Synthesis and Physical Properties of the Antimalarial Agent 4-(2-Bromo-4,5-dimethoxyphenyl)-1,1,7,7-tetraethyldiethylenetriamine (RC-12) (WR-27,653)

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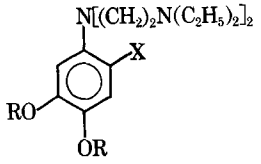
Several synthetic routes to the antimalarial agent 4-(2-bromo-4,5-dimethoxyphenyl)-1,1,7,7-tetraethyldiethylenetriamine (RC-12) (WR-27,653) were evaluated. The preferred scheme involved alkylation of 4-aminoveratrole with excess 2-chlorotriethylamine in dimethylformamide at 140–145° to give 4-(4,5-dimethoxyphenyl)-1,1,7,7-tetraethyldiethylenetriamine, which was brominated in acetic acid to give the desired product. The physical and biological properties of the drug are summarized.

SEVERAL DECADES ago it was discovered that certain pyrocatechol amine derivatives possess interesting antimalarial properties (1, 2). Among them, dimeplasmin (I*a*) was reported to be as active as pamaquine against trophozoite-induced *Plasmodium cathemerium* and *P. relictum*, and to be substantially less toxic for the mouse, rat, dog, and cat. However, dimeplasmin was clearly less active than quinine in controlling parasitemia and fever resulting from *P. falciparum* infections in man (3–5). Results



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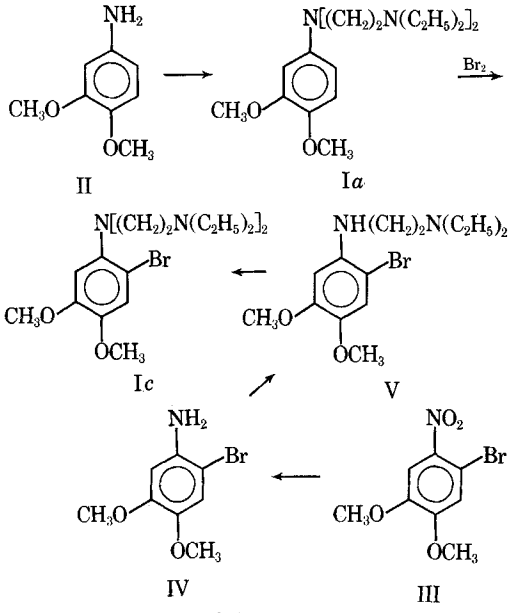
a, X = H; R = CH_3 (dimeplasmin)
b, X = Br; R = CH_2CH_3 (diapromin)
c, X = Br; R = CH_3 (RC-12)

of limited clinical studies with the related compound diapromin (I*b*) were likewise disappointing (6). Therefore, interest in this class of compounds waned.

More recently, interest in the antimalarial properties of the pyrocatechol amines was reawakened by the demonstration that 4-(2-bromo-4,5-dimethoxyphenyl)-1,1,7,7-tetraethyldiethylenetriamine (I*c*) (RC-12) had a pronounced effect on the exo-erythrocytic stages of *P. cathemerium* in canaries (7, 8). This observation stimulated an appraisal of the antimalarial effects of this agent against *P. cynomolgi* in the monkey (9). Although the drug *per se* had little promise as a schizonticidal or suppressive agent in monkeys, it showed significant promise as a prophylactic or radical curative agent. These authors concluded that RC-12 might find use when: (*a*) the combination of chloroquine–primaquine is not effective in causal prophylaxis, as appears to be the case where there is chloroquine resistance; (*b*) there is a need for a curative agent which can produce benefits in less than 10–14 days; and (*c*) there are fears of enhanced susceptibility to the hematotoxicity of primaquine (9). In anticipation that RC-12 might prove useful in connection with malaria problems encountered by the armed forces in Southeast Asia, the U. S. Army Medical Research and Development Command requested that suitable methods be developed for the laboratory synthesis and characterization of this substance. (Scheme I.)



