discussion of the work of Greene and Voskuyl, which overlaps ours to a certain extent, will be given later.

Acknowledgments.—We wish to acknowledge gratefully a grant from the Alexander Dallas Bache Fund of the National Academy of Sciences which made possible the use of the platinum resistance thermometer for measuring temperature. We are also indebted to Mr. R. L. Slobod and Mr. J. L. Gabbard for experimental coöperation.

Summary

1. Water containing normal Lake Michigan oxygen and pure protium has been prepared by an exchange reaction in the gas phase: $H_2 + HOD = HD + HOH$. The density of this water has been found to be 15.5 ± 0.1 p. p. m. lighter than normal Lake Michigan water. From this the ratio of hydrogen atoms to deuterium atoms was calculated to be 6970.

2. Water containing normal Atlantic Ocean oxygen and pure protium was prepared by the same method. The difference in density between this and normal Atlantic Ocean water was found to be 15.7 ±0.2 p. p. m., corresponding to a H/D ratio of 6900.

3. Deuterium-free hydrogen was burned with atmospheric oxygen and the density of the water produced was compared with that of water containing normal Lake Michigan oxygen and pure protium. The atmospheric oxygen water was found to be 6.6 p. p. m. heavier, which corresponds to a greater atomic weight of oxygen in air of 0.000119 ± 0.000002 atomic weight units.

4. The chemical atomic weight of hydrogen as calculated from our new H/D ratio is 1.0080 instead of the value of 1.0081 recently adopted by the International Committee on Atomic Weights.

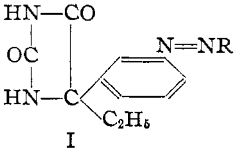
EVANSTON, ILLINOIS RECEIVED MAY 4, 1939

[CONTRIBUTION NO. 155 FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING, THE UNIVERSITY OF TEXAS]

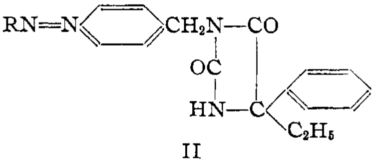
The Synthesis of Colored Derivatives of Nirvanol. II. N-Benzyl Azo Compounds[1]

BY SHIRLEY POWELL LINGO[2] WITH HENRY R. HENZE

Last year there was reported[3] an attempt to convert the soporific called Nirvanol into colored derivatives (I) possessing dyestuff properties. Since it is generally believed that the activity of soporifics is related, at least in part, to the alkyl or aryl groups present in a molecule, it follows that attachment of an azo linkage might so affect the influence of the phenyl group in Nirvanol as to reduce or completely alter its activity. Therefore it was decided to leave intact the phenyl and ethyl groups at the 5-position of the hydantoin nucleus, and to introduce the azo grouping through attachment to a benzyl group linked to the amidic nitrogen of the heterocycle (II).



I



II

In this investigation the sodium salt of Nirvanol was condensed with *p*-nitrobenzyl chloride and the product reduced catalytically to form 3-*p*-aminobenzyl-5-ethyl-5-phenylhydantoin. The latter was diazotized and coupled, in turn, with dimethylaniline, β-naphthylamine, phenol, salicylic acid, α- and β-naphthol, and 1,5-dihydroxynaphthalene, respectively, to form colored azo derivatives. From alkaline solution the dyes derived from phenol, α-naphthol and salicylic acid impart a yellow or orange color, and the 1,5-dihydroxynaphthalene derivative a purple color, to silk or wool. The β-naphthol azo compound is too insoluble in dilute alkali to serve as a dyestuff for these fibers. Likewise, the β-naphthylamine derivative is too little soluble even in concentrated hydrochloric acid to impart color, whereas the azo dyestuff derived from dimethylaniline dyes silk or wool from an acid bath a light purple which is changed to yellow by contact with alkali.

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(1) Presented before the Division of Medicinal Chemistry at the 97th meeting of the American Chemical Society, April 3-7, 1939, at Baltimore, Md.

