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THE STRUCTURES OF ANHYDROBONELLIN AND BONELLIN, THE PHYSIOLOGICALLY ACTIVE PIGMENT FROM THE MARINE ECHIUROID BONELLIA VIRIDIS

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SUMMARY The structure of anhydrobonellin (II) has been proposed by spectroscopic techniques and confirmed by single crystal X-ray diffraction. The relationship of (II) to bonellin is clear and thus the structure of bonellin is certain.

In our previous communication[1] we reported on the extraction of the physiologically active pigment, bonellin, responsible for masculinisation in the marine echiuroid Bonellia viridis and on the basis of physical methods proposed (I) as the most probable structure for the compound.

During this investigation it was observed that the mass spectrum of bonellin contained a major fragmentation in which a molecule of water was eliminated in one step from the molecular ion[2] and it was therefore decided to investigate the chemical dehydration of bonellin. Treatment with cold concentrated sulphuric acid yielded an optically active dehydrated product, anhydrobonellin, which was purified as its crystalline methyl ester (ABME) m.p. 216-7°C. Analysis and mass spectrometry established a molecular formula of $C_{32}H_{34}N_4O_3$, which corresponded to a monomethyl ester of a dehydration product of bonellin. The visible spectrum of ABME $\lambda_{max.}^{CHCl_3}$ 676, 627, 572sh, 536, 502, 470sh and 404nm was shifted to higher wavelength relative to bonellin dimethyl ester (BDME) and a kinetic study of the dehydration indicated that the reaction took place in one step without any intermediate stages.†

Analysis of the ^1H and ^{13}C n.m.r. spectra of BDME and ABME (Table I) established the nature and site of the dehydration reaction. The assignments in Table I are made by comparisons with the spectra of known compounds[3] and the use of off-resonance proton decoupling. Several assignments have been confirmed using specific proton decoupling experiments.

Comparison of the two ^1H n.m.r. spectra indicated that one ester methyl group and one meso proton of BDME were absent in ABME. The ^{13}C n.m.r. spectra indicated that the signal due to one of the aliphatic ester carbonyls of BDME had moved downfield by 24 p.p.m. in ABME, to a position characteristic of a ketone and also that the signal of one of the meso carbon atoms of BDME had been displaced downfield by 14 p.p.m. in ABME and lacked a hydrogen atom. This information is consistent with one of the propionic acid groups in bonellin having cyclised onto the neighbouring C-15 meso position to form a cyclic ketone. Such cyclisations

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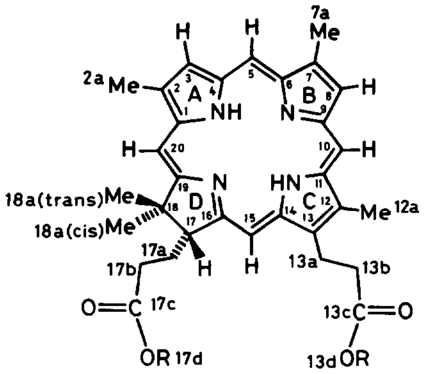
† We are indebted to Dr. P. Camilleri, University of Malta, for this information

are known for porphyrins and chlorins but usually require vigorous conditions.[4]

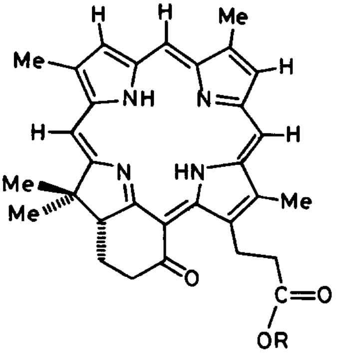
Structure (I) for bonellin has two propionic acid sidechains which could cyclise in this manner; one at C-17 attached to the saturated chlorin-like ring D and one at C-13 attached to the porphyrin-like ring C.

The changes in ^{13}C n.m.r. chemical shifts for the carbon atoms of ABME relative to BDME at 17a (+7.4), 17b (-6.0), 18a (cis) (-3.9) and 18a (trans) (+2.9 p.p.m.) on formation of the cyclic ketone are much larger than those observed for 13a (+1.9), 13b (-0.9) and 12a (+0.4 p.p.m.), and thus it is the C-17 propionic acid sidechain which has cyclised leading to structure II for anhydrobonellin.



