4. The chlorophyll derivatives found in the stomach walls have a definite erythropoietic effect when administered by mouth to certain animals.

ANTIOCH COLLEGE
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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CINCINNATI]

The Action of Nitrous Acid on Phenyl-beta-naphtholaminomethane. II

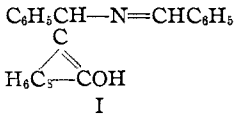
BY NZEER AHMED AND MARTHA G. HEMPHILL WITH FRANCIS EARL RAY

In a recent paper[1] it was reported that the action of nitrous acid on phenyl-β-naphtholaminomethane produced an aliphatic diazo compound. Further work has shown that the reaction is much more complicated than was at first supposed[2] and we now find that the supposed diazo compound was, in fact, a mixture of two compounds.

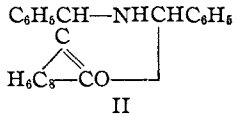
The material obtained by treatment of phenyl-β-naphtholaminomethane with nitrous acid turned red on the addition of alkali. When, however, this material was carefully recrystallized from a mixture of acetone and alcohol at room temperature a *neutral* compound, unaffected by alkali, was obtained which melted at 163°. The optical activity was due entirely to this substance.

This compound, melting at 163°, gave Liebermann's test for the nitroso group. On hydrolysis with hydrochloric acid benzaldehyde, which was identified by means of its phenylhydrazone, and the hydrochloride of phenyl-β-naphtholaminomethane were obtained.

There have been two formulas assigned to the condensation product (which melts at 150°) resulting from the reaction of benzaldehyde, β-naphthol and ammonia. One is a Schiff base or benzylidine, I, and the other is an iso-oxazine structure, II.



I



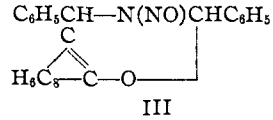
II

If formula I is correct the compound has a free naphtholic group and should methylate on treatment with alkali and methyl sulfate. This methoxy compound has been prepared by an entirely different synthesis by Ray and Moomaw[3] and melts at 98°. Even the most drastic treatment with methyl sulfate failed to convert the condensation product into this known Schiff base.

On the other hand, the condensation product (melting at 150°) reacted with acetic anhydride to give a compound melting at 170°. This compound on hydrolysis gave an acetyl derivative of phenyl-β-naphtholaminomethane. On methylating this the N-acetylphenyl-α-(β-methoxynaphthyl)-aminomethane previously prepared by Ray and Moomaw[3] was obtained. This showed that the compound melting at 170° was the N-acetyl derivative of II.

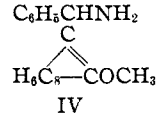
Formula II represents a secondary amine and should form an acetyl derivative but no methoxy compound. A secondary amine of this type should also form an N-nitroso derivative.

To test this hypothesis, the condensation product was treated with nitrous acid and a high yield of the pure compound melting at 163° was obtained with no trace of the salt-forming material. This synthesis, together with the decomposition products, and the absence of a free naphtholic group, establishes the constitution of this compound as the N-nitroso derivative of 1,3-diphenyl-4,2-β-naphtho-iso-oxazine, III. It also confirms II as the formula for the condensation product. The formula given in "Organic Syntheses"[4] is, therefore, incorrect.

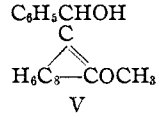


III

As was shown in a previous paper the replacement of the naphtholic group by the methoxy stabilizes the amine, IV. When, however, this methoxy amine was treated with nitrous acid the alcohol only was obtained, V.[3]



IV



V

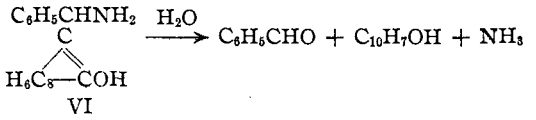
(1) F. E. Ray, THIS JOURNAL, **54**, 295 (1932).
(2) F. E. Ray, *ibid.*, **54**, 4753 (1932).
(3) Ray and Moomaw, *ibid.*, **55**, 3837 (1933).
(4) "Organic Syntheses," Coll. Vol. I, 1932, p. 372; *cf.* Littman and Brode, THIS JOURNAL, **52**, 1655 (1930).

In an attempt to prepare the aliphatic diazo compound the urethan of IV was treated with nitrous acid and a light lemon colored compound was obtained that formed beautiful orange laminae when treated with potassium hydroxide. Analyses, melting point and properties showed this to be identical with the dinitro-β-naphthol of Wallach and Wichelhaus.[5a]

Following the suggestion of Professor W. A. Noyes, phenyl-β-naphtholaminomethane was treated with concentrated nitric acid and the same dinitronaphthol was obtained. This proved to be identical with the salt-forming compound recovered from the reaction between nitrous acid and phenyl-β-naphtholaminomethane.

The product that previously had been thought to be a diazo compound was, therefore, a mixture of N-nitroso-1,3-diphenyl-4,2-β-naphtho-iso-oxazine, III, and 1,6-dinitro-2-naphthol. The optical activity was due entirely to the iso-oxazine.

