refluxed under argon for 1 h. A workup similar to that described in the synthesis of **6** followed by chromatography gave **9** (198 mg, 94%) as a foam: ^{1}H NMR ($CDCl_3$) δ 2.03 (3 H, s, Ac), 2.06 (6 H, s, Ac), 3.20 (6 H, s, N^2-CH_3), 4.27 (3 H, m, 4',5'-H), 5.35–6.10 (3 H, m, 1',2',3'-H), 7.43 (1 H, s, 8-H).

Anal. Calcd for $C_{18}H_{23}O_8N_5 \cdot {}^1/_3H_2O$: C, 48.76; H, 5.38; N, 15.79. Found: C, 48.50; H, 5.10; N, 15.69.

Method B. A mixture of **11** (112 mg, 0.2 mmol), TBTH (0.25 mL, 0.9 mmol), and AIBN (8 mg, 0.05 mmol) in benzene (2 mL) was refluxed under argon for 3 h. The usual workup followed by chromatography gave **9** (83.2 mg, 95%) as foam.

Methylation of Guanosine by Trimethyl Phosphate in the Presence of Tetrabutylammonium Fluoride. A mixture of guanosine (283.2 mg, 1 mmol) and trimethyl phosphate (2 mL, 17.1 mmol) was dissolved in a 1 M THF solution of tetrabutylammonium fluoride (10 mL, 10 mmol). The resulting mixture was stirred at room temperature for 18 h and then diluted with dioxane to a volume of 20 mL. One-fifth of this solution was applied to Watman 3MM papers developed with 2-propanol–concentrated ammonia–H_2O (7:1:2, v/v/v). The strongest UV absorbing band of R_f 0.61 was eluted with water to give N^1-methylguanosine (2022 $A_{255.4}$, 64% calculated by using $\epsilon = 15.9 \times 10^3$). The aqueous solution was freeze-dried and analyzed by 270 MHz NMR: ^{1}H NMR (270 MHz, D_2O/DSS) δ 3.37 (3 H, s, N^1-CH_3), 3.83 (1 H, dd, $J_{5'a-5'b}$ = 12.6 Hz, $J_{5'a-4'}$ = 4.2 Hz, 5'-Ha), 3.92 (1 H, $J_{5'a-5'b}$ = 12.6 Hz, $J_{5'b-4'}$ = 3.1 Hz, 5'-Hb), 4.23 (1 H, dd, $J_{3'-4'}$ = 7.5 Hz, $J_{2'-3'}$ = 3.9 Hz, 3'-H), 4.42 (1 H, dd, $J_{2'-3'}$ = 3.9 Hz, $J_{1'-2'}$ = 5.5 Hz, 2'-H), 5.85 (1 H, d, $J_{1'-2'}$ = 5.5 Hz, 1'-H), 7.93 (1 H, s, 8-H); UV (H_2O) λ_{max} 255.4 nm, λ_{min} 227 nm, sh 268 nm. The positions of λ_{max} and sh in the UV spectra of this material did not change essentially over the pH range 7–12.

N^2-Methylguanosine (MMG). Compound **6** (127 mg, 0.3 mmol) was dissolved in pyridine (9 mL), and 0.5 M sodium hydroxide (9 mL) was added. The solution was kept at room temperature for 20 min and then passed through a column of Dowex 50W X8 (pyridinium form, 13 mL). The column was washed successively with water (50 mL) and 10% aqueous pyridine (40 mL). The eluate and washings were combined and evaporated under reduced pressure. The residue was coevaporated several times with water to remove the last traces of pyridine. Crystallization of the residue from hot water (30 mL) gave MMG (69 mg, 73%): This material did not give a clear melting pattern as reported by Robins.[7] Softening began at ca. 179 °C and from then gradual decomposition was observed until ca. 230 °C: ^{1}H NMR ($CDCl_3$) δ 2.81 (3 H, s, N^2-CH_3), 3.68 (2 H, m, 5'-H), 3.98 (1, m, 4'-H), 4.55 (1 H, m, 2'-H), 5.67 (1 H, d, J = 6 Hz, 1'-H), 7.7 (1 H, s, 8-H).

Anal. $C_{11}H_{15}O_5N_5 \cdot {}^3/_4H_2O$: C, 42.51; H, 5.35; N, 22.53. Found: C, 42.21; H, 5.38; N, 22.60.

N^2,N^2-Dimethylguanosine (DMG). This compound was synthesized in a manner similar to that described for the synthesis of MMG and was recrystallized from water. DMG: mp 238–239 °C dec (lit.[6] mp 242 °C): ^{1}H NMR (d_6-DMSO–D_2O) δ 3.02 (6 H, s, N^2-CH_3), 3.79 (1 H, m, 4'-H), 4.07 (1 H, m, 3'-H), 4.44 (1 H, m, 2'-H), 5.61 (1 H, d, J = 5.4 Hz, 1'-H), 7.77 (1 H, s, 8-H).

Anal. Calcd for $C_{12}H_{17}O_5N_5$: C, 46.30; H, 5.50; N, 22.50. Found: C, 46.13; H, 5.47; N, 22.55.

Registry No. **3**, 6979-94-8; **4**, 130469-17-9; **6**, 4395-48-6; **8**, 130469-18-0; **9**, 73196-87-9; **10**, 130469-15-7; **11**, 130469-16-8; BDTF, 57842-27-0; DMG, 2140-67-2; MMG, 2140-77-4; guanosine, 118-00-3; N^1-methylguanosine, 2140-65-0.

New Diterpenoids from the Caribbean Gorgonian *Eunicea calyculata*. Photochemical Interconversion of the Cembrene and Cubitene Skeletons

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Seven new diterpenoids of the cubitane and cembrane classes (**1**–**7**) have been isolated from the Caribbean sea whip *Eunicea calyculata* (Ellis and Solander). The structures of these new compounds were assigned on the basis of spectral studies and an interconversion of the major metabolites via a photochemically induced 1,3-acyl migration. The first transformation of a cembrene to a cubitene diterpenoid is reported.

