Tridachiapyrones: Propionate-Derived Metabolites from the Sacoglossan Mollusc *Tridachia crispata*[1]

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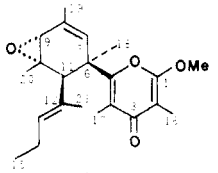
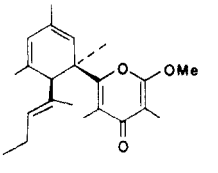
Ten related C-22 and C-25 metabolites, eight of them new and designated tridachiapyrones, have been isolated from the sacoglossan mollusc *Tridachia crispata* collected in Jamaica. The tridachiapyrones have propionate-derived skeletons and have in common a γ-pyrone moiety. The new metabolites are related to tridachione and crispatene that possess, respectively, photochemically related cyclohexadiene and bicyclo[3.1.0]hexene skeletal features. Structures were determined from ^1H and ^{13}C NMR data. Several of the tridachiapyrones exhibit cytotoxicity against lymphocytic leukemia cells.

Investigation of possible chemical defense resources of the shell-less and hence, vulnerable, opisthobranch molluscs has resulted in the recovery of a spectrum of novel metabolites.[2] The origins of these metabolites are varied. Thus, sea hares sesquester chemicals from their algal diet,[2a,b,g,3] while some nudibranchs store sponge metabolites.[2b,f,4] On the other hand, sacoglossan molluscs assimilate chloroplasts from siphonaceous marine algae and maintain these organelles in their own tissues where they carry out photosynthesis.[5] Studies by Faulkner and collaborators[6-8] of two such sacoglossans, *Tridachiella diomedea* and *Tridachia crispata*, resulted in the identification of a novel group of propionate-derived γ-pyrones, namely **1** and **2** from the former and **3** and **4** from the latter. In vitro experiments by Ireland and Faulkner[8] and



1



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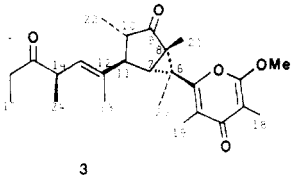

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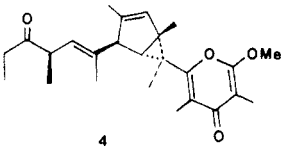

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in vivo experiments by Ireland and Scheuer[9] demonstrated that the cyclohexadiene ring system of **2** was converted photochemically to the bicyclohexene ring system of **4**. Since there are many instances of chemical variation related to geographical location in other mollusks dependent on an algal diet, we decided to investigate the metabolites of *T. crispata* from Jamaica for comparison with those from specimens collected at Belize and Panama by Ireland and Faulkner. We have isolated the known metabolites **3** and **4** obtained earlier from *T. crispata* as the major metabolites, but in addition we have isolated in trace amounts eight new related γ-pyrones and their structures are described in this paper. The chemistry of *T. crispata* from Jamaica illustrates the presence of propionate homologues in specimens from one location.

Table I

compd	formula	M^+	IR, cm^{-1}	UV: λ nm (ε)
5	$C_{25}H_{34}O_4$	398	1715, 1660, 1585	255 (11 800)
6	$C_{25}H_{34}O_4$	398	1715, 1660, 1585	253 (11 900)
7	$C_{25}H_{34}O_5$	414	1715, 1655, 1585	254 (5890)
8	$C_{25}H_{34}O_6$	430.24209	3450, 1712, 1660, 1585	255 (5900)
9	$C_{22}H_{28}O_4$	356	1690, 1660, 1585	255 (5800), 225 (7500)
10	$C_{22}H_{28}O_5$	372	1725, 1690, 1665, 1595	255 (5890), 225 (7400)
11	$C_{25}H_{32}O_5$	412	1710, 1660, 1650, 1590	248 (11 900)
12	$C_{25}H_{32}O_5$	412	1710, 1660, 1650, 1590	248 (11 900), 255 (5900)

Crispatone (**3**) and crispatene (**4**) were identified by spectral comparisons with literature values.[8] The molecular formulas for the eight new metabolites (see Table I) were established by high-resolution mass spectrometry (**8**), low-resolution mass measurements combined with ^{13}C and ^1H NMR, indicating the assigned carbon and proton count (**5**, **11**, **12**), or low-resolution mass measurements with proton count from ^1H NMR (**6**, **7**, **9**, **10**). Infrared and ultraviolet data provided supporting evidence for the oxygen content of all compounds for which high-resolution mass data were not available (see Table I). The presence of the α-methoxy-β,β′-dimethyl-γ-pyrone moiety A, characteristic of **1**–**4**[9] was confirmed for all the new metabolites by IR (1660, 1585 cm^{-1}), UV (λ 250 nm (ε 5900)), ^1H NMR data (see Tables II and III), and, in some instances, ^{13}C data (**5**, **11**, **12**; see Table IV). ^1H NMR assignments for the γ-pyrone methyls and ^{13}C values for all of the γ-pyrone carbons are based on analogy with literature values.[10] ^1H

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(2) For some recent references see: (a) Faulkner, D. J. *Nat. Prod. Rep.* **1984**, *1*, 251. (b) Faulkner, D. J.; Ghiselin, M. T. *Mar. Ecol. Prog. Ser.* **1983**, *13*, 295. (c) Gustafson, K.; Anderson, R. J.; Chen, M. H. M.; Clardy, J.; Hochlowski, J. *Tetrahedron Lett.* **1984**, 11. (d) Ayer, S. A.; Hellou, J.; Tischler, M.; Andersen, R. J. *Tetrahedron Lett.* **1984**, 141. (e) Ireland, C. M.; Biskupiak, J. E.; Hite, G. J.; Rapposch, M.; Scheuer, P. J.; Ruble, J. R. *J. Org. Chem.* **1984**, *49*, 559. (f) Hochlowski, J. E.; Walker, R. P.; Ireland, C.; Faulkner, D. J. *J. Org. Chem.* **1982**, *47*, 88. (g) Schmitz, F. J.; Michaud, D. P.; Schmidt, P. G. *J. Am. Chem. Soc.* **1982**, *104*, 6415.

