The hydrogenation was complete within 2 hr. and the catalyst was removed by filtration. The filtrate was evaporated to dryness and the residue was redissolved in 40 ml. of water and made alkaline with concd. ammonium hydroxide. The aqueous solution was then extracted with chloroform. The chloroform solution, after being dried over anhydrous magnesium sulfate, was filtered and distilled to dryness. The residue was dissolved in 25 ml. of absolute ethanol. Hydrogen chloride gas was passed through the alcoholic solution for a few minutes. Light brown needles separated upon cooling the solution in the refrigerator; yield, 0.16 g. (46.5%), m.p. 178–182°. The crude product was recrystallized from absolute ethanol to give tan needles, m.p. 179–182°.

Anal. Calcd. for $C_{11}H_{17}N_5.2HCl$: C, 45.21; H, 6.55; N, 23.97; Cl, 24.27. Found: C, 44.59; H, 6.38; N, 24.42; Cl, 24.00.

PHILADELPHIA, PA.

[CONTRIBUTION FROM THE LABORATORY OF CHEMICAL PHARMACOLOGY, NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH, PUBLIC HEALTH SERVICE, U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE]

Phenazine Syntheses. X.[1a] 2,8-Disubstituted Phenazines Made as Intermediates for New Vital Stains, Together with Two New Vital Stains Related to Neutral Red

DONALD L. VIVIAN,[1b] RICHARD M. HOGART, AND MORRIS BELKIN

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The synthesis and some of the properties of a homologous series of 2-alkylamino-8-chlorophenazines are described, together with the preparation of 2,8-dibromophenazine and two new vital stains related to neutral red: 2-methylamino-8-*n*-propylaminophenazine, and 2,8-bis(methylamino)-3-methylphenazine.

Earlier work[2,3] had shown that 2,8-diaminophenazines, when converted to their hydrochlorides or other salts, possessed the property of "localized" or "particulate" staining of living cells,[4] such as is exhibited by Neutral Red, which is the hydrochloride of 2-amino-8-dimethylamino-3-methylphenazine. It was likewise shown that this ability to act as a vital stain was peculiar to these 2,8-disubstituted phenazines, and was not possessed by their 2,7-disubstituted analogs. (In the report which follows, the free bases alone will be described, with the understanding that it is the hydrochloride or similar salt that actually produces the vital staining.)

It was originally proposed, therefore, to prepare a series of 2,8-bis(alkylamino)phenazines, in order to study their vital staining ability, as well as their other properties. The planned method of preparation involved replacement of both halogens of 2,8-dihalophenazines by amino or alkylamino groups, using the same method of relatively high-temperature sealed-tube reactions as was earlier found feasible with monohalogenated phenazines.[3] When primary alkylamines were used in this procedure, however, it was found that the results were not completely satisfactory with 2,8-dichlorophenazine, for a mixture of difficultly separable compounds always resulted. The chief reasons for the multiplicity of products are probably the following: (1) The greater activity of the secondary amines which result from replacement of the first chlorine. (2) Instability of some of the reaction products at the relatively high temperatures necessary for replacement of the second chlorine. (3) The tenacity with which a small portion of the chlorine is retained, possibly due to complex formation.

Because of these complex mixtures resulting from the higher temperature reactions necessary with the dichloro compounds, complete purification has been achieved only with two 2,8-bis(alkylamino) phenazines. This paper, therefore, deals chiefly with products in which only one of the two chlorines has been replaced. These are readily obtained from the 2,8-dichlorophenazine by lower-temperature reactions than those which yield the mixtures already referred to, for it has been found that the first chlorine atom can be replaced at a much lower temperature than is required to replace the second one. Thus, replacement of the first chlorine by the more reactive amines, such as methylamine, can be effected by long reaction at as low a temperature as 100°.

In later work it is planned to proceed with the original idea of making a series of both symmetrically and unsymmetrically substituted 2,8-bis-(alkylamino) phenazines, attempting to overcome some of the difficulties encountered with 2,8-dichlorophenazine by taking advantage of the greater reactivity of the bromine atoms in the 2,8-dibromophenazine described below, as well as by determining whether long-continued reaction will ultimately result in complete replacement of all of the chlorine in 2,8-dichlorophenazine. It is also planned to study the action of secondary amines on 2,8-dihalophenazines.

