Synthesis and Biological Evaluation of Cembranolide Analogs Containing Cyclic Ethers

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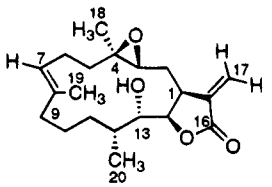
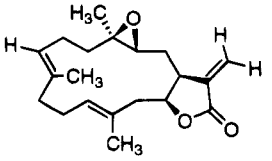
In 1984 Schmitz *et al.*[1] examined the sesquiterpenoid and diterpenoid content of the Caribbean gorgonian *Eunicea succinea* collected in St. Croix, U.S. Virgin Islands, and reported the isolation and structure determination by X-ray methods of the cembranolide diterpenoid 12,13-bisepieupalmerin (**1**). In 1990, as part of an effort to isolate novel anticancer agents from marine invertebrates, we reexamined the components of *E. succinea* from Puerto Rico and obtained a new bioactive cembranolide named euniolide (**2**) together with large amounts of the hydroxyl derivative **1**.[2] These cembranolides, which displayed significant *in vitro* cytotoxicity against CHO-K1 cells, were later found to be active pharmacologically on the nicotinic acetylcholine receptor (AChR).[3,4] During a recent reevaluation of their toxicity against several human tumor cell lines, we found that compound **1** displays potent cytotoxicity toward human CCRF-CEM T-cell leukemia (ED_{50} = 0.15 μg/mL), while **2** was distinctly less cytotoxic. With the ultimate goal



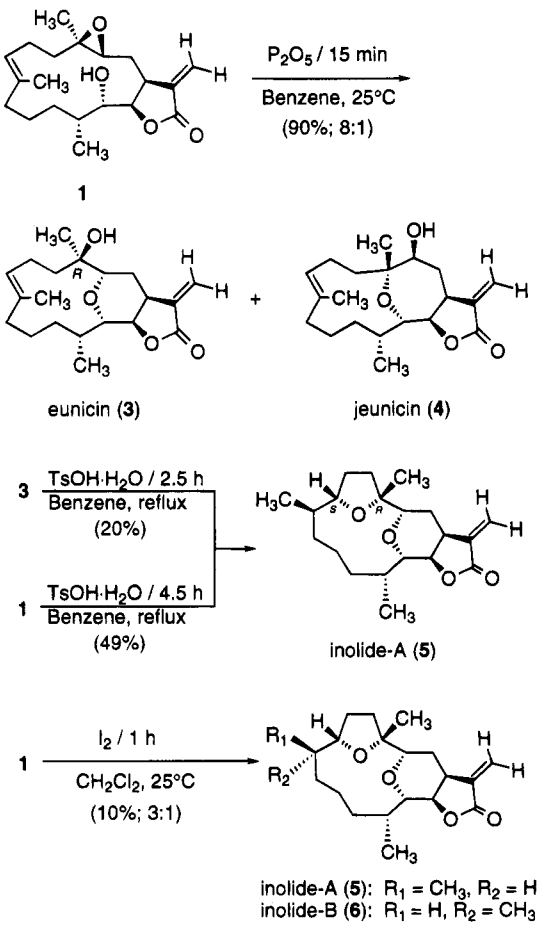
12,13-bisepieupalmerin (**1**)



euniolide (**2**)

of developing functional analogs of this agent for potential use in chemotherapy, we have had a long-standing interest in transformations of the C-13 hydroxyl group,[2] and we now report our studies on transformations of this group leading to the synthesis of inolide-A (**5**) and several other cytotoxic unnatural products characterized by the presence of cyclic ethers. Polyfunctionalized cyclic ether groupings such as these are present in a wide group of strongly antitumor substances isolated from different species of marine invertebrate and microbial organisms.[5]

Scheme 1



We thus elected to carry out the semisynthesis of cembranolides **3**, **5**, **8**, and **9**, with the aim not only of providing material for anticancer testing but also of obtaining information on structure–activity relationships in this area.

