New Bicyclic Diterpenoids from the Caribbean Gorgonian Octocoral *Eunicea calyculata*

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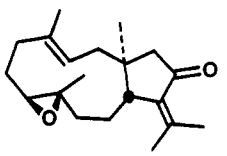
Two new bicyclic diterpenoids, 1 and 2, of the dolabellane ring system have been isolated from the Caribbean sea whip, *Eunicea calyculata*. The structures of these new compounds were assigned on the basis of chemical and spectral studies and particularly proton difference decoupling (DDS) and nuclear Overhauser enhancement difference spectroscopy (NOEDS). The facile BF_3-induced cyclization of one of these bicyclic diterpenoids to yield tricyclic compounds of the clavularane (dolastane) ring system illustrates the chemical relationship between these two diterpenoid classes.

Marine octocorals of the order Gorgonacea (Cnidaria), commonly referred to as the sea whips and sea fans, 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 several chemical investigations of *Eunicea* species have been reported. Two classes of diterpenoid molecules have been isolated. Cembrane derivatives are by far the most common; in total, some 7 cembranoids have been reported[2] from 6 of the 15 varieties of *Eunicea* found in the Caribbean Sea.[3] Fuscol, a diterpenoid structurally similar to the elemane sesquiterpenoids,[4] represents the sole noncembranoid diterpene isolated from this genus.

In this paper we report the structures of two new bicyclic diterpenoids, 1 and 2, which were isolated from the Caribbean gorgonian *E. calyculata* (Ellis and Solander). These bicyclic metabolites are new examples of diterpenoids of the dolabellane ring system,[5] which was first isolated from the opisthobranch mollusc *Dolabella californica*.[5,6] Later, these compounds were recognized as metabolites of brown seaweeds of the family Dictyotaceae.[7,8] The isolation of dolabellane diterpenoids from *E. calyculata* represents an extension of the biosynthetic capability of sea whips of the genus *Eunicea*, and of cnidarians in general.



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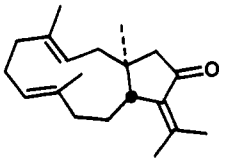

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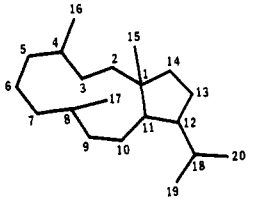

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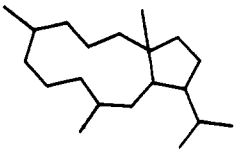

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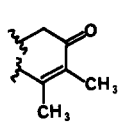
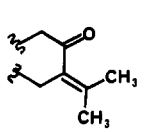
E. calyculata was collected in deeper waters (-30 m) along the barrier reef of Belize and in the Bahama Islands. Freshly collected animals were either stored in 2-propanol or frozen and subsequently extracted with chloroform/methanol (2:1). Diterpenoids 1 and 2 were purified by standard silica gel chromatography of the crude extract, followed by high-performance liquid chromatography (HPLC) of several of the relatively nonpolar fractions. Among the complex mixtures of secondary metabolites observed,[9] the diterpenoids, 1 and 2, were by far the major components, comprising 9 and 12% of the organic extract, respectively.

The epoxide 1 crystallized from diethyl ether after HPLC purification and analyzed for $C_{20}H_{30}O_2$ by high-resolution mass and ^{13}C NMR spectrometry. The two oxygen atoms in 1 were readily assigned to a trisubstituted epoxide and an α,β-unsaturated ketone constellation by evaluation of spectral information. ^{13}C NMR bands (Table I) at δ 60.4 (s) and 65.7 (d), in conjunction with ^1H NMR resonances at δ 2.91 (1 H, br d, J = 8.5) and δ 1.36 (3 H, s), illustrated 1 to possess a methyl-substituted epoxide group. ^{13}C NMR bands at δ 206.4 (s), 149.0 (s), and 135.9 (s) [or 137.6 (s)], infrared absorptions at 1700 and 1615 cm^{-1}, and UV absorption at 254 nm (ϵ 9200) indicated that 1 possessed a fully substituted α,β-unsaturated ketone chromophore. Further consideration of the NMR characteristics of 1 showed the molecule to possess an additional nonconjugated olefin and three olefinic methyl groups, two of which were unusually deshielded [δ 2.26 (3 H, s) and 1.93 (3 H, s)]. These data required that the fully substituted α,β-unsaturated ketone bear two methyl groups in one of two possible configurations: a vicinally disubstituted endocyclic olefin as in a, or a geminally disubstituted exocyclic olefin as in b. Comparison of the spectral



(a)



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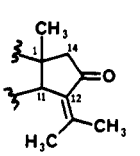

(c)

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(7) Sun, H. H.; Fenical, W. *Phytochemistry* **1979**, *18*, 340.

(8) Amico, V.; Oriente, G.; Piattelli, M.; Tringali, C.; Fattorusso, E.; Magno, S.; Mayol, L. *Tetrahedron* **1980**, *36*, 1409.

(9) A complex mixture of monocyclic metabolites, some of the cembrane class, were also isolated.

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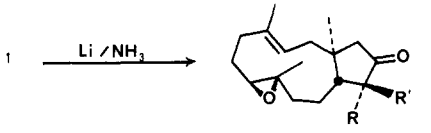
data with numerous model systems[10] clearly indicated that 1 possessed the α-isopropylidenyl ketone functionality as in b. Furthermore, the chemical shift for the carbonyl carbon in the ^{13}C NMR spectrum of 1 (206.4 ppm), the infrared absorption at 1700 cm^{-1}, and the UV absorption data were virtually identical with those data reported for 2-isopropylidenylcyclopentanone,[11] thus allowing the further assignment of this chromophore in a five-membered ring.

