Pergamon

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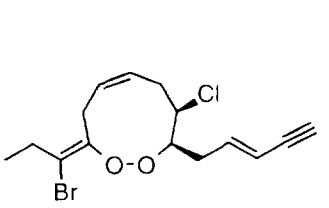
Two Cytotoxic 3,6-Epidioxy Fatty Acids from an Indonesian Sponge, *Plakortis* sp.[1]

Toshio Ichiba and Paul J. Scheuer*
Department of Chemistry, University of Hawaii at Manoa
2545 The Mall, Honolulu, Hawaii 96822

Michelle Kelly-Borges[2]
Harbor Branch Oceanographic Institution, Inc.,[3]
Division of Biomedical Marine Research,
5600 North U. S. 1, Fort Pierce, Florida 34948

Abstract: Two 3,6-epidioxy fatty acids, manadic acids A (**8**) and B (**9**), have been isolated from an undescribed species of a sponge, *Plakortis* sp., collected in Indonesia. Their structures and absolute configurations have been determined. Both compounds are moderately active against various antitumor cell lines.

Epidioxy compounds occur rather commonly in marine natural products, particularly in sponges of the genera *Plakortis*,[4] *Chondrilla*,[5] *Xestospongia*,[6] and *Chondrosia*.[7] Fatty acids are most often encountered, but alkenynes e. g. rhodophytin (**1**) from the red alga *Laurencia yamada*,[8] terpenoids, e. g. sigmosceptrellin-A (**2**) from the sponge *Sigmosceptrella laevis*,[9] and sterol peroxides **3** from the sponge *Axinella cannabina*[10] have also been reported. With the exception of a single 3,5-epidioxy fatty acid **4** from a sponge, *Plakortis zyggompha*,[4a] all others are 3,6-epidioxy compounds.



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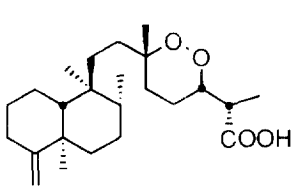

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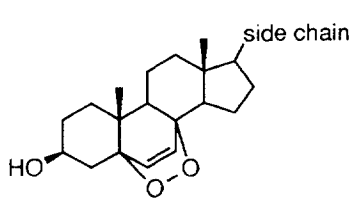

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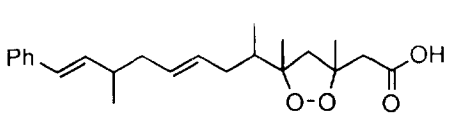

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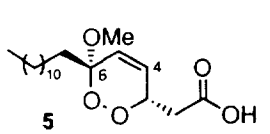

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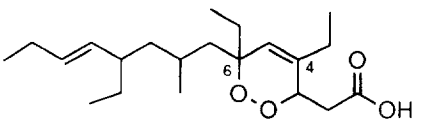
All of those that have so far been reported are either 6-methoxy-4-ene derivatives, e. g. **5**,[4b] or 4,6-

dialkyl acids with or without a C4 olefin, e. g. **6**[4c] or **7**.[4a] In addition, the genus *Plakortis* has produced many complex adducts of the basic epidioxy fatty acid.[11]

We now report two 3,6-epidioxy fatty acids that belong to a new structural type, possessing both 4-alkyl and 6-methoxy substituents. Furthermore, we determined the absolute stereochemistry of these two compounds by using the method recently described by Ohtani *et al.*[12]



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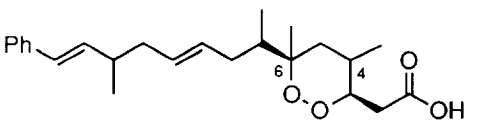

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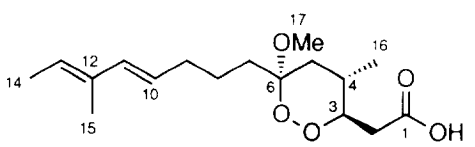
Results and Discussion

Manadic acid A (**8**) [3(*S*),6(*S*)-epidioxy-4(*S*),12-dimethyl-6-methoxy-tetradeca-10(*E*),12(*E*)-dienoic acid] and manadic acid B(**9**) [3(*S*),6(*S*)-epidioxy-4(*S*),12-dimethyl-6-methoxypentadeca-10(*E*),12(*E*)-dienoic acid] were isolated from a sponge, *Plakortis* sp., collected near Manado, Sulawesi, Indonesia, in October, 1992.[12]



