X-Ray Molecular and Crystal Structure of Imidazolopyrroloquinoline, a Main Reaction Product of Pyrroloquinolinequinone (PQQ) and L-Tryptophan *in Vitro*

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The chemical structure of imidazolopyrroloquinoline (IPQ), a main reaction product of pyrroloquinolinequinone and L-tryptophan *in vitro*, has been determined by X-ray crystal analysis. The IPQ molecules exist in keto form, having three neutral carboxyl groups, and they form continuous and prominent stacking structures in a crystal which are stabilized by van der Waals, dipole-dipole and electrostatic interactions. There are three crystallographically independent dimethyl sulfoxide solvents in a crystal, and they stabilize the stacking layers of IPQ molecules by intermolecular hydrogen bonds between neighboring oxygen atoms.

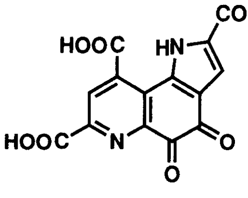
Key words imidazolopyrroloquinoline; PQQ adduct; tryptophan; X-ray analysis; crystal structure

In addition to its biological activities[1)] as a prosthetic group of various kinds of quinoproteins and as a growth stimulating substance for microorganisms, pyrroloquinolinequinone (PQQ) catalyzes the nonenzymatic reaction of a biomolecule to various products *in vitro*.[2)] For example, PQQ catalyzes the oxidative decarboxylation or oxidative dealdolation of amino acids *in vitro*.[3)] On the other hand, PQQ itself is known to be gradually converted into biologically active compounds by adduct formation with amino acids as these nonenzymatic catalytic cycles progress.[3,4)]

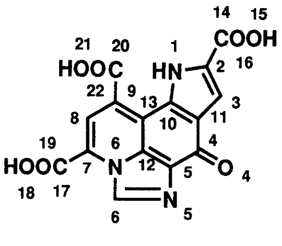
In a series elucidating the *in vitro* reaction mechanism of PQQ and L-tryptophan (Trp), we have investigated their molecular interaction[5)] and the structures of the reaction products. Recently, we analyzed the crystal structure of the main reaction product by the X-ray diffraction method, and showed the structure of imidazolopyrroloquinoline (IPQ).[6)] This paper deals with the details of its molecular and crystal structure.

Experimental

Preparation of the Reaction Products of PQQ and Trp The reaction solution (50 ml) consisted of PQQ (30 μmol) and Trp (300 μmol) in 50mM phosphate buffer (pH 6.5). The solution was stirred at 30 °C for 24 h under an aerobic condition in a dark room. The reaction mixture was separated from the unreacted Trp and PQQ by elution through a DEAE Cellulofine A200 column (0—2 M linear gradient of triethylaminoacetate). The fraction of the reaction product was further subjected to elution through an octadecyl silica (ODS) column (5—8% linear gradient of acetonitrile in 10 mM triethylaminoacetate, pH 7.0), leading to the isolation of the main reaction product of PQQ and Trp; the purity was confirmed by HPLC using a reverse-phase column (Cosmosil ODS 5C18).



PQQ



IPQ

X-Ray Crystal Analysis Single crystals of the main product (dark orange-colored plates) were obtained from a dimethyl sulfoxide (DMSO) solution at room temperature. Since the crystals become opaque (deterioration of crystallinity) in the air, they were sealed in glass capillaries containing a mother liquid. Crystal data are as follows: $C_{15}H_7N_3O_7 \cdot 3(CH_3)_2SO$, $M=575.62$, triclinic, space group $P\bar{1}$, $a=13.451(1)$ Å, $b=14.697(2)$ Å, $c=7.308(1)$ Å, $\alpha=91.26(1)°$, $\beta=103.37(1)°$, $\gamma=66.79(1)°$, $V=1287.9(3)$ Å^3 (by least-squares refinement on diffractometer angles for 20 automatically centered reflections), $Z=2$, $D_x=1.484$ g·cm^{-3}, $\lambda(\mathrm{Cu}K_\alpha)=1.5418$ Å, $\mu(\mathrm{Cu}K_\alpha)=3.164$ mm^{-1}, $F(000)=600$.

