TABLE I.—RESULTS OF TESTS OF LARVICIDAL ACTIVITY OF VARIOUS AGENTS

Drug	Concn., %	Percentages of Larvae Dead or Immotile 10 Min.	30 Min.	1 Hr.	2 Hr.	24 Hr.
Iodine (as free iodine in Lugol's solution)	0.055	100	100	100	100	100
	0.05	82	94	98	[b]	88
	0.025	53	65	75	75	80
	Control[a]	0	0	0	0	0
Nicotine	0.25	0	0	0	0	100
	0.1	75	75	63	75	92
	Control	0	0	0	0	0
Nicotine salicylate	0.16	44	57	75	82	[b]
	Control	0	0	0	0	[b]
Phenol	0.05	13	0	0	63	100
	0.025	0	0	0	50	0
	Control	0	0	0	0	0
Creosote	0.05	0	38	[b]	[b]	50
	Control	0	0	[b]	[b]	10
Glaucarubin	0.05	0	7	7	43	0
	Control	0	0	0	10	0

[a] Larvae in aqueous suspension. [b] Readings not taken.

Additional tests were performed to determine the effects of blood constituents on the activity of PCMX. Results obtained with *in vitro* studies reveal that the agent's effectiveness is reduced by these substances.

TABLE II.—RESULTS OF TESTS OF LARVICIDAL ACTIVITY OF *p*-CHLORO-*m*-XYLENOL USING PROPYLENE GLYCOL 5% AS SOLUBILIZING AGENT[a]

Concn. of PCMX, %	10 Min.	30 Min.	1 Hr.	2 Hr.	24 Hr.
0.05	83	100	100	100	100
0.025	80	90	90	[b]	[b]
0.012	29	67	96	98	100
0.006	0	0	0	10	100
Control	0	0	0	0	10

[a] Results expressed as percentages of larvae dead or immotile. [b] Readings not taken.

TABLE III.—EFFECTS OF SOLUBILIZING AGENTS ON THE ACTIVITY OF PCMX

Solubilizing Agent	Concn. of Solubilizing Agent, %	Effect on Percentage Kill
Polysorbate 80[a]	0.5	Reduced
Dioctyl sodium sulfosuccinate	0.125	Reduced
Sodium lauryl sulfate	0.25	Reduced
Castile soap	0.25	Reduced
Triton X-100	0.50	Reduced
Polyethylene glycols 400, 1540, 4000, 6000	10.0	Reduced
Propylene glycol	10.0	Increased
Propylene glycol	5.0	No effect

[a] Marketed as Tween 80 by Atlas Chemical Industries, Inc., Wilmington, Del.

The external use of PCMX as a germicidal agent has been without reported ill effects to man or animals. Blood and tissue concentrations, up to 4 mg. per cent, have been recorded by Zondek and Finkelstein following administration to humans by intramuscular and percutaneous routes (6). In view of the reported low toxicity of the drug, it appears desirable to obtain data on blood levels required for larvicidal activity in the body. In our preliminary *in vivo* studies in dogs, doses of 200 to 400 mg./Kg., by oral and intramuscular administration, were nephrotoxic. Further work on systemic use of PCMX in dogs and guinea pigs is in progress.

In view of the drug's high rate of absorption by percutaneous administration, the use of PCMX in treatment of cutaneous larva migrans is a possibility. Also of interest is application of the compound as an area spray where man and animals may be exposed to infectious larvae of parasites.

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Fluorometric Study of Antihistamines

By RICHARD E. JENSEN and RONALD T. PFLAUM

The reactions of 16 antihistamines with hydrogen peroxide and the fluorescent characteristics of the products are reported.

ANTIHISTAMINES containing an alkylamino substituent in the 2-position on a pyridine nucleus react with cyanogen bromide to yield fluorescent products (1). This reaction is the basis of a fluorometric method for the detection and determination of some of these antihistamines.

In the present work, the reactions of 16 antihistamines with a series of oxidants were studied. The fluorescence characteristics of the products formed upon treatment with hydrogen peroxide form the basis of this report. The corresponding studies on the cyanogen bromide systems were carried out for comparative purposes.

Excitation and fluorescence wavelengths and

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relative intensities are reported which tend to show a correlation between fluorescence and chemical structure.

EXPERIMENTAL

All fluorescence spectra were obtained with an Aminco-Bowman spectrophotofluorometer equipped with an Osram xenon lamp source and a photomultiplier detector. The slits in the monochromator were arranged as follows: excitation beam, 1/8, 1/16, and 1/8 in.; fluorescent beam, 1/8, 1/16, 1/8, and 1/16 in. Quartz cells, 10 mm. square, were used.

The antihistamines were obtained from the sources listed in Table I. Stock solutions were prepared by dissolving accurately weighed samples of the antihistamine salts of approximately 50 mg. in 50 ml. of distilled deionized water.

