Metabolites of the Burrowing Sponge *Siphonodictyon coralliphagum*

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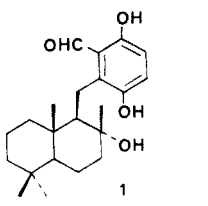
Received May 28, 1986

The burrowing sponge *Siphonodictyon coralliphagum* was obtained from several Caribbean and Pacific locations and the metabolites from each collection were compared. Six new metabolites, siphonodictyal C (**4**), siphonodictyal D (**5**), siphonodictyal E (**6**), siphonodictyol G (**7**), siphonodictyol H (**8**), and siphonodictyoic acid (**9**), are described and the structure of siphonodictyal B is revised from **2** to **3**. The antimicrobial properties of the metabolites are reported.

Siphonodictyon coralliphagum (Rützler)[1] is a bright yellow sponge that burrows into corals. *S. coralliphagum* from the Caribbean was described as having four distinct morphological forms, f. *typica*, f. *tubulosa*, f. *obruta*, and f. *incrustans*, all belonging to one population.[1] *S. coralliphagum* f. *typica* is found burrowing into living coral heads with the major portion of the sponge inside the coral head and only the oscular chimney protruding. In order to prevent overgrowth by coral polyps, the sponge maintains a 5–10-mm "dead zone" around the base of the oscular chimney. We have proposed that the coral polyps are killed by the action of toxic secondary metabolites (siphonodictyals) which are carried to the base of the oscular chimney in a mucus exudation.[2] We have previously reported the structural elucidation of siphonodictyal A (**1**) and siphonodictyal B (**2**) from the oscular chimneys of *S. coralliphagum* collected at Lighthouse and Glover Reefs, Belize.[3] We have also described the differences in metabolite content of several collections of *S. coralliphagum* from both Caribbean and Pacific locations, with particular reference to differences between f. *typica* from living coral heads and f. *tubulosa* found in dead coral rubble.[2] In this paper we report a revision of the structure of siphonodictyal B from **2** to **3** and the structural elucidations of siphonodictyals C–E (**4**–**6**), siphonodictyols G (**7**) and H (**8**), and siphonodictyoic acid (**9**).



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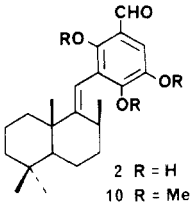

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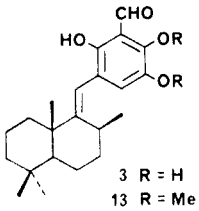
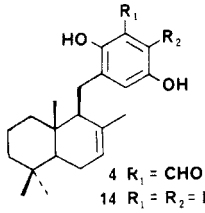
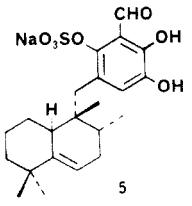
10 R = Me



3 R = H
13 R = Me



4 R_1 = CHO R_2 = OH
14 R_1 = R_2 = H



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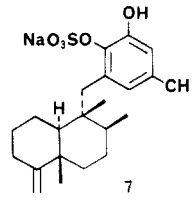

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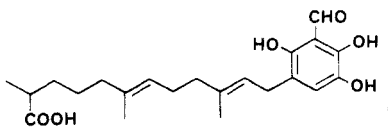

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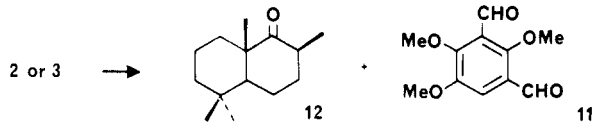
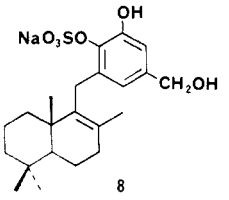
Scheme I. Products Formed by Ozonolysis of the Trimethyl Ether of Siphonodictyal B (Reference 3)



12 11



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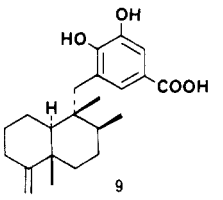

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Eight samples of *Siphonodictyon coralliphagum* f. *typica* and six samples of *S. coralliphagum* f. *tubulosa* were collected at Carrie Bow Cay, Belize. The six samples of f. *tubulosa* were considered indistinguishable by TLC and ^{1}H NMR spectroscopy and were combined. Of the eight samples of f. *typica*, one sample was too small to analyze, and the remaining samples were combined into three groups, two (A and B) of three samples each and one (C) unique sample. Table I lists the quantities of each metabolite obtained from the different samples of *S. coralliphagum*. The collection location and the status of the coral substrate [(L)iving or (D)ead] are recorded together with the yield of each metabolite. The higher concentrations of metabolites were always associated with collections that comprised only the oscular chimneys of sponges from living coral heads but this may only reflect the variation in inorganic material found in the various portions of the sponge.

Siphonodictyal B was isolated from all samples of *S. coralliphagum* f. *typica* from the Caribbean. The structure had previously been determined by ozonolysis of the corresponding trimethyl ether **10** to obtain 2,4,5-trimeth-

(1) Rützler, K. *Smithsonian Contr. Zoo.* **1971**, *77*, 1.
(2) Sullivan, B. W.; Faulkner, D. J. In *New Perspectives in Sponge Biology*; Smithsonian Inst. Press: Washington, D. C., in preparation.
(3) Sullivan, B.; Djura, P.; McIntyre, D. E.; Faulkner, D. J. *Tetrahedron* **1981**, *37*, 979.