Scheme I

General methods for the preparation of various pyrocatechol amines were described many years ago by Schulemann and Kropp (1), but to our knowledge the synthesis and properties of I*c* have not been described in the literature. In the present work several synthetic approaches to I*c* were evaluated. Among them, the following route proved to be most useful: alkylation of 4-aminoveratrole (II) with excess 2-chlorotriethylamine in dimethylformamide at 140–145° gave, in 57% yield, 4-(4,5-dimethoxyphenyl)-1,1,7,7-tetraethyldiethylenetriamine (dimeplasmin) (I*a*) which was 98–100% homogeneous by vapor phase chromatographic analysis. Attempted bromination of I*a* with bromine in carbon tetrachloride or with *N*-bromosuccinimide was unsuccessful; however, bromine in acetic acid at 55-60° afforded

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essentially homogeneous *Ic* in 41% yield. The material thus obtained was identical in all respects with an authentic sample of RC-12 base. The amounts of *Ia* and *Ic* formed in these reactions, as indicated by GLC analyses, were considerably larger than he amounts of pure materials isolated, and the use of more sophisticated distillation equipment would undoubtedly increase yields substantially.

Alternatively, alkylation of 2-bromo-4,5-dimethoxyaniline (IV) (10) with a slight excess of 2-chlorotriethylamine at 90–100° for 2.5 hr. (1) gave a mixture of 37% unchanged aniline (IV), 58% of the monoalkylated product (V), and 5% of *Ic* based on vapor phase chromatographic analysis. Extending the heating time to 27 hr. gave a mixture of 90% V and 10% *Ic*. Treatment of the latter mixture with 2-chlorotriethylamine at 130–140° for 10 hr. increased the amount of *Ic* in the mixture only to 25%. An attempt to bisalkylate IV directly with an excess of 2-chlorotriethylamine at 140–150° for 18 hr. gave a complex mixture which contained between six and eight components based on VPC analysis. The 2-bromo-4,5-dimethoxyaniline (IV) (10) used as starting material was prepared by bromination of either 4-amino-1,2-dimethoxyaniline or the corresponding acetamide, or preferably by catalytic reduction of 4-bromo-5-nitro-1,2-dimethoxybenzene (III) (11).

The infrared and NMR spectra of RC-12 appear quite normal. The absence of any obvious coupling of the aromatic protons, *cf.* clean doublet at 6.71, 6.91 p.p.m. in the NMR spectrum (in CCl_4 with tetramethylsilane as internal standard), supports the existence of 2 *para* protons and thus the structural orientation as proposed (*Ic*).

The ultraviolet absorption spectrum of RC-12 is similar to that of simpler analogous structures (Table I). In methanol, the spectrum has a secondary band (*13*) at lower wavelength and lower intensity than the corresponding amine IV. This shift to lower wavelength reflects the well known phenomenon of inhibition of resonance interaction between the tertiary aromatic amine and the benzene ring by the bromine in the *ortho* position (12). The primary band is not resolved. The authors have observed that bromobenzenes substituted in the *ortho* position by very weak donor groups characteristically show intense absorption at lower wavelength, in which this band may not be resolved.

The spectra of the cationic forms of compounds IV and *Ic* should be like that of 4-bromoveratrole, since the cation cannot resonate with the ring. This is evident for compound IV which shows a characteristic shift with acid to lower wavelength, with band wavelengths and intensities similar to those of 4-bromoveratrole. RC-12 (*Ic*), on the other hand, shifts very little with acid, indicating that the aromatic amine has not been affected. Attempts to protonate the aromatic amine in very strong acid were only partially successful. In 25% concentrated sulfuric acid, the secondary band shifted several millimicrons, and resolution of the primary band occurred. In still stronger acid, a slight further shift occurred, but other effects appeared also, including darkening of the solutions.

The basicity of the aromatic amine in RC-12 is thus seen to be strongly decreased as a result of its structural orientation. The ionization constants determined experimentally are in accord with this conclusion. Introduction of bromine *ortho* to the amine in II reduces the pK'a from 4.6 to 2.6 (IV) (Table I). Introduction of the basic side chain also decreases the basicity of the aromatic amine, as indicated by a pK'a of about −0.1 for compound *Ia*, determined spectrophotometrically. Both these effects would be operative in *Ic*. The predicted pK'a is as low as −2, a value beneath the pH to which the compound could be subjected without development of other effects.

EXPERIMENTAL

Melting points are corrected. Vapor phase chromatograms were run on an F & M No. 810 instrument using a 6-ft. × 1/4-in. 5% SE-30 on Anakrom ABS 90-100 mesh column at 250° with a nitrogen carrier gas flow rate of 60 ml./min.

