Muamvatin, a Novel Tricyclic Spiro Ketal from the Fijian Mollusc *Siphonaria normalis*

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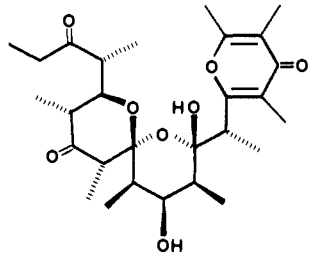
Abstract: An unusual polypropionate metabolite, muamvatin (**2**), was isolated from the Fijian mollusc *Siphonaria normalis*. Muamvatin represents the first example of a naturally occurring 2,4,6-trioxaadamantane ring skeleton. The structure of **2** was determined by interpretation of spectral data which included the use of a recently described two-dimensional indirect $^{13}C-^{1}H$ polarization transfer NMR experiment to trace the complete carbon-carbon connectivity of the molecule.

Pulmonate molluscs of the genus *Siphonaria* are known for their characteristic production of polypropionate metabolites containing either a hydroxylated pyrone[2,3] or a ketal[4] functionality. Siphonarin A (**1**) represented the first example of a bicyclic spiro ketal system obtained from a siphonariid.[5] In this paper we describe



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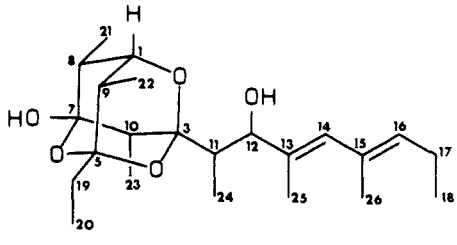
the structural elucidation of muamvatin (**2**), from the Fijian *S. normalis*, which possesses an unusual trioxaadamantane ring skeleton. The carbon-carbon connectivity of **2** was unambiguously assigned on the basis of the results of a two-dimensional $^{13}C-^{1}H$ polarization transfer NMR experiment (XCORFE) recently reported by Reynolds.[6] The technique, whereby one can indirectly



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trace the complete carbon skeleton of a molecule, has the ability to (1) distinguish between $^{2}J_{CH}$ and $^{3}J_{CH}$ connectivities based on vicinal proton coupling patterns and (2) detect carbon-hydrogen coupling through nonprotonated carbons and heteroatoms. Since the method is more sensitive than the two-dimensional INADEQUATE experiment, it can be used on smaller amounts of samples where the $^{13}C-^{13}C$ connectivity sequence would not be practical.

Frozen specimens of *S. normalis*, collected intertidally at Muamvatu, Fiji, were lyophilized and partitioned according to the Kupchan scheme.[7] The majority of muamvatin was located in the CCl_4 fraction. This material was chromatographed on Bio-Beads S-X12 (hexanes/CH_2Cl_2, 7:3) and silica gel (TMP/EtOAc, 7:3) to yield a mixture of muamvatin and sterols. Trituration with acetonitrile, followed by HPLC of the acetonitrile solubles on Partisil (TMP/EtOAc, 7:3), furnished 65.2 mg of **2** as a colorless oil.

Table I. ^{1}H, $^{13}C-^{1}H$, and Long-Range $^{13}C-^{1}H$ NMR Chemical Shift Assignments for Muamvatin (**2**)[a]

position	$\delta^{1}H$ (multiplicity)[d]	$\delta^{13}C$	$^{13}C-^{1}H$ long-range correlations
1	3.88 (1 H, br s)	78.7	C1-H22,[b] C1-H21[b]
3		105.2	C3-H24,[b] C3-H23,[b] C3-H1[b]
5		102.1	C5-H1[b]
7		97.5	C7-H23,[b] C7-H1[b]
8	1.97 (1 H, q, J = 7.03)	43.0	C8-H21,[c] C8-H1[c]
9	1.69 (1 H, q, J = 6.93)	37.7	C9-H22,[c] C9-H1[c]
10	2.10 (1 H, q, J = 6.74)	35.0	C10-H23,[c] C10-H11[c]
11	1.97 (1 H, dq, J = 9.02, 7.18)	40.7	C11-H12,[c] C11-H24[b,c]
12	4.40 (1 H, d, J = 9.02)	79.4	C12-H25,[b] C12-H24,[b] C12-H14[b]
13		134.6	C13-H25,[b] C13-H12[b]
14	5.87 (1 H, br s)	132.7	C14-H26,[b] C14-H25[b]
15		131.6	C15-H26,[b] C15-H14[b]
16	5.32 (1 H, br t, J = 7.19)	132.1	
17	2.10 (2 H, dq, J = 7.19, 7.52)	21.4	C17-H18[c]
18	0.98 (3 H, t, J = 7.52)	14.1	C18-H17[c]
19	1.69 (1 H, dq, J = 14.34, 7.46)	29.9	C19-H20[c]
	1.56 (1 H, dq, J = 14.34, 7.46)		
20	0.96 (3 H, t, J = 7.46)	5.8	C20-H19[c]
21	1.15 (3 H, d, J = 7.03)	13.2	C21-H8[c]
22	1.18 (3 H, d, J = 7.93)	13.2	C22-H9[c]
23	1.03 (3 H, d, J = 6.74)	6.6	C23-H10[c]
24	0.73 (3 H, d, J = 7.18)	10.4	C24-H11[c]
25	1.76 (3 H, s)	12.3	C25-H14[b]
26	1.72 (3 H, s)	16.7	

