Stereochemistry of Antiinflammatory Marine Sesterterpenes

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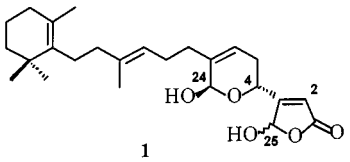
The absolute configuration of antiinflammatory marine sesterterpenes belonging to the manoalide and cacospongionolide structural type has been determined by comparison of the CD spectra of the natural compounds with those of analogues that have been stereoselectively synthesized. In the derived acetates the relative stereochemistry of the stereogenic centres in the pyranofuranone moiety is assigned from ^{1}H-NMR data and the absolute configuration from CD spectra. The absolute configuration of the naturally occurring marine sesterterpenes thorectolide monoacetate, manoalide monoacetate, petrosaspongiolide M and cavernosolide has been determined.

Introduction

New anti-inflammatory compounds that are potent inhibitors of phospholipase A_2 have been isolated in recent years from marine invertebrates.[1] Among these, a class of biologically active sesterterpenes containing a pyranofuranone substructure has been isolated from soft sponges. In respect to the pharmacophoric pyranofuranone moiety, these sesterterpenes can be grouped into two subclasses, exemplified by manoalide[2] (**1**; for the configuration at C-24 vide infra) and cacospongionolide B[3] (**2**), differing by the presence of the hemiacetal moiety in the dihydropyran ring.



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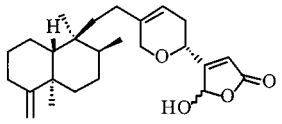

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Despite the wide interest in the bioactivities of these compounds, their absolute configuration has been elucidated only in a few cases. The absolute configuration at C-4 of manoalide, which is by far the most studied of these compounds, has been established by reduction to "manoalide diol'' (vide infra) and comparison of the CD spectrum of the latter with that of the synthetic (*S*)-enantiomer, while the absolute configuration of other related manoalide compounds, such as luffarin-A,[4] remains to be elucidated. On the other hand, the absolute configuration of several cacospongionolides was assigned only recently, by a very indirect way, using the modified Mosher method.[5]

We report here studies on the CD spectra of these sesterterpenes in comparison with those of simple manoalide and cacospongionolide synthetic analogues containing the same pyranofuranone chromophore as the natural products, which enable the absolute configuration at C-4 (manoalide numbering[6]) to be inferred in both subclasses of compounds. The absolute configuration of some naturally occurring monoacetates has also been derived from these studies.

Results and Discussion

Chiroptical Properties of 2(5H)-Furanones

Several studies have established that the ring atoms of 2(5*H*)-furanones are coplanar[7] and thus the observed chiroptical properties of chiral compounds containing this chromophore are to be ascribed to the perturbation induced by the surrounding stereogenic centres.

On studying the CD spectra of 2(5*H*)-furanones containing a stereogenic centre at C-5, Uchida and Kuriyama[8] have reported that while the sign of the n-π* Cotton effect is easily influenced by the contribution of the asymmetry external to the ring, the chirality at the γ-carbon atom of the butenolide ring is the sign-determining factor for the π-π* transition. Recently, Gawronski, Feringa et al.[9] have proposed a helicity rule for 2(5*H*)-furanones that correlates the absolute configuration at C-5 to the sign of the Cotton effects of the n-π* and π-π* transitions. The configurational rule has been very recently tested on a number of 3- and/or 4-heterosubstituted 2(5*H*)-furanones.[7]

In respect to the above studies, both manoalide (**1**) and cacospongionolide B (**2**) display a 2(5*H*)-furanone chromophore having more complex features. In fact, in both compounds, as well as in their companions, there is not only the simultaneous presence of two allylic stereogenic centres, but also the fact that only one of them (C-4) is fixed, with

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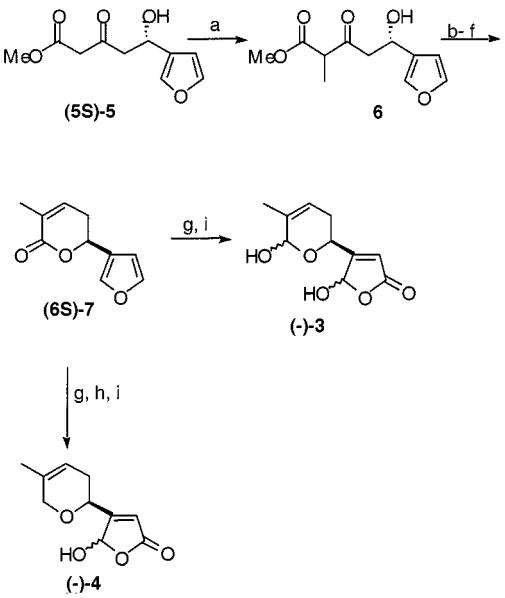
the butenolide ring γ-carbon being present as a mixture of epimers. For this reason the reported rules[7,8,9] cannot be applied. Indeed, Uchida and Kuriyama[8] have reported that in compounds in which there are two allylic stereogenic centres, the sign of the π-π* Cotton effect does not obey their empirical rule, while Gawronski, Feringa et al.[9] did not report any example of this type.