The readily available cembranolide 12,13-bisepieupalmerin (**1**) had been directly converted to eunicin (**3**) via transannular back-side attack of the C-13 hydroxyl group at C-3 of the epoxide.[2] The 3,13-oxabridge γ-lactone structure **3**, which has also been found in specimens of *E. succinea* collected throughout the Caribbean Sea, was proposed initially on chemical and spectral grounds and confirmed by X-ray crystallographic analysis of the corresponding iodoacetate.[6,7] Conversion of **3** to **5** or **8** might be accomplished through concomitant intramolecular nucleophilic attack of a hydroxyl group on an activated functional group in the chain (Schemes 1 and 2). Our expectations that this approach could lead to 4,7-oxabridged cembranolide analogs were based in part on our recent discovery of five new cembranolides possessing this functionality.[8] The isolation of these strongly cytotoxic and highly functionalized natural products (named uprolides D–G) suggested that a hydroxyl group located at C-4 could be converted to a 4,7-oxabridged product by intramolecular attack on a carbocation at C-7. This approach was realized as described below.

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Table 1. ^{1}H-NMR (300-MHz) and ^{13}C-NMR (75-MHz) Spectral Data of Cembranolide Analogs 5, 8, and 9 in $CDCl_3$[a]

	inolide-A (5)		inolene (8)		inolene oxide (9)	
position	^{1}H intgr mult (J)	^{13}C	^{1}H intgr mult (J)	^{13}C	^{1}H intgr mult (J)	^{13}C
1	3.34 (1H, m)	38.1 (d)	3.32 (1H, br m)	38.5 (d)	3.42 (1H, m)	37.9 (d)
2	1.97 (1H, d, 13.5)	27.3 (t)	1.98 (1H, m)	27.7 (t)	2.00 (1H, m)	27.2 (t)
2′	1.77 (1H, dd)	–	1.71 (1H, m)	–	1.82 (1H, m)	–
3	3.30 (1H, dd, 1.8, 12.3)	79.8 (d)	3.35 (1H, dd, 1.9, 12.1)	78.7 (d)	3.34 (1H, dd, 1.8, 13.5)	81.8 (d)
4	–	84.3 (s)	–	85.7 (s)	–	86.1 (s)
5	2.14 (1H, m)	29.9 (t)	2.01 (1H, m)	28.4 (t)	2.19 (1H, m)	31.1 (t)
5′	1.44 (1H, m)	–	1.35 (1H, m)	–	1.42 (1H, m)	–
6	1.98 (1H, m)	30.4 (t)	2.05 (1H, m)	29.2 (t)	2.12 (1H, m)	32.5 (t)
6′	1.91 (1H, m)	–	2.03 (1H, m)	–	2.10 (1H, m)	–
7	3.74 (1H, dd, 6.6, 11.4)	84.8 (d)	4.55 (1H, br m)	81.5 (d)	4.39 (1H, t, 7.8)	79.9 (d)
8	1.52 (1H, m)	36.8 (d)	–	129.2 (s)	–	64.4 (s)
9	1.54 (1H, m)	22.0 (t)	5.27 (1H, dd, 1.8, 9.6)	126.5 (d)	3.30 (1H, d, 9.0)	60.1 (d)
9′	1.28 (1H, m)	–	–	–	–	–
10	1.60 (1H, m)	30.5 (t)	2.18 (1H, m)	22.7 (t)	1.75 (1H, m)	21.8 (t)
10′	1.10 (1H, m)	–	1.96 (1H, m)	–	1.58 (1H, m)	–
11	1.62 (1H, m)	35.5 (t)	1.92 (1H, m)	35.5 (t)	1.84 (1H, m)	33.9 (t)
11′	1.37 (1H, m)	–	1.57 (1H, m)	–	1.80 (1H, m)	–
12	2.10 (1H, m)	31.8 (d)	2.02 (1H, m)	31.5 (d)	2.18 (1H, m)	31.1 (d)
13	3.04 (1H, dd, 1.2, 9.9)	83.2 (d)	2.92 (1H, d, 9.6)	84.6 (d)	2.99 (1H, d, 9.9)	84.8 (d)
14	4.45 (1H, t, 8.4)	73.7 (d)	4.32 (1H, t, 8.7)	73.9 (d)	4.45 (1H, t, 8.7)	73.6 (d)
15	–	136.5 (s)	–	136.5 (s)	–	136.1 (s)
16	–	170.1 (s)	–	170.2 (s)	–	169.9 (s)
17	6.38 (1H, d, 3.6)	121.2 (t)	6.39 (1H, d, 3.6)	121.1 (t)	6.46 (1H, d, 3.3)	121.5 (t)
17′	5.53 (1H, d, 3.3)	–	5.54 (1H, d, 3.3)	–	5.60 (1H, d, 3.0)	–
Me18	1.13 (3H, s)	25.5 (q)	1.17 (3H, s)	25.2 (q)	1.25 (3H, s)	25.4 (q)
Me19	0.83 (3H, d, 6.6)	19.1 (q)	1.46 (3H, s)	13.2 (q)	1.25 (3H, s)	16.0 (q)
Me20	0.98 (3H, d, 7.2)	12.4 (q)	0.78 (3H, d, 7.2)	9.2 (q)	0.94 (3H, d, 7.2)	10.6 (q)