Marine octocorals of the order Gorgonacea, the sea whips and sea fans (phylum Cnidaria), are recognized as a rich source of biologically active and structurally unique secondary metabolites.[1] In the Caribbean Sea, sea whips of the genus *Eunicea* (Family Plexauridae) are particularly abundant and form a major component of the shallow water invertebrate fauna.[2] Early chemical investigations of *Eunicea* species, beginning in the early 1960s, showed these animals to be a chemically complex resource for cembrene lactones.[3] More recent studies of *Eunicea* species, based upon a chemotaxonomic approach, have shown that this gorgonian genus produces a wider diversity of secondary metabolites.[4-7]

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Table I. ^1H and ^{13}C NMR Spectral Assignments for Calyculones D–G (1–4)[a,b]

	1		2		3		4	
C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
1	2.10–2.04 (1 H, m)[c]	27.5	2.13–2.01 (1 H, m)[c]	28.4	2.24 (1 H, m)[c]	27.8	2.09–2.01 (1 H, m)[c]	28.7
2	1.41 (1 H, m)[c]	34.8	1.48 (1 H, m)[c]	33.5	1.54 (1 H, dddd, 14.1, 10.3, 3.8, 3.8)	32.8	1.45 (1 H, m)[c]	35.9
	1.27 (1 H, dddd, 13.8, 7.0, 7.0, 4.5)		1.35 (1 H, m)[c]		1.28 (1 H, dddd, 14.0, 7.0, 6.5, 3.2)		1.08 (1 H, m)[c]	
3	2.15–2.10 (1 H, m)[c] 1.97 (1 H, m)[c]	23.9	2.13–2.01 (2 H, m)[c]	24.9	2.20–2.11 (1 H, m)[c] 1.98 (1 H, m)[c]	23.5	2.05–1.93 (2 H, m)[c]	25.7
4	4.98 (1 H, dd, 9.5, 4.8)	127.6	5.03 (1 H, dd, 7.6, 7.6)	129.6	4.87 (1 H, dd, 10.2, 4.3)	127.9	5.02 (1 H, br dd, 7.3, 7.3)	127.6
5		133.2		132.8		132.5		134.6
6	2.22–2.15 (2 H, m)[c]	39.2	2.27 (1 H, m)[c,d] 2.25–2.18 (1 H, m)[c,d]	39.3	2.20–2.11 (1 H, m)[c,d] 2.06 (1 H, m)[c,d]	38.0	2.14–1.93 (2 H, m)[c,d]	37.0
7	2.29 (1 H, m)[c]	28.8	2.25–2.18 (1 H, m)[c,d]	28.4	2.32 (1 H, ddd, 13.4, 13.1, 2.1)	27.4	2.36 (1 H, m)[c]	28.3
	2.15–2.08 (1 H, m)[c]		2.16–2.13 (1 H, m)[c,d]		1.93 (1 H, ddd, 13.4, 6.6, 2.1)[d]		2.14–1.93 (1 H, m)[c,d]	
8		144.5		146.2[f]		147.3[f]		147.3[f]
9	4.95 (1 H, d, 9.6)	122.2	4.92 (1 H, d, 10.7)	122.0	5.32 (1 H, d, 10.1)	120.4	5.50 (1 H, d, 10.5)	119.9
10	4.02 (1 H, d, 9.6)	57.7	3.99 (1 H, d, 10.7)	59.3	3.82 (1 H, d, 10.1)	59.3	4.05 (1 H, d, 10.5)	56.5
11		211.2		209.5		210.2		209.9
12	2.48 (1 H, dd, 12.7, 6.5) 2.00 (1 H, dd, 12.7, 7.7)	49.1	2.98 (1 H, dd, 15.9, 7.7) 2.13–2.01 (1 H, m)[c]	47.1	2.46 (1 H, dd, 14.3, 7.2) 2.04 (1 H, dd, 14.3, 6.6)	47.6	2.43 (1 H, dd, 14.5, 9.3) 2.24 (1 H, dd, 14.5, 4.3)	48.4
13	0.95 (3 H, d, 6.6)	20.8	0.97 (3 H, d, 6.8)	21.1[g]	0.91 (3 H, d, 6.9)	20.7[g]	0.98 (3 H, d, 6.8)	21.1[g]
14	1.60 (3 H, br s)	15.3	1.57 (3 H, br s)	15.2	1.72 (3 H, br s)	16.8	1.62 (3 H, br s)	17.3
15	2.76 (1 H, hep, 6.9)	29.4	2.83 (1 H, hep, 7.0)	29.5	2.27 (1 H, m)[c]	33.2	2.31 (1 H, hep, 6.8)	33.0
16	1.08 (3 H, d, 7.0)[e]	21.1[g]	1.12 (3 H, d, 7.0)[e]	21.4[g]	1.07 (3 H, d, 6.9)[e]	21.6[g]	1.13 (3 H, d, 6.9)[e]	21.7[g]
17	0.95 (3 H, d, 6.9)[e]	21.6[g]	1.00 (3 H, d, 6.9)[e]	21.9[g]	1.03 (3 H, d, 6.8)[e]	23.4[g]	1.03 (3 H, d, 6.8)[e]	23.4[g]
18		143.7		143.2[f]		143.9[f]		144.1[f]
19	1.69 (3 H, br s)	21.1	1.72 (3 H, br s)	21.9[g]	1.69 (3 H, br s)	21.2[g]	1.67 (3 H, br s)	22.0[g]
20	4.88 (1 H, br dd, 1.5, 1.5)	112.8	4.91 (1 H, br s)	112.9	4.81 (1 H, br dd, 1.5, 1.5)	112.3	4.82 (1 H, br dd, 1.5, 1.5)	112.2
	4.75 (1 H, br s)		4.77 (1 H, br s)		4.73 (1 H, br s)		4.74 (1 H, br s)	

[a] ^1H NMR spectra weree recorded in $CDCl_3$ solution at 360 MHz. Assignments for 1 are on the basis of COSY and RCT experiments. Assignments of others were aided by COSY NMR experiments. J values are reported in hertz, and the chemical shifts are given in δ units (ppm downfield from Me_4Si). [b] ^{13}C NMR spectra were recorded in $CDCl_3$ solution at 50 MHz. Chemical shifts are given in δ units (ppm downfield from Me_4Si). Attached proton counts (not shown) were obtained by DEPT experiments. Assignments for 1 were aided by XHCORR and COLOC experiments. Assignments of 2–4 were aided by comparison with 1. [c] Coupling constants were not determined. [d–g] Signals within a column may be reversed.

