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# Scalemic 12-Hydroxyambliofuran and 12-Acetoxyambliofuran, Five Tetracyclic Furanoditerpenes and a Furanosesterterpene from *Spongia* sp.†

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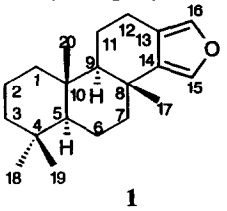
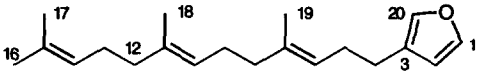
**Abstract.** Eight new compounds were isolated from a Western Australian sponge *Spongia* sp., including 12-hydroxyambliofuran (**5**), its acetate ester **6**, five new tetracyclic diterpenes **7** - **11** and the epoxyfuranosesterterpene carboxylic acid **12**. Compounds **5** and **6**, each isolated as a 3:1 mixture of enantiomers as determined by a modified Mosher's ester method and chiral lanthanide NMR shift studies, are rare examples of scalemic marine natural products.

## Introduction.

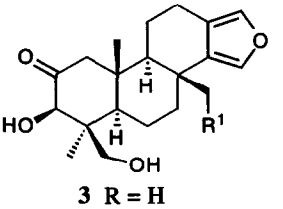
Sponges (Porifera) of the genus *Spongia* are known to produce diterpenes with structures based on the hypothetical tetracyclic parent, spongian. For example, spongia-13(16),14-diene (**1**) was first reported in 1980 by Capelle and coworkers from *Spongia* sp.[1] and is related to its putative linear precursor, ambliofuran (**2**), first obtained from *Dysidea amblia*.[2] Ketones **3** and **4** are representative of tetracyclic spongian diterpenes which have undergone further oxidation.[3]



**1**



**2**



**3** R = H
**4** R = OH

Several other analogs of this class are known,[1,3-6] however, the absolute configuration has only been determined in one example.[3] Recent evidence suggests that sesquiterpene[7-9] and diterpene marine natural products[10] can occur in both enantiomeric modifications. In our investigations of biosynthesis of

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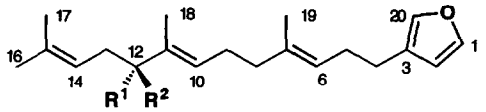
marine natural products we considered the possibility that simple linear precursors of tetracyclic diterpenes, such as **2**, may undergo cyclization by different enzymes to give either the 'normal' 5α,10β-tetracyclic diterpenes, represented by **1**, or the antipodal stereoisomer of **1** prior to further oxidative modification. We report here the isolation of eight new compounds; (*S*)-12-hydroxyambliofuran (**5**), (*S*)-12-acetoxyambliofuran (**6**), the tetracyclic furanoditerpenes **7-11**, the linear furanosesterterpene carboxylic acid **12**, together with **1**[1] and **2**.[2] The tetracyclic compound **7** has the unexceptional 5α,10β-sterol configuration, however, to our surprise, compounds **5** and **6** were each shown to occur as a 3:1 mixture of enantiomers with predominantly (12*S*) configuration. These are rare examples of marine natural products with scalemic composition - the presence of both enantiomers in non-racemic proportions.*

**Results and Discussion.**

A specimen of *Spongia* sp. (93-01-008) was collected in Exmouth Gulf, Western Australia, and extracted twice with $CH_2Cl_2$/MeOH (4:1) and evaporated. Thin layer chromatography of this extract revealed a mixture rich in non-sterol terpenoids, visualized by vanillin-$H_2SO_4$ as intense pink-purple spots. Partitioning of the extract between EtOAc and water, followed by purification of the organic extract by repeated flash chromatography and HPLC on silica gel using hexane/EtOAc solvent mixtures, afforded ten pure compounds: the known natural products **1-2**; along with eight novel compounds **5-12**.




**2** $R^1 = R^2 = H$
**5** $R^1 = H$, $R^2 = OH$
**6** $R^1 = H$, $R^2 = OAc$
**13** $R^1 = H$, $R^2 = O$-(*R*)-MTPA
**14** $R^1 = H$, $R^2 = O$-(*S*)-MTPA

Ambliofuran (**2**) was obtained by silica gel chromatography from the least polar fractions as a colorless oil (0.015% dry weight) and identified by comparison of $^1H$ NMR and $^{13}C$ NMR data with published values.[2] The more polar fractions were purified by HPLC (Dynamax, silica gel, hexane/EtOAc), to afford two compounds which showed similarities in their NMR spectra to ambliofuran. Compound **6** (colorless oil, 0.11% dry weight, $[\alpha]_D$ -10.2°, *c* 2.8, $CHCl_3$) produced a molecular ion in the high resolution mass spectrum at 344.2334 ($M^+$, Δmmu 1.7) implying a formula of $C_{22}H_{32}O_3$. $^1H$ NMR revealed three furan protons (δ 6.26, br s, 1H; 7.20, br s, 1H; 7.33, br s, 1H) and three vinyl protons (δ 4.99, br t, *J* = 7.0 Hz, 1H; 5.16, br t, *J* = 7.0 Hz, 1H; 5.40, br t, *J* = 7.0 Hz,

* James Brewster, of Purdue University, first proposed the term 'scalemic' for mixtures of enantiomers that *are not* racemic. This term has been used to succinctly describe mixtures of enantiomers with compositions other than 50:50 or 100:0.[11]

1H). The numbering system used, herein, for **5**, **6**, and **12** relates to geranylgeraniol. Four vinylic methyl signals in compound **6** were associated with three trisubstituted olefinic bonds, two with *E* geometry as indicated by the high field $^{13}$C-NMR shifts (δ 12.0, q, C-18; 16.0, s,C-19).[12] The major difference in the $^{1}$H and $^{13}$C NMR spectra of **6** from that of **2**, was an additional acetoxyl substituent (δ 2.01, s, 3H; 21.2, q; 170.1, s), also evident in the IR spectrum (υ 1735 cm$^{-1}$), and the downfield methine signal (δ 5.09, t, $J$ = 7.0 Hz, 1H). In order to determine the position of this substituent, COSY, HETCOR and HMBC experiments were used (Table 1). In the COSY spectrum of **6**, a correlation from H-12 (δ 5.09) to the methylene protons at C-13 (δ 2.22, m, 1H; 2.35, m, 1H), and from the vinyl proton H-14 (δ 4.99) to both the terminal methyl group C-16 (δ 1.67) as well as the C-13 methylene protons, established that the acetoxyl group was at the 12-position. The assignment was fully supported by long-range correlations in the HMBC spectrum (Table 1).