[a] Assignments were aided by ^{1}H–^{1}H COSY, spin splitting patterns, selective decoupling experiments, comparison of J values, heteronuclear chemical shift correlation methods, carbon atom multiplicities, and chemical shift values. The δ values are in ppm and are referenced to the residual $CHCl_3$ signal (7.26 and 77.0 ppm, respectively).

Treatment of alcohol **1** with anhydrous P_2O_5 afforded mostly the tetrahydropyran eunicin (**3**, 80%) and a small amount of jeunicin (**4**, 10%, Scheme 1).[9–11] Although the reaction can also be performed in a catalytic manner we found that the best yields were obtained with 1 equiv of P_2O_5. **3** was formed in only 33% yield when **1** was treated with I_2 in the presence of pyridine in CH_2Cl_2, but the yield of **4** was better (15%).

The conversion of **3** into a tetracyclic system was first effected using *p*-toluenesulfonic acid ($TsOH\cdot H_2O$) in refluxing benzene for 2.5 h to give inolide-A (**5**) in 20% yield. However, the best yield (49%) of **5** was obtained when **1** was treated with $TsOH\cdot H_2O$ at 80 °C in benzene. The structure of **5** was deduced from its spectra. Its stereochemistry was confirmed using NOE data which showed that the C-18 and C-19 proton signals were enhanced when the C-7 proton was irradiated, and the signals at C-13 and C-18 were enhanced when the C-3 proton was irradiated. The structure of **5** was confirmed by X-ray crystallography including its absolute configuration. The full ^{1}H and ^{13}C NMR spectral assignments from two-dimensional proton–proton and proton–carbon NMR spectroscopy are shown in Table 1. Attack of $TsOH\cdot H_2O$ on **3** at C-8 thus occurs from the less-hindered α face of the ring followed by the intramolecular attack, also from the α face, of the C-4 hydroxyl group on the carbocation generated by the acid treatment. The C-4,8 six-membered cyclic ether was not observed.

When the tandem cyclization of **1** was carried out with I_2 (in the absence of pyridine) in CH_2Cl_2 at 25 °C for 1 h, a complex mixture of products was obtained from which diastereoisomers inolide-A (**5**) and inolide-B (**6**), in 7 and 3% isolated yields, respectively, were recovered (Scheme 1). Thus both C-8 epimers occur, but only one configuration is found at C-7. The structure of **6** was deduced from its spectra. Its stereochemistry around the oxolane moiety was established from a NOESY experiment which showed that the C-3, C-7, and C-13 proton signals were within NOE distance of the Me-18 protons. Interestingly, of the twenty carbons in **6** only thirteen were detected clearly in the ^{13}C NMR spectrum as sharp signals which could be assigned to the three small semirigid rings of the cembrane carbon skeleton. The remaining seven resonance lines were quite broad signals of very low intensity. This peculiarity, which was not observed in inolide-A (**5**), suggests that the coalescence temperature for interconversion of different conformations of the large ring in inolide-B (**6**) is near the NMR probe temperature.

Epoxidation of **3** with *m*-chloroperoxybenzoic acid (*m*-CPBA) at 25 °C in benzene afforded the desired epoxycembranolides **7** as a 1:1 mixture of two geometrical isomers in 96% yield (Scheme 2). The structure of each diastereoisomer was deduced, after separation by high-performance liquid chromatography (HPLC), from its spectra. Initial attempts to rearrange and dehydrate the crude epoxide mixture with $TsOH\cdot H_2O$ at 25 °C in benzene were encouraging. The desired 8-hydroxy 4,7-oxolane intermediate was formed followed by elimination in the presence of acid to produce the corresponding polycyclic olefin **8** in 28% yield as solely the *E* isomer. The structural identity of inolene (**8**) was established through the concerted application of two-dimensional NMR spectroscopy which included COSY, CSCMBB, and NOESY experiments (see Table 1). Assignment of the olefin geometry was made possible from the signals in the ^{13}C NMR spectrum, which showed a significant