(2) From the Ph.D. thesis of S. P. Lingo in June, 1939.

(3) Spurlock with Henze, THIS JOURNAL, **60**, 3005 (1938).

Experimental

Preparation of *p*-Nitrobenzyl Chloride.—This compound was prepared in satisfactory yield by nitration of benzyl chloride following the procedure of Alway[4]; m. p. 72.5–72.8°.

Preparation of 3-(*p*-Nitrobenzyl)-5-ethyl-5-phenylhydantoin.—A solution of sodium methylate was prepared by action of 23 g. (1 gram atom) of freshly cut sodium with 200 cc. of methanol. To this was added 204 g. (1.0 mole) of Nirvanol[3] (m. p. 198.0–198.5°) dissolved in about 1300 cc. of hot methanol, and the mixture was heated for fifteen hours to accomplish complete reaction. Then, to the clear but slightly yellow colored solution was added 171.5 g. (1.0 mole) of *p*-nitrobenzyl chloride; at first the color of the mixture turned to green, then darkened to a deep brown without turbidity. This solution was heated for seven hours; precipitation of solid material, first noticed at the end of one hour, continued during the next three hours. After standing for twenty-four hours, the solid was separated, washed free of sodium chloride, and dried. The alcoholic solution when diluted with water yielded additional reaction product as well as 9.0 g. of unchanged Nirvanol. The total yield (319 g.) of the nitro compound, of m. p. 170–170.5°, represented 98.5% of the theoretical quantity based on the Nirvanol which reacted. The product was recrystallized from ethyl alcohol and acetone, yielding broad, transparent, colorless needles of m. p. 177.0–177.5° (corr.). The nitrobenzyl derivative is insoluble in water, only slightly soluble in methyl and ethyl alcohols, and is fairly soluble in acetone.

Anal. Calcd. for $C_{18}H_{17}N_3O_4$: N, 12.38. Found: N, 12.16.

The configuration of 3-(*p*-nitrobenzyl)-5-ethyl-5-phenylhydantoin is predicated on the analogous conversion of 5-ethyl-5-phenylhydantoin into 3-methyl-5-ethyl-5-phenylhydantoin.[5]

Preparation of 3-(*p*-Aminobenzyl)-5-ethyl-5-phenylhydantoin.—From 33.9 g. of 3-(*p*-nitrobenzyl)-5-ethyl-5-phenylhydantoin (partially dissolved in 100 cc. of ethanol) was obtained 29.8 g. of the pure amine (96% yield) by reduction using hydrogen under twenty atmospheres pressure with Raney nickel as catalyst at 100°. The 3-(*p*-aminobenzyl) derivative was obtained in the form of colorless, diamond-shaped platelets by adding water to a hot alcohol–acetone solution; m. p. 171.7° (corr.).

Anal. Calcd. for $C_{18}H_{19}N_3O_2$: C, 69.88; H, 6.19; N, 13.59. Found: C, 70.07; H, 6.06; N, 13.53.

Preparation of 3-(Dimethylaniline-*p*-azobenzyl)-5-ethyl-5-phenylhydantoin.—Three and nine-hundredths grams (0.01 mole) of 3-(*p*-aminobenzyl)-5-ethyl-5-phenylhydantoin was dissolved in 100 cc. of water containing 6 cc. of concd. hydrochloric acid; this solution was cooled in ice and treated with a cold solution of 0.69 g. (0.01 mole) of sodium nitrite dissolved in the least volume of water, and the whole mixture allowed to stand for thirty minutes. Then 0.60 g. (0.01 mole) of urea was added to destroy any unreacted nitrous acid and the mixture was stirred during twenty minutes.

To this diazotized solution was added 1.21 g. (0.01 mole) of freshly distilled dimethylaniline causing a yellow color to develop. Ethyl alcohol (about 90 cc.) was added in quantity sufficient to make the solution homogeneous; the solution developed a blood-red color. The addition of about 7 g. of sodium acetate caused an orange-colored solid to separate; the mixture stood overnight.