0022-3263/86/1951-4568$01.50/0

Table I. Distribution of Metabolites from *Siphonodictyon* Samples (Percent Dry Weight)

collection location	sponge form	coral status	1	3	4	5	6	7	8	9
Belize	unknown	L	0.12	0.90						
Belize	*typica* A	L		0.11			0.03	0.12		0.01
	B	L		0.21			0.03	0.04		
Belize	C	L		0.20			0.04	0.09		
Belize	*tubulosa*	D						0.09		
Bahamas	*typica*	L		3.41			0.25	0.51		
Bahamas	*tubulosa*	D					0.05	0.07		
Ponape	unknown	L			0.21	0.51	0.10		0.08	
Kwajalein	unknown	D			0.23		0.05			

oxyphthaldialdehyde (**11**) and the ketone **12**. These two components could have been derived from either structures **2** or **3** (Scheme I). We had previously selected structure **2** on the basis of calculated ^{1}H NMR chemical shift values[4] but we were aware that the predicted values became less accurate as the degree of substitution about an aromatic ring increased. A more reliable method to determine the substituent pattern about an aromatic ring is to measure the nuclear Overhauser effect (NOE) between adjacent substituents in the ^{1}H NMR spectrum of a suitable derivative. Siphonodictyal B (**3**) was treated with methyl iodide and potassium carbonate in refluxing acetone for 8 h to obtain the dimethoxy derivative **13**. Strong nuclear Overhauser effects were observed between the aldehyde proton signal at δ 10.9 and both the hydroxyl signal at 11.42 and the methoxyl signal at 3.99. Irradiation of the aromatic proton signal at δ 7.01 caused enhancement of the olefinic proton signal at 6.13 and the second methoxy signal at 3.83. Only structure **13** is compatible with these data and the structure of siphonodictyal B must therefore be reassigned to **3**.

Siphonodictyal C (**4**) is an isomer of siphonodictyal B (**3**) that was isolated from two samples of *Siphonodictyon* from Ponape and Kwajalein in the Pacific Ocean. Comparison of their ^{13}C NMR spectra [**3**, 149.6 (s), 144.8 (s), 142.9 (s), 127.7 (s), 125.2 (d), 116.6 (s)] showed that the substitution pattern about the aromatic ring of **4** was identical with that of **3**. The UV spectrum of **4** [368 nm (ϵ 1600), 276 nm (ϵ 5800), 228 nm (sh, ϵ 7900), 209 nm (ϵ 11 800)] differed significantly from that of siphonodictyal B (**3**) and indicated that the olefinic bond was not conjugated to the aromatic ring. The structure of the sesquiterpene portion of siphonodictyal C (**4**) was elucidated by interpretation of spectral data. The ^{13}C NMR spectrum contained, in addition to the aromatic signals, two olefinic signals at δ 136.5 (s) and 122.9 (d) and signals in the ^{1}H NMR spectrum at δ 5.32 (br s, 1 H) and 1.48 (br s, 3 H) indicated the presence of a trisubstituted olefin with a methyl substituent. Comparison of the ^{1}H NMR and ^{13}C NMR data of siphonodictyal C (**4**) with those of isozonarol (**14**)[5] confirmed that they had the same bicyclic ring system and relative stereochemistry.

Siphonodictyal D (**5**), mp 131–132 °C, was isolated from a specimen of *Siphonodictyon* collected from Ponape. The molecular formula, $C_{22}H_{29}O_7SNa$, was determined by high resolution FAB mass spectrometry. Both the ^{1}H and ^{13}C NMR spectra were difficult to interpret due to the low intensity and broadness of many of the signals.[6] A suitable crystal was therefore selected for a single-crystal x-ray diffraction experiment. A computer-generated perspective drawing of the final X-ray model of siphonodicytal D (**5**) is shown in Figure 1. Hydrogens, the sodium counterion, and the waters of crystallization are omitted for clarity. The X-ray experiment was unable to distinguish between the enantiomers, and no absolute configuration is implied. The A ring is in a chair conformation, and the B ring, constrained by the 5,6-double bond, is in a half-chair conformation. The most unusual feature of this molecule is that the secondary methyl group at C-8 is axial to the B ring, the opposite geometry to that predicted by biosynthetic theory.

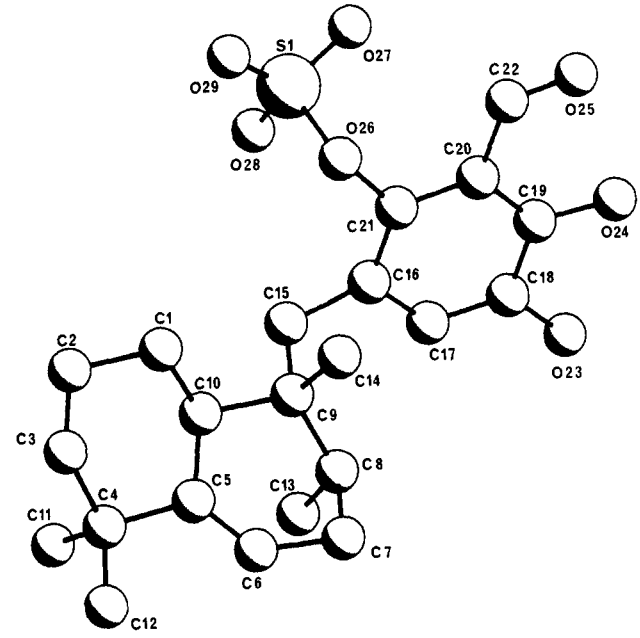


Figure 1. Computer-generated perspective drawing of the final X-ray model of siphonodictyal D (**5**).

Siphonodictyal E (**6**) was obtained in low yield from a majority of the specimens of *S. coralliphagum*. The molecular formula $C_{22}H_{30}O_6$ was determined by high resolution mass spectrometry. The UV and ^{13}C NMR spectra suggested that the same aromatic ring system was present in both siphonodictyal E (**6**) and siphonodictyal C (**4**). The sesquiterpene portion of the molecule contained two trisubstituted olefinic bonds [δ 137.5 (s), 135.8 (s), 123.9 (2 × d)] and a carboxylic acid group [δ 180.8 (s)], which indicated that this portion of the molecule was acyclic. The ^{1}H NMR spectrum contained two olefinic methyl signals at δ 1.57 (s, 3 H) and 1.70 (s, 3 H) and an aliphatic methyl signal at 1.14 (d, 3 H, J = 7 Hz) coupled to a proton signal at 2.41 (m, 1 H), which was assigned to a proton adjacent to the carboxylic acid group. These data sug-

(4) Jackman, L. M.; Sternhell, S. *Applications of NMR Spectroscopy in Organic Chemistry*, 2nd ed.; Pergamon Press: Oxford, 1969; p 202.