I

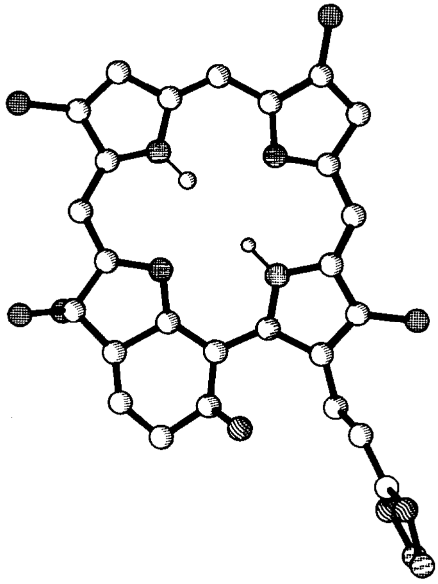
R = H bonellin
R = Me bonellin DME

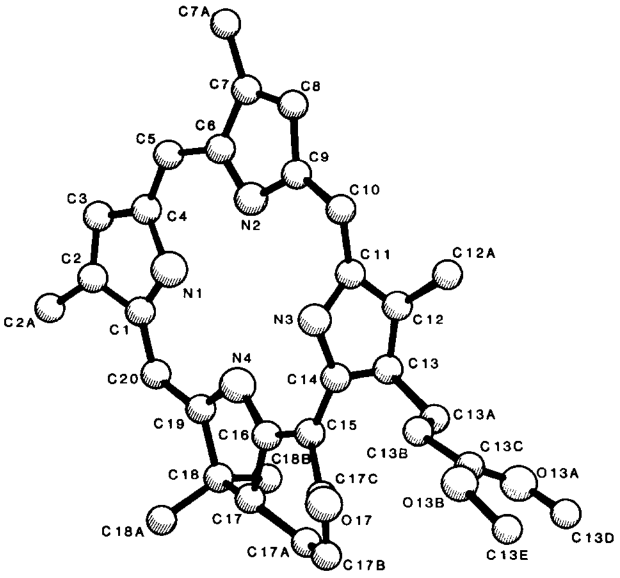


II

R = H anhydrobonellin
R = Me anhydrobonellin ME

Complete confirmation of structure II has been obtained by X-ray diffraction studies on a single crystal of anhydrobonellin methyl ester. The gross sample of ABME consisted of two polymorphs, monoclinic rhombs and triclinic prisms. The first m.p. 167-9°C was monoclinic $P2_1/n$, Z = 8, a not determined, b = 18.3Å, c = 29.4Å, β = 88.0°, and proved the sample to be at least partly racemic. The second, m.p. 223-5°C has crystallographic data M_r = 522.3. Triclinic, $P\bar{1}$. a = 13.96 (2), b = 10.50 (1), c = 10.24 (1) Å, α = 86.35 (5), β = 109.62 (5), γ = 105.21 (5°) from diffractometer measurements (Mo $K\bar{\alpha}$ radiation). V = 1364.5Å^3, Z = 2, D_c = 1.27 g cm^{-3}, F(000) = 556, μ = 0.47 cm^{-1}. The compound was recrystallised from ether/pentane. Data were collected from hk0-8 with θ_{max} = 22.5° on a Stoe STADI-2 2-circle diffractometer (graphite monochromated Mo $K\bar{\alpha}$ radiation). This gave 3112 data of which 1741 unique reflexions with $I > 3\sigma(I)$ were used in subsequent calculations. Lorentz and polarization correction (but none for extinction or absorption) were applied, and the data scaled by a Wilson plot. The structure was solved by direct phasing methods with the SHELX-76 system of crystallographic programs,[5] which was used for all calculations. Complex neutral atomic scattering factors were taken from International Tables for X-ray Crystallography.[6] Full-matrix least squares refinement (unit weights) including isotropic hydrogen atoms converged at R = 0.06 for 1741 observed reflexions ($R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$); R_w = 0.06. In the final cycle all shifts in parameters were less than their standard deviations. The ester group was found to be disordered but all other atoms, including the imine hydrogens were located. Bond distances in the ring agree well with those in porphyrins.[*] Two different computer generated projections of anhydrobonellin methyl ester are shown below. The first clearly shows the overall structure as in (II) whilst the second gives an indication of the shape of the molecule, particularly the puckering of the 6-membered ketone ring and the arrangement of the gem-dimethyl groups, one of which (18B) is almost at right angles to the plane of the macrocyclic ring.





This research constitutes a confirmation of the structure I for bonellin which we proposed as the most probable structure in our previous communication.(1) The only point of uncertainty which remains is the absolute configuration at C-17 in bonellin, as it appears from the centrosymmetric space groups that partial racemisation of this centre takes place during the cyclisation reaction.

One of us (AFP) thanks the I.U.C. for financial help.

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[*] The atomic coordinates are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW. Any request should be accompanied by the full literature citation for this paper.

TABLE I

N.M.R. Data for Bonellin Dimethyl Ester and Anhydrobonellin Methyl Ester

	^{1}H n.m.r. spectra ($CDCl_3$)(τ)		^{13}C n.m.r. spectra ($CDCl_3$)(p.p.m.)	
	BDME	ABME	BDME	ABME
Sidechains				
2a	6.58	6.85 or 6.92	13.4	12.9
7a	6.61	6.85 or 6.92	13.4	13.0
12a	6.62	6.85 or 6.92	11.2	11.4
18a (cis)	7.94	7.95	32.0	27.9
18a (trans)	8.26	8.70	23.4	26.3
17a	7.42 - 7.80 (4Hm)	6.97 - 7.50 (4Hm)	31.8	39.2
17b			27.6	21.6
17c	-	-	173.8 or 174.2	198.5
17d	6.42 or 6.52	-	51.5 or 51.7	-
13a	5.86t	6.16m	21.5	23.4
13b	6.91t	6.64m	36.6	35.5
13c	-	-	173.8 or 174.2	175.9
13d	6.42 or 6.52	6.49	51.5 or 51.7	51.5
Saturated ring				
16	-	-	165.7 or 172.1	168.2 or 173.8
17	5.6 br t	6.10br m	57.8	56.8
18	-	-	49.7	49.1
19	-	-	165.7 or 172.1	168.2 or 173.8
Meso				
5	0.40	0.87	102.1 or 102.5	108.5 or 105.4
10	0.50	1.06	102.1 or 102.5	108.5 or 105.4
15	1.15	-	91.2 or 93.0	106.4
20	1.28	1.86	91.2 or 93.0	91.0
Aromatics				
3	1.24br	1.66br	126.3 or 130.3	127.4 or 130.7
8	1.48br	1.86br	126.3 or 130.3	127.4 or 130.7
other 10C atoms	-	-	131.6 - 151.5	130.8 - 153.1
NH	12.50br	broad	-	-

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