 5247−5252.

(13) Migliuolo, A.; Piccialli, V.; Sica, D. *Steroids* **1992**, *57*, 344−347.

(14) (a) Aiello, A.; Fattorusso, E.; Menna, M.; Carnuccio, R.; Iuvone, T. *Steroids* **1995**, *60*, 666−673. (b) Anjaneyulu, V.; Nageswara Rao, K.; Suresh Babu, J.; Kobayashi, M. *Indian J. Chem.* **1994**, *33B*, 144−147.

(15) Stott, K.; Stonehouse, J.; Keeler, J.; Hwang, T.; Shaka, A. J. *J. Am. Chem. Soc.* **1995**, *117*, 4199−4200.

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