The Structure of Commercial Pyronin B[1]

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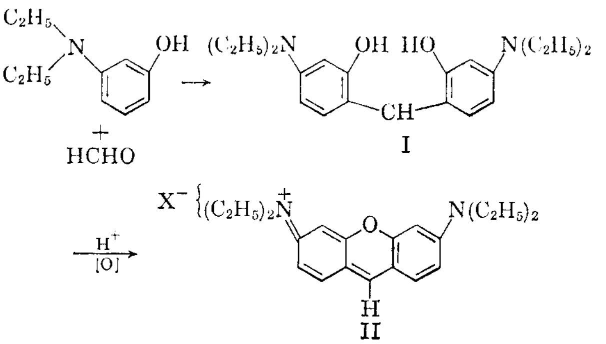
Pyronin B (Color Index 45010),[2] a xanthene dye, has been studied under a variety of circumstances: as a carcinogenic agent,[3a] as a mutagenic agent,[3b] and as a stain for bacteria, molds,[3c] and ribonucleic acids.[3d] Interest in Pyronin B in the cancer chemotherapy program of the National Institutes of Health required a pure sample of the dye as a standard and a crude commercial sample of the dye seemed like a logical source for this although the purification has not been described in the literature. Exploratory experiments indicated that commercial material could not be represented by the simple molecular structure (II) predicted by its synthesis.

Pyronin B is prepared from *meta-N,N*-diethylaminophenol and formaldehyde according to the following scheme.[4a,b]

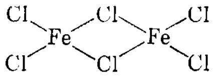


Combustion of a sample of the crude commercial dye gives an ash which is not due to adulteration by inorganic salts as washing with water did not change the ash content; spectrographic analysis indicated the ash was due to iron.

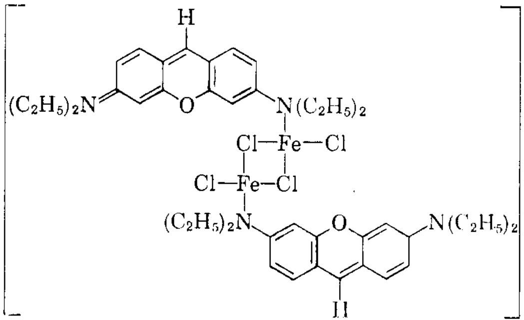
Paper strip electrophoresis in pH 2 buffer exhibits four colored components which migrate to the cathode. Examination of the strips in a recording densitometer showed absorption maxima for two of the spots in the 550-mμ region.

Dialysis against aqueous ethanol (1:1) separates the crude into two components. The nondialyzable material has an ultraviolet absorption maximum at 555 mμ ($E^{1\%}_{1\text{ cm.}}$ 1219), but its solution in aqueous alcohol lacks the characteristic red fluorescence of Pyronin B and it was not examined further. Concentration of the dialysate gave material which had the typical red fluorescense in aqueous alcohol and had an ultraviolet absorption maximum at 542 mμ ($E^{1\%}_{1\text{ cm.}}$ 951). Recrystallization of this latter material from ethyl acetate gives metallic green needles, $\lambda_{\max}^{50\%\text{ alc.}}$ 555 mμ, $E^{1\%}_{1\text{ cm.}}$ 2300. The crystalline material on combustion gives an ash of 15.5%, consequently the product cannot be completely represented by structure II.

Biehringer[4] obtained the uncomplexed dye by performing the oxidation (I→II) with chloranil or nitrous acid. When he carried out the oxidation with stannic chloride, a tin complex of the dye was obtained. The iron in our material could originate from an oxidizing agent such as ferric chloride used in the commercial preparation of the dye.



III



IV

Elemental analysis establishes the emperical formula as $C_{21}H_{27}N_2Cl_4OFe$, and indicates a Pyronin B molecule combined with ferric chloride. Polarography shows a strongly complexed trivalent Fe. Titration with base yields an equivalent weight of 134 (theory for replacement of four chloride ions is 130). Three equivalents of chloride ion are easily replaced by hydroxyl. A fourth equivalent can be replaced only above pH 10 where precipitation of ferric hydroxide indicates a breakup of the complex. That each iron has one chloride ion more strongly bound than the others suggests the bridged structure of Fe_2Cl_6 (III). A nitrogen in the Pyronin B nucleus could replace one of the chlorides around the iron. Since the crystal

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[1] This work was done under Contract SA-43-ph-1948 with the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health.