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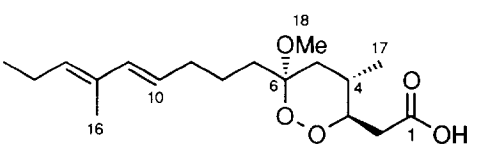

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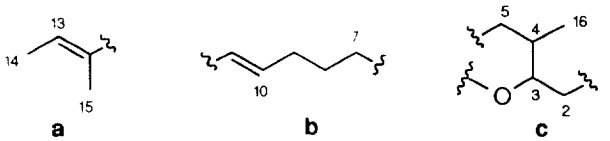
A sponge (130 g, dry), an undescribed *Plakortis* sp., collected in Indonesia in 1992, was freeze-dried and extracted with ethanol. The EtOH extract was concentrated to dryness. The residual solid of the EtOH extract was extracted with CH_2Cl_2/EtOH (5:1) to yield 9.8 g of a non-polar extract. A portion (1.0 g) of the non-polar extract was separated with bioassay-guided (Gram-positive bacterium *Staphylococcus aureus*) fractionation by high speed counter-current chromatography, yielding two compounds, manadic acid A, **8** (45.6 mg) and manadic acid B, **9** (5.2 mg), as colorless oils.

An IR band at 1060 cm^{-1} indicated the presence of a peroxide. The ^{13}C NMR spectrum of compound **8** showed seventeen carbon signals including one carboxylic acid (δ 176.3), four olefinic carbons (δ 124.9 - 135.5), one ketal (δ 103.6), three methyl groups, one methoxy (δ 48.1), and one carbon signal bearing oxygen (δ 78.9). An IR band at 1710 cm^{-1} confirmed the carboxyl group. A molecular formula $C_{17}H_{28}O_5$ was deduced from ^1H NMR, ^{13}C NMR, DEPT, and IR spectral data, and was confirmed by HREIMS data on the highest fragment ion at *m/z* 264 (M^+-O-MeOH) and by HRFABMS data at *m/z* 295 (M^+H-H_2O) of the methyl ester.

COSY correlations allowed establishment of three partial structures (**a**-**c**). A UV band at 234 nm characteristic of a trisubstituted diene connected **a** and **b**, which was confirmed by HMBC correlations between

C13 and H11, and C12 and H10. Furthermore, HMBC correlations from ketal carbon C6 to H_25, H_27, and methoxy H_317 combined part structures **b** and **c** through a ketal. Carboxyl C1 was attached to C2 based on an HMBC correlation between C1 and H_22. Finally, C3 and C6 were connected through a peroxy bond to complete the structure of compound **8**.



The relative stereochemistry of the 6-membered ring in **8** was established by a 1D NOE experiment. NOE's were observed from Me16 to MeO17 and to H_22. These NOE's confirm the relative stereochemistry as illustrated. The geometry of C12-C13 was determined to be *E* by an NOE between Me14 and Me15 and supported by the chemical shifts of the vinyl methyls, C15 and C16. Geometry of C10-C11 was also *E* on the basis of a coupling constant of 15.6 Hz between H10 and H11.

^1H and ^{13}C NMR spectra of **8** and **9** were almost superimposable, except for the terminal olefin portion (Tables 1 and 2). A molecular formula of $C_{18}H_{30}O_5$ was secured for **9** by HREIMS of its methyl ester (*m/z* 308, M^+-MeOH). It differed by CH_2 from compound **8**, thereby suggesting that **9** has an additional methylene at the diene terminus. The ^1H NMR spectrum clearly indicated that one methyl signal couples to a methylene proton, which in turn couples to olefin proton H13. This confirmed that compound **9** terminates in propylidene rather than ethylidene as does **8**.

