The Physiological Evaluation of a New Analog of the Tropane Alkaloids

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by

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This paper is dedicated to my parents, Alexander J. and Ellen M. Kaczmarczyk, who have made my education possible.

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Abstract

A new analog of the tropane alkaloids, 3-methyl-1,5-diphenyl-9,3-oxazatricyclo(3.3.1.$0^{2,4}$)nonan-7-one, was synthesized. Since this hetero-tricyclic ketone is structurally similar to atropine and scopolamine, it was predicted that this new compound would exhibit parasympatholytic action. Following the preparation of the hydrobromide salt of the new analog, its physiological activity was established, in part, through a pharmacological screening. In order to determine whether or not the synthetic alkaloid would produce parasympatholytic action, its effect on mydriasis, antisialagogue activity, prevention of intestinal spasm, lacrimation, acetylcholine-induced contraction of isolated ileum of the rat, and gastrointestinal propulsion was examined. This novel analog, however, failed to exhibit the predicted parasympatholytic activity, inasmuch as it displayed only central nervous system excitation.

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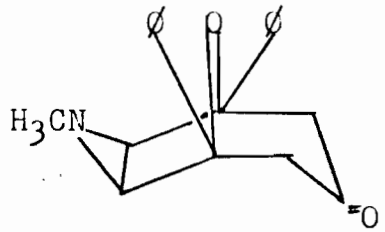
Introduction

The hydrobromide salt of a novel analog of the tropane alkaloids was prepared.



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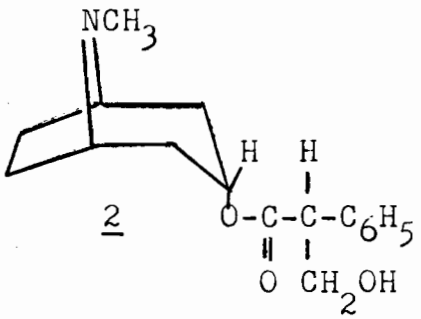
$\emptyset = C_6H_5$

The hetero-tricyclic ketone, 3-methyl-1,5-diphenyl-9,3-oxazatricyclo(3.3.1.$0^{2,4}$)nonan-7-one 1, structurally resembles the naturally-occuring tropane alkaloids, atropine 2 and scopolamine 3.



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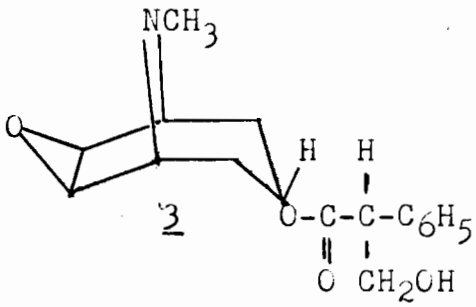

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In fact, the novel compound 1 is curiously similar, in terms of its structural configuration, to scopolamine 3, except for the interchanging of the oxygen atom and the N-methyl moiety (Turner and Lutz, 1968). However, there are two additional structural features which should not be eschewed: first, the new analog 1 lacks the tropic acid ester found in both atropine and scopolamine; second, compound 1 contains two phenyl groups which are not seen in the naturally-occuring alkaloids.

Correlation between Chemical Structure and Parasympatholytic Activity

There exists a myriad of parasympatholytic compounds, all sharing certain characteristics in their chemical structures. First of all, in the overwhelming majority of cases, they contain a substituted ammonium group or, less frequently, a sulfonium or a phosphorous group with a positively charged central atom (N,S,P) which is ordinarily called a cation head. No less characteristic of parasympatholytic compounds is the presence in their structure of one or, more often, two cyclic moieties which are somewhat removed from the cation head. Sometimes one or both cyclic moieties are replaced by rather long alkyl substituents. The chain which connects the cation head and the cyclic moieties consists ordinarily of three to six atoms of which one or, less frequently, two may not be carbon atoms. In addition, in the most active substances, there is a hydroxyl group which is connected most often to the same carbon atom to which the cyclic moieties are connected or to an adjacent atom (Kuznetsov, 1965).

It was natural to assume that the role of the cation head, with its positive charge, consists of attraction of the negatively charged field (anion center) of the choline receptor. The cation head seemingly begins the process of adsorption of the substance by the receptor. Following the attraction of the oppositely charged groups, the weaker dipole-dipole and van der Waals forces go into action; their multiplicity, especially in the case of parasympatholytic compounds, provides great stability of the complex of the substance and the receptor. It is clear that in

such an interaction, not only the presence of a positive charge at the central atom of the cation head, but also the size and shape of the cation head is of vital importance (Kuznetsov, 1965).

In the hetero-tricyclic ketone 1, as in all ammonium compounds, the presence of a positive charge on the nitrogen atom is determined by the degree of electrolytic disassociation which, for a constant pH, is, in turn, determined by the basicity of the substance. Inasmuch as the basicity of different amino derivatives varies over a rather broad range, the degree of their ionization under the conditions of an organism ranges from several percent up to one hundred percent. It is clear that the more ionized particles of the parasympatholytic compound which are in solution and which have a positive charge at the nitrogen atom, the greater will be the probability of their coming in contact with the anion center of the receptor and of the formation of a complex with it. In addition, the stability of the complex which has been formed should depend on the basicity of the compound, inasmuch as it is known that the weaker a base is, the easier it is to hydrolize its salt. Thus, high basicity should favor the parasympatholytic activity of a substance (Kuznetsov, 1965).

Mechanism of Interaction of Atropine-like Substances with Choline Receptors

Since there is a correlation between the activity and chemical structures of the various compounds which exhibit parasympatholytic activity, it could be possible that a single mechanism of physiological action is responsible for the observed

properties of these drugs. As early as 1937, Clark showed that acetylcholine combines with specific receptors (which were later termed cholinoreceptors) on the surface of the effector cell. He was the first to demonstrate the antagonism between acetylcholine and atropine and some other substances, advancing the hypothesis of concurrent antagonism between structurally similar and physiologically active substances. The antagonism of these substances is due to the reversibility of their interaction with the receptor, whereby one substance can displace another from its complex with a receptor, depending on its affinity for the receptor and its concentration. The magnitude of the action of a physiologically active substance, according to Clark, is proportional to the number of receptors engaged by it (Kuznetsov, 1965). In other words, atropine successfully competes with acetylcholine for a receptor with which it will react, thus preventing acetylcholine from forming a complex with that choline receptor.

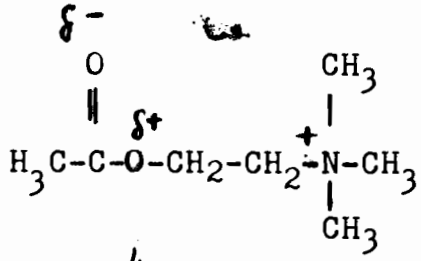
Parasympatholytic action is viewed by Pfeiffer (1948), Guggenheim (1951), and Lands (1951) as a blockade of the cholinoreceptors, following the saturation of their surfaces with molecules of a parasympatholytic. Parasympatholytics possess a more stable connection with the receptor and a larger molecular size so that they are capable, according to the above-named authors, of mechanically or of electrostatically inactivating both the receptors engaged by them and the adjacent receptors, thereby rendering them unavailable to the acetylcholine molecule. This accounts for the large molar capacity of atropine, one

molecule of which is known to block the action of several molecules of acetylcholine (Kuznetsov, 1965). Although the steric hindrance produced by the larger molecular size of the parasympatholytic compound would contribute to the mechanical inactivation of a receptor, the effect of this larger molecular size in relation to the electrostatic inactivation of a receptor is not as apparent. In fact, according to Coulomb's law, a larger molecular size should reduce the force of the electrostatic attraction between the parasympatholytic molecule and the receptor in that the force of electrostatic attraction is inversely proportional to the square of the distance between the charges which, in this case, would be increased by the larger molecular size.

In recent years, the emphasis which Clark placed on the importance of affinity has come under serious attack in that Clark suggests that the physiological activity of a substance is solely dependent on its affinity for the cholinoreceptor (Kuznetsov, 1965). If one considers the fact that the ability of a compound to both combine with a receptor and to cause a response which is characteristic of that receptor is as varied as the number of compound displaying physiological activity, one must, therefore, look more carefully at the concepts of affinity and internal, or intrinsic, activity. Affinity may be regarded as the attraction for and the recognition of a receptor by a physiologically active compound which leads to the formation of a complex between that compound and the receptor. Internal, or intrinsic, activity simulates the physiological activity which is brought about by the interaction of a mediator such as,

acetycholine, with the receptor, possibly through an alteration of the structure of the receptor (Kuschinsky and Lullmann, 1973).

