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CHEMICAL STUDIES OF MARINE INVERTEBRATES. IV.[1a] TERPENOIDS LXII.[1b]
EUNICELLIN, A DITERPENOID OF THE GORGONIAN EUNICELLA STRICTA.
X-RAY DIFFRACTION ANALYSIS OF EUNICELLIN DIBROMIDE.

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Repeated silica gel column fractionation of the petroleum ether extract of dried Eunicella stricta (collected in 45-75 feet off Banyuls-sur-Mer, France)[2] afforded in 0.005% yield (based on dry animal weight) a new compound, eunicellin (I), m.p. 186-188°, $\{\alpha\}_D$-36°, after several crystallisations from ether-petroleum ether.

The structure of eunicellin (I) is based on an X-ray diffraction analysis of the dibromide (II). The formula is in agreement with and can, in part, be deduced from the physical-chemical studies described below. The stereochemistry illustrated in Figs 1 and 2 was derived from the X-ray analysis alone.

The empirical formula $C_{28}H_{42}O_9$ of eunicellin was established by elementary analysis and by high resolution mass spectrometry (molecular ion at m/e 522.28298). The mass spectrum showed the consecutive loss of four fragments of mass 60, suggesting the presence of four acetate groups. Their presence was established by the NMR spectrum of (I), showing four singlets at 0.79 and 0.92 ppm (assignable to an isopropyl group on the basis of double resonance experiments), two singlets of 3H each at 1.56 and 1.59 ppm (assignable to methyls on carbon bearing oxygen rather than methyls on double bond on the basis of solvent effect), and two absorptions 1H each at 5.27 and 5.43 ppm (assignable to $C=CH_2$). No proton was exchangeable with D_2O. The UV spectrum of (I) showed the absence of any conjugated chromophore. The IR spectrum, while confirming the presence of acetates, showed no ν_{OH} band. Eunicellin displayed no Cotton effect and hence was devoid of carbonyl functions. Eight of the nine oxygen atoms of (I) having been located as acetates, the last one must necessarily be involved in an ether linkage.

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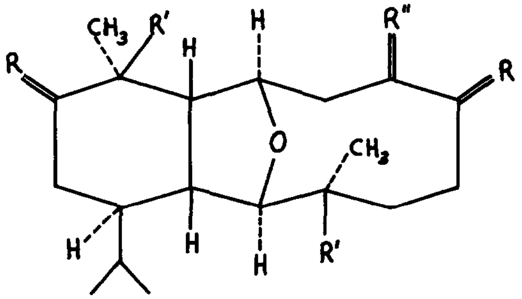
The presence of the C=CH_2 group in eunicellin was confirmed by ozonolysis of I to yield noreunicellone (III), ($C_{27}H_{40}O_{10}$; M-60 peak at m/e 464) in which the presence of all four acetates was confirmed by its NMR spectrum. No conclusion regarding ring size could be derived from the broad $\nu_{C=O}$ band in the IR spectrum. The failure of generating a conjugated ketone from III by acid or basic conditions indicated that no acetate was β to the carbonyl function.

Catalytic hydrogenation of I with Pd on $BaSO_4$ in ethyl acetate afforded dihydroeunicellin (IV), m.p. 151-153° ($C_{28}H_{44}O_{10}$; M^+ at m/e 524), which showed no chemical evidence of further unsaturation; consequently, barring the presence of a non-reducible double bond, eunicellin (I) had to be tricyclic, one of the rings involving the ether function.

The parent tetrol V (m.p. 209-211°, $C_{20}H_{34}O_5$; M^+ at m/e 354) was obtained by LAH reduction (2 hours reflux in ether) or basic hydrolysis (10% NaOH in MeOH) of eunicellin (I). Since acetylation of V yielded a monoacetate ($C_{22}H_{36}O_6$; m.p. 208-212°) and a diacetate (VI) ($C_{24}H_{38}O_7$; m.p. 160-163°) while oxidation of V with CrO_3 in pyridine afforded a diketone (VII)($C_{20}H_{30}O_5$; m.p. 186-189°), it follows that two of the four acetates of (I) are secondary and the other tertiary. The IR spectrum of VII exhibited $\nu_{C=O}$ at 1705 cm^{-1}. thus establishing that both keto groups in VII and hence both secondary acetates of eunicellin (I) are located on six-membered or larger rings.

Important structural evidence was deduced from the periodate oxidation of the tetrol V, since it yielded an unstable compound ($C_{20}H_{32}O_5$; M^+ at m/e 352) which contained both a methylketone and an aldehyde, as evidenced by its NMR spectrum (1H absorption at 9.66 ppm and 3H singlet at 2.31 ppm) as well as its IR spectrum showing bands at 2740, 1725 and 1715 cm^{-1}. This proves the presence in eunicellin of the sequence -CHOAc-C(CH_3)OAc- on a ring of 6 or more carbon atoms.



