phate of different concentration were mixed with equal volumes of toluidin blue (0.5×10^{-4} *M*). Under these conditions no precipitate was formed. The results are given in Fig. 1. The absorption maximum shifted from 630 mμ, in water, to 530 mμ in hexametaphosphate.

Defining metachromasy as ε at 530 mμ/ε at 630 mμ, the maximum metachromasy was obtained at a concentration of hexametaphosphate[7] which was 8 times that of the dye. It decreased in very low and in high concentration (Table I). Trimetaphosphate gave a very slight metachromatic reaction which may have been due to some contaminating hexametaphosphate. Ortho-, pyro-, tri-phosphate and adenosine triphosphate gave no metachromasy. Nucleic acid and salts (potassium sulfate) inhibited metachromasy.

According to Michaelis and Granick[1a] metachromasy is due to a polymerization of the dye. This suggests the following explanation for the case of hexametaphosphate. When many molecules of dye are joined on the same molecule of hexametaphosphate they can be considered as polymerized, not directly but through the metaphosphate. When the concentration of metaphosphate is increased, the probability of molecules of dye to be joined on the same molecule of hexametaphosphate decreases and there is less metachromasy. When there is an excess of dye, the free molecules of dye not being metachromatic, metachromasy also decreases. That the maximum metachromasy is not obtained for equimolarity may be explained by the dissociation of the toluidin blue–hexametaphosphate compound.

TABLE I

Toluidin blue 0.25×10^{-4} *M* in (*p*H between 6.5 and 7)	Metachromasy ε 530 mμ / ε 630 mμ
Water	0.23
2×10^{-5} *M* hexametaphosphate	.46
4×10^{-5} *M* hexametaphosphate	1.24
1×10^{-4} *M* hexametaphosphate	3.52
2×10^{-4} *M* hexametaphosphate	3.59
4×10^{-4} *M* hexametaphosphate	3.31
8×10^{-4} *M* hexametaphosphate	2.97
4×10^{-3} *M* hexametaphosphate	2.17
2×10^{-2} *M* hexametaphosphate	1.16
5×10^{-2} *M* hexametaphosphate	0.73
1×10^{-1} *M* hexametaphosphate	.50
2×10^{-4} *M* hexa. + 1.25×10^{-4} *M* K_2SO_4	2.00
2×10^{-4} *M* hexa. + 1.25×10^{-3} *M* K_2SO_4	1.56
2×10^{-4} *M* hexa. + 1.25×10^{-2} *M* K_2SO_4	0.84
2×10^{-4} *M* hexa. + 1.25×10^{-1} *M* K_2SO_4	.26
2.5×10^{-5} *M* trimetaphosphate	.22
2.5×10^{-4} *M* trimetaphosphate	.26
2.5×10^{-3} *M* trimetaphosphate	.31
0.5×10^{-3} *M* orthophosphate	.21
$.5 \times 10^{-3}$ *M* pyrophosphate	.21
$.5 \times 10^{-3}$ *M* triphosphate	.24
$.5 \times 10^{-4}$ *M* triphosphate	.23
$.5 \times 10^{-3}$ *M* adenosine triphosphate	.22

