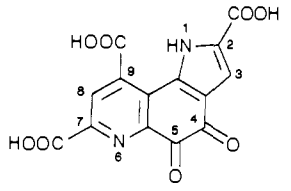
Molecular and Crystal Structure of PQQ (Methoxatin), a Novel Coenzyme of Quinoproteins: Extensive Stacking Character and Metal Ion Interaction

Toshimasa Ishida,*[1a] Mitsunobu Doi,[1a] Koutarou Tomita,[1a] Hiroki Hayashi,[1a] Masatoshi Inoue,[1a] and Teizi Urakami[1b]

Contribution from the Laboratory of Physical Chemistry, Osaka University of Pharmaceutical Sciences, 2-10-65 Kawai, Matsubara, Osaka 580, Japan, and the Niigata Research Laboratory, Mitsubishi Gas Chemical Company, Inc., Niigata 950-31, Japan. *Received December 27, 1988*

Abstract: The crystal structure of PQQ, crystallized as pentahydrated disodium salt, was determined by the X-ray diffraction method. Two crystallographically independent PQQ molecules show quite similar conformations, and the crystal structure is stabilized by formations of the coordination column formed by the arrangement of four independent sodium ions as well as the extensive hydrogen-bond networks via ten independent waters of crystallization. The most important insights obtained concerning the PQQ molecule are its significant stacking formation and the chelating site of metal ions. The prominent stacking formation between PQQ molecules is stabilized by van der Waals forces and dipole–dipole and electrostatic coupling interactions; the dipole moment and atomic charge distribution of the PQQ molecule are calculated by a quantum-chemical MNDO method. The stacking ability of this molecule is proved to be important to the formation of the charge-transfer complex with aromatic amino acids. However, it is clear from this coordination mode with sodium ions that the PQQ molecule has two discrete chelating sites for metal ions. The biological significance of the PQQ molecule participation has been discussed on the basis of the results obtained.

PQQ (methoxatin) [pyrroloquinoline quinone; 4,5-dihydro-4,5-dioxo-1*H*-pyrrolo[2,3-*f*]quinoline-2,7,9-tricarboxylic acid] has



been receiving increasing interest since its structure determination in 1979,[2] because it is a novel coenzyme of a new class of bacterial dehydrogenases (quinoproteins) which are neither flavin nor nicotinamide dependent.[3] These enzymes[4] need the PQQ coenzyme in order to metabolize the methane derivatives which form their sole source of carbon and metabolic energy. Recently, it was established that PQQ also occurs in mammalian enzymes as a covalently bound coenzyme.[5]

However, the PQQ molecule itself has been found to function as a growth factor or a growth-stimulating factor for microorganisms[6] and to foster the oxidation reactions of amines, alcohols, thiols, and amino acids.[7] These biological functions could all be based on chemical properties inherent in the PQQ molecule. To understand its biological behavior, therefore, it is of great importance to elucidate the exact physicochemical characters of the

Table I. Summary of Crystal Data and Intensity Collection

formula	$C_{14}H_4N_2O_8^{2-}\cdot 2Na^+\cdot 5H_2O$
M_r	464.27
space group	$P\bar{1}$
a, Å	13.044 (6)
b, Å	18.474 (7)
c, Å	8.415 (3)
α, deg	103.78 (4)
β, deg	110.31 (4)
γ, deg	97.62 (6)
V, Å^3	1795 (2)
Z	4
D (measd), g·cm^{-3}	1.724 (2)
D(calcd), g·cm^{-3}	1.718
absorpt coeff, cm^{-1}	17.36
F (000)	952
T of data collection, °C	20
scan speed in 2θ, deg·min^{-1}	3
scan range in ω, deg	1.0 + 0.15 tan θ
data range measd, deg	$2° \le 2\theta \le 130°$
data collected	$\pm h,\pm k,l$
no. of unique data measd	6118
no. of variables	672
coeff used for refinement	
a	0.19147
b	−0.06412
c	0.00858
R	0.076
R_w	0.095
S	1.245

(1) (a) Osaka University of Pharmaceutical Sciences. (b) Mitsubishi Gas Chemical Co., Inc.

(2) (a) Salisbury, S. A.; Forrest, H. S.; Cruse, W. B. T.; Kennard, O. *Nature* **1979**, *280*, 843–844. (b) Duine, J. A.; Frank, J. Jzn.; Van Zeeland, J. K. *FEBS Lett.* **1979**, *108*, 443–446.

(3) Duine, J. A.; Frank, J. Jzn. *Trends Biochem. Sci.* **1981**, *6*, 278–280.

(4) (a) De Beer, R.; Duine, J. A.; Frank, J. Jzn.; Large, P. J. *Biochim. Biophys. Acta* **1980**, *622*, 370–374. (b) Ameyama, M.; Matsushita, K.; Ohno, Y.; Shinagawa, E.; Adachi, O. *FEBS Lett.* **1981**, *130*, 179–183. (c) Hommes, R. W. J.; Postma, P. W.; Neijssel, O. M.; Tempest, D. W.; Dokter, P.; Duine, J. A. *FEMS Microbiol. Lett.* **1984**, *24*, 329–333. (d) Duine, J. A.; Frank, J. Jzn.; Berkhout, M. P. J. *FEBS Lett.* **1984**, *168*, 217–221. (e) Ameyama, M.; Nonobe, M.; Hayashi, M.; Shinagawa, E.; Matsushita, K.; Adachi, O. *Agric. Biol. Chem.* **1985**, *49*, 1227–1231. (f) Ameyama, M.; Shinagawa, E.; Matsushita, K.; Adachi, O. *Agric. Biol. Chem.* **1985**, *49*, 1001–1010. (g) Kawai, F.; Yamanaka, H.; Ameyama, M.; Shinagawa, E.; Matsushita, K.; Adachi, O. *Agric. Biol. Chem.* **1985**, *49*, 1071–1076. (h) Duine, J. A.; Frank, J. Jzn.; Jongejan, J. A. *FEMS Microbiol. Rev.* **1986**, *32*, 165–178. (i) Ameyama, M.; Nonobe, M.; Shinagawa, E.; Matsushita, K.; Takimoto, K.; Adachi, O. *Agric. Biol. Chem.* **1986**, *50*, 49–57. (j) Neijssel, O. M. *Microbiol. Sci.* **1987**, *4*, 87–90. (k) Van der Meer, R. A.; Jongejan, J. A.; Duine, J. A. *FEBS Lett.* **1987**, *221*, 299–304. (l) Van der Meer, R. A.; Duine, J. A. *FEBS Lett.* **1988**, *235*, 194–200.

(5) (a) Lobenstein-Verbeek, C. L.; Jongejan, J. A.; Frank, J. Jzn.; Duine, J. A. *FEBS Lett.* **1984**, *170*, 305–309. (b) Ameyama, M.; Shinagawa, E.; Matsushita, K.; Takimoto, K.; Nakashima, K.; Adachi, O. *Agric. Biol. Chem.* **1985**, *49*, 3623–3626.

(6) (a) Shimao, M.; Yamamoto, H.; Ninomiya, K.; Kato, K.; Adachi, O.; Ameyama, M.; Sakazawa, C. *Agric. Biol. Chem.* **1984**, *48*, 2873–2876. (b) Ameyama, M.; Shinagawa, E.; Matsushita, K.; Adachi, O. *Agric. Biol. Chem.* **1984**, *48*, 2909–2911. (c) Ameyama, M.; Shinagawa, E.; Matsushita, K.; Adachi, O. *Agric. Biol. Chem.* **1984**, *48*, 3099–3107. (d) Hanauske-Abel, H. M.; Tschank, G.; Gunzler, V.; Baader, E.; Gallop, P. *FEBS Lett.* **1987**, *214*, 236–243. (e) Linton, J. D.; Woodard, S.; Gouldney, D. G. *Appl. Microbiol. Biotechnol.* **1987**, *25*, 357–361.

