Pintoxolanes A – C, Highly Functionalized 3,14-Oxa-bridged Cembranoids from the Caribbean Gorgonian Coral *Eunicea pinta*

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Three new cembrane-based diterpenes possessing the rare 3,14-oxa-bridged moiety were isolated from the hexane extract of a southwestern Caribbean gorgonian octocoral, *Eunicea pinta*, together with seven known cembranoid diterpenes. The structures of pintoxolanes A – C (**1** – **3**, resp.), including their relative configurations, were deduced from spectroscopic data and chemical-correlation experiments. Experimental evidence hinting to a plausible biogenesis of the 3,14-oxalane moiety is provided. Preliminary cytotoxicity data for compounds **1** – **3** toward human cancer cell lines are also provided.

Introduction. – In 2002, our research group examined the $CHCl_3$ extract of the southwestern Caribbean gorgonian octocoral *Eunicea pinta* (BAYER & DEICHMANN, 1958) collected in San Andrés Island, Colombia, and reported the isolation and structure elucidation of eight new γ-cembranolide-type diterpenes by spectroscopic analysis, X-ray diffraction analysis, and chemical conversion [1]. By far, the majority of the secondary metabolites then identified were 3,4-epoxy-α-methylidene-γ-cembranolides that showed strong cytotoxic activities in *in vitro* tests against the NCI 60 cell-line tumor panel. Since that time, no further chemical investigations on the secondary-metabolite composition of this uncommon gorgonian species have been reported [2]. We have now examined in detail the diterpene cembranolide mixture occurring in the hexane extract of this gorgonian octocoral and found that it consisted primarily of seven previously well-characterized cembranes on the basis of chemical and spectroscopic data, and by comparison with literature data, namely, pseudoplexauric acid methyl ester (**4**), (−)-eunicenone (**5**), 12-epieupalmerone (**6**), (1*S*,3*R*,4*R*,7*E*,11*E*)-3,4;15,17-diepoxycembra-7,11-diene (**7**), succinolide (**8**), euniolide (**9**), and uprolide H (**10**) [1][3]. We also found three new compounds, trivially named pintoxolanes A – C (**1** – **3**, resp.), characterized by the presence of an unusual 3,14-oxa bridge across the 14-membered carbocyclic cembrane skeleton and a methyl 2-prop-2-enoate appendage at C(1)[1]). Literature search using *SciFinder*® and *MarinLit*® revealed only two naturally occurring marine metabolites with a 3,14-oxa bridge across the 14-membered carbocyclic cembrane skeleton [4]. These conspicuous structural features suggest a close structural relationship between the new compounds described and several *Eunicea* 3,4-epoxy-α-methylidene-γ-cembranolides. Herein, we report the results of this investigation.

[1]) Arbitrary numbering. For systematic names, see the *Exper. Part.*

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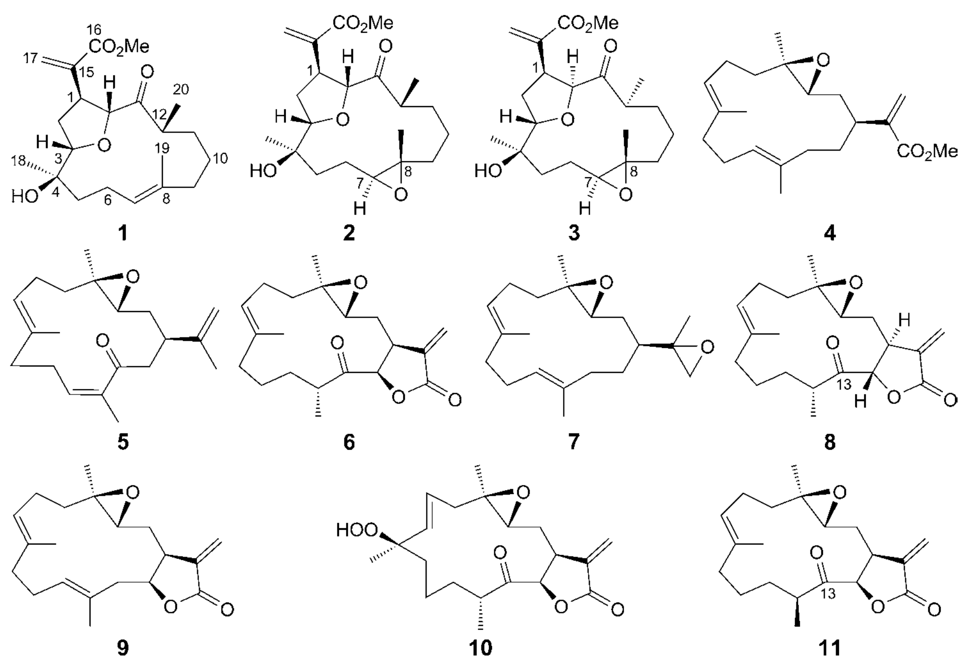


Results and Discussion. – Freeze-dried specimens of *E. pinta* were extracted as described in [1]. After removal of the organic solvents, the thick brown-green paste left behind was suspended in H_2O and then extracted exhaustively with hexane. The hexane-soluble fraction was chromatographed over SiO_2 using hexane/acetone mixtures of increasing polarity. Subsequent purification of the most promising fractions, on the basis of TLC and NMR analyses, using *Bio-Beads SX-3* and SiO_2 columns resulted in the isolation of pintoxolanes A–C (**1**–**3**, resp.). Identification of the known cembranolides **4**–**10** co-isolated during the course of this investigation was based on comparison of their physical properties ($[\alpha]_D$, IR, UV, NMR, and MS) with those in the literature [1][3].