(1)(a) Paper IX. *J. Org. Chem.*, 21, 1188 (1956).
(1)(b) Present address: College of Pharmacy, University of Arizona, Tucson, Ariz.
(2) D. L. Vivian and M. Belkin, *Nature*, 178, 154 (1956).
(3) D. L. Vivian, *J. Org. Chem.*, 21, 565 (1956).
(4) M. Belkin and M. J. Shear, *Am. J. Cancer*, 24, 483 (1937).

TABLE I

PREPARATION OF CRUDE 2-ALKYLAMINO-8-CHLOROPHENAZINES

	From		Temp. of Bomb Furnace	Duration of Heating	Yield of Crude, %
	2,8-Dichlorophenazine,	1.5 g.			
A	Sodium acetate, anhydrous	1.5 g.	98–105°	160 hr.	25 (0.25 g.)
	Methylamine, 40% aqueous solution	15 cc.			
	2,8-Dichlorophenazine,	1.5 g.			
A	Sodium acetate, anhydrous	1.5 g.	98–105°	300 hr.	40 (0.40 g.)
	Methylamine, 40% aqueous solution	15 cc.			
	2,8-Dichlorophenazine,	10 g.			
A	Sodium acetate, anhydrous	10 g.	98–105°	300 hr.	50 (4.87 g.)
	Methylamine, 40% aqueous solution	100 cc.			
	2,8-Dichlorophenazine,	2.5 g.			
B	Sodium acetate, anhydrous	2.5 g.	98–105°	320 hr.	35 (0.91 g.)
	Ethylamine, 66% aqueous solution	25 cc.			
	2,8-Dichlorophenazine	2.5 g.			
C	Sodium acetate, anhydrous	2.5 g.	180°	22 hr.	56 (1.54 g.)
	n-Propylamine, aqueous solution, approximately 30%	25 cc.			
	2,8-Dichlorophenazine	2.5 g.			
C	Sodium acetate, anhydrous	2.5 g.	195°	24 hr.	43 (1.16 g.)
	n-Propylamine	10 cc.			
	2,8-Dichlorophenazine	2.5 g.			
D	Sodium acetate, anhydrous	2.5 g.	180°	22 hr.	67 (1.82 g.)
	Isopropylamine, aqueous solution, approximately 30%	25 cc.			
	2,8-Dichlorophenazine	2.5 g.			
E	Sodium acetate, anhydrous	2.5 g.	167–170°	40 hr.	10 (0.29 g.)
	n-Amylamine	12.5 cc			
	2,8-Dichlorophenazine	2.5 g.			
E	Sodium acetate, anhydrous	2.5 g.	195–199°	24 hr.	42 (1.27 g.)
	n-Amylamine	12.5 cc.			
	2,8-Dichlorophenazine	2.5 g.			
F	Sodium acetate, anhydrous	2.5 g.	195–198°	24 hr.	42 (1.32 g.)
	n-Hexylamine	6 cc.			
	2,8-Dichlorophenazine	2.5 g.			
G	Sodium acetate, anhydrous	2.5 g.	198–205°	24 hr.	54 (1.77 g.)
	n-Heptylamine	9 cc.			

TABLE II

PROPERTIES AND ANALYSES OF PURE 2-ALKYLAMINO-8-CHLOROPHENAZINES[a]