(7) (a) Ohshiro, Y.; Itoh, S.; Kurokawa, K.; Kato, J.; Hirano, T.; Agawa, T. *Tetrahedron Lett.* **1983**, *24*, 3465–3468. (b) Itoh, S.; Kato, N.; Ohshiro, Y.; Agawa, T. *Tetrahedron Lett.* **1984**, *25*, 4753–4756. (c) Itoh, S.; Mure, M.; Ohshiro, Y.; Agawa, T. *Tetrahedron Lett.* **1985**, *26*, 4225–4228. (d) Itoh, S.; Kato, N.; Ohshiro, Y.; Agawa, T. *Chem. Lett.* **1985**, 135–136. (e) Itoh, S.; Mure, M.; Ohshiro, Y. *Yukagaku* **1987**, *36*, 882–883. (f) Itoh, S.; Mure, M.; Ohshiro, Y. *J. Chem. Soc., Chem. Commun.* **1987**, 1580–1581.

0002-7863/89/1511-6822$01.50/0 © 1989 American Chemical Society

PQQ molecule such as its molecular dimension and atomic charge distribution; hence, this structural study of the PQQ molecule has been carried out. This paper deals with the molecular and crystal structures of PQQ crystallized as hydrated sodium salt. This is the first report on the crystal structure of the intact PQQ molecule to our knowledge, although the structures of its 5-(2-oxopropyl)[2a,8] and 5-(2,4-dinitrophenylhydrazine)[9] adducts have already been reported.

Experimental Section

Materials and Crystallization. A free form of the PQQ molecule was purchased from Kanto Kagaku Co. Other materials were commercial preparations (reagent grade) and were used without further purification. After many attempts to crystallize PQQ, single crystals suitable for X-ray analysis were finally obtained by the slow evaporation of the following phosphate buffer solution at 20 °C: PQQ was dissolved in 1/15 M phosphate buffer (NaH_2PO_4/Na_2HPO_4) to give a 0.2 M solution. The pH value was then adjusted to about 5.0 by addition of 1 N HCl solution. Rectangular platelet crystals appeared after a few weeks; the pH value of the solution was 5.9 The measurements of the thermogravimetry (TG), difference thermal analyses (DTA), and X-ray fluorescence spectroscopy showed that the crystals contained two sodium ions and five waters of crystallization per PQQ molecule. In order to prevent the release of the waters of crystallization during the X-ray work, the crystals were sealed in glass capillaries containing the mother liquid. Crystal sizes of ca. 0.2 × 0.1 × 0.5 mm^3 were used for data collection.

Crystal Data and Intensity Collection. Details of crystal data and the intensity collection parameters are summarized in Table I. The unit-cell dimensions were determined by a least-squares fit of 2θ angles for 25 reflections ($30° < 2\theta < 50°$) measured by graphite-monochromated Cu Kα radiation (λ = 1.5418 Å) on an automated Rigaku AFC-5 diffractometer. The crystal density was measured by the flotation method using a CCl_4–C_6H_6 mixture. The Z value, which was calculated from M_r, unit-cell volume (V), and the measured crystal density, equaled 4, implying the presence of two crystallographically independent sets for the chemical formula of $PQQ^{2-}\cdot 2Na^+\cdot 5H_2O$ in an asymmetric unit. The $\omega - 2\theta$ scan technique was employed for the intensity recording, where the background was counted for 5 s at both extremes of the reflection peak. Four standard reflections were monitored for every 100 reflection intervals throughout the data collection, showing a random variation of ±0.4% with no significant trends. The observed intensities were corrected for the Lorentz and polarization effects. Correction of the absorption was also performed with an empirical method based on the ϕ scan along the longest crystal side (c axis).

Structure Solution and Refinement. The structure was solved by the combination of heavy-atom and direct methods (program MULTAN 87).[10] The positional parameters of the non-hydrogen atoms obtained were refined by a full-matrix least-squares method with isotropic thermal parameters and then by a block-diagonal least-squares method with anisotropic parameters. The positions of the geometrically reasonable hydrogen atoms were calculated, all of which, except three water molecules, were also verified on a difference Fourier map, and included in subsequent refinements with isotropic thermal factors. The function minimized was $\sum w(|F_o| - |F_c|)^2$, where $|F_o|$ and $|F_c|$ represent the observed and calculated structure amplitudes, respectively. The weighting scheme used was as follows: $w = a$ for $|F_o| = 0.0$ and $w = 1.0/[\sigma(F_o)^2 + b|F_o| + c|F_o|^2]$ for $|F_o| > 0.0$, where $\sigma(F_o)^2$ represents the standard deviation of the reflection intensity on the basis of counting statistics. The coefficients, a–c, were refined by a least-squares method, on the basis of the statistics of $\sigma(F_o)^2$ and $(|F_o| - |F_c|)^2$, and the values used in the final stage are given in Table I. Final R ($=\sum(|F_o| - |F_c|)/\sum|F_o|$), R_w ($=[\sum w(|F_o| - |F_c|)^2/\sum w|F_o|^2]^{1/2}$) for 6118 observed reflections, and goodness of fit S ($=[\sum w(|F_o| - |F_c|)^2/(M - N)]^{1/2}$, where M = number of observed reflections and N = number of variables used for refinement) are also tabulated. None of the positional parameters for non-hydrogen atoms shifted by more than one-fifth from their standard deviations, and the maximum electron density in the final difference Fourier map was 0.35 e·Å^{-3}. The final positional parameters and their isotropic temperature factors, which were calculated from the anisotropic factors with $B_{iso} = (4/3)(B_{11}a^2 + B_{22}b^2 + B_{33}c^2 + 2abB_{12}\cos\gamma + 2acB_{13}\cos\beta + 2bcB_{23}\cos\alpha)$, of non-hydrogen atoms are given in Table II. The UNICS programs[11] were used for all crystallographic computations, and the atomic scattering factors were taken from ref 12.

Table II. Final Atomic Coordinates with ESD in Parentheses and Isotropic Temperature Factors