A single crystal with the dimensions $0.1\times0.1\times0.3$ mm, was used for X-ray diffraction data collection on a Rigaku AFC-5 diffractometer employing graphite-monochromated CuK_α radiation. A total of 3698 independent reflections were collected with an $\omega-2\theta$ scan mode at 293 K; the parameters for data collection are scan speed (in ω) $=16°$ min^{-1}, scan range (in ω) $=(1.15+0.15\tan\theta)°$ and data range measured $= 2°\leq 2\theta \leq 130°$. The weak X-ray intensities $[F_o^2 \leq \sigma(F_o^2)$ at the first scan$]$ were rescanned several times (up to 7 scans) to ensure good counting statistics. The stationary background counts for 5 s were recorded on each side of the reflections. Three standard reflections were monitored at an interval of every 100 reflections throughout the data collection and showed no significant deterioration (random fluctuation $<\pm3\%$). Intensities were corrected for Lorentz and polarization effects, and an empirical absorption correction using the ϕ scan was also applied; the transmission coefficient was in the range of 0.6 to 1.0.

The structure was determined by a direct method using the program MULTAN87,[7)] and was refined by the least-squares method (program SHELXL-93[8)]) with use of anisotropic temperature factors for non-H atoms. Ideal positions for the H atoms were calculated and included only in the calculation of structure factors. The function of $\Sigma w(F_o^2-F_c^2)^2$ was minimized, and $w=1/[\sigma^2(F_o^2)+(0.1432p)^2]$ was used in the final refinement, where $p=(F_o^2+2F_c^2)/3$. The present discrepancy indexes $R1$ $(\Sigma||F_o|-|F_c||/\Sigma|F_o|)$, $wR2$ $([\Sigma[w(F_o^2-F_c^2)^2]/\Sigma[w(F_o^2)^2]]^{1/2})$ and S $([\Sigma[w(F_o^2-F_c^2)^2]/(M-N)]^{1/2}$, where $M=$ no. of reflections and $N=$ no. of variables used for the refinement $(=335)$) are 0.0583, 0.1522 and 0.909 for 1575 independent reflections of $F_o^2\geq 2\sigma(F_o^2)$. None of the positional parameters for non-H atoms shifted by more than one-fifth from their standard deviations, and the maximum electron density in the final difference Fourier map was in the range of -0.37 to $0.50e$Å^{-3}. The final positional parameters and their isotropic temperature factors of non-H atoms are given in Table 1.[9)] The atomic charges, dipole moments, and other quantum chemical data were calculated by the MNDO program[10)] using the atomic coordinates of the present X-ray analysis or its related compounds.

The numerical calculations were performed at the Computation Center, Osaka University of Pharmaceutical Sciences.

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Table 1. Final Positional Atomic Coordinates and Their Isotropic Temperature Parameters of Non-H atoms, with Their e.s.d.s in Parentheses

