Proceedings
of the
Society
for
Experimental Biology and Medicine

VOL. 99 NOVEMBER, 1958 No. 2

SECTION MEETINGS

CLEVELAND, O.
Western Reserve University October 20, 1958
MINNESOTA
Mayo Foundation October 10, 1958

Convulsant Activities of Aminocyclanol Derivatives as Influenced by Stereochemical Configurations.* (24321)

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(Introduced by Emilio Weiss)
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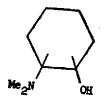
Recent studies(1) have indicated that unexpected physiological activity results from drug structures such as 1 in which a tertiary amino group and a hydroxylated function are



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located adjacent to one another on a cyclohexane ring. For example, the *cis*-isomer 1 has previously been shown(1) to inhibit the acetylcholinesterase-acetylcholine system, to produce neuromuscular blockade of the frog *rectus abdominis* preparation, and to inhibit conduction of the propagated impulse (to be published) in a standard desheathed bullfrog sciatic nerve. Consequently, it was of considerable interest to investigate physiological responses to the basic *cis* and *trans* isomers of the series starting with structure 1, for evidences of interference with CNS and/or neuromuscular phenomena in intact animals, and at the same time to extend the study to include the effects of esters of these aminoalcohols with acids which in themselves possess some intrinsic activity. The present report briefly describes the results of these preliminary studies on mice and cats, with emphasis on the role of stereochemistry of the drugs in determining the nature and strength of their actions, and in part details the results of altering the ring structure from the 6-membered cyclohexane ring to the 5-membered cyclopentane nucleus.

Materials and methods. Synthesis of drugs. The majority of the tertiary aminoalcohols, their quaternary iodide derivatives and acetates thereof were available from previous studies(2,3) of activity in the acetylcholinesterase system. The new esters with phenylacetic, diphenylacetic and benzilic acids were prepared *via* interaction of the aminoalcohols with the appropriate acid chlorides, and employed in animal work as the crystalline hydrochloride salts. The quaternary derivatives of the benzilic acid tertiary esters were ob-

* The opinions in this paper are those of the authors and do not necessarily reflect the views of the Navy Department or the naval service at large.

TABLE I. Results of Intravenous Injection of Aminoalcohol Derivatives into Cats and Mice.

Compound				Mice	Cats		
No.	Structure	R	R′	LD_{50}, mg/kg	Dose used, mg/kg	Nature of reaction	Nictitating membrane relaxed
1	I c	CH_3	H	163–164*	15–50	Convulsion, followed by depression	+
2	I t	CH_3	H	130	25–50	*Idem*	+
3	II c	CH_3	H	75	20	Fasciculations, collapse, areflexic	—
4	II t	CH_3	H	96–98	20	None	—
5	I c	C_2H_5	H	85	25–50	Quiescence, muscle hypotonicity	—
6	I t	C_2H_5	H	100	25–50	Excitation, muscle hypertonicity, convulsions	—
7	III c	CH_3	H	90	15	Collapse, flaccidity, areflexic	+
8	III t	CH_3	H	140	15	None	—

* Calculated as the free base. Used as the HCl salt to ensure solubility.

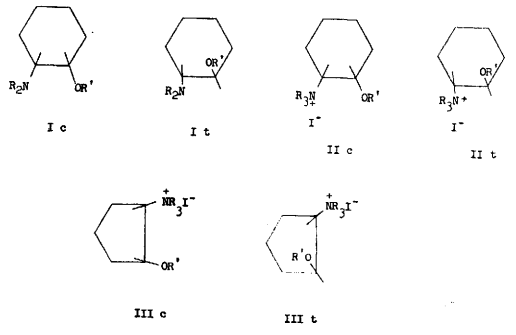
tained by courtesy of the J. R. Geigy A.-G. organization in Basel.

Animal testing. Orientation as to gross toxicity and symptomology produced by these drugs was obtained with the mouse. Aqueous solutions with pH adjusted near neutrality were administered *via* tail vein to animals in the 18-22 g weight range. LD_{50} determinations in mice were derived from dose-response curves in which at least 4 doses were employed, with 10 animals being injected at each dose. In the testing of the esters it was found that the dose-lethality curves have very steep slopes.

From the lethality levels in mice, sublethal doses were selected for the cat studies. In these experiments the drug solutions were injected into the greater saphenous vein, and the animals observed till return to normality. In certain of the tests in which one compound was assayed for protective activity against another, the injections were given in rapid succession through the same needle.

