Sesquiterpenoids of the Drimane Class from a Sponge of the Genus *Dysidea*

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Ten sesquiterpenoids, including seven new ones, have been isolated from an undescribed sponge of the genus *Dysidea*. Compounds **1**−**8** are sesquiterpenoids of the drimane class, while **9** and **10** are 12-norsesquiterpenoids of the same structural class. The structures of novel compounds have been determined by combined spectroscopic methods. These compounds exhibited moderate antimicrobial and enzyme inhibitory (Na^+/K^+-ATPase and PLA_2) activities.

Sesquiterpenoids of the drimane class are widely recognized as bioactive metabolites of terrestrial plants and marine animals such as mollusks and sponges.[1] Among sponges, these compounds have been frequently isolated from *Dysidea* spp.[2–4] We encountered a species of *Dysidea* in a reef habitat on Guam that commonly overgrows another sponge, *Cacospongia* sp., causing necrosis and damage to the *Cacospongia* sp. by destroying its basal portions. In field experiments, crude extracts of *Dysidea* sp. and its major metabolite 7-deacetoxyolepupuane caused necrosis in *Cacospongia* sp. when incorporated into agar strips at natural concentrations and placed in contact with *Cacospongia* sp. for 7 days.[5] In addition to its role in competition, 7-deacetoxyolepupuane deterred predation by a spongivorous fish, *Pomacanthus imperator,* illustrating the multiple ecological roles that a single secondary metabolite may play.[5]

We also found that the crude organic extract of *Dysidea* sp. exhibited potent antimicrobial activity against several strains of marine bacteria. Because of the multiple biological activities observed for the organic extract, we became interested in characterizing the complex mixture of sesquiterpenoids present in *Dysidea* sp. We isolated several sesquiterpenoids of the drimane class by chromatographic methods. Herein we report the structures and bioactivities of 10 compounds including seven new ones. Compounds **1**−**8** are sesquiterpenoids of the drimane class whose structural variations exist in the highly functionalized C ring, while **9** and **10** are unusual 12-norsesquiterpenoids of the same structural class. Several compounds exhibited moderate antimicrobial activity and/or inhibitory activity against Na^+/K^+-ATPase and PLA_2.

Results and Discussion

The sponge was collected at a site known as Sponge Mound in Apra Harbor, Guam (Mariana Islands),[6] and was lyophilized, macerated, and exhaustively extracted with CH_2Cl_2 and MeOH. The combined crude extracts were separated by silica vacuum flash chromatography using sequential mixtures of *n*-hexane and EtOAc as eluents. The presence of secondary metabolites in
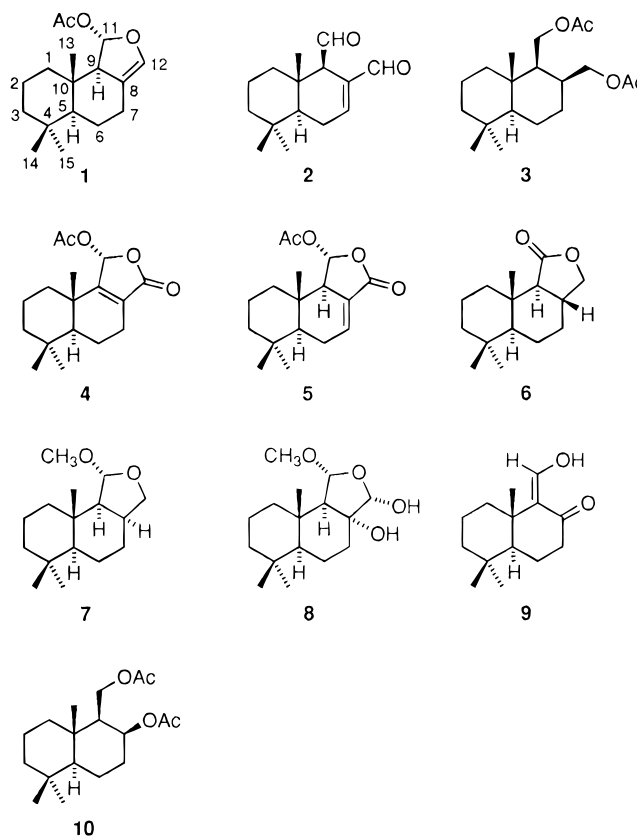
nonpolar and moderately polar fractions was recognized by ¹H-NMR analysis. Separation by silica and reversed-phase HPLC of these fractions yielded 10 compounds.