Proton NMR data yielded even more information that defined the substitution pattern of the α-isopropylidenylcyclopentanone group in 1. One bridgehead methyl group [δ 1.19 (3 H, s)], one allylic methine proton at δ 2.69 (1 H, br d, J = 12.6 Hz), and an AB double doublet at δ 2.38 and 2.12 (J = 18.6 Hz) were observed in the proton NMR spectrum of 1. These features could only be accommodated by the cyclopentanone constellation as fully defined in c. In order to confirm this assignment, epoxide 1 was treated with lithium in ammonia to selectively reduce the conjugated chromophore. Two non-UV-absorbing products, 5 and 6, were obtained; each compound was purified by HPLC and fully characterized. Both products showed simple cyclopentanone carbonyl infrared absorptions at 1735 cm^{-1}, and two new doublet methyl groups were present at high field in their ^1H NMR spectra. Irradiation of a complex one-proton band at high field in each spectrum caused the doublet methyl groups to collapse to singlet resonances, thereby proving their assignments in isopropyl groups.



5 R=H, R'=CH(CH3)2

6 R=CH(CH3)2, R'=H

Mass spectral and ^{13}C NMR data showed 1 to possess two carbocyclic rings. Since 1 could be defined as possessing a five-membered ring with no other substituents except for methyls and the epoxide in the second ring, the remaining ring had to be 11-membered. The dolabellanes are a known class of diterpenoids possessing a bicyclo[9.3.0] ring system as in 3. However, an alternative structure, 4, which is also regularly terpenoid, or other structures with irregular methyl substitution patterns in the 11-membered ring could not be excluded as possibilities for the carbon skeleton of 1. In order to position the trisubstituted olefin at C3–C4 with an olefinic methyl at C4, a proton NMR experiment involving difference decoupling spectroscopy[12] (DDS) was performed. While the trisubstituted olefin could be placed in numerous positions within the 11-membered ring, only in the C3–C4 position, as in 3, would this olefin be proximate to a methylene group (C2), which would be isolated by the quaternary center at C1. Figure 1 illustrates the results of difference decoupling of the lone olefin proton in 1, which appears at δ 5.43 (dd, J = 11.6 and 4.7 Hz). The methylene protons at C2 were very clearly defined by this experiment at δ 2.16 and 1.63 by the difference pattern illustrated in Figure 1a. The geminal coupling constant was measured as 11.8 Hz, and further couplings of 11.6 and 4.7 Hz to the olefin proton were measured, since they could be eliminated in the decoupled component. Using the observed coupling constants, a computer simulation (Figure 1b) yielded superimposable results. Based upon these data, epoxide 1 was proposed to possess the dolabellane skeleton as in 3, void, however, of stereochemical refinements.

In order to determine the relative spatial arrangements of all substituents and, at the same time, the conformation

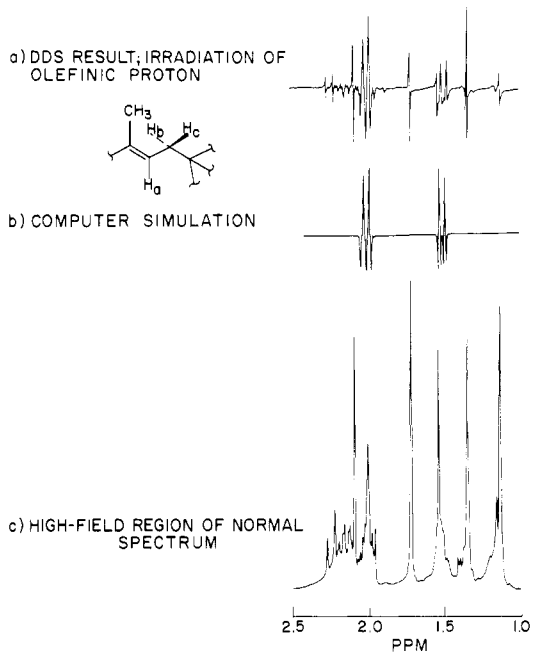
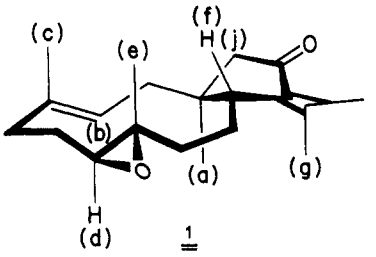


Figure 1. Results of a proton difference decoupling experiment (DDS) with epoxide 1 at 360 MHz in $CDCl_3$ solution. (a) The result of spectra subtraction after irradiation at δ 5.43. (Methyl resonances were incompletely nulled.) (b) The results of a computer simulation (using a Nicolet 1180E computer program) of the difference experiment with J_{ac} = 11.6, J_{ab} = 4.7, and J_{bc} = 11.8 Hz. (c) Normal spectrum showing the region between δ 1.0 and 2.5.



Proton(s) irradiated	Proton(s) enhanced	Estimated internuclear distances (Å)
a	b,d,j	1.6, 2.4, 2.4
b	a,d	1.6, 2.2
c	e,f	2.2, 2.2
d	a,b	2.4, 2.2
e	c,f	2.2, 2.4
f	c,e,g	2.2, 2.4, 2.2
g	f	2.2

Figure 2. Results of a nuclear Overhauser enhancement difference spectroscopy (NOEDS) experiment with epoxide 1. Estimated internuclear distances were obtained with Dreiding models. The proton labeled "j" represents the α proton only, which is within nOe proximity.