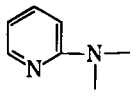
For the study of the effect of cyanogen bromide on antihistamines, 1 ml. of a freshly prepared saturated solution of cyanogen bromide was added to 1-ml. aliquots of the antihistamine stock solution. The mixtures were diluted to 10-ml. volume, allowed to stand for 30 minutes at room temperature, and then measured spectrofluorometrically.

In the study of the effect of hydrogen peroxide, 1 ml. of 3% hydrogen peroxide solution was added to 1-ml. aliquots of the antihistamine stock solution. The mixtures were diluted to 10 ml., heated in a 90° water bath for 30 minutes, and measured spectrofluorometrically.

RESULTS

The data obtained upon treatment of antihistamines with cyanogen bromide and hydrogen peroxide are summarized in Table I. Compounds are grouped according to structural formulation.

The first five antihistamines containing the alkylaminopyridyl grouping,



have an excitation wavelength of 340–345 mμ and a fluorescence maximum at 410 ± 3 mμ when treated with hydrogen peroxide. The situation is very similar for the cyanogen bromide system; excitation is 350 ± 5 mμ and a fluorescence at 415 ± 4 mμ. In every case, except for pyrilamine, the intensity of the fluorescence is much greater in the cyanogen bromide system. Thonzylamine, a 2-substituted pyrimidine derivative, reacts quite analogously to pyrilamine.

Compounds VII–X, containing a 2-picoline nucleus, in general developed a very weak fluorescence after treatment with cyanogen bromide. An excitation wavelength of 275–280 mμ resulted in fluorescence maxima between 388–467 mμ. The very weak intensity of the fluorescence may account for the fact that it had not been observed previously (1). Carbinoxamine (VII) exhibited only one set of fluorescence peaks, while the other compounds of this group revealed two sets of peaks upon treatment with hydrogen peroxide. This may be due to the ethereal linkage found only in carbinoxamine. Antazoline, a 2-substituted imidazole, reacts similarly to thonzylamine and to the compounds in the picoline group.

TABLE I.—FLUORESCENCE DATA ON ANTIHISTAMINES

	Antihistamine[g]	H_2O_2 System			CNBr System		
		Ex. mμ	Fl. mμ	Int.	Ex. mμ	Fl. mμ	Int.
I	Chlorothen[e] (Lederle Laboratories)	340	407	44	345	414	160
II	Methapyrilene[b] (Irwin, Neisler Co.)	345	409	86	350	412	361+[a]
III	Pyrilamine[c] (Dorsey Laboratories)	340	408	43	350	419	35
IV	Thenyldiamine[b] (Sterling Winthrop Laboratories)	345	412	96	355	414	369+[a]
V	Tripelennamine[b] (Ciba Pharmaceutical)	345	408	41	355	419	300+[a]
VI	Thonzylamine[b] (Warner-Lambert Laboratories)	330	407	5.4	345	419	0.6
VII	Carbinoxamine[c] (McNeil Laboratories)	335	394	3.6	275	467	0.3
VIII	Chlorpheniramine[c] (Schering Research)	350 360	436 446	4.0 3.7	280	447	3.0
IX	Doxylamine[d] (Wm. S. Merrell Co.)	370 345	449 436	2.8 2.2	280	388	0.1
X	Pheniramine[c] (Dorsey Laboratories)	330 370	352 457	7.2 5.8	275	434	0.4
XI	Antazoline[b] (Ciba Pharmaceutical)	330	415	2.8	350	410	0.6
XII	Chlorcyclizine[b] (Burroughs Wellcome)	345	451	1.5	...	...	...
XIII	Cyclizine[b] (Burroughs Wellcome)	305 335	417 449	1.3 1.0	...	...	...
XIV	Meclizine[f] (Chas. Pfizer & Co.)	310 345	420 444	1.4 1.4	...	...	...
XV	Diphenhydrazine[b] (Parke, Davis and Co.)	305 345	412 454	0.8 0.5	...	...	...
XVI	Promethazine[b] (Wyeth Laboratories)	345	384	6.6	320	449	2.4

[a] +, indicates that the intensity was greater than the instrument could detect using the described procedure and a 6 mμ band pass. [b] Weighed as the hydrochloride salt. [c] Weighed as the maleate salt. [d] Weighed as the succinate salt. [e] Weighed as the citrate salt. [f] Weighed as the dihydrochloride salt. [g] All concentrations are 100 mcg./ml.