The structures of three known metabolites, 7-deacetoxyolepupuane (**1**), polygodial (**2**), and a derivative (**3**), were determined by a combination of spectroscopic analysis and comparison with reported data for these compounds.[2–4,7] 7-Deacetoxyolepupuane, previously isolated from the nudibranch mollusks *Dendrodoris* spp.[8] and an Australian sponge of the genus *Dysidea,*[2] was the major metabolite; 1.9% of the dried sponge. Polygodial, a well-known bioactive constituent of terrestrial plants and marine mollusks,[1,7] was isolated for the first time from a sponge in this study. Compound **3** was isolated very recently from the sponge *D. fusca* collected from New Caledonia.[4]

Compound **4** was isolated as a colorless oil. The molecular formula for this compound was deduced as

Table 1. Proton NMR Assignments for Sesquiterpenoids **4**−**8**[a,b]

position	4	5	6	7	8
1ax	1.47 (m)	1.19 (ddd, 13.2, 13.2, 3.4)	1.07 (br ddd, 13.2, 13.2, 3.4)	1.04 (ddd, 13.7, 13.2, 3.9)	1.09 (ddd, 13.2, 13.2, 2.4)
1eq	1.53 (m)	1.61 (br d, 13.2)	2.43 (ddd, 13.2, 4.9, 3.4)	1.70 (m)	1.71 (ddd, 13.2, 4.9, 2.9)
2ax	1.63 (br dddd, 13.7, 13.7, 13.2, 2.9)	1.52 (m)	1.61 (ddddd, 13.7, 13.2, 13.2, 3.4, 3.4)	1.56 (m)	1.55 (m)
2eq	1.47 (m)	1.47 (m)	1.45 (m)	1.37 (m)	1.42 (m)
3ax	1.22 (ddd, 13.2, 13.2, 3.4)	1.21 (ddd, 13.7, 13.7, 3.4)	1.19 (m)	1.16 (ddd, 13.7, 13.2, 3.9)	1.17 (m)
3eq	1.47 (m)	1.47 (m)	1.42 (m)	1.39 (m)	1.42 (m)
5	1.32 (dd, 12.7, 2.0)	1.36 (dd, 11.7, 5.4)	0.90 (dd, 13.2, 2.9)	0.80 (dd, 12.2, 2.1)	0.96 (dd, 12.2, 2.0)
6ax	1.53 (m)	2.09 (dddd, 20.6, 11.7, 3.4, 3.4)	1.28 (dddd, 13.7, 13.2, 12.7, 3.9)	1.26 (dddd, 13.7, 13.2, 12.2, 4.4)	1.21 (m)
6eq	1.91 (br dd, 13.7, 6.8)	2.42 (dddd, 20.6, 5.4, 3.4, 3.4)	1.74 (dddd, 13.2, 3.4, 2.9, 2.9)	1.46 (dddd, 13.7, 2.9, 2.9, 2.1)	1.61 (m)
7ax	2.17 (dddd, 18.6, 11.2, 6.8, 1.5)		1.19 (m)	1.61 (m)	1.51 (ddd, 13.7, 13.7, 5.9)
7eq	2.40 (br dd, 18.6, 6.4)	6.90 (ddd, 3.4, 3.4, 3.4)	1.95 (dddd, 12.2, 3.4, 3.4, 2.9)	1.67 (m)	2.00 (ddd, 13.7, 3.9, 2.4)
8			2.34 (m)	2.59 (m)	
9		2.68 (m)	1.69 (d, 13.7)	1.64 (br d, 7.3)	1.80 (br s)
11	6.95 (dd, 1.9, 1.5)	6.43 (d, 5.9)		4.72 (br s)	4.66 (br s)
12			4.23 (dd, 8.3, 6.4) 3.66 (dd, 11.2, 8.3)	3.96 (dd, 9.3, 7.8) 3.56 (dd, 11.2, 7.8)	5.15 (br s)
13	1.16 (s)	0.84 (s)	0.98 (s)	0.90 (s)	0.87 (s)
14	0.88 (s)	0.91 (s)	0.81 (s)	0.82 (s)	0.80 (s)
15	0.94 (s)	0.90 (s)	0.85 (s)	0.86 (s)	0.89 (s)
OAc	2.11 (s)	2.12 (s)			
OMe				3.28 (s)	3.36 (s)

[a] Measured in CDCl₃ solutions at 500 MHz; δ in ppm (J in Hz); TMS as internal standard. [b] Assignments were aided by ^{1}H COSY, HMQC, and HMBC experiments.

Table 2. Carbon NMR Assignments for Compounds **4**−**10**[a,b]

no.	4	5	6	7	8	9	10
1	34.5 t	38.7 t	37.2 t	41.9 t	41.3 t	38.3 t	39.5 t
2	18.2 t	18.1 t	18.2 t	18.3 t	18.4 t	18.9 t	18.3 t
3	41.4 t	42.0 t	42.3 t	41.9 t	41.7 t	41.4 t	41.8 t
4	33.3 s	32.9 s	33.1 s	33.0 s	32.9 s	32.9 s	33.2 s
5	50.9 d	49.3 d	55.5 d	52.4 d	52.2 d	50.3 d	55.3 d
6	18.0 t	25.0 t	21.2 t	17.9 t	18.7 t	17.7 t	17.3 t
7	21.4 t	137.6 d	28.7 t	23.9 t	30.9 t	33.1 t	31.5 t
8	128.4 s	126.3 s	38.3 d	34.1 d	76.9 s	188.9 s	69.2 d
9	165.6 s	56.1 d	57.4 d	58.8 d	65.1 d	122.8 s	51.5 d
10	37.3 s	33.9 s	35.7 s	34.3 s	35.0 s	35.3 s	37.0 s
11	90.7 d	93.5 d	175.8 s	107.2 d	105.4 d	183.0 d	61.0 t
12	170.9 s	166.6 s	71.2 t	72.2 t	102.2 d		
13	21.7 q	14.2 q	15.5 q	16.0 q	15.3 q	25.0 q	13.0 q
14	21.4 q	21.2 q	21.2 q	22.0 q	21.9 q	21.3 q	21.7 q
15	33.3 q	33.0 q	33.5 q	33.5 q	33.5 q	33.3 q	33.6 q
OMe				54.3 q	54.3 q		
OAc	169.1 s	169.1 s					171.3 s
							170.5 s
	20.9 q	20.9 q					21.4 q
							21.0 q

