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THE METABOLITES OF THE MARINE MOLLUSCS *TRIDACHIELLA DIOMEDEA* AND *TRIDACHIA CRISPATA*

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Abstract—Tridachione (**1**) and 9,10-deoxytridachione (**2**) were isolated from *Tridachiella diomedea* collected from the Gulf of California. The corresponding mollusc from the Caribbean, *Tridachia crispata*, contained crispatone (**13**) and crispatene (**14**). The structures of tridachione (**1**) and crispatone (**13**) were determined by X-ray crystallographic studies. The structures of 9,10-deoxytridachione (**2**) and crispatene (**14**) were elucidated from spectral data and chemical interconversions. Photolysis of 9,10-deoxytridachione (**2**) gave a single photoproduct **18** having the bicyclohexene ring system of crispatene (**14**)

Studies of the chemistry of opisthobranch molluscs have shown them to be a most interesting group of invertebrates.[1] Unlike other molluscs, opisthobranchs lack the protection of an external shell and use other forms of defense against predators. One of the most common forms of defense is the use of toxic or noxious chemicals, often derived from a dietary source.[2] The sea hares consume large quantities of algae and can store the unsavory constituents from their algal diet in a digestive gland. The nudibranchs feed on sponges and have been shown to use sponge metabolites in their defensive secretions.[3] We have been particularly interested in the chemistry of a group of sacoglossans that contain functional chloroplasts derived from siphonous marine algae.[4] These sacoglossans appear to be almost independent of an external food source as they employ the photosynthetic ability of the chloroplasts to produce primary metabolites from dissolved inorganic carbonate. Since these sacoglossans lack both physical defense mechanisms and dietary-derived chemical defense mechanisms, we suspected that they might be capable of synthesizing the constituents of a chemical defense mechanism.

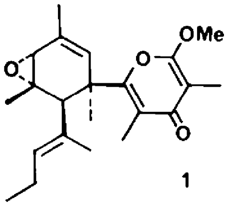
Tridachiella diomedea (Bergh),[5] commonly known as the "Mexican Dancer," was collected in shallow water (−2 m) in the Gulf of California and on the Pacific coast of Panama and El Salvador. *Tridachia crispata* (Mörch)[6] was collected in shallow water at Glover Reef, Belize and on the Caribbean coast of Panama. Despite the physically different habitats, these two sacoglossans are quite similar in appearance and behavior. In two recent communications,[7,8] we have reported the major metabolites of each sacoglossan. The metabolites were structurally dissimilar but had similar biosynthetic origins. In this paper we will describe structural elucidations and chemical reactions of the metabolites of *Tridachiella diomedea* and *Tridachia crispata*.

The metabolites of Tridachiella diomedea

The ether soluble material from an acetone extract of homogenized *T. diomedea* was chromatographed on silica gel to obtain tridachione (**1**) (~ 1.0 mg/animal) and 9,10-deoxytridachione (**2**) (~ 0.75 mg/animal). Tridachione (**1**) had the molecular formula $C_{22}H_{30}O_4$ indicating the presence of eight unsaturation equivalents. The ^{13}C NMR spectrum contained a CO carbon signal at δ 181.1 (s) and olefinic carbon signals at 161.0 (s), 160.1 (s), 132.5 (s), 131.6 (d), 129.0 (d), 128.5 (s), 118.4 (s) and 97.8 (s); tridachione (**1**) was therefore a tricyclic molecule. The presence of a α-methoxy-β,β'-dimethyl-γ-pyrone ring system could be inferred by comparing the ^{13}C NMR spectrum with that of spectinabilin (**3**)[9] (Table 1). The signals at δ 60.5 (s) and 54.7 (d) suggested the presence of a trisubstituted epoxide ring. Thus, the third ring must be carbocyclic.

The presence of the epoxide ring was confirmed by treatment of tridachione (**1**) with aqueous acid to obtain the diol **4**. The ^1H NMR spectrum of the diol **4** was similar to that of tridachione (**1**) with the exception of the presence of a new allylic α-OH proton signal at δ 5.48 (s, 1 H) in place of the α-epoxy proton signal of tridachione. Oxidation of the epoxide functionality in tridachione (**1**) with periodic acid in ether gave a keto-aldehyde **5** (IR 1710, 1680 cm^{-1}) having the molecular formula $C_{22}H_{30}O_5$. Since cleavage of the epoxide ring did not result in loss of C atoms from the molecule, the epoxide ring must be fused to the carbocyclic ring. The ^1H NMR spectrum of the keto-aldehyde **5** contained an aldehyde signal at δ 9.41 (s, 1 H), a vinyl proton signal at 6.92 (s, 1 H) due to the β-proton on an α,β-unsaturated aldehyde and a signal at 3.86 (s, 1 H) due to a proton on the α-carbon of a β,γ-unsaturated ketone. We did not observe the expected methyl ketone signal in the 2.0 ppm region; in retrospect, we must assume that the Me group was shielded by either the pyrone ring or an olefinic bond and gave a signal in the 1.7 1.86 ppm region.

With the exception of the signals due to the 1-methylbutene side-chain [δ 0.68 (t, 3 H, $J = 7$ Hz), 2.05 (m, 2 H), 5.18 (t, 1 H, $J = 7$ Hz)], the ^1H NMR spectrum of tridachione (**1**) consisted of seven three-proton singlets and three one-proton singlets. The lack of coupling between the remaining signals in the ^1H NMR spectrum of tridachione (**1**) prevented structural elucidation by interpretation of spectral data. Fortunately, treatment of tridachione (**1**) with boron trifluoride etherate in dry ether at 0° gave a crystalline isomeric diketone **6** whose structure and relative stereochemistry were determined by a single crystal X-ray diffraction study.[7] Since the boron trifluoride catalyzed rearrangement of cyclic epoxides to ketones has been shown to be a highly stereospecific reaction involving a suprafacial proton migration,[10] the



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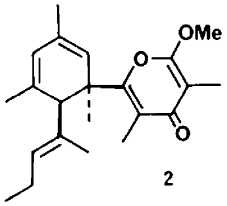

