Metabolites of *Siphonaria maura* from Costa Rica

Denise C. Manker and D. John Faulkner*

Scripps Institution of Oceanography (A-012F), University of California, San Diego, La Jolla, California 92093

Xe Chang-fu and Jon Clardy*

Department of Chemistry—Baker Laboratory, Cornell University, Ithaca, New York 14853-1301

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Specimens of *Siphonaria maura* from Jaco Beach, Costa Rica, contained the mildly antimicrobial maurapyrones A–D (**5**–**8**) and an unrelated polypropionate metabolite maurenone (**9**). The structure of racemic maurapyrone A (**5**) was determined by X-ray analysis. Maurapyrone B (**6**) is a racemic diastereoisomer of maurapyrone A (**5**) and maurapyrones C (**7**) and D (**8**) are likewise a pair of racemic diastereoisomers. The structure of maurenone (**9**) is defined as fully as possible from the spectral data.

Pulmonate molluscs of the genus *Siphonaria* closely resemble limpets. Both limpets and siphonariids live on intertidal rocks from which they forage microalgae, but only the siphonariids possess polypropionate metabolites.[1] Typical examples are diemenensin (**1**) from *Siphonaria diemenensis*,[1a] denticulatin A (**2**) from *S. denticulata*,[1b] and siphonarins A (**3**) and B (**4**) from both *S. zelandica* and *S. atra*[1c] (Chart I). Specimens of *Siphonaria maura*[2] from Costa Rica contained five new polypropionate metabolites, consisting of two pairs of racemic diastereoisomers, maurapyrones A–D (**5**–**8**) and an unrelated compound, maurenone (**9**).

Specimens (230) of *S. maura* from Jaco Beach, Costa Rica, were extracted with acetone. Repeated chromatography of the ethyl acetate soluble material from the acetone extract gave maurapyrone A (**5**; 3 mg), maurapyrone B (**6**; 4 mg), maurapyrone C (**7**; 1 mg), maurapyrone D (**8**; 2.5 mg), and maurenone (**9**; 5.3 mg). Although the diastereoisomeric bis-pyrones could be separated by LC using a normal-phase system, separation of the homologous pairs required a reversed-phase system.

Maurapyrone A (**5**) was obtained as a colorless crystalline solid, mp 110–112 °C. At first sight, the ^{1}H NMR spectrum appeared almost too simple for a compound of molecular formula $C_{26}H_{36}O_5$ and implied some element of symmetry. The infrared spectrum indicated the presence of a ketone (1730 cm^{-1}) and a γ-pyrone ring system (1650, 1600 cm^{-1}). The ultraviolet absorption at 259 nm (ϵ 17 000) was sufficiently intense to require two γ-pyrone rings per molecule. The four methyl signals at δ 1.86, 1.87, 1.90, and 1.91 (all s, 3 H) in the ^{1}H NMR spectrum were therefore assigned to β-methyl groups on two γ-pyrone rings. The remaining signals in the ^{1}H NMR spectrum were interpreted as an ethyl and an *n*-pentyl group attached to separate γ-pyrone rings that were joined by a 2,4-disubstituted 3-pentanone moiety. Spectroscopic studies failed to define the sterochemistry at the two chiral centers (C8 and C10) and an X-ray experiment was therefore performed.

Figure 1 is a computer-generated perspective drawing of the final X-ray model of maurapyrone A (**5**). Hydrogen atoms are omitted for clarity, and the material analyzed was a racemic mixture. The molecule, a 3-pentanone substituted on the 2- and 4-carbons with 6-alkyloxo-2-pyranyl groups, has an approximate mirror plane perpendicular to the 3-pentanone chain. The pyrone rings are roughly parallel, oriented in the same direction, and separated by 3.5 Å. The pyrone rings are oriented such that the pyrone oxygen is staggered with respect to the central carbonyl carbon and the methyl groups. The C9–C10–C11–C12 torsional angle is 127°, and the C6–C7–C8–C9 torsional angle is −115°. The ethyl side chain is in the plane of the ring, while the pentyl side chain is in a fully extended conformation with the best plane of the pentyl group orthogonal to the plane of the pyrone ring. Both side chains have large thermal parameters, and the molecular geometry in these regions deviates from generally accepted values.