Stereoselective Synthesis and CD Spectra of Manoalide and Cacospongionolide Methyl Analogues

The model compounds **3** and **4** were prepared in enantiomerically enriched forms, starting from chiral non racemic compound **5**,[10] through the exploitation of the synthetic sequence previously reported for the preparation of racemic analogues of manoalide and cacospongionolide B.[11,12] In Scheme 1 the synthesis of the (4*S*)-methyl analogues [(–)-**3** and (–)-**4**] of manoalide and cacospongionolide is outlined.



Scheme 1. **a:** LiOH·H_2O, MeI, THF, 40 °C; **b:** $Zn(BH_4)_2$, THF; **c:** 1 N NaOH, EtOH; **d:** 6 N HCl; **e:** Ac_2O, Py; **f:** DBU, $CHCl_3$; **g:** DIBAL, PhMe, –78 °C; **h:** Et_3SiH, BF_3Et_2O, –78 °C; **i:** $^1O_2$, CH_2Cl_2, –78 °C

The key intermediate (6*S*)-**7** was obtained in 36% yield based on (5*S*)-**5** and 92% e.e. determined by ^{1}H-NMR analysis in the presence of Eu(hfc)$_3$ as a shift reagent by integration of the signals relative to the C-4 furan proton. The compounds (+)-**3** and (+)-**4** were analogously obtained starting from (5*R*)-**5**.

The CD spectra of compounds (+)-**3**, (–)-**3**, (+)-**4** and (–)-**4** are reported in Table 1.

All the compounds show a CD curve which is conceivably the sum of the influences of both allylic stereogenic centres on the furanone chromophore, and whose sign depends upon the stereochemistry of the stereogenic centre in the pyranofuranone ring. When the absolute configuration at C-4 is *R* the curve is positive, while it is negative when the absolute configuration at that centre is *S*. Furthermore,

Table 1. CD data

Compound	CD (Θ)	
1	239 (7.8×10^3)	220 (7.3×10^3)
2	236 (s) (5.1×10^3)	214 (1.1×10^4)
(+)-3	242 (7.5×10^3)	219 (7.7×10^3)
(–)-3	242 (-7.2×10^3)	219 (-7.6×10^3)
(+)-4	239 (s) (3.6×10^3)	214 (9.5×10^3)
(–)-4	242 (s) (-3.2×10^3)	212 (-8.6×10^3)
8	236 (s) (4.6×10^3)	218 (1.1×10^4)
9	245 (1.9×10^4)	
10	260 (-6.8×10^2)	217 (2.6×10^4)
11	226 (2.3×10^3)	
12	231 (1.6×10^4)	
13	260 (-1.2×10^3)	216 (1.8×10^4)
(+)-14	244 (6.0×10^4)	
(–)-14	245 (-6.2×10^4)	
(+)-15	260 (-1.1×10^3)	220 (1.2×10^4)
(–)-15	264 (7.1×10^2)	216 (-1.4×10^4)
17	246 (3.9×10^3)	216 (-1.0×10^3)
18	254 (-1.2×10^3)	217 (5.8×10^3)
(+)-19	248 (4.9×10^3)	220 (-2.9×10^3)
(–)-19	247 (-4.9×10^3)	219 (2.7×10^3)
(+)-20	256 (-1.6×10^3)	216 (1.4×10^4)
(–)-20	254 (2.0×10^3)	215 (-1.8×10^4)
21	254 (-2.1×10^3)	219 (1.1×10^4)
23	246 (-5.2×10^3)	216 (1.8×10^3)
24	254 (1.9×10^3)	217 (-1.2×10^4)
25	243 (5.1×10^3)	218 (-2.2×10^3)
26	247 (2.7×10^3)	219 (-0.6×10^3)
27	246 (6.6×10^3)	218 (-8.5×10^3)
28	254 (-1.3×10^3)	2.18 (7.2×10^3)
29	250 (3.3×10^3)	218 (-4.3×10^3)
30	258 (-0.9×10^3)	223 (5.8×10^3)

two distinct Cotton effects were detected in the spectra of manoalide analogues [(+)-**3** and (–)-**3**], while in the spectra of cacospongionolide analogues [(+)-**4** and (–)-**4**] the Cotton effect at longer wavelength was partially obscured.

Absolute Configuration of Cacospongionolides

The relative configuration of the first cacospongionolide isolated (**8**)[13] has been established by X-ray analysis of its major monacetate (**9**).[14] The CD spectrum of **8** shows a positive curve (Figure 1, Table 1) that, if compared to the spectra of (+)-**4** and (–)-**4**, establishes a 4*R* absolute configuration and the whole absolute configuration as depicted in **8**. The CD spectrum of cacospongionolide B (**2**) behaves similarly, thus establishing the 4*R* stereochemistry also for **2**.