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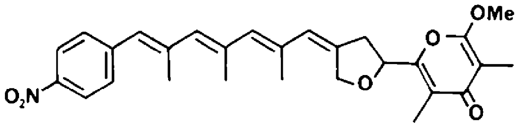

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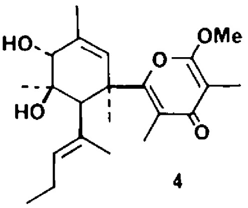

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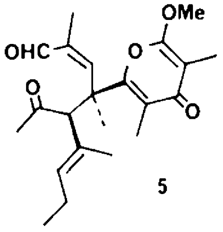

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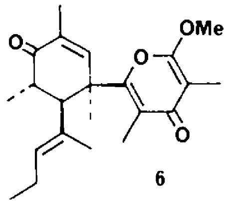

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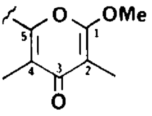
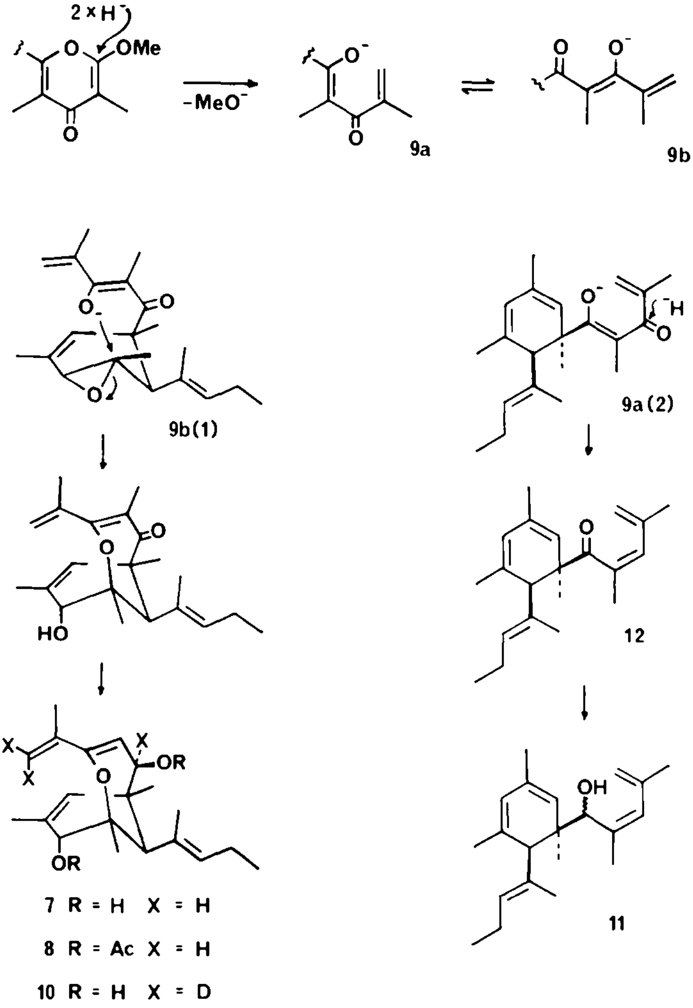
structure and stereochemistry of tridachione (**1**) were assigned as shown.

9,10-Deoxytridachione (**2**) had the molecular formula $C_{22}H_{30}O_3$. The spectral data suggested that the relationship of tridachione (**1**) to 9,10-deoxytridachione (**2**) was that of an epoxide to the corresponding olefin. In particular, the ^1H NMR spectrum of 9,10-deoxytridachione (**2**) contained an additional vinyl proton signal at either δ 5.56 (s, 1 H) or 5.65 (s, 1 H) and lacked an α-epoxy proton signal while the ^{13}C NMR spectrum contained two additional olefinic signals at the expense of two signals in the δ 50-65 region. The structure of 9,10-deoxytridachione (**2**) was confirmed by synthesis. Reduction of

Table 1. Comparison of the ^{13}C NMR data for the α-methoxy-γ-pyrone ring in spectinabilin (**3**), tridachione (**1**), 9,10-deoxytridachione (**2**), crispatone (**13**) and crispatene (**14**)



	3	1	2	13	14
C-1	162.1	161.0	161.7	162.2	162.0
C-2	99.1	97.8	98.8	100.0	99.2
C-3	180.6	181.8	181.8	181.1	181.2
C-4	119.9	118.4	120.0	121.0	120.1
C-5	155.2	160.1	161.1	157.7	159.9
OCH_3	55.3	57.6	59.6	55.0	54.9
CH_3-2	6.9	6.1	6.8	6.6	6.5
CH_3-4	9.4	11.6	12.2	10.5	12.7



Scheme 1. The reduction of tridachione (**1**) and 9,10-deoxytridachione (**2**) with lithium aluminum hydride

tridachione (**1**) with zinc dust in the presence of sodium iodide and sodium acetate in acetic acid[11] gave 9,10-deoxytridachione (**2**) identical in all respects to the natural product.

