AD-782 491

STRUCTURE-ACTIVITY RELATIONSHIPS ON COMPOUNDS HAVING NEURO-MUSCULAR ACTIVITY

Ted A. Loomis

Washington University

Prepared for:

Army Research Office-Durham

28 March 1974

Unclassified
Security Classification

ARO 11619.3-L

AD782491

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)
University of Washington

2a. REPORT SECURITY CLASSIFICATION
Unclassified

2b. GROUP
NA

3. REPORT TITLE
STRUCTURE-ACTIVITY RELATIONSHIPS ON COMPOUNDS HAVING NEURO-MUSCULAR ACTIVITY

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)
Final Report: 1 January 1972 - 31 December 1973

5. AUTHOR(S) (First name, middle initial, last name)
Ted A. Loomis

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
28 Mar 74	29	

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
DAHC19 72 G 0008	NA
b. PROJECT NO.	
c.	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d.	11619.3-L

DDC
RECEIVED
AUG 5 1974
C

10. DISTRIBUTION STATEMENT
Approved for public release; distribution unlimited.

11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
None	U. S. Army Research Office Box CM, Duke Station Durham, North Carolina 27706

13. ABSTRACT

This report deals with the biological activity of four different series of compounds which were developed to clarify the structure-activity relationships required for optimal biological activity. Three of the series were carbamates with potent antiesterase activity. These three series were (1) Bis symmetrical quaternary ammonium isoquinoline carbamates, (2) Bis unsymmetrical quaternary ammonium isoquinoline carbamates, and (3) Tetra and Octa quaternary ammonium isoquinoline carbamates. In the course of the study, a series of hemicholinium-like compounds was further developed and tested for both hemicholinium-like action as well as for antiesterase action. The latter are methyl pyridinio and 2 hydroxy ethyl dimethyl ammonio analogues.

14. KEY WORDS
Molecular structure
Biological compounds
Chemical reactions
Neuro-muscular activity
Carbamates
Ammonium compounds

The findings in this report are not to be construed as an official Department of the Army position, unless so designated by other authorized documents.

DD FORM 1 NOV 1473 REPLACES DD FORM 1473, 1 JAN 64, WHICH IS OBSOLETE FOR ARMY USE.

Unclassified
Security Classification

TABLE OF CONTENTS

I. Foreward: The compounds described in this study were synthesized by Mr. Harold Sommer at Edgewood Arsenal, Maryland. The current report deals with the biological activity of four different series of compounds which were developed to clarify the structure-activity relationships required for optimal biological activity. Three of the series were carbamates with potent antiesterase activity. These three series were (1) Bis symmetrical quaternary ammonium isoquinoline carbamates, (2) Bis unsymmetrical quaternary ammonium isoquinoline carbamates, and (3) Tetra and Octa quaternary ammonium isoquinoline carbamates. In the course of the study, a series of hemicholinium-like compounds was further developed and tested for both hemicholinium-like action as well as for antiesterase action. The latter are methyl pyridinio and 2 hydroxy ethyl dimethyl ammonio analogues.

GENERAL METHODS APPLIED THROUGHOUT THE STUDIES

(1) Anticholinesterase action was determined by the Michel ΔpH method (Michel, 1949) using the red blood cell buffer on semi-purified bovine red blood cell cholinesterase (Winthrop Chemical Company) using acetylcholine chloride as the substrate. Activity was determined by continuous recordings of the change in pH.

(2) The 24-hour LD_{50}'s were determined in 25-30 gram mice (male Swiss-Webster Strain) and were estimated according to the method of Litchfield and Wilcoxon (1949). Four to six animals were used for each dose. All compounds were administered to the mice by I.V. injection into a tail vein.

(3) The in vivo tests were conducted on a modified anterior tibial nerve-muscle preparation previously described by Loomis and Salafsky (1964). Sprague-Dawley rats of either sex, weighing 300-500 grams were anesthetised with pentobarbital (30mg/kg, ip). The trachea and an external jugular vein were cannulated. The anterior tibial branch of the left sciatic nerve was isolated close to its insertion in the anterior tibial muscle. Shielded electrodes were placed on the anterior tibial nerve and the nerve was transected proximal to the electrodes. The left femur was immobilized with metal clamps. The distal tendon on the anterior tibial muscle was transected at its insertion and attached to the core of a Sanbourn linear transformer (7DCDT-100) the output of which was recorded on a Brush (Mark 280) linear recorder. Stimuli were applied to the electrodes from a Tektronix stimulator (type 160-161-162) which was triggered by a precision electronic timer (Heath model EU-801-A). The output of the stimulator was continuously monitored with a digital pulse counter (Heath model EU-805). With this equipment precise interrupted tetanic stimuli were applied to the anterior tibial nerve at a frequency of 50 pulses/second for a duration of 0.5 seconds and repeated every 15 seconds. Each pulse was of 5 milliseconds duration at a supramaximal voltage of 2.5 volts. Control experiments indicated that the rat nerve-muscle preparation maintained maximal tetanic response to the tetanic stimuli for the duration of the experiments. All subsequent injections were made intravenously in the external jugular vein.

Ref: Michel, H.O. (1949), Jr. Lab. and Clinical Med. 34, 1564-1568.
Litchfield, J.T. and Wilcoxon, F (1949), Jr. Pharm. and Exp. Therap. 96, 99-113.
Loomis, T.A. and Salafsky, B. (1964), Jr. Pharm. and Exp. Therap. 144, 301-308.

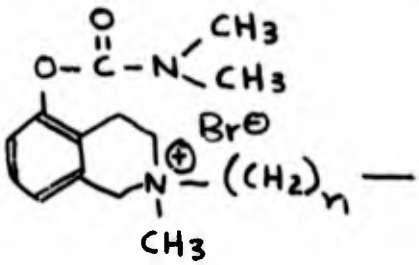
RESULTS AND CONCLUSIONS OF STUDIES ON THE BIS SYMMETRICAL ISOQUINOLINE CARBAMATES

The series of compounds in this group are shown in Figure 1. The only structural difference between the members of the series is in the length of the aliphatic chain (n) separating the quaternary nitrogen atoms. The PI_{50}'s (negative log molar concentration required to produce 50% inhibition of cholinesterase) were found to be between 3.88 and 7.92 for the various members of the series and the potency was directly related to n with the exception of n4. This is shown in Tables 1 and 2. The LD_{50}'s were indirectly related to the n numbers for those members of the series from n4 to n9 and directly related to the n numbers from n9 to n16. Therefore the compound with the greatest lethal potency was n9 (LD_{50}=.027 mg/kg). These data are shown in Tables 1 and 3. Table 4 shows the relationship between the PI_{50}'s and the LD_{50}'s for the series of compounds.

The most potent anitesterase compound is not the most potent lethal compound in the series. Optimal antiesterase activity and lethal potency were evident in compound n9.

The duration of action of the compounds measured as the half-life (of a standard dose of 100 µg/kg I.V.) of the effect of the compounds on the intact rat neuro-muscular preparation was determined for compounds n6, 8, 10 and 12. An example of the effect of a single I.V. injection of compound n8 is shown in Figure 2. Table 5 shows the relationship between the LD_{50} and the half-life of the neuro-muscular effect in the intact animal. The table shows a direct relationship between the LD_{50} and the half-life and that the relatively high lethal potency of the n8 and 10 compounds as compared to the n6 and 12 compounds is directly related to longer half-life of the n8 and 10 compounds. The half-life of action on the neuro-muscular system is related to the ability of the kidney to terminate the action of the compounds. This was demonstrated by measuring the half-life of n10 and 12 in normal and nephrectomyzed rats. These data are shown in Table 6.

