[CONTRIBUTION FROM THE WELLCOME RESEARCH LABORATORIES]

Dicarboxylic Acid Bis-β-tertiaryaminoalkyl Amides and their Quaternary Ammonium Salts as Curare Substitutes. III

BY ARTHUR P. PHILLIPS

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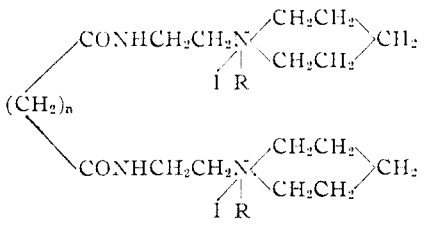
A series of bis-β-piperidinoethyl and β-pyrrolidinoethyl amides of aliphatic dicarboxylic acids and their quaternary ammonium salts have been made for pharmacological examination. Many of these compounds are powerful potentiators of the curare-like activity of diacetylcholine (succinylcholine) and also are strong antagonists of the curariform activity of *d*-tubocurarine chloride. In contrast to the earlier described bis-amides and their salts, in the current series the bis-tertiary-amino amides afford the most active compounds while their quaternary salts are relatively inactive.

This paper reports the preparation and properties of series of bis-β-piperidinoethyl and bis-β-pyrrolidinoethyl amides of aliphatic dicarboxylic acids. The bis-amides were obtained by refluxing the appropriate dicarboxylic acid methyl or ethyl esters with at least two molar equivalents of piperidinoethylamine or pyrrolidinoethylamine. Amides were made from the simple dicarboxylic acids, oxalic through sebacic, and bis-methiodides were also prepared in most cases. Usually both the tertiary aminoalkyl amides and their methiodides crystallized readily, were easily soluble in water, and were best recrystallized from mixtures of organic solvents. The bis-methiodides of some of the longer chain members of the series were glassy solids or viscous oils very reluctant to crystallize. The chemical and physical properties and analyses of these compounds are collected in Table I.

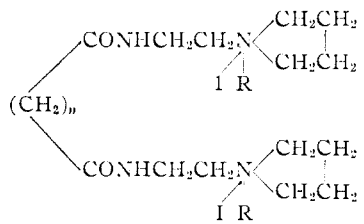
TABLE I

(A) AMIDES AND QUATERNARY AMMONIUM SALTS FROM PIPERIDINOETHYLAMINE AND ALIPHATIC DICARBOXYLIC ACIDS



n	R	M.p., °C.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
0		155–156	61.9	62.2	9.7	9.8
0	CH_3	286–288	36.3	35.7	6.1	5.9
			I, 42.7	I, 42.7		
0	C_2H_5	285–286	38.6	38.4	6.5	6.3
1		102–103	62.9	62.8	10.0	9.6
1	CH_3	176–177	37.5	37.6	6.3	6.0
2		150–151	63.8	63.9	10.1	10.2
2	CH_3	196–198	38.6	38.6	6.5	6.3
			I, 40.8	I, 40.8		
3		134–135	64.7	65.0	10.3	10.0
3	CH_3	136–137	39.6	39.6	6.7	6.4
4		140–141	65.6	65.6	10.4	10.4
4	CH_3	177–178	40.6	40.6	6.8	6.6
5		120–121	66.2	66.4	10.6	10.4
5	CH_3	Viscous oil	I, 38.2	I, 38.0		
7		116–117	67.7	68.0	10.8	10.7
7	CH_3	Viscous oil	43.3	43.8	7.3	7.3
8		135–136	68.2	68.3	11.0	11.1
8	CH_3	Viscous oil	44.1	44.2	7.4	7.7

(B) AMIDES AND QUATERNARY AMMONIUM SALTS FROM PYRROLIDINOETHYLAMINE AND ALIPHATIC DICARBOXYLIC ACIDS



0		163–164	59.5	59.7	9.3	9.0
0	CH_3	266–267	33.9	33.4	5.7	6.1
			I, 44.9	I, 44.9		
2		166–167	61.9	61.9	9.7	9.5
2	CH_3	169–170	36.4	36.1	6.1	5.9
			I, 42.7	I, 42.7		
4		171–172	63.8	64.0	10.1	10.2
4	CH_3	147–148	38.6	38.2	6.5	6.4
			I, 40.8	I, 40.6		
5		142–143	64.8	64.8	10.3	10.4
7		131–132	66.2	66.3	10.6	10.7

Piperidinoethylamine[1] was prepared both by the catalytic hydrogenation[2] of piperidinoacetonitrile[3] and also by the reaction of excess ethylenediamine with pentamethylene dibromide.[4] Pyrrolidinoethylamine[5] was obtained by the latter method using excess ethylenediamine and tetramethylene dibromide.