The α-methoxy-β,β'-dimethyl-γ-pyrone ring system was unaffected by most reagents but could be reduced with LAH. Reduction of tridachione (**1**) with LAH in refluxing ether gave the diol 7. The diol 7 had the molecular fomula $C_{21}H_{32}O_3$ which, together with the ^{1}H NMR data, indicated a reductive elimination of the OMe group. The ^{13}C NMR spectrum contained eight olefinic C signals including signals at δ 156.7 (s) and 99.8 (s) that could be assigned to an enol ether moiety and signals at 146.8 (s) and 108.2 (t), assigned to a terminal methylene group. The ^{13}C NMR spectrum contained additional signals at 84.5 (s), 76.8 (d) and 73.5 (d) that suggested the presence of a tertiary ether linkage and two secondary alcohols. This assignment was confirmed by the conversion of the diol 7 into the corresponding diacetate **8** using acetic anhydride in pyridine at 25°. Since the ^{13}C NMR spectrum contained eight olefinic carbons and the molecular formula demanded six unsaturation equivalents, the diol 7 must be bicyclic. The ^{1}H NMR spectrum of diol 7 contained terminal methylene proton signals at δ 4.82 (s, 1 H) and 5.07 (s, 1 H), two additional olefinic protons at 5.27 (t, 1 H, J = 7 Hz) and 5.76 (s, 1 H) and two α-OH proton signals at 3.59 (d, 1 H, J = 7 Hz) and 4.98 (s, 1 H), the latter signals being shifted to 4.80 (s, 1 H) and 6.18 (s, 1 H) in the coresponding acetate **8**. The proposed mechanism of formation of diol 7 is shown in Scheme 1. The epoxide ring was protected from hydride attack by the pyrone ring and the 1-methylbutene group. However, the enolate anion **9b** was ideally positioned for nucleophilic attack at C-10. The alternative reaction of enolate anion **9a** with the epoxide ring would have given a cross-conjugated dienol; however, the ultraviolet band at 240 nm was consistent with a conjugated dienol. In order to confirm the spectral interpretation and mechanistic assumptions, tridachione (**1**) was reduced with lithium aluminum deuteride in refluxing ether to obtain a d_3-diol **10**. As expected, the ^{1}H NMR spectrum of the d_3-diol **10** lacked the signals at δ 4.82, 4.98 and 5.07 that corresponded to the terminal methylene protons and the C-5 α-OH proton.

Reduction of 9,10-deoxytridachione (**2**) with LAH in refluxing ether gave a 1:1 mixture of two isomeric alcohols **11**. The alcohol mixture was oxidized with Jones' reagent to obtain a single conjugated dienone **12** (λ_{max} 275 nm). The production of a mixture of alcohols can be explained by assuming the reduction mechanism shown in Scheme 1. The intermediate **9** was reduced at C-3 (**9a**) rather than C-5 (**9b**) to obtain the conjugated dienone **12** as an intermediate. Reduction of dienone **12** gave both diastereoisomeric alcohols. The ^{1}H NMR spectrum of the mixture of alcohols contains two signals at δ 5.37 (s, 0.54 H) and 5.32 (s, 0.46 H) due to the proton at C-7, two signals at δ 2.51 (s, 0.46 H) and 2.48 (s, 0.54 H) due to the doubly allylic proton at C-11, the α-OH proton signal at 4.30 (s, 1 H), two terminal methylene proton signals at 4.88 (s, 1 H) and 5.03 (s, 1 H) and three additional olefinic signals at 5.55 (s, 1 H), 5.53 (s, 1 H) and 5.14 (m, 1 H). In the ^{1}H NMR spectrum of the dienone **12**, the β-vinyl proton signal was shifted downfield to δ 6.50 (s, 1 H) while the terminal methylene protons were at δ 5.37 (s, 1 H) and 5.44 (s, 1 H). The chemical shift of the β-vinyl proton signal suggested the *Z* stereochemistry about the $^3\Delta$-olefinic bond.[12]

The metabolites of Tridachia crispata

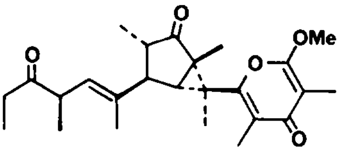
The ether-soluble material from the acetone extraction of homogenized *T. crispata* was chromatographed on silica gel to obtain crispatone (**13**) (~0.12 mg/animal) and crispatene (**14**) (~0.28 mg/animal). Crispatone (**13**) had the molecular formula $C_{25}H_{34}O_5$, which corresponds to the molecular formula of tridachione (**1**), plus an additional "propionate" unit. The IR spectrum contained two CO bands at 1725 and 1710 cm^{-1} together with the γ-pyrone bands at 1660 and 1590 cm^{-1}. The ^{13}C NMR spectrum (Table 1) and ^{1}H NMR signals at δ 1.83 (s, 3 H), 1.95 (s, 3 H) and 3.96 (s, 3 H) suggested the presence of the α-methoxy-β,β'-dimethyl-γ-pyrone ring system while the ^{1}H NMR spectrum indicated that the additional propionate unit had been added to the side chain to produce a 1,3-dimethyl-1-hexen-4-one side chain. Signals at δ 1.05 (t, 3 H, J = H z) and 2.49 (q, 2 H, J = 7 Hz) were assigned to the terminal ethyl ketone. A second methyl signal at δ 1.05 (d, 3 H, J = 7 Hz) was coupled to a methine at 3.48 (m, 1 H) which was in turn coupled to a vinyl proton at 5.38 (d, 1 H, J = 8 Hz); the chemical shift of the methine proton required that it be adjacent to the ethyl ketone. The Me group on the trisubstituted olefin gave a signal at δ 1.82 (bs, 3 H). The remaining signals in the ^{1}H NMR spectrum consisted of singlets at δ 1.20 (s, 3 H), 1.24 (s, 3 H), 1.87 (s, 1 H) and 2.40 (s, 1 H) together with a Me signal at 1.18 (d, 3 H, J = 7 Hz) coupled to a methine signal at 2.38 (q, 1 H, J = 7 Hz), assigned to a Me group on a carbon adjacent to carbonyl. These signals could not be assigned in any obvious manner to a bicyclohexanone ring system. The structure of crispatone (**13**) was therefore determined from single-crystal X-ray diffraction data.[8] The stereochemical arrangement of substituents at C-7, C-11 and C-10 resulted in a H–C(7)–C(11)–H dihedral angle of ~90° and a H–C(10)–C(11)–H dihedral angle of ~110°. Thus the H(C-7)–H(C-11) coupling constant was <0.5 c/s, resulting in a singlet at δ 1.89 for the cyclopropyl proton while the expected small H(C-11)–H(C-10) coupling would not be observed because of the almost identical chemical shifts of these protons. The ^{13}C NMR spectrum was assigned (Table 2) using J^r values to differentiate between certain signals.[13]