In order to further explore these two concepts, acetylcholine 4 will be employed as a model, since it is endowed with both the affinity for a receptor and the ability to elicit internal activity. The basis for acetylcholine's physiological activity is its chemical structure. Kuschinsky and Lullmann (1973) maintain that acetylcholine 4 contains three spatially separated centers which determine its physiological activity: a positively charged nitrogen, a carboxyl oxygen with a partial negative charge, and an esteratic oxygen carrying a partial positive charge.



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Although there are three reactive sites, these authors suggest that only two of the three are necessary to produce the characteristic physiological activity. That is to say, a reaction invariably occurs between the quaternary nitrogen and the receptor, whereas an additional reaction occurs either between the partially positive oxygen and the receptor or between the partially negative oxygen and the receptor. This indicates that the receptor possesses two active centers: an anion center

which probably determines its affinity and an esteratic center which may be responsible for its internal activity.

Some theories of receptors embrace the notion that ". . . affinity is caused by the interaction of the electric 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)." There still remains the unanswered question of what structural feature of the molecule is the determinant of its internal activity. Kuznetsov and other investigators speculate that the internal activity of acetylcholine (and other substances with similar physiological activity) depends on the structure of the cation head and ". . . is determined by the effectiveness of the positive charge of their onium group with respect to changes in a certain part of the electric field of the receptor (Kuznetsov, 1965)."

These theories can be better understood by examining the structure of the cholinoreceptor. It is possible that the active portion of the receptor consists of two parallel protein chains which are linked by ionic and hydrogen bonds. The acetylcholine molecule disrupts the bond of the anion center with the principal group of the receptor located on the other protein chain and forms a bond between its cation head and the anion center. Simultaneously, the hydrogen bond between the two protein chains may be broken by the ester function of the acetylcholine molecule, producing a break in the structure of the receptor protein. Consequently, the extracellular and intracellular concentrations of sodium and potassium ions can

be equalized. The acetylcholine molecule can somewhat strengthen its link with the protein chain through van der Waals forces, but its complex with the receptor will necessarily remain sufficiently unstable so that the processes which return the receptor to its original state can be carried out in the shortest time possible. Otherwise, the automatism of nerve transmission would be impaired (Kuznetsov, 1965).

Since the mechanism apparently involves a membrane-like structure, composed of protein and phospholipids, it is reasonable to assume that amino acid residues of the protein portion, as well as phosphate moieties of the phospholipid segment, of this structure are intimately associated with the process. This is the contention of Kuschinsky and Tullmann (1973) who assert that the reaction at the anion center occurs between carboxyl or phosphate moieties and a molecule of acetylcholine. On the other hand, the reaction at the esteratic center is between a molecule of acetylcholine and the hydroxyl groups of serine or tyrosine residues, in addition to a reaction which can occur between the acetylcholine and an imidazole nitrogen of a histidine residue. Furthermore, the involvement of two protein chains is supported by Kuschinsky and Tullmann (1973) in that they contend both the anion center and the serine and tyrosine residues of the esteratic center are contained within the structure of one protein chain while the imidazole nitrogen of the histidine residue is found as part of another protein chain.

Kuznetsov (1965) notes that the interaction of a parasympatholytic molecule with a cholinoreceptor partly resembles

the above description of the interaction of acetylcholine with a cholinoreceptor. The parasympatholytic molecule cleaves the ionic bond, as well as occasionally rupturing the hydrogen bond, between the protein chains. Because of the presence of functional groups such as, cyclic moieties and hydroxyl groups, not only is the range of the interaction of the parasympatholytic substance with the receptor broadened, but also the stability of the bond between them is substantially increased. Moreover, a point which Kuznetsov (1965) emphasizes is the ability of the parasympatholytic compound, with structural characteristics unlike those of acetylcholine, to form a bond with both protein chains of the cholinoreceptor, simultaneously "sewing them together" more firmly than in the case of the native protein. As a result of the structural integrity of the cholinoreceptor being preserved, there can be no alteration in ionic permeability; hence, there can be no depolarization of the membrane. In addition, since the stability of the complex comprised of the parasympatholytic compound and the cholinoreceptor is so great, the receptor is not available to acetylcholine and is thus excluded from participation in nerve transmission.

Determination of Physiological Activity

Pharmacological Screening

The initial step in establishing a profile of the physiological activity elicited by the test compound was a pharmacological screening. This procedure was employed in order to ensure that any physiological activity, in addition to the expected parasympatholytic activity which was predicted on the basis of a structural similarity to scopolamine, could be observed. The particular screening method which was chosen (Irwin, 1959) was selected because it is claimed that it can detect not only sedatives, hypnotics, tranquillizers, psychomotor stimulants, muscle relaxants, analgesics, and convulsants; but also neuromuscular blocking agents, atropine-like drugs, ganglion-blocking agents, anti-pyretics, peripheral vasodilators, and acetylcholine-like compounds (Vane, 1964).

Method

The screening was performed on groups of four mice, consisting of two males and two females. The weights ranged from 20 g to 30 g. The dose levels which were examined included 0.01 mg/kg, 0.1 mg/kg, 1.0 mg/kg, 10.0 mg/kg, 25.0 mg/kg, 50.0 mg/kg, and 100.0 mg/kg. All doses were given by intraperitoneal injections. Members of a control group conforming to the above conditions were injected intraperitoneally with an equivalent volume of a 0.85% saline solution.

The screening was accomplished by repeatedly employing the same basic technique. After intraperitoneally administering the test compound to two experimental mice and saline to two controls (N.B., unless otherwise noted, it should be assumed

that all mice are of the same sex), observations were made. The observations are assigned to the following major categories: awareness, mood, motor activity, excitation of the central nervous system, posture, motor inco-ordination, muscle tone, reflexes, and autonomic profile. Comparisons with the controls were made at 15, 30, 60, 90, and 120 minutes with the results recorded and scored according to the suggestions purported by Irwin (1959). The reader's attention is directed to Tables 1 and 2 for a listing of the subdivisions of the categories which were examined and to Irwin's published works (1959, 1962, 1963) for a detailed description of the screening technique which was used in effecting an evaluation of these parameters. A brief consideration of the method of scoring these parameters is needed so that any possible confusion may be averted. The various effects are assigned scores over an arbitrary rating scale from 0 to 8; 4 for a characteristic normally present, allowing increases to 8 for increases in the characteristic and decreases to 0 for diminution of the characteristic. Signs normally absent begin at 0, and the relative activity of the drug is recorded as an increase in score up to 8 (Vane, 1964).

Results

The pharmacological screening was performed once on each dose group; the results are compiled in Tables 1 and 2 in terms of the average of a given dose. At relatively low dosages, the only observed deviations from the normal were increased grooming, increased touch response, and increased pain response. It is not known if a one unit change is significant. If, however,

these observations are valid, the excessive grooming, increased touch response, and increased pain response may indicate central nervous system stimulation or sympathetic stimulation. In short, the data for the doses from 0.01 mg/kg to 25.0 mg/kg indicate that the compound exhibits stimulatory activity and not parasympatholytic as hypothesized.

The data for the higher doses (i.e., 50.0 mg/kg and 100.0 mg/kg) are equally as dubious as the data for the lower doses. That is, these observations were made at approximately 3 minutes after the intraperitoneal injection of the test compound when the laboratory animals were the victims of violent convulsions which would mask a host of physiological changes.

On the other hand, the increased passivity may be indicative of myorelaxation; the reduced spontaneous activity and reactivity may measure neuromuscular blockade; the deviations in both body posture and limb position may indicate neuromuscular blockade; the appearance of abnormal gait may manifest myorelaxation; the abrogation of the righting reflex may demonstrate the action of an agent causing synaptic blockade; and the absence of the ipsilateral flexor reflex may show that the test substance may be active in blocking synaptic transmission. These observations would indicate more strongly the expected presence of a parasympatholytic compound if there were a reduction in body and abdominal tones. It may be the case that myorelaxation did occur in these areas, but it perhaps was not detected because of an insufficient level of expertise in effecting such determinations. Nevertheless, it should be emphasized that the test compound does, in fact, display stimulatory action.

