[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, DUQUESNE UNIVERSITY]

Derivatives of 1-Thia-4,5-diazacyclohepta-2,4,6-triene. III. Isomeric Dibenzo-1-thia-4,5-diazacyclohepta-2,4,6-triene-1,1-dioxides and Dibenzo-1-thia-4,5-diazacyclohepta-2,4,6-triene-1,1,4-trioxides

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The isolation of three isomers of the previously described heterocyclic system containing either the azo or the azoxy function is described. Isomerization, oxidation and reduction experiments, as well as physical properties are utilized in the provisional assignment of conformations to the three isomers in each series. While two of each group of three isomers seem to be chair and boat conformations of the heterocyclic ring, the third isomer appears to be a novel seven-membered puckered ring in which a *trans* azo or azoxy group is part of the ring.

Repetition of the previously described[2] oxidation of di-(*o*-aminophenyl) sulfone by means of phenyliodoso acetate gave in addition to the already reported dibenzo-1-thia-4,5-diazacyclohepta-2,4,6-triene-1,1-dioxide (I) of melting point 170–171°,[3] another compound melting at 130°. This material was shown to be an isomer of I by analysis and by thermal conversion to the higher melting solid, and the two compounds are herewith designated IA and IB, respectively. As previously reported,[2] the oxidation of IA by peracetic acid gave dibenzo-1-thia-4,5-diazacyclohepta-2,4,6-triene-1,1,4-trioxide (II) of melting point 251–252°, and analogous oxidation of IB gave a mixture of the already known azoxy compound and an isomeric azoxy compound of melting point 239°. These two azoxy compounds are herewith designated IIA and IIB, respectively, and as in the case of IA and IB, the new isomer (IIB) could be isomerized thermally (and also photochemically) to IIA.

It was of interest to confirm the existence of the two isomeric compounds I by the reduction of the azoxy function of II to the azo function in I. To our surprise, the reduction of either IIA or IIB by means of lithium aluminum hydride or zinc in acetic acid gave neither IA or IB but rather a third isomer of I, a solid of melting point 193°, which is herewith designated as IC. The fact that IC is related structurally to IA was established by successful photochemical isomerization of IC to IA. The same isomerization could be accomplished by dissolving IC in cold sulfuric acid and precipitating IA upon dilution of the solution with ice. The oxidation of IC by means of peracetic acid gave IIA just as did the oxidation of IA.

The existence of three isomers of I implied the possible existence of three isomers of II, and the third isomer of II of melting point 250–251° (IIC) was indeed isolated when dibenzo-1-thia-4,5-diazacyclohepta-2,6-diene was subjected to oxidation with peracetic acid. While IIB and IIC have almost identical melting points they differ in several respects. Thus while IIA is a yellow solid which melts without decomposition, IIC is a white solid which undergoes complete decomposition upon melting. IIC is isomerized to IIA when heated in acetic acid solution and reduction of IIC by means of lithium aluminum hydride gives IC.

The above mentioned results prove the existence of three isomers in the azo series I and also in the azoxy series II. Assuming a non-planar structure of the heterocyclic ring in I and II, there can be visualized two isomers in which the heterocyclic ring assumes either a chair or a boat conformation. This hypothesis still does not account for the existence of the third pair of isomers. We wish to suggest at this time that the third pair of isomers represents a puckered, seven-membered ring in which the azo function exists in the *trans* form. Evidence in support of this suggestion is cited below. The three suggested conformations in the azo series I are represented schematically in Fig. 1.

Assuming the correctness of the proposed conformational differences between IA,B,C and IIA,B,C, our efforts were next directed toward the assignment of specific conformations to the individual isomers.

Examination of the ultraviolet spectra of IA,-B, C(Fig. 2) revealed a distinct feature differentiating IC from IA and IB. The 430 mμ band, the well recognized[4] R-band of azo compounds, was abnormally weak in IC (ϵ 14) while it was of normal intensity in IA and IB (ϵ 380–595). Since in negatively substituted azo compounds the intensity of the R band is generally higher in the *cis* isomer, the weak R band in IC favors the assignment of the *trans* azo structure to this isomer. Supporting this assignment is the above mentioned facile isomerization of IC to IA by ultraviolet light or by acid catalysis. In the course of both kinds of treatments the temporary breaking of the nitrogen–nitrogen double bond would permit the rearrangement to the *cis* azo structure which would be expected to be the more stable structure in a cyclic azo compound. Further support of the *trans* azo assignment to IC is the fact that the reduction of all three isomers of II gave exclusively this particular isomer. Badger[5] has shown that the reduction of both *cis*- and *trans*-azoxybenzene gives the *trans* azo system and explains these results in terms of the preferential *trans* elimination of water from the intermediate dihydroazoxy structure. The same explanation may account for the formation of IC from IIA,B,C on reduction with lithium aluminum hydride or zinc, even though IC is readily isomerized to IA.