Pintoxolane A (**1**) was isolated as a UV-active yellowish oil, the molecular formula of which, $C_{21}H_{32}O_5$, was established by HR-EI-MS (*m/z* 364.2256) and ^{13}C-NMR data implying six degrees of unsaturation. Its IR spectrum showed a broad absorption between 3600 and 3200 cm^{-1} (OH stretching), and strong absorptions at 1773 and 1718 cm^{-1}, consistent with the presence of an α,β-unsaturated ester and saturated ketone functions, respectively. That **1** contained a methyl 2-prop-2-enoate functionality was supported by the UV absorption around 200 nm and the two broad *singlet*s at δ(H) 6.28 and 5.71 (each 1 H), and sharp 3-H *singlet* at δ(H) 3.76 (CO_2Me) in the ^{1}H-NMR spectrum (*Table*). This assignment was supported by the ^{13}C-NMR (δ(C) 166.7 (*s*), 139.7 (*s*), 126.3 (*t*), and 51.9 (*q*)), and IR (1773 cm^{-1}) spectra. A C-atom signal at δ 213.3 confirmed the presence of a saturated ketone moiety in **1**, and two additional sp^2 C-atom signals at δ 131.8 (*s*) and 128.9 (*d*), together with a H-atom signal at δ 5.39 (br. *t*, *J* = 7.5, H–C(7)), hinted at a trisubstituted olefin. The ^{1}H-NMR spectrum also indicated the presence of three Me groups: one (δ(H) 1.12 (*s*, Me(18)) attached to a sp^3

Table. *^{1}H- and ^{13}C-NMR* (300 and 75 MHz) *Data for Pintoxolanes A – C* (**1** – **3**, resp.)[a]). Atom numbering as indicated in the *Formulae*.

Position	Pintoxolane A (**1**)		Pintoxolane B (**2**)		Pintoxolane C (**3**)	
	δ(H)	δ(C)[b])	δ(H)	δ(C)[b])	δ(H)	δ(C)[b])
1	3.30 (*q*, *J* = 7.5)	43.8 (*d*)	3.32 (*q*, *J* = 7.5)	43.3 (*d*)	3.79 – 3.81 (*m*)	41.5 (*d*)
2α	1.89 – 1.91 (*m*)	32.9 (*t*)	1.84 – 1.86 (*m*)	32.5 (*t*)	1.84 – 1.86 (*m*)	32.1 (*t*)
2β	1.89 – 1.91 (*m*)		1.84 – 1.86 (*m*)		1.84 – 1.86 (*m*)	
3	4.04 (*t*, *J* = 8.0)	86.5 (*d*)	4.03 (*t*, *J* = 7.8)	85.6 (*d*)	4.09 (*t*, *J* = 7.3)	87.2 (*d*)
4		73.1 (*s*)		72.9 (*s*)		73.5 (*s*)
5α	1.87 – 1.89 (*m*)	37.6 (*t*)	1.51 – 1.53 (*m*)	30.6 (*t*)	1.52 – 1.54 (*m*)	30.4 (*t*)
5β	1.58 – 1.61 (*m*)		1.51 – 1.53 (*m*)		1.52 – 1.54 (*m*)	
6α	2.19 – 2.21 (*m*)	22.6 (*t*)	1.27 – 1.29 (*m*)	22.1 (*t*)	1.57 – 1.59 (*m*)	22.7 (*t*)
6β	2.19 – 2.21 (*m*)		1.94 – 1.96 (*m*)		1.89 – 1.91 (*m*)	
7	5.39 (br. *t*, *J* = 7.5)	128.9 (*d*)	3.10 (*dd*, *J* = 9.5, 3.6)	64.9 (*d*)	2.81 – 2.83 (*m*)	65.5 (*d*)
8		131.8 (*s*)		59.9 (*s*)		59.5 (*s*)
9α	2.00 – 2.03 (*m*)	36.5 (*t*)	1.86 – 1.89 (*m*)	35.4 (*t*)	2.09 – 2.11 (*m*)	38.5 (*t*)
9β	2.00 – 2.03 (*m*)		1.86 – 1.89 (*m*)		2.12 – 2.14 (*m*)	
10α	1.62 – 1.64 (*m*)	21.7 (*t*)	1.86 – 1.89 (*m*)	21.6 (*t*)	1.94 – 1.96 (*m*)	21.0 (*t*)
10β	1.47 – 1.49 (*m*)		1.48 – 1.50 (*m*)		1.52 – 1.54 (*m*)	
11α	1.64 – 1.66 (*m*)	28.5 (*t*)	1.69 – 1.71 (*m*)	29.1 (*t*)	1.81 – 1.83 (*m*)	28.6 (*t*)
11β	1.14 – 1.16 (*m*)		1.27 – 1.29 (*m*)		1.47 – 1.49 (*m*)	
12	3.23 (*dq*, *J* = 6.8, 2.8)	39.0 (*d*)	3.13 – 3.15 (*m*)	39.6 (*d*)	3.20 – 3.22 (*m*)	43.3 (*d*)
13		213.3 (*s*)		212.2 (*s*)		212.5 (*s*)
14	4.37 (br. *d*, *J* = 7.9)	86.3 (*d*)	4.33 (br. *d*, *J* = 7.3)	85.8 (*d*)	4.48 (br. *d*, *J* = 3.5)	85.5 (*d*)
15		139.7 (*s*)		139.3 (*s*)		140.1 (*s*)
16		166.7 (*s*)		166.6 (*s*)		166.8 (*s*)
17α	6.28 (br. *s*)	126.3 (*t*)	6.19 (br. *s*)	126.2 (*t*)	6.30 (br. *s*)	125.3 (*t*)
17β	5.71 (br. *s*)		5.61 (br. *s*)		5.71 (br. *s*)	
18	1.12 (*s*)	24.5 (*q*)	1.03 (*s*)	25.4 (*q*)	1.03 (*s*)	22.3 (*q*)
19	1.58 (br. *s*)	15.2 (*q*)	1.13 (*s*)	16.6 (*q*)	1.30 (*s*)	16.4 (*q*)
20	0.99 (*d*, *J* = 6.8)	13.8 (*q*)	0.89 (*d*, *J* = 6.7)	14.2 (*q*)	1.08 (*d*, *J* = 7.0)	15.9 (*q*)
MeO	3.76 (*s*)	51.9 (*q*)	3.66 (*s*)	52.0 (*q*)	3.78 (*s*)	52.1 (*q*)