(5) **Isozonarol (14)**: ^{1}H NMR ($CDCl_3$) δ 0.88 (s, 3 H), 0.89 (s, 3 H), 0.91 (s, 3 H), 1.47 (br s, 3 H), 1.92 (m, 2 H), 2.34 (m, 1 H), 2.59 (m, 2 H), 4.66 (s, OH), 4.70 (s, OH), 5.38 (br s, 1 H) + 3 signals for aromatic protons; ^{13}C NMR (CD_3OD) δ 137.1 (s), 122.6 (d), 55.7 (d), 51.7 (d), 43.5 (t), 40.7 (t), 38.0 (s), 34.0 (s), 33.9 (q), 27.2 (t), 24.8 (t), 22.6 (q), 22.5 (q), 20.0 (t), 14.4 (q) + 6 signals for aromatic carbons: cf. Fenical, W.; Sims, J. J.; Squatrito, D.; Wing, R. M.; Radlick, P. *J. Org. Chem.* **1973**, *38*, 2383.

(6) Sullivan, B. W. Ph.D. Thesis, University of California, San Diego, 1985.

Table II. Comparison of ^{13}C NMR Data (Unassigned) of Sesquiterpene Portion of Siphonodictyol G (7), Siphonodictyoic Acid (9), Ilimaquinone (15), and Palauolide (16)

7[a]	9[a]	15[b]	16[b]
161.29 s	161.29 s	160.5 s	160.2 s
103.09 t	103.24 t	102.1 t	102.4 t
49.35 d	49.28 d	50.2 d	48.5 d
43.20 s	43.31 s	43.3 s	39.8 s
41.30 s	41.31 s	40.5 s	39.2 s
38.76 t	38.59 t	38.2 d	37.1 d
37.88 t	37.95 t	36.7 t	36.5 t
37.42 d	37.52 d	33.0 t	36.2 t
34.10 t	34.15 t	32.4 t	33.6 t
29.33 t	29.15 t	28.6 t	28.5 t
28.88 t	28.89 t	28.0 t	27.3 t
24.24 t	24.36 t	23.2 t	21.5 t
21.09 q	21.07 q	20.6 q	20.6 q
18.43 q	18.51 q	17.9 q	17.9 q
18.13 q	18.13 q	17.3 q	17.6 q

[a] CD_3OD. [b] $CDCl_3$.

gested a linear sesquiterpene side chain with one of the terminal methyl groups oxidized to a carboxylic acid. Proton-decoupling experiments confirmed the proposed structure. The signal at δ 2.41 was coupled to signals at 1.60 (m, 1 H) and 1.38 (m, 3 H) while a signal at 1.96 (t, 2 H, J = 7 Hz) was also coupled to the signal at 1.38. These data indicated the partial structure $CH_3CH(COOH)CH_2CH_2C(CH_3)=$. The olefinic proton signal at δ 5.27 (t, 1 H, J = 7 Hz) was coupled to the benzylic proton signal at 3.47 (d, 2 H, J = 7 Hz) and the second olefinic proton signal at 5.09 (t, 1 H, J = 7 Hz) was coupled to a two-proton signal at 2.10 (q, 2 H, J = 7 Hz), which was in turn coupled to a vinyl methylene signal at 2.04 (t, 2 H, J = 7 Hz). The two olefinic bonds were assigned the *E* stereochemistry on the basis of the chemical shifts of the ^{13}C NMR signals for the olefinic methyl groups at δ 15.9 (q) and 16.3 (q).

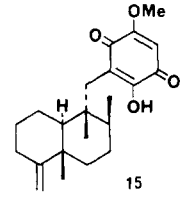
Siphonodictyol G (**7**) was found in most samples of *S. coralliphagum* from the Caribbean and had a molecular formula of $C_{22}H_{31}O_6SNa$ that was determined by FAB mass spectrometry. The presence of a sulfate group was suggested by the presence of IR bands at 1250 and 1050 cm^{-1}. The ^1H NMR signals at δ 6.83 (d, 1 H, J = 1.7 Hz) and 6.63 (d, 1 H, J = 1.7 Hz) and six signals in the ^{13}C NMR spectrum at δ 150.7 (s), 140.5 (s), 139.2 (s), 135.2 (s), 123.4 (d), and 115.3 (d) were indicative of a 1,2,3,5-tetrasubstituted aromatic ring. Signals at δ 4.50 (s, 2 H) in the ^1H NMR spectrum and at δ 64.9 (t) in the ^{13}C NMR spectrum were assigned to an isolated benzylic alcohol group. Irradiation of the benzylic signal at δ 4.50 caused a nuclear Overhauser enhancement of the aromatic proton signals at 6.63 and 6.83. The ^{13}C NMR chemical shift values were calculated[7] for the aromatic protons of all possible substitution patterns having an alkyl chain, a hydroxyl group, a sulfate group[8] and a benzylic alcohol flanked by two aromatic protons: the values for a 3-alkyl 4-*O*-sulfate 5-hydroxybenzyl alcohol [calcd δ 150.5 (s), 141.8 (s), 140.6 (s), 138.5 (s), 122.8 (d), 115.2 (d)] most closely agree with the observed values.

The ^1H NMR spectrum of siphonodictyol G (**7**) showed the presence of one secondary and two tertiary methyl groups [δ 0.86 (s, 3 H), 1.04 (d, 3 H, J = 7 Hz), 1.05 (s, 3 H)], an exocyclic methylene [δ 4.34 (br s, 1 H), 4.38 (br s, 1 H)], and an isolated benzylic methylene groups [δ 2.79 (d, 1 H, J = 14 Hz), 2.83 (d, 1 H, J = 14 Hz)]. The ^{13}C NMR data (Table II) suggested that the bicyclic carbon skeleton of siphonodictyol G was identical with those of ilimaquinone (**15**)[9] and palauolide (**16**).[10]

Table III. Comparison of ^{13}C NMR Data of Sesquiterpene Portion of Siphonodictyol H (8) with Compounds 17 and 18

C no.	8[a]	17[b]	18[b]
1	37.2 t	37.1	36.9
2	20.3 t	19.0	19.1
3	43.0 t	41.8	41.8
4	34.3 s	33.3	33.3
5	53.3 d	51.8	51.8
6	19.9 t	19.0	19.4
7	34.9 t	33.6	33.3
8	129.8 s	129.6	125.4
9	139.9 s	139.8	140.4
10	40.2 s	39.1	37.3
11	28.6 t	22.0	21.8
17[c]	20.5[d] q	19.4	20.1
18[c]	33.8 q	33.3	33.6
19[c]	22.2 q	21.7	21.6
20[c]	20.7[d] q	20.1	19.1

[a] CD_3OD. [b] $CHCl_3$. [c] Or equivalent methyl carbon on the sesquiterpene portion of 8. [d] Assignments may be reversed.