In this paper, we report the structures of seven new diterpenoids, 1–7, isolated from the Caribbean gorgonian *Eunicea calyculata* (Ellis and Solander) (Chart I). Compounds 1–4 (calyculones D–G) are ketones of the irregular diterpenoid cubitane class, precedented by cubitene, the first compound with this monocyclic skeleton isolated from the East African termite *Cubitermes umbratus*.[8] Compounds 5–7 are olefin geometrical isomers of 1,3,11-cembratrien-6-one. Although cembranes are the most frequently encountered class of metabolites from octocorals, compounds 5–7 possess different functionalities from other *Eunicea* cembranes. Calyculones D–G are precedented by our earlier study describing calyculones A–C from the same source.[5]

A unique observation in this investigation is the co-occurrence of both the cubitane and cembrane skeletons, each possessing similar functionalization. As part of the structure elucidation process, we have shown that the 6-ketocembratrienes reported here undergo a photochemical ring contraction reaction (a 1,3-acyl migration) to yield the corresponding 11-ketocubitenes. This is the first example of a 1,3-acyl migration in natural products and the first ring contraction reaction involving the cembrene skeleton. This observation leads us to speculate that a similar cembrene ring contraction reaction may also be responsible for production of the pseudopterane class of rearranged diterpenoids.[9]

Eunicea calyculata was collected in 1986, as part of an expedition to the Tobago Cays in the eastern Caribbean Sea. Freshly collected animals were air-dried, stored frozen, and subsequently exhaustively extracted with dichloromethane. Proton NMR analysis of the crude extract revealed the presence of unusually large amounts of triglycerides (>90% of the extract). Silica vacuum flash chromatography, followed by Sephadex LH-20 column chromatography of the nonpolar fractions, gave 250 mg (4% of the extract) of terpenoid-containing material. Four diterpenoids, calyculone D (1), (1*E*,3*E*,11*E*)-1,3,11-cembratrien-6-one (5) (1*Z*,3*Z*,11*E*)-1,3,11-cembratrien-6-one (6), and (1*E*,3*Z*,11*E*)-1,3,11-cembratrien-6-one (7), were isolated from this mixture by preparative HPLC (5% EtOAc in isooctane). Three additional diterpenoids, calyculones E–G (2–4), which were unsuccessfully purified by HPLC, were ultimately purified by $AgNO_3$-impregnated silica centrifugal chromatography (Chromatotron, 4–6% EtOAc in isooctane). Compounds 1–7 were isolated as less than 0.1% of the crude extract each. However, these compounds are thought to occur in larger quantities in the animal. We demonstrated that significant loss had occurred during evaporation processes due to the unexpectedly high volatilities of these compounds.

Calyculone D (1) was isolated as an oil (5% EtOAc in isooctane), which analyzed for $C_{20}H_{32}O$ by high resolution mass and ^{13}C NMR spectrometry. The carbonyl signal at δ 211.2 (C) in the ^{13}C NMR spectrum (Table I) and the

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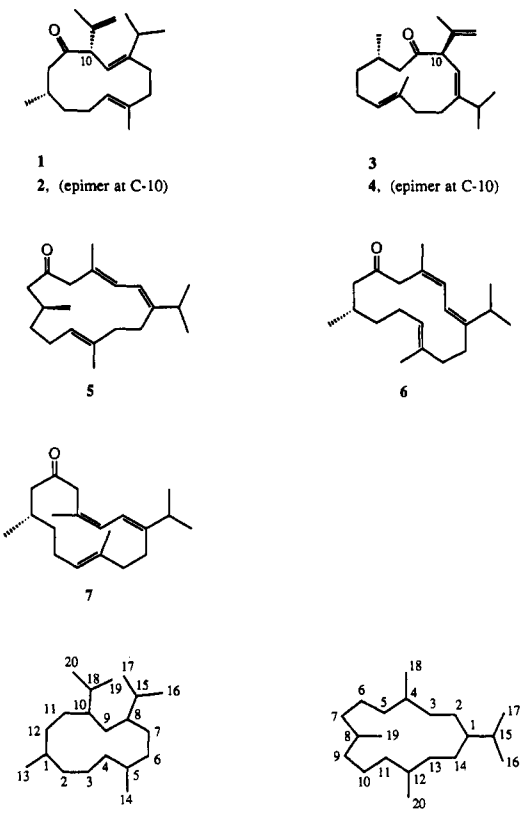
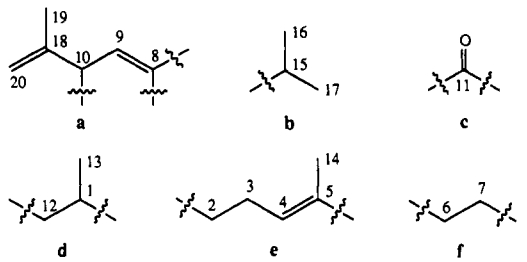
Chart I. New Metabolites from *Eunicea calyculata*



corresponding absorption band at 1715 cm^{-1} in the IR spectrum established the presence of a ketone, which accounted for the oxygen atom in the molecular formula. Six low-field carbon bands at δ 144.5 (C), 143.7 (C), 133.2 (C), 127.6 (CH), 122.2 (CH), and 112.8 (CH_2) in the ^{13}C NMR spectrum illustrated 1 to possess three double bonds, thus calyculone D was monocyclic. The lack of absorption in the UV spectrum showed that the chromophores (ketone and double bonds) in 1 were not conjugated. A combination of ^1H NMR COSY and ^1H relay coherence transfer (RCT)[10] NMR experiments showed the presence of several isolated spin systems (Table I). With the help of direct carbon–proton NMR correlation (XHCORR) data, all of the partial structures of compound 1 were confidently identified as a–f.



A long-range carbon–proton correlation (COLOC) experiment allowed the partial structures to be combined (Table II). The carbonyl carbon (c) was coupled to the protons at δ 4.95, 4.02, 2.48, and 2.00 (H-9, -10, -12, -12, respectively), allowing partial structure c to be connected to substructures a and d. The isopropyl carbon at δ 29.4 (CH) of partial structure b was observed to be coupled to the H-9 proton. At the same time, the C-8 olefinic carbon at δ 144.5 (C) of a was coupled to the methyl protons of

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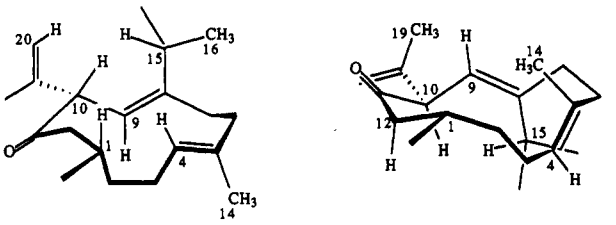
Table II. Results of the Two-Dimensional Carbon–Proton Long-Range Correlation Experiments (COLOC)[a,b] with Compounds 1 and 5

	correlations to protons at C	
C	1	5
1	12 (2.48), 13	
2	12 (2.00)	14 (2.55)
3		5 (3.20), 5 (2.88), 18
4	14	5 (3.20), 5 (2.88), 18
5	14	
6	14	5 (3.20), 5 (2.88), 7 (2.65), 7 (1.66)
7	9	
8	10, 16, 17	
9	10	
10	20 (4.88), 20 (4.75), 19	
11	9, 10, 12 (2.48), 12 (2.00)	
12	13	20
13	12 (2.48), 12 (2.00)	20
14	4	2
15	9, 16	2
16	17	
17	16	16
18	10, 19, 20 (4.75)	3, 5 (3.20)
19	20 (4.88), 20 (4.75)	
20		

