Physiological Characterization of a New Tropane Analog: 3-benzyl-1,5-diphenyl-9,3-exazatricyclo(3.3.1.$0^{2,4}$)nonan-7-one

Presented to the faculty of Lycoming College in partial fulfillment of the requirements for graduation with Departmental Honors

by

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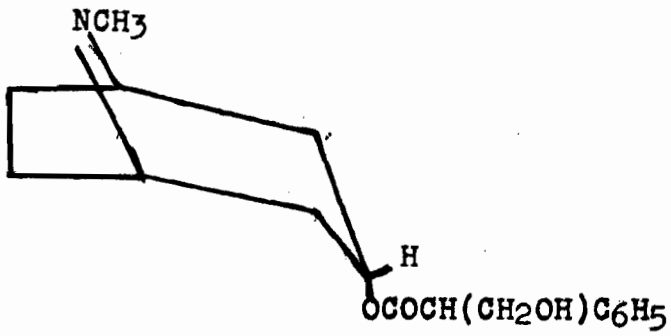
The compound under discussion is a synthetic member of the naturally occurring tropane alkaloids. Within this grouping there are two major subdividions, the solanaceous alkaloids and the coca alkaloids. Atropine (dl-hyoscyamine) 1 and scopolamine (hyoscine) 2a are the two best known of the solanaceous variety while cocaine 3 is the best known of the coca alkaloids.

1



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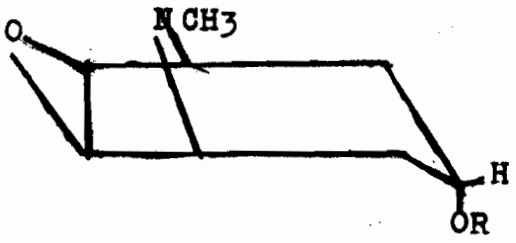
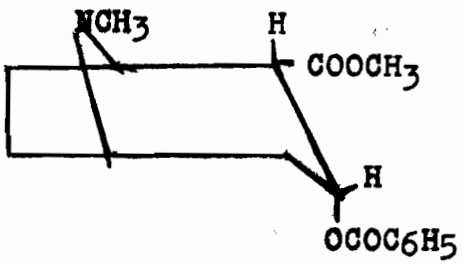


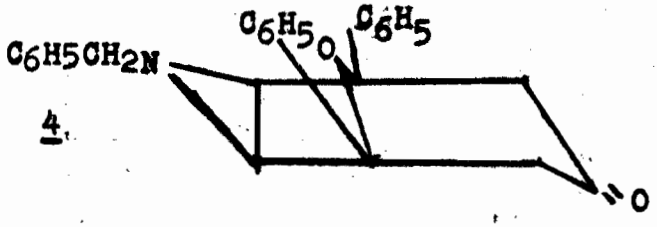
(a. R=COCH(CH2OH)C6H5)
(b. R=H)

3



The novel compound 3-benzyl-1,5-diphenyl-9,3-oxazatricyclo(3.3.1.$O^{2,4}$)nonan-7-one 4, which is under discussion, is most closely related to those compounds mentioned of the solanaceaus vaiety. This group of compounds are generally derived from plants belonging to the poatato family solanaceoae. Atropine is extracted, predominately, from the Atropa belladonna (deadly nightshade), Hyoscymus niger (black henbane), and Datura stramonium (thorn apple). Scopolamine is extracted predominately from Datura metel and Scopolia carniolica (Hamerslag, 1950).

The new heterotricyclic ketone 4 has structural similarity to the physiologically active scopine 2b. The structural configurations are similar with the exception of the interchanging of the ring oxygen and nitrogen (Turner and Lutz, 1968).



4

There is also great similarity between these compounds and the previously mentioned atropine and scopolamine.

Atropine 1 and scopolamine 2a exhibit a host of effects of both a peripheral and a central nervous system nature. These effects are attributed to the

affinity of atropine towards the receptors of the cholinergic synaptic units. The atropine molecule thereby blocks synaptic transmission in acetylcholine mediated neuronal systems, producing muscarinolytic effects (Goodman and Gilman, 1941).

Mechanisms of synaptic blockade

There are at least two principal theories explaining how this synaptic blockade occurs. Clark (1937) has indicated, through his experiments, that neuronal transmission occurs due to acetylcholine's ability to combine with very specific chemical receptor sites. He further expresses the view that the quantitative effects of any synaptic blocker or transmitter are proportional to the number of receptor sites with which these molecules can combine. This view is adopted by Lands (1951) in explaining the route of action of cholinolytic compounds. He views the blockade as a saturation of the receptor's surface by the cholinolytic substance. The substance, when in combination with the receptor, creates a highly stable complex. The stability of this complex suppresses the ability of the membrane to depolarize. It, when bonded to the receptor, renders the membrane, due to the stable

complex, inactive. The inactivity of this complex is explained by a more stable connection of molecule to receptor. The cholinolytic compounds generally have larger molecules than cholinomimetic compounds giving them the ability to protect, by their bulk, the bonds formed. These inactivating substances are capable of mechanically or electrostatically inactivating both the receptors directly attacked by them and adjacent receptors. This theory, however, has led to further work and a new postulation of activity.

This new proposition states that the effect of a substance on a receptor is due to the substance's affinity for the receptor and with its ability to stimulate the receptor, "internal activity". This group thinks "that affinity is caused by the interaction of the electron fields of the substance and receptor in the most general sense, while internal activity is caused by some specific part of the interaction of the fields" (Kuznetsov, 1965).

