until the precipitate is dissolved. The solution is cooled, 5 g. of potassium nitrite added and it is heated to boiling. About 2 g. of potassium bromide is added, the solution heated again to boiling, cooled and transferred to a separatory funnel. It is shaken out two or three times with chloroform in order to remove the free bromine. About 1 cc. of phenol is added and the shaking out continued with chloroform or carbon tetrachloride until all of the iodine is removed, which is evident when the chloroform has no color. From time to time more phenol may be required. The aqueous solution is filtered, heated to about 80° and the mercury precipitated with hydrogen sulfide. The precipitate is collected on a tared Gooch crucible, washed with alcohol, then carbon disulfide, again with alcohol and dried in an oven for an hour at 110°. The precipitate is weighed as mercuric sulfide.

This method has been used on known organic mercury compounds to which analyzed potassium iodide has been added with excellent results. Different experimenters have checked analyses with less then 0.3% error.

Summary

1. Several new halogen derivatives of resorcinsulfonephthalein have been made.

2. Mercury derivatives of a number of halogen compounds of resorcinsulfonephthalein have been prepared.

3. A satisfactory method for analysis for mercury in the presence of iodine is included.

4. Work in this field is being continued.

BALTIMORE, MARYLAND

[CONTRIBUTION FROM THE CHEMISTRY LABORATORY OF NORTHWESTERN UNIVERSITY DENTAL SCHOOL]

POLARIZED LIGHT AND COCAINE DECOMPOSITION

BY H. T. DAILEY[1] WITH H. C. BENEDICT

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Introduction

Macht and Anderson[2] report that polarized light has a selective action on the decomposition of cocaine, digitalis and quinine as measured by their pharmacological effects. Other chemical effects attributed to polarized light have been reported.[3] Semmens and Baly and Semmens[4] have found that polarized light accelerates the hydrolysis of starch by diastase. Macht announced a substantiation of this work.[5] These reports have been ques-

[1] A portion of a thesis submitted to the Faculty of Northwestern University Dental School in partial fulfilment of the requirements for the degree of Master of Science in Dentistry.

[2] Macht and Anderson, THIS JOURNAL, **49**, 2017 (1927).

[3] S. S. Bhatnagar, *Science*, **64**, 359 (1927).

[4] Semmens, *Nature*, **cxi**, 49 (1923); *J. Soc. Chem. Ind.*, **xlii**, 716 (1923); Baly and Semmens, *Proc. Roy. Soc. London*, **97**, 250 (1924).

[5] Macht, *Proc. Soc. Exptl. Biol. Med.*, **22**, 473 (1924).

tioned by Jones.[6] More recently the carefully controlled experiments of Bunker and Anderson[7] gave reason to doubt the validity of the conclusions of Semmens, Baly or Macht. In a note[8] Macht gave a preliminary report of the effect of polarized light on other alkaloids, all of which are optically active. Macht gives the work a practical turn by reporting that when a bottle of tincture of digitalis is wrapped in thin tissue paper the solution is more decomposed than that in an unwrapped bottle, due to the small amount of polarization of the light by the thin tissue paper.

He suggests as an explanation of this effect[2] that "if 'for every action there is a reaction' we may look at this phenomenon from the other end and argue that if a given solution can turn the plane of polarized light to the right or to the left, then *vice versa* we may expect polarized light also to react on the solution, and tend to produce some rearrangement in its molecules."

It seemed that an investigation of the effect of polarized light on a substance which did not contain an asymmetric carbon atom would be a test of Macht's theory. Procaine (β-diethylamino-ethyl *p*-aminobenzoate hydrochloride) suggested itself, as it can be tested in much the same way as cocaine. This was also interesting from a practical point of view because procaine is frequently obtained in wrapped bottles or vials.

Before beginning the work on procaine it was considered desirable to attempt the work of Macht and Anderson on cocaine. From this attempt the contrary conclusion is drawn that polarized light does not cause greater decomposition of cocaine solutions than non-polarized light of the same intensity. Experiments in which procaine solutions are irradiated with polarized and non-polarized light of the same intensity do not show any selective effect (unpublished work).

