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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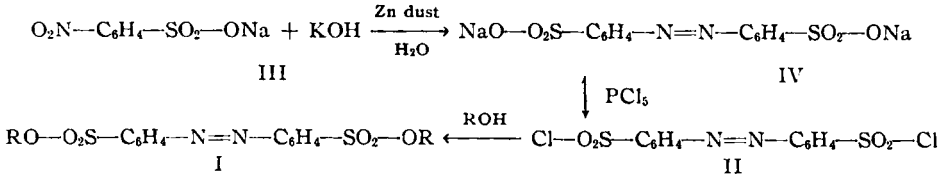

R = alkyl or aminoalkyl group.

EXPERIMENTAL

Sodium p-Azobenzene-4:4′-disulfonate.—Sodium *p*-nitrobenzenesulfonate (100 Gm.) and potassium hydroxide (140 Gm.) were dissolved in distilled water (1000 ml.) and the solution filtered. On the addition of potassium hydroxide, the temperature increased to 97°, and the solution turned a dark orange. While the mixture was being stirred mechanically and maintained at 103–104°, six portions (15 Gm. each) of zinc dust were added in 15-minute intervals until the solution became colorless. Before each new portion of zinc dust was added, the progress of the reduction was followed by "spotting" the reaction mixture on filter paper. A regular grading of the color, from a faint yellow (after the first addition of zinc dust) to orange (azoxy condition) until colorless (hydrazo condition), was observed.

The zinc residues were removed by filtration, washed with two 50-ml. portions of hot distilled water, and the washings added to the filtrate. The hydrazo compound oxidized rapidly to the azo compound on exposure to air as observed when the solution became deeply colored. The reaction was aided by raising the temperature to 80–90° and mechanically stirring for 3 hours or until the color had become constant (dark reddish orange). The completeness of the color change could be followed by colorimetric comparison.

The temperature of the solution was raised to 85° and carbon dioxide passed through the mixture for 2 hours. A white precipitate was formed, which was removed by filtration; the filtrate was concentrated and chilled. Small orange crystals (32 Gm.) of sodium *p*-azobenzene-4:4′-disulfonate were collected. Further concentration of the solution yielded more of the product.

Purification was accomplished by dissolving the crystals in a solution consisting of 25 ml. of water and 100 ml. of ethanol and recrystallizing. Yield, 78 Gm. (91.2%). (See *References* in Table I.)

p-Azobenzene-4:4′-disulfonyl Dichloride.—Anhydrous sodium *p*-azobenzene-4:4′-disulfonate (10 Gm.) was mixed with phosphorus pentachloride (20 Gm.) and heated to a temperature of 190° for 35 minutes. Partial softening, then liquefaction of the mass occurred after 10 minutes. The mass was stirred at intervals with a glass rod to insure a complete reaction. After the mass developed a constant dark red, it was slowly poured over ice while several small quantities of ice water were added to the reaction flask. Crude *p*-azobenzene-4:4′-disulfonyl dichloride that formed was broken up, isolated, and washed with ice cold distilled water until free of soluble materials, then dried, and melted at 217–218° uncorrected. The dichloride compound could be recrystallized from most organic solvents; benzene, ligroin, and ether were used. Crystallization from benzene yielded a product melting at 219–220° uncorrected. Yield, 8.9 Gm. (90.8%). (See *References* in Table I.)

Bis-(ethyl)-p-azobenzene-4:4′-disulfonate.—*p*-Azobenzene-4:4′-disulfonyl dichloride (1 Gm.) was dissolved in anhydrous benzene (140 ml.) and absolute ethanol (25 ml.). The solution was refluxed for 30 minutes, then evaporated under reduced pressure to one-half of its original volume and chilled. The orange crystalline flakes (0.53 Gm.) obtained by filtration were air dried. Further evaporation of solvent yielded more product (0.20 Gm.) which melted at 155–156° uncorrected. Drying under reduced pressure in the presence of a drying agent did not alter the melting point of the ester product. Yield, 0.73 Gm. (69.5%).

