Formation of Imidazolopyrroloquinoline as Main PQQ Adduct with Amino Acid *in Vitro*: X-ray Structural Evidence

Toshimasa Ishida,* Eiji Kawamoto, Yasuko In,
Takashi Amano, Jyutoku Kanayama, and Mitsunobu Doi

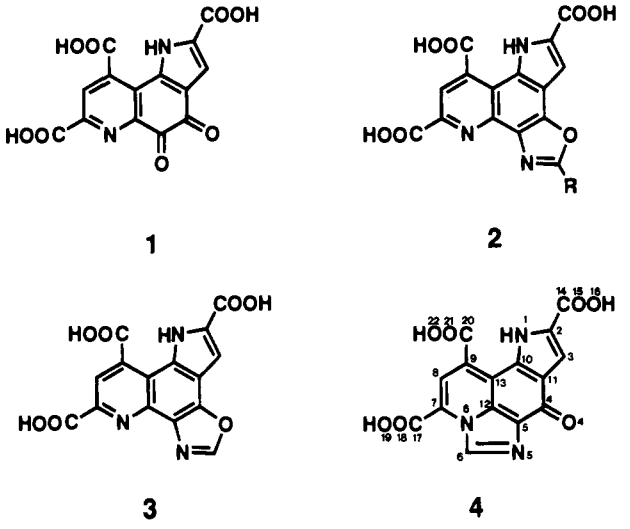
Department of Physical Chemistry
Osaka University of Pharmaceutical Sciences
2-10-65 Kawai, Matsubara, Osaka 580, Japan

Takashi Iwashita and Kyosuke Nomoto

Suntory Institute for Bioorganic Research
1-1-1 Shimamoto-cho, Mishima-gun, Osaka 618, Japan

Received November 4, 1994

Pyrroloquinolinequinone (PQQ, **1**) exhibits biological activity as a prosthetic group of various kinds of quinoproteins as well as a growth-stimulating substance for microorganisms.[1] It is also well-known that PQQ itself reacts nonenzymatically with biomolecules *in vitro* to form various products.[2] PQQ catalyzes *via* the Schiff base formation the oxidative decarboxylation or oxidative dealdolation of amino acid *in vitro*.[3] It has been generally accepted that, during the catalytic cycles, PQQ is converted into an inactive oxazolopyrroloquinoline (OPQ) derivative (**2**).[3,4] In a catalytic experiment of the reaction of PQQ with tryptophan under aerobic conditions *in vitro*, the spectral data of the major adduct obtained were found to be the same as those reported for OPQ (**3**);[3b] however, X-ray single-crystal analysis of the major adduct revealed that it is in fact imidazolopyrroloquinoline (**4**).



In vitro reaction of PQQ with L-tryptophan in phosphate buffer (pH 6.5) afforded three different PQQ adducts.[5] Among them, the major adduct showed the same molecular weight, quantitative HPLC profile, and spectroscopic data as those reported for **3**.[6] However, in the ^{1}H NMR spectrum, the chemical shift (9.23 ppm) assigned to the oxazole ring proton was rather in the low-field region, compared with the usually observed value (~8.10 ppm), and the peak corresponding to the carbonyl carbon was revealed at 173.17 ppm, in addition to three peaks of carboxyl carbons, in the ^{13}C-NMR measurement.

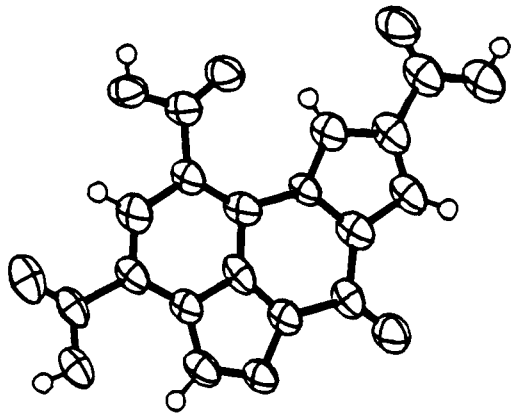


Figure 1. Perspective drawing of **4**, viewed perpendicular to the PQQ plane. Open circles represent hydrogen atoms.

