TABLE II

BIOLOGICAL ACTIVITY OF BRADYKININ ANALOGS

Analog	μg equivalent to 1 μg of bradykinin in guinea pig: Lung	Blood pressure
4-Sar bradykinin	1000	33
5-(5-NH_2Val) bradykinin	1000	50
5-D-Phe bradykinin	33	40
4-Homogly bradykinin	>40	70
Glycyl bradykinin	4	2–4
8-*m*-CF_3Phe bradykinin	2	0.67

ethyl acetate. The ethyl acetate solutions were evaporated, and the residues were suspended in 300 ml of 2 *N* HCl and refluxed for 12 hr. The solutions were evaporated to one-third volume and the pH was brought to 7 with concentrated NH_4OH. The precipitates were removed, washed with cold water, and dried. The L isomer melted at 210–211°, $[\alpha]^{24}D$ −14° (*c* 1, water) and the D isomer at 210–212°, $[\alpha]^{24}D$ +14° (*c* 1, water).

Carbobenzoxy-*m*-trifluoromethyl-L-phenylalanine.—The reaction of 8.5 g (0.0355 mole) of *m*-trifluoromethyl-L-phenylalanine with carbobenzoxy chloride gave 12.5 g (96%) of a white solid, mp 106–107°, $[\alpha]^{25}D$ −2° (*c* 1, methanol).

Anal. Calcd for $C_{18}H_{16}F_3NO_4$: C, 58.86; H, 4.40; N, 3.82. Found: C, 58.59; H, 4.41; N, 3.69.

Carbobenzoxy-*m*-trifluoromethyl-L-phenylalanine *p*-nitrophenyl ester was obtained as a cream-colored solid in 87% yield using *p*-nitrophenol and dicyclohexylcarbodiimide, mp 106–107°, $[\alpha]^{25}D$ −30° (*c* 1, methanol).

Anal. Calcd for $C_{24}H_{19}F_3N_2O_6$: C, 59.02; H, 3.92; N, 5.73. Found: C, 59.23; H, 3.98; N, 5.80.

Acknowledgment.—We wish to thank Mr. C. E. Childs and associates for the microanalyses and Dr. J. M. Vandenbelt and associates for the rotations reported.

2,8-Bis(substituted amino)phenothiazine 5,5-Dioxides[1]

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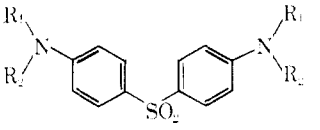
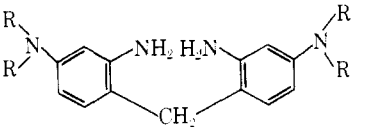
The synthesis of bis(4-aminophenyl) sulfone (Ia, 4,4′-diaminodiphenyl sulfone, DDS) and its acetylated derivative, Ib (DADDS), was reported in 1908.[2] Compounds of this type were found to possess antistreptococcal activity (approximately 30 times that of sulfanilamide) and have been used in the treatment of some common bacterial infectious diseases.[3] Later, DDS and its derivatives were recognized as being useful in the treatment of tuberculosis[4] and leprosy.[5] During the Second World War, the antimalarial activity of a number of compounds in this series was unveiled in screening processes.[6] In 1960, Archibald and Ross[7] reported the use of DDS in the treatment of falciparum and quartan malaria in natives living in hyperendemic areas. The importance of DDS and related compounds has recently been demonstrated by the fact that many chloroquine-resistant strains of malaria parasites did not show cross-resistance to DDS.[8,9] Structure activity relationship studies revealed that, in general, the antimalarial activity of compounds of this type is lost when tertiary amino groups or non-hydrogen-bonding substituents are present at the *para* (4 and 4′) positions or when the *p*-amino functions are changed to the *meta* (3 and 3′) positions. On the other hand, changing to small secondary amino or acylamido groups at the *para* positions[10] and/or certain substitution at the *ortho* (2 and 2′) positions[6] does not alter and may even enhance antimalarial activity.

