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Configurational Analysis of New Furanosesquiterpenes from *Dysidea herbacea*. Assignment of Absolute Stereochemistry.

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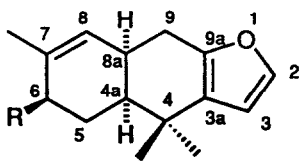
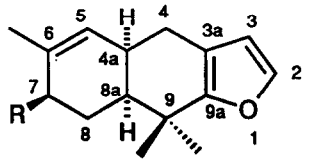
Abstract: Two new marine furanoterpenes, (+)-(4a*S*, 6*R*,8a*S*)-4,4,7-trimethyl-4,4a,5,6,8,8a,9-hexahydronaphtho[2,3-*b*]furan-6-yl acetate (**1**) and (+)-(4a*S*,7*R*,8a*S*)-6,9,9-trimethyl-4,4a,7,8,8a,9-hexahydronaphtho[2,3-*b*]furan-7-yl acetate (**2**) , were isolated from a sample of *Dysidea herbacea*, collected from Harrier Reef on the Great Barrier Reef, Australia. The enantiomer of **1**, (-)-(4a*R*, 6*S*,8a*R*)-4,4,7-trimethyl-4,4a,5,6,8,8a,9-hexahydronaphtho[2,3-*b*]furan-6-yl acetate (**17**) was found in another sample of *D. herbacea* collected nearby from Norman Reef. The structures were determined by spectroscopic methods, in particular 2D NMR, and the absolute configurations of the parent rings of **1** and **2** defined by chemical correlation and application of Kakisawa's modification of the Mosher ester method. Compounds **1** and **2** are diastereomers of acetates **5** and **6**, reported earlier from *D. herbacea*, but have opposite configuration with respect to the ring junctions. These results and others suggest *D. herbacea* may possess a dual capacity for antipodal cyclization pathways from a common achiral terpenoid precursor.

Introduction: Naturally occurring monoterpenes from terrestrial organisms are frequently found as both enantiomeric modifications. Antipodal sesquiterpenes and higher terpenes, however, are less common. Furodysinin, one of several rearranged furanosesquiterpenes, isolated from marine sponges of the genus *Dysidea*, has recently been shown to occur in both antipodal forms: (+)-furodysinin (**3**) or (-)-furodysinin (**7a**), depending on the species of *Dysidea* and its geographical location.[1-3] We now report the isolation and absolute stereostructure of two new furanosesquiterpene acetates, **1** and **2**, from *Dysidea herbacea* collected from Harrier Reef on the middle Great Barrier Reef (15° 23'S; 145° 45'E) and show that they belong to the (+)-furodysinin series. In addition we report (-)-**17**, the enantiomer of **1**, from the same species of sponge collected from Norman Reef (16° 51'S; 146° 00'E), less than 120 km away.





1 R = OAc
3 R = H
9 R = OH
11 R = -O-(*R*)-MTPA
12 R = -O-(*S*)-MTPA

2 R = OAc
4 R = H
10 R = OH
13 R = -O-(*R*)-MTPA
14 R = -O-(*S*)-MTPA

Results: The hexane soluble fraction of the total methanol extract of *Dysidea herbacea* was fractionated by repeated flash chromatography and HPLC to provide the trichloroleucine derivative, herbaceamide (**16**),[4] a mixture of furodysinin and furodysin (3:1 inseparable mixture, trace[1]), two non-polar acetates, **1** (m.p. 107-108°; $[\alpha]_D$ +61.3°, c= 0.57 $CHCl_3$) and **2** and their corresponding alcohols, **9** and **10**. Inspection of the NMR and mass spectroscopic data for **1** and **2** suggested that the compounds were isomeric 6-acetoxy derivatives of the respective parents furodysinin (**3**) and furodysin (**4**). The CI HRMS of compound **1** gave an MH^+ ion at m/z 275.1637, which provided the formula $C_{17}H_{22}O_3$ with seven degrees of unsaturation. The presence of an acetoxyl group was revealed by an IR band at 1737 cm^{-1} while the ^{13}C NMR data suggested a 2,3-disubstituted furan (δ 140.8 d, 108.1 d, 124.4 s, 147.0 s), further supported by ^1H NMR (δ 7.21, m, 1H; 6.22, d, J = 2.0 Hz, 1H). A trisubstituted alkene (131.4, d, 132.5, s) and three rings accounted for the remainder of the unsaturation.

Most of the ^{13}C NMR signals of **1** agreed well with those of furodysinin (**3**).[1] In particular, the relatively low field signal due to C3a (124.4, s) in **1** was diagnostic of C3a substitution by a quaternary carbon, which eliminated the isomeric furodysin (**4**) skeleton (*c.f.* 113.0, s[1]). Note that due to the systematic IUPAC ring nomenclature used here, the numbering schemes for furodysinin **3** and furodysin **4** skeletons differ, however, Figure 1 compares spectral data for analagous ^1H and ^{13}C signals in each row.