Apparatus

Macht and Anderson[2] used an apparatus in which they obtained polarized light by transmission through a stack of twelve glass plates at the polarizing angle and also with large Nicol prisms, 2.5 and 3.5 cm. in diameter. As we were unable to obtain as large a Nicol prism as this, experiments were tried with a stack of twelve photographic plates (8 in. by 10 in.). These did not give very complete polarization, as tested by a Nicol prism for extinction. It can be computed that 18% of the light transmitted is non-polarized when using twelve plates. Why do Macht and Anderson make careful measurements of the absolute units of energy when they do not indicate how much of this is polarized? An apparatus was therefore built using a plane plate of black Carrara glass (17 by 24 cm.) obtained from Bausch and Lomb. They use this plate in their strain finder for glassware. The plate was adjustable to any angle by a three-point suspension to attain the maximum of polarization. When properly adjusted the center of the plate was completely extinguished by a crossed Nicol prism and the rest

[6] Jones, *Ann. Bot.*, **39**, 651 (1925).

[7] Bunker and Anderson, *J. Biol. Chem.*, **77**, 473 (1928).

[8] Macht, *Science*, **66**, 653 (1927).

of the plate nearly so. The plate was inside a triangular blackened box (12 by 12 by $13^3/_8$ inches). In the two equal sides of the box six-inch holes were made to take the six-inch stove-pipe used to isolate the light system. In order to obviate any possible changes in the light by absorption in filters, the non-polarized end was balanced as to intensity with the polarized end by increasing its distance from the light source. At the end of each stove-pipe a removable upright was made, having a 0.75-inch hole to allow observation and to take a thermometer and having a shelf to hold the receptacle for the solution.

As a source of light a 400-watt tubular projection lamp rated at 9000 lumens was used. Because its filament lies in one plane, little light is wasted to each side. To diffuse the light a plate of ground glass was placed four inches from each side of the bulb. The intensity of the polarized and non-polarized ends was made the same within 3 or 4% by using a photometer made of two paraffin plates separated by a piece of tin foil. The experimental error of the photometer was determined on an optical bench before calibrating the apparatus. This photometer was placed in the position to be occupied by the receptacle on the polarized side and a 75-watt lamp was adjusted to balance. Holding the distance between the 75-watt lamp and the photometer constant, a position was found on the non-polarized side where a balance was attained. This was made the position of the non-polarized receptacle.

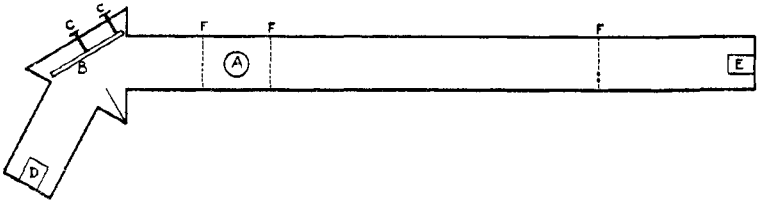

Fig. 1.—Diagram for irradiation device.

A, 400-watt projection lamp; *B*, black Carrara glass plate; *C*, screws to adjust angle of plate; *D*, shelf for receptacle on polarized side; *E*, shelf for receptacle on non-polarized side; *F*, ground plate glass.

The apparatus was so ventilated that the temperatures of the two ends were approximately that of the room, about 23–26° depending on the day, and were always the same within a half degree throughout an irradiation. Our apparatus was so constructed that the receptacles were about one inch away from the black surface to prevent heating by absorption. From the description of the Macht–Anderson apparatus it seems that the receptacles were placed directly on the blackened surface. Also the distance from the light to the polarized end was less than half the distance to the non-polarized end (see diagram). Therefore, if heating effects came into play, they would be stronger on the polarized side and would if anything enhance the effect he described and which we were unable to obtain.

A few experiments were tried using an "Ahrens" prism of 13 mm. aperture as a polarizing device. The light source was a 15-watt microscope lamp. The heat was absorbed in one experiment by a water cell, in another by a Corning heat-absorbing glass filter.

