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# Structure of (13αH,14βH)-Jeunicin

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## Abstract

$C_{20}H_{30}O_4$, $M_r = 334.5$, is orthorhombic, $P2_12_12_1$, with $a = 8.921$ (2), $b = 18.076$ (4), $c = 22.647$ (5) Å, $V = 3652.0$ Å$^3$ [at 113 (2) K]; $a = 8.962$ (4), $b = 18.30$ (1), $c = 22.96$ (1) Å, $V = 3765.5$ Å$^3$, $Z = 8$, $D_m = 1.184$, $D_x = 1.180$ Mg m$^{-3}$ (at 295 K). The compound is a toxic cembranolide isolated from the gorgonian *Eunicea mammosa*. The molecular structure and absolute configuration have been determined from 4191 X-ray intensity data. The final $R$ factor is 0.063. The compound is an epimer of jeunicin.

## Introduction

The diterpenes jeunicin (I) (van der Helm, Enwall, Weinheimer, Karns & Ciereszko, 1976) and eunicin (II) (Hossain, Nicholas & van der Helm, 1968) have been isolated as the major cembranolides in the gorgonian *Eunicea mammosa* from Jamaica and Bimini, respectively. Both are found to be cytotoxic against the National Cancer Institute's KB cell line. Because the crude extract of the Bimini gorgonian showed confirmed antineoplastic activity in NCI's *in vivo* bioassay against P-388 lymphocytic leukemia, we have subjected it to systematic fractionation with guidance at each stage by bioassays in NCI's *in vitro* KB and LE cell lines. This approach has led to the isolation of, in addition to eunicin. a second cytotoxic cembranolide in minor amounts. It is shown to have the structure of (13αH,14βH)-jeunicin (III).

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(I)    (II)    (III)
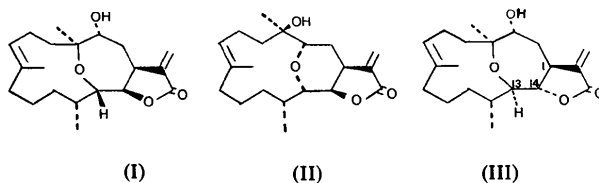
The bioactive material is contained in the chloroform phase. This fraction is further refined by three successive partitions using hexane, carbon tetrachloride and chloroform *vs* 20, 25 and 35% water in methanol respectively.

Chromatography of the residue from this phase on Florisil in benzene/ethyl acetate, and re-chromatography of the active fraction on silica gel using acetone/hexane affords (13αH,14βH)-jeunicin, followed closely by eunicin.

(13αH,14βH)-Jeunicin (III) melts at 420–420.5 K; $[\alpha]_D^{20°C} = -31.1°$ ($c = 3.0$ g dm$^{-3}$, CHCl$_3$); $C_{20}H_{30}O_4$ (calc.: C 71.82, H 9.04%; found: C 71.74, H 8.93%), $m/e$ 334 ($M^+$), IR (KBr): 3560, 3520 (–OH), 1760 (C=O), and 1665 cm$^{-1}$ (C=C). Its $^1$H NMR spectrum (CDCl$_3$) shows methyl signals at $\delta$ 1.01, $d$, $J = 7$ Hz (Me–C–H); 1.25, $s$ (Me–C–O) and 1.57, $bs$ (Me–C=C). One proton absorption appears at $\delta$ 3.22, $m$ (H-1); 3.27, $t$, $J = 8$ Hz (H-13, coupled to H-14 by NMDR); 3.69, $dd$, $J = 8$, 10 Hz (H-14, coupled to H-1 by NMDR); 3.90, $dd$, $J = 3$, 5 Hz (H-3); 5.26, $bd$, $J = 10$ Hz (H-7, coupled to 8-Me by NMDR); 5.41, $d$, $J = 3$ Hz and 6.13, $d$, $J = 3$ Hz (H$_2$C=C, both coupled to H-1 by NMDR).

The spectral data demonstrate the presence of an α-methylenic lactone, a methyl-substituted olefin, a cyclic ether and an alcohol function. A 14-membered cembrane ring is suggested by the nature of the six C

atoms (three lactone, three methyls) which cannot be part of the single carbocycle required. Of the two types of cembranolides already isolated from *E. mammosa* (I and II), the $^1$H NMR spectrum of the new compound shows greater similarity to that of jeunicin (I), particularly in respect to the doublet character of the vinyl proton signal (H-7). In eunicin this signal appears as a triplet.

