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STRUCTURAL AND STEREOCHEMICAL STUDIES ON MARINE NORTERPENE CYCLIC PEROXIDES, PART 2[1]

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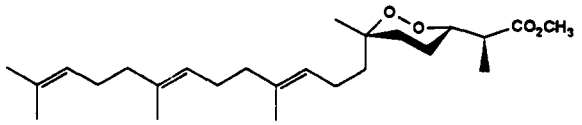
ABSTRACT.—Two new norsesterterpene cyclic peroxides, **4** and **5**, isomeric with *enantio*-sigmosceptrellin A [**3**], have been isolated as their methyl esters **7** and **8** from *Mycale ancorina*. The major isomer **4** was identified as the endocyclic double bond isomer of *enantio*-sigmosceptrellin A [**3**] by detailed spectroscopic analysis and correlation with co-occurring **3**. The minor component **5** represents the first reported example of a norsesterterpene cyclized to a labdane-type bicyclic unit.

Reports of sesterterpenes as natural products (1) have become more numerous over recent years, particularly since the emergence of marine natural products chemistry. Sesterterpenes isolated from marine organisms (1) are invariably modified by the loss or addition of one or more carbon units. During our investigations into antimicrobially active secondary metabolites from marine sponges, we have identified a number of norsesterterpene cyclic peroxides (2,3). These have included compounds possessing acyclic [**1**], monocyclic [**2**], and bicyclic [**3**] carbon skeletons. In this report we describe the isolation and structural elucidation of two isomers of *enantio*-sigmosceptrellin A [**3**], one of which is the first representative of a biosynthetically related labdane-type norsesterterpene.

RESULTS AND DISCUSSION

Exhaustive extraction of a specimen of *Mycale ancorina* Whitelegge (Poes-



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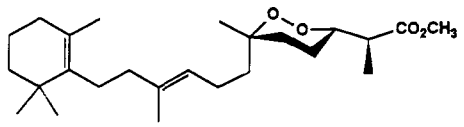

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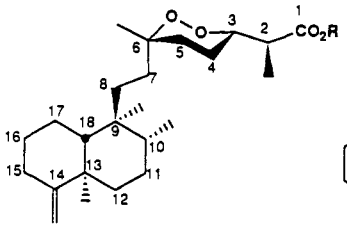
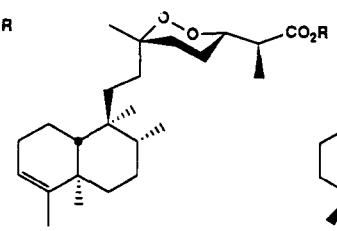
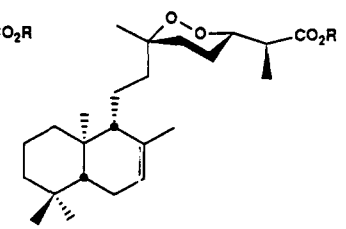


3 R=H
6 R=CH_3



4 R=H
7 R=CH_3



5 R=H
8 R=CH_3

[1]For Part 1, see Capon and MacLeod (2).

ciloscleridа, Mycalidae, Subgenus *Aegogropila*) followed by methylation with CH_2N_2 and chromatography on $AgNO_3$ impregnated silica yielded **6**, the methyl ester of *enantio*-sigmosceptrellin A [**3**], together with a mixture of two isomers, **7** and **8**. Careful hplc of this mixture permitted resolution of the major isomer **7** from the minor isomer **8**. Comparison of the ^{1}H- and ^{13}C-nmr spectral data for the methylated extract with that of pure **6**, **7**, and **8** confirmed that these compounds were present in the original extract and that they were not artifacts arising during purification on $AgNO_3$ impregnated silica.