	Compound	Crystalline Form and Color	M.P.[b]	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
A	2-Chloro-8-methylaminophenazine	Dull red microcrystals	254–255	$C_{13}H_{10}ClN_3$[c]	64.1	64.0	4.13	4.26
B	2-Chloro-8-ethylaminophenazine	Brownish red microcrystals	210–211	$C_{14}H_{12}ClN_3$	65.3	65.1	4.69	4.86
C	2-Chloro-8-*n*-propylaminophenazine	Orange-red microcrystals	190–191	$C_{15}H_{14}ClN_3$	66.4	66.5	5.16	5.42
D	2-Chloro-8-isopropylaminophenazine	Brownish red plates	170–171	$C_{15}H_{14}ClN_3$	66.4	66.7	5.16	5.40
E	2-*n*-Amyl-8-chlorophenazine	Dull red microcrystals	140–142	$C_{17}H_{18}ClN_3$	68.1	67.8	6.05	6.24
F	2-Chloro-8-*n*-hexylaminophenazine	Dark red prisms	161–162[d]	$C_{18}H_{20}ClN_3$	68.9	69.1	6.42	6.75
G	2-Chloro-8-*n*-heptylaminophenazine	Aggregates of orange microcrystals	108–109	$C_{19}H_{22}ClN_3$	69.6	69.9	6.76	6.74

[a] All were recrystallized from *n*-heptane. [b] Corrected. [c] Additional analysis: Cl, Calcd.: 14.54; Found: 14.52. [d] Verified by melting point repetition.

The preparation of all the dihalophenazines described herein was effected by the usual ring-closure through the nitro group.[5] It was originally planned to synthesize 2,8-bis(alkylamino)phenazines with two different alkylamino groups by taking advantage of the greater lability of an iodo-group, and 2-chloro-8-iodophenazine was hence prepared, as described below. Use of this compound proved to be unnecessary when it was found

(5) H. C. Waterman and D. L. Vivian, *J. Org. Chem.*, 14, 289 (1949).

that the two chlorine atoms of 2,8-dichlorophenazine differed greatly in reactivity, as already mentioned. Another compound described below, 5,5′-dichloro-2′-methoxy-2-nitrodiphenylamine was also found to be superfluous. It was prepared as a proposed intermediate for 2,8-dichlorophenazine because it had been found that when halogen was *ortho* or *para* to the imino group of 2-nitrodiphenylamine, as in 4′-chloro-2-nitrodiphenylamine, for example, a portion of the halogen was lost on ring closure, and the resulting mixture of unsubstituted phenazine with monohalophenazine was extremely difficult to purify.[6] Such a mixture has now been found not to form when 4,4′-dichloro-2-nitrodiphenylamine is subjected to ring closure; in this instance no chlorine is lost in the ring closure.

EXPERIMENTAL

2-Chloro-8-iodophenazine. (a) *5′-Chloro-5-iodo-2′-methoxy-2-nitrodiphenylamine.* A mixture of 14.7 g. of 1,2-dinitro-4-iodobenzene[7] and 31.5 g. of 5-chloro-2-anisidine was melted, with stirring, and heating and stirring were continued for about 40 hr. at 55–60°. The mixture resulting was then steam-distilled until no more product passed over, leaving a semi-solid residue weighing 25.5 g. after drying. Repeated recrystallization from 70% alcohol (Norit), gave reddish-orange microcrystals, melting at 152–154°.[8]

Anal.[9] Calcd. for $C_{13}H_{10}ClIN_2O_3$: C, 38.6; H, 2.49. Found: C, 38.6; H, 2.74.

(b) *2-Chloro-8-iodophenazine.* There was heated in an open flask immersed in an oil-bath at 260–265°, an intimate mixture of 1.5 g. once-recrystallized 5′-chloro-5-iodo-2′-methoxy-2-nitrodiphenylamine, 2.2 g. of ferrous oxalate dihydrate, and 22.5 g. of granulated lead. The internal temperature rose to a maximum of 273° within 12 min., then after 1 min. the temperature began to drop, and the flask was withdrawn from the bath. Sublimation from the entire reaction mixture at 0.01 mm., from a bath at 265–275°, gave 0.2 g. of product. Recrystallized from benzene, this gave pale yellow needles, melting at 157–159°.

Anal. Calcd. for $C_{12}H_6ClIN_2$: C, 42.3; H, 1.76. Found: C, 42.2; H, 1.78.