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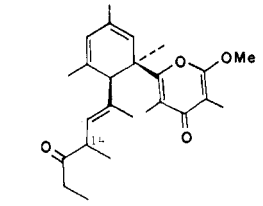
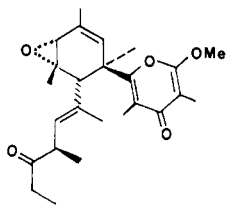
Table II. 300-MHz ^1H NMR Data (δ) in $CDCl_3$ of Crispatone (3), Crispatene (4), and the Tridachiapyrones 5–8, 11, and 12

	3	4	5	6	7	8	11	12
H-7	1.89 (br s)	1.41 (br s)	5.61 (br s)	5.64 (br s)	5.50 (br s)	5.47 (br s)	6.60 (br s)	6.63 (br s)
H-9		5.39 (br s)	5.71 (br s)	5.69 (br s)	3.0 (br s)	3.16 (br s)		
H-10	2.37 (q), 7.2							
H-11	2.40 (br s)	2.80 (br s)	2.80 (br s)	2.78 (br s)	3.40 (br s)	2.99 (q), 2.0		
H-13	5.37 (br d), 8	5.26 (br d), 8	5.07 (br d), 9.5	4.97 (br d), 9.8	5.41 (br d), 9.2	4.42 (dq), 7, 2	4.96 (br d), 8	4.99 (br d), 8
H-14	3.45 (dq), 8, 7	3.45 (dq), 8, 7	3.20 (dq), 9.5, 7	3.16 (dq), 9.8, 6.5	3.50 (m)	2.78 (quintet), 7	3.39 (dq), 8, 7	3.37 (dq), 8, 7
H-15								
H-16	2.47 (m)	2.47 (m)	2.11 (m)	2.34 (m)	2.46 (m)	2.48 (m)	2.32 (m)	2.41 (m)
H-17	1.05 (t), 7	1.04 (t), 7	0.88 (t), 7	0.98 (t), 7.1	1.05 (t), 7	1.03 (t), 7.2	0.99 (t), 7	1.01 (t), 7
H-18	1.83 (s)	1.84 (s)	1.81 (s)	1.80 (s)	1.85 (s)	1.86 (s)	1.85 (s)	1.84 (s)
H-19	1.95 (s)	1.95 (s)	2.10 (s)	2.08 (s)	2.02 (s)	2.25 (s)	1.85 (s)	1.84 (s)
H-20	1.20 (s)	1.11 (s)	1.42 (s)	1.43 (s)	1.17 (s)	1.45 (s)	1.63 (s)	1.66 (s)
H-21	1.24 (s)	1.20 (s)	1.80 (br s)	1.79 (br s)	1.95 (br s)	1.53 (br s)	1.94 (br s)	1.95 (br s)
H-22	1.05 (d), 7.2	1.61 (br s)	1.75 (br s)	1.69 (br s)	1.32 (s)	1.56 (s)	1.72 (s)	1.72 (s)
H-23	1.83 (br s)	1.58 (br s)	1.44 (br s)	1.46 (br s)	1.58 (br s)	4.92 (t), 2 4.84 (t), 2	1.70 (br s)	1.67 (br s)
H-24	1.17 (d), 7	1.16 (d), 7	1.05 (d), 7	0.75 (d), 6.5	1.20 (d), 7	1.13 (d), 7	1.17 (d), 7	1.08 (d), 7
OMe	3.95 (s)	3.94 (s)	3.98 (s)	3.97 (s)	3.98 (s)	3.94 (s)	3.96 (s)	3.93 (s)

NMR assignments for the γ-pyrone methyls in 8 and 9 (see below) were confirmed by NOE results.



5 (14R*)
6 (14S*)



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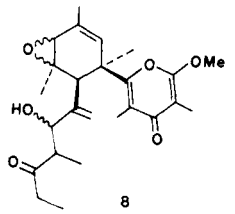

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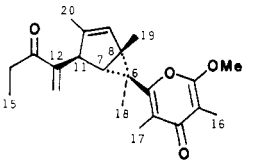

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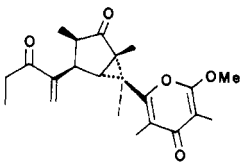

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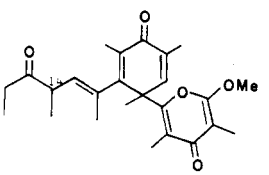
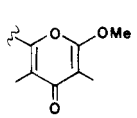
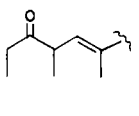


11 (14R*)
12 (14S*)

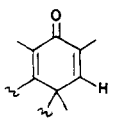
Five of the compounds, 5–7, 11, and 12, have in common the side chain B. In each instance the terminal ethyl



A



B



C

ketone of B was substantiated by IR absorption at 1715 cm^{-1} and typical triplet and quartet ^1H NMR signals at approximately 1 and 2.5 ppm. In most cases the allylic methine proton of B was sharpened on irradiation of the methylene resonance, confirming linkage of the terminal propionyl group to the methine carbon. The remainder of partial formula B was established by decoupling.

(10) Kakinuma, K.; Hanson, C. A.; Rinehart, K. L. *Tetrahedron* **1976**, *32*, 217.