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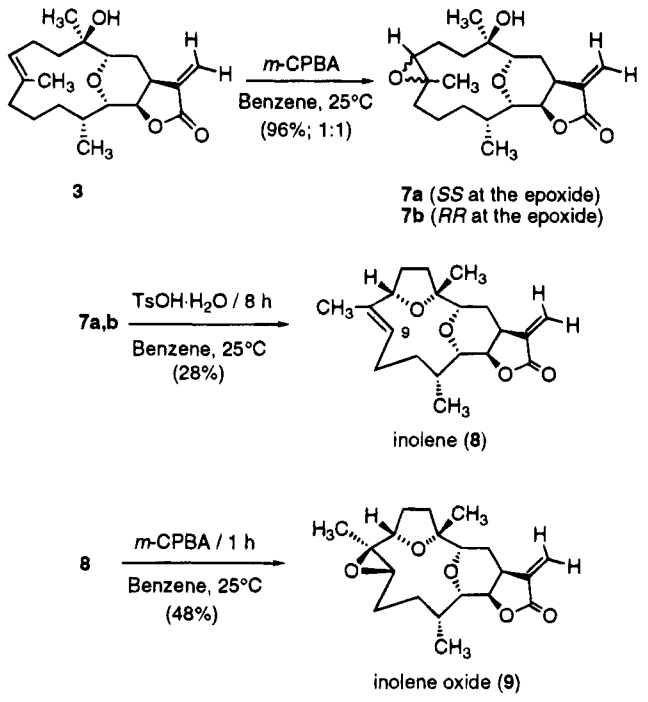
Table 2. *In Vitro* Inhibitory Activity of the Cembranolides against Several Human Tumor Cell Lines

compound	cancer cell line: colon cancer HCT 116 ED_{50} (μg/mL)	T-cell leukemia CCRF-CEM ED_{50} (μg/mL)	breast adenocarcinoma MCF-7 ED_{50} (μg/mL)
12,13-bisepieupalmerin (**1**)	3.1	0.15	1.0
euniolide (**2**)	43.4	5.6	21.7
eunicin (**3**)	5.0	0.06	3.4
inolide-A (**5**)	3.7	0.01	5.0
inolide-B (**6**)	4.6	0.07	3.1
inolene (**8**)	–	0.06	–
inolene oxide (**9**)	–	1.24	–

Scheme 2



shielding of the Me-19 group (δ 13.2) caused by vicinal carbons in the same way as in *trans*-polyisoprene.[12] Moreover, the lack of an NOE between the olefinic H-9 proton and the Me-19 group, the presence of a strong NOE between the Me-19 and Me-20 groups, and the unusually strong upfield shift experienced by the C-20 atoms due to shielding from the nearby Δ^8 olefin in the NMR spectra (see Table 1) were consistent with the *E* orientation of the double bond. Although the starting epoxycembranolides **7a** and **7b** were identified and characterized separately, attempts to perform the reaction with each individual geometrical isomer were not pursued.

Addition of *m*-CPBA to a solution of inolene (**8**) in benzene at 25 °C gave a 48% isolated yield of inolene oxide (**9**, Scheme 2) as a single geometrical isomer, as determined by analysis of ^1H and ^{13}C NMR spectra (Table 1). Attack of *m*-CPBA on **8** thus occurs from the less hindered β face of the trisubstituted double bond.

Biological Evaluation. The biological activities of **1**–**3**, **5**, **6**, **8**, and **9** were measured initially in terms of their cytotoxicity against human colon cancer (HCT 116), T-cell leukemia (CCRF-CEM), and breast adenocarcinoma (MCF-7) cells. Table 2 is a summary of the key results from the comparative study of these compounds.

It is apparent that as a group these cembranolides are strongly cytotoxic against the human leukemia cell line. All of the synthetic congeners of 12,13-bisepieupalmerin (**1**) were found to exhibit moderate to strong human T-cell leukemia cell growth inhibition ranging from ED_{50} 1.24 to 0.01 μg/mL. All of the analogs except **9** inhibited cell growth better than **1**, with the best being inolide-A (**5**). It is interesting that the most active compounds **3**, **5**, **6**, and **8** have the same tetrahydropyran ring and most of the same structural features. **9**, the only other compound with most of these features, may be less active due to a conformational change in the large ring.