Results. The results of animal experiments with the simple tertiary *cis* and *trans* aminoalcohols under study, together with the corresponding findings on certain of their quaternized derivatives, are summarized in Table I below. The basic structures referred to in the Table are summarized in the formulae I-III.

Basic Structures



The experimental observations were next extended to several series of *cis* and *trans* esters of the previously studied aminoalcohols, with the results summarized in Table II.

Several interesting features emerge from the tabulated data. Of particular interest in the mouse LD_{50} data is the observation that with the exception of isomer pair 1-2, each of the *cis* (c) isomers is more toxic than its corresponding *trans* (t) isomer. This is true even though reaction to the drug and mode of death varied considerably from pair to pair. All produced convulsions, but these were generally of a complicated pattern and probably due to several different causes. The quaternary amines, in general, caused weakness and collapse sometimes accompanied by fasciculations, and presumably the convulsion was a terminal event subsequent to impairment of the respiratory musculature. The tertiary aminocyclohexanols and their phenylacetic, diphenylacetic and benzilic esters produced, as the initial event, typical tonic-clonic convulsions, presumably of CNS origin. The remaining compounds produced such complicated mixtures of general excitement, muscle hypertonicity, isolated limb paralysis, respiratory embarrassment and convulsions that the primary cause of the convulsion could not be ascertained by gross observation.

As an initial step in a program to clarify

TABLE II. Biological Activities of Esters of the Basic Aminoalcohol Structures I-III.

Compound				Mice	Cats		
No.	Structure	R	R′	LD_{50}, mg/kg	Dose used, mg/kg	Nature of reaction	Nictitating membrane relaxed
9	II c	CH_3	CH_3CO	51–52	20	Salivation, sweating, muscle hypertonicity	+
10	II t	CH_3	CH_3CO	76	20	Mild depression	—
11	III c	CH_3	CH_3CO		20	Flaccid paralysis	+
12	III t	CH_3	CH_3CO	140	20	Mild depression	—
13	I c*	CH_3	$C_6H_5CH_2CO$	25†	10–25	Severe tonic convulsions	—
14	I c*	CH_3	$(C_6H_5)_2CHCO$	21	10	Tonic and clonic convulsions, running motions	—
15	I t*	CH_3	$(C_6H_5)_2CHCO$	21	15	*Idem*	—
16	I c*	CH_3	$(C_6H_5)_2C(OH)CO$	17–18	5	Salivation, mild paralysis, nystagmus	—
17	I t*	CH_3	$(C_6H_5)_2C(OH)CO$	24	2–10	Tonic convulsion	—
18	II c	CH_3	$(C_6H_5)_2C(OH)CO$	11–12	7–11	Mild lethargy	+
19	II t	CH_3	$(C_6H_5)_2C(OH)CO$	20–21	15	" "	+
20	I t*	C_2H_5	$p\text{-}CH_3OC_6H_4CH_2CO$	37	15	Convulsion, running motions	—

* Used as the HCl salt.

† Result by courtesy of Dr. J. R. Tureman, Dept. of Pharmacology, Howard Univ. Med. School.

the mechanism of action of these agents the drugs were administered in sublethal doses to cats. Although complicating actions were still seen, the picture was much clearer. In the tables those cats listed as convulsing showed classic tonic-clonic reactions frequently accompanied by strong running motions. In those animals listed as showing flaccidity and collapse the response was similar to that seen after administration of a depolarizing neuromuscular blocking agent. In these studies, too, the striking correlation of structure and potency was noted. With the exception of the pair 1-2, in which at equivalent doses the convulsions produced by the *cis* were a little less severe than those produced by the *trans* isomer, all *cis* isomers were more potent than the corresponding *trans* forms. This is most dramatically exemplified in the compound pairs 3-4 (quaternary) and 7-8 (quaternary), wherein each *cis* isomer is quite active in producing symptomology, whereas the corresponding *trans* isomer is virtually *inactive* at the same dose level. That this behavior may result from differential actions of stereoisomers at the *same* receptor centers can perhaps be inferred from an experiment in the cat in which it was found that 30 mg/kg of the relatively inactive *trans* quaternary compound 8 completely protected the animal against the normally powerful action of 15 mg/kg of the *cis* quaternary compound 7, when 8 was administered intravenously immediately before 7. A repetition of this experiment in the cyclohexane series with administration of 30 mg/kg of the *trans* compound 4 followed immediately by 20 mg/kg of the *cis* derivative 3 resulted in complete protection against paralytic symptoms. In both assays of protective action in the cat, however, the combined action of the two drugs appeared to be greater than the individual effects of the isomers on the nictitating membranes.