Bis-symmetrical Isoquinoline Carbamates



No.	n	M.W. and H_2O amt.	True M.W.
5476	3	679.53 ½ mole	670.52
5475	4	693.56 ½ mole	684.55
5479	5	716.59 1 mole	698.57
5480	6	730.62 1 mole	712.60
5481	7	740.15 ¾ mole	726.63
4874	8	749.66 ½ mole	740.65
5482	9	772.70 1 mole	754.68
4870	10	786.72 1 mole	768.71
5483	11	796.26 ¾ mole	782.74
5484	12	819.28 1¼ mole	796.76
5485	16	861.88 ½ mole	852.87

Figure 1

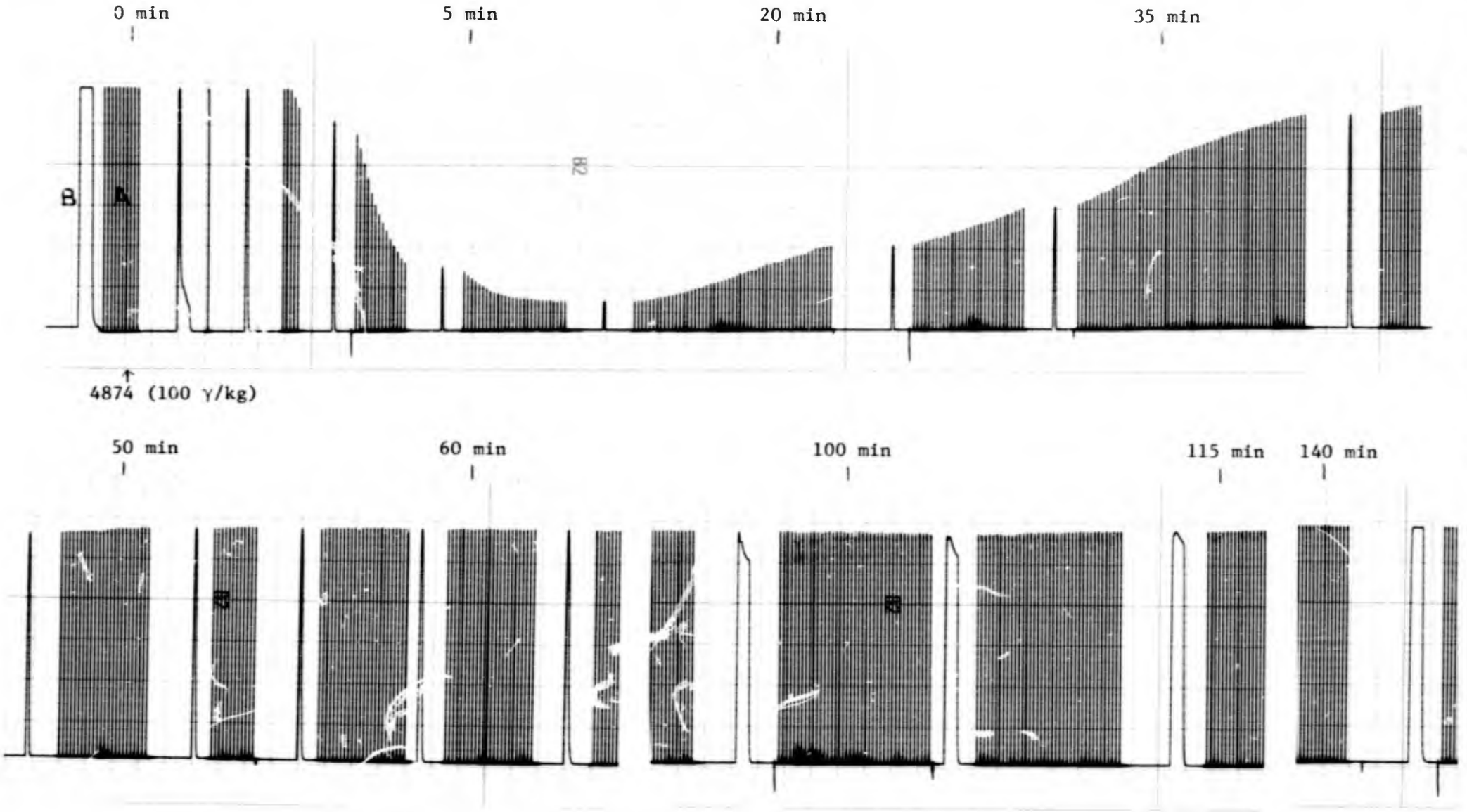


Figure 2

Effect of 4874(C8) on the Intact Anterior Tibial Muscle of the Rat: Interrupted tetanic stimulation, 50/sec, given for 0.5 sec every 15 sec. Pulse duration = 5 msec, voltage = 2.5 V. Paper speed = 0.05 mm/sec (A) and 5 mm/sec (B).

BIS-SYMMETRICAL ISOQUINOLINE SERIES

Number	n	PI50	LD50 (mg/kg)
5476	3	4.17	5.3
5475	4	3.88	10.5
5479	5	4.71	1.3
5480	6	5.80	0.22
5481	7	6.40	0.055
4874	8	7.04	0.034
5482	9	7.18	0.027
4870	10	7.66	0.042
5483	11	7.55	0.10
5484	12	7.77	0.15
5485	16	7.92	0.44