Elimination of the methoxy group follows the usual course of the general reaction, in which 2′-alkoxy groups are always eliminated in preference to hydrogen.[10]

5,5′-Dichloro-2′-methoxy-2-nitrodiphenylamine. A solution of 40.6 g. of 4-chloro-1,2-dinitrobenzene[11] and 94.6 g. of 5-chloro-2-anisidine in 500 cc. of alcohol was refluxed for 66 hr., and then distilled with steam until no more material passed over. There remained a residue of about 60 g. of nearly black, somewhat soft material. Recrystallization from alcohol gave about 25 g. of brick-red product. Two more recrystallizations from alcohol gave small orange rods, melting at 148–149°.

Anal. Calcd. for $C_{13}H_{10}Cl_2N_2O_3$: C, 49.9; H, 3.22. Found: C, 49.9; H, 3.39.

2,8-Dichlorophenazine. The intermediate 4,4′-dichloro-2-nitrodiphenylamine was prepared by Blom[12] using amyl alcohol as solvent. In the present work, a mixture of 3000 g. of *p*-chloroaniline, 4500 g. of 2,5-dichloronitrobenzene, and 4700 g. of sodium acetate was heated for 18 hr. in an oil bath at 200–220°. After steam distillation of all volatile material, the product on air-drying weighed 5300 g., and was used without further purification. When 100 g. of this crude material was intimately mixed with 1000 g. of granulated lead, and the mixture stirred by hand while being heated in an open beaker immersed in an oil bath at 250–260°, strong fumes were emitted. (Condensation of these fumes proved not worthwhile, as relatively little product was recovered.) Vacuum sublimation from the whole reaction mixture at about 0.01 mm. from an oil bath at 250–260° gave 20 g. of crude 2,8-dichlorophenazine.

When 160 g. of this crude was recrystallized from benzene, there was obtained 60 g. of pure 2,8-dichlorophenazine,[13] melting at 232–234°.

Anal. Calcd. for $C_{12}H_6Cl_2N_2$: C, 57.9; H, 2.43; Cl, 28.4. Found: C, 57.8; H, 2.54; Cl, 28.3.

Recrystallizing the intermediate before closing the ring, as well as using a mixture of ferrous oxalate dihydrate and lead, instead of lead alone, did not give a yield sufficiently larger to compensate for the additional time and material required.

2,8-Dibromophenazine. The intermediate 4,4′-dibromo-2-nitrodiphenylamine[14] was synthesized by heating a mixture of 50 g. of 2,5-dibromonitrobenzene, 60 g. of *p*-bromoaniline, and 50 g. of anhydrous sodium acetate for 18 hr. in an oil bath at 175–180°. The residue after exhaustive steam distillation and washing with water weighed about 60 g., and melted at 140–145°. This crude material was used for the preparation of the phenazine without further purification. A mixture of 16.0 g. of the crude material, 20.8 g. of ferrous oxalate dihydrate, and 240 g. of granulated lead was heated for 9.5 min. in an oil bath at 260–265°, during which the internal temperature rose to a maximum of 266°. Sublimation from the entire reaction mixture at about 0.01 mm., with the oil bath at about 250–260°, gave 4.2 g. of crude product. This, when recrystallized from 75 cc. of benzene gave 2.6 g. of lemon-yellow platelets, melting at 226–228°.

Anal. Calcd. for $C_{12}H_6Br_2N_2$: C, 42.6; H, 1.79. Found: C, 42.5; H, 1.92.

Unlike the 2,8-dichlorophenazine, this 2,8-dibromophenazine could not be prepared satisfactorily by the use of lead alone, for when the ferrous oxalate was omitted the internal temperature rose to a maximum of 360° (external bath at 250°), and only 0.41 g. of very crude material was obtained from 5.0 g. of intermediate and 50 g. of granulated lead.

2,8-Bis(methylamino)-3-methylphenazine. a. *4-Bromo-4′-chloro-5-methyl-2-nitrodiphenylamine.* A mixture of 40 g. each of 2,5-dibromo-4-nitrotoluene[15] *p*-chloroaniline, and anhydrous sodium acetate was heated for 18 hr. in an oil bath at 210–220°. Complete steam distillation and washing with water gave 43 g. of brownish-red product. Twice recrystallized from alcohol, this formed small orange needles, melting at 160–161°.

