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Synthesis of cytotoxic cembranolide analogues via acid-induced opening of oxiranes☆

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Abstract—A large series of analogues of *Eunicea* cembranolides **1**–**3** were synthesized with the purpose of evaluating their cytotoxicity against 60 human cancer cell-lines. Most of the analogues were as active as the lead compounds and a few displayed increased cytotoxicity. Their syntheses were based on the specific reactivity and stereochemistry of the epoxide substructure and involved highly regio- and diastereoselective acid-induced chemical transformations. © 2000 Elsevier Science Ltd. All rights reserved.

1. Introduction

Gorgonian cembranolides belonging to the genus *Eunicea* are easily accessible and contain highly reactive functions which make them suitable templates for the synthesis of analogues possessing uncommon structural features and interesting biological properties.[1,2] Naturally occurring *Eunicea* cembranoids such as euniolide (**1**),[3] 12,13-bisepieupalmerin (**2**),[4] and eupalmerin (**3**)[5] have shown strong in vitro antitumor activity (Fig. 1). We have synthesized a large series of unusual analogues of cembranolides **1**–**3** with the purpose of evaluating their cytotoxicity against 60 human cancer cell-lines. Our studies with α-methylene-γ-lactones **1**–**3** aimed at establishing the feasibility of obtaining unusual cembranoid systems of increased cytotoxicity using simple functional group interconversions in concise fashion. The complete structural assignment of all the synthetic cembranoid analogues described in the present work was accomplished on the basis of comprehensive 1D and 2D NMR experiments involving ^{1}H–^{1}H COSY, DEPT, NOESY, ^{1}H–^{13}C COSY (CSCMBB), selective INEPT, HMQC and HMBC measurements. In most cases, these 2D NMR spectra provided both the structure and the complete and unambiguous proton and carbon atom assignments. Since starting materials **1**–**3** were optically pure without ambiguity in the absolute configuration, the absolute configurations of the synthetic analogues are as depicted. Finally, the molecular structures of cembranolide analogues **13**, **17**, and **32** were confirmed by single-crystal X-ray crystallography.

2. Results and discussion

2.1. Transformations of euniolide (1)

This abundant compound is generally regarded as the biosynthetic precursor of cembranolides **2** and **3**.[2] Our first attempt at structural modification of euniolide (**1**) was to rearrange the epoxide functionality to form a carbonyl group at the C-3 position (Eq. (1)). To arrive at ketone **4** advantage was taken of the known chemoselectivity with which a C-3,4 epoxide function such as that of **1** reacts with various Lewis acid promoters to give predominantly the C-4(*R*) diastereomer.[6] Thus, treating euniolide (**1**) with phosphorus pentoxide in refluxing benzene for 1 h delivered a 37:22:1 mixture of ketones 4(*R*)-euniolone (**4**), 4(*S*)-euniolone (**5**), and lactone **6** in 54% overall yield. Surprisingly, under these reaction conditions the C-7,8 double bond isomerized in lactone **6** giving the (*Z*)-Δ^{7} olefin in 1% isolated yield. These reaction conditions also led to a large amount of an intractable mixture of highly polar compounds accounting for the remainder of the starting material **1**. A combination of coupling constant analysis and NOESY spectra established the relative stereochemistry of 4(*R*)-euniolone as shown in structure **4**, which showed that it had the same relative stereochemistry as **5** at all centers except at C-4. The large coupling (11.7 Hz) observed between H-1 (δ_H 3.62) and H-2 (δ_H 2.51) suggests that they are anti-parallel; therefore H-2 is β-oriented in **4**. In addition to producing NOEs at H-5 (δ_H 1.51) and H-5′ (δ_H 1.85), Me-18 (δ_H 0.94) produced NOEs at H-2 also.

☆ Taken in part from the graduation theses of I. C. Piña (1995) and A. L. Acosta (1997).

Keywords: coelenterates; epoxides; ethers; cytotoxins.

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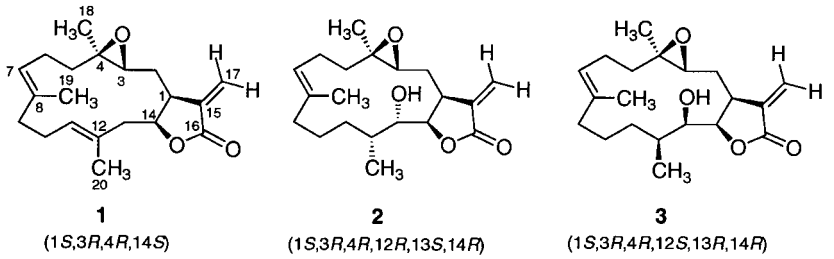


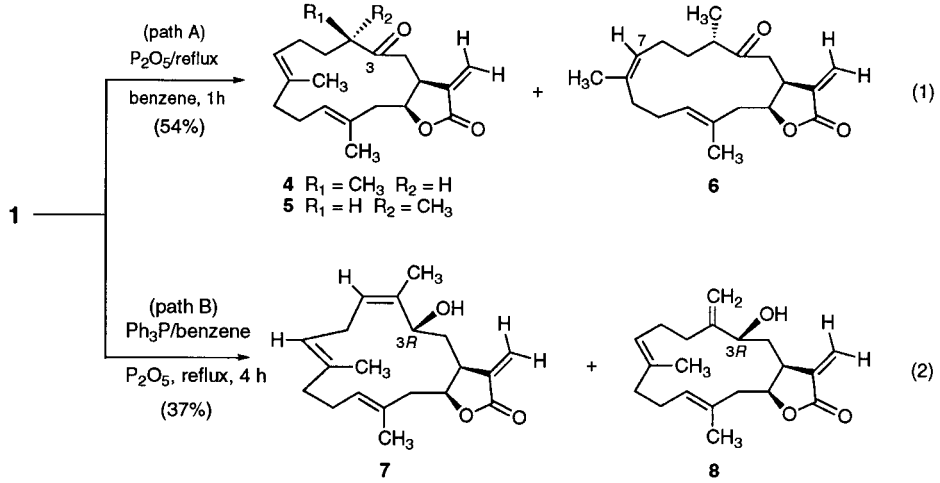
Figure 1. Absolute structures of euniolide (**1**), 12,13-bisepieupalmerin (**2**), and eupalmerin (**3**).

This NOE between Me-18 and H-2 indicates that the Me-18 is on the same side as H-2, making the former β-oriented in **4**. The NOESY spectrum of **4** also showed key correlations between H-1/H-11, H-1/H-14, H-1/H-17, H-2′/H-11, H-11/H-13, and H-14/Me-20. From the foregoing discussion, 4(*R*)-euniolone has the relative stereochemistry depicted in **4**. An NOE between H-7 and Me-19 in lactone **6** established that the Δ^7 double bond has (*Z*)-geometry. When the epoxide ring opening in **1** was tried with phosphorus pentoxide in the presence of triphenylphosphine the formation of ketones **4**–**6** was completely suppressed, but the reaction could not be driven to completion (Eq. (2)). In this way, an easily separable 1.5:1 mixture of tetraene alcohols **7** and **8**, respectively, was obtained in 37% yield. The HREI-MS, IR, and NMR spectral data of **7** and **8** indicated that these compounds possess similar molecular formula and functionality. The ^{13}C NMR spectrum of **7**, which also revealed the lack of an epoxide moiety, displayed signals for three methyl groups with the methyl resonance ascribed to Me-18 occurring downfield of 20 ppm. This indicated the (*Z*)-configuration of the Δ^4 trisubstituted double bond. Signals for only two methyl groups were observed in the ^{13}C NMR spectrum of **8**. The olefinic methylene carbon resonating at 112.2 ppm correlated to the two broad singlets at δ 4.99 and 4.94 assigned as the C-18 *exo*-methylene. The relative stereochemistry of C-3 in alcohols **7** and **8** was again determined with the aid of NOESY spectra. The doublet of doublet absorbed at δ 4.05 in **7** correlated to the oxygenated methine carbon at 71.2 ppm and thus was assigned as H-3. The strong NOESY correlation between H-3 and the Me-18 group suggested a similar relative stereochemistry for C-3 as euniolide (**1**). Likewise, the doublet of doublet resonating at δ 4.20, which was assigned as the C-3 oxymethine in **8**, showed a strong NOESY correlation with one of the C-17 (δ_H 5.69) and one of the C-18 (δ_H 4.94) *exo*-methylene protons which suggested the same relative stereochemistry at that position as **7**. A more promising result was achieved when **1** was treated with $BF_3{\cdot}O(C_2H_5)_2$ in benzene at 0°C for 1.5 h. Under these conditions, the desired euniolones **4** and **5** were formed in reasonable yield (62%) as a 3:1 mixture, respectively.

Exposure of **1** to a mixture of triphenylphosphine and iodine in CH_2Cl_2 at rt led to a complex product mixture from which the main product (1*S*,3*E*,7*E*,11*E*,14*S*)-cembra-3,7,11, 15(17)-tetraen-16,14-olide (**9**) could be isolated in modest yield (33%), (Eq. (3)). The HREI-MS data for **9** suggested the molecular formula $C_{20}H_{28}O_2$ and seven degrees of unsaturation. The IR and NMR spectra indicated the lack of hydroxyl and epoxy functionalities. On the other hand, the ^1H and ^{13}C NMR showed the presence of three methyl substituted trisubstituted double bonds. In the ^{13}C NMR spectrum these methyl resonances occur upfield of 20 ppm indicating the (*E*)-configuration of the trisubstituted double bonds. These conditions also led to the formation of lactones **10**–**12** in 18, 15, and 6% isolated yields, respectively.

Further experimentation revealed that the introduction of $CHCl_3$ as co-solvent, after 4.5 h at 0°C, suppressed the formation of tetraene **9** giving instead a 7:1:1:1.5:1:1 mixture of products **4**, **7**, **8**, and **12**–**14** in 81% overall yield (Eq. (4)).

The formation of an oxa bridge function at the C-4/C-8 position of **10**, **11**, and **13** and at the C-4/C-7 position of



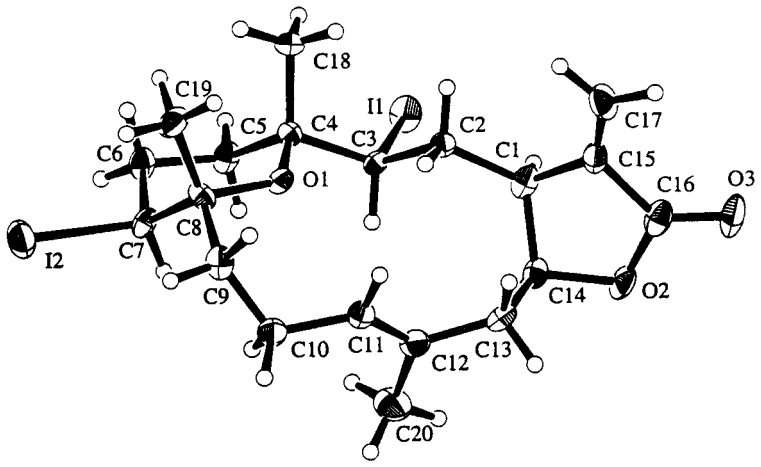
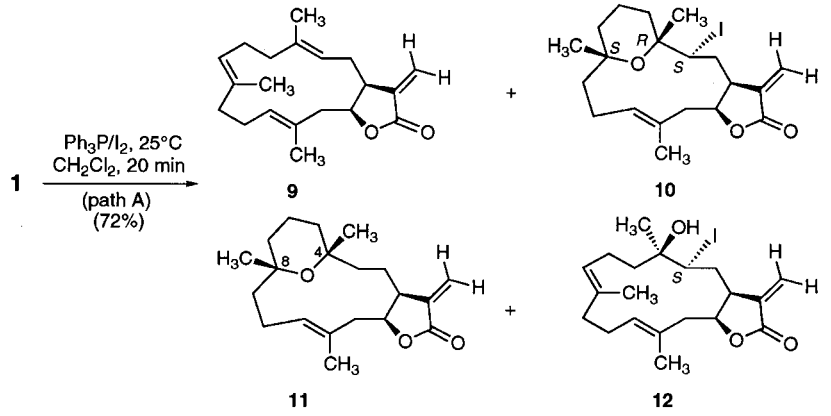


Figure 2. Crystalline structure of 4, 8-oxabridged cembranolide **13**.