[a] Measured in CDCl₃ solutions. [b]Assignments were aided by DEPT, HMQC, and HMBC experiments.

$C_{17}H_{24}O_4$ by a combination of HRMS and ^{13}C-NMR analysis. The presence of an α,β-unsaturated lactone was readily recognized by signals of quaternary carbons at δ 170.9, 165.6, and 128.4 in the ^{13}C-NMR spectrum. This interpretation was supported by a strong absorption band at 1760 cm⁻¹ in the IR spectrum and an absorption maximum at 221 nm (log ϵ 3.65) in the UV spectrum. The signal for a methine carbon at δ 90.7 and a corresponding proton signal at δ 6.95 (1H, dd, J = 1.9, 1.5 Hz) in the NMR data revealed the presence of an acetal functionality (Tables 1 and 2).

With the aid of this information, the structure of **4** was determined by a combination of ^{1}H COSY, HMQC, HMBC, and NOESY experiments. Long-range correlations of H-5, H-6, Me-13, Me-14, and Me-15 with neighboring carbons were particularly helpful in determining the structure of the A and B rings. The presence of a lactone ring and the attachment of an acetoxyl group to it were secured by long-range correlations of the acetal proton at δ 6.95 in the ^{1}H-NMR spectrum with both of the carbonyl carbons in the lactone and acetoxyl groups. The acetoxyl-bearing lactone ring could be arranged in two ways; the lactone carbonyl and acetal carbons could be located at either C-11 or C-12. Assignments of the former to C-12 and the latter to C-11, respectively, were established on the basis of the chemical shifts of C-8 and C-9 determined by HMBC experiments; C-8 and C-9, δ 128.4 and 165.6, respectively (Table 2). The orientation of the AB ring junction was determined as *trans* by NOE correlations of H-5 and Me-13 with ring protons as well as the splitting pattern of H-5 at δ 1.32 (1H, dd, J = 12.7, 2.0 Hz) in the ^{1}H-NMR spectrum. Similarily the 11R^* configuration was assigned on the basis of a NOE correlation between H-11 and Me-13.

Compound **5** was isolated as a colorless oil. The molecular formula for this compound was established as $C_{17}H_{24}O_4$, identical with that of **4**, by a combination of HRMS and ^{13}C-NMR spectrometry. Spectral data for this compound were very similar to those obtained for **4**. The only significant difference in the ^{13}C-NMR spectrum was the replacement of the C-9 quaternary carbon at δ 165.6 of **4** by a methine carbon at δ 56.1 and the replacement of the C-7 methylene at δ 21.4 by an olefinic carbon at δ 137.6. The corresponding difference was also observed in the ^{1}H-NMR spectrum in which the signal for a new olefinic proton appeared at δ 6.90 (1H, ddd, J = 3.4, 3.4, 3.4 Hz). These differences were accommodated by a shift of the double bond from C-8 in **4** to C-7 in **5** and were confirmed by 2-D NMR experiments. The long-range correlations of H-9 with C-7 and C-13 were especially useful for this assignment. The relative configurations of the asymmetric carbon centers in ring C were assigned as 9R^*,11R^* by NOESY correlations between H-5 and H-9 and also between the H-11 and Me-13 protons.

The molecular formula for compound **6**, isolated as a white solid, was established as $C_{15}H_{24}O_2$ by combined HRMS and ^{13}C-NMR analysis. The presence of a lactone ring was readily recognized by the signal for a

quaternary carbon at δ 175.8 in the ^{13}C-NMR spectrum and an absorption band at 1765 cm^{-1} in the IR spectrum. The structure of this compound was determined by combined 2-D NMR methods. The position of the carbonyl carbon was assigned to C-11 on the basis of HMBC correlations of this carbon with H-9 and H-12. In addition to the *trans* AB ring junction, **6** possessed additional asymmetric carbon centers at C-8 and C-9. The relative configurations of these centers were assigned as 8R^*,9S^* on the basis of the large coupling ($J_{8,9}$ = 13.7 Hz) between these protons and a strong 1,3-diaxial NOE correlation between H-8 and Me-13.