		Awareness			Mood					Motor Activity			CNS Excitation					Posture		Motor Incoord.		Muscle Tone				Reflexes		Autonomic		
Mg/Kg Dosage (4 animals/dose)	Normal Score	Alertness	Visual Placing	Passivity	Stereotype	Grooming	Vocalization	Restlessness	Irritability	Fearfulness	Reactivity (Envir.)	Spontaneous Activity	Touch Response	Pain Response	Startle Response	Straub Response	Tremors	Twitches	Convulsions	Body Posture	Limb Position	Staggering Gait	Abnormal Gait	Righting Reflex	Limb Tone	Grip Strength	Body Sag	Body Tone	Abdominal Tone	Pinna	Corneal	IPR	Writhing	Palpebral Opening	Urination	Salivation	Skin Color
0.01	4	4	4	0	0	5	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	0	4	0	0	4
0.1	4	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	0	4	0	0	4
1.0	4	4	4	0	0	4	0	0	0	0	4	4	5	4	0	0	0	0	0	4	4	0	0	0	4	4	0	4	4	4	4	0	4	0	0	4
10.0	4	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	0	4	0	0	4
25.0	4	4	4	0	0	4	0	0	0	0	4	4	5	5	0	0	0	0	0	4	4	0	0	0	4	4	0	4	4	4	4	0	4	0	0	4

Albino | ♀ and ♂ | IP | 20 - 30 g | 0.85% Saline

	Awareness				Mood					Motor Activity				CNS Excitation					Posture		Motor Incoord.			Muscle Tone					Reflexes			Autonomic				
Mg/Kg Dosage (4 animals/dose)	Alertness	Visual Placing	Passivity	Stereotype	Grooming	Vocalization	Restlessness	Irritability	Fearfulness	Reactivity (Envir.)	Spontaneous Activity	Touch Response	Pain Response	Startle Response	Straub Response	Tremors	Twitches	Convulsions	Body Posture	Limb Position	Staggering Gait	Abnormal Gait	Righting Reflex	Limb Tone	Grip Strength	Body Sag	Body Tone	Abdominal Tone	Pinna	Corneal	IFR	Writhing	Palpebral Opening	Urination	Salivation	Skin Color
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	0	0	4
50.0	4	0	8	0	4	0	0	0	0	0	0	0	0	0	0	4	0	6	0	0	0	1	8	6	2	0	6	4	4	4	0	0	4	1	0	4
100.0	2	0	8	0	0	0	0	0	0	0	0	0	0	0	0	4	1	6	2	2	0	8	8	6	3	0	5	5	0	0	0	0	4	0	0	4

Acute Toxicity

Method

Twelve female mice whose weights ranged between 20 g and 30 g were employed in the determination of the acute toxicity of the test compound. The testing began at 50 mg/kg, since the pharmacological screening had shown that death results at both 50 mg/kg and 60 mg/kg. The test compound was administered intraperitoneally to four mice of a given dose group. There were three doses which were studied: 50 mg/kg, 55 mg/kg, and 60 mg/kg. After receiving the test compound, the mice were placed in separate litter-free cages. Twenty-four hours later, the number of deaths was recorded. The data which are contained in Table 3 were utilized in the calculation of the LD_{50_1} for the intraperitoneal injection of the test substance. Using the method of Reed and Muench (1938), a statistically valid calculation of the LD_{50} can be made with an extremely small sample. This method involves addition of mortalities and survivals for the various groups of animals and computation of the LD_{50} from the cumulative mortalities and survivals which bracket the 50% point (Diehl, 1973). The following formula which is an adaptation of this method was employed in the calculation of the LD_{50_1}.

$$\log LD_{50} = \log (\text{dose} < LD_{50}) + \frac{50 - (\%\ \text{mortality} < 50\%)}{(\%\ \text{mortality} > 50\%) - (\%\ \text{mortality} < 50\%)} \times \log (\text{dose increment})$$

Results

The data presented in Table 3 were used in the calculation of the LD_{50_1} by the method of Reed and Muench (1938) to give an LD_{50_1} of 56.6 mg/kg.

Table 3. Acute Toxicity Data

		Actual		Cumulative		
Dose	Mice Used	Living	Dead	Living	Dead	% Mortality
50 mg/kg	4	3	1	7	1	12.5
55 mg/kg	4	3	1	4	2	33.3
60 mg/kg	4	1	3	1	5	83.3

Hepatotoxicty

Method

This simple test for hepatotoxicity makes use of the fact that the damaged liver does not metabolize, or detoxify, drugs such as, pentobarbital sodium, thus prolonging the effect of the drugs (Turner, 1965).

Six male albino mice weighing between 20 g and 30 g were given subcutaneously 0.05 ml of a 0.2% solution of the test compound in propylene glycol per 10 g of body weight. Twenty-four hours later, 45 mg/kg of pentobarbital sodium was administered intraperitoneally and the duration of sleep measured. Two controls were given an equivalent volume of propylene glycol and subsequently injected intraperitoneally with 45 mg/kg of pentobarbital sodium. The mean sleeping-time is 9.0 $\pm$ 7.3 minutes for the control mice. A sleeping-time of 23 minutes or more is selected as being significantly prolonged, indicating possible liver damage (Turner, 1965).

Results

The initial data (Table 4) are ambiguous in that some mice either had no sleep or had sleep lasting as long as 75 minutes. After repeating the procedure, it was found that the replication data (Table 5) did not differ greatly from the data in Table 4. However, if one examines the mean sleep-times for the data of Table 4, one will notice that the experimental mice exhibited a mean sleep-time of 25.67 minutes which may suggest that liver damage had occurred. On the other hand, if the mean sleep-times of Table 5 are scrutinized, one will observe that the controls had a mean sleep-time (26.00 minutes)

which was two-fold larger than that of the experimental mice (13.00 minutes). Nevertheless, an entirely different interpretation of the data is possible if all the data are combined and the mean sleep-times calculated. Upon doing so, the mean sleep-time of the controls becomes 13.00 minutes, whereas the mean sleep-time of the experimental mice becomes 19.33 minutes. This may indicate that the test substance does not produce liver damage.

Mydriasis

Method

Two adult rabbits, a male and a female, were used to test for mydriasis. Two drops of a 2.0% solution of the test compound were placed in the left eye of each rabbit. The right eye of each rabbit served as a control. The pupillary diameter of each eye was measured at 15 minute intervals. The pupillary diameters were determined by restraining the rabbit in a stockade and then measuring the diameter of both pupils with a transparent metric rule. This was facilitated by using a hand-held magnifying lense.

Results

No significant change in pupillary diameter was observed in the experimental (left) eye of either rabbit when it was compared with the control (right) eye of that rabbit. The reader is referred to Table 6 for actual pupillary diameters.

Table 6. Pupillary Measurements of Rabbits used in Testing for Mydriasis

Rabbit	Time (Min.)	Left Diameter (cm.)	Right Diameter (cm.)
Male	15	.70	.70
	30	.65	.70
	45	.70	.70
	60	.70	.70
Female	15	.60	.60
	30	.60	.65
	45	.65	.60
	60	.60	.60

Antisialagogue Activity

Method

The blockade of salivation in rats weighing between 225 g and 270 g was studied. Because of the limited number of rats available for experimentation, only one rat per dose was used. The test compound was given at 40 mg/kg and 80 mg/kg by intraperitoneal injection. Five minutes later a 10 mg/kg intraperitoneal injection of methacholine was administered to induce salivation. The interim between injections of the test compound and the methacholine was increased to 15 minutes when the test compound was administered intraperitoneally at 100 mg/kg and 200 mg/kg. Comparisons were made with a control which received an intraperitoneal injection of a saline solution before being injected with 10 mg/kg of methacholine. Gross observations were used to determine if salivation had been blocked, as all-or-none responses were recorded.

Results

The results of the testing are compiled in Table 7. Even with the increased amount of time interposed between injections of the test compound and the methacholine and with doses as large as 100 mg/kg and 200 mg/kg (which eventually produced death), the synthetic analog did not effect the blockade of salivation. In fact, in all cases the salivation was as copious as that manifested by the control.

Table 7. Results of Testing for Antisialagogue Activity
(+=salivation)

Rat	Dose Test Compound	Interim Between Injections	Response
Control	--	5 min.	+
Expt. 1	40 mg/kg	5 min.	+
Expt. 2	80 mg/kg	5 min.	+
Expt. 3	100 mg/kg	15 min.	+
Expt. 4	200 mg/kg	15 min.	+

Prevention of Intestinal Spasm

Method

Four fasted, albino mice weighing between 20 g and 25 g were given 50 mg/kg of a 0.2% solution of the test compound by stomach tube. One half hour after administering the test substance, the animals were anesthetized by an intraperitoneal injection of 10 mg/kg of pentobarbital sodium (Turner, 1965). When the animals no longer responded to a vigorous tail pinch, the abdominal cavity was opened, exposing the intestines. Methacholine (0.2 ml containing 0.25 μg) was applied topically via pipette to the intestines in order to produce an intestinal spasm. The intestines were observed for a three-second period. Although the primary concern was observing prevention of intestinal spasm, gross observations were made for not only a reduction in intensity of the spasm, but also for a restriction of the spasm to a limited area. Comparisons were made by gross observation with the exposed intestines of a control that received an equivalent volume of distilled water by stomach tube.

