Structure of Neutral Red and Other 2,8-Substituted Aminophenazines

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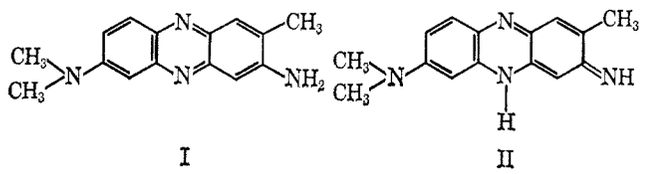
In view of discrepancies in the literature, the structures of neutral red and other 2,8-substituted aminophenazines are assigned by comparing their spectral characteristics with those of model compounds, 2,8-diamino- and 2-amino-8-dimethylaminophenazine, which are prepared by the oxidative cyclization of corresponding diphenylamine derivatives. Neutral red is confirmed as 2-amino-8-dimethylamino-3-methylphenazine and is found to exist in the amino form.

In connection with previous work on the affinity of neutral red for the exocrine pancreas,[1,2] several 2,8-substituted aminophenazines were synthesized to correlate pancreatic affinity to chemical structure. Since neutral red and other 2,8-substituted aminophenazines have been considered by various workers to have both imino and amino structures, the application of modern spectral techniques to elucidate the neutral red structure is warranted.[3]

Neutral red was first prepared by Witt[4] by condensation of *p*-nitrosodimethylaniline and 2,4-diaminotoluene in aqueous medium and oxidation of the resulting toluylene blue by ferric chloride. Phillips[5] prepared neutral red iodide for vital staining by a modified procedure of Witt. Purification and structural elucidation for the above preparations were lacking. Vivian,[6] using oxidative cyclization of 4-bromo-4′-N,N-dimethylamino-5-methyl-2-nitrodiphenylamine in the presence of ferrous oxalate and granulated lead, prepared 2-bromo-3-methyl-8-N,N-dimethylaminophenazine, which on treatment with ammonium hydroxide should have yielded neutral red. However, this product differs from commercial preparations[7] in melting point, solubility, fluorescence in ether, coloration with concentrated sulfuric acid, and in its staining properties. Similar discrepancies were noted with 2-amino-8-dimethylaminophenazine.[6] Based on keto–enol tautomerism of 1,3,4-trimethyl-2-phenazinol,[8] neutral red is assumed to exist in amino and imino forms (I and II[9]). The present investigation was designed to refine



I II

Witt's procedure for preparing neutral red for maximum yield and to redefine its structure. Emphasis in this work is placed on tautomerism of neutral red so that the mechanism of neutral red granule formation in the pancreas will be better understood in terms of protein–dye interaction. Several related phenazines were prepared for comparison.

Experimental Section

Toluylene Blue.—Toluylene blue chloride was prepared by the procedure of Phillips.[5] The pH of the reaction mixture, however, was adjusted to 4.8 in the initial stages. Toluylene blue chloride (7.5 g, 75% as anhydrous free base) melted with decomposition at 184–188°. When dried *in vacuo* at 60° over phosphorus pentoxide, the crystals turned green and the loss of weight varied from 8.7 to 10.7%. When these crystals were exposed to air, stable, blue crystals were obtained. The free base was unstable.

Anal.[10] Calcd for $C_{15}H_{19}ClN_4$: C, 61.95; H, 6.59; Cl, 12.19; N, 19.27. Found (immediately after drying): C, 61.90; H, 6.73; Cl, 12.14; N, 19.15.

Neutral Red.[11]—The formation of neutral red was best when an aqueous solution of toluylene blue chloride was heated at 70–80° with pH maintained at 7.0. The pure, free base of neutral red precipitates in the reaction mixture under these conditions. The vacuum-dried product was directly crystallized from ethyl acetate, thus avoiding the purification by chromatography normally needed for reactions conducted at low pH. Neutral red (72%) melted at 280–282°.