	R	R'	R"
I	(H)OAc	OAc	CH_2
II	(H)OAc	OAc	(Br)CH_2Br
III	(H)OAc	OAc	O
IV	(H)OAc	OAc	(H)CH_2
V	(H)OH	OH	CH_2
VI	(H)OAc	OH	CH_2
VII	O	OH	CH_2

Simultaneously with the chemical investigations an X-ray diffraction study of the dibromide (II) was initiated. X-ray analysis was thought to be a particularly suitable technique in view of the very small quantity of material available.

The dibromide $C_{28}H_{42}O_9Br_2$ crystallised as small colourless, irregular prisms m.p. 211-213°. The crystals were monoclinic with cell dimensions: a = 20.1±1, b = 11.15±2, c = 15.34±6Å, β = 116.4°±4, Z = 4, Dc = 1.42 gm/cc. Copper radiation (λ = 1.5418Å). The space group C2 was assigned from absent reflexions and the successful solution of the structure in the non-centric space group.

Rotation and Weissenberg photographs were taken about the a and b axes and the intensities of 2550 reflexions estimated by eye from the h0ℓ to h8ℓ layer-line photographs. The nine scale factors were included as variables in the refinement.

The three-dimensional sharpened Patterson map gave clear evidence for the space group C2 and the location of the two bromine atoms. These were used to derive phases for the first electron density map and the positions of all 37 light atoms (excluding hydrogens) in the empirical formula were found in four cycles.

Initially all atoms were treated as carbons and their coordinates assigned solely from peak heights and criteria based on reasonable neighbouring distances. The oxygen atoms were gradually identified from peaks with heights greater than 8 e./Å^3. The complete molecule was located by connecting the nearest atomic neighbours. No chemical assumptions were used but the resultant formula (II) contained all elements deduced from the chemical studies.

The atomic positions were refined through one cycle of full matrix, and six cycles of block diagonal calculations with anisotropically vibrating bromine atoms. The final reliability factor was 13.2%. A difference electron density map calculated at this stage showed only a few peaks height above 1 e./Å^3 (1.2 e./Å^3 max) and several around 0.7 e./Å^3, attributable to hydrogen atoms.

Figs. 1a and 1b illustrate the interatomic distances and angles in eunicellin dibromide. Although the standard deviations in atomic positions are comparatively large the bond lengths and angles agree well with expected values and confirm the assignment of the structure. Thermal parameters are satisfactory and range from B $\sim$ 3Å^2 for the inner, more tightly bound atoms to B $\sim$ 6Å^2 for the methyl groups at the periphery.

The stereochemistry of the molecule is shown in Fig. 2 and it should be noted that it also applies to eunicellin (I) itself, since zinc debromination of the dibromide (II) regenerated the parent compound (I). The six-membered ring is in the chair form and is cis fused to the 10-membered ring. The acetate groups are planar. The strain introduced by the ether bridge is symmetrically distributed. Full details of the structure analysis, as well as of the chemical and NMR data, will be published elsewhere.

Biogenetically, the carbon skeleton of eunicellin is unexceptional since it is made up of four isoprene units linked head to tail. However, structurally, it represents an unusual diterpenoid and a search for similar products from marine organisms continues in our laboratories.

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REFERENCES

1. (a) Part III: W. Rüdiger, W. Klose, B. Tursch, H. Budzikiewicz, *Annalen*, in press.
   (b) Part LXI: P. Sengupta, A.K. Chakraborty, A.M. Duffield, L.J. Durham and C. Djerassi, *Tetrahedron*, *24*, 1205 (1968).

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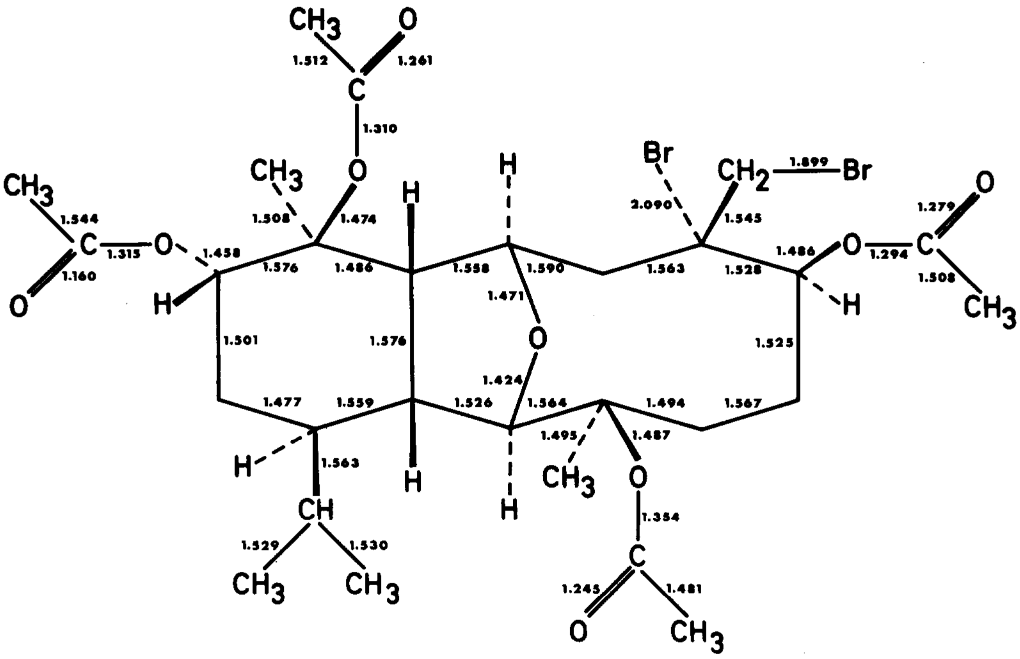
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Fig 1a