Table 1

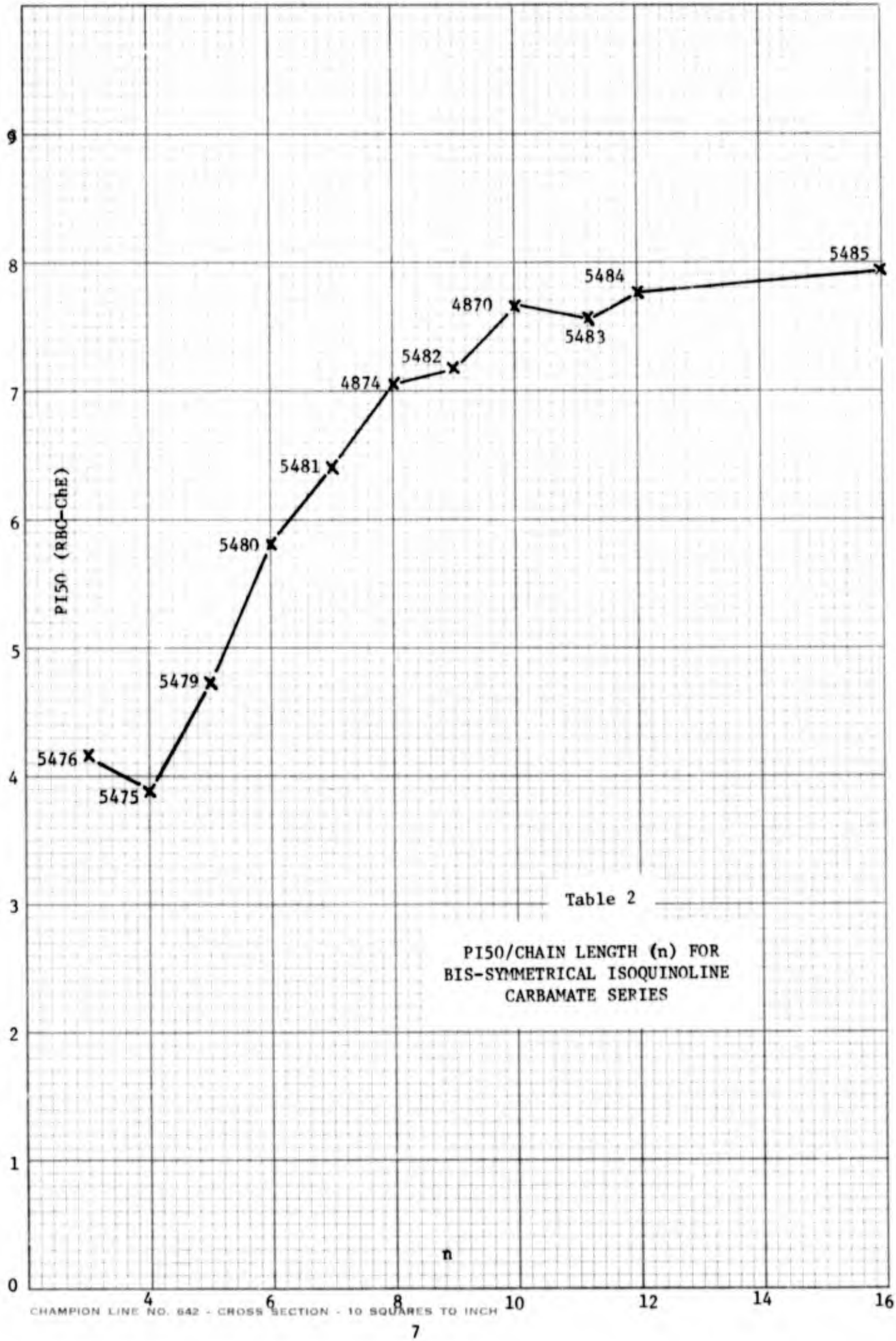


Table 2

PI50/CHAIN LENGTH (n) FOR BIS-SYMMETRICAL ISOQUINOLINE CARBAMATE SERIES

Table 3

LD50/CHAIN LENGTH(n) FOR
BIS-SYMMETRICAL ISOQUINOLINE
CARBAMATE SERIES

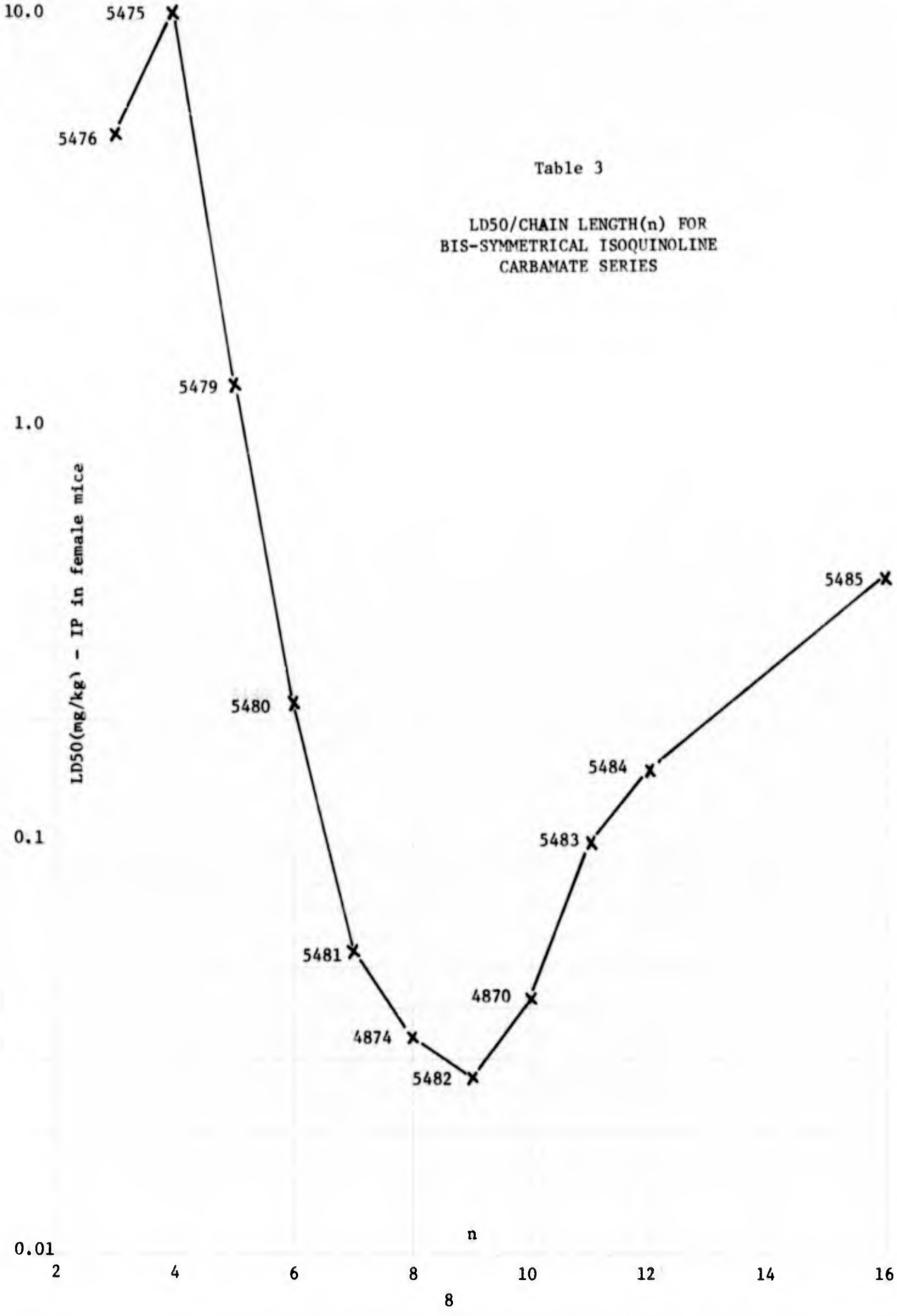
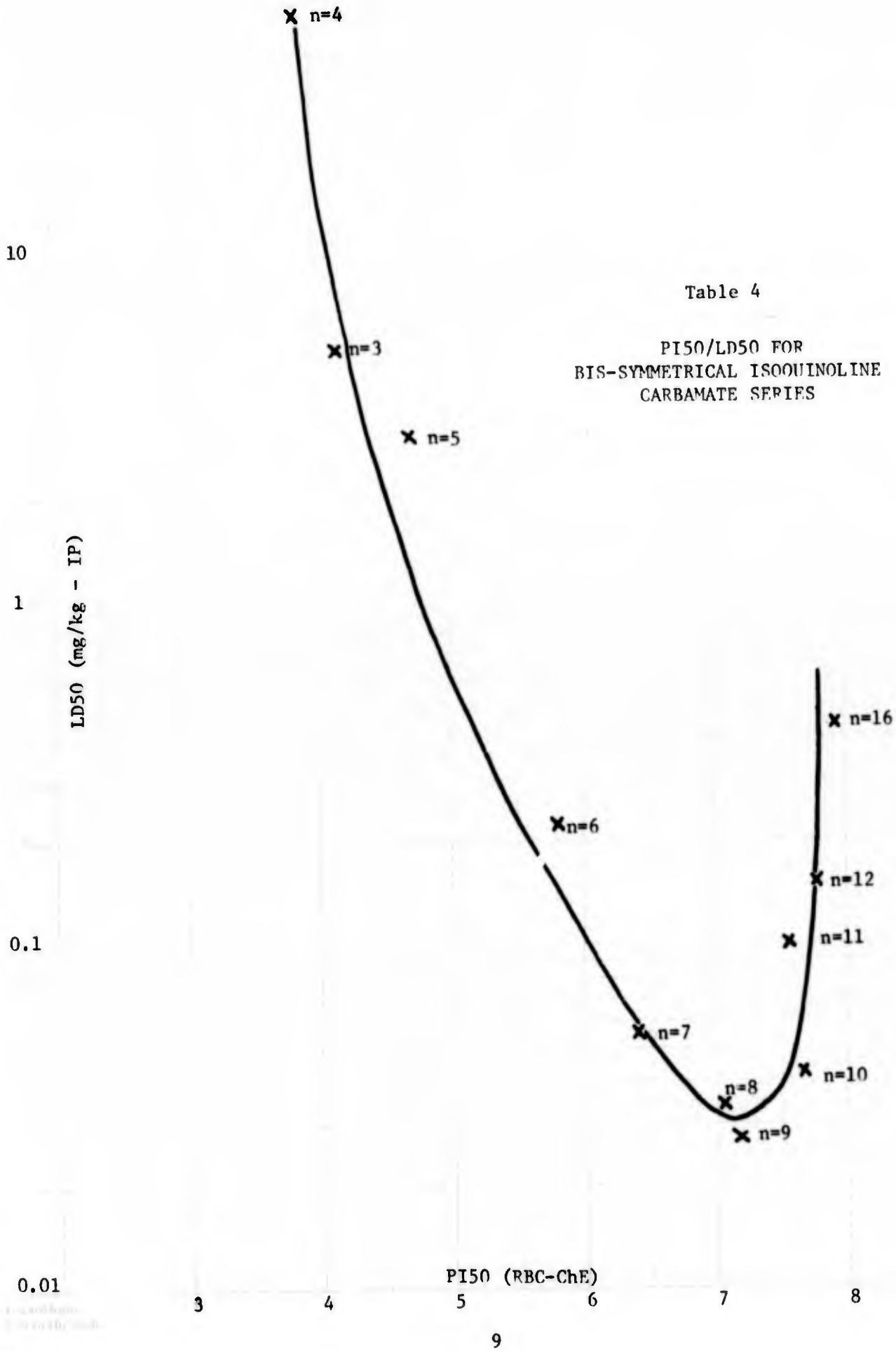