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(1) From the Ph.D. thesis of Y.L.C.

(2) H. H. Szmant and R. L. Lapinski, THIS JOURNAL, **78**, 458 (1956).

(3) Extensive purification raised the melting point to 174°.

(4) R. E. Gillam and E. S. Stern, "Electronic Absorption Spectroscopy," Edward Arnold, London, 1954.

(5) G. M. Badger, R. G. Buttery and G. E. Lewis, *J. Chem. Soc.*, 2143 (1953).

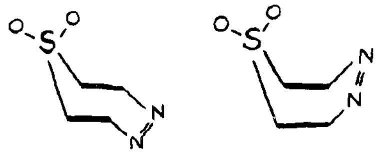
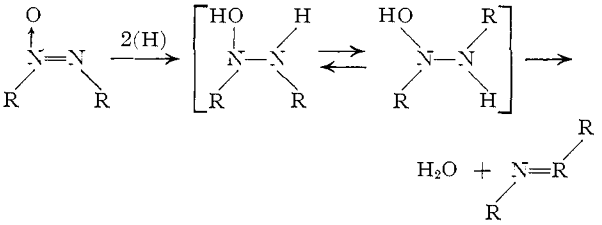


Fig. 1.—Conformations of I. Heavy lines indicate fusion of benzene rings.

The assignment of the conformations to IA and IB is more difficult in the absence of additional physical data,[6] but provisionally the higher melting and more stable isomer IA is assigned the *cis* azo chair structure. In this connection it was found that IB was oxidized 13 times faster than IA by perbenzoic acid. Badger[5] has shown that the greater rate of oxidation of *cis*-azobenzene as compared to *trans*-azobenzene (50–80 fold difference) can be accounted for by considering the greater electron-availability in the *cis* azo linkage. In the case of IA and IB, both compounds are assumed to be *cis* azo structures and apparently the electron-withdrawing sulfone group is more effective in the chair structure.



The comparison of the behavior of the isomeric II compounds at their respective melting points reveals that IIA is the most stable compound since it is the only isomer which does not decompose. It is of interest that IIB decomposes violently with evolution of brown gas, while IIC decomposes to a tar without noticeable gas evolution. The relative stability of IIA suggests that it may have the *cis*-azoxy chair configuration. The oxidation of IA to IIA was reported in the preceding publication[2] and the formation of a mixture of IIA and IIB by peracetic acid oxidation of IB was mentioned above. These results indicate that IA and IIA are configurationally related, and the fact that IB isomerizes to IA upon heating explains why the oxidation of IB gives some IIA in addition to IIB. A mixture of IIA and IIB was also obtained when di-(*o*-aminophenyl) sulfone was oxidized by peracetic acid. The conclusion that IB and IIB are configurationally related also is supported by the selective oxidation of IB to IIB by perbenzoic acid. The success of the last mentioned experiment is in line with the experiences of Badger[5] who demonstrated that perbenzoic acid oxidizes *cis*-azobenzene without isomerization while peracetic acid also brings about isomerization.

In an attempt to bring about a controlled reduction of the azoxy to the azo linkage, IIB was treated with deactivated nickel (see Experimental) but in place of the expected IB the only product which could be isolated from this reaction was II-A. Since this experiment was performed without application of heat it demonstrates the relatively greater stability of IIA.

