Crystal Structure of the Chromophore from the Fluorescent Peptide Produced by Iron-deficient *Azotobacter vinelandii*

By JAMES L. CORBIN*
(*C. F. Kettering Laboratory, Yellow Springs, Ohio* 45387)
and ISABELLA L. KARLE and JEROME KARLE
(*Naval Research Laboratory, Washington, D.C.* 20390)

Summary The structure of the chromophoric unit of the fluorescent peptide formed by low-iron *Azotobacter* cultures has now been determined by the application of *X*-ray crystallography to a methylated derivative.

BULEN AND LECOMTE described[1] the isolation of a yellow-green fluorescent peptide from iron-deficient cultures of *Azotobacter vinelandii*. They reported that acid hydrolysis of this peptide yielded, in addition to some uncommon amino-acids, a chromophoric substance which retained the spectral properties of the parent peptide.

In relation to studies on iron deficiency in relation to bacterial nitrogen fixation, this chromophore has now been investigated further. It was found to have the formula $C_{14}H_{11}N_3O_5$ and yielded a trimethylated derivative ($C_{17}H_{17}N_3O_5$) upon prolonged treatment with diazomethane. The structure of this methyl derivative was obtained by *X*-ray crystallography and is shown stereoscopically in the Figure and in the Scheme as (**1b**). The chromophore is thus represented as (**1a**).

Although (**1b**) crystallized in transparent, well-formed prisms which were stable in a humid environment, they readily turned to powder when the atmospheric humidity decreased. Accordingly, the diffraction data were collected from a wet crystal sealed in a capillary. The structure analysis showed that there are four molecules of H_2O per molecule of methyl chromophore in the crystal. The material crystallizes in a centrosymmetric triclinic cell. For ease in data collection and indexing, a face-centred unit cell, space group $F\bar{1}$, with eight molecular units was chosen. The cell parameters are $a = 9{\cdot}12 \pm 0{\cdot}02$, $b = 19{\cdot}94 \pm 0{\cdot}03$, $c = 21{\cdot}17 \pm 0{\cdot}03$ Å, $\alpha = 93{\cdot}00 \pm 0{\cdot}25$, $\beta = 92{\cdot}76 \pm 0{\cdot}25$, and $\gamma = 97{\cdot}97 \pm 0{\cdot}25°$. Visual estimates were made of 2758 independent reflections recorded by the multiple-film Weissenberg method using Cu-K_α radiation.

The structure was readily solved by means of the symbolic-addition procedure[2] for obtaining phases directly from the measured *X*-ray intensities. The tentative identification of the N and O atoms was established by least-squares refinement in which all non-H atoms were

assumed to be C and the behaviour of the apparent thermal parameters was examined. The identification was confirmed by examination of the interatomic distances as the chemical degradation experiments had been performed and structures can now be assigned to these products (see Scheme). Mild alkaline treatment of the methyl derivative

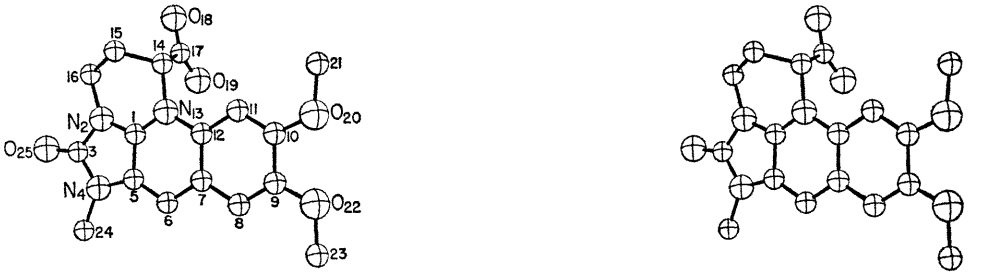
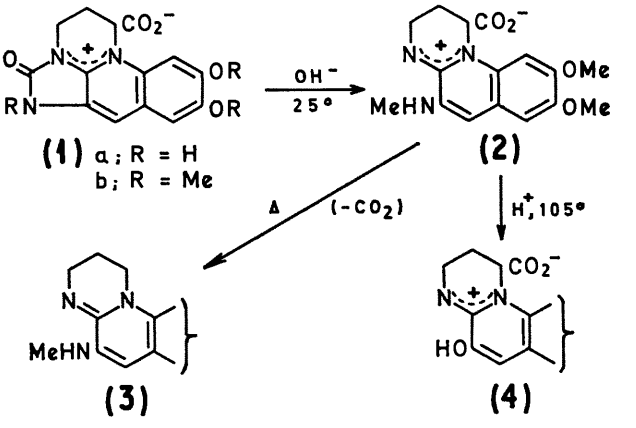