Two of the new metabolites, tridachiapyrone-A (5) and isotridachiapyrone-A (6),[11] proved to be identical with 9,10-deoxytridachione (2) except for the addition of a propionyl group in the side chain. In addition to spectral data confirming the presence of partial structures A and B, ^1H and ^{13}C data of 5 matched closely that of 2 for H and C-6 to C-11. Likewise, the NMR signals for H and C-20 to C-22 in 5 matched the analogous signals in 2, i.e., H and C-18 to C-20 (see Tables II–IV), thus suggesting that these two compounds possess the same cyclohexadiene framework.[12] UV absorption at 255 nm with an extinction coefficient equal to that of 2 provided additional evidence for the cyclohexadiene structure. The cyclohexadiene substitution pattern was established by demonstrating that H-11 was coupling to both H-9 and H-22 (J = 1.5 Hz), while both H-7 and H-9 exhibited small coupling (J = 1.5 Hz) to H-21. Nuclear Overhauser enhancements of the H-7 and H-11 signals were observed upon irradiation of H-20, thus confirming inclusion of the quaternary carbon C-6 in the cyclohexadiene ring and revealing a cis arrangement of H-11 and the quaternary methyl (H-20) at C-6. The similarlity in chemical shift for H-11 in 2 and 4–6 also argues for the same relative configurations at C-6 and C-11 in these compounds (see also arguments below the relative stereochemistry of 6 and 7). A 12*E* configuration was confirmed by observation of a NOE of the H-14 signal upon irradiation of H-23. The ^1H chemical shift for the methyl group at C-14 in 5 is similar to that of the corresponding methyl group in 3 and 4, while in the C-14 epimer 6 this methyl signal is observed at significantly higher field. Hence, 5 is assigned the same configuration at C-14, i.e., (*R**) as 3 and 4.

Isotridachiapyrone-A (6), $C_{25}H_{34}O_4$, showed IR, UV, and mass spectral fragmentation virtually identical with that of 5, and the ^1H NMR spectra of the two compounds were the same except for minor differences associated with some side chain protons. Decoupling established the same ^1H connectivities as in 5. Similarly, the *E* geometry for C-12 and C-13 and a cis arrangement for H-11 and the C-6 methyl group was confirmed by the same NOE's as observed for 5. Also, the ^{13}C data for 6 and 2 at C-6 and C-11 are virtually identical (47.43, 47.6 and 59.67, 59.6 ppm,

(11) The names of these two compounds have been reversed relative to that given in ref 1. This is to allow consistency in assigning the iso designation to the 14*S** isomer in this series of compounds.

(12) The multiplicities for the carbon resonances at 46.7 (C-6) and 55.3 ppm (C-10) have been inadvertently incorrectly reported in ref 6 and 8 as d and s, respectively, for tridachione and should be reversed: Faulkner, D. J., private communication. With this correction all of the C-6 and C-10 chemical shifts in this series of compounds are compatible.

Table III. NMR Data of 1, 2, 9, and 10

	^{13}C			^{1}H							
	1[a]		2[a]	1[a]		2[a]		9[b]		10[b]	
C											
1	161.0	s	161.7								
2	97.8	s	98.8								
3	181.8	s	181.8								
4	118.4	s	120.0								
5	160.1	s	161.1								
6	46.7	s	47.6								
7	129.0[c]		124.3[f]	6.10	s	5.56	s	1.41	br s	1.83	br s
8	132.5	s	134.9[g]								
9	54.7	d	122.4[f]	3.11	s	5.65	s	5.45	br s		
10	60.5	s	132.2[g]							2.50	dq, 8.2, 7.1
11	55.3	d	59.6	2.91	s	2.71	s	3.52	br s	2.80	br d, 8.2
12	128.5	s	131.0[g]								
13	131.6[c]	d	127.8[g]	5.18	t, 7	5.09	t, 7				
14	31.2	t	26.9	2.08	m			2.80	q, 7	2.80	q, 7.1
15	12.0[d]	q	13.7[h]	0.68	t, 7	0.69	t, 7	1.15	t, 7	1.13	t, 7.1
16	6.1	q	6.8	2.07	s	1.81	s	1.84	s	1.82	s
17	11.6[d]	q	12.2[h]	2.0	s	2.03	s	1.98	s	1.95	s
18	12.9[d]	q	13.8[h]	1.32	s	1.32	s	1.19	s	1.26	s
19	20.2[e]	q	21.1[i]	1.80	s	1.69	s	1.16	s	1.25	s
20	21.5[e]	q	21.5[i]	1.36	s	1.76		1.58	br s	1.05	d, 7.1
21	21.8[e]	q	22.3[i]	1.60	s	1.42	s	5.72	s	5.92	s
								6.21	s	6.22	s
OMe	57.6		59.6	3.93	s	3.95	s	3.96	s	3.96	s

[a] Data from ref 8; ^{13}C NMR at 20 MHz in $CDCl_3$; ^{1}H NMR at 220 MHz in $CDCl_3$. [b] This work, 300 MHz, $CDCl_3$. [c–i] Assignments with identical superscripts may be interchanged.

