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The Unusual Presence of Hydroxylated Furanosesquiterpenes in the Deep Ocean Tunicate *Ritterella rete*. Chemical Interconversions and Absolute Stereochemistry.

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Abstract

Six new dendrolasin-type hydroxylated sesquiterpenes have been isolated from the cytotoxic extracts of the marine tunicate *Ritterella rete*. The absolute stereochemistry of the new compounds was determined by a combination of spectroscopic and chemical correlations, including derivatization with a recently introduced NMR reagent. This is the first time furanoterpenes have been isolated from a marine tunicate.

Keywords: Ritterella Rete; Furans; Marine Metabolites; Stereochemistry.

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1. Introduction

Marine tunicates (phylum Chordata; subphylum Urochordata) have been the focus of attention for many investigators in recent years because they have been found to be the source of a wide variety of biologically active substances [1]. Many of these metabolites are nitrogen-containing compounds derived from amino acids, such as acyclic and cyclic peptides, aromatic alkaloids, etc [2]. Although some non-nitrogenous metabolites have also been isolated, terpenoid compounds have seldom been reported from tunicates [1,2].

During the course of our search for bioactive metabolites from marine organisms, we selected the tunicate *Ritterella rete* for chemical study because it was a species that had not been studied and is collected at an unusual ocean depth (300 m). We therefore felt that this constituted a potentially remarkable biotope scarcely studied in terms of its chemical potential. In addition, its crude extracts showed strong antifungal (1 mg/disk against *Fusarium oxysporum*, *Helminthosporium sativum*, *Phytophthora hevean*, *Botrytis cinerea*, and *Pyricularia oryzae*) and cytotoxic (IC_{50} = 1μg/mL against P388) activities in addition to strong toxicity against *Artemia salina*. We now report the isolation of six new dendrolasin-type sesquiterpenes from those extracts: four compounds (**1–4**) are characterized by the presence of a furan moiety and the other two (**5** and **6**) by a γ-butyrolactone ring.

2. Results and Discussion

2.1 Isolation and General Structures

The tunicate *Ritterella rete* was collected by dredging at an average depth of 300 m on the ridges of a sea mount in a relatively poor environment for marine life (just a few specimens of stylasters, crinoids, and sponges), at the coral reefs in New Caledonia, and these were subsequently extracted with methanol. Partitioning of the crude extracts between water and solvents of increasing polarity gave hexane and dichloromethane fractions rich in sesquiterpenes which were repeatedly submitted to flash column chromatography using silica gel (hexane/ethyl acetate gradient system). Normal phase HPLC of the resulting fractions

using a μ-Porasil column (hexane/ethyl acetate solvent mixtures) yielded the new oxygenated sesquiterpenes **1–6** in a pure form (Figure 1).

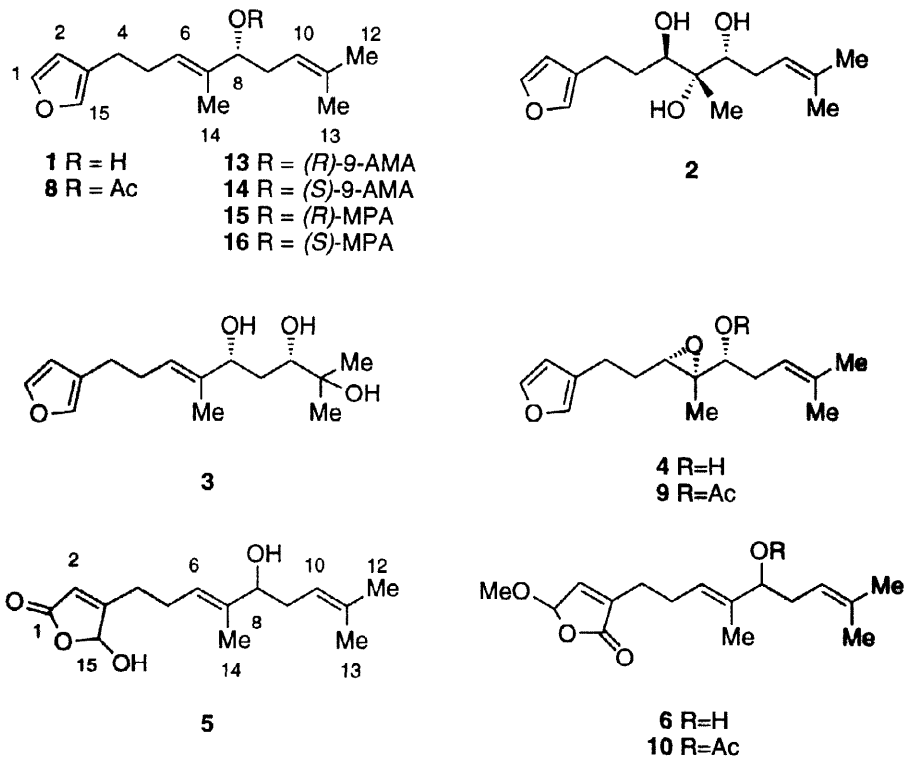


Figure 1. Compounds **1-6** and their derivatives.

The major component, 8-hydroxydendrolasin (**1**), was obtained from both the hexane and dichloromethane fractions as a colourless oil [110 mg, [α] +15.5, (*c* $4 \cdot 10^{-3}$, MeOH)]. Its HREIMS showed the molecular ion at 234.1624 amu, corresponding to a molecular formula of $C_{15}H_{22}O_2$ (M^+, Δ 0.4 mmu), that indicates the existence of five double bond equivalents. The ^1H NMR spectrum indicated, as major features, the presence of a monosubstituted furyl system (three protons at δ 7.34, 7.21, and 6.28; substitution at position 3); two olefinic hydrogens (δ 5.38 and 5.05); one methine group attached to oxygen (δ 3.94); three methylene groups (δ 2.48, 2.29, and 2.23) and three vinylic methyl groups (δ 1.72, 1.64, and 1.61). The multiplicities of the signals, together with ^1H-^1H COSY experiments, allowed us to establish the connectivities between these substructures, clearly suggesting a dendrolasin (**7**) [3] type skeleton with one hydroxy group at position 8. A series of MS experiments [including (+) HRFAB in *m*-NBA and LREI, Figure 2], together with the ^{13}C and DEPT NMR spectra confirmed the furanosesquiterpene structure and established the geometry of the Δ^6 double

bond as *E* (δ 11.3, C-14) [4,5]. Furthermore, acetylation of **1** (Ac_2O/pyridine) exclusively gave acetate **8** that, when compared to **1**, showed the expected downfield shift of H-8 to δ 4.90 and the disappearance of the hydroxy IR band (3700–3400 cm^{-1}). So, the above data provide clear evidence that **1** is 8-hydroxydendrolasin.

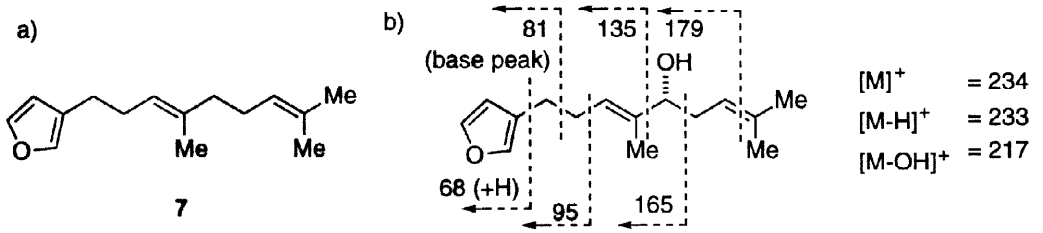


Figure 2. a) Dendrolasin skeleton (7). b) LREIMS fragmentations of compound **1**.

Two other compounds (**2** and **3**) were also found in the same partition fractions in smaller amounts. Both showed spectroscopic data with marked similarities to those of 8-hydroxydendrolasin (**1**), with clear indications that additional hydroxy groups are present in the same framework.