[a] Experiments were performed at 50 MHz in $CDCl_3$ solutions. Parameters were optimized for couplings of 6 Hz. [b] The numbers in parentheses are the ^1H NMR chemical shifts of the protons that correlate.



	proton (s) irradiated	proton (s) enhanced	measured enhancement (%)
1	10	1, 15, 20 (4.75)[a]	2.5, 12.5, 3.8
	14	9	4.9
	15	10, 16	13.4, 2.2
	16	15	5.6
2	12α (2.98)[a]	10	3.3
	10	15	8.6
	14	9	1.1
	15	10	8.2
	19	9, 10	3.0, 2.3

[a] Chemical shifts of the corresponding protons.

Figure 1. Results of ^1H NMR nuclear Overhauser enhancement difference spectroscopy (NOEDS) experiments with compounds 1 and 2.

δ 1.08 (H-16) and 0.95 (H-17). Thus, the connection between a and b was established. The methylene carbons at δ 39.2 and 28.8 in partial structure f were found to be coupled to the protons at δ 1.60 (H-14) and 4.95 (H-9), respectively, indicating that f was connected to the substructures a and e. Finally, connection between d and e was accomplished by observation of coupling between the C-2 carbon at δ 34.8 and the H-12 proton at δ 2.00. This assignment was supported by the high field shifts, δ 1.41 and 1.27, assigned to the H-2 proton resonances. Thus, the structure of 1 was assigned as a diterpene of the rare cubitane class.

Calyculone D possesses two double bonds (Δ^4 and Δ^8) and two asymmetric carbon centers (C-1 and C-10). The high field chemical shift of the C-14 methyl group (δ 15.3) in the ^{13}C NMR spectrum indicated an *E* configuration for

Table III. ^1H and ^{13}C NMR Assignments for *Eunicea* Cembratrienes 5–7[a,b]

	5		6		7	
C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
1		148.6		145.3		147.9
2	6.07 (1 H, d, 11.2)	118.7	5.91 (1 H, br d, 11.5)	119.4	6.14 (1 H, d, 10.5)	119.1
3	6.23 (1 H, br d, 11.2)	126.8	6.29 (1 H, br d, 11.5)	123.6[e]	6.08 (1 H, br d, 10.9)	127.3[e]
4		128.5		128.8		128.3
5	3.20 (1 H, d, 12.7)	54.4	3.22 (1 H, br d, 13.9)	50.5	3.72 (1 H, d, 13.7)	48.0
	2.88 (1 H, br d, 12.7)		3.17 (1 H, br d, 13.9)		2.64 (1 H, br d, 13.7)	
6		211.2		211.0		212.4
7	2.65 (1 H, dd, 12.6, 4.1)	48.8	2.32–1.98 (2 H, m)[c]	46.0	2.57 (1 H, dd, 12.6, 4.5)	46.1
	1.66 (1 H, m)[c]				1.62 (1 H, m)[c]	
8	2.15–1.95 (1 H, m)[c]	28.4	2.32–1.98 (1 H, m)[c]	27.6	2.22–1.89 (1 H, m)[c]	27.2
9	1.49 (1 H, m)[c]	37.3	1.27–1.20 (2 H, m)[c]	35.8[f]	1.40 (1 H, dddd, 13.7, 12.0, 3.7, 3.7)	38.5[f]
	1.21 (1 H, dddd, 13.7, 8.5, 6.8, 3.5)				1.15 (1 H, m)[c]	
10	2.15–1.95 (2 H, m)[c]	24.7	2.32–1.98 (2 H, m)[c]	24.6	2.22–1.89 (2 H, m)[c]	24.4
11	5.10 (1 H, br dd, 7.3, 6.8)	125.9	5.07 (1 H, br ddd, 7.7, 7.6, 1.1)	126.2[e]	4.73 (1 H, br ddd, 10.4, 2.0, 1.1)	125.5[e]
12		136.0		134.2		132.8
13	2.23 (1 H, m)[c]	37.9	2.32–1.98 (2 H, m)[c]	36.9[f]	2.22–1.89 (2 H, m)[c]	37.3[f]
	2.15–1.95 (1 H, m)[c]					
14	2.55 (1 H, m)[c]	29.8	2.32–1.98 (2 H, m)[c]	27.2	2.72 (1 H, ddd, 13.1, 12.8, 3.2)	27.8
	2.23 (1 H, m)[c]				2.22–1.89 (1 H, m)[c]	
15	2.39 (1 H, hep, 6.9)	33.5	3.04 (1 H, hep, 6.9)	29.4	2.31 (1 H, hep, 6.9)	32.7
16	1.12 (3 H, d, 6.8)[d]	21.8[g]	1.06 (3 H, d, 7.1)[d]	21.2[g]	1.09 (3 H, d, 6.8)[d]	21.1[g]
17	1.07 (3 H, d, 6.8)[d]	22.9[g]	1.04 (3 H, d, 7.1)[d]	21.6[g]	1.04 (3 H, d, 6.9)[d]	23.6[g]
18	1.71 (3 H, br s)	16.4	1.76 (3 H, br s)	24.1	1.71 (3 H, br s)	23.5[g]
19	0.87 (3 H, d, 6.8)	20.0	0.84 (3 H, d, 6.3)	19.4[g]	0.83 (3 H, d, 6.7)	20.4[g]
20	1.58 (3 H, br s)	18.1	1.60 (3 H, br s)	15.4	1.62 (3 H, br s)	16.1

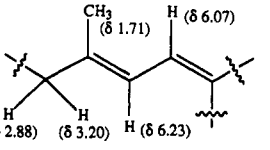
[a] ^1H NMR spectra were recorded in $CDCl_3$ at 360 MHz. Assignments for 5 were aided by COSY and RCT experiments. Assignments for 6 and 7 were aided by COSY experiments. *J* values were reported in hertz and chemical shifts are given in δ units (ppm downfield from Me_4Si). [b] ^{13}C NMR spectra were recorded in $CDCl_3$ at 50 MHz. Attached proton counts (not shown) were obtained by DEPT experiments. Assignments for 5 were aided by XHCORR and COLOC experiments. Assignments for 6 and 7 were aided by comparison with 5. Chemical shifts are given in δ units (ppm downfield from Me_4Si). [c] Coupling constants were not measured. [d–g] Signals within a column may be reversed.