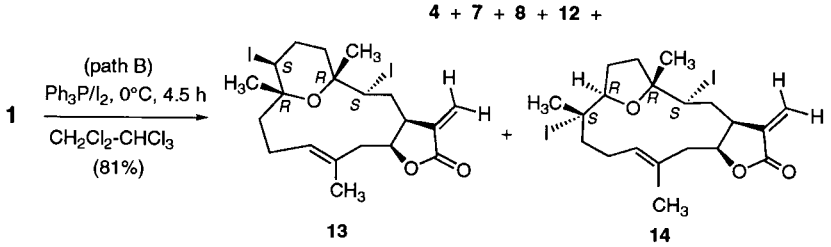


(3)

product **14** was a pleasant surprise. Another surprise was that certain analogues (i.e. **10** and **12**–**14**) contained iodine. The formation of the bridged ether cores of **13** and **14** must proceed via epoxide ring opening in **1** upon nucleophilic attack at C-3 with inversion of configuration of an iodide anion to give iodohydrin **12**. The latter undergoes conversion to diiodides **13** and **14** via intramolecular nucleophilic attack, from the α and β face, respectively, of the C-4 hydroxyl group on an activated Δ^7 olefin at either position. Consecutive C–I bond reductions of **13** should furnish pyranethers **10** and **11**, respectively. While these conversions are likely to involve solvent deprotonations by free radical intermediates the mechanistic details of the conversions **1**→**12**→**13**→**10**→**11** were not probed further. The structural assignments for **9**–**14** rest firmly on spectroscopic grounds (^{1}H and ^{13}C/DEPT, ^{1}H–^{1}H COSY, CSCMBB, 2D-NOE, selective INEPT, and HREI-MS studies) with the X-ray crystallographic analysis of tricyclic lactone **13** proving particularly informative (Fig. 2).[7] Unfortunately, the ^{1}H NMR spectrum of **11** in $CDCl_3$ solution gave signals with co-incident chemical shift at δ 1.13 (s, 6H) for Me-18 and Me-19, thus the relative stereochemistry at C-4 and C-8 of pyranethers **10** and **11** was established from the 2D-NOE spectrum of **10**. This experiment, which revealed the Me-18/Me-19 connectivity, indicated that these methyl groups had the same β-orientation as in cembranolide analogue **13**.

2.2. Transformations of 12,13-bisepieupalmerin (2)

Conversion of **2** to diepoxide **15** was accomplished in 81% isolated yield with *m*-CPBA in benzene at 25°C for 1.5 h (Eq. (5)). The oxidation proceeded to completion with no traces of the alternate C-7(*R*),8(*R*) diastereomer detected. Cembranolide **15**, having the 7(*S*),8(*S*) configuration, was identical in all respects to the natural product 12,13-bisepieupalmerin epoxide isolated previously from the



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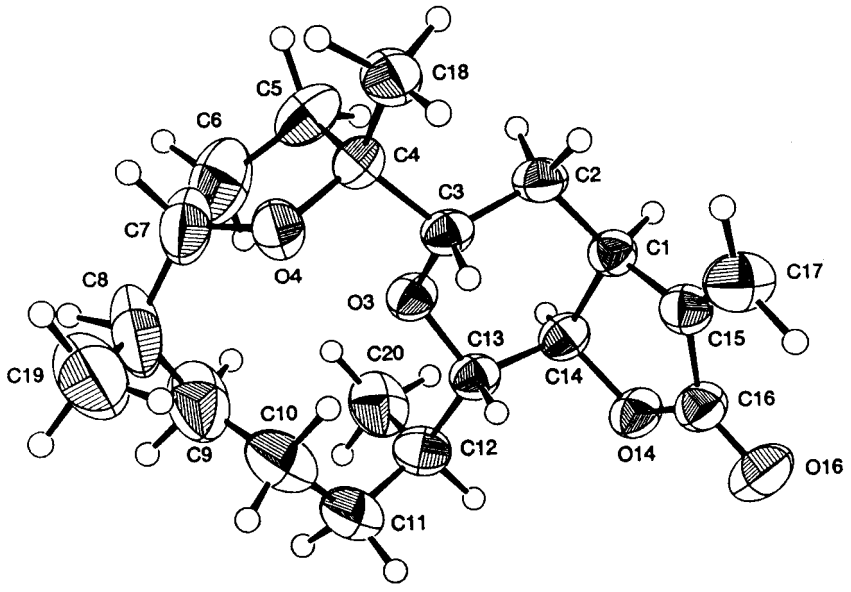
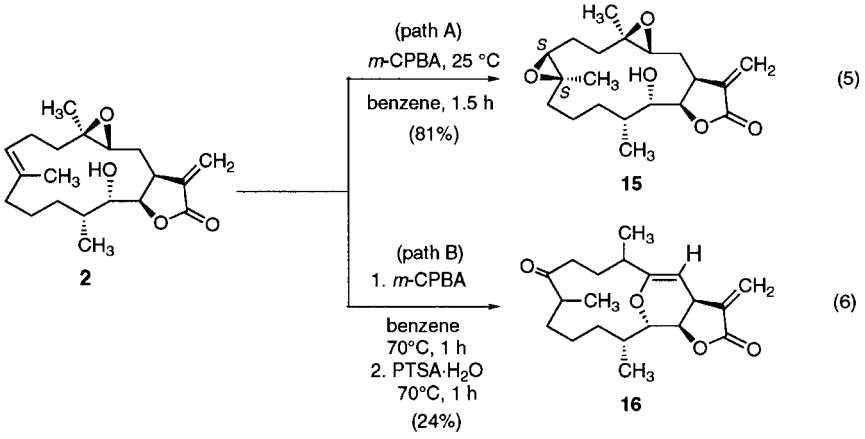


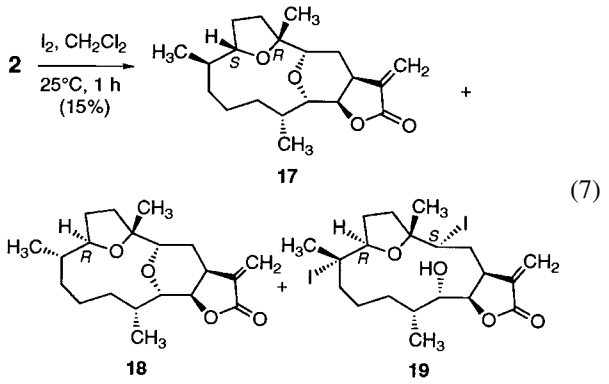
Figure 3. ORTEP drawing of inolide-A (**17**).

gorgonian *E. succinea*.[8] It should be noted that the peracid oxidation of **2** in CH_2Cl_2 at 25°C for 3 h produced roughly a 2:1 mixture of diepoxides the major one of which, after separation by HPLC, was identical to the natural product. Following epoxidation to generate **15**, the fate of this diepoxide in strongly acidic media was explored. Thus, exposure of **2** to *m*-CPBA in hot benzene, followed by treatment with *p*-toluenesulfonic acid hydrate (PTSA·H_2O) for 1 h, gave a complex product mixture from which only pyranether **16** could be isolated (Eq. (6)). In this instance, protic acid-catalyzed rearrangement of the epoxide groups followed by intramolecular ring closure and concomitant loss of water generated the 2,3-dihydro-4*H*-pyran moiety. While we were unable to isolate additional diastereomers of **16**, their formation is highly probable and may explain why the isolated yield of this reaction amounted to only 24%. In agreement with the proposed structure, the HREI-MS data for **16** suggested the molecular formula $C_{20}H_{28}O_4$ and seven degrees of unsaturation. The ^{1}H NMR spectrum indicated the presence of three secondary methyl groups at δ 1.09, 0.98, and 0.95 (each d, 3H) and a highfield-shifted olefinic proton [δ 4.85 (d, 1H, J=4.5 Hz, H-2)] which, according to a ^{1}H–^{13}C HETCOR experiment, correlated with the carbon resonance at δ 93.2 (d, C-2). The ^{13}C NMR spectrum revealed the presence of ketone and lactone carbonyls at δ 215.8 (s) and 169.9 (s), respectively, and a downfield-shifted quaternary olefinic carbon bearing oxygen [δ 162.1 (s, C-3)] that showed through selective INEPT NMR experiments two- and three-bond ^{1}H–^{13}C couplings to H-2 and Me-18. The latter data suggested that C-3 participated in the formation of an ethereal linkage. Unfortunately, the absence of significant NOEs at Me-18 and Me-19 (or H-4 and H-8) in pyranether **16** precluded our assignment of the relative stereochemistry at C-4 and C-8.

In accord with our previous results, treatment of 12,13-bisepieupalmerin (**2**) with iodine in CH_2Cl_2 at 25°C for 1 h yielded a complex mixture of products from which



tetracyclic furanethers inolide-A (**17**) and inolide-B (**18**) were isolated in 7 and 3% yields, respectively (Eq. (7)).[9]

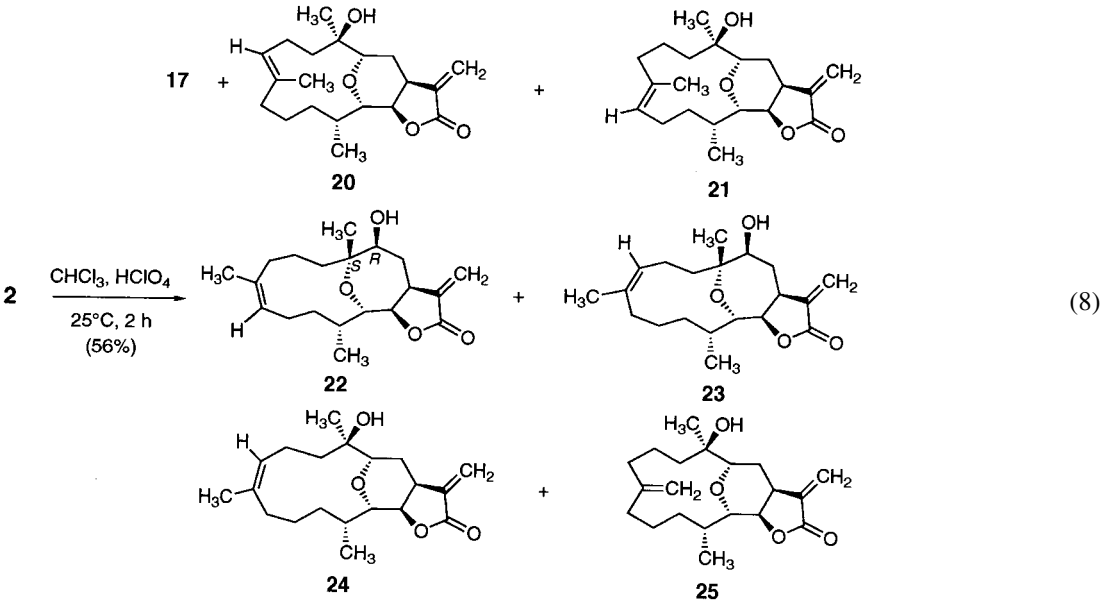


(7)

This time, however, tricyclic diiodide **19** was also isolated in 5% yield. The HREI-MS and NMR data of **19** indicated similar functionality as furanether **14**. The downfield-shifted chemical shifts of carbons C-3 (δ 42.5, d) and C-8 (δ 52.7, s) in the ^{13}C NMR spectrum suggested the location of the iodine atoms. NOEs were detected in $CDCl_3$ solution between H-7, H-3, and H-1 of furanether **19**, which indicated that these protons were all on the same (α) face of the molecule. The structure of inolide-A (**17**) including its absolute configuration was confirmed by X-ray crystallography and is presented in Fig. 3. Interestingly, in the ^{13}C NMR spectra of cyclic ethers **18** and **19**, many of the resonances were quite broad signals of very low intensity. This peculiarity, which was not observed in inolide-A (**17**), is a consequence of slowly interconverting conformers. In comparison to **17**, the most obvious difference in the ^{1}H NMR spectrum of **18** (in $CDCl_3$ solution) was that the signal for H-7 had shifted from δ 3.74 in inolide-A (**17**) to δ 4.12 in inolide-B (**18**). In view of these observations we conclude that the C-7(*S*) configuration we reported earlier for inolide-B should be revised to the C-7(*R*) configuration shown in **18**.[9] In sharp contrast to our previous findings with euniolide (**1**), none of the corresponding C-4,8 pyranethers are formed during these I_2-mediated cyclizations. On the other hand, the formation of diiodide **19** is fully compatible with the corresponding reaction in the euniolide series (**1**→**14**).

Exposure of **2** to perchloric acid in chloroform at 25°C for 2 h led, after acidolytic cleavage of the epoxide, to complex product mixtures (Eq. (8)) from which inolide-A (**17**) was obtained as the major product but in slightly higher isolated yield (20%). The separation of cembranolides **20**–**25** was achieved successfully by silica gel column chromatography followed by normal-phase HPLC. As in the case of our previous reactions with iodine, cyclic ether formation was the chief reaction pathway here, but in sharp contrast, olefin isomerization was a major reaction pathway as well. The spectroscopic properties for analogue **20** were identical with those reported for eunicin, a major secondary metabolite isolated from *E. succinea*.[10] Additional information on the relative position of the trisubstituted double bonds in lactones **21**–**24** were obtained from HMQC and HMBC NMR experiments. For instance, in (*Z*)-Δ^7 eunicin (**24**), the methylene protons H-5 (δ_H 1.60) showing ^1J-HMQC correlations with the methylene carbon resonating at 41.6 ppm (C-5) displayed ^3J-HMBC correlations with the olefin carbon resonated at 125.4 ppm (d, C-7). In turn, C-5 correlated to the C-18 methyl group absorbed at δ 1.14. This was further supported by the HMBC correlation of the C-3 proton signal (δ_H 3.46) with C-5. The Me-19 signals in the ^{13}C NMR spectra of cembranolide analogues **22**–**24** (δ 26.0, 25.4, and 23.8, respectively) were at low field which indicated that the trisubstituted double bonds are *cis* with respect to the continuous chain of carbons. The Me-19 signal of (*E*)-Δ^8 eunicin (**21**), on the other hand, was at high field (δ 17.3) indicating that the Δ^8 olefin has the (*E*)-configuration.