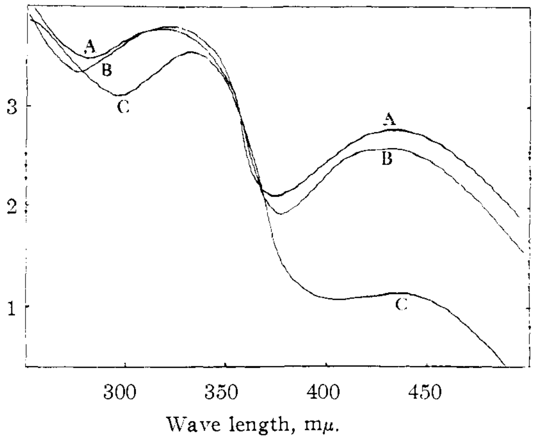


Fig. 2.—Ultraviolet spectra of IA (curve A), IB (curve B), and III (curve C).

The assignment of the chair and boat conformations to IIA and IIB, respectively, leaves the *trans*-azoxy conformation for the IIC isomer. It was hoped to confirm this assignment by the perbenzoic acid oxidation of IC to IIC but unfortunately the oxidation of IC gave exclusively IIA. When the kinetics of this reaction was examined it was found that IC is subject to a very rapid isomerization to IA with the loss of one equivalent of perbenzoic acid. The isomerized product could be isolated when the oxidation was stopped at that point, and when the oxidation was allowed to continue it proceeded at a rate essentially equal to that of IA. The mechanism of isomerization of IC to IA with loss of perbenzoic acid is not understood at this time and is subject to additional study.

The successful isomerization of IC to IA by concentrated sulfuric acid prompted the examination of possible isomerization of IIC under the same conditions. Neither IIC nor the other two azoxy compounds were found to be isomerized when dissolved in concentrated sulfuric acid in the cold and were recovered when the solutions were poured on ice. The failure of IIC to be isomerized can be ascribed to the protonation of the azoxy group at the oxygen atom without disturbing the nitrogen–nitrogen double bond.

Experimental[7]

Preparation and Isolation of IA and IB.—A mixture of 5 g. (0.02 mole) of di-(*o*-aminophenyl) sulfone, 5.8 g. (0.02 mole) of phenyliodoso acetate and 500 cc. of dry toluene was allowed to stand at room temperature for 5 days. A brown precipitate, 0.8 g., was filtered, washed with hot toluene and discarded since it did not melt at 350°. The filtrate and washings were concentrated *in vacuo* and the residue was crystallized from methanol to give 2.2 g. of dark red crystals, m.p. 164–169°; yield 42%. Three crystallizations from isopropyl alcohol gave orange crystals, m.p. 174°, which did not depress the m.p. of the previously reported[2] IA. Concentration of the filtrates yielded 2.1 g. (40%) of dark orange crystals, m.p. 125–130°, which were purified to constant m.p. 130.5–131.5° by crystallization from methanol. Chromatography of this material (IB) on alumina gave only one band.

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[6] The measurement of the dipole moments of the compounds under discussion is planned for the near future.

[7] All melting points are uncorrected. Microanalyses by Drs. Strauss and Weiler, Oxford, England, and by Dr. Bernhardt, Mülheim, Germany.

Anal. Calcd. for $C_{12}H_8O_2N_2S$: C, 59.0; H, 3.30; S, 13.11; N, 11.47. Found: C, 59.4; H, 4.07; S, 13.3; N, 11.40.

Since IB upon heating is converted to IA the molecular weight of the latter was determined using the Rast method. The experimentally found molecular weight of 233 compares favorably with the calculated value of 244.

The relative yield of IA seemed to increase when the ratio of phenyliodoso acetate to the diamine was increased. Thus from the reaction of 15.0 g. of phenyliodoso acetate and 8 g. of di-(*o*-aminophenyl) sulfone there was isolated 70% of IA and 21% of IB.

A mixture of IA and IB gave a melting point range 129–148°.

Isomerization of IB to IA.—A solution of 300 mg. of IB in 12 cc. of acetic acid was refluxed for 13 hr. The solution was concentrated to a volume of 4 cc. and the cold residue was diluted by dropwise addition of water. The orange precipitate was filtered, washed with water and dried to give 210 mg. of solid, m.p. 169–173°. Crystallization from isopropyl alcohol gave orange crystals, m.p. 173–174°, which did not depress the m.p. of IA.

Attempts to effect isomerization of IB by irradiation with ultraviolet light were unsuccessful. Also attempted isomerizations of IA by either thermal or photochemical means failed.

IA and IB were recovered in nearly quantitative yields when solutions in cold concd. sulfuric acid were poured on ice.