Compound **2** was isolated as a pale yellow oil. Its mass spectrum shows a small molecular ion peak at *m/z* = 268 ($C_{15}H_{24}O_4$, M^+) along with a more intense peak at *m/z*= 250 ($[M–H_2O]^+$; obs. HREIMS 250.1570, calcd. 250.1569 for $C_{15}H_{22}O_3$), thus indicating a molecular formula of $C_{15}H_{24}O_4$ and four double bond equivalents for **2**. The proton and carbon chemical shifts of the skeletal fragment between positions 6 and 8 show the major differences when compared to those of **1**, suggesting that the Δ^6 double bond present in **1** is dihydroxylated in **2** (see Table 1).

The location of those two hydroxyl groups at C-6 and C-7 was confirmed by HMBC experiments which established the connectivities between the oxymethine proton H-6 (δ 3.93, dd, *J*= 11.4, 2.0 Hz) and carbons C-7 and C-14 [δ 75.7 (s) and δ 19.6 (q), respectively] and between oxymethine proton H-8 (δ 3.71, dd, *J*= 9.7, 3.3 Hz) and the methyl carbon C-14. Thus, compound **2** is 6,7,8-trihydroxydendrolasin, without stereochemical assignment at this point.

Table 1.

^{1}H and ^{13}C NMR ($CDCl_3$) data for furanosesquiterpenes **1–4**.

	^{13}C δ ppm (mult)				^{1}H δ ppm (mult, *J* in Hz)			
# C	1[b]	2[a]	3[a]	4[c]	1[d]	2[d]	3[e]	4[f]
1	142.4 (d)	142.9 (d)	142.6 (d)	142.9 (d)	7.34 (s)	7.27 (s)	7.34 (s)	7.36 (s)
2	110.8 (d)	111.0 (d)	111.0 (d)	110.8 (d)	6.28 (s)	6.30 (s)	6.26 (s)	6.29 (s)
3	124.5 (t)	123.8 (s)	124.8 (s)	123.9 (s)				
4	27.8 (t)	22.1 (t)	28.1 (t)	21.8 (t)	2.48 (m)	2.78 (m)	2.47 (m)	2.58 (m)
						2.57 (m)		
5	24.5 (t)	32.3 (t)	24.6 (t)	28.6 (t)	2.29 (m)	2.26 (m)	2.31 (m)	1.82 (m)
						1.92 (m)		
6	125.2 (d)	67.4 (d)	125.6 (d)	59.1 (d)	5.38 (t, 6.9)	3.93 (dd,	5.52 (t, 6.9)	3.06 (t, 6.2)
						11.4, 2.0)		
7	137.3 (s)[g]	75.7 (s)	135.6 (s)	62.6 (s)				
8	77.1 (d)	74.2 (d)	78.5 (d)	73.0 (d)	3.94 (t, 6.7)	3.71 (dd,	4.00 (dd,	3.59 (dd,
						9.7, 3.3)	5.6, 3.7)	8.0, 3.8)
9	33.8 (t)	29.4 (t)	39.2 (t)	31.4 (t)	2.23 (m)	2.21 (m)	2.09 (m)	2.27 (br)
							1.93 (m)	2.13 (br)
10	120.1 (s)	120.0 (d)	81.3 (d)	119.8 (d)	5.05 (t, 7.1)	5.18 (t, 7.5)	4.53 (t, 7.7)	5.20 (t, 1.2)
11	137.4 (s)[g]	136.8 (s)	82.8 (s)	134.1 (s)				
12	24.5 (q)	25.9 (q)	27.7 (q)[g]	25.8 (q)	1.72 (s)	1.75 (s)	1.24 (s)	1.72 (s)
13	17.7 (q)	17.9 (q)	21.4 (q)[g]	17.9 (q)	1.64 (s)	1.65 (s)	1.27 (s)	1.63 (s)
14	11.3 (q)	19.6 (q)	11.5 (q)	14.3 (q)	1.61 (s)	1.32 (s)	1.58 (s)	1.26 (s)
15	138.6 (d)	139.3 (d)	138.9 (d)	139.0 (d)	7.21 (s)	7.35 (t, 1.1)	7.20 (s)	7.25 (s)

The NMR spectra were recorded at [a]62.83, [b]75.42 and [c]125.75 MHz for ^{13}C and [d]250.13, [e]300.13 and [f]500.13 MHz for ^{1}H.
[g]Assignments may be interchangeable.

The ^{1}H and ^{13}C NMR spectra of compound **3** differ from those of **1** only in the signals corresponding to the fragment between C-10 and C-13 (Table 1). All the evidence suggests that **3** also has the same skeleton as **1**, but in this case it is the Δ^{10} double bond present in **1** that is dihydroxylated. This proposal was corroborated by ^{1}H-^{1}H COSY NMR correlations between the diasterotopic methylene protons at δ 2.09 and 1.93 (m, H-9) and the two oxymethine protons at δ 4.53 (t, *J*= 7.7 Hz, H-10) and δ 4.00 (dd, *J*= 5.6, 3.7 Hz, H-8). Furthermore, the carbon chemical shifts of C-10 at δ 81.3 (d), C-11 at δ 82.8 (s) and those corresponding to the C-12 and C-13 methyl groups at δ 27.7 and 21.4 are in good agreement with data reported for the $Me_2C(OH)$-CH(OH) fragment present in compounds such as lobanes [6] and 4,5-epoxyxeniaphyllan-14,15-diol [7]. The ^{13}C signal generated by the methyl group at position 14 (δ 11.5) also allows us to assign an *E* geometry for the Δ^{6} double bond.

The LREIMS of **3** showed the higher mass peak at *m/z*= 250 ($C_{15}H_{22}O_3$). The NMR data unambiguously indicate the presence of 4 oxygen atoms, and this is in agreement with the aforementioned molecular ion, which corresponds to the $[M–H_2O]^+$ fragment. Consequently, compound **3** is 8,10,11-trihydroxydendrolasin.

Compound **4** was isolated from the hexane partition. After purification by HPLC on a μ-Porasil column (hexane/ethyl acetate 9:1) it showed an $[M + H]^+$ ion (*m/z* = 251.1647, Δ 0.0 mmu of calcd. value) in the positive ion mode HRFABMS, indicative of a molecular formula of $C_{15}H_{23}O_3$ and five degrees of unsaturation.

The most obvious structural difference between **1** and **4** is the change in hybridization of the carbons at positions 6 and 7 (from sp^2 to sp^3). However, in this case the chemical shifts suggest that the Δ^{6} double bond underwent epoxidation instead of dihydroxylation. Thus the existence of an epoxide group in **4** was deduced from the carbon resonances at δ 59.1 (d, C-6) and 62.6 (s, C-7) and its exact location was inferred by HMBC experiments: the oxymethine proton H-6 at δ 3.06 (t, *J*= 6.2 Hz) correlated with the carbons C-5, C-7 and C-8 [δ 28.6 (t), 62.6 (s) and 73.0 (d) respectively], while the methyl protons at position 14 [δ 1.26 (s)] displayed cross-peaks with carbons C-6, C-7 and C-8. The *trans* geometry of the trisubstituted epoxide group was based on both a NOE enhancement observed between the oxymethine protons H-6 and H-8 and on the characteristic ^{13}C chemical shifts [i.e. δ 14.32 (q), C-14] [8]. Therefore, the 6,7-epoxy-8-hydroxydendrolasin structure was assigned to compound **4**, that yielded a single acetate **9** when treated with Ac_2O/pyridine. This acetate,

when compared to **4**, showed the expected downfield shift of H-8 to δ 4.53.

Two other metabolites, compounds **5** and **6**, were also isolated from the dichloromethane partition fraction. Analysis of their spectroscopic data suggests that they share the same carbon framework as **1–4**, but include a γ-butyrolactone moiety instead of the furan ring found in **1–4**.

Table 2.