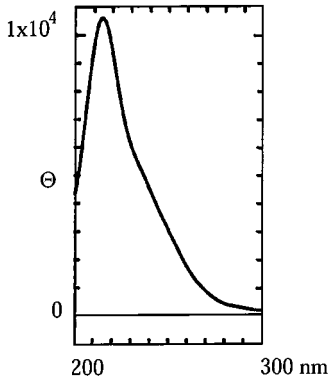
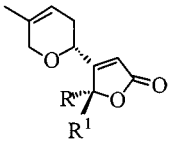
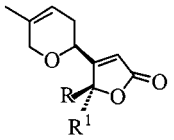


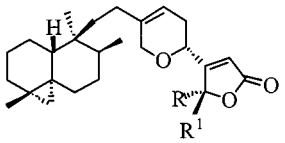
Figure 1. CD spectrum of cacospongionolide (**8**).



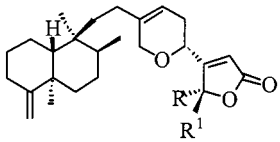
(+)-**4**: R, R^1=H, OH
(+)-**14**: R= H; R^1= OAc
(+)-**15**: R= OAc; R^1= H



(-)-**4**: R, R^1= H, OH
(-)-**14**: R= H; R^1= OAc
(-)-**15**: R= OAc; R^1= H;



8: R and R^1= H, OH
9: R=H; R^1= OAc
10: R=OAc; R^1=H



2: R, R^1= H, OH
11: R=R^1= H
12: R=H; R^1= OAc
13: R=OAc; R^1=H

Table 2. Selected ^{1}H-NMR data ($CDCl_3$) of acetates

Compound	δ_{H-2}	δ_{H-25}
9	6.16	6.94
10	6.09	7.04
12	6.16	6.94
13	6.09	7.04
(+)-14	6.16	6.95
(+)-15	6.09	7.05
(–)-14	6.16	6.95
(–)-15	6.09	7.05
17	6.20	6.97
18	6.12	7.06
(+)-19	6.21	6.97
(+)-20	6.13	7.06
(–)-19	6.20	6.97
(–)-20	6.13	7.06
23	6.20	6.97
24	6.10	7.11
22	6.08	7.05
27	6.13	6.94
28	6.06	6.99
29	6.14	6.92
30	6.03	7.02

Furthermore, in order to eliminate the influence in the CD spectrum of the butenolide ring γ-carbon, **2** was reduced with $NaBH_4$[15] to afford 25-deoxycacospongionolide B (**11**), whose relative stereochemistry was established by X-ray analysis.[16] The CD spectrum of **11** shows a single positive curve in the π-π* zone (Table 1).

Acetylation of both **8** and **2** affords major (**9** and **12**) and minor (**10** and **13**) acetates. The relative stereochemistry of these compounds can be inferred from NMR data when compared with the data of **9**, whose relative configuration has been established by X-ray analysis, as mentioned above. Of particular diagnostic importance are the chemical shift values of the C-2 and C-25 protons (Table 2); the latter resonates below δ = 7 in the major acetates, in which the C-4/C-25 relative configuration is *anti*, and above δ = 7 in the minor acetates, in which the C-4/C-25 relative configuration is *syn*.

Acetylation of (+)-**4** and (–)-**4** afforded the four acetates (+)-**14**, (+)-**15** and (–)-**14**, (–)-**15**, and in each couple the predominant major acetates are the analogues (+)-**14** and (–)-**14**, which have the same *anti* relative stereochemistry at C-4 and C-25 as in **9** (comparison of the ^{1}H-NMR spectra, Table 2). The CD spectra of the acetates show a behaviour that is dependent on the stereochemistry at C-25 (Table 1). The major acetates of compounds **9**, **12** and (+)-**14** show CD spectra with a single positive curve, while the C-25 epimers **10**, **13** and (+)-**15** show a small negative curve at longer wavelength and a positive one in the zone near 200 nm [e.g. (+)-**14** and (+)-**15**, Figure 2].

The spectra of (–)-**14** and (–)-**15** show CD curves opposite to those of (+)-**14** and (+)-**15**, as expected (Figure 2). Thus, the CD spectra of the above compounds allow the absolute configuration both at C-4 and C-25 to be established simultaneously.