The cyclization of **2** also proceeds with phosphorus pentoxide, as reported in our earlier work,[9] but the current procedure furnishes a larger diversity of cembranolide analogues albeit in slightly lower overall yield (71%), (Eq. (9)). Besides the cyclization substrates **20**, **24**, and **25**



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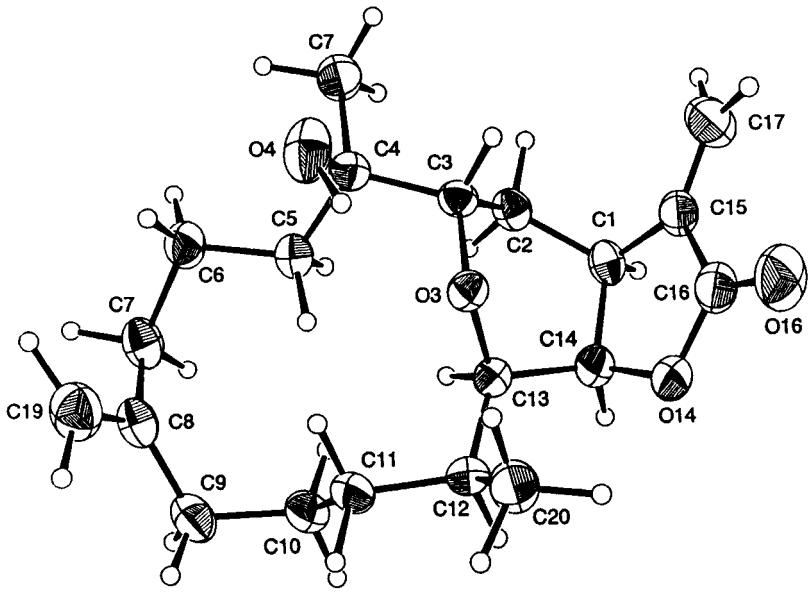
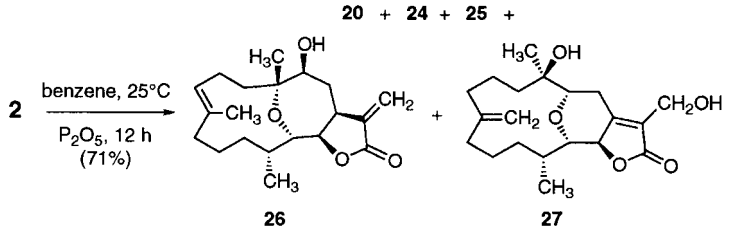


Figure 4. The X-ray structure of cembranolide **32**.



(9)

which we had obtained previously, this reaction also delivered lower yields of cyclized materials **26** and **27**. As was the case for eunicin (**20**), the spectroscopic properties for analogue **26** were identical with those reported for jeunicin, a minor secondary metabolite isolated from *E. succinea*.[11] The physical and spectral data of **27** were somewhat reminiscent of those of pyranether **25**. The HREI-MS spectral data for **27** suggested the molecular formula $C_{20}H_{30}O_5$ and six degrees of unsaturation. The ^{13}C NMR spectrum exhibited key signals at δ 173.8 (s, C-16), 163.9 (s, C-1), 123.2 (s, C-15), 77.8 (d, C-14), and 54.9 (t, C-17) whose chemical shift values and multiplicity confirmed the presence of a butenolide moiety bearing an α-hydroxylmethyl group.

2.3. Transformations of eupalmerin (3)

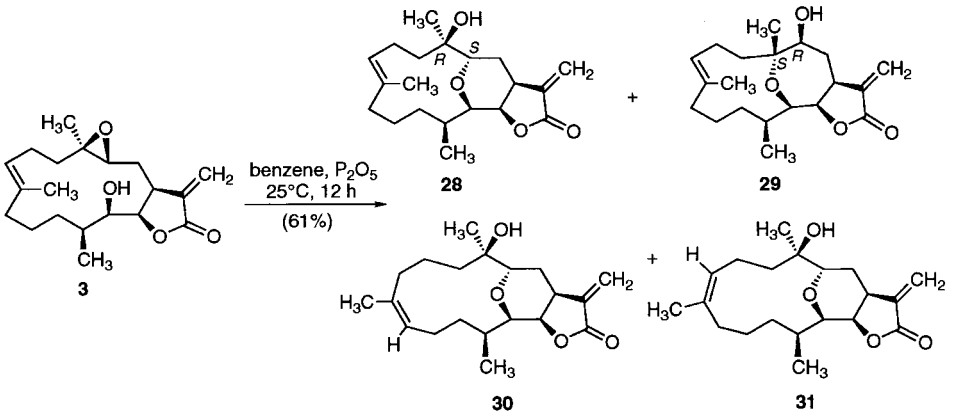
Analogues of eupalmerin (**3**), which has the same constitution as 12,13-bisepieupalmerin (**2**) but opposite stereochemistry at C-12 and C-13, were prepared next. Treatment of eupalmerin (**3**) with phosphorus pentoxide in benzene at 25°C for 12 h afforded a 3:1:2:2 mixture of cyclic ethers **28**–**31** in 61% overall yield favoring the pyranethers in a 7:1 ratio (Eq. (10)). The HREI-MS, NMR, and IR data of analogues **28**–**31** indicated similar molecular formula and functionality as **20**–**24**. Compound **28** was shown to be the C-12 epimer of cueunicin,[12] a minor secondary metabolite isolated from *E. succinea* from Curaçao, whereas 12,13-bisepijeunicin (**29**) was likewise identified as the C-12,14 bisepimer of (13αH, 14βH)-jeunicin isolated from specimens of *E. succinea* collected in Bimini.[13] The relative position of the trisubstituted double bond in each of these analogues was established from extensive NMR spectral comparisons with cembranolide analogues **20**–**24**. The Me-19 signal of cyclic ethers **28** and **29** was at high field (each at δ 15.6) whereas that of **30** and **31** resonated at low field (δ 24.2 and 23.7, respectively). This indicated that the trisubstituted olefin in each pair of isomers had the (*E*)- and (*Z*)-configuration, respectively. The proton on the oxygenated carbon C-3 of **28**, **30** and **31** did not produce NOESY correlations with the neighboring Me-18 α-methyl group nor the H-13 oxymethine signal indicating the β-orientation of the former proton.

The epoxide ring opening in eupalmerin (**3**) was likewise affected with PTSA·H_2O in benzene at 25°C for 6 h, giving a 16:1:1:1 mixture of 12-epicueunicin (**28**) and α-methylene lactones **32**–**34** in 42% overall yield (Eq. (11)). By using PTSA·H_2O the formation of cembranolide analogues containing the oxepane ring is completely suppressed and pyranethers such as **28** and **32** thrive as the predominant cyclized products. The HREI-MS and IR data of **32** indicated similar molecular formula and functionality as bisepimer **25**. This was further supported by the NMR data that displayed signals for only two methyl groups and suggested the lack of epoxide functionality. The olefinic methylene carbon resonating at 113.5 ppm correlated to the two broad singlets at δ 4.94 and 4.86 assigned as the

Table 1. Comparison of in vitro cytotoxicities of compounds **1–3** with those of selected analogues (the cytotoxicities described for compound **3** are actually those of its acetate derivative)

Compd	Effects on human cancer cell growth IC_{50} (μg/mL)[a]													
	Leukemia		Prostrate carcinoma		Melanoma		Breast carcinoma		CNS carcinoma	Ovarian carcinoma		Colon carcinoma		Lung carcinoma
	CCRF-CEM	MOLT-4	PC-3	DU-145	LOX-IMV1	MALME-3M	MCF7	MCF7 ADR-RES	SNB-75	IGROV1	OVCAR-3	HCT-116	COLO 205	NCI-H522
1	5	NT[b]	NT	NT	NT	NT	22	NT	NT	NT	NT	43	NT	NT
2	0.1	2	13	6	4	11	1	5	15	2	11	3	3	2
3	0.3	2	2	1	2	16	2	1	2	1	2	1	2	1
7	>100	18	42	49	27	NT	26	27	31	18	34	27	22	17
11	0.8	6	12	15	12	NT	13	8	12	6	9	8	6	6
12	1	2	6	8	0.02	5	4	6	12	3	11	4	7	2
13	0.2	0.8	4	3	1	NT	1	0.9	6	1	1	3	2	1
15	1	1	7	6	NT	NT	2	8	7	5	4	2	3	1
17	0.01	1	1	1	1	2	2	1	1	1	1	1	2	1
18	0.07	NT	NT	NT	NT	NT	3	NT	NT	NT	NT	5	NT	NT
19	0.1	1	4	2	1	0.5	1	1	0.4	1	1	3	2	0.06
20	5	NT	NT	NT	NT	NT	3	NT	NT	NT	NT	5	NT	NT
21	0.8	2	12	13	14	NT	7	8	10	1	14	13	3	4
27	34	>50	>50	>50	>50	>50	>50	>50	>50	25	>50	>50	>50	28
33	0.3	1	9	4	3	6	3	8	12	2	6	6	1	1

[a] The IC_{50} is the drug concentration that reduced cell number by 50%.
[b] Not tested.



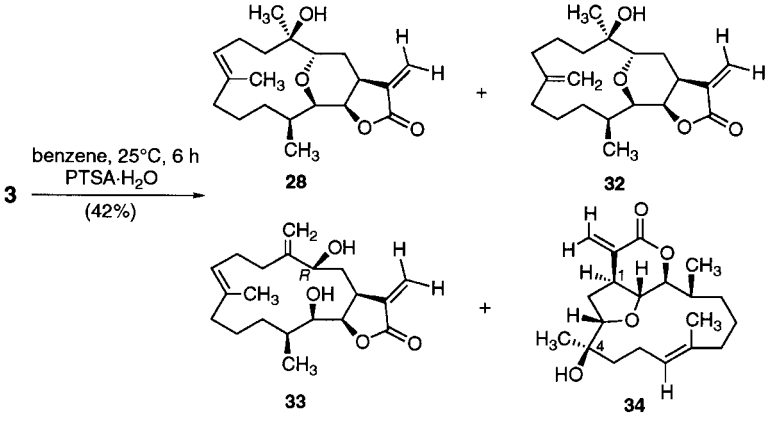
(10)

C-19 *exo*-methylene. This was confirmed through the ^{2}J-HMBC coupling of the C-19 methylene protons with the quaternary olefinic carbon at 148.0 ppm.

The stereochemistry of cembranolide **32** was confirmed by X-ray analysis and is shown in Fig. 4. Interestingly, acid-promoted rearrangement of the epoxide produced allylic alcohol **33** as the only isomer of this class. The IR and NMR data of **33** indicated the lack of epoxide functionality and displayed signals for a similar functionality as alcohol **8**, namely, hydroxyl and a nearby *exo*-methylene group. The olefinic methylene carbon at 113.9 ppm correlated to a two proton broad singlet at δ 4.95 assigned as the C-18 *exo*-methylene. The C-18 methylene protons also showed ^{2}J-HMBC and ^{3}J-HMBC correlations with the quaternary olefinic carbon at 152.4 ppm (C-4) and the oxygenated methine carbon at 71.2 ppm (C-3), respectively. The relative stereochemistry of C-3 in **33** was assigned similar to that of **8** on the basis of a comparison of the splitting and coupling of H-3. A most intriguing finding of the present investigation was the isolation, albeit in rather low isolated yield (2%), of known eupalmerolide (**34**).[14] This structurally complex analogue analyzed for $C_{20}H_{30}O_4$ and showed bands for α-methylene-δ-lactone and hydroxyl groups in its IR spectrum. The ^{13}C NMR spectrum showed the presence of a lactone carbonyl [δ 163.8 (C-16)], one quaternary carbon bearing oxygen [δ 72.9 (C-4)], three oxymethine carbons [δ 88.4 (C-3), 84.1 (C-13), and 78.7 (C-14)], two double bonds: one trisubstituted [δ 133.3 (s, C-8), 128.4 (d, C-7)] and one terminal [δ 136.9 (s, C-15), 126.0 (t, C-17)], two methine carbons [δ 40.7 (C-1) and 35.9 (C-12)], six methylene carbons [δ 37.6 (C-9), 36.6 (C-5), 29.4 (C-2), 29.2 (C-11), 23.3 (C-10), and 22.3 (C-6)], and three methyl groups [δ 25.8 (C-18), 16.5 (C-19), and 15.7 (C-20)]. Most likely, its formation entails tandem acid-mediated ester hydrolysis and nucleophilic attack at C-3 by the C-14 hydroxyl followed by lactonization of the ensuing δ-hydroxy carboxylic acid to give the δ-lactone array.