^{1}H and ^{13}C NMR ($CDCl_3$) data for the γ-butyrolactone sesquiterpenes **5** and **6**.

	^{13}C δ ppm (mult)		^{1}H δ ppm (mult, *J* in Hz)	
# C	**5**[a]	**6**[b]	**5**[a]	**6**[b]
1	171.0 (s)	102.4 (d)		5.72 (d, 1.3)
2	118.0 (d)	142.3 (d)	5.86 (s)	6.76 (d, 1.3)
3	168.9 (s)	138.1 (s)[c]		
4	24.8 (t)	25.1 (t)	1.58 (m)	2.39 (m)
5	27.3 (t)	25.2 (t)	2.42 (m)	2.32 (m)
6	123.8 (d)	123.9 (d)	5.42(t, 6.1)	5.37 (t,6.2)
7	138.8 (s)[c]	138.8 (s)[c]		
8	76.7 (d)	76.9 (d)	3.96 (t, 6.6)	3.98 (t, 6.4)
9	34.2 (t)	34.3 (t)	2.39 (m)	2.21 (m)
			2.30 (m)	
10	119.6 (d)	119.9 (d)	5.07 (t ,6.3)	5.07 (t, 6.6)
11	135.6 (s)[c]	135.0 (s)		
12	25.9 (q)	25.9 (q)	1.70 (s)	1.72 (s)
13	18.0 (q)	18.0 (q)	1.60 (s)	1.64 (s)
14	12.2 (q)	11.9 (q)	1.60 (s)	1.62 (s)
15	98.9 (d)	171.3 (s)	5.95 (s)	
OMe		56.9 (q)	3.36 (s)	3.56 (s)

[a]The NMR spectra were recorded at 62.83 MHz for ^{13}C and 250.13 MHz for ^{1}H.

[b]The NMR spectra were recorded at 125.75 MHz for ^{13}C and 500.13 MHz for ^{1}H.

[c]Assignments may be interchangeable.

The LRFABMS (positive ion mode) of compound **5** shows an intense ion at *m/z*= 249 ($[M-H_2O+H]^+$; obs. HRFABMS 249.1478, calcd. 249.1491 for $C_{15}H_{21}O_3$), revealing a molecular formula of $C_{15}H_{22}O_4$ with five degrees of unsaturation. NMR analysis (^1H, ^{13}C, DEPT, ^1H-^1H COSY, and HMQC) of **5** and comparison of the data with those of **1** reveals that they share the same C_4 to C_{14} substructure (Table 2). The γ-hydroxy-β-substituted-α,β-unsaturated-γ-lactone group in compound **5** was deduced from characteristic chemical shifts: a lactone carbonyl group at δ 171.0 (s, C-1); a hemiacetal carbon at δ 98.9 (d, C-15) attached to the hemiacetal hydrogen at δ 5.95 (s, H-15); two olefinic carbons at δ 168.9 (s, C-3) and δ 118.0 (d, C-2), the latter bonded to the olefinic proton at δ 5.86 (s, H-2). So, compound **5** contains a lactone ring identical to that previously found in other marine metabolites such as luffariellolide [9], hydroxymokupalide [10], and other sesterterpenes [11].

Finally, compound **6** shows spectroscopic data suggesting a structure quite similar to that of **5**, but with a modified ring substitution pattern. Its LRFABMS (positive ion mode) shows a small molecular ion peak at *m/z*= 281 ($[M+H]^+$) and an intense peak at *m/z*= 263 ($[M-H_2O+H]^+$, obs. HRFABMS 263.1639, calcd. 263.1647 for $C_{16}H_{23}O_3$), indicative of a molecular formula for this compound of $C_{16}H_{24}O_4$. The γ-methoxy-α-substituted-α,β-unsaturated-γ-lactone group was deduced from the characteristic NMR signals at δ 171.3 (s, C-15); δ 142.3 (d, C-2)/δ 6.76 (d, 1H, *J*= 1.3 Hz); δ 138.1 (s, C-3); δ 102.4 (d, C-1)/δ 5.72 (d, 1H, *J*= 1.3 Hz), and δ 56.9 (q, OMe)/δ 3.56 (s, 3H). This moiety has also been found in other metabolites such as ambliolide, isolated from the sponge *Dysidea amblia* [12], that shows analogous data. In order to unambiguously establish the regiochemistry of this molecule, a simple derivatization was carried out. Acetylation of **6** gave monoacetate **10**, characterized by the downfield shift observed for H-8 (from δ 3.94 to δ 5.09), confirming that the hydroxy group of **6** is linked to C-8 and the methoxy group at C-1.

2.2 Absolute Stereochemistry

The determination of the absolute configuration of the chiral centres of terpenes **1–6** was carried out by a combination of spectroscopic (NMR) analysis and chemical interconversions, including derivatization with *(R)*- and *(S)*-9-AMA [2-(9-anthryl)-2-methoxyacetic acid, **11**] and *(R)*- and *(S)*-MPA (**12**), selective Sharpless

epoxidation, carbonate and acetonide ring formation, and Payne rearrangement. These different strategies for the elucidation of the structure were often limited by the small amount of sample available.

The NMR Approach: The Absolute Configuration of 1. Firstly, we established the absolute configuration at carbon C-8 of compound **1** by means of NMR using the recently introduced chiral auxiliary reagents (Figure 3), *(R)*- and *(S)*-2-(9-anthryl)-2-methoxyacetic acids [13] (**11**, 9-AMA) and the commercially available MPA (**12**), employed for comparative purposes. In this approach, compound **1** was separately esterified with the *(R)* and *(S)* enantiomers of **11** and **12** and the ^{1}H NMR spectra of the resulting pairs of diastereoisomers were compared with the model established [13,14]. The Δδ signs and values (For a given proton, $\Delta\delta^{RS}$ is the difference in the chemical shifts between (R) and (S) derivatives) obtained for the ester pairs **13/14** and **15/16** are shown in Figure 3 and imply an *(R)* absolute configuration for C-8. Thus, the complete structure of **1** was determined to be *(8R)*-8-hydroxydendrolasin.

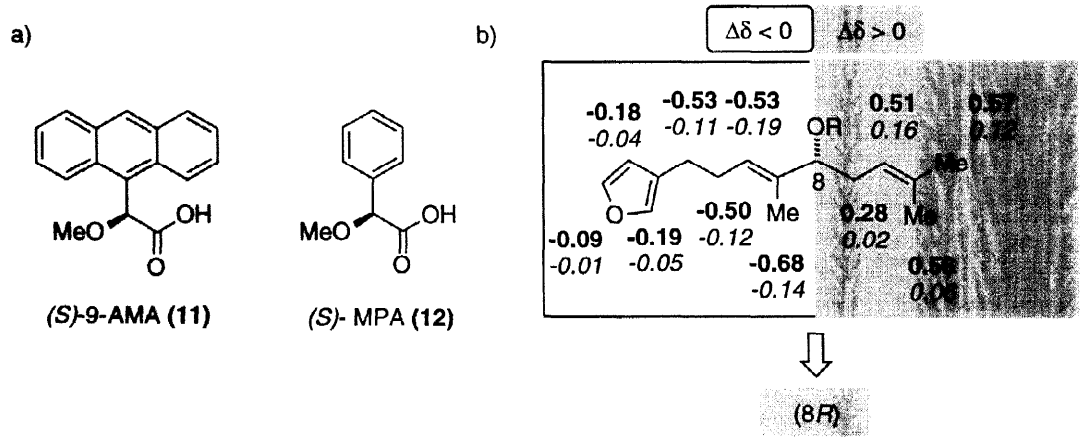


Figure 3. a) NMR reagents 9-AMA (11) and MPA (12). b) $\Delta\delta^{RS}$ values from the 9-AMA (**bold**) and MPA (*italic*) esters of **1**.

It is worth mentioning that the Δδ values obtained with 9-AMA (**11**) are markedly higher than those obtained with MPA (**12**), stressing the clear superiority of the former reagent over the latter when used as CDAs for the determination of the absolute configuration by NMR [13,15].

