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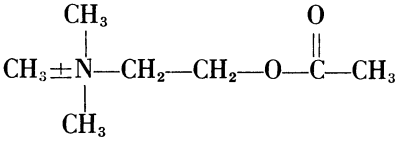
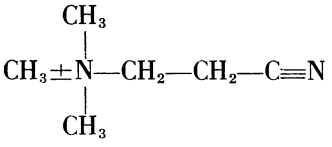
Introduction

In recent years there has developed a keen interest in the limits through which chemical constitutional changes in biological potent drugs may be carried without great loss of their characteristic pharmacological activity. In this regard the present study concerns itself with the effect of replacing the acetate by a cyano group in acetylcholine. This type of constitutional change is illustrated below:



Acetylcholine



Cyano (CN) analogue
β-Trimethylammonium
propionitrile iodide

According to a recent hypothesis suggested by Pfeiffer (1948) requirement for maximal muscarinic activity "depends on adjacent prosthetic oxygen atoms at a distance of approximately 5.0 Å and 7.0 Å, respectively, from one or more methyl groups attached to nitrogen." Of further interest in this regard are the articles by Welsh (1950) and Schueler (1950). Data are given in the article by Welsh (1950) in which the ether and ketonic oxygen groups are independently replaced by the methylene group, the remaining oxygen group in each compound thus retaining essentially the identical distance as is found in acetylcholine. Welsh's data show the effects of these changes in regard to the depressant effects of the materials on the heart of the clam, *Venus mercenaria*. Schueler (1950) reversed the carboxyl group in acetylcholine to give methyl (β-trimethylammonium) propionate and showed this compound to be strikingly similar, quantitatively as well as qualitatively, to acetylcholine in a number of its effects. Upon examining models of the above compounds studied by the above authors it was noted that there was one or more methyl groups attached to a nitrogen

group which is a cation at a distance of 5 to 7 Å from a polarized group containing an electrophilic carbon bound to a nucleophilic hetero-atom. This relationship is better shown in table 1.

In view of these considerations the (CN) analogue was prepared and studied in various pharmacological preparations.

Table 1

Maximal Inter-Atomic Distances of Acetylcholine and Related Compounds

	Distance in Å from the center of onium ion to the center of the nucleophilic hetero-atom
$CH_3CH_2-(CH_3)_2\overset{+}{N}CH_2CH_2O\overset{O}{\overset{\|}{C}}CH_3$	7
$(CH_3)_3\overset{+}{P}CH_2CH_2O\overset{O}{\overset{\|}{C}}CH_3$	7
$(CH_3)_3\overset{+}{N}CH_2CH_2\overset{O}{\overset{\|}{C}}OCH_3$	5
$(CH_3)_3\overset{+}{N}-CH_2CH_2O\overset{O}{\overset{\|}{C}}NH_2$	7
$(CH_3)_3\overset{+}{N}CH_2CH_2CN$	6.5

These inter-atomic distances were measured by means of Fischer-Hirschfelder-Taylor atomic models.

Experimental

β-Trimethylammonium propionitrile Iodide: Acrylonitrile was allowed to react with dimethylamine in the cold. The β-dimethylaminopropionitrile produced was fractionally distilled and then reacted with methyl iodide as described by Terent'ev and Kost (1947).

Acute Toxicity: Toxicity experiments were carried out on white mice, using the method of probits following intraperitoneal administration in physiological saline.

Circulatory System: These experiments were performed on four dogs anesthetized with nembutal solution 36 mg/kg. intravenously. One common carotid was cannulated and blood pressure recorded from a mercury manometer writing on a smoked drum. The (CN) analogue and acetylcholine bromide were dissolved in physiological saline and injected in a constant volume, via the femoral vein.

Isolated Gut: Guinea pig ileum strips, approximately two cm. in length were suspended in a muscle cup of 25 ml. capacity arranged

for ready renewal of the bath medium, which consisted of oxygenated Tyrode's solution. After a suitable period for the adjustment of the gut strip toward a constant amplitude of spontaneous contraction, the drugs were injected into the bath as solutions in the Tyrode's medium.

Salivation: Dogs were anesthetized with nembutal, 36 mg/kg. intravenously, Wharton's duct was cannulated and the chorda tympani sectioned. The drugs, dissolved in physiological saline, were injected into a femoral vein, a constant injection volume was maintained.

Results

Acute Toxicity (table 2): At all dose levels used in determining the acute toxicity of the (CN) analogue the mice exhibited extreme salivation, lacrimation, defecation and urination followed by muscular tremors which developed into clonic convulsions. Death was apparently due to respiratory arrest, when the chest was opened immediately upon cessation of respiration a feeble, rapid heartbeat was observed. All animals which died following intraperitoneal administration of the drug did so within thirty minutes.

Table 2.