Table 4

PI50/LD50 FOR
BIS-SYMMETRICAL ISOQUINOLINE
CARBAMATE SERIES



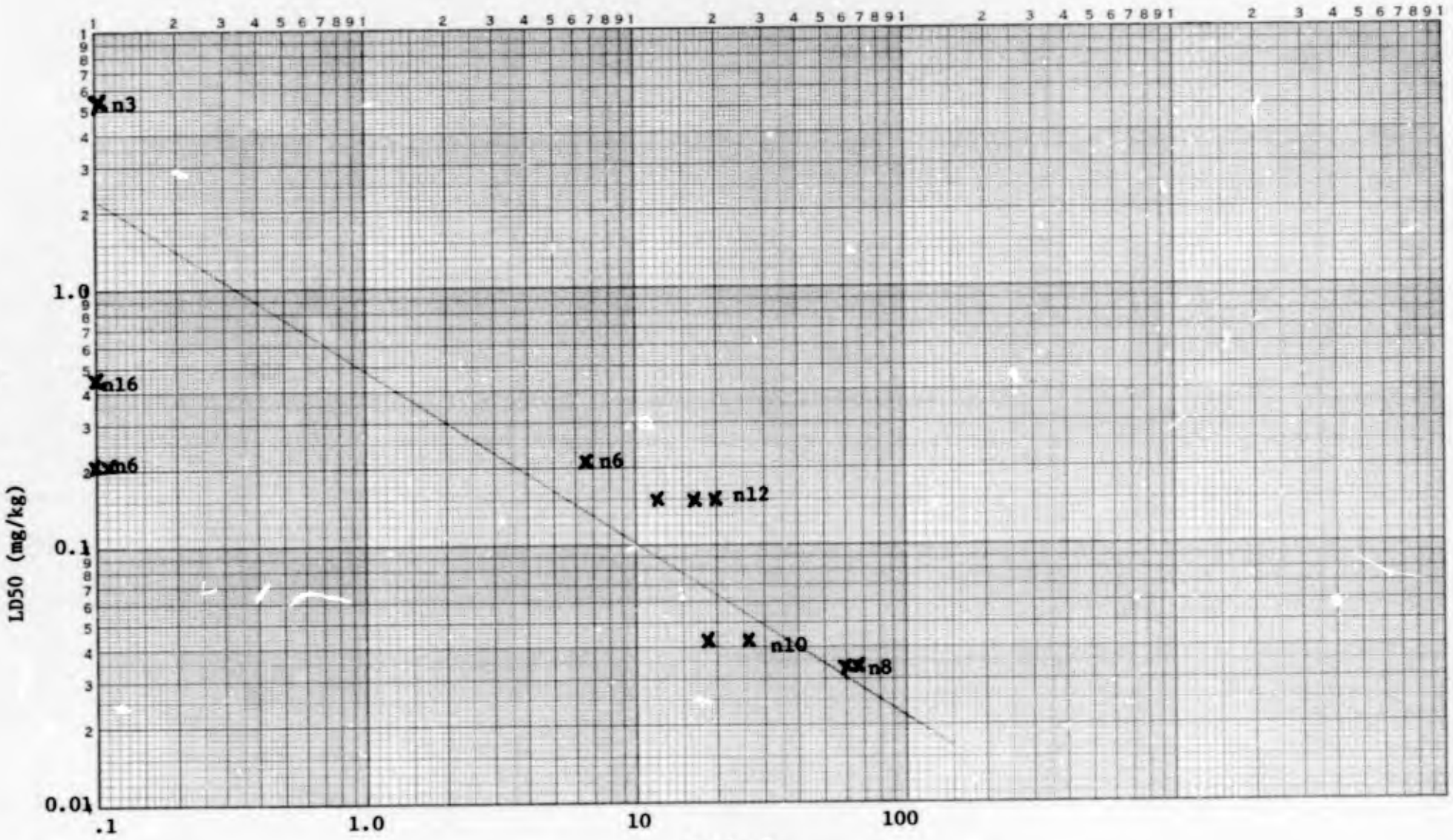


Table 5
Half-Life of Neuromuscular Effect (min)
Each X represents 1 animal.

Duration of Action of 4870 (n10) and 5484 (n12) in Nephrectomized vs. Control Rats. Dose = 100 µg/kg

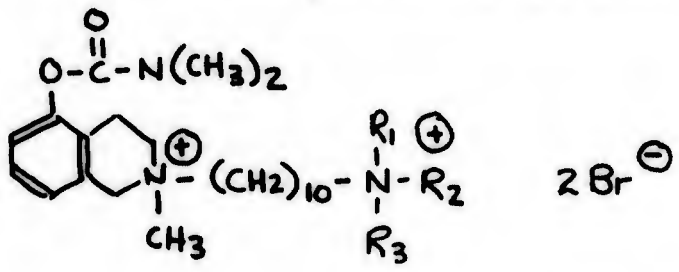
	1/2 Life (min) Control animals	1/2 Life (min) Nephrectomized animals	Ratio: Control/Nephrectomized
4870 n10	18.8	80.0	
	26.5	140.0	
	30.5	75.0	
	22.2	110.0	
	24.5 (Ave.)	101.2 (Ave.)	1/4.1
5484 n12	12.5	45.0	
	20.2	67.5	
	16.8	50.0	
	16.5 (Ave.)	54.0 (Ave.)	1/3.2

Table 6

RESULTS AND CONCLUSIONS OF STUDIES ON THE BIS UNSYMMETRICAL ISOQUINOLINE CARBAMATES

The series of compounds in this group are shown in Figure 3. The inter nitrogen distance is the same for all members of this series, i.e. n=10. The members of the series differ in the bulk of the substituted quaternary nitrogen which is not in the isoquinoline ring. Table 7 shows the PI_{50}'s and the LD_{50}'s for the members of the series. The PI_{50}'s varied over the small range of 6.40 to 7.34 and the LD_{50}'s varied from .061 mg/kg to .145 mg/kg. Notable is the fact that the trimethyl and the triethyl members of the series showed identical LD_{50}'s (.061mg/kg) but the PI_{50}'s were 6.4 and 7.06 respectively. Also the dimethyl ethanol and the dimethyl propanol compounds had different LD_{50}'s (.12 and .60 mg/kg respectively) but had similar PI_{50}'s (6.63 and 6.69 respectively). Therefore, there was no consistant relationship between the bulk as measured by the total molecular weight of the $R_1 + R_2 + R_3$ radicals on the nitrogen, and the lethality or the antiesterase activity. (Figures 4 and 5).