**Table 1**. NMR Data for the Acetate **6**.[a]

| Position | $^{13}$C NMR δ (mult.) | $^{1}$H NMR δ (mult., $J$ Hz, integral) | HMBC Correlation |
|---|---|---|---|
| 1 | 142.5 (d) | 7.33 (br s, 1H) | C-2, C-3, C-20 |
| 2 | 111.0 (d) | 6.26 (br s, 1H) | C-1, C-3, C-20 |
| 3 | 124.9 (s) | | |
| 4 | 25.0 (t) | 2.44 (t, 7.0, 2H) | C-2, C-2, C-3, C-5, C-6, C-20 |
| 5 | 28.5 (t) | 2.24 (t, 7.0, 2H) | C-3, C-4, C-6, C-7 |
| 6 | 124.1 (d) | 5.16 (t, 7.0, 1H) | C-8, C-19 |
| 7 | 135.3 (s) | | |
| 8 | 39.1 (t) | 2.00 (m, 2H) | |
| 9 | 26.2 (t) | 2.10 (t, 7.0, 2H) | C-8, C-10, C-11 |
| 10 | 128.1 (d) | 5.40 (t, 7.0, 1H) | C-12, C-18 |
| 11 | 132.8 (s) | | |
| 12 | 79.0 (d) | 5.09 (t, 7.0, 1H) | C=O, C-10, C-14 (w), C-18 |
| 13 | 31.7 (t) | 2.22 (m, 1H) | C-14 (w) |
| | | 2.35 (m, 1H) | C-12 (w), C-14 (w) |
| 14 | 119.4 (d) | 4.99 (t, 7.0, 1H) | C-17 (w) |
| 15 | 133.8 (s) | | |
| 16 | 25.6 (q) | 1.67 (s, 3H) | C-14, C-15 |
| 17 | 17.8 (q) | 1.60 (s, 3H) | C-15 |
| 19 | 12.0 (q) | 1.60 (s, 3H) | C-10, C-11, C-12 |
| 19 | 16.0 (q) | 1.58 (s, 3H) | C-6, C-7 |
| 20 | 138.8 (d) | 7.20 (br s, 1H) | C-1, C-2, C-3, |
| $CH_3COO$ | 170.1 (s) | | |
| $CH_3COO$ | 21.2 (q) | 2.01 (s, 3H) | C=O |

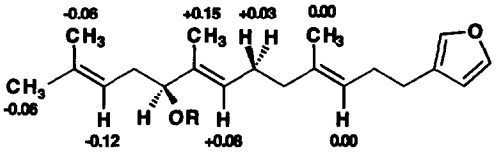
[a]Recorded in $CDCl_3$. HMBC experiment optimized for $J_{C\text{-}H}$ = 8 Hz, (w) indicates weak correlations.

The other, more polar, compound **5** was isolated as a colorless oil (0.025% dry weight, $[\alpha]_D$ -10.1°, *c* 0.77, $CHCl_3$) and produced a molecular ion in the high resolution mass spectrum at 302.2241 ($M^+$, Δmmu 0.5) implying a formula of $C_{20}H_{30}O_2$. The $^{13}$C and $^{1}$H NMR spectra of **5** were very similar to those of **6**, but lacked the acetoxyl group signals. A downfield methine proton (δ 3.97, dd, $J$

= 7.5, 5.7 Hz, 1H) and the presence of an O-H stretching band in the IR spectrum (υ 3445 $cm^{-1}$), suggested that **5** was the secondary alcohol corresponding to the acetate **6**. In order to confirm the structure of **5**, a sample of the natural product **6** was hydrolyzed (aq. NaOH, MeOH, 72%), to afford an alcohol which was identical with **5** by $^1H$ and $^{13}C$ NMR.

Both **5** and **6** were optically active. In order to determine the configuration at C-5, we used the modified Mosher method.[13] A sample of the acetate **6** was hydrolyzed to the alcohol, partitioned and separately esterified with (*R*) and (*S*) methoxytrifluoromethylphenylacetic acid (MTPA). The $^1H$ NMR spectra of the MTPA esters showed an unexpected feature: each ester was present as a 3:1 mixture of diastereoisomers, with minor peaks in the spectrum of the (*R*)-MTPA ester corresponding to the major peaks in the spectrum of the (*S*)-MTPA ester, and *vice versa*, and *it is concluded that the natural product must be a mixture of enantiomers at C-12.* $^1H$ NMR signals of the esters were assigned by interpretation of the COSY spectra and by comparison with the acetate **6**, and Δδ (δ*S*-δ*R*) values calculated[13] for the major diastereomers **13** and **14** (Figure 1). Signals to the right of the acyloxy substituent, as drawn for structure **13**, showed a positive Δδ, whilst those to the left showed a negative Δδ. Interpretation of these data according to literature[13] predicts that the major diastereomers, **13** and **14**, have the (12*S*) configuration.

**Figure 1.** Calculated Δδ (δ*S*-δ*R*) values from the MTPA esters **13** and **14**




In order to confirm that the acetate **6** was produced as a mixture of enantiomers, $^1H$ NMR spectra were obtained in the presence of Eu((+)-hfc)$_3$ chiral shift reagent.[14] At a 5:1 molar ratio of Eu((+)-hfc)$_3$ to **6** each methyl group was resolved into two peaks, the integration of which confirmed the 3:1 ratio of enantiomers.

Preparation of the (*R*)-MTPA ester from the naturally occurring alcohol **5** yielded the same 3:1 mixture of diastereoisomers, the $^1H$ NMR spectrum being identical to that of the product derived from the acetate **6**. Thus we can conclude that both **5** and **6** were isolated as scalemic mixtures, with a 50% enantiomeric excess of the (12*S*) enantiomer.

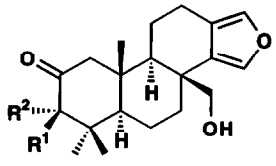
The other component of the least polar fractions was spongia-13(16),14-diene (**1**), isolated after flash chromatography, as a colorless solid (0.054% dry weight). $^1H$ and $^{13}C$ NMR data were identical to those reported.[1,5] The more polar components of the extract were purified by HPLC (Dynamax, silica gel, hexane/EtOAc) to afford compounds **7-11**. Compound **7** was obtained as a colorless solid (0.18% dry weight). A high resolution E.I. mass spectrum indicated a molecular ion at 332.1987 ($M^+$, Δmmu 0.1), providing the formula $C_{20}H_{28}O_4$. The IR spectrum indicated the presence of hydroxyl