The largest difference in the ^{13}C-NMR spectrum of **1** and **3** was the substitution of signals for a methylene group in **3** for a oxymethine group in **1** (74.0, d) together with the addition of an acetoxyl group (2.04, s, 3H; 21.1, q, 170.9, s). The location of the acetoxyl group and complete proton assignments for **1** were obtained by analysis of COSY and HETCOR experiments together with ^1H-^1H coupling constant analysis (Figure 1). The acetoxyl bearing carbon signal of C6 (74.0, d) was correlated to a H6 signal in the ^1H-NMR spectrum (5.42, br dd, J = 9.8, 7.0 Hz, 1H). This was further coupled to the H5 vicinal methylene group (2.15 ddt, 1H, J = 12.5, 7.0, 2.0 Hz, 1H; 1.20 ddd, J = 14.0, 12.5, 9.8 Hz, 1H) which were contiguous with the ring junction protons H4a (1.72, ddd, J = 14.0, 3.0, 2.0 Hz, 1H) and H8a (2.70, m, 1H).

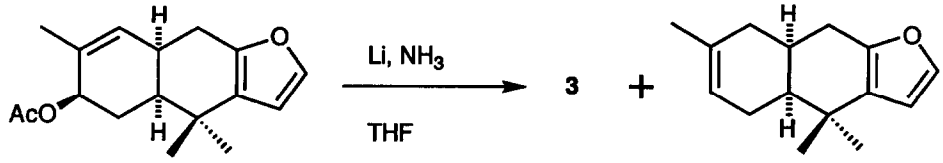
Compound **2**, $C_{17}H_{22}O_3$ (oil) was found to be isomeric with **1** by analysis of data from EI HRMS (m/z, M^+ 274.1594), Δmmu 2.5) and was assigned the regioisomeric furodysin skeleton by comparison of IR, ^1H NMR and particularly, the ^{13}C NMR data, with those of furodysin.[1] The exceptionally low field C3a signal (156.2, s) indicated substitution at C9a by a quaternary carbon. The remaining spectroscopic data for the **2** were almost identical with those of **1**. Like **1**, compound **2** is dextrorotatory ($[\alpha]_D$ +41.0°, c 1.19, $CHCl_3$).

The relative stereochemistry of **1** and **2** was determined by ^1H coupling constant analysis and interpretation of the phase sensitive NOESY spectra (t_m 1.5 s) (Figure 1). A strong NOESY correlation between H4a and H8a found in **1** together with a small vicinal coupling (J = 3 Hz) confirmed a *cis* ring junction, while a *pseudo*-equatorial 6-OAc conformation was indicated by the vicinal coupling constants for the allylic proton H6 in **1** (5.42, br dd, J = 9.8, 7.0 Hz). The cyclohexene ring in **1** adopts the same half chair conformation as furodysinin[5] as evidenced by a diagnostic 'W' coupling between H8a and H5α (J = 2 Hz) and nOe correlations between the *syn*-protons H6 and H4a. This conformer, presumably, is favored over an alternate *pseudo*-axial OAc conformer due to relief of gauche strain against the vinylic methyl group. The second ring also favours a half chair conformation with H9β in a *pseudo*-axial position, as shown by an nOe correlations between H9β and H5β. A similar analysis of **2** produces the same conformation.

Atom #	Compound 1			Atom #	Compound 2		
	^{13}C δ (mult.)	^{1}H, δ (mult., *J* (Hz), integration)	NOESY		^{13}C δ (mult.)	^{1}H, δ (mult., *J* (Hz), integration)	NOESY
1	-	-		3	109.7 (d)	6.10 (d, *J* = 2.0, 1H)	2
2	140.8 (d)	7.21 (m_s, 1H)	3	2	140.7 (d)	7.23 (d, *J* = 2.0, 1H)	3
3	108.1 (d)	6.22 (d, *J* = 2.0, 1H)	2, 4β-Me	1	-	-	
3a	124.4 (s)	-		9a	156.1 (s)	-	
4	33.2 (s)	-		9	34.6 (s)	-	
4a	43.5 (d)	1.72 (ddd, *J* = 14.0, 3.0, 2.0, 1H)	4α-Me, 6, 8a	8a	44.5 (d)	1.78 (ddd, *J* = 14.0, 3.0, 2.0, 1H)	4a, 7, 9α-Me
5 (α)	26.0 (t)	2.15 (ddt, *J* = 12.5, 7.0, 2.0, 1H)	5β, 4β-Me, 6	8 (α)	26.2 (t)	2.19 (ddt, *J* = 12.2, 7.0, 2.0, 1H)	7, 8β, 9β-Me
(β)		1.30 (ddd, *J* = 14.0, 12.5, 9.8, 1H)	5α, 9β	(β)		1.39 (ddd, *J* = 14.0, 12.2, 9.6, 1H)	4β, 8α
6	74.0 (d)	5.42 (br dd, *J* = 9.8, 7.0, 1H)	4a, 5α, 7-Me	7	74.0 (d)	5.42 (br dd, *J* = 9.6, 7.0, 1H)	6-Me, 8α, 8a
7	132.5 (s)	-		6	132.0 (s)	-	
8	131.4 (d)	5.85 (dm, *J* = 6.0, 1H)	7-Me, 8a	5	131.7 (d)	5.83 (dm *J* = 6.0, 1H)	4a, 6-Me
8a	31.2 (d)	2.70 (m, 1H)	4a, 4α-Me, 8	4a	31.5 (d)	2.60 (m, 1H)	5, 8a, 9α-Me
9 (α)	27.2 (t)	2.78 (dd, *J* = 15.5, 6.4, 1H)	9β	4 (α)	26.9 (t)	2.60 (dd, *J* = 17.2, 6.0, 1H)	4β
(β)		2.40 (dd, *J* = 15.5, 10.0, 1H)	5β, 9α,	(β)		2.30 (dd, *J* = 17.2, 12.2, 1H)	4α, 8β
9a	147.0 (s)	-		3a	112.9 (s)	-	
4α-Me	32.5 (q)	1.17 (s, 3H)	4a, 8a	9α-Me	30.4 (q)	1.26 (s, 3H)	4a, 8a
4β-Me	26.2 (q)	1.20 (s, 3H)	3, 5α	9β-Me	23.7 (q)	1.24 (s, 3H)	8α
7-Me	18.9 (q)	1.64 (br s, 3H)	6, 8	6-Me	18.9 (q)	1.64 (br s, 3H)	5, 7
Ac Me	21.1 (q)	2.05 (s, 3H)		AcMe	21.2 (q)	2.05 (s, 3H)	
AcC=O	170.9 (s)			AcC=O	170.9 (s)		

