are not in accord with rapid hydrolysis of I to α-acetaminocinnamic acid and L-diiodotyrosine but can be explained by postulating rapid reversible formation of a 1:1 complex of I and pepsin (eq. 1). For such a scheme,[5] $O.D._0$ is defined by eq. 3, and the concentration of the EI complex, [EI], by eq. 4. Equations 3 and 4 permit calculation of $\Delta\epsilon$ for each experimental point as a function of assumed K_I values; the true K_I should provide an identical $\Delta\epsilon$ for all points. This procedure relies on the difference between two quantities of similar magnitude ($O.D._0$ and $[I]_0\epsilon_I$ of eq. 3).[6] Table I presents the results of four such calculations

Table I. Spectrophotometric Determination of the Dissociation Constant of the Complex between Pepsin and N-(α-Acetaminocinnamoyl)-L-diiodotyrosine

	No. of points	$K_I \times 10^5$, M 30	8	3	0.3
$\Delta\epsilon$	43	1444	719	534	410
Standard deviation, %		±37	±18	±14	±24
$\Delta\epsilon$	13	1012	640	538	474
Standard deviation, %		±15	±5.2	±5.8	±12

for all 43 points and for the 13 points in which ($O.D._0 - [I]_0\epsilon_I$) was greatest.[7] A value for K_I in the vicinity of $3\text{–}8 \times 10^{-5}$ M best fits the data but it appears unlikely that the present system can define K_I more closely.[8]

$$EI \rightleftharpoons E + I \quad K_I = [E][I]/[EI] \tag{1}$$

$$[E]_0 = [E] + [EI] \quad [I]_0 = [I] + [EI] \tag{2}$$

$$O.D._0 = [I]_0\epsilon_I + [EI](\epsilon_{EI} - \epsilon_I - \epsilon_E) \tag{3}$$

$$[EI] = ([I]_0 + [E]_0 + K_I - \sqrt{([I]_0 + [E]_0 + K_I)^2 - 4[E]_0[I]_0})/2 \tag{4}$$

$$\Delta\epsilon = \epsilon_{EI} - \epsilon_I - \epsilon_E \tag{5}$$

Solutions of N-(α-acetaminocinnamoyl)-L-tyrosine (II) in the presence of pepsin exhibit the same effects as solutions of I, but on a reduced scale.[9] The assumption that $\Delta\epsilon$ is the same[10] for II as for I permits direct calculation of K_{II} from the experimental data and eq. 3 and 4. For $\Delta\epsilon = 540$, $K_{II} = 2.7 \pm 0.5 \times 10^{-3}$ M; for $\Delta\epsilon = 640$, $K_{II} = 3.3 \pm 0.6 \times 10^{-3}$ M. The value for K_{II} is not highly sensitive to the assumed $\Delta\epsilon$ and is remarkably similar to that for the Michaelis constant ($\sim 2 \times 10^{-3}$ M) of the pepsin substrate, N-acetyl-L-phenylalanyl-L-tyrosine (AcPheTyr).[11–13]

(5) (a) In these experiments, 3.0 ml. of enzyme solution was placed in a cuvette and allowed to attain thermal equilibrium, the optical density was set at zero, 100 μl. of a methanolic solution of I added, and the $O.D._0$ recorded. (b) ϵ_{315} for pepsin is ~180.

(6) The largest value for ($O.D._0 - [I]_0\epsilon_I$) was ~0.1, with $O.D._0$ = ~0.6.

(7) Calculated and observed values of $O.D._0$ were in excellent agreement for all 43 points with $K_I = 8 \times 10^{-5}$ M, $\Delta\epsilon = 640$, or $K_I = 3 \times 10^{-5}$ M, $\Delta\epsilon = 538$.

(8) (a) K. Conrow, G. D. Johnson, and R. E. Bowen, *J. Am. Chem. Soc.*, **86**, 1025 (1964), have discussed difficulties in the calculation of association constants from spectrophotometric data. (b) K_I is not small enough to make the method useful as a titration of pepsin.

(9) (a) II had m.p. 216–219°, $[\alpha]^{27}D$ +41.2° (*c* 3, pyridine), λ_{max} 282 (ε 20,650), λ_{min} 241 (ε 6815) (spectrum in 3% methanol, pH 2). Reference 2 gives m.p. 217–218°, $[\alpha]^{20}D$ +47.1° for II. (b) Baker (ref. 1) has shown that N-acetyl-L-phenylalanyl-L-diiodotyrosine is a much better substrate than N-acetyl-L-phenylalanyl-L-tyrosine for pepsin.