(10) The exceptionally deshielded *syn*-methyl groups in α-isopropylidenyl ketones are highly characteristic of this chromophore. For (+)-pulegone, see: Pouchert, C. J.; Campbell, J. R. "The Aldrich Library of NMR Spectra"; Aldrich Chemical Co., Inc.: Milwaukee, WI, 1974; Vol. II, pp 140.

(11) 2-Isopropylidenylcyclopentanone shows infrared absorption at 1706 ($\nu_{C=O}$) and 1633 cm^{-1} ($\nu_{C=O}$) [see Erskine, R. L.; Waight, E. S. *J. Chem. Soc.* **1960**, 3425], ultraviolet absorption at 252 nm ($\log^{10} \epsilon$ 3.56) [see French, H. S.; Wiley, L. *J. Am. Chem. Soc* **1949**, *71*, 3702], and ^{13}C NMR bands at 204.6, 130.2, and 144.5 ppm, respectively, for the carbonyl, and α and β exocyclic carbon atoms [see Marr, D. H.; Stothers, J. B. *Can. J. Chem.* **1965**, *43*, 596].

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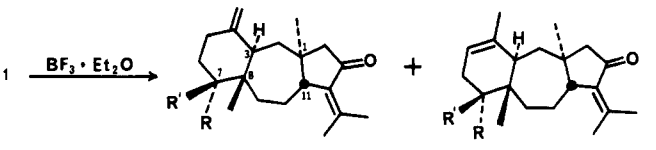
Table I. ^1H and ^{13}C NMR Assignments for the *Eunicea calyculata* Metabolites and the Two Key Derivatives[a,b]

	epoxide 1		triene 2		*exo*-7		*endo*-8	
C no.	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
1		41.0		40.9		39.6		40.1
2α	1.63 (1 H, dd, J = 11.8, 4.7)	39.8[e]	1.60 (1 H, dd, J = 11.7, 5.0)	40.1[e]	c	42.3	c	41.1
2β	2.16 (1 H, dd, J = 11.6, 11.8)		2.13 (1 H, dd, J = 11.7, 11.2)					
3	5.43 (1 H, dd, J = 11.6, 4.7)	124.7	5.24 (1 H, dd, J = 5.0, 11.2)	124.8	c	47.0	c	47.3
4		137.6[d]		137.9[d]		149.4[d]		135.5[d]
5α					2.41 (1 H, m)			
5β	c	36.9[e]	c	38.1[e]	2.35 (1 H, m)	35.5[e]	5.35 (1 H, br s)	120.3
6α					1.83 (1 H, m)		2.28 (1 H, dd, J = 5.8, 3.0)	
6β	c	27.5[f]	c	27.9[f]	1.52 (1 H, m)	35.3[e]	2.00 (1 H, dd, J = 10.0, 2.0)	33.5[e]
7α	2.91 (1 H, br d, J = 8.5)	65.7	4.93 (1 H, br d, J = 10.6)	130.3	3.36 (1 H, dd, J = 4.5, 11.7)	77.2	3.48 (1 H, dd, J = 6.1, 10.3)	74.7
8		60.4		131.4[d]		44.6		41.4
9	c	38.0[e]	c	39.8[e]	c	32.2[e]	c	32.2[e]
10	c	22.9[f]	c	24.2[f]	c	27.5	c	27.4
11β	2.69 (1 H, br d, J = 12.6)	42.1	2.83 (1 H, br d, J = 12.2)	41.4	2.93 (1 H, m)	49.3	3.04 (1 H, m)	49.1
12		135.9[d]		135.4[d]		136.1		136.1[d]
13		206.4		206.7		206.2		206.2
14α	2.38 (1 H, d, J = 18.6)	54.3	2.36 (1 H, d, J = 18.4)	54.7	2.28 (1 H, d, J = 15.3)	58.6	2.20 (1 H, d, J = 15.8)	58.2
14β	2.12 (1 H, d, J = 18.6)		2.10 (1 H, d, J = 18.3)		2.02 (1 H, d, J = 15.4)		2.02 (1 H, d, J = 15.6)	
15α	1.19 (3 H, s)	23.3	1.23 (3 H, s)	23.1	1.03 (3 H, s)	19.4	1.00 (3 H, s)	19.3
16	1.57 (3 H, br s)	15.7	1.44 (3 H, br s)	16.1	4.83 (1 H, d, J = 1.2), 4.63 (1 H, s)	108.0	1.64 (3 H, d, J = 0.8)	22.9
17β	1.36 (3 H, s)	17.7	1.64 (3 H, br s)	15.5	0.82 (3 H, s)	9.5	0.92 (3 H, s)	9.6
18		149.0		147.8		148.2[d]		148.4
19	1.93 (3 H, s)	25.0	1.83 (3 H, s)	24.4	1.89 (3 H, d, J = 2.1 to δ 2.93)	24.6	1.91 (3 H, d, J = 2.0 to δ 3.04)	24.6
20	2.26 (3 H, s)	21.8	2.22 (3 H, s)	21.3	2.22 (3 H, d, J = 2.4 to δ 2.93)	22.8	2.22 (3 H, d, J = 2.4 to δ 3.04)	22.8

[a] ^1H NMR spectra were recorded in $CDCl_3$ at 360 MHz. Assignments were aided by spin-decoupling and DDS experiments. J values are reported in hertz, and the chemical shifts are given in δ units (parts per million downfield from Me_4Si). [b] ^{13}C NMR spectra were recorded at 50 MHz in $CDCl_3$. Multiplicities were obtained by single-frequency off-resonance decoupling, and assignments were made on the basis of J_R values when applicable and/or a comparison to models. The δ values are in parts per million downfield from Me_4Si. [c] Nonassignable proton resonances. [d-f] Signals within a column may be reversed.