Oxidation of IB.—Ten cc. of 40% peracetic acid was added over a period of 30 minutes to a solution of 1 g. of IB in 35 cc. of acetic acid. The mixture was stirred and cooled by means of an ice-bath during the addition, and then was allowed to stand at room temperature for 15 hr. Faintly yellow needles precipitated from the solution and they were filtered, washed with methanol and dried to give 850 mg. (80%) of solid, m.p. 212–218° dec. The filtrate was poured on ice and gave an additional 210 mg. of the same solid. The combined crystals were purified by dissolving in a minimum volume of hot dioxane, addition of approximately 100 cc. of water, and slow cooling. The resulting product gave a constant m.p. 243–245° dec. Admixture with IIA gave m.p. 244–251°, and admixture with IIB gave m.p. 242–244°. A portion of the product on heating in glacial acetic acid for 14 hr. was converted quantitatively to IIA, m.p. and mixture m.p. 250–251°.

Oxidation of IB with 30% hydrogen peroxide in refluxing acetone gave the same results, but oxidation of IB with peracetic acid in refluxing acid gave directly a quantitative yield of IIA, m.p. and mixture m.p. 251–252°.

Oxidation of IA to IIA.—A solution of 50 mg. of IA in 10 cc. of hot acetic acid was treated with 1 cc. of 40% peracetic acid. The solution was refluxed for 10 hr. and then poured on ice to give greenish-yellow needles, m.p. 247–249°. Crystallization from acetic acid gave pure IIA, m.p. and mixture m.p. 251–252°.

Preparation of IIA and IIB.—A solution of 2.5 g. of di-(*o*-aminophenyl)sulfone in 35 cc. of acetic acid and 10 cc. of benzene was cooled below 10° and stirred while a solution of 10 cc. of 40% peracetic acid in 50 cc. of acetic acid was added dropwise over 40 hr. The colorless solution turned brown. Mechanical stirring was extended for another 24 hr. at room temperature. The reaction mixture on cooling in an ice-bath deposited dark needles which were filtered, washed with methanol and water. The solid, 2.1 g., m.p. 235–241° dec., was fractionally crystallized from acetone. The first fraction, 1.8 g. (69%), consisted of white needles, m.p. 235° dec., while the second fraction, 0.3 g., consisted of yellow-green needles, m.p. 235–245°. The first fraction was crystallized repeatedly from acetone and gave white needles of constant m.p. 239° (violent decomposition with evolution of brown gas). This material is considered to be IIB.

Anal. Calcd. for $C_{12}H_8O_3N_2S$: S, 12.31; N, 10.8; mol. wt., 260. Found: S, 11.95; N, 10.8; mol. wt. (Rast), 247.

The mother liquor was poured into water and gave 0.5 g. of green-yellow needles, m.p. 238–245°. This material was combined with the second fraction from the above crystallization and the solids (30%) were treated with charcoal in acetic acid solution. The purified product, m.p. 251–252°, did not depress the m.p. of IIA, and the ultraviolet spectra of the two materials were identical. The mol. wt. of IIA was found to be 244.

Isomerization of IIB to IIA. (A).—A solution of 15 mg. of IIB in acetic acid was refluxed 48 hr. and poured on ice. The product, 13 mg. (87%), was crystallized from acetic acid to give green-yellow needles, m.p. 250–251°, which were identical with IIA. A reflux period of 30 hr. was also sufficient to cause the isomerization of IIB to IIA. When in addition to acetic acid there was present also a small amount of hydrochloric acid (1 cc. of concd. hydrochloric acid in 20 cc. of acetic acid), the yield of IIA was only 60% and an unidentified brown gelatinous precipitate was produced.

(B).—A solution of 50 mg. of IIB in 50 cc. of acetic acid was irradiated for 3 days in a quartz vessel by means of an ultraviolet lamp. The solution turned brown and when poured on ice there was obtained 42 mg. (84%) of solid, m.p. 241–244°. A charcoal treatment in acetic acid and an additional crystallization from the same solvent gave pure IIA, m.p. and mixture m.p. 251–252°.

(C).—A solution of 130 mg. of IIB in 180 cc. of toluene was added to two scoops of deactivated Raney nickel[8] covered by toluene. The mixture changed from the original slight yellow color to a green-yellow color upon shaking, and after 5 hr. it was filtered and the solvent was evaporated *in vacuo*. The residual solid, 100 mg. (78%), m.p. 237–239°, was recrystallized from methanol to give pure IIA, m.p. and mixture m.p. 251–252°.

