

152. X-Ray Molecular Structure of a Red Bilin Derivative from *Chlorella protothecoides*

4th Communication on Chlorophyll Catabolism¹⁾

by Norbert Engel and Albert Gossauer*

Institut für Organische Chemie der Universität Freiburg i. Ü., Pérolles, CH-1700 Fribourg

and Karl Gruber and Christoph Kratky

Institut für Physikalische Chemie der Universität Graz, Heinrichstrasse 28, A-8010 Graz

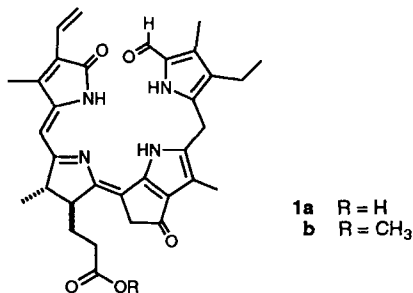
(29.IV.93)

The molecular structure of a chlorophyll *a* catabolite isolated from the culture medium of bleached *Chlorella protothecoides* cells has been determined by X-ray diffraction; the crystal structure shows a distorted helical arrangement of the pyrrole rings and consists of symmetrical dimers located at a crystallographic diad, which are stabilized by six intermolecular H-bonds.

1. Introduction. – In the investigation of the enzymatic degradation of chlorophylls – like in many other domains of molecular biology – the study of a particular microorganism, in this case the microalgae *Chlorella protothecoides*, facilitates substantially the understanding of the same process in more complicated pluricellular organisms. Thus, the elucidation of the structure of the pigments excreted in the culture medium during the process of bleaching of *C. protothecoides* cells, when this green algae is grown in a medium rich in glucose but poor in nitrogen [2], has revealed them as products of degradation of chlorophyll *a* [3] [4] similar to the so-called *RP-14* pigment which has been isolated from dark-bleached excised primary leaves of barley (*Hordeum vulgare* cv. GERBEL) [5].

The present communication deals with an X-ray diffraction study of the molecular structure of the main product of chlorophyll *a* catabolism isolated from *C. protothecoides* which confirms the structure suggested earlier on the basis of analytical data [3].

2. Results and Discussion. – The pigment isolated from the culture medium **1a** was transformed into the corresponding methyl ester **1b** through successive reaction with



¹⁾ Part 3: [1].

carbonyldiimidazole and MeOH in dry THF [3]. Red, needle-shaped crystals were obtained by diffusion of hexane vapour into a solution of **1b** in AcOEt.

The overall molecular conformation is characterized by a distorted left-handed helical arrangement of the pyrrole rings. The angles between least-squares planes through the rings *A*, *D*, and *C* are 30.3° (*A*–*D*) and 22.4° (*D*–*C*). They are larger than the corresponding angles in biliverdin (19.5° and 9.8°, respectively) [6], resulting in a higher helix pitch. The interruption of the conjugated system at the C(10) methylene apparently leads to a distortion of the helical conformation, which manifests itself by a large angle between least-squares planes through ring *B* and *C* (62.2°). The vinyl and the formyl groups lie almost exactly in the plane of the corresponding pyrrole rings and are likely to be involved in the conjugated system of the chromophore.

The structure determination was complicated by disorder of the propionic ester chain, which points into a channel running along the crystallographic *c*-axis. Presumably, the disorder is due to the loss of solvent molecules incorporated into these channels upon crystallization. Some residual density was observed in this region and accounted for by including two pseudo atoms (C) with isotropic a.d.p.'s.

In the crystal, molecules of **1b** occur as symmetric dimers located at the crystallographic diad along the *c*-axis (see the Fig.). These dimers are stabilized by six intermolecular H-bonds between the pyrrolic H-atoms and the carbonyl O-atoms. The crystal structure consists of cylindrical stacks of these dimers with the cylinder axis parallel to *c*. There are no intermolecular H-bonds in addition to the six H-bonds stabilizing the symmetric dimers. A result of this type of packing are the solvent-channels mentioned above.