of the 11-membered ring, we employed ^1H NMR experiments involving nuclear Overhauser enhancement difference spectroscopy (NOEDS).[13] The results of these experiments are summarized in Figure 2. Since no enhancements of the epoxide or olefin protons were observed when the associated methyl resonances were irradiated, these groups were assigned as trans and *E*, respectively. The spatial arrangement of both methyls in the 11-membered ring and the bridgehead proton on the β face of the molecule were clearly established. Furthermore, the bridgehead methyl, the olefin proton, and the epoxide proton were found to be within nOe proximity on the opposite, α face, of the molecule. These results indicated that 1 adopts a crown conformation, in complete analogy with the corresponding 10-membered ring system (*all-E*)-germacradiene.[14]

The reactivity of (*E,E*)-1,5-germacradienes and of the corresponding monoepoxides toward acid-catalyzed transannular cyclization has been extensively investigated. The propensity of this reaction illustrates the ease of adoption of a favorable transition state from the crown conformation.[15] In order to illustrate these same features of gross structure and conformation, we investigated the capacity of 1 to undergo a similar regioselective transannular cyclization. Treatment of epoxide 1 with $BF_3 \cdot Et_2O$ in Et_2O yielded the isomeric tricyclic alcohols, 7 and 8, as



7 R=H, R'=OH
9 R=H, R'=OAc
11 R=R'=O
14 R=H, R'=R(+)-OMTPA
15 R=H, R'=S(-)-OMTPA

8 R=H, R'=OH
10 R=H, R'=OAc

the major products. Analysis of the structures of these isomers involved the interpretation of both ^{13}C and ^1H NMR data, which are outlined in Table I. Both compounds were readily recognized as secondary alcohols via

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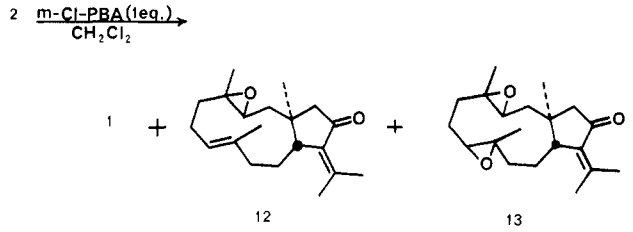
(14) Allen, F. H.; Rogers, D. *Chem. Commun.* **1967**, 588.
(15) Brown, E. D.; Sutherland, J. K. *Chem. Commun.* **1968**, 1060. For a review, see: Sutherland, J. K. *Tetrahedron* **1974**, *30*, 1651.

their facile acetylation to yield the secondary acetates, **9** and **10**. The alcohols were extensively investigated by NMR methods, and their gross structures were confirmed by spin-decoupling experiments (Table I). Oxidation of alcohol **7** with pyridinium chlorochromate yielded the ketone **11**. Upon oxidation, the methyl resonance at δ 0.82 (3 H, s) in **7** shifted downfield to δ 1.11 (3 H, s) in **11**, thus establishing the resonance at δ 0.82 in **7** as that due to the bridgehead methyl at C8, adjacent to the secondary alcohol. As in the case of the epoxide **1**, the conformation and relative stereochemistry of **7** was established by nuclear Overhauser enhancement difference experiments involving the irradiation of selected proton resonances. Irradiation of the C8-substituted methyl group (δ 0.82, 3 H, s) resulted in significant enhancement of the allylic bridgehead methine proton at C11 but did not enhance the proton at C3. This experiment established the cyclohexane ring as trans-fused and showed the newly formed bridgehead methyl (at C8) to be a β substituent. These data confirmed the structures of **7** and **8**, thus illustrating that **1** does undergo a regioselective cyclization analogous to that observed in the germacrene epoxide.

The corresponding triene **2** was isolated from slightly less polar fractions from the initial chromatography and purified by HPLC. The triene analyzed for $C_{20}H_{30}O$ by high-resolution mass spectrometry and showed infrared and UV absorptions that clearly defined the analogous α-isopropylidenylcyclopentanone group as in **1**. The NMR features of the triene were also analogous to **1**, except that the NMR bands of the epoxide were replaced in **2** by those of an additional trisubstituted olefin. Based upon this very favorable comparison, triene **2** was formulated as the C7–C8 olefin precursor to **1**. Treatment of **2** with 1 equiv of *m*-chloroperbenzoic acid yielded a predictable[16] mixture of two monoepoxides, **1** and **12**, and diepoxide **13**. The major synthetic monoepoxide obtained was identical in all respects, including its optical properties, with the natural product, **1**.



Having established the structures and relative stereochemistries of the natural products, **1** and **2**, and of the cyclization products, **7** and **8**, we next defined the absolute configurations of these compounds using Mosher's ^{19}F NMR method.[17] Treatment of the tricyclic alcohol **7** with both (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl (MTPA) chloride and the (*S*)-(−)-acid chloride in pyridine yielded the esters **14** and **15**, respectively. The ^{19}F chemical shift of the trifluoromethyl group in each derivative was measured at 188 MHz and was found to be 5.89 ppm for **14** and 6.21 ppm (relative to external trifluoroacetic acid) for **15**. Mosher's model predicts that the (*S*)-MTPA ester will show a CF_3 resonance downfield relative to the (*R*)-MTPA ester if the secondary alcohol possesses the *S* configuration. Our results indicate that the configuration at C7 in **7** is *S*. Based upon the regioselective transformation of **1** to **7**, the absolute configuration and structure of **1** were fully defined as 7(*S*),8(*S*)-epoxy-13-keto-1-(*S*),11(*R*)-dolabell-3(*E*),12(18)-diene. Correspondingly, the triene **2** was fully characterized as 13-keto-1(*S*),11(*R*)-dolabell-3(*E*),7(*E*),12(18)-triene.

