New Acetogenin Peroxides from the Indian Sponge *Acarnus bicladotylota*

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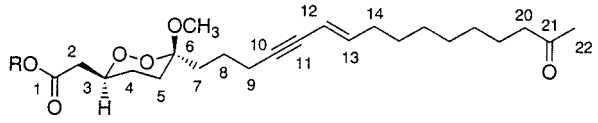
Two new cyclic peroxides (**1** and **2**) and the known metabolite **3** have been found in the organic extract of the Indian sponge *Acarnus bicladotylota*. The structure of the new products has been assured by chemical and spectroscopic methods. The absolute stereochemistry of **1**−**3** has been determined by Mosher's method on the semisynthetic derivative **4**.

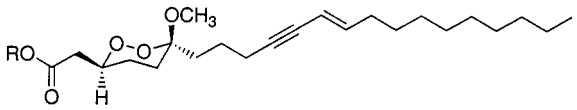
Cyclic peroxides are rather unstable metabolites that have been isolated often from marine organisms, particularly sponges of the genus *Plakortis*.[1−5] Biogenetically, the carbon skeleton of internal peroxides can derive from different metabolic pathways, although straight or branched acetogenins are most often encountered.[6,7] Most of the endoperoxides, furthermore, exhibit interesting biological properties, among which the antimicrobial activity seems to be the most promising.[6,7]

Here, we wish to report the isolation and structure elucidation of two new acetogenin peroxides (**1** and **2**), named peroxyacarnoic acids C and D, from the sponge *Acarnus bicladotylota*, Hoshino. Besides **1** and **2**, the sponge extract contained the related peroxyacarnoic acid A (**3**), which has been previously described by Kashman and co-workers from the Red Sea sponge *Acarnus* cf. *bergquistae*.[8] Compounds **1**−**3**, which occurred as free carboxylic acids in the fresh sponge extract, have been isolated as esters **1a**−**3a** by repeated chromatographies (Experimental Section). The absolute stereochemistry of **1a**−**3a** has been determined by applying Mosher's method to the alcohol **4** that was prepared by reduction of the major sponge metabolite, peroxyacarnoic acid A (**3**).[9]

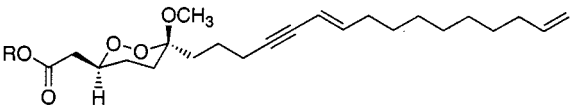
The molecular formula of **1a** was established as $C_{24}H_{38}O_6$ on the basis of a parent ion in the CIMS (*m*/*z* 440 $[M + NH_4]^+$) consistent with the ^{13}C NMR data (Table 1). The ^1H NMR spectrum of **1a** showed two well-defined double-doublets at δ 2.39 and 2.52 (H_2-2) that were both coupled to the downshifted methine signal at δ 4.49 (H-3). The coupling constants and the chemical shift of this spin system strongly suggested the presence of a peroxycyclic fragment.[1,2] The structure of the dioxane ring was further supported by two cross-related methylene systems (H_2-4, δ 1.78 and 1.65; H_2-5, δ 1.84 and 1.62) in the COSY spectrum. The ^{13}C NMR data, moreover, showed the presence of an ester function (δ 170.5, C-1) and two oxygen-bearing carbons at δ 76.8 (C-3) and 102.3 (C-6). This latter signal, together with the resonance of the methoxy group at δ 3.25 in the ^1H NMR spectrum, suggested the peroxyketal function of **1a**. This assignment was totally in agreement with both $^1H-^{13}C$ correlations and literature data.[8,9] In particular, HMBC correlations from C-6 (δ 102.3) to H_2-5 (δ 1.84 and 1.62), H_2-7 (δ 1.42), and the methoxyl group at δ 3.25 justified joining the peroxycycle to the remaining part of the molecule through the ketal carbon.