Anal. Calcd for $C_{15}H_{16}N_4$: C, 71.40; H, 6.39; N, 22.21. Found: C, 71.36; H, 6.42; N, 22.13.

Acetylation with acetic anhydride was carried out in pyridine. Neutral red free base was dissolved in acetic anhydride–pyridine (1:1, v/v) and heated in boiling water for about 1.5 hr. The solvent was evaporated and the residue was dried over P_2O_5. The acetyl derivative was purified by dissolving this crude residue in 0.1 *N* hydrochloric acid, filtering, and precipitating the base with alkali. The dried base was then chromatographed[12] and the major, orange band was collected. It crystallized from ethyl acetate, mp 236°.

Anal. Calcd for $C_{17}H_{20}N_4O_2$ (as monohydrate): C, 65.36; H, 6.45; N, 17.94. Found: C, 65.23; H, 6.34; N, 17.90.

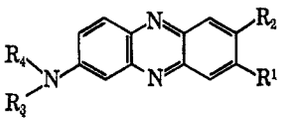
In Table I are listed phenazines prepared during the course of this investigation. Phenazines III to VI were obtained by the procedure described under neutral red while VI and VII were synthesized by oxidative cyclizations of the corresponding diphenylamine derivatives according to the general procedure of Holliman.[13] The intermediates, 4-dimethylamino-2′,4′-dinitrodiphenylamine[14] and the 4-amino-2′,4′-dinitrodiphenylamine,[15] were reduced and the amines were refluxed with nitrobenzene. Using 50% ethanol for diazotization in Browning's[16] procedure higher yields of VIII were obtained.

Spectral Studies.—The ultraviolet and visible spectra were recorded on a Bausch and Lomb Spectronic 505 recording spectrophotometer. Optical density measurements at λ_{max} were made in a Carl Zeiss spectrophotometer. The infrared spectra were obtained on a Beckman IR-8 spectrophotometer, using potassium bromide disks and Nujol mull. The nmr spectra obtained in trifluoroacetic acid were recorded on a Varian Associates Model A-60 nmr spectrometer, operating at 60 Mcps.

(1) W. S. Morgan, *Quart. J. Microscop. Sci.*, **94**, 141 (1953).
(2) W. S. Morgan, *ibid.*, **94**, 269 (1953).
(3) In this paper only the free bases are described.
(4) O. N. Witt, *Ber.*, **12**, 933 (1879).
(5) M. Phillips and B. Cohen, *Stain Technol.*, **2**, 74 (1927).
(6) D. L. Vivian, *J. Org. Chem.*, **21**, 565 (1956).
(7) D. L. Vivian and M. Belkin, *Nature*, **178**, 154 (1956).
(8) W. John, *Angew. Chem.*, **59**, 188 (1947); W. John, W. Emte, and E. Maue, *Chem. Abstr.*, **41**, 6391 (1947).
(9) D. L. Vivian, *Nature*, **188**, 746 (1960).
(10) Analyses were performed by A. Bernhardt, Mikroanalytisches Laboratorium im Max-Planck-Institut, Mülheim, Germany.
(11) Attempts to reproduce Vivian's procedure were unsuccessful.
(12) Activated alumina (90% Al_2O_3) purchased from Matheson Scientific Co., Cleveland, Ohio, is used in all chromatography. Solvent system is chloroform–benzene–ethanol (500:475:25, v/v).
(13) A. Gray, G. Gaertner, and F. G. Holliman, *Tetrahedron Letters*, No. 7, 24 (1959).
(14) E. Lellman and F. Mack, *Ber.*, **23**, 2739 (1890).
(15) R. Nietzki and O. Ernst, *ibid.*, **23**, 1852 (1890).
(16) See footnote *d* in Table I.