[a]) NMR spectra were recorded in $CDCl_3$ at 25°. Chemical shift values are given in ppm and are referenced to the residual $CHCl_3$ (δ(H) 7.26) or $CDCl_3$ (δ(C) 77.0) signal. Assignments were achieved by ^{1}H,^{1}H-COSY, HSQC, HMBC, and NOESY experiments. [b]) Multiplicities were obtained from DEPT-135 NMR experiments.

quaternary C-atom bearing O-atom (δ(C) 73.1 (*s*, C(4)), one (δ(H) 1.58 (*s*, Me(19)) at an sp^2 quaternary C-atom, and one (δ(H) 0.99 (*d*, *J* = 6.8, Me(20)) connected to a somewhat deshielded tertiary C-atom (δ(C) 39.0 (*d*, C(12)). A ^{1}H,^{13}C-HSQC NMR experiment correlated the H-atom resonances at δ(H) 4.04 (*t*, *J* = 8.0, 1 H) and 4.37 (br. *d*, *J* = 7.9, 1 H), ascribed to H–C(3) and H–C(14), respectively, to the corresponding C-atom resonances at δ(C) 86.5 (C(3)) and 86.3 (C(14)), respectively. These combined ^{1}H- and ^{13}C-NMR data suggested that C(3) and C(14) participate in the formation of an ether linkage. The nature of these low-field signals was consistent with the presence of an oxolane ring system in **1**. ^{1}H,^{1}H-COSY, HSQC, and ^{1}H,^{13}C-HMBC NMR techniques (*Fig. 1*) were employed extensively to position the methyl 2-prop-2-enoate, alcohol, olefin, ketone, and oxolane moieties in the cembrane ring of

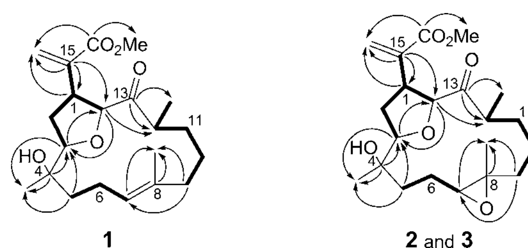


Fig. 1. *Partial structures for pintoxolanes A–C* (**1**–**3**, resp.) *generated from* $^1H,^1H$*-COSY* (—), *HSQC, and HMB* (H→C) *correlations*

pintoxolane A (**1**). Applying these combined NMR methods resulted in the assignment of all H-atoms and C-atoms as compiled in the *Table* and allowed the overall planar structure for pintoxolane A (**1**) to be deduced.

Since our repeated attempts to obtain suitable crystals for X-ray diffraction were unsuccessful, the relative configuration of **1** was assigned primarily on the basis of NOESY NMR data and J values in the ^{1}H-NMR spectrum (*Table*). The large coupling ($^2J(1,14) = 7.9$) observed between H–C(1) (δ(H) 3.30) and H–C(14) (δ(H) 4.37), combined with the lack of NOE couplings, suggested an *anti*-orientation for this H-atom pair. Strong NOE cross-peaks between H–C(1) and Me(18), as well as significant through-space interactions between H–C(7) (δ(H) 5.39), and both Me(18) (δ(H) 1.12) and H–C(12) (δ(H) 3.23), placed all of these groups on the α face. Most informative was a series of pronounced NOESY correlations among H–C(14), and H–C(3) (δ(H) 4.04), H_β–C(17) (δ(H) 5.71), and Me(20) (δ(H) 0.99), consistent with their orientation on the opposite (top) face of the molecule (*Fig. 2*). Furthermore, CH_2(6) (δ(H) 2.19–2.21) exhibited intense NOEs with both Me(19) (δ(H) 1.58) and H–C(3), indicating that these H-atoms are also on the same β face of the molecule. The (E)-geometry of the trisubstituted C=C bond was also deduced from the NOE correlation of Me(19) (δ(H) 1.58 (br. *s*, 3 H)) with CH_2(6) (δ(H) 2.19–2.21 (*m*, 2 H)), but not with olefinic H-atom H–C(7) (δ(H) 5.39 (br. *t*, $J = 7.5$)), and also the upper field chemical shift of

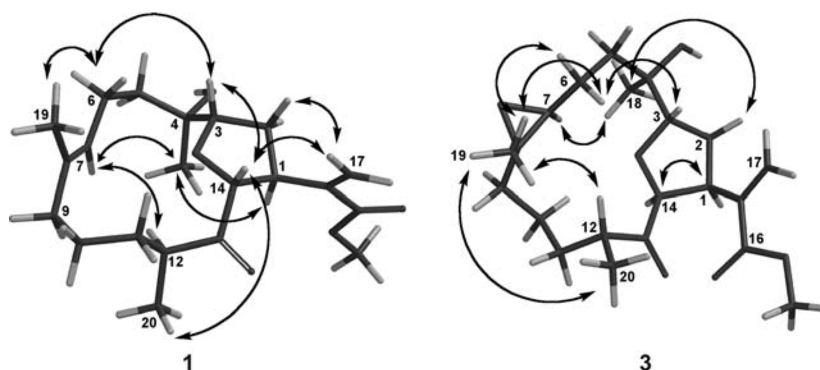
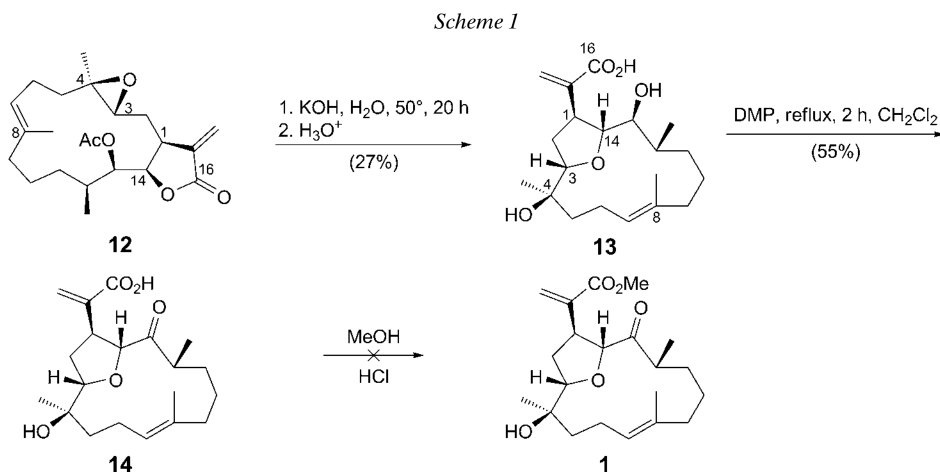