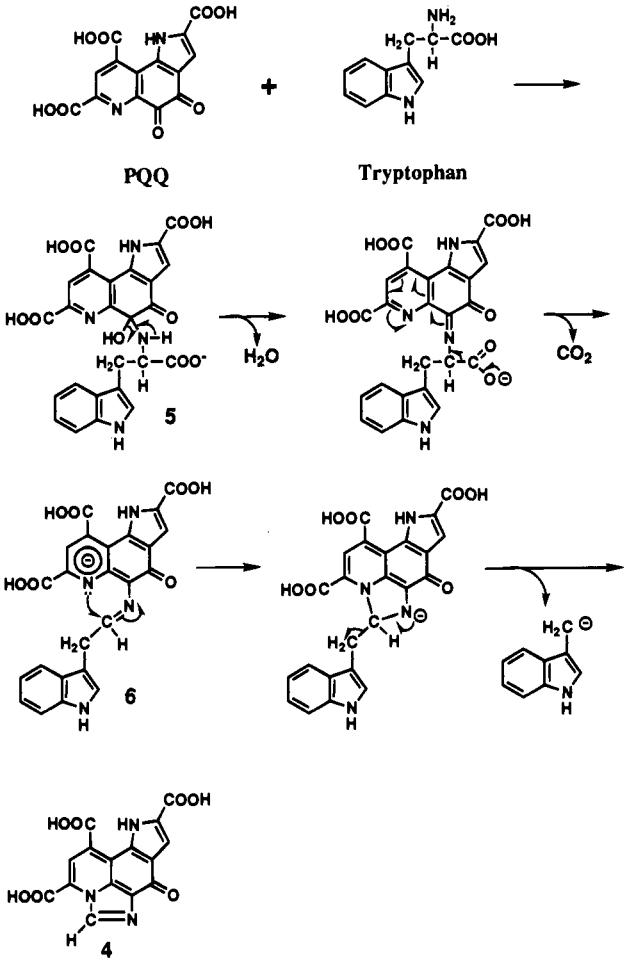


Figure 2. Proposed reaction mechanism for tryptophan conversion and **4** formation.

(1) (a) Gallop, P. M.; Paz, M. A.; Fluckiger, R.; Kagan, H. M. *Trends Biol. Sci.* **1989**, *14*, 343. (b) Duine, J. A.; Jongejan, J. A. *Vitamins Horm. (N.Y.)* **1989**, *45*, 223. (c) Duine, A. A.; van der Meer, R. A.; Groen, B. W. *Annu. Rev. Nutr.* **1990**, *10*, 297. (d) Duine, J. A. *Eur. J. Biochem.* **1991**, 200, 271.

(2) (a) Duine, J. A.; Frank, J. J.; Jongejan, J. A. *Adv. Enzymol.* **1986**, *59*, 169. (b) Ohshiro, Y.; Itoh, S. *Yuki Gosei Kagaku* **1989**, *47*, 855.

(3) (a) Adachi, O.; Okamoto, K.; Shinagawa, E.; Matsushita, K.; Ameyama, M. *BioFactors* **1988**, *1*, 251. (b) Itoh, S.; Mure, M.; Suzuki, A.; Murao, H.; Ohshiro, Y. *J. Chem. Soc., Perkin Trans. 2* **1992**, 1245.

(4) (a) Sleath, P. R.; Noar, J. B.; Eberlein, G. A.; Bruice, T. C. *J. Am. Chem. Soc.* **1985**, *107*, 3328. (b) Van Kleef, M. A. G.; Jongejan, J. A.; Duine, J. A. *Eur. J. Biochem.* **1989**, *183*, 41.

(5) The reaction solution (50 mL) consisted of PQQ (30 μmol) and L-tryptophan (300 μmol) in 50 mM phosphate buffer (pH 6.5). The reaction was stirred for 24 h at 30 °C under aerobic conditions in a dark room. Separation of the reaction mixture by elution through a DEAE Cellulofine A200 column yielded the PQQ adduct, in addition to unreacted Trp and PQQ (0−2 M linear gradient of triethylammonium acetate). The adduct fraction was further separated into three different adducts by elution through an ODS column.

0002-7863/95/1517-3278$09.00/0

In order to confirm the structure, thus, it was crystallized as needles from dimethyl sulfoxide at room temperature (20 °C) by slow evaporation and was subjected to X-ray diffraction.[7]

The major adduct formed upon reaction of PQQ with tryptophan or glycine has been reported to be **3**;[3] the present X-ray analysis clearly showed that this adduct has the chemical structure of **4** (Figure 1). As judged from bond lengths and angles, the three carboxyl groups are all in the neutral state, with the bond lengths of 1.20 Å for C=O and 1.31−1.32 Å for C−O(H), and the C4−O4 (1.22 Å) taking a keto form. Considering these results and that the results of ^{1}H NMR, mass, and UV−vis spectroscopies and elemental analysis of **4** were the same as those reported for **3**,[3b,4] we concluded that the major adduct formed upon reaction of PQQ with tryptophan is **4**.