A comparison of selected signals in the ^{1}H- and ^{13}C-nmr spectra of **7** with those of **6** (Table 1) confirmed that **7** possessed the same cyclic peroxide moiety as **6**. Further-

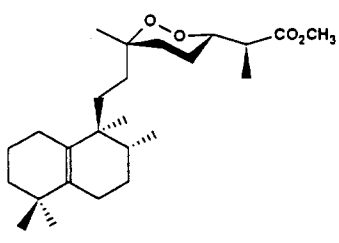
TABLE 1. Selected ^{13}C- and ^{1}H-nmr ($CDCl_3$) Shifts for Marine Norterpene Peroxides

Carbon No.	Compounds			
	6[a]	**7**	**8**	**9**
1	174.1	174.3	174.4	174.3
2	42.5	42.6	42.7	42.5
3	81.0	81.1	81.2	80.9
4	22.5	22.6	22.7	22.4
5	32.4	32.3	32.2	32.1
6	80.1	80.2	80.3	80,3
O-CH_3	51.8	51.8	51.9	51.9
2-CH_3	12.4	12.5	12.7	12.4
6-CH_3	24.0	24.0	23.9	24.0
2-H	2.56	2.55	2.57	2.60
3-H	4.23	4.23	4.24	4.24
O-CH_3	3.69	3.69	3.69	3.69
2-CH_3	1.13	1.13	1.13	1.14
6-CH_3	1.06	1.08	1.12	1.09

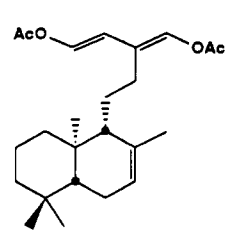
[a]Capon and MacLeod (2).

more, the observation of ^{1}H- and ^{13}C-nmr resonances in **7** consistent with a trisubstituted double bond (δ 5.18, 1H, bs; 1.60, 3H, s: ppm 144.6, s; 120.2, d), rather than the disubstituted exocyclic double bond seen in **6**, suggested that **7** was the endocyclic double bond isomer of *enantio*-sigmosceptrellin A methyl ester [**6**], as shown. This was confirmed by acid catalyzed isomerization of **6** to give a product identical in all respects, including optical activity, to **7**, together with the rearranged tetrasubstituted double bond isomer **9**.

The remaining minor component **8** clearly possessed the same cyclic peroxide moiety as **6** and **7** (Table 1). The bicyclic portion was, however, of the labdane rather than clerodane-type as evidenced by both ^{1}H- and ^{13}C-nmr comparisons (4) with the labdane **10** (Table 2). This correlation clearly revealed that **8** possessed a *trans*-labdane type



9



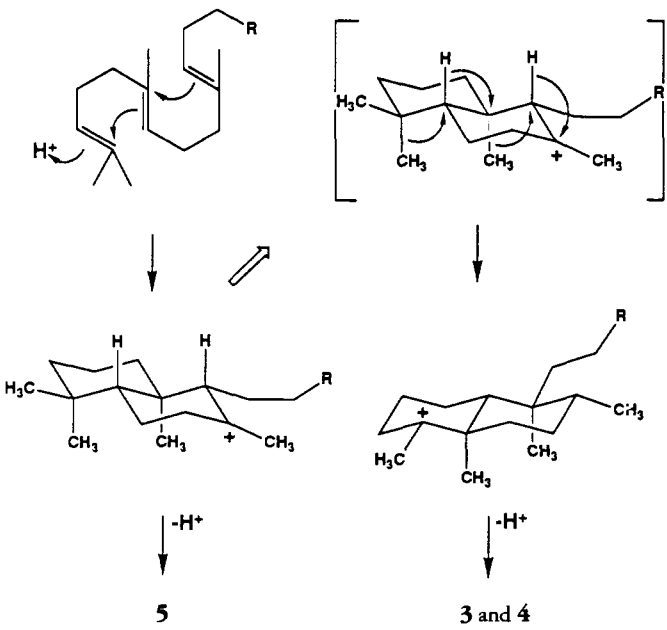
10

TABLE 2. Selected ^{13}C- and ^{1}H-nmr ($CDCl_3$) Shifts for **10**[a,b] and **8**