2.4. Biological studies

The compounds listed in Table 1 were tested in the NCI's 60 cell-line human tumor screen. The results with 14 representative cell-lines (CCRF-CEM, MOLT-4, PC-3, DU-145, LOX-IMV1, MALME-3M, MCF7, MCF7 ADR-RES, SNB-75, IGROV1, OVCAR-3, HCT-116, COLO 205, and NCI-H522) are shown. In comparative testing against these human tumor cell-lines, euniolide (**1**) and 12,13-bisepieupalmerin (**2**) exhibited IC_{50} values of 0.1–43 μg/mL. The acetate derivative of eupalmerin (**3**) exhibited IC_{50} values from 0.3–16 μg/mL. A cursory run through Table 1 indicates that almost all of the synthetic analogues are strongly cytotoxic suggesting that chemical modifications involving the epoxide substructure of cembranolides **1**–**3** are not generally deleterious to their cytotoxicity. The notable exception to this is **27**, which does not feature an α-methylene-γ-lactone functionality, and thus is less toxic than its prototype in this bioassay. Moreover, several analogues (**13**, **17**, and **19**) showed a characteristic pattern of differential cytotoxicity and were approximately equipotent (e.g. mean panel $GI_{50's} \backsim 1$–2 nM). COMPARE pattern-recognition analyses of the mean-graph profiles of these



(11)

analogues did not reveal any significant correlations to the profiles of reference antitumor compounds contained in the NCI's standard agent database.[15] This suggests that these modified cembranolide analogues may act by a novel mechanism of action since their activity profiles did not match compounds whose mechanism of action are known. Among the 12,13-bisepieupalmerin (**2**) series, for instance, a significantly enhanced cytotoxic activity was generally obtained by the introduction of cyclic ether linkages across the cembrane skeleton. Thus, compound **17** is up to 15 times more cytotoxic against the T-cell leukemia (CCRF-CEM) cells than prototype **2** (compounds **2** and **17**, $IC_{50's}$=0.15 and 0.01 μg/mL, respectively). Furthermore, lactone diiodide **19**, having undergone preliminary evaluation in the NCI's in vitro antitumor screening system, has been selected by the Biological Evaluation Committee for further in vivo anticancer studies which begin with the Hollow Fiber Assay.[16] Considering these early observations, analogues of this series appear to be attractive targets for the development of antitumor drugs. While further pharmacological evaluation of this series of compounds in relevant in vitro tests is still in progress at this stage, already some active analogues have displayed more potency than their precursors.

3. Experimental

3.1. General experimental procedures

Infrared spectra were determined as thin films and were referenced to polystyrene. ^{1}H NMR, ^{13}C NMR, DEPT, HMQC, HMBC, ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY (CSCMBB), 2D-NOESY, and selective INEPT spectra were recorded either at 300 and 500 MHz for ^{1}H or at 75 and 125 MHz for ^{13}C. Column chromatography was carried out with silica gel (35–75 mesh). HPLC was performed using a 10 μm silica gel Partisil 10 semipreparative column (9.4 mm×50 cm). Reactions were monitored by TLC on silica gel plates (0.25 mm) and visualized using UV light and I_2 vapors. Reagents from commercial suppliers were used as provided. Yields refer to chromatographically and spectroscopically pure materials.

3.2. Extraction and isolation of *Eunicea* γ-cembranolides 1–3

The Caribbean gorgonian octocorals *E. succinea* and *E. mammosa* were collected at 25 m depth by SCUBA from Mona Island, Puerto Rico. The gorgonians were stored at 0°C immediately after collection, then were frozen at −10°C upon arrival, freeze-dried, and kept frozen until extraction. The dried *E. succinea* (2.5 kg) was blended with MeOH:$CHCl_3$ (1:1) and after filtration the crude extract was evaporated under vacuum to yield a residue (322.9 g) which was partitioned between hexane and H_2O. The hexane extract was concentrated to yield 170.9 g of a dark green oily residue, which was later dissolved in toluene and filtered. The resulting filtrate was concentrated (168.9 g), loaded onto a large size exclusion column (Bio-Beads SX-3), and eluted with toluene. The combined terpenoid-rich fractions (TLC guided) were concentrated to a dark yellow oil (118.6 g) and chromatographed over a large silica gel column (3 kg) using 30% EtOAc in hexane. From this column 14 fractions were obtained the less polar of which consisted of complex mixtures of unidentified sterols and fatty acid derivatives, and the known cembranolide diterpene euniolide (**1**) (24 g). The more polar portion of the extract was divided roughly into fractions 6–14 on the basis of TLC analyses. From some of these fractions several known cembranolides such as 12,13-bisepieupalmerin (**2**) (5.0 g) and eupalmerin (**3**) (2.5 g) were identified. The identification of **1**–**3** was accomplished through detailed comparisons with the physical and chemical data previously reported for these compounds.[3–5] A similar extraction procedure was followed with dried *E. mammosa* specimens.

3.3. Reaction of euniolide (1) with phosphorus pentoxide

3.3.1. Path A. A mixture of **1** (863 mg, 2.73 mmol) and P_2O_5 (442 mg, 3.11 mmol) in dry benzene (50 mL) was refluxed gently for 1 h before it was quenched with saturated $NaHCO_3$ (50 mL). The reaction mixture was extracted with benzene (3×50 mL), concentrated, and purified by column chromatography on silica gel (22 g, 1:9 (v/v) ethyl acetate in hexane) to give 4(*R*)-euniolone (**4**, 294 mg, 34%), 4(*S*)-euniolone (**5**, 173 mg, 20%), lactone **6** (8 mg, 1%), and 370 mg of an intractable mixture of polar compounds.

3.3.2. Data for 4. White crystalline solid; $[\alpha]_D^{25}=-21.7°$ (*c* 4.4, $CHCl_3$); IR (neat) 2967, 2929, 2858, 1755, 1703, 1665, 1392, 1375, 1273, 1247, 1168, 1099, 969, 946, 815 cm^{-1}; UV (MeOH) λ_{max} 208 nm (ϵ 10900); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.62 (ddd, 1H, *J*=1.8, 6.9, 11.7 Hz, H-1), 2.51 (dd, 1H, *J*=11.7, 18.6 Hz, H-2), 3.13 (dd, 1H, *J*=2.1, 18.6 Hz, H-2′), 2.49 (m, 1H, H-4), 1.51 (m, 1H, H-5), 1.85 (m, 1H, H-5′), 1.91 (m, 1H, H-6), 2.03 (m, 1H, H-6′), 4.87 (br t, 1H, *J*=6.3 Hz, H-7), 2.13 (m, 2H, H-9), 2.16 (m, 2H, H-10), 5.04 (br t, 1H, *J*=7.5 Hz, H-11), 2.25 (dd, 1H, *J*=12.3, 15.0 Hz, H-13), 2.69 (br d, 1H, *J*=15.0 Hz, H-13′), 4.70 (ddd, 1H, *J*=4.2, 6.9, 12.3 Hz, H-14), 5.71 (br t, 1H, *J*=1.2 Hz, H-17), 6.14 (dd, 1H, *J*=0.9, 1.8 Hz, H-17′), 0.94 (d, 3H, *J*=6.9 Hz, Me-18), 1.46 (s, 3H, Me-19), 1.64 (s, 3H, Me-20); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 36.1 (d, C-1), 41.9 (t, C-2), 212.0 (s, C-3), 45.1 (d, C-4), 31.9 (t, C-5), 25.6 (t, C-6), 124.7 (d, C-7), 135.5 (s, C-8), 39.0 (t, C-9), 24.6 (t, C-10), 128.5 (d, C-11), 129.2 (s, C-12), 40.3 (t, C-13), 76.6 (d, C-14), 139.7 (s, C-15), 169.9 (s, C-16), 124.2 (t, C-17), 17.4 (q, C-18), 14.3 (q, C-19), 15.9 (q, C-20); HREI-MS *m/z* $[M]^+$ calcd for $C_{20}H_{28}O_3$ 316.2038, found 316.2034, 316 (3), 301 (1), 298 (3), 197 (5), 121 (5), 107 (14), 84 (78), 67 (14), 49 (100).

3.3.3. Data for 5. White crystalline solid; $[\alpha]_D^{25}=-4.1°$ (*c* 4.6, $CHCl_3$); IR (neat) 2962, 2929, 2856, 1755, 1693, 1660, 1460, 1365, 1273, 1169, 1005, 946 cm^{-1}; UV (MeOH) λ_{max} 207 nm (ϵ 15600); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.61 (m, 1H, H-1), 2.49 (dd, 1H, *J*=11.4, 16.8 Hz, H-2), 2.98 (dd, 1H, *J*=2.7, 16.8 Hz, H-2′), 2.43 (m, 1H, H-4), 1.51 (m, 1H, H-5), 1.70 (m, 1H, H-5′), 1.90 (m, 1H, H-6), 2.25 (m, 1H, H-6′), 5.01 (br t, 1H, *J*=7.5 Hz, H-7), 1.94 (m, 1H, H-9), 2.21 (m, 1H, H-9′), 2.25 (m, 2H, H-10), 5.04 (br t, 1H, *J*=7.8 Hz, H-11), 2.28 (m, 1H, H-13), 2.68 (br d, 1H, *J*=15.0 Hz, H-13′), 4.76 (ddd, 1H, *J*=3.9, 6.9, 11.4 Hz, H-14), 5.59 (d, 1H, *J*=1.5 Hz, H-17), 6.16 (d, 1H, *J*=1.8 Hz, H-17′), 1.01 (d, 3H, *J*=7.2 Hz, Me-18), 1.54 (s,

3H, Me-19), 1.65 (s, 3H, Me-20); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 36.8 (d, C-1), 36.7 (t, C-2), 212.4 (s, C-3), 47.9 (d, C-4), 34.3 (t, C-5), 26.4 (t, C-6), 124.2 (d, C-7), 135.7 (s, C-8), 39.3 (t, C-9), 24.5 (t, C-10), 127.9 (d, C-11), 129.6 (s, C-12), 39.8 (t, C-13), 77.2 (d, C-14), 139.2 (s, C-15), 169.8 (s, C-16), 123.8 (t, C-17), 17.2 (q, C-18), 15.0 (q, C-19), 16.4 (q, C-20); HREI-MS *m/z* $[M]^+$ calcd for $C_{20}H_{28}O_3$ 316.2038, found 316.2040, 316 (14), 301 (3), 298 (14), 283 (5), 192 (11), 179 (13), 167 (14), 164 (20), 163 (19), 134 (25), 119 (32), 107 (56), 81 (65), 69 (73), 53 (78), 41 (100).

3.3.4. Data for 6. Yellowish oil; $[\alpha]_D^{25}=-50.7°$ (*c* 1.3, $CHCl_3$); IR (neat) 2954, 2925, 2855, 1763, 1709, 1456, 1376, 1261, 1165, 1001, 816, 757 cm^{-1}; UV (MeOH) λ_{max} 216 nm (ϵ 12100); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.57 (ddd, 1H, *J*=1.0, 3.9, 7.0 Hz, H-1), 2.54 (dd, 1H, *J*=7.1, 11.1 Hz, H-2), 3.01 (dd, 1H, *J*=1.2, 11.1 Hz, H-2′), 2.46 (m, 1H, H-4), 1.49 (m, 1H, H-5), 1.80 (m, 1H, H-5′), 1.81 (m, 1H, H-6), 1.93 (m, 1H, H-6′), 5.15 (br d, 1H, *J*=4.4 Hz, H-7), 1.19 (m, 1H, H-9), 2.21 (m, 1H, H-9′), 1.94 (m, 1H, H-10), 2.33 (m, 1H, H-10′), 5.25 (br d, 1H, *J*=6.4 Hz, H-11), 2.44 (m, 1H, H-13), 2.73 (br d, 1H, *J*=8.6 Hz, H-13′), 4.74 (ddd, 1H, *J*=2.4, 3.9, 7.1 Hz, H-14), 5.77 (br t, 1H, *J*=0.7 Hz, H-17), 6.19 (dd, 1H, *J*=0.6, 0.9 Hz, H-17′), 1.05 (d, 3H, *J*=4.3 Hz, Me-18), 1.67 (s, 3H, Me-19), 1.67 (s, 3H, Me-20); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 36.1 (d, C-1), 34.8 (t, C-2), 212.0 (s, C-3), 48.7 (d, C-4), 34.9 (t, C-5), 26.6 (t, C-6), 125.4 (d, C-7), 133.6 (s, C-8), 31.0 (t, C-9), 24.2 (t, C-10), 128.6 (d, C-11), 128.9 (s, C-12), 40.8 (t, C-13), 76.8 (d, C-14), 139.8 (s, C-15), 169.9 (s, C-16), 124.3 (t, C-17), 17.9 (q, C-18), 22.3 (q, C-19), 15.7 (q, C-20); HREI-MS *m/z* $[M]^+$ calcd for $C_{20}H_{28}O_3$ 316.2038, found 316.2032, 316 (21), 301 (4), 298 (17), 284 (13), 192 (10), 179 (15), 164 (20), 163 (21), 134 (28), 119 (35), 107 (61), 95 (50), 81 (69), 69 (87), 55 (100).

3.3.5. Path B. A mixture of **1** (181 mg, 0.57 mmol) and $(Ph)_3P$ (150 mg, 0.57 mmol) in dry benzene (15 mL) was treated with anhydrous P_2O_5 (300 mg, 2.1 mmol), allowed to stir at 80°C for 4 h, and concentrated. The residue obtained was chromatographed on silica gel (25 g, 1:4 (v/v) ethyl acetate in hexane) to give 40 mg (22%) of **7** and 27 mg (15%) of **8**.