[a] Spectra recorded on JEOL JNM-FX270, Bruker WM-500, and IBM NR/200 FTNMR spectrometers in $CDCl_3$ or CD_2Cl_2; the 2D pulse sequences used were those furnished in the IBM software manual. [b] Correlations observed when the $^{13}C-^{1}H$ long-range couplings were set for maximum sensitivity at J = 9.5 Hz. [c] Correlations observed when the couplings were set for J = 4 Hz. [d] Coupling constants J are in hertz.

Muamvatin (**2**) displayed a small MH^+ ion in the chemical-ionization mass spectrum (CIMS) at m/z 395; a base peak at m/z 377 and a fragment ion at m/z 359 corresponded to the successive loss of two molecules of water. High-resolution FAB mass

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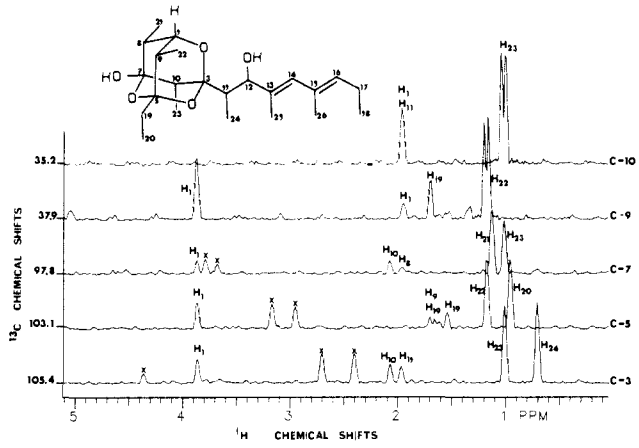
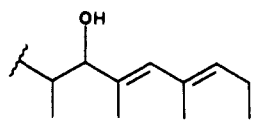


Figure 1. Traces from the two-dimensional XCORFE spectrum of muamvatin (**2**). The traces were taken parallel to the proton chemical shift axis at carbon chemical shifts corresponding to carbons 3, 5, 7, 9, and 10. Artifact peaks are denoted by an X.

measurement of the base peak indicated a formula of $C_{23}H_{37}O_4$ from which the molecular formula $C_{23}H_{38}O_5$ could be deduced. The CIMS also exhibited a fragment ion at m/z 285 for allylic cleavage of the C_8H_{13} diene portion of the side chain. The presence of this diene system was also confirmed by a UV absorption at 236 nm (ϵ 10 244).

All 38 hydrogens were observed in the ^{1}H NMR spectrum of **2**, the propionate nature of the molecule being implied by the presence of 8 methyl groups. An ethyl group, an isolated methine bearing a methyl, and the spin systems **a** and **b** could be deduced