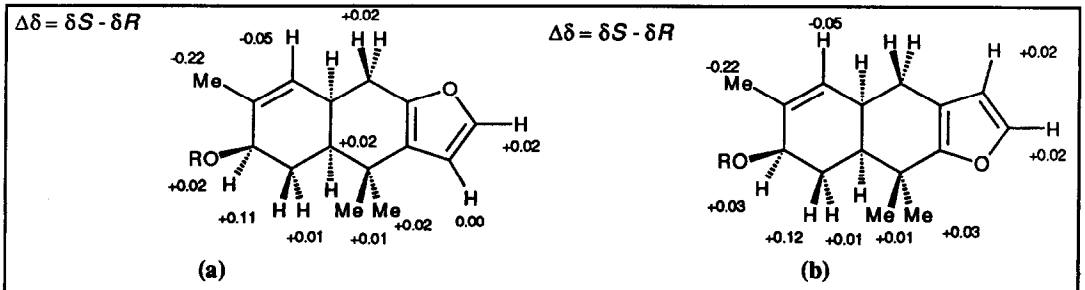
Figure 1 ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) of acetates **1** and **2** in $CDCl_3$. ^{13}C multiplicities were determined from DEPT experiments and assignments are based on interpretation of COSY, NOESY and HETCOR experiments. Note the numbering schemes are different as a consequence of usage of IUPAC ring nomenclature for the different heterocyclic parents. See Experimental for IUPAC systematic names.

The absolute configurations of **1** and **2** were determined as follows. Reductive deacetoxylation of **1** (Li, NH_3, THF, -50 °C, 20 min) gave (+)-furodysinin (**3**) and the allylic rearrangement product **8**,[1] isolated as an inseparable mixture (3:1). The absolute stereochemistry of (-)-furodysinin (**7a**) and (-)-furodysin (**7b**) have been established by total synthesis from (+)-9-bromocamphor,[7,8] therefore, the absolute configuration of **1** must be (4a*S*, 8a*S*).



1 **8**

The unavoidable presence of **8** in the purified Li-NH_3 reduction product of **1** compromised interpretation of the sign of rotation of **3** in the mixture, so an independent method was used to confirm stereochemistry. The absolute stereochemical assignments of **1** were secured by application of the modified Mosher's method.[9,10]. Acetates **1** and **2** were separately hydrolyzed (NaOH, MeOH aq., 23 °C) to give the corresponding alcohols **9** and **10**, each of which was partitioned and esterified independently with excess (*R*)- or (*S*)-methoxytrifluoromethylphenylacetic acid (MPTA, DCC, THF, 23 °C) and the products purified by HPLC (Dynamax silica, 5:95 EtOAc-hexane) to give four MPTA esters, **11-14**. A single diastereoisomer was obtained from each reaction with MTPA acid, proving that each of the corresponding alcohols and acetates were optically pure. Pairwise comparison of signals in the ^1H NMR spectra of the (*R*) and (*S*)-MTPA esters (**11/12**; **13/14**) derived from each alcohol revealed that protons positioned clockwise around the carbocyclic rings from the acyl group showed a negative $\Delta\delta$ ($\Delta\delta = \delta_S - \delta_R$) and those positioned counterclockwise gave a positive $\Delta\delta$ (Figure 2). Interpretation of these data according to the modified Mosher's ester method[9,10] predicts that acetate **1** has the (4a*S*, 6*R*, 8*S*) configuration and acetate **2** has the analogous (4a*S*,7*R*,8a*S*) configuration as drawn. This agrees with the ring junction stereochemistry inferred from the Li-NH_3 reduction and both **1** and **2** can now be placed in the (+)-furodysinin and (+)-furodysin series, respectively.