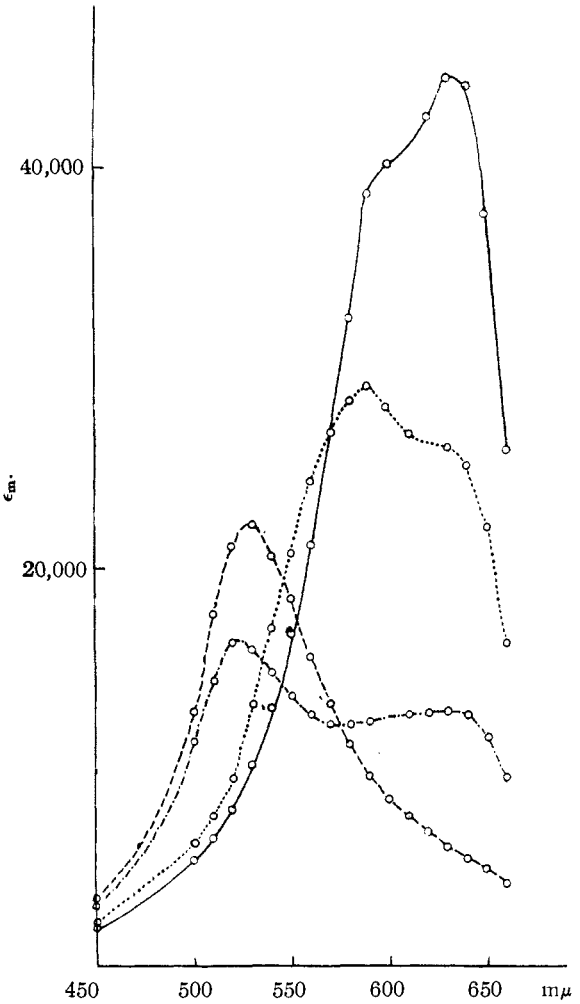

Fig. 1.—Toluidin blue 0.25×10^{-4} *M*: ——, in water; — — — —, 0.4×10^{-4} hexametph.; — — — —, 2×10^{-4} hexametph.; ----------, 1×10^{-1} hexametph.

As shown by the data, the metachromatic reaction in this series of phosphoric acid compounds is specific and very sensitive for hexametaphosphate.

DEPARTMENT OF BIOLOGICAL CHEMISTRY
WASHINGTON UNIVERSITY SCHOOL OF MEDICINE
ST. LOUIS, MISSOURI RECEIVED JULY 23, 1947

NEW COMPOUNDS

4-Nitro-6,9-dichloroacridine[1]

5-Chloro-2′-nitrodiphenylamine-2-carboxylic Acid.—The Ullmann[2] method of diphenylamine synthesis was

(1) The work described herein was carried out under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Illinois.

(2) Ullmann, *Ber.*, **36**, 2383 (1903).

employed. A mixture of 22 g. of 2,4-dichlorobenzoic acid, 24 g. of *o*-nitroaniline, 17 g. of anhydrous potassium carbonate, 0.8 g. of copper bronze and 35 ml. of *n*-hexanol was heated under reflux with stirring for five hours. The mixture was steam-distilled, the residue was filtered, and the filtrate was acidified with 12 *N* hydrochloric acid. The precipitate obtained on acidification was collected and washed with water. Recrystallization of the moist product from ethanol–acetone gave 12.8 g. (35%) of bright orange crystals, m. p. 281°.

Anal. Calcd. for $C_{13}H_9ClN_2O_4$: C, 53.33; H, 3.08. Found: C, 53.22; H, 3.19.

4-Nitro-6-chloroacridone.—The substituted diphenylamine (5 g.) and 10 ml. of concentrated sulfuric acid were heated on the steam-bath for six hours.[3] The warm mixture was poured carefully down the wall of a 150-ml. beaker into 80 ml. of boiling water. The precipitate thus formed was boiled with dilute sodium carbonate solution, collected on a filter and washed with water. Recrystallization of the product from glacial acetic acid gave 3.75 g. (75%) of yellow powder, m. p. 312° (dec. at 321°).

Anal. Calcd. for $C_{13}H_7ClN_2O_3$: C, 56.82; H, 2.57. Found: C, 56.60; H, 2.71.

4-Nitro-6,9-dichloroacridine.—Ten grams of 5-chloro-2'-nitrodiphenylamine-2-carboxylic acid and 35 ml. of phosphorus oxychloride were heated with stirring for two hours at 135°. The mixture was poured onto 100 g. of ice and aqueous ammonia. The precipitate which formed was recrystallized from ethanolic ammonia solution to yield 8.5 g. (85%) of yellow powder, m. p. 205°. 4-Nitro-6,9-dichloroacridine was also prepared by treating 4-nitro-6-chloroacridone with a mixture of phosphorus pentachloride and phosphorus oxychloride. The product was found to be readily susceptible to hydrolysis, with regeneration of the acridone.

Anal. Calcd. for $C_{13}H_6Cl_2N_2O_2$: C, 53.23; H, 2.06. Found: C, 53.09; H, 2.26.

(3) "Organic Syntheses," **19**, 6 (1939).

NOYES CHEMICAL LABORATORY
UNIVERSITY OF ILLINOIS — NELSON J. LEONARD
URBANA, ILLINOIS — LEONARD C. SMITH

RECEIVED JUNE 30, 1947

Choline Cholanate

Pure cholanic acid (3.62 g.), prepared by Clemmensen reduction of 3,12-diketocholanic acid, was refluxed two and one-half hours with 25 cc. of thionyl chloride.

The crystalline acid chloride so obtained was treated according to a preparatory method of Fourneau and Page[1] with 5 cc. of β-iodoethanol.[2] The reactants were mixed and allowed to stand at room temperature for three hours with occasional warming in the water-bath to redissolve the crystalline mass. After this time the reaction mixture was taken up in ether and the ether layer washed at 0° successively with dilute sodium carbonate, hydrochloric acid and water until neutral. Evaporation of the dried ether solution produced an oily residue which crystallized on addition of a drop of methanol.