**Table 2.** NMR Data for Compounds **7** and **8**.[a]

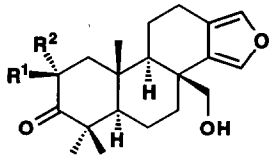
| Position | Compound **7** | | | Compound **8** | | |
|---|---|---|---|---|---|---|
| | $^{13}$C NMR δ (mult.) | $^{1}$H NMR δ (mult., *J* Hz, integral) | COLOC Correlations | $^{13}$C NMR δ (mult.) | $^{1}$H NMR δ (mult., *J* Hz, integral) | COLOC Correlations |
| 1 α | 52.9 (t) | 2.08 (br d, 12.1, 1H) | C2, C9, C10, C20 | 53.2 (t) | 2.17 (d, 17.3, 1H) | C2, C9, C10, C20 |
| β | | 2.59 (d, 12.1, 1H) | C2, C3, C5, C10 | | 2.47 (d, 17.3, 1H) | C2, C10 |
| 2 | 210.4 (s) | | | 212.6 (s) | | |
| 3 | 82.7 (d) | 3.83 (br s, 1H) | C2, C4, C18, C19 | 80.2 (d) | 4.19 (s, 1H) | C2, C4, C18, C19 |
| 4 | 45.3 (s) | | | 41.8 (s) | | |
| 5 | 54.7 (d) | 1.60 (m, 1H) | | 52.9 (d) | 1.25 (m, 1H) | C7 (w), C10, C19 (w), C20 |
| 6 | 17.6 (t) | 1.60 (m, 2H) | | 19.6 (t) | 1.55 (m, 2H) | |
| 7 α | 34.0 (t) | 1.32 (m, 1H) | | 33.5 (t) | 1.25 (m, 1H) | |
| β | | 2.46 (m, 1H) | C5, C8, C9 | | 2.39 (m, 1H) | |
| 8 | 40.4 (s) | | | 40.1 (s) | | |
| 9 | 55.9 (d) | 1.65 (m, 1H) | | 56.3 (d) | 1.42 (br d, 11.8 1H) | C1, C10, (C11, 12, 14 w), C20 |
| 10 | 43.3 (s) | | | 39.6 (s) | | |
| 11 | 18.0 (t) | 1.70 (m, 2H) | C8, C10 | 18.1 (t) | 1.55 (m, 1H) | C13 (w) |
| | | | | | 1.68 (m, 1H) | |
| 12 | 19.9 (t) | 2.46 (m, 1H) | C11 (w) | 20.1 (t) | 2.46 (m, 1H) | |
| | | 2.75 (m, 1H) | | | 2.74 (dd, 16.6, 6.3, 1H) | |
| 13 | 119.3 (s) | | | 119.3 (s) | | |
| 14 | 129.4 (s) | | | 129.1 (s) | | |
| 15 | 137.9 (d) | 7.13 (d, 1.4, 1H) | | 137.9 (d) | 7.12 (br s, 1H) | |
| 16 | 137.3 (d) | 7.10 (br d, 1.4, 1H) | | 137.3 (d) | 7.09 (br s, 1H) | |
| 17 a | 61.7 (t) | 3.37 (br d, 10.9, 1H) | C7 | 61.5 (t) | 3.39 (br d, 11.0, 1H) | C7 |
| b | | 3.71 (d, 10.9, 1H) | | | 3.78 (d, 11.0, 1H) | C7 (w), C14 |
| 18 | 29.0 (q) | 1.12 (s, 3H) | C3, C4, C5, C19 | 23.1 (q) | 0.79 (s, 3H) | C3, C4, C5, C19 |
| 19 | 16.1 (q) | 0.66 (s, 3H) | C3, C4, C5, C18 | 24.7 (q) | 1.06 (s, 3H) | C3, C4, C5, C18 |
| 20 | 17.4 (q) | 0.79 (s, 3H) | C1, C5, C9, C10 | 19.3 (q) | 1.14 (s, 3H) | C1, C9, C10 |

[a] Recorded in $CDCl_3$. COLOC experiment optimised for $J_{C\text{-}H}$ = 10 Hz, (w) indicates weak correlations.

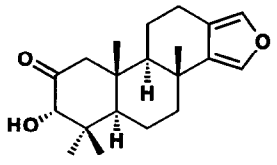
($\upsilon$ 3480 $cm^{-1}$) and ketone carbonyl ($\upsilon$ 1710 $cm^{-1}$) groups. Structural assignment was accomplished with the aid of COSY, HETCOR and COLOC NMR experiments (Table 2), and established an oxidized tetracyclic spongian diterpene structure. The 3,4-disubstituted furan ring was evident from the $^1H$ NMR spectrum ($\delta$ 7.10, br d, 1H; 7.13 br d, 1H), along with three methyl groups and a hydroxymethylene ($\delta$ 3.37, br d, $J$ = 10.9 Hz, 1H; 3.71, d, $J$ = 10.9 Hz, 1H; 61.7, t). Comparison of the furan $^{13}C$ chemical shifts with those for reported compounds,[3] allowed the hydroxymethylene group to be assigned to C-17. In particular, the C-14 resonance for compound **7** ($\delta$ 129.4, s) is at higher field than compounds bearing a methyl group at C-17 (eg. 136.4 for compound **11**). This assignment was also supported by observation of a W-coupling from H-17a ($\delta$ 3.37) to H-7$\alpha$ ($\delta$ 1.32, m, 1H) in the COSY spectrum and the COLOC experiment which revealed a long-range correlation from the geminal H-17b ($\delta$ 3.71) to the furan carbon C-14 ($\delta$ 129.4, s). The A-ring of compound **7** contained both the carbonyl ($\delta$ 210.4, s) and a hydroxymethine ($\delta$ 3.83, br s, 1H; 82.7, d). Conformational analysis of the A-ring revealed a chair conformation (Figure 2) as evidenced by the following NOEDS experiments. Irradiation of C-18 gave a nOe to H-3$\alpha$, while the C-19 methyl showed a nOe to the C-20 methyl and a small nOe to the C-18 methyl. The C-20 methyl also exhibited a nOe to H-17b and to H-1$\beta$. A small ($J$ = *ca.* 1 Hz) coupling between H-3$\alpha$ to H-1$\alpha$ across the keto group ($^1H$ NMR and COSY) supported diaxial disposition of these protons.[15] Hence, ring A adopts a chair conformation with the 3$\beta$-hydroxyl group and C-18 methyl group in equatorial positions and compound **7** is 3$\beta$,17-dihydroxyspongia-13(16),14-dien-2-one.




**7** $R^1$ = OH, $R^2$ = H
**8** $R^1$ = H, $R^2$ = OH




**9** $R^1$ = H, $R^2$ = OH
**10** $R^1$ = OH, $R^2$ = H



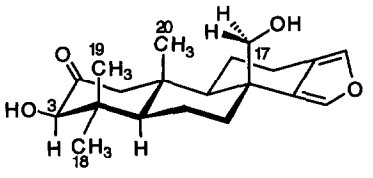

**11**

Compound **8** (colorless solid, 0.20% dry weight) was isomeric with **7** ($C_{20}H_{28}O_4$, $M^+$ 332.1970, $\Delta$mmu 1.8). Analysis of the NMR data obtained from COSY, HETCOR and COLOC experiments (Table 2) suggested that **8** must be the C-3 epimer of compound **7**. A series of nOe difference experiments established the relative stereochemistry of the A-ring. A strong nOe observed from the C-20 methyl group to H-3 requires ring A to assume a boat conformation (Figure 2) with the 3-hydroxyl in a pseudo-equatorial 'prow' position. The methyl at C-20 also exhibited a nOe to H-17b ($\delta$ 3.78) and nOe were also observed from the C-19 methyl to H-3 and from C-18 methyl to C19 methyl. Thus compound **8** is 3$\alpha$,17-dihydroxyspongia-13(16),14-dien-2-one.

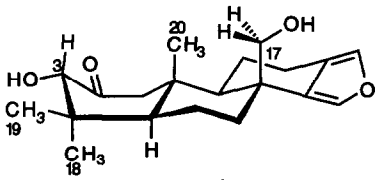
Two other isomeric spongian diterpenes, **9** (0.042% dry weight) and **10** (0.023% dry weight), were isolated. Comparison of the NMR data with that for compounds **7** and **8**, suggested that compound **9** differed only in the substitution of the A-ring, with transposition of hydroxyl and carbonyl

groups at C-2 and C-3. The downfield hydroxymethine proton was no longer a singlet but instead a doublet of doublets (δ 4.58, dd, $J$ = 12.4, 6.6 Hz, 1H), which was shown in the COSY spectrum to be coupled to a methylene group (δ 2.60, dd, $J$ = 12.2, 6.6 Hz, 1H; 1.18, obscured m, 1H). The relative stereochemistry of **9** was determined by interpretation of a NOESY experiment, which suggested a chair conformation for the A-ring, with the 2-hydroxyl substituent in an equatorial position. Strong axial-axial correlations was observed between H-2 (δ 4.58) and both the C-20 and C-19 methyl groups (δ 1.22 and 1.14 respectively). A nOe was also seen between the C-20 methyl and H-17b (δ 3.84, d, $J$ = 10.8 Hz, 1H). Thus compound **9** is 2α,17-dihydroxy-spongia-13(16),14-dien-3-one.

