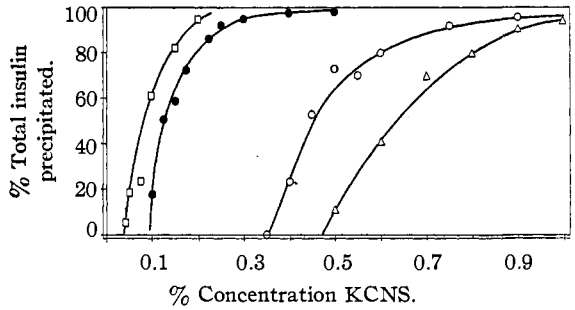

Fig. 3.—Influence of pH on the precipitating action of KCNS on 0.5% insulin at 25°: △, pH 1.6; ○, pH 2.5; ●, pH 3.5; □, pH 3.9.

experimental results can be expressed by the salting-out equation[7]

$$\log S = \beta' - K_s'(\Gamma/2)$$

where S is the solubility of the protein in g. per liter and $\Gamma/2$ the ionic strength. However, unlike typical salting-out phenomena, the determined values of K_s' were not independent of pH and temperature but decreased as either of these factors was decreased.

The values of β' showed a gradual increase as the pH decreased. From the variations of K_s' it was concluded that factors other than those which govern the salting-out of proteins by concentrated salt solutions partake in the present precipitation reaction. The most important of these is specific combination between protein and thiocyanate, as evidenced by the following findings: (1) the shift in pH as revealed by the addition of thiocyanate to unbuffered insulin solutions at pH 3.0 or higher; (2) the shift in electrophoretic mobility at pH 5.8, observed by Volkin,[2] and (3) the failure of other univalent anions, such as chloride, to cause the precipitation of insulin in equal or 5 times higher salt concentrations. Another reason for the failure of the salting-out equation to apply strictly to the present system is the change in the degree of association of the insulin monomer with changing pH, and ionic strength.[4]

The present findings are in full agreement with the assumption that the precipitating action of thiocyanate is directed primarily toward the tri- or tetrameric form, I_3 or I_4 (considering I as the 12,000 molecular weight unit),[4] and that any factor which shifts the molecular equilibrium toward the aggregated state likewise promotes precipitation by thiocyanate. These factors are:[3,4] (1) increase in protein concentration, (2) increase in pH above pH 2, (3) decrease in temperature and (4) increase in ionic strength. The results of other types of measurements on the effect of thiocyanate on insulin[8] are in agreement with this view.

(7) Cohn and Edsall, "Proteins, Amino Acids and Peptides," New York, N. Y., 1943.

(8) Fredericq and Neurath, THIS JOURNAL, 72, 2684 (1950).

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Preparation of 2′-Nitro-4′-methoxy-5-chlorodiphenylamine-2-carboxylic Acid

BY DAVID SHAPIRO

In contradistinction with the claim of Knunyants and Benevolenskaya,[1] the preparation of the above acid from 2,4-dichlorobenzoic acid and 3-nitro-4-aminoanisole gave only poor yields, and the synthesis from 4-chloroanthranilic acid and 3-nitro-4-bromoanisole,[2] suffers from the difficult accessibility of the starting material. The acid can be prepared conveniently by nitration of 4′-methoxy-5-chlorodiphenylamine-2-carboxylic acid which is a commercial intermediate in the Atabrine synthesis.

The solution of 69.5 g. of 4′-methoxy-5-chlorodiphenylamine-2-carboxylic acid in 550 cc. of glacial acetic acid, was cooled with stirring to 6° and slowly treated with a mixture of 19 cc. of nitric acid (sp. gr. 1.402) and 50 cc. of glacial acetic acid. The temperature was slowly raised to 50° and kept at this level, until the mixture became brick colored. Cold water was added and the red crystals were collected and washed with water (60 g., 75%). The acid crystallizes from 40 parts of butanol, melts at 270–272°, and shows no depression of the m. p. with a sample prepared according to the Russian authors.[1]

Anal. Calcd. for $C_{14}H_{11}ClN_2O_5$: N, 8.7. Found: N, 8.7.

(1) Knunyants and Benevolenskaya, *J. Gen. Chem.* (*U. S. S. R.*). **10**, 1415 (1940) (*C.A.*, **35**, 3642 (1941)).

(2) Samant, *Ber.*, **75**, 1008 (1942).

