solution to cool overnight in a refrigerator. The crystalline material was collected on a filter and dried, 23.8 mg., m.p. 205–7°. The material was again recrystallized to give a slightly yellow colored solid, m.p. 205–7°.

Anal. Calcd. for $C_{18}H_{16}O_7$: C, 62.77; H, 4.69. Found: C, 61.94; H, 4.81.

On admixture of methylated hydrolyzed dactylin with 3,4′-dihydroxy-5,7,3′-trimethoxyflavone, no depression of melting point was observed. Infrared spectra of the two samples were identical in every respect as shown in Fig. 1.

Acknowledgment. The author wishes to thank Dr. Richard Kuhn, who graciously supplied samples that allowed a rapid solution to this problem.

CINCINNATI, OHIO

[CONTRIBUTION FROM KAY-FRIES CHEMICALS, INC.]

Preparation of Cytosine

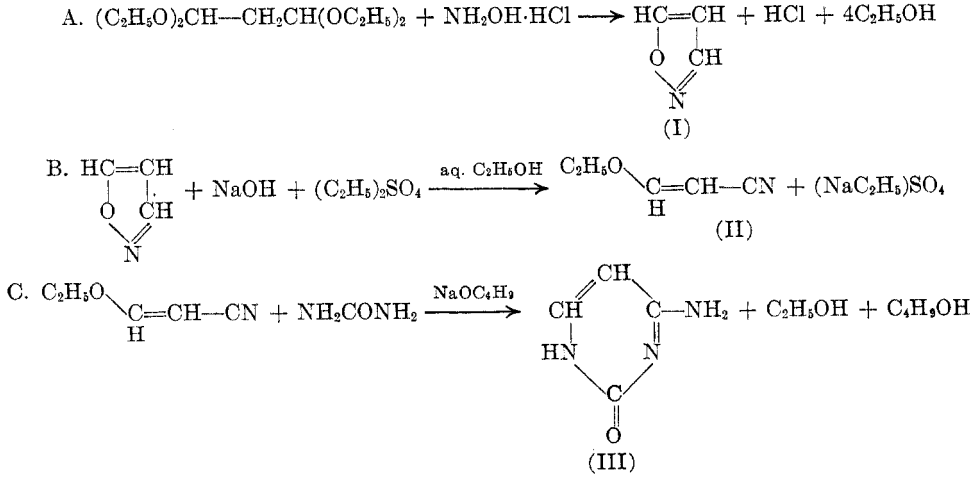
PETER J. TARSIO AND LEONARD NICHOLL

Received July 31, 1956

A new method for the preparation of cytosine is described. Isoxazole is prepared by the reaction of malonaldehyde tetraethyl acetal with hydroxylamine hydrochloride. β-ethoxyacrylonitrile is prepared by reaction of isoxazole with diethyl sulfate in alkaline solution. Cytosine is prepared by condensing β-ethoxyacrylonitrile with urea in a sodium alcoholate solution.

In the development of certain work in this laboratory, cytosine was desired. It therefore became necessary to prepare cytosine in sufficient quantities so that further reactions of it could be studied. The preparation of cytosine from uracil, thiouracel, dithiouracil, and cyanoacetal has been reported.[1–4] The procedures and results obtained by the above methods did not seem suitable for our purposes, since yields are invariably low and the processing of the necessary intermediates is involved and time consuming. Consequently, an alternative method of preparation of cytosine was developed.

This method employed the following sequence of reactions:



Isoxazole (I) was obtained in 70% yield from 1,1,3,3-tetraethoxypropane (malonaldehyde acetal) and hydroxylamine hydrochloride. The reaction of osoxazole with diethyl sulfate and sodium hydroxide to form β-ethoxyacrylonitrile (II) proceeded to give a yield of 85–90% of a mixture of β-ethoxyacrylonitrile and cyanacetaldehyde acetal. The condensation of β-ethoxyacrylonitrile with urea in a refluxing sodium butylate solution resulted in a 43% yield of cytosine (III). All the steps were characterized by the absence of by-products except in the case of β-ethoxyacrylonitrile which invariably contained varying amounts of cyanoacetaldehyde acetal. The β-ethoxyacrylonitrile was obtained substantially pure by dealcoholating a mixture containing the β-ethoxyacrylonitrile and cyanoacetaldehyde acetal with heat at atmospheric pressure.