Table IV. ^{13}C NMR Data of Crispatone (3), Crispatene (4), and Tridachiapyrones 6, 11, and 12[a]

	3		4		6		11		12	
C										
1	162.20	s	162.17	s	161.90	s	161.85	s	161.94	s
2	99.84	s	99.45	s	96.95	s	99.95	s	100.02	s
3	181.06	s	181.46	s	181.10	s	180.27	s	180.54	s
4	120.94	s	126.34	s	119.79	s	121.94	s	122.16	s
5	157.42	s	159.5	s	161.20	s	158.05	s	158.05	s
6	42.45	s	40.68	s	47.43	s	48.13	s	48.62	s
7	37.63	d	36.76	d	124.19	d	146.21	d	146.78	d
8	31.92	s	31.88	s	137.71	s	132.19[e]	s	132.20[g]	s
9	215.51	s	129.29	d	122.94	d	186.05	s	186.03	s
10	49.14	d	143.25	s	134.10	s	133.35[e]	s	133.28[g]	s
11	51.32	d	58.16	d	59.67	d	156.33	s	156.13	s
12	137.04	s	137.67	s	136.08	s	134.27[e]	s	133.71[g]	s
13	126.88	d	126.46	d	128.70	d	130.19	d	130.58	d
14	46.02	d	46.07	d	45.52	d	46.10	d	46.46	d
15	211.95	s	212.28	s	212.21	s	210.91	s	210.26	s
16	33.81	t	33.65	t	34.29	t	33.75	t	34.58	t
17	*b*		*c*		*d*		*f*		*h*	
18	6.82	q	6.76	q	6.72	q	6.97	q	7.03	q
19	10.87	q	12.92	q	12.24	q	12.68	q	12.70	q
20	*b*		*c*		*d*		*f*		*h*	
21	*b*		*c*		*d*		*f*		*h*	
22	*b*		*c*		*d*		*f*		*h*	
23	*b*		*c*		*d*		*f*		*h*	
24	*b*		*c*		*d*		*f*		*h*	
OMe	55.36	q	55.08	q	55.38	q	55.45	q	55.94	q

[a] At 75.4 MHz in $CDCl_3$; multiplicities established by DEPT experiments.[18] [b] Signals, all quartets, not assigned: δ 16.8, 16.0, 14.0, 11.4, 10.87, 7.9. [c] Signals, all quartets, not assigned: δ 16.59, 13.6, 13.35, 12.9, 10.7, 7.9. [d] Signals, all quartets, not assigned: δ 27.0, 22.38, 21.6, 16.1, 14.2, 8.0. [e] Assignments may be interchanged. [f] Signals, all quartets, not assigned: δ 24.2, 17.6, 16.7, 15.8, 9.0, 7.6. [g] Assignments may be interchanged. [h] Signals, all quartets, not assigned: δ 24.5, 17.5, 16.5, 15.9, 9.2, 7.8.

respectively), providing additional evidence for identity of configuration at these centers in these two compounds[13] and, by inference, in **5**. The configuration at C-14 is assigned as (*S**) on the basis of significant chemical shift differences of key resonances compared to those of the known compounds **3** and **4** (as well as to other related compounds reported in this paper). Thus, the proton absorption for the methyl group (H-24) attached to C-14 in **6** is markedly upfield from that of the corresponding methyls of **3** and **4** (as well as **5**, **7**, and **11**) (see Table I). Also, the absorption for C-14 in **6** is upfield by ~0.4 ppm from that of **3**, **4**, and **11**, whose C-14 resonances are essentially invariant in spite of structural differences in their cyclic moieties.

Tridachiapyrone-C (**7**), $C_{25}H_{34}O_5$, contained one more oxygen than **5** or **6**, possessed the diagnostic spectral data for γ-pyrone A and the side chain B, but showed UV absorption at 254 nm of only half the intensity of **5** and **6**, signaling the absence of a conjugated diene. The principal differences between the spectrum of **7** and **5** or **6** were upfield shifts of H-9 and H-22 in **7**, corresponding to the presence of a methyl-substituted epoxide instead of a vinyl methyl feature. Indeed, the H-9 and H-22 signals in **7** closely matched the corresponding signals (H-9 and H-20) for the epoxide group in **1**, indicating that **7** was a 14-propionyl analogue of **1**. Coupling between H-9 and H-11, H-7, and H-21 was observed, providing evidence for the substituted cyclohexene ring. Observation of an allylic coupling (1 Hz) between H-13 and H-11 established the location of the side chain on the cyclohexene ring. Nuclear Overhauser enhancement of both H-9 and H-11 upon irradiation of the methyl signlet at 1.32 ppm confirmed the H-22 resonance assignment and provided evidence for an all-cis arrangement for these groups. Biogenetic considerations and observations of NOE of H-7 by irradiation of the methyl singlet signal at 1.17 ppm (H-20) provided grounds for locating this quaternary methyl group at C-6. The 12*E* configuration was assigned on the basis of a NOE observed between H-23 (irradiated) and H-14. The configuration at C-14 is assumed to be the same as in crispatene since the chemical shifts of the protons associated with the side chain in these two compounds are very similar. However, at C-11 we propose that the configuration

(13) For sensitivity of ^{13}C chemical shift to configurational differences, see, for example: Levy, G. C.; Lichter, R. L.; Nelson, G. L. "Carbon-13 Nuclear Magnetic Resonance spectroscopy"; Wiley: New York, 1980; p 194 ff.

is epimeric to that of the closely related compound 1 as well as the similar compounds 5, 6, and 8. That is, in 7, H-11 and methyl-20 are trans to each other. This assignment is based on the marked downfield shift of H-11 and upfield shift of the quaternary methyl at C-6 in 7 relative to 1 as well as to 5, 6, and 8. While the absence of NOE cannot be used to assign stereochemistry, we think that in comparing this series of similar compounds, i.e., 5–8, the absence of NOE between H-11 and H-20 for only compound 7 is supportive of the configuration assigned on the basis of chemical shift differences.