No attempt was made to have a non-polarized control. The polarized light treated portion was compared with a portion which had been kept in the ice box during the irradiation. As the ice box was dark and cold and the polarized portion was warm (room temperature about 24°) and light, this gives the greatest chance for the results to agree with those of Macht and Anderson. However, the goldfish anesthesia times are approximately the same (see Table II).

We also made a few tests with the light coming down perpendicularly to the surface of the solution. This is the manner in which many of the experiments of Macht and Anderson were run. It was suggested that we might not be obtaining the results they report because the decomposition was a surface effect, due to some sort of an oriented surface layer on which the polarized light would have a selective effect. The results of our tests do not bear out this suggestion, but may overcome an objection that we did not use the same apparatus as that of Macht and Anderson.

Methods of Testing the Solutions

The two methods used by Macht and Anderson[2] were used by us to determine the extent of decomposition of the cocaine; namely, the goldfish anesthesia time and the growth of *Lupinus albus*. They give no details of either method. We followed the technique given by Adams, Rideal, Burnett, Jenkins and Dreger[9] on goldfish.

The goldfish were kept in Alberen sinks, the temperature of the water being 20° plus or minus 2°. During a test the temperature of the solution was maintained at 20° plus or minus 0.2°. Three goldfish were used in each experiment in 250 cc. of solution in a 600-cc. beaker. The time of anesthesia was taken as that given by Adams and co-workers.[9] "When no amount of pressure (on tail or fins) will cause the fish to move." At this point the fish were usually on their sides and had stopped breathing—to all appearances dead. If removed at once, 98% of the fish will entirely recover. Common goldfish about 2 to 3 inches long were used, as supplied by the Auburndale Goldfish Co., Chicago.

Whereas Adams and co-workers[9] found that with alkyl esters of *p*-aminobenzoic acid the goldfish could be used repeatedly after allowing a week for recuperation, we found that they could not be used a second time with cocaine. When the fish were used a week after one exposure no checks could be obtained. The time of anesthesia was longer by five to ten minutes and the individual times seemed to vary more. Fresh distilled water was made and a fresh supply of cocaine was obtained; neither of these changes gave any better results. A fresh supply of goldfish gave us checks and consistent results.

The following test was applied: two goldfish were anesthetized in a 1:5000 cocaine solution in about fourteen minutes. The fish were allowed to recuperate for two hours and again placed in a fresh 1:5000 cocaine solution. They were still swimming normally at the end of an hour. This suggests that unless fresh fish were used throughout, erroneous results might be obtained. It seems that goldfish develop a tolerance for cocaine and unless specially mentioned all the experiments reported were done on fresh fish.

To check our technique with that of Adams and co-workers,[9] tests were made on one of the anesthetics they used at two dilutions. The average

[9] Adams, Rideal, Burnett, Jenkins and Dreger, THIS JOURNAL, **48**, 1758 (1926).

anesthesia time of 8 and 9 fish checked their average time within 0.2 and 0.3 minute.

TESTS ON *n*-BUTYL *p*-AMINOBENZOATE

Concn., *M*	Time, minutes										Av.	Adams[9] Av.
0.0001	6	6.75	7.75	4.5	5.75	6.75	6.75	7	7	9	6.7	7.0
0.0005	16	16.5	26.5	17.5	23	20.5	21.0	22			20.5	20.7

We are thus reasonably certain that our technique of using the goldfish is essentially the same as theirs.

Another possible error may be introduced by the water used. If doubly distilled water is used, distilled the second time from alkaline permanganate as recommended by Adams and co-workers,[9] much longer times are required for cocaine anesthesia than when ordinary distilled water is used. That this was due to traces of tin from the block tin condenser was shown by boiling some of the doubly distilled water with a piece of cleaned block tin for two hours. A cocaine solution made with this water gave an anesthesia time nearly as short as that of ordinary distilled water and much shorter than the original water. Some of the doubly distilled water was boiled without the tin for two hours; a solution made with this water gave an anesthesia time the same as the original. We hope to look further into this effect.

The results of tests on 108 fish are tabulated in Tables I and II. About 50 more fish were used to obtain an anesthesia time for the non-irradiated solutions. As this time did not appreciably differ from either the polarized or the non-polarized times for periods of irradiation up to three hours, and as it is not essential to the proposition, it is not necessary to include this data.