Chart I

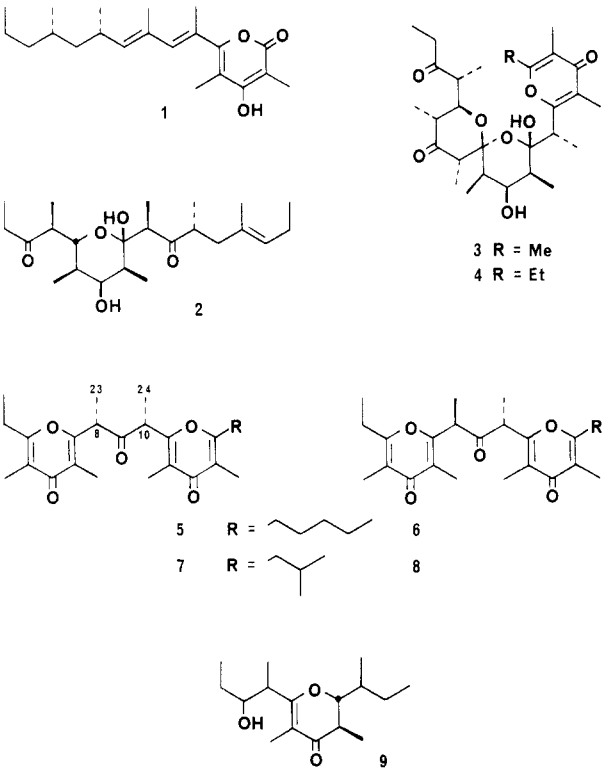

Table I. Selected 360-MHz ^{1}H NMR Data for Maurapyrones A–D (5–8)

HC locant	5	6	7	8	
8 or 10	3.985	3.805	3.975	3.80	(q, 1 H, J = 7.3 Hz)
	3.993	3.815	3.987	3.82	(q, 1 H, J = 7.3 Hz)
23 or 24	1.425	1.358	1.425	1.35	(d, 3 H, J = 7.3 Hz)
	1.43	1.357	1.43	1.35	(d, 3 H, J = 7.3 Hz)

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(2) Keen, M. "Sea Shells of Tropical West America. Marine Molluscs from Baja California to Peru", 2nd ed.; Stanford University Press, Stanford, CA, 1971; p 852, entry 2422.

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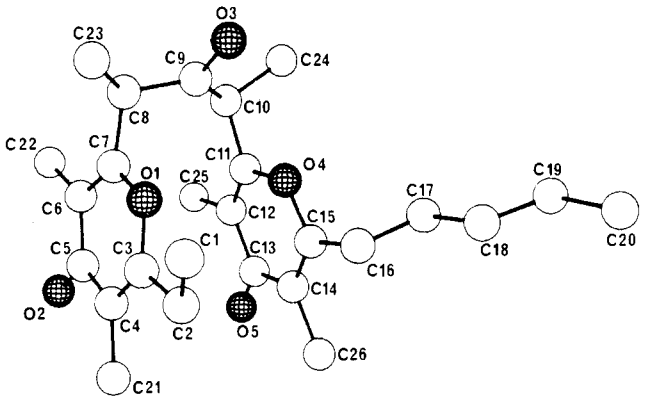

Figure 1. A computer-generated perspective drawing of the final X-ray model of maurapyrone A (**5**). Hydrogens are omitted for clarity. The absolute configuration drawn is meaningless since maurapyrone A is racemic.

Maurapyrone B (**6**) is a stereoisomer of maurapyrone A (**5**). Their spectral data, particularly the ^1H NMR spectra, were almost identical. Since mauropyrone A (**5**) has the racemic 8*S**,10*R** stereochemistry, maurapyrone B (**6**) is assigned the alternative 8*S**,10*S** stereochemistry and is also racemic. This structural assignment was confirmed when it was observed that each of the pure maurapyrones slowly isomerized to a mixture of diastereoisomers.

Maurapyrones C (**7**) and D (**8**) are a similar pair of racemic diastereoisomers of molecular formula $C_{25}H_{34}O_5$. The ^1H NMR spectra indicated that the *n*-pentyl side chain in **5** and **6** was replaced by an isobutyl side chain in **7** and **8**. Comparison of the chemical shifts for the H-8, H-10, H_3-23, and H_3-24 signals (Table I) allowed assignment of the 8*S**,10*R** stereochemistry to maurapyrone C (**7**) and the 8*S**,10*S** stereochemistry to maurapyrone D (**8**).

Maurenone (**9**) is an oil of molecular formula $C_{16}H_{28}O_3$. Although maurenone (**9**) is not closely related to the maurapyrones, the presence of six methyl signals in the ^1H NMR spectrum suggested a polypropionate structure. The presence of a β-alkoxy-α,β-dialkyl-α,β-unsaturated ketone was indicated by infrared bands at 1655 and 1610 cm^{-1} and an ultraviolet absorption at 276 nm. Maurenone also contained a hydroxyl group (IR 3490 cm^{-1}). The structure of maurenone was elucidated from ^1H NMR data that was assigned as shown in Table II. The relative stereochemistry at C8 and C9 was defined by the magnitude of the coupling constant $J_{8,9}$ = 12.3 Hz. No other stereochemical details could be defined from the spectral data. The ^{13}C NMR spectrum is completely in accord with the proposed structure.

