Structure of Peunicin

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Abstract. $C_{20}H_{26}O_4$, $M_r = 330 \cdot 4$, orthorhombic, $P2_12_12_1$, $a = 9 \cdot 5920 (6)$, $b = 11 \cdot 6344 (10)$, $c = 15 \cdot 6859 (15)$ Å, $V = 1750 \cdot 5$ Å³ at 113 ± 2 K, $Z = 4$, $D_c = 1 \cdot 213$, $D_m = 1 \cdot 223$ Mg m⁻³ at 298 K. The compound is a toxic cembranolide extracted from the gorgonian *Eunicea succinea*. The molecular structure and absolute configuration are determined from X-ray data (2059 reflections measured at 113 K using Cu $K\alpha$ radiation). The final R factor is $0 \cdot 038$.

Introduction. A new marine cembranolide, peunicin $\{C_{20}H_{26}O_4$; m.p. 448–449 K; $[\alpha]_D^{24\,°C} +93 \cdot 6°$, $c = 7 \cdot 8$ g/ml, CHCl₃; $R_f = 0 \cdot 55$ [benzene/ethyl acetate (3/1), silica gel H]$\}$, was isolated as the major component from the *n*-hexane extract of *Eunicea succinea* which was collected off the Atlantic coast of Panama (Chang, 1977). Peunicin is one among several cembranolides isolated from the *Eunicea mammosa-succinea* complex (Ciereszko & Karns, 1973). The compound shows anticiliary action (Hadfield & Ciereszko, 1978) and is cytoxic. Peunicin is less active than other cembranolides in this series. The effective doses for 50% inhibition (ED_{50}) of the *in vitro* KB and PS cell lines are the relatively high concentrations of 30 and 32 μg/ml respectively.

The IR spectrum of peunicin shows the existence of a γ-lactone (1770 cm⁻¹) and an α,β-unsaturated ketone (1675 cm⁻¹). The latter feature is also indicated by the strong UV absorption at 241·3 nm (methanol, $\varepsilon = 1 \cdot 27 \times 10^4$). A tentative structural assignment was made on the basis of the observed PMR and CMR spectrum (Chang, 1977).

Peunicin was recrystallized from an ethanol solution as transparent orthorhombic crystals. A crystal of dimensions $0 \cdot 3 \times 0 \cdot 2 \times 0 \cdot 1$ 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 a low-temperature apparatus. 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 -2θ values of 48 reflections, distributed throughout all quadrants of the reciprocal lattice, and measured at 113 ± 2 K using Cu $K\alpha_1$ radiation ($\lambda = 1 \cdot 54051$ Å). Intensities of all 2059 unique reflections with $2\theta \leq$

144° were measured at 113 ± 2 K using Cu $K\bar{\alpha}$ radiation ($\lambda = 1 \cdot 5418$ Å) and employing θ–2θ scan techniques with a variable scan width of $(0 \cdot 9 + 0 \cdot 09 \tan \theta)°$. The maximum scan time was 60 s with $\frac{2}{3}$ of the time used in scanning the peak and $\frac{1}{6}$ for each of the two backgrounds. Out of the total, 176 reflections were considered unobserved $[I \leq 2\sigma(I)]$ and were left out of the least-squares calculations. Lorentz and polarization corrections were applied to the data. No absorption correction was made.

The structure was determined by direct methods using tangent refinement (Karle & Karle, 1966) with the program *MULTAN* (Germain, Main & Woolfson, 1971). The structure was refined by least-squares methods using anisotropic thermal parameters for the C and O atoms. H atoms were located from a difference Fourier map and their parameters were refined isotropically. The final R factor ($R = \sum ||kF_o| - |F_c||/\sum |kF_o|$) is $0 \cdot 032$ for 1868 reflections included in the least-squares calculations, and $0 \cdot 038$ for all 2059 reflections. All least-squares refinements were performed by using a block-diagonal least-squares program (Ahmed, 1966) in which the quantity $\sum W_F(|kF_o| -$