In figure 1b, page 5, it can be seen that the anion receptor site attracts electrostatically the cation head of the acetylcholine molecule. These anion receptor sites are represented in the figure as negatively charged humps on straight protein chains, the

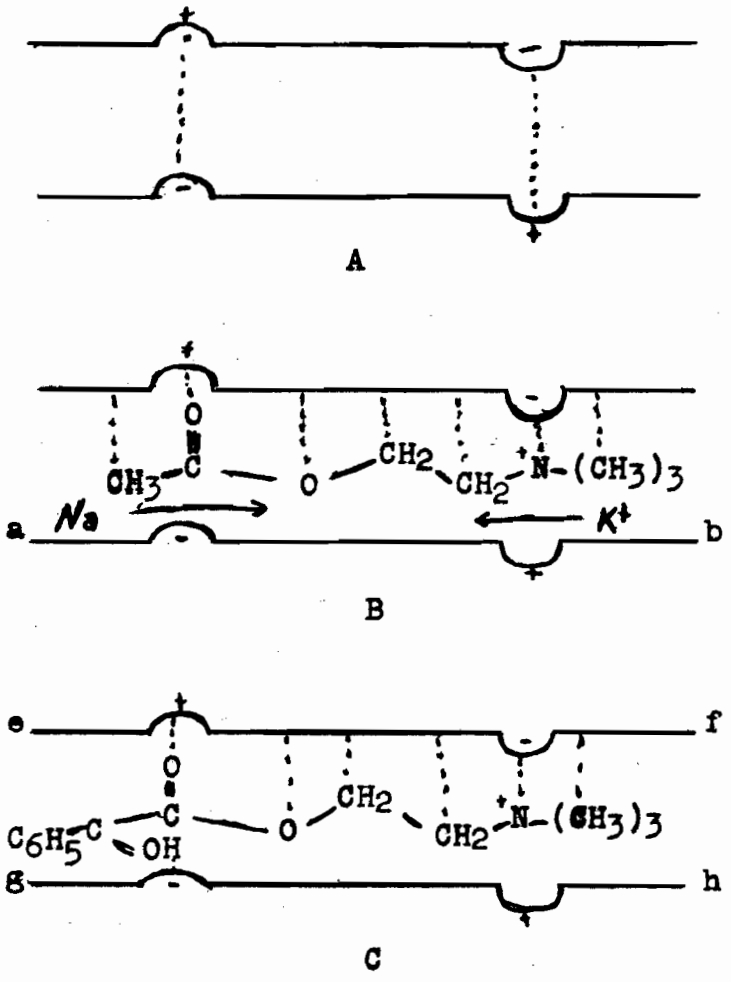


Fig. 1 Schematic representation of the interaction of acetylcholine and a cholinolytic wth a recepter. (Kuznetsov, 1965).

horizontal lines. A positively charged component of the receptor then attracts the oxygen of the carbonyl in the ester linkage of the molecule. These bonds are strengthened by Van der Waals forces, but as can be seen there is no interaction with the opposing protein chain, a-b. This produces a rather unstable complex which is easily degraded by cholinesterase. This is not to say that the receptor actually resembles this representation in nature but rather these are theoretical representations that may aid in picturing the interaction of the molecule and receptor (Kuznetsov, 1965).

Figure 1c illustrates a cholinolytic substance's interaction with the protein chains. As can be seen there are differences in the structure of the compound used as a cholinolytic substance and acetylcholine. These functional groups will be dealt with later. It can be seen, by the structure of this compound when placed in the diagram, that there may be electrostatic interaction between the anionic receptor and the cation head, electrostatic attraction between cation receptor or positively polarized protein and the oxygen of the carbonyl. In addition, there could be Van der Waals forces between the molecule and both protein chains, e-f and g-h. An extremely stable complex

is formed, which may be attributed to the cyclic structures which bond to both protein chains. On the one hand, acetylcholine causes a change in the receptor. This results in the membrane being depolarized, causing the propagation of the impulse. The cholinolytic compound is inert, or, due to its dual bondings, causes no change in the receptor, and thus no membrane permeability change. Exactly how this occurs is still in doubt (Kuznetsov, 1965).

Those areas of chemical structure which have been found to be, or are thought to be, of significance in producing cholinolytic activity will now be discussed. There are several characteristics which appear critical for the manifestation of this activity: a cation head, one or more cyclic structures removed slightly from this head, the main chain of the molecule, a complex ester grouping, and a hydroxyl group. (Gymermek and Nador, 1957). The importance of these groups will be discussed as they relate to the theoretical material just presented. In discussing the theoretical aspects of these various structures, the heterotricyclic compound 3-benzyl-1,5-diphenyl-9,3-oxazatricyclo(3.3.1.$0^{2,4}$)nonan-7-one will be used as the model, and only substituents which it has, will be

discussed in depth.

The presence of the cation head appears crucial in the exhibition of cholinolytic properties. The specific central atom of the cation head is usually nitrogen but may be sulfur or phosphorus. As was described previously, this cation head ionically bonds to the anion receptor site. This bonding is theorized to be the initial instant of adsorption of the compound by the receptor. The diagram, figure 1, indicates that the size and shape of the cation head complex effects the cholinolytic activity. This same consideration is present in cholinomimetic compounds also, for certain types of cation heads allow stronger bonding. When the central atom, N, in compounds similar to the heterotricyclic compound under discussion, is bound in a cycle, there is optimal cholinolytic effect reached by the N-methyl moiety. "The reason for this phenomenon obviously lies in the fact that the elements of the cyclic structure themselves occupy a sufficiently large space beside the atom of nitrogen" (Kuznetsov, 1965). The test compound obviously does not have the N-methyl group described, but instead has a N-benzyl group which should obstruct the activity if the theory is sound.

There appears to be a significant effect on the cholinolytic properties of a compound if various cyclic moieties are present in the substance. In those compounds where there is an amino group present, such as this one, the exact position of the cyclic structures does not appear to be a major concern. There is evidence that suggests there may be increased cholinolytic activity if the cyclic structures are on the third carbon from the amino group. In cases of esters of amino alcohols the position of the cyclic groups are more crucial than they are in other compounds.(Gymermek and Nador, 1957).

The presence of cyclic moieties in compounds without the aforementioned cation head can produce cholinolytic activity. This is illustrated by the 3,3-dimethylbutyl ester of benzilic acid 5.

5 $(C_6H_5)_2C(OH)COOCH_2CH_2C(CH_3)_3$

A general rule for increased cholinolytic activity is that the compound must contain two cyclic structures on the same carbon atom. There are, however, a large number of compounds wherein this generalization does not hold. There does appear to be a correlation

between high cholinolytic activity and the presence of at least one phenyl group, slightly removed from the cation head.

The increased effect, due to the cyclic structures, is apparently caused by the additional strength of the Van der Waals forces from the compound to the receptor. This stronger bonding causes the receptor to be blocked from the acetylcholine molecules.

The basic chain of the compound has been the topic of much discussion. There have been some people, such as Ing (1949), who believe that the maximum anticholinergic activity is obtained by a main chain of five atoms. There are others who would try to predict the exact size in Angstroms which yields maximum action. This is an unresolved topic of theory. In a structure such as 4 the conformation of the main chain is fixed in the chair form. There can be no deviation in chain length in this compound and in comparing this compound with atropine, this topic of description would appear fruitless.

