NOTES

Induced Oxidation of *p*-Benzophenone

By Merchant L. Cushing

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During preliminary experiments on the effect of *o*-benzoquinone on the tyrosinase-catalyzed oxidation of *p*-cresol the effect of the *p*-benzoquinone was also tried. In view of the well-known stability of aqueous solutions of *p*-benzoquinone the results were unexpected.

It was found that in the presence of a substrate which was being oxidized *p*-benzoquinone was also oxidized with the consumption of one atom of oxygen per mole of quinone. When only enzyme but no substrate was present *p*-benzoquinone was not oxidized. If the quinone was added to the system after the substrate had been completely oxidized with the normal consumption of oxygen (*e.g.*, 3 atoms per mole for *p*-cresol) no oxidation of the *p*-benzoquinone took place. Thus it appears that some intermediate form of the oxidized substrate is able to induce the oxidation of *p*-benzoquinone.

This reaction displayed another peculiar feature in that the *p*-benzoquinone had to be added in the *solid* form in order for this phenomenon to be observed. All attempts to find this reaction with aqueous solutions of *p*-benzoquinone, no matter how freshly prepared, failed.

The nature of the substrate appeared to have no influence on the induced oxidation of solid *p*-benzoquinone as the use of *p*-cresol, catechol, 4-chlorocatechol and 4,5-dichlorocatechol all showed a normal oxygen consumption plus one atom per mole of *p*-benzoquinone.

Table I shows a typical result of the oxidation of a mixture of *p*-cresol and solid *p*-benzoquinone.

Table I

Oxidation of *p*-Cresol + Solid *p*-Benzoquinone in the Presence of Tyrosinase

*p*H 6.48 (citrate–phosphate buffer); enzyme strength 2.5 cresolase units[1] per ml., 25°

p-Cresol, mg.	Quinone, mg.	Oxygen consumption, cu. mm. Calculated *p*-Cresol	Quinone	Obsd. total
0.01	10	3.1	1030	1003
0.1	10	31	1030	1060
1.0	10	310	1030	1370
2.0	10	620	1030	1690
4.0	10	1240	1030	2315

Fresh enzyme was added whenever the oxygen consumption had nearly ceased until the addition of fresh enzyme caused no further oxygen consumption. The measurements were made by use of the Warburg respirometer, according to the technique of Graubard and Nelson.[2] The enzyme was obtained from the common mushroom, *Psalliota campestris*. It was purified by precipitation with trichloroacetic acid from the press juice, taken up in water, precipitated with chilled acetone, redispersed in water, absorbed on alumina, eluted with secondary sodium phosphate, and dialyzed against distilled water.

The author does not intend to continue this work and has submitted these data with the hope of provoking further investigation.

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(1) Mark H. Adams and J. M. Nelson, This Journal, **60**, 2472 (1938).

(2) Mark Graubard and J. M. Nelson, *J. Biol. Chem.*, **111**, 757 (1935).

Brown Condensation Products from Acetaldehyde and Aliphatic Amines

By J. F. Carson and Harold S. Olcott

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Previous papers from this Laboratory have shown that the reaction of acetaldehyde with proteins[1] has many features in common with the reaction of glucose with proteins.[2] In an effort to elucidate the mechanisms of these "browning" reactions, studies are being extended to simple model systems.[3] This report describes some reactions of acetaldehyde with several aliphatic amines and ammonia and, in particular, the properties of the brown water-soluble polymers resulting. Reactions were conducted at 3–25° in aqueous solutions held at *p*H 6–7 with phosphate buffers.

The rates of browning, measured colorimetrically, for methylamine, ethylamine and *n*-butylamine with acetaldehyde were very similar and significantly greater than the rates of browning of primary aliphatic amines containing only one hydrogen atom on the α-carbon atom, such as cyclohexylamine, isopropylamine and *sec*-butylamine, and were much faster than the rate for ammonia–acetaldehyde combinations. For example, in aqueous solution, 0.5 *M* in amine and in acetaldehyde, buffered at *p*H 6.75–6.80 with phosphate at 25°, the times in hours to reach the same brown color[4] for methylamine, cyclohexylamine, isopropylamine, *sec*-butylamine and ammonia were 1, 2.5, 7, 8 and 18 hours, respectively. Under the same conditions, mixtures of aliphatic secondary amines with acetaldehyde did not yield water-soluble brown products but deposited yellow ether-soluble resins. With primary amines the browning reaction was very sensitive to *p*H, the rate increasing rapidly with increasing *p*H between *p*H 5 and 10. Below *p*H 5, very little color development was observed; at *p*H 10 or above, yellow, water-insoluble, ether-soluble resins precipitated. The rate of browning

(1) A. Mohammad, H. S. Olcott and H. Fraenkel-Conrat, *Arch. Biochem.*, **24**, 270 (1949).