Fig. 2. *Energy-minimized molecular models of pintoxolane A* (**1**) *and pintoxolane C* (**3**) *with diagnostic ROESY* (↔) *correlations*

C(19) (δ(C) 15.2) [5]. The MacSpartan' 04 molecular-modeling program was used to calculate the conformer distribution for each possible structure; key interproton distances and dihedral angles were then determined for the lowest-energy conformer [6]. Structures displaying calculated internuclear distances longer than *ca.* 3.0 Å were not considered further. These correlations, all of which were consistent on the basis of a molecular-modeling study, were sufficient to confidently establish the overall relative configuration for **1** as shown. Thus, after careful evaluation of all of the data gathered, the observed NOESY correlations, and coupling constants were consistent only with the depicted structure.

We have previously reported on the synthesis of analogs of *Eunicea* γ-cembranolides containing medium ring ethers *via* saponification and have applied this method to the semi-synthesis of pintoxolane A acid derivative **13** using eupalmerin acetate (**12**) as starting material (*Scheme 1*) [7][8]. We found that treatment of **12** with a large excess of aqueous KOH gave 3,14-eupalmeroic acid (**13**) in modest yield [8]. *Dess–Martin* oxidation of the secondary alcohol afforded ketone **14** in good yield. Further attempts to transform **14** to natural product **1**, including acid-catalyzed *Fisher* esterification and the use of methylating agents such as CH_2N_2, $(Me)_2SO_4$, and MeI under a variety of reaction conditions failed. Instead, they led to the consumption of starting material and formation of a large number of intractable products. Notwithstanding, the ^{1}H- and ^{13}C-NMR signals of **14** assigned to atoms in positions 1, 3, 4, 12, and 14 were virtually identical to those for pintoxolane A (**1**), and because similar NOEs were observed in these compounds, it was assumed that the relative configuration of **14** at these centers was the same as that of **1**. The absolute configuration of eupalmerin acetate (**12**) has already been established [9], and inasmuch as **1** and **14** have identical relative configurations at all common stereogenic centers and comparable optical rotations in $CHCl_3$ solution ($[\alpha]_D = -30.3$ ($c = 1.3$, $CHCl_3$) and -33.3 ($c = 1.2$, $CHCl_3$), resp.), it is quite likely that they also have the same absolute configuration. All other spectroscopic data for **14** support the relative configuration assigned to **1**, namely, (1*S**,3*S**,4*R**,12*S**,14*R**).

Scheme 1



HR-EI-MS established the molecular formula for pintoxolane B (**2**) as $C_{21}H_{32}O_6$, *i.e.*, one O-atom more than in **1**. The six O-atoms in **2** were readily assigned to an ester, and a ketone, an alcohol, a trisubstituted epoxide, and oxolane ring by evaluation of spectroscopic information. Furthermore, it was found that the NMR data of **2** were very similar to those of **1**. ^{13}C-NMR signals (*Table*) at δ(C) 64.9 (*d*, C(7)) and 59.9 (*s*, C(8)) in conjunction with ^{1}H-NMR resonances at δ(H) 3.10 (*dd*, $J = 9.5$, 3.6, H–C(7)) and 1.13 (*s*, Me(19)) evidenced the presence of a Me-substituted epoxide group. From 2D-NMR data, including ^{1}H,^{1}H-COSY, HSQC, and HMBC, compound **2** was shown to possess many of the same structural features as **1**. Comparison of the ^{13}C-NMR data of the two compounds revealed that the major differences between them lay in the vicinity of the C(7),(8) junction (*Table*). On the basis of ^{1}H,^{1}H-couplings observed in the ^{1}H-NMR spectrum and overall HMBC spectroscopic data of **2** (*Fig. 1*), it was apparent that the trisubstituted epoxide group had to involve C(7) and C(8). The HMBC spectrum of **2** showed correlations between C(8) (δ(C) 59.9 (*s*)) and CH_2(9) (δ(H) 1.86–1.89), as well as from C(7) (δ(C) 64.9 (*d*)) to CH_2(6) (δ(H) 1.94–1.96 and 1.27–1.29), which allowed the unambiguous positioning of the epoxy group across C(7) and C(8). The relative configurations of **2** at C(1), C(3), C(4), C(12) and C(14) were identical to those of **1** on the basis of comparable NOE interactions (*Fig. 2*) and interproton coupling patterns [6]. For C(7) and C(8), the (7*S**,8*S**) relative configuration was deduced, on the basis of ^{13}C-NMR similarities with closely related 7,8-epoxycembrane models, the multiplicity, and coupling constants of H–C(7) (δ(H) 3.10 (*dd*, $J = 9.5$, 3.6)), as well as strong NOE effects observed between H–C(3) (δ(H) 4.03), CH_2(2) (δ(H) 1.84–1.86), and H–C(14) (δ(H) 4.33), between Me(19) (δ(H) 1.13) and Me(20) (δ(H) 0.89), and also between Me(18) (δ(H) 1.03) and H–C(7) (δ(H) 3.10) [10]. Microscale oxidation of **1** at 25° with *m*CPBA (3-chloroperbenzoic acid) in $CDCl_3$ yielded a mixture of compounds, the major one of which was shown to be epoxide **2** by comparison of TLC R_f value, MS, UV, IR, $[\alpha]_D$, and ^{1}H-, and ^{13}C-NMR spectra.