This leads to the topic of greatest controversy in the predicting of substituents increasing anticholinergic effects. The presence of the ester group is an unsolved and much discussed topic. This

controversy is particularly troublesome because , in early research with atropine-like substances, activity was known only in compounds containing this group. At that time the ester linkage was considered the most important single substituent. Today this is still a widely purported belief and the research appears inconclusive. It does appear, however, that this group is not absolutely necessary for cholinolytic action. There is the debate as to it being a sufficient cause, however.(Kuznetsov, 1965).

In discussing the theories of pharmacological action it was concluded from Figure 1 that the ester group is hypothesized to participate in the bonding between molecule and receptor. The electronegative oxygen of the carbonyl is attracted to the positive field of the active surface of the receptor.

It is hypothesized by Welsh and Taub (1950) that this bonding occurs through hydrogen bonding. Compound 4 does not have this ester carbonyl, but does have the ketone carbonyl which should polarize and can be involved in hydrogen bonding.

There is the opinion expressed by Kuznetsov (1965) that the bonding properties of the ester

function are of a minor consequence. He feels that the main function of this grouping is in its influence on the molecule's conformation. This conformation determines the effectiveness of the interaction with the receptor by the main groups. This idea is not important in a cyclic structure such as atropine, scopolamine, or compound 4. The cyclization of these compounds fixes the conformation permitting the carbonyl group to be free.

The presence of, and even more specifically, the exact position of the hydroxyl group appears to be of great importance in increasing the cholinolytic action of a compound. In amino alcohols, a derivative of which is under discussion, it appears that the presence of a hydroxyl group on the carbon atom gamma to the nitrogen yields optimal effects. This is calculated to be from 4 to 7 Å in length. The presence of the hydroxyl group on the same or adjacent carbon atom in relation to the cyclic moieties also appears to be of great significance. The cyclic structures are responsible for protecting the hydrogen bonding from decomposing agents. Lands (1951) theorizes that this hydroxyl group is in position to form hydrogen bonds to the protein receptor. The hydroxyl group appears

neither necessary nor sufficient to cause cholinolytic action, although it does increase a molecule's effect greatly. Compound 4 does not contain the hydroxyl group but contains instead free oxygens, one in a cyclic ring, the other as a carbonyl. Both of these are available for participating in hydrogen bonding. In comparing the structure of the novel heterotricyclic molecule to those structures that appear to yield maximum cholinolytic activity, it seems that the molecule may not exhibit as potent effects as atropine or scopolamine. There is no ester function in this new molecule, although the free carbonyl should yield similar bonding. The two cyclic radicals removed from the cation head, that do exist, are not on the same or an adjacent carbon. There is, however, one phenyl group on each of two carbons, slightly removed from the cation head, and as was mentioned earlier, this appears to be a generalized rule for the increasing of anticholinergic effects. The cation head is tertiary, with respect to carbon atoms, but the free benzyl group seems as though it would be inhibitory as to cholinolytic potency. There is no hydroxyl group in the compound, although it was mentioned that the carbonyl and the cyclic oxygen might act in a similar manner.

Physiological Activity

Atropine and scopolamine inhibit the secretion of tears, sweat, saliva, and secretions of the pancreas and the gastrointestinal tract. These effects occur only in cases where glands of the above mentioned secretions are innervated by cholinergic systems.

Atropine and atropine-like compounds, in general, have an antispasmodic action on smooth muscle. They antagonize the stimulant action of acetylcholine on smooth muscle innervated by parasympathetic nerves. In this regard atropine and its analogs have a marked and important effect on the eye muscles. The eye muscles are innervated by the third cranial nerve, fibers of which are cholinergic. When applied locally or systemically in large doses, it blocks transmission at the nerve endings thereby producing mydriasis and cycloplegia. These effects may be for several days duration, and are not readily overcome by presently used antimydriatics.

In reference to heart rate, atropine causes initial bradycardia, then a more pronounced acceleration. This tachycardia is greater, occurs earlier, and lasts longer, as the dose of atropine is increased. The

initial slowing is said to be due to atropine stimulating the cardioinhibitory center in the medulla. This change in heart rate presents an interesting method of prenatal investigation. Atropine rapidly passes across the placenta, and the intravenous injection of this compound into the mother should produce tachycardia in the fetus. This occurs 10 to 15 minutes after injection if the placenta is normal (Hellman et al., 1963).

Atropine causes initial stimulation of the higher cerebral centers and then a marked depression. The ultimate depression is so characteristic that, at toxic doses, death is due to medullary paralysis which causes respiratory cessation. Not all areas of the brain are affected by this stimulatory action as is indicated by the sedative action of atropine on the tremors of Parkinsonism. Scopolamine, on the other hand, exhibits an initial and marked depression.

The nature of peripheral action in the two compounds also seems to differ. Scopolamine is the stronger blocking agent for the iris, ciliary body, and certain secretory glands. The action of atropine is more pronounced and more prolonged than that of sco-

polamine on the heart, intestine, and bronchiolar musculature.

Man has exhibited the ability to adapt to continuous injections of atropine and develop tolerance. This quality has been noted in patients receiving atropine for Parkinson's disease.

Atropine is absorbed rapidly by the gastrointestinal tract. It can also be absorbed by the eye and to a limited extent by the skin. Once in the circulatory system atropine is distributed rapidly throughout the body. Once dispersed in this manner most of the alkaloid is converted, by hydrolysis to tropine and tropic acid. The remaining amount is excreted through the kidneys (Gosselin et al., 1960).

This vast myriad of effects is the major drawback of the natural alkaloids. The search for less general cholinolytics is the basis for further research in the area of synthetic alkaloids. It is hoped that more specific action can be found in one of these compounds.

Physiological Experiments

A. General Screening Procedure

The physiological testing began with a blind screening procedure laid out by Irwin (1959). This procedure was chosen because of its simplicity, inexpensive nature, and veracity. Although the compound under discussion, due to its chemical similarity to other compounds of a parasympatholytic nature, is believed to exhibit similar properties, a general testing program was needed to outline the real properties of the drug. The purpose of this series of tests is to indicate the general nature of the drug and indicate the path further investigation should take.