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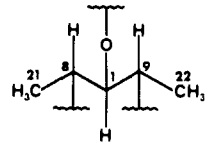
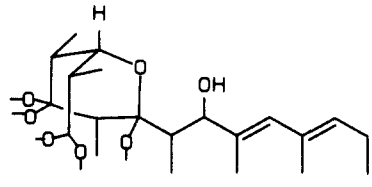


b

from ^{1}H-^{1}H spin-decoupling (Table I) and COSY experiments. One terminus of the molecule was defined by an ethyl group at δ 0.98 (3 H, t, J = 7.52 Hz) and 2.10 (2 H, dq, J = 7.19, 7.52 Hz) attached to a trisubstituted diene system at δ 5.32 (1 H, br t, J = 7.19 Hz) as in **a**. The other end of the molecule also encompassed an ethyl group [δ 0.96, (3 H, t, J = 7.46 Hz), 1.69 and 1.56 (1 H, dq, J = 14.34, 7.46 Hz)] bonded to a carbon-bearing oxygen. Although the connection in **b** from C-8 to C-1 to C-9 could not be seen from ^{1}H-^{1}H spin-decoupling studies, this sequence was clearly evident from the COSY spectrum. In addition, two exchangeable hydroxyl protons were present in the ^{1}H NMR spectrum at δ 4.40 and 2.86. A ^{13}C-^{1}H chemical shift correlation NMR experiment provided definitive assignments for all protonated carbons as shown in Table I. Five additional quaternary carbons were discernible in the ^{13}C NMR spectrum and were assigned to two fully substituted alkene carbons at δ 134.6 and 131.6 and three ketal carbons at δ 105.2, 102.1, and 97.5.

All elements of the molecular formula could now be accounted for; however, the data did not permit an unambiguous structure assignment. ^{13}C-^{1}H long-range coupling NMR experiments permitted expansion of the fragment ions to give partial structure **c** (see Table I) but failed to distinguish between the ketal carbons.



c

Table II. Indirect ^{13}C-^{1}H Polarization Transfer NMR Data for Muamvatin (**2**)[a]

C position	two-bond ^{1}H-C-^{13}C-H coupling pattern to H	two-bond ^{1}H-C-^{13}C coupling to H[c]	three-bond ^{1}H-C-C-^{13}C coupling to H[d]
1	8, 9, singlets[b]		21, 22
3		10, 11	1, 12, 23, 24
5		9, 19	1, 20, 22
7		8, 10	1, 21, 23
8	1 singlet,[b] 21 doublet		9
9	1 singlet,[b] 22 doublet		8, 19
10	23, doublet		8, 11
11	12, 24, doublets		
12	11, br singlet		24, 25
13		12, 25	OH
14			12, 25
15		26	17
16			18, 26
17	16, 18, triplets		
18	17, quartet		16
19	20, triplet		
20	19, quartet		
21	8, quartet		1
22	9, quartet		1
23	10, quartet		
24	11, quartet		12
25			12, 14
26			14, 16

[a] 97 mg of **2** in 0.5 mL of CD_2Cl_2 in a 5-mm tube; recorded on a Varian XL-400 spectrometer for 20 h. [b] These $^3J_{HH}$ couplings were too small to be observed and therefore appear as singlets in the spectrum. [c] Both two- and three-bond correlations between protons and non-protonated carbons appear as singlets. [d] Three-bond connectivites appear as singlets.

The specific placement of the ketals and thus the structure of muamvatin were unequivocally assigned to **2** by a two-dimensional indirect ^{13}C-^{1}H polarization transfer NMR experiment. The results shown in Table II, in addition to confirming partial structure **c**, now allowed complete assignment of the hemiketal to C-7 and connections of the C-5 ketal oxygens to both C-7 and C-3. A one-dimensional sample plot of five of the trioxaadamantane carbons is shown in Figure 1. Two-bond connections from H-22 to C-9 and H-23 to C-10 clearly appear as doublets due to their vicinal proton-proton coupling while the three-bond relationships from protons 8 and 19 to C-9 and protons 8 and 11 to C-10 appear as singlets. The ethyl proton (H-19) is unmistakably linked to only the ketal at C-5 while C-3 shows coupling to both H-10 and H-11, thereby establishing the side-chain junction. Since C-7 also exhibits coupling to H-10, and all three ketal carbons demonstrate three-bond connectivities to H-1, the structure of muamvatin can only be drawn as in **2**.