Atom	x	y	z	U_{eq}
N(1)	0.7060 (5)	−0.1843 (5)	−0.0266 (9)	0.038 (2)
C(2)	0.7988 (7)	−0.1774 (6)	0.082 (1)	0.038 (2)
C(3)	0.7851 (6)	−0.0800 (6)	0.074 (1)	0.042 (2)
C(4)	0.6228 (6)	0.0821 (5)	−0.102 (1)	0.032 (2)
O(4)	0.6648 (5)	0.1403 (4)	−0.0440 (9)	0.056 (2)
C(5)	0.5150 (6)	0.1076 (5)	−0.228 (1)	0.033 (2)
N(5)	0.4375 (5)	0.1999 (4)	−0.309 (1)	0.038 (2)
C(6)	0.3523 (7)	0.1842 (6)	−0.409 (1)	0.044 (2)
N(6)	0.3675 (5)	0.0855 (4)	−0.3963 (9)	0.030 (2)
C(7)	0.3037 (6)	0.0327 (5)	−0.470 (1)	0.033 (2)
C(8)	0.3492 (7)	−0.0657 (6)	−0.427 (1)	0.038 (2)
C(9)	0.4582 (6)	−0.1204 (5)	−0.308 (1)	0.034 (2)
C(10)	0.6314 (6)	−0.0937 (5)	−0.110 (1)	0.031 (2)
C(11)	0.6798 (6)	−0.0255 (5)	−0.047 (1)	0.033 (2)
C(12)	0.4730 (6)	0.0374 (5)	−0.281 (1)	0.031 (2)
C(13)	0.5223 (6)	−0.0669 (5)	−0.233 (1)	0.033 (2)
C(14)	0.8868 (7)	−0.2693 (7)	0.185 (1)	0.050 (3)
O(15)	0.9744 (5)	−0.2563 (4)	0.2874 (8)	0.055 (2)
O(16)	0.8795 (5)	−0.3480 (5)	0.171 (1)	0.069 (2)
C(17)	0.1881 (6)	0.0815 (6)	−0.592 (1)	0.043 (2)
O(18)	0.1576 (4)	0.1763 (4)	−0.6358 (8)	0.051 (2)
O(19)	0.1316 (5)	0.0350 (4)	−0.643 (1)	0.068 (2)
C(20)	0.4961 (7)	−0.2300 (6)	−0.272 (1)	0.041 (2)
O(21)	0.4176 (5)	−0.2604 (4)	−0.3484 (8)	0.052 (2)
O(22)	0.5864 (5)	−0.2839 (4)	−0.1828 (9)	0.060 (2)
DMSO				
S(1)	0.8682 (2)	0.2483 (2)	0.0946 (5)	0.074 (1)
O(1)	0.9696 (5)	0.2727 (4)	0.155 (1)	0.074 (2)
C(1)	0.9112 (9)	0.1393 (8)	−0.026 (2)	0.080 (4)
C(1′)	0.8515 (9)	0.1980 (9)	0.298 (2)	0.094 (4)
S(2)	0.1858 (2)	0.4815 (2)	−0.6358 (4)	0.070 (1)
O(2)	0.1387 (6)	0.5795 (4)	−0.553 (1)	0.082 (2)
C(2)	0.179 (1)	0.5108 (8)	−0.869 (2)	0.089 (4)
C(2′)	0.0838 (9)	0.4320 (8)	−0.678 (2)	0.089 (4)
S(3)	0.4414 (2)	0.4772 (2)	−0.3200 (4)	0.0537 (8)
O(3)	0.4601 (6)	0.5648 (4)	−0.233 (1)	0.076 (2)
C(3)	0.3583 (8)	0.4509 (7)	−0.188 (1)	0.057 (3)
C(3′)	0.5693 (8)	0.3739 (7)	−0.234 (1)	0.071 (3)

$U_{eq} = 1/3 \times \Sigma_i \Sigma_j U_{ij} \cdot a_i^* \cdot a_j^* \cdot \mathbf{a}_i \cdot \mathbf{a}_j$.

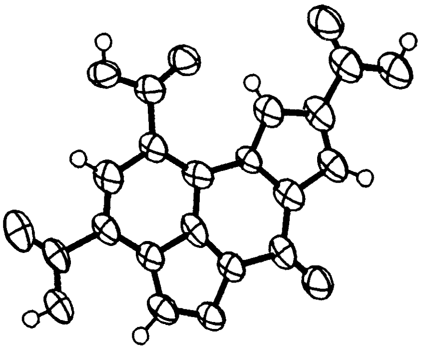

Fig. 1. IPQ Molecular Conformation, Perpendicular to the Aromatic Plane

Open circles represent H atoms.