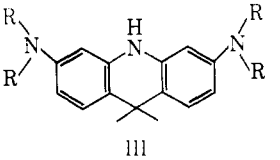


Ia, R_1, R_2 = H
b, R_1 = H; R_2 = CH_3CO

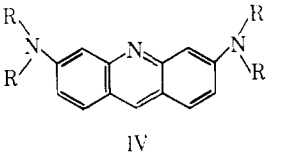
Two 2,4-bis(substituted amino)diphenylmethanes (IIa and IIb) were shown to possess antimalarial activity in avian malaria. Billman, *et al.*,[15] proposed that these compounds could actually be considered as precursors of the acridine derivative (IV), since it has been reported that compounds of type II could conceivably be deaminated to give the intermediate dihydroacridines (III), which are readily oxidized by oxygen or ferric chloride to form IV.[16]



IIa, R = CH_3
b, R = C_2H_5



III



IV

A number of phenothiazines are known to display a variety of physiological activities. Methylene blue, a tetramethyl derivative of Lauth's violet (3,6-diaminophenothiazonium chloride), was found to have some

(1) This investigation was supported by the Walter Reed Army Institute of Research (WRAIR), Walter Reed Army Medical Center, Department of the Army, and Headquarters, U.S. Army Medical Research and Development Command, Office of the Surgeon General, Contract No. DA-49-193-MD-2788. This paper is Contribution No. 132 from the Army Research Program on Malaria.

(2) E. Fromm and I. Whitman, *Ber.*, **41**, 2264 (1908).

(3) (a) G. A. H. Buttle, D. Stephenson, S. Smith, T. Dewing, and G. E. Foster, *Lancet*, **1**, 1331 (1937); (b) E. Fourneau, I. Tréfouël, F. Nitti, D. Bovet, and M. I. Tréfouël, *Compt. Rend.*, **204**, 1763 (1937).

(4) (a) W. H. Feldmann, H. C. Hinshaw, and H. E. Moses, *Proc. Staff Meetings Mayo Clinic*, **15**, 695 (1940); (b) N. Rist, F. Block, and V. Hamon, *Ann. Inst. Pasteur*, **64**, 203 (1940); (c) G. W. Raiziss, *Science*, **98**, 350 (1943).

(5) (a) G. H. Faget, R. C. Pogge, F. A. Johansen, J. F. Dinan, B. M. Prejean, and C. G. Eccles, *Public Health Rept. (U. S.)*, **58**, 1729 (1943); (b) R. G. Cochrane, *Brit. Med. J.*, **2**, 1220 (1952); (c) J. A. Doull, *Intern. J. Leprosy*, **22**, 377 (1954).

(6) F. Y. Wiselogle, "A Survey of Antimalarial Drugs, 1941–1945," Vol. II, Part 1, J. W. Edwards, Ann Arbor, Mich., 1946, pp 760–766, 895–896.

(7) H. M. Archibald and C. M. Ross, *J. Trop. Med. Hyg.*, **63**, 25 (1960).

(8) *Cf.* (a) A. B. G. Laing, *ibid.*, **68**, 251 (1965); (b) W. Peters, *Exptl. Parasitol.*, **17**, 80 (1965).

(9) DDS, but not quinine, chloroquine, quinacrine, or pyrimethamine, inhibits competitively the incorporation of *p*-aminobenzoic acid into folic acid. See ref 10–13 and other literature cited therein.

(10) H. Heymann and L. F. Fieser, *J. Am. Chem. Soc.*, **67**, 1979 (1945).

(11) J. Greenberg, *J. Pharmacol. Exptl. Therap.*, **97**, 238 (1949).

(12) A. Bishop, *Parasitology*, **53**, 10 (1963).

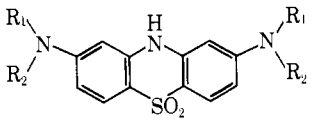
(13) J. Hill, *Exptl. Chemotherapy*, **1**, 547 (1963).

(14) E. F. Elslager and D. F. Worth, *Nature*, **205**, 630 (1965).