Preparation of 2-Bromo-4,5-dimethoxyaniline (IV)—A solution of 13.1 Gm. (0.05 mole) of 4-bromo-5-nitro-1,2-dimethoxybenzene (Eastman Kodak) in 150 ml. of tetrahydrofuran was hydrogenated over 2 Gm. of Raney nickel at 25° and an initial pressure of 49.5 psig. The catalyst was removed by filtration and the solvent removed *in vacuo*. The residue was taken up in ether, the ether solution was filtered to remove some insoluble material, and dry hydrogen chloride was bubbled into the ether filtrate. The white precipitate was removed by filtration, washed with ether, and dried *in vacuo* at 50°

TABLE I—ULTRAVIOLET[a] AND pK'a DATA ON RC-12 AND INTERMEDIATES

	Solvent	λ	ε	λ	ε	pK'a[b]
4-Bromoveratrole	MeOH	283	2,800	232	8,900	...
II	MeOH	292	3,000	236	7,900	4.6
ortho-Bromoaniline	MeOH	293	2,600	236	8,200	
IV·HCl	MeOH, 0.05 *N* in NaOH	304	4,100	237	8,800	2.6
	MeOH, 0.05 *N* in HCl	284	3,300	234	9,200	
Ia	MeOH	309	2,800	256	10,900	7.6, 9.1 ~−0.1[c]
Ic	MeOH	292	3,300	Unresolved		7.7, 9.2
	MeOH, 0.05 *N* in HCl	290	3,300	Unresolved		
	MeOH, 25% Conc. H_2SO_4	286	3,600	238	8,500	

[a] Ultraviolet spectra were determined on a Cary model A recording spectrophotometer. [b] By potentiometric titration in 50% methanol (v/v). [c] By spectrophotometric determination in 20% methanol.

to give 10.2 Gm. (76%) of 2-bromo-4,5-dimethoxyaniline hydrochloride, m.p. 203-204° dec. A portion recrystallized from ethanol gave m.p. 202.5–203.5° dec.

Anal.—Calcd. for $C_8H_{10}BrNO_2 \cdot HCl$: C, 35.78; H, 4.13; N, 5.22. Found: C, 36.11; H, 4.30; N, 5.10.

Preparation of 4-(4,5-Dimethoxyphenyl)-1,1,7,7-tetraethyldiethylenetriamine (Ia)—A mixture of 99.2 Gm. (0.647 mole) of 4-aminoveratrole (Hi Laboratories), 387.7 Gm. (2.59 moles) of freshly distilled chlorotriethylamine (generated from the hydrochloride salt),[1] and 165 ml. of dimethylformamide was heated with stirring for 20.5 hr.[2] by means of an oil bath kept at 140–145°. After cooling to room temperature, the reaction mixture was treated with 10% aqueous sodium hydroxide solution[3] and chloroform.[4] The chloroform extract was dried over sodium sulfate and the chloroform removed on a rotary evaporator. The resulting oil was suspended in 10% aqueous sodium hydroxide solution[5] and extracted with ether.[6] The ether solution was dried over sodium sulfate and the ether removed on a rotary evaporator. The residual crude product (196 Gm.) was distilled under vacuum through a 6-in. Vigreaux column[7] to give 128.8 Gm. (57%) of 4-(4,5-dimethoxyphenyl)-1,1,7,7-tetraethyldiethylenetriamine, b.p. 163–165°/0.17–0.20 mm., shown by vapor phase chromatography to be 98–100% homogeneous.

Anal.—Calcd. for $C_{20}H_{37}N_3O_2$: C, 68.33; H, 10.61; N, 11.95. Found: C, 67.99; H, 10.54; N, 11.89.

Preparation of 4-(2-Bromo-4,5-dimethoxyphenyl)-1,1,7,7-tetraethyldiethylenetriamine (Ic)—To a solution of 82.2 Gm. (0.234 mole) of 4-(4,5-dimethoxyphenyl)-1,1,7,7-tetraethyldiethylenetriamine in 500 ml. of glacial acetic acid was added dropwise with stirring at room temperature 41.0 Gm. (0.256 mole) of bromine.[8] The addition required 50 min., and the final temperature of the solution was 41°. The mixture was stirred for 1 hr. at room temperature and then for 1 hr. at 55–60°. The acetic acid was removed on the rotary evaporator. The cooled residue was made alkaline with a 50% aqueous sodium hydroxide solution[9] and extracted first with ether and then with benzene.[10] The extracts were washed with water[11] and dried over sodium sulfate. The solvents were removed *in vacuo* leaving about 82 Gm. of crude product which was subjected to distillation with an oil diffusion pump.[12] After removing the forerun up to 150°/0.005 mm., two fractions, b.p. 150–151°/.005–.001 mm. (28.0 Gm.), were collected which were shown by vapor phase chromatography to contain about 95% of Ic. Two additional fractions, b.p. 149–150°/<.001 mm. (28.5 Gm.) and 151.5–149°/.001– <.001 mm. (13.1 Gm.), were collected and shown to be essentially homogeneous Ic (98–99%). These two fractions represent a yield of 41%. The product is a yellow-brown viscous liquid with a retention time of 12.25 min. under the conditions of vapor phase chromatography indicated above.