Reduction of IIA and IIB to IC. (A) Lithium Aluminum Hydride.—A suspension of 350 mg. of IIA in 80 cc. of purified, warm dioxane was added slowly to a solution of 470 mg. of lithium aluminum hydride in 180 cc. of ether. An atmosphere of nitrogen was maintained throughout the reaction. Ten cc. of hot dioxane was used to wash down residual IIA from the addition funnel, and the mixture was allowed to remain for 25 hr. while agitated by a stream of nitrogen. The reaction mixture was hydrolyzed by addition of 50 cc. of 50% methanol followed by 100 cc. of 3% sulfuric acid. The resulting mixture was concentrated *in vacuo* while keeping the temperature below 35°. After removal of approximately 120 cc. of distillate, the solution deposited a yellow solid which was filtered and washed with water. The product, 270 mg., m.p. 183–187°, was crystallized from isopropyl alcohol to give IC, yellow needles, m.p. 194–196°.

Anal. Calcd. for $C_{12}H_8O_2N_2S$: C, 59.00; H, 3.30; N, 11.47; S, 13.11; mol. wt., 244. Found: C, 58.82; H, 3.80; N, 11.35; S, 13.00; mol. wt. (Rast), 244.

Using the same procedure the reduction of IIB gave 83% of IC, m.p. and mixture m.p. 194–196°. In another experiment in which the amount of dilute sulfuric acid was in excess of the amount required to neutralize the reaction mixture and the temperature during the distillation was raised to 100°, IA was obtained instead of IC. IA was identified by m.p. and mixture m.p. with authentic IA.

(B) Zinc Dust.—A solution of 100 mg. of IIA in 15 cc. of acetic acid was warmed on a hot plate while 1 g. of zinc dust was added in small portions. The mixture was filtered after 20 minutes and the solid was washed with 3-cc. portions of boiling acetic acid. The combined filtrate and washings were poured into a large volume of water and a solid was collected. The crude product was charcoaled and crystallized from methanol to give 60 mg. (64%) of yellow needles, m.p. 185–190°. Additional crystallization gave m.p. and mixture m.p. 194–196°.

The same procedure was applied to 150 mg. of IIB and gave 100 mg. (79%) of IC.

Isomerization of IC to IA.—A solution of 30 mg. of IC in 30 cc. of ethanol was irradiated in a quartz vessel for one week by means of an ultraviolet lamp. The solution was poured on ice and the resulting solid, 28 mg. (94%), m.p. 169–172°, was recrystallized from ethanol to give pure IA, m.p. and mixture m.p. 173–174°.

The same reaction was followed spectrophotometrically using a 1.134×10^{-3} M solution of IC and determining the ϵ at 430 $m\mu$. The initial value of ϵ agreed with that reported for IC and upon irradiation, the ϵ gradually approached the value of 550.

The isomerization of IC to IA also could be achieved by refluxing an ethanolic solution for 40 hr. However, the product was isolated in a yield of only 65–75%.

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[8] H. Hauptmann and B. Wladislaw, This Journal, **72**, 707 (1950).

IC, 20 mg., was dissolved in cold concd. sulfuric acid and the resulting red solution was immediately poured on ice. There was isolated 17 mg. (85%) of crude IA, m.p. 157–167°, which upon crystallization from ethanol gave m.p. and mixture m.p. 173–174°.

Oxidation of IC to IIA.—A solution of 50 mg. of IC in 10 cc. of acetic acid was warmed with 2 cc. of 40% peracetic acid and the temperature was raised gradually to reflux. The color was observed to change from yellow to dark orange and finally to green-yellow. After a reflux period of 2 hr. the solution was diluted with an equal volume of water and gave 40 mg. (78%) of solid, m.p. 245–250°. One crystallization from acetic acid gave pure IIA, m.p. and mixture m.p. 250–252°.