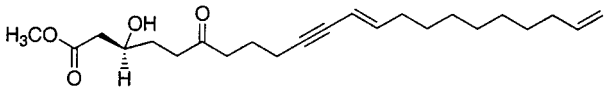
1 R = H
1a R = CH_3



2 R = H
2a R = CH_3



3 R = H
3a R = CH_3



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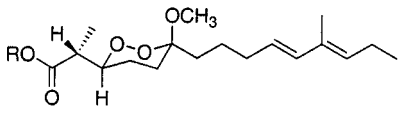

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In agreement with the IR absorption at 1714 cm^{-1}, ^{13}C NMR spectrum also showed a carbonyl signal at δ 209.8 (C-21). This resonance was attributed to a terminal methyl-ketone based on the presence of the downfield methyl group signal at δ 2.12 (H_2-22) in the ^1H NMR spectrum. The partial structure was unequivocally supported by long-range correlation from C-21 to H_2-19 (δ 1.63), H_2-20 (δ 2.41), and H_3-22 (δ 2.12). The remaining NMR data of **1a**

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Table 1. NMR Data[a] for Compounds **1a** and **2a** ($CDCl_3$, 500 and 125 MHz) (Numbering in Agreement with Ref 8)

	1		**2**	
position	$\delta_{^1H}$	$\delta_{^{13}C}$	$\delta_{^1H}$	$\delta_{^{13}C}$
1		170.5, s		170.5, s
2	2.52, dd, 16.1 and 7.7 Hz 2.39, dd, 16.1 and 5.8 Hz	38.3, t	2.52, dd, 16.0 and 7.7 Hz 2.39, dd, 16.0 and 5.8 Hz	38.3, t
3	4.49, m	76.8, d	4.49, m	76.8, d
4	1.78, m 1.65, m	25.1, t	1.73, m 1.64, m	25.1, t
5	1.84, m 1.62, m	30.3, t	1.89, m 1.64, m	30.4, t
6		102.3, s		102.7, s
7	1.42, m[b]	28.2, t	1.39, m	29.6, t
8	1.47, m[b]	28.7, t	1.21−1.50, m	29.6, t
9	2.28, t, 7.1 Hz	19.3, t	1.21−1.50, m	29.6, t
10		89.1, s		29.6, t
11		79.0, s		29.6, t
12	5.45, bd, 15.7 Hz	110.8, d	1.21−1.50, m	29.6, t
13	6.00, dt, 15.7 and 7.7 Hz	141.9, d	1.21−1.50, m	29.6, t
14	2.08, bt, 6.9 Hz	32.8, t	1.21−1.50, m	29.6, t
15	1.45, m	22.2, t	1.21−1.50, m	29.6, t
16	1.65, m 1.35, m	30.2, t	1.21−1.50, m	29.6, t
17	1.30, m[b]	28.7, t	1.21−1.50, m	29.6, t
18	1.30, m[b]	28.8, t	1.21−1.50, m	29.6, t
19	1.63, m	28.7, t	1.21−1.50, m	29.6, t
20	2.41, t, 7.4 Hz	43.7, t	1.21−1.50, m	32.6, t
21		209.8, s	1.21−1.50, m	22.8, t
22	2.12, s	29.0, q	0.88, m	14.1, q
Me (ester)	3.70, s	51.9, q	3.70, s	51.9, q
Me (acetal)	3.25, s	48.5, q	3.26, s	48.4, q

[a] NMR assignments were supported by ^1H, ^{13}C, DEPT, HMBC, and HMQC experiments. [b] Interchangeable values.

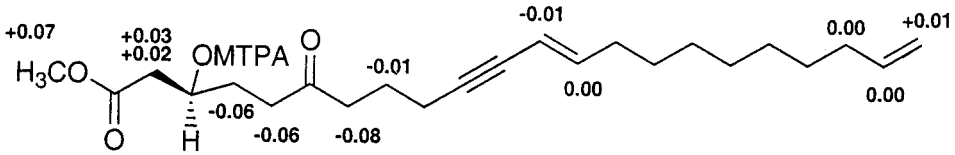
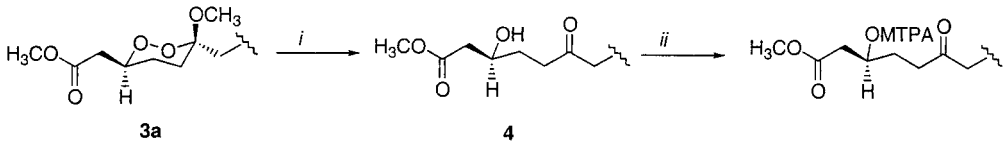


Figure 1. $\Delta\delta$ ($\delta_S - \delta_R$) of MTPA derivatives of **4**.

