Two New Norsesterterpene Cyclic Peroxides from a Marine Sponge, Mycale (Carmia) cf. spongiosa

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TWO NEW NORSESTERTERPENE CYCLIC PEROXIDES FROM A MARINE SPONGE, *MYCALE (CARMIA)* CF. *SPONGIOSA*

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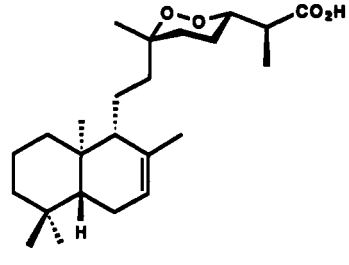
ABSTRACT.—Two new isomeric norsesterterpene cyclic peroxides **3** and **5** have been isolated from an Australian marine sponge, *Mycale (Carmia)* cf. *spongiosa*, and their structures have been determined by spectroscopic analysis and chemical degradation.

Norsesterterpene cyclic peroxides such as **1** (1) and **2** (2) represent a class of secondary metabolites unique to marine sponges. In recent years we have reported (1–3) a range of structural variants, including acyclic, monocyclic, and bicyclic examples. As a consequence, spectroscopic methods for assigning relative stereochemistries about the three asymmetric centers in the cyclic peroxide/acid termini were developed (2). This report describes two new norsesterterpene cyclic peroxides **3** and **5** incorporating a hitherto undescribed relative stereochemistry about C-2, C-3, and C-6. The numbering system applied to **3** and **5** is in accord with that previously used for related norsesterterpene cyclic peroxides (3).

RESULTS AND DISCUSSION

A specimen of sponge, *Mycale (Carmia)* cf. *spongiosa* (order Poecilosclerida, family Mycalidae, Dendy, 1986) collected by scuba at a depth of 20 m off Wasp Head, Durras, on the mid-south coast of New South Wales, was shown to possess an EtOH extract that significantly inhibited growth of the bacterium *Bacillus subtilis* and the yeast *Saccharomyces cerevisae*. Solvent partitioning of the concentrated crude extract resulted in localization of the antimicrobial activity in a CH_2Cl_2-soluble fraction. Rapid filtration of this material through a short column of silica resolved the inactive nonpolar from active polar components. The latter were in turn methylated with CH_2N_2 and subjected to hplc to return two isomeric methyl esters **4** and **6**.



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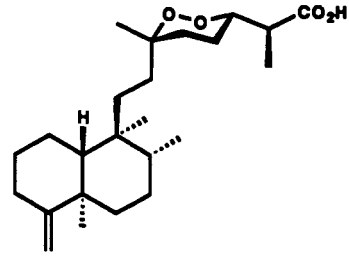

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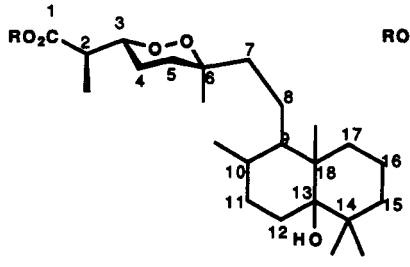
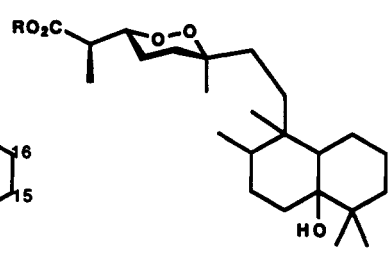