For tridachiapyrone-D, 8, spectral data confirmed the presence of partial structure A, a hydroxyl group (IR, 3450 cm^{-1}; one exchangeable proton in ^1H NMR), and a side chain somewhat different from that present in 7. Broad, one-proton singlet absorptions at 5.47 and 3.16 ppm that corresponded closely to those of H-7 and H-9 in 7 and were coupled to each other ($J \simeq 1$ Hz) and also to a vinlylic methyl signal at 1.53 ppm (Me-21) indicated the presence of a cyclohexadiene epoxide ring as in 7. Nuclear Overhauser enhancement of H-9 upon irradiation of the epoxide methyl (H-22; 1.56 ppm) and a 2.1-Hz *W* coupling between H-9 and H-11 (2.99 ppm) supported this partial structure. Similarly, NOE of H-11 upon irradiation of H-20 (1.45 ppm) provided evidence for completion of the cyclohexadiene epoxide ring in 8 as in 7 and a cis arrangement of H-11 and H-20. A slight NOE was also observed for H-9 upon irradiation of the 2.25 ppm methyl signal (this confirms that this downfield methyl signal arises from the γ-pyrone C-4 methyl). However, examination of models indicates that both the *W* coupling between H-9 and H-11 and the NOE of H-9 by H-20 appear possible in one of the conformations of either a 9α,10α- or a 9β,10β-epoxide. Hence the epoxide configuration cannot be assigned.

Coupling of H-11 (J = 2.1 Hz) with the two terminal methylene protons (H-23, H-23′; 4.92, 4.84 ppm) established the joining of a side chain at C-11 as indicated in formula 8. The remainder of the side chain was confirmed by decoupling. The methine proton at 2.75 ppm was coupled slightly to a two-proton multiplet at 2.48, which was coupled to the methyl triplet at 1.03 ppm (H-17), thus confirming the connection of the propionyl group. The relative configurations at C-13 and C-14 were not established.

Tridachiapyrone-E (9) showed typical IR and UV and NMR absorptions for partial structure A. Comparison of the appropriate ^1H NMR absorptions for 9 and crispatene (4) revealed that these compounds possessed the same cyclic structures, cf. H-7, H-9, H-11, H-16 to H-20 and OMe in 9 with H-7, H-9, H-11, H-18 to H-22 and OMe in 4. The chemical shift difference for H-11 in 9 and 4 may be attributed to the difference in the side chains in the two compounds. Coupling of the H-11 proton, 3.52 ppm, to one of the terminal methylene protons, H-21 (5.72 ppm), provided evidence for connection of the side chain at this position, while the chemical shift of H-21 and H-21′ revealed their presence in an α,β-unsaturated ketone system. The latter was corroborated by IR absorption at 1690 cm^{-1} and UV absorption at 225 nm. A slight sharpening of the H-20 signal upon irradiation of the H-11 signal also supported the assigned structure 9. The remaining proton signals in the spectrum of 9, 1.15 (t) and 2.80 (q) ppm, indicated a terminal ethyl group attached to a carbonyl group corresponding to the remainder of the side chain. The lack of any noticeable coupling between H-7 and H-11 is attributed to a nearly 90° dihedral angle between these protons as in 4. A small *W* coupling (1 Hz) observed between H-7 and H-18 indicated a trans relationship between these groups as in 4, and hence the overall stereochemistry shown for 9 was confirmed. Irradiation of the 1.98 ppm methyl signal produced a slight NOE for one of the exocyclic methylene protons (6.21 ppm), thereby confirming the C-4 methyl signal assignment.

Tridachiapyrone-F (10) contains one more oxygen than 9. Comparison of the ^1H NMR data of 10 with that of 3 provided a basis for postulating the same cyclic skeletons for these two compounds, cf. H-7, H-10, H-11, H-16 to H-20 and OMe for 10 with H-7, H-10, H-11, H-18 to H-22 and OMe in 3. Minor differences in the H-10 and H-11 chemical shifts are readily attributable to differences in their side chains or C-10 configurations. Infrared absorption attributable to the α,β-cyclopropylcyclopentanone feature is observed in both compounds at 1725 cm^{-1}. The side chain for 10 was confirmed by decoupling and comparison of the proton chemical shifts of H-14 and H-15 and H-21 and H-21′ with those in 9. As was the case for 3 and 9, no coupling was observed between H-7 and H-11 and hence the stereochemistry at these centers is assumed to be the same in all three compounds. An 8.2-Hz coupling was observed between H-10 and H-11, and hence a cis 10,11 arrangement is assigned in contrast to the trans arrangement present in crispatone (3).

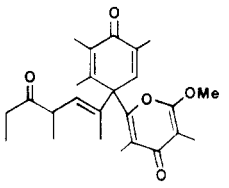
Tridachiapyrone-B (11) showed the typical IR and UV absorptions for partial structures A and B (see Table I), and these features were confirmed by comparing ^1H NMR absorptions (supported by decoupling) and ^{13}C resonances with analogous ones in compounds 3–7 (see Tables II and IV) (cf. H-13 to H-19 and OMe in 11 vs. 3–7; C-1 to C-5; C-12 to C-19 and OMe in 11 vs. 3-5). The remaining carbons of 11 could be arranged to form partial structure C the basis of the following data: UV absorption at 248 nm, an IR absorption at 1650 cm^{-1}, and carbonyl carbon absorption at 186 ppm in the ^{13}C NMR indicated a cross-conjugated ketone. The low-field position of the second olefinic proton resonance, 6.60 ppm, and its small coupling with a vinyl methyl group, 1.94 ppm, indicated an α-methyl-α,β-unsaturated ketone as in C. The second double bond and vinyl methyl group are attested to by a ^1H NMR singlet at 1.72 ppm and the carbon resonances assigned to C-10 and C-11 (see Table IV). This left only two carbons to be placed in the structure, an sp^3 quaternary carbon [48.13 (s) ppm] and a quaternary methyl (1.63 ppm), and these were added to form the methyl-substituted cyclohexadienone structure C. Both the ^1H chemical shift for this quaternary methyl and the ^{13}C shift for the quaternary carbon are in good agreement with the corresponding shifts for the methyl (1.70 ppm) and C-4 quaternary carbon (44.9 ppm) reported[14,15] for the structurally related 4-methyl-4-phenylcyclohexadienone. Joining of partial structures A and B to the open bonds of C in the same relative positions in which they occur in 1, 2, and 5–8 yields structure 11. Irradiation of the 1.63 ppm signal (H-20) resulted in an NOE of the 6.60 ppm signal (H-7) in support of this structure.