FIGURE. *A stereo-representation‡ of the configuration of the methylated chromophore from* A. vinelandii.

least-squares refinement progressed. Difference electron-density maps revealed the positions of 12 of the 17 H atoms in the methyl chromophore and also showed large anisotropic thermal motions about the peripheral atoms C(23), C(24), O(19), and O(25). So far, only isotropic refinement has been performed and resulted in an R factor of 16%. The methyl chromophore is a zwitterion with the positive charge probably divided between N(2) and N(13) and the negative charge on the carboxylate ion. The two C–O distances in the carboxylate ion are equal, 1·22 Å, and the two C(1)–N^+ distances are 1·35 and 1·30 Å (σ = 0·01 Å), based solely on the least-squares refinement at this stage. The molecule is characterized by two planes (see Figure). All atoms (excluding H atoms) except the carboxylate ion and C(15) lie essentially in one plane with the greatest deviation being 0·06 Å below for the C atom in one of the methoxy-groups and 0·13 Å above and 0·11 Å below for C(16) and C(14), respectively. Atom C(15) is 0·68 Å above this plane. The carboxylate ion and the attached C(14), coplanar to within 0·005 Å, lie in a plane perpendicular to the plane of the rings. This configuration results in a minimum intramolecular distance between N^+(13) and O^-(19) which is only 2·64 Å. Since the crystal is centrosymmetric, molecules exist as racemates. The crystal structure is layered, with each layer of organic molecules separated by a layer of H_2O molecules. Hydrogen bonds link the H_2O molecules and, in addition, there are two hydrogen bonds between H_2O molecules and the organic moiety, O(19) · · · W(1) at 2·69 Å and O(25) · · · W(4) at 2·79 Å.

Prior to this crystal-structure determination, some (**1b**) gave, upon neutralization, carbon dioxide and a new compound $C_{16}H_{19}N_3O_4$ (**2**).† Thermal decomposition of (**2**) resulted in decarboxylation and formation of $C_{15}H_{19}N_3O_2$ (**3**), while acid hydrolysis gave methylamine and $C_{15}H_{16}N_2O_5$ (**4**).



SCHEME

The unusual heterocyclic structure of the chromophore does not appear to fit any naturally occurring system known to us. Another compound, 2-*N*,6-*N*-di-(2,3-dihydroxybenzoyl)-L-lysine, is also formed[3] by *A. vinelandii* under the same conditions as the fluorescent peptide, but there is no structural similarity beyond the *o*-diphenol groups.

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† (**1a**) was recovered unchanged under these same reaction conditions.

‡ The drawing was made by computer from a program prepared by C. K. Johnson, of Oak Ridge National Laboratory. It should be viewed with a stereoscope.

[1] W. A. Bulen and J. R. LeComte, *Biochem. Biophys. Res. Comm.*, 1962, **9**, 523.
[2] See *e.g.*, J. Karle and I. L. Karle, *Acta Cryst.*, 1966, **21**, 849; I. L. Karle and J. Karle, *ibid.*, 1963, **16**, 969.
[3] J. L. Corbin and W. A. Bulen, *Biochemistry*, 1969, **8**, 757.