Pintoxolane C (**3**) turned out to be an isomer of **2**. The molecular formula, $C_{21}H_{32}O_6$, was established by HR-EI-MS and ^{13}C-NMR data (*Table*). The IR spectrum of **3** exhibited absorptions for OH (3455 cm^{-1}), ester (1769 cm^{-1}), ketone (1717 cm^{-1}), olefin (1625 cm^{-1}), and epoxide (1247 cm^{-1}) functionalities, the same structural features found in **2**. Like **2**, compound **3** was UV-active and showed two broad *singlet*s at δ(H) 6.30 and 5.71 (each 1 H), and a sharp 3-H *singlet* at δ(H) 3.78 (CO_2Me) in the ^{1}H-NMR spectrum (*Table*), consistent with the presence of an α,β-unsaturated methyl ester. Comparison of the NMR spectra of **3** with those of **2** revealed that these molecules are indeed structurally similar. The ^{1}H-NMR spectrum of **3** closely matched the data for pintoxolane B (**2**) except different chemical shifts for H–C(7) (δ(H) 2.81–2.83 (*m*)) and H–C(12) (δ(H) 3.20–3.22 (*m*)), and the signals for H–C(1), H–C(14), Me(19), and Me(20) had shifted from δ(H) 3.32 (*q*, $J = 7.5$, 1 H), 4.33 (br. *d*, $J = 7.3$, 1 H), 1.13 (*s*, 3 H), and 0.89 (*d*, $J = 6.7$, 3 H), respectively in compound **2** to δ(H) 3.79–3.81 (*m*, 1 H), 4.48 (br. *d*, $J = 3.5$, 1 H), 1.30 (*s*, 3 H), and 1.08 (*d*, $J = 7.0$, 3 H) in **3**, respectively. The overall planar structure of **3** was fully established by analyzing the ^{1}H,^{1}H-COSY, HSQC, and $^{2,3}J$(C,H) HMBC correlations (*Fig. 1*). On the basis of these combined data, the identity and locations of the substituents on the cembrane ring, as well as the unsaturation pattern, were assumed to be unaltered in **3**. Comparison of ^{1}H,^{1}H-COSY and overall NMR data of **2** and **3** strongly indicated that all of these

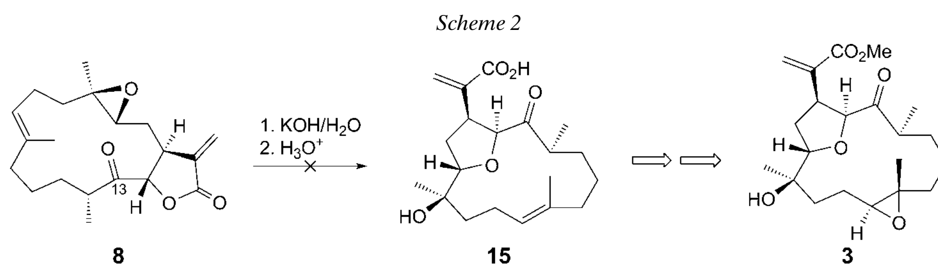
subtle differences had to be associated with a change of the relative configuration at both C(12) and C(14) of **3**.

Using NOESY to probe this portion of **3**, we observed a series of informative correlations that enabled us to investigate the relative configuration of the seven stereogenic C-atoms (*Fig. 2*). Whereas the Me(18) H-atoms exhibited NOE correlations with H–C(7) and H_α–C(2), H–C(14) correlated only with H–C(1). The vinylic Me(19) H-atoms also exhibited additional correlations that included H–C(12), CH_2(6), and Me(20). An additional set of correlations was detected between H–C(3), and H_β–C(6) and H_β–(2), but not with H–C(14). That both C(3) and C(14) have the (*S**)-configuration was supported by the (3.5 Hz) small coupling constant observed between H–C(3) and H–C(14), consistent with the *trans*-orientation shown in structure **3** ($^3J_{trans}$ is always notably smaller than $^3J_{cis}$ in five-membered rings) [11]. On the basis of these data, compound **3** was determined to be the C(12), C(14) diastereoisomer of pintoxolane B (**2**). Thus, the relative configuration of pintoxolane C (**3**) is proposed as (1*S**,3*S**,4*R**,7*S**,8*S**,12*R**,14*S**). Furthermore, we speculate that **3** represents the absolute structure of pintoxolane C on the basis of biogenetic considerations (*Schemes 1 and 2*) and optical-rotation data.

The facile rearrangement of eupalmerin acetate (**12**) to **13** (*Scheme 1*), together with the co-occurrence of compounds **1**–**3** with many 3,4-epoxy-γ-cembranolides not unlike **12** within the same specimen of *E. pinta*, provide circumstantial support that the pintoxolanes might be synthesized *in vivo* from these abundant γ-lactones. Indeed, it is tempting to speculate that one such 3,4-epoxy-γ-cembranolide, succinolide (**8**), might serve as a precursor to pintoxolane C (**3**) *via* an analogous tandem saponification–oxacyclization sequence (*Scheme 2*). Yet, all of our attempts to rearrange succinolide (**8**) to 3,14-oxa-bridged intermediate **15** using an assortment of reaction conditions were unsuccessful. Parallel attempts to directly rearrange another 3,4-epoxy-13-oxo-γ-cembranolide avaiable from a previous investigation, eupalmerone (**11**) [3c], to pintoxolane A carboxylic acid (**14**) also failed. Thus, we hypothesize that the oxo functionality at C(13) in succinolide (**8**) and eupalmerone (**11**) halts the tandem saponification–oxacyclization sequence.