3 R=H
4 R=Me



5 R=H
6 R=Me

The major component **4** ($C_{25}H_{44}O_5$) displayed signals in its ^{1}H- and ^{13}C-nmr spectra consistent with the C-1–C-7 portion of the known norditerpene cyclic peroxide **7** (2) (Tables 1 and 2). Significantly, a methylene resonance at 38.2 ppm (C-7) and a methyl resonance at 20.5 ppm (6-Me) were indicative of an axial 6-Me. That the substituent on the cyclic peroxide ring at C-3 was equatorial was confirmed by interpretation of the C-3 oxymethine proton spin system (δ 4.22, ddd, $J = 4, 8, 8$ Hz). Unlike **7** for which the 2-Me ^{1}H-nmr resonance was at δ 1.23, in the case of **4** this signal appeared at δ 1.14. It has previously been established (2) that the chemical shift for the 2-Me resonance can be used to define the relative stereochemistry about C-2 and C-3 in systems of this type. Thus in cases where an erythro (2*R*, 3*R* or 2*S*, 3*S*) arrangement occurs the shift of this secondary methyl is δ 1.14, whereas in threo (2*R*, 3*S* or 2*S*, 3*R*) configurations these protons resonate at δ 1.24. This observation presumably indicates conformational preferences for rotamers about the C-2–C-3 bond. Consequently, the relative stereochemistry about C-2 and C-3 in **4** must be erythro. This arrangement in combination with that of an axial 6-Me represents a new relative stereochemistry about the cyclic peroxy/acid terminus (C-1–C-7).

TABLE 1. Selected ^{13}C Resonances for **4** and the Known Norditerpene **7**.

Carbon	Compound	
	4	**7**[a]
C-1	174.6	174.1
C-2	42.7	42.9
C-3	81.8	81.3
C-4	22.2	23.4
C-5	32.2	31.9
C-6	81.2	80.0
C-7	38.2	39.6
2-Me	12.9	13.5
6-Me	20.5	20.5
OMe	51.8	51.7

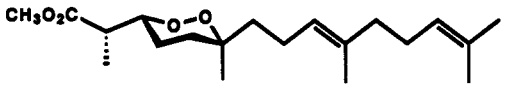
[a]Data for compound **7** are from Capon and MacLeod (2).

Having accounted for two degrees of unsaturation and in the absence of additional sp^2 carbon resonances, the unassigned portion of **4** was bicyclic, with the remaining oxygen present as a tertiary OH (s, 77.3 ppm). Also present were three tertiary methyls (δ 0.80, 0.82, 0.96) and a secondary methyl (0.80, d, $J = 8$ Hz). As none of the tertiary methyls was significantly deshielded, the tertiary OH could not be attached to a car-

TABLE 2. Selected ^{1}H-nmr Resonances for **4**, **6**, and the Known Norditerpene **7**.

Proton	Compound		
	4	**6**	**7**[a]
H-2	2.66 (dq, 8, 8 Hz)	2.56 (dq, 8, 8 Hz)	2.65 (dq, 7, 7 Hz)
H-3	4.22 (ddd, 4, 8, 8 Hz)	4.22 (bddd, 4, 8, 8 Hz)	4.12 (ddd, 4, 8, 8 Hz)
2-Me	1.14 (d, 8 Hz)	1.14 (d, 8 Hz)	1.23 (d, 8 Hz)
6-Me	1.26 (s)	1.29 (s)	1.30 (s)

[a]Data for compound **7** are from Capon and MacLeod (2).

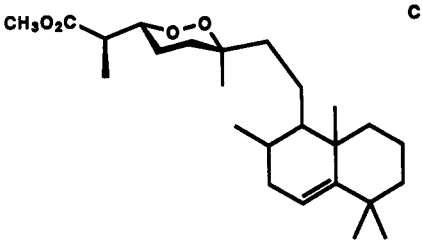


7

bon bearing a methyl. Dehydration of **4** with oxalic acid in C_6H_6 yielded under forcing conditions a single product **8** incorporating a trisubstituted double bond [146.5 (s), 114.3 (d) ppm] and an unaltered C-1 to C-7 unit. The ^{1}H-nmr spectrum of **8** also revealed three tertiary methyls (δ 0.77, 1.00, 1.04) and a secondary methyl (0.76, d, $J = 6$ Hz). These observations are consistent with **3**, **4**, and **8** incorporating the labdane-type bicyclic ring system as shown. Although a vast array of oxygenated labdane diterpenes have been reported from terrestrial sources, oxygenation at the bridgehead adjacent to the geminal dimethyl moiety (C-13 in the adopted norsesterterpene numbering and C-5 in conventional diterpene numbering) is unprecedented. Due to lack of material, the stereochemistry about C-9, C-10, C-13, and C-18 in the bicyclic unit of **3** and its derivatives remains undetermined.