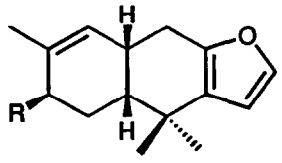


(a) (b)

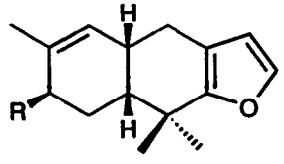
Fig. 2 ^1H NMR $\Delta\delta$ values for (a) (*R*)-and (*S*)-MTPA esters, **11** and **12**, derived from acetate **1** and (b) esters **13** and **14**, derived from acetate **2**.

Several additional samples of *Dysidea herbacea* were collected at the same time from the Great Barrier Reef. Examination by TLC and ^1H NMR of the hexane soluble fractions of the sponge extracts showed the

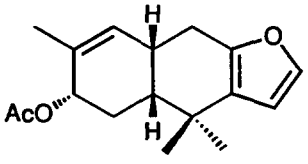
presence of furanoterpenes including furodysin, furodysinin and nakafuran-8 and nakafuran-9.[11] One extract from a sample (90-05-041) collected at Norman Reef (16° 51'S; 146° 00'E) gave an extract that exhibited ^{1}H NMR and TLC properties characteristic of the 6β-acetoxyfurodysin /furodysinin mixture. This extract was separated by HPLC to provided two isomeric compounds, for which HPLC retention times, ^{1}H NMR, ^{13}C-NMR and MS spectra were identical to those of **1** and **2**, respectively. Although the major isomer was obtained pure, the minor isomer was contaminated with an inseparable unknown furanoterpene. Careful determination of the specific rotation for the major pure isomer revealed that *the $[\alpha]_D$ was opposite in sign and equal in magnitude* to that of **1**. Clearly, the new major isomer is (-)-**17**, the enantiomer of **1** of optical purity *ca.* 100%, and it is most likely that the minor isomer **18** corresponds to the enantiomer of **2**.



5 R = β-OAc
7a R = H



6 R = β- OAc
7b R = H



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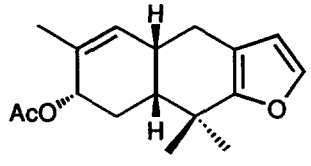

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Discussion: Two compounds, **5** and **6**, were isolated from *Dysidea herbacea* by Wells *et al.*, from Gladstone, near the southern Great Barrier Reef (23° 50'S; 151° 16'E). The absolute configurations of **5** and **6** were not determined, however, we show here they are diastereomers of **1** and **2** with opposite ring junction chirality.[6] Compounds **5** and **6** have ^{1}H NMR spectra almost identical to their respective stereoisomers **1** and **2**, however other properties are different. The ^{13}C NMR signal due to C6 in **1** (δ 74.0, d) occurs downfiled in **5** (δ 79.1, d). The specific rotations of **1** and **2** are opposite in sign, but unequal in magnitude to those of **5** and **6** ($[\alpha]_D$ -41° $CHCl_3$ and $[\alpha]_D$ -32°, CCl_4, respectively). Considering the pronounced dextrorotation exhibited by (+)-(4a*S*,8a*S*)-furodysinin (**3**) and its derivative **1**, the absolute configuration of diastereoisomer **5** is assigned as (4a*R*, 6*R*, 8*R*), which places it in the (-)-*ent*-furodysinin series. A similar argument establishes the configuration of compound **6** as (4a*R*,7*R*,8*R*) and relates this compound to (-)-*ent*-furodysin.[8] It is noteworthy that the signs of optical rotation of the two isomeric 6-acetoxyfurodysinins, **1** and **5**, correlate well with the C4a-8a stereochemistry and conform to the general trend noted by Crews *et al.*[3]

The stereostructure of natural product **9**, obtained by hydrolysis of acetate **1**, matches that proposed for a minor alcohol isolated by Wells *et al* ($[\alpha]_D$ +2.0, $CHCl_3$, referenced as compound number 20 in their paper)[6] from another Australian sample of *Dysidea herbacea*, however, the reported ^{1}H NMR data for their compound 20 do not agree with those of **9** or **10**. As the structure of **9** is established unambiguously, Well's compound 20 may correspond to yet a third diastereomer of 6-hydroxyfurodysinin, however, the reported data

are incomplete and the arguments presented for the stereochemistry do not rigorously exclude other hydroxyfurodysinin stereoisomers, for example, a *trans* fused ring system.