The molecular formula of **7** was established as $C_{16}H_{28}O_2$ by HRMS and ^{13}C-NMR spectrometry. NMR data for this compound were similar to those obtained for **6**. The only significant difference in the ^{13}C-NMR data was the replacement of the carbonyl carbon at C-11 (δ 175.8) of **6** by an acetal (δ 107.2) and a methoxy (δ 54.3) group in **7**. Corresponding changes were also observed in the ^{1}H-NMR spectrum in that signals of the new acetal and methoxy protons were found at δ 4.72 (1H, br s) and 3.28 (3H, s), respectively. The structure of **7** was determined by a combination of ^{1}H COSY, HMQC, and HMBC experiments. The ether linkage between C-11 and C-12 was established by long-range correlations between these carbons and attached protons; C-11 and H-12, C-12 and H-11. Similarly, the attachment of the methoxy group to C-11 was confirmed by a long-range correlation between the methoxy proton and C-11.

Compound **7** possessed three neighboring asymmetric centers at C-8, C-9, and C-11. The stereochemistry of these centers was assigned on the basis of NOESY data. Spatial proximity between C-12 and C-13 was revealed by a NOE correlation between one (δ 3.56) of the H-12 methylene and the Me-13 protons. In addition, a NOE correlation was observed between H-5 and H-9. A molecular model study showed that, to exhibit these NOE correlations, the BC ring junction must be *cis* oriented; H-8α, H-9α. The β orientation of H-11 was also determined by a NOE correlation between this proton and Me-13. Thus, the relative configurations of the asymmetric centers of the C ring were assigned as 8R^*,9S^*,11R^*. A literature survey revealed that the hemiacetal corresponding to **7** has been isolated from a sponge of the genus *Dysidea*[3] and recently from *D. fusca*.[4] The 11S^* configuration originally suggested[3] was revised to 11R^* on the basis of NOE analysis.[4] Comparison of spectral data showed good agreement between **7** and the hemiacetal.[3,4]

Compound **8** was isolated as a colorless gum. The HRCIMS for this compound, obtained by using NH$_3$ as a carrier gas, showed a quasi-molecular ion peak at *m/z* 284.2225 that was interpreted as $C_{16}H_{30}NO_3$ by mass calculation and ^{13}C-NMR analysis. Observation of four oxygen-bearing carbons at δ 105.4 (CH), 102.2 (CH), 76.9 (C), and 54.3 (CH$_3$) in the ^{13}C-NMR spectrum and a broad absorption band at 3450 cm^{-1} in the IR spectrum, however, suggested that **8** contained an additional hydroxyl group. Therefore, the molecular formula of **8** was thought to be $C_{16}H_{28}O_4$. The NMR data for **8** were reminiscent of those obtained for **7**. A combination of 2-D NMR data revealed that **8** possessed the same A and B rings as **7**. Replacement of signals of C-8 and C-12, observed at δ 34.1 (CH) and 72.2 (CH$_2$),

respectively, in the ^{13}C-NMR spectrum of **7** by downfield signals at δ 102.2 (CH) and 76.9 (C) in **8** revealed that hydroxyl groups were attached to these carbons (Table 2). Thus, the C-12 oxymethylene of **7** was converted to an acetal (or hemiacetal) in **8**, while the C-8 methine was converted to a quaternary carbinol group. Supporting information for these assignments was obtained by HMBC experiments in that H-9 exhibited long-range correlations with these carbons. Thus, compound **8** contained two acetal or hemiacetal carbons at C-11 and C-12. A long-range correlation of C-11 with methoxy protons revealed that C-11 and C-12 were the methoxy acetal and hemiacetal carbons, respectively. Thus, the structure of **8** was determined as the 8,12-dihydroxy derivative of **7**.

In addition to the trans AB ring junction, **8** possessed neighboring asymmetric centers at C-8, C-9, C-10, and C-11. A spatial proximity among H-1eq, H-11, and Me-13 was found by mutual NOE correlations among these protons. An additional NOE correlation was also observed between H-12 and Me-13. A three-dimensional model study revealed that, to exhibit these correlations, the BC ring junction must be *cis* oriented. In addition, both H-11 and H-12 must be β-oriented to ring C. This interpretation was further supported by NOE correlations of H-9 with H-1ax and H-5. Thus, the relative configurations of the C ring of **8** were defined as 8R^*,9R^*,11R^*,12S^*.

