[CONTRIBUTION FROM THE FERTILIZER INVESTIGATIONS UNIT OF THE BUREAU OF CHEMISTRY AND SOILS, U. S. DEPARTMENT OF AGRICULTURE]

Action of Alkali on 2,4-Diethoxypyrimidine and the Application of the Reaction to a New Synthesis of Cytosine

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The Refractive Indices of Some Pyrimidines

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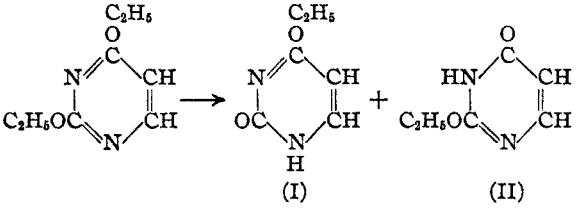
Two different types of reactions have been utilized for the synthesis of pyrimidine nucleosides: (1) the metathetical reaction between an acetobromo sugar and the metal salt of a pyrimidine[1] containing a lactam grouping and (2) the interaction of 2,4-dialkoxypyrimidines with acetobromoglucose resulting in one case in the introduction of the sugar in the desired (1) position.[2] Although a considerable amount of work has been done on the former reaction, the results, considered on the basis of the synthesis of products analogous in structure to uridine or cytidine, have not been very successful; it is doubtful that the sugar in any of the products synthesized is attached to the (1) position of the pyrimidine cycle. The investigators were handicapped by the lack of methods for the synthesis of appropriately substituted pyrimidines and much of the work was of necessity done with pyrimidines containing more than one reactive grouping with the resultant tendency toward the formation of mixtures which are difficult to separate. It appears that some of the difficulties in this reaction can now be overcome by the use as an intermediate of 1,2-dihydro-2-keto-4-ethoxypyrimidine (I). It has the distinct advantage of probably being susceptible to attack by acetobromo sugars only at the (1,2) position. The synthesis of this pyrimidine is the subject of this communication.

Recently it has been shown[3] that 2,4-dialkoxy-5-bromopyrimidines can be attacked by alkali. In the particular case of 2,4-dimethoxy-5-bromopyrimidine the end product of the reaction was α-bromo-β-methylisoureidoacrylic acid, which was readily cyclicized by acid to give the lactam 1,2-dihydro-4-keto-2-methoxy-5-bromopyrimidine. By analogy one would also expect 2,4-diethoxypyrimidine to be susceptible to the action of alkali and this has been found to be the case. It is readily hydrolyzed by hot alcoholic sodium hydroxide to yield a mixture consisting of approximately equal amounts of the sodium salts of 1,2-dihydro-2-keto-4-ethoxypyrimidine (I) and 3,4-dihydro-4-keto-2-ethoxypyrimidine (II).[4] Fortunately the former, which is the more desirable product, was easily obtained in the pure



(I) (II)

state by taking advantage of the considerable solubility differential of the sodium salts in alcohol. No ring fission of the lactams occurred under the conditions to which 2,4-diethoxypyrimidine had been subjected, in contrast to the behavior of the lactam formed from 2,4-dimethoxy-5-bromopyrimidine under a much milder treatment. The direct isolation of the lactams in the former is additional evidence in favor of the mechanism previously suggested for the hydrolysis of the latter to the open chain compound. It is interesting to note that the rate of reaction of alkali with the following pyrimidines is in the order: 2,4-diethoxy-5-bromo > 2,4-diethoxy > 2,4-dimethoxy.

Both of the lactams, 1,2-dihydro-2-keto-4-ethoxy and 3,4-dihydro-4-keto-2-ethoxypyrimidine, interact with ammonia at 120° to give practically quantitative yields of cytosine and isocytosine, respectively. The rate of reaction of the former with ammonia was appreciably greater

(1) For references to this work, which has mainly been carried on by Fischer and Levene and their co-workers, see Levene and Bass, "Nucleic Acids," Chemical Catalog Co., Inc., New York, 1931, p. 149.

(2) Hilbert and Johnson, THIS JOURNAL, **52**, 4489 (1930); see also Schmidt-Nickels and Johnson, *ibid.*, **52**, 4511 (1930) and Hilbert, *ibid.*, **56**, 190 (1934), for attempts to prepare 1-glucosidothymine and 1-glucosidocytosine.

(3) Hilbert and Jansen, *ibid.*, **56**, 134 (1934).

(4) Preliminary information concerning the allocation of the ethoxy group in (I) and (II) was obtained by their different types of Wheeler-Johnson color reaction [see Hilbert and Jansen, THIS JOURNAL, **56**, 134 (1934)]. Their structures were subsequently definitely established by their conversion to cytosine and isocytosine, respectively.

than that of the latter. This reaction is a practical means for the preparation of cytosine and although the same number of steps are involved as in the method described by Hilbert and Johnson,[5] who used 2-chloro-4-aminopyrimidine as an intermediate, it appears to be superior. Perhaps more important is the application of the principle of this reaction to the synthesis of the amino type of nucleoside on which, because of the lack of methods, little work has been done.