the Δ^4 double bond. The stereochemistries of the other asymmetric centers were determined by ^1H NMR NOEDS experiments summarized in Figure 1. Irradiation of the H-15 proton (δ 2.76) enhanced the H-10 proton by 13.4%. Also, irradiation of the H-10 proton enhanced the protons at H-1, -15, and -20 by 2.5, 12.5, and 3.8%, respectively. Finally, the H-9 proton was enhanced by irradiation of the H-14 methyl protons (4.9%). Thus, the stereochemistries of the asymmetric centers of calyculone D (1) were confidently assigned as 4*E*,8*Z*,1*S** and 10*S** (asterisk indicating relative stereochemistry only).

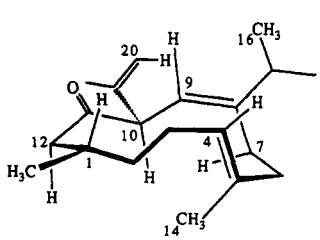
A related compound, calyculone E (2), was isolated as an oil that also analyzed for $C_{20}H_{32}O$ by high resolution mass and ^{13}C NMR spectrometry. NMR spectral data for 2 were very similar to those obtained from 1. The only significant difference was the downfield shift of one of the C-12 protons by 0.50 ppm in the ^1H NMR spectrum of 2 (Table I). Therefore, compound 2 was concluded to be a stereochemical and/or geometrical isomer of 1. A ^1H NMR NOEDS experiment allowed the stereochemistries of the asymmetric centers to be defined (Figure 1). Several enhancements among key protons revealed that calyculone E has the same olefin geometries as calyculone D. Since both compounds have only two asymmetric carbon centers (C-1 and -10), 2 must be a diastereomer of 1. Photochemical interconversion studies (discussed in the final section) showed that calyculone E was the epimer of calyculone D at C-10. The overall relative configurations of 2 are thus 4*E*,8*Z*,1*S** and 10*R**.

Two other related metabolites, calyculones F (3) and G (4), were also isolated as oils. Spectral data for these compounds were also highly compatible with those derived from compounds 1 and 2. Proton COSY NMR experiments again showed that these compounds were diastereomers or geometrical isomes of 1 and 2. Proton NMR NOEDS experiments established the stereochemistries of the asymmetric centers (Figure 2). NOE enhancements among the C-9 and C-16 protons revealed that these compounds possessed *E* configurations at the Δ^8 olefin position, in contrast to the *Z* configurations (determined by NOE between the C-10 and C-15 protons) of the Δ^8 double bonds in calyculones D and E. Further NOE studies showed that as in the case of compounds 1 and 2, calyculones F and G are epimers at C-10. Thus, the stereochemistry of calyculone F was confidently assigned as 4*E*,8*E*,1*S** and 10*R**, while calyculone G was assigned the relative configuration 4*E*,8*E*,1*S** and 10*S**.

In addition to the cubitanes, the crude extract contained metabolites of the cembrane skeleton. The major metabolite, (1*E*,3*E*,11*E*)-cembra-1,3,11-triene-6-one (5), was isolated as an oil by preparative HPLC (5% EtOAc in isooctane) and found to analyze for $C_{20}H_{32}O$ by high resolution mass and ^{13}C NMR spectrometry. Comparison of the ^1H and ^{13}C NMR spectra showed some similarities between cembrene 5 and calyculone D (1). However, there were several significant differences in the spectral data (Table III). The most apparent difference was the replacement of the isopropylene group in 1 by an olefinic methyl group (δ 1.71). Also, in contrast to the lack of UV absorption from calyculone D, the UV spectrum of 5 showed maxima at 254 and 248 nm. Careful examination of ^1H and ^{13}C NMR spectra revealed 5 to possess the same partial structures b–f of calyculone D (1). However, a combination of ^1H NMR COSY and RCT experiments showed that the partial structure a of 1 was replaced by a new 1,1,4,4-tetrasubstituted diene unit in 5.



A combination of XHCORR and COLOC ^{13}C NMR experiments (all of the carbon and proton resonances were



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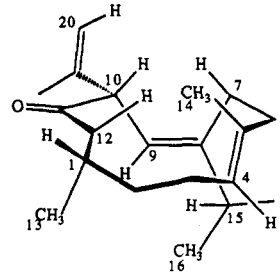


4

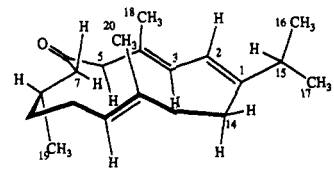
	proton (s) irradiated	proton (s) enhanced	measured enhancement (%)
3	9	1, 16, 20(4.73)[a]	b, 2.3, 1.1
	10	7α(2.32)[a], 12α(2.46)[a], 20(4.73)[a]	b, 3.1, 2.3
	12α(2.46)[a]	10	5.4
	14	10	2.3
	16	9	4.4
4	9	16, 20(4.74)[a]	4.8, 2.3
	10	12β(2.43)[a], 20(4.74)[a]	b, 2.9
	16	9, 15	4.6, b

[a] Chemical shifts of the corresponding protons. [b] Due to overlapping proton signals, enhancements were not measured.