Table 1. *Positional parameters* ($\times 10^4$) *and B or $B_{eq}$ for the non-H atoms*

| | x | y | z | B (Å$^2$) |
|---|---|---|---|---|
| (a) Molecule 1 | | | | |
| C(11) | 9748 (4) | −1082 (2) | 468 (1) | 1·44 (5) |
| C(21) | 10452 (4) | −318 (2) | 539 (1) | 1·44 (5) |
| C(31) | 9481 (4) | 207 (2) | 899 (2) | 1·57 (5) |
| C(41) | 10121 (4) | 982 (2) | 1019 (2) | 2·21 (6) |
| C(51) | 9018 (4) | 1431 (2) | 1398 (2) | 2·20 (6) |
| C(61) | 9012 (5) | 2271 (2) | 1294 (2) | 2·65 (7) |
| C(71) | 8367 (5) | 2505 (2) | 695 (2) | 2·85 (7) |
| C(81) | 6890 (4) | 2140 (2) | 550 (2) | 2·34 (6) |
| C(91) | 6826 (4) | 1603 (2) | 145 (2) | 2·32 (6) |
| C(101) | 5558 (5) | 1102 (2) | 13 (2) | 2·75 (7) |
| C(111) | 5985 (4) | 272 (2) | −12 (2) | 2·05 (6) |
| C(121) | 6805 (4) | −63 (2) | 523 (1) | 1·56 (5) |
| C(131) | 6953 (4) | −913 (2) | 395 (1) | 1·30 (5) |
| C(141) | 8389 (4) | −1110 (2) | 63 (1) | 1·40 (5) |
| C(151) | 11103 (4) | −1535 (2) | 310 (1) | 1·48 (5) |
| C(161) | 12390 (4) | −1152 (2) | 586 (2) | 1·72 (5) |
| C(171) | 11242 (4) | −2149 (2) | 2 (2) | 2·15 (6) |
| C(181) | 11669 (5) | 980 (3) | 1309 (2) | 3·52 (21)* |
| C(191) | 5577 (5) | 2384 (2) | 925 (2) | 2·55 (7) |
| C(201) | 5984 (4) | 78 (2) | 1100 (2) | 1·93 (6) |
| O(11) | 11949 (3) | −475 (1) | 784 (1) | 1·70 (9)* |
| O(21) | 13666 (3) | −1365 (1) | 647 (1) | 1·96 (9)* |
| O(31) | 8230 (2) | 328 (1) | 505 (1) | 1·34 (8)* |
| O(41) | 6938 (3) | −1362 (1) | 912 (1) | 1·61 (9)* |
| (b) Molecule 2 | | | | |
| C(12) | 7207 (4) | 2016 (2) | 7222 (1) | 1·35 (5) |
| C(22) | 6399 (4) | 2743 (2) | 7106 (1) | 1·40 (5) |
| C(32) | 7307 (4) | 3265 (2) | 6718 (1) | 1·46 (5) |
| C(42) | 6564 (4) | 4017 (2) | 6582 (2) | 1·78 (5) |
| C(52) | 7463 (4) | 4435 (2) | 6108 (2) | 1·72 (5) |
| C(62) | 7384 (4) | 5283 (2) | 6143 (2) | 1·94 (6) |
| C(72) | 8256 (4) | 5612 (2) | 6675 (2) | 1·99 (6) |
| C(82) | 9784 (4) | 5263 (2) | 6746 (2) | 1·72 (5) |
| C(92) | 10052 (4) | 4825 (2) | 7210 (1) | 1·67 (5) |
| C(102) | 11385 (4) | 4336 (2) | 7312 (2) | 1·80 (5) |
| C(112) | 10985 (4) | 3536 (2) | 7489 (1) | 1·61 (6) |
| C(122) | 10030 (4) | 3077 (2) | 7056 (1) | 1·31 (5) |
| C(132) | 9986 (4) | 2261 (2) | 7296 (1) | 1·42 (5) |
| C(142) | 8561 (4) | 2057 (2) | 7632 (1) | 1·40 (5) |
| C(152) | 5925 (4) | 1515 (2) | 7372 (2) | 1·65 (5) |
| C(162) | 4602 (4) | 1828 (2) | 7053 (2) | 1·70 (5) |
| C(172) | 5852 (5) | 903 (2) | 7689 (2) | 2·59 (7) |
| C(182) | 4927 (4) | 3973 (2) | 6393 (2) | 2·73 (17)* |
| C(192) | 10859 (4) | 5396 (2) | 6245 (2) | 1·90 (6) |
| C(202) | 10686 (4) | 3110 (2) | 6434 (2) | 1·74 (5) |
| O(12) | 4966 (3) | 2510 (1) | 6852 (1) | 1·64 (9)* |
| O(22) | 3388 (3) | 1557 (1) | 6970 (1) | 2·21 (11)* |
| O(32) | 8580 (2) | 3443 (1) | 7084 (1) | 1·36 (8)* |
| O(42) | 10157 (2) | 1713 (1) | 6844 (1) | 1·51 (9)* |

* $B_{eq} = \frac{1}{3}$ trace $\tilde{\mathbf{B}}$.