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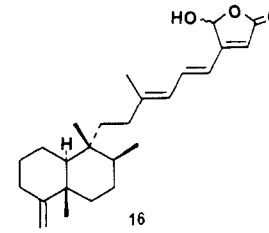

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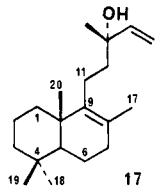
Siphonodictyol H (**8**) was obtained in low yield from the Ponape specimen of *S. coralliphagum* and had the molecular formula $C_{22}H_{31}O_6SNa$, isomeric with siphonodictyol G (**7**). The ^1H NMR spectrum contains signals at δ 6.69 (d, 1 H, J = 1.8 Hz) and 6.56 (d, 1 H, J = 1.8 Hz), assigned to meta protons on a 1,3,4,5-tetrasubstituted aromatic ring. The ^{13}C NMR data for siphonodictyol H [δ 151.3 (s), 139.9 (s), 139.0 (s), 138.9 (s), 137.6 (s), 129.8 (s), 119.5 (d), 115.4 (d)] confirmed the presence of a tetrasubstituted aromatic ring and a tetrasubstituted olefinic bond. Again, irradiation of the $-CH_2OH$ signal at δ 4.32 in the ^1H NMR spectrum caused a nuclear Overhauser enhancement of both aromatic signals at 6.69 and 6.56 and comparison of the calculated and observed ^{13}C NMR chemical shift values indicated that siphonodictyol H (**8**) had the same aromatic ring substitution pattern as siphonodictyol G (**7**). The ^1H NMR spectrum contained four methyl signals at δ 0.85 (s, 3 H), 0.91 (s, 3 H), 1.00 (s, 3 H), and 1.45 (br s, 3 H) and signals at 3.41 (d, 1 H, J = 18 Hz) and 3.50 (d, 1 H, J = 18 Hz) due to a methylene group that is situated between the aromatic ring and a tetrasubstituted olefin in the bicyclic ring system. Comparison of the ^{13}C NMR data (Table III) of siphonodictyol H (**8**) with those of model compounds **17**[11] and **18**[12] strongly suggested that they have the same structure and relative stereochemistry for the bicyclic ring system.

Siphonodictyoic acid (**9**) has the molecular formula $C_{22}H_{28}O_4$. The ^1H NMR signals at δ 7.46 (d, 1 H, J = 1.9 Hz) and 7.42 (d, 1 H, J = 1.9 Hz) were assigned to meta-

(7) Wehrli, F. W.; Wirthlin, T. *Interpretation of Carbon-13 NMR Spectra*; Heyden: London, 1978.
(8) Ragan, M. A. *Can. J. Chem.* **1978**, *56*, 2681.
(9) Luibrand, R. T.; Erdman, T. R.; Vollmer, J. J.; Scheuer, P. J.; Finer, J.; Clardy, J. *Tetrahedron* **1979**, *35*, 609.
(10) Sullivan, B.; Faulkner, D. J. *Tetrahedron Lett.* **1982**, *23*, 907.
(11) Imamura, P. M.; Marsaioli, A. J.; Barata, L. E. S.; Ruveda, E. A. *Phytochem.* **1977**, *16*, 1842.
(12) Bastard, J.; Duc, D. K.; Fetizon, M.; Francis, M. J.; Grant, P. K.; Weavers, R. T.; Kaneko, C.; Baddeley, G. V.; Bernassau, J. M.; Burfitt, I. R.; Wovkulich, P. M.; Wenkert, E. *J. Nat. Prod.* **1984**, *47*, 592.



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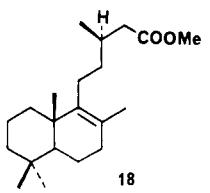

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substituted aromatic protons. The ^{13}C NMR spectrum contained a signal at δ 169.7 (s) due to a carboxylic acid group, six aromatic carbons signals at 151.2 (s), 145.3 (s), 135.6 (s), 128.2 (s), 126.8 (d), and 118.0 (d) and signals at 161.3 (s) and 103.2 (t) due to an exocyclic methylene group. Comparison of the observed NMR chemical shift values for the aromatic protons and carbons with the calculated values for five possible nonsymmetrical structures clearly favored the proposed structure. Comparison of the ^{13}C NMR data (Table II) with those of siphonodictyol G (**7**) required the same structure and relative stereochemistry for the sesquiterpenoid portion of each molecule.

We had previously reported that both siphonodictyal A (**1**) and siphonodictyal B (**3**) inhibited the growth of *Staphylococcus aureus* and *Bacillus subtilis* in a standard disk assay. Both siphonodictyals C (**4**) and D (**5**) were significantly more active than siphonodictyal B (**3**) against *S. aureus* and *B. subtilis* and also inhibited the marine bacterium *Vibrio anguillarum*. Siphonodictyols G (**7**) and H (**8**) were slightly less active than siphonodictyal B (**3**) against *S. aureus* and *B. subtilis* while siphonodictyal E (**6**) was completely inactive in all assays. Studies directed toward elucidation of a functional role for these metabolites are discussed in detail elsewhere.[2]

Experimental Section

Collection, Extraction, and Purification. *Siphonodictyon coralliphagum* f. *typica* was collected by hand using SCUBA at Chub Cay, the Bahamas (−20 m), in August, 1983, and Carrie Bow Cay, Belize (−20 m), in November, 1983. The Bahamas sample consisted of only the oscular chimneys, while the Belize samples included both oscular chimneys and sponge from the interior of the coral heads.