Stereospecific Sharpless Epoxidation. Once the absolute configuration of chiral allylic alcohol **1** was known, we correlated it with the other components of *Ritterella rete.* In this way, stereospecific Sharpless reaction [16] of **1** with *t*-butyl hydroperoxide (THBP) in the presence of Ti(O-*i*-Pr)$_4$ and (+)-diethyl tartrate [(*L*)-(+)-(DET)] yielded a single product that was found to be identical to natural epoxide **4.**

The Sharpless model for this asymmetric epoxidation is shown in Figure 4 and predicts the transformation of the substrate into the *threo* derivative by kinetic control. The absolute configurations of carbons C-6 and C-7 in **4** were therefore assigned as *(S)* and *(R)* respectively.

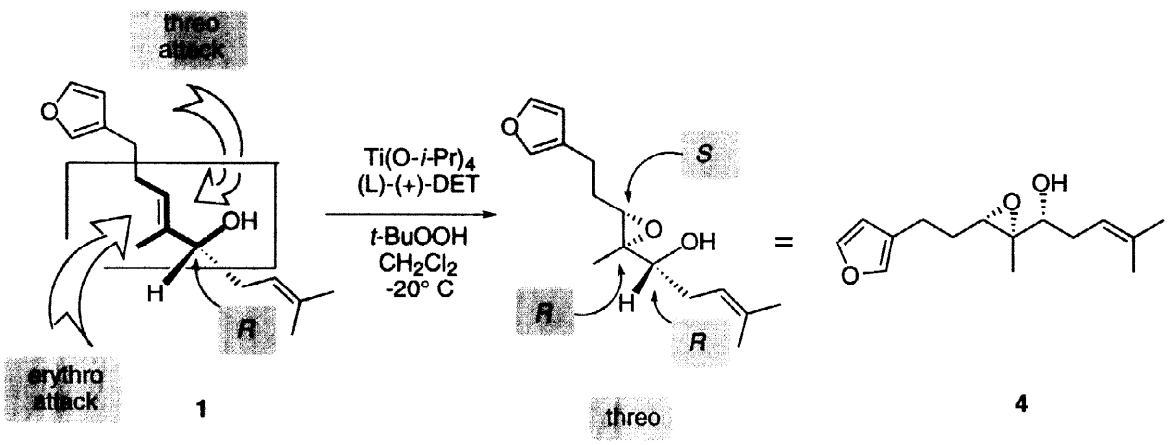


Figure 4. Stereospecific Sharpless epoxidation of compound **1**.

***m*-CPBA Epoxidation.** In a similar way to that described above, compound **1** was submitted to epoxidation with *m*-chloroperbenzoic acid (*m*-CPBA) in CH_2Cl_2. A mixture of several mono- and diepoxides resulted without any apparent selectivity. However, epoxide **4,** its diastereomer **17,** and regioisomers **18** and **19** could be isolated and identified in the reaction mixture (Figure 5). The regiochemistry of **17** was deduced from its NMR data. With this information on hand, only one possible stereochemistry remained for **17,** which was therefore unequivocally established as (6*R*,7*S*,8*R*).

The regiochemistry of **18** and **19** were established analogously by spectroscopic studies, and their absolute stereochemistry by chemical correlation with **3**. Compound **18** decomposed on standing in commercial chloroform to give natural triol **3**.

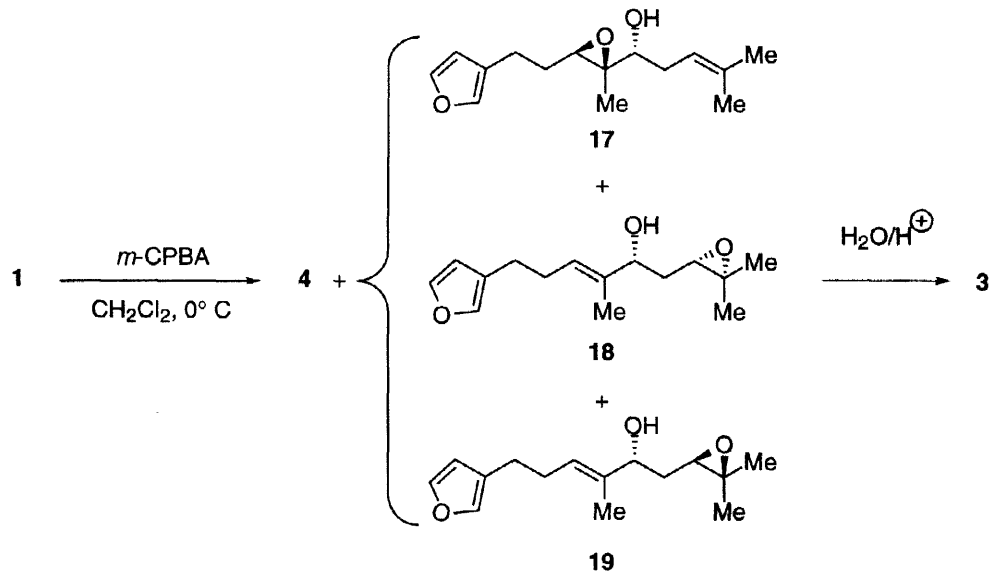


Figure 5. *m*-CPBA Epoxidation of compound **1**.

Carbonate and Ketal Ring Formations. In order to establish the stereochemistry of the 1,3-diol system of **2** and **3**, NMR analysis of certain cyclic derivatives was carried out. Thus, treatment of **3** with dimethylcarbonate led to the formation of **21**, which was found to have the ring substituents in a *cis* relationship by NOESY experiments (Figure 6). Stereospecific chemical conversion of **1** into **3** was easily achieved (*via* **18**: 1. *m*-CPBA; 2. H^+/H_2O; Figure 5), indicating that the absolute configuration at C-8 of **3** is (8*R*), and therefore the configuration of the remaining chiral centre should be assigned as (10*S*). If we assume that an acid-catalyzed *anti* opening of the epoxide ring of **18** occurred in the chloroform solution, the absolute configuration of the chiral centre at position 10 in **18** can be directly assigned as (10*S*), and that of its diastereoisomer **19** as (10*R*).

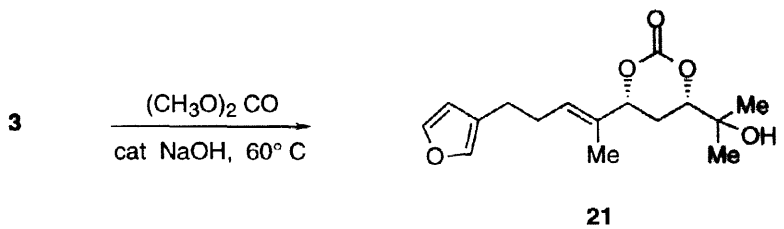


Figure 6. Treatment of **3** with dimethylcarbonate.

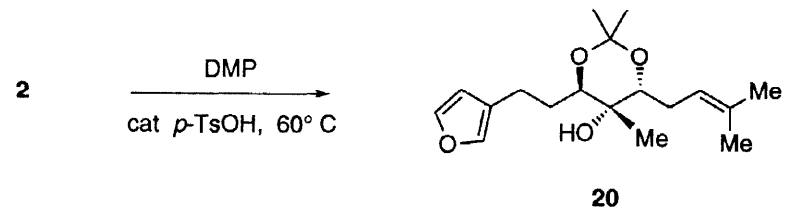


Figure 7. Reaction of **2** with DMP.

On the other hand, triol **2** was transformed into its acetonide (**20**) by reaction with 2,2-dimethoxypropane. The ^{13}C NMR signals of the methyl groups of the ketal were used as diagnostic signals [17] to determine the *syn* or *anti* relative stereochemistry at positions 6 and 8. In this way, comparison of the chemical shifts with well established models (Figure 7) [17] led to the assignment of a (6*R**,8*R**) relative stereochemistry for those centres.