Results

The results are presented in Table 8. The synthetic alkaloid being evaluated did not prevent the induced spasm, reduce the intensity of the spasm, nor restrict the spasm to a limited area when compared with the response of a control which received distilled water by stomach tube instead of the test compound.

Table 8. Prevention of Intestinal Spasm Data
(+=unaffected intestinal spasm)

Mouse	Weight (g)	Vol. Test Compound	Spasmodic Response
Control	20	--	+
Expt. 1	21	0.52 ml	+
Expt. 2	24	0.60 ml	+
Expt. 3	20	0.50 ml	+
Expt. 4	25	0.62 ml	+

Lacrimation

Method

Adult rats whose weights ranged between 375 g and 555 g were employed in an attempt to demonstrate blockade of lacrimation produced by carbamylcholine, a parasympathomimetic agent. An incandescent light was placed in close proximity to the rat's tail in order to cause vasodialation so that the test compound could be injected into the tail vein. The doses studied included 20 mg/kg, 100 mg/kg, and 200 mg/kg; the duration of intravenous injection injection was approximately 20 seconds. A dose of 0.5 mg/kg of carbamylcholine was given by intraperitoneal injection 15 minutes after the administration of the test compound. A control which received an intravenous injection of saline (into the tail vein) before being given an intraperitoneal injection of carbamylcholine was used for comparisons. The appearance of a yellowish-brown spot on a cotton swab when pressed against the eye within 3 minutes after administering the carbamylcholine is regarded as a positive response (Turner, 1965). This is accompanied by an increased flow of tears which can be detected by gross observation.

Results

The results obtained by using the above method are given in Table 9. As compared with a control that received saline prior to being injected with carbamylcholine, the experimental rats showed neither a blockade of lacrimation nor a diminished volume of tear flow.

Table 9. Lacrimation Data
(+=lacrimation)

Rat	Weight (g.)	Vol. Test	Vol. Carbamylcholine	Response
Control	510	--	0.26 ml	+
Expt. 1	555	0.56 ml	0.28 ml	+
Expt. 2	375	1.88 ml	0.19 ml	+
Expt. 3	505	5.05 ml	0.25 ml	+

Acetylcholine-Induced Contraction in the Isolated Ileum of the Rat

Method

A rat was sacrificed by cervical dislocation after which the abdominal cavity was opened quickly, and a segment of the ileum was removed. The tissue was placed in an aerated bath of Tyrode's solution kept at 37° C. A segment, 1 to 2 cm in length, was ligated and cut from the remaining tissue. This ligated segment of ileum was secured to an anchor in a specially designed muscle chamber, as well as to a myograph that was connected to a physiograph. The temperature of the muscle chamber was maintained at 37° C by being suspended in a water bath. The system was designed in such a way that the Tyrode's solution could be aerated, as well as drained and replenished, without altering the tension on the intestinal segment. In addition, the design allowed acetycholine and/or any test substance to be injected through a small opening in the top of the muscle chamber.

A series of spontaneous contractions were recorded (paper speed 0.5 cm/sec.). With continued recording, acetylcholine was added to the Tyrode's solution, bringing the concentration of acetylcholine to 0.1 μg/ml. The muscle chamber was then emptied, and fresh Tyrode's solution was added. Spontaneous contractions were again recorded. While recording, acetylcholine (0.1 μg/ml) was added to the Tyrode's solution immediately after which the new analog (16 μg/ml) was introduced into the muscle chamber. The above procedure was repeated with scopolamine substituted for the test compound. The procedure was

later performed as follows: after a brief recording of spontaneous contractions and a reaffirmation of the character of the acetylcholine-induced contraction, the Tyrode's solution was drained and the chamber filled with fresh Tyrode's solution. Spontaneous contractions were again recorded. At this point, the effect of adding the test compound (16 μg/ml) immediately followed by introducing the acetylcholine (0.1 μg/ml) was recorded.

Results

Figure 1a shows the spontaneous contractions followed by an acetylcholine-induced contraction. The effect of adding the test compound immediately after the introduction of acetylcholine can be seen in Figure 1b. In this case, no apparent alteration in the acetylcholine-induced contraction was recorded. However, when the procedure was repeated, the test compound appeared to have caused a slight reduction in the intensity of the acetylcholine-induced contraction (Figure 1c). Although the procedure was repeated again, the previously discribed results were not obtained. Instead, the test compound appeared to have blocked the smooth muscles response to acetylcholine (Figure 1d). This apparent blockade of an acetylcholine-induced contraction (Figure 1d) does not resemble the recorded activity of scopolamine (Figure 1e), a compound known to be a parasympatholytic agent. In addition, it appears as though the order of the introduction of the test compound and the acetylcholine is not crucial in that Figure 1f which represents the introduction of the test compound followed by the addition of the acetylcholine is not significantly different from Figure 1b.

Although ambiguous results were obtained, one, nevertheless, can conclude that the test compound does not exhibit parasympatholytic activity. More importantly, this novel analog does not appear to display parasympathomimetic activity, nor does it appear to have any effect on smooth muscle.