Methods

Fourteen male mice of body weight 23 to 29 grams were used in the test. They were placed in seven groups of two mice each. The groups were tested at 10, 30, 50, 100, 200, 300, and 500 mg of test substance per kg of body weight. All mice were injected intraperitoneally (IP). The mice were kept in groups of two, in separate cages during the testing, both mice in the same cage received the same dosage. While observations were being made however they were placed in a different cage thereby producing a novel environment.

Each mouse received three injections on succes-

sive days. The injections were given via a .85% saline solution. For dosages greater than 100mg/kg, a 2% solution of the test substance was used and for doses of less than 100 mg/kg, a .2% solution was used. The time of onset and termination of effects were recorded in tabular form using numbers from 0 to 8 to designate intensity of the effect. Table 1 gives the averages over the three day interval for each dosage level. A base score for abnormal signs was 0 with an 8 maximum. Four was used as a baseline in instances where the normal could be depressed or stimulated. The scores were given subjectively by the experimenter but there was always at least one other person present to collaborate the results. The scoring was done in accordance with the procedure of Irwin and his description.(Irwin, 1959).

Results

The program began with a behavioral profile indicating the awareness, mood, and motor activity of the animal. The alertness of the mice was depressed. The resulting stupor was enhanced by increased dosages. Also, at higher dosages the animals appeared abnormally passive, able to be placed in unaccustomed positions. These findings suggest central depression and possibly myorelaxation,

3-benzyl-1,5-diphenyl-6,8-oxazatricyclo[3.3.1.0^{3,7}]nonan-7-one

TABLE 1.

TEST: CNS ACTIVITY AND ACUTE TOXICITY SCREEN

SPECIES	SEX	ROUTE	WEIGHT	VEHICLE		
MOUSE	MALE	IP	23-29 gms	.85% saline	.2%	☒ sol. ☐ insol.
					2.0%	☒ sol. ☐ insol.

mg./kg Dose	AWARENESS: Alertness	Visual Placing	Passivity	Stereotypy	MOOD: Grooming	Vocalization	Restlessness	Irritability (Aggress)	Fearfulness	MOTOR ACTIVITY: Reactivity (Envir.)	Spontaneous Activity	Touch Response	Pain Response	CNS EXCITATION: Startle Response	Straub Tail	Tremors	Twitches	Convulsions	POSTURE: Body Posture	Limb Position	MOTOR INCOORD.: Staggering Gait	Abnormal Gait	Righting Reflex	MUSCLE TONE: Limb Tone	Grip Strength	Body Sag	Body Tone	Abdominal Tone	REFLEXES: Pinna	Corneal	Iris	AUTONOMIC: Writhing	Pupil Size	Palpebral Opening	Exophthalmos	Urination	Salivation	Piloerection	Hypothermia	Skin Color	Heart Rate	Respir. Rate	MISC.: Lacrimation	LD_{50}
NORMAL SCORE	4	4	0	0	4	0	0	0	0	4	4	4	4	0	0	0	0	0	4	4	0	0	0	4	4	0	4	4	4	4	4	0	4	4	0	0	0	0	0	4	4	4	0	0
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10	4		0	0	4	0	0	0	0	5	4	4	4	0	0	0	0	0	4	4	0	0	0	4	4	0	4	4				0		2	0	0	0	0		4			0	
30	3		0	0	4	0	0	0	0	4	4	3	3	0	0	0	0	0	4	4	0	0	0	4	4	0	4	4				0		2	0	0	0	0		4				0
0	3		1	0	4	0	0	0	0	4	4	3	3	0	0	0	2	0	4	4	0	0	0	4	4	0	4	4				0		2	0	0	0	0		4				0
0	4		2	0	4	0	0	0	0	3	3	2	2	0	0	0	3	0	3	3	0	3	0	4	4	1	4	4				0		3	0	0	0	0		4				0

paralysis, or anesthesia. There were, however no indications of stereotypic movements which would aid in the confirmation of the possible central depression hypothesis.

The mice did not evidence excessive grooming, vocalizations, restlessness, irritability, or fearfulness, all signs of sympathetic or central stimulation. The experimenter did notice apparent grooming movements at very high dosages which may have been vomiting motions instead.

Motor activity was measured by four indications: reactivity to a novel environment, spontaneous activity, touch response, pain response. At low levels of the test material the mice appeared inquisitive toward their new environment but the higher the dosage, the less exploratory behavior, until they became totally unreactive at 300 mg/kg. There was incrementally less spontaneous activity, less reaction to being touched, and less indication of pain. The decrease in spontaneous activity and reactivity to a novel environment could indicate central nervous system depression and ganglia and neuromuscular blockade. The increased reactivity that occurred at low dosages could be just stimulation of a new situation overwhelming the effects of the minimal dosage. The increasing lack of response to pain may signify analgesia, sedation, and central de-

pression. The unreactivity the mice exhibited toward being touched may evince anesthetic activity.

The second phase is a neurological profile which consists of a series of tests on central excitation, motor incoordination, and muscle tone. The startle response, reaction to loud noise, was normal at low dosages but was elevated at higher intake levels. There was a normal Straub tail response. Tremor and convulsions resulted from injections of 100mg/kg or greater. The animals' posture was also affected only at higher dosages, showing a deterioration of body and limb posture. This deviation in body and limb position may indicate neuromuscular blockade or central disturbance.

Motor incoordination was exhibited only at elevated dosages. The animals began to stagger and walk abnormally at 100 mg/kg or greater. Despite this apparent difficulty, the righting reflex was normal until 200 mg/kg. The high scores recorded here may indicate central nervous depression or an agent causing synaptic blockade in some part of the nervous system.

The section on muscle tone was very difficult to judge thereby weakening the reliability of the results, but here again there was the hint of neuromuscular blockade

and central depression. The areas of scrutiny here were limb, abdominal, and body tone, body sag, and grip strength.

The third area considered in the screening was the autonomic profile. The general areas here were optical, secretory, and general signs. The optical signs used were the size of the palbebral opening and indication of exopthalmos, bulging of the eye. The size of the palbebral opening rendered varying information. At the 10 mg/kg dosage there was apparent widening of the opening, but at increased dosage levels there was incremental narrowing of the opening. This can be explained in many ways for this widening indicates sympathomimetic activity if accompanied by other signs. There was no sign of exopthalmos exhibited at any time. If there had been any corroborating evidence yielded here, more credence could have been given the findings on the low dosage effects on the palbebral opening.