Anal.—Calcd. for $C_{16}H_{18}N_2O_6S_2$: C, 48.24; H, 4.51. Found: C, 47.84; H, 4.47.

Bis-(diethylaminoethyl)-p-azobenzene-4:4′-disulfonate.—*p*-Azobenzene-4:4′-disulfonyl dichloride (2 Gm.) was dissolved in anhydrous benzene (350

TABLE I.—DERIVATIVES OF AZOBENZENEDISULFONIC ACID R—O_2S—C_6H_4—N=N—C_6H_4—SO_2—R

R Group	Isomer	M.p., °C. Uncorrected	Formula	Calcd., % C	Calcd., % H	Found, % C	Found, % H	Ref.
NaO—	*para*	...	$C_{12}H_8N_2Na_2O_6S_2$	...	...	...	...	(1, 3, 8–11, 15–17)
Cl—	*para*	219–220°	$C_{12}H_8Cl_2N_2O_4S_2$	...	...	...	...	(1, 7, 15, 17)
CH_3O—	*para*	201.5 to 202°	$C_{14}H_{14}N_2O_6S_2$	45.47	3.78	45.10	3.89	...
CH_3CH_2O—	*para*	155–156°	$C_{16}H_{18}N_2O_6S_2$	48.24	4.51	47.84	4.47	...
$CH_3CH_2CH_2O$—	*para*	161–162°	$C_{18}H_{22}N_2O_6S_2$	50.70	5.16	50.78	5.27	...
$CH_3(CH_2)_3O$—	*para*	156–157°	$C_{20}H_{26}N_2O_6S_2$	52.84	5.76	52.20	5.66	...
$(CH_3CH_2)_2$-NCH$_2$CH$_2$O— HCl	*para*	183.5 to 184°	$C_{24}H_{38}Cl_2N_2O_6S_2$	46.99	5.89	47.43	6.51	...
NaO—	*meta*	...	$C_{12}H_8N_2Na_2O_6S_2$	...	...	...	...	(1, 8, 12–14)
Cl—	*meta*	164–166°	$C_{12}H_8Cl_2N_2O_4S_2$	...	...	...	...	(1, 8, 12)
CH_3O—	*meta*	136–137°	$C_{14}H_{14}N_2O_6S_2$	45.47	3.78	45.09	3.77	...
CH_3CH_2O—	*meta*	102–103°	$C_{16}H_{18}N_2O_6S_2$	48.24	4.51	47.82	4.63	(8) ...
$CH_3CH_2CH_2O$—	*meta*	79–80°	$C_{18}H_{22}N_2O_6S_2$	50.70	5.16	50.61	5.20	...
$CH_3(CH_2)_3O$—	*meta*	75–76°	$C_{20}H_{26}N_2O_6S_2$	52.84	5.76	52.70	5.68	...
$[CH_3(CH_2)_3]_2$-NCH$_2$CH$_2$O— HCl	*meta*	154–155°	$C_{32}H_{56}Cl_2N_2O_6S_2$	52.98	7.44	53.37	7.60	...

ml.). The solution was warmed to 65°, and diethylaminoethanol (1.24 Gm.) in anhydrous benzene (50 ml.) was slowly added dropwise; no visible reaction was observed. The reaction mixture was refluxed for 4 hours at 65°. After this time, the hydrochloride salt which had precipitated was removed by filtration, washed with two 50-ml. portions of anhydrous benzene, and dissolved in absolute methanol (100 ml.). Anhydrous ether was added until the solution became slightly cloudy; the solution was then stored in a refrigerator (6°) for 24 hours. The product was recrystallized twice using the above procedure. The reddish orange crystals which formed (1.03 Gm.) melted at 184–185° uncorrected. The product was not subjected to further purification. After drying under reduced pressure in the presence of a drying agent, the product melted at 184–185° uncorrected. Yield, 1.02 Gm. (25%).