(15) J. H. Billman, D. G. Thomas, M. Hedbek, G. Schroenhaer, D. K. Barnes, J. Nemec, P. Trix, and E. Cleland, *J. Org. Chem.*, **11**, 773 (1946).

(16) A. Biehringer, *J. Prakt. Chem.*, [2] **54**, 217 (1896).

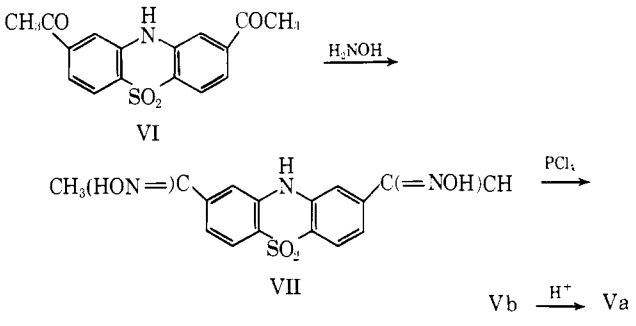
beneficial effects on patients suffering from malaria.[17] It is thus apparent that the synthesis and biological evaluation of 2,8-bis(substituted amino)phenothiazine 5,5-dioxides (V), which possess the combined structural features of I, II, and IV, should be of considerable interest.



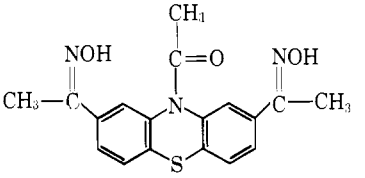
Va, R_1, R_2 = H
b, R_1 = H; R_2 = $COCH_3$
c, R_1 = H; R_2 = C_2H_5
d, R_1 = H; R_2 = CHO
e, R_1 = H; R_2 = CH_3
f, R_1 = CH_3; R_2 = CHO
g, R_1, R_2 = CH_3

Two synthetic routes to 2,8-diaminophenothiazine 5,5-dioxide (Va) have been reported. Michels and Amstutz[18] prepared Va from phenothiazine by an eight-step synthesis involving a Curtius rearrangement. A new synthesis of Va by prolonged heating of bis(2,4-diaminophenyl) sulfone with zinc chloride in dilute hydrochloric acid was reported by Bradbury and Smith.[19] Since the first procedure is rather lengthy and the second method could not be repeated in our laboratory,[20] a modification of Michels' and Amstutz's synthesis using a Beckmann rearrangement was examined. Our procedure consists of the conversion of the dioxime[21] (VII) of 2,8-diacetylphenothiazine 5,5-dioxide[18] (VI) to the corresponding diacetamido derivative (Vb) with phosphorus pentachloride. The desired 2,8-diaminophenothiazine 5,5-dioxide (Va) was readily obtained by acid hydrolysis of Vb. Thus, the present synthesis (shown in Scheme I) provides an easy access to Va and its related compounds.

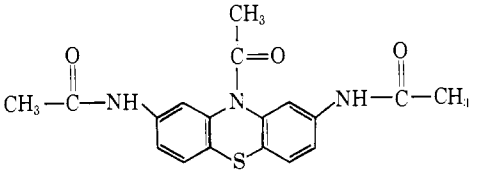
SCHEME I



The alternate synthetic approach, which involves a Beckmann rearrangement of the dioxime of 2,8,10-triacetylphenothiazine (VIII) prior to the oxidation step, was also attempted. Although the rearrangement was carried out successfully, difficulty was encountered during the oxidation of the rearranged product (IX)[18] to the corresponding sulfone.



VIII



IX

Reduction of the acetamido derivative (Vb) with borane in tetrahydrofuran readily yielded 2,8-bis(ethylamino)phenothiazine 5,5-dioxide (Vc). The corresponding methylamino compound (Ve) was prepared from the formamido derivative (Vd) in a similar manner. Formylation of Ve with acetic formic anhydride followed by reduction of the resulting compound (Vf) by borane afforded 2,8-bis(dimethylamino)phenothiazine 5,5-dioxide (Vg).