TABLE I

PHENAZINES

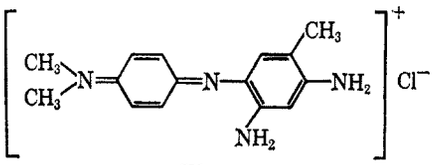


Compound	Reactants	Yield, %	Mp, °C	Formula	—Carbon, %— Calcd	Found	—Hydrogen, %— Calcd	Found	—Nitrogen, %— Calcd	Found
III, $R_1 = NH_2$; $R_2 = CH_3$; $R_3 = R_4 = C_2H_5$	*p*-Nitrosodiethylaniline + 2,4-diaminotoluene	58	225[a]	$C_{17}H_{20}N_4$	72.82	72.70	7.19	7.29	19.99	19.72
IV, $R_1 = NH_2$; $R_2 = CH_3$; $R_3 = CH_3$; $R_4 = C_2H_5$	*p*-Nitroso-N-ethyl-N-methylaniline + 2,4-diaminotoluene	52	214	$C_{16}H_{18}N_4$	72.15	72.30	6.81	6.84	21.04	20.86
V, $R_1 = NH_2$; $R_2 = OCH_3$; $R_3 = R_4 = CH_3$	*p*-Nitrosodimethylaniline + 2,4-diaminoanisole	52	260	$C_{15}H_{16}N_4O$	67.14	67.10	6.01	6.02	20.88	20.86
VI, $R_1 = NH_2$; $R_2 = H$; $R_3 = R_4 = CH_3$	*p*-Nitrosodimethylaniline + *m*-phenylenediamine also 4-dimethylamino-2′,4′-dinitrodiphenylamine	14[b]	252	$C_{14}H_{14}N_4$	70.56	70.26	5.92	5.91	23.52	23.25
		24	252	$C_{14}H_{14}N_4$	70.56	70.16	5.92	6.10	23.52	23.23
VII, $R_1 = NH_2$; $R_2 = H$; $R_3 = R_4 = H$	4-Amino-2′,4′-dinitrodiphenylamine	24	284[c]	$C_{12}H_{10}N_4$	68.55	68.33	4.79	5.11	26.65	25.92
VIII, $R_1 = H$; $R_2 = CH_3$; $R_3 = R_4 = CH_3$	Neutral red + $NaNO_2$ in ethanolic HCl	70	171[d]	$C_{15}H_{15}N_3$	75.92	75.93	6.37	6.31	17.71	17.67

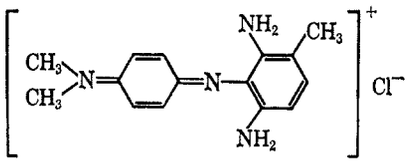
[a] Lit. mp 180–181°: I. V. Alexandrov, *Zh. Nauch. i Prikl. Fotog. i Kinematogr.*, **2**, 432 (1957); *Chem. Abstr.*, **52**, 9823 (1957); see Results and Discussion. [b] During purification by chromatography, another major component was isolated. This material was crystallized from benzene to yield 18% of dark red crystals, mp 302–304°. Elemental analyses correspond to 8(2)-dimethylaminophenazine. *Anal.* Calcd for $C_{14}H_{13}N_3$: C, 75.31; H, 5.87; N, 18.82. Found: C, 75.50; H, 5.68; N, 18.77. [c] H. Otomasu, *Chem. Pharm. Bull.* (Tokyo), **6**, 77 (1958). [d] Lit. mp 168–169°: C. H. Browning, *Proc. Roy. Soc.* (London), **B93**, 359 (1922).

Results and Discussion

The elemental analyses and the condensing positions of 2,4-diaminotoluene with *p*-nitrosodimethylaniline demonstrate that toluylene blue chloride may exist in one of the following structures: IX or X. Structure IX is assigned to toluylene blue chloride because the 5 position of 2,4-diaminotoluene is less hindered than the 3 position and because neutral red, the oxidative product of toluylene blue is obtained in high yields (72%) whereas the proximity of amino groups in structure X warrants the formation of four isomeric phenazines, XI to XIV.