The mechanism of this reaction is as follows: phenyl-β-naphtholaminomethane, VI, first decomposes to form benzaldehyde, β-naphthol and ammonia. The benzaldehyde thus liberated reacts with a molecule of unchanged amine to give the condensation product, II. Nitrous acid then reacts with this to form the nitroso derivative, III.



The β-naphthol formed in the decomposition is nitrated at the same time to give the other principal product of the reaction.

The urethan of IV suffered oxidation and removal of the methoxy group and so gave, with nitrous acid, the same dinitro-β-naphthol.

Experimental Part

1,3-Diphenyl-4,2-β-naphtho-iso-oxazine, II, and phenyl-β-naphtholaminomethane were prepared according to the directions of M. Betti.[4]

N-Nitroso-1,3-diphenyl-4,2-β-naphtho-iso-oxazine, III, was prepared by suspending 1 g. of the condensation product, II, in 10 cc. of absolute ether and passing dry nitrous anhydride into the mixture at −20° for one hour. The ether was evaporated and the material recrystallized by dissolving it in acetone, filtering and adding an equal volume of alcohol. After some hours, light sulfur yellow rhombic crystals separated, melting at 163°; yield 0.8 g. This proved to be identical with the compound obtained in much smaller yield from the reaction between phenyl-β-naphtholaminomethane and nitrous acid.

(5) (a) Wallach and Wichelhaus, *Ber.*, **3**, 846 (1870); (b) Graebe and Drews, *ibid.*, **17**, 1171 (1884).

Anal. Calcd. for $C_{24}H_{18}N_2O_2$: C, 78.7; H, 4.9; N, 7.65. Found: C, 78.4, 78.2; H, 4.9, 5.3; N, 7.2, 7.7.

This compound was insoluble in acid and alkali, and only sparingly soluble in alcohol. It gave Liebermann's[6] nitrosamine test. When heated for two hours with 1-1 alcoholic hydrochloric acid, the compound was hydrolyzed and on cooling the hydrochloride of phenyl-β-naphtholaminomethane separated. The filtrate was neutralized and warmed with phenylhydrazine. On standing benzaldehyde phenylhydrazone separated. It was identified by its melting point, 156°, and by a mixed melting point with an authentic sample.

Urethan of Phenyl-β-naphtholaminomethane.—This compound was prepared by refluxing for one hour a 10% ethereal solution of the amine with an excess of ethyl chlorocarbonate in the presence of sodium bicarbonate. The ether solution was filtered and evaporated. The original residue consisted of sodium bicarbonate, sodium chloride and some urethan. The inorganic material was dissolved out with hot water and the urethan recrystallized from alcohol. It melted at 201°; yield 40–50%.

Urethan of Phenyl-α-(β-methoxynaphthyl)-aminomethane.—The urethan described above was methylated by treating 10 g. with 30 cc. of 25% potassium hydroxide and 20 cc. of ethyl alcohol, cooling to 0° and adding, with vigorous stirring, 4 cc. of methyl sulfate. The temperature was raised slowly to 40° and held at this point for forty-five minutes. After cooling the material was filtered and recrystallized from alcohol; yield 55–60%, melting at 132°.

Anal. Calcd. for $C_{21}H_{21}NO_3$: C, 75.23; H, 6.26. Found: C, 75.0; H, 5.87.

Potassium Salt of 1,6-Dinitro-2-naphthol.—The methylated urethan was suspended in five times its weight of toluene and dry nitrous anhydride was passed in for three hours, the material gradually going into solution. The excess nitrous anhydride was removed by blowing air through the solution. The toluene was then extracted with 25% potassium hydroxide. This potassium salt was recrystallized from water in which it is only moderately soluble. It separated in shimmering orange colored laminae. It did not melt, but when heated over 400° decomposed in the melting point tube with a puff of smoke.

Another method for isolating this salt was to steam distil the toluene and recrystallize the residue from dilute potassium hydroxide. An aqueous solution of the potassium salt was treated with silver nitrate and precipitated the deep red silver salt.

Anal. Calcd. for $C_{10}H_5N_2O_6Ag$: Ag, 31.7. Found: Ag, 31.3.

The silver salt was treated with ethyl iodide in the cold and the product recrystallized from alcohol: long white needles, m. p. 138.5°, were obtained. Graebe and Drews[5b] give 138° as the melting point of 1,6-dinitro-2-ethoxynaphthalene.

The free acid was obtained by dissolving the potassium salt in hot water and acidifying with acetic acid. Microscopic pale yellow crystals separated which melted at 194° with decomposition. The literature[5] gives the melting point of 1,6-dinitro-2-naphthol as 195°.

(6) Liebermann, *Ber.*, **7**, 248 (1874).

Anal. Calcd. for $C_{10}H_6N_2O_5$: C, 51.28; H, 2.56; N, 11.97. Found: C, 51.22; H, 2.51; N, 11.6.