Preliminary, antimalarial evaluation results[22] indicated that compounds of type V did not show activity against *Plasmodium berghei* in mice. Apparently the *rigid* phenothiazine dioxide ring system failed to retain the original antimalarial activity exhibited by DDS and related compounds. Bis(2,4-diacetamidophenyl) sulfone,[19] prepared in connection with the attempted cyclization to Va by the method of Bradbury and Smith,[19] was also inactive.

Experimental Section[23]

Dioxime of 2,8-Diacetylphenothiazine 5,5-Dioxide (VII).—A mixture of 3.15 g (0.01 mole) of 2,8-diacetylphenothiazine 5,5-dioxide (VI), 1.8 g (0.026 mole) of hydroxylamine hydrochloride, 200 ml of absolute ethanol, and 100 ml of pyridine was heated under reflux for 3 hr. After evaporation of part of the solvent by a stream of air, the reaction mixture was diluted with 600 ml of water and filtered by suction to give 3.05 g (88% yield) of VII, mp 320–323° dec. From tetrahydrofuran–methanol, the analytically pure dioxime crystallized as white needles, mp 324–325° dec.

Anal. Calcd for $C_{16}H_{15}N_3O_4S$ (345.4): C, 55.6; H, 4.38; N, 12.2. Found: C, 55.6; H, 4.58; N, 12.0.

2,8,10-Triacetylphenothiazine Dioxime (VIII).—A mixture of 5.5 g (0.017 mole) of 2,8,10-triacetylphenothiazine,[18] 4.0 g (0.06 mole) of hydroxylamine hydrochloride, 250 ml of absolute ethanol, and 25 ml of pyridine was heated under reflux for 20 min. The yield of VIII was 5.6 g (93%), mp 222–224°. On recrystallization from methanol, the pure dioxime was obtained as white needles, mp 224–225°.

Anal. Calcd for $C_{18}H_{17}N_3O_3S$ (355.4): C, 60.8; H, 4.82; N, 11.8. Found: C, 60.5; H, 5.12; N, 11.5.

Beckmann Rearrangement of VII.—The dioxime VII (3.0 g, 0.009 mole) in 300 ml of anhydrous tetrahydrofuran was treated

(17) P. Guttmann and P. Ehrlich, *Berlin Klin. Wochschr.*, **28**, 953 (1891).
(18) J. G. Michels and E. D. Amstutz, *J. Am. Chem. Soc.*, **72**, 888 (1950).
(19) H. Bradbury and F. J. Smith, *J. Chem. Soc.*, 793 (1956).
(20) According to the procedure given by Bradbury and Smith,[19] the tetramino sulfone was refluxed in a saturated solution of $ZnCl_2$ in 2 *N* HCl at below 120° for 72 hr. Our attempts to repeat this work yielded only decomposition products. Using reaction temperatures higher than 120° (a saturated $ZnCl_2$ solution boils higher than 120°) also failed to yield the desired product.
(21) A number of oximes of acetyl- or ketophenothiazines have already been reported in the literature. See, for example, (a) R. Baltzly, M. Harfenist, and F. J. Webb, *J. Am. Chem. Soc.*, **68**, 2673 (1946); (b) A. Burger and A. C. Schmalz, *J. Org. Chem.*, **19**, 1841 (1954); (c) G. Cauquil and A. Casadevall, *Compt. Rend.*, **240**, 1784 (1955); (d) G. Cauquil and A. Casadevall, *Bull. Soc. Chim. France*, 768 (1955); (e) G. Cauquil, E. Casadevall, and A. Casadevall, *Compt. Rend.*, **243**, 159 (1956); (f) G. Cauquil, A. Casadevall, and E. Casadevall, *Bull. Soc. Chim. France*, 1049 (1960).
(22) Antimalarial testing results were provided by Dr. David P. Jacobus of WRAIR.
(23) All melting points were taken on a Thomas–Hoover melting point apparatus. The ultraviolet absorption spectra were determined with a Beckman DK-2 spectrophotometer, and the infrared spectra were taken with a Perkin-Elmer Infracord.

with 4.4 g (0.02 mole) of PCl_5 at room temperature. The dioxime gradually went into solution, and the reaction mixture, after being allowed to stand at room temperature for 1 day, was poured into 400 ml of cold water; tetrahydrofuran was removed by a stream of air. The crude product was collected by filtration, washed with water, and dried in air. On recrystallization from acetone–methanol there was obtained 1.5 g (50%) of 2,8-diacetamidophenothiazine 5,5-dioxide (Vb) as white crystals, mp 382–384°.