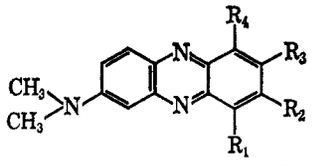


IX



X

The nmr spectrum of toluylene blue chloride in trifluoroacetic acid has single peaks corresponding to four aromatic protons at 7.57, the aromatic proton adjacent to the methyl group at 6.63, the aromatic proton adjacent to the amino groups at 6.23, the exchangeable amino protons at 4.03, the dimethylamino protons at 3.45, and the C-methyl protons at 2.32 ppm. These assignments based on chemical shifts, intensities, and splittings are consistent with the structure proposed (IX). The absence of splitting between the aromatic proton adjacent to the methyl group and that adjacent to the amino group rules out the structure X.



XI, $R_1 = R_2 = H$; $R_3 = CH_3$; $R_4 = NH_2$
XII, $R_1 = NH_2$; $R_2 = R_3 = H$; $R_4 = CH_3$
XIII, $R_1 = NH_2$; $R_2 = CH_3$; $R_3 = R_4 = H$
XIV, $R_1 = CH_3$; $R_2 = R_3 = H$; $R_4 = NH_2$

In proving the structure of neutral red, two model compounds, 2,8-diaminophenazine and 2-amino-8-dimethylaminophenazine, are prepared by oxidative cyclizations of 4,2′,4′-triaminodiphenylamine and 4-dimethylamino-2′,4′-diaminodiphenylamine, respectively, in nitrobenzene. The 2-amino-8-dimethylaminophenazine is also prepared, like neutral red, in aqueous medium by allowing *m*-phenylenediamine to react with *p*-nitrosodimethylaniline and heating the resulting toluylene blue analog to 70°. These compounds are characterized by their melting points, elemental analyses, ultraviolet and visible spectra (Table II), comparisons of infrared and nmr spectra, and their staining properties.[17]

The spectra of 1-amino- and 2-aminophenazine are similar, although protonation of the 1-aminophenazine causes greater shifts to longer wavelengths in the visible region than that of 2-aminophenazine.[18] The introduction of a second amino group in the 8 position of the phenazine ring decreases the complexity of the 2-aminophenazine spectrum. 2,8-Diaminophenazine in absolute alcohol has two peaks, one at 273 and another at 446 mμ. In nonpolar solvents, the ultraviolet peak undergoes only minor changes while the visible peak is shifted considerably to shorter wavelengths. The effect of protonation on this compound is comparable with that of 2-aminophenazine. The spectra of 2-amino-8-dimethylaminophenazine and neutral

(17) W. S. Morgan and J. Fernando, to be published.
(18) See footnote *a* in Table II.

TABLE II

ULTRAVIOLET AND VISIBLE SPECTRA OF PHENAZINES

Phenazine	Solvent	λ_{max}, mμ	Log ε	λ_{max}, mμ	Log ε	λ_{max}, mμ	Log ε	λ_{max}, mμ	Log ε
1-Amino[a]	96% ethanol	240	4.50	290	4.54	365	3.60	500	3.29
						372	3.64		
	0.1 *N* HCl	250	4.80	302	4.17	368	4.00	635	2.97
2-Amino[a]	96% ethanol	230	4.43	275	4.71	362	3.82	468	3.88
	0.1 *N* HCl	231	4.52	281	4.66	383	4.04	518	4.08
2,8-Diamino (VII)	Abs ethanol			273	4.85			446	4.38
	Dioxane			270	4.81			430	4.30
	Water (pH 6.88)			270	4.72			440	4.09
								503	4.29
	0.1 *N* HCl			272	4.56			506	4.45
2-Amino-8-dimethylamino (VI)	Abs ethanol			278	4.80			462	4.39
	Benzene			279	4.74			440	4.31
	0.1 *N* HCl			274	4.60			534	4.55
2-Amino-8-dimethylamino-3-methyl (I) (neutral red)	Abs ethanol			279	4.83			460	4.41
	Benzene			279	4.74			436	4.31
	Dioxane			277	4.81			440	4.31
	0.1 *N* HCl			272	4.61			532	4.54
2-Acetamido-8-dimethylamino-3-methyl	Dioxane	249	4.50	286	4.81	379	3.93	467	4.07
						395	3.95		
	0.1 *N* HCl	242	4.55	297	4.60	412	4.06	558	4.34
2-Amino-8-dimethylamino-3-methoxy (V)	0.1 *N* HCl			261	4.63			530	4.47
2-Amino-8-diethylamino-3-methyl (III)	Abs ethanol			279	4.81			466	4.40
	Benzene			280	4.70			438	4.25
	0.1 *N* HCl			278	4.58			538	4.52
2-Amino-8-N-ethyl-N-methylamino-3-methyl (IV)	0.1 *N* HCl			274	4.60			534	4.58
3-Methyl-8-dimethylamino (VIII)	Dioxane	236	4.41	284	4.77	360	3.77	469	3.98
	0.1 *N* HCl	237	4.49	298	4.56	388	3.83	560	4.11