atom	x	y	z	B_{eq}
Na1	0.5577 (1)	0.36977 (9)	0.9713 (2)	2.81 (5)
Na2	0.9215 (1)	0.15581 (9)	0.5139 (2)	2.53 (4)
Na3	0.7690 (1)	0.30645 (9)	0.8543 (2)	2.78 (5)
Na4	0.7818 (1)	0.17390 (9)	0.1108 (2)	2.82 (5)
N(1)	0.3331 (2)	0.3915 (2)	0.3604 (4)	1.76 (8)
C(2)	0.3057 (3)	0.4506 (2)	0.4610 (5)	1.8 (1)
C(3)	0.1949 (3)	0.4487 (2)	0.3745 (5)	2.2 (1)
C(4)	0.0410 (3)	0.3568 (2)	0.0795 (5)	2.0 (1)
O(4)	−0.0426 (2)	0.3776 (2)	0.0802 (4)	3.33 (9)
C(5)	0.0318 (3)	0.2922 (2)	−0.0820 (5)	1.9 (1)
O(5)	−0.0557 (2)	0.2709 (2)	−0.2109 (4)	2.80 (8)
N(6)	0.1063 (2)	0.1984 (2)	−0.2175 (4)	1.75 (8)
C(7)	0.1882 (3)	0.1632 (2)	−0.2266 (5)	1.6 (1)
C(8)	0.2952 (3)	0.1877 (2)	−0.0957 (5)	1.8 (1)
C(9)	0.3232 (3)	0.2513 (2)	0.0558 (5)	1.7 (1)
C(10)	0.2426 (3)	0.3526 (2)	0.2110 (4)	1.60 (9)
C(11)	0.1543 (3)	0.3874 (2)	0.2167 (5)	1.9 (1)
C(12)	0.1304 (3)	0.2573 (2)	−0.0745 (4)	1.7 (1)
C(13)	0.2365 (3)	0.2867 (2)	0.0682 (5)	1.6 (1)
C(14)	0.3863 (3)	0.5005 (2)	0.6357 (5)	2.0 (1)
O(15)	0.4851 (2)	0.4840 (2)	0.6851 (3)	2.57 (8)
O(16)	0.3609 (2)	0.5516 (2)	0.7248 (4)	3.23 (9)
C(17)	0.1580 (3)	0.0971 (2)	−0.3947 (5)	1.9 (1)
O(18)	0.2279 (2)	0.0598 (2)	−0.4059 (4)	3.08 (9)
O(19)	0.0621 (2)	0.0879 (1)	−0.5142 (4)	2.69 (8)
C(20)	0.4474 (3)	0.2756 (2)	0.1793 (5)	1.9 (1)
O(21)	0.4740 (2)	0.3021 (2)	0.3453 (3)	2.63 (8)
O(22)	0.5137 (2)	0.2646 (2)	0.1066 (4)	2.85 (9)
N(1)′	0.1716 (2)	0.1060 (2)	0.1456 (4)	1.74 (8)
C(2)′	0.2050 (3)	0.0462 (2)	0.0622 (5)	1.9 (1)
C(3)′	0.3138 (3)	0.0489 (2)	0.1645 (5)	2.3 (1)
C(4)′	0.4541 (3)	0.1442 (2)	0.4644 (5)	2.6 (1)
O(4)′	0.5351 (3)	0.1169 (2)	0.4915 (5)	5.2 (1)
C(5)′	0.4613 (3)	0.2192 (2)	0.6010 (5)	2.1 (1)
O(5)′	0.5505 (2)	0.2513 (2)	0.7227 (3)	2.61 (8)
N(6)′	0.3742 (2)	0.3093 (2)	0.7173 (4)	1.77 (8)
C(7)′	0.2869 (3)	0.3388 (2)	0.7172 (5)	1.7 (1)
C(8)′	0.1818 (3)	0.3098 (2)	0.5789 (5)	1.7 (1)
C(9)′	0.1627 (3)	0.2468 (2)	0.4327 (5)	1.7 (1)
C(10)′	0.2567 (3)	0.1475 (2)	0.2998 (5)	1.7 (1)
C(11)′	0.3482 (3)	0.1142 (2)	0.3165 (5)	2.3 (1)
C(12)′	0.3575 (3)	0.2490 (2)	0.5797 (4)	1.64 (9)
C(13)′	0.2547 (3)	0.2152 (2)	0.4324 (5)	1.67 (9)
C(14)′	0.1322 (3)	−0.0087 (2)	−0.1139 (5)	1.8 (1)
O(15)	0.0357 (2)	0.0076 (2)	−0.1875 (3)	2.71 (8)
O(16)′	0.1620 (2)	−0.0633 (1)	−0.1833 (4)	2.69 (8)
C(17)′	0.3082 (3)	0.4050 (2)	0.8821 (5)	1.8 (1)
O(18)′	0.2269 (2)	0.4310 (2)	0.8923 (4)	2.74 (8)
O(19)′	0.4061 (2)	0.4256 (2)	0.9967 (4)	2.95 (9)
C(20)′	0.0418 (3)	0.2214 (2)	0.2924 (5)	1.9 (1)
O(21)′	0.0236 (2)	0.1901 (2)	0.1326 (3)	2.50 (8)
O(22)′	−0.0329 (2)	0.2355 (1)	0.3484 (4)	2.20 (8)
O(1)W	0.8581 (2)	0.0795 (2)	0.6684 (4)	2.78 (9)
O(2)W	0.7286 (2)	0.3010 (2)	0.1166 (4)	2.84 (9)
O(3)W	0.6968 (2)	0.4220 (2)	0.8804 (4)	3.07 (9)
O(4)W	0.7435 (2)	0.1710 (2)	−0.1849 (4)	3.06 (9)
O(5)W	0.7415 (3)	0.1767 (2)	0.3879 (4)	3.7 (1)
O(6)W	0.8839 (3)	0.3642 (2)	0.4633 (4)	3.8 (1)
O(7)W	0.7040 (3)	0.3208 (2)	0.5562 (4)	3.9 (1)
O(8)W	0.0583 (3)	0.4837 (2)	0.6824 (6)	7.1 (2)
O(9)W	0.5820 (3)	0.1201 (3)	1.0114 (7)	7.6 (2)
O(10)W	0.5848 (4)	−0.0041 (3)	0.2568 (8)	11.2 (3)

Quantum-Chemical Calculation. The quantum-chemical parameters (dipole moment and direction, atomic charge) of the PQQ molecule in its neutral form were calculated by the MNDO program.[13] The atomic coordinates used for calculation were derived from the present X-ray

(8) Cruse, W. B. T.; Kennard, O.; Salisbury, S. A. *Acta Crystallogr.* **1980**, *B36*, 751–754.
(9) Van Koningsveld, H.; Jansen, J. C.; Jongejan, J. A.; Frank, J. Jzn.; Duine, J. A. *Acta Crystallogr.* **1985** *C41*, 89–92.
(10) Debaerdemaeker, T.; Germain, G.; Main, P.; Tate, C.; Woolfson, M. M. MULTAN 87, Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data, 1987.
(11) The Universal Crystallographic Computing System—Osaka; The Computation Center, Osaka University: Osaka, Japan, 1979.
(12) *International Tables for X-ray Crystallography*; Kynoch: Birmingham, England, 1974; Vol. IV.
(13) Dewar, M. J. S.; Thiel, W. *J. Am. Chem. Soc.* **1977**, *99*, 4899–4917.

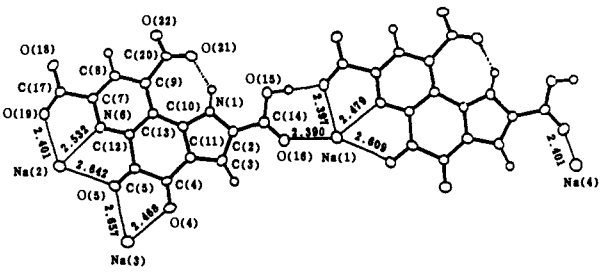


Figure 1. Molecular conformation of two crystallographically independent PQQ molecules [molecule A (left side), molecule B (right side)]. Thin lines represent the coordination mode of sodium ions with PQQ molecules. The coordination distance are also shown. The dotted lines represent intra- or intermolecular hydrogen bonds.

results. The stabilities of the respective electroic energies were used to verify the convergence in the iteration calculations.

The numerical calculations were performed on a Micro VAX II computer at the Computation Center, Osaka University of Pharmaceutical Sciences.

Absorption Spectra. The electronic absorption spectra of the PQQ molecule at 30 °C were recorded on a Jasco UNIDEC-610A spectrometer with 10-mm dual cells in the absence and presence of an aromatic amino acid. The aromatic amino acids used were the methyl esters of L-tryptophan, L-tyrosine, and L-phenylalanine because of their solubilities in the phosphate buffer solution. The solutions of 1×10^{-4} M (PQQ) and 4×10^{-4} to 1×10^{-3} M (aromatic amino acid) concentrations were prepared by dissolving each sample in a 1/15 M phosphate buffer (pH 6.8). After preparation, the sample solutions were kept in a dark room for 1 week and were then measured. Job plots for the PQQ–aromatic amino acid mixtures were performed with the summation of [PQQ] and [aromatic amino acid] as the constant ($=8 \times 10^{-4}$ M).

