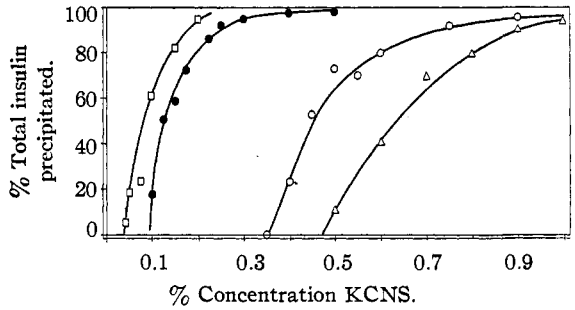

Fig. 3.—Influence of pH on the precipitating action of KCNS on 0.5% insulin at 25°: △, pH 1.6; ○, pH 2.5; ●, pH 3.5; □, pH 3.9.

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) Frederieq 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.