The bicyclic dolabellane skeleton has been proposed as a likely biosynthetic precursor[18] for tricyclic diterpenoids of the dolastane[19] and/or clavularane[18] types, which are illustrated in the structures **7** and **8**. Compounds first assigned the clavularane ring system were isolated from the octocoral *Clavularia inflata*[18] (order Stolonifera), which is related to *E. calyculata*. Our results clearly illustrate the chemical and structural relationship between these bicyclic and tricyclic diterpenoid ring systems.

Experimental Section

General. Infrared spectra were recorded on a Perkin-Elmer Model 137 spectrophotometer. Ultraviolet spectra were obtained in MeOH on a Beckman Acta XIV spectrophotometer. Proton NMR spectra were recorded in $CDCl_3$ solution on a 360-MHz spectrometer constructed from an Oxford narrow-bore magnet and a Nicolet Fourier transform data system by Dr. John M. Wright of the UCSD NMR Facility; all chemical shifts are reported with respect to Me_4Si (δ 0). Carbon-13 NMR spectra were recorded in $CDCl_3$ solution on a Nicolet-Oxford Magnetics 50 MHz Wide-Bore spectrometer; all chemical shifts are reported with respect to Me_4Si (δ 0). Low-resolution mass spectra were recorded at 70 eV on a Hewlett-Packard Model 5930A mass spectrometer. High-resolution mass measurements were supplied by Dr. A. Burlingame, University of California, Berkeley. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter with a 10-cm microcell. Melting points were determined on a Fisher-Johns apparatus and are reported uncorrected. All solvents used were either spectral grade or were distilled from glass prior to use. Final purifications of all metabolites and reaction products were achieved by preparative high-performance liquid chromatography on silica gel with mixtures of ethyl acetate and isooctane.

Collection and Extraction. *Eunicea calyculata* (Ellis and Solander) (collection B-79-46) was collected by hand using SCUBA at 25 to 30 m depth in May, 1979, along the barrier reef near Carrie Bow Cay, Belize. The collection was stored in isopropyl alcohol (IPA). Upon workup, the IPA was decanted, the animal was homogenized, and the gorgonian cake was repeatedly extracted with $CHCl_3$/MeOH (2:1). After filtration, the combined IPA and $CHCl_3$/MeOH extracts were evaporated under vacuum to give a residue, which was partitioned between saturated brine and $CHCl_3$. The $CHCl_3$ extract was subsequently dried over $MgSO_4$ and filtered, and the filtrate was evaporated to yield 17 of a crude organic extract (from 500 g, dry weight of the gorgonian). A second collection of *E. calyculata* was made in Sept., 1981 in the Bahamas. The gorgonian was again found in deeper waters and collected by hand using SCUBA. The animal was stored frozen, and an organic extract was produced as described above. From both collections, **1** and **2** were eluted from a silica gel column with 25 and 15% EtOAc in isooctane, respectively, and further purified by HPLC.

7(*S*),8(*S*)-Epoxy-13-keto-1(*S*),11(*R*)-dolabell-3(*E*),12-(18)-diene (*1*). The epoxide **1** crystallized from diethyl ether after purification by HPLC (μ-Porasil, with 30% EtOAc in isooctane). Repeated recrystallization gave 1.5 g (9% of the crude extract) of **1**, mp 147–149 °C. Epoxide **1** showed $[\alpha]^{20}_D$ +61° (*c* 1.29, $CHCl_3$) and exhibited the following spectral features: UV λ_{max} (MeOH) 254 nm (ϵ 9200); IR (CCl_4) 2960, 1700, 1615, 1440, 1370, 1270, 1230, 1180, 1040, 900 cm^{-1}; HRMS: M^+, *m/z* obsd 302.2274, $C_{20}H_{30}O_2$ requires 302.2246.

13-Keto-1(*S*),11(*R*)-dolabell-3(*E*),7(*E*),12(18)-triene (2). Triene **2** was isolated as an oil. Purification by HPLC (μ-Porasil

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with 10% EtOAc in isooctane) gave 2.0 g (12% of crude extract) of **2**. The triene showed $[\alpha]^{20}_D$ +31° (c 0.88, $CHCl_3$) and exhibited the following spectral features: UV λ_{max} (MeOH) 256 nm (ϵ 6900); IR (CCl_4) 2960, 1700, 1615, 1435, 1370, 1270, 1200, 1175, 890 cm^{-1}; HRMS *m/z* (relative intensity) for M^+, obsd 286.2296 (11), $C_{20}H_{30}O$ requires 286.2297, 271.2063 ($C_{19}H_{27}O$, 5), 163.1117 ($C_{11}H_{15}$, 39), 150.1047 ($C_{10}H_{14}$, 100), 149.0966 ($C_{10}H_{13}$, 39), 136.0883 (C_9H_{12}, 14), 107.0854 (C_8H_{11}, 42), 93.0707 (C_7H_9, 53).