EXPERIMENTAL

Isoxazole (I). Two hundred twenty grams (1.0 mole) of malonaldehyde tetraethyl acetal prepared by the method of

(1) G. E. Hilbert and T. B. Johnson, *J. Am. Chem. Soc.*, 52, 1152 (1930).

(2) D. J. Brown, *J. Soc. Chem. Ind. (London)*, 69, 353 (1950).

(3) G. Hitchings and P. Russell, *J. Biol. Chem.*, 177, 357 (1949).

(4) A. Bendich, H. Getler, and G. Brown, *J. Biol. Chem.*, 177, 565 (1949).

Copenhaver,[5] was added over a 3-hr. period to 76.5 (1.1 moles) of hydroxylamine hydrochloride in 500 ml. water at 60–70°. Heating was continued for an additional hour at 60–70°. The mixture was distilled collecting water, alcohol, and isoxazole to a vapor temperature of 95°.

The distillate was added dropwise to a well stirred saturated solution of an excess of 183 g. cadmium chloride in 150 ml. water. The curdy precipitate that resulted was suction filtered, washed with a little cold water, and sucked as dry as possible. The precipitate was suspended in water and heated to boiling, distilling out a mixture of isoxazole and water. The distillate, which had separated into two phases, was extracted with ether. The ether extract was dried with $CaCl_2$ and distilled. After removal of the ether, there was obtained 49.3 g. (70%) of isoxazole boiling at 93–95°. Speroni and Pino[6] report the boiling point of isoxazole as b_{760} 94.8°.

(Note: A reference to a similar procedure appeared during the preparation of the manuscript.[7])

β-ethoxyacrylonitrile (II). Instead of preparing pure isoxazole for the preparation of β-ethoxyacrylonitrile, it was found advantageous to employ the aqueous alcoholic solution of isoxazole as described under the preparation of isoxazole. Thus, to 561 g. of aqueous alcoholic isoxazole containing 88.5 g. of isoxazole (1.285 moles), determined by alkaline hydrolysis or by isolation with cadmium chloride, was added 570 g. (3.7 moles) of technical diethyl sulfate. The mixture was chilled to 5° with stirring and 606 g. of 24.45% sodium hydroxide (3.7 moles) solution was added over a 4-hr. period, maintaining reaction temperature at 5–10° with the use of an ice bath. Stirring was continued for 2 hr. at 5–10° after the caustic addition. The mixture was slowly warmed up, removing ether and alcohol through a 1.5-ft. packed column. The column was removed and the product removed by steam distillation. The product phase was separated from the aqueous layer, dried with $CaCl_2$ and distilled at reduced pressure. There was obtained 114 g. of product boiling from 80–90° at 12 mm., d_{20}^{20} 0.9463, n_D^{20} 1.4531. Based on nitrogen analysis, the fraction consists of 78.9% β-ethoxyacrylonitrile and 21.1% of cyanoacetaldehyde acetal.

The mixture was heated to boiling at atmospheric pressure until the evolution of alcohol ceased. After removal of residual alcohol under reduced pressure, 108.2 g. β-ethoxyacrylonitrile boiling at 90–91° (19 mm.), d_{20}^{20} 0.9437, n_D^{20} 1.4545, M_D 27.86 (calcd. 27.97) was obtained. Final yield of β-ethoxyacrylonitrile, 86.9% on isoxazole employed. McElvain and Clarke[8] report the following constants for β-ethoxyacrylonitrile b_8 71–72°C, n_D^{25} 1.4520, d_4^{25} 0.945.