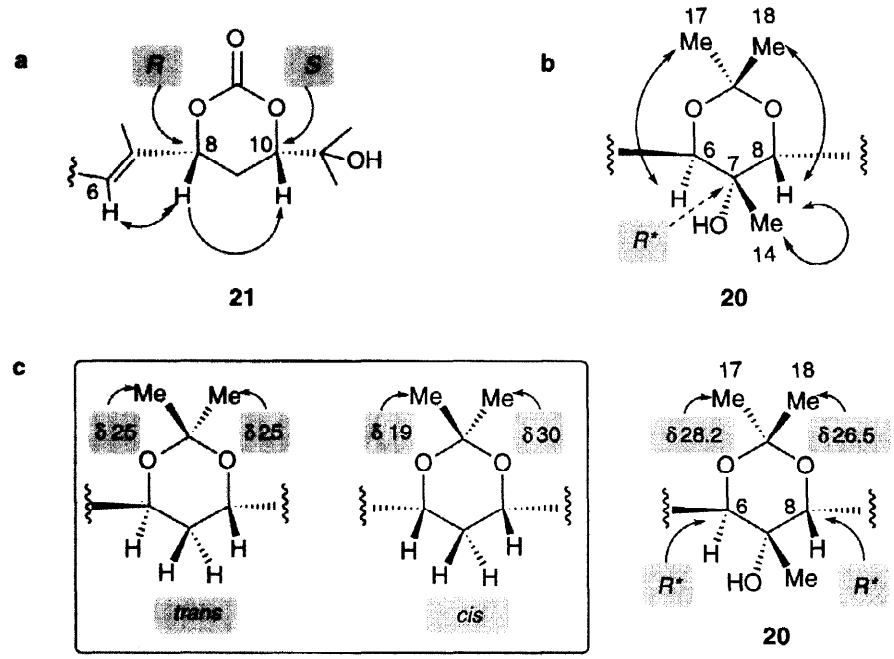


Figure 8. a) Selected NOE from NOESY experiments on carbonate **21**. b) Selected NOE from NOESY experiments on acetonide **20**. c) Diagnostic ^{13}C chemical shifts from *syn* and *anti* model compounds [17] and experimental values from acetonide **20**.

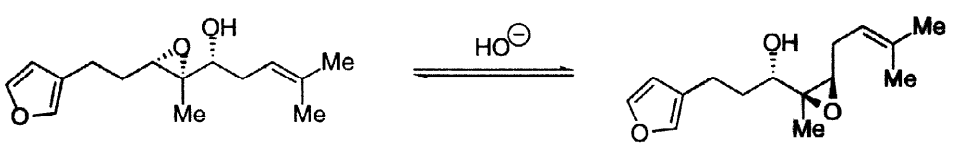
The (7*R**) configuration of the remaining centre was deduced by means of NOESY experiments (COSY and HMQC experiments were also performed in order to obtain fully assigned spectra), which revealed the through space interactions shown in Figure 8, the *anti*

relationship for the hydroxy groups at positions 6 and 8, and a (6*R**,7*R**,8*R**) relative stereochemistry for **2**.

The small amount of sample available did not allow us to perform any further transformations that could be useful to verify the absolute stereochemistry of **2**. The enantiomeric structure (6*S**,7*S**,8*S**) is also possible based on the data available. However, due to the fact that **1** is the major component and the most likely precursor for the other furanoterpenes isolated from *R. rete* (each of which possesses an (8*R*) absolute configuration), the (6*R*,7*R*,8*R*) absolute stereochemistry is the most reasonable choice for **2**.

Attempts to transform epoxide **4** into **2** or **20** through ring opening and concomitant ketal formation were unsuccessful. Treatment of **4** with mixtures of 2% H_2SO_4/wet acetone or 2% *p*-TsOH/2,2-dimethoxypropane/wet acetone led to complex mixtures in which neither **2** nor **20** were detected.

Payne Rearrangement of 4. As compound **4** is a 2,3-epoxy alcohol, we reasoned that it should undergo a Payne rearrangement in basic media. In fact, when **4** was allowed to stand at r.t. in a mixture of *t*-BuOH/0.5 M NaOH, an equilibrium between epoxide **4** and its regioisomer **22** (in a 1:1.7 ratio) was rapidly obtained (Figure 9). The mechanism of the reaction implies an inversion of configuration at C-7 producing a *cis* stereochemistry for **22**. Curiously, epoxide **22** was not detected, even in trace amounts, as a component of *Ritterella rete* .



4 **22**

Figure 9. Payne Rearrangement of **4**.

3. Final Remarks

The isolation from the tunicate *Ritterella rete* of furanoterpenes **1–6**, all with an identical skeleton but with different degrees and patterns of oxidation, raises the possibility

that **2–6** were artefacts of **1** generated during the extraction or other manipulative operations. This can be ruled out because ^{13}C signals characteristic for all the above metabolites were shown to be present in the original crude oils and all the metabolites were isolated in enantiomerically pure form. In addition, no such transformations were observed when the potential precursor **1** was submitted to the experimental conditions of extraction, isolation and storage used with the biological material. Also, when furan **1** was treated with $^1O_2$ (O_2/TPP/hν/CH_2Cl_2) in the presence of MeOH and/or H_2O, **5** and **6** were not detected. It is reasonable to imagine that the metabolites **2–6** were formed by the action of oxidative enzymes on the major component **1**. This could be originated from dendrolasin **7**, although the latter is not present in *R. rete*. A literature precedent for a similar case has been reported by Capon *et al.* who isolated a series of dendrolasin-type oxygenated sesquiterpenes from the marine sponge *Dictyodendrilla sp* [18].

Although a large number and variety of terpenes have been found in marine organisms, mainly in sponges [1], reports of the occurrence of terpenes in tunicates are scarce [2]. This paper constitutes the first report describing the isolation of furanoterpenes and sesquiterpenes from a tunicate. Nevertheless, this is not the first time a tunicate has been reported to be the source of compounds considered to be characteristic of other organisms: A few years ago, a diterpene belonging to the cembranoid family, typical of soft corals, was isolated from the non-contaminated ascidian *Stylena plicata* [19].

(8*R*)-8-hydroxydendrolasin (**1**) exhibited *in vitro* cytotoxic activity (IC_{50} = 1 μg/ml) against P388 tumour cells. Dendrolasin has also been found to be cytotoxic [20] and this coincidence could have some significance in terms of structure-activity.

4. Experimental Section

General Methods.- Optical rotations were measured in MeOH using a polarimeter with a sodium lamp operating at 598 nm. The NMR spectra were recorded at 250.13, 300.13 and 500.13 MHz for ^1H and 62.83, 75.42 and 125.75 MHz for ^{13}C, using $CDCl_3$ as solvent and internal standard. Multiplicities were obtained by DEPT. Semipreparative HPLC was carried out using Nucleosil C_{18} 300 x 8 mm reversed phase columns and a 300 x 7.8 mm μ-Porasil normal phase column and a differential refractometer and UV (254 nm) detectors.

Fast atom bombardment mass spectra (FABMS) were obtained using a mass spectrometer employing Xe atoms at 70 KeV in a thioglycerol matrix. HREIMS and LREIMS were obtained on a spectrometer operating at 70 eV.

Two-dimensional Experiments. Two-dimensional ^{1}H-^{13}C heteronuclear shift correlation (HMQC) spectra (512 x 2K) for directly bonded protons and carbons were obtained by accumulating 48 scans per t_1, with a relaxation delay of 1 s and J_{CH} = 130 Hz. The two-dimensional ^{1}H-^{13}C heteronuclear shift correlation (HMBC) spectrum (1024 x 2K) was obtained by accumulating 128 scans per t_1; the relaxation delay was 1.5 s and the value of J_{CH} selected was 9 Hz. The data were zero-filled to 1024 in F_1 and subjected to a QSINE transformation.

