[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING OF THE UNIVERSITY OF PENNSYLVANIA]

The Reaction of *ortho*-Quinones and *ortho*-Quinonimines with Primary Amines

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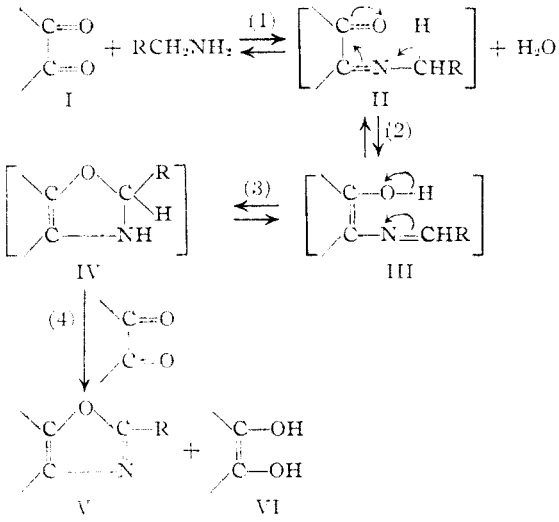
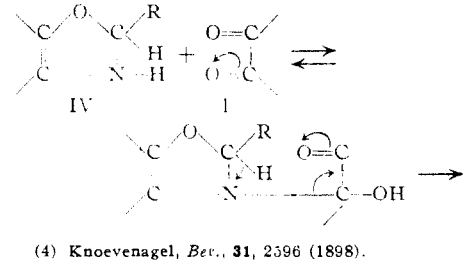
While the reactions of retenequinone and phenanthraquinone with ammonia to form the corresponding quinonimines have long been known,[2] no work has been reported on the behavior of these quinones with primary amines under similar conditions. We have found that certain primary amines react with retenequinone to form 2-substituted retenoxazoles. Only those amines which have two α-hydrogen atoms will react to form retenoxazoles. This is shown by the fact that isopropylamine and α-methylbenzylamine do not yield oxazoles, whereas n-butylamine, ethanolamine and benzylamine yield 2-substituted retenoxazoles.[3]

The following mechanism is proposed for this reaction



The formation of the Schiff base type of compound II is indicated by the fact that when the reaction was carried out in dry toluene water was liberated, and when the quinone was replaced by the quinonimine ammonia was formed with subsequent formation of a 2-subsituted retenoxazole. Evidence for the formation of III was obtained by adding hydrochloric acid at the proper time to a reaction mixture consisting of retenequinone and benzylamine in alcohol. Benzaldehyde was isolated as its semicarbazone from the hydrolysis products. The hydrolysis of III should also yield 9-amino-10-retenol. Therefore, if aminoretenol were condensed with an aldehyde to yield an oxazole, the above evidence would be confirmed. Since aminoretenol is very unstable, 9,10-aminophenanthrol was prepared instead and condensed with n-butyraldehyde and benzaldehyde, giving 2-propyl- and 2-phenylphenanthroxazoles, respectively.

Evidence for the course of reaction involved in ring closure is strongly in favor of the one postulated. Knoevenagel,[4] Mayer[5] and Stein and Day[6] have established the fact that active hydrogen compounds readily add to Schiff bases under suitable conditions. That the dihydroöxazole (IV) was dehydrogenated by the quinone could not be established directly due to the instability of retenehydroquinone. Satisfactory evidence for this step was obtained by adding p-benzoquinone to reaction mixtures of retenequinonimine and primary amines. Both hydroquinone and quinhydrone were isolated from these reactions.[7] If the reaction follows the proposed final step, a maximum yield of 50% would be expected when retenequinone and amine are used in equivalent amounts. The higher yields actually obtained may be explained by the ease with which retenehydroquinone undergoes air oxidation to retenequinone. The dehydrogenation may be represented as



(1) Present address, University of Pennsylvania, Philadelphia, Pa.

(2) Bamberger and Hooker, *Ann.*, **229**, 102 (1885); Pschorr, *Ber.*, **35**, 2729 (1902).

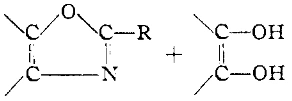
(3) Ethylenediamine is an exception for it reacts with retenequinone to form a pyrazine, Mason, *J. Chem. Soc.*, **63**, 1288 (1893).

(4) Knoevenagel, *Ber.*, **31**, 2596 (1898).