Acute Toxicity of (CN) Analogue of Acetylcholine

	Dose mg/kg.	Log Dose	No. Mice Used	No. Killed	% Kill	Probit
Intraperitoneal	40.0	1.60	20	1	5	3.35
	60.0	1.78	20	9	45	4.87
	80.0	1.90	20	19	95	6.65

LD_{50} (IP) = 63 mg/kg.

Effects on Circulatory System: The (CN) analogue in doses of ten to fifty microgms/kg. produced a depressor response in three dogs comparable to doses of two microgm/kg. of acetylcholine bromide. With the (CN) analogue, as well as with acetylcholine, the response was immediate in onset and of brief duration. The depressor effects of both drugs were blocked by atropinization, following which both agents exhibited comparable nicotinic-stimulant action in doses of 50 mg/kg.

Effects on Isolated Gut and Salivation: The (CN) analogue was found to be 1/1000 as potent as acetylcholine bromide in stimulating the guinea pig ileum (table 3 and figure I), but by plotting per cent contraction *vs* dose, the dose response curves were parallel and both

were blocked by the same concentration of atropine. The (CN) analogue was 1/300 as potent as acetylcholine bromide in stimulating salivary secretion on the dog.

Table 3.
Effects of the (CN) Analogue of the Guinea Pig Ileum

Dose of (CN) Analogue in mg.	Per Cent Total Contraction of Ileum	Dose of Acetylcholine in mg.	Per Cent Total Contraction of Ileum
1.000	20	0.001	11
2.000	40	0.002	28
3.000	56	0.005	58
4.000	81	0.010	88
5.000	98	0.500	100
Atropine 0.005 followed by 4.000	6	Atropine 0.005 followed by 0.005	7

Bath volume 25 ml.

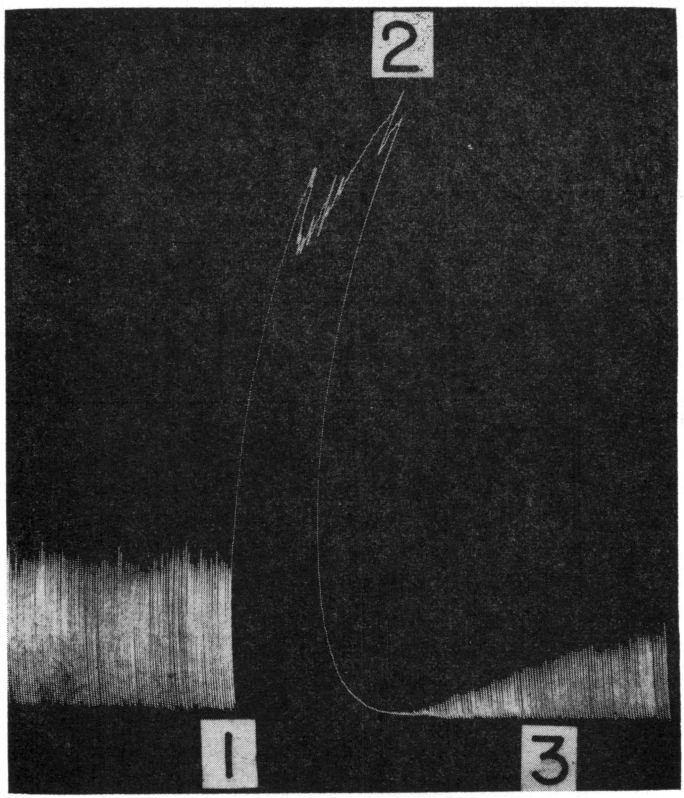


Figure 1. Effect of the (CN) Analogue on the Isolated Guinea Pig Ileum. 1—(CN) analogue 1.000 mg.; 2—atropine sulfate 0.010 mg.; 3—dose 1 repeated.

Discussion

The (CN) analogue of acetylcholine has been prepared and studied in a number of animal and isolated tissue preparations, where it has shown striking qualitative similarities to acetylcholine in all of its effects tested but it is quantitatively less potent. The studies of the effects of the (CN) analogue on the isolated ileum of the guinea pig, anesthetized dog blood pressure, and salivary secretion in the dog indicated it to possess both muscarinic and nicotinic activity.

The (CN) analogue may be looked upon as a modification of acetylcholine with respect to physiological actions. Thus to explain the actions of this compound it is necessary to modify the hypothesis suggested by Pfeiffer (1948) for maximal muscarinic activity. The modified hypothesis is as follows: For maximal muscarinic activity it is necessary to have an onium ion which is a strong cation at a maximal distance of 5 to 7 Å from a polarized group containing an electrophilic atom bound to a nucleophilic hetero-atom in which the substituents are small.

Acknowledgment is made to Edward V. Dietrich, Dr. Hugh Keasling and especially to Dr. F. W. Schueler from whose formulation of the general approach to the structure action relationships in this class of compounds, the author was lead to develop and study the (CN) analogue.

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