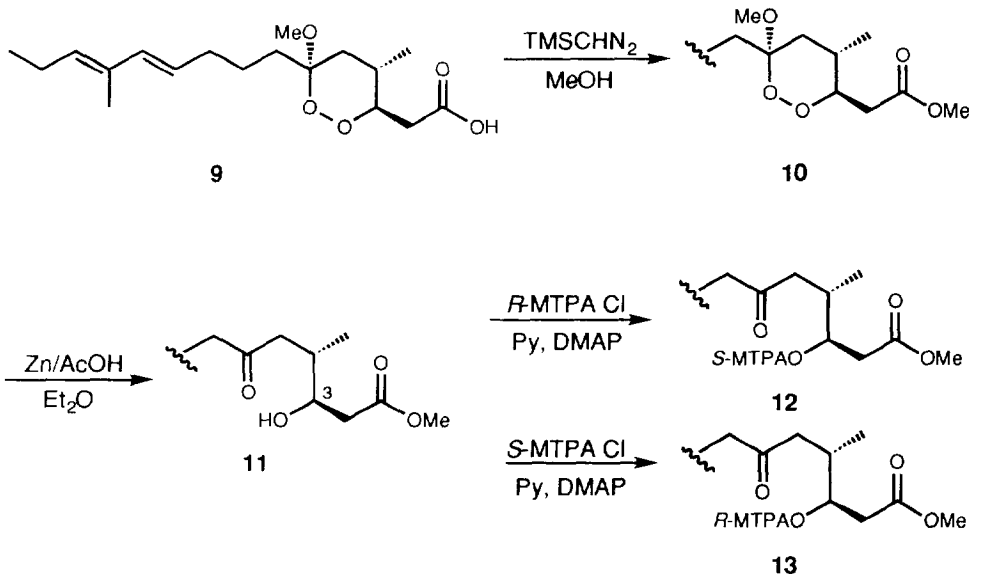
Table 1. ^1H NMR Data of Compounds **8** - **11**

	8		**9**		**10**		**11**	
	ppm	*J* (Hz)	ppm	*J* (Hz)	ppm	*J* (Hz)	ppm	*J* (Hz)
1								
2	2.51 dd	16.5, 8.9	2.54 dd	16.2, 8.7	2.50 dd	15.9, 8.6	2.64 dd	16.8, 4.2
	2.37 dd	16.5, 4.8	2.40 dd	16.2, 4.8	2.35 dd	15.9, 5.1	2.33 dd	16.8, 8.3
3	4.64 ddd	8.9, 4.8, 2.7	4.65 ddd	8.7, 4.8, 2.7	4.66 ddd	8.6, 5.1, 2.7	3.98 dt	8.3, 4.2
4	1.90 m		1.92 m		1.90 m		2.17 m	
5	1.82 dd	13.8, 5.4	1.85 dd	13.5, 5.4	1.80 m		2.43 m	
	1.74 dd	13.8, 3.0	1.76 dd	13.5, 2.7				
6								
7	1.30 m		1.62 m		1.63 m		2.41 t	7.4
	1.62 m		1.35 m		1.32 m			
8	1.41 m		1.43 m		1.41 m		1.68 quint	7.4
9	2.07 q	6.9	2.08 q	7.2	2.08 q	7.0	2.09 dt	7.4
10	5.47 dt	15.6, 6.9	5.50 dt	15.6, 7.2	5.46 dt	15.6, 7.0	5.47 dt	15.6, 7.4
11	6.04 d	15.6	6.05 d	15.6	6.04 d	15.6	6.04 d	15.6
12								
13	5.45 q	6.9	5.38 t	7.4	5.36 t	7.4	5.37 t	7.5
14	1.68 d	6.9	2.11 quint	7.4	2.12 quint	7.4	2.11 quint	7.5
15	1.69 s		0.96 t	7.4	0.97 t	7.4	0.97 t	7.5
16	1.09 d	7.2	1.70 s		1.70 s		1.70 s	
17	3.21 s		1.11 d	7.2	1.09 d	6.9	0.89 d	6.6
18			3.23 s		3.22 s		3.71 s	
19					3.70 s			

Table 2. ^{13}C NMR Data of Compounds **8** - **10**

	8	**9**	**10**
Carbon	ppm	ppm	ppm
1	176.3	175.2	171.0
2	35.2	35.0	35.2
3	78.9	78.9	79.1
4	28.5	28.7	28.7
5	36.9	37.0	37.0
6	103.6	130.6	103.5
7	32.4	32.4	32.7
8	23.1	23.1	23.0
9	32.7	32.8	32.4
10	126.0	126.3	126.3
11	135.5	135.6	135.5
12	134.2	132.8	132.7
13	124.9	132.8	132.7
14	12.0	21.4	21.3
15	13.7	14.1	14.1
16	14.2	12.3	12.2
17	48.1	14.2	14.2
18		48.2	48.1
19			51.9

The absolute stereochemistry of compound **9** was determined by the method recently described by Ohtani *et al.*[12] by transforming compound **9** into acyclic alcohol, **11**.