(10) The ultraviolet absorption of I at 315 mμ must arise almost exclusively from the α-acetaminocinnamoyl chromophore, since ϵ_{315} is 2630 for II and 3000 for α-acetaminocinnamic acid.

(11) L. E. Baker, *J. Biol. Chem.*, **211**, 701 (1954).

The general validity of the above interpretation of the spectroscopic studies is independently substantiated by kinetic evaluation of K_I from reactions in which I inhibits the pepsin-catalyzed hydrolysis of Ac-PheTyr or its carbobenzoxy analog, Z-PheTyr (Table II).[14] The apparent *noncompetitive* nature of the inhibition is very interesting, if substantiated by later work.

Table II. Kinetic Determination of the Dissociation Constant of the Complex between Pepsin and N-(α-Acetaminocinnamoyl)-L-diiodotyrosine[a]

Substrate	$[I] \times 10^5$, M	$K_0 \times 10^3$, M[b]	$k_0 \times 10^2$, sec.$^{-1}$[b]	$K_I \times 10^5$, M[c]
Z-PheTyr[d]	0[e]	0.214 ± 0.034[i]	1.24 ± 0.08[i]	
	3.86[f]	0.206 ± 0.040[i]	0.83 ± 0.05[i]	8.7 ± 2.5
Ac-PheTyr[d]	0[g]	1.95 ± 0.18[i]	4.66 ± 0.44[i]	
	3.86[h]	1.91 ± 0.06[i]	3.19 ± 0.11[i]	8.9 ± 0.7
	5.8[h]	1.28 ± 0.18[i]	1.96 ± 0.18[i]	10.3 ± 1.2

[a] All runs at 35°, pH 2, 3.4% methanol, $[E]_0 = 1.20\text{–}1.58 \times 10^{-5}$ M. [b] Evaluated from the equation $v = k_0[E][S]/(K_0 + [S])$. [c] Evaluated from the equation $K_I = [I]/(X/Y - 1)$, where X and Y are the slopes of the Lineweaver–Burk plots for the inhibited and uninhibited reactions, respectively, corrected for any difference in [E]. [d] All uncertainties are standard deviations. [e] $[S]_0 = 0.73\text{–}3.29 \times 10^{-4}$ M. [f] $[S]_0 = 1.02\text{–}3.05 \times 10^{-4}$ M. [g] $[S]_0 = 1.16\text{–}13.6 \times 10^{-4}$ M. [h] $[S]_0 = 2.4\text{–}9.7 \times 10^{-4}$ M. [i] Average of six independent Lineweaver–Burk plots of 7–12 points. [j] Average of three independent Lineweaver–Burk plots of 8–9 points.

We conclude that it is possible to observe spectroscopically a 1:1 complex of pepsin with N-(α-acetaminocinnamoyl)-L-diiodotyrosine, formation of which results in a diminution of the catalytic activity of the enzyme.[15] Further studies should afford valuable information on the interaction of simple dipeptides with pepsin and may provide a means of estimating the number of active sites per pepsin molecule.

Acknowledgment. This investigation was supported by Grant AM 08005-01 from the U. S. Public Health Service. Carol D. Silver carried out the computer programming. Professors Myron L. Bender and Allen Kropf provided helpful criticisms.

(12) M. S. Silver, J. L. Denburg, and J. J. Steffens, *J. Am. Chem. Soc.*, **87**, 886 (1965); see Table II.

(13) Likewise, K_I is nearly equal to K_m (7.5×10^{-5} M) for N-acetyl-L-phenylalanyl-L-diiodotyrosine and to K_i (8.0×10^{-5} M) for N-acetyl-D-phenylalanyl-L-diiodotyrosine, all at pH 2.0. The values for K_m and K_i were kindly provided to us by Dr. William T. Jackson of the University of Texas Medical Center, Houston.

(14) We suspect that the spectrophotometric method[12] employed in determining the kinetics may be breaking down in the experiments at $[I] = 5.8 \times 10^{-5}$ M, but we have not yet succeeded in pinpointing the difficulty.

(15) The present experiments indicate nothing about the nature of the binding in EI.