A solution of 50 mg. of IC in 5 cc. of acetic acid was treated dropwise with 5% solution of potassium permanganate in acetone. The addition was continued until the pink color persisted and then an additional 2-cc. volume of oxidizing solution was added. The mixture was warmed for two hours and allowed to stand until a crystalline precipitate began to appear. The mixture was then diluted with an equal volume of water and was filtered. The solid was extracted with isopropyl alcohol and the filtrate yielded upon concentration 20 mg. of solid, m.p. 250–252°. The product did not depress the m.p. of authentic IIA. The filtrate was poured into a large volume of water and the orange precipitate was crystallized to give IA, m.p. and mixture m.p. 173.5–174°.[9]

Preparation of Dibenzo-1-thia-4,5-diazacyclohepta-2,6-diene.—Di-(*o*-nitrophenyl) sulfide, 15 g., in 700 cc. of hot isopropyl alcohol was diluted with an equal volume of water and 42 g. of sodium acetate was dissolved in the mixture. The vigorously stirred solution was treated with 30 g. of zinc dust while a nitrogen atmosphere was maintained in the flask. The temperature was gradually raised to reflux (30 minutes) and maintained at reflux for 10 minutes. The reaction mixture was filtered rapidly and the filtrate was collected in a preheated 2-l. suction flask. Without changing the container the filtrate was concentrated by application of vacuum and 350–400 cc. of solvent was removed in this fashion. During all these operations care was taken to prevent excessive contact of the solution with air since the latter caused discoloration and separation of solid. The concentrated solution was diluted with water to a volume of 2 l. and, after refrigeration, there was isolated 8.5 g. (75%) of white crystals, m.p. 121° (with evolution of gas). Attempted crystallizations from alcohol caused discoloration of the product and in view of the failure to obtain satisfactory analysis of the previously prepared[10] cyclic hydrazo compounds the material from this experiment was used immediately for the preparation of IIC.

Preparation of IIC.—The hydrazo compound from the preceding experiment, 8.5 g., was dissolved in 30 cc. of acetic acid and the resulting solution was stirred and treated dropwise with a solution of 10 cc. of 40% peracetic acid in 100 cc. of acetic acid. An additional 20 cc. of 40% peracetic acid was added after 5 hr. When a white solid began to precipitate the mixture was first heated for 30 minutes on a hot plate, cooled and then filtered. The white solid was washed with methanol to give 7.0 g. (72%) of product, m.p. 243–247° dec. Crystallization from acetic acid raised the m.p. to 250–251° dec.

Anal. Calcd. for $C_{12}H_8O_3N_2S$: C, 55.38; H, 3.09; S, 12.31; N, 10.88; mol. wt., 260. Found: C, 55.13; H, 3.03; S, 12.28; N, 10.91; mol. wt. (Rast), 257.

Isomerization of IIC to IIA.—A solution of 50 mg. of IIC in 100 cc. of acetic acid was refluxed for 100 hr., treated with charcoal, filtered and cooled to give 35 mg. (70%) of green-yellow needles, m.p. 251–252° (no decomposition!). This material did not express the m.p. of IIA.

Neither irradiation by ultraviolet light nor dissolution in concentrated sulfuric acid were effective in the isomerization of IIC.

Reduction of IIC to IC. (A) Lithium Aluminum Hydride.—The procedure described for the reduction of IIA and IIB gave 390 mg. (92.5%) of crude IC, m.p. 190–192°, from 450 mg. IIC. A single crystallization from ethanol gave m.p. and mixture m.p. 194–196°.

(B) Zinc Dust.—The procedure described for the reduction of IIA and IIB was applied to 600 mg. of IIC and gave 490 mg. (87%) of IC.

Oxidation of IA, IB and IC by Perbenzoic Acid.—The perbenzoic acid was prepared as described in the literature[11] except that the chloroform solution was washed with 1% sodium bisulfite before washing with water.

A solution of 70 mg. of IA in 20 cc. of chloroform was treated with 3 cc. of chloroform containing 0.1807 mmole of perbenzoic acid per cc. The solution was allowed to stand at room temperature for 48 hr. and a change in color from orange to yellow was observed. The solution was washed with 10% sodium hydroxide, twice with water, and then was evaporated to dryness *in vacuo*. The residual yellow solid was treated with charcoal in methanolic solution and gave 70 mg. of yellow solid, m.p. 246–249°, which did not depress the m.p. of authentic IIA.

Application of the same procedure to 76 mg. of IB and 5 cc. of perbenzoic acid solution of the same concentration gave 65 mg. (80%) of crude product, m.p. 225–235° dec. Crystallization from methanol gave white needles, m.p. 239° (violent dec.), which did not depress the m.p. of authentic IIB.