There was no sign of any undue urination or salivation, two secretory signs, both indicative of muscarinic activity. The general signs used were writhing, piloerection, and skin color change. All these areas remained normal at all dosages, unless the writhing was masked by the convulsions at higher levels.

The last part of the examination was the LD_{50}. The apparent maximal toxicity level was between 300 and 500 mg/kg, for at 500 mg/kg all the mice died, at 300 mg/kg none died. At 300 mg/kg, however, all mice evidenced severe convulsions.

The results of these tests appear to indicate neuromuscular blockade and central depression as characteristic of the drug. This will have to be born out by further tests, however, for the findings are only generalizations. The repeated positive implication of the synaptic blockade symptoms confirms the expectations of the experimenter based on chemical structure alone.

This screening procedure yielded only qualitative information and the overall effectiveness and potency of the drug can not be estimated at this time. The drug now appears to be a cholinolytic one, and several of the confirmatory as well as quantitative procedures will be dealt with shortly (Lawrence and Bacherach, 1964).

B. LD_{50}

This experiment is a crucial one for it is impossible to conduct further research without knowing the dosage range at which the compound exhibits nontoxic effects. This procedure indicates the point at which one half of the total experimental group injected at one dosage dies. (Turner, 1965).

Method

Twelve female mice were used, their body weights ranging from 22 to 26 grams. The general screening procedure had indicated that the LD_{50} lay between 300 and 500 mg/kg. This more exact procedure therefore began at 325 mg/kg and increased at 25 mg/kg increments. Four mice were placed in each group, the members of the group all residing in one cage. Due to the type of test, only one dosage level was given on a single day, to spare as many animals as possible. The animals were injected at dosages of 325, 350, and 375 mg/kg with a 2% solution of the test substance. The solvent was a .85% saline solution.

Results

The results of the procedure indicated that at both 325 and 350 mg/kg the groups suffered two

deaths out of four mice. In the 375 mg/kg group all mice died. The results indicate a range for the LD_{50}. Due to lack of experimental animals and the purpose of the testing, these results appear exact enough. The LD_{50} of atropine injected into mice IP has been found to be 250 mg/kg. This lesser toxicity expressed by the novel heterotricyclic ketone, however, indicates nothing in respect to the pharmacological potency or overall usefulness of 3-benzyl-1,5-diphenyl-9,3-oxaza-tricyclo(3.3.1.$0^{2,4}$)nonan-7-one (Merck Index,1960).

C. Lacrimation

The purpose of this experiment was to aid in the characterization of the drug under discussion. This test is designed so that if the drug being tested has parasympatholytic properties it will yield a negative test. The lacrimal glands of the eye are innervated by the parasympathetic system. On injection of the drug the synapses of these glands are blocked, if the drug is anticholinergic, and lacrimation should not occur when carbamyl choline is injected. This drug, carbamyl choline, is commercially available as Isopto carbachol and is parasympathomimetic. It induces lacrimation and is used in the treatment of glaucoma. The procedure followed was similar to that of de Jongh et al. (1955).

Method

Thirteen Wistar rats, no sex differentiation was made, weighing between 170 and 225 gms. were used. De Jongh called for the rats to be kept at 36°C for ten minutes prior to injection of the test compound. This was not done: The rats were kept at about 25°C before injection. The prescribed increase in temperature was to cause vasodilation but longer periods of time were alotted during the experiment to counter-

balance the effects of the non vasodilative procedure. The animals were injected with the test compound via the tail vein. The volume of solution injected varied depending on the body weight of the rat but it was always approximately the directed, 1 ml/kg. The duration of the injection was from 17 to 26 seconds. Those rats injected with greater than 30 mg/kg were injected IP, for the injection was not feasible via the tail vein. Rat number 13 was not injected with the test substance but rather 0.10 ml of sterilized distilled water. This was to insure that ~~just simple~~ breaking of the skin did not induce lacrimation.

The dose of carbamyl choline was held at .5 mg/kg, given intraperitoneally, for each animal. This injection was to occur at 15,45, 75, and 105 minute intervals for each animal.A piece of cotton was then lightly touched to the eye of an injected animal 3 minutes after each injection. If a colored spot appeared on the cotton this was a positive sign. In other words the compound being tested would mot be blocking the synaptic units leading to the lacrimal glands, and would not be exhibiting parasympatholytic activity.

Two of the rats, 11 and 12, were used as control animals. They were injected only with carb-

amyl choline in order to ascertain visually what a normal response was to 0.5 mg/kg of the compound without any other drug present (de Jongh et al., 1955).

Results

The test began with the injection of rats 1 and 2 in Table 2. The rats were injected with 10 mg/kg of the test compound. The two rats died due an inadvertant overdose in the amount of carbamyl choline; ten times the prescribed quantity was injected. The LD_{50} of carbamyl choline, injected IV, in mice is 0.3 mg/kg. Before death, however, they evinced a great deal of lacrimation.

The procedure was followed correctly for the mice 3 through 10. The results achieved were less than conclusive in any quantitative way. As can be seen on the chart, the rats did not ever display complete parasympathetic blockade even at the 15 minute interval and were not tested at any stage later than 45 minutes. There was, however, an apparent difference in the degree and duration of lacrimation at the doses of 30 mg/kg IV and 100 mg/kg IP when compared to the control rats, 11 and 12, and the preceding rats at lower dosages. These observations cannot be used in any quan-

Table 2

Lacrimation Dosages and Observations

Rat number	Test Compound Group mg/kg	Body Weight	carbamyl choline ml. of 2% test	ml. of .75%	observations
1	10	170	.085	.110	Dead
2	10	177	.086	.120	Dead
3	10	173	.085	.010	no inhibition
4	10	181	.086	.010	no inhibition
5	30	173	.260	.010	slight inhib.
6	30	185	.270	.015	slight inhib.
7	50	185	.460	.010	no inhibition
8	50	178	.450	.010	no inhibition
9	100	201	1.000	.013	slight inhib.
10	100	206	1.000	.013	slight inhib.
11	0	225	.000	.015	
12	0	210	.000	.014	
13	0	193	.000	.10 H_2O	no lacrimation

titative way but there did seem to be an extremely weak cholinolytic activity. These effects were noted only at the 15 minute interval injection of carbamyl choline and were not present at the 45 minute interval injection. This would indicate, aside from weak cholinolytic activity, relatively transient action.