The solid was filtered, washed with water, and dried in an oven at 105°. The dried material was a very bulky orange powder with a tendency to develop a static electrical charge; yield 4.1 g. (93%). A portion of the solid was dissolved in hot acetone, to which water was added to incipient cloudiness. Set aside to cool, shiny plates separated; filtered, and dried at 105°, 3-(dimethylaniline-*p*-azobenzyl)-5-ethyl-5-phenylhydantoin melts at 228.5–229.0° (corr.). The configuration of this dyestuff is predicated upon the normal tendency of dimethylaniline to form azo derivatives by coupling in the position para to nitrogen. Difficulty was experienced in determining nitrogen by the micro Dumas method until a minute quantity of lead dioxide was added to the sample; neither copper oxide nor potassium chlorate was satisfactory.

Anal. Calcd. for $C_{26}H_{27}N_5O_2$: C, 70.72; H, 6.16; N, 15.87. Found: C, 70.58; H, 6.07; N, 15.73.

Preparation of 3-(β-Naphthylamine-*p*-azobenzyl)-5-ethyl-5-phenylhydantoin.—A solution of 3.09 g. (0.01 mole) of 3-(*p*-aminobenzyl)-5-ethyl-5-phenylhydantoin in 80 cc. of water containing 6 cc. of concd. hydrochloric acid was cooled in ice and diazotized with 0.69 g. (0.01 mole) of sodium nitrite; after twenty minutes the excess of the nitrite was destroyed by addition of 0.60 g. of urea. The diazotized amine was coupled with 1.43 g. (0.01 mole) of β-naphthylamine dissolved in 50 cc. of ethyl alcohol; a bright red solid separated immediately but the mixture was allowed to stand overnight. The product was filtered, washed with water, dried, and then weighed 4.60 g. (99% yield).

The unpurified dyestuff in a melting point tube undergoes a darkening in color and superficial fusion at about 100°, is tarry at about 110°, and is melted with evolution of gas at about 120°. A solution of the azo derivative in glacial acetic acid was poured into ice water to yield a semi-colloidal precipitate that could be coagulated by addition of a few drops of hydrochloric acid. The melting point behavior of the material was unchanged by this treatment. The dyestuff was dissolved in glacial acetic acid, to which some concd. hydrochloric acid had been added, and was reduced by addition of zinc dust. The reduction product was recrystallized from dilute methyl alcohol and from water–acetone mixture; m. p. 170°; mixed melting point with authentic sample of 3-(*p*-aminobenzyl)-5-ethyl-5-phenylhydantoin 171°.

A portion of the original product was heated in an evaporating dish over a free flame until the solid melted to a clear dark red liquid and the evolution of gas had ceased; on cooling a glass-like solid remained. The latter was pulverized and analyzed. Another portion of this heated material was dissolved in glacial acetic acid and reduced with zinc dust and concd. hydrochloric acid. After recrystallization from water–acetone, the melting point was 169°; mixed melting point with an authentic sample of 3-(*p*-aminobenzyl)-5-ethyl-5-phenylhydantoin 170–171°. Qualitative evidence of the recovery of 1,2-diaminonaphthalene from the fusion residue was obtained.

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(4) Alway, THIS JOURNAL, **24**, 1060 (1902).

(5) German Patent 611,057

Anal. Calcd. for $C_{25}H_{25}N_5O_2$: C, 72.55; H, 5.44; N, 15.15. Found: C, 72.82; H, 5.39; N, 15.21.

The structural configuration of this azo derivative is predicated upon the usual tendency of β-naphthylamine to couple in the α-position.