In an attempt to apply Witt's[7] method for the production of diazo compounds phenyl-β-naphtholaminomethane was treated with ten times its weight of concentrated nitric acid. After standing for one hour it was poured into water and the precipitated material was converted into the potassium salt of 1,6-dinitro-2-naphthol as previously described.

Benzoic acid was also formed in this reaction. It was isolated by acidifying the alkaline mother liquor and recrystallizing the precipitated material from hot water.

Acetyl Derivative of 1,3-Diphenyl-4,2-β-naptho-iso-oxazine.—This was obtained by warming 2.0 g. of the condensation product, II, with 10.0 cc. of acetic anhydride on the water-bath for two hours; yield, 1.6 g.; m. p. 170°.

Anal. Calcd. for $C_{26}H_{21}NO_2$: C, 82.3; H, 5.55; N, 3.69. Found: C, 82.0; H, 5.7; N, 4.1.

(7) Witt, *Ber.*, **42**, 2953 (1909).

Acetyl Derivative of Phenyl-β-naphtholaminomethane.—This was obtained by the hydrolysis of the above-mentioned compound with concentrated hydrochloric acid for six hours in the cold; m. p. 225°.

N-Acetyl-phenyl-α-(β-methoxynaphthyl)-aminomethane was obtained when the acetyl derivative of phenyl-β-naphtholaminomethane was methylated as previously described for the urethan. It melted at 186° and was identical with the compound prepared by Ray and Moomaw.[3] This shows that the acetyl group in the oxazine was attached to nitrogen as Ray and Moomaw prepared their acetyl compound from phenyl-α-(β-methoxy-naphthyl)-aminomethane.

Summary

The reaction between nitrous acid and phenyl-β-naphtholaminomethane leads to the formation of N-nitroso-1,3-diphenyl-4,2-β-naphtho-iso-oxazine and 1,6-dinitro-2-naphthol.

CINCINNATI, OHIO RECEIVED JUNE 27, 1934

[CONTRIBUTION FROM THE BIOCHEMICAL LABORATORY, STATE UNIVERSITY OF IOWA]

The Association of Fat-Soluble Vitamins and Antioxidants in Some Plant Tissues[1]

BY ELIZABETH M. BRADWAY AND H. A. MATTILL

In earlier papers[2] the evidence was reviewed that vitamin A or carotene, and especially vitamin E as it exists in foods, are easily destroyed by oxidation in the presence of autoxidizable substances, and that their survival in such association depends, in part at least, upon the protective action of naturally occurring or added inhibitors. The stability of carotene in different solvents under various conditions[3] and the vulnerability of vitamin E to catalyzed autoxidation of fats[4] have been the subjects of more recent studies. The proved presence of antioxidants in some vegetable oils and the isolation of an antioxidant from lettuce[5] which was separate and distinct from the vitamins and sterols, prompted the suggestion that such inhibitors might be the protective agencies in all plant tissues, whereby the labile fat-soluble vitamins are preserved in an environment otherwise favorable to oxidation.

If the presence of natural antioxidants is related to the abundance and stability of carotene or vitamin E in vegetable foods, it should be possible to demonstrate the existence of inhibitors especially in such vegetables as are rich sources of these vitamins or whose vitamin content is little diminished by drying or by exposure to other conditions favoring oxidation. Attempts to isolate naturally occurring antioxidants might also yield information on their chemical behavior perhaps on the particular form in which they exist in the plant, as well as on other constituents of plant oils. Carrots, the earliest known source of carotene, suffer no appreciable loss of their vitamin A value by autoclaving at 15 pounds pressure and subsequent drying at room temperature.[6] The tomato, whose vitamin A is especially stable,[7,8] seems also to contain vitamin E. These two vegetables were chosen for this study along with wheat germ oil whose content in vitamin E is the highest of any of the vegetable oils. The methods for separating the unsaponifiable constituents were essentially those employed in the study of lettuce[9] with appropriate modifications where necessary.

(1) Presented at the Philadelphia meeting of the American Society of Biological Chemists, April 29, 1932.

(2) (a) Cummings and Mattill, *J. Nutrition*, **3**, 421 (1931); (b) Olcovich and Mattill, *J. Biol. Chem.*, **91**, 105 (1931).

(3) Baumann and Steenbock, *ibid.*, **101**, 561 (1933); Turner, *ibid.*, **105**, 443 (1934).

(4) Waddell and Steenbock, *J. Nutrition*, **4**, 79 (1931).

(5) Olcott and Mattill, *J. Biol. Chem.*, **93**, 65 (1931).

(6) Steenbock and Boutwell, *J. Biol. Chem.*, **41**, 163 (1920).

(7) Sherman, Quinn, Day and Miller, *ibid.*, **78**, 293 (1928).

(8) Steenbock and Schrader, *J. Nutrition*, **4**, 267 (1931).

(9) Olcott and Mattill, *J. Biol. Chem.*, **93**, 59 (1931).