Table 1. *Observed and calculated Bijvoet differences*

$$DEL = \{[F^2(+) - F^2(-)]/\tfrac{1}{2}[F^2(+) + F^2(-)]\} \times 100$$
$$SF = [F^2(+) - F^2(-)]/\sigma(F^2)$$
$$F^2(+) = F^2(hkl), F^2(-) = F^2(h\bar{k}l)$$

| | | | DEL | | SF | |
			Observed	Calculated	Observed	Calculated
1	4	1	−7·4	−4·5	−0·7	−1·0
2	5	3	+0·4	+7·1	+0·1	+1·8
2	1	9	+4·1	+3·8	+0·9	+1·0
1	5	3	+1·3	+4·8	+0·3	+1·4
2	4	11	+4·7	+5·8	+0·3	+0·9
2	13	8	−1·3	−5·8	−0·1	−1·5
3	5	5	+1·3	+4·3	+0·1	+1·0
5	6	5	−4·2	−5·6	−0·5	−1·5
3	3	8	+10·2	+8·9	+0·8	+1·9
3	1	15	−0·1	+3·5	−0·0	+0·9
8	9	3	−1·0	−6·9	−0·1	−1·1
8	9	5	−16·5	−8·3	−0·5	−1·1
9	7	3	−6·0	−4·1	−0·9	−1·1
9	7	5	+15·0	+13·4	+0·4	+1·1
5	8	10	−10·6	−5·5	−0·7	−0·9

Table 2. Positional parameters ($\times 10^5$) for the non-hydrogen atoms

Standard deviations are in parentheses.

	x	y	z
O(1)	9135 (17)	32255 (12)	6175 (8)
O(2)	30446 (16)	17258 (14)	7460 (9)
O(3)	−9531 (16)	−17339 (12)	6040 (9)
O(4)	9524 (18)	40694 (13)	18996 (9)
C(1)	−1428 (21)	14156 (17)	9426 (12)
C(2)	1190 (22)	1199 (16)	10477 (12)
C(3)	−4677 (21)	−6062 (16)	3415 (12)
C(4)	−19551 (21)	−8993 (17)	2822 (12)
C(5)	−26423 (22)	−12373 (19)	−5501 (13)
C(6)	−16802 (23)	−17007 (19)	−12465 (13)
C(7)	−9211 (23)	−7802 (18)	−17414 (12)
C(8)	3257 (22)	−8779 (18)	−21100 (13)
C(9)	9731 (23)	1071 (19)	−25890 (12)
C(10)	23245 (22)	5513 (18)	−21696 (13)
C(11)	20237 (22)	11045 (17)	−13273 (12)
C(12)	25058 (20)	7975 (17)	−5572 (12)
C(13)	21943 (21)	15506 (17)	1853 (11)
C(14)	7446 (21)	20839 (16)	2722 (12)
C(15)	1402 (21)	21076 (17)	17390 (12)
C(16)	7141 (22)	32342 (17)	14765 (12)
C(17)	−632 (24)	18368 (17)	25528 (13)
C(18)	34575 (23)	−2040 (19)	−3749 (14)
C(19)	11875 (26)	−19581 (21)	−20798 (17)
C(20)	−29802 (24)	−4369 (21)	9310 (14)

$|F_c|^2$ was minimized. The weights of F's were determined from counting statistics (Ealick, van der Helm & Weinheimer, 1975). The atomic scattering factors for O and C atoms were taken from *International Tables for X-ray Crystallography* (1974a), and those of H from Stewart, Davidson & Simpson (1965).

The absolute configuration of the molecule was determined by comparing the calculated and observed differences in the intensities of 15 Friedel pairs (Bijvoet, Peerdeman & van Bommel, 1951), using the anomalous dispersion of Cu radiation by O atoms. The f' and f'' values for O atoms are taken from *International Tables for X-ray Crystallography* (1974b). The intensity differences for 14 reflections are in agreement with the absolute configuration shown in the figures. The Bijvoet parameters are listed in Table 1. The final positional parameters for non-hydrogen atoms are given in Table 2.* The atoms are numbered following the scheme outlined by Weinheimer, Matson, Hossain & van der Helm (1977). The bond distances and angles calculated on the basis of the final parameters are shown in Figs. 1 and 2 respectively. A stereoview of the single molecule is shown in Fig. 3.

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* Lists of structure factors, anisotropic thermal parameters and H atom parameters have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 34763 (15 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.
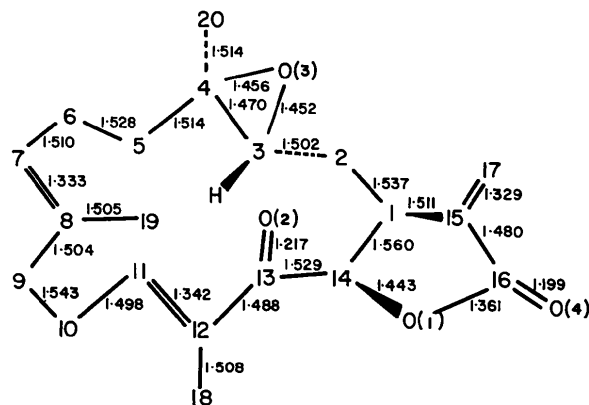