Anal.—Calcd. for $C_{20}H_{36}BrN_3O_2$: C, 55.81; H, 8.43; Br, 18.57; N, 9.76. Fraction b.p. 149–150°/<.001 mm. Found: C, 55.75; H, 8.41; Br, 18.75; N, 9.68. Fraction b.p. 151.5–149°/.001– <.001 mm. Found: C, 55.96; H, 8.61; Br, 18.80; N, 9.62.

The NMR spectrum of RC-12 in CCl_4 was determined on a Varian A-60 using tetramethylsilane as an internal standard. The peaks (p.p.m.) integrate for the following relative intensities: singlet 6.71 (1H), singlet 6.91 (1H), singlet 3.75 (6H), multiplet 3.00 (4H), multiplet 2.43 (12H), triplet 0.93 (12H).

[1] Shirley, D. A., "Preparation of Organic Intermediates," John Wiley & Sons, Inc., New York, N. Y., 1951.

[2] Attempted fusion of a model monoalkylated material with 2 equivalents of diethylaminoethyl chloride at 140° up to 14 hr. resulted in only 56% conversion to the bisalkylated material.

[3] About 200–300 ml. of 10% sodium hydroxide was used.

[4] The mixture was extracted four times with 250-ml. portions of chloroform.

[5] About 200 ml. was used. This is to remove any inorganic material which has been carried into the chloroform extract. The presence of small amounts of this material leads to difficulties in the distillation.

[6] Extracted four times with 250-ml. portions of ether.

[7] The pot was heated with an oil bath (silicone) maintained at 200–250°. The oil bath was heated by an Agit-Therm magnetic stirrer which also served to agitate a stirring bar in the distillation pot.

[8] The beaker used to weigh out the bromine was rinsed with 10 ml. of acetic acid and this was added to the bromine in the dropping funnel. Room temperature was approximately 26°.

[9] About 50 ml. was used.

[10] Two extractions using about 250 ml. of ether each followed by two extractions with 250-ml. portions of benzene.

[11] Two portions of approximately 250 ml. each.

[12] A 4-in. Vigreaux column heated with an electric heating tape was used for this distillation. The pot was heated (200–250°) and stirred as described in *Footnote 7*.

REFERENCES

(1) Schulemann, W., and Kropp, W., U. S. pat. 1,757,394 (May 6, 1930).
(2) Schönhöfer, F., "Chemotherapy," *Office Tech. Services Rept.*, PB-85033, 1948; FIAT, *Review of German Science*, 43.
(3) Green, R., *Lancet*, **217**, 1137(1929).
(4) Green, R., *Bull. Inst. Med. Res. Federation Malaya*, **28**, 29(1929).
(5) Sinton, J. A., *Indian J. Med. Res.*, **17**, 815(1930).
(6) Coppock, J. B. M., "The Pharmaceutical Industry in Germany During the Period 1939–1945," *Office Tech. Services Rept.*, PB-101472 1950; BIOS, Rept., No. 24.
(7) Schulemann, W., World Health Organization, unpublished report, 1963.
(8) Bruce-Chwatt, L. J., *Trans. Roy. Soc. Trop. Med. Hyg.*, **59**, 105(1965).
(9) Schmidt, L. H., Rossan, R. N., Fradkin, R., Woods, J., Schulemann, W., and Kratz, L., *Bull. World Health Organ.*, **34**, 783(1966).
(10) Simonsen, J. L., and Rau, M. G., *J. Chem. Soc.*, **1918**, 782.
(11) Pettit, G. R., and Piatak, D. M., *J. Org. Chem.*, **25**, 721(1960).
(12) Remington, W. R., *J. Am. Chem. Soc.*, **67**, 1838(1945).
(13) Doub, L., and Vandenbelt, J. M., *ibid.*, **69**, 2714 (1947).
(14) Parke, T. V., and Davis, W. W., *Anal. Chem.*, **26**, 642(1954).