The Bahamas sample (83-185, 15.0 g dry wt) was frozen immediately after collection and stored frozen for 4 months. The sponge was then steeped in methanol for 2 weeks at 4 °C. The solvent was decanted and the sponge was washed with fresh methanol. The combined methanol extracts were evaporated to dryness and then dissolved in water (400 mL). The aqueous material was extracted with hexane (2 × 400 mL), dichloromethane (2 × 400 mL), and ethyl acetate (3 × 400 mL). The individual extracts were dried over anhydrous sodium sulfate and the solvents evaporated to obtain 40 mg (0.26% dry wt) of the hexane extract, 60 mg (0.4% dry wt) of the dichloromethane extract, and 1.145 g (7.63% dry wt) of the ethyl acetate extract.

The ethyl acetate extract was chromatographed on Sephadex LH-20 by using a 1:1 mixture of methanol/dichloromethane as eluant to obtain siphonodictyal B (**3**, 512 mg, 3.41% dry wt), siphonodictyol G (7, 76 mg, 0.51% dry wt) and siphonodictyal E (**6**, 37 mg, 0.25% dry wt).

Eight samples were collected in Belize (83-258 A–H). Each sample was stored in methanol for 1–2 months and then worked up in an identical manner. The solvent was decanted and the sponge was washed with fresh methanol. The combined methanol extracts were evaporated to dryness and then dissolved in water (200–400 mL). The aqueous material was extracted with hexane (2 × 300 mL), dichloromethane (2 × 300 mL), and ethyl acetate (3 × 400 mL). The individual extracts were dried over anhydrous sodium sulfate and the solvents evaporated. Each extract was examined by ^1H NMR and those extracts that were identical were combined. The hexane and dichloromethane extracts contained very little material and were not further investigated. The following ethyl acetate extracts were combined: 83-258 A, G, and H, and 83-258 B, C, and D. Sample 83-258 E was too small for further analysis.

The ethyl acetate extract (83-285 A, G, and H, 366 mg, 1.16% dry wt) was chromatographed on Sephadex LH-20 by using a 1:1 mixture of methanol and dichloromethane as eluant to obtain siphonodictyal B (**8**, 67 mg, 0.21% dry wt). Siphonodictyol G (**7**, 12 mg, 0.04% dry wt) and siphonodictyal E (**6**, 10 mg, 0.03% dry wt) were present in two earlier fractions and were purified by thin layer chromatography on C_8 reversed-phase plates using methanol/water (7/3) as eluant.

The ethyl acetate extract (83-258 B, C, and D, 1.107 g, 1.49% dry wt) was chromatographed on Sephadex LH-20 by using a 1:1 mixture of methanol and dichloromethane as eluant. One pure compound, siphonodictyoic acid (**9**, 7 mg, 0.01% dry wt), was obtained from this column. A fraction containing siphonodictyal E (**6**, 25 mg, 0.03% dry wt) was further purified by thin layer chromatography on C_8 reversed-phase plates. Siphonodictyal B (**3**, 80 mg, 0.11% dry wt) could be crystallized from a fraction that also contained siphonodictyol G (**7**, 90 mg, 0.12% dry wt) that was purified by thin layer chromatography on C_8 reversed-phase plates using methanol/water (7/3) as eluant.

The ethyl acetate extract (83-258 F, 531 mg, 1.06 dry wt) was chromatographed as above to obtain siphonodictyal B (**3**, 100 mg, 0.20% dry wt), siphonodictyol G (**7**, 80 mg, 0.16% dry wt), and siphonodictyal E (**6**, 18 mg, 0.04% dry wt).

Siphonodictyon coralliphagum f. *tubulosa* was collected by hand using SCUBA at Carrie Bow Cay, Belize (−20 m), in November, 1983, and Bimini, the Bahamas (−20 m), in August, 1984. The portion of the sponge protruding from the coral head was collected.

Five samples were collected in Belize (83-256 A–E). Each sample was stored in methanol for 4 months and then worked up in an identical manner. The solvent was decanted and the sponge was soaked in fresh methanol for 1 day. The combined methanol extracts were evaporated to dryness and then dissolved in water (400 mL). The aqueous material was extracted with hexane (2 × 300 mL), dichloromethane (2 × 300 mL), and ethyl acetate (3 × 300 mL). The individual extracts were dried over anhydrous sodium sulfate and the solvents evaporated. Each extract was examined by ^1H NMR. The extracts from all five sponges were identical by ^1H NMR and were combined.

The ethyl acetate extract (83-256 A–E, 494 mg, 0.44% dry wt), which was found to contain the majority of the material, was chromatographed on Sephadex LH-20 using a 1:1 mixture of methanol/dichloromethane as eluant to obtain siphonodictyol G (**7**, 100 mg, 0.09% dry wt).

The Bahamas sample (84-090, 145.7 g dry wt) was frozen immediately after collection and stored frozen for 2 weeks prior to extraction. The sponge was then steeped in methanol at 4 °C for 1 week. The solvent was decanted and the sponge was soaked in fresh methanol for 1 day. The combined methanol extracts were evaporated to dryness and then dissolved in water (400 mL). The aqueous material was extracted with hexane (2 × 100 ML), dichloromethane (2 × 400 mL), and ethyl acetate (3 × 400 mL). The individual extracts were dried over anhydrous sodium sulfate and the solvents evaporated.

The ethyl acetate extract (643 mg, 0.44% dry wt), which contained the compounds of interest, was chromatographed on Sephadex LH-20 by using a 1:1 mixture of methanol/dichloromethane as eluant to obtain siphonodictyol G (**7**, 100 mg, 0.07% dry wt) and siphonodictyal E (**6**, 70 mg, 0.05% dry wt).

Siphonodictyon coralliphagum from the Pacific was collected from two locations, Ponape (−15 m) in January, 1983, and Nini Island, Kwajalein (−11 m) in December, 1983. The Ponape sample (83-076) consisted of only the portion of the sponge that was protruding from the coral heads, while the Kwajalein sample was mainly the sponge from inside the coral heads.

