Bonellin, a most Unusual Chlorin

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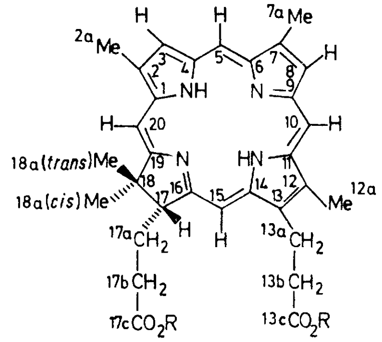
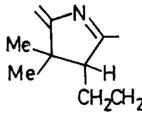
Summary The physiologically active pigment bonellin is shown to be a chlorin unrelated to chlorophyll and having many novel structural features.

Bonellin, the physiologically active[1] pigment of the echiuroid *Bonellia viridis* Rolando, has for over a century received attention from both biologists and chemists.[2] In 1939 Lederer[3] succeeded in isolating crystalline bonellin to which he assigned the formula $C_{31}H_{36}N_4O_4 \pm$ 2H and on the basis of its absorption spectrum suggested that it was related to mesopyrrochlorin. His further suggestion that the two remaining oxygen atoms be placed at C-13 and C-15 (owing to a presumed relationship to chlorophyll) is unacceptable as the absorption spectrum would no longer be that of a simple chlorin.[4] Despite this, the structure tentatively assigned has gradually gained such acceptance that bonellin is treated as a fully characterised natural product.[5] As part of a general programme concerned with physiologically active natural products of marine origin we have reinvestigated the problem and find that bonellin is a unique chlorin for which (**1**, R=H) is the most probable structure.

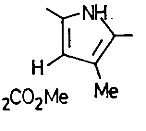


(1)

Bonellin was isolated from the proboscides of *B. viridis* and purified as the diethyl and dimethyl esters; from the latter a zinc complex was prepared. Both esters were always contaminated with a little copper derivative. The dimethyl ester (BDME) had m.p. 55 °C and an intense molecular ion at 554·2928 ± 0·0040 corresponding to $C_{33}H_{38}N_4O_4$. It had λ_{max} ($CHCl_3$) 641, 620 sh, 590, 539, 521, 494, 488, and 394 nm and λ_{max} (6% HCl) 631, 587 sh., 548 sh., 523, and 402 nm† in accord with the previous suggestion of a close relationship to mesopyrrochlorin.[3] The ^1H n.m.r. spectrum ($CDCl_3$) however showed several unusual features, perhaps the most striking of which was the absence of the usual doublet due to the C-18a methyl group.[6] Instead there were two sharp singlets (3H each) at τ 8·2 [C-18a(*trans*) as in other chlorins] and 7·88 [C-18a (*cis*)]. A *gem*-dimethyl grouping in the reduced ring is inferred and confirmed in that irradiation of the multiplet at τ 7·2—8·1 (17a and 17b) reduced the triplet at τ 5·6, due to H-17, to a singlet. Hence C-17 cannot be adjacent to any ring carbon atoms that bear a proton and the sub-unit (**2**) is unequivocally delineated. Such a unit is known in vitamin B_{12} but is unique in chlorins.[7] The next feature of note was the appearance of two signals (1H each) at τ 1·36 (d) and 1·18 (br. s), coupled allylically to two of the three 'aromatic' methyl groups at τ 6·52—6·56. The position is strikingly similar to that of deuteroporphyrin IX[8] (DP IX) and the sub-units (**3**) and (**4**) must be present, another unique feature for a naturally occurring chlorin.[7] The standard sub-unit (**5**) is also present, as shown by the remaining 'aromatic' methyl group and the methylene signals at τ 6·85 and 5·8. There is the standard pattern for the *meso*-protons of a C-15 unsubstituted chlorin with two peaks (1H each) at τ 0·34 and 0·44 plus another pair at τ 1·09 and 1·22.[9] Bonellin is the first naturally occurring chlorin to lack a C-15 substituent. The ^1H spectrum of BDME and bonellin diethyl ester were almost superimposable except that the signals at τ 6·36 and 6·46 (2 × $-CO_2Me$) were replaced by signals at τ 5·88 (2H), 5·98 (2H), 8·83 (3H), and 8·9 (3H) (2 × CO_2CH_2Me). This evidence, coupled with the mass spectral data for bonellin and its esters, proves that bonellin is a dicarboxylic acid.