[a] A. Gray and F. G. Holliman, *Tetrahedron*, **18**, 1095 (1962).

red in a given solvent are almost identical and when compared to that of 2,8-diaminophenazine, undergo bathochromic shifts in both regions, the visible peak exhibiting greater shift. The spectral similarity of neutral red to the 2,8-disubstituted aminophenazines is evidence for its substituent positions. The 3-methyl group, as expected, has very little effect on the spectrum[19] of 2-amino-8-dimethylaminophenazine (VI) while the more active methoxy group in that position (V), brings about a distinct hypsochromic shift in the ultraviolet region. Removal or acetylation of the primary amino group of neutral red produces multiplicity of peaks and the spectra are analogous to mono-substituted phenazines. Among the other phenazines prepared, the 2-amino-8-diethylamino-3-methylphenazine (III) deserves special mention. The visible spectrum of this compound in 0.1 *N* hydrochloric acid is in agreement with those of neutral red and the 2-amino-8-N-ethyl-N-methylamino-3-methylphenazine (IV), but differs from the previously reported value of λ_{max} 545 mμ.[16] The melting point of this compound (225°) also differs from the one reported (180–181°). Our product, however, is identified by its elemental analyses, by comparisons of ultraviolet, visible, and infrared spectra with other 2,8-substituted aminophenazines, and by its pancreatic affinity.

The infrared spectra of neutral red in Nujol and in potassium bromide are consistent with those of phenazine, 2,8-diaminophenazine, and 2-amino-8-dimethylaminophenazine. A strong band at 812 cm^{-1} in neutral red, attributed to out-of-plane CH-deformation vibrations arising from two adjacent ring hydrogens corresponding to 1,2,4 substitution,[20] is comparable to similar bands at 820 and 806 cm^{-1} in 2,8-diamino and 2-amino-8-dimethylamino derivatives. Phenazine itself has strong absorption at 750 and 740 cm^{-1}, corresponding to four adjacent hydrogens and a medium band at 818 cm^{-1}. In view of the anomalies in structural assignments of neutral red, particular attention was paid to N–H stretching absorption bands of neutral red occurring at 3330 and 3220 and to those of 2,8-diaminophenazine and 2-amino-8-dimethylaminophenazine occurring at 3350 and 3190 ± 10 cm^{-1}. Heterocyclic amines exist predominantly in amino form with the exception of the benzo derivative of 9-aminoacridine, although 9-aminoacridine itself exists as an amine.[21] In the above benzo derivative a peak at 3448 is attributed to secondary amine (ring nitrogen) and a peak at 3298 cm^{-1} is assigned to exocyclic imine structure.[22] Considering that heterocyclic secondary amines like indole, carbazole, and pyrole absorb at 3490 ± 10 cm^{-1},[23] that thionine which contains both amine and imine structures gives rise to peaks at 3430, 3300, and 3240 cm^{-1}, and that 3,6-diaminoacridine which exists in the amino form absorbs at 3360 and 3160 cm^{-1}, it is evident that neutral red and the other 2,8-substituted aminophenazines have a primary amine structure in the solid state.