On the basis of these results primarily, inolide-A (**5**) and inolene (**8**) were examined in the National Cancer Institute cell line screen, and their differential cytotoxicity pattern was evaluated by the COMPARE algorithm.[13] Interestingly, most of the human leukemia cell lines recently incorporated into the NCI panel (CCRF-CEM, HL-60(TB), K-562, MOLT-4, RPMI-8226 and SR) were among the most sensitive (e.g., GI_{50} 10^{-7}–10^{-8}). Against this NCI human cancer cell line subpanel, their negative $\log_{10}$ GI_{50} values[14] range to over 7 and represent an interesting selection of human cancer types. Aside from their novel structures, compounds **5** and **8** may offer some hope for improving future human cancer treatment. On the other hand, compounds **5**, **7a**, and **8** proved inactive in the NCI test for agents active against the human immunodeficiency virus (HIV).

Experimental Section

General Methods. ^1H and ^{13}C NMR were measured at 300 and 75 MHz, respectively, with a General Electric QE-300 spectrometer. Infrared (IR) spectra were determined in a Nicolet 600 FT-IR spectrophotometer, and ultraviolet (UV) spectra were recorded in a Hewlett-Packard Chem Station 8452A spectrometer. High-performance liquid chromatography (HPLC) separations were performed on a Zorbax C-8 semipreparative column (9.4 mm × 25 cm) using a flow rate of 2mL/min. All separations were monitored simultaneously by refractive index and UV absorption. Optical rotations were recorded on a Perkin-Elmer polarimeter (Model 243B). "Dried and concentrated" refers to removal of residual quantities of water with anhydrous Na_2SO_4 followed by evaporation of solvent on a rotary evaporator. Brine refers to a saturated aqueous solution of sodium chloride. Column (flash) chromatography was performed on silica gel (35–75 mesh), and TLC analyses were carried out using glass precoated silica gel plates.

Materials. All solvents and volatile reagents used were either spectral grade or were distilled from glass prior to use. Diethyl ether (ether) was distilled from sodium/benzophenone; benzene and CH_2Cl_2 were distilled from CaH_2. Other reagents were purchased from Aldrich.

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Extraction and Isolation of 12,13-Bisepieupalmerin (1).[2] Minced and freeze-dried *E. succinea* (352.6 g) collected at Palomino Island, Puerto Rico in May 1990 was extracted exhaustively with 3.5 L of $CHCl_3$–MeOH (1:1). The dried residue after filtration and concentration amounted to *ca.* 47.0 g and was partitioned between hexane and water giving, after subsequent rotary evaporation, 24.0 g of lipids as a viscous dark green oil. The hexane extract was dissolved in toluene (10 mL) and passed through a Bio-Beads SX-2 (toluene) column. The fractions eluting last contained all the diterpenoids and were combined on the basis of TLC analyses. After concentration *in vacuo* the oily mixture (5.72 g) was purified by column chromatography on silica gel (170 g) using mixtures of ethyl acetate–hexane of increasing polarity. The less polar portion of the lipids was fractionated roughly into fractions A through G: fraction A [euniolide (**2**); 2.04 g; 0.58%] and fraction G [12,13-bisepieupalmerin (**1**); 302 mg; 0.085%].

Eunicin (3) and Jeunicin (4). Path A. To a mixture of 515 mg (1.54 mmol, 0.01 equiv) of 12,13-bisepieupalmerin (**1**) in 30 mL of dry benzene at 25 °C was added 277 mg (1.95 mmol) of anhydrous P_2O_5. The mixture was stirred for 15 min before it was quenched with 30 mL of 0.5 N $NaHCO_3$. Chloroform was added, and the solution was washed with brine, dried, and concentrated. Purification by column chromatography on silica gel (20 g, 1:4 (v/v) ethyl acetate in hexane) gave 415 mg (80%) of **3** and 50 mg (10%) of **4**; all solution spectral data were identical with those obtained from authentic material.[9-11]

Path B. To a solution of 144 mg (0.43 mmol) of **1** and 5 mL of pyridine in dry CH_2Cl_2 at 25 °C was added 119 mg (0.47 mmol) of iodine in 25 mL of CH_2Cl_2, dropwise over 1 h. The reaction mixture was concentrated and purified by column chromatography on silica gel (10 g, 1:4 (v/v) ethyl acetate in hexane) to give 47 mg (33%) of **3** and 21 mg (15%) of **4**. An undetermined amount of unreacted **1** was also recovered.