Figure 1. Smooth Muscle Responses of the Isolated Ileum of the Rat

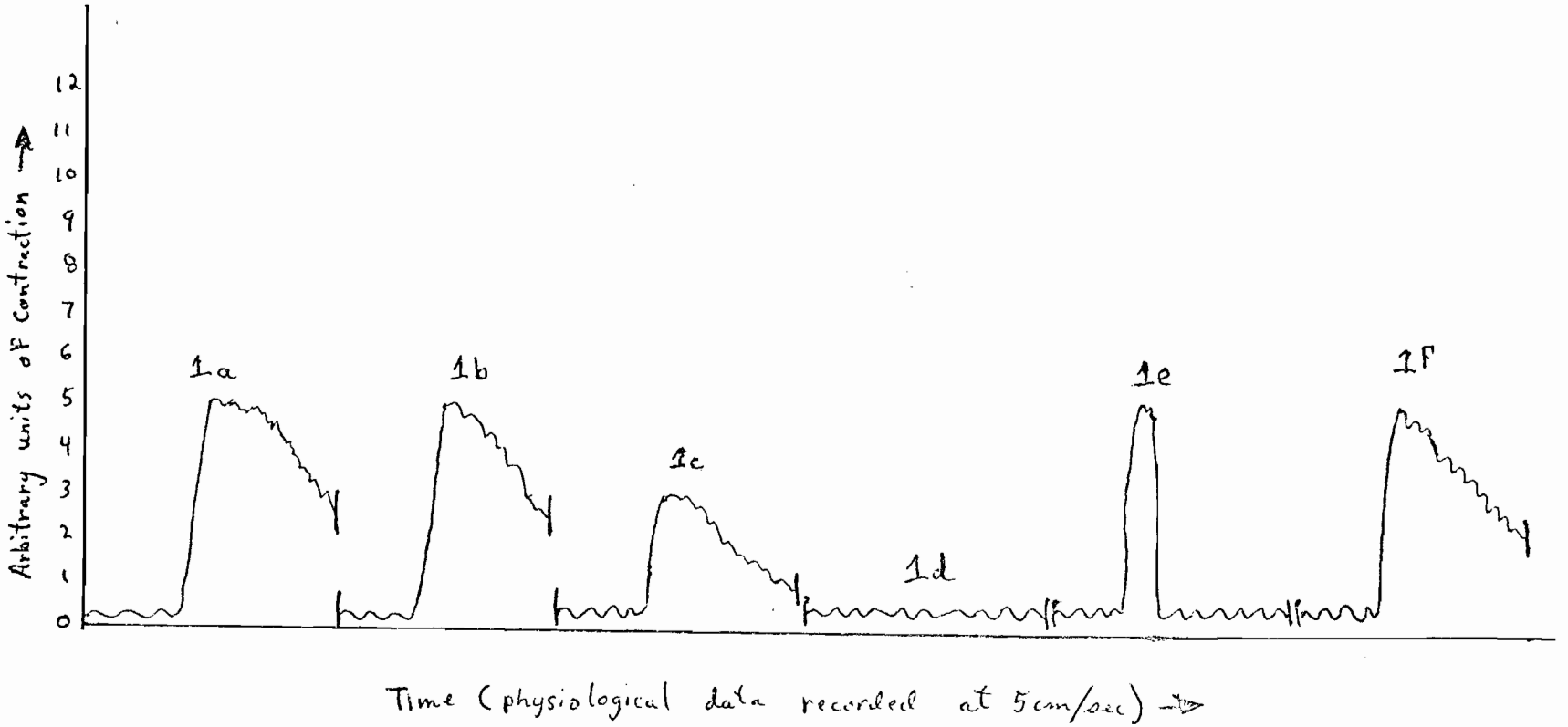


Figure 1a Spontaneous Contractions followed by an Acetylcholine-Induced Contraction.
1b Spontaneous Contractions followed by the Addition of Acetylcholine and the Test Compound which had no effect on the Acetylcholine-Induced Contraction.
1c Spontaneous Contractions followed by an Acetylcholine-Induced Contraction which was Apparently Reduced in Intensity by the Addition of the Test Compound.
1d Spontaneous Contractions and the Apparent Prevention of an Acetylcholine-Induced Contraction by the Test Compound.
1e Spontaneous Contractions and an Acetylcholine-Induced Contraction that was blocked by the Addition of Scopolamine.
1f Spontaneous Contractions followed by the Addition of the Test Compound and then Acetylcholine, resulting in an Acetylcholine-Induced Contraction.

Gastrointestinal Propulsion

Method

Six adult, female albino mice were fasted overnight. Four mice were then injected intraperitoneally with 0.1 ml of a 0.2% solution of the test compound per 10 g of body mass. The remaining two mice which received an intraperitoneal injection of saline (0.1 ml/10g) were used as controls. Exactly one hour later, 0.3 ml of an aqueous suspension of 10% charcoal and 5% gum acacia was given by stomach tube as a food substitute that would not be metabolized. The mice were killed two hours later by cervical dislocation. The gastrointestinal tract was then exised from the cardia to the anus and examined for the presence of the black suspension. A quantal criterion for antispasmodic activity was employed: control mice which were injected with saline have black ceca, those mice to which received an antispasmodic have ceca that are free of charcoal (Turner, 1965).

Results

The analog under scrutiny failed to inhibit peristalsis, inasmuch as the ceca of all four mice that received the test compound contained charcoal. In other words, the test compound does not show any antispasmodic activity.

Analeptic Properties

Method

Since the test compound may produce excitation of the central nervous system as indicated by the pharmacological screening; in addition to the fact that some mice did not succumb to the barbiturate administered during the hepatotoxicity testing, it was hypothesized that the compound may possess analeptic properties. One of the primary uses of an analeptic is a function of its restorative action; that is, analeptics are employed to revive a patient who has taken a lethal overdose of a hypnotic drug such as, a barbiturate (Turner, 1965).

Groups of six adult mice (20 g to 30 g in weight) were given an intraperitoneal dose of 130 mg/kg of pentobarbital sodium (Turner, 1965) immediately after which they received a subcutaneous injection of the test compound. The test compound was administered in doses of 120 mg/kg, 180 mg/kg, 240 mg/kg, 300 mg/kg, and 360 mg/kg. The number of survivors in each group was recorded 24 hours later.

Results

The results of the testing are presented in Table 10. The only mice which did not die as a result of the lethal dose of pentobarbital sodium were those in the group which received a dose of 300 mg/kg of the test compound. That is to say, a 33.3% survival rate was obtained because the stimulatory effects of the hetero-tricyclic ketone offset the depressive action of the pentobarbital sodium. However, all of the mice in the group receiving a dose of 360 mg/kg of the test compound died probably because of the toxicity of the test compound and not as a result of the depressive action of the pentobarbital sodium.

This point is supported by the types of behavior prior to death. The mice which received 120 mg/kg to 240 mg/kg of the test compound exhibited a flaccid paralysis prior to death. However, the mice receiving 360 mg/kg of the test compound displayed tetanic paralysis prior to death. In addition, those mice that survived (300 mg/kg of test compound) were victims of tetanic paralyses from which they eventually recovered.

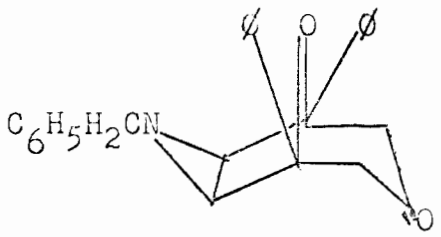
Table 10. Analeptic Properties Data

Dose Test Compound	Number Survivors	Per Cent Survival	Behavior
120 mg/kg	0	0	Flaccid paralysis
180 mg/kg	0	0	Flaccid paralysis
240 mg/kg	0	0	Flaccid paralysis
300 mg/kg	2	33.3	Tetanic paralysis
360 mg/kg	0	0	Tetanic paralysis

Discussion

If one examines the data obtained from the pharmacological screening (Tables 1 and 2) and the various tests in an attempt to construct a profile of the novel, hetero-tricyclic ketone's physiological activity, one can conclude that such a profile would include central nervous system excitation. Although various interpretations of the data collected during the blind screening procedure are possible, most interpretations would be of nebulous merit, since the presence of tremors and convulsions could overshadow other physiological changes. Moreover, the tremors, convulsions, and paralysis could be conducive to the making of faulty inferences. Paralysis, for example, might be misinterpreted as excessive passivity.

The test compound produces central nervous excitation as demonstrated by the 33% survival rate which was found during the exploration of possible analeptic properties. However, the compound does not exhibit the predicted parasympatholytic activity; that is, it failed to induce mydriasis, to manifest antisialogogue activity, to prevent intestinal spasm, to abrogate lacrimation, to block an induced contraction in the isolated ileum of the rat, and to inhibit gastrointestinal propulsion. The lack of parasympatholytic properties is most surprising since a similar compound, 3-benzyl-1,5-diphenyl-9,3-oxazatricyclo ($3.3.1.0^{2,4}$) nonan-7-one 5, possesses weak and transient cholinolytic properties (Kramer, 1972).



$\emptyset = C_6H_5$

5

As can be seen, the only structural difference between the compounds 1 and 5 is that analog 1 contains an N-methyl moiety, whereas the analog 5 contains an N-benzyl moiety. One can, therefore, infer that the different physiological activities of these two analogs are a result of the variation in cation heads. Although the N-methyl group should contribute significantly to increased parasympatholytic action (Kuznetsov, 1965), the data collected fails to support this claim. This seemingly inexplicable situation may be rationalized if one considers the steric hindrance which could result from the combination of three cyclic moieties per molecule. In other words despite a possible reduction in affinity, the N-benzyl analog could, with its three bulky phenyl groups, mechanically or electrostatically prevent the receptors from reacting with acetylcholine. The feasability of this occurring is enhanced by the fact that the phenyl groups are one of the primary anchor groups which, through Van der Waals forces, can strengthen the bond between the parasympatholytic molecule and the receptor (Kuznetsov, 1965). Since this argument eschews the importance of the positioning of the cyclic moieties, it is subject to critical attack.

The reversal of the N-methyl moiety and the oxygen bridge, as contrasted with that of scopolamine, may contribute to a

reduction or an absence of parasympatholytic activity in the N-methyl analog. This structural alteration may be more crucial than one would assume in that it constitutes a relocation of the cation head with respect to the oxygen atoms of the molecule. That is to say, this interchange of an oxygen atom and an N-methyl nitrogen group could impair greatly the interaction of the compound with the amino acid residues of the protein structure of the receptor, thereby causing a diminution of the compound's physiological activity. In addition, this contention may seem more plausible in light of the predictions made by Ing (1949), who was concerned with the size of the main chain of the molecule, as well as in view of the hypothesis advanced by others who have attempted to calculate the maximal distances in angstroms between functional groups. This indicates that there can be little or no modification of the configuration of the molecule, if one is to ensure maximal parasympatholytic activity.

Although the importance of the esteratic linkage has been a moot point, the ester function has been demonstrated not to be necessary for the manifestation of parasympatholytic activity (Rama Sastry, 1970). Obviously, the synthetic alkaloid is not esterified. This may explain, in part, the absence of parasympatholytic activity. The ester may effect optimal physiological action in atropine-like compounds through its ability to influence the comformation of the parasympatholytic molecule which, in turn, determines the extent of the interaction between the major bonding groups (the cation head and the cyclic moieties) and the receptor (Rama Sastry, 1970).