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An Improved Procedure for the Condensation of Potassium Phthalimide with Organic Halides

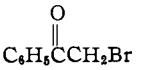
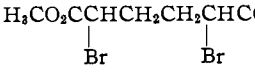
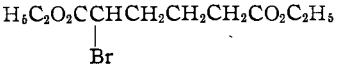
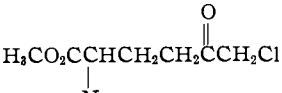
BY JOHN C. SHEEHAN AND WILLIAM A. BOLHOFER[1]

In the usual method of conducting the Gabriel condensation, potassium phthalimide and the organic halide are heated together without solvent or in the presence of a non-polar, high-boiling solvent (such as xylene). The insolubility of potassium phthalimide under these conditions hinders the reaction, necessitating prolonged heating (two to twenty-four hours) at relatively high temperatures (100–150°). This results in lowered yields and impure products.

We have found that by carrying out the condensations in dimethylformamide, in which potassium phthalimide is appreciably soluble, a mild exother-

(1) Swift Amino Acid Fellow, 1947–1949.

TABLE I

Halide	Product	M. p., °C.	% Yield in dimethylformamide	% Yield by other methods
	Phthalimidoacetophenone	165–167	92	54[a]
	Dimethyl α,δ-diphthalimidoadipate	160–185[b]	90	31[c]
	Diethyl α-phthalimidoadipate[d]	Liquid[e]	95	...
	Methyl α,ε-diphthalimido-δ-oxocaproate[g]	143–144	89	...

[a] Recorded m. p. 167°; Gabriel, *Ber.*, **41**, 1132 (1908). [b] The product is a mixture of stereoisomers. [c] This yield was obtained by us when the reaction was carried out in xylene according to the published directions for the preparation of the corresponding diethyl ester; ref. 3. [d] Reaction carried out by Dr. Charles Mumaw. [e] Purified by evaporative distillation, $n^{25}D$ 1.5140. *Anal.* Calcd. for $C_{19}H_{23}O_6N$: C, 63.14; H, 6.70; N, 3.88. Found: C, 62.90; H, 6.50; N, 3.89. [f] Sheehan and Bolhofer, THIS JOURNAL, **72**, 2469 (1950). A recrystallized sample was used in this experiment. [g] An analysis and the properties of this compound are given in ref. *f*.

mic reaction starts spontaneously at room temperature and appears to be essentially complete in ten minutes with the more reactive halides. Products of high purity can usually be isolated in over 90% yield by chloroform extraction of the reaction mixture after dilution with water; dimethylformamide remains in the aqueous phase. The dark-colored reaction tars frequently accompanying the Gabriel reaction are completely absent.

Listed in Table I are four products prepared by the dimethylformamide method in connection with problems current in this Laboratory. Preliminary experiments indicate that the method is applicable to less reactive halides if the reaction temperature is raised. The procedure may be illustrated by the preparation of phthalimidoacetophenone and the conversion of dimethyl α,δ-dibromoadipate to the corresponding diphthalimido compound in 90% yield (in xylene the yield was 31%).

In order to obtain α,δ-diaminoadipic acid two methods were employed for removal of the phthaloyl groups, vigorous acid hydrolysis and cleavage with hydrazine. It is interesting to note that hydrazine apparently did not affect the ester groups under the reaction conditions. Since the purity of diaminoadipic acid cannot be estimated from the melting point, the corresponding dimethyl ester dihydrochloride was made in 94% yield.

Experimental[2]

Phthalimidoacetophenone.—Potassium phthalimide (5.0 g., 0.027 mole) was added to a solution of 5.0 g. (0.0253 mole) of phenacyl bromide (Eastman Kodak Co.) in 20 ml. of dimethylformamide (du Pont). The reaction was slightly exothermic, the temperature rising to 55° in five minutes. Stirring was continued for thirty minutes, and the temperature dropped slowly to 25°. After the addition of 30 ml. of chloroform, the mixture was poured into 100 ml. of water. The aqueous phase was separated and extracted with two 10-ml. portions of chloroform. The combined chloroform extract was washed with 20 ml. of 0.2 *N* sodium hydroxide (to remove unreacted phthalimide) and 20 ml. of water. After drying (sodium sulfate) the chloroform was removed. The crystalline residue was triturated with 40 ml. of ether, and 6.1 g. (92%) of phthalimidoacetophenone was collected by filtration; m. p. 165–167°.

Dimethyl α,δ-Diphthalimidoadipate.—Dimethyl α,δ-dibromoadipate was prepared in 88.9% yield by the method of Stephen and Weizmann.[3] This is essentially the same process described in "Organic Synthesis"[4] for the preparation of the diethyl ester.