We were fortunate in having the assistance of Mr. V. F. Lang of Northwestern University Medical School in running tests on the *Lupinus albus*, as he has been using the method under Dr. C. A. Dragstedt for a year. The solutions were brought to him labeled A, B and C. This assured that the results obtained were not influenced by any preconceived idea of the results expected. The technique employed was that of Macht and Livingston.[10] All of the following steps are carried out in the dark at 20° plus or minus 1°. Seeds of *Lupinus albus* are sprouted in water overnight, and are then placed with the hilum down in moist sphagnum moss for three days. The roots are then measured from a characteristic brown ring to the tip. Seeds with roots of approximately the same length are placed in a series of three-inch test-tubes. The average of ten seedlings is considered a test. A standard is obtained by putting a half and half mixture of Shive's solution,[11] a nutrient solution containing $Ca(NO_3)_2$, $MgSO_4$ and KH_2PO_4 and distilled water

[10] Macht and Livingston, *J. Gen. Physiol.*, **4**, 573 (1922).

[11] Shive, *Physiol. Research*, **i**, 327 (1915).

in the ten test-tubes containing the seeds. The average increase in length in mm. in twenty-four hours is considered 100% growth. The unknown solution is tested by mixing it with an equal volume of Shive's solution, treating ten plants with this mixture and comparing the average growth with that of the standard. Results are reported in percentage of standard growth. The concentrations of cocaine given are those of the original solution. The mixture used to grow the seedlings will thus be half as concentrated.

It should be noted that as the cocaine is decomposed it will have less anesthetic effect and the time of anesthesia for goldfish will be lengthened. On the other hand, as the cocaine has little effect on the growth of *Lupinus albus* and the decomposition products have a great effect,[10] the growth of the plants should be inhibited if there were appreciable decomposition.

In fact it can be computed from the data given by Macht,[10] on the relative growth of *Lupinus albus* in different concentrations of cocaine and its decomposition products, benzoic acid, methyl alcohol and ecgonine hydrochloride that, considering all the lethal effect to be due to the benzoic acid, 17% decomposition of a 1:1000 cocaine solution (0.0029 molar) would completely inhibit the growth and that 1.7% decomposition will give a growth index of 66–67%. Macht[2] finds an index of 75 after the solution has been irradiated for three hours. This would correspond to about 1% decomposition. Yet he obtains a change in the anesthesia time of about 400%.

Experimental Data

In Table I are given the results of the tests on cocaine solutions using the black Carrara glass plate as the polarizing device. Besides the individual times and the average times of anesthesia, the average deviation is included. The percentage increase in time of anesthesia due to the polarized light was obtained by subtracting the average non-polarized time from the average polarized time and dividing by the non-polarized time, results being expressed in percentage. If the non-polarized time is greater than the polarized, the results are negative.

The anesthesia time gradually increases with time of irradiation. This bears out the known fact that cocaine hydrochloride solutions are decomposed by light.

Table II is made up in the same form as Table I. It gives the results of experiments in which the polarized light was perpendicular to the surface of the solution and in which the polarized radiation was obtained by an "Ahrens" prism. In these experiments no non-polarized control was used but the results are compared with a portion of the solution which was kept in the ice box.

In the last experiment if the growth of one seedling which was obviously a "sport" had been omitted from the calculation, the growth index of the

TABLE I

COCAINE HYDROCHLORIDE IN DISTILLED WATER AT 20 ± 0.2°

Concn. / Radiation	1:5000 1 hour Pol.	1:5000 1 hour Non.	1:5000 3 hours Pol.	1:5000 3 hours Non.	1:5000 5 hours Pol.	1:5000 5 hours Non.	1:5000[a] 6 hours Pol.	1:5000[a] 6 hours Non.
Anesthesia time of individual goldfish	11.0	11.0	15.0	18.0	16.0	15.5	15.0	19.0
	10.7	11.2	14.0	16.0	18.5	18.5	15.0	27.0
	11.2	10.0	13.5	16.5	18.5	18.5	15.0	27.0
	11.5	12.5	11.5	11.5				
	10.7	12.0	12.5	12.0				
	12.5	10.5	13.0	14.5				
			16.0	10.5				
			16.0	12.0				
			18.0	14.0				
Average	11.29	11.2	14.38	13.8	17.6	17.5	15.0	24.1
Av. deviation	0.47	0.70	1.65	2.13	1.13	1.33	0.0	3.43
% increase in time	0.8		3.62		0.57		−37.5	

[a] Old fish.