Scheme 1. Preparation of MTPA Derivatives from **3a**[9]



i. Zn/AcOH in Et_2O, rt, 18 h; *ii.* MTPA-Cl in dry pyridine, rt overnight.

were very similar to those recently described for compound **3a**[8] and indicated the presence of an enyne-containing alkyl chain (δ 89.1, C-10; δ 79.0, C-11; δ 110.8, C-12; δ 141.9, C-13). Comparison of the spectral data of **1a** and **3a** confirmed the gross structure of **1** and the *E* stereochemistry of the 12,13-double bond (J = 15.7 Hz).

NOESY data of **1a** proved that the relative stereochemistries of the major substituents on the 1,2-dioxane ring were both equatorial. Accordingly, NOEs were observed between H-3 (δ 4.49) and H-5_{ax} (δ 1.62), as well as between the methoxy ketal group (δ 3.25) and H-4_{ax} (δ 1.65). As expected, the same relative configuration was demonstrated for **3a**, thus confirming the literature assignment[8] that had been based on comparison of the NMR data of **3a** with those of peroxyplakoric acid methyl esters (e.g., **5**).[10]

The structure of peroxyacarnoic acid D methyl ester (**2a**) was very similar to that of **1a** and **3a**, except for the alkyl chain that was completely saturated in **2a**. Comparison of the spectral data suggested the depicted stereochemistry of **2a**. This geometry was further confirmed by NOESY correlations.

To determine the absolute stereochemistry of the dioxane ring, the major sponge metabolite, peroxyacarnoic acid A (**3**), was converted into the β-hydroxy ester **4** by reductive cleavage of the peroxyketal function (Scheme 1).[9] $\Delta\delta$ analysis of the MTPA derivatives of **4** (Figure 1) supported a 3*S* configuration for the alcohol, thus suggesting that the absolute stereochemistry of **3a** was 3*S*,6*R*. Since compounds **1a**−**3a** showed both the same relative stereochemistry of the cyclic part and similar polarimetric values ($[\alpha]_D$ −12.1, −10.1, and −9.5 for **1a**, **2a**, and **3a**, respectively), we assumed that compounds **1** and **2** have the same absolute configuration (3*S*,6*R*).

In conclusion, the extract of the Indian sponge contained three metabolically related endoperoxides possessing a C-22 alkyl chain. Similar products have been recently reported from another Indo-Pacific sponge, *Acarnus* cf. Bergquistae.[7] The limited amount of isolated products prevented the assessment of biological activities.

Experimental Section

General Methods. 1D and 2D NMR spectra were recorded on Bruker AMX 500 and Bruker AMX 300 instruments. The

$CHCl_3$ resonances at δ 7.26 and 77.0 were used as internal references. MS spectra were obtained by "Servizio di Spettrometria di Massa" of Naples. Infrared spectra were recorded with a Bio-Rad FTS-7 FT/IR spectrophotometer. Optical rotations were determined with a JASCO DIP-370 polarimeter.

Animal Material. The specimen of *A. bicladotylota*, brightly orange in life, was collected on a sandy bottom at a depth of 20 m in Muttom, Kerala (India). The sponge was thickly encrusted with fingerlike branches arising from the thickest part. Sand grains and other foreign objects were heavily incorporated into the interior of the specimen. The surface appeared rugged and irregularly conulose. The ectosomal skeleton showed a felt-work of tylotes (tylota head spined terminally), whereas the endosomal skeleton showed plumose bands of styles in thicker parts of the specimen and erect in encrusting parts. Spongin, pale yellow in color, is seen at the nodes on plumose columns. A voucher specimen (TRI-21, 1998) is kept at NIO, Goa (India).