Biological Material. Specimens of the tunicate *Ritterella rete* [21] (sample reference: UA-343) were collected by dredging at a depth of 300 m on the sea mount "Azteke Bank" on the Norfolk Ridge to the south of New Caledonia, and stored at –5°C. Voucher samples, identified by Dr. Francoise Monniot, are kept in the Museum National d'Histoire Naturelle in Paris under reference A1 RIT 20.

Extraction and Isolation.- Specimens of the freeze-dried tunicate were first lyophilized (3.1 kg), homogeneized in MeOH (4 x 2.5 L), and then the solvent evaporated to dryness under reduced pressure. The crude extract (109 g) was partitioned between 10% aqueous MeOH (400 mL) and hexane (2 x 400 mL). Water was added to the polar fraction until the mixture became 40% aqueous MeOH and this was then extracted with CH_2Cl_2 (3 x 400 mL). After the extraction, MeOH was removed from the aqueous methanol layer and the resulting aqueous fraction extracted with *n*-BuOH saturated with water (7 x 400 mL). After evaporation, the combined organic layers yielded 5.0 g (hexanes), 14.6 g (CH_2Cl_2), and 80.4 g (*n*-BuOH) of products. The viscous oil (14.6 g) obtained from the CH_2Cl_2 fraction was purified by repeated flash column chromatography (silica gel 230–400 mesh, eluting with hexanes/AcOEt mixtures of increasing polarity) to give compounds **1** (110 mg), **2** (4.5 mg) and **3** (3.2 mg), which were purified by normal phase HPLC on a μ-Porasil column eluted with hexane/AcOEt (8:2), and compounds **5** (4.5 mg) and **6** (10 mg), which were separated and purified by reversed phase HPLC on a Nucleosil C_{18} column eluting with MeOH/H_2O (7:3). In a similar way, flash column chromatography and normal phase HPLC on a μ-Porasil column eluting with hexane/AcOEt (9:1) allowed the isolation of compounds **1** (11 mg), **2**

(10 mg), **3** (3 mg) and **4** (8 mg) from the hexane fraction (5.0 g).

(8*R*)-8-Hydroxydendrolasin (1): colourless oil; [α] +15.5 (*c* $4 \cdot 10^{-3}$, MeOH); UV (MeOH) λ_{max} (log ε) 274 (1.03), 210 (2.53) nm; IR (dry film) υ_{max} 3400–3600 (OH) cm^{-1}; ^1H NMR and ^{13}C NMR (see Table 1); LREIMS *m/z*: (relative intensity) 234 (1), 233 (2), 217 (6), 165 (57), 135 (18), 119 (16), 81 (100); HREIMS M^+ 234.1620 calcd for $C_{15}H_{22}O_2$, found 234.1624.

(6*R,7*R**,8*R**)-6,7,8-Trihydroxydendrolasin (2)**: pale yellow oil; [α] −14 (*c* $1.2 \cdot 10^{-3}$, MeOH); UV (MeOH) λ_{max} (log ε) 274 (2.18), 228 (2.77) nm; IR (dry film) υ_{max} 3461 (OH) cm^{-1}; ^1H NMR and ^{13}C NMR (see Table 1); LREIMS *m/z* (relative intensity): 268 ($[M]^+$, 2), 250 ($[M-H_2O]^+$, 7), 217 (6), 201 (4), 199 (10), 187 (11), 125 (42), 99 (11), 95 (49), 81 (100), 69 (54), 67 (21), 55 (41); HREIMS $[M-H_2O]^+$ 250.1569 calcd for $C_{15}H_{22}O_3$, found 250.1570.

(8*R*,10*S*)-8,10,11-Trihydroxydendrolasin (3): pale yellow oil; [α] +14 (*c* $3.5 \cdot 10^{-3}$, MeOH); IR (dry film) υ_{max} 3400–3600 (OH) cm^{-1}; ^1H NMR and ^{13}C NMR (see Table 1); LREIMS *m/z*: (relative intensity) 251 ($[M-OH]^+$, 3), 250 ($[M-H_2O]^+$, 18), 232 ($[M-2H_2O]^+$, 6), 209 (5), 179 (5), 135 (17), 95 (22), 81 (100), 67 (15), 59 (19).

(6*S*,7*R*,8*R*)-6,7-Epoxy-8-hydroxydendrolasin (4): yellow oil; [α] +52.8 (*c* $5 \cdot 10^{-3}$, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 272 (10.0), 244 (35.82) nm; IR (dry film) υ_{max} 3423 (OH) cm^{-1}; ^1H NMR and ^{13}C NMR (see Table 1); LREIMS *m/z*: (relative intensity) 232 ($[M - H_2O]^+$), 217 (5), 149 (33), 97 (74), 81 (100); HRFABMS $[M+H]^+$ 251.1647 calcd for $C_{15}H_{23}O_3$, found 251.1647; $[M-H_2O+H]^+$ 233.1541 calcd for $C_{15}H_{21}O_2$, found 233.1541.

8,15-Dihydroxy-γ-butyrolactone dendrolasin (5): yellow oil; [α] −5.4 (*c* $2.2 \cdot 10^{-3}$, MeOH); UV (MeOH) λ_{max} (log ε) 280 (2.10), 234 (2.59) nm; IR (dry film) υ_{max} 3400–3300 (OH) cm^{-1}; ^1H NMR and ^{13}C NMR (see Table 2); LRFABMS (positive ion mode) *m/z*: 357 ([M+thioglycerol]$^+$, 22); 249 ($[M-H_2O]^+$, 35); 217 ([M–MeOH]$^+$, 100), 181 (76); 126 (42); HRFABMS $[M-H_2O+H]^+$ 249.1490 calcd for $C_{15}H_{21}O_3$, found 249.1478.

8-Hydroxy-1-methoxy-γ-butyrolactone dendrolasin (6): yellow oil; [α] –20 (*c* $1 \cdot 10^{-3}$, MeOH); UV ($CHCl_3$) λ_{max} (log ε) 278 (2.4), 232 (2.8) nm; IR (dry film) υ_{max} 3343 (OH), 2364, 1562, 1325, 1112, 996, 845, 796 cm^{-1}; ^1H NMR and ^{13}C NMR (see Table 2); LRFABMS (positive ion mode) *m/z*: 371[M+thioglycerol]$^+$, 281 [M+H]$^+$, 263 ([M–H_2O+H]$^+$ 100), 279 (16); 231 (25); 205 (67), 149 (44), 147 (35); HRFABMS [M–H_2O+H]$^+$ 263.1647 calcd for $C_{16}H_{23}O_3$, found 263.1639.

Epoxidation of 1 to 4: An oven-dried 5 mL two-necked round-bottomed flask equipped with a magnetic stirrer bar, thermometer and nitrogen inlet, was charged with activated 4A molecular sieves and 1.5 mL of dry CH_2Cl_2 and cooled to –20 °C. To the stirred mixture L-(+) diethyl tartrate (2.0 mg, $9.9 \cdot 10^{-3}$ mmol), Ti (O-*i* -Pr)$_4$ (2.3 mg, $8.25 \cdot 10^{-3}$ mmol), and TBHP (0.97 mL, 0.49 mmol, 5 M in isooctane) were added, and the reaction mixture maintained at –20 °C for 30 min. A solution of compound **1** (38.8 mg, 0.165 mmol) in 2.5 mL of dry CH_2Cl_2 was added dropwise over a period of 15 min and the mixture was stirred at –20 °C for 8 h. After that time, the reaction mixture was warmed to 0 °C, quenched with 2 mL of water and stirred for 30–60 min, while allowing it to warm to room temperature. Hydrolysis of the tartrate was then effected by the addition of 1.5 mL of 30% aqueous NaOH solution saturated with sodium chloride and vigorous stirring of the mixture until the separation of two phases occurred. The lower organic phase was removed and the aqueous phase extracted twice with CH_2Cl_2 (2 x 5 mL). The combined extracts were dried with $MgSO_4$, concentrated and purified by normal phase HPLC (μ-Porasil column; hexane/AcOEt 9:1) to afford 8 mg of compound **4**.