Table 2. Some Selected Bonding Parameters

Bond length (Å)			
C(4)–O(4)	1.22 (1)	C(5)–N(5)	1.38 (1)
N(5)–C(6)	1.31 (1)	C(6)–N(6)	1.38 (1)
C(2)–C(14)	1.47 (1)	C(14)–O(15)	1.32 (1)
C(14)–O(16)	1.20 (1)	C(7)–C(17)	1.50 (1)
C(17)–O(18)	1.31 (1)	C(17)–O(19)	1.20 (1)
C(9)–C(20)	1.49 (1)	C(20)–O(21)	1.31 (1)
C(20)–O(22)	1.20 (1)		
Bond angle (°)			
O(4)–C(4)–C(5)	125.6 (5)	O(4)–C(4)–C(11)	122.4 (5)
C(5)–C(4)–C(11)	111.9 (5)	C(4)–C(5)–N(5)	128.9 (5)
C(4)–C(5)–C(12)	121.6 (5)	N(5)–C(5)–C(12)	109.5 (5)
C(5)–N(5)–C(6)	105.4 (5)	N(5)–C(6)–N(6)	112.9 (5)
C(6)–N(6)–C(7)	135.0 (5)	C(6)–N(6)–C(12)	104.6 (5)
C(7)–N(6)–C(12)	120.4 (5)	C(2)–C(14)–O(15)	113.4 (5)
C(2)–C(14)–O(16)	122.4 (5)	O(15)–C(14)–O(16)	124.2 (5)
C(7)–C(17)–O(18)	113.6 (5)	C(7)–C(17)–O(19)	121.0 (5)
O(18)–C(17)–O(19)	125.4 (5)	C(9)–C(20)–O(21)	111.9 (5)
C(9)–C(20)–O(22)	124.6 (5)	O(21)–C(19)–O(22)	123.5 (5)
Torsion angles (°)			
N(1)–C(2)–C(14)–O(15)	−180.0 (9)	N(1)–C(2)–C(14)–O(16)	1.5 (9)
C(3)–C(2)–C(14)–O(15)	−2.7 (7)	C(3)–C(2)–C(14)–O(16)	179 (1)
N(6)–C(7)–C(17)–O(18)	5.7 (6)	N(6)–C(7)–C(17)–O(19)	−174 (1)
C(8)–C(7)–C(17)–O(18)	−174.7 (9)	C(8)–C(7)–C(17)–O(19)	5.3 (8)
C(8)–C(9)–C(20)–O(21)	−4.5 (7)	C(8)–C(9)–C(20)–O(22)	177 (1)
C(13)–C(9)–C(20)–O(21)	175.6 (9)	C(13)–C(9)–C(20)–O(22)	−3.1 (8)
C(5)–N(5)–C(6)–N(6)	1.1 (7)		

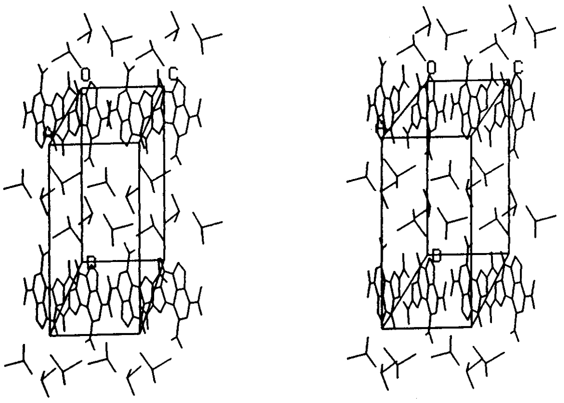

Fig. 2. Stereoscopic Drawing of IPQ Crystal Structure, Viewed along the *a*-Axis

Results and Discussion

Molecular Structure The molecular conformation of the main reaction product is shown in Fig. 1, and some selected bond lengths, bond angles, and torsion angles are summarized in Table 2. Because of the limited observed intensities due to small crystal size and relatively poor crystallinity, the bonding parameters were not as accurate as usual. However, we believe that the low accuracy does not significantly influence the description of the structural characteristics, because these bonding parameters are all in the range accepted for general organic compounds.[11]

The present X-ray crystal analysis clearly showed that the main *in vitro* reaction product of PQQ and Trp is IPQ. As judged from the bond lengths and angles, three carboxyl groups are all in a neutral state, with the C(4)–O(4) taking a keto form, although H atoms attached to respective carboxyl groups were not clearly observed on the electron density map. The structure of IPQ is essentially planar with a fluctuation from −0.023(8) Å [C(10) atom] to 0.021(9) Å [C(3) atom], and three carboxyl groups and an O(4) atom are almost coplanar with the aromatic ring [the deviation <0.05 Å], thus, the resonance state is expanded over the whole structure; respective carboxyl groups assume a dihedral angle of 1.5(4)°—5.9(3)° with respect to the aromatic ring.