Anal. Calcd. for $C_{13}H_{10}BrClN_2O_2$: C, 45.2; H, 3.14. Found: C, 45.8; H, 3.18.

b. *2-Bromo-8-chloro-3-methylphenazine.* A mixture of 2.0 g. of the crude, unrecrystallized 4-bromo-4′-chloro-5-methyl-2-nitrodiphenylamine, 2.6 g. of ferrous oxalate dihydrate and 30 g. of granulated lead was heated in an oil bath at 260–270° until the internal temperature reached a maximum of 255°. This required 10 min. Sublimation at about 0.01 mm. from the same oil bath gave 0.49 g. of product.

(6) D. L. Vivian and J. L. Hartwell, *J. Org. Chem.*, **18**, 1065 (1954).

(7) F. Ullmann, *Ber.*, **34**, 2179 (1902).

(8) All melting points given by the authors are corrected.

(9) Microanalyses by the Microanalytical Laboratories of the National Institutes of Health, under the direction of Dr. W. C. Alford.

(10) H. C. Waterman and D. L. Vivian, *J. Org. Chem.*, **14**, 291 (1949).

(11) H. F. J. Lorang, *Rec. trav. chim.*, **47**, 187 (1928).

(12) A. V. Blom, *Helv. Chim. Acta*, **4**, 1038 (1921).

(13) P. V. Chernetskii and A. I. Kiprianov, *Zhur Obscheĭ Khim.*, **23**, 1743 (1953); H. Otomasu, *Pharm. Bull Japan*, **3**, 365 (1955).

(14) British patent **738,013** (1955).

(15) J. B. Cohen and H. D. Dakin, *J. Chem. Soc.*, **79**, 1130 (1901).

Recrystallized from benzene, this gave yellow needles melting at 223–225°.

Anal. Calcd. for $C_{13}H_8BrClN_2$: C, 50.8; H, 2.62. Found: C, 50.9; H, 2.83.

c. *2,8-Bis(methylamino)-3-methylphenazine.* In a bomb tube was put a mixture of 0.88 g. recrystallized 2-bromo-8-chloro-3-methylphenazine, 4 cc. of a 40% aqueous solution of methylamine, and about 0.1 g. of cuprous chloride. The tube was sealed and heated about 20 hr. in a bomb oven at 170°. A very dark red solid resulted, which was extracted in a Soxhlet apparatus with a minimum of benzene, and the resulting solution put through a column of basic alumina 14 mm. in diameter by 140 mm. in length. Three zones resulted: a black layer on top, a dark red zone in the middle, and a lighter red zone on the bottom. The black portion was removed by spatula, and the bottom zone eluted with benzene. Soxhlet extraction of the middle dark red zone with ether, followed by evaporation of the latter, gave 0.1 g. of reddish-brown microcrystals. These melted, with gradual decomposition, at 205–210°. Because no good solvent for recrystallization was found, the product was analyzed directly.

Anal. Calcd. for $C_{15}H_{16}N_4$: C, 71.4; H, 6.39. Found: C, 71.6; H, 6.36.

This compound was dissolved in dilute hydrochloric acid, and the solution diluted to a concentration of 1 to 40,000 with Hank's basal salt solution, to provide a properly buffered saline medium for living cells. When the *p*H was adjusted to 7.2 by the addition of sodium hydroxide, the resultant solution stained Sarcoma 37 ascites tumor cells very well, in the same manner as is shown by neutral red, and with little indication of toxicity.

2-Methylamino-8-n-propylaminophenazine. (a) *2-Chloro-8-n-propylaminophenazine.* A mixture of 2.5 g. of 2,8-dichlorophenazine (recrystallized, and ground to pass an 80-mesh sieve), 2.5 g. of anhydrous sodium acetate, and 10 cc. of *n*-propylamine was heated for 24 hr. in a sealed tube, in a bomb oven at 195°. The contents of the tube were dried on the steam bath, and then put into benzene solution by Soxhlet extraction. Passage through a column of basic alumina 37 mm. in diameter by 165 mm. long gave three zones, plus a small black layer at the top. The product desired was in the middle zone, dark purple in color. This zone was mechanically separated, and exhausted by Soxhlet extraction with ether. Evaporation of the ether gave 1.16 g. of dark red product. When this was recrystallized from 75% methanol it formed orange-red microcrystals, melting at 190–191°.