3.3.6. Data for 7. Colorless oil; $[\alpha]_D^{25}=-1.4°$ (*c* 1.7, $CHCl_3$); IR (neat) 3478, 2962, 2912, 2853, 1761, 1661, 1436, 1261, 1161, 1086, 1019, 802, 757 cm^{-1}; UV ($CHCl_3$) λ_{max} 246 nm (ϵ 1000); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.31 (m, 1H, H-1), 1.39 (m, 1H, H-2), 2.07 (m, 1H, H-2′), 4.05 (dd, 1H, *J*=2.7, 11.1 Hz, H-3), 5.54 (br t, 1H, *J*=9.3 Hz, H-5), 2.39 (m, 1H, H-6), 3.64 (m, 1H, H-6′), 5.06 (br d, 1H, *J*=9.6 Hz, H-7), 2.12 (m, 2H, H-9), 2.03 (m, 1H, H-10), 2.35 (m, 1H, H-10′), 5.12 (br d, 1H, *J*=8.7 Hz, H-11), 2.38 (m, 1H, H-13), 2.67 (br d, 1H, *J*=15.3 Hz, H-13′), 4.71 (ddd, 1H, *J*=4.2, 6.3, 11.1 Hz, H-14), 5.71 (br s, 1H, H-17), 6.26 (br s, 1H, H-17′), 1.59 (s, 3H, Me-18), 1.55 (s, 3H, Me-19), 1.65 (s, 3H, Me-20); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 39.0 (d, C-1), 30.6 (t, C-2), 71.2 (d, C-3), 128.4 (s, C-4), 126.1 (d, C-5), 26.2 (t, C-6), 125.1 (d, C-7), 138.7 (s, C-8), 38.6 (t, C-9), 24.4 (t, C-10), 129.7 (d, C-11), 133.1 (s, C-12), 39.7 (t, C-13), 78.2 (d, C-14), 138.8 (s, C-15), 170.4 (s, C-16), 122.6 (t, C-17), 23.1 (q, C-18), 14.8 (q, C-19), 15.6 (q, C-20); HREI-MS *m/z* $[M]^+$ calcd for $C_{20}H_{28}O_3$ 316.2038, found 316.2032, 316 (2), 298 (30), 278 (40), 277 (100), 201 (15), 157 (39), 145 (39), 133 (88), 119 (65), 107 (77), 93 (73), 91 (59).

3.3.7. Data for 8. Colorless oil; $[\alpha]_D^{25}=-0.43°$ (*c* 0.74, $CHCl_3$); IR (neat) 3456, 3008, 2960, 2924, 2853, 1766, 1662, 1437, 1261, 1163, 1093, 1026, 815, 802 cm^{-1}; UV ($CHCl_3$) λ_{max} 246 nm (ϵ 1000); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.44 (m, 1H, H-1), 1.54 (m, 1H, H-2), 1.94 (m, 1H, H-2′), 4.20 (dd, 1H, *J*=1.8, 10.8 Hz, H-3), 2.08 (m, 1H, H-5), 2.28 (m, 1H, H-5′), 2.10 (m, 1H, H-6), 2.25 (m, 1H, H-6′), 5.22 (br t, 1H, *J*=3.9 Hz, H-7), 2.08 (m, 2H, H-9), 2.13 (m, 1H, H-10), 2.22 (m, 1H, H-10′), 5.03 (br t, 1H, *J*=6.0 Hz, H-11), 2.33 (dd, 1H, *J*=10.8, 15.3 Hz, H-13), 2.52 (br d, 1H, *J*=15.3 Hz, H-13′), 4.74 (m, 1H, H-14), 5.69 (d, 1H, *J*=2.1 Hz, H-17), 6.25 (d, 1H, *J*=2.4 Hz, H-17′), 4.94 (br s, 1H, H-18), 4.99 (br s, 1H, H-18′), 1.56 (s, 3H, Me-19), 1.65 (s, 3H, Me-20); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 39.6 (d, C-1), 32.9 (t, C-2), 72.5 (d, C-3), 151.6 (s, C-4), 31.6 (t, C-5), 26.8 (t, C-6), 125.2 (d, C-7), 134.9 (s, C-8), 38.8 (t, C-9), 24.7 (t, C-10), 128.9 (d, C-11), 129.7 (s, C-12), 40.8 (t, C-13), 78.4 (d, C-14), 139.3 (s, C-15), 170.4 (s, C-16), 121.7 (t, C-17), 112.2 (t, C-18), 15.7 (q, C-19), 16.2 (q, C-20); HREI-MS *m/z* $[M]^+$ calcd for $C_{20}H_{28}O_3$ 316.2038, found 316.2049, 316 (2), 278 (6), 277 (14), 221 (12), 149 (28), 111 (30), 97 (46), 81 (60), 69 (95), 57 (100).

3.4. Reaction of euniolide (1) with $BF_3{\cdot}O(C_2H_5)_2$

Freshly distilled $BF_3{\cdot}O(C_2H_5)_2$ (0.2 mL, 1.28 mmol) was added under N_2 to a solution of **1** (368 mg, 1.16 mmol) in dry benzene (10 mL) kept at 0°C. After stirring for 1.5 h at 0°C the reaction was quenched with H_2O (2 mL) and concentrated. The residue obtained was purified by silica gel chromatography (18 g, 1:9 (v/v) ethyl acetate in hexane) to yield **4** (169 mg, 46% yield) and **5** (59 mg, 16% yield).

3.5. Reaction of euniolide (1) with $(Ph)_3P/I_2$

3.5.1. Path A. To a solution of Ph_3P (247 mg, 0.94 mmol) and iodine (239 mg, 0.94 mmol) in CH_2Cl_2 (25 mL) at 25°C was added **1** (298 mg, 0.94 mmol) dissolved in 10 mL of CH_2Cl_2, at once. After 20 min at 25°C the mixture was poured over aqueous $NaHCO_3$, extracted with $CHCl_3$ (3×50 mL), and concentrated. The residue was purified by silica gel chromatography (10 g, 3:7 (v/v) ethyl acetate in hexane) followed by normal-phase HPLC (Partisil 10, elution with 1:99 (v/v) 2-propanol in hexane) to give 93 mg (33%) of (1*S*,3*E*,7*E*,11*E*,14*S*)-cembra-3,7,11,15(17)-tetraen-16,14-olide (**9**), 75 mg (18%) of **10**, 45 mg (15%) of **11**, and 25 mg (6%) of **12**.

3.5.2. Data for 9. Colorless oil; $[\alpha]_D^{25}=+31.4°$ (*c* 8.5, $CHCl_3$); IR (neat) 2961, 2925, 2852, 1768, 1666, 1441, 1329, 1265, 1158, 1100, 983, 936, 813 cm^{-1}; UV (MeOH) λ_{max} 208 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.09 (m, 1H, H-1), 2.22 (m, 2H, H-2), 4.90 (br t, 1H, *J*=6.6 Hz, H-3), 1.97 (m, 1H, H-5), 2.13 (m, 1H, H-5′), 2.12 (m, 2H, H-6), 4.83 (br t, 1H, *J*=6.0 Hz, H-7), 2.14 (m, 2H, H-9), 2.12 (m, 2H, H-10), 4.99 (br t, 1H, *J*=6.6 Hz, H-11), 2.24 (m, 1H, H-13), 2.35 (dd, 1H, *J*=6.9, 15.6 Hz, H-13′), 4.67 (m, 1H, H-14), 5.53 (d, 1H, *J*=2.4 Hz, H-17), 6.18 (d, 1H,

J=2.4 Hz, H-17′), 1.67 (s, 3H, Me-18), 1.56 (s, 3H, Me-19), 1.59 (s, 3H, Me-20); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 45.1 (d, C-1), 27.8 (t, C-2), 126.4 (d, C-3), 133.3 (s, C-4), 38.8 (t, C-5), 24.3 (t, C-6), 124.9 (d, C-7), 136.2 (s, C-8), 39.1 (t, C-9), 24.5 (t, C-10), 121.5 (d, C-11), 130.5 (s, C-12), 39.3 (t, C-13), 80.6 (d, C-14), 140.4 (s, C-15), 170.3 (s, C-16), 120.0 (t, C-17), 16.7 (q, C-18), 15.8 (q, C-19), 15.5 (q, C-20); HREI-MS m/z $[M]^+$ calcd for $C_{20}H_{28}O_2$ 300.2089, found 300.2094, 300 (5), 285 (17), 192 (10), 147 (16), 135 (18), 121 (35), 108 (34), 107 (45), 93 (55), 81 (51), 68 (100).

3.5.3. Data for 10. Colorless oil; $[\alpha]_D^{21}=-40.0°$ (c 6.8, $CHCl_3$); IR (neat) 2960, 2934, 2870, 1770, 1669, 1436, 1377, 1292, 1105, 1040, 974 cm^{-1}; UV (MeOH) λ_{max} 208 nm; ^{1}H NMR (C_6D_6, 300 MHz) δ 3.63 (m, 1H, H-1), 3.72 (d, 1H, J=12.6 Hz, H-3), 1.00 (m, 1H, H-5), 2.10 (m, 1H, H-5′), 5.14 (br d, 1H, J=9.3 Hz, H-11), 4.81 (m, 1H, H-14), 5.49 (d, 1H, J=3.3 Hz, H-17), 6.24 (d, 1H, J=3.3 Hz, H-17′), 1.35 (s, 3H, Me-18), 1.17 (s, 3H, Me-19), 1.67 (s, 3H, Me-20); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 46.9 (d, C-1), 29.4 (t, C-2), 50.5 (d, C-3), 74.8 (s, C-4), 39.4 (t, C-5), 16.6 (t, C-6), 32.3 (t, C-7), 74.6 (s, C-8), 42.8 (t, C-9), 22.8 (t, C-10), 135.3 (d, C-11), 122.4 (s, C-12), 43.4 (t, C-13), 76.5 (d, C-14), 137.9 (s, C-15), 170.2 (s, C-16), 119.6 (t, C-17), 19.5 (q, C-18), 27.9 (q, C-19), 15.5 (q, C-20); HREI-MS m/z $[M]^+$ calcd for $C_{20}H_{29}IO_3$ 444.1117, found 444.1164, 444 (8), 318 (12), 317 (51), 299 (36), 271 (6), 191 (12), 121 (40), 93 (52), 81 (100), 69 (70).

3.5.4. Data for 11. White solid; $[\alpha]_D^{24}=-33.0°$ (c 1.7, $CHCl_3$); IR (neat) 2966, 2935, 2866, 1765, 1456, 1373, 1290, 1261, 1120, 1042, 963 cm^{-1}; UV (MeOH) λ_{max} 206 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.96 (m, 1H, H-1), 1.60 (m, 1H, H-2), 2.05 (m, 1H, H-2′), 1.23 (m, 2H, H-3), 1.10 (m, 1H, H-5), 1.41 (m, 1H, H-5′), 1.50 (m, 1H, H-6), 1.78 (m, 1H, H-6′), 1.05 (m, 1H, H-7), 1.46 (m, 1H, H-7′), 1.35 (m, 1H, H-9), 1.51 (m, 1H, H-9′), 1.78 (m, 1H, H-10), 2.20 (m, 1H, H-10′), 5.17 (br d, 1H, J=9.3 Hz, H-11), 2.28 (m, 2H, H-13), 4.82 (m, 1H, H-14), 5.51 (d, 1H, J=2.7 Hz, H-17), 6.21 (d, 1H, J=3.0 Hz, H-17′), 1.13 (s, 3H, Me-18), 1.13 (s, 3H, Me-19), 1.67 (s, 3H, Me-20); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 45.8 (d, C-1), 19.3 (t, C-2), 43.8 (t, C-3), 72.5 (s, C-4), 32.2 (t, C-5), 16.1 (t, C-6), 37.5 (t, C-7), 71.4 (s, C-8), 42.9 (t, C-9), 23.1 (t, C-10), 134.7 (d, C-11), 121.8 (s, C-12), 43.2 (t, C-13), 77.9 (d, C-14), 138.8 (s, C-15), 170.8 (s, C-16), 120.2 (t, C-17), 24.1 (q, C-18), 28.4 (q, C-19), 15.5 (q, C-20); HREI-MS m/z $[M]^+$ calcd for $C_{20}H_{30}O_3$ 318.2195, found 318.2204, 318 (5), 300 (5), 285 (3), 217 (4), 147 (9), 121 (22), 95 (35), 81 (48), 69 (40), 55 (48), 43 (100).

3.5.5. Data for 12. Yellowish oil; $[\alpha]_D^{24}=-51.3°$ (c 2.6, $CHCl_3$); IR (neat) 3431, 2957, 2924, 2854, 1761, 1666, 1455, 1378, 1261, 1099, 984, 800, 757 cm^{-1}; UV (MeOH) λ_{max} 208 and 268 nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.33 (m, 1H, H-1), 1.97 (m, 1H, H-2), 2.56 (dd, 1H, J=5.8, 9.5 Hz, H-2′), 3.59 (d, 1H, J=6.9 Hz, H-3), 1.88 (m, 1H, H-5), 2.07 (m, 1H, H-5′), 2.08 (m, 1H, H-6), 2.26 (m, 1H, H-6′), 4.88 (br d, 1H, J=5.7 Hz, H-7), 2.11 (m, 2H, H-9), 2.02 (m, 1H, H-10), 2.39 (m, 1H, H-10′), 5.34 (br d, 1H, J=5.7 Hz, H-11), 2.16 (m, 1H, H-13), 2.50 (dd, 1H, J=3.7, 9.3 Hz, H-13′), 4.58 (dd, 1H, J=3.7, 6.7 Hz, H-14), 5.62 (d, 1H, J=1.3 Hz, H-17), 6.19 (d, 1H, J=1.4 Hz, H-17′), 1.31 (s, 3H, Me-18), 1.73 (s, 3H, Me-19), 1.55 (s, 3H, Me-20); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 39.6 (d, C-1), 33.2 (t, C-2), 44.1 (d, C-3), 76.0 (s, C-4), 40.9 (t, C-5), 24.0 (t, C-6), 126.7 (d, C-7), 138.3 (s, C-8), 37.8 (t, C-9), 22.7 (t, C-10), 124.4 (d, C-11), 131.8 (s, C-12), 39.4 (t, C-13), 79.7 (d, C-14), 139.3 (s, C-15), 170.1 (s, C-16), 120.4 (t, C-17), 23.4 (q, C-18), 15.4 (q, C-19), 15.1 (q, C-20); HREI-MS m/z $[M-HI]^+$ calcd for $C_{20}H_{28}O_3$ 316.2038, found 316.2027, 316 (3), 299 (11), 254 (100), 217 (3), 177 (5), 128 (24), 127 (29), 107 (23), 93 (26), 81 (32), 67 (21).