The reported rotations of furodysinin isolated from geographically diverse sponges vary in sign and magnitude. *Dysidea tupha* of the East Pyrenean Mediterranean contains (-)-*ent*-furodysinin (**8**) ($[\alpha]_D$ -47.8°, $CHCl_3$), as does *D. herbacea* from Fiji (-61°, $CDCl_3$).[3] Conversely, dextrorotatory (+)-furodysinin (**4**) was found in *D. herbacea* collected near Sydney, Australia, (+64°, $CHCl_3$)[1] The differences in magnitude of these optical rotations may be due to heterogeneous enantiomeric composition, or scalemic mixtures.† Although measurement errors in reported optical rotations are not uncommon, we cannot preclude the possibility that furodysinin isolated from different geographical locations may have optical purity less than 100% and consist of mixtures of enantiomers, varying along a continuum of composition from pure **3** to pure **7a**.* Scalemic terrestrial natural products, and more recently, marine natural products, has several precedents and several chiral monoterpenes of heterogeneous enantiomeric composition are known from terrestrial sources. Terpene cyclases have been isolated from immature sage, *Salvia officinalis*, and shown to be capable of producing (+)-α-pinene and (-)-α-pinene independently from geranyl pyrophosphate with no cross reactivity.[13] Cimino *et al.* have reported the isolation of bicyclic and tricyclic *ent*-diterpenes from opistobranch molluscs *Archidoris* spp.[14] Finally, we have found the diterpene, 5'-hydroxyambliofuran, in a Western Australian *Spongia* sp. as a 3:1 mixture of enantiomers, [15] Although enantiomeric furodysinins have been described from disparate geographical locations, the present study shows that they may be produced by the same species of sponge collected from the same location (*Dysidea herbacea* from two collection sites separated by less than 120 km).

Faulkner and coworkers have demonstrated that sponge cells isolated from *Dysidea herbacea* contain only sesquiterpenes and no trichloroleucine metabolites and, conversely, trichloroleucine metabolites are sequestered only by the symbiont *Oscillatoria* sp.[16] These observations, together with the foregoing discussion, strongly suggests that *Dysidea herbacea* sponge cells from certain collections have the enzymic capacity to catalyze antipodal modes of cyclization of farnesyl pyrophosphate leading to enantiomeric furodysinin series. Since, it is highly unlikely that one enzyme could be responsible for generating antipodal furodysinin skeleta, it would appear that *Dysidea herbacea* contains at least two types of cyclases leading to enantiomeric products from a suitable precursor, in turn derived from farnesyl pyrophosphate, analogous to those found in *Salvia* sp.[13] A candidate for the furodysinin precursor, (+)-spirodysin (**15**), has been described from *D. herbacea*. Lewis acid ($BF_3.Et_2O$) catalyzed cyclization of **15** gave furodysinin and furodysin (optical rotations of the products were not reported).[17] Biosynthetic oxidation and acetylation of furodysinin would furnish **1** and **2**. We have found **1** and **2** in strictly homochiral form, however, this is not unexpected as kinetic resolution at one or more of the later enzyme catalyzed steps may provide a high level of diastereoselection.

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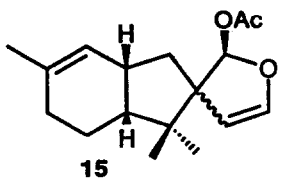
† James Brewster, of Purdue University, first proposed the term 'scalemic' for non-racemic mixtures of enantiomers. This term has been adopted by several chemists[12] and is appropriate here to describe such natural products.

* It should be noted that the samples of (+)-furodysinin from Sydney[1] and (-)-furodysinin from Fiji were purified by crystallization from hexane, introducing the possibility of resolution of optically pure enantiomers from different *partially* racemic mixtures, thus giving the same magnitudes of $[\alpha]_D$'s but of opposite signs. Furodysinin from the Mediterranean sponge, *D. tupha*, on the other hand, was purified by HPLC and the measured $[\alpha]_D$ of this sample may reflect native enantiomeric composition.

This is consistent with our findings of optically pure, but enantiomeric (+)-**1** and (-)-**17** in different samples of *Dysidea herbacea*[18].



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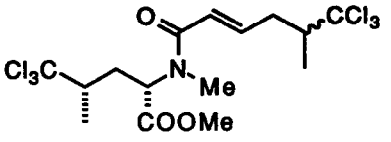

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Conclusion Compounds **1**, **2**, and their enantiomers **17** and **18** were isolated from two samples of *Dysidea herbacea* and shown to be diastereomeric with the reported compounds, **5** and **6**, respectively. The configurational relationships among these six isomers were rigorously established by $[\alpha]_D$, NMR and chemical correlation. Biosynthesis of the putative precursor **15** of the parent compounds **3**, **4**, **7a** and **7b** fixes the ring junction configuration by cyclization of farnesyl pyrophosphate, prior to commitment to the subsequent rearrangement, oxidation and acetylation to give **1** and **2** or their enantiomers **17** and **18**. In the latter case, antipodal (-)-*ent*-spirodysin, or an equivalent spirofuranosesquiterpene, may be expected to be found in suitable specimens of *Dysidea herbacea* which also express (–)-*ent*-furodysinin **7a** or its derivatives.