Reduction of Epoxide 1 To Yield 5 and 6. To a stirred solution of excess lithium in ammonia (20 mL) (dry ice–acetone bath) and anhydrous diethyl ether (10 mL) was added 85 mg (0.28 mmol) of **1** in 5 mL of diethyl ether. After 20 min, NH_4Cl was slowly added, and the solution was allowed to warm to room temperature. When the ammonia had evaporated (2 h), the reaction mixture was washed with 5% HCl (2 × 20 mL), followed by 5% $NaHCO_3$, dried ($MgSO_4$), and filtered, and the filtrate was evaporated to give a mixture of two major products, **5** (25 mg, 29% from **1**) and **6** (12 mg, 14% from **1**), which were separated by HPLC (μ-Porasil, with 20% EtOAc in isooctane). These products were fully characterized. Compound **5** showed $[\alpha]^{20}_D$ -126.0° (c 0.86, $CHCl_3$) and exhibited the following spectral features: IR (CCl_4) 2960, 2920, 1735, 1560, 1455, 1370, 1270 cm^{-1}; MS, M^+ *m/z* 304 for $C_{20}H_{32}O_2$ (low resolution); ^1H NMR ($CDCl_3$) δ 5.44 (1 H, br d, *J* = 10.3 Hz), 2.74 (1 H, dd, *J* = 2.4 and 10.8 Hz), 2.0 (1 H, m), 1.68 (3 H, s), 1.36 (3 H, s), 1.19 (3 H, d, *J* = 6.9 Hz), 0.99 (3 H, s), 0.93 (3 H, d, *J* = 6.9 Hz). The stereochemistry of the isopropyl group in **5** was established as β based on results from NOEDS. The doublet methyl group at δ 0.93 was shown to be within nOe proximity to the methyls at δ 0.99, 1.36, and 1.68, which are assigned to the other isopropyl methyl, epoxide methyl, and olefin methyl, respectively. Compound **6** showed $[\alpha]^{20}_D$ +10.3° (c 0.63, $CHCl_3$) and exhibited the following spectral features: IR (CCl_4) 2960, 1735, 1540, 1465, 1320, 1270, 1250, 1030, 970 cm^{-1}; MS, M^+ *m/z* 304 for $C_{20}H_{32}O_2$ (low resolution); ^1H NMR ($CDCl_3$) δ 5.31 (1 H, br d, *J* = 11.2 Hz), 2.81 (1 H, dd, *J* = 11.0 and 1.8 Hz), 1.62 (3 H, s), 1.44 (3 H, s), 1.23 (3 H, s), 1.07 (3 H, d, *J* = 7.0 Hz), 0.97 (3 H, d, *J* = 7.0 Hz).

DDS and NOEDS Experiments. The decoupling difference and nOe difference spectroscopy experiments were performed in general as outlined by Hall and Sanders.[12] However, in these experiments, since the decoupler used was not under computer control, the various irradiations were manually performed sequentially. All samples prepared for NOEDS were degassed by bubbling Ar through the solution for 45–60 min and then sealed around the cap with parafilm. Solutions were made up in $CDCl_3$ such that, after degassing and a loss of a significant volume of $CDCl_3$, the final concentration was 0.03–0.05 M.

Transannular Cyclization of 1 with BF_3·Et_2O. Epoxide **1** (200 mg, 0.66 mmol) was dissolved in 5 mL of anhydrous diethyl ether, and the mixture was cooled to 0 °C. To this mixture was added 0.1 mL of BF_3–etherate (distilled) with stirring. After 15 min, distilled water (10 mL) and diethyl ether were added. The ether layer was separated and washed with 5% $NaHCO_3$ (2 × 20 mL) and then dried over $MgSO_4$ and filtered, and the filtrate was evaporated to yield a mixture of products. Purification by HPLC (μ-Porasil with 30% EtOAc in isooctane) gave as the major products starting material, **1** (52.0 mg, 26%), **7** (34.2 mg, 17% from **1**), and **8** (39.6 mg, 20% from **1**). Products **7** and **8** were fully characterized. Compound **7** showed $[\alpha]^{20}_D$ -34° (c 0.27 $CHCl_3$) and exhibited the following spectral features: UV λ_{max} (MeOH) 251 nm (ϵ 9200); IR (CCl_4) 3550, 2920, 1705, 1650, 1540, 1440, 1380, 1250, 1200, 1030, 1000 cm^{-1}; HRMS, M^+ *m/z* (relative intensity) obsd 302.2253 (31), $C_{20}H_{30}O_2$ requires 302.2246, 269.1897 ($M^+ - H_2O$, $M^+ - CH_3$, $C_{19}H_{25}O$, 4), 107.0862 (C_8H_{11}, 37), 105.0704 (C_8H_9, 29), 95.0865 (C_7H_{11}, 35), 91.0545 (C_7H_7, 41), 83.0496 (C_5H_7, 100). Compound **8** crystallized from EtOAc, mp 132–134 °C and gave $[\alpha]^{20}_D$ -51° (c 0.74, $CHCl_3$) and exhibited the following spectral characteristics: UV λ_{max} (MeOH) 252 nm (ϵ 6100); IR (CCl_4) 3550, 2920, 1705, 1615, 1440, 1375, 1245, 1200, 1155, 1080 cm^{-1}; HRMS, M^+ *m/z* (relative intensity) obsd 302.2234 (100), $C_{20}H_{30}O_2$ requires 302.2246, 284.2123 ($M^+ - H_2O$, $C_{20}H_{28}O$, 7), 269.1912 ($M^+ - H_2O$, CH_3, $C_{19}H_{25}O$, 9), 204.1499 ($C_{14}H_{20}O$, 39), 149.0962 ($C_{10}H_{13}O$, 35), 123.0811 ($C_8H_{11}O$, 34).