Results and Discussion

Molecular Structure of PQQ. A schematic illustration of two crystallographically independent PQQ molecules (molecule A and B, respectively) is presented in Figure 1 together with the atomic numbering scheme used in this paper. The dotted lines represent intra- and intermolecular hydrogen bonds. The tables of bond lengths and angles have been deposited as supplementary material. There is no significant difference between these bonding parameters; the maximum difference is 0.028 Å for the C(4)–C(11) bond length and 1.6° for the C(4)–C(11)–C(10) bond angle. As judged from the bond lengths and angles, two carboxyl groups attached to the pyridine ring of the PQQ molecule assume anionic forms to neutralize the sodium cations, while the group attached to the pyrrole ring is in a neutral state.

It is of interest to consider why the carboxyl groups attached to the pyridine ring assume their anionic forms more easily than the one attached to the pyrrole ring. The reason for this can also be used to explain why the trimethyl esters of the PQQ molecule are initially hydrolyzed at the 7- and 9-positions.[14] The answer to this question can be estimated from the atomic charge distribution of the PQQ molecule. Figure 2 shows the results calculated by the quantum-chemical MNDO method. Although the atomic charge distributions are similar among the three neutral carboxyl groups, a large difference in the net charges between the pyrrole and pyridine rings can be observed: the former ring has the net charge of −0.3552 e [summation of N(1), C(2), C(3), C(10), and C(11) atomic net charges], while the latter shows a charge of −0.1699 e [summation of N(6), C(7), C(8), C(9), C(12), and C(13) net charges]. Thus, the energetic stabilization of the carboxyl group through the resonance effect with respective aromatic rings could be more significant for the carboxyl group at the 2-position than that at the 7- or 9-position. This consideration is also suggested by the following fact: The bond length of C(7)–C(17) [1.519 (5) Å for molecule A and 1.527 (5) Å for B] or C(9)–C(20) [1.524 (5) Å for A and 1.532 (5) Å for B] is significantly longer than that of C(2)–C(14) [1.463 (5) Å for A and 1.476 (5) Å for B], and the latter length is in the range of a partial double bond.[15]

(14) Itoh, S.; Ohshiro, Y. *Chem. Exp.* **1986**, *5*, 315–318.

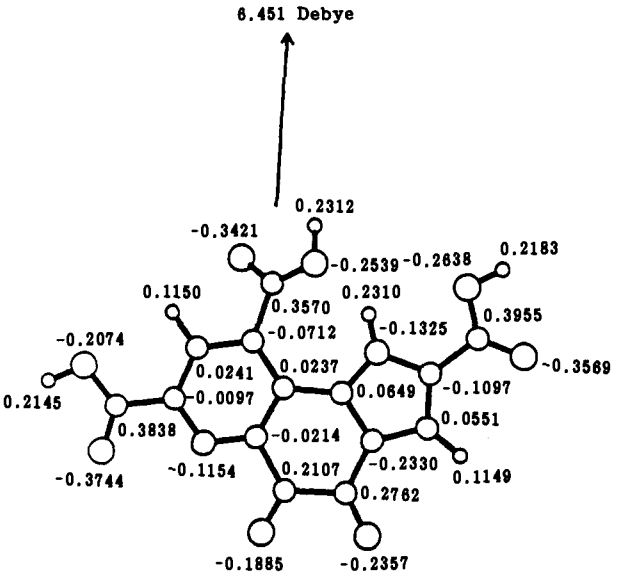


Figure 2. Atomic net charges and dipole moment of a neutral PQQ molecule. The direction of the dipole moment is almost parallel to the PQQ aromatic ring. PQQ has a total energy of −112147.09 kcal·mol^{-1}, a HOMO energy of −221.72 kcal·mol^{-1}, and a LUMO energy of −46.79 kcal·mol^{-1}.

Two keto bonds, C(4)=O(4) and C(5)=O(5), of the quinone ring take the shortened double bond lengths [1.204 (5) and 1.205 (5) Å for A and 1.205 (6) and 1.206 (5) Å for B, respectively], as is usually observed in the conjugated system. The localization of these bond lengths showing a partial triple bond character makes the C(4)–C(5) [1.537 (5) Å for A and 1.543 Å for B], C(4)–C(11) [1.456 (5) Å for A and 1.428 (6) Å for B], and C(5)–C(12) [1.503 (5) Å for A and 1.498 (5) Å for B] bond lengths longer than the usual aromatic C–C bond. The C(4)–C(5) bond length in particular is in a partial single bond region.[15]

The equations for the least-squares planes and their dihedral angles of the PQQ aromatic ring portions are also given as supplementary material. Although both of the PQQ molecules essentially showed reasonable planarities, a noticeable difference was observed in the deviations from their best-fit planes: the mean root square deviations were 0.008 Å for A and 0.014 Å for B.

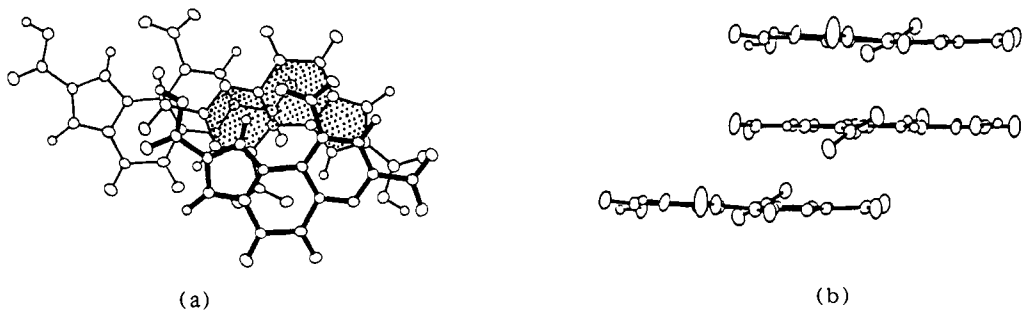
While the two carboxyl groups at the 2- and 7-positions are almost coplanar with a PQQ aromatic ring, that at the 9-position inclines significantly toward the PQQ plane [the dihedral angles are 34.4 (2)° and 30.7 (2)° for A and B, respectively].

Molecules A and B both form an intramolecular hydrogen bond between the N(1) and O(21) atoms and are directly linked together by an intermolecular O(15)–H···O(19) hydrogen-bond formation.

As might be expected from Figure 1 and the above-mentioned results, molecules A and B both show very similar conformations and bonding parameters. The major difference between them is the planarity of the PQQ aromatic ring and the interaction mode with sodium ions. These factors could be the reasons why the present crystal has two independent PQQ molecules.

Coordination of Sodium Ions to PQQ Molecules. Four independent sodium ions were included in the crystal structure; each of these was closely coordinated with the polar atoms of the PQQ molecules and waters of crystallization (discussed later). Of these coordinations, the direct coordination mode with the PQQ molecules is shown in Figure 1, along with the coordination distances (in angstroms). Two important coordination modes can be found. One mode, which was observed in both molecules, is the coordination of a sodium ion [Na(1) or Na(2)] to O(5), N(6), and O(19) atoms, thus newly forming two five-membered chelate rings via a sodium ion with the O(5)–Na–N(6) and N(6)–Na–O(19) bond angles of 61.7 (1)° and 64.8 (1)° for molecule A and 62.8

(15) *International Tables for X-ray Crystallography*; D. Reidel: Dordrecht, Holland, 1983; Vol. III.



