Table 1. Results of x-ray powder diffraction of pecoraite with copper radiation and nickel filter.

Probable hkl	Observed d (Å)	Intensity ($\sqrt{2}$ scale)
002	7.43	8
020	4.50	5
004	3.66	6
130	2.620	5
$20\bar{2}$	}	Diffuse band
202	2.447	4
204	P*	Diffuse
060	1.529	6
$40\bar{2}$	1.303	Diffuse band

* Present but not measurable.

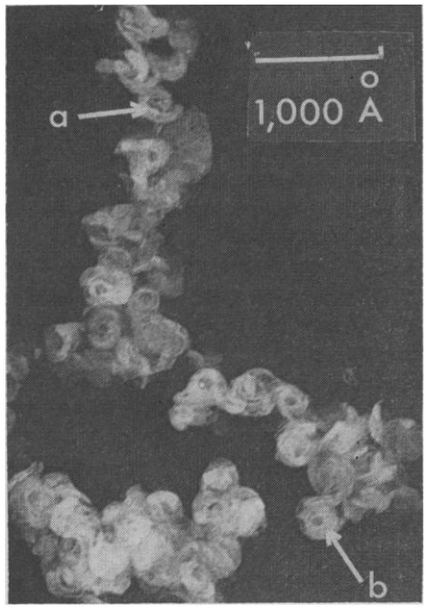

Fig. 1. Electron micrograph of pecoraite showing aggregates of curved plates: *a*, coils; *b*, spirals.

0.5; Al_2O_3, 1.4; H_2O^+, 9.7; H_2O^-, 4.1; CaO, 0.4; a total of 99.3 percent. Deducting the CaO value, which is equivalent to about 1 percent of the phosphate cassidyite (*1*), and the adsorbed water (H_2O^-) the formula becomes (*5*)

$$\left[Ni_{5.41}Mg_{0.10}Fe^{2+}_{0.08}Al_{0.22}\right]\left[Si_{4.05}\right]O_{10}(OH)_8$$

The x-ray powder diffraction pattern (obtained with copper radiation and nickel filter) is very similar to that of clinochrysotile, except for the line broadening due to the extremely fine grained nature of the material (Table 1). Indexing is by analogy with data for clinochrysotile of Whittaker and Zussman (*6*). Electron microscopy confirms the small particle size and shows that some of the particles form as curved plates; others have started to coil and some have formed complete spirals (Fig. 1). The average thickness of the plates [calculated from line-breadth measurements on the x-ray powder photographs and diffractometer traces with the equation of Wilson (*7*)] is 74 Å and 68 Å, respectively. Direct measurement on the electron micrograph gives 70(±5) Å in those areas free of overlapping segments of coils and spirals. This value will be determined more accurately when high-resolution stereo pairs are available.

Pecoraite is associated with major quantities of maghemite and goethite and lesser amounts of cassidyite, reevesite, and adventitious quartz blown into the cracks.

Pecoraite was formed during the mechanical and chemical disintegration of the meteorite fragments as they lay on the desert floor. The cyclical process of extreme heating during the day and slower cooling after sundown and at night, integrated over a long period of time, caused the meteorite fragments to crack. Sand grains blown into the cracks helped to widen them and were themselves crushed. Rainfall during the monsoon season became entrapped in the cracks, and on heating in the morning sun the cracks behaved like a whole system of small hydrothermal bombs in which pecoraite was slowly formed by the reaction of nickel and silica. At the same time goethite, maghemite, and the other phases were formed as the iron alloy was decomposed.

We anticipated from crystal chemical considerations that nickel analogs of many magnesium silicates should exist under natural conditions, and many poorly characterized nickel silicates have been described. Pecoraite is the second naturally occurring hydrous nickel silicate to be established firmly; the first was pimelite, the nickel member of the montmorillonite series (*8*). Ostrowicki (*9*) has found a phase with a *d*-spacing of 9.9 to 10.4 Å. We anticipate that others will be found.