Anal. Calcd. for: C_5H_7NO: N, 14.42. Found: N, 14.15.

Cytosine (III). To a cooled solution of 23 g. of sodium (1 g.-atom) in 690 ml. dry butanol, was added 60 g. (1 mole) of dry urea and 97.0 g. (1.0 mole) of β-ethoxyacrylonitrile. The mixture was refluxed (112–115°) for 2 hr. and cooled to 20°. Sulfuric acid (128.0 g.) in 1250 ml. water was added and the mixture was stirred for 0.5 hr. The aqueous layer was separated from the butanol, heated to 80° and 2500 ml. alcohol was added. The mixture was chilled to 0° and the crude cytosine sulfate filtered off. The cytosine sulfate was added to 1 l. of H_2O and alkalized with concentrated ammonium hydroxide until the mixture was slightly alkaline to alkacid paper. The crude cytosine was filtered, added to 1 l. of water, and clarified with charcoal. On cooling there were obtained colorless plates of cytosine. Concentration of the mother liquor yielded an additional amount of cytosine. There was obtained a total of 48.5 g. of cytosine. Yield, 43.7% based on β-ethoxyacrylonitrile employed. Melting point 305° (browns), 319–323° (decomp.) The infrared spectrum of the compound obtained was identical with a known sample of cytosine[9].

Anal. Calcd. for $C_4H_5N_3O$: N, 37.82. Found: N, 37.1.

WEST HAVERSTRAW, N. Y.

(5) U.S. Patent **2,527,533** (Gen. Aniline & Film Corp., Oct. 31, 1950).

(6) G. Speroni and P. Pino, *Proc. XIth Intern. Congr. Pure and Applied Chem. (London)* **2**, 311 (1947).

(7) R. Justoni and R. Pessina, *Gazz. chim. ital.*, **85**, 34–40 (1955). [*Chem. Abstr.* **50**, 4127d.]

(8) S. McElvain and R. Clarke, *J. Am. Chem. Soc.*, **69**, 2657 (1947).

(9) Private communication, Dr. J. S. Fox, Sloan-Kettering Institute for Cancer Research.

[CONTRIBUTION FROM THE DEPARTMENT OF ORGANIC CHEMISTRY, RADIUM INSTITUTE, UNIVERSITY OF PARIS]

New Fluorine-containing Aromatics as Potential Carcinostats

NG. PH. BUU-HOÏ, N. D. XUONG, AND R. RIPS

Received August 20, 1956

A large number of fluorine-containing aromatic acids, nitriles, and ketones have been synthesized from various fluoro aromatics for biological testing as potential carcinostatic agents.

3-Fluorotyrosine (Pardinon) has been found to inhibit the initiation and development of various tumors in animals, such as grafts of Jensen sarcomas in rats and tumors induced in mice through injection or painting with 3,4-benzpyrene.[1] Further, the same compound and 3-fluoro-4-hydroxyphenylacetic acid (Capacin) have found therapeutic use against hyperthyreosis.[2] Both these biological effects have recently been accounted for on the grounds of an antagonism toward aromatic acids and their metabolites.[3] With this in mind, a large number of new compounds more or less related to that type of molecular structure have now been prepared for biological investigation for possible carcinostatic activity and inhibitory effects on the pituitary secretions.

o-Fluoroanisole readily underwent Friedel-Crafts succinoylation to β-(3-fluoro-4-methoxybenzoyl)-

(1) May and Litzka, *Zeitschr. Krebsforsch.*, **48**, 376 (1939).

(2) May, *Die Basedowsche Krankheit, Jod und Fluor*, Editio Cantor (Aulendorf), 1950.

(3) Buu-Hoï, Symposium on Chemotherapy of Cancer (Oslo, 1956); *Acta Unio Intern. contra Cancrum*, in press.