One might expect the extended conjugation in structure 11 to give rise to longer wavelength UV absorption than is observed, 248 nm. However, due to steric crowding, the side chain may not be able to achieve the coplanarity needed for this extended conjunction. The observation of a NOE between the H-13 proton and the methyl signal at 1.63 (H-20) supports this argument. The C-12,C-13 geometry was assigned as *E* on the basis of the NOE observed between H-23 (1.70 ppm) and H-14 (3.39 ppm). The configuration at C-14 in 11 is assigned as *R** as in 3

(14) Zimmerman, H. E.; Jones, G. *J. Am. Chem. Soc.* **1970**, *92*, 2753.
(15) Gramlich, W. *Ann. Chem.* **1979**, 121.

and 4 since the chemical shift for C-14 in all three of these metabolites is virtually identical, while the 12, the C-14 epimer of 11, this resonance occurs 0.3 ppm farther downfield. Likewise, the chemical shift of the methyl group at C-14 (H-24) in 11 absorbs close to the value observed for 3 and 4, while that of H-24 in 12 is shifted upfield a little.

An alternate structure 11′ could also be proposed for 11, since the 1.63 ppm methyl signal assigned to H-20 in 11 is also very compatible with that of a vinyl methyl group. However, the NOE for both H-7 and H-13 upon irradiation of the 1.63 ppm signal only seems compatible with structure 11. Structure 11 also is a more likely rearrangement product from a precursor such as 7.



11′

Isotridachiapyrone-B (12) has the same formula as 11, and the IR and ^{13}C NMR spectra of these compounds are also virtually identical (see Tables II and IV). The ^{1}H NMR spectra for 12 and 11 were also nearly identical, the most notable differences occurring for H-16 and H-24; see Table II). Hence, it was concluded that 12 and11 were epimeric at C-14. The 12*E* geometry was confirmed by observation of an NOE of H-14 upon irradiation of H-23. The *S** configuration is assigned to C-14 following arguments made above for the stereochemistry at C-14 in 11; i.e., the chemical shift of C-14 for 12 is noticeably different from that of 3, 4, and 11, which all have the 14*R* configurations. Also, the proton chemical shift of the methyl at C-14 occurs upfield from that of 11.

Crispatene (3), crispatone (4), and all of the tridachiapyrones except 9 and 10 were tested for cytotoxicity against lymphocytic leukemia (PS).[16] The active compounds (ED_{50}'s) were as follows: 3 (7.2); 4 (3.7); 5 (5); 8 (3.1); 11 (6).

Experimental Section[17]

Extraction and Isolation Procedures. Freshly thawed molluscs, *T. crispata* (80 animals), collected and shipped frozen from Discovery Bay, Jamaica, in Jan 1979, were allowed to soak in acetone (4 L) for 24 h. The acetone solution was concentrated and the concentrate diluted with H_2O and extracted with ether (2 × 3 L). The ether soluble portion (6.5 g) of the acetone extracts was chromatographed extensively to yield 11 compounds as outlined in Figure 1.

Crispatone (3): 6.5 mg, colorless oil; IR ($CHCl_3$) 2735, 2712, 1660, 1660, 1590 cm^{-1}; ^{1}H and ^{13}C NMR data, Tables II and IV; low-resolution mass spectrum (70 eV) *m/z* (relative intensity) 414

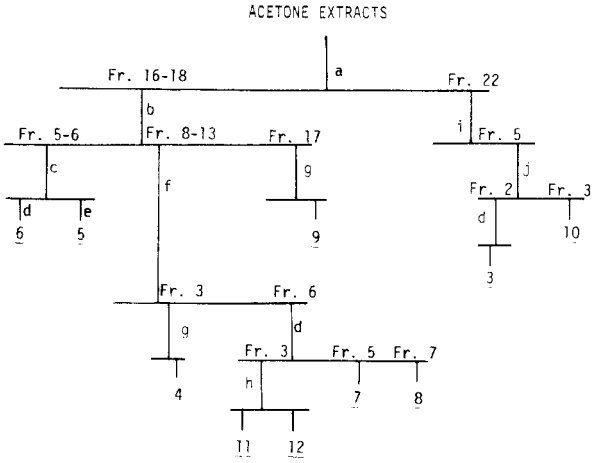


Figure 1. Fractionation of *T. crispata* extracts. Key: (a) SiO_2; acetone–hexane (6:94) with step increase in acetone; (b) SiO_2, hexane–acetone (9:1 → 75:25); (c) HPLC, SiO_2, hexane–acetone (75:25); (d) HPLC, C_{18}, MeOH–H_2O (75:25); (e) HPLC, SiO_2, MeOH–H_2O (73:27); (f) SiO_2, hexane–acetone (7:3); (g) HPLC, C_{18}, MeOH–H_2O (8:2); (h) HPLC, C_{18}, MeOH–H_2O (42:58); (i) SiO_2, hexane–acetone (6:4); (j) HPLC, SiO_2, hexane–acetone (65:35).

(14), 358 (10), 357 (7), 305 (1), 289 (3), 249 (41), 189 (62), 134 (100); HRMS for $C_{25}H_{34}O_5$, obsd 414.24325, calcd 414.2406.