Concn. / Radiation	1:5000 7 hours Pol.	1:5000 7 hours Non.	1:5000 12 hours Pol.	1:5000 12 hours Non.	1:1000 2 hours Pol.	1:1000 2 hours Non.	1:1000 4 hours Pol.	1:1000 4 hours Non.	1:500 3.5 hours Pol.	1:500 3.5 hours Non.
Anesthesia time of individual goldfish	19.5	19.5	24.0	29.0	15.7	14.5	6.7	8.5	9.0	9.2
	24.5	25.0	29.0	29.7	15.7	15.7	10.5	9.5	9.5	9.7
	26.0	30.0	34.0	34.0	15.7	15.7	10.5	12.5	10.0	9.7
Average	23.3	24.8	29.0	30.9	15.7	15.3	8.2	10.1	9.5	9.5
Av. deviation	2.56	3.56	3.3	2.0	0.0	0.5	1.7	1.5	0.3	0.2
% increase	−6.0		−6.1		3.2		−9.0		−0.8	

TABLE II

COCAINE HYDROCHLORIDE IN DISTILLED WATER AT 20 ± 0.2° USING VARIOUS METHODS FOR OBTAINING POLARIZED LIGHT

Concn. / Radiation	1:1000[a] 4 hours Pol.	1:1000[a] 4 hours Non.	1:500[a] 3 hours Pol.	1:500[a] 3 hours Non.	1:5000[a] 5 hours Pol.	1:5000[a] 5 hours Non.	1:5000[a] 6.5 hours Pol.	1:5000[a] 6.5 hours Non.
Anesthesia time of individual goldfish	6.5	9.2	11.0	7.2	26.5	25.5	30.0	37.0
	10.5	10.5	14.0	11.5	30.0	30.0	35.0	41.0
	10.5, 11.7	11.0	16.0	11.5	38.0	38.0	59.0	65.0
Average	9.8	10.1	13.6	10.0	31.5	31.1	41.0	47.6
Av. deviation	1.3	0.6	1.8	1.8	4.3	4.6	11.8	8.2
% increase	−3.0		36.0		1.3		−13.8	

[a] In all of these tests the polarized light was perpendicular to the surface of the solution.

Concn. / Radiation	1:1000[a] 2.5 hours Pol.	1:1000[a] 2.5 hours Control	1:1000[b] 3 hours Pol.	1:1000[b] 3 hours Control
Anesthesia time of individual goldfish	13.0	12.0	11.0	13.0
	15.2	16.5	12.0	14.5
	16.5	16.5	13.5	29.2
Average	14.9	15.0	12.0	15.5
Av. deviation	1.3	2.0	1.0	5.8
% increase	0.67		−22.6	

[a] Solution in a plane-sided cell, light polarized by an "Ahrens" prism using a 15 watt lamp.

[b] Solution in beaker in same apparatus.

TABLE III

GROWTH OF 120 LUPINUS ALBUS AS REPORTED BY V. F. LANG

Radiation	Pol.	Non.	Normal	Shive's	Concn.
2.5 hours	96	88	104	100	1:1000
10 hours	126	99.9	102	100	1:1000
10 hours	80	90	90	100	1:500

polarized side would have been 86.5% instead of 80%. It has been the experience of Dr. C. A. Dragstedt that variations of 10% or more are frequently obtained and that unless this difference is consistently obtained it is without significance.[12]

Table IV summarizes the results of Tables I and II, giving the average times of each experiment. We have included the average of the averages, regardless of time of irradiation or concentration. If there were a consistently longer time due to the polarized light, this should show up. However, this average shows a slightly longer time for the non-polarized light, and the average percentage increase in time due to the polarized light is −3.48%. These results are probably within the experimental error of the method.