Maurapyrones A–D (**5–8**) exhibited mild antimicrobial activity against the marine bacterium *Vibrio anguillarum*. In an effort to obtain more of these compounds for feeding inhibition assays, additional collections of *Siphonaria maura* were made at Punta Judas, Costa Rica (30 km south of Jaco Beach), and Punta Mita, Nr. Puerto Vallarta, Mexico. The Punta Judas specimens of *S. maura* were devoid of polypropionate compounds, while those from Punta Mita contain different polypropionate metabolites.

Experimental Section

Specimens (230) of *Siphonaria maura* (average length = 23 mm) were collected intertidally at Jaco Beach, Costa Rica. The animals were stored in acetone at 4 °C for 4 weeks. The acetone was decanted and evaporated to obtain an aqueous phase, which was extracted with ethyl acetate (4 × 50 mL). The organic extracts were combined, dried over sodium sulfate, and evaporated to yield a green oil (220 mg). The oil was chromatographed on silica by using eluants of increasing polarity from hexane to ether to ethyl acetate. Fractions eluted with ethyl acetate contained the UV active compounds **5–9**. A mixture of compounds **5** and **7** were rechromatographed by LC on Partisil using 2:9:9 isopropyl alcohol–hexane–ethyl acetate as eluant and then by LC on C-18 ODS using 25% water in methanol as eluant to obtain maurapyrone A (**5**; 3 mg) and maurapyrone C (**7**; 1 mg). Another fraction containing a mixture of compounds **6** and **8** was rechromatographed first by LC on Partisil using 2:19:19 isopropyl alcohol–hexane–ethyl acetate as eluant and then by LC on C-18 ODS using 15% water in methanol as eluant to obtain maurapyrone B (**6**; 4 mg) and maurapyrone D (**8**; 2.5 mg). The fraction containing compound **9** was rechromatographed by LC on Partisil using 30% ethyl acetate in hexane as eluant to obtain maurenone (**9**; 5.3 mg).

Table II. 360-MHz ^1H NMR Data for Maurenone (9)

HC locant	chem shift, ppm	mult	integration	*J*, Hz
1	0.95	t	3 H	7.4
2	1.40	m	2 H	
3	3.65	ddd	1 H	7.8, 6.5, 3.3
4	2.78	qd	1 H	6.9, 6.5
8	2.49	dq	1 H	12.3, 7
9	3.80	dd	1 H	12.3, 3
10	1.74	m	1 H	
11	1.5	m	2 H	
12	0.98	t	3 H	7.5
13	1.22	d	3 H	6.9
14	1.74	s	3 H	
15	1.08	d	3 H	6.9
16	1.05	d	3 H	6.9

After standing at 4 °C for 2 months, pure samples of compounds **5–8** had isomerized into their respective equilibrium mixtures (~1:1).

Maurapyrone A (5): mp 110–112 °C; IR ($CHCl_3$) 1730, 1650, 1600 cm^{-1}; UV (MeOH) 259 nm (ε 17 000); ^1H NMR ($CDCl_3$) δ 0.88 (t, 3 H, *J* = 7.3 Hz), 1.15 (t, 3 H, *J* = 7.9 Hz), 1.3 (m, 4 H), 1.43 (d, 3 H, *J* = 7.3 Hz), 1.425 (d, 3 H, *J* = 7.3 Hz), 1.55 (m, 2 H), 1.862 (s, 3 H), 1.87 (s, 3 H), 1.904 (s, 3 H), 1.91 (s, 3 H), 2.38 (m, 1 H), 2.47 (m, 1 H), 2.55 (m, 2 H), 3.985 (q, 1 H, *J* = 7.3 Hz), 3.993 (q, 1 H, *J* = 7.3 Hz); mass spectrum, *m/z* 428.2565, $C_{26}H_{36}O_5$ requires 428.2563.