[2] Color Index, 2nd ed., The American Association of Textile Chemists and Colorists, Lowell Technicological Institute, Lowell, Mass., Vol. 3, p. 3382 (1956).

[3] (a) R. Willheim and A. C. Ivy, *Gastroenterology*, **23**, 1 (1953); (b) B. C. Auerbach, *Am. Nat.*, **89**, 241 (1955); (c) F. Aiquel and F. J. Herrero, *Arch. Farm. y Bioquim. Tucuman*, **4**, 181 (1948); (d) B. Biswas, *Current Sci.*, **22**, 346 (1953), E. Taft, *Expt. Cell Research*, **2**, 312 (1951), L. Abolins, *Expt. Cell Research*, **3**, 1 (1952).

[4] (a) J. Biehringer, *Ber.*, **27**, 3299 (1894); (b) J. Biehringer, *J. Prakt. Chem.*, **54**, 217 (1896).

symmetry established by X-ray analysis requires the molecule to have a center of symmetry, two Pyronin B molecules would have to replace diagonally opposite chlorides in the iron dimer. On the basis of these data we propose the structure IV for Pyronin B. Symmetry and spatial considerations in the unit cell of the crystal eliminate all other chemically logical structures.

From the dialysis experiments, the differential solubility of the two major components of the crude dye in ethyl acetate suggested a simplified extraction procedure for the isolation of pure Pyronin B on a large scale. Extraction of 500 g. of crude dye in a Soxhlet extractor with ethyl acetate gave 120 g. of product which on re-extraction with ethyl acetate gave analytically pure Pyronin B, m.p. 176–178°, $\lambda_{\max}^{50\%\text{ alc.}}$ 555 mμ, $E_{1\text{ cm.}}^{1\%}$ 2324. The purity by phase solubility is 99.8 ± 0.2%.

The absorption spectra of the dye was examined in 50% aqueous ethanol in a Beckmann DU spectrophotometer and a Cary recording spectrophotometer. It was found that solutions of the dye of concentrations greater than 10 mg./l. do no obey Beer's law. Principal absorption maximum is at 555 mμ with another at 521 mμ, and multiple absorption from 350 mμ to 210 mμ In addition, a fluorescent emission maximum appears at 580 mμ and an activation maximum at 530 mμ. These latter maxima give rise to erroneous results when measurements are made with the Beckmann DU spectrophotometer.

Experimental

Five hundred grams of commercial Pyronin B[5] was extracted in a Soxhlet extractor with 3-1. portions of ethyl acetate over a period of 14 days. The solvent was changed after the second, fifth, and ninth day. Each extract was worked up separately by concentrating to 500 ml., cooling in ice, filtering the product, washing with ether, and drying. In this way, 120 g. of product was obtained in four crops; 8 g. $\lambda_{\max}^{50\%\ C_2H_5OH}$ 555 mμ, $E_{1\text{ cm.}}^{1\%}$ 2185; 34 g. $\lambda_{\max}^{50\%\ C_2H_5OH}$ 555 mμ $E_{1\text{ cm}}^{1\%}$ 2207; 51 g. $\lambda_{\max}^{50\%\ C_2H_5OH}$ 555 mμ, $E_{1\text{ cm.}}^{1\%}$ 2383; 27 g., $\lambda_{\max}^{50\%\ C_2H_5OH}$ 555 mμ, $E_{1\text{ cm.}}^{1\%}$ 2290.