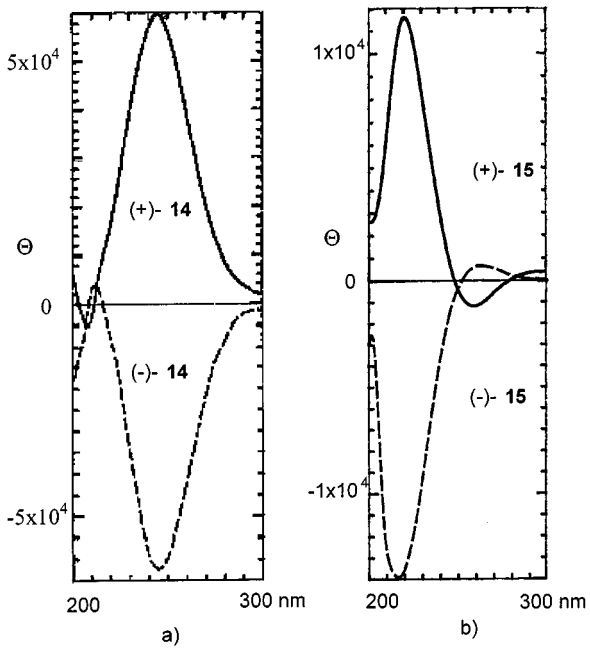
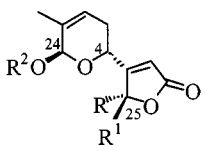


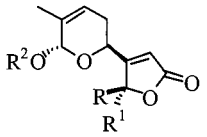
Figure 2. CD spectra of diacetates (+)-**14** and (+)-**15** and of their enantiomers (–)-**14** and (–)-**15**.

Absolute Configuration of Manoalide and Related Naturally Occurring Monoacetates

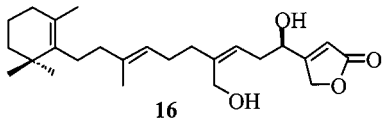
The absolute configuration at C-4 in manoalide (**1**) was established by Amoo et al.[17] to be *R* by reduction to manoalide diol (**16**) and comparison of the CD spectrum of the latter with that of the synthetic (*S*)-enantiomer prepared from 2-deoxy-D-ribose. The same result was later obtained by the Horeau method and the modified Mosher method.[18]



(+)-**3**: R, R^1=H, OH; R^2=H
(+)-**19**: R= H; R^1= OAc; R^2= Ac
(+)-**20**: R= OAc; R^1= H; R^2= Ac



(-)-**3**: R, R^1= H, OH; R^2= H
(-)-**19**: R= H; R^1= OAc; R^2= Ac
(-)-**20**: R= OAc; R^1= H; R^2= Ac
23: R= R^2= H; R^1= OAc
24: R= OAc; R^1=R^2= H



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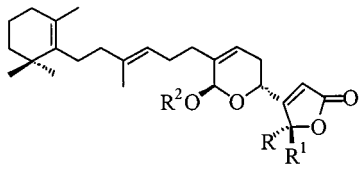
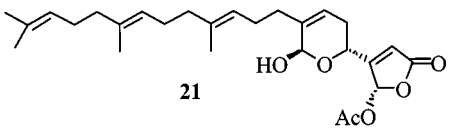


1: R, R^1= H, OH; R^2= H
17: R= H; R^1= OAc; R^2= Ac
18: R= OAc; R^1= H; R^2= Ac
22: R= OAc; R^1= R^2= H



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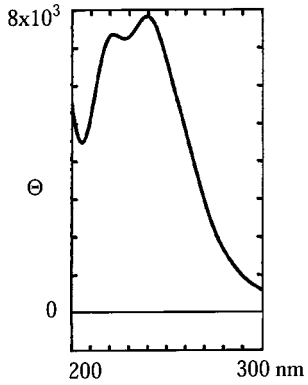


Figure 3. CD spectrum of manoalide (**1**).

The CD spectrum of manoalide (Figure 3) shows a positive curve similar to that of the 4*R* analogue (+)-**3** (Table 1), confirming the finding that the sign of the curve in this type of compound is determined by the stereochemistry at C-4. Moreover, acetylation of manoalide affords a major, less polar compound (**17**) and a minor acetate (**18**),[19] which were correlated to the acetates of cacospongionolides by NMR data. As for the cacospongionolides, the C-25 chemical shift value (Table 2) establishes the C-4/C-25 relative stereochemistry. The CD spectra of the two acetates (Figure 4) resemble those of the acetates of cacospongionolides and are identical to those of the two acetates prepared from the 4*R* analogue [(+)-**19** and (+)-**20**] and opposite to those arising from the 4*S* analogue [(–)-**19** and (–)-**20**] (Table 1).

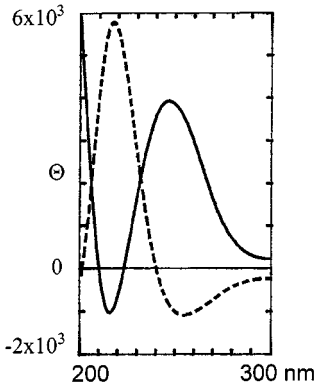


Figure 4. CD spectra of manoalide diacetates **17** (continuous line) and **18** (dotted line).