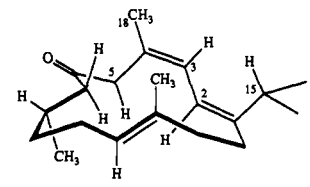
Figure 2. Results of ^{1}H NMR nuclear Overhauser enhancement difference spectroscopy (NOEDS) experiments with compounds **3** and **4**.

unambiguously assigned, Tables II and III) confidently connected all of the partial structures and thus revealed the full planar structure of **5**. Cembratriene **5** has three double bonds (Δ^1, Δ^3, and Δ^{11}) and an asymmetric carbon at C-8. The high field ^{13}C chemical shift of the C-20 carbon signal (δ 18.1) indicated the *E* configuration for the Δ^{11} double bond. The configurations of the other centers were determined by a ^{1}H NMR NOEDS experiment (Figure 3). Irradiation of the C-2 proton enhanced the proton signals at C-15, -16, and -17 by 3.7, 2.5, and 3.4%, respectively. At the same time, the signal of the C-18 methyl protons was enhanced by 4.9%. The signal of the C-2 proton was enhanced by irradiation of both the C-16 and -18 methyl protons (5.2 and 8.3%, respectively). On the other hand, irradiation of the C-3 proton enhanced the signals of the C-5α and C-14α protons by 2.5 and 9.8%, respectively. In addition, the C-3 proton signal was enhanced by the irradiation of the C-5α proton (5.1%). Therefore, the configuration of the diene was assigned as 1*E* and 3*E*. Although the enhancement was not large (1.1%), irradiation of the C-19 methyl protons consistently enhanced the C-3 proton, indicating that the orientation of the C-19 methyl is axial to the plane of molecule. The absolute stereochemistry at C-8 is unknown. However, since the cembrenes have been interconverted to the calyculones (discussion to follow), the stereochemistry at this center is identical with that at C-1 in the calyculones. Thus, the full structure of compound **5** was determined as (1*E*,3*E*,11*E*)-cembra-1,3,11-trien-6-one.

Two other related cembratrienes **6** and **7** were also isolated as oils. The spectral data of these compounds were very similar to those from cembratriene **5**. Proton NMR COSY data, in particular, showed the same proton correlations as in **5**. However, there were significant differences in the chemical shifts of a few key protons (for example, the C-5, C-14, and C-15 proton resonances, Table III), suggesting that compounds **6** and **7** were isomeric to cembratriene **5**. Proton NMR NOEDS studies again allowed the stereochemistries of the double bonds and the asymmetric center to be assigned. NOE studies revealed that, in contrast to the 1*E*,3*E* configuration for **5**, compounds



5



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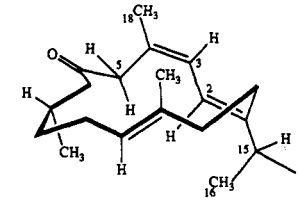

7

	proton (s) irradiated	proton (s) enhanced	measured enhancement (%)
5	2	15, 16, 17, 18	3.7, 2.5, 3.4, 4.9
	3	5α(3.20)[a], 14α(2.55)[a]	2.5, 9.8
	5α	3	5.1
	7β	18	5.7
	16	2, 15	5.2, 3.7
	18	2	8.3
	19	3	1.1
6	2	5[b]	4.3
	3	15	6.2
	5[b]	2	7.5
	15	3	21.8
	18	3	8.4
7	5α(3.72)[a]	2	13.2
	16	2	2.5
	18	3, 5β(2.64)[a]	5.2, 4.5
	19	5α(3.72)[a]	4.0

[a] Chemical shifts of the corresponding protons. [b] Both methylene protons were irradiated.

Figure 3. Results of ^{1}H NMR nuclear Overhauser enhancement difference spectroscopy (NOEDS) measurements with compounds **5–7**.

6 and **7** possessed the 1*Z*,3*Z* and 1*E*,3*Z* diene configurations, respectively (Figure 3). Thus, **6** and **7** were identified as (1*Z*,3*Z*,11*E*)-cembra-1,3,11-trien-6-one and (1*E*,3*Z*,11*E*)-cembra-1,3,11-trien-6-one, respectively.

The origin of the irregular terpenoid skeleton of the cubitanes has been an intriguing question. Prestwich and co-workers suggested two possible routes for the formation of this monocyclic skeleton: cyclization of an irregular acyclic diterpene or recyclization of an open-chain intermediate formed by an oxidative cleavage of a cembrane precursor.[8] Comparison of the structures of compounds **1–7** revealed striking similarities in functionalization, suggesting they had a common intermediate or were interconvertable. The presence of the ketone and 1,3-diene chromophores in the cembratrienes **5–7** suggested the possibility of the photochemical process. It is well known that β,γ-unsaturated ketones readily undergo 1,3-acyl migrations under photochemical conditions.[11,12] Irradiation of cembratriene **5** with a medium pressure Hg lamp (3 h) resulted in the rapid loss of starting material and the generation of complex mixtures (Figure 4). Purification by HPLC showed that calyculone D (**1**) and cembratrienes **6** and **7** were produced in 8.8, 10.6, and 11.5% yields,

(11) For a review of the photochemical 1,3-acyl migration and related reactions, see: Hixson, S. S.; Mariano, P. S.; Zimmerman, H. E. *Chem. Rev.* **1973**, *73*, 531.

(12) For some of recent examples of utilizing the 1,3-acyl migration in organic synthesis, see (a) Uyehara, T.; Kabasawa, Y.; Furuta, T.; Kato, T. *Tetrahedron Lett.* **1985**, *26*, 2343. (b) Uyehara, T.; Kabasawa, Y.; Kato, T.; Furuta, T. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 539. (c) Uyehara, T.; Furuta, T.; Kabasawa, Y.; Yamada, J.; Kato, T.; Yamamoto, Y. *J. Org. Chem.* **1988**, *53*, 3669.

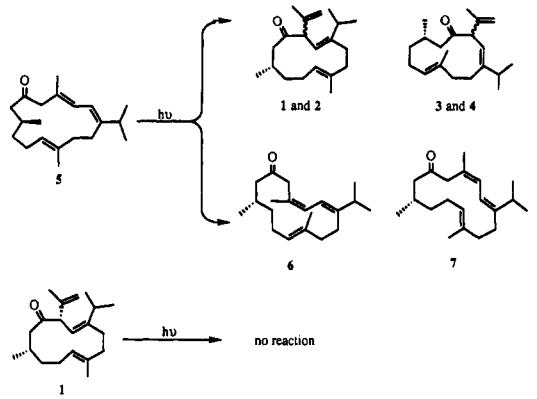

Figure 4. Photochemical reactions of compounds 1 and 5.

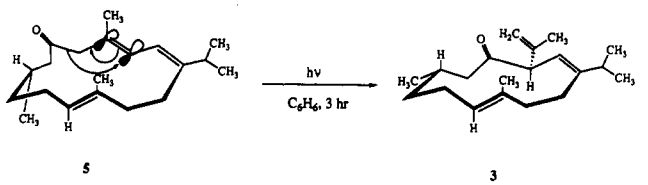

Figure 5. Photochemical concerted synfacial 1,3-acyl migration for the conversion of cembratriene 5 to calyculone F (3).

respectively. Calyculones E–G (2–4) were also produced in 6.2, 10.6, and 15.9% yields. The potential reversability of this photoprocess was also investigated. Photolysis of calyculone D (1) was performed under the same conditions as above. After 8 h of continuous irradiation, no detectable conversion to other products was observed.