3.5.6. Path B. To a mixture of iodine (351 mg, 1.38 mmol) and Ph_3P (363 mg, 1.38 mmol) stirred in CH_2Cl_2 at 0°C (30 mL) for 0.5 h was added dropwise a solution of **1** (0.44 g, 1.38 mmol) in a mixture of 1:1 $CH_2Cl_2/CHCl_3$ (30 mL) over 5 min. After stirring at 0°C for 4.5 h the reaction mixture was poured over 0.1N $NaHSO_3$ (40 mL), extracted with $CHCl_3$ (3×30 mL), and concentrated. The resulting oil was chromatographed on silica gel (20 g, elution with 15% ethyl acetate in hexane) to provide 93 mg (15%) of **12**, 26 mg (6%) and 27 mg (6%), respectively, of alcohols **7** and **8**, and 295 mg of a mixture of **4**, **13** and **14**. Subsequent purification of the mixture by reversed-phase HPLC (silica gel ODS, elution with 15% water in MeOH, flow rate 1.5 mL/min, λ 220 nm) furnished 185 mg (42%) of ketone **4**, 48 mg (6%) of pyranether **13**, and 47 mg (6%) of furanether **14**.

3.5.7. Data for 13. Crystalline solid; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.25 (d, 1H, J=3.3 Hz), 5.48 (d, 1H, J=3.1 Hz), 5.10 (br d, 1H, J=9.6 Hz), 4.80 (dd, 1H, J=7.3, 11.6 Hz), 4.20 (dd, 1H, J=3.7, 12.9 Hz), 3.68 (d, 1H, J=12.5 Hz), 3.58 (m, 1H), 1.69 (s, 3H), 1.49 (s, 3H), 1.42 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.0, 137.6, 134.8, 122.8, 119.9, 77.6, 76.4, 75.4, 48.7, 46.8, 43.6, 43.4, 40.9, 33.0, 30.9, 29.5, 26.0, 22.5, 19.3, 15.5; HREI-MS m/z $[M]^+$ calcd for $C_{20}H_{28}I_2O_3$ 570.0119, found 570.0100, 570 (3), 443 (22), 425 (9), 316 (14), 315 (13), 298 (16), 297 (20), 253 (100), 221 (6), 177 (7), 149 (20), 127 (34), 109 (49), 81 (58), 55 (63). The structural assignment to **13** was corroborated by X-ray crystallographic analysis (Fig. 2).

3.5.8. Data for 14. White solid; $[\alpha]_D^{24}=+21.1°$ (c 0.9, $CHCl_3$); IR (neat) 2952, 2924, 2853, 1770, 1455, 1380, 1296, 1254, 1100, 1074, 1023, 930, 811 cm^{-1}; UV (MeOH) λ_{max} 208 nm (ϵ 26100); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.56 (m, 1H, H-1), 2.02 (m, 1H, H-2), 2.30 (m, 1H, H-2′), 3.80 (d, 1H, J=7.6 Hz, H-3), 2.02 (m, 1H, H-5), 2.17 (m, 1H, H-5′), 1.97 (m, 1H, H-6), 2.13 (m, 1H, H-6′), 4.18 (dd, 1H, J=3.2, 5.3 Hz, H-7), 1.71 (dd, 1H, J=5.4, 9.2 Hz, H-9), 2.27 (dd, 1H, J=6.1, 9.2 Hz, H-9′), 2.12 (m, 1H, H-10), 2.46 (m, 1H, H-10′), 5.31 (br d, 1H, J=5.9 Hz, H-11), 2.34 (m, 2H, H-13), 4.85 (t, 1H, J=5.4 Hz, H-14), 5.51 (d, 1H, J=1.8 Hz, H-17), 6.27 (d, 1H, J=2.0 Hz, H-17′), 1.40 (s, 3H, Me-18), 1.89 (s, 3H, Me-19), 1.79 (s, 3H, Me-20); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 47.0 (d, C-1), 30.7 (t, C-2), 44.8 (d, C-3), 87.4 (s, C-4), 41.0 (t, C-5), 29.9 (t, C-6), 81.9 (d, C-7), 58.2 (s, C-8), 44.7 (t, C-9), 26.6 (t, C-10), 132.1 (d, C-11), 126.5 (s, C-12), 43.8 (t, C-13), 76.2 (d, C-14), 137.5 (s, C-15), 169.9 (s, C-16), 120.2 (t, C-17), 21.7 (q, C-18), 28.5 (q, C-19), 15.6 (q, C-20); HREI-MS m/z $[M-I]^+$ calcd for $C_{20}H_{28}IO_3$ 443.1083, found 443.1077, 443 (6), 316 (6), 315 (5), 314

(9), 279 (7), 254 (29), 167 (16), 149 (60), 95 (34), 81 (47), 71 (57), 57 (100).

3.6. Reaction of 12,13-bisepieupalmerin (2) with *m*-chloroperbenzoic acid

3.6.1. Path A. A solution of 12,13-bisepieupalmerin (202 mg, 0.60 mmol) in dry benzene (30 mL) was stirred vigorously with *m*-CPBA (150 mg, 1.3 meq) at rt for 1.5 h and concentrated to leave a residue that was chromatographed on silica gel (10 g). Elution with 30% ethyl acetate in hexane yielded 171 mg (81%) of diepoxide **15** as a colorless oil. Compound **15** was identical in all respects to natural product 12,13-bisepieupalmerin epoxide.[8]

3.6.2. Path B. A solution of **2** (411 mg, 1.23 mmol) and *m*-CPBA (213 mg, 1.23 mmol) in dry benzene (25 mL) was warmed to 70°C, stirred for 1 h, treated with *p*-toluenesulfonic acid hydrate (10 mg), and stirred for another hour before being cooled and diluted with saturated $NaHCO_3$ solution. Following extraction with chloroform (3×30 mL), the organic layers were dried and concentrated to leave a crude oil (461 mg) that was purified by means of normal-phase HPLC (Partisil-10, elution with 7% 2-propanol in hexane) to give **16** (98 mg, 24%).

3.6.3. Data for 16. Colorless oil; IR (neat) 2965, 2929, 2918, 2877, 1761, 1707, 1462, 1381, 1318, 1251, 1221, 1171, 1118, 1030, 1018 cm^{-1}; UV (MeOH) λ_{max} 208 and 302 nm; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.57 (m, 1H, H-1), 4.85 (d, 1H, J=4.5 Hz, H-2), 2.35 (dd, 1H, J=3.6, 10.5 Hz, H-6), 2.42 (m, 1H, H-6′), 2.77 (m, 1H, H-8), 3.27 (dd, 1H, J=2.1, 9.9 Hz, H-13), 4.60 (dd, 1H, J=8.1, 9.9 Hz, H-14), 5.61 (d, 1H, J=3.3 Hz, H-17), 6.32 (d, 1H, J=3.6 Hz, H-17′), 1.09 (d, 3H, J=6.9 Hz, Me-18), 0.98 (d, 3H, J=6.9 Hz, Me-19), 0.95 (d, 3H, J=7.5 Hz, Me-20); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 36.3 (d, C-1), 93.2 (d, C-2), 162.1 (s, C-3), 34.4 (d, C-4), 31.1 (t, C-5), 41.1 (t, C-6), 215.8 (s, C-7), 43.6 (d, C-8), 33.7 (t, C-9), 21.9 (t, C-10), 32.6 (t, C-11), 29.9 (d, C-12), 80.5 (d, C-13), 72.4 (d, C-14), 138.0 (s, C-15), 169.9 (s, C-16), 123.1 (t, C-17), 19.4 (q, C-18), 18.2 (q, C-19), 11.0 (q, C-20); HREI-MS m/z $[M]^+$ calcd for $C_{20}H_{28}O_4$ 332.1988, found 332.2000, 332 (26), 261 (23), 256 (4), 219 (2), 191 (8), 149 (6), 137 (14), 121 (9), 95 (17), 81 (100).

3.7. Reaction of 12,13-bisepieupalmerin (2) with iodine[9]

A solution of iodine (246 mg, 0.97 mmol) in CH_2Cl_2 (30 mL) was added dropwise over 45 min to a magnetically stirred solution of **2** (300 mg, 0.90 mmol) in CH_2Cl_2 (50 mL). The mixture was stirred at 25°C for 1 h, concentrated, and the resulting oil was chromatographed on silica gel (20 g, 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%) of inolide-A (**17**) and 9 mg (3%) of inolide-B (**18**) as white solids, and 25 mg (5%) of diiodide **19**. The structure of **17** was corroborated by X-ray crystallographic analysis (Fig. 3).

3.7.1. Data for 18. White solid; $[\alpha]_D^{21}=-36.7°$ (c 1.8, MeOH); IR (neat) 2982, 2899, 2873, 1762, 1461, 1296, 1114, 1097, 1005, 947 cm^{-1}; UV (MeOH) λ_{max} 210 nm; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.37 (m, 1H, H-1), 3.28 (d, 1H, J=11.7 Hz, H-3), 4.12 (m, 1H, H-7), 2.96 (d, 1H, J=9.9 Hz, H-13), 4.45 (t, 1H, J=8.7 Hz, H-14), 5.53 (d, 1H, J=2.7 Hz, H-17), 6.38 (d, 1H, J=3.0 Hz, H-17′), 1.16 (s, 3H, Me-18), 0.77 (d, 3H, J=7.2 Hz, Me-19), 1.08 (d, 3H, J=7.2 Hz, Me-20); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 38.2 (d, C-1), 27.2 (t, C-2), 81.3 (d, C-3), 84.1 (s, C-4), 30.4 (t, C-5), 30.5 (t, C-6), 85.0 (d, C-7), 35.2 (d, C-8), 26.9 (t, C-9), 32.4 (t, C-10), 34.8 (t, C-11), 31.4 (d, C-12), 82.8 (d, C-13), 73.5 (d, C-14), 136.5 (s, C-15), 170.2 (s, C-16), 121.2 (t, C-17), 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).

3.7.2. Data for 19. Colorless oil; $[\alpha]_D^{21}=+0.2°$ (c 6.1, $CHCl_3$); IR (neat) 2964, 2947, 2924, 2871, 1755, 1668, 1446, 1378, 1282, 1147, 1075, 1019, 927, 804 cm^{-1}; UV (MeOH) λ_{max} 214 nm; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.33 (m, 1H, H-1), 1.95 (m, 1H, H-2), 2.65 (dd, 1H, J=10.5, 15.9 Hz, H-2′), 4.05 (d, 1H, J=11.1 Hz, H-3), 4.38 (dd, 1H, J=4.5, 11.1 Hz, H-7), 4.21 (br s, 1H, H-13), 4.44 (t, 1H, J=6.3 Hz, H-14), 5.62 (d, 1H, J=1.2 Hz, H-17), 6.16 (d, 1H, J=1.8 Hz, H-17′), 1.29 (s, 3H, Me-18), 1.97 (s, 3H, Me-19), 0.89 (d, 3H, J=6.6 Hz, Me-20); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 41.5 (d, C-1), 39.3 (t, C-2), 42.5 (d, C-3), 86.2 (s, C-4), 41.2 (t, C-5), 31.0 (t, C-6), 82.7 (d, C-7), 52.7 (s, C-8), 42.0 (t, C-9), 23.7 (t, C-10), 31.1 (t, C-11), 31.8 (d, C-12), 82.2 (d, C-14), 140.7 (s, C-15), 170.0 (s, C-16), 120.4 (t, C-17), 21.4 (q, C-18), 29.0 (q, C-19), 14.9 (q, C-20) (the resonance line ascribable to C-13 could not be detected); HREI-MS m/z $[M-I]^+$ calcd for $C_{20}H_{30}IO_4$ 461.1189, found 461.1180, 461 (32), 443 (12), 334 (16), 333 (16), 316 (13), 315 (21), 297 (10), 249 (5), 189 (9), 109 (54), 95 (44), 81 (81), 69 (68), 55 (100).

3.8. Reaction of 12,13-bisepieupalmerin (2) with perchloric acid

A solution of **2** (968 mg, 2.9 mmol) in chloroform (50 mL) containing perchloric acid (6 drops) was stirred vigorously at rt for 2 h, treated with saturated $NaHCO_3$ solution, and extracted with $CHCl_3$ (3×30 mL). The combined organic layers were dried and concentrated to leave a residue that was chromatographed on silica gel (40 g, elution with 1:8:11 (v/v) 2-propanol/$CHCl_3$/hexane) followed by normal-phase HPLC (Partisil-10, elution with 1:19 (v/v) 2-propanol in hexane) giving 194 mg (20%) of inolide-A (**17**),[9] 58 mg (6%) of eunicin (**20**),[10] 60 mg (6%) of (*E*)-Δ^8 eunicin (**21**), 21 mg (2%) of (*Z*)-Δ^8 jeunicin (**22**), 19 mg (2%) of (*Z*)-Δ^7 jeunicin (**23**), 78 mg (8%) of (*Z*)-Δ^7 eunicin (**24**), and 117 mg (12%) of **25**.