The results of this test are described in the literature in terms of the ED_{50}, the dose necessary to prevent the appearance of a colored spot in half of the animals after 105 minutes. The recognized dose of atropine required to do this is only 3.9 mg/kg.

Table 2

Lacrimation Dosages and Observations

Rat number	Test Compound Group mg/kg	Body Weight	carbamyl choline ml. of 2% test	ml. of .75%	observations
1	10	170	.085	.110	Dead
2	10	177	.086	.120	Dead
3	10	173	.085	.010	no inhibition
4	10	181	.086	.010	no inhibition
5	30	173	.260	.010	slight inhib.
6	30	185	.270	.015	slight inhib.
7	50	185	.460	.010	no inhibition
8	50	178	.450	.010	no inhibition
9	100	201	1.000	.013	slight inhib.
10	100	206	1.000	.013	slight inhib.
11	0	225	.000	.015	
12	0	210	.000	.014	
13	0	193	.000	.10 H_2O	no lacrimation

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This figure indicates that the test drug is at best 10 times less potent than atropine in this particular test.

To interpret these results is very difficult. They do, however, coincide with the screening procedure diagnosis in that the drug appears to exhibit at least slight parasympathetic properties and that the effects are transitory in nature. In the screening procedure the animals also appeared to return to normal activity in a relatively short time.

D. Mammalian Smooth Muscle

This test is another in the general procedures for characterization of the compound. It differs from the previous tests. The drug has shown properties, although moderately, of an apparent parasympathetic blockade. This enables the use of a more specific test. The test is designed to monitor specifically, the drug's inhibtion of acetylcholine, the parasympathetic synaptic transmission agent. The intestine of the guinea pig and rat is an organ of smooth muscle exhibiting spontaneous rhythmicity and innervated by the parasympathetic system.(Turner, 1965).

Method

A male guinea pig weighing 315 gms and a male albino Wistar rat of 335 gms were used. Both animals were not fed for 32 hours previous to their sacrifice in order to minimize fecal wastes in the intestine. The animals were sacrificed by a sharp blow to the nape of the neck. These animals were not used on the same day, but the procedure was the same except where indicated to be different. After sacrifice, the abdomens were opened as quickly as possible and the intestine was cut free just below the pylorus and

just above the colon. The small intestine was then placed in a finger bowl containing Tyrode's solution and a segment of approximately 2.5 cm was cut free from the lower end. This segment was then bound at both ends and placed in the apparatus.

The apparatus consisted of a 250 ml beaker containing 100 ml of Tyrodé's solution aerated constantly. Below this, in the case of the rat but not the guinea pig, there was a heater which kept the water at approximately 39 °C. Above this entire mechanism was an A myograph joined to the physiograph. The paper speed of the physiograph was set at 5 cm/sec. The small strip of intestine was then attached to the bottom of the aeration rod and to the lever of the myograph, the strip being suspended in the solution. Care was taken not to overdistend the smooth muscle strip, as this ruins its contractile properties. The amplitude of the recorder was adjusted until the contraction peaks were recorded at 3 cm in height. This occurred only for the rat. The experimenter was unable to record any contraction from several strips of the guinea pig intestine. Even with the addition of 10 drops of 1.5:10,000 solution of acetylcholine no response was obtained. The

rat intestine, on the other hand, exhibited contractile properties. After a baseline contraction rate had been established, the acetylcholine was added dropwise. Two drops were added in total and the results were recorded. The water bath was then washed out and the tissue's contractile rate was allowed to stabalize. Two drops of acetylcholine were then added again but this time the test compound was added dropwise immediately afterward.

Results

The results of this test again seem to indicate transient and weak cholinolytic properties. Two drops of 1:500 of the test compound were added and there was a short lived, 12 seconds, and incomplete inhibition, 67 %. For total inhibition there was required six drops of the compound. When compared to the inhibitory strength of atropine, this is weak. Atropine has 1000 times the potency of acetylcholine.

Food Consumption

This procedure is designed to determine any subacute toxicity effects of the compound. The continued druging of the animal permits the detection of any long range toxicity effects of a compound. The body weights, food consumption, and fecal wastes are keys to these effects on the body.

Method

Six adult female mice between 27 and 29 gms were used. Two were in a control group and the other four were in two groups receiving 30 and 50 mg/kg injections, respectively. Food was given the animals for 2 hours each day. The injections occurred 1 hour before feeding time. The animals were weighed before and after the feeding period. The difference in weight was considered the amount of food consumed. The fecal pellets were counted at the end of each 22 hour period and the amount of waste was thereby calculated. The injections were given subcutaneously. This procedure was followed for twelve days.(Janssen, 1961).

Results

The results of this test indicated nothing

in terms of long range effects. After an initial drop in weight, as was to be expected on the feeding schedule, the animals gained the weight back and ended at approximately the same weight as they had begun the test.

F. Inhibition of Perphenazine-induced Catatonic Reaction

This procedure is a more specific, and also a quantitative analysis, of cholinolytic compounds than those preceding it. Morpurgo (1955) analyzed many anti-parkinson drugs with respect to their inhibition of phenothiazine-induced catatonic reactions. She did this testing in the hope that it could provide a useful test in the screening of antiparkinson drugs. The results she achieved appeared to confirm these hopes and the reliability of the test has been quite good. "The term catatonic reaction is used to describe the failure to correct spontaneously an unphysiological posture of the body" (Morpurgo, 1955). It was found that perphenazine was one of the most useful phenothiazine derivatives for the induction of the catatonic reaction due to its rapid aetion and constancy of results. This reaction is very similar to symptoms exhibited by patients having Parkinson's disease.

Method

Eight male albino Wistar rats weighing between 225 and 275 gms were used. They were placed in four groups of two rats each. The groups of rats were first injected with 0,5,10, and 30 mg/kg of the test compound. In the 5 mg/kg group, a 0.2% solution was

used and in the higher dosages a 2% solution was employed. A time period of fifteen minutes was alloted for the drug to circulate, and the animals all then received an injection of 5 mg/kg of perphenazine (Trilafon by Schering). The animals were placed in separate cages and observations were made at fifteen minute intervals. The degree of inhibition was calculated in a subjective manner, as the percent of inhibition of the experimental animals, in relation to those animals given only the perphenazine injection.