**Figure 2.** Conformations of Compounds **7** and **8**.




**7**




**8**

Comparison of the $^1H$ and $^{13}C$ NMR of compound **10** with that for compounds **7-9** indicated that **10** was the C-2 epimer of **9**. Unfortunately, both **9** and **10** proved to be rather unstable, compound **10** being extensively decomposed after overnight $^{13}C$ NMR acquisition which prevented complete characterization.

A fifth tetracyclic diterpene **11** was isolated (colorless solid, 0.059% dry weight). A high resolution mass spectrum provided the formula $C_{20}H_{28}O_3$ ($M^+$ 316.2050, Δmmu 1.2). The $^1H$ and $^{13}C$ NMR data revealed that **11** was only hydroxylated in the A-ring, as shown by the presence of a C-17 methyl (δ 1.24, s, 3H; 25.4, q) and the C-14 chemical shift (δ 136.4, s). Comparison of NMR data with those of compounds **7** and **8**, and to data from compounds previously reported in the literature,[3] implied that compound **11** has the 3α-hydroxyl substituent. The structural and NMR assignments were fully supported by long-range correlations in the HMBC spectrum (Table 3).

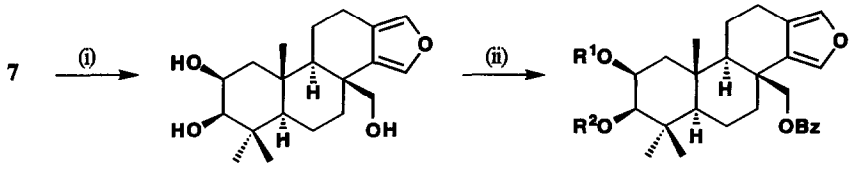
In order to determine the absolute configuration of the spongian diterpenes **7-11**, several benzoate derivatives suitable for circular dichroism studies were prepared (Scheme 1). Reduction of compound **7** provided the triol **15** as the sole product formed by α side equatorial hydride attack. Analysis of $^1H$ NMR coupling constants for H-2 (δ 4.04, ddd, $J$ = 4.0, 3.4, 3.4 Hz, 1H) and H-3 (δ 3.14, d, $J$ = 4.0 Hz, 1H), confirmed that the product has the 2β,3β-configuration. Benzoylation of **15** (BzCl, py, 55°) yielded a mixture of the tribenzoate **16** and the two dibenzoates **17** and **18**, which was separated by HPLC. Exciton coupling between the dibenzoate chromophores is predicted to give a bisignate circular dichroism spectrum whose helicity is correlated with the sign of longer wavelength Cotton effect produced by Davydov splitting.[16]

**Table 3.** NMR Data for Compound **11**.[a]

| Position | $^{13}$C NMR δ (mult.) | $^{1}$H NMR δ (mult., *J* Hz, integral) | HMBC Correlation |
|---|---|---|---|
| 1 α | 53.9 (t) | 2.18 (d, 17.7, 1H) | C-2, C-9 |
| β | | 2.57 (d, 17.7, 1H) | C-2 |
| 2 | 212.9 (s) | | |
| 3 | 80.1 (d) | 4.36 (s, 1H) | C-2, C-4, C-19 |
| 4 | 42.0 (s) | | |
| 5 | 53.6 (d) | 1.22 (m, 1H) | |
| 6 | 20.5 (t) | 1.70 (m, 2H) | |
| 7 α | 39.9 (t) | 2.12 (dt, 12.4, 3.0, 1H) | |
| β | | 1.60 (m, 1H) | |
| 8 | 34.1 (s) | | |
| 9 | 56.4 (d) | 1.30 (m, 1H) | |
| 10 | 39.6 (s) | | |
| 11 | 19.3 (t) | 1.65 (m, 2H) | |
| 12 | 20.7 (t) | 2.44 (dddd, 16.1, 12.0, 7.2, 1.6, 1H) | |
| | | 2.79 (dd, 16.1, 5.8, 1H) | C-9 |
| 13 | 119.2 (s) | | |
| 14 | 136.4 (s) | | |
| 15 | 135.2 (d) | 7.09 (br s, 1H) | |
| 16 | 136.9 (d) | 7.04 (br s, 1H) | |
| 17 | 25.4 (q) | 1.24 (s, 3H) | C-7, C-14 |
| 18 | 24.7 (q) | 0.83 (s, 3H) | C-3, C-4, C-5, C-19 |
| 19 | 23.6 (q) | 1.16 (s, 3H) | C-3, C-4, C-5, C-18 |
| 20 | 19.1 (q) | 1.25 (s, 3H) | C-1 |

[a]Recorded in $CDCl_3$. HMBC experiment optimized for $J_{C\text{-}H}$ = 8 Hz, (w) indicates weak correlations.

The measured CD spectrum of the tribenzoate revealed a positive bisignate Cotton effect (Δε -3.4, 220 nm; +21.6, 235). Although the CD spectrum of **16** is somewhat asymmetrical, due to overlapping dichroism from the furan chromophore (λ 220 nm), the CD spectra of each dibenzoate **17** and **18** showed relatively negligible contributions from long range exciton coupling to the C-17 benzoate group. Analysis of coupling constants for **16** requires a chair conformation for the A-ring, with the 2-OBz in an axial position and the 3-OBz equatorial.



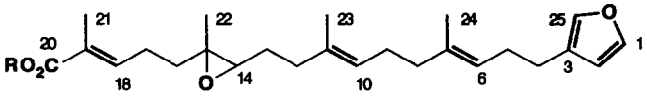

**15**

**16** $R^1$ = Bz, $R^2$ = Bz
**17** $R^1$ = H, $R^2$ = Bz
**18** $R^1$ = Bz, $R^2$ = H

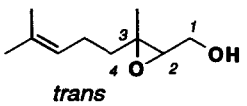
**Scheme 1.** Reagents and Conditions: (i) $NaBH_4$, MeOH, 20°, 5 mins; (ii) BzCl, py, 55°, 60 mins.