A mixture of 69 g. (0.21 mole) of dimethyl α,δ-dibromoadipate, 87 g. (0.47 mole) of potassium phthalimide and 260 ml. of dimethylformamide was heated to 90° (a mild exothermic reaction starts at 50°) and maintained at this temperature for forty minutes. The cooled reaction mixture was diluted with 300 ml. of chloroform and poured into 1200 ml. of water. The chloroform layer was separated, and the aqueous phase was extracted twice with 100 ml. of chloroform. The combined chloroform extract was washed with 200 ml. of cold 0.1 *N* sodium hydroxide, 200 ml. of water, and dried over sodium sulfate. The chloroform was removed by concentration under reduced pressure to the point of incipient crystallization; the immediate addition of 300 ml. of ether induced a rapid crystallization. The ether-washed product weighed 87 g. (90.2%) and melted over the range 160–185°.

After three recrystallizations from ethyl acetate and one from benzene, an apparently pure stereoisomer was obtained which melted at 210.7–211.4°.

Anal. Calcd. for $C_{24}H_{20}N_2O_8$: C, 62.06; H, 4.34; N, 6.03. Found: C, 62.33; H, 4.35; N, 5.95.

α,δ-Diaminoadipic Acid. A. By Acid Hydrolysis.—A mixture of 100 ml. of 48% hydrobromic acid, 100 ml. of glacial acetic acid and 50 g. of dimethyl α,δ-diphthalimidoadipate (m. p. 160–185°) was heated under reflux until a clear solution resulted (ten days). On cooling, most of the phthalic acid crystallized. After filtration, the filtrate and water washes were concentrated under reduced pressure practically to dryness. The residue was dissolved

(2) All melting points are corrected. We are indebted to Mr. S. M. Nagy and his associates for the microanalyses.

(3) Stephen and Weizmann, *J. Chem. Soc.*, **103**, 274 (1913).

(4) Guha and Sankaran, "Organic Syntheses," Vol. 26, John Wiley and Sons, Inc., New York, N. Y., 1946, p. 57.

in 100 ml. of water, filtered, and neutralized with concentrated ammonia. After crystallization at 0° for twelve hours, 17.3 g. (91.2%) of α,δ-diaminoadipic acid was obtained. The product did not have a sharp melting point but sintered and charred at about 300°. It was soluble in acids and bases but insoluble in water.

B. By Treatment with Hydrazine.—A mixture of 4.64 g. of dimethyl α,δ-diphthalamidoadipate (m. p. 160–185°, 0.01 mole), 50 ml. of methanol and 1.2 ml. of an 85% aqueous hydrazine hydrate solution (0.02 mole) was heated under reflux for one hour. After cooling, 25 ml. of water was added and the methanol was removed by concentration under reduced pressure. Concentrated hydrochloric acid (25 ml.) was added to the residual aqueous suspension and the mixture was heated under reflux for one hour. After cooling to 0°, crystalline phthalhydrazide was removed by filtration. The filtrate was then concentrated under reduced pressure to remove most of the hydrochloric acid and the moist residue was dissolved in 50 ml. of water. A small amount of insoluble matter was removed by filtration and the clear filtrate was neutralized with 2 *N* sodium hydroxide. After cooling at 0° for twelve hours, 1.4 g. (79.5%) of α,δ-diaminoadipic acid was obtained.

Dimethyl α,δ-Diaminoadipate Dihydrochloride.—By the Fischer esterification method using methanol and hydrogen chloride, 15 g. of α,δ-diaminoadipic acid (from the acid hydrolysis) was converted to 21.2 g. (94%), m. p. 203–205° dec., of dimethyl α,δ-diaminoadipate dihydrochloride. A sample for analysis was obtained by recrystallization from methanol–ether; m. p. 206–207° dec.

Anal. Calcd. for $C_8H_{18}O_4N_2Cl_2$: C, 34.67; H, 6.55; N, 10.11. Found: C, 34.36; H, 6.59; N, 10.15.

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Metalation of Thianaphthene by *n*-Butyllithium

BY DAVID A. SHIRLEY AND MARGARET D. CAMERON[1]