Crispatene (4): 15 mg, colorless oil; IR (neat) 1715, 1660, 1615, 1590 cm^{-1}; ^{1}H and ^{13}C NMR data, Tables II and IV; low-resolution mass spectrum (70 eV) *m/z* (relative intensity), 398 (M^+, 36), 383 (28), 342 (23), 341 (65), 313 (56), 297 (34), 281 (15), 273 (10), 253 (56), 237 (47), 225 (36), 182 (40), 159 (39), 145 (33), 133 (54), 129 (41), 125 (26), 119 (41), 115 (47), 105 (57), 91 (71), 85 (20), 83 (79), 57 (100).

Tridachiapyrone-A (5): 1.1 mg, colorless oil; UV (MeOH) λ_{max} 255 nm (ϵ 11 800); IR (neat) 2980, 2915, 1860, 1715, 1660, 1600, 1585, 1460, 1405, 1325, 1310, 1250, 1160, 985 cm^{-1}; ^{1}H NMR data, Table II; low-resolution mass spectrum (70 eV) *m/z* (relative intensity) 398 [M^+, (20)], 384 (52), 341 (35), 313 (100), 297 (17), 281 (14), 273 (6), 259 (21), 253 (34), 237 (29), 225 (22), 209 (11), 197 (17), 179 (23), 155 (24), 153 (9), 129 (19), 115 (20), 105 (21), 91 (25), 83 (25), 57 (28).

Isotridachiapyrone-A (6): 1.5 mg, colorless oil; UV (MeOH) λ_{max} 253 nm (ϵ 11 900); IR ($CHCl_3$) 2990, 2930, 2860, 1715, 1660, 1600, 1585, 1460, 1410, 1375, 1315, 1250, 1165, 1100, 990 cm^{-1}; ^{1}H and ^{13}C NMR data, Tables II and IV; low-resolution mass spectrum (70 eV) *m/z* (relative intensity) 398 [M^+, (7)], 383 (21), 341 (15), 313 (41), 273 (3), 267 (6), 253 (18), 237 (17), 225 (14), 199 (14), 179 (22), 171 (18), 155 (26), 153 (11), 149 (28), 143 (22), 135 (12), 129 (40), 125 (17), 119 (22), 115 (41), 111 (26), 105 (35), 97 (28), 91 (48), 85 (31), 83 (84), 73 (42), 69 (44), 57 (100).

Tridachiapyrone-C (7): 1.5 mg, colorless oil; UV (MeOH) λ_{max} 254 nm (ϵ 5890); IR (neat) 2990, 2930, 2860, 1715, 1655, 1600, 1585, 1460, 1410, 1380, 1330, 1240, 1170, 980 cm^{-1}; ^{1}H NMR data, Table II; low-resolution mass spectrum (12 eV) *m/z* (relative intensity) 414 [M^+, (3)], 399 (2), 357 (5), 329 (5), 289 (1), 273 (5), 246 (16), 245 (100), 233 (3), 227 (21), 199 (16), 170 (31), 155 (9), 153 (5), 142 (8), 125 (22), 57 (10).

Tridachiapyrone-D (8): 0.7 mg, colorless oil; UV (MeOH) λ_{max} 255 nm (ϵ 5900); IR (neat) 3450, 2980, 2860, 1712, 1660, 1600, 1585, 1460, 1415, 1380, 1375, 1260, 1180, 1110, 980, 900, 885 cm^{-1}; ^{1}H NMR data, Table II; high-resolution mass spectrum, observed *m/z* (composition, interpretation, calculated millimass) 430.24209 ($C_{25}H_{34}O_6$, M^+, 430.2355), 412.2235 ($C_{25}H_{32}O_5$, $M^+ - H_2O$, 412.2250), 299.16785 ($C_{19}H_{23}O_3$, $M^+ - C_6H_{11}O_3$, 299.16472), 153.05363 ($C_8H_9O_3$, $M^+ - C_{17}H_{25}O_3$, 153.05517), 141.09030 ($C_8H_{13}O_2$, $M^+ - C_{17}H_{21}O_4$, 141.09156), 123.07868 ($C_8H_{11}O$, $M^+ - C_{17}H_{23}O_5$), 123.08099), 115.07465 ($C_6H_{11}O_2$, $M^+ - C_{19}H_{23}O_4$, 115.07591), 97.06567 (C_6H_9O, $M^+ - C_{19}H_{25}O_5$, 97.06534), 85.06498 (C_5H_9O, $M^+ - C_{20}H_{25}O_5$, 85.06534).

Tridachiapyrone-E (9): 0.8 mg, colorless oil; UV (MeOH) λ_{max} 255, 225 nm (ϵ 5800, 7400); IR (neat) 2980, 2950, 2860, 1690, 1660, 1585, 1460, 1415, 1380, 1330, 1260, 1160, 1170, 1040, 990, 870 cm^{-1}; ^{1}H NMR data, Table III; low-resolution mass spectrum

(16) Gueran, R. I.; Greenberg, N. H.; Macdonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3* **1972**, *3* (2), 1–103. Effective doses (ED_{50}) in the tissue culture tests are expressed as concentrations (μg/mL) of test material in the growth medium that causes 50% inhibition of cell growth. "Active" materials display an ED_{50} 20 μg/mL. PS refers to in vitro lymphocytic leukemia.

(17) ^{1}H NMR spectra were recorded at 300 MHz and ^{13}C NMR spectra at 75.4 MHz, MHz, all in $CDCl_3$, on a Varian XL-300 spectrometer; chemical shifts are reported in parts per million downfield from internal tetramethylsilane. Other instrumental and general experimental conditions are as described earlier: Schmitz, F. J.; Lakshmi, V. J.; Powell, D. R.; van der Helm, D. *J. Org. Chem.* **1984**, *49*, 241.

(18) Doddrell, D. M.; Pegg, D. T.; Bendall, M. R. *J. Magn. Reson.* **1982**, *48*, 323.