(a) (b)

Figure 3. Stacking interaction modes between PQQ molecules A and B. Two up-and-down stacked molecules B, which are related by a symmetry operation of $x,y,z + 1$, are projected perpendicular (a) and parallel (b) to the central molecule A. The molecule A in (a) is shaded.

(1)° and 65.7 (1)° for molecule B. The other mode is formed only in molecule A by the chelation of a sodium ion to the O(4) and O(5) atoms with a O(4)–Na–O(5) bond angle of 63.2 (1)°. These results demonstrate that the PQQ molecule possesses two metal-binding sites: a primary site in the pocket formed by the O(5), N(6), and O(19) atoms and a secondary site in the pocket formed by the O(4) and O(5) atoms. In the crystal structure of a related 5-(2-oxopropyl)-PQQ molecule,[8] a water molecule is located at the former site; furthermore, the coordination of the copper ion to the former site [N(6) and O(19) atoms] has been shown in a series of Cu(PQQ)(bipyridine or terpyridine) ternary complexes.[16] Thus, it could be assumed that a bivalent metal ion such as Cu^{2+} or Mn^{2+} is also coordinated with these binding sites.

Recently it has been demonstrated[5a,17] that the PQQ molecule functions together with copper ions as a prosthetic group in copper-requiring amine oxidase, which is widespread in plants, microorganisms, and animals. This oxidase catalyzes the oxidation deamination of amine by accepting two electrons from the amine and transferring them to the dioxygen.[18] The present coordination modes provide information on the possible interaction between the copper ion and the PQQ molecule. The direct coordination of the copper ion to O(5) and/or O(4), which are reduction atoms of the PQQ molecule, would be advantageous in catalyzing the electron transfer from the substrate to the PQQ molecule. In this respect, it would be worthwhile to note that flavin, which is a similar coenzyme for oxidation/reduction catalysis, also has two separate metal-binding sites in its flavoquinoid system, and such a metal-binding species is necessary in some cases to allow electron transfer to occur.[19]

Stacking Interaction of PQQ Molecules. One of the most pronounced structural features observed in the crystal structure is the extensive stacking formation between molecules A and B. Figure 3 shows the stacking mode, where the two up-and-down stacked molecules B are projected perpendicular (a) or parallel (b) the central molecule A. As is obvious from this figure, molecules A and B are almost related to each other by a center of symmetry. There are many short contact pairs within 3.5 Å; the atomic pairs less than 3.4 Å are listed in Table III. The dihedral angles are 2.13 (8)° for both the lower and upper pairs, and the averaged interplanar spacing in the overlapping area is 3.360 Å for the upper pair and 3.302 Å for the lower one; both of these interplanar distance are shorter than the normal van der Waals separation distance (3.4 Å). In addition to the normal van der Waals forces, therefore, this infinite stacking formation could be further stabilized by the dipole–dipole and electrostatic interactions. As can be seen in Figure 2, the PQQ molecule has a large dipole moment (=6.451 D) almost parallel to the aromatic ring. Since molecules A and B are related to each other by a pseudocenter of symmetry, the directions of their dipole moments are almost coupled. Furthermore, many of the short contact pairs listed in Table III occur between two atoms capable of forming electron-rich/electron-deficient pairs.

Table III. Short Contacts (Å) between PQQ Molecules and Their Standard Deviations in Parentheses

molecule A	molecule B	distance
	Upper Pair	
C(2)	C(7)	3.365 (5)
C(4)	O(21)	3.207 (5)
C(15)	O(21)	2.922 (5)
C(9)	C(10)	3.364 (5)
C(10)	C(9)	3.306 (5)
C(12)	O(21)	2.963 (5)
C(13)	O(21)	3.373 (5)
C(14)	C(17)	3.343 (5)
O(16)	C(17)	3.377 (5)
C(17)	O(15)	3.305 (5)
O(21)	C(4)	3.308 (5)
O(21)	C(5)	2.958 (5)
O(21)	O(5)	3.380 (4)
O(21)	C(12)	3.126 (5)
	Lower Pair	
N(6)	C(8)	3.233 (5)
C(8)	N(6)	3.283 (5)
C(10)	O(18)	3.298 (5)
C(13)	C(17)	3.195 (5)
C(13)	O(18)	3.340 (5)
C(17)	C(13)	3.244 (5)
O(18)	C(10)	3.360 (5)
O(19)	C(9)	3.249 (5)
O(19)	C(20)	3.254 (5)
O(22)	O(5)	3.385 (4)

It is of interest to note that the atomic coefficients of the highest occupied molecular orbital (HOMO) and the lowest unoccupied one (LUMO) in the PQQ molecule have mostly the same signs in the short contact atomic pairs, and they are interactive due to the coupling of their orbitals. This means that the HOMO–LUMO interaction is also an important factor in the stability of these stacking formations.

The stacking character of the PQQ molecule may reveal how the PQQ coenzyme is recognized by quinoproteins and is fixed at their active sites. Before considering the possible mechanism, it is important to note that the flavin coenzyme, which also undergoes a similar stacking interaction by its molecular association,[20] interacts strongly with the aromatic amino acids,[21] and this in-

(16) (a) Suzuki, S.; Sakurai, T.; Itoh, S.; Ohshiro, Y. *Inorg. Chem.* **1988**, *27*, 591–592. (b) Suzuki, S.; Sakurai, T.; Itoh, S.; Ohshiro, Y. *Nippon Kagaku Kaishi* **1988**, 421–424. (c) Suzuki, S.; Sakurai, M.; Itoh, S.; Ohshiro, Y. *Chem. Lett.* **1988**, 777–780.

(17) (a) Ameyama, M.; Hayashi, M.; Matsushita, K.; Shinagawa, E.; Adachi, O. *Agric. Biol. Chem.* **1984**, *48*, 561–565. (b) Williamson, P. R.; Moog, R. S.; Dooley, D. M.; Kagan, H. M. *J. Biol. Chem.* **1986**, *261*, 16302–16305. (c) Van der Meer, R. A.; Jongejan, J. A.; Frank, J. Jzn.; Duine, J. A. *FEBS Lett.* **1986**, *206*, 111–114. (d) Glatz, Z.; Kovar, J.; Macholan, L.; Pec, P. *Biochem. J.* **1987**, *242*, 603–606. (e) Knowles, P. F.; Pandeya, K. B.; Rius, F. X.; Spencer, C. M.; Moog, R. S.; McGuirl, M. A.; Dooley, D. M. *Biochem. J.* **1987**, *241*, 603–608.

(18) Mondovi, B., Ed. *Structure and Functions of Amine Oxidases*; CRC Press: Boca Raton, FL, 1985.

(19) (a) Wade, T. D.; Fritchie, C. J., Jr. *J. Biol. Chem.* **1973**, *248*, 2337–2343. (b) Garland, W. T., Jr.; Fritchie, C. J., Jr. *J. Biol. Chem.* **1974**, *249*, 2228–2234. (c) Clarke, M. J.; Dowling, M. G.; Garafolo, A. R.; Brennan, T. F. *J. Biol. Chem.* **1980**, *255*, 3472–3481.

(20) Wang, M.; Fritchie, C. J., Jr. *Acta Crystallogr.* **1973**, *B29*, 2040–2045.