The relative stereochemistry of the trioxaadamantane ring constituents was determined by interpretation of proton coupling constants and the results of a two-dimensional NOE experiment. The fact that there is zero coupling between H-8, H-1, and H-9 in the one-dimensional ^{1}H NMR spectrum, but that there is an obvious connection between these same protons in the 2D COSY spectrum, suggests that they must have an axial–equatorial–axial relationship, respectively, as in **b**. An NOE between protons 8 and 9, plus the adamantane ring distortion due to the shorter carbon–oxygen bond lengths, supports this assignment. The relative positions of the hydrogens are also similar to those in two model systems: the 2-aza-6-oxaadamantane **3** where J_{BX} = 1.5 Hz while J_{AX} = 4 Hz[8] and the 2,4-dioxaadamantane **4** which exhibits zero coupling between H-8e and H-10′ but does show a "W" coupling of 1.88 Hz between the H-8a and H-10 protons.[9] These results, combined with the rigidity of the trioxaadamantane ring system, force the ethyl group, the C-7 hydroxyl, and the diene side chain to assume equatorial positions. The presence of an NOE between H-10 and the C-21 methyl group indicates that the C-23

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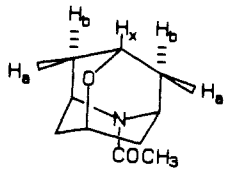

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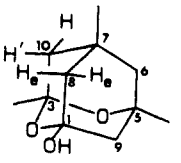

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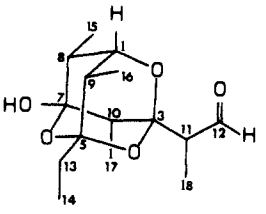

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methyl group is axial. The *E* geometry of the two trisubstituted olefins was established from the ^{13}C NMR shifts of the vinyl methyls at δ 12.3 and 16.7.[10] The relative stereochemistry at carbons 11 and 12 was not determined.

Pyridinium chlorochromate (PCC) oxidation[11] of muamvatin (**2**) gave one product, **5**, after loss of the diene portion of the side chain. The ^1H NMR spectrum of **5** contained an aldehydic proton signal at δ 10.06; in addition, the proton on C-11 had shifted from δ 1.97 in **2** to δ 2.57, and the C-24 methyl protons now resonated at δ 1.08 in the reaction product. Otherwise, the ^1H NMR spectrum of **5** was identical with that of the natural product. High-resolution FABMS data secured the formula of $C_{15}H_{24}O_5$, and this, in conjunction with the ^{13}C NMR data, confirmed the structure of the reaction product as **5**. The unexpected formation of **5** as the product instead of the simple oxidation of an allylic alcohol to a conjugated ketone may be explained by the presence of water and a small amount of chromic acid in the reaction mixture.[12] The chromic acid may electrophilically attack the allylic double bond to give a carbonium ion which could then react with another molecule of water to form a diol. Chromic acid attack on the diol to form a cyclic chromate ester and subsequent cleavage would give aldehyde **5**.

Muamvatin represents the first example of a tricyclic ketal to be isolated from the siphonariid molluscs; it is also the first reported naturally occurring 2,4,6-trioxaadamantane. Muamvatin, analogous to siphonarin A[5] and the denticulatins, cyclic hemiketals from *Siphonaria denticulata*,[4] exhibited no antibiotic activity against common test organisms. In fact, it is likely that the pyrone metabolites isolated from this family of molluscs are responsible for their antibiotic activity.[2,3] Although the role of **2** and other polypropionate metabolites remains in question, it is possible that these compounds are associated with the trail-following habits of *Siphonaria*.[13] The structure determination of this highly cyclized metabolite also demonstrates the utility of the two-dimensional ^{13}C-^1H polarization transfer NMR experiment, XCORFE, to indirectly establish the carbon skeleton of a molecule.

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Experimental Section

Infrared spectra were recorded on a Beckman Microlab 620MX spectrophotometer. UV spectra were recorded on a Beckman DU-8 spectrophotometer. Optical rotations were taken on a Perkin-Elmer 241MC polarimeter. Chemical-ionization and FAB mass spectra were recorded on Varian MAT 112 and MAT 731 spectrometers, respectively. ^1H and ^{13}C NMR spectra were obtained on JEOL JNM-FX270, Bruker WM-500, and IBM NR/200 FTNMR spectrometers; chemical shifts are reported relative to Me_4Si. A Varian XL-400 spectrometer having a carbon radio frequency field strength of 21 kHz (12 kHz for protons) operating at a probe temperature of 14 °C was used for the XCORFE experiment. The data matrix was 14 206.4 Hz (4096 points) in the carbon domain by 2469.1 Hz (256 increments) in the proton domain. One hundred and twenty-eight transients were collected at a pulse repetition rate of 2.144 s for a total acquisition time of about 20 h. The data matrix was zero filled to 8192 by 512, and a Lorentzian to Gaussian transformation was applied before Fourier transformation. The exponential time constants were 35 and 12 ms, and the Gaussian time constants were 55 and 35 ms for the carbon and proton dimensions, respectively.