A solution of 66 mg. (0.2712 mmole) of IC in 20 cc. of chloroform was mixed with 4 cc. of chloroform containing a total of 0.386 mmole of perbenzoic acid. The color of the solution changed instantly from yellow to dark orange and immediate iodimetric titration of an aliquot showed the loss of 0.27 mmole of perbenzoic acid. The solution was shaken with 10% sodium hydroxide 15 minutes after mixing, and the washed chloroform layer was evaporated to give 66 mg. of product which was obtained as orange cubes from methanol, m.p. 173–174°. This material did not depress the m.p. of IA.

In another experiment in which IC was allowed to react at room temperature with 2.5 equivalents of perbenzoic acid there was isolated after 50 hr. a quantitative yield of IIA.

Kinetics of the Oxidation of IA, IB and IC by Perbenzoic Acid. General Procedure.[5]—An accurately weighed sample of I was dissolved in reagent grade chloroform and the solution was thermostated at 25.0 ± 0.1° in a 25-cc. volumetric flask coated with black enamel. A stock solution of freshly prepared perbenzoic acid in chloroform (approximately 0.20 mmole perbenzoic acid per cc.) was also thermostated. The time at which one-half of the perbenzoic acid solution was added to the solution of I was taken as zero time, and the mixture was rapidly diluted to the 25 cc. mark and shaken. Approximately 1.5 equivalents of perbenzoic acid were used in each oxidation of I. The first aliquot was titrated as promptly as possible upon mixing and subsequent aliquots were titrated at suitable intervals. The initial concentration of perbenzoic acid was determined by extrapolation. A sample of perbenzoic acid solution of equal concentration was kept side by side with the kinetic sample and the autodecomposition of perbenzoic acid was also followed by iodimetric titration in order to correct the observed loss of perbenzoic acid in the kinetic experiments. The reactions were followed for at least 50% oxidation of I and second-order rate constants were calculated.

Oxidation of IA.—The solution contained 0.4725 mmole of IA and 0.5862 mmole of perbenzoic acid. Aliquots of 2.0 cc. were titrated with 0.00493 *N* sodium thiosulfate.

Time, min.	Titer, cc. Oxid.	Titer, cc. Control	$k \times 10^2$ ./mole min.
0	19.00	19.05	..
4.1	18.98	19.05	..
100	18.10	19.05	2.73
215	17.69	19.05	1.91
480	15.80	17.85	2.18
632	14.47	16.91	2.09
1112	12.32	15.70	1.99
1360	11.19	15.41	2.57
1807	10.47	14.82	2.23

Av. $k \times 10^2 = 2.2 \pm 0.3$ l./mole min.

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[9] The permanganate oxidation of IC is apparently accompanied by isomerization of IC to IA, and the latter is then oxidized to IIA. The isomerization of IC to IA by means of permanganate (without oxidation) was demonstrated in an independent experiment and is subject to additional study.

[10] H. H. Szmant and R. L. Lapinski, THIS JOURNAL, **75**, 6338 (1953).

[11] A. I. Vogel, "Practical Organic Chemistry," Longmans, Green and Co., London, 1948, p. 766.

Oxidation of IB.—The solution contained 0.1600 mmole of IB and 0.1875 mmole of perbenzoic acid. The average rate constant was found to be 0.29 ± 0.03 l./mole-min.

Oxidation of IC.—The solution contained 0.4355 mmole of IC and 0.9035 mmole of perbenzoic acid. After an almost instantaneous loss of one equivalent of perbenzoic acid the rate of oxidation approached that of the rate of oxidation of IA, and during the interval of 400–800 minutes the rate of oxidation coincided with the rate reported above for IC.