TABLE IV

SUMMARY OF RESULTS

Radiation time	Concn.	Pol.	Non.	% Increase in time due to pol. light, %	Remarks
1	1:5000	11.29	11.2	0.8	
2	1:1000	15.75	15.3	3.2	
2.5	1:1000	14.9	15.0	0.67	Ahrens prism
3	1:5000	14.38	13.8	3.62	
3	1:500	13.6	10.0	36.0	Perpendicular light
3	1:1000	12.0	15.5	−22.6	Ahrens prism
3.5	1:500	9.5	9.58	− 0.8	
4	1:1000	9.2	10.1	− 9.0	
4.0	1:1000	9.8	10.1	− 3.0	Perpendicular light
5.0	1:5000	17.6	17.5	0.57	
6.0	1:5000	15.0	24.1	−37.5	Re-used goldfish
6.5	1:5000	41.0	47.6	−13.8	Perpendicular light
7.0	1:5000	23.3	24.8	− 6.0	
12.0	1:5000	29.0	30.9	− 6.1	
5.0	1:5000	31.5	31.1	1.3	Perpendicular light
Average of av.		17.8	19.1	Total −52.2	Av. −3.48

It will be noticed that in one or two experiments the solutions irradiated with polarized light have a slightly longer anesthesia time than the specimen treated with non-polarized light. However, these results are not obtained consistently, and as many or more tests are found which would reverse the conclusion.

[12] Personal communication.

Summary and Conclusions

1. A study of the relative effect of polarized and non-polarized light on the decomposition of cocaine has been made, in which (a) several concentrations of cocaine hydrochloride were used; (b) two methods of obtaining a polarized radiation were used; (c) the times of irradiation ranged from one to twelve hours; (d) the effect was tested on goldfish and *Lupinus albus*.

2. The conclusion is drawn that polarized radiations have no selective action in the decomposition of cocaine hydrochloride.

3. Cocaine is slowly decomposed by light.

4. Obvious sources of error which we have tried to eliminate are: the variability of individual goldfish, the tolerance that goldfish develop to cocaine and the effect of traces of impurity in the water.

CHICAGO, ILLINOIS

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF KITASATO INSTITUTE]

THE SYNTHESIS OF CERTAIN ACRIDINE COMPOUNDS

BY KONOMU MATSUMURA

RECEIVED JUNE 22, 1928 PUBLISHED MARCH 6, 1929

In continuation of work on the preparation of certain acridine derivatives for bactericidal examination,[1] this paper deals with the preparation of the following compounds.

V. 9-Amino-3,6-dimethoxy-acridinium-methyl Chloride

9-Amino derivatives of acridine compounds are now attracting considerable interest in the field of medicine. Of these, Rivanol (2-ethoxy-6,9-diamino-acridine-hydrochloride) is the only product now employed in practice. The compound described here was prepared as a further contribution to the study of this kind of compounds.

Diphenylmethane, prepared from benzyl chloride, benzene and aluminum chloride by Radziewanowski's method,[2] was first converted into 3,6-diamino-acridone (I) by nitration, oxidation, and subsequent reduction by Schöpff's[3] and Staedel's[4] methods, respectively, with a little modification. 3,6-Diamino-acridone was then converted into 9-amino-3,6-dimethoxy-acridine (II) by hydrolysis with 50% sulfuric acid, methylation with dimethyl sulfate, chlorination with phosphorus pentachloride and subsequent amidation with ammonia. 9-Amino-3,6-dimethoxy-acridine on acetylation, treatment with *p*-toluene-methyl-sulfonate, and subsequent hydrolysis with hydrochloric acid, gave 9-amino-3,6-dimethoxy-acridinium-methyl chloride (IV).

[1] Matsumura, THIS JOURNAL, **49**, 810 (1927).

[2] Radziewanowski, *Ber.*, **28**, 1136 (1895).

[3] M. Schöpff, *ibid.*, **27**, 2318 (1894).

[4] Staedel, *Ann.*, **218**, 339 (1883)