Hydrogenation of **4** returned the triol ester **9** which was in turn subjected to a Horeau determination of absolute stereochemistry about the C-3 secondary hydroxyl (2). Because of the small scale on which this analysis was performed, together with the low optical yield (3%, $[\alpha]_D + 1.5$), the assignment of a 2*R*,3*R*,6*S* absolute stereochemistry is tentative.

The methyl ester **6** of the minor component **5** possessed ^{1}H-nmr resonances fully consistent with the peroxy/ester terminal (C-1–C-7) observed in **4** (Table 2). Thus, interpretation of the ^{1}H-nmr spin system for the C-3 peroxymethine proton (ddd, $J = 4$, 8, 8 Hz) and the chemical shift for the 2-Me (δ 1.14) confirmed a common C-2 and C-3 relative stereochemistry with **4**. A combination of instrumental limitations together with a small and slowly decomposing sample necessitated pursuing a degradative approach to solving the structure. Although we were unable to acquire a ^{13}C-nmr spec-



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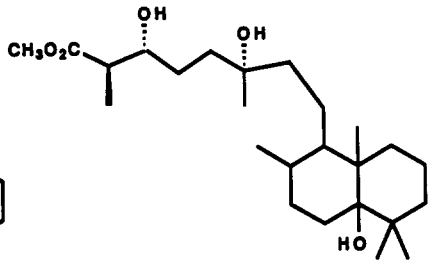

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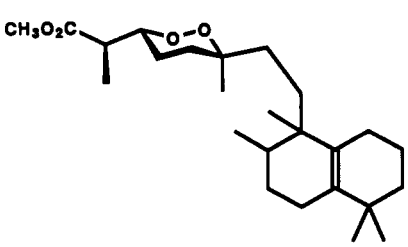

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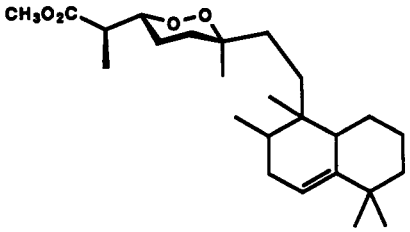

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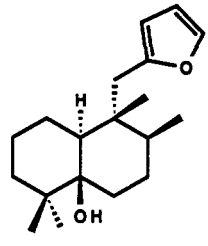
trum for **6**, it was possible to assign an axial 6-Me from the ^{1}H-nmr shift of the 6-Me resonance. Examination of the ^{1}H-nmr spectra for model compounds (1–3) revealed that axial 6-Me's in systems of this type resonate at ~ δ 1.30 while equatorial 6-Me's appear at < δ 1.13. The shift for the 6-Me in **6** (δ 1.29) is consistent with an axial methyl substituent. Insufficient material precluded an independent assessment of absolute stereochemistry about C-2, C-3, and C-6 in **6**. Compound **5** and its derivatives are arbitrarily represented with the same absolute stereochemistry about C-2, C-3, and C-6 as **3**.

As with **4** the remaining portion of **6** was bicyclic and incorporated a tertiary OH not attached to a carbon bearing any of three tertiary methyls (δ 0.75, 0.86, 0.98). Unlike **4**, dehydration of **6** with oxalic acid in C_6H_6 yielded two products in a ratio of 2:1, both of which contained the intact peroxy/ester termini (C-1–C-7). The major component **10** displayed no olefinic proton resonances and was attributed a tetrasubstituted olefinic structure. The minor component **11** contained a trisubstituted double bond (δ 5.43, m) bearing no olefinic methyls and differed from **8**.