Ultraviolet Spectra.—The ultraviolet spectra were determined in 95% ethanol by means of a Beckman DU spectrophotometer. The absorption curves are reproduced in Fig. 2.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, IOWA STATE COLLEGE]

Structures of Thiamine in Basic Solution[1]

BY GEORGE D. MAIER AND DAVID E. METZLER

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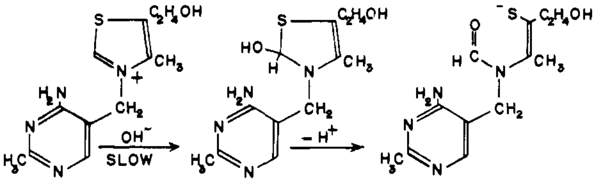
Thiamine in basic solution is instantly converted to a yellow form which fades within a few minutes in water but which persists for several hours in methanol. The *p*H dependence of the zero-time concentration of this yellow form indicates that it arises by the simultaneous loss of two protons from the neutral form of thiamine with an apparent average *pK* value of 11.6 at 19°. In methanol the two dissociation steps are distinct and an intermediate can be detected. This intermediate non-ionic form of thiamine has been isolated as a white crystalline solid. It apparently arises through the intramolecular addition of the amino group of thiamine to the thiazolium ring with loss of one proton; opening of the thiazole ring and loss of a second proton produce the yellow form. The *p*H dependence indicates that fading of the yellow color results from formation of the pseudo-base and open-ring thiol forms. The possible biochemical significance of these unstable forms of thiamine is considered.

Thiamine (I) reacts slowly with hydroxyl ions to form a pseudo-base II and a thiol form III in which the thiazole ring has been opened.[2–4] In addition, it has been known for many years that thiamine in alkaline solutions develops a transient yellow color.[5,6] We have investigated this latter phenomenon in the belief that it might shed some light on the mechanism of the catalytic action of this vitamin.



I II III

Pseudo-Base and Colorless Thiol Forms.—Figure 1, curve A shows the ultraviolet absorption spectrum of the neutral form of thiamine (I) and (curve B) that of thiamine at *p*H 10.4, 90 minutes after its addition to this basic buffer. The latter represents the spectrum of the thiol, III, which forms slowly over a period of about an hour at this *p*H. An identical spectrum is observed for the crystalline sodium salt of III[6] dissolved in 0.02 *N* KOH. (Addition of base to simpler thiazolium salts such as 3,4-dimethyl-5-(2-hydroxyethyl)-thiazolium chloride causes very similar absorbancy increases at all wave lengths.)

The two reactions by which the neutral form of thiamine is converted to the thiol form III can be regarded as formally equivalent to the stepwise dissociation of a diprotic acid with characteristic acid dissociation constants which can be measured titrimetrically. Williams and Ruehle[2] have reported the apparent pK_{av} (average of the apparent *pK*'s for the two steps) of 9.0, and Watanabe and Asahi,[4] of 9.33 at 25°. We have estimated pK_{av} spectrophotometrically as 9.3 for thiamine and 10.3 for the simpler 3,4-dimethyl-5-(2-hydroxyethyl)- and 3-benzyl-4-methyl-5-(2-hydroxyethyl)-thiazolium salts.

The Yellow Form of Thiamine.—The addition of thiamine to solutions of *p*H 11 and above leads to the instantaneous production of a yellow color, which fades rapidly in aqueous media,[5] but which is clearly visible, depending on the basicity, for an hour or more in methanol. Figure 1C shows the spectrum of thiamine in 0.1 *N* NaOH extrapolated to zero time (see Experimental) and Fig. 4A the spectrum in methanolic KOH. Since these spectra are not further changed at higher base concentrations, we believe that they represent a single substance. No other forms of thiamine absorb light above 325 mμ; hence we have used the absorbancy at 349 mμ to measure the concentration of this yellow form in mixtures. In water the yellow form decays within several minutes to a substance having a spectrum identical to that of the thiol form III (Fig. 1B). A similar but much slower decay occurs in methanol. The change leading to color formation appears to be completely and rapidly reversible as indicated by the quenching of the yellow color and the immediate appearance of the spectrum of thiamine hydrochloride upon addition of hydrochloric acid to a solution containing the yellow form. The yellow color can then be regenerated quantitatively (97% or more of the original amount) by addition of more base.[5]

Figure 2 shows the increase in zero time absorbancy at 349 mμ with increasing *p*H of an aqueous

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[2] R. R. Williams and A. E. Ruehle, THIS JOURNAL, **57**, 1856 (1935).

[3] H. T. Clarke and S. Gurin, *ibid.*, **57**, 1876 (1935).

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[5] R. A. Peters and J. H. L. Philpot, *Proc. Roy. Soc.* (*London*), **B113**, 48 (1933).

[6] O. Zima and R. R. Williams, *Ber.*, **73**, 941 (1940).