(2) A. Mohammad, H. Fraenkel-Conrat and H. S. Olcott, *ibid.*, **24**, 157 (1949).

(3) J. F. Carson, This Journal, **75**, 4300, 4337 (1953).

(4) For this comparison, a reading of 100 on the Klett–Summerson colorimeter with a green filter was used. The same qualitative order was observed throughout later stages of browning.

TABLE I

BROWN CONDENSATION PRODUCTS FROM REACTION OF ACETALDEHYDE AND CROTONALDEHYDE WITH ALIPHATIC PRIMARY AMINES AND AMMONIA

Amine	Aldehyde	Mol. ratio aldehyde/amine	Analyses, % C	H	N	Calcd. for 1 amine + 4 ald. − 2 H_2O, % C	H	N	G. polymer/basic groups[b]
Ammonia	Acetaldehyde	2.0	61.0	8.51	9.02	61.1	9.62	8.91	
Methylamine	Acetaldehyde	2.0	62.4	8.87	8.86	63.1	10.0	8.18	383
Ethylamine	Acetaldehyde	1.0	63.8	9.07	7.66	64.8	10.3	7.56	450
Ethylamine	Acetaldehyde	2.3	64.3	9.08	7.71	64.8	10.3	7.56	441
Ethylamine	Acetaldehyde	4.0	64.3	8.91	7.78	64.8	10.3	7.56	
n-Butylamine	Acetaldehyde	2.0	67.2	9.81	6.65	67.6	10.80	6.57	480
Cyclohexylamine	Acetaldehyde	2.5	68.7	9.18	5.32	70.2	10.5	5.85	
Ethylamine	Crotonaldehyde	2.0	65.1	8.79	7.02	64.8[a]	10.3[a]	7.56[a]	

[a] Calculated for 2 moles of crotonaldehyde and one of ethylamine with no loss of water. [b] By titration of aqueous solution of polymer from *p*H 6.8 to 11.0.

was inhibited by sodium sulfite but complete prevention of color development was attained only with one mole of sulfite per mole of aldehyde.

In Table I are given the analytical properties of brown products isolated from the reaction of acetaldehyde with ammonia and with several aliphatic primary amines, and one product obtained by reaction of crotonaldehyde with ethylamine, all prepared in aqueous solution as described in the Experimental section. The dried products were brown, hygroscopic, bitter powders, soluble in water, methanol and ethanol but insoluble in ether, acetone or ethyl acetate. They did not reduce hot Fehling solution. Analyses of the acetaldehyde–amine products indicated an average composition best represented by a combination of four aldehyde molecules with one amine molecule accompanied by the loss of approximately two molecules of water.[5] The greatest deviation, aside from hydrogen content which was always low, was found in the polymer prepared from cyclohexylamine. The approximate agreement in analyses among the products prepared with three different ratios of acetaldehyde to ethylamine shows that the average composition of the polymers does not depend on the ratios of reacting substances.

The product obtained from crotonaldehyde and ethylamine had a composition indicating that this polymer is formed by reaction of aldehyde with amine in an approximate ratio of two to one instead of four to one as with acetaldehyde. The development of a brown color with crotonaldehyde and amine is faster than the corresponding reaction of acetaldehyde on an equimolar basis.

Potentiometric titration of several of the polymers between *p*H 6 and 11 indicated that less than half of the nitrogen is basic, the non-titratable nitrogen probably being in the form —C=N—.

In the ultraviolet (Cary recording spectrophotometer) the polymers in methanol solution showed maxima at 272–277 mμ superimposed on generalized absorption. Addition of alkali blurred the maximum; acid restored it reversibly. Catalytic hydrogenation in methanol or in acetic acid with platinum oxide or with palladium-on-carbon resulted in very slow uptake of hydrogen and no observable change in color or in ultraviolet absorption spectra. Reaction with sodium borohydride in aqueous solution was vigorous, but the isolated product showed no appreciable decrease in brown color.

Attempts to fractionate the polymers by partition chromatography on silica gel with butanol–water systems failed. The brown material moved as a broad band. The non-homogeneity of the ethylamine–acetaldehyde product was demonstrated by two-dimensional filter paper ionophoresis in a solution buffered with borate at *p*H 9.2 according to the procedure of Kunkel and Tiselius.[6] The colored material moved as a single broad spot, but on rotation of the paper 90° the initial movement of the color described a broad curved path. The direction of movement indicated that the isoelectric point was above *p*H 9.2. Preliminary estimates by the Signer isothermal distillation technique in methanol indicated that the average molecular weight was considerably less than 1000.

We thank L. M. White and G. Secor for the analytical data, G. F. Bailey for the ultraviolet absorption spectra and James Pence for the ionophoresis measurements.