The photochemical process we observed results in the conversion of cembratriene 5 to all compounds observed in the natural extract. This observation suggests that a photochemical ring contraction, occurring in nature, is responsible for the biosynthesis of the calyculones isolated from *Eunicea calyculata*. Although this remains unproven, Caribbean gorgonians are functionally photosynthetic organisms that contain endosymbiotic algae known as zooxanthellae. Since considerable sunlight reaches these animals, it is entirely conceivable that photochemical processes could proceed at slow rates. The photoprocesses observed appear complex and to be a combination of photoisomerization of the 1,3-diene followed by 1,3-acyl migration. Photolysis of 5 leads to the diene isomers 6 and 7, demonstrating that photoisomerization has occurred. Based upon the products obtained, and upon the demonstrated photostability of calyculone D (1), we suggest that the ring contraction reaction occurs via a concerted synfacial 1,3-acyl migration (Figure 5). Numerous examples exist in which similar photochemical acyl migrations have been observed and utilized to perform synthetic ring contraction reactions.[12] On the basis of the preferred ring conformations as determined by NOE studies (Figure 3), the stereochemical result of the ring contraction reaction should be predictable. In a concerted process, the 1*E*,3*E* isomer 5 yields calyculone F (3) (Figure 5). By analogy, cembratriene 6 (1*Z*,3*Z*) would lead directly to calyculone D (1), and cembratriene 7 (1*E*,3*Z*) would lead to calyculone G (4). Conspicuously absent in the natural and photoproduct mixture is the cembratriene isomer with the 1*Z*,3*E* configuration. This isomer appears to be formed from 5 during the photoisomerization process but is predicted to be rapidly converted to calyculone E (2). The origin of calyculones A–C, which possess an epoxide functionality at C-11, is predicted to be analogous to that described here involving a 6-keto-11,12-epoxycembradiene precursor.

Experimental Section

General. Proton NMR, COSY, and RCT spectra were recorded in $CDCl_3$ solutions at 360 MHz. All chemical shifts are reported with respect to internal Me_4Si. NOE difference spectroscopy (NOEDS) experiments were performed in general as outlined by Hall and Sanders.[13] Carbon-13 NMR, direct (XHCORR), and long-range (COLOC) carbon–proton correlation spectra were recorded in $CDCl_3$ solutions at 50 MHz. All chemical shifts are reported with respect to Me_4Si. Both high- and low-resolution mass measurements were provided by the Mass Spectrometry Service Laboratory, University of Minnesota. Optical rotations were measured in the indicated solutions with a 10-cm microcell. All solvents used either were spectral grade or were distilled from glass prior to use.

Collection and Extraction. *Eunicea calyculata* (Ellis and Solander) (specimen number CI86227)[14] was collected by hand using SCUBA at 20 to 25 m depth in July, 1986, along the offshore islands of the Tobago Cays, eastern Caribbean Sea. The collection was surface air-dried in the shade and immediately frozen. The gorgonian was next repeatedly extracted with CH_2Cl_2 and the combined extracts were evaporated to yield 6.7 g of crude organic extract (from 1 kg, dry weight of the gorgonian). The extract was separated by silica vacuum flash chromatography using sequential mixtures of EtOAc and isooctane as elutants. Analysis of the flash chromatographic fractions by ^1H NMR showed the presence of secondary metabolites in the fraction eluted with 10% EtOAc in isooctane. Separation by Sephadex LH-20 chromatography (isooctane/$CHCl_3$/EtOH = 2:1:0.5) gave 250 mg of a terpenoid-rich mixture. Compounds 1, 5, 6, and 7 were subsequently isolated by preparative HPLC (5% EtOAc in isooctane). Due to similar silica polarities, compounds 2–4 could not be separated by HPLC. The latter were isolated by preparative TLC (Chromatotron, 4–6% EtOAc in isooctane) using silica–gypsum–$AgNO_3$ (5% by weight) as the adsorbant.

Calyculone D (1). Compound 1 was isolated as an oil by HPLC (5% EtOAc in isooctane). The extract yielded 49 mg (0.8% of the extract) of calyculone D, which showed $[\alpha]_D$ +198° (*c* 1.0, $CHCl_3$) and the following spectral features: HRMS M^+, *m/z* obsd 288.2447, $C_{20}H_{32}O$ required 288.2455; low-resolution MS *m/z* (rel intensity) 288 (100), 273 (29), 255 (12), 245 (83), 227 (35), 219 (21), 203 (29), 189 (57), 177 (61), 165 (43), 163 (47), 161 (51), 151 (33); IR (film) 2960, 2940, 1715, 1650, 1455, 1385, 890 cm^{-1}; UV (MeOH) no λ_{max}.

Calyculone E (2). Compound 2 was isolated as an oil by centrifugal $AgNO_3$–silica TLC (Chromatotron, 6% EtOAc in isooctane). The extract gave 12 mg (0.2% of the extract) of calyculone E, which exhibited $[\alpha]_D$ −359° (*c* 0.5, $CHCl_3$) and the following spectral features: HRMS M^+, *m/z* obsd 288.2463, $C_{20}H_{32}O$ required 288.2455; low-resolution MS *m/z* (rel intensity) 288 (10), 245 (6), 189 (11), 176 (12), 149 (11), 136 (100), 133 (13); IR (film) 2960, 2930, 2870, 1715, 1645, 1450, 1370, 1285, 890 cm^{-1}; UV (MeOH) no λ_{max}.

Calyculone F (3). Compound 3 was isolated by centrifugal $AgNO_3$–silica TLC (Chromatotron, 4% EtOAc in isooctane). The extract gave 11 mg (0.2% of the extract) of calyculone F, which showed $[\alpha]_D$ +143° (*c* 0.4, $CHCl_3$) and the following spectral features: HRMS M^+, *m/z* obsd 288.2474, $C_{20}H_{32}O$ required 288.2455; low-resolution MS *m/z* (rel intensity) 288 (54), 273 (25), 245 (68), 277 (29), 217 (10), 203 (15), 189 (84), 176 (100), 165 (71), 163 (34), 161 (58); IR (film) 2960, 2930, 2870, 1715, 1640, 1460, 1380, 890 cm^{-1}.