The Kwajalein sample (84-030, 55.1 g dry wt) was frozen immediately after collection. The sample was then placed in methanol and stored at 4 °C for 1 month. The solvent was decanted and the sponge was washed with fresh methanol. The combined methanol extracts were evaporated to an aqueous suspension (500 mL). The aqueous material was extracted with hexane (2 × 30 mL), dichloromethane (2 × 300 mL), and ethyl acetate (3 × 300 mL). The individual extracts were dried over anhydrous sodium sulfate and the solvents evaporated. Each extract was examined by ^1H NMR.

The ethyl acetate extract (485 mg, 0.88% dry wt) was chromatographed on Sephadex LH-20 by using a 1:1 mixture of methanol/dichloromethane as eluant to obtain siphonodictyal C (4, 127 mg, 0.23% dry wt) and siphonodictyal E (6, 30 mg, 0.05% dry wt).

The Ponape sample (83-076, 245.9 g dry wt) contained large quantities of mucus (12.3 g dry wt) which was collected separately. Both the sponge and the mucus were frozen immediately after collection. The frozen sponge was lyophilized and the dried sponge extracted successively with dichloromethane (2 × 1 L), ethyl acetate (2 × 1 L), and methanol (3 × 1 L). The methanol extract (44.7 g, 18.19% dry wt) contained the compounds of interest and was dissolved in water (400 mL). The aqueous material was extracted with ethyl acetate (5 × 300 mL) to obtain a yellow gum (6.784 g, 2.76% dry wt).

The ethyl acetate extract (3.392 g) was chromatographed on Sephadex LH-20 by using a 1:1 mixture of methanol/dichloromethane as eluant to obtain siphonodictyal C (4, 260 mg, 0.11% dry wt) and siphonodictyal D (5, 650 mg, 0.26 dry wt). A fraction containing siphonodictyal E (6, 120 mg, 0.05% dry wt) was purified by thin layer chromatography on C_8 reversed-phase plates. Siphonodictyol H (8, 105 mg, 0.04% dry wt) was present with the corresponding aldehyde as a 3:1 mixture. Purification by thin layer chromatography on C_8 reversed-phase plates resulted in the loss of the aldehyde.

The frozen mucus was lyophilized and the dried material dissolved in water (100 mL). The aqueous material was extracted with ethyl acetate (3 × 100 mL) to obtain a brown gum (60 mg, 0.49% dry wt). Analysis of the ^1H NMR spectrum of this extract indicated that it contained both siphonodictyals C (4) and D (5).

Siphonodictyal C (4): $[\alpha]_{578}$ −23.60° (c 0.47, MeOH), $[\alpha]_{546}$ −30.47 (c 0.47, MeOH); UV (MeOH) 368 nm (ε 1600), 276 (5800), 228 sh (7900), 209 (11 800); UV (MeOH + KOH) 406 nm (ε 1800), 291 (4470), 244 (7840), 212 (16 360); IR (KBr) 3500 br, 1645, 1240, 1040 cm^{-1}; ^1H NMR ($CDCl_3$ + CD_3OD) δ 0.88 (s, 3 H), 0.89 (s, 3 H), 0.93 (s, 3 H), 1.48 (br s, 3 H), 1.94 (m, 2 H), 2.28 (m, 1 H), 2.48 (dd, 1 H, J = 3, 15 Hz), 2.60 (dd, 1 H, J = 9, 15 Hz), 5.32 (br s, 1 H), 7.01 (s, 1 H), 10.17 (s, 1 H); ^{13}C NMR (CD_3OD) δ 198.1 (d), 149.1 (s), 144.8 (s), 143.7 (s), 136.5 (s), 130.5 (s), 123.4 (d), 122.9 (d), 116.6 (s), 56.4 (d), 51.6 (d), 43.4 (t), 40.8 (t) 37.9 (s), 33.9 (s), 33.8 (q), 27.2 (q), 24.7 (t), 22.6 (q), 22.4 (q), 19.9 (t), 14.4 (q); high resolution mass measurement, observed m/z 358.2122, $C_{22}H_{30}O_4$ requires 358.2144.

Siphonodictyal D (5): mp 131–132 °C; $[\alpha]_{478}$ −56.54°, $[\alpha]_{546}$ −67.65° (c 1.12, MeOH); UV (MeOH) 367 nm (ε 2700), 276 (9350), 228 sh (12 360) 214 (14 110); UV (MeOH + KOH) 401 nm (ε 2965), 288 (6620), 244 (13 165), 212 (17 530); IR (KBr) 3450 br, 1650, 1230, 1050 cm^{-1}; ^1H NMR ($CDCl_3$ + CD_3OD) δ 0.76 (br s, 3 H), 1.10 (d, 3 H, J = 7 Hz), 1.08 (s, 6 H), 5.41 (br s, 1 H), 6.92 (br s, 1 H), 10.28 (s, 1 H); ^{13}C NMR (CD_3OD) δ 194.6 (d), 155.7 (s), 150.0 (s), 148.8 (s), 137.7 (s), 130.8 (d), 118.7 (s), 117.2 (d), 112.4 (s), 44.6 (d), 43.2 (d), 40.1 (s), 38.6 (s), 35.2, 33.2 (t), 31.5, 31.3, 31.0 (q), 28.0, 24.8 (t), 23.0 (q), 16.8 (q); high resolution fast atom bombardment mass measurement, observed 483.1427 $(M + H + Na)^+$, $C_{22}H_{29}O_7SNa_2$ requires 483.1429.

Siphonodictyal E (6): UV (MeOH) 366 nm (ε 1600), 276 (5500); IR (KBr) 3400 br, 1710, 1650 cm^{-1}; ^1H NMR ($CDCl_3$ + CD_3OD) δ 1.14 (d, 3 H, J = 7 Hz), 1.38 (m, 3 H), 1.57 (br s, 3 H), 1.60 (m, 1 H), 1.70 (br s, 3 H), 1.96 (t, 2 H, J = 7 Hz), 2.04 (t, 2 H, J = 7 Hz), 2.10 (q, 2 H, J = 7 Hz), 2.41 (m, 1 H), 3.47 (d, 2 H, J = 7 Hz), 5.09 (t, 1 H, J = 7 Hz), 5.27 (t, 1 H, J = 7 Hz), 6.96 (s, 1 H), 10.27 (s, 1 H); ^{13}C NMR (CD_3OD) δ 198.0 (d), 180.8 (s), 149.4 (s), 145.0 (s), 143.9 (s), 137.5 (s), 135.8 (s), 128.0 (s), 125.5 (d), 123.9 (d), 123.9 (d), 116.6 (s), 40.8 (t), 40.5 (d), 40.5 (t), 34.4 (t), 28.3 (t), 27.5 (t), 26.5 (t), 17.6 (q), 16.3 (q), 15.9 (q); high-resolution mass measurement, observed m/z 390.2076, $C_{22}H_{30}O_6$ requires 390.2042.