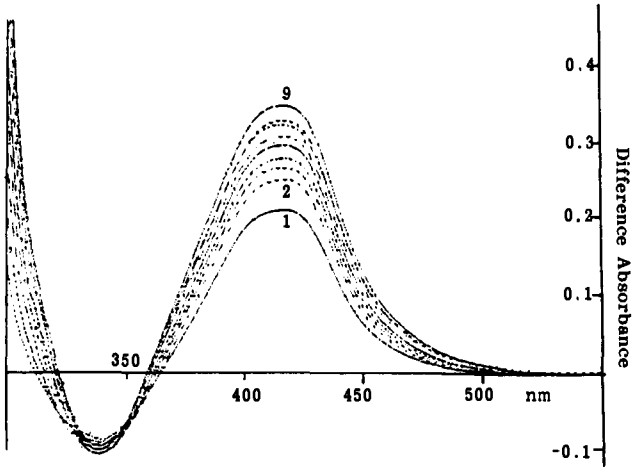


Figure 4. Difference absorption spectra for mixtures of PQQ and L-tryptophan methyl ester dissolved in 1/15 M phosphate buffer (pH 6.8) at 30 °C. The concentration of PQQ was maintained at 1×10^{-4} M, while that of tryptophan was varied from 4×10^{-4} M (1) to 2×10^{-3} M (9) by 2×10^{-4} M increments. Spectra were obtained by subtracting the absorbance of PQQ from that of the respective mixtures.

teraction is one of the essential binding forces between the coenzyme and apoenzyme.[22] Furthermore, this interaction plays an important role in the acceleration of the reaction of the substrate.[23] Similarly, it is possible that the PQQ molecule forms an intense stacking formation with an aromatic amino acid. In fact, the absorption spectra measurements showed the characteristic charge-transfer band due to the PQQ–aromatic amino acid interaction, as exemplified by the PQQ–tryptophan system in Figure 4. Since the maximum of the Job plot[25] occurs at the 0.5 M fraction of each PQQ with a tryptophan, tyrosine, or phenylalanine mixture, the 1:1 complex formation exists in these pairs. The association constant (K, mol^{-1}) and molar extinction coefficient (ϵ, $L \cdot mol^{-1} \cdot cm^{-1}$) of each complex pair are as follows:[26] λ_{max} = 418 nm, K = 2615, and ϵ = 8222 for PQQ–tryptophan; λ_{max} = 412 nm, K = 837, and ϵ = 2801 for PQQ–tyrosine; λ_{max} = 412 nm, K = 685, and ϵ = 2466 for PQQ–phenylalanine. Although the PQQ molecule itself has been reported to catalyze the oxidative decarboxylations of tryptophan and phenylalanine under mild conditions,[7b] a similar charge-transfer band was also observed in the skatole–PQQ system. Thus, the above-mentioned spectral behavior could result from the interaction between the intact molecules.

The intense interaction of the PQQ molecule with aromatic amino acid could also be responsible for the binding and/or catalytic reaction of the PQQ coenzyme with quinoprotein.

Crystal Structure. A stereoscopic view of the crystal packing is shown in Figure 5, where the sodium ions and waters of crystallization are represented by the filled and open circles, respectively. The infinite stacking layers of the PQQ molecules run along the c axis. The sodium ions and waters of crystallization are located among these layers, helping to stabilize the layers by

Table IV. Coordination Distances (Å) of Sodium Ions with Their ESD in Parentheses

Na ion at x,y,z	atom[a]	at symmetry operation	distance
Na1	O(16)	$1-x,1-y,2-z$	2.390 (4)
Na1	O(22)	$x,y,1+z$	2.572 (3)
Na1	O(5)′	x,y,z	2.609 (3)
Na1	N(6)	x,y,z	2.479 (4)
Na1	O(19)′	x,y,z	2.397 (3)
Na1	O(2)W	$x,y,z,1+z$	2.771 (3)
Na1	O(3)W	x,y,z	2.379 (4)
Na2	O(5)	$1+x,y,1+z$	2.643 (3)
Na2	N(6)	$1+x,y,1+z$	2.532 (4)
Na2	O(19)	$1+x,y,1+z$	2.401 (3)
Na2	O(16)′	$1-x,-y,-z$	2.643 (3)
Na2	O(22)′	$1+x,y,z$	2.405 (3)
Na2	O(1)W	x,y,z	2.382 (3)
Na2	O(5)W	x,y,z	2.343 (4)
Na3	O(4)	$1+x,y,1+z$	2.468 (4)
Na3	O(5)	$1+x,y,1+z$	2.657 (3)
Na3	O(5)	x,y,z	2.625 (3)
Na3	O(2)W	$x,y,1+z$	2.461 (3)
Na3	O(3)W	x,y,z	2.438 (4)
Na3	O(4)W	$x,y,1+z$	2.407 (4)
Na3	O(7)W	x,y,z	2.446 (4)
Na4	O(16)′	$1-x,-y,-z$	2.401 (3)
Na4	O(22)′	$1+x,y,z$	2.443 (3)
Na4	O(2)W	x,y,z	2.529 (3)
Na4	O(4)W	x,y,z	2.348 (4)
Na4	O(5)W	x,y,z	2.553 (4)
Na4	O(9)W	$x,y,-1+z$	2.425 (5)

[a] The primed atoms correspond to molecule B, and the suffix letter W represents the water of crystallization.

Table V. Possible Hydrogen-Bond Distances (Å) with Their ESD in Parentheses[a]

donor atom at x,y,z	acceptor atom	at symmetry operation	distance
N(1)	O(21)	x,y,z	2.645 (4)
O(15)	O(19)′	$1-x,1-y,2-z$	2.565 (4)
N(1)′	O(21)′	x,y,z	2.623 (4)
O(15)′	O(19)	$-x,-y,-1-z$	2.472 (4)
O(1)W	O(18)	$1-x,-y,-z$	2.751 (4)
O(1)W	O(15)′	$1+x,y,1+z$	2.869 (4)
O(2)W	O(22)	x,y,z	2.762 (4)
O(2)W	O(5)′	$x,y,-1+z$	3.142 (4)
O(2)W	O(4)W	x,y,z	3.132 (4)
O(2)W	O(6)W	x,y,z	2.761 (5)
O(3)W	O(15)	x,y,z	3.197 (4)
O(3)W	O(5)′	x,y,z	3.162 (4)
O(3)W	O(18)′	$1-x,1-y,2-z$	2.741 (4)
O(3)W	O(7)W	x,y,z	2.957 (5)
O(4)W	O(5)	$1+x,y,z$	3.156 (5)
O(4)W	O(4)′	$x,y,-1+z$	2.941 (5)
O(4)W	O(5)′	$x,y,-1+z$	3.054 (4)
O(4)W	O(1)W	$x,y,-1+z$	2.751 (4)
O(5)W	O(16)′	$1-x,-y,-z$	3.070 (5)
O(5)W	O(22)′	$1+x,y,z$	3.156 (5)
O(5)W	O(7)W	x,y,z	2.891 (5)
O(5)W	O(9)W	$x,y,-1+z$	2.955 (6)
O(6)W	O(22)′	$1+x,y,z$	2.842 (4)
O(6)W	O(8)W	$1+x,y,z$	2.696 (6)
O(7)W	O(21)	x,y,z	2.830 (5)
O(7)W	O(5)′	x,y,z	3.121 (4)
O(7)W	O(6)W	x,y,z	2.799 (5)
O(8)W	O(4)	$-x,1-y,-1-z$	2.930 (6)
O(8)W	O(18)′	x,y,z	2.756 (5)
O(8)W	O(8)W	$-x,1-y,-1-z$	3.160 (6)
O(9)W	O(22)	$x,y,1+z$	2.943 (6)
O(9)W	O(10)W	$1-x,-y,1-z$	2.733 (8)
O(10)W	O(18)	$1-x,-y,-z$	2.786 (7)
O(10)W	O(4)′	x,y,z	2.914 (7)

[a] The primed atoms belong to molecule B, and the suffix leter W implies the water of crystallization.