Additional evidence concerning the structure of neutral red and related phenazines is provided by their

(19) Z. P. Penyugalov, Z. V. Pushkareva, R. K. L. Batulina, and E. P. Darienko, *Zh. Obshch. Khim.*, **34**, 1956 (1964).

(20) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd ed, John Wiley and Sons, Inc., New York, N. Y., 1958, p 78.

(21) A. R. Katritzky and A. P. Ambler in "Physical Methods in Heterocyclic Chemistry," Vol. II, A. R. Katritzky, Ed., Academic Press Inc., New York, N. Y., 1963, p 323.

(22) S. F. Mason, *J. Chem. Soc.*, 1281 (1959).

(23) Reference 22, p 251.

TABLE III[a]

NUCLEAR MAGNETIC RESONANCE SPECTRAL DATA

Compd	H_1	H_3	H_4	H_6	H_7	H_9	NH_2	$N(CH_3)_2$	CH_3	OCH_3
VII	7.20[b]	7.67[c]	8.17[d]	8.17[d]	7.67[c]	7.20[b]	4.03	...	...	...
VI	7.09[b]	7.58[c]	8.17[d]	8.20[d]	7.92[c]	7.30[b]	4.03	3.48	...	...
I	7.14	...	7.95	8.14[d]	7.80[c]	7.06[b]	4.05	3.53	2.57	...
V	7.20	...	7.50	8.20[d]	7.83[c]	7.32[b]	4.07	3.50	...	4.30

[a] Chemical shifts are reported in parts per million (ppm) from tetramethylsilane in trifluoroacetic acid solvent. [b] Doublet, J = 2.0 cps. [c] Doublet of doublets, J = 9.5 and 2.0 cps. [d] Doublet, J = 9.5 cps.

nmr spectra. The spin–spin splitting patterns are readily interpreted. However, it is necessary to consider the model compound, 2,8-diaminophenazine (VII), to make assignments based on chemical shifts owing to the solvent interaction of trifluoroacetic acid. An analysis of the ABC pattern of this compound (VII) allows assignment of the 1 and 9 protons to the high-field absorption and the 4 and 6 protons to the low-field absorption. An absorption of exchangeable protons occurs at 4.0–4.1 ppm. The phenazines studied were 2-amino-8-dimethylaminophenazine (VI), neutral red (I), and 2-amino-3-methoxy-8-dimethylaminophenazine (V). The observed chemical shifts and coupling constants are given in Table III.

Tautomerism of hydroxyl derivatives of aromatic N-heterocyclics has been extensively investigated by means of ultraviolet spectroscopy showing that 2- and 4-hydroxyacridine[24] and 2-hydroxyphenazine[25] on passing from nonaqueous to aqueous alcohol or water solutions undergo marked spectral changes. Of particular interest is the existence of two stable tautomers (one dark yellow and another dark purple) of 1,3,4-trimethyl-2-hydroxyphenazine,[8] which is used as the basis of hypothesis for amino–imino tautomers in neutral red. The tautomerism of amino derivatives of aromatic N heterocyclics was critically surveyed and it was concluded that these compounds exist predominantly in the amino form.[26] When neutral red was recrystallized from benzene, ethyl acetate, ethyl alcohol, and methyl alcohol, the resulting solids were identical in melting points (279–282°) and spectral characteristics in polar and nonpolar solvents (Table II).