(5) Mayer, *Bull. Soc. Chim.*, **33**, 157, 395, 498 (1905).

(6) Stein and Day, THIS JOURNAL, **64**, 2569 (1942).

(7) The rate of reaction between the primary amine and p-benzoquinone is greater than the rate of reaction between the amine and retenequinone, making it impractical to separate any hydroquinone from the mixture of products. However, when retenequinone was replaced by its isoster, retenequinonimine, the rates were favorably reversed.



V VI

When retenequinone is replaced by retenequinonimine, the reactions proceed more rapidly and give higher yields of oxazoles. The formation of the latter rather than imidazoles indicates that the initial condensation takes place at the imino group. The fact that higher yields are obtained may be attributed to the liberation of ammonia in place of water, thus eliminating any secondary hydrolysis reactions.

The reaction of phenanthraquinone with benzylamine gave small amounts (9%) of 2-phenylphenanthroxazole and 1-benzyl-2-phenyl-phenanthrimidazole (2.7%). Benzaldehyde was recovered in the steam distillate from this run as its phenylhydrazone and semicarbazone.[8] The use of phenanthraquinonimine in place of the quinone gave better yields of 2-phenylphenanthroxazole and the reaction with butylamine gave a 35% yield of 2-propylphenanthroxazole as contrasted with the complete failure of the reaction when the quinone was used.

Experimental

All of the melting points represent corrected values and check the literature values unless otherwise stated.

Retenequinone.—This compound was prepared from retene by the method of Kreps and Day,[9] m. p. 197–199°.

Phenanthraquinone was prepared from phenanthrene according to the directions of Graebe[10] and the purification was carried out by the method of Courtot.[11] It was then recrystallized from 50% acetic acid; yields 55–60%; m. p. 208–209.5°.

Retenequinonimine.—The quinonimine was prepared from retenequinone and ammonia according to Bamberger and Hooker[2] and recrystallized from warm (50°) dry alcohol saturated with ammonia; yields 50–60%; m. p. 107–108°.

Phenanthraquinonimine.—The method of Pschorr[2] was used for this preparation. The crude product was recrystallized from warm (50°) dry alcohol saturated with ammonia; yields 70–75%; m. p. 156–157°.

9,10-Aminophenanthrol Hydrochloride.—One gram (0.0048 mole) of phenanthraquinonimine was added to 100 cc. of dry alcohol, which had been previously flushed with nitrogen, and warmed in a stream of nitrogen until solution was effected. The solution was diluted with 100 cc. of air-free water and 0.9 g. (0.0048 mole) of sodium hydrosulfite added. When the solution became practically colorless, the nitrogen was replaced with dry hydrogen chloride. The aminophenanthrol hydrochloride precipitated as a white, fluffy compound; yield 1.1 g. (92%). It has no melting point, but becomes red and starts to decompose around 120°.[12]

Anal. Calcd. for $C_{14}H_{12}NOCl$: N, 5.70. Found: N, 5.53.

Reactions of Retenequinone with Amines.—A series of experiments were carried out to determine the optimum temperature conditions. In general 5 g. (0.019 mole) of retenequinone and 2.1 cc. (0.019 mole) of benzylamine were added to the dry solvent (alcohol, toluene, cymene or nitrotoluene) and the mixture either allowed to stand or heated for a definite period of time. After cooling, the 2-phenylretenoxazole was removed by filtration. Concentration of the filtrate usually gave more of the oxazole. The product was then recrystallized from dry alcohol and the pure product obtained as white, fibrous needles, m. p. 174–176°. Mixed melting point determinations with a sample prepared by Stein and Day[6] showed no depression. In some cases where the crude product was contaminated with gummy material, the reaction mixture was steam distilled and the residue so obtained then recrystallized from dry alcohol. At room temperature the reaction proceeds very slowly. The best yields (70–80%) were obtained by heating the reaction mixtures at 75–100° for three hours. Heating for longer periods of time or at higher temperatures did not increase the yields.

Experiments were then carried out to determine the effect of a wholly aliphatic amine, *n*-butylamine, and of an aromatic-aliphatic type, benzylamine, on the rate of the reaction. The quinone and amines were taken in 0.019 molar quantities and 100 cc. of dry alcohol was used as the solvent for each experiment. The procedure was as described above, except that it was found more convenient in the reactions with *n*-butylamine to evaporate the reaction mixtures to dryness and recrystallize the residue from alcohol until colorless crystals of 2-propylretenoxazole were obtained; m. p. 100–101°. Mixed melting point determinations with a sample prepared by Stein and Day[6] showed no depression. It was found that at 78° maximum yields (62%) of 2-propylretenoxazole were obtained in eight to eleven hours while maximum yields of 2-phenylretenoxazole were obtained in three hours. It should be pointed out that the presence of the phenyl group in benzylamine would be expected to facilitate the hydrogen shift noted earlier and thus increase the rate of oxazole formation.