(21) (a) Slifkin, M. A. *Charge Transfer Interactions of Biomolecules*; Academic Press: New York, 1971; pp 132–171. (b) McCormick, D. B. *Photochem. Photobiol.* **1977**, *26*, 169–182.

(22) (a) Inoue, M.; Shibata, M.; Kondo, Y.; Ishida, T. *Biochemistry* **1981**, *20*, 2936–2945. (b) Inoue, M.; Okuda, Y.; Ishida, T.; Nakagaki, M. *Arch. Biochem. Biophys.* **1983**, *227*, 52–70.

(23) Ishida, T.; Itoh, M.; Horiuchi, M.; Yamashita, S.; Doi, M.; Inoue, M.; Mizunoya, Y.; Tona, Y.; Okada, A. *Chem. Pharm. Bull.* **1986**, *34*, 1853–1864.

(24) It is important to note that these absorption bands are dependent on time. The absorbances increased almost linearly until ca. 1 week after preparation; they also varied with the temperature measured. Thus, in addition to the usual charge-transfer complex formation, there may be a possibility of forming a species such as an adduct of both molecules. We are now investigating these reactions in detail.

(25) Job, P. *Comput. Rend.* **1925**, *180*, 928–930.

(26) These data were obtained with $A/[D]_o = -KA + K[A]_o\epsilon$, where A is the absorbance at maximum wavelength, λ_{max}, of the band of the complex and $[A]_o$ and $[D]_o$ denote the initial molar concentrations of PQQ and each aromatic amino acid, respectively. (a) Faster, R.; Hammick, D. L.; Wardley, A. A. *J. Chem. Soc.* **1953**, 3817–3820. (b) Benesi, H. A.; Hildebrand, J. H. *J. Am. Chem. Soc.* **1949**, *71*, 2703–2707.

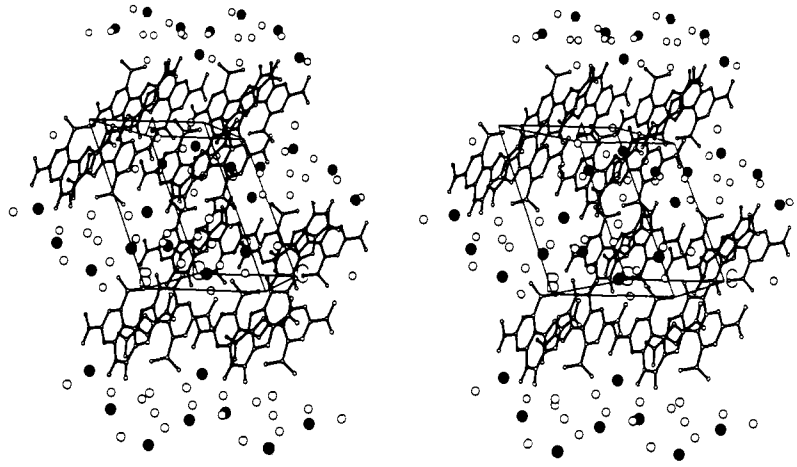


Figure 5. Stereoscopic view of crystal packing. Filled and open circles represent sodium ions and waters of crystallization, respectively.

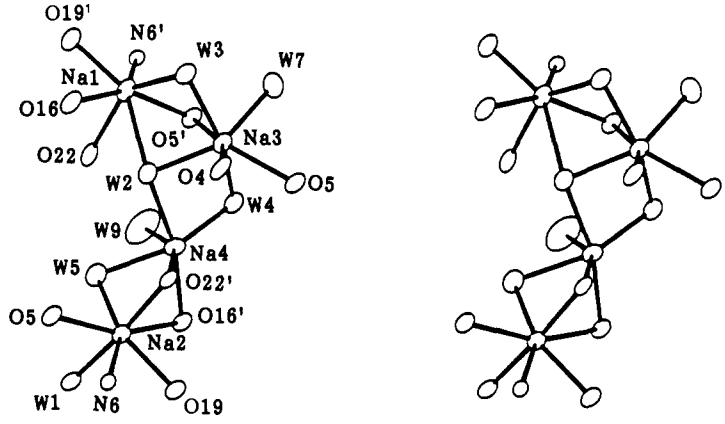


Figure 6. Stereoscopic view of the coordination mode of sodium ions. The primed atoms belong to molecule B. The letters W show the waters of crystallization.

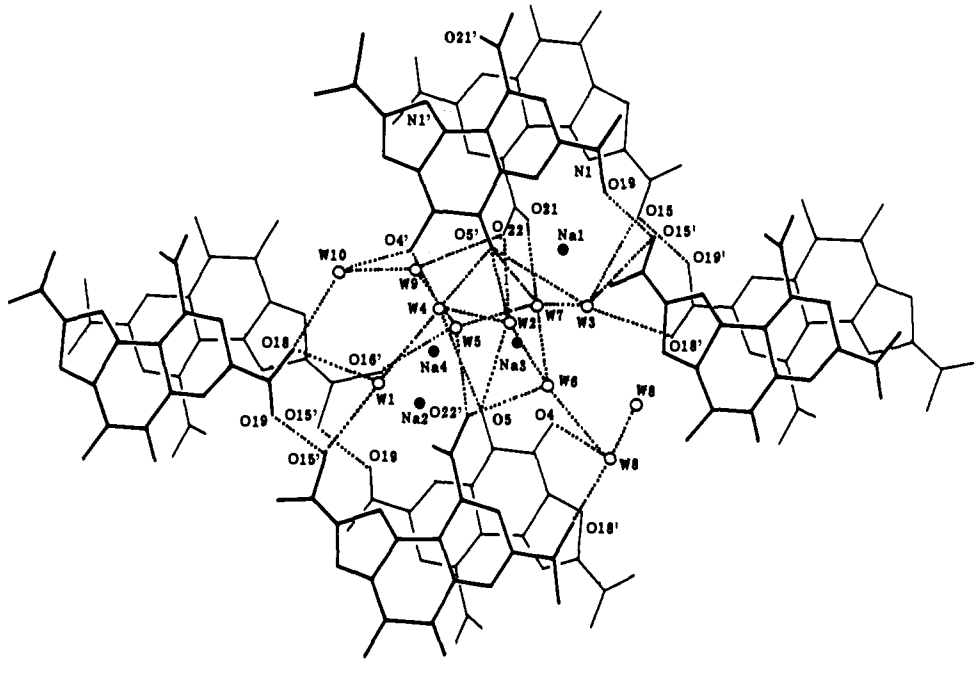


Figure 7. Schematic hydrogen-bonding network formed in the crystal structure. The dotted lines represent possible hydrogen bonds. The coordinations in which sodium ions (filled circles) participate are omitted for the sake of clarity. The primed atoms show those of molecule B, and the letters W represent the water oxygen atoms.

coordination and/or hydrogen-bond formations with the PQQ polar atoms. It is a characteristic of the crystal structure that about half of the unit-cell volume is occupied by these sodium ions and water molecules.

One notable feature observed in the crystal is that the arrangement of the four independent sodium ions forms a continuous and L-shaped column of the coordination shell, a part of which is shown in Figure 6. The coordination distances are listed in

Table IV; their bond angles are available in the supplementary material. These Na-O or Na-N distances are normal compared to those found in other sodium-coordinated compounds.[15] Each sodium ion coordinates with six (Na4) or seven (Na1-Na3) surrounding ligands. The coordination geometry could be essentially described as a tetragonally distorted octahedron, being bound to four (Na4) or five (Na1-Na3) ligands in an approximately equatorial plane and two others as a distorted axial coordination.