Maurapyrone B (6): wax; IR ($CHCl_3$) 1730, 1650, 1600 cm^{-1}; UV (MeOH) 257 nm (ε 19 700); ^1H NMR δ 0.91 (t, 3 H, *J* = 7.3 Hz), 1.16 (t, 3 H, *J* = 7.6 Hz), 1.3 (m, 4 H), 1.357 (d, 3 H, *J* = 6.9 Hz), 1.358 (d, 3 H, *J* = 6.9 Hz), 1.57 (m, 2 H), 1.835 (s, 3 H), 1.84 (s, 3 H), 1.94 (s, 6 H), 2.54 (t, 2 H, *J* = 7.6 Hz), 2.58 (q, 2 H, *J* = 7.6 Hz), 3.805 (q, 1 H, *J* = 6.9 Hz), 3.815 (q, 1 H, *J* = 6.9 Hz); mass spectrum, *m/z* 428.2561, $C_{26}H_{36}O_5$ requires 428.2563.

Maurapyrone C (7): wax; IR ($CHCl_3$) 1730, 1650, 1600 cm^{-1}; UV (MeOH) 254 nm (ε 21 600); ^1H NMR δ 0.91 (d, 6 H, *J* = 6.3 Hz), 1.14 (t, 3 H, *J* = 7.3 Hz), 1.425 (d, 3 H, *J* = 6.9 Hz), 1.43 (d, 3 H, *J* = 6.9 Hz), 1.87 (s, 6 H), 1.91 (s, 3 H), 1.915 (s, 3 H), 2.22 (m, 1 H), 2.42 (dd, 1 H, *J* = 7.5, 3.1 Hz), 2.46 (dd, 1 H, *J* = 7.5, 3.8 Hz), 2.56 (m, 2 H), 3.975 (q, 1 H, *J* = 6.7 Hz), 3.987 (q, 1 H, *J* = 6.9 Hz); mass spectrum, *m/z* 414.2408, $C_{25}H_{34}O_5$ requires 414.2406.

Maurapyrone D (8): oil; IR ($CHCl_3$) 1730, 1650, 1600 cm^{-1}; UV (MeOH) 253 nm (ε 17 900); ^1H NMR δ 0.93 (d, 3 H, *J* = 7.3 Hz), 0.96 (d, 3 H, *J* = 7.3 Hz), 1.18 (t, 3 H, *J* = 7.3 Hz), 1.35 (d, 6 H, *J* = 7.3 Hz), 1.835 (s, 3 H), 1.84 (s, 3 H), 1.94 (s, 3 H), 1.948 (s, 3 H), 2.0 (m, 1 H), 2.41 (m, 2 H), 2.57 (q, 2 H, *J* = 7.3 Hz), 3.80 (q, 1 H, *J* = 7.3 Hz), 3.82 (q, 1 H, *J* = 7.3 Hz); mass spectrum, *m/z* 414.2400, $C_{25}H_{34}O_5$ requires 414.2406.

Maurenone (9): oil; IR ($CHCl_3$) 3490, 1655, 1610, 1460 cm^{-1}; UV (MeOH) 276 nm (ε 13 400); ^1H NMR ($CDCl_3$) δ 0.95 (t, 3 H, *J* = 7.4 Hz), 0.98 (t, 3 H, *J* = 7.5 Hz), 1.05 (d, 3 H, *J* = 6.9 Hz), 1.08 (d, 3 H, *J* = 6.9 Hz), 1.22 (d, 3 H, *J* = 6.9 Hz), 1.40 (m, 2 H), 1.74 (s, 3 H), 2.49 (m, 1 H, *J* = 12.3, 7 Hz), 2.78 (dq, 1 H, *J* = 7, 6.5 Hz), 3.65 (m, 1 H), 3.8 (dd, 1 H, *J* = 12.3, 3.2 Hz); ^{13}C NMR ($CDCl_3$) δ 9.4 (q), 10.3 (q), 10.7 (q), 11.8 (q), 13.2 (q), 16.2 (q), 22.0 (t), 28.0 (t), 35.1 (d), 40.6 (d), 41.6 (d), 75.2 (d), 87.0 (d), 172.9 (s), 208.2 (s); mass spectrum, *m/z* 268.2033, $C_{16}H_{28}O_3$ requires 268.2038.

Single-Crystal X-ray Diffraction Analysis of Maurapyrone A (5): Suitable crystals were obtained from ether/hexane