At this point a comment about the stereochemistry at C-24 in manoalide and related compounds is warranted. There is evidence that this hemiacetal carbon occurs as a single epimer in all compounds containing this moiety. In fact, in 25-deoxy derivatives, such as luffariolide D,[20] or in 25-acetylated derivatives, such as manoalide monoacetate[21] and thorectolide monoacetate,[21,15] the ^{13}C-NMR spectra show a single resonance for C-24. Moreover, manoalide[2] or manoalide-related compounds, such as luffariellin A,[22] yield on monoacetylation or diacetylation only two diastereomeric acetates, which should be epimeric at C-25 and therefore consist of a single epimer at C-24. We argue that in all these compounds the 25-OH group should be in a quasi-axial orientation because of the well-known anomeric effect.[23,24] On the other hand, the quasi-axial nature of the C-4 proton was deduced from the coupling constants with the C-5 protons, thus establishing the *anti* relative stereochemistry between the C-4 and C-24 substituents. Evidence confirming this view, based on nOe experiments, has been reported for thorectolide monoacetate.[15]

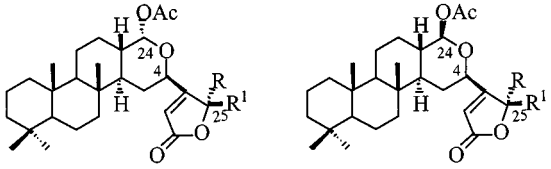
Thorectolide monoacetate (**21**) has been isolated from two different sponge species,[21,15] one of which also yielded manoalide monoacetate (**22**).[21] The latter compound, upon acetylation, affords a diacetate that we identify from the reported NMR data as the minor manoalide diacetate **18**, thus establishing the relative stereochemistry in **22**. In the original paper[21] the absolute configuration at C-4 and C-25 was not established. However, since **22** co-occurs with **21**, whose absolute configuration at C-4 was later established to be *R*,[15] it is reasonable to assume that manoalide monoacetate also has the same absolute configuration at C-4 and therefore **22** represents its absolute configuration.

The absolute configuration at C-4 and C-24 for thorectolide monoacetate (**21**) had been established, while the configuration at C-25 remained to be clarified.[15] The reported NMR data[21,15] establish that **21** has the same relative configuration at C-4/C-25 as **22** (Table 2) and that, accordingly, **21** represents the absolute configuration. Confirmation

comes from the CD spectrum of thorectolide monoacetate, which is very similar to that of the minor manoalide diacetate **18** (Table 1). On the other hand, the question as to whether C-24 is acetylated or not has no influence on the CD spectrum since monoacetates prepared from the analogue (–)-**3** (**23** and **24**) show the same CD spectra as those of the corresponding diacetates (–)-**19** and (–)-**20**, respectively (Table 1).

Absolute Configuration of Petrosaspongiolide M and Cavernosolide

Petrosaspongiolide M (**25**)[25] and cavernosolide (**26**)[26] are tetracyclic sesterterpenes related to manoalide for which only the relative stereochemistry on the tetracyclic nucleus has been established by NMR data.



25: R, R^1= H, OH
27: R= OAc; R^1= H
28: R= H; R^1= OAc

26: R, R^1= H, OH
29: R= OAc; R^1= H
30: R= H; R^1= OAc

Both compounds display a *trans*-fused tetrahydropyran ring bearing acetal functions at C-24 that have opposite stereochemistry and are blocked as acetates. The CD spectra of **25** and **26** are very similar (Table 1), suggesting the same absolute configuration. However, these CD spectra are different from that of manoalide in that they show a positive curve at longer wavelength while the curve is negative at the shorter one. This behaviour is reminiscent of the major diacetate of manoalide and of its methyl analogue (Table 1). On these grounds it is reasonable to conclude that both compounds have a C-4 *R* absolute configuration. Furthermore, acetylation of **25** and **26** affords diacetates **27**, **28** and **29**, **30**, respectively, whose CD spectra are very similar to the corresponding diacetates of manoalide and its analogues (Table 1), thus establishing their absolute configuration as depicted in **27**–**30**.

Conclusion

The present studies establish that the absolute configuration at C-4 in manoalide- and cacospongionolide-type compounds can be simply determined from the sign of the CD spectrum of the intact molecules. The absolute configuration at the butenolide ring γ-carbon in the derived acetates can also be determined from the CD spectra. In the latter case, the relative configuration between the stereogenic centres on the pyran and butenolide rings can be inferred from the chemical shift value of the butenolide ring γ-proton. Finally, it is interesting to note that the absolute configuration at C-4 is *R* in all the natural compounds so far examined.