Compound **9** was isolated as a white solid that was analyzed for $C_{14}H_{22}O_2$ by HRMS and ^{13}C-NMR spectrometry. In addition to the lack of one carbon in the molecular formula, spectral data for this compound were different from the other sesquiterpenoids in many aspects. The ^{13}C-NMR spectrum of **9** showed three downfield signals at δ 188.9 (C), 183.0 (CH), and 122.8 (C) (Table 2). Although the former two carbons were initially thought to be an unsaturated ketone and aldehyde, respectively, the occurrence of only one signal in the olefinic region of the ^{13}C-NMR spectrum suggested the presence of an enolized β-dicarbonyl functionality. Supporting evidence for this interpretation included strong absorption bands at 3500 (broad, −OH) and 1620 (C=O stretching of β-hydroxy-α,β-unsaturated ketone) cm^{-1} in the IR spectrum and an absorption maximum at 294 nm (log ε 3.34) in the UV spectrum. In addition, the ^{1}H-NMR spectrum showed a D$_2$O exchangeable signal at δ 15.36 (1H, d, J = 4.4 Hz) and another downfield signal at δ 8.60 (1H, d, J = 4.4 Hz), which were coupled to each other (Table 3). Thus, compound **9** possessed an enolized β-dicarbonyl group stabilized by hydrogen bonding between the carbonyl oxygen and enolic hydroxyl proton.

The structural elucidation of **9** was aided by a combination of ^{1}H COSY, HMQC, and HMBC experiments. Long-range correlations of three methyl protons at δ 1.15 (Me-13), 0.91 (Me-15), and 0.86 (Me-14) with adjacent carbons were particularly helpful for determining the structure of the A and B rings. The presence of an α,β-unsaturated carbonyl group was confirmed by HMBC correlations between the olefinic proton at δ 8.60 and quaternary carbons at δ 188.9 and 122.8. An additional long-range correlation of the latter carbon with Me-13 enabled us to assign the location of the β-hydroxy-α,β-unsaturated carbonyl moiety to C-8, C-9, and C-11, which was further supported by a three-bond

Table 3. Proton NMR Assignments for Norsesquiterpenoids **9** and **10**[a,b]

position	9	10
1ax	1.30 (ddd, 12.7, 12.7, 3.7)	1.04 (ddd, 13.2, 13.2, 3.4)
1eq	2.01 (br d, 12.7)	1.72 (br dd, 13.2, 3.4)
2ax	1.63 (m)	1.58 (ddddd, 13.7, 13.7, 13.2, 3.4, 3.4)
2eq	1.53 (m)	1.41 (m)
3ax	1.20 (m)	1.16 (ddd, 13.7, 13.7, 3.4)
3eq	1.45 (dddd, 13.6, 3.3, 3.3, 1.5)	1.41 (m)
5	1.16 (dd, 12.0, 1.8)	0.90 (dd, 11.7, 2.0)
6ax	1.58 (m)	1.41 (m)
6eq	1.80 (br ddd, 13.6, 7.7, 1.8)	1.48 (m)
7ax	2.39 (ddd, 19.8, 11.0, 7.7)	1.48 (m)
7eq	2.48 (ddd, 19.8, 7.0, 1.8)	1.99 (br ddd, 13.2, 2.9, 2.9)
8		5.12 (ddd, 3.4, 2.9, 2.9)
9		1.53 (ddd, 10.7, 3.9, 3.4)
11	8.60 (d, 4.4)	4.14 (dd, 10.7, 3.9) 4.01 (dd, 10.7, 10.7)
13	1.15 (d, 0.7)	1.00 (s)
14	0.86 (s)	0.84 (s)
15	0.91 (s)	0.86 (s)
OAc		2.03 (s, C−8), 2.00 (s, C−11)
OH	15.36 (d, 4.4)	

[a] Measured in $CDCl_3$ solutions at 500 MHz; δ in ppm (J in Hz); TMS as internal standard. [b] Assignments were aided by ^1H COSY, HMQC, and HMBC experiments.

correlation of the carbon at δ 188.9 with H-6eq. Of the two possible arrangements of the unsaturated carbonyl group, that is, either a ketone and an enol or an enol and an aldehyde at C-8 and C-11, respectively, the former was proved to be correct because the coupling constant between the enol and olefinic protons ($J = 4.4$ Hz) was much larger than the homoallylic coupling expected from the latter arrangement. Thus, the structure of **9** was determined as a 12-norsesquiterpenoid of the drimane class.

The molecular formula of **10**, a white solid, was deduced as $C_{18}H_{30}O_4$ by HRCIMS and ^{13}C-NMR analysis. The presence of two acetoxyl groups, readily recognized by the NMR data, suggested that **10** was also a norsesquiterpenoid. Careful examination of the NMR data revealed that **10** was structurally closely related to **3**. In addition to the spectral changes due to the loss of a carbon, the only significant difference in the ^{13}C-NMR data was the replacement of one of the oxymethylene carbons at δ 63.9 (C-12) and 62.7 (C-8) by an oxymethine carbon at δ 69.2. A corresponding difference was also observed in the ^1H-NMR spectrum in that a new downfield signal was found at δ 5.12 (1H, ddd, $J = 3.4, 3.9, 2.9$ Hz). Therefore, an oxymethylene group in **3** must be lost in compound **10**. The structure of **10** was defined with the aid of combined 2-D NMR methods. The ^1H COSY experiment showed that an upfield proton signal at δ 1.53 was coupled with all of the oxymethylene and oxymethine signals at δ 5.12, 4.14, and 4.01. This proton was assigned as H-9 because the carbon at δ 51.4 bearing this proton showed a long-range correlation with Me-13 in the HMBC experiment. Therefore, two acetoxyl groups were attached to C-8 and C-11 of the 12-nordrimane skeleton. Supporting evidence for this interpretation was the HMBC correlations of H-11 with C-8 and C-9. In addition to the *trans* AB ring junction, the relative configurations of asymmetric carbon centers of **10** were assigned as $8S^*,9S^*$ on the basis of a NOESY correlation of H-9 with H-5 together with the small vicinal coupling ($J_{8,9} = 3.4$ Hz). Thus, the structure of **10** was determined as a diacetoxylated 12-norsesquiterpenoid of the drimane class.