solution. Preliminary X-ray photographs displayed monoclinic symmetry, and precise lattice constants of a = 38.725 (8) Å, b = 10.727 (2) Å, c = 13.231 (4) Å, and β = 67.88 (2)° were determined from a least-squares analysis of 15 diffractometer measured 2θ values. The crystal density (~1.12 g/cm^3) indicated that eight molecules of composition $C_{26}H_{36}O_5$ were in this unit cell. Systematic absences were consistent with either space group Cc or $C2/c$. The latter choice ultimately proved to be the correct one through successful refinement. This requires that **5** be a racemic mixture. All unique diffraction maxima with $2\theta \leq 114°$ were collected on a four-circle computer-controlled diffractometer using graphite monochromated Cu K$\bar{\alpha}$ radiation (1.54178 Å) and variable speed, 1° ω scans. Of the 3434 reflections collected in this fashion, only 1538 (45%) were judged observed ($|F_o| \geq 3\sigma(F_o)$) and used in subsequent calculations.[3] A phasing model was found uneventfully using the MULTAN family of programs, and all non-hydrogen atoms were located on E-syntheses. Hydrogens were located on difference syntheses following partial refinement. Block diagonal least-squares refinements with anisotropic non-hydrogen atoms and isotropic hydrogens have converged to a standard crystallographic residual of 0.0597 for the observed reflections. A final difference map showed no anomalous electron density. Additional crystallographic details are available and are described in the supplementary material paragraph at the end of this manuscript.

(3) All crystallographic calculations were done on a PRIME 9950 computer operated by the Cornell Chemistry Computing Facility. Principal programs employed were: REDUCE and UNIQUE, data reduction programs by M. E. Leonowicz, Cornell University, 1978; MULTAN 78, MULTAN 80, and RANTAN 80, systems of computer program for the automatic solution of crystal structures from X-ray diffraction data (locally modified to perform all Fourier calculations including Patterson syntheses) written by P. Main, S. E Hull, L. Lessinger, G. Germain, J. P. Declercq, and M. M. Woolfson, University of York, England, 1978 and 1980; BLS78A, an anisotropic block diagonal least-squares refinement written by K. Hirotsu and E. Arnold, Cornell University, 1980; PLUTO78, a crystallographic illustration program by W. D. S. Motherwell, Cambridge Crystallographic Data Centre, 1980; BOND, a program to calculate molecular parameters and prepare tables written by K. Hirotsu and G. Van Duyne, Cornell University, 1985.

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Registry No. **5**, 100082-10-8; **6**, 100046-03-5; **7**, 100046-04-6; **8**, 100046-05-7; **9**, 100046-02-4.

Supplementary Material Available: Tables of fractional coordinates, thermal parameters, interatomic distances, and interatomic angles for **5** (4 pages). Ordering information is given on any current masthead page.

Mechanism of Acid-Catalyzed Anomerization of Methyl D-Glucopyranosides

James L. Jensen,* Sho-Chein Tsuang, and Arif H. Uslan[1]

Chemistry Department, California State University, Long Beach, California 90840

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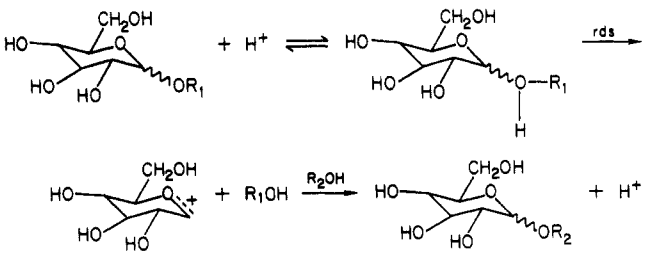
The anomerization of methyl D-glucopyranosides catalyzed by D_2SO_4 in Me_2SO/CD_3OD solvent was monitored by proton magnetic resonance and by changes in optical rotation. The rate of anomerization was found to be zero order in methanol, yet methanol (or a similar nucleophilic species) was required to effect anomerization. It has been shown that the solvent plays a dominant role in controlling the stereochemistry of reactions at the anomeric carbon; however, the transition-state interactions must be between solvent cage molecules and substrate and are thus solvational forces rather than a more direct nucleophilic participation inside the solvent cage. Evidence is presented supporting the notion that glucoside hydroxyls orient solvent cage molecules to assist attaining a suitable transition-state structure.

The process by which glycosides hydrolyze or exchange algyons has received much attention, and the mechanism generally cited for this process is A-1; for the glucosides, exocyclic C–O bond cleavage has always been observed.[2,3]

For example, Capon[4] has shown that acid-catalyzed anomerization of methyl D-glucopyranosides in methanol-d_4 occurs with incorporation of a solvent CD_3O moiety at the anomeric carbon. This requires in Scheme I that R_1OH (CH_3OD in the Capon experiment) not to be able

Scheme I



to produce the anomeric product. In other words, CH_3OD either recombines with the oxycarbocation to reform the reactant anomer or becomes lost in bulk solvent. This is perhaps not a surprising result when methanol is the solvent.

The purpose of the present investigation is to separate the effects of methanol acting as solvent and as reagent,

(1) Present address: Faculty of Chemistry, Ege University, Bornova-Izmir, Turkey.

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