The effective doses for 50% inhibition of the *in vitro* KB and LE cell lines by (13αH,14βH)-jeunicin (NSC 304687) are 25 and 7·5 µg ml$^{-1}$ respectively.

(13αH,14βH)-Jeunicin was recrystallized by slow evaporation at room temperature from 95% ethanol. An orthorhombic crystal of dimensions 0·132 × 0·220 × 0·440 mm was used for data collection and for unit-cell determination. All X-ray measurements were carried out on a Nonius CAD-4 automatic diffractometer fitted with low-temperature equipment in which the crystal was in a stream of cold nitrogen gas. The space group was uniquely determined to be $P2_12_12_1$ from systematic absences. The unit-cell dimensions were determined from the $+2\theta$ and $-2\theta$ values of 43 reflections, distributed throughout reciprocal space using Cu $K\alpha_1$ radiation ($\lambda = 1·54051$ Å). The intensities of 4191 reflections [3758 reflections had $I > 2\sigma(I)$] with $2\theta < 150°$ were measured at 113 (2) K using Ni-filtered Cu $K\alpha$ radiation ($\lambda = 1·5418$ Å) and $\theta$–$2\theta$ scans. Other data-collection parameters have been described previously (Chang, Ciereszko, Hossain & van der Helm, 1980). Lorentz and polarization corrections were applied, but no absorption corrections were made.

The crystal structure was determined by direct methods using tangent refinement (Karle & Karle, 1966) with the program *MULTAN* (Germain, Main & Woolfson, 1971). The structure was refined using block-diagonal least-squares methods (Ahmed, 1966). Because the data were taken at low temperature only the O atoms and the C(18) atoms were refined with anisotropic thermal parameters. All other C atoms were refined with isotropic thermal parameters. H atoms were located from difference Fourier syntheses and their parameters were refined with isotropic temperature factors. The refinement was terminated when all shifts were less than half the corresponding standard deviations. Scattering factors for C and O atoms were taken from *International Tables for X-ray Crystallography* (1974, p. 73), and those for H atoms from Stewart, Davidson & Simpson (1965). The R value for all data (4191) based on the final parameters (Table 1)* ($R = \sum ||kF_o| - |F_c||/\sum |kF_o|$) was 0·063. The weights of $F$ in the least-squares calculations were calculated from $\sigma(I)$ which were determined from counting statistics (Ealick, van der Helm & Weinheimer, 1975).

The absolute configuration was determined with Bijvoet differences (Bijvoet, Peerdeman & Van Bommel, 1951) in two experiments, once using the anomalous dispersion of oxygen ($f' = 0·047, f'' =$

0·032) for Cu radiation and secondly using the anomalous dispersion of C ($f'$ = 0·035, $f''$ = 0·021) and O ($f'$ = 0·090, $f''$ = 0·073) for Cr radiation (*International Tables for X-ray Crystallography*, 1974, p. 149). In each experiment, after structure refinement, a set of reflections was chosen, which were the most sensitive to the anomalous-dispersion effects, based on the largest values for $||F_+|^2 - |F_-|^2|_{(calc)}/\sigma(I)_{obs}$. The selected set of reflections were measured repeatedly in all octants. For the experiment with Cu radiation 16 of 18 reflections, and for Cr radiation 13 of 15 reflections indicated the absolute configuration shown in the figures.

## Discussion

The bond distances and angles are given in Figs. 1 and 2, and a stereoscopic view of one of the two independent molecules is shown in Fig. 3. The molecule belongs to the *β*-configurational series (Weinheimer, Matson, Hossain & van der Helm, 1977), and is epimeric to jeunicin (van der Helm *et al.*, 1976) at positions 13 and 14. The opposite configuration at position 14 means that the methylene-substituted *γ*-lactone ring is *trans*-fused to the cembrane ring in the present compound rather than *cis*-fused as is observed in jeunicin.