Crispatene (**14**) had the molecular formula $C_{25}H_{34}O_4$. The IR spectrum indicated that crispatene (**14**) contained the γ-pyrone ring (1660, 1590 cm^{-1}) and the ketone (1710 cm^{-1}) in the side chain but lacked the ketone in the 5-membered ring. The ^{13}C NMR spectrum contained signals (Table 1) that could be assigned to the α-methoxy-β,β'-dimethyl-γ-pyrone system. The ^{1}H NMR spectrum contained signals at δ 1.04 (d, 3 H, J = 7 Hz), 2.47 (m, 2 H), 1.16 (d, 3 H, J = 7 Hz), 3.45 (m, 1 H), 5.26 (d, 1 H, J = 9 Hz) and 1.61 (bs, 3 H) that were assigned to the 1,3-dimethyl-1-hexen-4-one side chain. Comparison of the spectral data of crispatene (**14**) with that of crispatone (**13**) led to the suggestion that both contained the same basic carbon skeleton but that crispatene contained a 9,10-olefinic bond. The ^{1}H NMR spectrum contained a vinyl proton signal at δ 5.39 (s, 1 H), a vinyl Me signal at 1.58 (s, 3 H), a signal at 2.80 (s, 1 H) due to the bis-allylic proton at C-11, two methyl signals at 1.11 (s, 3 H) and 1.20 (s, 3 H), and a signal at 1.41 (s, 1 H) due to the cyclopropyl proton. The lack of coupling between the C-11 and C-7 protons was expected, provided that the stereochemistry about the bicyclic ring system is the same as for crispatone (**13**). The ^{13}C NMR spectrum was assigned as shown in Table 2. The similar chemical shifts for the cyclopropyl carbon atoms suggested that both compounds had an identical substitution pattern and stereochemistry about the cyclopropyl ring.

The photochemical relationship of 9,10-deoxytridachione (**2**) *and crispatene* (**14**)

We proposed[7] that the metabolites of *T. diomedea*, tridachione (**1**) and 9,10-deoxytridachione (**2**) had been derived by polyketide condensation of seven "propionate" units. The metabolites of *T. crispata*, crispatone (**13**) and crispatene (**14**) could therefore be derived from eight "propionate" units. Whereas the formation of both the γ-pyrone ring and the cyclohexadiene ring in 9,10-deoxytridachione (**2**) can be explained by orthodox polyketide condensation reactions, the formation of the bicyclo[3,1,0]hexane ring in crispatene (**14**) would require either abnormal condensation reactions or a molecular rearrangement. However, molecules of the type **15** could be the precursor of both 9,10-deoxytridachione (**2**) and crispatene (**14**). A disrotatory thermal cyclization of the (6*E*, 8*Z*, 10*E*)-hexatriene or a conrotatory photochemical cyclization of the (6*E*, 8*Z*, 10*Z*)-hexatriene could give rise to 9,10-deoxytridachione (**2**)[14a] while a $[{}_{\pi}4a + {}_{\pi}2_{s}]$ photochemical cycloaddition reaction of the (6*E*, 8*Z*, 10*Z*)-hexatriene could produce crispatene (**14**).[14b] In the natural systems we would expect these reactions to be enzyme-mediated in order to produce optically active products. However, the possibility of photochemical reactions occurring in organisms that are known for their photosynthetic abilities is a most attractive hypothesis. We therefore attempted to show a photochemical relationship between the ring systems of 9,10-deoxytridachione (**2**) and crispatene (**14**).

The photochemical rearrangement of a 1,3-cyclohexadiene to obtain the corresponding bicyclo[3,1,0]hex-2-ene has been described by Barton and



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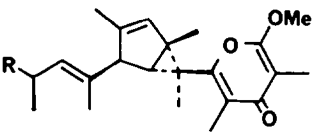
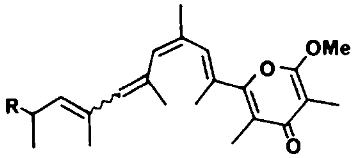


14 R = C_2H_5CO **18** R = H



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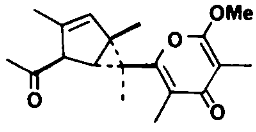

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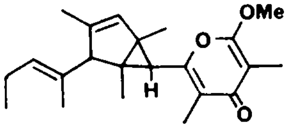

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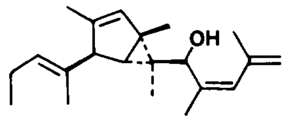

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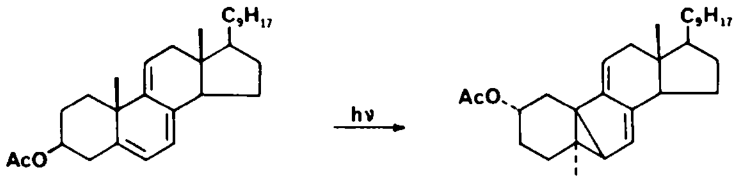

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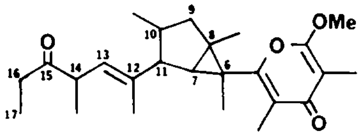
Table 2. Comparison of selected signals from the ^{13}C NMR spectra of crispatone (13) and crispatene (14).



	13	14		13	14
C-6	42.2*	40.5*	C-12	137.0	137.3
C-7	37.4	36.5	C-13	126.6	126.4
C-8	31.9*	31.7*	C-14	45.7	45.8
C-9	216.0	129.1	C-15	211.0	211.5
C-10	48.8	143.0	C-16	33.5	33.4
C-11	51.0	58.0	C-17	7.8	7.6

*These signals may be exchanged.