Experiments were finally carried out to determine the effect of the ratio of the reactants on the yields when the time was kept constant. These reactions were run in dry alcohol as described above. It was found that increasing the concentration of one or the other of the reactants had no appreciable effect on the yields.

Reaction of Retenequinone with α-Methylbenzylamine.—The reaction of retenequinone with α-methylbenzyl-

(8) The ease with which benzaldehyde splits out in this case is interesting in view of the fact that in a similar run with retenequinone it was necessary to add hydrochloric acid before any benzaldehyde could be isolated. This would indicate that the intermediate (III) formed in the second step is more readily hydrolyzed in the case of phenanthraquinone than with retenequinone and might explain the lower yields of oxazoles obtained.

(9) Kreps and Day, *J. Org. Chem.*, **6**, 140 (1941).

(10) Graebe, *Ann.*, **167**, 140 (1873).

(11) Courtot, *Ann. Chim.*, [10] **14**, 69 (1930).

(12) The method of Pschorr (*Ber.*, **35**, 2729 (1902)) and Schmitt ([illegible] **35**, 3129 [illegible]) [illegible] compound.

amine was carried out under a variety of conditions, but only intractable gums were obtained from which no oxazole could be isolated. Extraction of these gums with alcoholic hydrochloric acid followed by treating the acid extract with hydroxylamine hydrochloride and excess sodium acetate yielded acetophenone oxime; m. p. 58°. A mixed melting point determination with an authentic sample gave no depression.

Similar results were obtained with isopropylamine. The identification of a ketone in these mixtures is definite proof that the initial condensation between the quinone and amine did occur. The fact that no oxazole could be isolated suggests that there must be two α-hydrogen atoms in the amine for oxazole formation to take place.

Reaction of Retenequinone with Ethanolamine. Preparation of 2-Hydroxymethylretenoxazole.—Five grams (0.019 mole) of retenequinone and 1.2 cc. (0.019 mole) of ethanolamine were added to 50 cc. of dry alcohol and refluxed for six hours. The solution was evaporated to dryness and the gummy residue triturated with dilute hydrochloric acid until it had entirely solidified. The solid residue was recrystallized from dioxane and finally from alcohol; colorless needles, m. p. 187.5–189°; yield 2.4 g. (41%). This compound has not been reported previously.

Anal. Calcd. for $C_{20}H_{19}NO_2$: C, 78.65; H, 6.27; N, 4.59. Found: C, 78.61; H, 6.32; N, 4.40.

To confirm the identity of the above compound, a sample was converted into 2-acetoxymethylretenoxazole by refluxing with acetic anhydride and pouring the solution into cold water. The conversion was nearly quantitative; m. p. 134.5–136°.

Anal. Calcd. for $C_{22}H_{21}NO_3$: C, 76.06; H, 6.09; N, 4.09. Found: C, 75.90; H, 6.15; N, 4.00.

Reaction of Retenequinone with Ethylenediamine. Preparation of 12-Methyl-6-isopropylphenanthrapyrazine.—Five grams (0.019 mole) of retenequinone and 1.4 cc. (0.019 mole) of 95% ethylenediamine were dissolved in 50 cc. of warm toluene and refluxed for six hours. The toluene was removed by steam distillation and the residue extracted with a mixture of alcohol and ether. The extract was evaporated and the residue recrystallized from dry alcohol until a colorless product was obtained; yield 2.8 g. (52%); m. p. 126–126.5°.

Anal. Calcd. for $C_{21}H_{18}N_2$: N, 9.79. Found: N, 9.61.

Study of the Course of Reaction. Step 1. The Reaction of Retenequinonimine with Benzylamine. One gram (0.0038 mole) of retenequinonimine and 0.42 cc. of benzylamine were dissolved in 50 cc. of dry toluene and refluxed for four hours. The evolved ammonia was collected in 4% boric acid solution and titrated with hydrochloric acid in the presence of methyl red; ammonia evolved 0.003 mole. The reaction mixture was steam distilled and the residue recrystallized from alcohol; yield of 2-phenylretenoxazole 1.11 g. (84%); m. p. 174–176°.