Extraction and Purification. The sponge (85 g dry wt) was immediately extracted with acetone (3 × 250 mL). After removing the organic solvent, the aqueous residue was diluted with fresh water (70 mL) and partitioned against Et_2O (3 × 50 mL). The ether extract (487 mg) was fractionated by LH-20 ($CHCl_3$/MeOH, 1:1). The fractions of interest were methylated by CH_2N_2 in Et_2O, and the resulting reaction mixture was purified with a SiO_2 column to give **1a** (0.9 mg), **2a** (2.0 mg), and **3a** (2.9 mg).

Methyl ester of peroxyacarnoic acids C (1a): obtained as a pale yellow powder ($C_{24}H_{38}O_6$); $[\alpha]_D$ −12.1° (*c* 0.09, $CHCl_3$); UV (EtOH) λ_{max} 206 (ϵ 46 000) and 230 (ϵ 32 000) nm; IR (liquid film) ν_{max} 2927, 2857, 1738, 1714, 1359, 1066 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) are reported in Table 1; CIMS (NH_3) *m/z* 440 $[M + NH_4]^+$ (60), 408 (10, $[M + NH_4]^+ - O_2$), 310 (25), 252 (30), 191 (100), 173 (50); EIMS *m/z* 196 (20), 173 (30), 169 (80), 85 (100); HRCIMS (NH_3) *m/z* 440.3019 [calcd for $C_{24}H_{42}NO_6$ ($M + NH_4^+$), 440.3012].

Methyl ester of peroxyacarnoic acids D (2a): colorless powder ($C_{24}H_{46}O_5$); $[\alpha]_D$ −10.1° (*c* 0.2, $CHCl_3$); IR (liquid film) ν_{max} 2927, 2875, 1738, 1066 cm^{-1}; ^1H and ^{13}C NMR ($CDCl_3$) are reported in Table 1; CIMS (NH_3) *m/z* 432 (65) $[M + NH_4]^+$, 400 (10, $[M + NH_4]^+ - O_2$), 383 (40), 367 (40), 302 (35), 191 (75), 176 (100); EIMS *m/z* 382 (12, $M - O_2$), 189 (20), 174 (100); HRCIMS (NH_3) *m/z* 426.3227 [calcd for $C_{24}H_{44}NO_5$ ($M + NH_4^+$), 426.3219].

Compound 3a: pale yellow oil ($C_{24}H_{38}O_5$); $[\alpha]_D$ −9.5° (*c* 0.2, $CHCl_3$); IR ν_{max} (liquid film) 2934, 2865, 1746, 1058 cm^{-1}; UV, ^1H NMR, and ^{13}C NMR ($CDCl_3$) are identical to those reported in ref 9; CIMS (NH_3) *m/z* 424 (50) $[M + NH_4]^+$, 292 (10, $[M + NH_4]^+ - O_2$), 302 (15), 294 (40), 131 (90), 176 (100); EIMS *m/z* 196 (15), 173 (35), 169 (100).

Reductive Cleavage of 3a. The ester **3a** (2 mg) in 1 mL of Et_2O was reacted with 20 μL of AcOH and 11 mg of Zn. The heterogeneous solution was stirred vigorously for 18 h at room temperature and then filtered. The clear filtrate was dried at reduced pressure and purified on a Si gel column (petroleum ether/Et_2O, 80:20) to give 1.2 mg of **4**.

Compound 4: ^1H NMR (500 MHz, $CDCl_3$) δ 5.98 (1H, m, H-13), 5.80 (1H, m, H-21), 5.46 (1H, d, *J* = 15.6 Hz, H-12), 4.98 (1H, dd, *J* = 17.0 and 1.2 Hz, H-22a), 4.93 (1H, d, *J* = 10.4 Hz, H-22b), 4.05 (1H, m, H-3), 3.71 (3H, s, OCH_3), 2.59 (1H, dd, *J* = 15.8 and 3.1 Hz, H-2a), 2.46 (1H, m, H-2b), 2.35 (2H, t, *J* = 7.2 Hz, H_2-5 or H_2-7), 2.27 (2H, m, H_2-7 or H_2-5), 2.02 (m), 1.75−1.21 (m).

