[CONTRIBUTION FROM THE WELLCOME RESEARCH LABORATORIES]

Some N,N′-Bis-tertiaryaminoacetyl-α,ω-polymethylene Diamines and their Quaternary Ammonium Salts as Curare Substitutes. V

BY ARTHUR P. PHILLIPS

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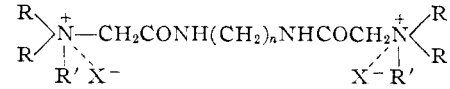
Some N,N′-bis-tertiaryaminoacetyl-α,ω-polymethylene diamines and certain of their quaternary ammonium salts have been prepared by the reaction of the N,N′-bis-chloroacetylamides with secondary amines, and by the subsequent quaternization of the bis-tertiary amines so obtained. These compounds were made for testing as curare-like drugs and as potentiators of the curare-like action of the well-known succinylcholine.

In the search for synthetic curare substitutes modeled after decamethylene-1,10-bis-trimethylammonium salts[1-3] very potent agents were discovered among a series of bis-quaternary salts of dicarboxylic acid bis-aminoethyl esters.[4,5] In the aminoester series, as in the polymethylene type compounds, maximum neuromuscular blocking potency was attained when a chain of ten atoms separated the terminal ammonium groups. Thus succinylcholine[4-6] was the most powerful curare-like member of the ester series although the glutaric and adipic analogs were only slightly less potent.

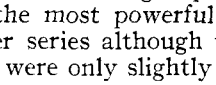
Modification of the profitable bis-amino ester line led to the preparation of several analogous series of dicarboxylic acid bis-amino *amides* and their quaternary salts.[7-9] These amino-amides and their salts were found to be relatively devoid of curare-like activity, in contrast with the analogously constituted amino ester salts, but they were found to be powerful prolongers of the curare-like activity of succinylcholine. This potentiating action of the bis-amino amides was even less sensitive to changes in chain length than was the curare-like activity in the bis-amino esters, although maximum potency usually was found in the succinic, glutaric, adipic members. End group structure, too, was less specific in the bis-amino amides. Succinylcholine potentiating ability was found in both the bis-tertiary amino amides and in their quaternary ammonium salts. In certain series the bis-quaternary salts were more powerful extenders than the bis-tertiary amines, while in other series the bis-tertiary amines were more effective than their quaternary salts.

The present work deals with several new types of bis-amino amides and derived bis-quaternary salts which have been made for testing both for curare-like activity and for the ability to potentiate the curare-like activity of succinylcholine. These new bis amides were designed to resemble somewhat the earlier types of bis amides and it was hoped that the pharmacological properties of the new compounds would emulate the desirable activities of the old. The new amides, which might be termed "inverse" amides as related to the older series, can be illustrated by the general structural formula I.



I

n = 2,3,6, etc.



pyrrolidino, piperidino or morpholino

R′X = —, HCl, or CH_3I

The compounds were prepared by the following sequence of reactions: (1) suitable diamines were diacylated with chloroacetyl chloride; (2) the bis-chloroacetyl compounds were heated with an excess of secondary (or tertiary) amine to give the bis-tertiaryaminoacetylamine (or bis-quaternary salt); (3) the bis-tertiaryaminoacetylamides were quaternized with methyl iodide.

The diamines used were ethylenediamine (n = 2), trimethylenediamine (n = 3), hexamethylenediamine (n = 6), *p*-phenylenediamine and piperazine. The bis-chloroacetyl derivatives of ethylenediamine,[10] trimethylenediamine,[11] *p*-phenylenediamine,[12] and piperazine[13] are known.

The end-group amines trimethylamine, diethylamine, pyrrolidine, piperidine and morpholine were combined with the bis-chloroacetylamides.

Pharmacology.—The members of the current series of "inverse" bis-aminoamides were inactive as neuromuscular blocking agents. Most of them strongly potentiated the neuromuscular blocking action of succinylcholine[4-6] as had the other types of bis-aminoamides described earlier.[7-9] The most potent members of both the old and new series, in doses of 0.1 mg./kg., prolonged by 100% the duration of the neuromuscular block produced by succinylcholine in anesthetized cats and dogs. The L.D.50 toxicities (i.v. in mice) of the present compounds fell in the range of 150–200 mg./kg. as compared with a range of 3–20 mg./kg. for the older series.