Therefore the absolute configuration for the tribenzoate **16** and the corresponding natural product **7** is 2*S*, 3*R*, as shown. This corresponds to the normal '5α,10β' absolute configuration, common to all sterols and most polycyclic diterpenes with the exception of the *ent* kaurenes,[17] and is consistent with the findings of Kazlauskus *et al.* for earlier spongian derivatives.[3]

The most abundant component of the extract was compound **12**, obtained as an optically active colorless oil (0.24% dry weight, $[\alpha]_D$ +8.7°, *c* 6.0, $CHCl_3$). A high resolution E.I. mass spectrum indicated a molecular ion at 400.2617 ($M^+$, Δmmu 0.3), providing the formula $C_{25}H_{36}O_4$, requiring eight degrees of unsaturation. Examination of the NMR spectra suggested a linear furanosesterterpene with a trisubstituted epoxide (δ 60.3, s; 63.2, d, $^1J_{C\text{-}H}$ = 165 Hz). A 3-substituted furan ring was evident in the $^1H$ NMR spectrum as three broadened singlets at δ 6.25, 7.18 and 7.30. Structural assignment was accomplished with the aid of COSY, HETCOR and COLOC NMR experiments (Table 4). Long-range correlations in the COLOC spectrum, from the 15-methyl protons to C-16, and from H-18 to C-16, allowed placement of the epoxide at C-14, C-15. An α,β-unsaturated carboxylic acid was suggested by the IR spectrum (υ 1690 $cm^{-1}$), a broad downfield singlet in the $^1H$ NMR spectrum at δ 11.50, and the corresponding carbonyl resonance in the $^{13}C$ NMR spectrum (δ 173.1, s). Methylation of **12** with diazomethane (diethyl ether, 0°, 83%) afforded the methyl ester **19** (δ 3.73, s, 3H, OMe; 168.4, s, C20). The COLOC spectrum showed long-range correlations from the carbonyl group in **12** to the olefinic bond (δ 6.86, t, *J* = 7.7 Hz H-18; 143.6, d, C-18; 127.4, s, C-19), placing the carboxyl group at C-20.

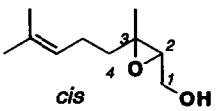



**12** R = H
**19** R = Me




*trans*

**20**




*cis*

**21**

The remaining two degrees of unsaturation were assigned to two tri-substituted double bonds at C-10,11 (δ 5.15, m; 133.8, s; 124.7, d) and C-6,7 (δ 5.15, m; 135.4, s; 123.7, d). The remainder of the COLOC correlations are in full agreement with the proposed linear furanosesterterpene. Sufficient quantities of **12** were available to confirm all $^{13}C$ assignments by 2D INADEQUATE (Table 4). The *E* configuration of the three olefinic groups follows from the high field $^{13}C$-NMR chemical shifts of the vinyl methyl signals (δ 11.8, q, C-21; 15.8, q, C-23; 15.9, q, C-24).[12] A *trans* geometry of the trisubstituted epoxide **12** was established using the following analogous argument.

**Table 4.** $^{1}H$ NMR and $^{13}C$ NMR Data for the Sesterterpene **12**.[a]

| Position | $^{13}C$ NMR δ (mult.) | $^{1}H$ NMR δ (mult., *J* Hz, integral) | COLOC Correlation[b] | 2D INADEQUATE Correlation[c] |
|---|---|---|---|---|
| 1 | 142.3 (d) | 7.30 (bs, 1H) | C-2, C-3 (w), C-25 | |
| 2 | 110.9 (d) | 6.25 (bs, 1H) | C-1, C-3 (w), C-25 | |
| 3 | 124.8 (s) | | | |
| 4 | 24.9 (t) | 2.42 (t, 7.8, 2H) | C-2, C-3, C-5, C-6, C-25 | C-5 |
| 5 | 28.3 (t) | 2.24 (m, 2H) | C-3, C-4, C-6, C-7 | C-4, C-6 |
| 6 | 123.7 (d) | 5.15 (m, 1H) | C-4, C-5, C-8 | C-5 |
| 7 | 135.4 (s) | | | |
| 8 | 39.4 (t) | 1.99 (m, 2H) | C-6, C-7, C-9, C-10 | C-9 |
| 9 | 26.4 (t) | 2.08 (m, 2H) | C-7 (w), C-8 | C-8, C-10 |
| 10 | 124.7 (d) | 5.15 (m, 1H) | C-8, C-9, C-12 | C-9 |
| 11 | 133.8 (s) | | | C-23 |
| 12 | 36.0 (t) | 2.10 (m, 2H) | C-10, C-11,C-13, C-14 , C-23 | C-13 |
| 13 | 27.0 (t) | 1.64 (m, 2H) | | C-12, C-14 |
| 14 | 63.2 (d) | 2.73 (t, 6.1, 1H) | C-13 | C-13, C-15 |
| 15 | 60.3 (s) | | | C-14, C-16 |
| 16 | 37.0 (t) | 1.72 (m, 1H) | | C-15, C-17 |
| | | 1.60 (m, 1H) | | |
| 17 | 24.4 (t) | 2.30 (m, 2H) | C-15, C-16, C-18, C-19 | C-16, C-18 |
| 18 | 143.6 (d) | 6.86 (t, 7.7, 1H) | C-16, C-17, C-19, C-21 | C-17 |
| 19 | 127.4 (s) | | | |
| 20 | 173.1 (s) | 11.50 (bs, 1H) | | |
| 21 | 11.8 (q) | 1.83 (s, 3H) | C-18, C-19, C-20 | |
| 22 | 16.3 (q) | 1.26 (s, 3H) | C-14, C-15, C-16 | |
| 23 | 15.8 (q) | 1.60 (s, 3H) | C-10, C-11, C-12 | C-11 |
| 24 | 15.9 (q) | 1.57 (s, 3H) | C-6, C-7, C-8 | |
| 25 | 138.6 (d) | 7.18 (bs, 1H) | C-1, C-2 (w), C-3 | |

[a]Recorded in $CDCl_3$; [b]Optimized for $J_{C-H}$ = 10 Hz, weak correlations (w) .[c] Optimized for $^{1}J_{CC}$145 Hz.

Steric compression of the methyl group substituted on the epoxide in **12** (δ 16.3, q, C-22) by the *syn*, eclipsed C-13 methylene group should result in a higher field shift for methyl signals of *trans* methyl epoxides compared to the *cis* isomers. Comparison of $^{13}C$ NMR signals of **12** (Table 5) with those of the diastereomeric *trans* geraniol 2,3-epoxide (**20**, δ 17.4, q, C-3 Me) and *cis* nerol 2,3-epoxide (**21**, δ 21.9, q, C-3 Me)* shows an upfield shift of 4.5 ppm for the C-3 methyl group in **20**, similar to **12**, hence, supporting a *trans* configuration for **12**. Conversely, a Δδ 5.4 ppm upfield shift is seen for the C-4 methylene group in **21**. Attempts to open the epoxide ring of **19** with a variety of nucleophiles were unsuccessful and the absolute configuration of **12** remains to be determined.

* Prepared by mCPBA (1.0 equiv) epoxidation of geraniol or nerol, followed by separation of the faster eluting 2,3-epoxide by flash chromatography (silica, 40:60 EtOAc/*n*-hexane). Compounds were identified by comparision with literature data[18] and unambiguous $^{13}C$ NMR assignments were obtained by analysis of COSY, DEPT and HETCOR experiments.