Compounds **1**–**3** were sent to *NCI* and tested at a single dose of 10 μM against a human three-cell panel consisting of MCF 7 (breast), NCI-H460 (non-small cell lung), and SF-268 (CNS). Compound **2** displayed growth percentages of 74, 106, and 119, respectively, whereas compound **3** showed 102, 98, and 117% inhibition against the same cells. Compound **1**, on the other hand, exhibited significant growth inhibition with smaller percentages of 3, 0, and 79, respectively. Pintoxolane A (**1**) was, therefore,

Scheme 2



selected for further *in vitro* testing in the NCI-60 DTP human tumor cell line screen conducted at five concentration levels. These tests revealed that compound **1** was active against essentially all cancer cells with GI_{50} values at the 10^{-5} level. For instance, the GI_{50} values against the MCF 7, NCI-H460, and SF-268 cell lines were 43.5, 50.5, and 39.4 μM, respectively. Only one cell line was inhibited with a GI_{50} value within the order of 10^{-6} M (SR (leukemia, 5.1 μM)).

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Experimental Part

General. All solvents used were either of spectral grade or were distilled from glass prior to use. The percentage yields of compounds **1**–**3** are based on the weight of the dry hexane extract. TLC: Glass pre-coated SiO_2 plates; spots visualized using a UV lamp at λ 254 nm or by exposure to I_2 vapor. Column chromatography (CC): silica gel (SiO_2, 35–75 mesh). Optical rotations: *Autopol IV* automatic polarimeter. UV Spectra: *Shimadzu UV-2401 PC* UV/VIS spectrophotometers; λ_{max} (log ε) in nm. IR Spectra: *Nicolet Magna FT-IR 750* spectrometer; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR spectra: *Bruker DRX-500* FT-NMR spectrometer. MS: Recorded at the Mass Spectrometry Laboratory of the University of Illinois at Urbana–Champaign with a *Waters 70-VSE* double focusing sector instrument; in *m*/*z*.

Animal Material. The gorgonian specimen, collected by Dr. *Juan A. Sánchez* in May 1996, belongs to a deep-water form of *Eunicea pinta* (BAYER & DEICHMANN, 1958). This species, which appears as openly tall branched colonies, was described in [1].

Collection, *Extraction*, *and Isolation*. Fresh specimens of the gorgonian coral *Eunicea pinta* (phylum Cnidaria; class, Anthozoa; subclass, Gorgonia; order, Alcyonaria; family, Plexauridae) were collected by hand using SCUBA at depths of 110–140 ft off San Andrés Island, Colombia. A voucher specimen (No. EPSAI-1996) was deposited with the Chemistry Department of the University of Puerto Rico, Río Piedras Campus. The organism was frozen and lyophilized prior to extraction. The dry specimens (2.2 kg) were cut into small pieces and extracted using a mixture of $CHCl_3$/MeOH (1:1) (2×4 l). After filtration, the crude extract was concentrated and stored under vacuum to yield a brown-green gum (247 g) that was suspended in H_2O (2 l) and extracted with hexane (3×2 l). Concentration under reduced pressure yielded 173.9 g of the hexane extract as a dark-brown oil that was chromatographed over SiO_2 (1.0 kg) using mixtures of hexane/acetone of increasing polarity (0–100%). A total of 24 fractions, *Frs. I–XXIV* were generated on the basis of TLC and ^{1}H-NMR analyses.

Purification of *Fr. IV* (10 g) by size-exclusion chromatography over *Bio-Beads SX-3* with toluene as eluent led to five fractions, the last of which was fractionated consecutively over SiO_2 (200 g) with $CHCl_3$ and then SiO_2 (4.0 g) using a mixture of AcOEt/acetone/$CHCl_3$ 4:1:95 to afford *pintoxolane A* (**1**; 111 mg, 0.064%). *Fr. V* (5.1 g), *VI* (5.0 mg), and *VII* (5.5 mg) were pooled together and purified further by size-exclusion chromatography as described above, to yield ten fractions. Purification of the last two fractions (4.0 g) by CC (SiO_2 (200 g); 10% AcOEt/hexane) afforded nine additional subfractions, *Subfrs. A–I*. Subsequent purification of the penultimate *Subfr. H* (2.7 g) by CC (SiO_2 (80 g); 5% AcOEt/$CHCl_3$, and then SiO_2 (13 g); 5% i-PrOH/hexane) yielded *pintoxolane B* (**2**; 76 mg, 0.044%) along with *pintoxolane C* (**3**; 9.3 mg, 0.005%).

Further purification of the hexane extract afforded seven known compounds, namely, *succinolide* (**8**; 3.0 g, 1.7%), *(1*S*,3*R*,4*R*,7*E*,11*E*)-3,4;15,17-diepoxycembra-7,11-diene* (**7**; 22 mg, 0.01%), *(−)-eunicenone* (**5**; 43 mg, 0.02%), *12-epieupalmerone* (**6**; 4.5 g, 2.6%), *euniolide* (**9**; 1.7 g, 1.0%), *pseudoplexauric acid methyl ester* (**4**; 38 mg, 0.02%), and *uprolide H* (**10**; 33 mg, 0.02%) [1][3].