Experimental Section

General Remarks: All reactions involving air-sensitive materials were performed using oven-dried glassware under an atmosphere of dry nitrogen. Anhydrous Et_2O, toluene, CH_2Cl_2 and pentane were freshly distilled from CaH_2; THF was distilled from $LiAlH_4$ and then from sodium and benzophenone. iPr_2NH was distilled from CaH_2, MeOH from KOH. NMR spectra were recorded on a Bruker DRX 400 spectrometer (400.135 MHz for ^1H and 100.03 MHz for ^{13}C) and a Bruker AM 250 spectrometer (250.13 MHz for ^1H and 62.89 MHz for ^{13}C). Chemical shifts are given in ppm on the (δ) scale; for the spectra in $CDCl_3$, the $CHCl_3$ signal was used as the internal standard (δ = 7.26 ^{1} H, δ = 77.0 ^{13}C). *J* values are given in Hz. Column chromatographic separations were carried out using Silica gel 60 (70–230 mesh and 230–400 mesh, Merck). Optical rotations were measured at the sodium D line (589 nm) at room temperature with a JASCO DIP 1000 polarimeter. HPLC separations were carried out with a Waters apparatus equipped with a differential refractometer. The UV and CD spectra were obtained with a JASCO model J 700 spectropolarimeter. The spectra were recorded for 10^{-4} M EtOH solutions in air, in the 200–500 nm range. All the compounds showed UV absorptions for the π-π* transition near or below 200 nm and thus were not recorded because of the cut-off of the solvent.

Manoalide was purified from a natural extract received from Professor D. J. Faulkner, La Jolla, by HPLC on a ODS-Z column (CH_3CN). Petrosaspongiolide M and Thorectolide monoacetate were received from Prof. L. Gomez-Paloma (Salerno) and Dr. M. L. Bourguet-Kondracki, respectively, and used without further purification.

Methyl 5-[Furan-3-yl]-5-hydroxy-2-methyl-3-oxopentanoate (6): A mixture of (5*S*)-**5** (1.42 g, 6.68 mmol, 91% e.e.), dry THF (6 mL), solid $LiOH·H_2O$ (0.28 g, 6.68 mmol) and CH_3I (10 mmol) was stirred at 40 °C until the starting material had disappeared (ca. 1 h). After dilution with Et_2O (100 mL) the organic phase was washed with 0.1 N HCl (2 × 5 mL) and brine (4 × 5 mL). After removal of the solvent the crude product was purified by silica gel column chromatography. Elution with $CHCl_3$/Et_2O mixtures afforded pure **6** as a mixture of diastereomers (very dense oil, 70% yield). ^1H NMR (250 MHz, $CDCl_3$): δ = 7.38 (s, 1 H), 7.37 (s, 1 H), 6.37 (s, 1 H), 5.14 (m, 1 H), 3.72 (s, 3 H), 3.56 (dq, 1 H, *J* = 7.0 Hz), 2.96 (m, 2 H), 1.34 (d, 3 H, *J* = 7.1 Hz). – $C_{11}H_{14}O_5$ (226.08): calcd. C 58.40, H 6.24; found C 58.33, H 6.34.

(6*S*)-6-[(Furan-3-yl)-3-methyl-5,6-dihydropyran-2-one [(6*S*)-7]: A solution of **6** (1.8 g, 8.0 mmol) in dry Et_2O (116 mL) was added under N_2 to a solution of $Zn(BH_4)_2$ in dry Et_2O (166 mL; 0.2 M) at 0 °C. Stirring was continued for 1 h, then water (50 mL) was added and, after 0.5 h, the mixture was carefully acidified with 0.1 N HCl. After the usual workup, 1.8 g of crude (5-furan-3-yl)-3,5-dihydroxy-2-methylpentanoic acid methyl ester was obtained as a complex diastereoisomeric mixture. The latter mixture, dissolved in EtOH (32 mL), was submitted to base-catalyzed hydrolysis by treatment with 1 N NaOH (8.6 mL) for 1 h at room temperature. After acidification with 6 N HCl, the usual workup afforded crude (5-furan-3-yl)-3,5-dihydroxy-2-methylpentanoic acid (1.6 g) as a diastereomeric mixture. This mixture was directly submitted to treatment with acetic anhydride (3.8 mL, 40 mmol) in dry pyridine

(6.4 mL, 80 mmol) for 1 h at room temperature to give crude (6*S*)-(furan-3-yl)-4-acetoxy-3-methyltetrahydropyran-2-one. Compound (6*S*)-**7** was finally obtained by base-catalyzed elimination involving reaction of the above acetyl-lactone dissolved in $CHCl_3$ (3 mL) with DBU until the starting material had disappeared. Usual workup and silica gel column chromatography ($CHCl_3$) afforded (6*S*)-**7** (oil, 516 mg, 2.9 mmol) [36% yield based on (5*S*)-**5**]. $[\alpha]_D^{25}$ = –71.0 (*c* = 0.2, $CHCl_3$, 92% e.e.). – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.43 (s, 1 H), 7.37 (s, 1 H), 6.58 (m, 1 H), 6.40 (s, 1 H), 5.36 (dd, 1 H, J_1 = 4.5 Hz, J_2 = 11.2 Hz), 2.6–2.3 (m, 2 H), 1.92 (s, 3 H). – ^{13}C NMR (400 MHz, $CDCl_3$): δ = 165.3, 143.6, 139.8, 138.5, 128.7, 123.4, 108.5, 72.4, 30.4, 16.9. – $C_{10}H_{10}O_3$ (178.06): calcd. C 67.41, H 5.66; found C 67.46, H 5.57.