Neither the bis-tertiaryamino amides nor their bis-methiodides showed any significant curare-like activity. However, the tertiary amino amides were powerful potentiators of the curare-like activity of diacetylcholine[6,7] (succinylcholine). In this respect the bis-β-piperidinoethyl succinamide was about five times as active as the bis-β-dimethylaminoethyl succinamide bis-methiodide reported earlier.[8] Surprisingly, the bis-methiodides of the piperidinoethyl amides were virtually inactive as potentiators of diacetylcholine. Activity in the bis-piperidino amide series was present in all members above the oxalic derivative, thus in malonic through sebacic, but was maximal in the succinic, glutaric, adipic group. In the pyrrolidinoethyl

(1) S. Gabriel, *Ber.*, **24**, 1110 (1891).
(2) C. F. Winans and H. Adkins, THIS JOURNAL, **55**, 4167 (1933).
(3) E. Knoevenagel, *Ber.*, **37**, 4073 (1904).
(4) V. J. van Alphen, *Rec. trav. chim.*, **56**, 529 (1937).
(5) V. J. van Alphen, *ibid.*, **58**, 1105 (1939).
(6) A. P. Phillips, THIS JOURNAL, **71**, 3264 (1949).
(7) J. C. Castillo and E. J. deBeer, *J. Pharmacol. Exp. Therap.*, **99**, 458 (1950).
(8) A. P. Phillips, THIS JOURNAL, **73**, 5822 (1951).

amides, activities were about one-half those of the analogous piperidino amides, while maximum activity was approached at a longer chain length than in the latter series.

These bis-amides also are powerful antagonists to the block of neuromuscular transmission produced by *d*-tubocurarine chloride, and rapidly reverse the paralysis induced by the latter. Roughly similar relationships between structure and activity, in the bis-amides, hold for the two principal kinds of activity, potentiation of diacetylcholine and antagonism of *d*-tubocurarine.

A detailed pharmacological report will be published elsewhere.

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Experimental

Bis-aminoalkyl Amides.—A mixture of 1 mole of dicarboxylic ester and 2.5 moles of piperidinoethylamine was refluxed in a metal-bath at 190° for a period of from four to 24 hours. Excess amine and alcohol, formed in the reaction, were removed *in vacuo*. Upon cooling the product usually crystallized. Purification was accomplished by recrystallization from ethyl acetate or methanol–ethyl acetate mixtures. Yields were between 60 and 90%.

Bis-methiodides.—Brief refluxing of a methanol solution of the bis-β-tertiary amino amides with excess of methyl iodide usually gave nearly quantitative yields of the bis-methiodides. These were purified by recrystallization from methanol or methanol–ethyl acetate mixtures.

The above outline illustrates the preparative methods used. Details for all compounds appear in Table I. All melting points are uncorrected.

TUCKAHOE 7, NEW YORK

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF CALIFORNIA AND THE RADIATION LABORATORY OF THE UNIVERSITY OF CALIFORNIA]

The Regeneration of Squalene from its Solid Hexahydrochloride

BY WILLIAM G. DAUBEN, H. LEON BRADLOW, N. K. FREEMAN, DAVID KRITCHEVSKY AND MARTHA KIRK

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It has been found that natural squalene is of a homogeneous trialkylethylene bond structure but that squalene which has been regenerated from its solid hexahydrochloride possesses both the trialkylethylene and the unsymmetrical dialkylethylene types of bond structures. It is estimated that between 20-40% of this latter type of structure can be present. Methyl group determination by oxidation has been shown to be unreliable. It has been found that squalene can be chromatographed on "Quilon" treated paper and can be separated from cholesterol by this method.

The recent interest[1,2] in the early postulate of Robinson[3] that the triterpene squalene could act as a precursor of cholesterol has prompted us to initiate the preparation of the compound labeled with C^{14}. Before this could be done, it has been necessary to reinvestigate certain phases of the chemistry of this hydrocarbon. Heilbron and his collaborators[4] have studied, in detail, the isolation and purification of squalene. They found that the solid hexahydrochloride which is easily prepared and purified is a convenient intermediate to employ in the purification of the compound. They reported that at least two isomeric hexahydrochlorides could be obtained but upon removal of the acid both solids yielded what was apparently the same compound. Since it has been demonstrated by various investigators[5,6] that many natural terpenes occur as a mixture of carbon–carbon double bond isomers of the isopropylidene and isopropenyl type, it was deemed important to investigate this problem with regard to natural and regenerated squalene.

Natural squalene, obtained from a commercial source,[7] was redistilled and converted into its hexahydrochloride and then regenerated by heating with pyridine as described by Heilbron.[4] The infrared spectra of the materials are shown in Fig. 1.

The infrared spectrum of squalene has been published previously[8] but no mention of the source of the material was given. The results in Fig. 1 are consistent with the accepted structure of squalene, in which all of the double bonds can be regarded as trialkylethylenes, *viz.*, $RR'C{=}CHR''$. A characteristic absorption band for this class of alkenes is found in the range from 12.0 to 12.5 μ,[5,9,10] In light of the recent spectroscopic investigation of acyclic terpenes by Barnard and his collaborators[6] it is clear that the fraction of double bonds having the alternate configuration $RR'C{=}CH_2$, is quite small and may well be zero. The characteristic band for this latter structure is more sharply defined at about 11.25μ[5,9,10] and according to Barnard, *et al.*,[6] the molar extinction coefficient of this band is four to five times as great as that of the 12μ band of the trialkylethylenes. Using this estimated ratio of intrinsic intensities, an upper limit of about 3% was set by them on the amount of $RR'C{=}CH_2$ in purified natural terpenes. Squalene conforms with this result.

The spectrum of squalene regenerated from the hexahydrochloride differs from that of natural material. A strong new absorption band appears

(1) K. Bloch, "Recent Progress in Hormone Research," Vol. VI, Academic Press, Inc., New York 10, N. Y., 1951, p. 111 ff.

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