Succinylcholine potentiating potency was even less sensitive to changes in chain length with the present series of amides than with the earlier se-

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(2) W. D. M. Paton and E. J. Zaimis, *Nature*, **161**, 718 (1948); *ibid.*, **162**, 810 (1948).

(3) J. C. Castillo, A. P. Phillips and E. J. de Beer, *J. Pharmacol. Exp. Therap.*, **97**, 150 (1949).

(4) D. Bovet, *et al.*, *Rend. Ist. Super. Sanita*, **12**, 1 (1949).

(5) A. P. Phillips, THIS JOURNAL, **71**, 3264 (1949).

(6) J. C. Castillo and E. J. de Beer, *J. Pharmacol. Exp. Therap.*, **99**, 458 (1950).

(7) A. P. Phillips, THIS JOURNAL, **73**, 5822 (1951).

(8) A. P. Phillips, *ibid.*, **74**, 4320 (1952).

(9) A. P. Phillips, *ibid.*, **75**, 2774 (1953).

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(11) J. von Braun, F. Dengel and A. Jacob, *Ber.*, **70B**, 994 (1937).

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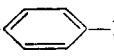
Table I

Polymethylene-α,ω-bis-tertiaryaminoacetylamides and their Quaternary Ammonium Salts

$$R_2\overset{+}{N}—CH_2CONH(CH_2)_nNHCOCH_2\overset{+}{N}R_2$$
$$R' \quad X^- \qquad\qquad X^- \quad R'$$

Compd. No.	R_2N	R′X	M.p., °C.	Formula	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found
				(A) $n = 2$				
1	Dimethylamino	CH_3Cl	270–271	$C_{12}H_{28}Cl_2N_4O_2$	43.5	43.5	8.5	8.5
2	Diethylamino		85–86	$C_{14}H_{30}N_4O_2$	58.7	58.4	10.5	10.1
3	Diethylamino	CH_3I	210–211	$C_{16}H_{36}I_2N_4O_2$	33.7	34.1	6.4	6.7
4	Pyrrolidino		115–116	$C_{14}H_{26}N_4O_2$	59.6	59.2	9.3	9.1
5	Pyrrolidino	CH_3I	227–228	$C_{16}H_{32}I_2N_4O_2$	34.0	33.8	5.7	5.5
6	Piperidino		138–139	$C_{16}H_{30}N_4O_2$	61.9	61.9	9.8	10.0
7	Morpholino		191–192	$C_{14}H_{26}N_4O_4$	53.4	53.5	8.3	8.1
				(B) $n = 3$				
8	Dimethylamino	CH_3Cl	238–239	$C_{13}H_{30}Cl_2N_4O_2$	45.2	45.4	8.8	8.9
9	Diethylamino	HCl	155–156	$C_{15}H_{34}Cl_2N_4O_2$	48.2	48.2	9.2	9.3
10	Diethylamino	CH_3I	151–152	$C_{17}H_{38}I_2N_4O_2$	34.9	35.1	6.6	6.8
11	Piperidino		83–84	$C_{17}H_{32}N_4O_2$	63.0	63.1	9.9	9.8
12	Piperidino	CH_3I	203–204	$C_{19}H_{38}I_2N_4O_2$	37.5	37.5	6.3	6.1
13	Morpholino		153–154	$C_{15}H_{28}N_4O_4$	54.9	54.6	8.6	8.3
14	Morpholino	CH_3I	182–183	$C_{17}H_{34}I_2N_4O_4$	33.3	33.1	5.6	5.5
				(C) $n = 6$				
15	Dimethylamino	CH_3Cl	232–233	$C_{16}H_{36}Cl_2N_4O_2$	49.6	49.3	9.4	9.4
					Cl, 18.3	Cl, 18.2		
16	Diethylamino		61–62	$C_{18}H_{38}N_4O_2$	63.1	62.9	11.2	11.1
17	Diethylamino	CH_3I	116–118	$C_{20}H_{44}I_2N_4O_2$	38.4	38.8	7.1	7.2
18	Pyrrolidino		82–83	$C_{18}H_{34}N_4O_2$	63.9	63.9	10.1	10.0
19	Pyrrolidino	CH_3I	137–138	$C_{20}H_{40}I_2N_4O_2$	38.6	38.6	6.5	6.7
20	Piperidino		108–109	$C_{20}H_{38}N_4O_2$	65.6	65.8	10.4	10.1
21	Piperidino	CH_3I	175–176	$C_{22}H_{44}I_2N_4O_2$	40.6	40.3	6.8	6.7
22	Morpholino		85–86	$C_{18}H_{34}N_4O_4$	58.4	58.5	9.3	8.9
23	Morpholino	CH_3I	175–176	$C_{20}H_{40}I_2N_4O_4$	36.7	36.4	6.2	6.1
				(D) $-NH(CH_2)_nNH-$ replaced by piperazino-1,4-				
24	Dimethylamino	CH_3Cl	290 (dec.) sinters from 245°	$C_{14}H_{30}Cl_2N_4O_2 \cdot H_2O$	44.8	44.7	8.6	8.3
				(E) $-NH(CH_2)_nNH-$ replaced by 				
25	Dimethylamino	CH_3Cl	296–298 (dec.)	$C_{16}H_{28}Cl_2N_4O_2$	50.6	50.6	7.4	7.2