Inolide-A (5) and Inolide-B (6). Path A. A solution of 64 mg (0.19 mmol) of eunicin (**3**) and 36 mg (0.19 mmol) of *p*-toluenesulfonic acid ($TsOH·H_2O$) in 15 mL of dry benzene was stirred at reflux for 2.5 h and then concentrated *in vacuo*. Chromatography of the residue on silica gel (10 g) (elution with 1:3 (v/v) ethyl acetate in hexane) gave 13 mg (20%) of **5** as a colorless oil: IR (neat) 2964, 2915, 1758, 1458, 1301, 1112, 1056, 1002 cm^{-1}; UV (MeOH) λ_{max} 210 nm; $[\alpha]^{21}_D$ −23.8° (*c* 6.3, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 1); HREI-MS *m/z* (M^+) calcd for $C_{20}H_{30}O_4$ 334.2145, found 334.2147, 334 (58), 316 (6), 278 (8), 182 (14), 153 (40), 108 (38), 85 (92), 69 (86), 55 (100), 41 (85).

Path B. A solution of 12,13-bisepieupalmerin (**1**) (234 mg, 0.70 mmol) in dry benzene (25 mL) was treated with $TsOH·H_2O$ (133 mg, 0.70 mmol), allowed to stir at reflux for 4.5 h, and poured into a saturated $NaHCO_3$ solution. The product was extracted into ether, dried, concentrated, and chromatographed on silica gel (elution with 1:4 (v/v) ethyl acetate in hexane). There was isolated 115 mg (49%) of **5** as a colorless oil. Upon prolonged storage at −10 °C, **5** slowly crystallized. Recrystallization from MeOH–water mixtures produced crystals amenable to a single-crystal X-ray diffraction study.

Path C. To a solution of **1** (300 mg, 0.90 mmol) in dry CH_2Cl_2 (50 mL) at 25 °C was added iodine (246 mg, 0.97 mmol) dissolved in 30 mL of CH_2Cl_2, dropwise over 45 min. After stirring for 1 h, the reaction solution was evaporated to dryness. The residue was purified by gravity chromatography on silica gel (elution with chloroform) followed by normal-phase HPLC (Partisil 10, elution with 1:19 (v/v) 2-propanol in hexane) to give 22 mg (7%) and 9 mg (3%) of **5** and **6**, respectively. Data for inolide-B (**6**): IR (neat) 2982, 2899, 2873, 1762, 1461, 1296, 1114, 1097, 1005, 947 cm^{-1}; UV (MeOH) λ_{max} 210 nm; $[\alpha]^{21}_D$ −36.7° (*c* 1.8, MeOH); ^1H NMR ($CDCl_3$, 300 MHz) δ 6.38 (1H, d, *J* = 3 Hz, H-17), 5.53 (1H, d, *J* = 2.7 Hz, H-17'), 4.45 (1H, t, *J* = 8.7 Hz, H-14), 4.12 (1H, m, H-7), 3.37 (1H, br m, H-1), 3.28 (1H, d, *J* = 11.7 Hz, H-3), 2.96 (1H, d, *J* = 9.9 Hz, H-13), 2.20–1.23 (complex multiplet), 1.16 (3H, s, Me-18), 1.08 (3H, d, *J* = 7.2 Hz, Me-20), 0.77 (3H, d, *J* = 7.2 Hz, Me-19); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.2 (s, C-16), 136.5 (s, C-15), 121.2 (t, C-17), 85.0 (d, C-7), 84.1 (s, C-4), 82.8 (d, C-13), 81.3 (d, C-3), 73.5 (d, C-14), 38.2 (d, C-1), 35.2 (d, C-8), 34.8 (t, C-11), 32.4 (t, C-10), 31.4 (d, C-12), 30.5 (t, C-6), 30.4 (t, C-5), 27.2 (t, C-2), 26.9 (t, C-9), 25.7 (q, C-18) (the signals for C-19 and C-20 were not detected); HREI-MS *m/z* (M^+) calcd for $C_{20}H_{30}O_4$ 334.2145, found 334.2147, 334 (50), 316 (6), 278 (7), 182 (13), 153 (34), 107 (26), 95 (43), 85 (77), 81 (90), 69 (100), 55 (85).