Siphonodictyol G (7): $[\alpha]_{578}$ −24.92°, $[\alpha]_{546}$ −28.02° (c 0.65, MeOH); UV (MeOH) 282 nm (ε 1440), 276 (1390), 220 sh (6875), 212 (8340); UV (MeOH + KOH) 299 nm (ε 1700), 284 (1550), 244 (2950), 218 (10 275); IR (KBr) 3450 br, 1250, 1050 cm^{-1}; ^1H NMR ($CDCl_3$ + CD_3OD) δ 0.86 (s, 3 H), 1.04 (d, 3 H, J = 7 Hz), 1.05 (s, 3 H), 2.79 (d, 1 H, J = 14 Hz), 2.83 (d, 1 H, J = 14 Hz), 4.34 (br s, 1 H), 4.38 (br s, 1 H), 4.50 (s, 2 H), 6.63 (d, 1 H, J = 1.7 Hz), 6.83 (d, 1 H, J = 1.7 Hz); ^{13}C NMR (CD_3OD) δ 161.3 (s), 150.7 (s), 140.5 (s), 139.2 (s), 135.2 (s), 123.4 (d), 115.3 (d), 103.1 (t), 64.9 (t), 49.4 (d), 43.2 (s), 41.3 (s), 38.8 (t), 37.9 (t), 37.4 (d), 34.1 (t), 29.3 (t), 28.9 (t), 24.2 (t), 21.1 (q), 18.4 (q), 18.1 (q); high resolution fast atom bombardment mass measurement, observed m/z 469.1633, $C_{22}H_{31}O_6SNa_2$ $(M + H + Na)^+$ requires 469.1367.

Siphonodictyol H (8): no rotation observed; UV (MeOH) 280 nm (ε 1550), 274 (1500), 213 (9100); UV (MeOH + KOH) 292 nm (ε 1575), 282 (1650), 244 (3100), 218 (10 900); IR (KBr) 3450 br, 1250, 1045 cm^{-1}; ^1H NMR ($CDCl_3$ + CD_3OD) δ 0.85 (s, 3 H), 0.91 (s, 3 H), 1.00 (s, 3 H), 1.45 (br s, 3 H), 2.07 (dd, 1 H, J = 6, 17 Hz), 2.17 (m, 1 H), 3.41 (d, 1 H, J = 18 Hz), 3.50 (d, 1 H, J = 18 Hz), 4.50 (s, 2 H), 6.56 (d, 1 H, J = 1.8 Hz), 6.69 (d, 1 H, J = 1.8 Hz); ^{13}C NMR (CD_3OD) δ 151.3 (s), 139.9 (s), 138.9 (s), 137.6 (s), 129.8 (s), 119.5 (d), 114.9 (d), 65.2 (t), 53.3 (d), 43.0 (t), 40.2 (s), 37.2 (t), 34.9 (t), 34.3 (s), 33.8 (q), 28.6 (t), 22.2 (q), 20.7 (q), 20.5 (q), 20.3 (t), 19.9 (t); high resolution fast atom bombardment mass measurement, observed m/z 469.1633 $(M + H + Na)^+$, $C_{22}H_{31}O_6SNa_2$ requires 469.1637.

Siphonodictyoic acid (9): $[\alpha]_{578}$ −5.69°, $[\alpha]_{546}$ −12.60° (c 0.25, MeOH); UV (MeOH) 294 nm (ε 1650), 245 (3340), 216 (8400); UV (MeOH + KOH) 295 nm (ε 1640), 244 (3710, sh), 216 (8800); IR (KBr) 3450 br, 1710, 1250, 1050 cm^{-1}; ^1H NMR ($CDCl_3$ + CD_3OD) δ 0.87 (s, 3 H), 1.06 (s, 3 H), 1.07 (d, 3 H, J = 7 Hz), 2.19 (m, 2 H), 2.33 (m, 1 H), 2.82 (d, 1 H, J = 14 Hz), 2.89 (d, 1 H, J = 14 Hz), 4.36 (br s, 1 H), 4.39 (br s, 1 H), 7.42 (d, 1 H, J = 1.9 Hz), 7.46 (d, 1 H, J = 1.9 Hz); ^{13}C NMR (CD_3OD) δ 169.7 (s), 161.3 (s), 151.2 (s), 145.3 (s), 135.6 (s), 128.2 (s), 126.7 (d), 118.0 (d), 103.2 (t), 49.3 (d), 43.3 (s), 41.3 (s), 38.6 (t), 38.0 (t), 37.5 (d), 34.2 (t), 29.2 (t), 28.9 (t), 24.4 (t), 21.1 (q), 18.5 (q), 18.1 (q); high resolution mass measurement, observed m/z 358.2145, $C_{22}H_{30}O_4$ requires 358.2144.

Single-Crystal X-ray Analysis of Siphonodictyal D (5). Yellow cubic crystals suitable for X-ray analysis were formed by slow evaporation of an aqueous ethanol solution. Preliminary X-ray photographs displayed orthorhombic symmetry, and lattice constants of a = 8.460 (3), b = 11.867 (3), and c = 25.003 (5) Å resulted from a least-squares fit of 15 diffractometer-measured 2θ values. The systematic extinctions, crystal density (~1.33 g/mL), and optical activity were uniquely accommodated by space group $P22_12_1$ (alternate setting of $P2_12_12$) with a unit of composition $C_{22}H_{29}O_7SNa{\cdot}3H_2O$ forming the asymmetric unit. All unique diffraction maxima with $2\theta \leq 114°$ were collected on a computer-controlled four-circle diffractometer using variable speed, 1° ω scans and graphite-monochromated Cu Kᾱ radiation (1.54178 Å). Of the 1965 reflections that were recorded in this fashion, 1391 (71%) were judged observed ($F_o \geq 3\sigma(F_o)$) after correction for Lorentz, polarization, and background effects.[13] A phasing model was found with a multisolution tangent formula approach, and the first E-synthesis revealed essentially all of the non-hydrogen atoms. Hydrogen atoms were located in a ΔF-synthesis after partial refinement of the non-hydrogen atoms. Block-diagonal least-squares refinements have converged to the current crystallographic residual of 0.085 for the observed reflections. In retrospect, this relatively high value is attributed to the loss of waters of hydration from the crystal. Further crystallographic information is available and is described in the paragraph entitled supplementary material available at the end of this paper.