Bis-unsymmetrical Isoquinoline Carbamates



No.	M. W.	R_1	R_2	R_3
5471	635	$-CH_2-CH_3$	$-CH_2-CH_3$	$-CH_2-CH_3$
5443	747	$-CH_3$	$-(CH_2)_{11}-CH_3$	$-CH_3$
5492	593	$-CH_3$	$-CH_3$	$-CH_3$
5493	618	$-CH_3$	$-CH_2CN$	$-CH_3$
5474	623	$-CH_3$	$-CH_2CH_2OH$	$-CH_3$
5494	637	$-CH_3$	$-(CH_2)_3-OH$	$-CH_3$
5506	653	$-CH_3$	$-CH_2CH_2OH$	$-CH_2CH_2OH$

Figure 3

BIS-UNSYMMETRICAL ISOQUINOLINE SERIES

Number	PI50	LD50(mg/kg)
5471	7.06	0.061
5443	7.34	0.66
5492	6.40	0.061
5493	6.52	0.145
5474	6.63	0.12
5494	6.69	0.60
5506	6.65	0.072

Table 7

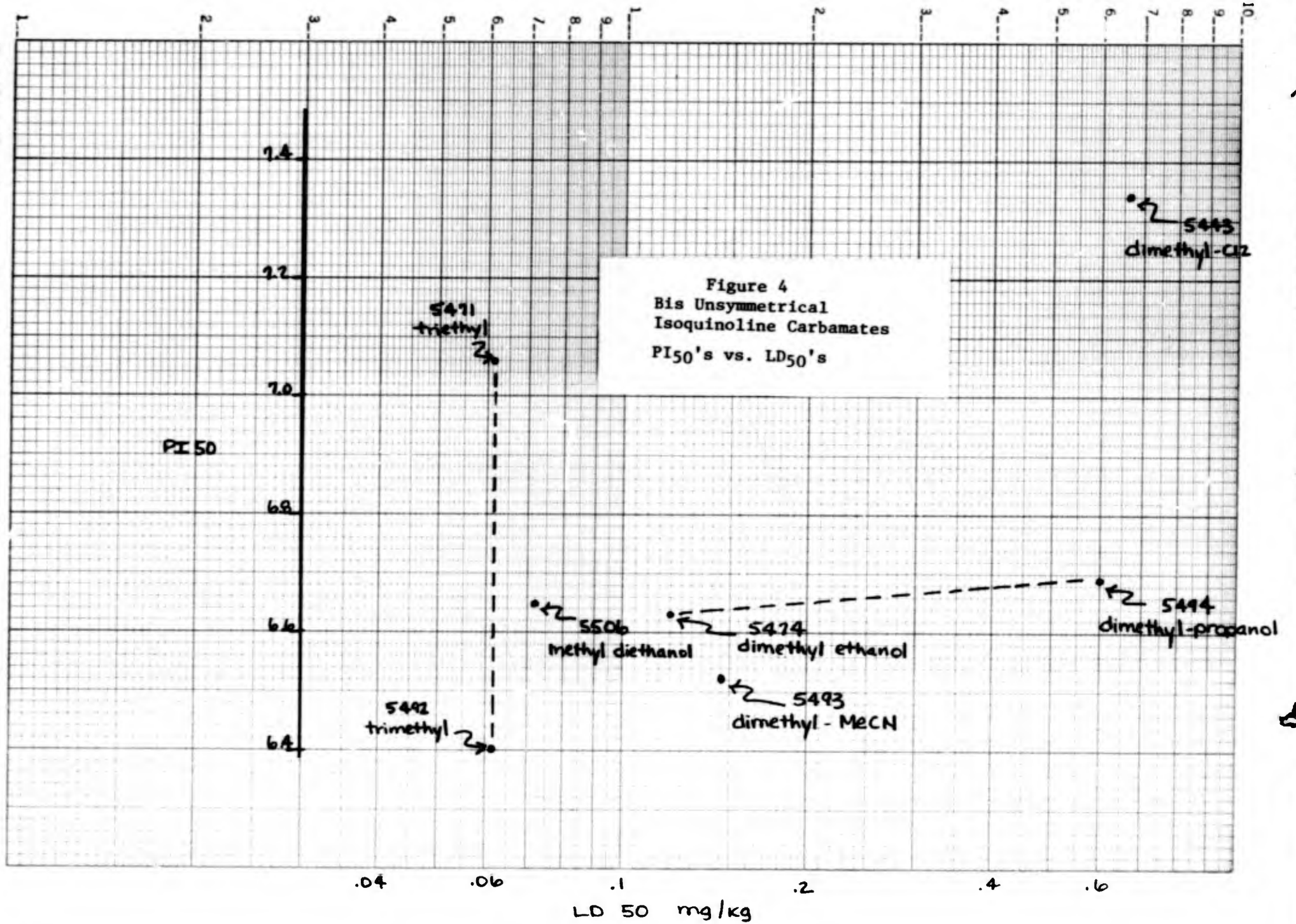
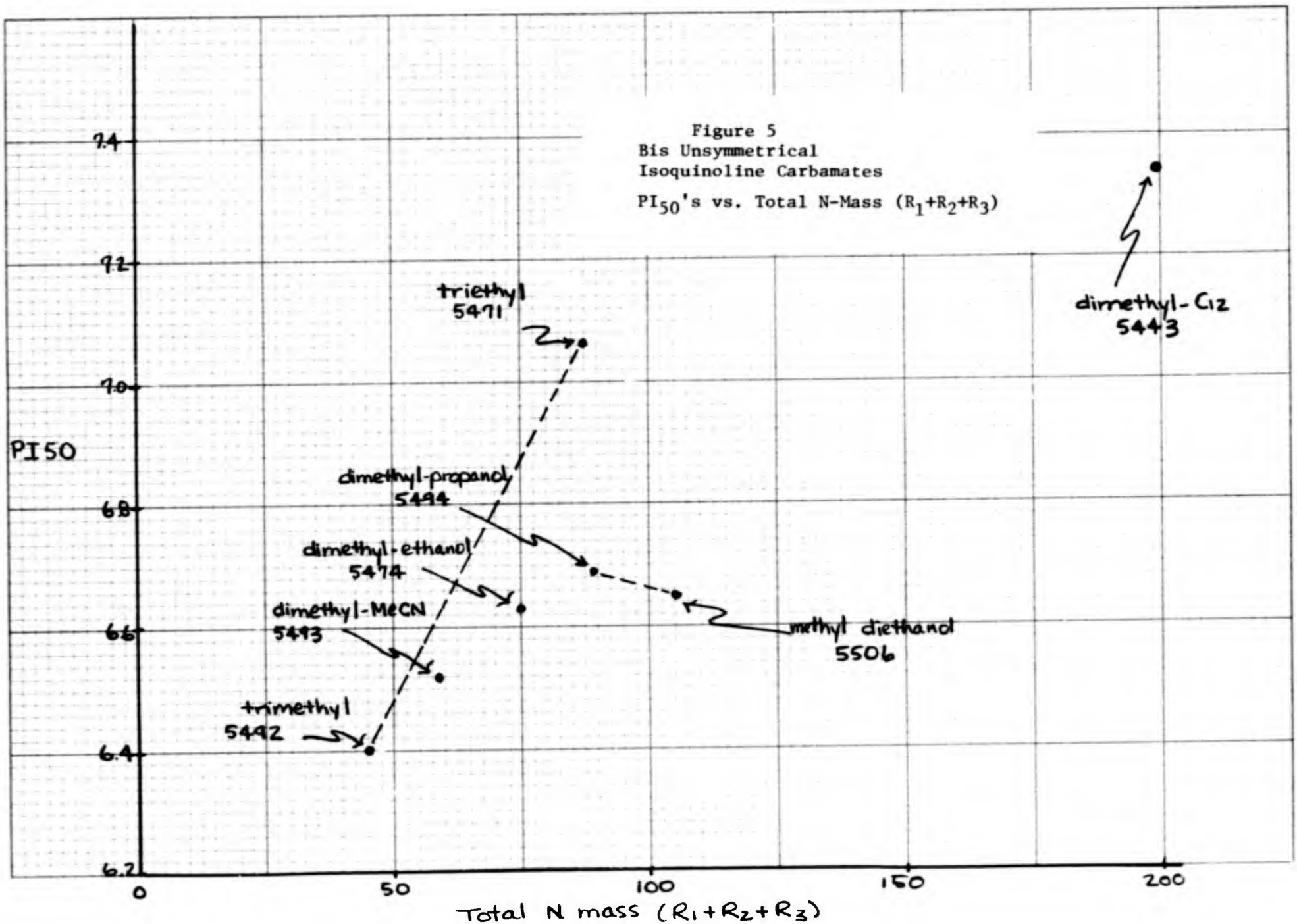