Kende,[15] who photolyzed dehydroergosterol acetate (**16**) in benzene solution to obtain photodehydroergosterol (**17**). Similar rearrangements in the Vitamin D series and in simple model compounds have been studied by Dauben *et al.*,[16] Ullman *et al.*,[17] and Havinga *et al.*[18] Using the conditions of Barton and Kende, a solution of 9,10-deoxytridachione (**2**) in benzene was photolyzed using a 450 watt Hanovia lamp for 3 hr to obtain a single photoproduct **18** in 85% yield. The ^{1}H NMR spectrum of the photoproduct **18** was, with the exception of signals assigned to the side chain, remarkably similar to the ^{1}H NMR spectrum of crispatene (**14**). In particular, the spectrum of the photoproduct **18** contained only two vinyl proton signals at δ 5.33 (s, 1 H) [cf. 5.39 in crispatene] and 5.30 (t, 1 H, J = 7 Hz) that were assigned to the cyclopentene and side-chain vinyl protons respectively. The bis-allylic C-11 proton at δ 2.75 (s, 1 H) and the C-7 cyclopropyl proton at 1.42 (s, 1 H) both appeared as singlets, as had the C-11 and C-7 protons in crispatone (**13**) and crispatene (**14**). The Me signals at C-6 and C-8 occurred at identical chemical shifts [δ 1.11 (s, 3 H) and 1.20 (s, 3 H)] in both the photoproduct **18** and crispatene (**14**), suggesting an identical substitution pattern and stereochemistry in both molecules. In order to confirm that the bicyclo[3,1,0]hex-2-ene ring system in photoproduct **18** was identical to that of crispatene (**14**), both molecules were carefully ozonized in ethyl acetate solution at −78°, followed by workup with zinc in acetic acid, to obtain the same major product, the methyl ketone **19**. The two samples of ketone **19** not only had identical spectral and chromatographic properties but had identical optical rotations.

Despite the biosynthetic relationship and similarities in spectral data between crispatone (**13**) and crispatene (**14**), it could be argued that the photochemical rearrangement of 9,10-deoxytridachione (**2**) had proceeded *via* a di-π-methane rearrangement to obtain structure **20**,[19] in which both the cyclopropyl proton and the bis-allylic proton signals were necessarily singlets. This structure could be ruled out by reduction of the photoproduct **18** with LAH in refluxing ether to obtain a single secondary alcohol **21** that contained a conjugated diene system (UV 228 nm). The ^{1}H NMR spectrum of alcohol **21** contained an α-OH proton signal at δ 4.40 that appeared as a singlet, with slight broadening due to allylic coupling with the olefinic proton signal at δ 5.65. Thus the carbinol functionality was unlikely to be adjacent to the cyclopropyl carbon bearing hydrogen.

An accepted mechanism for the photochemical conversion of 1,3-cyclohexadienes to bicyclo-[3,1,0]hex-2-enes involves a hexatriene intermediate.[20] The expected intermediate in the photolysis of 9,10-deoxytridachione (**2**) is the non-chiral tetraene **15** (10*Z*, R = H), which can undergo a stereo-specific [$_{\pi}4_a + _{\pi}2_s$] cycloaddition to give the photoproduct **18**. However, the retention of optical activity during photolysis and the absence of other products expected from the photolysis of the tetraene argue against that mechanism. We propose that the photochemical rearrangement of 9,10-deoxytridachione (**2**) has occurred through the more direct [$_{\sigma}2_a + _{\pi}2_a$] mechanism.[14c]

Subsequent to the completion of this study, one of the authors (C.I.) showed that a related sacoglossan, *Placobranchus ocellatus*, contained both 9,10-deoxytridachione (**2**) and the photoproduct **18** and provided some preliminary data supporting the occurrence of the photochemical rearrangement *in vivo*.[21]

EXPERIMENTAL

IR spectra were recorded on a Perkin–Elmer Model 137 spectrophotometer. UV spectra were recorded on a Perkin–Elmer Model 124 double-beam spectrophotometer. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter, using a 10 cm microcell. ^{1}H NMR spectra were recorded on a Varian HR-220 NMR spectrometer, and ^{13}C spectra were recorded on a Varian CFT-20 NMR spectrometer; all chemical shifts are reported with respect to Me_4Si ($\delta = 0$). Low-resolution mass spectra were recorded on a Hewlett-Packard 5930A mass spectrometer. High-resolution mass spectra were supplied by the mass spectrometry service at UCLA. Melting points were measured on a Fisher-Johns apparatus and are uncorrected. All solvents used were either spectral grade or distilled prior to use.

Collection and extraction of Tridachiella diomedea

Specimens of *T. diomedea* were collected by hand (0 to −4 m) at Bahia Concepcion (26°43′N, 111°55′W), Bahia de Los Angeles (20°N, 113°33′W), Isla San José (24°52′N, 111°35′W) and Isla Espiritu Santo (24°31′N, 110°23′W). All specimens contained the same metabolites. The animals were stored in acetone for one week then homogenized in acetone. The resulting suspension was filtered and the acetone evaporated under vacuum to obtain an oily residue that was partitioned between ether and water. The combined ether extracts were dried over Na_2SO_4 and the solvent evaporated to obtain an oil. The oil was chromatographed on silica gel using eluants of increasing polarity from hexane through ether to EtOAc. Fractions eluted with 1:1 hexane–ether contained **2** (~0.75 mg/animal) while elution with 70% ether in hexane gave **1** (~1.0 mg/animal).

Tridachione (**1**). $[\alpha]_D$ −113.3° (c 0.06, $CHCl_3$); IR ($CHCl_3$) 1660, 1590, 890 cm^{-1}; UV (MeOH) 257 nm (ε 6,000), ^{1}H NMR ($CDCl_3$) δ 0.68 (t, 3 H, J = 7 Hz), 1.32 (s, 3 H), 1.36 (s, 3 H), 1.60 (s, 3 H), 1.80 (s, 3 H), 2.01 (s, 3 H), 2.05 (m, 2 H), 2.07 (s, 3 H), 2.91 (s, 1 H), 3.11 (s, 1 H), 3.93 (s, 3 H), 5.18 (t, 1 H, J = 7 Hz), 6.10 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 181.1 (s), 161.0 (s), 160.1 (s), 132.5 (s), 131.6 (d), 129.0 (d), 128.5 (s), 118.4 (s), 97.8 (s), 60.5 (s), 57.6 (q), 55.3 (s), 54.7 (d), 46.7 (d), 31.2 (t), 21.8 (q), 21.5 (q), 20.2 (q), 12.9 (q), 12.0 (q), 11.6 (q), 6.1 (q); mass spectrum, *m/e*, 358 (M^+) 343, 315, 283, 254, 222, 155; HRMS, Obs: 358.2139, $C_{22}H_{30}O_4$ Requires: 358.2144.