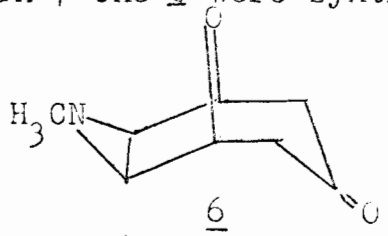
In summary, the observed physiological activity produced by this hetero-tricyclic ketone 1 can only be described as central nervous system excitation which is affirmed by the observations made during the pharmacological screening, by the absence of sleep in some animals seen during the testing for hepatotoxicity, and by the compound's analeptic properties. The compound's physiological activity may be attributable to the transposition of the N-methyl moiety and the oxygen bridge, as well as to the absence of an esteratic linkage. In addition, the presence of the phenyl groups at positions 1 and 5 may contribute to the manifestation of the observed physiological action. Furthermore, if one compares compound 1 with both acetylcholine 4 and scopolamine 3, one can see that the carbonyl function of compound 1 is more similar to that of acetylcholine 4 than it is to scopolamine 3 in that compound 1 has a free carbonyl group. This free carbonyl group may interact with the esteratic center of the choline receptor, thereby confering a limited amount of intrinsic activity upon the compound. Moreover, since compound 1 produces tremors, convulsions, and paralysis, the site of the stimulatory action of this compound may be located either at the motor centers of the brain or at the synapses of the affected striated muscle. It should be emphasized that although the synthetic alkaloid is a stimulant, it does not have any effect on smooth muscle. There is a possibility that the observed physiological properties of this analog are due to impurities in the hydrobromide salt used in the evaluation of this compound. Because of the extremely hydroscopic nature of the salt which made the isolation of the

salt from solution very difficult, it was decided to abort any attempts to recrystallize the compound and use it as it was collected.

Future Work

The first point of interest which should be studied is the physiological effect of the formation of the tropic ester of compound 1. That means the ketone would have to reduced to an alcohol and then reacted with tropic acid (Kaczmarczyk, 1973). After the completion of the synthetic chemistry, a detailed physiological investigation should be conducted.

The effect of the reversal of the N-methyl moiety and the oxygen atom could be demonstrated if 3-methyl-9,3-oxazatricyclo $(3.3.1.0^{2,4})$nonan-7-one 6 were synthesized.



6

Once the ketone 6 has been successfully prepared, it should be reduced to the corresponding alcohol and esterified with topic acid (Kaczmarczyk, 1973). This compound would then differ from scopolamine 3 only in the position of the N-methyl group and the oxygen atom, thereby affording an excellent opportunity to explore the importance of the positioning of the aforementioned N-methyl moiety and oxygen atom.

In addition, compound 6 should be esterified with acetic acid and its physiological activity compared with that of compound 6 esterified with tropic acid. The acetate ester, since it resembles the ester portion of acetylcholine 4, may contribute to stimulatory action, whereas the tropic ester may enhance blockade as it is the ester found in both atropine 2 and scopolamine 3.

Appendix 1

History of Natural Sources

The tropane alkaloids are widely distributed in nature, especially in plants belonging to the order Solanaceae. Atropine (dl-hyoscyamine) 2 and scopolamine (l-hyoscine) 3, the most important and best known of the naturally-occuring alkaloids, are derived from Atropa belladonna (deadly nightshade), Hyoscyamus niger (black henbane), and Datura stramonium (jamestown weed, jimson weed, thron apple, or stink weed) (Rama Sastry, 1970; Cullumbine, 1967; Goodman and Gilman, 1952; Hamerslag, 1950). Although the aforementioned solanaceous plants contain mainly l-hyoscyamine, atropine is formed upon extraction when l-hyoscyamine racemizes to the more stable form, dl-hyoscyamine (Cullumbine, 1967). Scopolamine, however, is obtained predominately from Datura metel and Scopolia carniolia (Jenkins et al, 1967). In addition, it is important to note that the relative amounts of these two alkaloids differ not only from species to species, but also vary with the part, the location or origin, and the age of the plant from which they are extracted (Hamerslag, 1950).

Since the plants from which these compounds are derived are found the world over, these drugs have enjoyed a long and rich history which is affirmed by allusions to them in the Ebers papyrus, the Greek herbal of Dioscorides, and the Grand Herbier. One of the earliest applications of the solanaceous alkaloids is credited to the Greeks who used Datura at the oracular shrine of Apollo at Delphi where the prietess, Pythia, who was seated on a tripod, would reply to questions with incoherent words which

were uttered in divine ecstasy. Pythia was intoxicated by inhalation of fumes from burning leaves of Datura. A common use of Datura was to facilitate robbery or conspiracy: Indian courtesans placed Datura in their visitors' wine so that their victims could be robbed with ease. As recently as 1908, Datura was to play a key role in a nefarious performance that included a plot to stupefy and murder the European Garrison at Hanoi, Viet Nam (Rama Sastry, 1970).

The practice of habitually smoking the leaves of Datura stamonium for pleasure has been witnessed in various parts of Africa and India. Moreover, the leaves are frequently mixed with other intoxicants such as, cannabis, alcohol, or tobacco, producing an inebriating concoction. The American Indians averted these time consuming practices without compromising the desired effects by simply chewing the leaves of the plant. In addition, several forms of Datura were employed by the American Indians in their religious ceremonies as a means of promoting visions and as a way of providing for intoxication of the young braves of the tribe in order to ensure that they could withstand the painful initiation ceremonies (Hollister, 1968).

The most animated and amusing account describing the ostensible effects of Datura stramonium occurred in 1676 among the British troops bivouacked in Jamestown, Virginia:

> They had cooked the plant as food, and for several days many behaved in a strange and comical manner, some taking eleven days to recover and having amnesia for the entire event. The narcotic properties of the Jamestown, or Jimson, weed soon became known among the colonists, though it was not news to the Indians (Hollister, 1968). . . .

Similarly, the pharmacological properties of the shrub Hyoscyamus niger did not go unnoticed for as early as the first century, the Romans were well aware that the black variety of Hyoscyamus caused insanity. Later, during the Middle Ages, the plant was used by sourcerers as a potion for conjuring up demons. Its biological distribution is further demonstrated by the fact that a species of henabene with dry, felt-like leaves flourished in Egypt and surrounding lands.

Leaves of Sekaran, as it was termed by the Arabs, were smoked in order to produce a state of inebriation (Hollister, 1968).

Atropa belladonna did not escape the annals of history nor the pages of great literature. Barton and Castle express this sentiment with their assertion that Atropa belladonna was alluded to by Shakespeare in the speech of Banquo to Macbeth (Goodman and Gilman, 1952):

> Or have we eaten of the insane root,
> That takes the reason prisoner?

The history of Atropa belladonna does not begin with Shakespeare for the Ancient Hindus employed galenical preparations of belladonna in the practice of medicine. However, the properties of the plant were not always utilized with noble intentions, inasmuch as the professional poisoners of the Middle Ages often used deadly nightshade to produce prolonged and obscure intoxication (Goodman and Gilman, 1952). Goodman and Gilman (1952) remind us that this prompted Linne to name the shrub Atropa belladonna, after Atropos, the eldest of the Three Fates, who severs the thread of life. It is interesting to note that the

word "belladonna" is itself an indication of the antiquity of Atropa belladonna in that it means "beautiful lady." That is, in order to induce dialation of the pupils, a sign of comeliness, Venetian women would instill a decoction of belladonna into their eyes. Evidently, this was a small price to pay for beauty, despite the disturbance of vision (Goodman and Gilman, 1952; Cullumbine, 1967).

Appendix 2

Synthesis of the Test Compound

meso-1,2-Dibenzoyl-1,2-dibromoethane

The procedure which was delineated in the literaure[17,18,19] was employed. A melting point of 179-180° C was observed (lit mp 178.5-179.0° C).

trans-1-Methyl-2,3-dibenzoylaziridine

The compound was prepared as it was described in the literature[17,18]. Ten g (0.025 mole) of meso-1,2-dibenzoyl-1,2-dibromoethane gave 5.37 g (0.02 mole) of aziridine (80% yield) which melted at 85-87° C (lit mp 88-88.5° C).

3-Methyl-1,5-diphenyl-9,3-oxazatricyclo[3.3.1.$0^{2,4}$]nonan-7-one,1

This hetero-tricyclic ketone was obtained by modifying the previously rported procedure[12].