(2)



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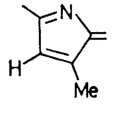

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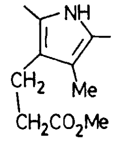

(5)

The units (**2**)—(**5**) may, of course, be joined *via* the *meso*-carbon atoms in a large variety of ways but, on biogenetic grounds structure (**1**, R=Me) is the most probable, bonellin not being directly related to chlorophyll but produced by degradation and reductive alkylation of protoporphyrin IX.

The ^{13}C n.m.r.‡ spectrum of BDME received close study, all assignments being supported by the off-resonance spectrum. Signals for all the carbon atoms were observed, and most assigned by comparison§ with the spectra of either DP IX[10] or various chlorins.[11] Such comparisons leave no doubt that units (**2**)—(**5**) are present in BDME and cast some light on their arrangement.

Two 'aromatic' methyl groups appear at 13·4 (C-2a, C-7a) and one at 11·2 p.p.m. (C-12a) (13·4 and 11·5 p.p.m. for DP IX). Ring atoms C-3 and C-8 at 130·2 and 126·1 p.p.m. also correspond closely with DP IX (128·0 p.p.m.). C-17 and C-18 give signals at 57·7 and 49·7 p.p.m. (corresponding respectively to 54·5 for *trans*-octaethylchlorin and 49·2 p.p.m. for other chlorins[11]). Most importantly, C-18 was

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† Solutions of BDME do not obey Beer's Law and hence intensities are not given here.

‡ Spectra run at high dilution in $CDCl_3$ and values given in p.p.m. downfield of Me_4Si.

§ Details of the comparisons used will be given in the full paper.

shown to be quaternary and C-17 tertiary. The *gem*-dimethyl carbon atoms give resonances at 23·4 (18a, *cis*) and 31·8 p.p.m. (18a, *trans*). These stereo-assignments are in line with those given by Scott[12] to the similar grouping in dicyanocobalaminamide and were confirmed by single frequency decoupling experiments using the well separated frequencies of the aliphatic methyl groups. The signals for the propionate groups attached to reduced and non-reduced rings compare very closely with the corresponding groups in DP IX and a wide range of chlorins. Signals due to C-16 and C-19 are found at 164·8 p.p.m. and one of three signals at 171·3, 173·4, and 173·9 p.p.m. (*cf.* 159·5—168·5 p.p.m., and 167·1—173·4 p.p.m. for a wide range of chlorins[11]). An important feature of the ^{13}C n.m.r. spectrum is that the signals for the *meso*-carbon atoms are found in two groups, C-15 and C-20 at 91·2 and 93·0 p.p.m.¶ and C-5 and C-10 at 102·1 and 102·5 p.p.m. (*cf.* 92·5 and 98·3 p.p.m., respectively, for *trans*-octaethylchlorin[11]). The values for C-15 and C-20 are as expected but those for C-5 and C-10 are *ca.* 4 p.p.m. downfield, as compared with octaethylchlorin. A very recent ^{13}C n.m.r. examination of porphyrins[13] shows that *meso*-carbon atoms flanked by two protons give signals at *ca.* 104 p.p.m. compared with *ca.* 96 p.p.m. when flanked by two simple alkyl groups.[10] Further, in DP IX, the signals due to C-5 and C-10 are *ca.* 2·5—3·5 p.p.m. downfield of C-15 and C-20 and the same trend would be anticipated in the electronically similar chlorins. We conclude that neither C-5 nor C-10 in BDME can be flanked by either two methyl groups or two hydrogen atoms (at C-3 and C-7 or at C-8 and C-12) and take this as strong support for (**1**) as compared with alternative structures, particularly the biogenetically plausible one in which alkylation has occurred at C-12 rather than C-18.

The mass spectrum of bonellin is in full accord with (**4**), fragment ions due to losses of methyl and water being particularly noteworthy.[14]

Clearly bonellin is the first of a new type of relatively simple tetrapyrrolic macrocycles of high physiological activity. The similarity of structure to that postulated for sirohydrochlorin[15] is noteworthy.

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¶ As yet these signals have not been definitely assigned between C-15 and C-20 nor between C-5 and C-10.

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