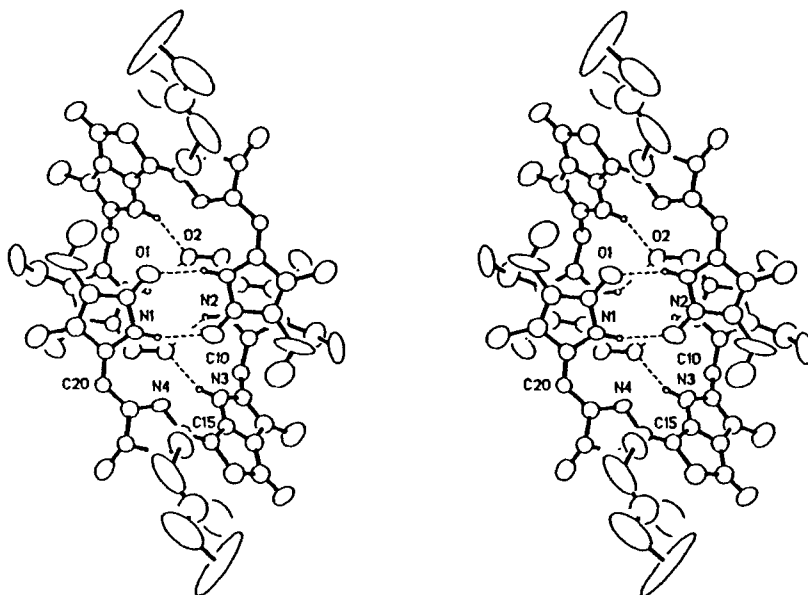


Figure. Stereoscopic representation of the dimer of **1b** projected approximately along the twofold axis. H-Bonds are represented as dotted lines. H-Atoms not involved in H-bonding have been omitted for clarity. Anisotropic atomic displacement ellipsoids have been drawn at the 50% probability level.

Although pigment **1b** is optically active, the absolute configuration at the chiral centers of the molecule could not be determined from the diffraction data; thus the absolute configuration at the chiral centers of the molecule is assumed to be the same as in chlorophyll *a* [7–10].

The present X-ray molecular structure determination is the first one of a natural product of chlorophyll *a* catabolism and confirms the structure already suggested by spectroscopic methods [3] and corroborated by partial synthesis from methyl pyropheophorbide *a* [4].

This work has been supported by the *Swiss National Science Foundation* (project No. 20-30236.90) and by the *Austrian Science Foundation* (project No. 8371).

Experimental Part

X-Ray Diffraction. Crystal data: orthorhombic space group $P2_12_12_1$, with cell dimensions: $a = 18.995(2)$ Å, $b = 23.831(3)$ Å, $c = 7.403(1)$ Å; $V = 3351.1(11)$ Å³, $Z = 4$, $\rho_c = 1.155$ g/cm³, $F(000) = 1240$. Intensity data were collected using monochromatic CuK_α radiation ($\lambda = 1.5418$) at 88(2) K on a *Siemens P4* diffractometer for 3384 reflections ($2^\circ \leq 2\theta \leq 113.5^\circ$), of which 3168 were unique ($R_{\text{int}} = 2.14\%$). A semi-empirical absorption and volume (Ψ -scan) correction was performed. The structure was solved by direct methods, 1718 reflections with $F > 4\sigma(F)$ were used for refinement. Anisotropic atomic displacement parameters (a.d.p.'s) were refined for the four N-atoms and most of the peripheral atoms; scattering factors were taken from the *International Tables for X-Ray Crystallography* [11]. The R index after full-matrix refinement [12] was 10.2% ($R_w = 8.36\%$, $1/\sigma^2$ weights) for a data-to-parameter ratio of 6.0:1. H-Atoms attached to N(1), N(2), and N(3) were observed from difference electron density *Fourier* syntheses; the same is true for some of the Me protons. The positions of the remaining H-atoms were calculated on the basis of stereochemical plausibility. During the refinement, H-atoms were treated as 'riding' on the respective non-H-atom, with fixed isotropic a.d.p.'s. Refinement was terminated when A/σ for all parameters refined was considerably below 1, with the exception of the parameters of the pseudo atoms included to account for the disordered solvent density. These atoms showed A/σ values up to 3.0.

Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the *Cambridge Crystallographic Data Centre*.

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