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23 May 1969

Azurin: X-ray Data for Crystals from Pseudomonas denitrificans

Abstract. *Azurin, a blue-colored copper protein, from a soil bacteria,* Pseudomonas denitrificans, *has been crystallized and its molecular weight of 16,000 was confirmed by means of x-ray diffraction.*

Azurins are the simplest copper proteins known. They contain only one copper atom per molecule and have low molecular weight (about 14,000 in a single polypeptide chain) (*1*). However, their optical absorption and electronic spin resonance structure are almost identical to much more complex copper proteins, such as ceruloplasmin (*2*) which contains eight copper atoms per molecule, with a molecular weight of about 140,000 and eight subunit polypeptide chains (*3*). The purpose of this study is to obtain detailed structural data applicable to all copper proteins and their biological roles.

We used azurin from *Pseudomonas denitrificans* (a soil bacteria) which was purified by the procedure of Suzuki and Iwasaki (*4*). The protein was not pure, as indicated by the absorption spectrum. Nevertheless, azurin could be crystallized in three different ways. (i) Slow addition of solid ammonium sulfate with slow evaporation of water gave both discrete and continuous changes in ammonium sulfate concentration. (ii) A freshly prepared hot gelatin solution (0.7 percent by weight, U.S.P. gelatin in distilled water) was allowed to cool and solidify in the bottom of a U-shaped polyvinylchloride tube, and a saturated solution of ammonium sulfate was placed at one side of the gelatin plug to diffuse across to the azurin solution placed on the other side. Azurin crystals formed in a few days, and the crystals and the liquid

and solid contents of the tube were removed by cutting the tube. (iii) A saturated solution of ammonium sulfate was placed in a test tube, and the less dense azurin solution was added to the top of the ammonium sulfate solution forming a separate layer. Azurin crystals formed after several days. The crystals formed in these three processes were always blue-colored needles of square cross-section from 0.1 to 2.0 mm long, their width was about 1/20th of their length. Much smaller crystals were reported by Suzuki and Iwasaki (*4*).

X-ray diffraction photographs were taken with a precession camera with nickel-filtered CuKα radiation (wavelength, 1.542 Å). The photographs taken with the x-ray beam coincident with the direction of elongation of the crystal show 4*mm* symmetry, and the photographs taken with the x-ray beam perpendicular to the elongation axis and the side of the cross section show *mm* symmetry. The Laue group is 4/*mmm*, the former photographs are of the *hk*0 reciprocal lattice plane, and the latter are of the 0*kl* or *h*0*l* reciprocal lattice plane (which are identical). The cell dimensions are $a = b = 53.2$ Å and $c = 101$ Å. The only extinctions observed are: 00*l* present only for $l = 4n$. This observation, together with the Laue group, defines the space group as 91 $P4_122$ (or its enantiomorph 95 $P4_322$). This space group is quite rare. This is also the first report of crystal data for a copper protein. There are eight equal asymmetric units in this space group, and therefore the volume of the asymmetric unit containing azurin and solvent of crystallization is about 36,000 Å^3. The most frequent ratio of volume to molecular weight is 2.2 Å^3/dalton (*5*), and this gives a molecular weight of 16,000 daltons, which is in agreement with the observed value of 16,300 (*4*).

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Biochemistry at 100°C: Explosive Secretory Discharge of Bombardier Beetles (Brachinus)

Abstract. *The defensive chemical spray of bombardier beetles is ejected at 100°C, with a heat content of about 0.2 calorie per milligram.*

More than 100 years ago, the entomologist J. O. Westwood, quoting a traveler returned from South America, wrote that certain large beetles of the genus *Brachinus* "on being seized . . . immediately . . . play off their artillery, burning . . . the flesh to such a degree, that only few specimens (can) be captured with the naked hand" (*1*). *Brachinus* is a widely distributed genus of Carabidae, whose members have long been known to naturalists as bombardier beetles. Their "artillery" is a defensive spray, ejected from a pair of glands that open at the tip of the abdomen. The spray is visible as a fine mist, and the discharge occurs with an audible detonation (*2*).