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Experimental:

General: All compounds, unless otherwise specified, were characterized using chromatographically and spectroscopically pure samples. Optical rotations were measured on a JASCO DIP-370 spectropolarimeter using a 1 dm microcell under stated conditions. NMR spectra were recorded at 300 MHz for ^{1}H, and 75 MHz for ^{13}C. ^{1}H NMR and ^{13}C NMR are referenced to the signals of $CHCl_3$ at 7.26 and $CDCl_3$ at 77.00 ppm, respectively. Multiplicities of ^{13}C spectra were assigned by DEPT experiments. Standard NMR FT pulse sequences were employed for DEPT, magnitude COSY, HETCOR and phase sensitive NOESY experiments. FTIR spectra were recorded on an IBM IR /32 instrument. Mass spectra were provided by the University of Minnesota Chemistry Department Mass Spectrometry Service Laboratory. TLC was carried out on Merck Kieselgel 60F_{254} plates and visualized with 1% vanillin-EtOH-H_2SO_4. All solvents were distilled in glass before use.

Isolation of **1** and **2** from Two Samples of *Dysidea herbacea*

i. *Dysidea herbacea* (sample: DEB-1) was collected from Harrier Reef (Great Barrier Reef, Australia, 15° 23'S; 145° 45'E, May 1990) by hand using SCUBA at -3 to -10 meters, frozen immediately and extracted as described previously .[4] The ^{1}H NMR spectrum of the non-polar fractions revealed two major

furanosesquiterpenes together with traces of furodysinin and furodysin (3:1 ratio, less than 1.5 mg). The hexane soluble material, obtained by solvent partitioning, was purified by repeated flash chromatography over silica and Florisil, and the non-polar fractions (pink stain, vanillin-H_2SO_4) were further purified by HPLC (silica, 2:98 EtOAc- hexane) to provide (+)-6-acetoxyfurodysinin (**1**, 2.9 % w/w of hexane extract) and (+)-6-acetoxyfurodysin (**2** , 1.0 %). More polar fractions, including the CCl_4 partition, provided herbaceamide (**16**)[4].and a 3:1 mixture of the alcohols **9** and **10** (19.5 mg, 2.1%).

ii. *D. herbacea* (sample: 90-05-041, 32.5 g wet weight) was collected from Norman Reef (Great Barrier Reef, 16° 51'S; 146° 00'E, May 1990) by hand using SCUBA at -3 to -10 meters and frozen immediately. The frozen sample was extracted with methanol (100 mL) overnight at 25°C and twice more with homogenization. The filtered solution was extracted with hexane (3x 150 mL) and the combined hexane extracts were dried and evaporated to give a dark green oil which was purified as described above to provide the enantiomers (-)-**17** and (-)-**18** and several other non-polar furanosesquiterpenes.

(+)-(4a*S*, 6*R*, 8a*S*)-4,4,7-Trimethyl-4,4a,5,6,8,8a,9-hexahydronaphtho[2,3-*b*]furan-6-yl acetate (**1**)
$C_{17}H_{22}O_3$; m.p. 107-108 °C (from hexane); $[\alpha]_D$ +61.3° (c = 0.57, $CHCl_3$); IR (neat) υ_{max} 1737 cm^{-1}; ^1H NMR ($CDCl_3$) see Figure 1; ^{13}C NMR ($CDCl_3$) see Figure 1; MS (CI, CH_4) m/z 275 (MH^+, 38%), 232 (60), 215 (100); HRMS (C.I.) found 275.1637, $C_{17}H_{23}O_3$ requires 274.1647.

(+)-(4a*S*,7*R*,8a*S*)-6,9,9-Trimethyl-4,4a,7,8,8a,9-hexahydronaphtho[2,3-*b*]furan-7-yl acetate (**2**)
$C_{17}H_{22}O_3$; oil $[\alpha]_D$ +40.0° (c = 0.59, $CHCl_3$); IR (neat) υ_{max} 1735 cm^{-1}; ^1H NMR ($CDCl_3$) see Figure 1; ^{13}C NMR ($CDCl_3$) see Figure 1; MS (FAB) m/z 274 (M^+, 3%), 215 (57); HRMS (FAB) found 274.1594, $C_{17}H_{22}O_3$ requires 274.1569.

(-)-(4a*R* 6*S*,8a*R*)-4,4,7-Trimethyl-4,4a,5,6,8,8a,9-hexahydronaphtho[2,3-*b*]furan-6-yl acetate (**17**)
$C_{17}H_{22}O_3$; oil $[\alpha]_D$ -60.6° (c = 2.45, $CHCl_3$); FTIR, MS, ^1H NMR and ^{13}C-NMR data identical to those of **1**.