Comparison of ^{1}H-nmr shifts for the methyl substituents to the bicyclic unit in **6**, **10**, and **11** with those reported for ambliol B [**12**] (4–6), its dehydration products **13** and **14** (4), and other related model compounds (3,7,8), suggested a common carbon framework. Although the ^{1}H-nmr correlation between **6**, **10**, and **11** with ambliol B [**12**] and its dehydration products **13** and **14** is better than that with the stereoisomeric ambliol C [**15**] and its dehydration product **16**, the relative stereochemistry about C-9, C-10, C-13, and C-18 in **5** and its derivatives remains unassigned.



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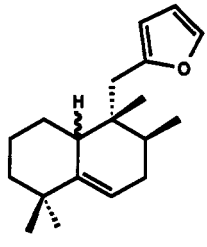

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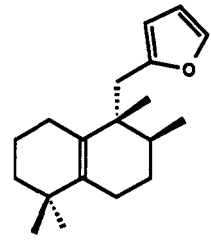

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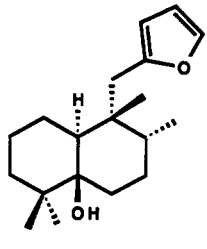

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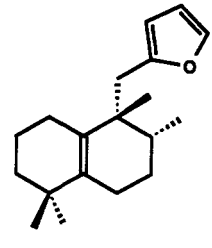

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Biosynthetically, all the known marine norterpene cyclic peroxides could be derived from suitable conjugated diene ($\Delta^{3,5}$) precursors via addition of oxygen. The geometry of these dienes would in turn define the relative stereochemistry about C-3 and C-6 in the peroxy functionalities. A 3*E*, 5*E* geometry would result in an axial C-6 alkyl chain substituent whereas a 3*E*, 5*Z* geometry would return an equatorially oriented C-6 alkyl chain. The unusual bicyclic subunit proposed for **5** can be rationalized as an intermediate form between "labdane" [**1**] and "clerodane" [**2**] analogues. It is sobering to note that, in addition to providing a range of stereoisomeric cyclic peroxides, marine sponges appear capable of operating in either enantiomeric series. As yet no evidence has been put forward to support the co-occurrence of enantiomeric cyclic peroxides moieties, although it is questionable whether such a situation would be recognized unless specifically addressed.

EXPERIMENTAL

For general experimental details see Capon and Barrow (9).

COLLECTION AND ISOLATION.—A specimen of *Mycale* (*Carmia*) cf. *spongiosa* (type locality Port Phillip, Vic., syntypes NMV G2430-2451, Reg. No. Z4966, 40 g) was collected by hand (scuba) at a depth of 10 m off South Duras on the mid-south coast of New South Wales, Australia. A voucher specimen (Z4966) is lodged with the Australian Museum, Sydney. The freshly collected, extremely fragile, and heavily mucus-laden specimen was diced, stored in EtOH, and packed in dry ice for transport. Prolonged storage was at −3°. The crude EtOH extract was screened and found to inhibit the growth of *B. subtilis* and *S. cerevisae*. The active constituents partitioned into a lipid-soluble (CH_2Cl_2) fraction and were further resolved by rapid elution through a short column of silica. ^{1}H-nmr analysis of the crude active mixture confirmed the presence of norterpene cyclic peroxides, present in low yield. To facilitate isolation, the crude active fraction was methylated with CH_2N_2 and subjected to hplc (25% Et_2O/hexane at 2.0 ml/min through a 10μ 10 cm × 0.8 cm silica RCM cartridge) to yield, in increasing order of polarity, the cyclic peroxide methyl esters **4** (32 mg, 0.08%) and **6** (4 mg, 0.01%). Neither of the methyl esters displayed antimicrobial activity. It has previously been observed (1–3) that, while the free acids of marine norterpene cyclic peroxides display antimicrobial activity, the methyl esters are inactive.