Isolation. Approximately 3500 specimens of *S. normalis* were collected by hand in the intertidal zone at Muamvatu, Fiji, and frozen. The animals were lyophilized (455-g dry weight) and extracted with MeOH. The MeOH was decanted and evaporated to a watery residue which was then partitioned successively with 10% aqueous MeOH/hexanes, 20% aqueous MeOH/CCl_4, and 40% aqueous MeOH/$CHCl_3$. The majority of muamvatin was concentrated in the CCl_4 extract, although small amounts could also be recovered from both the hexanes and $CHCl_3$ partitions. The appropriate fractions were chromtographed twice on Bio-Beads S-X12 (hexanes/CH_2Cl_2, 7:3) to give 1.5 g of a brown oil. Further purification of the oil on silica gel (TMP/EtOAc, 7:3) followed by trituration with acetonitrile to remove the bulk of the sterols gave 314 mg of a pale-green solid. Final separation by HPLC (Whatman Partisil columns, TMP/EtOAc, 7:3) yielded 65.2 mg of **2** as a colorless oil (0.01% dry weight).

Muamvatin (2): $[\alpha]^{25}_D$ +61.1° (*c* 0.175, CH_2Cl_2); IR ($CHCl_3$) 3440, 2930, 1455, 1120, 1005 cm^{-1}; UV (CH_2Cl_2) λ_{max} 236 nm (ϵ 10244); ^1H and ^{13}C NMR ($CDCl_3$) see Table I; high-resolution FABMS, observed *m/z* 377.2708, $C_{23}H_{38}O_5$ requires 377.2692.

PCC Oxidation of 2 to 5. PCC (22 mg) and NaOAc (16 mg) were suspended in CH_2Cl_2 (10 mL), and the mixture was refluxed. A solution of **2** (10 mg) in CH_2Cl_2 (2 mL) was added and the reflux continued for 24 h. The reaction mixture was filtered through a silica Sep-Pak and purified by HPLC (Partisil, TMP/EtOAc, 7:3) to give **5** as an oil: 2.6 mg, 36%; $[\alpha]^{25}_D$ +50.2° (*c* 0.0917, CH_2Cl_2); IR ($CHCl_3$) 3565, 2910, 2850, 1724, 1675, 1450, 1117, 989, 970 cm^{-1}; ^1H NMR ($CDCl_3$, 270 MHz) δ 10.06 (1 H, s), 3.81 (1 H, br s), 2.60 (1 H, br s, D_2O exch), 2.57 (1 H, q, *J* = 6.59 Hz), 2.04 (1 H, q, *J* = 7.25 Hz), 1.94 (1 H, br q, *J* = 7.25 Hz), 1.74 (1 H, q, *J* = 7.25 Hz), 1.67 (1 H, q, *J* = 7.25 Hz), 1.60 (1 H, q, *J* = 7.25 Hz), 1.14 (3 H, d, *J* = 7.25 Hz), 1.08 (3 H, d, *J* = 6.59 Hz), 1.06 (3 H, d, *J* = 7.25 Hz), 1.03 (3 H, d, *J* = 7.25 Hz), 0.97 (3 H, t, *J* = 7.25 Hz); ^{13}C NMR ($CDCl_3$, 67.8 MHz) δ 203.3 (d), 105.4 (s), 103.1 (s), 97.2 (s), 78.9 (d), 50.5 (d), 43.1 (d), 37.4 (d), 34.4 (d), 29.6 (t), 13.4 (q), 13.1 (q), 7.0 (q), 6.6 (q), 5.8 (q); high-resolution FABMS, observed *m/z* 285.1692, $C_{15}H_{24}O_5$ requires 285.1702.

Acknowledgment. We thank Dr. Uday Raj and the Institute of Marine Resources, University of the South Pacific, for use of their facilities. This study was supported in part by a grant from the National Institutes of Health to C.M.I. (CA 36622). D.M.R. was supported in part by a NCI Graduate Training Stipend in Cancer Chemotherapy (3 T32 CA09038-05S1). The Varian XL-400 spectrometer was purchased through grants supplied by the National Science Foundation (CHE-8314771) and the National Institutes of Health (1S10RR01539).

Registry No. 2, 104013-99-2; **5**, 103982-29-2.