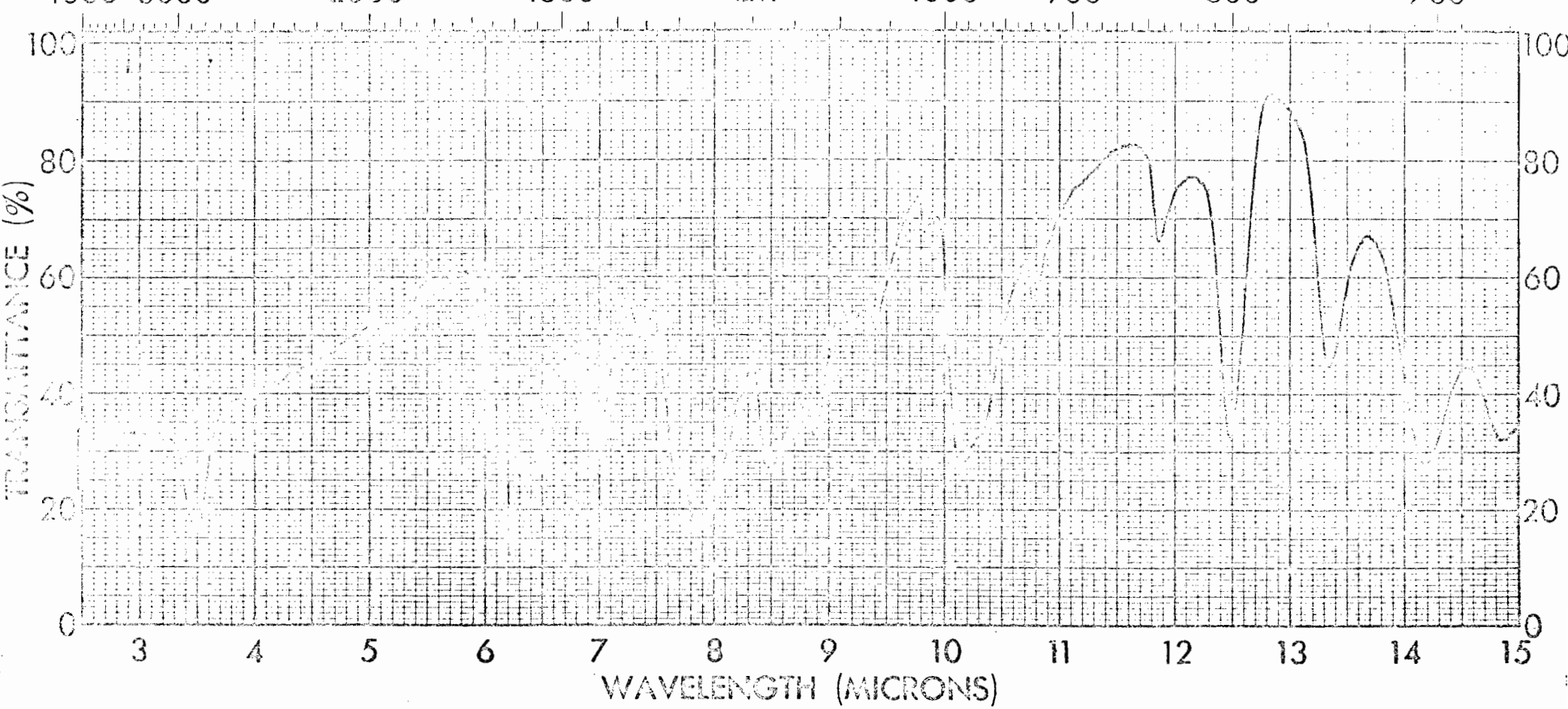
To 5.0 g (0.019 mole) of trans-1-methyl-2,3-dibenzoylaziridine in 188 ml of refluxing absolute ethanol was added, with stirring, 62 ml of refluxing absolute ethanol containing 1.0 g of sodium and 5 ml of acetone. The solution was refluxed for two minutes and then cooled in an ice bath. Ice was added to the solution. Precipitation resulted, and the product was collected by filtration (3.66 g; 63.2% yield). The product was recrystallized three times from 95% ethanol; mp 124-124.5° C; ir 1680 m^{-1}; nmr ($CDCl_3$) δ 1.97 (S,3), 2.18 (S,2), 2.65 (S) and 2.68 (S) 4H , 7.07-7.57 (M,10).

3-Methyl-1,5-diphenyl-9,3-oxazatricyclo[3.3.1.$0^{2,4}$]nonan-7-one Hydrobromide

To a stirred solution of 1 g (0.003 mole) of 3-methyl-1,5-diphenyl-9,3-oxazatricyclo[3.3.1.$0^{2,4}$]nonan-7-one in 50 ml of anhydrous diethyl ether cooled to 0° C was added dropwise to 30 ml

of a saturated anhydrous hydrogen bromide solution. The product was collected in a dry atmosphere, using a "glove box". The dry atmosphere was created by passing nitrogen through two six inch drying tubes containing molecular sieves before entering the "glove box". Further protection against the possible presence of any wator vapor was afforded by a large evaporating dish liberally filled with phosphorous pentoxide. The "glove box" was then periodically swept with nitrogen.

Although several attempts were made to recrystallize the compound in the "glove box," purification of the salt was abandoned because of the extreme hydroscopic nature of the compound. The compound sublimes at 134-137° C.



SPECTRUM NO.	ORIGIN	LEGEND	REMARKS
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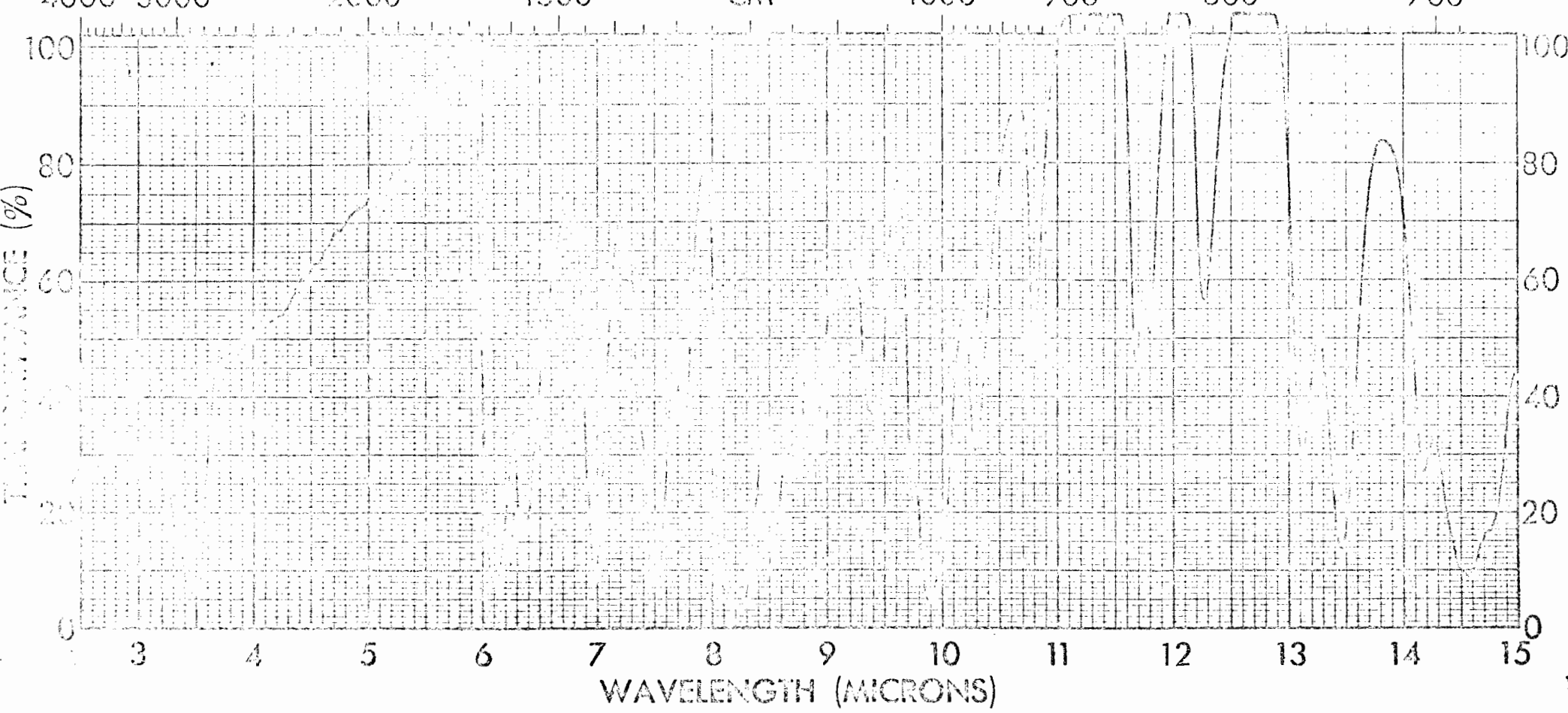
mull - 1/2 - dibutyl-	PURITY ~95°C	2.	
1/2 - Silicone thane	PHASE mull	DATE 5/20/74	
	THICKNESS	OPERATOR	