Step 2. Reaction of Retenequinone with Benzylamine.—Five grams (0.019 mole) of retenequinone was dissolved in 300 cc. of boiling absolute alcohol and 4.2 cc. (0.038 mole) of benzylamine added. The solution was refluxed and as soon as the solution turned dark an excess of 1:3 hydrochloric acid was added slowly. The solution was refluxed for an additional hour, cooled and filtered. The filtrate was then diluted with water and extracted with ether. The ether was removed by distillation and the residue steam distilled. Benzaldehyde was recovered from the steam distillate as its semicarbazone, m. p. 220–223°, and its phenylhydrazone, m. p. 157–158°. A similar run with phenanthraquinone and benzylamine also yielded benzaldehyde.

Reaction of 9,10-Aminophenanthrol with Benzaldehyde.—One gram (0.004 mole) of aminophenanthrol hydrochloride, 0.34 g. (0.004 mole) of sodium acetate and 0.41 cc. (0.004 mole) of benzaldehyde were added to 60 cc. of dry alcohol and refluxed for two and one-half hours. On cooling, 0.75 g. (63%) of 2-pheuylphenanthroxazole separated. It was recrystallized from alcohol; m. p. 207–208°.

Reaction of 9,10-Aminophenanthrol with *n*-Butyraldehyde. The above experiment was carried out with *n*-butyraldehyde in place of benzaldehyde and the solution refluxed for nine hours; yield of 2-propylphenanthroxazole 0.67 g. (94%). After recrystallization from alcohol and water it melted at 84–86°.

Step 4. Reaction of Retenequinonimine with *n*-Butylamine in the Presence of *p*-Benzoquinone.—Two grams (0.0076 mole) of retenequinonimine and 0.84 cc. (0.0076 mole) of *n*-butylamine were dissolved in 20 cc. of warm toluene and the solution heated. As soon as the solution became dark an equivalent of *p*-benzoquinone in 10 cc. of dry toluene was added. White needles of hydroquinone soon separated. After recrystallization from water the hydroquinone melted at 171.8–172.3°. A mixed melting point determination with an authentic sample showed no depression; m. p. of diacetyl derivative, 122.2–122.5°.

The above experiment was repeated with two equivalents of *p*-benzoquinone and quinhydrone instead of hydroquinone separated from the solution. It was recrystallized from water; m. p. 168–170°.

In both of the above experiments 2-propylretenoxazole was isolated from the filtrates by removing the toluene with steam and recrystallizing the residue from alcohol and water; m. p. 100–101°.

Reaction of Retenequinonimine with *n*-Butylamine.—Two grams (0.0076 mole) of retenequinonimine and 0.8 cc. (0.0076 mole) of *n*-butylamine were added to 50 cc. of dry alcohol and refluxed for eight hours. The solution was evaporated and the residue recrystallized from alcohol and water with the aid of decolorizing carbon; yield 1.6 g. (70%); m. p. 100–101°.

Reaction of Retenequinonimine with Benzylamine.—This experiment is recorded under Step 1 in the experimental work on the course of the reaction.

Reaction of Phenanthraquinone with Benzylamine. (a) **In Toluene.**—Five grams (0.024 mole) of phenanthraquinone and 2.6 cc. (0.024 mole) of benzylamine were dissolved in 50 cc. of hot toluene and refluxed for three hours. Filtration of the reaction mixture gave 3.7 g. of an intractable, brown solid. The filtrate was steam distilled and the distillate tested for benzaldehyde. Both the phenylhydrazone (m. p. 156–158°) and semicarbazone (m. p. 220–222°) of benzaldehyde were isolated. The residue from the steam distillation was fractionally crystallized from a mixture of dry alcohol and benzene. The least

soluble fraction (0.65 g., 9%) was recrystallized from alcohol and proved to be 2-phenylphenanthroxazole, m. p. 206–207°. The more soluble fraction (0.25 g., 2.7%) was 1-benzyl-2-phenylphenanthrimidazole; m. p. 241–241.5°. This compound has not been previously reported.

Anal. Calcd. for $C_{28}H_{20}N_2$: C, 87.46; H, 5.24; N, 7.27. Found: C, 87.69; H, 5.30; N, 7.12.