Methylation of Siphonodictyal B (3). Excess methyl iodide was added to a solution of siphonodictyal B (3, 40 mg, 0.11 mmol) in acetone (10 mL) containing potassium carbonate (25 mg). The reaction mixture was stirred at reflux temperature for 8 h. The

(13) All crystallographic calculations were done on a Prime 850 computer operated by the Cornell 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 programs 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; DIRDIF written by P. T. Beurskens et al., University of Nijmegen, Netherlands, 1981; MITHRIL, an automatic solution package written by C. J. Gilmore, University of Glasgow, Scotland, 1983; 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, 1978; and BOND, a program to calculate molecular parameters and prepare tables written by K. Hirotsu, Cornell University, 1978.

cooled reaction mixture was poured into ice and acidified with 3 N hydrochloric acid, and the products were extracted with ethyl acetate (3 × 20 mL). The crude product was purified by LC on Partisil by using 15% ethyl acetate in hexane as eluant to obtain the dimethoxy derivative **13** (22 mg, 51% yield): oil, ^{1}H NMR ($CDCl_3$) δ 0.88 (s, 3 H), 0.88 (d, 3 H, J = 7 Hz), 0.91 (s, 3 H), 1.19 (s, 3 H), 2.65 (m, 1 H), 3.83 (s, 3 H), 3.99 (s, 3 H), 6.13 (s, 1 H), 7.01 (s, 1 H), 10.29 (s, 1 H), 11.42 (s, 1 H).

Acknowledgment. We sincerely thank Dr. Klaus Rützler, Smithsonian Institution, for identifying all sponge samples and for his interest in this project. We gratefully acknowledge a generous gift of isozonarol from Professor William H. Fenical. Antimicrobial screening was performed by Mary Kay Harper. This research was supported by the California Sea Grant College Program (NA85AA-D-SG140, R/MP30), the New York Sea Grant Program, the National Institutes of Health (AI11969 to D.J.F.), and the National Science Foundation (INT14133 to J.C.).

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

Practical Routes to Two Functionalized Decalones for the Synthesis of Quassinoids

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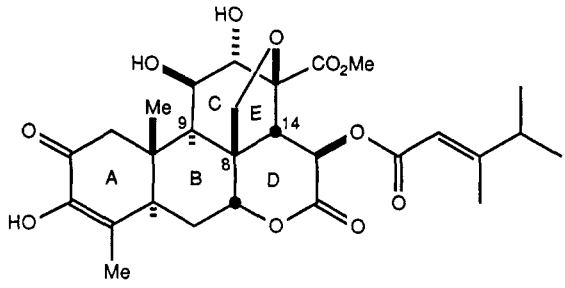
Received April 28, 1986

The chiral keto alcohol **6a** was prepared from (*S*)-(+)-carvone. Because two steps in this process gave only modest yields of isolated materials, an alternative route was developed. Racemic keto alcohol **6b** was prepared from enedione *rac*-**13a** by a more efficient process.

We have recently reported[1] a strategy for the synthesis of quassinoids,[2] in particular bruceantin (**1**), that employs



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a Claisen rearrangement to set the C_8, C_9, C_{14} stereochemistry and utilizes three successive ring closures (C → CE → CED) to realize the pentacyclic model *rac*-**5** (Scheme I).[3] While this model study provided important information regarding the elaboration of the CDE ring system, it did not provide latent functionality in ring A for eventual transformation into the substitution pattern present in ring A of bruceantin. Our goal was to prepare chiral, nonracemic keto alcohol **6a** that would ultimately lead to (−)-bruceantin and to be able to prepare **6a** with sufficient ease and in ample quantity to realize our synthetic goal. This paper details our efforts toward this end.

We have reported[4] that (+)-6-epi-α-cyperone (**7**) can be prepared in 67% yield by the Mueller lithium–bronze reduction of (+)-carvone, followed by annulation of the resultant enolate with ethyl vinyl ketone and subsequent KOH–MeOH dehydration. This 10-g scale reaction proved amenable to scale up (200–300 g), providing enone **7** (40% yield, 91% purity) along with 19% recovered dihydrocarvone, which could be recycled. The contaminant (GLC analysis) present in enone **7**, although not identified, was presumably *ent*-cyperone.[5] The presence of this substance did not adversely affect subsequent transformations and was eventually removed by crystallization. Successful double lithium–bronze reduction[6] of enone **7** to alcohol **8a** required the expected formation of the trans ring junction (*t*-BuOH proton source) and stereoselective α-axial protonation of the resultant enolate (EtOH proton source) prior to reduction of the intermediate saturated ketone. This procedure provided a complex mixture of products wherein alcohol **8a** was the major component. Alternatively, stepwise reduction proved successful. Lithium–bronze reduction (*t*-BuOH) of enone **7** afforded a mixture of three ketones from which **8b** (% ds = 85) could be isolated in 47% yield by crystallization. Unfortunately, the residual amount of ketone **8b**, while still the major component remaining in the mother liquors, could not be separated from the other isomers. Two of the minor components were presumed to be *cis*-decalones. The inordinately high percentage of cis isomer formed in the enone reduction is a result of the steric hindrance of the isopropenyl group associated with protonation of the intermediate anion radical of enone **7** from the α face.[7]

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(3) All structures are the enantiomers shown, unless specified otherwise (racemate = rac; enantiomer = ent). Quassinoid numbering is employed.

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