Product of Reaction of Acetaldehyde with Ethylamine.—Ethylamine (5 g., 0.11 mole) was dissolved in 50 ml. of water, neutralized to *p*H 8 with hydrochloric acid, cooled to 3° and added to a cold solution of 20 g. of acetaldehyde (0.45 mole) in 25 ml. of molar phosphate buffer (*p*H 7.0) and 100 ml. of water; final *p*H 6.0. The solution was diluted to 250 ml. and held at 3°, at which temperature it turned yellow in 30 minutes and deep amber in 6 hours. After five days, the deep red-brown solution was concentrated *in vacuo* (< 25°) to about 50 ml., and 500 ml. of absolute ethanol was added to precipitate salts. The filtrate from the salt precipitate was concentrated *in vacuo* to 50 ml. and additional salt was removed by adding 600 ml. of absolute ethanol and filtering. The filtered solution was again concentrated *in vacuo* to 50 ml., diluted to 300 ml. with water, and de-ashing completed by successive treatments with the cation exchanger, Dowex 50 (Dow Chemical Co.),[7] and the anion exchanger, Amberlite IR-4B (Rohm and Haas),[7] to give a final solution with *p*H 7.5. The product was obtained by lyophilization as a brown amorphous powder, yield 14.8 g. (59% of the combined weight of acetaldehyde and ethylamine employed). For analyses, the material was dried *in vacuo* at 25° for 60 hours over phosphorus pentoxide.

Anal.: C, 64.3; H, 8.91; N, 7.78.

(5) M. L. Wolfrom, *et al.*, THIS JOURNAL, **75**, 1013 (1953), have presented evidence suggesting that glucose–glycine polymers and xylose– and arabinose–glycine polymers are best represented by a 1:1 reaction of sugar and amino acid accompanied by partial dehydration and decarboxylation.

(6) H. G. Kunkel and A. Tiselius, *J. Gen. Physiol.*, **35**, 89 (1951).

(7) Mention of this product by name does not constitute endorsement over similar products made by other manufacturers.

The condensations were also performed at room temperature, in which case the concentrations of aldehyde and amine were one-third to one-half of those above.

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(8) Agricultural Research Service, U. S. Department of Agriculture. Article not copyrighted.

The Meerwein Reaction with *p*-Nitrostyrene and 2-Vinylpyridine

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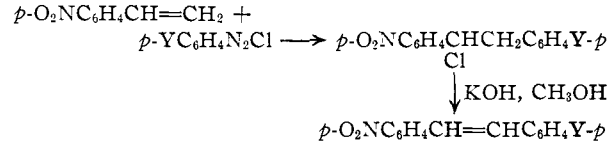
Since the unsaturated linkages in *p*-nitrostyrene[2] and 2-vinylpyridine[3] resemble simple unsaturated carbonyl compounds in their electrophilic character, it was of interest to investigate the Meerwein reaction with these compounds. Brunner and Kustatscher[4] have reported that styrene and α,β-dimethylstyrene react with diazotized aniline, *p*-methoxyaniline, *p*-chloroaniline and *p*-nitroaniline to yield the corresponding stilbenes in low yield (13 to 41%).

In the present research the following series of reactions was performed with *p*-nitrostyrene.



A similar series was investigated for 2-vinylpyridine. The method of Brunner and Kustatscher[4] was generally used for the Meerwein reactions. The products obtained could not be distilled *in vacuo* without decomposition and hence were purified by successive recrystallizations, which generally resulted in low yields. The purified materials contained the elements of hydrogen chloride and proved to be α-chlorobibenzyls and 1-chloro-1-(2-pyridyl)-2-phenylethanes. These were then converted to stilbenes or stilbazoles by dehydrohalogenation.

Experimental

***p*-Nitrostyrene.**—This material was prepared in 65% yield by the method of Strassburg, Gregg and Walling.[5]

α,4′-Dichloro-4-nitrobibenzyl.—The method used was that of Brunner and Kustatscher.[4] After coupling, the reaction solution was steam distilled to remove the acetone and dichloroacetone after which a dark viscous oil remained. The oil was extracted with ether and the extract was washed with water and dried. After removal of the solvent the residue was purified by repeated recrystallizations from alcohol. From 14.9 g. (0.10 mole) of *p*-nitrostyrene, 1.3 g. (4%) of fine white needles were obtained; m.p. 94.5–95° dec.

Anal. Calcd. for $C_{14}H_{11}NO_2Cl_2$: C, 56.77; H, 3.75. Found: C, 56.68; H, 3.93.

4-Chloro-4′-nitrostilbene.—The α,4′-dichloro-4-nitrobibenzyl (1.4 g., 0.005 mole) was dehydrohalogenated using potassium hydroxide (0.85 g.) in methanol (13 ml.). The product was recrystallized from alcohol; yield 0.4 g. (33%), m.p. 184–185° dec. (lit.[6] 186–187°).