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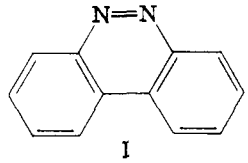
Silver and Palladium Complexes of 5,6-Benzocinnoline and 3,8-Dimethyl-5,6-diaza-1,10-phenanthroline

Sir:

Evidence for the participation of the azo group in metal chelate interaction has been shown by several workers[1] for aromatic azo compounds. Substituent

groups seem to be necessary as azobenzene shows only limited interaction with common transition metals under comparable conditions.[2] A compound which is somewhat similar to *cis*-azobenzene in basicity and reactivity,[3] but incapable of rearrangement to a *trans* isomer, is 5,6-benzocinnoline (I). While aqueous or alcoholic solutions of azobenzene showed no noticeable interaction with either palladium chloride or silver



I

nitrate, 5,6-benzocinnoline gave immediate yellow precipitates with both metal salts. The precipitates were infusible to 320° and only showed discoloration at this temperature. *Anal.* Calcd. for $Ag(C_{12}H_8N_2)NO_3$[4a]: C, 41.25; H. 2.29; N, 12.02; Ag, 30.62. Found: C, 41.95; H, 2.26; N, 11.61; Ag, 30.57. Calcd. for $Pd(C_{12}H_8N_2)_2Cl_2$: C, 53.60; H, 2.98; N, 10.42; Pd, 19.80; Cl, 13.20. Found: C, 54.69; H, 2.98; N, 10.40; Pd, 19.18; Cl, 11.98.[4b] The silver complex was soluble in dimethylformamide but the palladium complex was insoluble in all solvents tested.

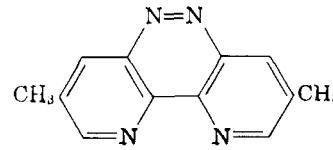
The chlorplatinate derivative of benzocinnoline was also prepared. *Anal.* Calcd. for $(C_{12}H_9N_2)_2PtCl_6$: C, 37.62; H, 2.09; N, 7.32; Pt, 25.52; Cl, 27.40. Found: C, 38.47; H, 2.51; N, 7.38; Pt, 24.66; Cl, 27.04. The decomposition point of this compound was >300°.

The chloroplatinate was made by mixing dilute hydrochloric acid solutions of benzocinnoline and chloroplatinic acid. Filtration followed by dilute acid wash gave a yellow crystalline material which was dried in a desiccator over H_2SO_4. Under the mild preparative conditions, one would not expect a change in the coordination sphere of the chloroplatinate ion. Potassium tetrachloroplatinate(II) gave no noticeable reaction after 2 days under comparable conditions.

These analyses indicate that benzocinnoline was actually coordinated with silver and palladium while in the case of platinum only salt formation occurred. A different analysis would be expected for the silver and palladium compounds if a salt linkage was operative.

The infrared spectra of the three organometallic compounds were quite similar, all showing strong absorption bands at 13.1 and 14.0μ and weaker absorptions at 6.3, 9.0, and 7.8 μ. These absorptions could all be found in approximately the same location in both benzocinnoline and its hydrochloride salt.

This study was extended through the preparation of a molecule similar to benzocinnoline but having additional coordinating possibilities. The compound proposed for synthesis was 3,8-dimethyl-5,6-diaza-1,10-phenanthroline (II).



II

The synthesis was initiated with 5-methyl-2-aminopyridine. This compound was nitrated to give directly 3-nitro-5-methyl-2-hydroxypyridine, m.p. 252–256°,[5] in 52% yield. This direct route to the hydroxy compound was accomplished by mixing the aminopyridine, concentrated sulfuric acid, and 1 mole excess of concentrated nitric acid together at 10° and then adding this mixture slowly to hot (70°) concentrated sulfuric acid. The hydroxypyridine was then converted to 2-chloro-3-nitro-5-methylpyridine, m.p. 46°, with phosphorous oxychloride.[5] The 2-chloro-3-nitro-5-methylpyridine was coupled with activated copper[6] in Spectrograde dimethylformamide to give a 31% yield of 5,5-dimethyl-3,3-dinitro-2,2-bipyridine, m.p. 195–196°. *Anal.* Calcd. for $C_{12}H_{10}N_4O_4$: C, 52.54; H, 3.65; N, 20.44. Found: C, 52.48; H, 3.70; N, 19.86. The bipyridine derivative was only soluble in concentrated hydrochloric acid and showed infrared absorption peaks at 7.41, 6.42, 6.52, 12.41, 11.21, 12.29, 12.57, 9.65, 8.02, and 12.92 μ in order of decreasing intensity and similar to the absorption spectra of 2,2′-dinitrobiphenyl. The dinitrobipyridine was reduced with hydrogen in alkaline ethanol using palladium (10%) on carbon as a catalyst[7] to give the desired 3,8-dimethyl-5,6-diaza-1,10-phenanthroline, m.p. 265–266°, in 52% yield. This product was soluble in dilute acid and had infrared absorption peaks at 8.42, 6.63, 7.52, 13.41, 14.53, 7.70, 8.59, 10.44, 11.08, 5.98, 6.20, 6.46, and 13.98 μ in order of decreasing intensity.