Among those compounds causing convulsions in cats the most dramatic contrast between actions of a *cis-trans* pair was seen in the diethyl pair 5 and 6. Both compounds were active, but the *cis* isomer caused a mild depression with muscle hypotonicity and lethargy while the *trans* isomer produced excitation, muscle tremors, rigidity and after about 15 minutes severe and prolonged tonic-clonic convulsions. Among the other pairs causing convulsions the reaction was qualitatively similar for the 2 isomers with, except for pair 1-2, the *cis* isomer slightly more potent in action than the *trans*. Quantitation of the convulsant effects of the tertiary aminocyclohexanols and their esters by means of the mouse LD_{50} data reveals that in addition to the general *cis-trans* relationship potency is affected by esterification. The activity se-

quence is alcohols<phenylacetates<diphenylacetates<benzilates.

Summary and conclusions. Generally, it has been found that *cis* and *trans* isomers of 1,2-tertiary aminocyclanols and their esters, studied largely in the cyclohexane series, are capable of exhibiting convulsant activity in the mouse and the cat. From graded activities in the mouse it is observed that with few exceptions each *cis* isomer of a given *cis-trans* pair is more potent than its corresponding *trans* isomer. Within the tertiary amino compounds as a class, it appears that convulsant activity in the mouse, which is roughly paralleled in the cat, runs in the increasing sequence alcohols<phenylacetate esters<diphenylacetate esters≤benzilate esters. The *cis* quaternary alcohols 3 and 7 are active in cats and mice, whereas the *trans* isomers 4 and 8 are strikingly inactive in cats. Further, the probability that active and inactive members of a quaternary *cis-trans* pair (*e.g.* 7 and 8, and 4 and 3) reach the same receptor centers in the cat is indicated by the observations that 8 protects against the action of 7, and 4 against 3. Finally, the quaternary *cis* and *trans* benzilates 18 and 19 appear to show strong species specificity, with high activity in mice (*cis* better than *trans*) and low activity in cats.

1. Friess, S. L., *J. Am. Chem. Soc.*, 1957, v79, 3269.
2. Baldridge, H. D., McCarville, W. J., Friess, S. L., *J. Am. Chem. Soc.*, 1955, v77, 739.
3. Friess, S. L., Baldridge, H. D., *ibid.*, 1956, v78, 2482.

Received June 5, 1958. P.S.E.B.M., 1958, v99.

Effect of Homologous Spinal Cord in Freund's Adjuvant on Cockerel Comb, Testicular and Body Growth.* (24322)

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During investigations concerning production of experimental allergic encephalomyelitis, Lipton and Steigman found (unpublished) that 4-week-old White Rock chickens were suitable hosts for production of the disease, both clinically and histologically, when injected with homologous spinal cord in Freund's adjuvant. Subsequent experiments were designed to determine the influence of age of chicken on its susceptibility. Casual observation revealed that injected groups had paler and smaller combs as well as smaller body weights than uninjected controls. The differences were most striking in birds injected at 3 to 7 days of age, and less so in birds injected at 14 days.

Methods. For Freund water-in-oil emulsions, *M. butyricum* was suspended in an Aracel-light mineral oil mixture to a concentration of 4 mg/ml. A local poultry provided adult chicken heads and necks from which spinal cord tissue was selected. Liver tissue was obtained from normal chickens killed in this laboratory. The tissue was suspended in 0.5% phenol water at 30% concentration and permitted to incubate 4-6 hours at room temperature. Emulsions were made by adding the aqueous phase to butyricum-oil suspension in a 1:1 proportion and emulsifying by a hypodermic syringe. In the group receiving *M. butyricum* alone, organisms were suspended in phenol solution in concentration of 2 mg/ml. White leghorn cockerels from a single source were used. All were received within 24 hours after hatching and living, attenuated Newcastle disease vaccine was instilled into the conjunctiva upon receipt. Diet was "starter mash" (Aubrey Feed Mills, Louisville) and water *ad lib.* Each bird received 0.7 ml of material subcutaneously divided into 7 sites. Seven-day-old chicks were injected,

* Aided by grants from National Multiple Sclerosis Soc. and N.I.H.

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