Carbon No.	Compounds		Proton No.	Compounds	
	10	**8**		**10**	**8**
9	55.2	55.3	11-H	5.42	5.39
10	135.1	135.5			
11	122.6	122.0	10-CH_3	1.81	1.72
12	24.0	23.8			
13	50.4	50.2	14-CH_3	0.87	0.86
14	33.1	33.0			
15	42.5	42.3	14-CH_3	0.87	0.88
16	19.0	18.8			
17	39.3	39.2	18-CH_3	0.73	0.78
18	37.0	37.0			
10-CH_3	22.0	21.8			
14-CH_3	33.2	33.2			
14-CH_3	21.9	22.0			
18-CH_3	13.6	13.6			

[a]Numbering as for *enantio*-sigmosceptrellin A [**3**].
[b]Capon *et al.* (4).

bicyclic unit with the relative stereochemistry as shown. It has been demonstrated (5) that for a large number of labdane diterpenes in which the asymmetric ring system is separated by two methylene groups from an asymmetric center in the side chain, the optical rotation of the two portions are approximately additive if no interactions occur between these two centers. In this way, the contribution to the molecular rotation of **8** due to the cyclic peroxide moiety C1 to C7 can be assessed to be either $-244°$ (2*S*,3*S*,6*S*) or $+244°$ (2*R*,3*R*,6*R*) based on measured values (2) for **1** and **2** in which this is the only chiral unit. Similarly, the contribution due to the bicyclic unit in **8** can be expected to be between either $+17°$ and $+90°$ [five examples in the labdane series (6,7)] or $-31°$ and $-84°$ [four examples in the *ent*-labdane series (4,8,9)]. As the ob-



SCHEME 1.

served [M]D for **8** is −361°, this would support a 2*S*,3*S*,6*S*,9*R*,13*R*,18*S* stereochemistry (numbering as for *enantio*-sigmosceptrellin A, **3**). Such an absolute stereochemistry is consistent with a common biosynthetic sequence leading to the bicyclic ring systems in the marine natural products **3**, **4**, and **5** (Scheme 1).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H-nmr (200 MHz) and ^{13}C-nmr (50 MHz) spectra were recorded on either a JEOL JNM-FX-200 or a VARIAN XL-200-E spectrometer. Electron impact mass spectra were obtained on a V.G. Micromass 7070F instrument at 70eV, with chemical ionization mass spectra being recorded on the same instrument using NH_3 as the reagent gas. High resolution accurate mass measurements were determined under electron impact conditions on an AEI MS 902 mass spectrometer. Optical rotations were recorded on a Perkin-Elmer 121 polarimeter.

COLLECTION, EXTRACTION AND ISOLATION.—Sponge specimens were collected by hand (SCUBA) at a depth of 20 to 25 m. The fresh specimens were immersed in EtOH, packed in dry ice, transported to the laboratory, and stored at −20°. Type specimens are deposited at the Australian Museum, Sydney, under the registry number Z4969.

A specimen of *M. ancorina* (173 gm dry wt, Z4969) was exhaustively extracted with EtOH to yield a gum that was, in turn, triturated with CH_2Cl_2. After methylation with CH_2N_2, the lipid soluble material was subjected to rapid filtration through 5% $AgNO_3$ impregnated silica to yield the methyl ester of *enantio*-sigmosceptrellin A (**6**, 460 mg, 0.26% dry wt) and a two component mixture of isomers. The latter material was resolved by hplc (elution with 2.5% EtOAc/hexane through a 10 cm×0.8 cm 5μ silica radial compression column) into the endocyclic double bond isomer **7** (110 mg) and the labdane norsesterterpene **8** (30 mg).

CYCLIC PEROXIDE ESTER **7**.—A stable colorless oil (0.06% dry wt of Z4969): [α]D −106.7° (*c* 3.3, $CHCl_3$); ^{1}H nmr ($CDCl_3$) δ 0.73 (s, 3H), 0.80 (d, 3H, *J*=6.OHz), 1.00 (s, 3H), 1.08 (s, 3H), 1.14 (d, 3H, *J*=7.OHz), 1.60 (s, 3H), 2.55 (dq, 1H, *J*=7.0, 7.0Hz), 3.69 (s, 3H), 4.23 (bm, 1H), 5.18 (bs, 1H); ^{13}C nmr ($CDCl_3$) 12.5 (q), 15.8 (q), 18.0 (q), 18.3 (t), 18.5 (q), 19.9 (q), 22.6 (t), 24.0 (q), 26.9 (t), 27.2 (t), 27.4 (t), 31.3 (t), 32.3 (t), 36.2 (d), 36.8 (t), 38.1 (s), 38.4 (s), 42.6 (d), 46.4 (d), 51.8 (q), 80.2 (s), 81.1 (d), 120.2 (d), 144.6 (s), 174.2 ppm (s); eims *m/z* 406 (M^+, <1%), 388 (3), 375 (2), 373 (3), 357 (1), 191 (45), 189 (53), 95 (100); hrms 388.2977 (M^+-H_2O requires 388.2977, $C_{25}H_{40}O_3$).