Lithium-ammonia Reduction of Acetate **1**. (+)-Furodysinin (**3**).
A solution of acetate **1** (8.1 mg, 0.03 mmol) in diethyl ether (2.5 mL) was added to the deep blue solution formed by adding lithium wire (18 mg, 2.57 mmol) to liquid ammonia (10 mL). The mixture was stirred and, after the ammonia had evaporated, quenched with water and extracted with ether. The combined diethyl ether extracts were dried ($MgSO_4$), evaporated and purified by silica flash chromatography (hexane) to obtain a 3:1 mixture of (+)-furodysinin **3**, along with the double bond isomer, **8**. $C_{15}H_{20}O[\alpha]_D$ +30° (c= 0.87, $CHCl_3$), ^1H NMR ($CDCl_3$, major component) δ, Identical with literature values for furodysinin.[1]

Hydrolysis of **1**: (4a*S*,6*R*,8a*S*)-4,4,7-Trimethyl-4,4a,5,6,8,8a,9-hexahydronaphtho[2,3-*b*]furan-6-ol (**9**)
A solution of **1** (6.8 mg, 25 μmol) in methanol (1 mL) was treated with 2M aqueous sodium hydroxide (100 μL) and stirred at 25 °C for 16 hrs. Methanol was evaporated under reduced pressure and the residue chromatographed on silica gel, (10:90 EtOAc-hexane, pipet tube), to yield **9** as a colorless oil (4.5 mg, 78%). $C_{15}H_{20}O_2$; $[\alpha]_D$ +80.2° (c = 0.29, $CHCl_3$); IR (neat) υ_{max} 3340 cm^{-1} br; ^1H NMR ($CDCl_3$) δ 1.17 (s, 3H), 1.21 (s, 3H), 1.26 (ddd, *J* = 13.6, 12.4, 9.9 Hz, 1H), 1.67 (ddd, *J* = 13.6, 3.6, 2.0 Hz, 1H), 1.77 (br s, 3H), 2.14 (ddt, *J* = 12.4, 6.7, 2.0 Hz, 1H), 2.36 (dd, *J* = 16.0, 10.0 Hz, 1H), 2.67 (m, 1H), 2.77 (dd, *J* = 16.0, 6.3 Hz, 1H), 4.18 (br dd, *J* = 9.9, 6.7 Hz, 1H), 5.73 (dm, *J* = 6.3 Hz, 1H), 6.23 (d, *J* = 1.9 Hz, 1H), 7.22 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 18.8, 26.3, 27.5, 30.2, 31.6, 32.6, 33.1, 44.0, 72.2, 108.1, 124.5, 129.4, 136.0, 140.7, 147.1;

EIMS m/z 232 (M^+, 4%), 214 (M^+-H_2O, 27), 199 (M^+-H_2O-CH_3, 32); HRMS (EI) found m/z 232.1476 (M^+), $C_{15}H_{20}O_2$ requires 232.1463.

(4a*S*,7*R*,8a*S*)-6,9,9-Trimethyl-4,4a,7,8,8a,9-hexahydronaphtho[2,3-*b*]furan-7-ol (**10**)

Acetate **2** was hydrolyzed under conditions similar to those described above for **1**. Purification of the crude product gave **10** as a colorless oil (5.9 mg, quantitative).

$C_{15}H_{20}O_2$; $[\alpha]_D$ +66.4° (c = 0.39, $CHCl_3$); IR (neat) υ_{max} 3180 br; ^1H NMR ($CDCl_3$) δ 1.23 (s, 3H), 1.28 (s, 3H), 1.34 (ddd *J* = 13.7, 12.5, 10.0 Hz, 1H), 1.74 (m, 1H), 1.77 (br s, 3H), 2.17 (ddt, *J* = 12.5, 6.8, 2.0 Hz, 1H), 2.26 (dd, *J* = 17.5, 12.4 Hz, 1H), 2.59 (dd, 17.5, 5.9 Hz, 1H), 2.59 (m, 1H), 4.18 (br dd, *J* = 10.0, 6.8 Hz, 1H), 5.72 (dm, *J* = 5.6 Hz, 1H), 6.10 (d, *J* = 1.7 Hz, 1H), 7.22 (d, *J* = 1.7 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 18.9, 23.8, 27.2, 30.4, 30.4, 32.0, 34.5, 45.0, 72.3, 109.7, 113.0, 129.8, 135.4, 140.6, 156.3; MS (E.I.) m/z 232 (M^+, 3%), 214 (M^+-H_2O, 15), 199 (M^+-H_2O-CH_3, 32); HRMS (EI) found m/z 232.1489 (M^+), $C_{15}H_{20}O_2$ requires 232.1463; 214.1362, $C_{15}H_{18}O$ requires 214.1358.

Acylation of **9**: (*R*)-MTPA ester (**11**)

A mixture of excess (*R*)-methoxytrifluorophenylacetic (MTPA) acid (21 mg) and DCC (13.1 mg) in dry dichloromethane (1 mL) was stirred at 25°C for *ca.* 30 mins. To the resulting suspension was added a solution of the alcohol, **9** (2.2 mg, 9.5 μmol) in dry dichloromethane (0.5 mL). After stirring for 24 hours the reaction mixture was concentrated under reduced pressure and the residue chromatographed on basic alumina (EtOAc:hexane, 5:95, pipet tube). Examination of the crude product by ^1H NMR showed a single product and no evidence of other diastereomeric esters. The crude product was purified by HPLC (Dynamax 10 mm, silica, 5:95 EtOAc: hexane) to afford the (*R*)-MTPA ester derivative, **11** (2.4 mg, 56%).