**Table 5.** Selected $^{13}C$ NMR Data ($CDCl_3$) for Epoxides; *trans*- **12**, **20** and *cis* **21**.

| **20** (*trans*) | | **21** (*cis*) | | **12** (*trans*) | |
|---|---|---|---|---|---|
| Position | δ, mult | Position | δ, mult. | Position | δ, mult. |
| 1 | 61.0 (t) | 1 | 60.8 (t) | 13 | 27.0 (t) |
| 2 | 63.1 (d) | 2 | 64.4 (d) | 14 | 63.2 (d) |
| 3 | 60.9 (s) | 3 | 61.3 (s) | 15 | 60.3 (s) |
| 4 | 38.3 (t) | 4 | 32.9 (t) | 16 | 37.0 (t) |
| 3-Me | 17.4 (q) | 3-Me | 21.9 (q) | 22 | 16.3 (q) |

Compounds **5** and **6** are rare examples of marine natural products that occur as a scalemic mixtures.[19] The origin of this property is not clear, however, brief examination of the structure of **6** and **7** suggests several alternate explanations. First, the possibility that **5** is the product of free radical non-enzymic autoxidation of ambliofuran (**2**) can be excluded as this would produce racemic hydroperoxides and alcohols. No evidence was found for hydroperoxidation of the natural products in the extract. Second, it is possible the compounds are produced from ambliofuran (**2**) by the action of an oxidase that lacks stereochemical discrimination. Enzymic reactions that produce chiral products from achiral precursors usually result in high enantiomeric excesses due to strict fidelity of asymmetric induction in the active sites. A single promiscuous oxidase would be an exception to this trend and seems unlikely, but the possibility of two oxidases with different stereochemical specificity is plausible. Cases of enantiomeric natural product biosynthesis are known which proceed within the same organism using the same achiral enantiotopic substrate, but employ enzymes with antipodal stereochemical preference. For example, the enantiomers of α-pinene found in immature sage are produced by distinctly different cyclase enzymes from the same precursor and are found as scalemic mixtures.[20] We[7] and others[9] have shown that marine furanosesquiterpenes do occur in both enantiomeric forms. Lastly, it is possibility that **5** is produced in homochiral form only to be partially racemized to a scalemic mixture. Non-enzymatic isomerization, is discounted for reasons mentioned above and an enzymatic route, again, would invoke the need for an indiscriminate enzyme. We cannot exclude the possibility that each enantiomer of **5** is produced by oxidation of **2** with two different enzymes in *Spongia* sp., one with (*S*) specificity and another with (*R*), followed by acylation to the scalemic acetates **6**.

We propose that stereochemically indiscriminate oxidation may be more commonplace than generally assumed and play a part in the enzymic oxidation of some marine terpenoids that are chiral due to the presence of a single stereocenter. This has not been generally tested in chiral marine natural products and can only be disproved through reporting of compounds with rigorous determination of enantiomeric excess (% ee) in addition to optical activity.

## Experimental Section.

Optical rotations were measured on a JASCO DIP-370 spectropolarimeter. NMR spectra were recorded on a General Electric QE-300 spectrometer at 300 MHz for $^1H$, and 75.4 MHz for $^{13}C$. $^1H$ NMR and $^{13}C$ NMR spectra are referenced to $CDCl_3$ solvent signals at 7.26 and 77.0 ppm respectively. Multiplicities of $^{13}C$ spectra were assigned by DEPT experiments. Standard pulse sequences (General Electric Instruments) were employed for DEPT, magnitude COSY, NOESY, HETCOR, COLOC and 2D INADEQUATE experiments. HMBC experiments were performed on a Varian Gemini 400 MHz NMR spectrometer. 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 0.2 mm silica $60F_{254}$ plates (Merck 9375), and developed with vanillin-EtOH-1% $H_2SO_4$. All solvents were distilled in glass before use.

*Collection and Extraction:* The sponge *Spongia* sp. (93-01-008) was collected in 1993 by hand using SCUBA at a depth of -5 meters in Exmouth Gulf, Australia and frozen at -20°C until required. A voucher specimen is archived at the University of California, Davis, Department of Chemistry. Lyophilized animals (148.0 g) were extracted with 4:1 $CH_2Cl_2$/MeOH (2 × 600 mL). The combined extracts were evaporated and the residue partitioned between water (400 mL) and EtOAc (400 mL). The aqueous phase was extracted with EtOAc (100 mL) and the combined organic extracts evaporated to give a yellow oil (5.75 g). A portion (2.00 g) was partitioned by flash chromatography (silica gel, 40mm × 320 mm, stepwise gradient elution hexane/EtOAc 10:90 to 75:25) to afford six fractions. Purification of the first fraction by flash chromatography (silica gel, hexane) gave spongia-13(16),14-diene (**1**) and ambliofuran (**2**). The more polar fractions were purified by HPLC (Dynamax silica, 10mm × 250 mm, hexane/EtOAc solvent mixtures) to afford compounds **5-12**.

*Spongia-13(16),14-diene* (**1**): colorless solid (27.9 mg, 0.054% dry weight of sponge); $^1H$ and $^{13}C$ NMR ($CDCl_3$) data identical to those reported.[1,5]

*Ambliofuran* (**2**): colorless oil (8.0 mg, 0.015%); $^1H$ and $^{13}C$ NMR ($CDCl_3$) data identical to those reported.[2]

*12-Hydroxyambliofuran* (**5**): colorless oil (12.7 mg, 0.025%); $C_{20}H_{30}O_2$; $[\alpha]_D$ -10.1° (*c* 0.77, $CHCl_3$); IR (film) $\upsilon_{max}$ 3445 (br) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 1.59 (s, 3H), 1.62 (s, 3H), 1.64 (s, 3H), 1.72 (s, 3H), 2.02 (m, 2H), 2.11 (m, 3H), 2.25 (m, 3H), 2.45 (t, *J* = 7.0 Hz, 2H), 3.97 (dd, *J* = 7.5, 5.7 Hz, 1H), 5.09 (br t, *J* = 7.0 Hz, 1H), 5.17 (br t, *J* = 7.0 Hz, 1H), 5.37 (br t, *J* = 7.0 Hz, 1H), 6.21 (br s, 1H), 6.27 (br s, 1H), 7.34 (br s, 1H); $^{13}C$ NMR ($CDCl_3$) δ 11.7 (q), 16.0 (q), 18.0 (q), 25.0 (t), 25.9 (q), 26.1 (t), 28.4 (t), 34.2 (t), 39.3 (t), 77.2 (d), 111.0 (d), 120.2 (d), 123.9 (d), 124.9 (s), 125.9 (d), 134.6 (s), 135.5 (s), 136.8 (s), 138.8 (d), 142.5 (d); MS (E.I.) 302 ($M^+$, 1%), 284 (32); HRMS found 302.2241, $C_{20}H_{30}O_2$ requires 302.2246.

*12-Acetoxyambliofuran* (**6**): colorless oil (58.2 mg, 0.11%); $C_{22}H_{32}O_3$; $[\alpha]_D$ -10.2° (*c* 2.8, $CHCl_3$); IR (film) $\upsilon_{max}$ 2920 $cm^{-1}$, 2855, 1735, 1370, 1240, 1025; $^1H$ and $^{13}C$ NMR see Table 1; MS (E.I.) 344 ($M^+$, 1%), 300 (3), 284 ($M^+$ - AcOH, 4), HRMS found 344.2334, $C_{22}H_{32}O_3$ requires 344.2351.

*3β,17-Dihydroxyspongia-13(16),14-dien-2-one* (**7**): colorless solid (96.9 mg, 0.18%); $C_{20}H_{28}O_4$; m.p. 165-167.5 °C (from hexane: ethyl acetate); $[\alpha]_D$ -8.1° (c = 1.7, $CHCl_3$); UV (MeCN) $\lambda_{max}$ 217 nm (ε 4900); IR (film) $\upsilon_{max}$ 3480 (br), 2950, 2850, 1710, 1395, 1035, 965, 890 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR see Table 2; MS (E.I.) 332 ($M^+$, 22%), 301 (100), 283 (20); HRMS found 332.1987, $C_{20}H_{28}O_4$ requires 332.1988.