The overall conformation of the two molecules is similar as can be seen from the torsion angles given in Table 2. There are, however, differences in confor-

### Table 2. *Torsion angles* (°)

E.s.d.'s are between 0·6 and 1·0°.

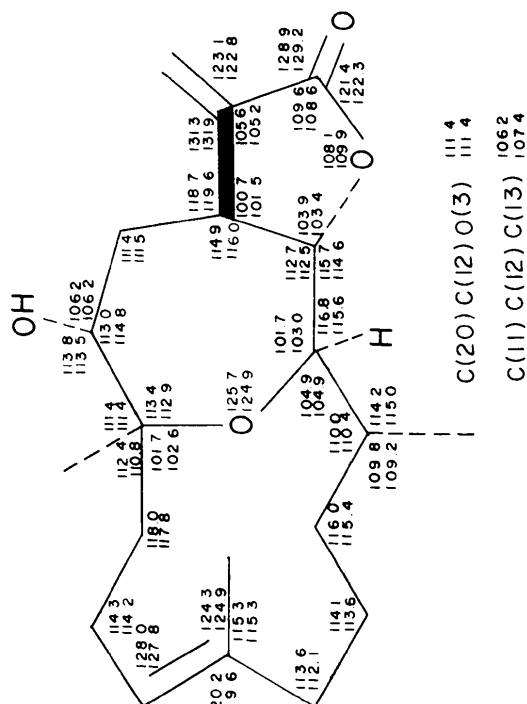|  | Molecule 1 | Molecule 2 |
|---|---|---|
| C(1)–C(2)–C(3)–C(4) | 177 | 180 |
| C(2)–C(3)–C(4)–C(5) | −179 | −171 |
| C(3)–C(4)–C(5)–C(6) | −149 | −151 |
| C(4)–C(5)–C(6)–C(7) | 70 | 71 |
| C(5)–C(6)–C(7)–C(8) | 48 | 45 |
| C(6)–C(7)–C(8)–C(9) | −106 | −111 |
| C(7)–C(8)–C(9)–C(10) | 169 | 169 |
| C(8)–C(9)–C(10)–C(11) | −130 | −130 |
| C(9)–C(10)–C(11)–C(12) | 54 | 60 |
| C(10)–C(11)–C(12)–C(13) | 175 | 172 |
| C(11)–C(12)–C(13)–C(14) | 90 | 100 |
| C(12)–C(13)–C(14)–C(1) | 75 | 69 |
| C(13)–C(14)–C(1)–C(2) | −85 | −86 |
| C(14)–C(1)–C(2)–C(3) | 71 | 71 |
| C(1)–C(2)–O(1)–C(16) | 29 | 28 |
| C(2)–O(1)–C(16)–C(15) | −12 | −10 |
| O(1)–C(16)–C(15)–C(1) | −11 | −12 |
| C(16)–C(15)–C(1)–C(2) | 28 | 28 |
| C(15)–C(1)–C(2)–O(1) | −34 | −33 |
| C(2)–C(3)–O(3)–C(12) | 97 | 96 |
| C(3)–O(3)–C(12)–C(13) | −64 | −70 |
| O(3)–C(12)–C(13)–C(14) | −21 | −13 |
| C(1)–C(2)–C(3)–O(3) | −69 | −66 |



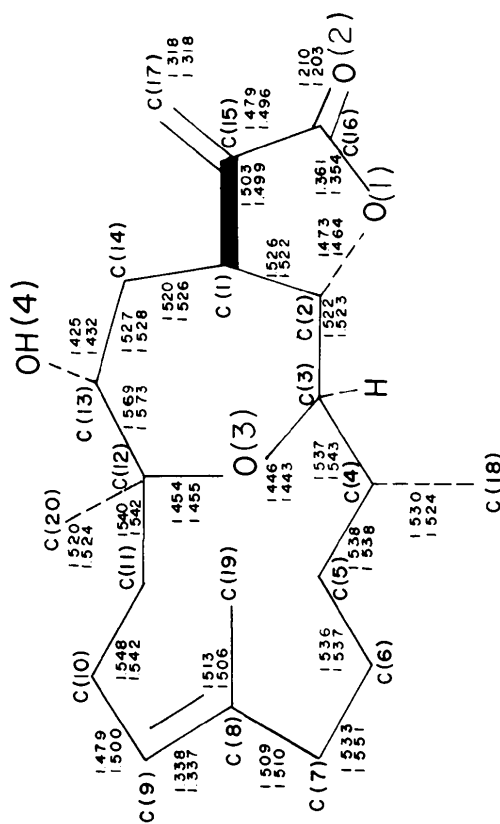Fig. 2. Bond angles (°). E.s.d.'s are between 0·3 and 0·4°.