α-(7*S*),(8*S*)-Epoxyeunicin (7a) and β-(7*R*),(8*R*)-Epoxyeunicin (7b). A solution of eunicin (**3**) (564 mg, 1.69 mmol) in dry benzene (25 mL) was treated with *m*-CPBA (340 mg, 1.97 mmol), stirred at 25 °C for 45 min, poured into a saturated $NaHCO_3$ solution, and extracted with ether. The combined organic extracts were washed with brine, dried, and concentrated, and the residue was purified by flash chromatography on silica gel (elution with 1:4 (v/v) ethyl acetate in hexane) to afford 571 mg (96%) of a 1:1 mixture of epoxycembranolides **7a** and **7b**. A small portion of the mixture was separated by reversed-phase HPLC (Ultrasphere-ODS; elution with 3:7 (v/v) water in MeOH). Data for **7a**: IR (neat) 3446, 3015, 2962, 2877, 1760, 1459, 1379, 1256, 1106 cm^{-1}; UV (MeOH) λ_{max} 210 nm; $[\alpha]^{26}_D$ −124.6° (*c* 6.7, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) δ 6.41 (1H, d, *J* = 3.6 Hz, H-17), 5.65 (1H, d, *J* = 3.3 Hz, H-17'), 4.45 (1H, dd, *J* = 7.6, 9.7 Hz, H-14), 3.44 (1H, m, H-1), 3.28 (1H, dd, *J* = 2.4, 11.4 Hz, H-3), 3.03 (1H, d, *J* = 9.9 Hz, H-13), 2.75 (1H, d, 9.6 Hz, H-7), 2.21 (1H, dt, *J* = 1.9, 14.4 Hz, H-2), 2.05–1.61 (complex multiplet), 1.52–1.27 (complex multiplet), 1.20 (6H, s, Me-18 and Me-19), 1.02–0.90 (2H, m), 0.87 (3H, d, *J* = 6.9 Hz, Me-20); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.0 (s, C-16), 136.5 (s, C-15), 121.6 (t, C-17), 78.0 (d, C-13), 73.8 (s, C-4), 73.2 (d, C-3), 72.9 (d, C-14), 66.8 (d, C-7), 61.2 (s, C-8), 38.4 (t, C-9), 38.3 (d, C-1), 35.6 (t, C-10), 33.0 (d, C-12), 29.4 (t, C-11), 24.3 (t, C-2), 24.0 (q, C-18), 22.0 (t, C-5), 19.3 (t, C-6), 17.0 (q, C-19), 13.9 (q, C-20); HREI-MS *m/z* (M^+) calcd for $C_{20}H_{30}O_5$ 350.2094, found 350.2107, 350 (1), 332 (5), 307 (4), 289 (4), 181 (7), 164 (14), 111 (12), 85 (82), 67 (19), 55 (43), 43 (100). Data for **7b**: IR (neat) 3471, 3097, 2960, 2933, 2877, 1772, 1461, 1384, 1285, 1236, 1213, 1107 cm^{-1}; UV (MeOH) λ_{max} 210 nm; $[\alpha]^{26}_D$ −43.4° (*c* 5.9, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) δ 6.40 (1H, d, *J* = 3.6 Hz, H-17), 5.67 (1H, d, *J* = 3.3 Hz, H-17'), 4.53 (1H, t, *J* = 8.7 Hz, H-14), 3.42 (1H, m, H-1), 3.30 (1H, d, *J* = 11.4 Hz, H-3), 3.05 (1H, d, *J* = 9.9 Hz, H-13), 2.61 (1H, dd, *J* = 2.4, 11.1 Hz, H-7), 2.24 (1H, br dt, *J* = 14.1 Hz, H-2), 2.07–1.29 (complex multiplet), 1.25 (3H, s, Me-18), 1.20 (3H, s, Me-19), 0.92 (3H, d, *J* = 6.9 Hz, Me-20), 0.87 (1H, t, *J* = 6.9 Hz, H-5'); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.0 (s, C-16), 135.7 (s, C-15), 122.2 (t, C-17), 77.1 (d, C-13), 74.0 (d, C-3), 73.4 (s, C-4), 72.6 (d, C-14), 60.5 (d, C-7), 59.8 (s, C-8), 37.9 (d, C-1), 35.6 (t, C-9), 34.3 (t, C-10), 32.3 (d, C-12), 32.2 (t, C-11), 24.2 (t, C-2), 23.9 (q, C-18), 21.0 (t, C-6), 20.1 (q, C-19), 18.8 (t, C-5), 14.9 (q, C-20); HREI-MS *m/z* (M^+) calcd for $C_{20}H_{30}O_5$ 350.2094, found 350.2091, 350 (2), 332 (15), 304 (6), 261 (5), 181 (14), 164 (21), 135 (20), 111 (32), 95 (48), 85 (92), 55 (100), 53 (51).