*3α,17-Dihydroxyspongia-13(16),14-dien-2-one* (**8**): colorless solid (105.4 mg, 0.20%); $C_{20}H_{28}O_4$; $[\alpha]_D$ +64.5° (c = 1.7, $CHCl_3$); UV (MeCN) $\lambda_{max}$ 218 nm (ε 4300); IR (film) $\upsilon_{max}$ 3420 (br), 2945, 1710, 1040, 890 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR see Table 2; MS (E.I.) 332 ($M^+$, 15%), 301 (60), 283 (10); HRMS found 332.1970, $C_{20}H_{28}O_4$ requires 332.1988.

*2α,17-Dihydroxyspongia-13(16),14-dien-3-one* (**9**): colorless oil (21.4 mg, 0.042%); $C_{20}H_{28}O_4$; $^1H$ NMR ($CDCl_3$) δ 1.14 (s, 3H), 1.16 (s, 3H), 1.18 (m obscured, 1H), 1.22 (s, 3H), 1.30 (m, 1H), 1.35(m,1H), 1.43 (dd, *J* = 11.8, 2.5 Hz, 1H), 1.75 (m, 4H), 2.51 (m, 1H), 2.52 (m, 1H), 2.60 (dd, *J* = 12.2, 6.6 Hz, 1H), 2.79 (dd, *J* = 16.4, 6.1 Hz, 1H), 3.71 (dd, *J* = 10.8, 1.6 Hz, 1H), 3.84 (d, *J* = 10.8 Hz, 1H), 4.58 (dd, *J* = 12.4, 6.6 Hz, 1H), 7.17 (br s, 1H), 7.17 (br s, 1H); $^{13}C$ NMR δ 17.3 (q), 17.9 (t), 18.8 (t), 20.0 (t), 21.3 (q), 24.5 (q), 34.2 (t), 37.9 (s), 40.5 (s), 47.5 (s), 49.2 (t), 56.0 (d), 57.9 (d), 62.1 (t), 69.2 (d), 119.6 (s), 129.5 (s), 137.3 (d), 138.3 (d), 215.8 (s).

*2β,17-Dihydroxyspongia-13(16),14-dien-3-one* (**10**): colorless oil (12.1 mg, 0.023%); $C_{20}H_{28}O_4$; $^1H$ NMR ($CDCl_3$) δ 0.78 (s, 3H), 1.16 (s, 3H), 1.17 (s, 3H), 1.20-1.80 (m, 8H), 2.50 (m, 2H), 2.81 (m, 1H), 3.42 (dd, *J* = 11.0, 1.3 Hz, 1H), 3.79 (d, *J* = 12.0 Hz, 1H), 3.88 (d, *J* = 12.0 Hz, 1H), 4.59 (dd, *J* = 11.6, 6.7 Hz, 1H), 7.19 (br s, 1H), 7.21 (br s, 1H); $^{13}C$ NMR δ 18.5, 19.0, 19.4, 19.5, 20.3, 29.6, 33.1, 36.8, 40.1, 45.4, 50.8, 51.9, 55.2, 61.5, 68.5, 119.6, 129.4, 137.2, 138.4, 218.9.
*3α-Hydroxyspongia-13(16),14-dien-3-one* (**11**): colorless crystalline solid (30.5 mg, 0.059%); $C_{20}H_{28}O_3$; $^1H$ and $^{13}C$ NMR see Table 3; HRMS (E.I.) found 316.2050, $C_{20}H_{28}O_3$ requires 316.2038.

*Epoxide* (**12**): colorless oil (125.0 mg, 0.24%); $C_{25}H_{36}O_4$; $[\alpha]_D$ +8.7° (c = 6.0, $CHCl_3$); IR (film) $\upsilon_{max}$ 3000 (br), 2925, 2855, 1690, 1645, 1285, 875 $cm^{-1}$; $^1H$ NMR and $^{13}C$ NMR see Table 4; MS (E.I.) 400 ($M^+$, 4%); HRMS found 400.2617, $C_{25}H_{36}O_4$ requires 400.2614.

*Hydrolysis of Acetate* **6**: A solution of **6** (18.4 mg, 55.1 mmol) in methanol (1.0 mL) was treated with aqueous sodium hydroxide (2.0 M, 0.2 mL) and stirred at 25°C for 16 hours. The reaction mixture was evaporated to dryness and the residue purified by flash chromatography (silica, 12mm 50mm,

hexane/EtOAc 90:10) to afford the alcohol **5** as a colorless oil (11.7 mg, 72%): $[\alpha]_D$ -7.9° (*c* 0.68, $CHCl_3$); $^1H$ NMR and $^{13}C$ NMR data identical with the natural product.

*Preparation of (R)-MTPA Ester* **13** *from Alcohol* **5**: A solution of the alcohol **5** (derived from **6**) (6.9 mg, 22.8 mmol) in $CH_2Cl_2$ (0.5 mL) was treated with (*R*)-MTPA acid (13.0 mg, 55.5 mmol), DCC (12.0 mg, 58.2 mmol) and DMAP (*cs.* 0.1 mg). After stirring at 25°C for 3 days the reaction mixture was evaporated and the residue eluted through basic alumina (pipet column, hexane/EtOAc 95:5) and silica gel (pipet column, hexane/EtOAc 95:5) to afford the (*R*)-MTPA ester derivative (**13**) as a colorless oil (10.6 mg, 90%): $^1H$ NMR for major diastereoisomer **13** ($CDCl_3$) δ 1.48 (s, 3H), 1.58 (s, 3H), 1.60 (s, 3H), 1.69 (s, 3H), 2.00 (m, 2H), 2.09 (m, 2H), 2.25 (m, 3H), 2.45 (m, 3H), 3.54 (s, 3H), 5.04 (br t, *J* = 7.0 Hz, 1H), 5.16 (br t, *J* = 7.0 Hz, 1H), 5.33 (dd, *J* = 8.4, 5.9 Hz, 1H), 5.46 (br t, *J* = 7.0 Hz, 1H), 6.27 (br s, 1H), 7.21 (br s, 1H), 7.34 (br s, 1H), 7.35-7.55 (m, 5H). See also Figure 1.

*Preparation of (S)-MTPA Ester* **14** *from Alcohol* **5**: Following a similar procedure to that detailed above using the alcohol **5** (derived from **6**) (4.8 mg, 15.9 mmol) and (*S*)-MTPA acid afforded the (*S*)-MTPA ester derivative (**14**) as a colorless oil (7.8 mg, 95%): $^1H$ NMR for major diastereoisomer **14** ($CDCl_3$) δ 1.54 (s, 3H), 1.58 (s, 3H), 1.63 (s, 6H), 2.00 (m, 2H), 2.12 (m, 2H), 2.25 (m, 3H), 2.45 (m, 3H), 3.52 (s, 3H), 4.92 (br t, *J* = 7.0 Hz, 1H), 5.16 (br t, *J* = 7.0 Hz, 1H), 5.37 (dd, *J* = 8.4, 5.9 Hz, 1H), 5.54 (br t, *J* = 7.0 Hz, 1H), 6.27 (br s, 1H), 7.21 (br s, 1H), 7.34 (br s, 1H), 7.35-7.55 (m, 5H). See also Figure 1.