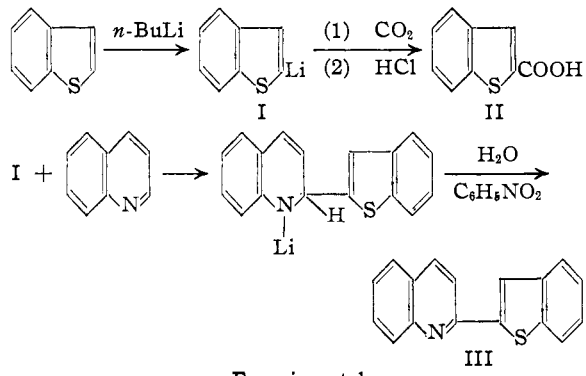
Thianaphthene previously has been metalated using ethylmagnesium bromide, sodamide, sodium metal and mercuric acetate. Weissgerber and Kruber[2] found that thianaphthene and ethylmagnesium bromide refluxed in dimethylaniline gave a 24% yield of the 2-derivative, and that sodamide and thianaphthene in xylene at 120° gave a 25–30% yield of each of the 2- and 2,3-sodio derivatives; Schönberg and co-workers[3] found that thianaphthene and powdered sodium in ether gave 2-thianaphthenylsodium in 56% yield after several days standing. Challenger and Miller[4] obtained the 2,3- and the 3-mercuric acetates by the action of mercuric acetate on thianaphthene.

In connection with a study of the chemistry of thianaphthene, its metalation with *n*-butyllithium was found to yield 54.6% 2-thianaphthenyllithium (I) after one hour at the temperature of refluxing ether. The position of the lithium substituent was determined by treatment of the thianaphthenyllithium with solid carbon dioxide followed by acidification to yield thianaphthene-2-carboxylic acid (II).

(1) Eli Lilly Research Fellow, 1949-1950.
(2) Weissgerber and Kruber, *Ber.*, **53**, 1551 (1920).
(3) Schönberg, Petersen and Kaltschmidt, *ibid.*, **66**, 233 (1933).
(4) Challenger and Miller, *J. Chem. Soc.*, 1005 (1939).

The 2-thianaphthenyllithium formed 2-(2′-thianaphthenyl)-quinoline (III) when treated with quinoline, hydrolyzed and the intermediate 1,2-dihydro-2-(2′-thianaphthenyl)-quinoline oxidized.

These reactions are analogous to an earlier series carried out on thiophene by Gilman and Shirley.[5]



Experimental

Metalation of Thianaphthene.—An ethereal solution of *n*-butyllithium was prepared by the gradual addition with stirring of 295 g. (2.17 moles) of *n*-butyl bromide in 400 ml. of dry ether to 36.1 g. (5.35 moles) of lithium wire cut into 5–10 mm. lengths and suspended in 700 ml. of dry ether. The mixture was heated under reflux with continued stirring for one hour. A solution of 134 g. (1.00 mole) of thianaphthene in 300 ml. of dry ether was added, with stirring, to the filtered *n*-butyllithium solution and the mixture refluxed for 45 minutes.

Four-fifths of the resulting solution of 2-thianaphthenyllithium (I) was poured over a slurry of solid carbon dioxide and dry ether. After the evaporation of the carbon dioxide, 500 ml. of water was added, and the aqueous layer separated and acidified with concentrated hydrochloric acid. The precipitated solid was removed by filtration and recrystallized from dilute methanol, giving 77.5 g. (54.6% based on thianaphthene) of 2-thianaphthenecarboxylic acid melting at 236–236.5°. After a second recrystallization the acid melted at 237° and formed an amide which melted at 177°. Mayer[6] gives the melting point of thianaphthene-2-carboxylic acid (II) prepared by ring closure as 236° as do Schönberg, Petersen and Kaltschmidt[3] and Weissgerber and Kruber[2] who prepared the acid from 2-thianaphthenylsodium. The latter investigators proved the structure of the acid and prepared the amide, giving its melting point as 177°.

2-(2′-Thianaphthenyl)-quinoline (III).—To the remaining one-fifth of the 2-thianaphthenyllithium (I) solution, 25.8 g. (0.20 mole) of quinoline in 150 ml. of dry ether was added and the mixture stirred under reflux for one hour. The mixture was then hydrolyzed with 100 ml. of water and 20 ml. of nitrobenzene was added for the oxidation of the 1,2-dihydro-2-(2′-thianaphthenyl)-quinoline. After stirring for fifteen minutes the ethereal layer was separated, dried over anhydrous magnesium sulfate and distilled. The solid residue was recrystallized from ethyl alcohol and then from petroleum ether (b. p. 80–110°) to give 25.7 g. (49.4% based on thianaphthene) of yellow needles of 2-(2′-thianaphthenyl)-quinoline (III), m. p. 189.8–189.9° (cor.).

Anal. Calcd. for $C_{17}H_{11}NS$: N, 5.36. Found: N, 5.39.

A **picrate** prepared from ethereal solutions of picric acid and 2-(2′-thianaphthenyl)-quinoline melted at 221.5–222°.

(5) Gilman and Shirley, THIS JOURNAL, **71**, 1870 (1949).
(6) Mayer, *et al.*, *Ann.*, **488**, 259 (1931).