Acetylation of 7 and 8 To Give 9 and 10. To 5 mg of compound, dissolved in 2 mL of dry pyridine, excess acetic anhydride was added with stirring. The reaction mixture was stirred at room temperature overnight and then quenched with ice and extracted with diethyl ether (2 × 20 mL). The combined ether layers were washed with 5% HCl (2 × 20 mL), water, and 5% $NaHCO_3$ (20 mL), dried over $MgSO_4$, and filtered,and the filtrate was evaporated to yield, quantitatively, the crude ester. Both **9** and **10** were purified by HPLC (μ-Porasil with 20% EtOAc in isooctane). Acetate **9** showed $[\alpha]^{20}_D$ -50° (c 0.3, $CHCl_3$) and exhibited the following spectral features: UV λ_{max} (MeOH) 252 (ϵ 11 700); IR ($CHCl_3$) 2950, 1735, 1705, 1610 cm^{-1}; MS: M^+ *m/z* 344 for $C_{22}H_{32}O_3$ (low resolution); ^1H NMR ($CDCl_3$) δ 4.86 (1 H, s), 4.65 (1 H, br s), 4.63 (1 H, dd, *J* = 11.8 and 4.5 Hz), 2.91 (1 H, br m), 2.22 (3 H, d, *J* = 2.4 Hz to δ 2.91), 2.08 (3 H, s), 1.89 (3 H, d, *J* = 1.9 Hz to δ 2.91), 1.01 (3 H, s), 0.91 (3 H, s). Acetate **10** showed $[\alpha]^{20}_D$ -33° (c 0.1, $CHCl_3$) and exhibited the following spectral features: UV λ_{max} (MeOH) 251 nm (ϵ 7700); IR ($CHCl_3$) 2950, 1735, 1705, 1605 cm^{-1}; MS, M^+ *m/z* 344 for $C_{22}H_{32}O_3$ (low resolution); ^1H NMR ($CDCl_3$) δ 5.35 (1 H, br s), 4.73 (1 H, dd, *J* = 10.4 and 6.2 Hz), 3.02 (1 H, br m), 2.22 (3 H, d, *J* = 2.0 Hz to δ 3.02), 2.07 (3 H, s), 1.90 (3 H, d, *J* = 1.5 Hz to δ 3.02), 1.64 (3 H, s), 1.01 (3 H, s), 0.98 (3 H, s).

Oxidation of 7 To Yield Ketone 11. One equivalent of pyridinium chlorochromate was added to a stirred solution of **7** (10 mg) in CH_2Cl_2 (2 mL) at room temperature. After 2 h, the reaction mixture was diluted with CH_2Cl_2 (15 mL) and filtered through a small silica gel column to yield ketone **11** as an oil in quantitative yield. Ketone **11** showed $[\alpha]^{20}_D$ +23.1° (c 0.51, $CHCl_3$) and exhibited the following spectral features: UV λ_{max} (MeOH) 252 nm (ϵ 7000); IR ($CHCl_3$) 2920, 1705, 1620; MS, M^+ *m/z* 300 for $C_{20}H_{28}O_2$ (low resolution); ^1H NMR ($CDCl_3$) δ 5.05 (1 H, s), 4.88 (1 H, s), 2.91 (1 H, br m), 2.22 (3 H, d, *J* = 1.8 Hz to δ 2.91), 1.90 (3 H, d, *J* = 1.5 Hz to δ 2.91), 1.11 (3 H, s), 0.99 (3 H, s).

Epoxidation of 2. To a stirred solution of **2** (61.2 mg, 0.21 mmol) in 10 mL of CH_2Cl_2 buffered with anhydrous Na_2HPO_4 (buffering was necessary in order to prevent transannular cyclizations) was added 1.2 equiv of *m*-chloroperbenzoic acid. After stirring for 2 h at room temperature, the CH_2Cl_2 layer was washed with 10% sodium sulfite (2 × 20 mL), 5% $NaHCO_3$ (2 × 20 mL), and, finally, with brine. After drying over $MgSO_4$ and filtration and evaporation of the CH_2Cl_2, the resulting oil was chromatographed by HPLC (45% EtOAc in isooctane). The reaction mixture gave two monoepoxides, **1** (29 mg, 45% yield from **2**) and **12** (21 mg, 32% yield from **2**), and diepoxide **13** (5 mg, 7% yield from **2**). The major product of the reaction was identical with the natural product, **1** ($[\alpha]^{20}_D$ +59° (c 0.48, $CHCl_3$)). Monoepoxide **12** showed $[\alpha]^{20}_D$ -54.1° (c 0.93, $CHCl_3$) and exhibited the following spectral features: UV λ_{max} (MeOH) 255 nm (ϵ 5700); IR ($CHCl_3$) 2950, 1700, 1610, 1460 cm^{-1}; HRMS, M^+ *m/z* (relative intensity) obsd 302.2233 (3), $C_{20}H_{30}O_2$ requires 302.2246, 163.1117 ($C_{11}C_{15}O$, 34), 149.0964 ($C_{10}H_{13}O$, 93), 135.0809 ($C_9H_{11}O$, 72), 95.0860 (C_7H_{11}, 70), 93.0705 (C_7H_9, 94); ^1H NMR ($CDCl_3$) δ 5.12 (1 H, br d, *J* = 11.1 Hz), 2.99 (1 H, dd, *J* = 11.1 and 2.9 Hz), 2.94 (1 H, br d, *J* = 11.9 Hz), 2.41 (1 H, d, *J* = 18.2 Hz), 2.20 (3 H, s), 2.11 (1 H, d, *J* = 18.4 Hz), 1.84 (3 H, s), 1.73 (3 H, s), 1.41 (3 H, s), 1.14 (3 H, s). Diepoxide **13** showed $[\alpha]^{20}_D$ -26.5° (c 0.83, $CHCl_3$) and exhibited the following spectral features: UV λ_{max} (MeOH) 253 nm (ϵ 6100); IR ($CHCl_3$) 2920, 1705, 1610, 1450, 1380 cm^{-1}; HRMS, M^+ *m/z* (relative intensity) obsd 318.2210 (45), $C_{20}H_{30}O_3$ requires 318.2195, 149.0968 ($C_{10}H_{13}O$, 100), 136.0892 ($C_9H_{12}O$, 71); ^1H NMR ($CDCl_3$) δ 3.07 (1 H, dd, *J* = 13.9 and 2.7 Hz), 2.97 (1 H, br d, *J* = 7.7 Hz), 2.82 (1 H, br d, *J* = 12.5 Hz), 2.45 (1 H, d, *J* = 18.3 Hz), 2.24 (3 H, s), 1.95 (3 H, s), 1.43 (3 H, s), 1.34 (3 H, s), 1.22 (3 H, s).