Metabolites possessing norsesquiterpene carbon skeletons have been occasionally isolated from both marine and terrestrial organisms. Among compounds of the drimane class, 11-norsesquiterpenoids have been isolated from the folk medicinal plant *Polygonium hydropiper*.[9] To the best of our knowledge, however, **9** and **10** are the first examples of 12-norsesquiterpenoids of the drimane class as natural products.

Some of the marine-derived drimane sesquiterpenoids have been reported to exhibit antibacterial, antifungal, and cytotoxic activities.[2] In our antimicrobial assays, **2** exhibited potent inhibition (IC_{50} 11.4 μM) of the bioluminescence reaction of *Photobacterium leiognathi*, a symbiotic luminous bacterium of tropical fish, while **1**, **6**, **7**, and **10** exhibited weaker inhibition (IC_{50} 90− 145 μM). In a method using filter paper disks, **2** was active (inhibition zone, 8 mm) against the same strain at a concentration of 10 μg/mL. In addition, **2**, **4**, and **6** exhibited moderate inhibition of Na^+/K^+-ATPase (IC_{50} 82, 98, and 45 μM for **2**, **4**, and **6**, respectively). Compound **6** was also weakly active against PLA_2 (IC_{50} 113 μM). All other compounds were inactive in the same tests.

In addition to their pharmacological activities, drimane sesquiterpenoids exhibit antifeedant activity against potential predators.[1] Polygodial (**2**), famous for its hot taste to humans, is reported to exhibit potent antifeedant activity against both terrestrial insects and carnivorous fish.[10−12] Crude extracts of the *Dysidea* sp. and pure 7-deacetoxyolepupuane (**1**) also showed antifeedant activity to reef fishes.[5] The other compounds have not yet been tested as antifeedants.

Experimental Section

General Experimental Procedures. NMR spectra were recorded in $CDCl_3$ solutions on a Varian Unity-500 spectrometer. Proton and carbon NMR spectra were measured at 500 and 125 MHz, respectively. All of the chemical shifts were recorded with respect to internal Me_4Si. IR spectra were recorded on a Mattson GALAXY spectrophotometer. UV spectra were obtained in MeOH using a Milton-Roy spectrophotometer. Mass spectra were provided by the Mass Spectrometry Facility, Department of Chemistry, University of California, Riverside. The optical rotations were measured on a JASCO digital polarimeter using a 5-cm cell. Melting points were measured on a Fisher-Johns apparatus and are reported uncorrected. All solvents used were spectral grade or were distilled from glass prior to use.

Animal Material. The specimens for extraction and taxonomic identification were collected by hand using scuba at 20−25 m depth in July 1996, off the western shore of Guam, at a site known as the Sponge Mound in Apra Harbor, Guam.[6] The voucher specimens are deposited at the British Museum of Natural History (no. 1997.5.13.1). Morphological characters of the specimens are similar to those of the type species *D. fragilis* Montague. The sponge had an encrusting morphology covering areas of 4×3 in. and producing upright lobes. Color of both the exterior and interior was dark gray in life and deep brown in spirit. The texture is friable, delicate, and easily torn. The surface is strongly conulose, each conule having a single, apical primary fiber extending from it and accessary lateral conules and fibers. As a result of the heavy content of coring

material in the primary fibers, the sponge surface appears to have a whitish tracery. Oscules are not evident in the specimens, and the surface membrane between conules is smooth macroscopically but supports a thin superficial layer of uniformly sized sand grains. This layer is enhanced to 100 μm deep on conule surfaces and tips. The skeleton has a rectangular arrangement with simple primary fibers oriented to the conules, of even diameter (60–120 μm) throughout the body of the sponge and packed with sand grains, leaving very little clear spongin evident. Fiber construction is stratified, and secondary fibers are oriented at right angles to the primaries, unbranched and cored to half the fiber diameter. The tissue is evenly infiltrated with pigment cells and shows no collagen enhancement near the surface or around canals. There are no evident algal or cyanobacterial symbionts. Choanocyte chambers are oval, 40 μm in longest dimension.