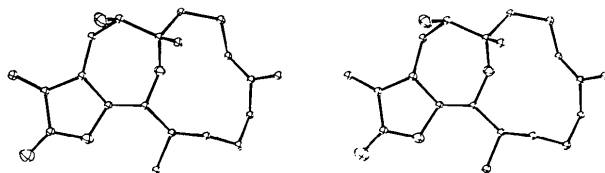Fig. 1. Atom numbering and bond distances (Å). E.s.d.'s are between 0·004 and 0·006 Å.

Fig. 3. Stereoscopic view of one of the independent molecules (Johnson, 1965).

### Table 3. *Least-squares planes*

(a) Plane through C(11)–C(141)

(molecule 1) $4.417x + 3.765y - 19.084z = 2.738$

Plane through C(12)–C(142)

(molecule 2) $-3.698x + 5.284y + 19.499z = 12.336$

Deviations from planes (Å) (e.s.d.'s 0·004 Å)

|       | Mol. 1 | Mol. 2 |       | Mol. 1 | Mol. 2 |
|-------|--------|--------|-------|--------|--------|
| C(1)  | 0·267  | 0·147  | C(8)  | 0·061  | −0·019 |
| C(2)  | 0·730  | 0·604  | C(9)  | 0·604  | 0·556  |
| C(3)  | −0·188 | −0·213 | C(10) | 0·107  | 0·003  |
| C(4)  | 0·158  | 0·194  | C(11) | 0·031  | 0·074  |
| C(5)  | −0·884 | −0·842 | C(12) | −0·754 | −0·660 |
| C(6)  | −0·372 | −0·296 | C(13) | −0·764 | −0·607 |
| C(7)  | 0·574  | 0·592  | C(14) | 0·429  | 0·467  |

(b) Plane through C(11) C(21) C(151) C(161) and O(11)

$1.825x + 5.534y - 21.035z = 0.396$

Plane through C(12) C(22) C(152) C(162) and O(12)

$-2.451x + 5.864y + 20.479z = 14.402$

Deviations from planes (Å) (e.s.d.'s 0·004 Å)

|       | Mol. 1 | Mol. 2 |       | Mol. 1 | Mol. 2 |
|-------|--------|--------|-------|--------|--------|
| C(1)  | −0·200 | −0·196 | O(1)  | −0·127 | −0·114 |
| C(2)  | 0·202  | 0·191  | C(17) | 0·463  | 0·440  |
| C(15) | 0·129  | 0·132  | O(2)  | −0·018 | −0·045 |
| C(16) | −0·005 | −0·013 |       |        |        |

mation as indicated by the distances of the atoms from the least-squares plane (Table 3) through the 14-membered rings for the two molecules. Also the angles between these least-squares planes and the ones through the γ-lactone ring differ and are calculated to be 18·6° in molecule 1 and 9·1° in molecule 2.

The *cis*-fused γ-lactone rings in cembranolides are quite common, but *trans*-fusion, observed in the present structure, has only been found in one previous structure, lobophytolide (Tursch, Braekman, Daloze, Herin & Karlsson, 1974), an α-series cembranolide from an Indonesian soft coral. The γ-lactone ring in lobophytolide is considerably more planar (Karlsson, 1977) (r.m.s. deviation: 0·054 Å) than those in

(13αH,14βH)-jeunicin (r.m.s. deviation 0·151 Å in molecule 1 and 0·145 Å in molecule 2). The γ-lactone rings in (13αH,14βH)-jeunicin are in the half-chair conformation, with the twofold axis passing through C(16) and the mid-point of C(1)–C(2). This is indicated by $\Delta C_2 = [\sum_m (\psi_i - \psi_{i'})^2 / m]^{1/2}$ for C(1)–C(2) which is 1° for both molecules (Duax & Norton, 1975), and by the pseudorotation parameters $\Delta$ and $\psi_m$ (Altona, Geise & Romers, 1968) which are −1·1 and 2·6, and 34·9 and 34·0°, for molecules 1 and 2 respectively {tan $\Delta/2 = [(\psi_2 + \psi_4) - (\psi_1 + \psi_3)]/3.0777 \psi_o$ and $\psi_o = \psi_m \cos \Delta/2$}. Weak intermolecular hydrogen bonding exists between O(41) and O(21) (1 + x,y,z), 2·980 Å, and O(42) and O(22) (x − 1,y,z), 2·910 Å.

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