Preparation of Mosher Derivatives. The alcohol **4** was divided in two fractions, each of which was dissolved in 0.3 mL of dry pyridine and reacted with either (+)- or (−)-MTPA-Cl. The reactions were stirred at room temperature for 12 h and then quenched by MeOH (1.5 mL). After removing the organic solvent under a N_2 stream, the residues were purified on a Si gel column ($CHCl_3$/MeOH, 99:1), to give (*R*)- and (*S*)-MTPA esters from (*S*)- and (*R*)-MTPA chlorides, respectively.

(*S*)-MTPA ester of 4: (0.4 mg) ^1H NMR (500 MHz, $CDCl_3$) δ 5.95 (1H, m, H-13), 5.79 (1H, m, H-21), 5.12 (1H, m H-3), 4.99 (1H, bd, *J* = 15.4 Hz, H-22a), 4.93 (1H, d, *J* = 11.7 Hz, H-22b), 3.66 (3H, s, OCH_3), 2.70 (1H, dd, *J* = 16.1 and 8.2 Hz, H-2a), 2.60 (1H, dd, *J* = 16.1 and 4.9 Hz, H-2b), 2.35 (1H, m, H-4a), 2.26 (4H, m, H_2-7 and H_2-5), 2.03 (m), 1.83 (1H, m, H-4b), 1.70−1.56 (m), 1.24 (m).

(*R*)-MTPA ester of 4: (0.4 mg) ^1H NMR (500 MHz, $CDCl_3$) δ 5.96 (1H, m, H-13), 5.79 (1H, m, H-21), 4.98 (1H, d, *J* = 17.0 Hz, H-22a), 4.93 (1H, d, *J* = 10.4 Hz, H-22b), 3.59 (3H, s, OCH_3), 2.67 (1H, dd, *J* = 16.1 and 8.2 Hz, H-2a), 2.58 (1H, dd, *J* = 16.1 and 4.9 Hz, H-2b), 2.41 (1H, m, H-4a), 2.34 (2H, t, *J* = 7.1 Hz, H_2-7), 2.27 (2H, m, H_2-5), 2.03 (m), 1.93 (1H, m, H-4b), 1.70−1.56 (m), 1.23 (m).

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References and Notes

(1) Fontana, A.; Ishibashi, M.; Kobayashi, J. *Tetrahedron* **1998**, *54*, 2041−2048.
(2) Fontana, A.; Ishibashi, M.; Shigemori, H.; Kobayashi, J. *J. Nat. Prod.* **1998**, *61*, 1427−1429.
(3) Murayama, T.; Ohizumi, Y.; Nakamura, H.; Sasaki, T.; Kobayashi, J. *Experientia* **1989**, *45*, 898−899.
(4) Stierle, D. B.; Faulkner, D. J. *J. Org. Chem.* **1980**, *45*, 3396−3401.
(5) Rudi, A.; Talpir, R.; Kashman, Y.; Benayahu, Y.; Schleyer, M. *J. Nat. Prod.* **1993**, *56*, 2178−2182.
(6) Faulkner, D. J. *Nat. Prod. Rep.* **2000**, *17*, 7−55.
(7) Casteel, D. A. *Nat. Prod. Rep.* **1992**, *9*, 289−312.
(8) Yosief, T.; Rudi, A.; Wolde-ab, Y.; Kashman, Y. *J. Nat. Prod.* **1998**, *61*, 491−493.
(9) Ichiba, T.; Scheuer, P. J.; Kelly-Borges, M. *Tetrahedron* **1995**, *51*, 12195−12202.
(10) Kobayashi, M.; Kondo, K.; Kitagawa, I. *Chem. Pharm. Bull.* **1993**, *41*, 1324−1326.

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