A proposed mechanism for the formation of **4** is shown in Figure 2. The amine oxidation by PQQ has been demonstrated to proceed *via* an ionic mechanism involving a carbinolamine-type intermediate (**5**).[10] After formation of the imine by direct dehydration, followed by decarboxylation, ring closure of the Schiff base *via* the N6 atom results in the formation of **4**. Since ring closure *via* the O4 atom gives **3**, the present result suggests that, for reaction with the Cα atom of a Schiff base, the reactivity of the N6 atom is superior to the reactivity of the O4 atom. Since the adduct formed upon reaction of PQQ with amino acid exhibits a more potent growth-stimulating effect in microorganisms than the PQQ itself and may be involved in the regulation of the catalysis of quinoprotein,[3a] the present result is useful when considering the biological role of PQQ and its adduct.

Supplementary Material Available: X-ray diffraction data including atomic coordinates, anisotropic thermal parameters, bond lengths, bond angles, and torsion angles for **4** (7 pages); listing of observed and calculated structure factors for **4** (9 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

JA943583J

(6) The elemental analysis results and spectroscopic data of the main adduct are the same as those reported for **3**: mass, *m/z* (FD) 342 (MH^+); UV−vis (λ_{max}/nm, in 0.5 M phosphate buffer, pH 7), 251 (log ϵ = 4.36), 276 (4.35), 422 (4.17); ^{1}H NMR (ppm in [$^2H_6$]Me_2SO_4, as a standard value of ^{1}H of Me_2SO_4 = 2.50 ppm), 7.25 (1 H, s, 3-H), 8.29 (1 H, s, 9-H), 9.23 (1 H, s, 6-H), 12.94 (1 H, s, 1-NH). The quantitative HPLC (ODS column) results showed that the retention time for the present adduct was the same as that reported for the adduct (**3**) synthesized by reaction of PQQ with glycine.[3b]

(7) Crystal data: $C_{15}H_7N_3O_7 3(CH_3)_2SO$, M_r = 875.624, triclinic, space group $P\bar{1}$, a = 13.451(1) Å, b = 14.697(2) Å, c = 7.308(1) Å, α = 91.26(1)°, β = 103.37(1)°, γ = 66.79(1)°, V = 1287.9(3) Å^3, Z = 2, D_{calcd} = 1.484 g cm^{-3}, λ(Cu Kα) = 1.5418 Å, μ(Cu Kα) = 3.16 mm^{-1}, F(000) = 600. A single crystal of dimensions 0.1 × 0.1 × 0.3 mm was used for X-ray diffraction data collection on a Rigaku AFC-5 diffractometer employing graphite-monochromated Cu Kα radiation. A total of 3698 reflections within 2θ = 130° were collected with an $\omega-2\theta$ scan mode. 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 0.6−1.0). Structure determination and refinement were carried out using 1575 independent reflections of $|F_o| > 4\sigma(F_o)$. The structure was finally determined by a direct method using the program MULTAN87[8] and refined by the least-squares method (program SHELXL-93[9]) with use of the anisotropic temperature factors for non-H atoms. Ideal positions for H atoms were calculated and fixed; they were included only in the calculation of structure factors. The present discrepancy indexes R_1, wR_2, and S are 0.058, 0.231, and 0.868, respectively.

(8) Debaerdemaeker, T.; Germain, G.; Main, P.; Tate, C.; Woolfson, M. M. *MULTAN87, a System of Computer Programs for the Automatic Solution of Crystal Structures from X-Ray Diffraction Data*; Universities of York, England, and Louvain, Belgium: 1987.

(9) Sheldrick, G. M. *J. Appl. Crystallogr.*, submitted.

(10) (a) Rodriguez, E. J.; Bruice, T. C. *J. Am. Chem. Soc.* **1989**, *111*, 7947. (b) Mure, M.; Itoh, S.; Ohshiro, Y. *Tetrahedron Lett.* **1989**, *30*, 6875. (c) Itoh, S.; Mure, M.; Ogino, M.; Ohshiro, Y. *J. Org. Chem.* **1991**, *56*, 6857.