The dibromide, α,α'-dibromo-4′-chloro-4-nitrobibenzyl was obtained by the method of Buckles, Hausman and Wheeler[7] and recrystallized from ethanol; yield 13%, m.p. 195–206° dec.

Anal. Calcd. for $C_{14}H_{10}NO_2Br_2Cl$: C, 40.08; H, 2.40. Found: C, 40.14; H, 2.69.

α-Chloro-4′-methyl-4-nitrobibenzyl.—The procedure of Koelsch[8] was used for the reaction of *p*-methylbenzenediazonium chloride with *p*-nitrostyrene. The coupling product was fractionated using a mercury vapor pump. The fraction (150–180°, oil-bath temperature), obtained by distillation with an alembic type flask, was redistilled using a Hickman vacuum still to give 5.0 g. (9%) of an orange viscous liquid which boiled at 122–123° (1 mm.) (oil-bath temperature).

Anal. Calcd. for $C_{15}H_{14}NO_2Cl$: C, 65.33; H, 5.12. Found: C, 65.28; H, 4.78.

α-Chloro-4,4′-dinitrobibenzyl.—Using the procedure of Brunner and Kustatscher,[4] 14.9 g. (0.1 mole) of *p*-nitrostyrene was caused to react with a solution of *p*-nitrobenzenediazonium chloride. The product was a dark brown oil. Repeated recrystallizations from alcohol yielded white needles which melted at 133–134° dec., yield 1.2 g. (4%).

Anal. Calcd. for $C_{14}H_{11}N_2O_4Cl$: C, 54.82; H, 3.62. Found: C, 55.12; H, 4.01.

4,4′-Dinitrostilbene.—Dehydrohalogenation of α-chloro-4,4′-dinitrobibenzyl was carried out with methanolic potassium hydroxide as described above. After recrystallization from dioxane, the product melted at 291–292° dec. (lit.[6] 294–295°), yield 59%.

1-Chloro-1-(2-pyridyl)-2-(4-chlorophenyl)-ethane.—*p*-Chlorobenzenediazonium chloride, prepared from 12.7 g. (0.1 mole) of *p*-chloroaniline, was caused to react as described above with 10.5 g. (0.1 mole) of freshly distilled 2-vinylpyridine. The dark brown oil which separated after steam distillation could not be obtained as a crystalline material. However, when the product was distilled under reduced pressure, 5.0 g. (20%) of a yellow viscous oil was obtained, b.p. 150–158° (1 mm.). Carbon and hydrogen values indicated that some dehydrohalogenation had occurred during the distillation.

4′-Chloro-2-stilbazole.—The above product was dehydrohalogenated as described earlier. White platelets were obtained on crystallization from alcohol; yield 61%, m.p. 82.5–83° (lit.[9] 83–84°).

1-Chloro-1-(2-pyridyl)-2-(*p*-tolyl)-ethane.—The product from the Meerwein coupling of *p*-methylbenzenediazonium chloride with 21.4 g. (0.2 mole) of 2-vinylpyridine was a dark oil and it was necessary to distil the product in a manner similar to that described for α-chloro-4′-methyl-4-nitrobibenzyl. The fraction obtained at 127–133° (1 mm.) (bath temperature), when redistilled in a Hickman still, yielded 24.8 g. (54%) of an orange liquid, b.p. 123–124° (1 mm.) (bath temperature). Carbon and hydrogen values indicated that some dehydrohalogenation had occurred during the distillation.

4′-Methyl-2-stilbazole.—A sample of the above 1-chloro-1-(2-pyridyl)-2-(*p*-tolyl)-ethane (1.6 g., 0.006 mole) was dehydrohalogenated with alcoholic potassium hydroxide. The 4′-methyl-2-stilbazole melted at 83–84° (lit.[10] 87°), yield 1.1 g. (82%).

1-Chloro-1-(2-pyridyl)-2-(4-nitrophenyl)-ethane.—When 10.5 g. (0.1 mole) of 2-vinylpyridine was caused to react with *p*-nitrobenzenediazonium chloride, a dark brown oil was isolated from the reaction mixture. Treatment with alcohol and recrystallization of the solid which separated gave 4.0 g. (15%) of white needles, m.p. 100.5–101° dec.

Anal. Calcd. for $C_{13}H_{11}N_2O_2Cl$: C, 59.43; H, 4.22. Found: C, 59.12; H, 4.32.

4′-Nitro-2-stilbazole.—1-Chloro-1-(2-pyridyl)-2-(4-nitrophenyl)-ethane (3.0 g., 0.011 mole) was dehydrohalogenated with alcoholic potassium hydroxide to give the correspond-

(1) Based on a thesis submitted August, 1952, by C. M. I. to the Graduate School of the University of Missouri in partial fulfillment of the requirements for the M.A. degree.

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