Preparation of 3-(Phenol-*p*-azobenzyl)-5-ethyl-5-phenylhydantoin.—This compound could be obtained by coupling the diazotized amine with a solution of phenol in sodium hydroxide and neutralizing the hydrochloric acid formed by means of sodium carbonate, but a slightly better yield of purer product was obtained by proceding similarly to the preparation of the dimethylaniline analog. Thus, 3.09 g. (0.01 mole) of 3-(*p*-aminobenzyl)-5-ethyl-5-phenylhydantoin was diazotized, the excess nitrite decomposed with urea, and to the mixture was added 0.94 g. (0.01 mole) of redistilled phenol, causing the production of a light yellow color. Seven grams of sodium acetate was added and a dull yellow solid separated, but the mixture was allowed to stand overnight before filtration. The solid was washed with water and dried at 105°; the yield was 3.73 g., representing 90% of the theoretical. The dyestuff could be crystallized from acetone–water in the form of clusters of needle-like platelets, m. p. 245–247° (corr.), with gas evolution subsequent to fusion.

Anal. Calcd. for $C_{24}H_{22}N_4O_3$: C, 69.55; H, 5.35; N, 13.52. Found: C, 69.59; H, 5.35; N, 13.46.

The structure assigned to this azo dyestuff is based upon the normal tendency of phenol to couple para to the hydroxyl group.

Preparation of 3-(α-Naphthol-*p*-azobenzyl)-5-ethyl-5-phenylhydantoin.—Diazotization of 3.09 g. (0.01 mole) of 3-(*p*-aminobenzyl)-5-ethyl-5-phenylhydantoin was carried out as usual and the solution poured into one of 1.04 g. (0.01 mole) of α-naphthol in 200 cc. of water containing 4.0 g. of sodium hydroxide. The deep-red colored solution was permitted to stand for one hour before adding a mixture of 15 cc. of concd. hydrochloric acid in 300 cc. of water, with stirring, to precipitate the azo compound. A purple-red flocculent mass was obtained by filtration, which, after washing and drying at 105°, weighed 4.36 g. (94% yield).

The dyestuff behaved as follows upon heating in a melting point tube: began to change color and to sinter at about 120°, formed a tarry sirup at about 140°, had melted sufficiently at 148° to begin a viscous flow down the side of the tube, at about 150° gas evolution began, causing pronounced expansion in volume of the mass. The solid was purified, without change in melting point behavior, by solution in benzene from which it was precipitated by petroleum ether addition. It is a brilliant red substance in finely powdered form, but is much darker in appearance in larger particles. The azo derivative is soluble in the common organic solvents, except petroleum benzine, insoluble in water, and dissolves slowly in 5% sodium hydroxide solution.

Anal. Calcd. for $C_{28}H_{24}N_4O_3$: C, 72.40; H, 5.21; N, 12.06. Found: C, 71.94; H, 5.17; N, 12.17.

The configuration assigned to this compound is based on the usual coupling behavior of α-naphthol, which is para to the hydroxyl group.

Preparation of 3-(β-Naphthol-*p*-azobenzyl)-5-ethyl-5-phenylhydantoin.—The method for preparation of this compound followed that of the phenol derivative. From 3.09 g. (0.01 mole) of the amine and 1.44 g. (0.01 mole) of β-naphthol in alcohol, 4.36 g. (94% yield) of bright red dyestuff was obtained. The product was recrystallized from diluted acetone and from ethyl acetate in the form of dark red crystals, m. p. 212–213° (corr.). Here, too, difficulty was had in analyzing for nitrogen content until a trace of potassium chlorate was incorporated with the sample.

Anal. Calcd. for $C_{28}H_{24}N_4O_3$: C, 72.40; H, 5.21; N, 12.06. Found: C, 72.54; H, 5.22; N, 11.99.

Since it is normal for β-naphthol to form azo compounds by coupling in the α-position, that structure is assigned to this derivative.