Figure 4
Bis Unsymmetrical
Isoquinoline Carbamates
PI_{50}'s vs. LD_{50}'s



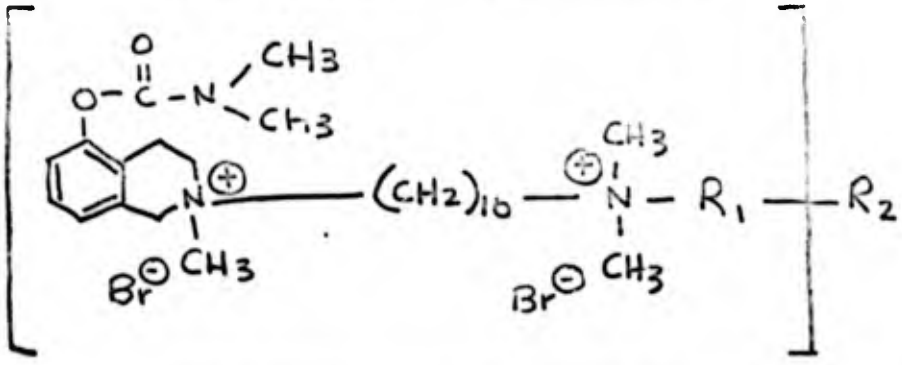
RESULTS AND CONCLUSIONS OF STUDIES ON THE "TETRA" AND "OCTA" QUATERNARY AMMONIUM ISOQUINOLINE CARBAMATES

The series of compounds in this group are shown in Figure 6. The figure also shows the molecular weights of the members of the series. The compounds have relatively high molecular weights compared to the previous series of symmetrical and unsymmetrical isoquinoline carbamates. Those members of the series having four quaternary nitrogen atoms have molecular weights of 1185 to 1333. It will be noted that the "octa" compound has a molecular weight of 3165.

Table 8 lists the LD_{50}'s and the PI_{50}'s for each member of the series. Figure 7 shows a direct relationship between the chain length (R_1+R_2) for the members of the series and the PI_{50}'s. Since the LD_{50}'s for the various members of the series of "tetra" compounds were similar (.050 to .063 mg/kg), chain length did not significantly affect the lethality of the members of this series (Figure 8). Figure 9 shows the relationship between the LD_{50}'s and the PI_{50}'s for the "tetra" compounds.

The molecularly large "octa" compound had a PI_{50} that was an order of magnitude greater than the PI_{50} of some of the "tetra" compounds. The "octa" compound has antiesterase potency equal to that of the most potent symmetrical isoquinoline series of compounds. In contrast to this the lethal potency of the "octa" compound is low (.32 mg/kg) compared to that of the "tetra" compounds (.050 to .063 mg/kg). This may be the result of impaired translocation to effector sites in the intact animal because of the molecular bulk of the "octa" compound.

"TETRA" AND "OCTA" QUATERNARY AMMONIUM ISOQUINOLINE CARBAMATES



No.	M. W.	R_1	R_2	H_2O
5540	1185.02	$-CH_2-$	—	—
5579	1232.08	$-CH_2-$	$-CH_2-$	2 moles
5581	1247.09	$-CH_2-CH=$	—	2 moles
5582	1249.11	$-(CH_2)_2-$	—	2 moles
5583	1286.17	$-(CH_2)_3-$	—	2½ moles
5584	1305.21	$-(CH_2)_4-$	—	2 moles
5585	1333.27	$-(CH_2)_5-$	—	2 moles
5586	3165.15			6 moles

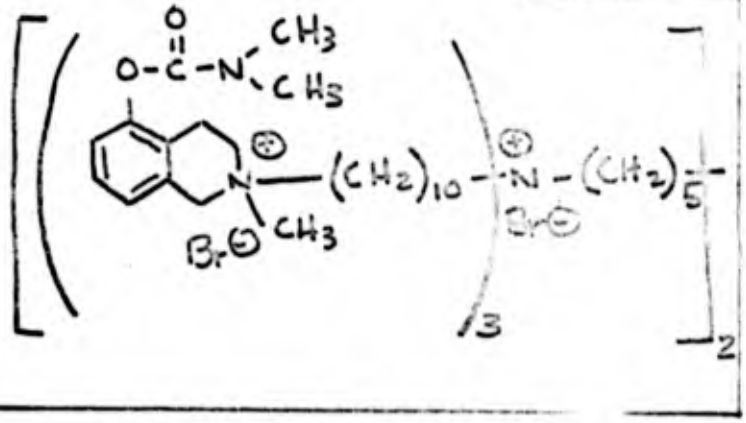


Figure 6

"TETRA" AND "OCTA" COMPOUNDS

		M.W.	LD50 (mg/kg)*	PI50
5540	ethane	1185.02	0.056	6.95
5579	propane	1232.08	0.063	6.82
5581	butene	1247.09	0.045	6.76
5582	butane	1249.11	0.063	6.99
5583	hexane	1286.17	0.050	7.07
5584	octane	1305.21	0.050	7.44
5585	decane	1333.27	0.053	7.51
5586	octaquat-ernary	3165.15	0.32	7.80

* Data from Edgewood Arsenal, Md., Toxicity Screening Branch. Agents administered I.V.