(6*R*)-6-[(Furan-3-yl)-3-methyl-5,6-dihydropyran-2-one [(6*R*)-7]: The same sequence afforded (6*R*)-**7** by the employment of (5*R*)-**5** as starting material. $[\alpha]_D^{25}$ = +70.2 (*c* = 1.8, $CHCl_3$, 90% e.e.). ^{1}H-NMR and ^{13}C-NMR data as reported above.

(4*S*)-Manoalide Methyl Analogue [(–)-3]: The enantiomerically enriched target compound (–)-**3** was prepared from (6*S*)-**7** according to the synthetic sequence reported in ref.[12] (–)-**3** $[\alpha]_D^{25}$ = –122.9 (*c* = 1.7, MeOH). – ^{1}H NMR (400 MHz, MeOH): δ = 6.13 (br. s, 1 H), 6.04 (s, 1 H), 5.67 (br. s, 1 H), 5.16 (br. s, 1 H), 4.83 (ovlp. with water in MeOH, 1 H), 2.25 (bm, 2 H), 1.75 (s, 3 H). – ^{13}C NMR (400 MHz, $CDCl_3$): δ = 173.0, 171.3, 135.5, 122.2, 117.2, 99.4, 93.3, 64.5, 30.4, 19.3. – $C_{10}H_{12}O_5$ (212.07): calcd. C 56.60, H 5.70; found C 56.68, H 5.64.

(4*R*)-Manoalide Methyl Analogue [(+)-3]: $[\alpha]_D^{25}$ = +106.4 (*c* = 1.1, MeOH). – ^{1}H-NMR and ^{13}C-NMR data as reported above.

(4*S*)-Cacospongionolide Methyl Analogue [(–)-4]: The enantiomerically enriched target compound (–)-**4** was prepared from (6*S*)-**7** according to the synthetic sequence reported in ref.[12] (–)-**4** $[\alpha]_D^{25}$ = –126.8 (*c* = 0.6, $CHCl_3$). – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.15 (s, 1 H), 6.04 (s, 1 H), 5.54 (br. s, 1 H), 4.42 (bt, 1 H, *J* = 7.0 Hz), 4.12 (ABq, 2 H, *J* = 14.1 Hz), 2.27 (bm, 2 H), 1.63 (s, 1 H). – ^{13}C NMR (400 MHz, $CDCl_3$): δ = 171.5, 169.2, 134.7, 119.2, 118.6, 99.2, 70.8, 70.6, 30.6, 20.0. – $C_{10}H_{12}O_4$ (196.07): calcd. C 61.22, H 6.16; found C 61.33, H 6.11.

(4*R*)-Cacospongionolide Methyl Analogue [(+)-4]: $[\alpha]_D^{25}$ = +126.6 (*c* = 0.6, $CHCl_3$). – ^{1}H-NMR and ^{13}C-NMR data as reported above.

Acetates of Cacospongionolide Methyl Analogue (+)-14, (+)-15, (–)-14, (–)-15: The acetates (+)-**14**, (+)-**15**, and (–)-**14**, (–)-**15** were prepared from (+)-**4** and (–)-**4**, respectively, by standard acetylation with Ac_2O/Py. After the usual workup, the diastereomeric mixtures were separated by HPLC on a Spherisorb S5 W analytical column using *n*-hexane/ethyl acetate (8:2) as the eluent.

(+)-**14**, $[\alpha]_D^{25}$ = +127.4 (*c* = 0.3, $CHCl_3$). – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.95 (s, 1 H), 6.16 (d, 1 H, *J* = 1.5), 5.53 (m, 1 H), 4.34 (bd, 1 H, *J* = 8.8 Hz), 4.13 (m, 2 H), 2.21 (m, 2 H), 2.16 (s, 3 H), 1.56 (s, 3 H). – ^{13}C NMR (400 MHz, $CDCl_3$): δ = 168.8, 166.2, 160.5, 133.5, 118.0, 116.8, 92.3, 69.3, 69.0, 29.5, 20.6, 18.4. – EIMS, *m/z:* 196 (M^+ – 42 m.u.).

(+)-**15**, $[\alpha]_D^{25}$ = +78.0 (*c* = 0.1, $CHCl_3$). – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.05 (s, 1 H), 6.09 (s, 1 H), 5.53 (m, 1 H), 4.31 (m, 1 H), 4.10 (m, 2 H), 2.28 (m, 2 H), 2.17 (s, 3 H), 1.52 (s, 3 H). – ^{13}C NMR (400 MHz, $CDCl_3$): δ = 169.4, 166.1, 160.4, 133.7, 118.9, 116.7, 92.8, 69.1, 69.4, 28.7, 20.6, 18.5. – EIMS, *m/z:* 196 (M^+ – 42 m.u.).

(–)-**14**, $[\alpha]_D^{25}$ = – 130.2 (c = 0.2, $CHCl_3$) ^{1}H- and ^{13}C-NMR spectra identical to (+)-**14**.