The n.m.r. spectra showed three peaks having absorption at 553, 528, and 166 c.p.s. (τ 0.79, 1.20, and 7.23), respectively.[8] The area ratio obtained from integration was 1 (553):1 (528):3 (166).

The solubility, n.m.r., infrared absorption, and analysis (Calcd. for $C_{12}H_{10}N_4$: C, 68.57; H, 4.67; N, 26.67. Found: C, 68.48; H, 5.06; N, 26.45) were all in agreement with the proposed structure. It may be noted that no evidence of hydrate formation, common to 1,10-phenanthroline, appeared at any time during isolation or analysis.

When dilute acidic solutions of 3,8-dimethyl-5,6-diaza-1,10-phenanthroline were mixed with similar acidic solutions of some of the common transition metals, only slight color changes were observable. $FeSO_4$ gave an amber solution while cobalt, nickel, and copper nitrate gave solutions hardly distinguishable from that of original salt solutions. The color change became much more significant when alcoholic solutions were used in place of aqueous solutions.

(1) J. A. J. Jarvis, *Acta Cryst.*, **14**, 961 (1961); A. K. Piskunov, D. N. Shigorin, V. I. Smirnova, and B. I. Stepanov, *Dokl. Akad. Nauk SSSR*, 130, 1284 (1960).

(2) R. H. Nuttal, E. R. Roberts, and D. W. A. Sharp, *J. Chem. Soc.*, 2854 (1962); M. S. Kharasch and T. A. Ashford, *J. Am. Chem. Soc.*, **58**, 1733 (1936).

(3) G. E. Lewis, *J. Org. Chem.*, **25**, 2193 (1960).

(4) (a) All analyses were done by the Alfred Bernhardt Laboratories, Max-Planck-Institute, West Germany. (b) Owing to the interference of palladium with the chlorine determination, this analysis is reported with reservation.

(5) J. H. Boyer and W. Schoen, *J. Am. Chem. Soc.*, **78**, 423 (1956).

(6) "Organic Synthesis," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 339.

(7) H. Stetter and M. S. Schwartz, *Chem. Ber.*, **90**, 1349 (1957).

(8) Samples run using a Varian n.m.r. spectrophotometer.

Solutions of palladium chloride and silver nitrate gave immediate yellow precipitates when mixed with dilute hydrochloric acid and nitric acid solutions of the diazaphenanthroline, respectively. *Anal.* Calcd. for $Ag_3(C_{12}H_{10}N_4)_4(NO_3)_3$: C, 42.64; H, 2.96; N, 19.70; Ag, 24.02. Found: C, 42.93; H, 3.14; N, 19.22; Ag, 24.06. Calcd. for $Pd(C_{12}H_{10}N_4)Cl_2$: C, 37.16; H, 2.58; N, 14.46; Cl, 18.33; Pd, 27.46. Found: C, 37.61; H, 2.77; N, 14.76; Cl, 15.75[4b]; Pd, 26.70 (from residue).

The ligand to metal ratio for the silver complex (which was the same in three separate preparations) is in contrast to that found with benzocinnoline presented in this paper but is in agreement with the results obtained by other workers with azobenzene.[2] In one preparation the sample was stirred 24 hr. before filtration. In this case there was no significant change in the elemental analysis.[9] These complexes were all infusible (>300°) materials. The silver complex was soluble in hot dimethylformamide, but the palladium complex was insoluble in this solvent or nitrobenzene at the boiling point. The infrared spectra of these two compounds were very similar to that of the ligand itself. The low solubility of the palladium complex and 1:1 ligand:metal ratio suggest a polymeric-type compound which could occur if both the azo group and the "methine chromophore" are interacting with the metals. Benzocinnoline gave a 2:1 ligand ratio for the palladium complex where polymer formation is not likely.