Extraction and Isolation. The freshly collected animals were lyophilized (dry wt 25 g), macerated, and repeatedly extracted with CH_2Cl_2 and MeOH. The combined crude extracts (3.6 g) were separated by silica vacuum flash chromatography by using gradient mixtures of *n*-hexane and EtOAc as eluents. Fractions eluted with nonpolar solvents (10–15% EtOAc–hexane) were combined and separated by semipreparative silica HPLC (YMC silica column, 12% EtOAc–hexane) to yield compounds **1**, **3**, and **7** in the order of **1**, **7** and **3**; 465.0, 31.2, and 2.2 mg for **1**, **3**, and **7**, respectively. Fractions eluted with slightly more polar solvents (20–25% EtOAc–hexane) were combined and subjected to silica HPLC (YMC silica column, 15% EtOAc–hexane) to yield **2**, **4**–**6**, **9**, and **10** in the order of **6**, **4**, **5**, **9**, **10**, and **2**. Final purification was made by reversed-phase HPLC (YMC *J* sphere ODS–H80 column, 100% CH_3CN) to isolate 6.7, 10.4, 9.1, 10.5, 6.3, and 16.0 mg for **2**, **4**–**6**, **9**, and **10**, respectively. Fractions eluted with moderately polar solvents (30–35% EtOAc–hexane) were separated by silica HPLC (15% EtOAc–hexane) and purified by reversed-phase HPLC (100% CH_3CN) using the same columns to yield **2** and **8**; 24.6 and 3.1 mg for **2** and **8**, respectively.

7-Deacetoxyolepupuane (1): white solid; mp 79–80 °C; $[\alpha]^{25}_D$ −153.9° (*c* 1.0, MeOH (lit.[2,3] $[\alpha]^{25}_D$ −166.7°);[2,3] HREIMS (M)$^+$ *m/z* 278.1871 (calcd for $C_{17}H_{26}O_3$, 278.1882).

Polygodial (2): white solid; mp 53–55 °C; $[\alpha]^{25}_D$ −123.4° (*c* 0.3, EtOH) (lit.[13,14] $[\alpha]^{25}_D$ −131°); IR (KBr) ν_{max} 3450 (broad, −OH), 2930, 2850, 1720 (−CHO), 1680 (unsaturated aldehyde), 1460, 1370, 1070 cm^{-1}; HR-CIMS (M + NH$_4$)$^+$ *m/z* 252.1949 (calcd for $C_{15}H_{26}NO_2$, 252.1963).

Compound 3: colorless oil; $[\alpha]^{25}_D$ +37.2° (*c* 0.1, MeOH) (lit.[3] $[\alpha]^{25}_D$ +37°); HRCIMS (M + NH$_4$)$^+$ *m/z* 342.2630 (calcd for $C_{19}H_{36}NO_4$, 342.2644).

Compound 4: colorless oil; $[\alpha]^{25}_D$ −7.2° (*c* 0.1, MeOH); UV (MeOH) λ_{max} 221 nm (log ϵ 3.65); IR (KBr) ν_{max} 2930, 2850, 1780 (unsaturated γ-lactone), 1760, 1210, 1010, 970 cm^{-1}; ^{1}H- and ^{13}C-NMR values, see Tables 1 and 2; NOESY correlations H-2ax/Me-13, H-2ax/-14, H-5/Me-15, H-6eq/Me-15, H-11/Me-13, Me-13/Me-14; HMBC correlations H-5/C-6, C-9, C-10; H-6eq/ C-5, C-7, C-8, C-10; H-7ax/C-6, C-8, C-9; H-7eq/C-5, C-6, C-8, C-9; H-11/C-8, C-12, C−OAc; Me-13/C-1, C-5, C-9,

C-10; Me-14/C-3, C-4, C-5; Me-15/C-3, C-4, C-5; HR-CIMS (M + NH$_4$)$^+$ *m/z* 310.2025 (calcd for $C_{17}H_{28}NO_4$, 310.2018).

Compound 5: colorless oil: $[\alpha]^{25}_D$ −40.1° (*c* 0.1, MeOH); UV (MeOH) λ_{max} 223 nm (log ϵ 3.78); IR (KBr) ν_{max} 2930, 2870, 1770, 1350, 1190, 960 cm^{-1}; ^{1}H- and ^{13}C-NMR values, see Tables 1 and 2; NOESY correlations H-1ax/H-9, H-2ax/Me-14, H-3ax/H-5, H-3ax/Me-15, H-5/H-9, H-5/Me-15, H-6ax/Me-14, H-6eq/Me-15, H-11/Me-13; HMBC correlations H-5/C-6, C-9, C-13, C-14, C-15; H-6ax/C-5; H-6eq/C-8, C-10; H-9/C-7, C-10, C-13; H-11/C-9, C-10, C-12, Ac; Me-13/C-1, C-5, C-9, C-10; Me-14/C-3, C-4, C-5; Me-15/C-3, C-4, C-5; HR-CIMS (M + NH$_4$)$^+$ *m/z* 310.2015 (calcd for $C_{17}H_{28}NO_4$, 310.2018).