The other significant feature of this structure is the very elaborate network of hydrogen bonds formed between the PQQ and water molecules, and the water molecules combined with the sodium ions form an extremely tightknit molecular packing of PQQ. The schematic hydrogen-bonding network is shown in Figure 7; possible hydrogen bonds are listed in Table V, as judged from their bond distances. Each of the 10 independent water molecules, as the electron-donor or -acceptor atom, participates in three to five hydrogen-bond formations with the polar atoms of neighboring PQQ or water molecules. As is obvious from Figures 5-7, the PQQ molecules exist in the heavily hydrated and sodium-coordinated state. Since half the volume of the unit cell is occupied by these sodium ions and water molecules, it would be reasonable to some extent to consider that the PQQ molecules also exhibit a similar environment in the solution state.[27] Thus, the packing mode of the PQQ molecules observed in this crystal structure may be useful when various behaviors of the molecules in aqueous solutions are being considered.

Supplementary Material Available: Tables of anisotropic thermal parameters of non-hydrogen atoms, atomic coordinates and isotropic thermal parameters of hydrogen atoms, bond lengths and angles, torsion angles, equations of least-squares best planes along with the atomic deviations from them and the dihedral angles between them, coordination angles concerning sodium ions, and short contacts less than 3.4 Å (11 pages). Ordering information is given on any current masthead page.

(27) Preliminary ^1H NMR experiments of PQQ in ^2H_2O solution have shown the existence of the stacking interaction, judging from the prominent upfield shifts of aromatic protons with increasing of concentration.

First Observation of a Helical Peptide Containing a Chiral Residue without a Preferred Screw Sense

G. Valle,[1a] M. Crisma,[1a] C. Toniolo,*[,1a] R. Beisswenger,[1b] A. Rieker,*[,1b] and G. Jung*[,1b]

Contribution from the Biopolymer Research Center, CNR, Department of Organic Chemistry, University of Padova, 35131 Padova, Italy, and the Institut für Organische Chemie, Universität Tübingen, D-7400 Tübingen, FRG. *Received January 12, 1989*

Abstract: The molecular and crystal structure of the fully blocked, *chiral* pentapeptide Ac-$(Aib)_2$-*S*-Iva-$(Aib)_2$-OMe was determined by X-ray diffraction. It was found that the two crystallographically independent molecules (A and B), aligned in an antiparallel arrangement in the asymmetric unit, differ essentially by the *handedness* of their 3_{10}-helical structure, left-handed for A while right-handed for B. To our knowledge, this is the first observation of a helical peptide containing a chiral residue without a preferred screw sense. Conformational energy computations on the same peptide support the view that various helical structures, including two close to those found in the crystal state, have comparable stabilities.

The C^α,C^α-*di*alkylated chiral α-amino acid isovaline (Iva)[2] is a constituent of the naturally occurring peptaibol mycotoxins where it is present either as the *R* (D) or as the *S* (L) enantiomer.[3-6] In this work, the *R*,*S* nomenclature has been used throughout to avoid ambiguities, since *S*-(+)-Iva can be formally derived from either L-Abu or D-Ala,[7,8] as illustrated in Figure 1.

Conformational energy calculations of Ac-*R*-Iva-NHMe have shown that several conformations, including the fully extended (C_5) and the *right*-handed $3_{10}/\alpha$-helical conformations, represent local minima of comparable energy.[9] These results are of interest for the following reasons: (i) peptides containing Iva, with its side chains of bulkiness intermediate between those of Aib and Deg, can adopt either the helical conformations characterizing Aib peptides[10-15] or the C_5 conformation typical of Deg peptides.[16] (ii) The more stable helical conformation for the *R*-Iva enantiomer has the same handedness (right-handed) as that exhibited by the C^α-*mono*alkylated *protein* α-amino acids with *S* chirality. Only a few X-ray diffraction structures were reported so far for open-chain derivatives: mClAc-*R*-Iva-OH, which adopts the C_5 conformation (additionally stabilized by a Cl···H-N intramolecular H-bond),[17] and Boc-*R*-Iva-*S*-Hyp(Bzl)-*S*-Ala-*S*-Phol and Boc-

(1) (a) University of Padova. (b) Universität Tübingen.

(2) Abbreviations: Abu, α-aminobutyric acid; Aib, α-aminoisobutyric acid or C^α,C^α-dimethylglycine; Iva, isovaline or C^α-methyl-C^α-ethylglycine; Deg, C^α,C^α-diethylglycine; Phol, phenylalaninol; Ac, acetyl; mClAc, monochloroacetyl; Boc, (*tert*-butyloxy)carbonyl; *p*BrBz, *p*-bromobenzoyl; PChd, 3,5-di-*tert*-butyl-4-oxo-1-phenyl-2,5-cyclohexadien-1-yl; Bzl, benzyl; OMe, methoxy; NHMe, methylamino; Tfa^-, trifluoroacetate; HOBt, 1-hydroxybenzotriazole; DCC, *N*,*N*′-dicyclohexylcarbodiimide.

(3) Brückner, H.; Nicholson, G. J.; Jung, G.; Kruse, K.; König, W. A. *Chromatographia* **1980**, *13*, 209-214.

(4) Brückner, H.; Bosch, I.; Graser, T.; Fürst, P. *J. Chromatogr.* **1987**, *395*, 569-590.

(5) Bullough, D. A.; Jackson, C. G.; Henderson, P. J. F.; Cottee, F. H.; Beechey, R. B.; Linnett, P. E. *Biochem. Int.* **1982**, *4*, 543-549.

(6) Bullough, D. A.; Jackson, C. G.; Henderson, P. J. F.; Beechey, R. B.; Linnett, P. E. *FEBS Lett.* **1982**, *145*, 258-262.

(7) Yamada, S. I.; Achiwa, K.; Terashima, S.; Mizuno, H.; Takamura, N.; Legrand, M. *Chem. Pharm. Bull.* **1969**, *17*, 2608-2613.

(8) Brückner, H. *Chromatographia* **1987**, *24*, 725-738.

(9) Benedetti, E.; Toniolo, C.; Hardy, P.; Barone, V.; Bavoso, A.; Di Blasio, B.; Grimaldi, P.; Lelj, F.; Pavone, V.; Pedone, C.; Bonora, G. M.; Lingham, I. *J. Am. Chem. Soc.* **1984**, *106*, 8146-8152.

(10) Marshall, G. In *Intra-Science Chemistry Reports*; Kharasch, N., Ed.; Gordon and Breach: New York, 1971; pp 305-316.

(11) Jung, G.; Brückner, H.; Schmitt, H. In *Structure and Activity of Natural Peptides*; Voelter, W.; Weitzel, G., Eds.; de Gruyter: Berlin, 1981; pp 75-114.

(12) Venkataram Prasad, B. V.; Balaram, P. *CRC Crit. Rev. Biochem.* **1984**, *16*, 307-348.

(13) Toniolo, C.; Bonora, G. M.; Bavoso, A.; Benedetti, E.; Di Blasio, B.; Pavone, V.; Pedone, C. *Biopolymers* **1983**, *22*, 205-215.

(14) Toniolo, C.; Benedetti, E.; Pedone, C. *Gazz. Chim. Ital.* **1986**, *116*, 355-359.

(15) Paterson, Y.; Rumsey, S. M.; Benedetti, E.; Némethy, G.; Scheraga, H. A. *J. Am. Chem. Soc.* **1981**, *103*, 2947-2955.

(16) Benedetti, E.; Barone, V.; Bavoso, A.; Di Blasio, B.; Lelj, F., Pavone, V.; Pedone, C.; Bonora, G. M.; Toniolo, C.; Leplawy, M. T.; Kaczmarek, K.; Redlinski, A. *Biopolymers* **1988**, *27*, 357-371.

0002-7863/89/1511-6828$01.50/0 © 1989 American Chemical Society