Table 8

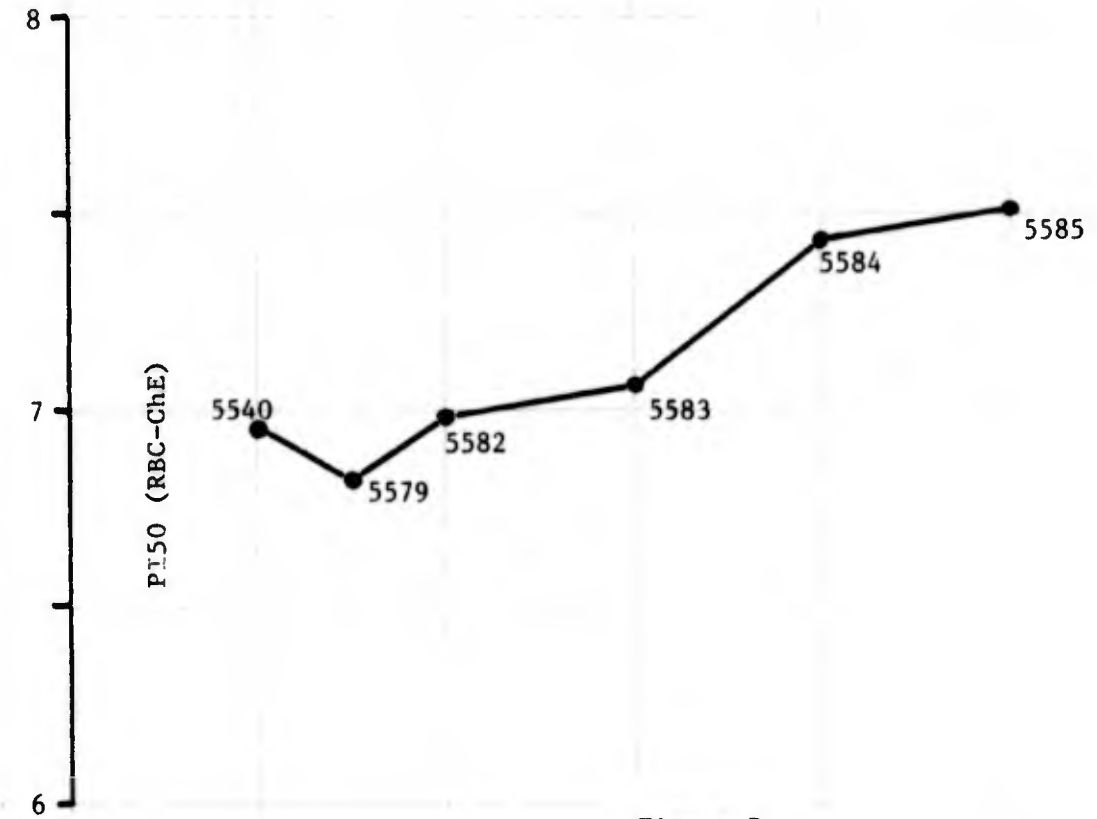


Figure 7

PI50 vs. CHAIN LENGTH (n)
FOR TETRA COMPOUNDS

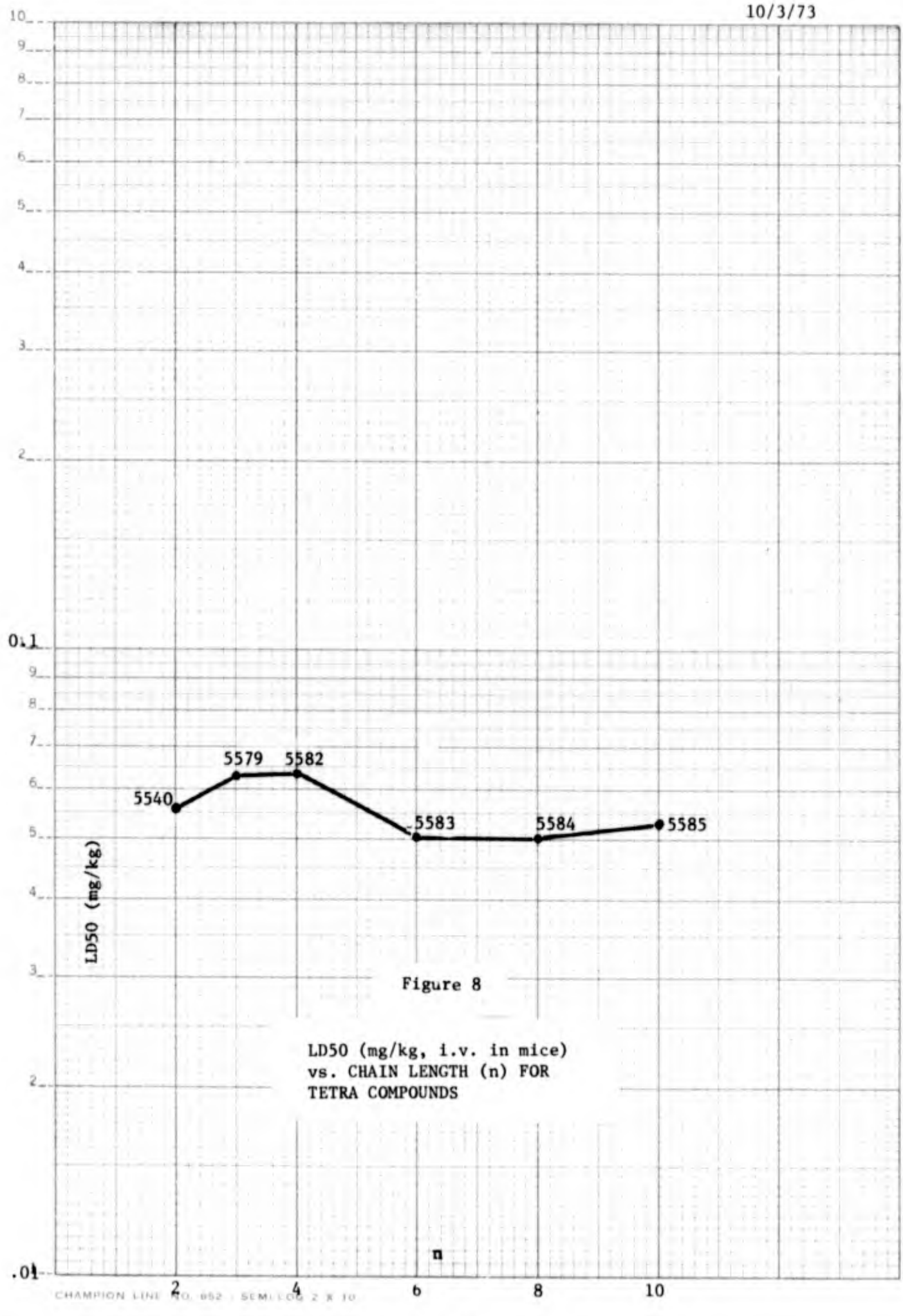


Figure 8

LD50 (mg/kg, i.v. in mice)
vs. CHAIN LENGTH (n) FOR
TETRA COMPOUNDS

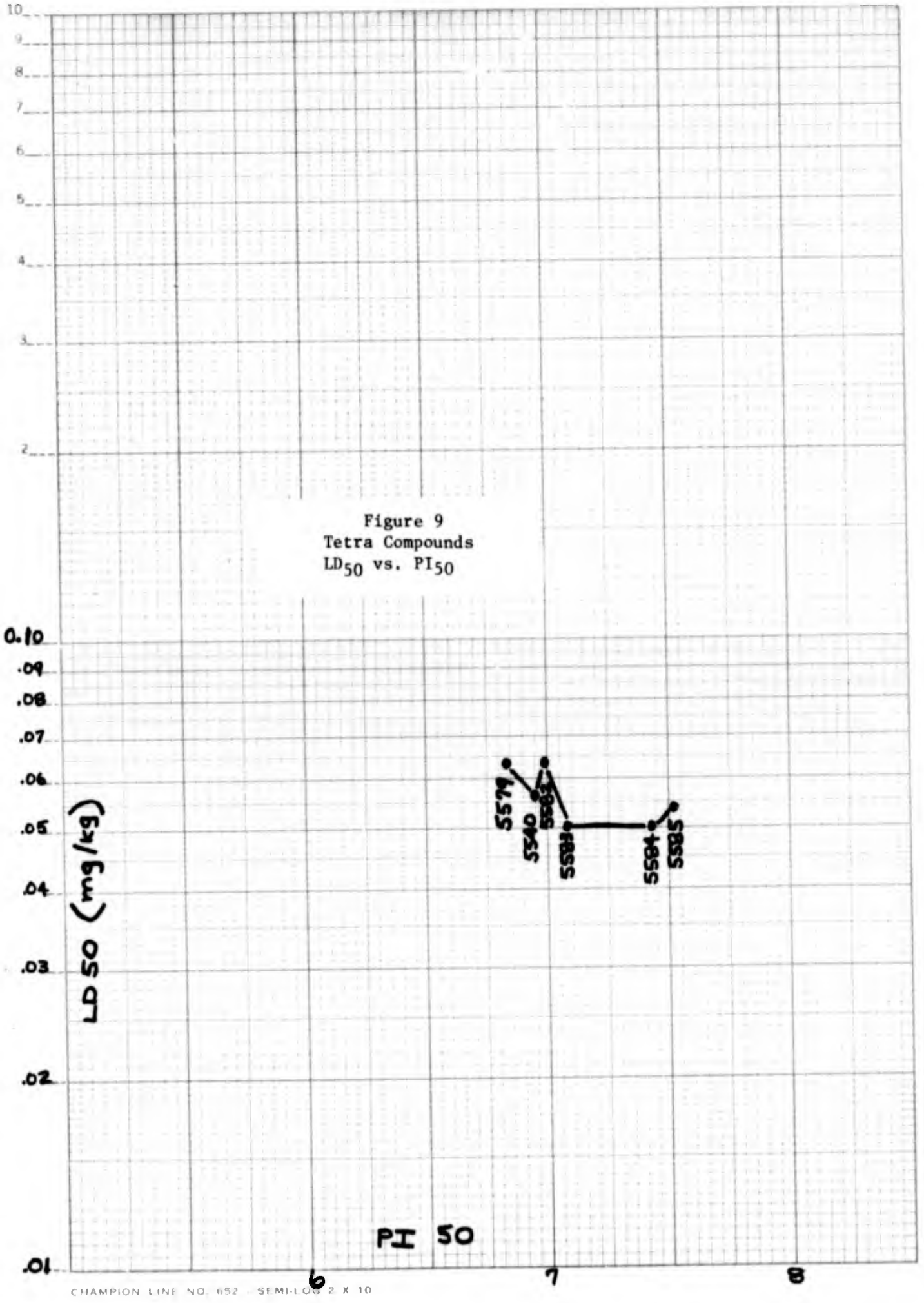
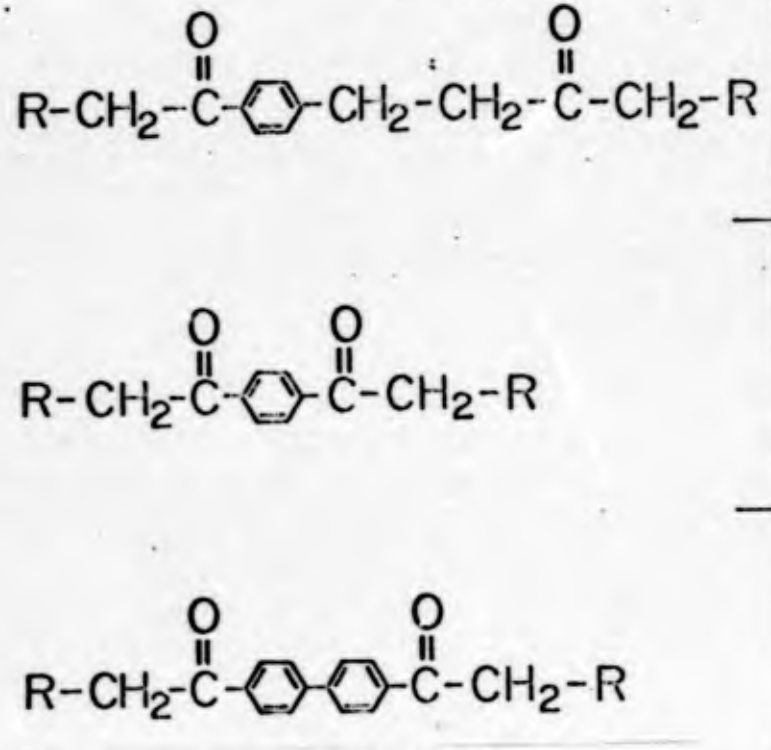


Figure 9
Tetra Compounds
LD_{50} vs. PI_{50}

ABSTRACTS OF STUDIES ON THE METHYL PYRIDINIO AND THE 2 HYDROXY ETHYL DIMETHYL AMMONIO ANALOGUES OF BUTANONE, PHENYLENE DIACETYL AND BIPHENACYL COMPOUNDS

The structures of the compounds in this series are shown in Figure 10. The following are abstracts of papers in press at the present time. The papers have been accepted and will appear early in 1974 in "Toxicology and Applied Pharmacology".



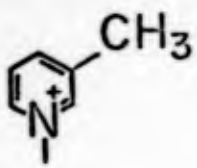
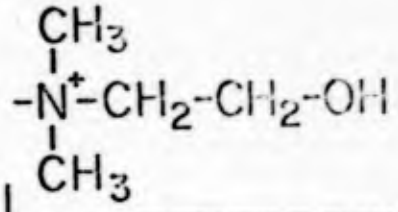
R =			
	I_a	II_a	III_a
	640μg[1] 7.27[2]	250μg[1] 6.98[2]	90μg[1] 4.37[3]
	I_b	II_b	III_b
	10.4mg[1] 5.96[2]	105μg[1] 6.22[2]	900μg[1] 4.34[4]
	I_c	II_c	III_c
	2.9mg[1] 8.12[2]	69μg[1] 8.00[2]	80μg[1] 4.40[5]

Figure 10
Structures, LD_{50}'s and PI_{50}'s for the methyl pyridinio and 2-hydroxy ethyl dimethyl ammonio compounds.

Identification of superscripts are:
1=LD_{50}'s, I.V. in mice; 2=PI_{50}'s; 3=PI_{24}; 4=PI_{27}; 5=PI_2

ABSTRACT

Lethalities, Antiesterase and Hemicholinium-like Actions of a Series of Bis-quaternary Ammonium Compounds, Loomis, T. A., Sommer, H. Z. and Collins, J. A. (1973) Toxicol. Appl. Pharmacol. 00, 00-00.