Each gland of *Brachinus* is a two-compartmented apparatus (Fig. 1). The inner compartment (reservoir) contains an aqueous solution of hydroquinones (hydroquinone and methylhydroquinone) and hydrogen peroxide, while the outer compartment (vestibule) contains a mixture of catalases and peroxidases (*3*). In order to effect a discharge, the beetle squeezes some reservoir fluid into the vestibule (*4*), thereby triggering what is essentially an instantaneous and explosive set of events: the catalases promote the decomposition of hydrogen peroxide, while the peroxidases force the oxidation of the hydroquinones to their respective quinones. Under pressure of the free oxygen, the mixture "pops" out. The active defensive principles of the secretion are the quinones generated in the discharge. They are strongly repellent to many predators (*2*, *5*). Bombardiers can spray repeatedly and in quick succession; as many as 29 discharges have been elicited from a single beetle (*2*). Through rotation of the abdominal tip they can eject the spray in virtually any direction, and they always aim it toward the foe (*2*).

Given the known concentration of the reactants (hydrogen peroxide, 25 percent; both hydroquinones, 10 percent) (*6*), as well as the thermodynamic properties of the reaction they undergo, it is possible to predict within certain limits the heat content and temperature of the spray. The chemical reaction

$$\text{hydroquinone (aq)} + H_2O_2\text{ (aq)} \longrightarrow \text{quinone (aq)} + 2H_2O\text{ (l)} \quad \text{(a)}$$

may be looked upon as the net result of the three steps (*7*):

$$\text{hydroquinone (aq)} \longrightarrow \text{quinone (aq)} + H_2\text{ (g)} \quad \text{(b)}$$

$$H_2O_2\text{ (aq)} \longrightarrow H_2O\text{ (l)} + \tfrac{1}{2}O_2\text{ (g)} \quad \text{(c)}$$

$$H_2\text{ (g)} + \tfrac{1}{2}O_2\text{ (g)} \longrightarrow H_2O\text{ (l)} \quad \text{(d)}$$

The enthalpy changes for (c) and (d) are known (*8*): $\Delta H_c = -22.6$ kcal/mole and $\Delta H_d = -68.3$ kcal/mole, both at 25°C. That for (b) is calculable from $\Delta H_b = -2F\ d(E/T)/d(1/T)$, where E is the standard electromagnetic force of the quinhydrone electrode, T is the absolute temperature, and F is the Faraday. From the known (*9*) temperature dependence of E, we then find $\Delta H_b = +42.4$ kcal/mole at 25°C. Thus, $\Delta H_a = -22.6 - 68.3 + 42.4 = -48.5$ kcal/mole at 25°C; that is, 48.5 kcal of heat are evolved for each mole of hydroquinone that undergoes the reaction (a). It is not anticipated that this would depend very much on the temperature at which reaction occurs (between 25° and 100°C) or on whether the reactant is hydroquinone itself or methylhydroquinone. In a reservoir solution of 10 percent hydroquinone and 25 percent hydrogen peroxide, there are 0.91 μmole of hydroquinone and 7.40 μmole

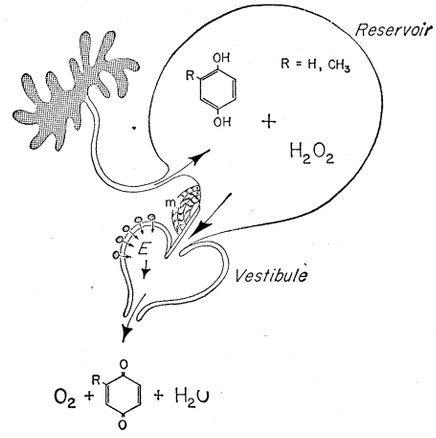

Fig. 1. Diagram of gland of bombardier beetle. The reservoir obtains its contents from a duct that drains an outlying cluster of secretory tissue. The enzymes (*E*) in the vestibule are secreted by cells on the wall of the vestibule itself. The muscle (*m*) controls the valve between the two compartments. Other details in text. [Based on Schildknecht *et al.* (*3*)]