Preparation of 3-(1,5-Dihydroxynaphthalene-*p*-azobenzyl)-5-ethyl-5-phenylhydantoin.—Diazotization of 3.09 g. (0.01 mole) of the amine, dissolved in 90 cc. of water containing 7 cc. of concd. hydrochloric acid, was accomplished in the usual manner with 0.76 g. (0.011 mole) of sodium nitrite. The diazotized material was poured into an alkaline solution of 1,5-dihydroxynaphthalene (m. p. 259–260°); the latter was obtained by dissolving 1.60 g. (0.01 mole) in 200 cc. of water containing 4 g. of sodium hydroxide, an amount slightly in excess of that of the hydrochloric acid used for diazotization so that the coupling occurred in basic solution. The development of color was immediate and quite intense, yielding finally a solution so deeply red as to be nearly opaque. The azo dyestuff was precipitated by dropwise addition of the basic solution to a mixture of 300 cc. of water and 20 cc. of concd. hydrochloric acid, utilizing mechanical stirring. A flocculent precipitate resulted, which was filtered, washed, and dried at 105° to a hard, red-black cake weighing 4.85 g. (100% yield).

Attempts at purification of the product were not very satisfactory; in general, an ether solution was shaken repeatedly with water, concentrated, and diluted with petroleum ether to reprecipitate the dyestuff. After drying over calcium chloride, the solid when heated darkened at about 140°, sintered at about 148–158° forming small tarry globules, the latter began a viscous flow at about 168°, and apparently became liquid at about 194°, above which temperature gas evolution caused pronounced expansion; after many and tedious attempts to dissolve out the more soluble portions of material, incipient fusion did not occur below 194° but decomposition with evolution of gas took place at 196°. Material of this latter melting point behavior was analyzed.

Anal. Calcd. for $C_{28}H_{24}N_4O_4$: C, 69.99; H, 5.03; N, 11.66. Found: C, 69.60; H, 5.07; N, 11.51.

The structure of this azo derivative is based upon the behavior of 1,5-dihydroxynaphthalene in coupling in the 2-position in forming azo dyes such as Diamond Black PV.

Preparation of 3-(Salicylic Acid-*p*-azobenzyl)-5-ethyl-5-phenylhydantoin.—A solution was made by dissolving 3.09 g. (0.01 mole) of 3-(*p*-aminobenzyl)-5-ethyl-5-phenylhydantoin in 80 cc. of water containing 7 cc. of concd. hydrochloric acid, cooled in ice and treated with 0.76 g. (0.011 mole) of sodium nitrite dissolved in 5 cc. of cold water. After standing for thirty minutes, the solution of diazonium salt was added with stirring to 1.38 g. (0.01

mole) of salicylic acid dissolved by 4 g. of sodium hydroxide in 200 cc. of water. The azo dyestuff was precipitated by dropping its alkaline solution slowly into a solution of 20 cc. of concd. hydrochloric acid in 300 cc. of water; vigorous stirring caused the separation of a flocculent precipitate. The latter was filtered, washed, and dried *in vacuo* for twenty-four hours. The weight, 4.4 g., represents a yield of 96% of the theoretical.

The resulting dyestuff was a light tan-colored solid which developed an extreme electrical charge when reduced to an amorphous powder by grinding. It exhibited the following behavior when heated: namely, its color deepened at 80–90°, and the solid began to melt at about 110° to a gummy solid. Above this temperature there was progressive softening, and at about 125–130° the material evolved a gas causing much expansion of the mass. No definite melting point or decomposition point could be recognized.

This azo derivative could not be recrystallized from any of the common organic solvents by dilution with water, for such addition of water caused separation of a gum which would not harden subsequently. It fuses in hot water at about 80°. The dyestuff was recrystallized from tetralin, yielding yellow-orange colored microscopic crystals. The latter could be dried at 110° and melted at 133–134°, although darkening occurred at about 125°; gas evolution took place above the melting point. Analysis was made of the recrystallized azo compound.

Anal. Calcd. for $C_{25}H_{22}N_4O_5$: C, 65.49; H, 4.84; N, 12.22. Found: C, 65.88; H, 4.77; N, 12.10.