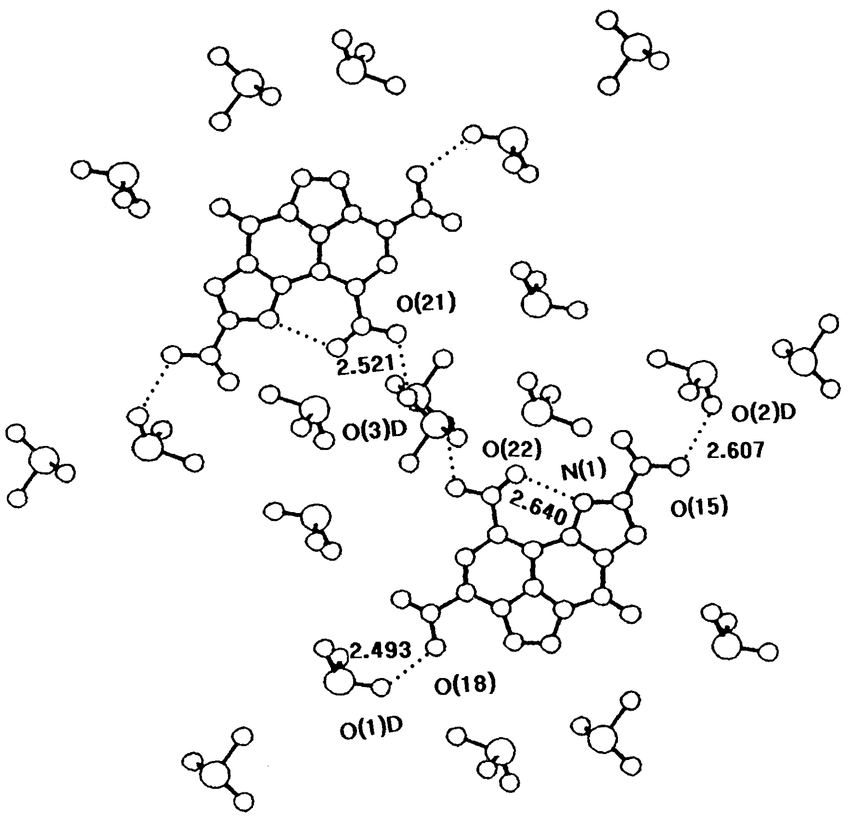

Fig. 3. Interaction of IPQ Molecules with DMSO Solvents, Viewed along the *c*-Axis

Dotted lines represent possible hydrogen bonds. The oxygen atoms of DMSO solvents are indicated as suffix letter D.

Crystal Structure The crystal packing of IPQ molecules is shown in Fig. 2, and the hydrogen bonds between the IPQ and DMSO molecules are shown in Fig. 3, where possible hydrogen bonds, together with their distances, are shown by dotted lines.

One of the most characteristic features in the crystal structure is the formation of extensive overlapping between the centrosymmetrically-related IPQ molecules, thus forming an infinite stacking layer of IPQ molecules along the *c*-axis. Respectively stacked IPQ molecules are antiparallelly arranged, and their average interplanar spacing is 3.35 Å, close to the minimum separation distance (=3.4 Å) between the usual aromatic rings. The molecular packing would be constructed by dipole-dipole and electrostatic interactions, in addition to the normal van der Waals forces, because the dipole moments are completely coupled due to the centrosymmetrically-related overlapping between the IPQ molecules. Also the electrostatic energy gave negative values of -3.1—-4.5 kcal/mol for the stacking pairs of upper and lower molecules with respect to the central one; the electrostatic energy was calculated by $332.0 \times \Sigma_i \Sigma_j q_i q_j / r_{ij}$, where q_i and q_j represent the *i*-th and *j*-th atomic net charges, calculated by the MNDO/2 method, in the stacking pair of $(\mathrm{IPQ})_i$-$(\mathrm{IPQ})_j$.

Another characteristic of the crystal structure is the alternative arrangement of respective layers comprising IPQ and solvent molecules, which run parallel to the *c*-axis, as shown in Fig. 2; the solvent layers are composed of the packing of three crystallographically independent DMSO molecules. It is interesting to note that the crystal is very liable to be cracked along the *c*-axis, and this would be due to the relatively loose packing of DMSO solvents among the stacking layers of IPQ molecules. The crystal structure is mainly stabilized by hydrogen bonds between the neighboring oxygen atoms of DMSO and IPQ molecules (Fig. 3): O(15)-H···O(2)D [=2.607(9) Å, O(18)-H···O(1)D [=2.493(9) Å], and O(21)-H···O(3)D [=2.521(9) Å.

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