Pintoxolane A (= *Methyl 2-[*rel-*(1*S*,2*R*,5*E*,10*R*,12*R*,13*S*)-2-Hydroxy-2,6,10-trimethyl-11-oxo-15-oxabicyclo[10.2.1]pentadec-5-en-13-yl]prop-2-enoate*; **1**). Yellowish oil. $[\alpha]_D^{20} = -30.3$ ($c = 1.3$, $CHCl_3$). UV (MeOH): 203 (4.0). IR (neat): 3600–3200 (br.), 2968, 2932, 2874, 1773, 1718, 1628, 1460, 1439, 1374, 1253, 1154, 818, 754. ^{1}H- and ^{13}C-NMR: see the *Table*. EI-MS: 364 (1, M^+), 255 (2), 197 (4), 165 (4), 121 (8), 109 (15), 95 (17), 93 (10), 87 (13), 84 (72), 83 (100), 69 (19), 55 (24). HR-EI-MS: 364.2256 (M^+, $C_{21}H_{32}O_5^+$; calc. 364.2250).

Pintoxolane B (= *Methyl 2-[*rel-*(1*S*,2*R*,5*S*,7*S*,11*R*,13*R*,14*S*)-2-Hydroxy-2,7,11-trimethyl-12-oxo-6,16-dioxatricyclo[11.2.1.0^{5,7}]hexadec-14-yl]prop-2-enoate*; **2**). Yellowish oil. $[\alpha]_D^{20} = -26.3$ ($c = 1.3$, $CHCl_3$). UV (MeOH): 202 (4.27). IR (neat): 3442, 2975, 2880, 1770, 1716, 1631, 1455, 1437, 1377, 1247, 1196, 1156, 1026, 915, 819, 730. ^{1}H- and ^{13}C-NMR: see the *Table*. EI-MS: 380 (3, M^+), 362 (5), 197 (6), 181 (12), 163 (10), 155 (12), 125 (10), 109 (19), 85 (66), 83 (100), 71 (12), 55 (25). HR-EI-MS: 380.2190 (M^+, $C_{21}H_{32}O_6^+$; calc. 380.2199).

Pintoxolane C (= *Methyl 2-[*rel-*(1*S*,2*R*,5*S*,7*S*,11*S*,13*S*,14*S*)-2-Hydroxy-2,7,11-trimethyl-12-oxo-6,16-dioxatricyclo[11.2.1.0^{5,7}]hexadec-14-yl]prop-2-enoate*; **3**). Yellowish oil. $[\alpha]_D^{20} = -18.2$ ($c = 1.0$, $CHCl_3$). UV (MeOH): 202 (4.27). IR (neat): 3455, 2932, 2872, 1769, 1717, 1625, 1456, 1433, 1376, 1247, 1196, 1141, 822, 739. ^{1}H- and ^{13}C-NMR: see the *Table*. EI-MS: 380 (4, M^+), 348 (7), 269 (13), 225 (20), 207 (27), 151 (34), 141 (47), 109 (65), 95 (78), 85 (85), 55 (100). HR-EI-MS: 380.2199 (M^+, $C_{21}H_{32}O_6^+$; calc. 380.2199).

3,14-Eupalmeroic Acid (= *2-[*rel-*(1*S*,2*R*,5*E*,10*R*,11*S*,12*R*,13*S*)-2,11-Dihydroxy-2,6,10-trimethyl-15-oxabicyclo[10.2.1]pentadec-5-en-13-yl]prop-2-enoic Acid*; **13**). The extraction of *eupalmerin acetate* (**12**) from the Caribbean gorgonian octocoral *E. mammosa* has been described in [7]. A suspension of **12** (553 mg, 1.47 mmol) in 3% aq. KOH (20 ml) was stirred vigorously at 50° for 20 h and then cooled, diluted with H_2O (50 ml), the reaction was quenched with 5N HCl until slightly acidic, and the mixture was extracted with Et_2O (2 × 15 ml). The combined org. extracts were washed with sat. NaCl, dried ($MgSO_4$), and concentrated. The white foam obtained was passed through a short column of SiO_2 (20 g) and eluted with an isocratic solvent mixture of $CHCl_3$/MeOH/AcOH 97:3:0.5 to afford 140 mg (27%) of pure **13** obtained as a colorless oil. The $[\alpha]_D^{20}$, IR, UV, MS, and ^{1}H- and ^{13}C-NMR data were identical in all respects to those reported in [8].

Pintoxolane A Carboxylic Acid (= *2-[*rel-*(1*S*,2*R*,5*E*,10*R*,12*R*,13*S*)-2-Hydroxy-2,6,10-trimethyl-11-oxo-15-oxabicyclo[10.2.1]pentadec-5-en-13-yl]prop-2-enoic Acid*; **14**). A mixture of **13** (31.4 mg, 0.089 mmol) and the *Dess–Martin* periodinane (DMP; 39.8 mg, 0.094 mmol) in anh. CH_2Cl_2 (10 ml) was stirred at 25° for 20 min before refluxing for 2 h. The cooled mixture was further diluted with CH_2Cl_2 (10 ml) and washed with H_2O (2 × 5 ml). Following evaporation of the dried ($MgSO_4$) solvent, purification of the oily residue was achieved by CC (SiO_2 (5 g); $CHCl_3$/MeOH/AcOH 97:3:0.5) to yield **14** (17.2 mg, 55%) as the sole product. Yellowish oil. $[\alpha]_D^{20} = -33.3$ ($c = 1.2$, $CHCl_3$). UV (MeOH): 203 (4.0). IR (neat): 3401, 2922, 2873, 1712, 1626, 1455, 1377, 1259, 1164, 1086, 755. ^{1}H-NMR ($CDCl_3$): 3.06–3.08 (*m*, H–C(1)); 2.06–2.10 (*m*, H_α–C(2)); 1.94–1.96 (*m*, H_β–C(2)); 3.99 (*t*, $J = 8.0$, H–C(3)); 1.83–1.85 (*m*, CH_2(5)); 2.42–2.44 (*m*, H_α–C(6)); 2.11–2.13 (*m*, H_β–C(6)); 5.32 (br. *t*, $J = 7.5$, H–C(7)); 2.11–2.13 (*m*, H_α–C(9)); 1.96–1.98 (*m*, H_β–C(9)); 1.66–1.68 (*m*, H_α–C(10)); 1.50–1.52 (*m*, H_β–C(10)); 1.96–1.98 (*m*, H_α–C(11)); 1.08–1.10 (*m*, H_β–C(11)); 3.06–3.08 (*m*, H–C(12)); 4.23 (*d*, $J = 9.7$, H–C(14)); 6.42 (br. *s*, H_α–C(17)); 5.83 (br. *s*, H_β–C(17)); 1.17 (*s*, Me(18)); 1.62 (br. *s*, Me(19)); 0.99 (*d*, $J = 6.5$, Me(20)). ^{13}C-NMR ($CDCl_3$): 44.6 (*d*, C(1)); 33.3 (*t*, C(2)); 86.3 (*d*, C(3)); 73.2 (*s*, C(4)); 37.2 (*t*, C(5)); 22.9 (*t*, C(6)); 128.5 (*d*, C(7)); 132.2 (*s*, C(8)); 37.9 (*t*, C(9)); 21.7 (*t*, C(10)); 30.1 (*t*, C(11)); 40.0 (*d*, C(12)); 213.3 (*s*, C(13)); 86.6 (*d*, C(14)); 138.2 (*s*, C(15)); 171.0 (*s*, C(16)); 129.0 (*t*, C(17)); 25.2 (*q*, C(18)); 15.3 (*q*, C(19)); 13.0 (*q*, C(20)). HR-ESI-MS: 373.2000 ($[M + Na]^+$, $C_{20}H_{30}NaO_5^+$; calc. 373.1991).