SPECTRUM NO. SAMPLE



SPECTRUM NO.	ORIGIN	LEGEND	REMARKS
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	THICKNESS	OPERATOR	

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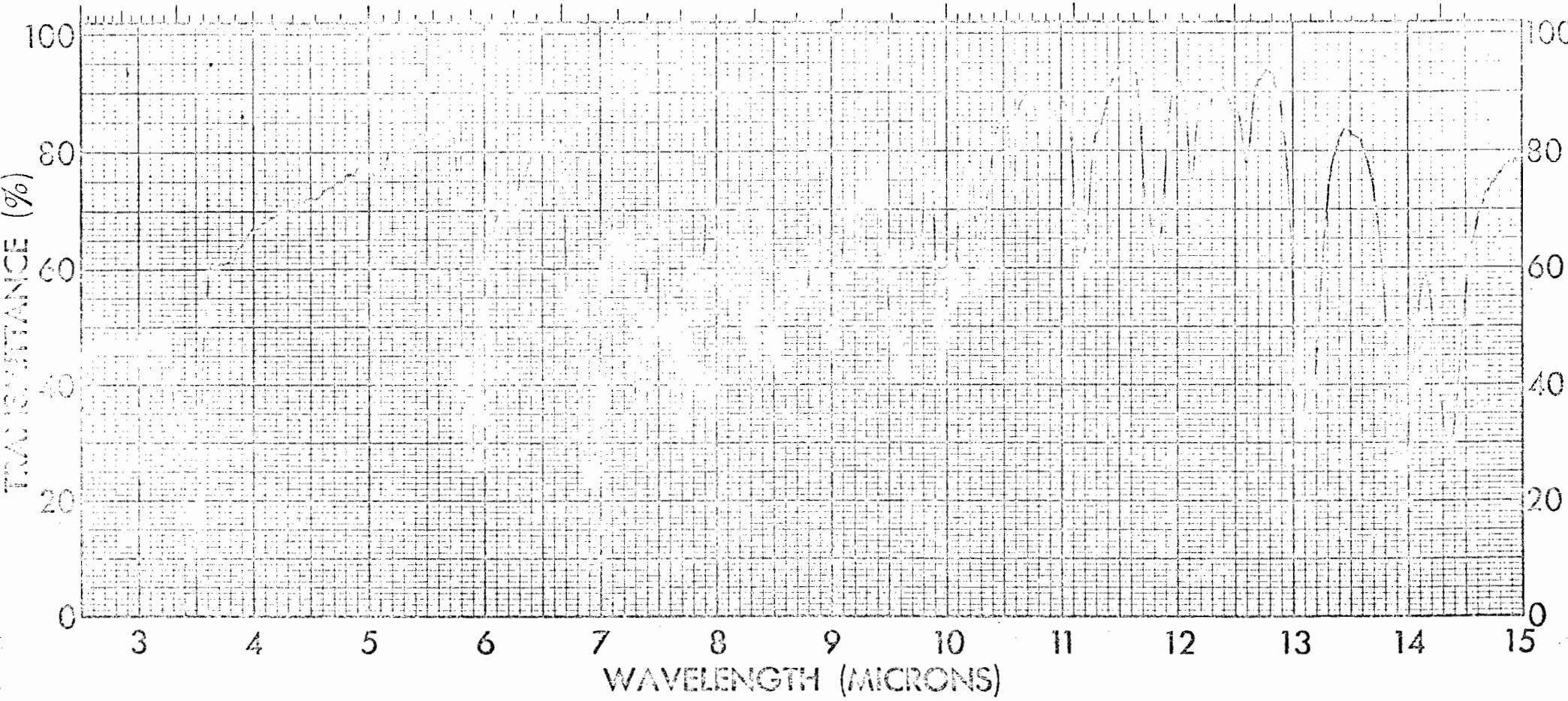

SPECTRUM NO. A	ORIGIN	LEGEND	REMARKS
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[illegible]	PURITY 124 - 12½° C [illegible]	2.	
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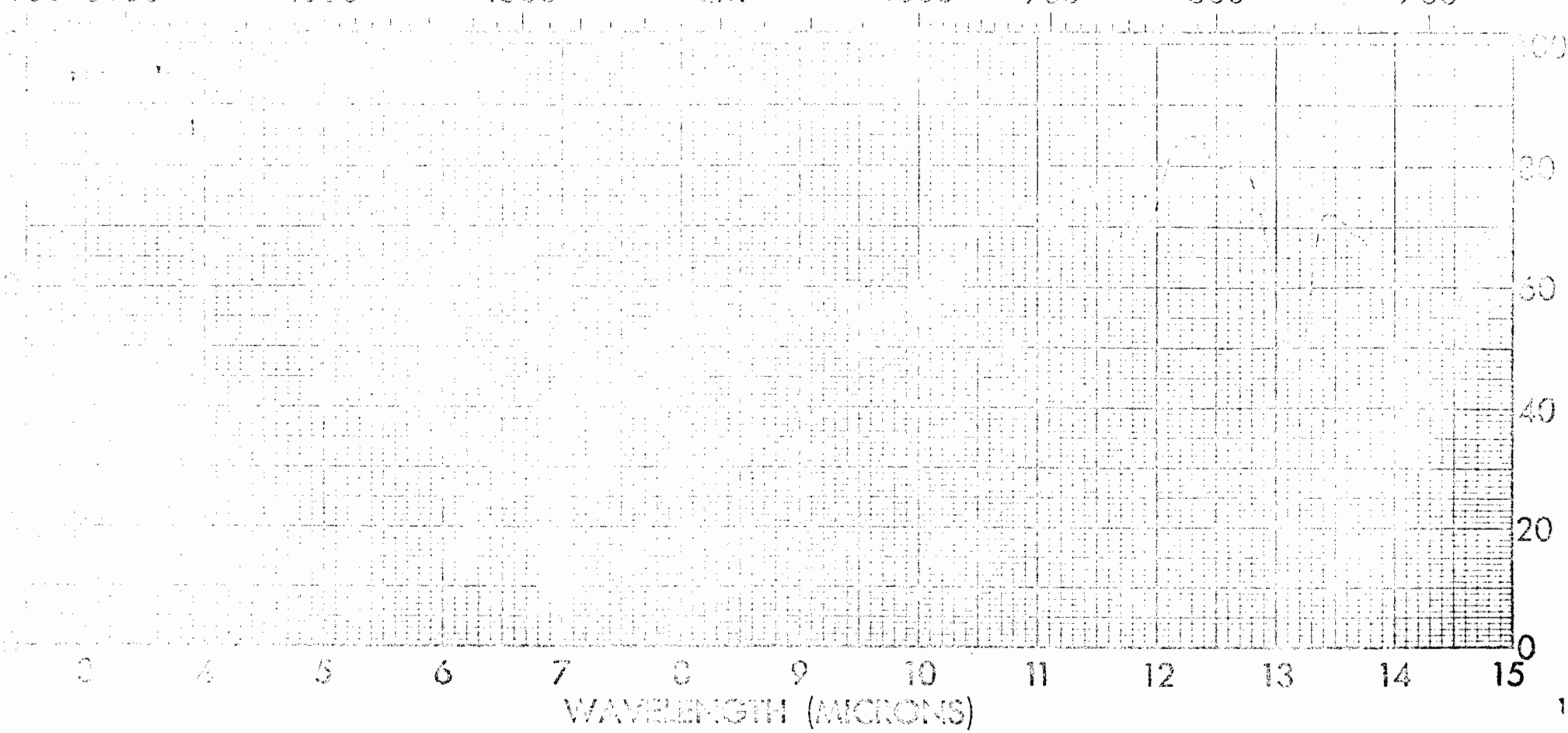

SAMPLE	ORIGIN	LEGEND	REMARKS
		1.	
	PURITY	2.	
	PHASE	DATE	
	THICKNESS	OPERATOR	

SPECTRUM NO.

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