This ester residue was transferred to a combustion tube and 10 cc. of an 18.7% benzene solution of trimethylamine was added. The tube was closed and heated in an autoclave at 120° for twenty-two hours.

Dilution of the reaction mixture with 3 volumes of ether precipitated 1.3 g. of leafy brown material which for the most part was the choline iodide ester of cholanic acid contaminated with some trimethylamine hydroiodide. This reaction product was filtered and dissolved in water. To the clear water solution was added an excess of sodium picrate. The bases so precipitated were recrystallized from dilute methanol and after five recrystallizations the picrate of the choline ester was obtained as a pure product (needles), m. p. 194 (dec.) cor.

Anal. Calcd. for $C_{35}H_{54}N_4O_9$: C, 62.29; H, 8.07. Found: C, 62.18; H, 8.09.

The authors are indebted to Merck and Company, Rahway, New Jersey, for the micro-analyses.

DEPARTMENT OF CHEMISTRY
FRICK CHEMICAL LABORATORY
PRINCETON UNIVERSITY — DEAN R. REXFORD[3]
PRINCETON, N. J. — EVERETT S. WALLIS

RECEIVED JUNE 23, 1947

(1) E. Fourneau and H. J. Page, *Bull. soc. chim.*, [4] **15**, 544–553 (1914).

(2) Louis Henry, *Rec. trav. chim.*, **20**, 253 (1901).

(3) Research Associate, Rockefeller Foundation Fellow.

α-(Di-*n*-amylaminomethyl)-2-methoxy-1-naphthalenemethanol Hydrochloride

This compound[1] was prepared from the corresponding amino ketone by reduction with aluminum isopropoxide as described earlier.[2] The yield of crude α-(di-*n*-amylaminomethyl)-2-methoxy-1-naphthalenemethanol hydrochloride was 72% based on bromo ketone, but purification was difficult and the yield of analytically pure hydrochloride, m. p. 136.5–137.5° (cor.), was 33%.

Anal. Calcd. for $C_{23}H_{36}O_2NCl$: C, 70.11; H, 9.21. Found: C, 70.03; H, 9.13.

The solubility at 23.5° is 0.61 g. in 100 ml. of saturated aqueous solution.

The hydrochloride was crystallized from ethyl acetate and from dioxane. From the latter it was obtained with one molecule of solvent of crystallization.

Anal. Calcd. for $C_{23}H_{36}O_2NCl \cdot C_4H_8O_2$: C, 67.26; H, 9.20. Found: C, 67.27; H, 9.03.

The dioxane was removed by heating at 70° in a Fischer pistol for two days.

(1) Under a contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and the University of California, Los Angeles. Its antimalarial activity will be found in "Survey of Antimalarial Drugs 1941–1945," Vol. II, Wiselogle, editor, J. W. Edwards, Ann Arbor, Michigan, 1946, p. 413.

(2) Jacobs, Winstein, Ralls, Robson, Henderson, Akawie, Florsheim, Seymour and Seil, *J. Org. Chem.*, **11**, 21 (1946).

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF CALIFORNIA — THOMAS L. JACOBS
LOS ANGELES 24, CALIFORNIA — RICHARD I. AKAWIE

RECEIVED JULY 12, 1947

Preparation of *p*-Phenylphenacyl Iodide, and Redetermination of the Melting Points of Some Phenacyl Halides

For a comparative study with the corresponding chloride and bromide, ***p*-phenylphenacyl iodide** was prepared as follows: To a solution of 2.3 g. of *p*-phenylphenacyl chloride in 30 cc. of dry acetone was added a solution of 1.7 g. of anhydrous sodium iodide in 20 cc. of the same solvent. The precipitate of sodium chloride formed at once was separated by filtration and rinsed with dry acetone. The acetone solutions, after evaporation of the solvent, gave 3.2 g. of crude product (corresponding to the theoretical amount), with a yellow color and darkening quickly when exposed to the air. After treatment with active charcoal in ethanol and two recrystallizations from ethanol the compound was obtained in the form of almost colorless needles and stable in the air, melting sharply at 104.4°.

Anal.[1] Calcd. for $C_{14}H_{11}OI$: I, 39.42. Found: I, 39.63, 39.35.

The same substance is obtained by the same procedure starting with *p*-phenylphenacyl bromide, or boiling the chloride or bromide in alcohol with potassium iodide.

(1) Micro-Carius, executed by Mr. H. W. Rzeppa in the Instituto Butantan, São Paulo.