Acid B (**9**) was converted with $TMSCHN_2$ in MeOH to methyl ester **10**. Esterification was confirmed by an IR band at 1730 cm^{-1}, a ^{13}C NMR signal at 171.0 ppm (Table 2), and a fragment ion at *m/z* 267 (M^+-CH_2COOMe) in the EI mass spectrum. The ketal function of **10** was transformed to a keto alcohol in **11** by treatment with Zn and acetic acid in ether.[5] An IR band at 1715 cm^{-1} confirmed presence of a ketone. Under these mild condition, formation of an α, β-unsaturated ester was avoided. An aliquot of **11** was treated with (-)-MTPA chloride and a catalytic amount of DMAP in dry pyridine; the resulting ester was purified by prep-TLC to yield (+)-MTPA ester **12**. Another aliquot of **11** was treated in the same way leading to *R*-MTPA ester **13**. ^1H NMR spectra of both esters were measured in chloroform-*d*, and the chemical shift differences were calculated ($\Delta=\delta_S-\delta_R$) (Fig. 1).

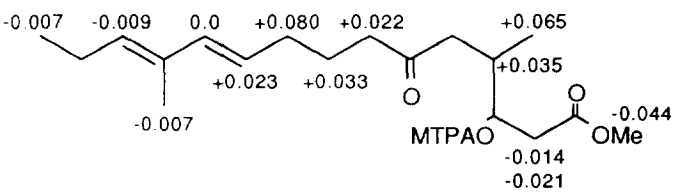


Fig. 1. Calculated chemical shift differences (in ppm) for MTPA esters.

Based on these values, the absolute stereochemistry of **11** at C3 was *S*. Since the relative stereochemistry of compound **9** is known, this result was applied to confirm the absolute stereochemistry at C3 (*S*), C4 (*S*), and C6 (*S*) in compound **9**. Both compounds **8** and **9** have positive optical rotation; therefore, manadic acid A (**8**) is likely to have the same absolute stereochemistry as manadic acid B (**9**).

Bioassay results are shown in Table 3.[13] Neither **8** nor **9** showed antiviral activity against HIV.

Table 3. Bioassay Data of Compounds **8** and **9**

	Assay (IC_{50} μg/mL)	**8**	**9**
Antitumor	P-388	0.5	0.5
	A-549	1	1
	HT-29	2	2
	MEL-28	5	2.5
Immumomodulatory	MLR	0.015	inactive
	LcV	0.5	inactive

Experimental Section

General; *Staphylococcus aureus* (ATTCC: 29213) was used as a guide for fractionation. High speed counter-current chromatography was carried out on an Ito Multi-Layer Coil Separator-Extractor (P. C. Inc., Potomac, MD).

A sponge (130 g, dry), collected in Indonesia in October, 1992,[14] was freeze-dried, and then extracted with EtOH (3 x 1.5 L). The EtOH extract was concentrated to dryness. CH_2Cl_2/EtOH (5:1, 100 mL) was added to the residual solid of the ethanolic extract; non-polar substances were extracted to yield 9.8 g of a brown oil. A portion (1.0 g) of the non-polar extract was separated with bioassay-guided (Gram-positive bacteria) fractionation by high speed counter-current chromatography, first with a solvent system of EtOAc/heptane/MeOH/H_2O 7:4:4:3, then heptane/MeCN/CH_2Cl_2 10:7:3, yielding **8** (45.6 mg) and **9** (5.2 mg), as colorless oils.

Manadic acid A (8). $[\alpha]_D^{18°}$ +83.9° (MeOH, *c* = 43.8); UV (MeOH): λ_{max} 232 nm (ε 30500); IR (CCl_4, NaCl): ν_{max} 3500-2400 cm^{-1} (br), 2950, 2930, 1710, 1430, 1300, 1270, 1070, 960; ^{13}C NMR (75 MHz, $CDCl_3$): see Table 2; ^1H NMR (300 MHz, $CDCl_3$): see Table 1; EIMS: *m/z* 264 (M^+-O-MeOH), 236; HREIMS: observed *m/z* 264.1738, required 264.1726 for $C_{16}H_{24}O_3$ (M^+-O-MeOH) (Δ 1.2 mmu); HRFAB *m/z* 295.1909, required 295.1910 for $C_{17}H_{27}O_4$ (M^+H-H_2O) (Δ 0.1 mmu).