Anal. Calcd for $C_{16}H_{15}N_3O_4S$ (345.4): C, 55.6; H, 4.38; N, 12.2; S, 9.28. Found: C, 55.5; H, 4.40; N, 12.2; S, 9.20.

Beckmann rearrangement of the dioxime (VIII) was carried out by a similar procedure to give a 73% yield of 2,8-diacetamido-10-acetylphenothiazine (IX), mp 297–299° (lit.[18] mp 301–302°).

2,8-Diaminophenothiazine 5,5-Dioxide (Va).—The diacetamide Vb (0.85 g, 0.0025 mole) was boiled with 100 ml of 18% HCl for 1.5 hr. The reaction mixture was filtered, and the cooled filtrate was neutralized with NH_4OH. The precipitate was collected by filtration, washed with water, and dried in air to give 0.52 g (95%) of the crude Va, mp 342–345° dec. On recrystallization from methanol, the pure amine was obtained as pale yellow needles, mp 348–350° dec (lit. 355–356°,[18] 339–342°[19]).

Anal. Calcd for $C_{12}H_{11}N_3O_2S$ (261.3): C, 55.1; H, 4.25; N, 16.1. Found: C, 54.9; H, 4.32; N, 15.7.

2,8-Bis(ethylamino)phenothiazine 5,5-Dioxide (Vc).—A suspension of 4.5 g (0.013 mole) of the diacetamide Vb in 120 ml of anhydrous tetrahydrofuran was treated with 65 ml of a 1 *M* solution of borane in the same solvent. The reaction mixture was heated under reflux for 1 hr in a hood, and water was cautiously introduced to decompose the excess borane. After dilution with 100 ml of water, the tetrahydrofuran was distilled, and an additional 300 ml of water was added. The precipitated solid was filtered by suction, washed with water, and allowed to dry in air to give 3.9 g (90%) of Vc, mp 270–273° dec. On recrystallization from methanol, analytically pure Vc was obtained as white needles: mp 275–278° dec; $\lambda_{max}^{ethanol}$ 268 mμ (ε 89,000), 312 mμ (ε 11,000). The infrared spectrum has maxima at 2.9, 6.2, 6.3, 6.7, 6.9, 8.2, and 8.8 μ.

Anal. Calcd for $C_{16}H_{19}N_3O_2S$ (317.4): C, 60.5; H, 6.03; N, 13.2. Found: C, 60.6; H, 5.98; N, 13.0.

2,8-Bis(methylamino)phenothiazine 5,5-Dioxide (Ve).—To an acetic formic anhydride solution (prepared by mixing 55 ml of 98–100% formic acid and 35 ml of acetic anhydride, and allowing the mixture to stand for 20 min before use) was added in small portions, 1.7 g (0.006 mole) of 2,8-diaminophenothiazine 5,5-dioxide (Va). The formylation mixture was stirred at room temperature for 4 hr, then diluted with 800 ml of water. The product was collected by filtration, washed with water, and dried in air to give 1.85 g of Vd, mp 392–395° dec. Compound Vd is a white solid, extremely insoluble in methanol and other common organic solvents. Its infrared spectrum showed the expected amide absorption bands at 6.0, 6.5, and 8.0 μ. The product thus obtained was used for the following reduction without purification.