(–)-**15**, $[\alpha]_D^{25}$ = –64.0 (*c* = 0.1, $CHCl_3$). ^{1}H- and ^{13}C-NMR spectra identical to (+)-**15**.

Diacetates of Manoalide Methyl Analogue (+)-19, (+)-20, (–)-19, (–)-20: The acetates (+)-**19**, (+)-**20**, (–)-**19** and (–)-**20** were prepared from (+)-**3** and (–)-**3**, respectively, as described above.

(+)-**19**, $[\alpha]_D^{25}$ = +128.6 (*c* = 0.6, $CHCl_3$). – ^{1}H NMR (400 MHz, $CHCl_3$): δ = 6.97 (s, 1 H), 6.27 (s, 1 H), 6.21 (d, 1 H, *J* = 5.7 Hz), 5.85 (br. s, 1 H), 4.68 (dd, 1 H, *J* = 11.5, 2.5 Hz), 2.28 (m, 2 H), 2.17 (s, 3 H), 2.13 (s, 3 H), 1.70 (s, 3 H). – ^{13}C NMR (400 MHz, $CDCl_3$): δ = 179.9, 169.0, 168.8, 131.3, 122.6, 118.9, 92.2, 90.2, 64.8, 28.7, 21.1, 20.6, 18.2. – EIMS, *m/z:* 236 (M^+ – AcOH).

(+)-**20**, $[\alpha]_D^{25}$ = +33.5 (*c* = 0.3, $CHCl_3$). – ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.06 (s, 1 H), 6.19 (s, 1 H), 6.13 (s, 1 H), 5.82 (br. s, 1 H), 4.67 (dd, 1 H, *J* = 10.7, 4.1 Hz), 2.33 (m, 2 H), 2.15 (s, 3 H), 2.09 (s, 3 H), 1.54 (s, 3 H). – ^{13}C NMR (400 MHz, $CDCl_3$): δ = 169.7, 169.0, 168.3, 164.5, 131.3, 122.6, 119.3, 92.7, 90.6, 64.2, 28.6, 21.0, 20.4, 18.2. – EIMS, *m/z:* 236 (M^+ – AcOH).

(–)-**19**, $[\alpha]_D^{25}$ = –130.4 (*c* = 0.5, $CHCl_3$). ^{1}H- and ^{13}C-NMR spectra identical to (+)-**19**.

(–)-**20**, $[\alpha]_D^{25}$ = –37.2 (*c* = 0.2, $CHCl_3$). ^{1}H- and ^{13}C-NMR spectra identical to (+)-**20**.

Monoacetates of (4*S*)-Manoalide Methyl Analogue 23 and 24: A solution of (–)-**3** (12 mg) in pyridine (0.1 mL) was treated with Ac_2O (3 μL) and the mixture was stirred at room temperature for 30 min. The mixture was poured into H_2O, extracted with AcOEt, and the crude product was purified by SiO_2 column chromatography (Et_2O/petroleum ether, 7:3) to give **23** (9 mg) and **24** (2.7 mg).

23: $[\alpha]_D^{25}$ = –111.3 (*c* = 0.6, $CHCl_3$). – ^{1}H NMR (250 MHz, $CDCl_3$): δ = 6.97 (s, 1 H), 6.20 (d, 1 H, *J* = 1.5 Hz), 5.68 (bd, 1 H, *J* = 3.5 Hz), 5.28 (s, 1 H), 4.85 (ddd, 1 H, *J* = 7.0, 2.7, 0.9 Hz), 3.06 (m, 1 H), 2.18 (m, 2 H), 2.16 (s, 3 H), 1.79 (s, 3 H). – ^{13}C NMR (250 MHz, $CDCl_3$): δ = 170.0, 168.9, 166.1, 133.7, 121.0, 118.0, 92.2, 92.1, 62.9, 29.7, 20.7, 18.9 . – EIMS, *m/z:* 236 (M^+ – H_2O).

24: $[\alpha]_D^{25}$ = –24.5 (*c* = 0.2, $CHCl_3$). – ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.11 (s, 1 H), 6.10 (d, 1 H, *J* = 0.8 Hz), 5.70 (br. s, 1 H), 5.23 (d, 1 H, *J* = 3.7 Hz), 4.75 (ddd, 1 H, *J* = 7.0, 2.7, 1.0), 2.85 (d, 1 H, *J* = 4.4), 2.27 (m, 2 H), 1.78 (d, 3 H, *J* = 0.9). – ^{13}C NMR (250 MHz, $CDCl_3$): δ = 168.9, 168.1, 165.5, 133.6, 121.1, 118.9, 92.8, 92.3, 61.8, 28.4, 20.7, 18.9. – EIMS, *m/z:* 236 (M^+ – H_2O).

Diacetates 9, 10, 12, 13, 27, 28, 29, 30: The diacetates of cacospongionolide (**9**, **10**),[9] cacospongionolide B (**12**, **13**),[3] petrosaspongiolide M (**27**, **28**),[25] and cavernosolide (**29**, **30**),[26] were prepared as previously described.

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