ries.[7,8] Compounds 6 and 20 of Table I ($n = 2$ and $n = 6$, respectively) were equally active and both were among the most potent of the group described here.

Variations in the end-group structure were more critical for activity, and the results obtained with the present series paralleled the findings with the older amides[7,8] in this regard. Thus the morpholino derivatives were inactive. The methiodides of simple dialkylamino derivatives were somewhat more active than the ditertiary amines. The most potent compounds had piperidino or pyrrolidino as end-groups, and for these types the bis-tertiary-aminoamides were from 5–20 times more active than the derived bis-methiodides as prolongers of succinylcholine.

Other useful activities have been observed with these amides. They resemble Prostigmine and Tensilon in their ability to antagonize *d*-tubocurarine chloride. Various members act as respiratory stimulants.

Acknowledgment.—The author is grateful to S. W. Blackman for the microanalyses included and to A. L. Wnuck for the pharmacological results summarized here.

Experimental

N,N′-Bis-chloroacetylhexamethylenediamine.—To a solution of 23 g. (0.2 mole) of chloroacetyl chloride in 500 cc. of absolute ether was added gradually with cooling and stirring 23 g. (0.2 mole) of hexamethylenediamine (as 70% aqueous solution). The addition took 0.5–1 hour and stirring was continued for 2 hours longer. A white pasty precipitate formed during the addition of the amine. After 2 hours the pasty solid was filtered off by suction and washed several times with small volumes of ether. The total crude yield of bis-amide and diamine dihydrochloride was 58 g. (100%). The crude mixture was heated with 100 cc. of water and on cooling gave 20 g. (70–75%) of bis-chloroacetylamide. After two additional recrystallizations from hot ethyl acetate the yield was 12–15 g. (50–55%) of amide melting at 131–132°.

Anal. Calcd. for $C_{10}H_{18}Cl_2N_2O_2$: C, 44.7; H, 6.7. Found: C, 44.9; H, 6.8.

The other bis-chloroacetylamides were made by similar procedures. Yields of pure products were usually 35–50%. Melting points corresponded with those reported in the literature.[10–13]

Preparation of the Bis-aminoacetylamides.—A mixture of 0.01 mole of the bis-chloroacetylamide and 0.06–0.1 mole of the amine, either in methanol solution or without solvent,

was heated for 2–4 hours at 100°. Any excess of unused amine and solvent was removed by evaporation *in vacuo*. The residue was basified with a small amount of cold 20% aqueous sodium hydroxide (to *p*H 11) and the precipitated solid bis-aminoamide was collected by suction and washed with a little cold water. The products were purified by recrystallization from ethyl acetate, hexane or mixtures of these solvents. Yields were usually above 90%.

The bis-tertiary-aminoacetylamides were quaternized by refluxing with methyl iodide in methanol for several hours. The bis-methiodides crystallized from methanol or methanol–ethyl acetate mixtures. Yields were all above 90%.

Details for all compounds appear in Table I.

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[CONTRIBUTION FROM THE CHEMICAL RESEARCH DIVISION OF THE SCHERING CORPORATION]

Antifungal Agents[1]

BY STEPHEN B. COAN AND DOMINICK PAPA

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A series of tertiary aliphatic and heterocyclic amines have been prepared and examined as antifungal agents for pathogenic molds and fungi. Certain structure–activity relationships are evident and are discussed.