The reduction of Vd was carried out in 100 ml of anhydrous tetrahydrofuran with 40 ml of 1 *M* borane in the same solvent. The reaction mixture was heated under reflux for 1.5 hr and worked up as described for its ethyl analog (Ve) to afford 1.5 g of Ve, mp 272–274° dec. The over-all yield from Va was 81%. On recrystallization from methanol, pure Ve was obtained as white needles: mp 275–277° dec; $\lambda_{max}^{ethanol}$ 267 mμ (ε 93,000), 311 mμ (ε 10,000); maxima in the infrared spectrum were at 2.9, 3.0, 6.2, 6.3, 6.7, 7.0, 8.2, and 8.9 μ.

Anal. Calcd for $C_{14}H_{15}N_3O_2S$ (289.4): C, 58.2; H, 5.23; N, 14.5. Found: C, 58.2; H, 5.30; N, 14.5.

2,8-Bis(dimethylamino)phenothiazine 5,5-Dioxide (Vg).—To a solution of 1.3 g (0.0045 mole) of Ve in 20 ml of 98% formic acid was slowly added 45 ml of mixed anhydride, prepared from 30 ml of formic acid and 15 ml of acetic acid as described above. After being stirred overnight at room temperature, the reaction mixture was poured slowly into 700 ml of water. The solid was collected by filtration, washed with water, and dried in the air to give 1.5 g of the diformamido intermediate Vf, mp 345–348° dec. It was extremely insoluble in methanol and was reduced without further purification in 50 ml of tetrahydrofuran by addition, in several portions, of 50 ml of 1 *M* borane in the same solvent. After being heated under reflux for 1 hr the reaction mixture was worked up in the same manner as for the preceding products to give 1.3 g (93%) of Vg, mp 361–364° dec. One recrystallization from dimethylformamide gave an analytically pure sample which melted at 363–367° dec: $\lambda_{max}^{ethanol}$ 272 mμ (ε 77,000), 315 mμ (ε 12,000).

Anal. Calcd for $C_{16}H_{19}N_3O_2S$ (317.4): C, 60.5; H, 6.03; N, 13.2. Found: C, 60.4; H, 6.18; N, 13.2.

Acknowledgments.—The authors wish to express their appreciation to Mrs. Margaret L. Rounds, Mr. John H. Gravatt, and Mr. Leland R. Lewis for their valuable assistance in performing analytical and instrumental measurements.

1,3-Diethyleneguanidines

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As part of a program dealing with the chlorination of isothiocyanates,[1] we have synthesized various 1,3-diethyleneguanidines, a new class of potent insect chemosterilants.

1,3-Diethyleneguanidines (I, $n = 1$) as well as bis-(1,3-diethyleneguanidines) (I, $n = 2$) were synthesized from isocyanide dichlorides (II) and 1,2-alkylenimines (aziridines) in the presence of triethylamine as hydrogen chloride scavenger.[2] The reaction proceeded particularly smoothly in the aromatic series and afforded compounds I in high purity. The infrared absorption spectra of I show as dominant features a very strong >C=N– absorption at approximately 1635 cm^{-1}, and a strong unassigned absorption at 1330–1340 cm^{-1}.

The isocyanide dichlorides employed in the above reactions were synthesized in the case of the aromatic representatives by chlorination of the corresponding isothiocyanates[1] or by reaction of the formanilides with thionyl chloride and sulfuryl chloride.[2c] Alkylene bis-(isocyanide dichlorides), which to our knowledge have not been previously reported,[3] were obtained by chlorination of the diisocyanates with phosphorus pentachloride in phosphorus oxychloride. This method,[2c] first described for the preparation of alkyl isocyanide dichlorides, gave only unseparable mixtures of chlorina-

(1) G. Ottmann and H. Hooks, *J. Org. Chem.*, **31**, 838 (1966).

(2) Reactions of isocyanide dichlorides with primary, secondary, and tertiary aliphatic or aromatic amines have been reported to some extent: (a) E. Sell and G. Zierold, *Ber.*, **7**, 1228 (1874); (b) G. M. Dyson and T. Harrington, *J. Chem. Soc.*, 121 (1910); (c) E. Kühle, *Angew. Chem.*, **74**, 861 (1962).

(3) Only *m*- and *p*-phenylene bis(isocyanide dichloride) are described in the corresponding aromatic series.[2c]