Semisynthesis of Pintoxolane B (**2**). A soln. of **1** (3.0 mg, 0.008 mmol) in dry benzene (1.0 ml) was treated with *m*CPBA (2.0 mg, 0.01 mmol), stirred at 25° for 45 min, poured into a sat. $NaHCO_3$ soln. (3 ml), and extracted with Et_2O (2 × 5 ml). The combined org. extracts were washed with brine, dried ($MgSO_4$), and concentrated, and the residue was purified by flash chromatography on SiO_2 (AcOEt/

hexane 1:4) to afford 2.9 mg (93%) of a 3:1 mixture of epoxides. All data (MS, UV, IR, $[\alpha]_D$, ^{1}H-, and ^{13}C-NMR and TLC R_f value) for the major isomer obtained were identical with those recorded from an authentic sample of **2**. An undetermined amount of unreacted **1** was also detected by TLC.

In Vitro *Screening of Compounds* **1**–**3** *against Human Tumor Cell Lines.* Each compound was exposed to 60 human tumor cell lines, including lung, colon, melanoma, prostate, ovarian, breast and kidney cancers at five different doses for 48 h. These cytotoxicity tests were conducted under the *NCI*'s *Developmental Therapeutics Program* (*DTP*) and were performed as described in [1]. Anticancer-activity screening by the DTP is conducted following the general protocol: most of the compounds screened have no antiproliferative activity (up to 85%). To avoid screening inactive compounds across all the cell lines, a prescreen is done using three highly sensitive cell lines (breast MCF-7, lung NCI-H640, and CNS SF-268). Antiproliferative activity must be seen in these cell lines in order to continue to the 60 cell line panel. The 60 different human tumor lines are incubated with five different doses of compound, and a sulforhodamine blue (SRB) assay is performed after 48 h to determine cytotoxicity. From the five point curve, the GI_{50} (growth inhibition by 50%) values are extrapolated. For the specific screening methods from the DTP website, visit: http://www.dtp.nci.nih.gov/branches/btb/ivclsp.html.

REFERENCES

[1] Y.-P. Shi, A. D. Rodríguez, C. L. Barnes, J. A. Sánchez, R. G. Raptis, P. Baran, *J. Nat. Prod.* **2002**, *65*, 1232.

[2] J. W. Blunt, B. R. Copp, R. A. Keyzers, M. H. G. Munro, M. R. Prinsep, *Nat. Prod. Rep.* **2013**, *30*, 237, and previous articles in this series.

[3] a) J. J. Morales, J. R. Espina, A. D. Rodríguez, *Tetrahedron* **1990**, *46*, 5889; b) A. D. Rodríguez, H. Dhasmana, *J. Nat. Prod*. **1993**, *56*, 564; c) A. D. Rodríguez, Y. Li, H. Dhasmana, C. L. Barnes, *J. Nat. Prod*. **1993**, *56*, 1101.

[4] M. Peniston, A. D. Rodríguez, *J. Nat. Prod*. **1991**, *54*, 1009; M. Kobayashi, E. Nakano, *J. Org. Chem*. **1990**, *55*, 1947.

[5] L. F. Johnson, W. C. Jankowsky, 'Carbon-13 NMR Spectra', John Wiley & Sons, New York, 1972; K. Pihlaja, E. Kleinpeter, 'Carbon-13 NMR Chemical Shifts in Structural and Stereochemical Analysis', VCH Publishers, Inc., New York, 1994.

[6] *Wavefunction, Inc.*, 18401 Von Karman Avenue, Suite 370, Irvine, CA 92612 USA.

[7] L. A. Fontán, W. Y. Yoshida, A. D. Rodríguez, *J. Org. Chem*. **1990**, *55*, 4956.

[8] A. D. Rodríguez, I. C. Piña, A. L. Acosta, C. Ramírez, J. J. Soto, *J. Org. Chem*. **2001**, *66*, 648.

[9] S. E. Ealick, D. Van der Helm, A. J. Weinheimer, *Acta Crystallogr., Sect. B.* **1975**, *31*, 1618; D. Van der Helm, S. E. Ealick, A. J. Weinheimer, *Cryst. Struct. Commun*. **1974**, *3*, 167.

[10] A. D. Rodríguez, I. C. Piña, C. L. Barnes, *J. Org. Chem*. **1995**, *60*, 8096.

[11] L. M. Jackman, S. Sternhell, 'Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry', Pergamon Press, Oxford, 1969.

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