Results

Again the drug did exhibit transient and moderate action. As is indicated in Table 3, the drug appeared to exhibit a noticeable effect after an hour at the 30 mg/kg dosage. The graphs in Figure 2 serve as a comparison between this compound and already known antiparkinson agents. It can be seen that the drug evinces extremely transient and weak effects when compared with the effects induced by much smaller amounts of several of the already existing compounds used in medicinal treatment.

Table 3

Dosages and observations of inhibition of perphenazine

rat number	body weight	mg/kg test compound	ml. test compound	dose of Trilafon	% inhib. at 1 hr.
1	275	0	0.000	0.280	control
2	250	0	0.000	0.250	control
3	225	5	0.56	0.225	0-5
4	230	5	0.58	0.225	0-5
5	235	10	0.12	0.230	10
6	236	10	0.12	0.230	15
7	255	30	0.38	0.250	30
8	267	30	0.42	0.255	30

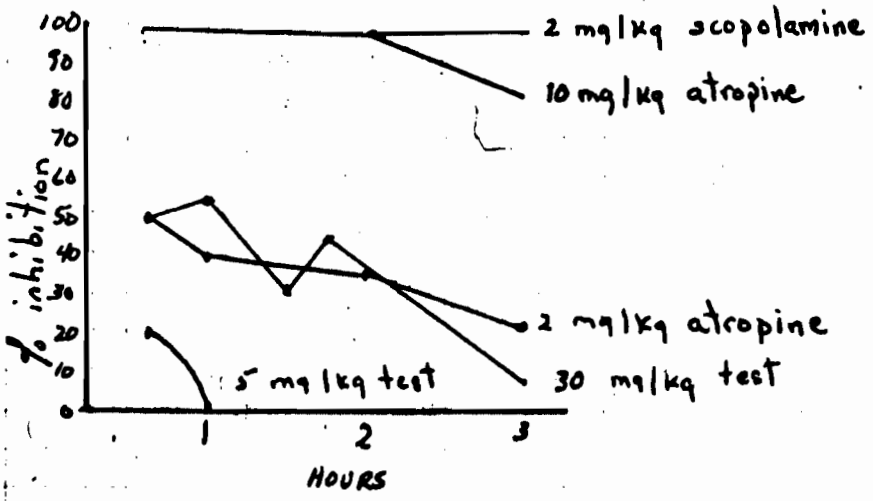


Fig. 2 Comparison of the % inhibition of the pherphenazine-induced catatonic reaction of the test compound with already used compounds.

Discussion

The data gathered in the testing of this compound would indicate it to be of weak and transient cholinolytic activity. The general screening procedure indicated hints of some other action, but due to the inhibition of acetylcholine exhibited by the drug, this must be discounted. These deviations were explained at the time as injection shock and general excitement. This reaction may have come also from almost total sympathetic input after the parasympathetic synaptic blockade and before muscular blockade occurred. In every instance, the compound did display this anticholinergic action and, although in many cases it was neither quantitative nor precise, the preponderance of data indicate that it is parasympatholytic in nature.

In a quantitative sense, the compound does not display the potency of atropine or scopolamine, but the present theoretical orientation on the pharmacological action caused by the structures of these compounds would indicate this to be the case. As was discussed previously, there are many substituent groups which the compound lacks or has in a position that does not increase its affinity for the synaptic receptor sites.

Previously, it was mentioned how the compound could bond with the receptor, and it appears that there are several pharmacological actions of the compound which are not as were theorized. The ketone carbonyl group of the test compound would seem a logical point to dispute with the previously offered bonding representation. There would be less hydrogen bonding here than with the hydroxyl group that was characterized as a group that greatly increased the molecule's affinity for the receptor. The presence of the bulky cation head may, as was mentioned earlier, be a detriment to cholinolytic activity. Finally, the lack of cyclic substituents in the positions theorized as useful, in increasing cholinolytic action, may also be an obstacle. (Kuznetsov, 1965).

This lack of potency and duration of effect, however, does not in itself provide a medicinally ineffectual drug. There was a need for compounds other than atropine that exhibited weaker and more temporary effects in producing mydriasis. This, however, has been remedied and there are now many compounds that are used as such. There is still a need for compounds exhibiting weaker action than atropine in the inhibition of cholinergic action in other areas of the body. (Kuznetsov, 1965).

Many atropine-like substances, especially the synthetic compounds, exhibit specific effects on individual organs. Therefore, if a compound has the structural promise, it should be tested more specifically. In future work concerning this drug or in evaluation of those compounds derived from it, specific action should be evaluated more closely.

Future Work

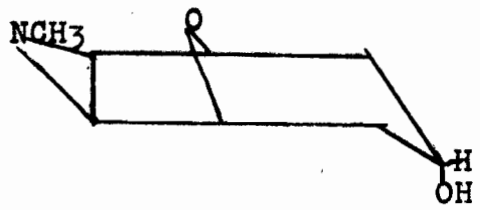
The compound under discussion introduces an interesting opportunity in the structural and theoretical analysis of cholinergic receptors and their blockers. The interchanging of the ring nitrogen and oxygen, in relation to scopine, introduces a new concern to the theory. How, if at all, will this effect neuronal transmission? It would seem an occasion to add to the present understanding of the theory involving the receptor, to synthesize and test a number of similar compounds.

The first change that could be executed is the substitution of the N-benzyl group by a N-methyl group. This could be accomplished by the use of methylamine in order to synthesize trans-1-methyl-2,3-dibenzoylaziridine (Turner, 1965). The change would remove the bulky benzyl group that theory states separates the cation head from the receptor, and introduce a less bulky methyl group. This methyl group is theorized to be the most advantageous substituent on the nitrogen for increased cholinolytic activity (Kuznetsov, 1965).

A second change could be the synthesis of the methyl chloride salt instead of the hydrochloride salt (Turner, 1968). "In the transition from tertiary to

quaternary compounds the cholinolytic activity (in the area of nerve endings), as a rule, increases strongly" (Gymermek and Nador, 1957). This is reasonable when the structure of acetylcholine is reviewed. This quaternary compound is stereochemically adapted to the receptor. It is logical that the dimethylammonium group in the hypothesized compound would appear to increase its bonding stability.