*Preparation of (R)-MTPA Ester* **13** *from Alcohol* **5** *(Natural Product)*: Following a similar procedure to that detailed above using the natural product **5** (1.6 mg, 5.3 mmol) and (*R*)-MTPA acid afforded the (*R*)-MTPA ester derivative (**13**) as a colorless oil (1.7 mg, 62%): $^1H$ NMR data identical to product produced from the acetate **6**.

*Preparation of Methyl Ester* **19**: A solution of the carboxylic acid **12** (11.0 mg) in ether (1.5 mL) was cooled to 0° and treated with excess ethereal diazomethane. After stirring for 1 hr the ether was evaporated under a stream of dry nitrogen to give a colorless oil. Purification by column chromatography (silica gel, hexane/EtOAc 9:1) afforded the methyl ester **19** as a colorless oil (9.5 mg, 83%): IR (film) $\upsilon_{max}$ 1715 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 1.26 (s, 3H), 1.58 (s, 3H), 1.61 (s, 3H), 1.84 (s, 3H), 1.70 (m, 4H), 2.25 (m, 4H), 2.05 (m, 6H), 2.44 (t, *J* = 7.5 Hz, 2H), 2.71 (t, *J* = 6.2 Hz, 1H), 3.73 (s, 3H), 5.16 (m, 2H), 6.27 (br s, 1H), 6.73 (br t, *J* = 7.5 Hz, 1H), 7.20 (br s, 1H), 7.33 (br s, 1H); $^{13}C$ NMR ($CDCl_3$) δ 12.3 (q), 15.9 (q), 16.0 (q), 16.5 (q), 24.3 (t), 25.0 (t), 26.5 (t), 27.2 (t), 28.4 (t), 36.2 (t), 37.3 (t), 39.6 (t), 51.7 (q), 60.2 (s), 63.2 (d), 111.0 (d), 123.8 (d), 124.8 (d), 124.9 (s), 127.9 (s), 133.9 (s), 135.5 (s), 138.8 (d), 141.3 (d), 142.5 (d), 168.4 (s); MS (E.I) 414 ($M^+$, 10%); HRMS found 414.2775, $C_{26}H_{38}O_4$ requires 414.2770.

*Reduction of Ketone* **7**: A solution of **7** (10.3 mg, 0.03 mmol) in methanol (1.0 mL) was treated with excess $NaBH_4$. After 5 mins the reaction mixture was blown down to dryness under a stream of

nitrogen. The residue was purified by column chromatography (silica gel, hexane/EtOAc 1:1) to afford the triol **15** as a colorless solid (6.5 mg, 63%): $[\alpha]_D$ -15.2° (*c* 0.42, MeOH); IR (film) $\upsilon_{max}$ 3395, 1044, 755 $cm^{-1}$; $^1H$ NMR ($CD_3OD$) δ 0.99 (s, 3H), 1.03 (s, 3H), 1.20 (m, 2H), 1.23 (s, 3H), 1.25 (m, 2H), 1.63 (m, 2H), 1.72 (m, 1H), 1.82 (m, 1H), 2.24 (dd, *J* = 14.1, 3.1 Hz, 1H), 2.46 (m, 2H), 2.77 (dd, *J* =16.2, 6.1 Hz, 1H), 3.14 (d, *J* = 4.0 Hz, 1H), 3.43 (dd, *J* = 11.0, 1.0 Hz, 1H), 3.92 (d, *J* = 11.0 Hz, 1H), 4.03 (ddd, *J* = 4.0, 4.0, 3.1 Hz, 1H), 7.10 (br s, 1H), 7.12 (br s, 1H); $^{13}C$ NMR ($CD_3OD$) δ 17.7 (q), 18.5 (q), 19.0 (2 × t), 21.5 (t), 30.0 (q), 36.2 (t), 38.3 (s), 39.5 (s), 41.4 (s), 45.4 (t), 57.2 (d), 59.1 (d), 63.0 (t), 72.1 (d), 79.4 (d), 121.1 (s), 131.8 (s), 138.1 (d), 139.1 (d); MS (FAB) 335 ($MH^+$, 10%); HRMS found 335.2232, $C_{20}H_{31}O_4$ requires 335.2222.

*Benzoylation of Triol* **15**: A solution of the triol **15** (6.1 mg, 0.018 mmol) in pyridine (0.5 mL) was treated with benzoyl chloride (50 μL) and the solution heated at 55° for 1 hr. Excess pyridine was removed under high vacuum and the residue purified by column chromatography (silica gel, hexane/EtOAc 4:1) followed by HPLC (Dynamax, silica gel, hexane/EtOAc 85:15) to afford the tribenzoate **16** (4.1 mg, 35%), and the dibenzoates **17** (0.8 mg, 8%) and **18** (0.6 mg, 6%), all as colorless glasses:

*Tribenzoate* **16**: CD (dioxane) 220 nm (Δε -3.4), 224 (0); 235 (+21.6); $^1H$ NMR ($CDCl_3$) δ 1.03 (s, 3H), 1.38 (s, 3H), 1.43 (s, 3H), 1.20-1.90 (m, 8H), 2.54 (m, 3H), 2.87 (dd, *J* = 16.2, 5.8 Hz, 1H), 4.35 (d, *J* = 10.8 Hz, 1H), 4.82 (d, *J* = 10.8 Hz, 1H), 5.03 (d, *J* = 4.2 Hz, 1H), 5.79 (ddd, *J* = 4.2, 3.5, 3.5 Hz, 1H), 7.12 (br s, 1H), 7.20 (br s, 1H), 7.30-7.60 (m, 9H), 7.96 (m, 6H).

*Dibenzoate* **17**: CD (dioxane) 235 nm (Δε +3.6); $^1H$ NMR ($CDCl_3$) δ 0.97 (s, 3H), 1.30 (s, 3H), 1.43 (s, 3H), 1.20-1.90 (m, 8H), 2.50 (m, 3H), 2.87 (dd, *J* = 16.7, 6.4 Hz, 1H), 4.35 (m, 2H), 4.82 (d, *J* = 10.8 Hz, 1H), 4.88 (d, *J* = 3.7 Hz, 1H), 7.12 (br s, 1H), 7.18 (br s, 1H), 7.52 (m, 6H), 7.99 (m, 2H), 8.11 (m, 2H).

*Dibenzoate* **18**: CD (dioxane) 228 nm (Δε -3.5); $^1H$ NMR ($CDCl_3$) δ 1.11 (s, 3H), 1.17 (s, 3H), 1.29 (s, 3H), 1.20-1.90 (m, 8H), 2.50 (m, 3H), 2.84 (dd, *J* = 16.4, 6.4 Hz, 1H), 3.47 (dd, *J* = 7.8, 4.3 Hz, 1H), 4.33 (d, *J* = 10.7 Hz, 1H), 4.76 (d, *J* = 10.7 Hz, 1H), 5.55 (m, 1H), 7.10 (br s, 1H), 7.18 (br s, 1H), 7.50 (m, 6H), 8.00 (m, 4H).

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