The remaining antihistamines all give fluorescent products with hydrogen peroxide. The cyclizines, *N*-substituted pyrazines, are similar fluorometrically to the substituted picolines. Promethazine, an *N*-substituted phenthiazine, appears to fluoresce upon treatment with both hydrogen peroxide and cyanogen bromide. Promethazine hydrochloride undergoes a rapid decomposition in water resulting in a highly colored solution and a definite increase in acidity. It is possible that the observed fluorescence was due to a decomposition product in the reaction mixture.

Four oxidizing agents, potassium bromate, potassium bi-iodate, potassium periodate, and potassium persulfate were investigated in addition to hydrogen peroxide. Hydrogen peroxide provided the highest intensity of fluorescence when thenyldiamine was treated with equivalent amounts of each oxidant. The fluorescence intensity decreased in the order: $KIO_4 > KH(IO_3)_2 > KBrO_3 > K_2S_2O_8$.

Investigations are being continued on the antihistamines with special attention given to the quenching of the cyanogen bromide fluorescence and regeneration using hydrogen peroxide. The quantitative dependence of antihistamine concentration on fluorescence intensity and the effect of hydrogen ion concentration will be studied. Attempts will be made to determine the mechanisms of the reactions.

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Synthesis of Some Alkyl and Aminoalkyl Esters of Azobenzenedisulfonic Acid

By WILBERT G. WALTER* and TONY E. JONES

Two homologous series of dialkyl esters ranging from dimethyl to dibutyl of *p*- and *m*-azobenzenedisulfonic acid were synthesized for the first time. In addition, the hydrochloride salts of bis-diethylaminoethyl ester of *p*-azobenzene-4:4′-disulfonic acid and bis-2-di-*n*-butylaminoethyl ester of *m*-azobenzene-3:3′-disulfonic acid are reported.

EXCEPT FOR esters, some derivatives of azobenzenedisulfonic acid have been reported. In 1930, some isomeric azobenzenedisulfonchloramides (1) were made for consideration as possible antibacterial agents similar to the chloramine-T type compounds. Later in 1937, azobenzenedisulfonamide (2), with substituted amide groups such as 2-pyridyl and 2-thiazolyl which are known to be associated with sulfonamide drugs, was studied. Also, iodo derivatives of *p*-azobenzenedisulfonamide were prepared for study as radiopaque materials (3).

The purpose of the work reported here was to synthesize and investigate chemically some alkyl and aminoalkyl esters of *p*- and *m*-azobenzenedisulfonic acid. The objective was of interest because compounds having physiological activity might be produced. Azobenzenedisulfonic acid is conceived to undergo reduction to give aminobenzenesulfonic acid, since compounds such as prontosil (2′,4′-diaminoazobenzene-4-sulfonamide) (4) and azobenzene (5) are known to be reduced to give sulfanilamide and aniline, respectively. Aminobenzenesulfonic acid is a chemical isostere of aminobenzoic acid whose esters constitute an important class of local anesthetic compounds; it is also structurally related to the sulfonamide type drugs. By esterifying with alcohols that are found attached to active local anesthetic compounds of aminobenzoic acid—*e.g.*, diethylaminoethanol (procaine), ethanol (benzocaine), propanol (propaesin), *n*-butanol (butesin), compounds with activity of a similar nature might be produced. While these structure-activity relationships can be drawn about the derivatives, the products have not been subjected to bioassay to substantiate these inferences.

A similar method which Stern (1) used to prepare *m*-azobenzene-3:3′-disulfonic acid is also used here to prepare the *para* and *meta* isomers. In 1939, he reported a procedure satisfactory for preparing potassium *m*-azobenzene-3:3′-disulfonate, whereas the *para* isomer was prepared with more difficulty and by a different method—the reaction of fuming sulfuric acid on azobenzene. Although other methods (6) were tried during the course of this work, they gave poor yields or a mixture of azo products. The methods tried included the reduction of sodium nitrobenzenesulfonate with sodium amalgam and the dissolution of azobenzene in fuming sulfuric acid. The reaction of chlorosulfonic acid on azobenzene also proved unsatisfactory for preparing azobenzenedisulfonyl chloride (7).

The procedure employed to prepare the ester derivatives is as follows. Reduction and coupling of sodium nitrobenzenesulfonate (III) using zinc dust and potassium hydroxide to give sodium azobenzenedisulfonate (IV) (8), and conversion of this salt with phosphorus pentachloride to the acid dichloride (II), followed by esterification with the respective alcohol in anhydrous benzene.

Because an individual discussion on the preparation of each ester or isomeric compound would involve duplication, it was decided to describe in detail the preparation of four compounds representative of the group: sodium *p*-azobenzene-4:4′-disulfonate, *p*-azobenzene-4:4′-disulfonyl chloride, bis-(ethyl)-*p*-azobenzene-4:4′-disulfonate, and bis(diethylaminoethyl)-*p*-azobenzene-4:4′-disulfonate. References leading to compounds related to this work are given in Table I.

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