CYCLIC PEROXIDE METHYL ESTER **4**.—A stable colorless oil: $[\alpha]_D$ −22.6 (c = 3.1, $CHCl_3$); found $[M]^+$ 424.3205 ($C_{25}H_{44}O_5$ requires 424.3189); ^{1}H nmr ($CDCl_3$) δ 0.80 (d, J = 8 Hz, 10-Me), 0.80, 0.82, 0.96 (3s, 14-Me, 14-Me, 18-Me), 1.14 (d, J = 8 Hz, 2-Me), 1.26 (s, 6-Me), 2.26 (ddd, J = 4, 12, 12 Hz, H-12 axial), 2.66 (dq, J = 8, 8 Hz, H-2), 3.69 (s, OMe), 4.22 (ddd, J = 4, 8, 8 Hz, H-3); ^{1}H nmr (C_6D_6) δ 0.87 (d, J = 6 Hz, 10-Me), 0.79, 0.80, 1.00 (3s, 14-, 14-, 18-Me), 0.97 (d, J = 8 Hz, 2-Me), 1.29 (s, 6-Me), 2.45 (m, H-12 axial), 2.58 (dq, J = 8, 8 Hz, H-2), 3.35 (s, OMe), 4.28 (bm, H-3); ^{13}C nmr ($CDCl_3$) 12.9 (q), 15.8 (q), 20.5 (q), 22.0 (t), 22.1 (q), 22.5 (t), 24.8 (q), 25.6 (t), 27.2 (q), 27.2 (2t), 27.8 (t), 32.2 (t), 34.1 (t), 36.2 (d), 38.0 (s), 38.2 (t), 39.3 (t), 41.6 (d), 42.7 (d), 51.8 (q), 77.3 (s), 81.2 (s), 81.8 (d), 174.6 ppm (s); ms *m/z* $[M]^+$ 424, 406, 388, 375, 209 (100), 191.

CYCLIC PEROXIDE METHYL ESTER **6**.—A moderately unstable, colorless oil: $[\alpha]_D$ −45 (c = 0.9, $CHCl_3$); found $[M]^+$ 424.3180 ($C_{25}H_{44}O_5$ requires 424.3189); ^{1}H nmr ($CDCl_3$) δ 0.75 (s, 9-Me), 0.86, 0.98 (2s, 14, 14-Me), 0.93 (d, J = 8 Hz, 10-Me), 1.14 (d, J = 8 Hz, 2-Me), 1.29 (s, 6-Me), 2.56 (dq, J = 8, 8 Hz, H-2), 3.69 (s, OMe), 4.22 (bddd, J = 4, 8, 8 Hz, H-3); ^{1}H nmr (C_6D_6) δ 0.73 (s, 9-Me), 0.77, 0.96 (2s, 14, 14-Me), 0.94 (d, J = 6 Hz, 10-Me), 0.96 (d, J = 7 Hz, 2-Me), 1.32 (s, 6-Me), 2.51 (dq, J = 8, 8 Hz, H-2), 3.36 (s, OMe), 4.31 (bddd, J = 4, 8, 8 Hz, H-3); ms *m/z* $[M]^+$ 424, 406, 388, 375, 209 (100), 191.

DEHYDRATION OF **4**.—To a sample of **4** (9 mg) in dry C_6H_6 (2 ml) was added 20 mg of freshly sublimed oxalic acid. The reaction mixture was stirred under anhydrous conditions at 90° for 19 h, during which time the C_6H_6 was allowed to evaporate. The solid reaction mixture was then dissolved in 25% Et_2O/hexane and eluted through a small plug of silica to remove oxalic acid, and the eluate was concentrated under reduced pressure to return a quantitative yield of **8** as a stable colorless oil: $[\alpha]_D$ −69 (c = 0.85, $CHCl_3$); found $[M]^+$ 406.3080 ($C_{25}H_{42}O_4$ requires 406.3083); ^{1}H nmr ($CDCl_3$) δ 0.76 (d, J = 6 Hz, 10-Me), 0.77, 1.00, 1.04 (3s, 18-, 14-, 14-Me), 1.15 (d, J = 8 Hz, 2-Me), 1.28 (s, 6-Me), 2.57 (dq, J = 8, 8 Hz, H-2), 3.70 (s, OMe), 4.24 (m, H-3), 5.32 (m, H-12); ^{1}H nmr (C_6D_6) δ 0.79 (d, J = 7 Hz, 10-Me), 0.75, 1.05, 1.09 (3s, 18-, 14-, 14-Me), 0.98 (d, J = 7 Hz, 2-Me), 1.25 (s, 6-Me), 2.51 (dq, J = 8, 8 Hz, H-2), 3.37 (s, OMe), 4.25 (m, H-3), 5.39 (m); ^{13}C nmr ($CDCl_3$) 12.8 (q), 14.6 (q), 20.4 (q), 22.0 (q), 22.9 (q), 22.9 (t), 27.6 (t), 29.5 (q), 29.7 (t), 30.8 (t), 32.2 (t), 32.8 (d), 33.9 (t),