Anal. Calcd. for $C_{15}H_{14}ClN_3$: C, 66.4; H, 5.16. Found: C, 66.5; H, 5.42.

(b) *2-Methylamino-8-n-propylaminophenazine.* An intimate mixture was made of 0.45 g. of recrystallized and finely ground 2-chloro-8-*n*-propylaminophenazine and 0.45 g. of anhydrous sodium acetate, and to this was added 10 cc. of a 40% solution of aqueous methylamine. The whole was sealed in a bomb tube, and heated for 16 hr. in a bomb furnace 174–178°. The same procedure as above gave three zones on basic alumina, plus a small dark upper band. Mechanical separation of the bottom, nearly black zone, followed by extraction of it with ether, and evaporation of the solvent, gave 0.12 g. of deep-red microcrystals, melting at 155–160°, with decomposition. This material resisted all attempts at recrystallization, and was hence analyzed directly.

Anal. Calcd. for $C_{16}H_{18}N_4$: C, 72.7; H, 6.80. Found: C, 72.3; H, 6.93.

This compound, when treated in the same manner as already detailed for 2,8-bis(methylamino)-3-methylphenazine, stained ascites tumor cells similarly.

2-Alkylamino-8-chlorophenazines. In general, these compounds were prepared by bomb tube reactions carried out as with the 2-chloro-8-*n*-propylaminophenazine already described, starting in all instances with 2,8-dichlorophenazine, recrystallized and ground to pass an 80-mesh screen. It was found, though, that the methyl- and ethylamines were so much more reactive than their higher homologs that replacement of the first chlorine of the 2,8-dichlorophenazine could be carried out at 100°, while the amines from propyl on up required a considerably higher temperature. The time required was not carefully determined, but was judged roughly by the appearance of the bomb tube contents as time went on. Elimination of the unused portion of the amine after the reaction's completion was carried out by washing with water for the amines up to *n*-amylamine, and by steam distillation for higher homologs. All separations were carried out by extracting the whole reaction mixture with benzene in a Soxhlet apparatus, chromatographing the resulting solutions on basic alumina, separating the darkest red zone mechanically, and isolating the product by extraction of this zone with ether. The crude 2-alkylamino-8-chlorophenazines were then recrystallized from *n*-heptane. It was found that sodium acetate gave better results than did ammonium acetate, cupric acetate, or no catalyst at all, and so a weight of sodium acetate equal to that of the 2,8-dichlorophenazine was arbitrarily taken when the dichloro compound was heated with the various primary amines. The following tables summarize the results. The first table gives the yields of crude products, and shows some variations in yield obtained by such changes in reactions conditions as different temperatures, different lengths of heating, and use of anhydrous or aqueous amine. All reactions with the same amine were marked by the same letter. The second table deals with the properties and analyses of the pure monoalkylamines.

BETHESDA 14, MD.

[COMMUNICATION NO. 2092 FROM THE KODAK RESEARCH LABORATORIES, EASTMAN KODAK CO.]

The Structure of Certain Polyazaindenes. VII. 4-Amino-6-methyl-1,3,3a,7-tetrazaindene and Its Derivatives[1a]

G. A. REYNOLDS AND J. A. VANALLAN

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The synthesis of a number of new amino tetrazaindenes is described.

In connection with the determination of structure of some tetrazaindenes,[1b] we had occasion to synthesize a number of 4-aminotetrazaindenes. These amines were synthesized by reaction of 4-chloro-6-methyl-1,3,3a,7-tetrazaindene (I) and the

(1a) The name of J. A. VanAllan as co-author of Part V in this series [*J. Org. Chem.*, **25** 361 (1960)] was inadvertently omitted.