(b) **In Glacial Acetic Acid.**—The same quantities of quinone and amine were dissolved in 25 cc. of glacial acetic acid, refluxed for three hours and filtered while hot. The brown residue (3.1 g.) was digested with hot dioxane and subsequently with hot nitrobenzene. This left a green residue of phenanthroxazine; yield 2.2 g. (48%); m. p. > 360°.

Anal. Calcd. for $C_{28}H_{17}NO$: N, 3.66. Found: N, 3.46.

The original filtrate on cooling deposited 2-phenylphenanthroxazole which was recrystallized from alcohol; yield 1 g. (14%); m. p. 206–207°. Water was added to the filtrate from the oxazole, precipitating a gum. The latter was dissolved in a hot mixture of acetone and ether, from which 1-benzyl-2-phenylphenanthrimidazole separated on cooling. It was recrystallized from alcohol; yield 0.4 g. (4%); m. p. 241–241.5°.

Reaction of Phenanthraquinone with *n*-Butylamine in Toluene.—Although this reaction was carried out under various conditions, only intractable brown solids and gums were obtained.

Reaction of Phenanthraquinonimine with Benzylamine.—One gram (0.0048 mole) of phenanthraquinonimine and 0.53 cc. (0.0048 mole) of benzylamine were dissolved in 50 cc. of hot toluene and refluxed for three hours. The solution was steam distilled and the residue recrystallized from alcohol; yield of 2-phenylphenanthroxazole 0.83 g. (59%); m. p. 205–206°. No imidazole could be isolated.

Reaction of Phenanthraquinonimine with *n*-Butylamine.—Two grams (0.0097 mole) of phenanthraquinonimine and 0.96 cc. (0.0097 mole) of *n*-butylamine were dissolved in 35 cc. of hot toluene and refluxed for eight hours. After steam distillation, the residue was dissolved in a mixture of dry alcohol and benzene. A small amount (0.1 g.) of a red, amorphous solid remained. The solution was evaporated and the residue recrystallized from alcohol; yield of 2-propylphenanthroxazole 0.9 g. (35%); m. p. 85–86°. A mixed melting point determination with a sample prepared by Stein and Day[6] showed no depression.

Summary

1. It has been shown that primary amines which have two hydrogen atoms on the alpha-carbon atom react with retenequinone and phenanthraquinone (or their isosters, the quinonimines) to form the corresponding oxazoles.

2. The course of the reaction has been shown to consist of the following probable steps: (1) an aldol-type of condensation between the quinone and the amine; (2) a shift of hydrogen in the two adjacent triad systems from carbon to oxygen; (3) addition of OH across a —N=CH— linkage; and (4) oxidation of a dihydroöxazole (by a quinone) to an oxazole.

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The Fluorescence of Vitamin A

BY HARRY SOBOTKA,[1] SUSAN KANN AND ERICH LOEWENSTEIN

The fading green fluorescence of vitamin A under ultraviolet irradiation has been used for its histochemical demonstration in slices of liver and other animal organs.[2] An attempt by one of us (E. L.) to utilize this fluorescence for the quantitative analysis of vitamin A solutions by a photoelectric fluorometer led to the observation that vitamin A preparations in alcoholic solution display upon irradiation an initial steep increase in fluorescence, followed by complete destruction of fluorescence during prolonged irradiation. In contrast to this, the fluorescence of vitamin A solutions in ether, chloroform, or benzene shows sometimes a small initial drop, but always assumes quickly a level of steady intensity which decreases but slowly.

The fluorescence of vitamin A in non-polar as well as in polar solvents is fairly proportional to its concentration over a range from 0.1–5.0 I.U./ml. under our experimental conditions. Although not quite as sensitive as the Carr–Price reaction, fluorescence may serve as a satisfactory basis for an analytical method. In concentrations below 0.1 I.U./ml. erratic results are often obtained. In polar solvents such as methanol, ethanol, or isobutanol fluorescence follows a curve such as "1" in Fig. 1. Symbatic curves are obtained for varying concentrations, the ordinates (intensity

(1) Supported by a Grant from Nutrition Foundation Inc. A report of the work was given at the Conference on vitamin research held at Gibson Island, Maryland, under the auspices of the A.A.A.S., July 20th, 1943.

(2) H. Popper, *Proc. Soc. Exptl. Biol. Med.*, **43**, 133 and 234 (1940); *Arch. Path.*, **31**, 766 (1941); H. Popper and R. Greenberg, *ibid.*, **32**, 11 (1941); R. Greenberg and H. Popper, *Am. J. Physiol.*, **134**, 114 (1941).