35.6 (s), 36.6 (s), 40.7 (d), 42.0 (t), 42.8 (d), 51.9 (q), 80.6 (s), 81.8 (d), 114.3 (d), 146.5 (s), 174.5 (s); ms *m/z* $[M]^+$ 406, 388, 375, 335, 301, 283, 241, 191 (100).

DEHYDRATION OF **6**.—Treatment of a sample of **6** (4 mg) with oxalic acid as described above for **4** yielded after workup a two-component mixture. These were resolved by hplc (3.0 ml/min 2% Et_2O/hexane on μ porasil) to return **10** and **11** as two stable colorless oils. Compound **10** (2 mg): $[\alpha]_D$ −25 ($c = 0.2$, $CHCl_3$); found $[M]^+$ 406.3072 ($C_{25}H_{42}O_4$ requires 406.3083); ^1H nmr ($CDCl_3$) δ 0.80 (d, $J = 8$ Hz, 10-Me), 0.80, 0.93, 0.96 (3s, 9-, 14-, 14-Me), 1.14 (d, $J = 8$ Hz, 2-Me), 1.29 (s, 6-Me), 2.58 (dq, $J = 8$, 8 Hz, H-2), 3.69 (s, OMe), 4.23 (bm, H-3); ms *m/z* $[M]^+$ 406, 191 (100). Compound **11** (1 mg): $[\alpha]_D$ −66 ($c = 0.1$, $CHCl_3$); found $[M]^+$ 406.3072, $C_{25}H_{42}O_4$ requires 406.3083; ^1H nmr ($CDCl_3$) δ 0.80 (d, $J = 8$ Hz, 10-Me), 0.62, 0.98, 1.05 (3s, 9, 14, 14-Me), 1.15 (d, $J = 8$ Hz, 2-Me), 1.25 (s, 6-Me), 2.59 (dq, $J = 8$, 8 Hz, H-2), 3.70 (s, OMe), 4.23 (m, H-3), 5.43 (m, H-12); ms *m/z* $[M]^+$ 406, 191 (100).

HOREAU ANALYSIS OF **3**.—To a sample of the methyl ester **4** (2.5 mg) in Et_2O (2 ml) was added 10% Pd/C (10 mg), and the resulting reaction mixture was stirred under an atmosphere of H_2 for 4 h to yield, after filtration through celite and concentration under reduced pressure, the triol ester **9** (1.8 mg, 72%): found $[M]^+$ 426.3346 ($C_{25}H_{46}O_5$ requires 426.3345); ^1H nmr ($CDCl_3$) δ 0.79, 0.80, 1.00 (3s, 14-, 14-, and 18-Me), 0.81 (d, $J = 8$ Hz, 10-Me), 1.13 (s, 6-Me), 1.19 (d, $J = 8$ Hz, 2-Me), 2.57 (dq, $J = 8$, 8 Hz, H-2), 3.71 (s, OMe), 3.71 (m, H-3). The triol ester **9** (1.8 mg) was in turn treated with a 12.5% solution of α-phenylbutyric anhydride in dry pyridine (23.2 μl, 2 equivalents), worked up, and analyzed as previously described (2) to yield a very slight excess of (+)-α-phenylbutyric acid (3% optical yield), $[\alpha]_D$ +1.5 ($c = 0.4$, $CHCl_3$).

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