Acetylation of **1**, **4** and **6**: Compound **1** (5 mg), **4** (3 mg) and **6** (3 mg), were dissolved in a mixture of Ac_2O (0.5 mL) and dry pyridine (0.5 mL) and the mixture left overnight at room temperature. The reaction mixture was then concentrated under reduced pressure and the crude material purified by column chromatography (silica gel; hexanes/AcOEt in increasing polarity) to give the mono-acetylated compounds **8** (3.5 mg), **9** (4 mg) and **10** (3.5 mg) respectively.

Compound **8**: ^1H NMR ($CDCl_3$, 300 MHz) δ 1.60 (3H, s, H-14), 1.65 (3H, s, H-12), 1.70 (3H, s, H-13), 2.06 (3H, s, OMe), 2.06 (2H, m, H-9), 2.31 (2H, m, H-5), 2.52 (2H, t, *J*= 6.9

Hz, H-4), 4.90 (1H, t, *J*= 5.8 Hz, H-8), 5.10 (1H, t, *J*= 6.7 Hz, H-10), 5.42 (1H, t, *J*= 6.9 Hz, H-6), 6.30 (1H, s, H-2), 7.21 (1H, s, H-15).

Compound **9**: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.25 (3H, s, H-14), 1.62 (3H, s, H-12), 1.69 (3H, s, H-13), 2.04 (3H, s, $\underline{CH_3}COO$), 1.70 (3H, s, H-13), 1.78 (2H, m, H-9), 2.33 (2H, m, H-5), 2.55 (2H, m, H-4), 2.98 (1H, t, *J*= 6.63 Hz, H-6), 4.53 (1H, t, *J*= 7.1 Hz, H-8), 5.06 (1H, t, *J*= 7.2 Hz, H-10), 6.28 (1H, s, H-2), 7.25 (1H, s, H-15), 7.36 (1H, s, H-1); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.6 (s, $CH_3\underline{C}OO$), 143.3 (d, C-1), 139.4 (d, C-15), 134.9 (s, C-11), 124.4 (s, C-3), 119.9 (d, C-10), 111.3 (d, C-2), 77.0 (d, C-8), 62.5 (d, C-6), 60.6 (s, C-7), 30.9 (t, C-9), 29.4 (t, C-5), 22.1 (t, C-4), 21.4 (q, C-12), 18.3 (q, C-13), 13.2 (q, C-14); LREIMS *m/z*: (relative intensity) 292 (M^+, 1), 232 ([M-HOAc]$^+$, 3), 125 (96), 81 (100), 71 (49), 69 (49).

Compound **10**: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.55 (6H, m, H-4, H-5 and H-9), 1.61 (3H, s, H-13), 1.68 (3H, s, H-12), 2.02 (3H, s, OMe), 3.55 (3H, s, OMe), 5.07 (1H, t, *J*= 6.4 Hz, H-10), 5.09 (1H, t, *J*= 6.2 Hz, H-8), 5.38 (1H, t, *J*= 6.6 Hz, H-6), 5.72 (1H, s, H-1), 6.76 (1H, s, H-2); LREIMS *m/z*: (relative intensity) 262 ([M-HOAc]$^+$, 1), 211 (29), 179 (25), 133 (27), 83 (98), 69 (100).

Preparation of 13–16: The esters **13–16** were prepared by the reaction of **1** (2 mg) with 1 equiv. of the *(R)* and the *(S)* enantiomers of acids **11** and **12** in the presence of DCC and DMAP (catalytic) [22] and purified by HPLC (μ–Porasil, hexane / EtOAc 96:4).

Compound **13**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.76 (3H, s, H-14), 1.49 (3H, s, H-13), 1.64 (3H, s, H-12), 1.74 (2H, m, H-5), 1.90 (2H, m, H-4), 2.09 (2H, m, H-9), 3.44 (3H, s, OMe), 4.74 (1H, t, *J*= 6.9 Hz, H-10), 4.88 (1H, t, *J*= 7.2 Hz, H-6), 5.09 (1H, t, *J*= 6.7 Hz, H-8), 6.07 (1H, s, H-2), 6.26 (1H, s, H_α), 6.99 (1H, s, H-15), 7.25 (1H, s, H-1), 7.50 (4H, m, Ar), 8.02 (2H, d, *J*= 8.0 Hz, Ar), 8.47 (1H, s, Ar), 8.56 (2H, d, *J*= 8.7 Hz, Ar).

Compound **14**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.93 (3H, s, H-13), 1.07 (3H, s, H-12), 1.44 (3H, s, H-14), 1.81 (2H, m, H-9), 2.24 (2H, q, H-5), 2.43 (2H, t, *J*= 7.5 Hz, H-4), 3.41 (3H, s, OMe), 4.23 (1H, t, *J*= 7.0 Hz, H-10), 5.12 (1H, t, *J*= 6.9 Hz, H-8), 5.41 (1H, t, *J*= 6.8

Hz, H-6), 6.22 (1H, s, CH_α), 6.25 (1H, s, H-2), 7.18 (1H, s, H-15), 7.34 (1H, s, H-1), 7.50 (4H, m, Ar), 8.02 (2H, d, *J*= 8.0 Hz, Ar), 8.48 (1H, s, Ar), 8.55 (2H, d, *J*= 8.6 Hz, Ar).

Compound **15**: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.33 (3H, s, H-14), 1.59 (3H, s, H-13), 1.66 (3H, s, H-12), 2.13 (2H, m, H-5), 2.17 (2H, m, H-9), 2.32 (2H, t, *J*= 7.6 Hz, H-4), 3.41 (3H, s, OMe), 4.73 (1H, s, CH_α), 4.96 (1H, t, *J*= 6.5 Hz, H-10), 5.14 (1H, t, *J*= 7.0 Hz, H-8), 5.24 (1H, t, *J*= 6.2 Hz, H-6), 6.21 (1H, s, H-2), 7.13 (1H, s, H-15), 7.32 (1H, s, H-1), 7.37 (5H, m, Ph).

Compound **16**: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.47 (3H, s, H-14), 1.53 (3H, s, H-13), 1.54 (3H, s, H-12), 2.19 (2H, m, H-9), 2.25 (2H, m, H-5), 2.43 (2H, t, *J*= 7.4 Hz, H-4), 3.41 (3H, s, OMe), 4.73 (1H, s, CH_α), 4.80 (1H, t, *J*= 6.6 Hz, H-10), 5.12 (1H, t, *J*= 6.9 Hz, H-8), 5.43 (1H, t, *J*= 7.5 Hz, H-6), 6.25 (1H, s, H-2), 7.18 (1H, s, H-15), 7.33 (1H, s, H-1), 7.46 (5H, m, Ph).

Reaction of 1 with *m*-CPBA: To a stirred solution of compound **1** (23 mg, $9.8\cdot10^{-2}$ mmol) in 3 mL of CH_2Cl_2 was added a solution of *m*-CPBA (34.7 mg, $2.0\cdot10^{-1}$ mmol) in 4 mL of CH_2Cl_2 at 0 ºC. After stirring for 48 h at that temperature, the mixture was washed with 10% aqueous $NaHCO_3$ and water, and the organic phase was dried ($MgSO_4$) and concentrated. The crude reaction product (15 mg) was purified by HPLC (normal phase μ-Porasil hexane/AcOEt 9:1) to give 5 mg of epoxide **4**, minor quantities of compounds **17** (0.5 mg), **18** (1.8 mg), and **19** (1.4 mg) and a mixture of diepoxides.

Compound **17**: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.63 (3H, s, H-13), 1.71 (3H, s, H-12), 1.80 (2H, m, H-5), 2.26 (2H, m, H-9), 2.55 (2H, m, H-4), 2.89 (1H, t, *J*= 5.8 Hz, H-6), 3.26 (1H, m, H-8), 5.09 (1H, t, *J*= 7.5 Hz, H-10), 6.28 (1H, s, H-2), 7.25 (1H, s, H-15), 7.35 (1H, s, H-1); LREIMS *m/z*: (relative intensity) 235 ($[M-CH_3]^+$, 3), 125 (96), 97 (31), 83 (100), 81 (48), 71 (43), 69 (53).