Solutions of neutral red in dioxane and in other organic solvents are yellow. The spectrum of neutral red in dioxane has maximum absorption at 277 and at 440 mμ. Addition of water up to 10% produces a bathochromic shift of 15–20 mμ in the visible peak. With increased amounts of water a new peak appears at the 536–540-mμ region with corresponding decrease in 440-mμ absorption. This conversion is complete when the solvent is 50% aqueous dioxane, at which time the solution is pink. A similar spectral change from 430 to 510 mμ takes place with 2,8-diaminophenazine between solutions of 30 and 60% aqueous dioxane. In both cases there are no changes in the positions of the ultraviolet peaks. The dielectric constants of dioxane–water mixtures which bring about such spectral changes in neutral red are between 18 and 27.[27] If these spectral changes are due to the formation of an imino tautomer of neutral red, brought about by a change in the dielectric constant of the medium, it would be expected that neutral red in ethyl alcohol, and more so in methyl alcohol, exists predominantly in the imino form. A similar conclusion can be drawn in the case of 2,8-diaminophenazine since dielectric constants of dioxane–water mixtures effecting spectral conversions are between 18 and 44. Alcoholic solutions of these two compounds, however, are yellow, have single visible peaks (Table II), and become pink on suitable dilution. The absorptions at 540 of neutral red and 510 mμ of 2,8-diaminophenazine are characteristic of the protonated species. Their appearance in aqueous alcohol and aqueous dioxane solutions independent of the dielectric constant of the medium, therefore, argues against tautomers in the above solutions but favors a pH effect. Spectral variations with pH indeed coincide with the changes described, and confirm the observation of Woislawski[28] who has used these spectral shifts for determining the ionization constant of neutral red. The 3-methyl-8-dimethylaminophenazine (VIII) in dioxane–water mixtures and at varying pH, gives continuous bathochromic shift from 468 to 560 mμ, presumably because the monocation of VIII is much less stable than that of neutral red or 2,8-diaminophenazine.[29] Based on infrared, nmr, ultraviolet, and visible spectral data, neutral red is represented by structure I.

The behavior of our synthetic neutral red is identical with that of the purified commercial neutral red.[30] They have the same melting point and low solubility in ether, and are green in solution with concentrated sulfuric acid. Ether solutions of both compounds exhibit green fluorescence. The 3-methyl-8-dimethylaminophenazine (mp 171°) is brown when dissolved in concentrated sulfuric acid. It is more soluble in ether than neutral red and the solution is yellow. It is of interest that the synthetic product of Vivian,[6,7] assumed to be neutral red, has the characteristics of 3-methyl-8-dimethylaminophenazine.

Registry No.—I, 366-13-2; 2-acetamido-8-dimethylamino-3-methylphenazine, 4607-75-4; III, 7704-36-1; IV, 7704-37-2; V, 7704-38-3; VI, 7704-39-4; VII, 7704-40-7; VIII, 4661-61-4; 8(2)-dimethylaminophenazine, 6494-69-5.

Acknowledgment.—Support of this work by Grants GM-10893-01 and CA-07766-02 from the National Institutes of Health and AT(11-1)-1290 from the Atomic Energy Commission is gratefully acknowledged. Miss Linda Ballard and Mrs. Alice Adams provided technical assistance.

(24) A. Albert and L. N. Short, *J. Chem. Soc.*, 760 (1945).
(25) G. M. Badger, R. S. Pearce, and R. Pettit, *ibid.*, 3204 (1951).
(26) S. J. Angyal and C. L. Angyal, *ibid.*, 1461 (1952).
(27) Values quoted from G. Akerlof and O. A. Short, *J. Am. Chem. Soc.*, **58**, 1241 (1936).
(28) S. Woislawski, *ibid.*, **75**, 5201 (1953).
(29) For a discussion of Kehrman's work on various cationic species of 2-amino- and 2,8-diaminophenazine, see D. E. Pearson, R. W. Brockman, W. E. Cole, C. M. Greer, and M. V. Sigal, in "Heterocyclic Compounds," Vol. 6, R. C. Elderfield, Ed., John Wiley and Sons, Inc., New York, N. Y., 1957, pp 661–666.
(30) The commercial neutral red chloride was obtained from British Drug House and was purified by chromatographing the base.