Synthesis of α-Methoxy-α-(trifluoromethyl)phenylacetyl Chlorides (MTPA-Cl). (+)-α-Methoxy-α-(trifluormethyl)-phenylacetic acid (1.0 g, Aldrich Chemical Co.), thionyl chloride (2 mL, distilled), and sodium chloride (10 mg) were combined and refluxed for 60 h. Excess thionyl chloride was removed by vacuum distillation (47 °C, 85 mm), and the remaining residue was distilled to give the acid chloride (83–85 °C, 8 mm). The (−)-MTPA-Cl was synthesized in the same manner.

Synthesis of Esters 14 and 15. To 8.3 mg of **7** in 2 mL of dry pyridine was added 1 equiv of (+)-MTPA-Cl in CCl_4 (1 mL). After stirring overnight at room temperature, ice was added and the mixture was extracted with diethyl ether (2 × 20 mL). The ether layer was washed with 5% HCl (2 × 20 mL) and 5% $NaHCO_3$, dried over $MgSO_4$, and filtered, and the filtrate was

evaporated to give 14 as an oil in quantitative yield. Compound 14 was purified by HPLC (μ-Porasil, 25% EtOAc in isooctane). The ^{19}F NMR chemical shift for the CF_3 group in ester 14, measured in $CDCl_3$ solution at 188 MHz, was determined to be 5.89 ppm (downfield from external trifluoroacetic acid in $CDCl_3$). Ester 15 was synthesized by reaction of 7 (10 mg) with (-)-MTPA-Cl and purified as described above. The CF_3 group for ester 15 gave a ^{19}F NMR chemical shift of 6.21 ppm.

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Registry No. 1, 82798-97-8; 2, 82798-98-9; 5, 82798-99-0; 6, 82838-28-6; 7, 82799-00-6; 8, 82799-01-7; 9, 82799-02-8; 10, 82799-03-9; 11, 82799-04-0; 12, 82799-05-1; 13, 82799-06-2; 14, 82799-07-3; 15, 82838-29-7; (+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid, 20445-31-2; (+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride, 20445-33-4; (-)-α-methoxy-α-(trifluoromethyl)phenylacetic acid, 17257-71-5; (-)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride, 39637-99-5.

Spectroscopic, Optical, and Crystallographic Properties of (*S*)-(+)-*cis*-6′-Bromo-*N*-formylnorreticuline

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A rotamer mixture of (*S*)-*N*-formyl-*N*-norreticuline (1) was brominated regioselectively in the 6′-position to afford the corresponding mixture of 6′-bromo rotamers (2) from which the thermodynamically less stable cis rotamer could easily be isolated by direct crystallization. This rotamer was stable in crystalline condition but equilibrated to a 1:2 cis–trans mixture on standing in solution. This equilibration was easily followed by observation of changes in optical rotation and the ratio of *N*-formyl protons in the NMR spectrum. Assignment of the cis structure to the rotamer isolated by crystallization was confirmed by a single-crystal X-ray analysis, which also confirmed the absolute configuration of this rotamer made independently from established stereochemical relationships. The crystals of 2 have space group $P2_1$ and cell dimensions a = 6.616 (1) Å, b = 10.985 (1) Å, c = 13.084 (2) Å and β = 95.61 (1)°. The structure was refined to an R factor of 4.3%.

The presence of rotamer pairs was repeatedly observed in the series of 1-benzyl-substituted *N*-formyl-1,2,3,4-tetrahydroisoquinolines (TIQ) by routine TLC and ^1H NMR analysis.[2-5] The latter method can be used to quantitate the individual amounts of cis and trans rotamers (cis = C_1–N bond cis to C=O of NCHO)[5] by the different chemical shifts of the *N*-formyl proton. Recently Olieman and van Koningsfeld[6] presented the first X-ray crystallographic study of the structure of an *N*-formyl-1-benzyl-1,2,3,4,5,8-hexahydroisoquinoline, a close relative of this class of compounds. We now supplement this information by describing the physical and crystallographic properties and particularly the optical behavior of another member of the class of TIQ.[4]

Table I

expt	time, min	cis–trans ratio[a]	α_{obsd}[b]	$[\alpha]^{26}D$, deg
1	5	83:17	0.742	87.5
2	30	54:46	0.686	80.9
3	60	41:59	0.657	77.5
4	180	33:67	0.636	74.9

[a] Determined by integration of the formyl proton NMR resonance in 0.85% solution in Me_2SO. [b] In 0.85% solution in Me_2SO.

Bromination of (*S*)-(+)-*N*-formyl-*N*-norreticuline (1), consisting of a rotamer mixture,[2] afforded a cis–trans rotamer mixture of the corresponding (*S*)-(+)-6′-bromo-*N*-

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