The combined product was re-extracted with two 3-1. portions of ethyl acetate. The first extraction (7 days) was cooled in ice, filtered, and washed with ether to give 68 g. of metallic green needles, m.p. 176–177°; paper strip electrophoresis[6] (pH 2 buffer, 200 v.)—single spot; phase solubility—slope 1 ± 0.5; $\lambda_{\max}^{50\%\ C_2H_5OH}$ 554 mμ, $E_{1\text{ cm.}}^{1\%}$ 2428.

Anal. Calcd. for $C_{42}H_{54}N_4Cl_8O_2Fe_2$ (1,042.28): C, 48.40; H, 5.22; N, 5.38; Cl, 27.22. Found: C, 49.04; H, 5.35; N, 6.66; Cl, 27.33.

The second extraction (14 days) on cooling gave 39 g. of product as green metallic needles, m.p. 176–178°; paper strip electrophoresis (pH 2 buffer, 200 v.)—single spot; phase solubility-slope 0.2 ± 0.2; $\lambda_{\max}^{50\%\ C_2H_5OH}$ 555 mμ, $E_{1\text{ cm.}}^{1\%}$ 2324.

Anal. Calcd. for $C_{42}H_{54}N_4Cl_8O_2Fe_2$ (1,042.28): C, 48.40; H, 5.22; N, 5.38; Cl, 27.22; Fe, 10.71. Found: C, 48.60; H, 5.13; N, 5.85; Cl, 27.60; Fe, 10.86.

Polarographic Analyses.—The half wave potential was determined on a Leeds and Northrup Electrochemograph Type E in 0.2 *M* potassium oxalate containing 0.004% gelatin against a saturated calomel electrode, $E_{1/2}$—0.25 V., i.d.—13.1 μA/mg. of iron added as Pyronin B, a standard Fe(III) solution under the same conditions, gave $E_{1/2}$—0.25V and i.d.—15.1 μA./mg. of iron.

X-Ray Diffraction.—Pyronin B crystallizes as needles, elongated along the *a* axis and developing the {010} and {011} forms. The ends of the needles are normally fractures rather than crystal faces. The crystals are quite opaque, being metallic green by reflected light. Observations on very thin crystals show them to be red by transmitted light.

The unit cell dimensions were determined from rotation and Weissenberg photographs around the *a* and *c* axes. The density was measured by floatation. Monoclinic, $a = 9.00$, $b = 20.84$, $C = 13.66$, $B = 108°$. Volume of the unit cell = 2562 Å.^{3}. Density calculated = 1.421, measured = 1.398. Systematic absences: *hol* for odd indices of *1*, and *oho* for odd indices, space group $P2_1/c$.

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[5] National Aniline Division of Allied Chemical and Dye Corp. certified dye content 34%.

[6] Spinco Model RB, Durrum type.

5-Pyrimidinecarboxylic Acid and Some of Its Derivatives

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The chemical literature seems to be devoid of suitable methods for the preparation of relatively large amounts of simple pyrimidines bearing a carboxy group or its derivative at position 5. In 1904 Gabriel and Colman[1] obtained a small amount of 5-pyrimidinecarboxylic acid during the course of degradative studies on quinazoline. Boarland and McOmie[2] converted this acid to the corresponding amide and methyl ester. Smith and Christensen[3] obtained the compound in 29% yield from the catalytic dehalogenation of 2,4-dichloro-5-pyrimidinecarboxylic acid ethyl ester using a palladium-carbon catalyst in an aqueous sodium hydroxide–ether system.

In our hands the ester was obtained in 60% yield by a modification of the Smith-Christensen procedure and was subsequently hydrolyzed to afford 5-pyrimidinecarboxylic acid. The acid was converted, *via* the acid chloride, to some amides whose counterpart in the pyridine series had been shown to possess biological activities. For example, treatment of a crude, ethereal solution of the acid chloride with diethylamine gave the *N,N*-diethylamide, analogous to "Coramine." A similar re-

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[1] S. Gabriel and J. Colman, *Ber.*, **37**, 3643 (1904).

[2] M. P. V. Boarland and J. F. W. McOmie, *J. Chem. Soc.*, 1218 (1951).

[3] V. H. Smith and B. E. Christensen, *J. Org. Chem.*, **20**, 829 (1955).