Compound 6: white solid: mp 75–76 °C; $[\alpha]^{25}_D$ +14.4° (*c* 0.3, MeOH); IR (KBr) ν_{max} 2950, 2860, 1765 (ester), 1460, 1350, 1150, 1090, 990 cm^{-1}; ^{1}H- and ^{13}C-NMR values, see Tables 1 and 2; NOESY correlations H-2ax/Me-13, H-2ax/Me-14, H-3ax/Me-15, H-3eq/Me-14, H-3eq/Me-15, H-5/H-9, H-6ax/Me-13, H-6ax/Me-14, H-6eq/ Me-15, H-8/Me-13, Me-13/Me-14; HMBC correlations H-2ax/C-1; H-7ax/C-6; H-8/C-12; H-9/C-1, C-7, C-8, C-10, C-11, C-12, C-13; H-12/C-7, C-8, C-9, C-11; Me-13/C-1, C-5, C-9, C-10; Me-14/C-3, C-4, C-5, C-14; Me-15/C-3, C-4, C-5, C-15; HREIMS (M)$^+$ *m/z* 236.1771 (calcd for $C_{15}H_{24}O_2$, 236.1776).

Compound 7: colorless oil; $[\alpha]^{25}_D$ −21.6° (*c* 0.3, MeOH); IR (KBr) ν_{max} 2930, 1460, 1370, 1240, 1120, 1050, 990 cm^{-1}; ^{1}H- and ^{13}C-NMR values, see Tables 1 and 2; NOESY correlations H-5/H-9, H-6ax/Me-13, H-8/ H-12 (δ 3.96), H-11/Me-13, H-11/OMe; H-12 (δ 3.56)/ Me-13, Me-13/Me-14; HMBC correlations H-1ax/C-13; H-6ax/C-5; H-9/C-1, C-10, C-11, C-13; H-11/C-8, C-9, C-12, C−OMe; H-12/C-7, C-8, C-9; Me-13/C-1, C-9, C-10; Me-14/C-3, C-4, C-14; Me-15/C-3, C-4, C-5, C-15; OMe/ C-11; HREIMS (M − OCH$_3$)$^+$ *m/z* 221.1908 (calcd for $C_{15}H_{25}O$, 221.1905).

Compound 8: colorless gum; $[\alpha]^{25}_D$ +2.8° (*c* 0.2, MeOH); IR (KBr) ν_{max} 3450 (broad, −OH), 2930, 2870, 1455, 1380, 1100, 1020 cm^{-1}; ^{1}H- and ^{13}C-NMR values, see Tables 1 and 2; NOESY correlations H-1ax/H-9; H-1eq/H-11, H-1eq/Me-13, H-2ax/Me-14, H-5/H-9, H-6ax/ H-12, H-6ax/Me-13, H-6ax/Me-14, H-6eq/Me-15, H-11/ Me-13, H-11/OMe, H-12/Me-13; HMBC correlations H-5/ C-4, C-6, C-7, C-10, C-13; H-7ax/C-6, C-8, C-12; H-7eq/ C-5, C-9; H-9/C-1, C-8, C-10, C-11, C-12, C-13; H-11/C-8, C-10, C-12, OMe; H-12/C-8, C-11; Me-13/C-1, C-5, C-9, C-10; Me-14/C-3, C-4, C-5, C-15; Me-15/C-3, C-4, C-5, C-14; OMe/C-11; HRCIMS (M + NH$_4$ − H$_2$O)$^+$ *m/z* 284.2217 (calcd for $C_{16}H_{30}NO_3$, 284.2225).

Compound 9: white solid; mp 79–80 °C; $[\alpha]^{25}_D$ +9.6° (*c* 0.1, MeOH); UV (MeOH) λ_{max} 294 nm (log ϵ 3.34); IR (KBr) ν_{max} 3500 (broad, −OH), 2930, 2870, 1620 (C=O stretching of β-hydroxy-α,β-unsaturated ketone), 1590, 1460, 1380, 1290, 1200, 940 cm^{-1}; ^{1}H- and ^{13}C-NMR values, see Tables 2 and 3; HMBC correlations H-6ax/ C-5; H-6eq/C-8; H-7ax/C-6, C-8; H-7eq/C-5, C-6, C-8; H-11/C-8, C-9; Me-13/C-1, C-5, C-9, C-10; Me-14/C-3, C-4, C-5, C-15; Me-15/C-3, C-4, C-5, C-14; HREIMS (M)$^+$ *m/z* 222.1614 (calcd for $C_{14}H_{22}O_2$, 222.1619).

Compound 10: white solid; mp 84–85 °C; $[\alpha]^{25}_D$ +36.0° (*c* 0.2, MeOH); IR (KBr) ν_{max} 2950, 2870, 1740, 1370, 1250, 1000 cm^{-1}; ^{1}H- and ^{13}C-NMR values, see Tables 2 and 3; HMBC correlations H-5/C-4, C-6; H-11/

C-8, C-9, 11-OAc; Me-13/C-1, C-5, C-9, C-10; Me-14/C-3, C-4, C-5; Me-15/C-3, C-4, C-5, C-14; HRCIMS (M + NH$_4$)$^+$ *m*/*z* 328.2494 (calcd for C$_{18}$H$_{34}$NO$_4$, 328.2487).

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