9,10-*Deoxytridachione* (**2**). $[\alpha]_D$ −194.8 (c, 0.27, $CHCl_3$); IR ($CHCl_3$) 1660, 1590 cm^{-1}; UV (MeOH) 257 nm (ε 12,000); ^{1}H NMR ($CDCl_3$) δ 0.69 (t, 3 H, J = 7 Hz), 1.32 (s, 3 H), 1.42 (s, 3 H), 1.69 (s, 3 H), 1.76 (s, 3 H), 1.81 (s, 3 H), 2.03 (s, 3 H), 2.71 (s, 1 H), 3.95 (s, 3 H), 5.09 (t, 1 H, J = 7 Hz), 5.56 (s, 1 H), 5.65 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 181.8, 161.7, 161.1, 134.9, 132.2, 131.0, 127.8, 124.3, 122.4, 120.0, 98.8, 59.6, 55.4, 47.6, 26.9, 22.3, 21.5, 21.1, 13.8, 13.7, 12.2, 6.8; mass spectrum, *m/e*, 342 (M^+) 327, 313, 199, 155; HRMS, Obs: 342.2199, $C_{22}H_{30}O_3$ requires 342.2195.

Collection and extraction of Tridachia crispata

Specimens of *T. crispata* were collected by hand (0 to −2 m) at Glover Reef, Belize (16°40′N, 87°45′W) and Galeta Island, Panama (9°23′N, 79°50′W). The animals were stored in acetone for one month then homogenized in acetone. The filtered extract was evaporated to obtain an oil that was partitioned between ether and water. The combined ether extracts were dried over sodium sulfate and the solvent evaporated. The resulting oil was chromatographed on silica gel. Elution with 50% ether in hexane gave fractions containing **14** (~0.28 mg/animal) followed by fractions containing **13** (~0.125 mg/animal).

Crispatone (**13**). $[\alpha]_D$—84.7° (c 0.03, $CHCl_3$); IR ($CHCl_3$) 1725, 1710, 1660, 1590 cm^{-1}; UV (MeOH) 257 nm (ε 5800); ^1H NMR ($CDCl_3$) δ 1.05 (t, 3 H, J = 7 Hz), 1.05 (d, 3 H, J = 7 Hz, 1.18 (d, 3 H, J = 7 Hz), 1.20 (s, 3 H), 1.24 (s, 3 H), 1.82 (s, 3 H), 1.83 (s, 3 H), 1.87 (s, 1 H), 1.95 (s, 3 H), 2.38 (q, 1 H, J = 7 Hz), 2.40 (s, 1 H), 2.49 (q, 2 H, J = 7 Hz), 3.48 (m, 1 H), 3.96 (s, 3 H), 5.38 (d, 1 H, J = 8 Hz); ^{13}C NMR ($CDCl_3$) δ 216.0 (s), 211.0 (s), 181.1 (s), 162.2 (s), 157.5 (s), 137.0 (s), 126.2 (d), 121.0 (s), 100.0 (s), 55.0 (q), 51.0 (d), 48.8 (d), 45.7 (d), 42.2 (s), 37.4 (d), 33.5 (t), 31.9 (s), 16.6 (q), 15.9 (q), 13.3 (2q), 11.0 (q), 10.5 (q), 7.8 (q), 6.6 (q); mass spectrum, *m/e*, 414 (M^+), 357, 304, 289, 249, 189, HRMS, Obs: 414.2392, $C_{25}H_{34}O_5$ requires 414.2406.

Crispatene (**14**). $[\alpha]_D$ 92.8 (c 0.12, $CHCl_3$); IR ($CHCl_3$) 1710, 1660, 1590 cm^{-1}; UV (MeOH) 256 nm (ε 5700); ^1H NMR ($CDCl_3$) δ 1.04 (t, 3 H, J = 7 Hz), 1.11 (s, 3 H), 1.16 (d, 3 H, J = 7 Hz), 1.20 (s, 3 H), 1.41 (s, 1 H), 1.58 (s, 3 H), 1.61 (s, 3 H), 1.84 (s, 3 H), 1.95 (s, 3 H), 2.47 (m, 2 H), 2.80 (s, 1 H), 3.45 (m, 1 H), 3.94 (s, 3 H), 5.26 (d, 1 H, J = 8 Hz), 5.39 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 211.5 (s), 181.2 (s), 162.0 (s), 159.9 (s), 143.0 (s), 137.3 (s), 129.1 (d), 126.4 (d), 120.1 (s), 99.2 (s), 58.0 (d), 54.9 (q), 45.8 (d), 40.5 (s), 36.5 (d), 33.4 (t), 31.7 (s), 17.6 (q), 16.3 (q), 13.7 (q), 13.3 (q), 13.1 (q), 12.7 (q), 7.6 (q), 6.5 (q); mass spectrum, *m/e* 398 (M^+) 382, 341, 313; HRMS, Obs: 398.244, $C_{25}H_{34}O_4$ requires 398.246.

Diol **4**. Conc. HNO_3 (3 drops) was added to a stirred soln of **1** (30 mg, 0.8 mmol) in glacial AcOH (2 mL). Soln was stirred at 25 for 30 min then quenched by pouring into 5% $NaHCO_3$aq. The organic material was extracted with $CHCl_3$ (3 × 15 mL). The combined extracts were dried over Na_2SO_4, and the solvent evaporated to obtain **4** (30 mg, 94% theoretical): IR ($CHCl_3$) 3600, 1660, 1590 cm^{-1}; UV (MeOH) 256 nm; ^1H NMR ($CDCl_3$) δ 0.82 (t, 3 H, J = 7 Hz), 1.25 (s, 3 H), 1.30 (s, 3 H), 1.69 (s, 3 H), 1.82 (s, 3 H), 1.89 (s, 3 H), 2.07 (s, 3 H), 2.75 (s, 1 H), 3.94 (s, 3 H), 5.30 (t, 1 H, J = 7 Hz), 5.48 (s, 1 H), 6.08 (s, 1 H); mass spectrum, *m/e*, 358 ($M-H_2O$), 343, 315, 222.