There are other possibilities of interest. The reduction of the ketone would yield a structure 6 similar to scopine 2b in that it would also contain a hydroxyl group on C-3. Compound 6 differs skelletally from 2b in that the ring oxygen and nitrogen are interchanged (Turner and Lutz, 1968).



6

The next proposed structure of interest is also an analog of scopine. It is the bisquaternary ammonium salt of 6. It would be synthesized by the attachment of two molecules of 6 by means of a polymethylene chain, n number of carbons in length. Kuznetsov

(1965) indicated the synthesis of such a molecule, the parent compound being atropine instead of 6. He also indicates an increase in curariform activity due to the chelating effect of the two cation centers attacking two anion centers of the choline receptor.

There are several other structures which would be of interest to theoreticians and possibly would have medicinal value. One is the ester of 6. It and its derivatives could be used to compare the properties of the esters and the alcohol derivatives now under discussion.

Appendix

Syntheses

The melting points were taken on an Eimer-Amend Melting Point Apparatus. The infrared absorption spectra were made on a Perkin-Elmer instrument, Model 137B.

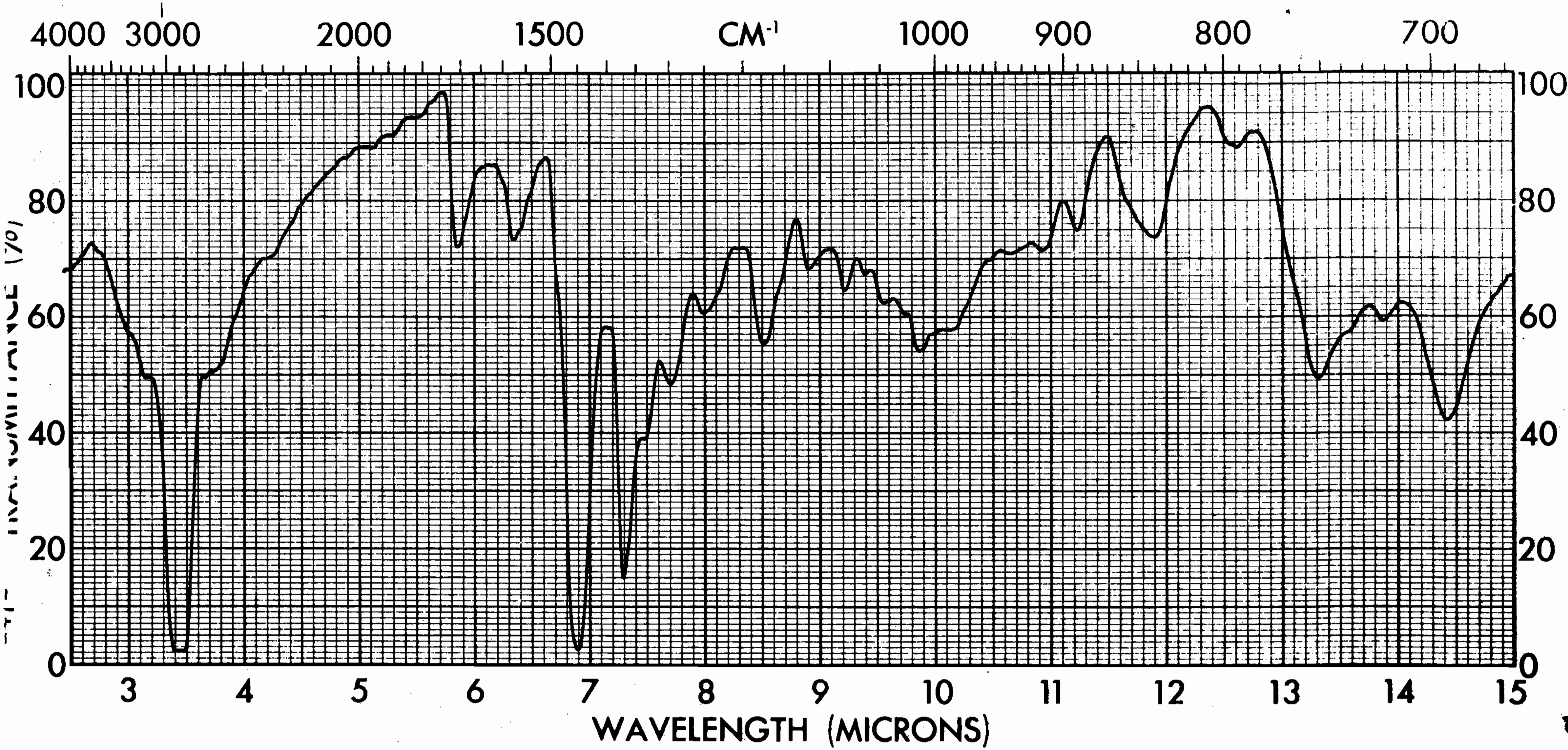
meso-1,2-dibenzoyl-1,2-dibromoethane. The literature preparation was followed and the melting point was 177.5-178.5° as compared to a literature value of 178.5-179°.(Turner, 1965).

trans-1-benzyl-2,3-dibenzoylaziridine. The procedure given in the literature was followed. The melting point was 132.5-133.5° as compared to the literature value of 134-136° (Turner, 1968).

3-benzyl-1,5-diphenyl-9,3-oxazatricyclo(3.3.1.$0^{2,4}$)nonan-7-one. This procedure was followed as in the literature except that care was taken not to use any stirring during the refluxing, as stirring prevented the reaction from occurring. The reason is unknown. The melting point was 140.5-143° as compared with the literature value of 143-144° (Turner, 1968).

3-benzyl-1,5-diphenyl-9,3-oxazatricyclo(3.3.1.$0^{2,4}$)nonan-7-one hydrochloride. The procedure was followed as was given in the literature. There was one problem, however.

When filtration was attempted, repeated decomposition occurred. Separation was achieved by decanting the solvent. It was found that if the hydrochloride salt was allowed to dry while in contact with glass, instead of the filter paper, no such decomposition was observed. The melting point was 112.5-116° as compared with 116-118° in the literature (Turner, 1968).



SPECTRUM NO. crude	ORIGIN	LEGEND	REMARKS
SAMPLE		1.	
-3-benzyl-1,5-diphenyl-	PURITY	2.	
9,3-oxazatricyclo[3.3.1.0^{2,4}]	PHASE Nujol	DATE 2/21/72	

SPECTRUM NO.
SAMPLE

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