Anal.—Calcd. for $C_{24}H_{36}N_2O_6S_2$: C, 46.99; H, 5.89. Found: C, 47.43; H, 6.51.

CONCLUSION

The synthesis and chemical investigation of some alkyl and aminoalkyl esters of *p*- and *m*-azobenzenedisulfonic acid have been reported. Two homologous series of dialkyl esters ranging from dimethyl to dibutyl and the hydrochloride salts of bis-(2-di - *n* - butylaminoethyl) - *m* - azobenzene - 3:3′-disulfonate and bis-(diethylaminoethyl)-*p*-azobenzene-4:4′-disulfonate are reported. Discussion and references leading to the preparation of *p*- and *m*-azobenzenedisulfonic acids and their chlorides, metallic salt, and amide derivatives are also presented. The compounds were not subjected to pharmacological evaluation.

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Stability Patterns of Vitamin A in Various Pharmaceutical Dosage Forms

By J. THURØ CARSTENSEN

Data presented show that the logarithm of the pseudo first-order rate constant for the degradation of vitamin A when moisture is abundantly present is linearly related to the water vapor pressure.

IT HAS BEEN established by us through numerous experiments that vitamin A in oil solution, when stored under an inert atmosphere, follows (*a*) a pseudo first-order degradation scheme and (*b*) lends itself to conventional Arrhenius plotting according to Garrett (1). This is exemplified in Figs. 1 and 2.

It has also been established that vitamin A acetate and palmitate encased in gelatin, acacia, and like substances, upon tableting into conventional uncoated tablets and/or chewable tablets, follow a similar pattern, provided that extrusion is allowed for. Examples of this are shown in Fig. 3.

While these facts are easily explained on a thermodynamical basis, *we have encountered the somewhat surprising phenomenon that in the case where moisture is abundantly present, the logarithm of the pseudo first-order rate constant* (k) *is linearly related to the water vapor pressure* (p). This holds true for water-micelle systems of vitamin A palmitate and also vitamin A acetate and palmitate beadlets in sugar coated tablets (see Figs. 4 and 5). To verify the better fit of a straight line (*e.g.*, the data in Fig. 4), the least square fit lines were drawn (*a*) with respect to $y = \log k = f(1/T)$ (T being absolute temperature) and (*b*) $y = \log k = f(p)$. The sum $\Sigma(y_i - \alpha x_i - \beta)^2 = S^2$ is not, in this case, of good comparative measurement because of the different abscissa; however, the relative standard deviation of the slopes (α) calculated in cases (*a*) and (*b*) give relative comparisons of which abscissa gives the best linear fit.

The values $S_\alpha/\alpha\,(1/T) = 0.485/3.463 = 0.14$ and $S_\alpha/\alpha\,(P) = 0.000479/0.0132 = 0.036$ demonstrate numerically what is obvious to visual inspection. The important qualitative fact is that in the case of $f\,(1/T)$ the intermediate points lie below the terminal line segment, whereas in case of $f\,(p)$ the points scatter, implying curvature for $f\,(1/T)$ but not for $f\,(p)$. There is no ready thermodynamic explana-

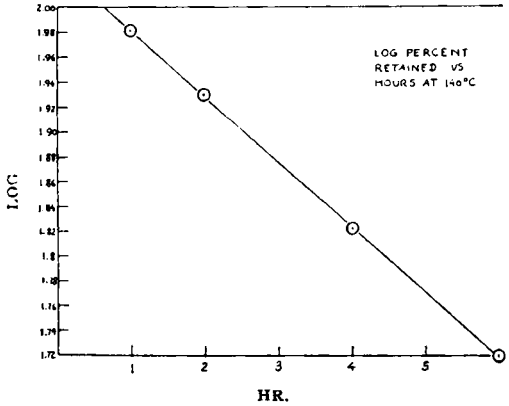

Fig. 1.—Vitamin A alcohol in Tween-Drew oil base, 500,000 units/ml., stored under nitrogen in glass bottles. Plot shows per cent potency retained as a function of hours at 140° C.

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