3.8.1. Data for 21. Colorless oil; $[\alpha]_D^{25}=-20.1°$ (c 3.2, $CHCl_3$); IR (neat) 3505, 2965, 2916, 2849, 1749, 1458, 1227, 1127, 1072, 1037, 991, 936, 817 cm^{-1}; UV (MeOH) λ_{max} 222 nm (ϵ 2800); ^1H NMR ($CDCl_3$, 300 MHz) δ 3.41 (m, 1H, H-1), 3.21 (d, 1H, J=12.0 Hz, H-3), 5.02 (t, 1H, J=7.8 Hz, H-9), 3.04 (d, 1H, J=9.6 Hz, H-13), 4.50 (t, 1H, J=8.7 Hz, H-14), 5.62 (d, 1H, J=3.3 Hz, H-17), 6.40 (d, 1H, J=3.6 Hz, H-17′), 1.13 (s, 3H, Me-18), 1.52 (s, 3H, Me-19), 0.89 (d, 3H, J=6.6 Hz, Me-20); ^{13}C NMR

($CDCl_3$, 75 MHz) δ 38.2 (d, C-1), 24.0 (t, C-2), 73.2 (d, C-3), 73.6 (s, C-4), 35.4 (t, C-5), 18.8 (t, C-6), 36.5 (t, C-7), 130.3 (s, C-8), 128.2 (d, C-9), 24.5 (t, C-10), 33.7 (t, C-11), 34.2 (d, C-12), 78.6 (d, C-13), 73.4 (d, C-14), 136.8 (s, C-15), 170.3 (s, C-16), 121.4 (t, C-17), 23.3 (q, C-18), 17.3 (q, C-19), 14.3 (q, C-20); HREI-MS *m/z* $[M]^+$ calcd for $C_{20}H_{30}O_4$ 334.2144, found 334.2144, 334 (43), 316 (19), 288 (6), 221 (9), 193 (33), 164 (15), 135 (18), 121 (33), 109 (45), 95 (63), 81 (100), 67 (47), 55 (79).

3.8.2. Data for 22. Colorless oil; IR (neat) 3445, 1751, 1463, 1095, 758 cm^{-1}; UV (MeOH) λ_{max} 222 nm (ϵ 3200); ^1H NMR ($CDCl_3$, 300 MHz) δ 3.59 (m, 1H, H-1), 3.59 (m, 1H, H-3), 5.13 (m, 1H, H-9), 3.59 (m, 1H, H-13), 4.62 (t, 1H, *J*=9.3 Hz, H-14), 5.57 (d, 1H, *J*=3.0 Hz, H-17), 6.27 (d, 1H, *J*=3.3 Hz, H-17′), 1.18 (s, 3H, Me-18), 1.70 (s, 3H, Me-19), 0.96 (d, 3H, *J*=6.3 Hz, Me-20); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 36.1 (d, C-1), 30.3 (t, C-2), 74.3 (d, C-3), 79.8 (s, C-4), 35.8 (t, C-5), 23.2 (t, C-6), 32.2 (t, C-7), 141.0 (s, C-8), 123.7 (d, C-9), 26.2 (t, C-10), 33.0 (t, C-11), 33.1 (d, C-12), 69.1 (d, C-13), 79.4 (d, C-14), 138.9 (s, C-15), 170.4 (s, C-16), 121.7 (t, C-17), 20.9 (q, C-18), 26.0 (q, C-19), 14.1 (q, C-20); HREI-MS *m/z* $[M]^+$ calcd for $C_{20}H_{30}O_4$ 334.2144, found 334.2131, 334 (14), 316 (10), 193 (12), 164 (12), 133 (15), 126 (14), 121 (31), 119 (22), 111 (21), 109 (33), 107 (42), 95 (65), 93 (56), 85 (66), 83 (100), 81 (89).

3.8.3. Data for 23. Colorless oil; $[\alpha]_D^{25}=-21.6°$ (*c* 1.9, $CHCl_3$); IR (neat) 3435, 2956, 2923, 2854, 1751, 1664, 1462, 1378, 1261, 1095, 1035, 803, 758 cm^{-1}; UV (MeOH) λ_{max} 222 nm (ϵ 2900); ^1H NMR ($CDCl_3$, 300 MHz) δ 3.67 (m, 1H, H-1), 3.59 (m, 1H, H-3), 5.28 (m, 1H, H-7), 3.80 (dd, 1H, *J*=1.8, 9.9 Hz, H-13), 4.64 (t, 1H, *J*=9.3 Hz, H-14), 5.61 (d, 1H, *J*=2.7 Hz, H-17), 6.28 (d, 1H, *J*=3.0 Hz, H-17′), 1.10 (s, 3H, Me-18), 1.72 (s, 3H, Me-19), 0.95 (d, 3H, *J*=6.6 Hz, Me-20); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 35.6 (d, C-1), 31.2 (t, C-2), 74.7 (d, C-3), 79.8 (s, C-4), 34.4 (t, C-5), 25.5 (t, C-6), 124.9 (d, C-7), 139.6 (s, C-8), 34.5 (t, C-9), 22.5 (t, C-10), 33.6 (t, C-11), 34.3 (d, C-12), 69.2 (d, C-13), 79.8 (d, C-14), 139.2 (s, C-15), 169.9 (s, C-16), 121.9 (t, C-17), 21.7 (q, C-18), 25.4 (q, C-19), 14.5 (q, C-20); HREI-MS *m/z* $[M]^+$ calcd for $C_{20}H_{30}O_4$ 334.2144, found 334.2133, 334 (2), 316 (2), 181 (3), 164 (3), 147 (4), 135 (4), 126 (9), 121 (11), 109 (11), 107 (12), 95 (25), 85 (65), 83 (100), 81 (42), 79 (18), 55 (31).

3.8.4. Data for 24. Colorless oil; $[\alpha]_D^{25}=-15.5°$ (*c* 15.4, $CHCl_3$); IR (neat) 3450, 2964, 2934, 2879, 1759, 1681, 1461, 1384, 1303, 1104, 1038, 1002, 951, 755 cm^{-1}; UV (MeOH) λ_{max} 203 nm (ϵ 30500); ^1H NMR ($CDCl_3$, 300 MHz) δ 3.40 (m, 1H, H-1), 3.46 (dd, 1H, *J*=1.2, 11.4 Hz, H-3), 1.60 (br m, 2H, H-5), 5.25 (dd, 1H, *J*=5.0, 10.0 Hz, H-7), 3.12 (d, 1H, *J*=9.6 Hz, H-13), 4.37 (t, 1H, *J*=8.7 Hz, H-14), 5.63 (d, 1H, *J*=3.3 Hz, H-17), 6.39 (d, 1H, *J*=3.6 Hz, H-17′), 1.14 (s, 3H, Me-18), 1.61 (s, 3H, Me-19), 0.89 (d, 3H, *J*=7. 2 Hz, Me-20); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 38.5 (d, C-1), 24.7 (t, C-2), 75.6 (d, C-3), 74.3 (s, C-4), 41.6 (t, C-5), 21.2 (t, C-6), 125.4 (d, C-7), 136.4 (s, C-8), 33.6 (t, C-9), 23.5 (t, C-10), 32.5 (t, C-11), 34.6 (d, C-12), 82.2 (d, C-13), 74.2 (d, C-14), 136.7 (s, C-15), 170.9 (s, C-16), 122.3 (t, C-17), 24.5 (q, C-18), 23.8 (q, C-19), 13.6 (q, C-20); HREI-MS *m/z* $[M]^+$ calcd for $C_{20}H_{30}O_4$ 334.2144, found 334.2130, 334 (9), 316 (9), 248 (6), 193 (4), 164 (8), 135 (6), 121 (19), 109 (21), 95 (33), 85 (65), 83 (100), 81 (45), 70 (35), 55 (44).

3.8.5. Data for 25. Colorless oil; $[\alpha]_D^{25}=-35.0°$ (*c* 1.9, $CHCl_3$); IR (neat) 3487, 2961, 2911, 2882, 2856, 1756, 1662, 1636, 1451, 1287, 1205, 1100, 999, 894, 815, 754 cm^{-1}; UV (MeOH) λ_{max} 207 nm (ϵ 5500); ^1H NMR ($CDCl_3$, 300 MHz) δ 3.41 (m, 1H, H-1), 3.20 (d, 1H, *J*=11.7 Hz, H-3), 3.05 (dd, 1H, *J*=2.7, 9.6 Hz, H-13), 4.55 (t, 1H, *J*=8.7 Hz, H-14), 5.61 (d, 1H, *J*=3.6 Hz, H-17), 6.38 (d, 1H, *J*=3.6 Hz, H-17′), 1.16 (s, 3H, Me-18), 4.73 (br s, 1H, H-19), 4.74 (br s, 1H, H-19′), 0.92 (d, 3H, *J*=6.6 Hz, Me-20); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 38.2 (d, C-1), 24.3 (t, C-2), 74.9 (d, C-3), 73.8 (s, C-4), 38.1 (t, C-5), 23.5 (t, C-6), 37.2 (t, C-7), 145.5 (s, C-8), 37.8 (t, C-9), 20.2 (t, C-10), 31.0 (t, C-11), 32.2 (d, C-12), 78.9 (d, C-13), 73.1 (d, C-14), 136.3 (s, C-15), 170.2 (s, C-16), 121.6 (t, C-17), 23.9 (q, C-18), 115.8 (t, C-19), 14.9 (q, C-20); HREI-MS *m/z* $[M]^+$ calcd for $C_{20}H_{30}O_4$ 334.2144, found 334.2176, 334 (62), 316 (58), 301 (8), 288 (10), 221 (19), 193 (16), 182 (27), 164 (41), 121 (74), 109 (83), 107 (73), 93 (59), 79 (92), 69 (73), 67 (88), 55 (100).

3.9. Reaction of 12,13-bisepieupalmerin (2) with phosphorus pentoxide

A solution of **2** (3.0 g, 9.1 mmol) in benzene (95 mL) was stirred at rt with anhydrous phosphorus pentoxide (1.7 g, 12 mmol) for 12 h, treated with saturated $NaHCO_3$ solution, and extracted with benzene (3×100 mL). Purification of the residue by chromatography on silica gel (80 g, elution with 1:6:13 (v/v) 2-propanol/CH_2Cl_2/hexane) followed by normal-phase HPLC (Partisil-10, elution with 15% 2-propanol in hexane) provided 1.4 g (45%) of eunicin (**20**),[10] 476 mg (16%) of **24**, 80 mg (3%) of **25**, 173 mg (6%) of jeunicin (**26**),[11] and 27 mg (1%) of butenolide **27**.

3.9.1. Data for 27. Colorless oil; $[\alpha]_D^{25}=+16.9°$ (*c* 0.53, $CHCl_3$); IR (neat) 3489, 2959, 2922, 2854, 1748, 1681, 1458, 1382, 1261, 1097, 1023, 758 cm^{-1}; UV (MeOH) λ_{max} 230 nm; ^1H NMR ($CDCl_3$, 300 MHz) δ 2.34 (t, 1H, *J*=7.5 Hz, H-2), 3.11 (dd, 1H, *J*=1.3, 8.0 Hz, H-2′), 3.21 (dd, 1H, *J*=1.3, 6.6 Hz, H-3), 3.15 (dd, 1H, *J*=1.9, 5.6 Hz, H-13), 4.60 (d, 1H, *J*=5.6 Hz, H-14), 4.42 (br s, 2H, H-17), 1.24 (s, 3H, Me-18), 4.76 (br s, 1H, H-19), 4.77 (br s, 1H, H-19′), 1.01 (d, 3H, *J*=4.1 Hz, Me-20), 1.60 (br s, exchangeable, –O*H*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 163.9 (s, C-1), 27.9 (t, C-2), 80.0 (d, C-3), 73.9 (s, C-4), 38.1 (t, C-5), 23.3 (t, C-6), 36.9 (t, C-7), 145.3 (s, C-8), 37.5 (t, C-9), 19.9 (t, C-10), 30.9 (t, C-11), 33.2 (d, C-12), 82.6 (d, C-13), 77.8 (d, C-14), 123.2 (s, C-15), 173.8 (s, C-16), 54.9 (t, C-17), 23.9 (q, C-18), 115.8 (t, C-19), 15.0 (q, C-20); HREI-MS *m/z* $[M]^+$ calcd for $C_{20}H_{30}O_5$ 350.2093, found 350.2089, 350 (8), 332 (20), 314 (6), 223 (19), 205 (15), 195 (15), 193 (18), 177 (33), 135 (37), 121 (33), 109 (67), 95 (70), 81 (100), 59 (57).