Calyculone G (4). Compound 4 was isolated as an oil by centrifugal $AgNO_3$–silica TLC (Chromatotron, 4% EtOAc in isooctane). The extract gave 15 mg (0.3% of the extract) of calyculone G, which showed $[\alpha]_D$ −107° (*c* 0.5, $CHCl_3$) and the following spectral features: HRMS M^+, *m/z* obsd 288.2460, $C_{20}H_{32}O$ required 288.2455; low-resolution MS *m/z* (rel intensity) 288 (24), 245 (27), 227 (11), 189 (100), 187 (17), 177 (18), 168 (25), 165 (25), 163 (11), 161 (21); IR (film) 2960, 2930, 2870, 1710, 1680,

(13) Hall, L. D.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1980**, *102*, 5703.
(14) Voucher specimens of *Eunicea calyculata* (Ellis and Solander) under the code number CI86-227 are on deposit in the octocoral collection at the National Museum of Natural History, Smithsonian Institution, Washington, DC.

1640, 1460, 1380, 1285, 890 cm^{-1}.

(1*E*,3*E*,11*E*)-Cembra-1,3,11-trien-6-one (5). The cembratriene **5** was isolated as an oil by HPLC (5% EtOAc in isooctane). The extract gave 51 mg (0.8% of the extract) of compound **5**, which showed $[\alpha]_D$ +353° (*c* 0.6, $CHCl_3$) and the following spectral features: HRMS M$^+$, *m/z* obsd 288.2443, $C_{20}H_{32}O$ required 288.2455; low-resolution MS *m/z* (rel intensity) 288 (24), 286 (46), 271 (11), 259 (12), 243 (15), 217 (18), 203 (100), 189 (20), 177 (45), 175 (35), 161 (40), 151 (59); IR (film) 2960, 2920, 1710, 1660, 1460, 1385 cm^{-1}; UV (MeOH) 254 nm (ε 28 500), 248 (28 000).

(1*Z*,3*Z*,11*E*)-Cembra-1,3,11-trien-6-one (6). The cembratriene **6** was isolated as an oil by HPLC (5% EtOAc in isooctane). The extract gave 10 mg (0.2% of the extract) of compound **6**, which showed $[\alpha]_D$ +23° (*c* 0.1, $CHCl_3$) and the following spectral features: HRMS M$^+$, *m/z* obsd 288.2440, $C_{20}H_{32}O$ required 288.2455; low-resolution MS *m/z* (rel intensity) 288 (25), 245 (4), 227 (3), 177 (3), 165 (3), 151 (4), 136 (97), 121 (100), 108 (22), 93 (77); IR (film) 2960, 2920, 1710, 1660, 1465, 1380 cm^{-1}; UV (MeOH) 248 nm (ε 21 000), 245 (21 000).

(1*E*,3*Z*,11*E*)-Cembra-1,3,11-trien-6-one (7). The cembratriene **7** was isolated as an oil by HPLC (5% EtOAc in isooctane). The extract yielded 11 mg (0.2% of the extract) of compound **7**, which showed $[\alpha]_D$ +283° (*c* 0.8, $CHCl_3$) and the following spectral features: HRMS M$^+$, *m/z* obsd 288.2467, $C_{20}H_{32}O$ required 288.2455; low-resolution MS *m/z* (rel intensity) 288 (100), 273 (13), 270 (19), 245 (50), 242 (24), 227 (31), 203 (12), 190 (12), 177 (40), 175 (26), 165 (40), 161 (46), 151 (30); IR (film) 2960, 2930, 1705, 1665, 1460, 1440, 1380, 865 cm^{-1}; UV (MeOH) 248 nm (ε 20 500), 243 (21 000).

Irradiation of (1*E*,3*E*,11*E*)-Cembra-1,3,11-trien-6-one (5). A solution of compound **5** (11.3 mg, 0.04 mmol) in benzene (15 mL) was placed in a covered quartz test tube within 10 cm of a water-cooled photolysis apparatus and irradiated for 3 h with light from a 450-W Hanovia lamp. After evaporating the solvent, separation by HPLC (5% EtOAc in isooctane) gave four compounds: **1** (1.0 mg, 8.8% yield), **5** (2.1 mg, 18.6%), **6** (1.2 mg, 10.6%), **7** (1.3 mg, 15.9%). The ^{1}H NMR spectra of these compounds were identical with those from the natural products. Compounds **2-4** were obtained as a mixture (3.7 mg). Chemical shifts of the key protons (signals in the region of δ 2.8-5.8 and signals of high field methyl protons) in the ^{1}H NMR spectrum were within ±0.002 ppm of the natural products. The compounds were quantified by integration of signals corresponding to their C-10 protons (δ 3.99 for **2**, 3.82 for **3**, 4.05 for **4**) in the ^{1}H NMR spectrum: **2** (0.7 mg, 6.2%), **3** (1.2 mg, 10.6%), **4** (1.8 mg, 15.9%).

Irradiation of Calyculone D (1). A solution of **1** (16.0 mg, 0.06 mmol) was dissolved in benzene (15 mL) and irradiated by using the same apparatus and procedure as described for compound **5**. After 8 h, the solvent was evaporated under vacuum and the ^{1}H NMR spectrum of the residue was recorded. The spectrum showed signals for only calyculone D, indicating that no reaction had occurred.

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Cibaric Acid, a New Fatty Acid Derivative Formed Enzymatically in Damaged Fruit Bodies of *Cantharellus cibarius* (Chanterelle)

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The secondary metabolites of both intact and damaged fruit bodies of the edible mushroom *Cantharellus cibarius* have been investigated. The fruit bodies originally contain several fatty acids, one of the most abundant being 14,15-dehydrocrepenyic acid which is present both as a free fatty acid and as the triglyceride. 14,15-Dehydrocrepenyic acid is proposed to be the precursor of a new fatty acid which is formed enzymatically in response to injury to the fruit bodies. The new compound, called cibaric acid (**1a**), was isolated, and the elucidation of its structure by spectroscopic methods is described.

Introduction

Fruit bodies of *Cantharellus cibarius* Fr. (chanterelles) are among the most popular edible wild mushrooms, at least in the north of Europe. The fruit bodies are common and easily distinguished from toxic species, their taste is palatable, and they are normally not attacked by parasites like insects and snails. Consequently, large amounts are consumed yearly. The question about the nutritive value of fruit bodies of *C. cibarius*, as well as a general concern for the public health, has stimulated several chemical investigations; the major volatile components have been identified[2] and the presence of fats and fatty acids,[3,4] carotenes,[5] and steroids[6] has been reported. Previous investigations of other mushrooms unattractive to parasites have shown that some of them (e.g. *Lactarius vellereus*,[7] *Agaricus xanthoderma*,[8] and *Paxillus atrotomentosus*[9])

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