Keto-aldehyde **5**. Periodic acid (50 mg, 0.22 mmol) was added to a stirred soln of **1** (29 mg, 0.08 mmol) in ether (15 mL) and the soln was boiled under reflux for 20 min. The cooled mixture was poured into water (10 mL), the ether layer separated and the aqueous phase extracted with ether (2 × 10 mL). The combined ether extracts were dried over Na_2SO_4, and the solvent evaporated to obtain **5** (27 mg, 90% theoretical): IR ($CHCl_3$) 1710, 1680, 1660, 1590 cm^{-1}; UV (MeOH) 253 nm; ^1H NMR ($CDCl_3$) δ 1.05 (t, 3 H, J = 7 Hz), 1.45 (s, 3 H), 1.70 (s, 3 H), 1.76 (s, 3 H), 1.84 (s, 3 H), 1.86 (s, 3 H), 2.02 (s, 3 H), 2.18 (m, 2 H), 3.86 (s, 1 H), 4.07 (s, 3 H), 5.66 (t, 1 H, J = 7 Hz), 6.92 (s, 1 H), 9.41 (s, 1 H); mass spectrum, *m/e*, 374 (M^+), 315, 249.

Diketone **6**. BF_3. etherate (400 μL) was added to a soln of **1** (20 mg, 0.06 mmol) in dry ether (5 mL) at 0° under N_2. After 1 min the reaction was quenched with water (1 mL). The ether phase was dried over Na_2SO_4 and the solvent evaporated to give **6** (8 mg, 40% theoretical) as crystals from hexane: mp 194–197 ; IR ($CHCl_3$) 1680, 1660, 1590 cm^{-1}; UV (MeOH) 251, 240 nm; ^1H NMR ($CDCl_3$) δ 0.98 (t, 3 H, J = 7 Hz), 1.00 (d, 3 H, J = 7 Hz), 1.25 (s, 3 H), 1.57 (s, 3 H), 1.84 (s, 3 H), 1.88 (s, 3 H), 2.06 (m, 2 H), 2.07 (s, 3 H), 2.43 (d, 1 H, J = 12 Hz), 2.65 (m, 1 H), 3.82 (s, 3 H), 5.36 (t, 1 H, J = 7 Hz), 6.50 (s, 1 H); mass spectrum, *m/e* 358 (M^+), 343, 326, 271, 249, 189.

Conversion of tridachione (1) *into 9,10-deoxytridachione* (2)

A soln of **1** (30 mg, 0.082 mmol) in AcOH (min. volume) was added to a cooled soln of NaI (21 mg, 0.142 mmol) and NaOAc (7 mg, 0.085 mmol) in AcOH (10 mL) and water (500 μL) at 0 . Zn dust (21 mg, 0.32 mmol) was added to the stirred soln. After 1½ hr the mixture was filtered and the solvent removed under vacuum. The residue was partitioned between ether (3 × 20 mL) and water (10 mL) and the combined ether extracts dried over Na_2SO_4 and evaporated to obtain **2** (15 mg, 52% theoretical), identical in all respects to the natural material.

Reduction of tridachione (1) *with lithium aluminum hydride*

LAH (70 mg) was added to a soln of **1** (50 mg, 0.14 mmol) in anhyd ether (20 mL) and the mixture was boiled under reflux for 1 hr under N_2. The mixture was cooled and excess reagent was destroyed by sequential dropwise addition of 5% NaOHaq (2 mL; caution!) and water (5 mL). The ether layer was filtered, dried over Na_2SO_4 and the solvent evaporated to obtain 7 (23 mg, 55% theoretical): IR ($CHCl_3$) 3600 cm^{-1}; UV (MeOH) 240 nm; ^1H NMR ($CDCl_3$) δ 0.93 (t, 3 H, J = 7 Hz), 1.27 (s, 3 H), 1.32 (s, 3 H), 1.50 (s, 3 H), 1.51 (s, 3 H), 1.61 (s, 3 H), 1.72 (s, 3 H), 2.05 (m, 2 H), 2.28 (s, 1 H), 3.59 (m, 1 H), 4.82 (s, 1 H), 4.98 (s, 1 H), 5.07 (s, 1 H), 5.27 (t, 1 H, J = 7 Hz), 5.76 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 156.7 (s), 146.8 (s), 135.7 (d), 134.2 (d), 132.6 (s), 131.3 (s), 108.2 (t), 99.8 (s), 84.5 (s), 76.8 (d), 73.5 (d), 61.8 (d), 46.7 (s), 21.0 (q), 20.3 (q), 19.2 (q; q; t), 14.1 (q), 13.3 (q), 8.6 (q); mass spectrum, *m/e*, 332 (M^+) 291, 189; HRMS, Obs: 332.235, $C_{21}H_{32}O_3$ requires 332.235.

Acetylation of diol 7. A soln of 7 (9 mg, 0.027 mmol) in AC_2O (1 mL) and pyridine (1 mL) was stirred at 25° for 24 hr. The reagents were removed under vacuum and the residue was partitioned between ether (2 × 10 mL) and water (5 mL). The combined ether extracts were dried over Na_2SO_4 and the solvent evaporated to obtain **8** (8 mg, 70% theoretical): ^1H NMR ($CDCl_3$) δ 0.94 (t, 3 H, J = 7 Hz), 1.19 (s, 3 H), 1.27 (s, 3 H), 1.48 (s, 3 H), 1.57 (s, 9 H), 2.04 (s, 3 H), 2.07 (s, 3 H), 2.36 (s, 1 H), 4.80 (s, 2 H), 5.12 (s, 1 H), 5.22 (t, 1 H, J = 7 Hz), 5.83 (s, 1 H), 6.18 (s, 1 H); mass spectrum, *m/e*, 416 (M^+), 373, 357, 333, 248.