Inolene (8). *p*-Toluenesulfonic acid hydrate (59 mg, 0.31 mmol) was added portionwise to a magnetically stirred solution of a 1:1 mixture of the epoxycembranolides **7a** and **7b** (110 mg, 0.31 mmol) in dry benzene (30 mL). After 8 h at 25 °C the reaction mixture was concentrated *in vacuo*. Chromatography of the residue on silica gel (elution with 3:7 (v/v) ethyl acetate in hexane) resulted in the isolation of 49 mg (45%) of unreacted starting epoxides and 16 mg (28% based on recovered starting material) of **8** as a colorless oil: IR (neat) 2961, 2937, 2875, 1761, 1453, 1300, 1112, 1048, 1003, 950 cm^{-1}; UV (MeOH) λ_{max} 210 nm; $[\alpha]^{26}_D$ +11.9° (*c* 8.8, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 1); HREI-MS *m/z* (M^+) calcd for $C_{20}H_{28}O_4$ 332.1988, found 332.1999, 332 (21), 314 (3), 290 (5), 256 (5), 163 (7), 109 (24), 95 (46), 81 (52), 67 (45), 55 (100).

Inolene Oxide (9). A solution of 16 mg (0.05 mmol) of inolene (**8**) and 15 mg (0.08 mmol) of *m*-CPBA in anhydrous benzene (15 mL) was stirred at 25 °C for 1 h. Removal of solvent and chromatography of the residue on silica gel (elution with 1:1 (v/v) ethyl acetate in hexane) afforded 8 mg (48%) of **9** as a colorless oil: IR (neat) 2962, 2934, 2860, 1764, 1378, 1302, 1259, 1112, 1099, 1052, 1005, 750 cm^{-1}; UV (MeOH) λ_{max} 210 nm; $[\alpha]^{26}_D$ −16.8° (*c* 2.5, $CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 75 MHz) (see Table 1); HREI-MS *m/z* (M^+) calcd for $C_{20}H_{28}O_5$ 348.1937, found 348.1947, 348 (33), 330 (6), 320 (5), 290 (33), 263 (5), 221 (6), 193 (9), 111 (71), 81 (54), 67 (60), 55 (100).

X-ray Structure Determination. Crystal Data for Inolide-A (5). [18]Crystallization of **5** from MeOH–water mixtures at low temperature yielded clear prisms of X-ray quality. X-ray diffraction data were collected on an Enraf-Nonius CAD4

diffractometer using Cu Kα radiation (λ = 1.54056 Å). The structure, which was solved by direct methods and completed by successive Fourier calculations, was refined by full-matrix least squares methods, with anisotropic thermal parameters for all non-H atoms. Following initial refinement, H atoms were located from a difference Fourier map. HO8 was refined with a fixed isotropic thermal parameter, and all remaining H atoms were included in the final model at calculated positions, riding on the connected atoms. The absolute configuration of the structure, known from other data, agreed with that calculated by refinement of the η parameter.[15] All calculations were performed with the NRCVAX program package of crystallographic programs.[16] Scattering factors were taken from the *International Tables for X-ray Crystallography*.[17,18]

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Supporting Information Available: Copies of ^{1}H and ^{13}C spectra (300 MHz and 75 MHz) for compounds **5**, **8**, and **9**, ORTEP structure for inolide-A (**5**) and the details of the X-ray experiment, crystal data, data collection, reduction and refinement, and the atomic parameters (9 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(18) The author has deposited atomic coordinates for this structure with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.