It is assumed that salicylic acid couples para to the hydroxyl group in forming this derivative in analogy to its behavior in forming Crumpsall Yellow.

Summary

1. Nirvanol has been converted into colored derivatives. The latter contain a substituted aryl-azo-benzyl grouping attached to the nitrogen in the 3-position of the heterocycle.

AUSTIN, TEXAS RECEIVED MAY 8, 1939

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE MALTBIE CHEMICAL CO.]

Sulfanilamido Derivatives of Heterocyclic Amines[1]

BY RUSSEL J. FOSBINDER AND L. A. WALTER

Since the discovery by Domagk[2] that compounds containing the sulfanilamido structure are more or less effective in combating streptococcic infections in animals, hundreds of modifications of the parent structure have been prepared and tested for anti-bacterial activity.

TABLE I

PROPERTIES OF THE NEW COMPOUNDS AND ANALYTICAL DATA

Compound[a]	M. p., °C.	Formula	% Nitrogen Calcd.	% Nitrogen Found[b]
2-*p*-Nitrobenzenesulfonamido-6-aminopyridine	228–230	$C_{11}H_{10}N_4O_4S$	19.04	18.75
2-Sulfanilamido-6-aminopyridine	204–206	$C_{11}H_{12}N_4O_2S$	21.20	20.93
2-N⁴-acetylsulfanilamido-6-aminopyridine	194–196[c] 237–239	$C_{13}H_{14}N_4O_3S$	18.29	18.19
2-*p*-Nitrobenzenesulfonamido-4-methylthiazole	197–199	$C_{10}H_9N_3O_4S_2$	14.04	13.91
2-N⁴-acetylsulfanilamido-4-methylthiazole	259–260	$C_{12}H_{13}N_3O_3S_2$	13.47	13.17
2-N⁴-acetylsulfanilamidothiazole	256–257	$C_{11}H_{11}N_3O_3S_2$	14.14	13.90
2-Sulfanilamido-4-methylthiazole	236–238	$C_{10}H_{11}N_3O_2S_2$	15.61	15.55
2-Sulfanilamidothiazole	194–196	$C_9H_9N_3O_2S_2$	16.46	16.31

[a] Nomenclature according to Crossley, Northey and Hultquist, THIS JOURNAL, **60**, 2217 (1938). [b] Kjeldahl. [c] Melts, then crystallizes and melts with decomposition at the higher temperature.

This paper describes sulfanilamido derivatives of the pyridine and thiazole series, some of which were prepared before the especially interesting announcement by Whitby[3] that 2-sulfanilamidopyridine was effective against pneumococcic infections.

Through the kindness of Dr. Ralph Mellon and Mr. Frank B. Cooper at Western Pennsylvania Hospital, Pittsburgh, several of the compounds were tested pharmacologically in mice to determine their anti-streptococcic and anti-pneumo-

TABLE II

PHARMACOLOGICAL ACTIVITY OF THE NEW COMPOUNDS

Compound	Effect Anti-streptococcic	Effect Anti-pneumococcic
2-N⁴-acetylsulfanilamido-6-aminopyridine	Slight	None
2-Sulfanilamido-6-aminopyridine	Good	Good
2-Sulfanilamido-4-methylthiazole	Good	Good
2-Sulfanilamidothiazole	Good	Good
2,6-Diamino-3-*p*-sulfonamidophenylazopyridine[a]	None	None
Sulfanilamide	Good	Fair
Sulfapyridine (2-sulfanilamidopyridine)	Good	Good

[a] Mietzsch and Klarer, U. S. Patent 2,148,705.

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[1] Presented before the Division of Medicinal Chemistry, A. C. S., Baltimore, April 6, 1939.

[2] Domagk, *Deut. Med. Wochschr.*, **61**, 250 (1935).

[3] Whitby, *Lancet*, **1**, 1210 (1938).