Fig. 1. Chemical structure, numbering scheme, and bond distances (Å) in peunicin. Estimated standard deviations range between 0·002 and 0·003 Å.
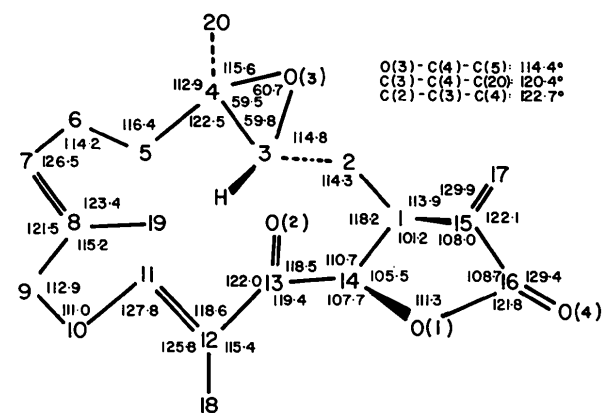

Fig. 2. Bond angles (°) in peunicin. Estimated standard deviations for all bonds: 0·2°.
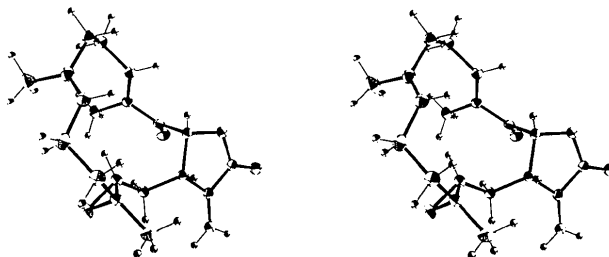

Fig. 3. Stereoview of a single molecule of peunicin. Thermal ellipsoids correspond to 50% probability (Johnson, 1965).

Discussion. The molecule consists of a 14-membered cembrane ring with a γ-lactone cis-fused at C(1) and C(14), and an epoxide trans-fused at C(3) and C(4). The two endocyclic double bonds at C(7)—C(8) and at C(11)—C(12) are both trans with respect to the cembrane ring. Peunicin differs from other known γ-lactone cembranolides such as eupalmerin acetate (EPA) (Ealick, van der Helm & Weinheimer, 1975), eunicin (Hossain, Nicholas & van der Helm, 1968), ieunicin (van der Helm, Enwall, Weinheimer, Karns & Ciereszko, 1976) and lobophytolide (Karlsson, 1977) in having an α,β-unsaturated ketone. The bond lengths

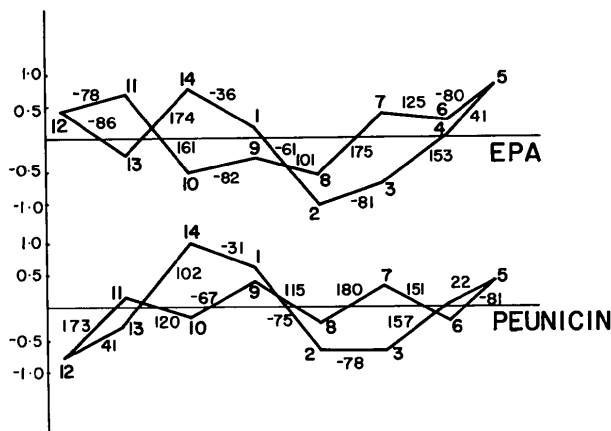Fig. 4. Torsion angles (°) and deviations (Å) from the least-squares plane in the cembrane ring in EPA and peunicin. Standard deviations of torsion angles range between 0·2 and 0·3°.

Table 3. *Deviations* (Å) *of atoms from the least-squares planes through the five atoms of the γ-lactone rings*

	EPA	Lobo-phytolide	Jeunicin	Peunicin
O(1)	0·104	0·030	0·127	0·097
C(1)	0·159	0·074	0·181	0·127
C(2)	−0·161	−0·064	−0·188	−0·137
C(15)	−0·105	−0·061	−0·115	−0·080
C(16)	0·004	0·021	0·004	−0·007
C(17)	−0·388	−0·271	−0·459	−0·285
O(4)	0·027	−0·044	0·015	0·009

and bond angles of peunicin are normal and compare well with those of the known cembranolides. Conformational features of peunicin are similar to those of EPA. Torsion angles in the cembrane ring in the two molecules are compared in Fig. 4. The only significant differences are in the region of the ketone group in peunicin. Fig. 4 also shows the deviations of individual atoms from the least-squares planes of the cembrane ring. The maximum deviation [atom C(2)] is 1·02 Å.