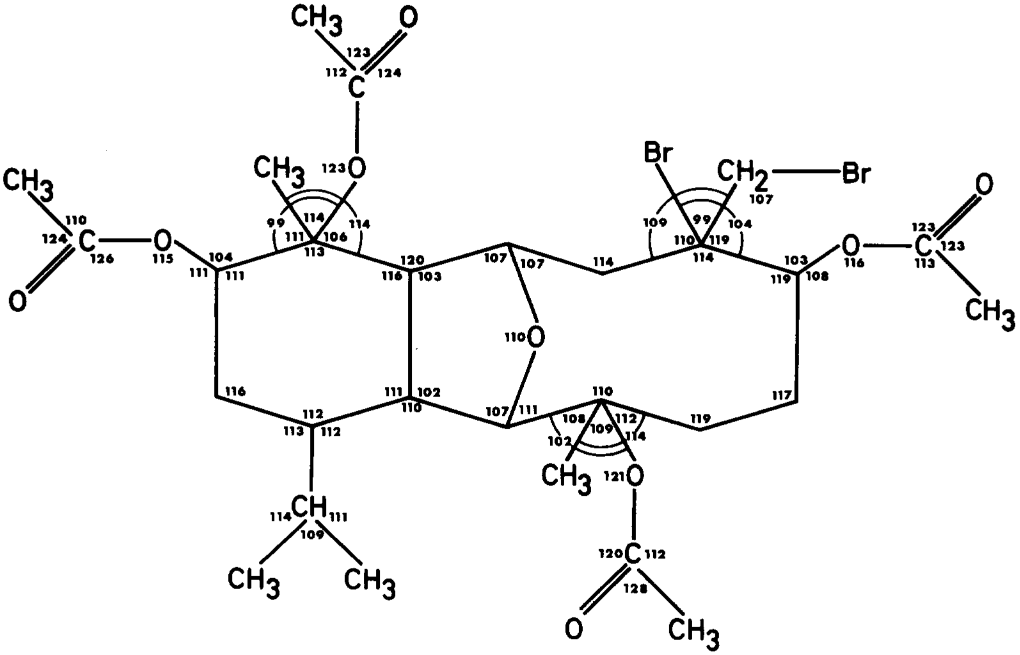
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Fig 1b

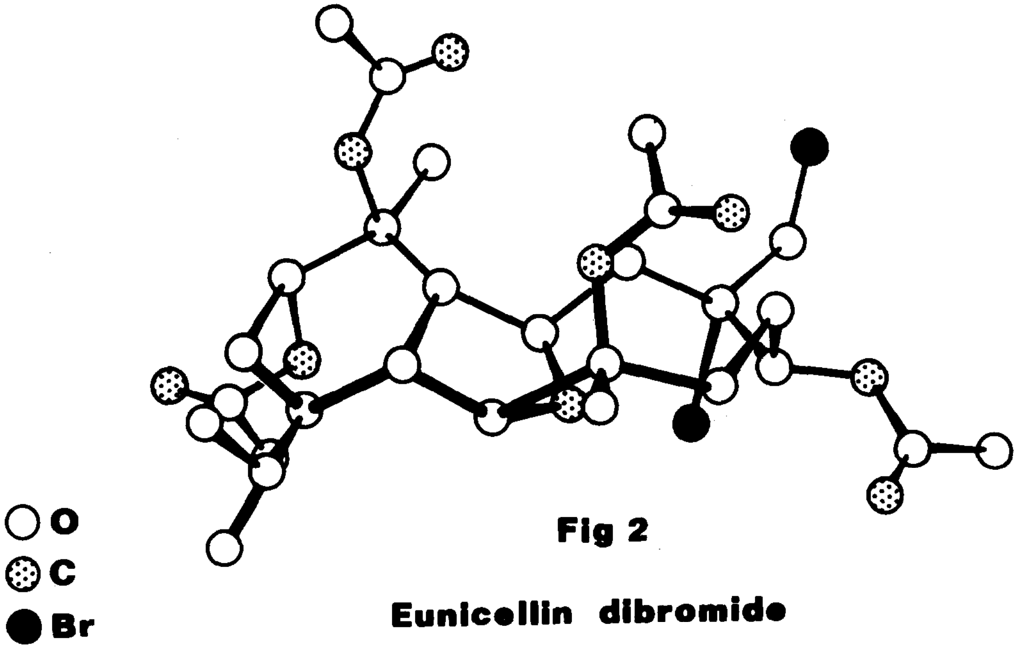

Fig 2

Eunicellin dibromide