3.10. Reaction of eupalmerin (3) with phosphorus pentoxide

A solution of **3** (616 mg, 1.84 mmol) in benzene (40 mL) was stirred at rt with anhydrous phosphorus pentoxide

(340 mg, 2.39 mmol) for 12 h, treated with saturated $NaHCO_3$ solution, and extracted with benzene (3× 100 mL). Purification of the residue by chromatography on silica gel (15 g, elution with 1:1 (v/v) ethyl acetate in hexane) followed by reversed-phase HPLC (Zorbax C8, elution with 30% water in MeOH) provided 142 mg (23%) of 12-epicueunicin (**28**), 45 mg (7%) of 12,13-bisepijeunicin (**29**), 102 mg (16%) of **30**, and 91 mg (15%) of **31**.

3.10.1. Data for 28. Colorless oil; $[\alpha]_D^{21}=-89.0°$ (*c* 3.9, $CHCl_3$); IR (neat) 3299, 2954, 2914, 2853, 1748, 1660, 1446, 1284, 1149, 1090, 1027, 991, 934 cm^{-1}; UV (MeOH) λ_{max} 210 nm (ϵ 15200); ^1H NMR ($CDCl_3$, 300 MHz) δ 3.42 (m, 1H, H-1), 3.52 (dd, 1H, *J*=4.2, 12.3 Hz, H-3), 5.19 (t, 1H, *J*=6.3 Hz, H-7), 3.58 (br d, 1H, *J*=3.6 Hz, H-13), 4.70 (dd, 1H, *J*=0.9, 9.0 Hz, H-14), 5.66 (d, 1H, *J*=2.4 Hz, H-17), 6.39 (d, 1H, *J*=2.7 Hz, H-17'), 1.13 (s, 3H, Me-18), 1.51 (s, 3H, Me-19), 0.94 (d, 3H, *J*=7.2 Hz, Me-20); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 34.8 (d, C-1), 26.0 (t, C-2), 76.5 (d, C-3), 74.4 (s, C-4), 44.7 (t, C-5), 24.2 (t, C-6), 129.3 (d, C-7), 129.7 (s, C-8), 39.4 (t, C-9), 22.8 (t, C-10), 29.1 (t, C-11), 35.5 (d, C-12), 73.0 (d, C-13), 76.3 (d, C-14), 137.8 (s, C-15), 170.3 (s, C-16), 122.9 (t, C-17), 20.5 (q, C-18), 15.6 (q, C-19), 16.2 (q, C-20); HREI-MS *m/z* $[M]^+$ calcd for $C_{20}H_{30}O_4$ 334.2144, found 334.2133, 334 (29), 316 (40), 193 (29), 182 (19), 177 (13), 164 (26), 153 (38), 121 (34), 109 (38), 95 (61), 81 (96), 67 (65), 55 (100).

3.10.2. Data for 29. Colorless oil; $[\alpha]_D^{21}=-122.2°$ (*c* 2.3, $CHCl_3$); IR (neat) 3443, 2952, 2929, 2854, 1733, 1660, 1456, 1406, 1287, 1260, 1162, 1099, 1052, 988, 937, 812 cm^{-1}; UV (MeOH) λ_{max} 210 nm (ϵ 5600); ^1H NMR ($CDCl_3$, 300 MHz) δ 3.44 (m, 1H, H-1), 3.58 (m, 1H, H-3), 5.22 (br d, 1H, *J*=11.1 Hz, H-7), 3.40 (br d, 1H, *J*=5.1 Hz, H-13), 4.74 (d, 1H, *J*=9.3 Hz, H-14), 5.56 (d, 1H, *J*=3.0 Hz, H-17), 6.37 (d, 1H, *J*=3.6 Hz, H-17′), 1.12 (s, 3H, Me-18), 1.59 (s, 3H, Me-19), 0.90 (d, 3H, *J*=6.9 Hz, Me-20); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 37.6 (d, C-1), 32.3 (t, C-2), 73.6 (d, C-3), 78.8 (s, C-4), 39.8 (t, C-5), 23.1 (t, C-6), 130.0 (d, C-7), 127.5 (s, C-8), 41.1 (t, C-9), 23.5 (t, C-10), 28.4 (t, C-11), 40.4 (d, C-12), 74.4 (d, C-13), 79.7 (d, C-14), 137.1 (s, C-15), 170.0 (s, C-16), 120.9 (t, C-17), 13.3 (q, C-18), 15.6 (q, C-19), 17.1 (q, C-20); HREI-MS *m/z* $[M]^+$ calcd for $C_{20}H_{30}O_4$ 334.2144, found 334.2158, 334 (21), 316 (14), 181 (12), 177 (12), 164 (17), 159 (12), 147 (18), 133 (21), 119 (30), 109 (31), 107 (41), 95 (62), 91 (42), 79 (61), 67 (57), 55 (100).

3.10.3. Data for 30. Colorless oil; $[\alpha]_D^{21}=-28.0°$ (*c* 2.5, $CHCl_3$); IR (neat) 3473, 2961, 2928, 2856, 1759, 1660, 1454, 1376, 1286, 1139, 1105, 1030, 934, 813, 756 cm^{-1}; UV (MeOH) λ_{max} 212 nm (ϵ 4500); ^1H NMR ($CDCl_3$, 300 MHz) δ 3.49 (m, 1H, H-1), 3.69 (dd, 1H, *J*=4.8, 12.9 Hz, H-3), 5.16 (t, 1H, *J*=7.5 Hz, H-9), 3.87 (br s, 1H, H-13), 4.69 (dd, 1H, *J*=1.5, 9.3 Hz, H-14), 5.65 (d, 1H, *J*=2.7 Hz, H-17), 6.44 (d, 1H, *J*=3.3 Hz, H-17′), 1.03 (s, 3H, Me-18), 1.66 (s, 3H, Me-19), 1.05 (d, 3H, *J*=6.6 Hz, Me-20); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 35.0 (d, C-1), 25.1 (t, C-2), 76.8 (d, C-3), 75.0 (s, C-4), 36.4 (t, C-5), 21.1 (t, C-6), 34.6 (t, C-7), 134.3 (s, C-8), 126.6 (d, C-9), 25.1 (t, C-10), 31.8 (t, C-11), 34.6 (d, C-12), 73.0 (d, C-13), 78.4 (d, C-14), 136.9 (s, C-15), 169.8 (s, C-16), 122.7 (t, C-17), 22.8 (q, C-18), 24.2 (q, C-19), 15.9 (q, C-20); HREI-MS *m/z* $[M]^+$ calcd for $C_{20}H_{30}O_4$ 334.2144, found 334.2146, 334 (13), 316 (30), 193 (17), 182 (12), 177 (10), 164 (15), 153 (12), 147 (14), 133 (19), 119 (31), 109 (42), 107 (38), 95 (57), 91 (40), 82 (24), 79 (68), 69 (55), 55 (100).

3.10.4. Data for 31. Colorless oil; $[\alpha]_D^{21}=-72.4°$ (*c* 2.1, $CHCl_3$); IR (neat) 3446, 2959, 2926, 2856, 1761, 1456, 1374, 1263, 1145, 1106, 1019 cm^{-1}; UV (MeOH) λ_{max} 212 nm (ϵ 12800); ^1H NMR ($CDCl_3$, 300 MHz) δ 3.44 (m, 1H, H-1), 3.58 (dd, 1H, *J*=3.0, 12.6 Hz, H-3), 5.17 (t, 1H, *J*=7.5 Hz, H-7), 3.83 (dd, 1H, *J*=1.2, 4.5 Hz, H-13), 4.72 (dd, 1H, *J*=1.2, 8.7 Hz, H-14), 5.69 (d, 1H, *J*=2.4 Hz, H-17), 6.41 (d, 1H, *J*=2.4 Hz, H-17′), 1.10 (s, 3H, Me-18), 1.66 (s, 3H, Me-19), 1.01 (d, 3H, *J*=7.2 Hz, Me-20); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 35.4 (d, C-1), 27.4 (t, C-2), 76.8 (d, C-3), 73.5 (s, C-4), 42.7 (t, C-5), 22.6 (t, C-6), 125.2 (d, C-7), 138.4 (s, C-8), 31.2 (t, C-9), 25.8 (t, C-10), 32.0 (t, C-11), 35.0 (d, C-12), 73.3 (d, C-13), 76.4 (d, C-14), 136.3 (s, C-15), 170.3 (s, C-16), 123.5 (t, C-17), 20.8 (q, C-18), 23.7 (q, C-19), 17.1 (q, C-20); HREI-MS *m/z* $[M]^+$ calcd for $C_{20}H_{30}O_4$ 334.2144, found 334.2189, 334 (21), 316 (25), 193 (25), 182 (16), 177 (11), 164 (24), 153 (23), 149 (22), 133 (20), 119 (28), 109 (19), 105 (18), 95 (64), 91 (20), 79 (44), 69 (71), 55 (100).

3.11. Reaction of eupalmerin (3) with *p*-toluenesulfonic acid hydrate

After a solution of **3** (700 mg, 2.1 mmol) in 30 mL of benzene was treated with 15 mg of PTSA·H_2O and stirred at rt for 6 h, the mixture was concentrated to afford a semisolid mass. The residue was layered on top of a 50×2.5 cm silica gel column eluted with increasing concentrations of ethyl acetate in hexane. Concentration of the major band gave 237 mg (34%) of 12-epicueunicin (**28**). Subsequent purification of the minor band by normal-phase HPLC (Partisil-10, elution with 20% 2-propanol in hexane) furnished 20 mg (3%) of **32**, 18 mg (3%) of **33**, and 15 mg (2%) of known eupalmerolide (**34**).[14]

3.11.1. Data for 32. Crystalline white solid; $[\alpha]_D^{21}=-45.0°$ (*c* 1.4, $CHCl_3$); IR (neat) 3544, 2932, 2905, 2855, 1751, 1405, 1366, 1298, 1201, 1138, 1096, 1001, 886 cm^{-1}; UV (MeOH) λ_{max} 212 nm (ϵ 4500); ^1H NMR ($CDCl_3$, 300 MHz) δ 3.46 (m, 1H, H-1), 3.65 (dd, 1H, *J*=5.7, 12.9 Hz, H-3), 3.83 (br s, 1H, H-13), 4.63 (dd, 1H, *J*=1.2, 9.3 Hz, H-14), 5.61 (d, 1H, *J*=3.0 Hz, H-17), 6.41 (d, 1H, *J*=3.0 Hz, H-17′), 0.97 (s, 3H, Me-18), 4.86 (br s, 1H, H-19), 4.94 (br s, 1H, H-19′), 1.06 (d, 3H, *J*=6.6 Hz, Me-20); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 35.1 (d, C-1), 24.6 (t, C-2), 77.8 (d, C-3), 74.5 (s, C-4), 32.8 (t, C-5), 22.3 (t, C-6), 33.8 (t, C-7), 148.0 (s, C-8), 38.1 (t, C-9), 22.1 (t, C-10), 33.5 (t, C-11), 35.3 (d, C-12), 70.4 (d, C-13), 79.4 (d, C-14), 136.4 (s, C-15), 169.7 (s, C-16), 122.3 (t, C-17), 20.3 (q, C-18), 113.5 (t, C-19), 15.8 (q, C-20); HREI-MS *m/z* $[M]^+$ calcd for $C_{20}H_{30}O_4$ 334.2144, found 334.2140, 334 (5), 316 (12), 195 (15), 193 (41), 182 (25), 177 (11), 164 (40), 153 (61), 149 (22), 135 (18), 121 (48), 119 (32), 109 (34), 107 (45), 95 (73), 93 (60), 81 (100), 79 (57), 67 (54), 55 (93). The structural assignment to **32** was corroborated by X-ray crystallographic analysis (Fig. 4).

3.11.2. Data for 33. Colorless oil; $[\alpha]_D^{21}=-13.3°$ (*c* 10.0, $CHCl_3$); IR (neat) 3451, 2928, 1754, 1445, 1272, 1159, 1124, 1022, 958, 755 cm^{-1}; UV (MeOH) λ_{max} 210 nm; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.40 (m, 1H, H-1), 4.21 (dd, 1H, *J*=3.6, 9.6 Hz, H-3), 5.57 (br t, 1H, *J*=6.6 Hz, H-7), 3.60 (dd, 1H, *J*=1.5, 8.1 Hz, H-13), 4.43 (dd, 1H, *J*=6.3, 8.1 Hz, H-14), 5.68 (d, 1H, *J*=0.9 Hz, H-17), 6.27 (d, 1H, *J*= 1.5 Hz, H-17′), 4.95 (br s, 2H, H-18), 1.56 (s, 3H, Me-19), 0.89 (d, 3H, *J*=6.9 Hz, Me-20); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 37.6 (d, C-1), 32.1 (t, C-2), 71.2 (d, C-3), 152.4 (s, C-4), 32.8 (t, C-5), 28.0 (t, C-6), 127.0 (d, C-7), 134.5 (s, C-8), 36.1 (t, C-9), 20.8 (t, C-10), 31.4 (t, C-11), 29.6 (d, C-12), 74.3 (d, C-13), 82.3 (d, C-14), 139.0 (s, C-15), 169.9 (s, C-16), 122.1 (t, C-17), 113.9 (t, C-18), 14.6 (q, C-19), 10.9 (q, C-20); HREI-MS *m/z* $[M]^+$ calcd for $C_{20}H_{30}O_4$ 334.2144, found 334.2143, 334 (6), 316 (17), 181 (22), 163 (11), 147 (13), 145 (11), 121 (32), 119 (26), 109 (23), 107 (38), 105 (37), 95 (59), 93 (80), 91 (44), 81 (94), 79 (57), 69 (49), 55 (100).

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