(9) This was also true for the palladium complex.

Acknowledgment. We wish to express our thanks to the Faculty Basic Research Committee and the Dow Corporation, who supported this work. Additional thanks are due to Dr. J. C. Fanning for helpful discussions and Dr. H. G. Spencer for n.m.r. spectra interpretation.

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Book Reviews

Kunstliche Organische Farbstoffe und Ihre Zwischenprodukte. By HANS RUDOLF SCHWEIZER, Dr. sc. techn. ETH. Springer-Verlag, Heidelberger Platz 3, Berlin-Wilmersdorf (West), Germany. 1964. xii + 542 pp. 16 × 24 cm. Price, DM 49,60.

In revising his earlier, two-volume work ("Künstliche Organische Farbstoffe," Verlag der Vereinigung der Chemiestudierenden der Eidgenossische Technische Hochschule, Zurich, 1959), the author has aimed at providing German-speaking students with a modern, comprehensive textbook on the chemistry of dyes and their intermediates. Comparison with other recent attempts in this direction, by Seidenfaden in 1957 and Schaeffer in 1963, shows decisive improvement. But the result is short of the goal even when the relative paucity of basic research in this field is taken into account. This failure may well be rooted in the catalog-like nature of the book, which includes topics of importance in Victorian times, but not now.

The book is organized in three sections: the application of dyes to textiles, or dyeing; intermediates; and colored compounds, including both dyes and pigments. The first section outlines dyeing technology, the various substrates and auxiliary products, as well as apparatus, but is so much a compendium of isolated and often irrelevant information that one gets the impression that the author has little more than second-hand knowledge in this field. This is disappointing since contemporary research calls much more for an understanding of the chemical and physical properties of dyes in solution and on substrates than mere search for new chromphoric systems.

The section on intermediates is adequately done. Sufficient mechanistic emphasis is given to awake some imagination on its utility in process rationalization, although this is not stressed.

The third and longest section presents dyes and pigments according to the classical approach. Such recent developments as reactive, 2:1 metal complex, phthalocyanine precursor, and polymerizable dyes are treated for the first time in a general textbook. Disappointingly, some space is given to pharmaceutical and insecticidal examples, when it more properly could have been devoted to the important areas of water-soluble sulfur, and azo and anthraquinone cationic dyes, which received little or no attention. Neither is there a serious attempt to correlate structure and properties, nor a sufficient explanation in modern terms of old terminology, *e.g.*, basic or acid dyes. Attention to these matters would have materially aided in arousing real interest for the subject in Hochschule chemistry students.

While the book is an improvement in the Germanic literature, it is less successful when compared to English-language competition. If an English translation were ever undertaken, a thorough revision would be indicated.

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Nuclear and Radiochemistry. Second Edition. By GERHART FRIEDLANDER, Senior Chemist, Brookhaven National Laboratory, JOSEPH W. KENNEDY, Late Professor of Chemistry, Washington University, and JULIAN MALCOLM MILLER, Professor of Chemistry, Columbia University. John Wiley and Sons, Inc., 605 Third Ave., New York, N. Y. 1964. xi + 585 pp. 17.5 × 24.5 cm. Price, $10.75.

Among other expectations of the postwar world—everyone owning his own helicopter, and so on—was the importance nuclear and radiochemistry was to have in the college and graduate chemistry curriculum. Unfortunately, the dilapidations of time have been such that despite the *de rigueur* inclusion of the usual chapter at the end of the usual textbook of freshman chemistry, the teaching of radiochemistry has become a chore that chemists expect the departments of physics to perform. So it comes about that a new edition of perhaps the best of the advanced texts of nuclear chemistry waits nearly a decade for publication, and its review is only reluctantly accepted by the Editor of the *Journal of the American Chemical Society*.

I shall confine myself to changes from the first edition (itself a revision of "Introduction to Radiochemistry," published in 1949): first, an increase in size by roughly a third (is the second edition ever smaller?) due in part to a thorough job of redesigning to give the book a much more attractive format. Professor J. M. Miller has replaced the late Joseph W. Kennedy of Washington University as author. Two new chapters have been added: "Nuclear