Compound **18**: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.29 and 1.32 (3H, each, s, H-13 and H-12), 1.63 (3H, s, H-14), 1.85 (2H, m, H-9), 2.32 (2H, m, H-5), 2.50 (2H, m, H-4), 2.82 (1H, dd, *J*= 7.6, 4.6 Hz, H-10), 4.27 (1H, t, *J*= 6.1 Hz, H-8), 5.51 (1H, t, *J*= 6.5 Hz, H-6),

6.28 (1H, s, H-2), 7.21 (1H, s, H-15), 7.34 (1H, s, H-1).

Compound **19**: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 1.23 and 1.26 (3H each, s, H-13 and H-12), 1.61 (3H, s, H-14), 1.83 (2H, m, H-9), 2.29 (2H, m, H-5), 2.47 (2H, m, H-4), 2.85 (1H, dd, *J*= 7.4, 4.7 Hz, H-10), 4.21 (1H, m, H-8), 5.48 (1H, t, *J*= 7.0 Hz, H-6), 6.26 (1H, s, H-2), 7.20 (1H, s, H-15), 7.33 (1H, s, H-1); LREIMS *m/z*: (relative intensity) 235 ($[M-CH_3]^+$, 4), 111 (96), 97 (57), 83 (80), 71 (100), 67 (28).

Reaction of 2 with 2,2-dimethoxypropane: A mixture of 4 mg ($1.4\cdot10^{-2}$ mmol) of **2**, 2 mL of acetone, 0.5 mL of 2,2-dimethoxypropane and a catalytic amount of *p*-TsOH in a 5 mL round-bottomed flask was refluxed for 2.3 h at 60 ºC. The reaction mixture was washed with aqueous NaOH solution (0.1%) and extracted four times with CH_2Cl_2. The combined organic phases were successively washed with water and brine, dried ($MgSO_4$) and concentrated to give 4.2 mg of compound **20**.

Compound **20**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.29 (3H, s, H-17), 1.34 (3H, s, H-18), 1.39 (3H, s, H-14), 1.63 (3H, s, H-13), 1.72 (3H, s, H-12), 1.84 (1H, m, H-5b), 2.28 (1H, m, H-9b), 2.37 (1H, m, H-5a), 2.48 (1H, m, H-9a), 2.58 (1H, m, H-4b), 2.77 (1H, m, H-4a), 3.80 (1H, dd, *J*= 10.1, 3.7 Hz, H-8), 3.85 (1H, dd, *J*= 11.3, 1.4 Hz, H-6), 5.19 (1H, t, *J*= 5.8 Hz, H-10), 6.28 (1H, s, H-2), 7.26 (1H, s, H-15), 7.35 (1H, s, H-1); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 142.9 (d, C-1), 136.3 (d, C-15), 133.7 (s, C-11), 123.6 (s, C-3), 120.7 (d, C-10), 111.1 (d, C-2), 107.2 (s, C-16), 84.8 (d, C-8), 83.2 (s, C-7), 62.2 (d, C-6), 32.8 (t, C-5), 28.4 (t, C-9), 28.2 (q, C-17), 26.5 (q, C-18), 25.7 (q, C-12), 21.7 (t, C-4), 19.7 (q, C-14), 18.0 (q, C-13); LRFABMS (positive ion mode) *m/z*: 331 ($[M+Na]^+$, 2), 214 ($[M-C_6H_7O+H]^+$, 3); LREIMS *m/z*: (relative intensity) 290 ($[M-H_2O]^+$, 1), 257 (24), 199 (17), 125 (28), 81 (100), 81 (48), 69 (37).

Reaction of 3 with dimethyl carbonate: A 5 mL flask, fitted with a distillation apparatus and a magnetic stirrer bar, was charged with compound **3** (2.5 mg, $9.3\cdot10^{-3}$ mmol), a catalytic amount of NaOH and 3 mL of dimethyl carbonate. This solution was heated at 60 ºC for 2.30 h under nitrogen and, after that time, the bath temperature was raised to 90 ºC for 5 min. The resulting suspension was washed with aqueous HOAc solution

(0.5%) and extracted four times with CH_2Cl_2. The combined organic phases were washed with water and brine, dried ($MgSO_4$) and concentrated to give 2.2 mg of compound **21**.

Compound **21**: ^1H NMR ($CDCl_3$, 500 MHz) δ 1.25 and 1.26 (3H, each, s, H-13 and H-12), 1.58 (3H, s, H-14), 2.04 (1H, m, H-9b), 2.17 (1H, m, H-9a), 2.30 (2H, m, H-5), 2.46 (2H, m, H-4), 4.44 (1H, dd, *J*= 10.4, 5.9 Hz, H-8), 4.83 (1H, dd, *J*= 6.3, 2.5 Hz, H-10), 5.46 (1H, t, *J*= 7.1 Hz, H-6), 6.26 (1H, s, H-2), 7.20 (1H, s, H-15), 7.33 (1H, s, H-1); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 156.7 (s), 142.6 (d), 138.9 (d), 133.7 (s), 123.6 (s), 120.7 (d), 111.1 (d), 107.2 (s), 84.8 (d), 83.2 (s), 62.2 (d), 32.8 (t), 28.4 (t), 28.2 (q), 26.5 (q), 25.7 (q), 21.7 (t), 19.7 (q), 18.0 (q); LREIMS *m/z*: (relative intensity) 279 ($[M-CH_3]^+$, 15), 111 (54), 97 (100), 71 (82), 67 (36).

Payne rearrangement of 4: The solvents required for this reaction (water and *t*-butyl alcohol) were deoxygenated prior to use by the rapid passage of nitrogen through the solvent for not less than 30 min. A solution of the epoxy alcohol **4** (9.2 mg, $3.6 \cdot 10^{-2}$ mmol) in 3 mL of *t*-butyl alcohol was added to 3 mL of 0.5 N aqueous NaOH solution, and stirred under an argon atmosphere for 2 h. Then it was neutralized with saturated aqueous NH_4Cl solution and sufficient water was added to produce the separation of two phases. The aqueous phase was extracted five times with $CHCl_3$, and the combined organic extracts were washed with water and brine, dried ($MgSO_4$). Evaporation of the solvent gave a mixture of compounds that was separated by normal phase HPLC (μ-Porasil column; hexane/AcOEt 9:1) to give 4.7 mg of compound **22** and 2.8 mg of compound **4**.

Compound **22**: [α] +8.6 (*c* $1.4 \cdot 10^{-3}$, $CHCl_3$) UV ($CHCl_3$) λ_{max} (log ε) 266 (2.41), 244 (2.56) nm. ^1H NMR ($CDCl_3$, 500 MHz) δ 1.29 (3H, s, H-14), 1.64 (3H, s, H-13a), 1.72 (3H, s, H-12), 1.62 (1H, m, H-5b), 1.83 (1H, m, H-5a), 2.16 (1H, m, H-9b), 2.38 (1H, m, H-9a), 2.53 (1H, m, H-4b), 2.64 (1H, m, H-4a), 3.04 (1H, t, *J*= 6.5 Hz, H-8), 3.64 (1H, dd, *J*= 8.9, 2.4 Hz, H-6), 5.14 (1H, t, *J*= 7.1 Hz, H-10), 6.29 (1H, s, H-2), 7.24 (1H, s, H-15), 7.36 (1H, s, H-1). LRFABMS (positive ion mode) *m/z*: 281 ($[M+Na+thioglycerol]^+$, 51), 249 ($[M-H]^+$, 14), 231 ($[M-H_2O+H]^+$, 10), 221 ($[M-2CH_3+H]^+$, 91).

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