(70 eV) m/z (relative intensity) 356 [M^+, (17)], 341 (13), 299 (20), 267 (13), 239 (25), 213 (17), 185 (32), 182 (82), 157 (16), 153 (33), 141 (16), 129 (25), 119 (31), 115 (31), 105 (17), 97 (26), 91 (35), 83 (54), 81 (16), 77 (25), 69 (33), 65 (14), 57 (100).

Tridachiapyrone-F (10): 1.2 mg, white powder; UV (MeOH) λ_{max} 255, 225 nm (ϵ 5800, 7400); IR ($CHCl_3$) 2960, 2850, 1725, 1690, 1665, 1595,1450, 1390, 1370, 1325, 1220, 1050, 990, 920 cm^{-1}; ^1H NMR data, Table III; low-resolution mass spectrum (70 eV) m/z (relative intensity) 372 [M^+, (35)], 357 (8), 315 (14), 297 (12), 283 (13), 269 (15), 255 (23), 241 (22), 227 (29), 220 (12), 201 (36), 189 (28), 182 (32), 173 (31), 171 (19), 161 (24), 159 (23) 155 (20), 153 (16), 134 (56), 133 (36), 128 (31), 115 (45), 105 (44), 91 (100), 77 (41), 57 (78).

Tridachiapyrone-B (11): 1.5 mg, colorless oil; UV (MeOH) λ_{max} 248 nm (ϵ 11 900); IR ($CHCl_3$) 3020, 2995, 2925, 2880, 1710, 1660, 1650, 1635, 1600, 1590, 1450, 1400, 1370, 1310, 1250, 1160, 1025, 975, 800 cm^{-1}; ^1H and ^{13}C NMR data, Tables II and IV; low-resolution mass spectrum (70 eV) m/z (relative intensity) 412 [M^+, (6)], 356 (20), 355 (4), 327 (12), 253 (12), 241 (15), 214 (13), 213 (43), 183 (17), 182 (91), 155 (10), 153 (10), 142 (15), 128 (13), 115 (16), 105 (15), 91 (33), 83 (87), 57 (100).

Isotridachiapyrone-B (12): 1.8 mg, colorless oil; UV, IR, and low-resolution mass spectrum same as for **11**; ^1H and ^{13}C NMR, Tables II and IV.

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Registry No. **3**, 70117-21-4; **4**, 79495-60-6; **5**, 99265-40-4; **6**, 99148-24-0; **7**, 99165-16-9; **8**, 99148-21-7; **9**, 99148-22-8; **10**, 99148-23-9; **11**, 99148-25-1; **12**, 99212-17-6.

Structure of Des(diserylglycyl)ferrirhodin, DDF, a Novel Siderophore from *Aspergillus ochraceous*

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Des(diserylglycyl)ferrirhodin, DDF, a novel ferric siderophore isolated from *Aspergillus ochraceous*, was identified as N^2-[N^2-[N^5-hydroxy-N^5-(*cis*-5-hydroxy-3-methyl-1-oxo-2-pentenyl)-L-ornithyl]-N^5-hydroxy-N^5-(*cis*-5-hydroxy-3-methyl-1-oxo-2-pentenyl)-L-ornithyl]-N^5-hydroxy-N^5-(*cis*-5-hydroxy-3-methyl-1-oxo-2-pentenyl)-L-ornithine. Evidence for the structure of the siderophore was obtained from ^1H and ^{13}C NMR of its deferri and gallium(III) complex forms, from synthesis of its *N*-acetyl and methyl ester derivatives, and from degradation studies. This is the first fungal siderophore with a linear tripeptide backbone.

Siderophores are compounds produced by microorganisms under an iron deficient condition to chelate and transport extracellular iron. *Aspergillus ochraceous* produces more than a dozen siderophores most of which belong to the ferrichrome family (asperchromes).[1-3] One of these compounds, named des(diserylglylcyl)ferrirhodin (DDF) (previously termed compound I) (1), was isolated and shown[1] to possess siderophore activity in tests carried out with *Arthrobacter flavescens* Jg-9. In recent studies, it is demonstrated that it can transport ^{59}Fe(III) to the producing organism as efficiently as ferrirubin, the major siderophore of the fungus. In this report we describe the structure determination of this siderophore, on the basis of various evidences including ^1H and ^{13}C NMR of its deferri and Ga(III) complex forms.

Structure Determination

Des(diserylglycyl)ferrirhodin, DDF (1), which is ninhydrin positive and cationic at low pH, is isolated from iron-starved cultures of *A. ochraceous* by a series of chromatographic procedures described earlier.[1,2] It crystallizes in thin red fibers or needles from a number of solvent systems including ethanol–ethyl acetate and dimethylformamide–acetronitrile, but the single crystals are not large enough for X-ray diffraction studies. On the basis of microanalysis, DDF and its deferri derivative are found to have the molecular formula $C_{33}H_{53}N_6O_{13}Fe$ and $C_{33}H_{56}N_6O_{13}$, respectively. A comparison of these elemental compositons shows that the Fe(III) to ligand ratio in DDF is 1:1. The visible absorption maximum of an aqueous solution of DDF at neutral pH is at 437 nm, which is typical of a ferric hydroxamate complex. The insensitivity of the absorption maximum to pH changes in the range of 7.0 to 2.0 also indicates that the ratio of Fe(III) to ligand is 1:1 and that DDF is a trihydroxamate compound.[4,5]

Quantitative reductive hydrolysis of 1 mol of DDF (1) with HI[6,7] produces 3 mol of L-ornithine. The absolute configuration of ornithine was confirmed by polarimetry. ^1H NMR data of deferri-DDF (**2**) (Table I) show the signal for three hydroxamic acid protons, which disappears in the spectra of Ga-deferri-DDF (**3**). The hydroxamic acid functions in the fungal siderophores are formed by N^5-

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