^1H NMR ($CDCl_3$) δ 1.18 (s, 3H), 1.20 (s, 3H), 1.30 (ddd, *J* = 13.8, 12.3, 9.6 Hz, 1H), 1.66 (br s, 3H), 1.75 (ddd, *J* = 13.8, 3.0, 2.0, 1H), 2.25 (ddt, *J* = 12.3, 6.9, 2.0 Hz, 1H), 2.35 (dd, *J* = 14.4, 8.4 Hz, 1H), 2.67-2.79 (m, 2H), 3.50 (s, 3H), 5.61 (br dd, *J* = 9.6, 6.9 Hz, 1H), 5.87 (dm, *J* = 6.0 Hz, 1H), 6.22 (d, *J* = 2.0 Hz, 1H), 7.22 (m, 1H), 7.38 (m, 3H), 7.51 (m, 2H); MS (EI) m/z 448 (M^+, 1%), 214 (30).

Acylation of **9**: (*S*)-MTPA ester (**12**)

Alcohol **9** (1.7 mg, 7.3 μmol) was treated with (*S*)-MTPA acid as described for **9**, above, to provide (*S*)-MTPA ester derivative **12** (1.9 mg, 58%).

^1H NMR ($CDCl_3$) δ 1.19 (s, 3H), 1.22 (s, 3H), 1.41 (ddd, *J* = 13.7, 12.2, 10.1 Hz, 1H), 1.44 (br s, 3H), 1.77 (ddd, *J* = 13.7, 3.0, 2.0 Hz, 1H), 2.26 (ddt, *J* = 12.2, 7.5, 2.0 Hz, 1H), 2.37 (dd, *J* = 14.5, 9.0 Hz, 1H), 2.66-2.81 (m, 2H), 3.55 (s, 3H), 5.63 (br dd, *J* = 10.1, 7.5 Hz, 1H), 5.82 (dm, *J* = 6.0 Hz, 1H), 6.22 (d, *J* = 2.0 Hz, 1H), 7.24 (m, 1H), 7.38 (m, 3H), 7.53 (m, 2H); MS (E.I.) m/z 448 (M^+, 1%), 214 (31).

Acylation of **10**: (*R*)-MTPA ester (**13**)

Alcohol **10** (2.8 mg, 12 μmol) was treated with (*R*)-MTPA acid as described for **9**, above, to provide (*R*)-MTPA ester derivative **13** (3.5 mg, 65%).

^1H NMR ($CDCl_3$) δ 1.24 (s, 3H), 1.26 (s, 3H), 1.38 (ddd, *J* = 13.7, 12.2, 9.8 Hz, 1H), 1.65 (br s, 3H), 1.81 (ddd, *J* = 13.7, 3.0, 2.0 Hz, 1H), 2.10-2.30 (m, 2H), 2.52-2.65 (m, 2H), 3.52 (s, 3H), 5.60 (br dd, *J* = 9.8, 6.7

Hz, 1H), 5.85 (dm, J = 6.0 Hz, 1H), 6.09 (d, J = 1.8 Hz, 1H), 7.22 (d, J= 1.8 Hz, 1H), 7.38 (m, 3H), 7.51 (m, 2H); MS (EI) m/z 448 (M^+, 4%), 214 (26).

Acylation of **10**: (*S*)-MTPA ester (**14**)

Alcohol **10** (2.5 mg, 11 μmol) was treated with (*S*)-MTPA acid as described for **9**, above, to provide (*S*)-MTPA ester derivative **14** (3.8 mg, 79%).

^{1}H NMR ($CDCl_3$) δ 1.25 (s, 3H), 1.28 (s, 3H), 1.43 (br s, 3H), 1.50 (ddd, J = 13.8, 12.0, 9.8 Hz, 1H), 1.82 (ddd, J = 13.8, 3.2, 2.2 Hz, 1H), 2.18-2.34 (m, 2H), 2.54-2.66 (m, 2H), 3.55 (s, 3H), 5.63 (br dd, J = 9.8, 6.7 Hz, 1H), 5.80 (dm, J = 5.8 Hz, 1H), 6.11 (d, J = 1.8 Hz, 1H), 7.24 (d, J = 1.8 Hz, 1H), 7.38 (m, 3H), 7.53 (m, 2H); MS (EI) m/z 448 (M^+, 14%), 214 (58).

References and Footnotes

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18. We have also analyzed *Dysidea herbacea* from a third location (Heron Island, Great Barrier Reef) by thin layer chromatography and found the furanosesquiterpene content varies greatly even for individual specimens occupying adjacent space on coral reefs.

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