With the advent of broad-spectrum antibiotics which somewhat indiscriminately destroy both malevolent and benevolent bacteria within the human host, the development of pathogenic fungal infections has received considerable attention.[2] Although there is apparently no completely satisfactory method for combatting fungal infections arising from *M. albicans* and *T. mentagrophytes*, whose overgrowth may be made possible by the destruction of competitive flora, the true incidence of the condition, specifically moniliasis, is still questionable.[2] Despite considerable work and the evolution of several theories, the mechanism whereby *M. albicans* acquires pathogenesis following antibiotic therapy is still obscure.

Despite the lack of agreement among clinicians regarding the need for antifungal agents specifically effective against pathogenic fungi, various types of fungicides are in clinical use or under investigation. Being cognizant of the anti-monilial properties of a group of steroidal amines and their quaternary salts[3] as well as the fungistatic and fungicidal action of certain long chain saturated and unsaturated fatty acids,[4] it was of interest to prepare and examine microbiologically a series of saturated and unsaturated tertiary amines and salts thereof.

The compounds prepared in this study are of the general formula $R_1NR_2R_3$ wherein R_1 is a saturated or unsaturated hydrocarbon group and $—NR_2R_3$ is a dialkylamino, pyrrolidino, piperidino and morpholino group. It is to be noted that these compounds combine the hydrocarbon moiety of the fatty acids[4] and the basic moiety of the steroidal amines.[3]

The tertiary amines were prepared by conventional methods, the procedure of choice being the reaction of the appropriate acid chloride and secondary amine to the intermediate amide (see Table I) followed by reduction of the amide with lithium aluminum hydride.[5]

The amides, tertiary amines and the acid addition and quaternary salts of the latter were submitted to an *in vitro* microbiological test to determine their activity against *Monilia albicans*, *T. mentagrophytes* (Table II) as well as *E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis* and *M. smegmatis*. The antifungal activities of the compounds were compared with two fungicides in clinical use, undecylenic acid and 6-(β-diethylaminoethoxy)-2-dimethylaminobenzothiazole dihydrochloride.[6] In order to obtain sufficient data for a structure correlation several known tertiary amines were prepared and examined.

All the compounds were essentially inactive against *E. coli* and *P. aeruginosa*. The amides exhibited activities which were neither of sufficient magnitude nor broadness of spectrum as to warrant reporting. From Table II certain relationships between structure and activity are manifest. It is of interest to note that within the confines of the optimum structures all compounds possessed antifungal activities greater than that of undecylenic acid and in most cases in excess of that possessed by Asterol.

Optimum activity resides in those compounds wherein R_1 = 10-undecenyl, and R_2 and R_3 contain a total of 4–6 carbon atoms (X, XIII, XIV, XV and XVI). Increasing the carbon chain in R_2 and R_3 to isobutyl (XI) or shortening to methyl (IX) markedly reduced activity.

Hydrogenation of the terminal double bond of compound X did not radically alter the antifungal activity (XIX) whereas transforming the ethylenic bond to an acetylenic link narrows the spectrum (XII). The pronounced activity of XIX was of interest since this investigation was originally based upon the supposition that the terminal unsaturation was a prerequisite for activity. Wyss, *et al.*,[4] demonstrated the rather singular properties of undecylenic acid as a fungicide.

Having somewhat empirically shown the effect

(1) Presented in abstract at The North Jersey Miniature Meeting in Newark, New Jersey, January 24, 1955.

(2) (a) M. J. Lipnik, A. M. Kligman and R. Strauss, *J. Investigative Dermat.*, **18**, 247 (1952); (b) W. I. Metzger, L. T. Wright and J. C. DiLorenzo, *J. Am. Med. Assoc.*, **155**, 352 (1954).

(3) (a) F. C. Kull, G. A. Castellano and R. L. Mayer, *J. Investigative Dermat.*, **21**, 227 (1953); (b) Hershel L. Herzog, Constance C. Payne and E. B. Hershberg, to be published.

(4) (a) O. Wyss, B. J. Ludwig and R. R. Joiner, *Arch. Biochem.*, **7**, 415 (1945); (b) J. Kimmig and H. Rieth, *Arzneim. Forsch.*, **3**, 267 (1953).

(5) The reduction (and isolation) was essentially that described by V. Micovic and M. Mihailovic, *J. Org. Chem.*, **18**, 1190 (1953).

(6) Asterol—Registered trade mark of Hoffmann-LaRoche, Inc.