Reduction of 9,10-deoxytridachione (2) *with lithium aluminum hydride*

LAH (20 mg) was added to a soln of **2** (14 mg, 0.04 mmol) in anhyd ether (10 mL) and the mixture was boiled under reflux for 1 hr under N_2. The mixture was worked up as described previously to obtain **11** (7 mg, 58% theoretical) as a 1:1 mixture of two diastereoisomers: IR ($CHCl_3$) 3600 cm^{-1}; UV (MeOH) 230 nm; ^1H NMR ($CDCl_3$) δ 0.91 (t, 3 H, J = 7 Hz), 1.16 (s, 3 H), 1.41 (s, 3 H), 1.57 (s, 3 H), 1.63 (s, 3 H), 1.70 (s, 3 H), 1.94 (m, 2 H), 2.48 (s, 0.54 H), 2.51 (s, 0.46 H), 4.30 (s, 1 H), 4.88 (s, 1 H), 5.03 (s, 1 H), 5.14 (m, 1 H), 5.32 (s, 0.46 H), 5.37 (s, 0.54 H), 5.53 (s, 1 H), 5.55 (s, 1 H); mass spectrum, *m/e*, 300 (M^+), 282, 267, 254, 229; HRMS, Obs: 300.246, $C_{21}H_{32}O$ requires 300.247.

Oxidation of diol **11**. Jones' reagent (40 μL, 0.048 mmol) was added dropwise to a stirred soln of **11** (7 mg, 0.023 mmol) in anhyd acetone (5 mL) at 0°C. After 10 min, the excess oxidant was destroyed with i-PrOH (1 2 drops) and the solvents removed under vacuum. The residue was partitioned between ether (3 × 20 mL) and water (20 mL). The combined ether extracts were dried over Na_2SO_4 and the solvent evaporated to obtain **12** (6 mg, 85% theoretical): UV (MeOH) 275, 238 nm; ^1H NMR ($CDCl_3$) δ 0.92 (t, 3 H, J = 7 Hz), 1.43 (s, 3 H), 1.73 (s, 9 H), 1.94 (s, 3 H), 1.98 (s, 3 H), 2.55 (s, 1 H), 5.23 (t, 1 H, J = 7 Hz), 5.37 (s, 1 H), 5.44 (s, 1 H), 5.54 (s, 1 H), 5.64 (s, 1 H); mass spectrum, *m/e*, 298 (M^+), 283.

Photolysis of 9,10-deoxytridachione (**2**). A soln of **2** (20 mg, 0.058 mmol) in benzene (500 mL) was placed in the well of a water-cooled photolysis apparatus (Ace Glass) and irradiated with light from a 450 W Hanovia lamp for 3 hr. Evaporation of the solvent gave an oil that was chromatographed on silica gel using ether as eluant to obtain a single photoproduct (**18**, 16 mg, 85% theoretical): ^1H NMR ($CDCl_3$) δ 0.98 (t, 3 H, J = 7 Hz), 1.11 (s, 3 H), 1.20 (s, 3 H), 1.42 (s, 1 H), 1.55 (s, 3 H), 1.57 (s, 3 H), 2.75 (s, 1 H), 3.96 (s, 3 H), 5.30 (t, 1 H, J = 7 Hz), 5.33 (s, 1 H); mass spectrum, *m/e*, 342 (M^+), 326, 313, 199; HRMS, Obs: 342.217, $C_{22}H_{30}O_3$ requires 342.219.

Ozonolysis of crispatene (**14**). A soln of O_3 in EtOAc was prepared by bubbling a mixture of O_3 in oxygen through EtOAc at − 78° until a blue-colored soln resulted. This soln was added dropwise to a stirred soln of **14** (9 mg, 0.002 mmol) in EtOAc (10 mL) at − 78° and the course of the reaction was followed by tlc on silica gel. As soon as all starting material had reacted, excess O_3 was removed in a stream of N_2 and the solvent was evaporated under vacuum.

AcOH (5 mL) and Zn dust (20 mg) were added to the ozonide and the mixture was stirred at 25° for 1 hr. The mixture was diluted with ether (25 mL), filtered and the solvent removed under vacuum to obtain an oil containing one major component. The oil was chromatographed on silica gel to obtain **19** (3 mg, 41% theoretical): $[\alpha]_D + 32°$ (*c* 0.02, $CHCl_3$); IR 1710, 1660, 1590 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.12 (s, 3 H), 1.25 (s, 3 H), 1.60 (s, 1 H), 1.72 (s, 3 H), 1.84 (s, 3 H), 1.98 (s, 3 H), 2.17 (s, 3 H), 3.09 (s, 1 H), 3.96 (s, 3 H), 5.56 (s, 1 H); mass spectrum, *m/e*, 316 (M^+), 301, 284, 275; HRMS, Obs: 316.168, $C_{19}H_{24}O_4$ Requires: 316.167.

Ozonolysis of photoproduct **18**. A soln of **18** (4 mg, 0.01 mmol) in EtOAc (5 mL) was subjected to ozonolysis using the procedure described above to obtain **19** (1.5 mg), identical in all respects to the sample obtained from crispatene.

Reduction of photoproduct **18** *with lithium aluminum hydride*

LAH (30 mg) was added to a soln of **18** (10 mg, 0.029 mmol) in anhyd ether (20 mL) and the mixture was boiled under reflux for 1 hr. The mixture was worked up as described for a previous LAH reduction to obtain **21** (5 mg, 57% theoretical): IR ($CHCl_3$) 3600 cm^{-1}; UV (MeOH) 228 nm; ^1H NMR ($CDCl_3$) δ 0.91 (s, 3 H), 0.97 (t, 3 H, J = 7 Hz), 1.22 (s, 1 H), 1.46 (s, 3 H), 1.52 (s, 3 H), 1.57 (s, 3 H), 1.58 (s, 3 H), 2.05 (m, 2 H), 2.65 (s, 1 H), 4.40 (s, 1 H), 4.92 (s, 1 H), 5.07 (s, 1 H), 5.25 (s, 1 H), 5.25 (t, 1 H, J = 7 Hz), 5.64 (s, 1 H), mass spectrum, *m/e*, 300 (M^+), 282, 267.

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