Manadic acid B (9). $[\alpha]_D^{18°}$ +130.3° (MeOH, *c* = 4.0); UV (MeOH) λ_{max} 234 nm (ε 13400); IR (CCl_4, NaCl): ν_{max} 3500-2400 cm^{-1} (br), 2950, 2920, 1710, 1430, 1300, 1060, 960; ^{13}C (75 MHz, $CDCl_3$): see Table 2; ^1H NMR (300 MHz, $CDCl_3$): see Table 1; EIMS: *m/z* 264, 248.

Methyl ester 10. A crude fraction of **9** (45.5 mg) was dissolved in 500 μL of MeOH, and 300 μL of $TMSCHN_2$ (Aldrich) was added dropwise; the reaction mixture was allowed to stand at rt overnight. After solvent removal by a stream of N_2, the products were purified by reversed phase HPLC to yield 17.5 mg of ester **10** as a colorless oil.

Methyl ester 10. UV (MeOH): λ_{max} 234 nm (ε 21500); IR ($CHCl_3$, NaCl): ν_{max} 2950 cm^{-1}, 1730, 1450, 1300, 1280, 1070, 965; ^{13}C NMR (75 MHz, $CDCl_3$): see Table 2; ^1H NMR (300 MHz, $CDCl_3$): see Table 1; EIMS: *m/z* 308 (M^+-MeOH), 292 (M^+-O-MeOH), 267 (M^+-CH_2CO_2Me), 249 (M^+-MeOH-CO_2Me); HREIMS: observed *m/z* 308.1975, required 308.1988 (Δ 1.3 mmu) for $C_{18}H_{28}O_4$ (M^+-MeOH).

Ring opening of 10. Methyl ester **10** in 1 mL of Et_2O was treated with 50 μL AcOH and ca. 50 mg Zn, then stirred vigorously for 6 h at rt. After confirmation of disappearence of the starting material by silica gel TLC, the solid was removed by filtration; the solvent was removed by N_2, kept under reduced pressure overnight, to yield 12.1 mg (75.8%) of a colorless oil, keto alcohol **11**.

Keto alcohol 11. IR ($CHCl_3$): ν_{max} 3450 cm^{-1}, 2960, 1730, 1715, 965; ^1H NMR (300 MHz, $CDCl_3$): see Table 2; HREIMS observed *m/z* 292.2051, required 292.2039 (Δ 1.2 mmu) for $C_{18}H_{28}O_3$ (M^+-H_2O).

Preparation of *S*-MTPA ester 12. Compound **11** (3.4 mg) was treated with (-)-MTPA chloride (Aldrich) (40 μL, 19.8 eq) in 0.5 mL of dry pyridine (distilled from CaH_2) with a catalytic amount of DMAP for 6 h at rt. After removal of the solvent under reduced pressure, the reaction mixture was applied to a prep-TLC (hexane/ EtOAc 3:1) to furnish 3.9 mg (67.4%) of *S*-MTPA ester **12** as a colorless oil.

Preparation of *R*-MTPA ester 13. Compound **11** (4.8 mg) was dissolved in 0.5 mL of dry pyridine, and 35 μL (8.9 eq) of (+)-MTPA chloride and a catalytic amount of DMAP was added; the mixture was allowed to stand at rt. overnight. The solvent was removed under reduced pressure; the product was purified by prep-TLC with hexane/EtOAc (3:1), yielding *R*-MTPA ester **13** (5.8 mg, 71.2 % yield) as a pale yellow oil.

Calculation of Δ (δ_S-δ_R). ^1H NMR spectra of **12** and **13** were measured on a 300 MHz high field NMR spectrometer, and chemical shifts were recorded in ppm. All signals were assigned based on decoupling experiments, then chemical shift differences were calculated by subtracting δ-values of *R*-MTPA ester from those of *S*-MTPA ester (Fig. 1).

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14. This sample was collected at a depth of 10-20 m from a rocky surface. The sponge formed a thick encrustation with a smooth surface and liver-like texture, and was light tan in life and in ethanol preservative. The sample contains ramdomly and densely distributed contriangulate diactines 90-100μm in length. The sample has been compared to *Plakortis lita* de Laubenfels from the West Central Pacific and *Plakortis simplex sensu* Topsent (1897) from Amboyne, Moluccas, Indonesia, but both possess triactines. *P.lita* and *P. simplex* also differ considerably in coloration of the sample, *lita* being dark reddish brown with a red interior and *simplex* dull dark blue with a yellowish interior. The sponge is an undescribed species of *Plakortis* (Homosclerophorida, Plakinidae). A voucher specimen has been deposited at the Harbor Branch Oceanographic Museum, Fort Pierce, Florida (Catalog No. 003: 892).

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