The γ-lactone ring deviates significantly from the ideal envelope conformation (the sum of the endocyclic torsion angles, $\sum |\tau|$, is 76°). Such deviations in γ-lactone conformations have been observed in a variety of compounds other than cembranolides (McPhail & Sim, 1973). The least-squares-plane parameters for the lactone ring in peunicin are given in Table 3 along with the relevant values for three other cembranolides. The tabulated results show that: (1) In all molecules, the lactone ring is distinctly non-planar. (2) The deviation from planarity is least in lobophytolide (r.m.s. deviation 0·054 Å), compared to that in EPA (r.m.s. deviation 0·121 Å), jeunicin (r.m.s. deviation 0·140 Å) and peunicin (r.m.s. deviation 0·101 Å). It may be mentioned that in lobophytolide, the γ-lactone is *trans*-fused with the cembrane ring while it is *cis*-fused in the other three molecules. (3) The *exo* methylene group [atom C(17)] is far out of the plane of the five-membered ring, whereas the carbonyl group lies close to the plane. The deviation of the methylene C is a maximum (0·45 Å) in jeunicin. In view of the suggestion (see, for instance, Lee *et al.*, 1977) that a lactone carrying an exocyclic methylene group is essential for biological activity, it would be of interest to investigate the correlation, if such exists, between the stereochemical features of the lactone as given in Table 3 and the biological activity of the molecules. It is relevant to mention that jeunicin is the most active in PS and KB tests among the cembranolides referred to here.

References

AHMED, F. R. (1966). SFLS program *NRC*-10. National Research Council, Ottawa, Canada.
BIJVOET, J. M., PEERDEMAN, A. F. & VAN BOMMEL, A. J. (1951). *Nature (London)*, **168**, 271–272.
CHANG, C. Y. (1977). Thesis, Univ. of Oklahoma.
CIERESZKO, L. S. & KARNS, T. K. B. (1973). *Biology and Geology of Coral Reefs*, Vol. II, *Biology*, Part 1, pp. 183–203. New York: Academic Press.
EALICK, S. E., VAN DER HELM, D. & WEINHEIMER, A. J. (1975). *Acta Cryst.* B**31**, 1618–1626.
GERMAIN, G., MAIN, P. & WOOLFSON, M. M. (1971). *Acta Cryst.* A**27**, 368–376.
HADFIELD, M. G. & CIERESZKO, L. S. (1978). *Drugs and Food from the Sea*, edited by P. N. KAUL & C. J. SINDERMANN, pp. 145–150. Univ. of Oklahoma Press.
HOSSAIN, M. B., NICHOLAS, A. F. & VAN DER HELM, D. (1968). *Chem. Commun.* pp. 385–386.
International Tables for X-ray Crystallography (1974*a*). Vol. IV, p. 73. Birmingham: Kynoch Press.
International Tables for X-ray Crystallography (1974*b*). Vol. IV, p. 149. Birmingham: Kynoch Press.
JOHNSON, C. K. (1965). *ORTEP*. Report ORNL-3794. Oak Ridge National Laboratory, Tennessee.
KARLE, J. & KARLE, I. L. (1966). *Acta Cryst.* **21**, 849–859.
KARLSSON, R. (1977). *Acta Cryst.* B**33**, 2032–2034.
LEE, K. H., HALL, I. H., MAR, E. C., STARNES, C. O., ELGEBALY, S. A., WADDELL, T. A., HADGRAFT, R. I., RUFFNER, C. A. & WEIDNER, I. (1977). *Science*, **196**, 533.
MCPHAIL, A. T. & SIM, G. A. (1973). *Tetrahedron*, **29**, 1751–1758.
STEWART, R. F., DAVIDSON, E. R. & SIMPSON, W. T. (1965). *J. Chem. Phys.* **42**, 3175–3187.
VAN DER HELM, D., ENWALL, E. L., WEINHEIMER, A. J., KARNS, T. K. B. & CIERESZKO, L. S. (1976). *Acta Cryst.* B**32**, 1558–1560.
WEINHEIMER, A. J., MATSON, J. A., HOSSAIN, M. B. & VAN DER HELM, D. (1977). *Tetrahedron Lett.* **34**, 2923–2926.