CYCLIC PEROXIDE ESTER **8**.—A stable colorless oil (0.007% dry wt of Z4969): [α]D −91° (*c* 0.4, $CHCl_3$); ^{1}H nmr ($CDCl_3$) δ 0.78 (s. 3H) 0.86 (s, 3H), 0.88 (s, 3H), 1.12 (s, 3H), 1.13 (d, 3H, *J*=6.OHz), 1.72 (bs, 3H), 2.57 (dq, 1H, *J*=6.OHz), 3.69 (s, 3H), 4.24 (bm, 1H), 5.39 (bm, 1H); ^{13}C nmr ($CDCl_3$) 12.7 (q), 13.6 (q), 18.8 (t), 20.9 (t), 21.8 (q), 22.0 (q), 22.7 (t), 23.8 (t), 23.9 (q), 32.2 (t), 33.0 (s), 33.2 (q), 37.0 (s), 37.5 (t), 39.2 (t), 42.3 (t), 42.7 (d), 50.2 (d), 51.9 (q), 55.3 (d), 80.3 (s), 81.2 (d), 122.0 (d), 135.5 (s), 174.4 ppm (s); eims *m/z* 388 (M^+-H_2O, 1%), 373 (2), 357 (2), 318 (2), 303 (4), 251 (5), 204 (100), 109 (80); hrms 388.2977 (M^+-H_2O requires 388.2977, $C_{25}H_{40}O_3$).

ACID CATALYZED REARRANGEMENT OF **6**.—A sample of **6** (50 mg) was dissolved in a mixture of MeOH-HCl-HOAc (1:1:1, 3 ml) and stirred at 70° for 3 h, after which the solvent was evaporated under reduced pressure, and the reaction product was chromatographed on 5% $AgNO_3$ impregnated silica (stepwise elution hexane to EtOAc). Recovered in order of increasing polarity were the rearranged tetrasubstituted double bond isomer **9** (10 mg), an endocyclic trisubstituted double bond isomer identical in all respects to authentic **7** (35 mg) and unreacted starting material **6** (5 mg). A second reaction allowed to progress for 15 h resulted in almost quantitative conversion of **6** to the rearranged product **9**, a stable colorless oil: [α]D −17.6° (*c* 3.2, $CHCl_3$); ^{1}H nmr ($CDCl_3$) δ 0.83 (s, 3H), 0.84 (d, 3H, *J*=6.OHz), 0.96 (s, 3H), 0.98 (s, 3H), 1.09 (s, 3H), 1.14 (d, 3H, *J*=8.OHz), 2.60 (dq, 1H, *J*=6.0, 6.OHz), 3.69 (s, 3H), 4.24 (bm, 1H); ^{13}C nmr ($CDCl_3$) 12.4 (q), 16.0 (q), 20.0 (t), 21.4 (q), 22.4 (t), 24.0 (q), 25.2 (t), 25.8 (t), 27.2 (t), 27.7 (q), 28.8 (t), 29.2 (q), 29.7 (t), 32.1 (t), 33.5 (d), 34.5 (s), 40.0 (t), 40.2 (s), 42.5 (d), 51.9 (q), 80.3 (s), 80.9 (d), 132.4 (s), 136.9 (s), 174.3 ppm (s); eims *m/z* 191 (100%); cims (NH_3) 424 (5%, $[M+NH_4]^+$), 407 (2, $[M+H]^+$), 389 (21), 319 (95), 303 (77), 245 (100), 191 (30).

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