Comparative data from mice and rats are presented concerning the lethalities, antiesterase and hemicholinium-like actions of a series of bis-quaternary ammonium compounds in which the positively charged nitrogens are connected by a phenacyl butanone (a), a phenylene diacetyl (b), or a biphenacyl (c) chain. Three analogues of each were studied. The analogues contain the ortho-pyridinio (L), the meta-pyridinio (II) or the 2-hydroxyethyldimethylammonio (III) moieties. A relatively simple method was developed for differentiating hemicholimium-like action from antiesterase action on the intact anterior tibial muscle preparation of the rat. All compounds of the III series are essentially of only a low order of activity as antiesterase agents but show hemicholinium-like action. Compounds of the I series are inactive when tested for hemicholimium-like action. The a, b, and c members of the I and II series are of similar antiesterase potency.

ABSTRACT

Two Active Structures for Hemicholinium-like Action, Blase, B. W., Loomis, T. A., Collins, J. A. and Sommer, H.Z. (1973) Toxicol, Appl. Pharmacol, 00,00-00.

The hemiketal structure has been reported as necessary for compounds to exhibit hemicholinium action. This report describes the hemicholinium action of two bis-quaternary compounds (i.e., 2-hydroxy ethyldimethylammonio derivatives) in which the positively charged nitrogens are connected by either the phenlene diacetyl chain or by the same chain in which the keto groups have been reduced. The former compound (IIIb) exists in a cyclic hemiketal or closed ring form and the latter (IIIb-OH) exists as the straight chain form. Studies on the intact rat anterior tibial nerve muscle preparation show that the compounds produce quantitatively similar effects and the both produce significant hemicholinium-like action. These results indicate that both the straight chain and the hemiketal forms are active structures for hemicholinium-like action.