The optical constants of the above pyrimidines are such as to permit ready differentiation of the various compounds; particularly cytosine hydrate is characterized by a small optic axial angle. The extreme birefringence necessarily arises from an approximate coplanar grouping of the atoms within a molecule and the parallel arrangement of such molecules within the crystal lattice. This is especially true of cytosine hydrate, $\alpha - \gamma = 0.337$, which moreover forms platy crystals with α normal to the extended surface parallel to which perfect cleavage obtains. Close similarity in molecular shapes of 1,2-dihydro-2-keto-4-ethoxy and 3,4-dihydro-4-keto-2-ethoxypyrimidine derivatives is indicated by these data. The extreme birefringence suggests that the arrangement of the atoms in the pyrimidine cycle is similar to that of the benzene ring.

We wish to express our appreciation to Dr. R. T. Milner and Mrs. M. S. Sherman for obtaining the microanalyses recorded.

Experimental

Preparations of 1,2-Dihydro-2-keto-4-ethoxypyrimidine and 3,4-Dihydro-4-keto-2-ethoxypyrimidine.—2,4-Diethoxypyrimidine[6] was prepared by the interaction of one mole of 2,4-dichloropyrimidine[5] and two moles of sodium ethylate in absolute alcohol. Although 2,4-diethoxypyrimidine has been prepared by one of us a number of times for other uses, the sodium salt of 1,2-dihydro-2-keto-4-ethoxypyrimidine was generally present only in traces. In one experiment, however, in which the conditions differed from the above by the use of a large excess of sodium ethylate and in a delay in the working up of the reaction mixture, a relatively large amount of the sodium salt was formed. It is thus well to emphasize that, for the preparation of 2,4-diethoxypyrimidine in good yield, these conditions be avoided.

In the study of the action of sodium hydroxide on 2,4-diethoxypyrimidine a considerable number of experiments were carried out in order to determine the optimum conditions of the reaction as well as discover a satisfactory method for the separation of the isomeric sodium salts. Ninety per cent. alcohol was found to be the best solvent. Alcohol containing a higher percentage of water was undesirable since this increased the solubility of the sodium salt of 1,2-dihydro-2-keto-4-ethoxypyrimidine to such an extent that it was difficult to isolate directly. As expected, increasing the concentration of the alcohol (95–99.8%) had an inhibiting effect on the rate of reaction.

The following procedure was found to give the best results. To a solution of 10.5 g. (0.45 mole) of sodium in 500 cc. of 90% ethyl alcohol was added 50 g. (0.30 mole) of 2,4-diethoxypyrimidine. After refluxing the clear solution for sixty hours on an oil-bath, the characteristic odor of 2,4-diethoxypyrimidine had disappeared and the reaction was completed.

Sodium Salt of 1,2-Dihydro-2-keto-4-ethoxypyrimidine.—The reaction was interrupted from time to time and the sodium salt, which separated on cooling to room temperature, was removed by filtering on a sintered glass funnel. After heating for ten hours 7.4 g. of the sodium salt was removed. The next eight hours of heating yielded 3.5 g. At the end of the reaction and upon cooling in an ice chest, 6.5 g. was removed; total yield of the crude salt 17.4 g.; further purification before conversion into 1,2-dihydro-2-keto-4-ethoxypyrimidine was unnecessary. It was purified for analysis by recrystallization from either alcohol or water; m. p. 305° (dec.).[7] It gave a Wheeler–Johnson color test similar to that of cytosine.

Anal. Calcd. for $C_6H_7O_2N_2Na$: C, 44.42; H, 4.35; N, 17.29; Na, 14.19. Found: C, 44.60; H, 4.57; N, 17.43; Na, 14.03.

The salt was dissolved in an excess of standardized sulfuric acid and titrated back with standardized sodium hydroxide using phenolphthalein as the indicator.

Neutral equivalent. Subs., 0.0585 g.: 3.47 cc. of 0.1029 *n* H_2SO_4. Calcd. for $C_6H_7ON_2 \cdot ONa$: 162.1. Found: 163.8.

The major portion of the sodium salt of 1,2-dihydro-2-keto-4-ethoxypyrimidine was recovered unchanged after boiling in sodium hydroxide solution for several hours. It was hydrolyzed by hydrochloric acid to give uracil.

Sodium Salt of 3,4-Dihydro-4-keto-2-ethoxypyrimidine.—The alcoholic filtrate from the sodium salt of 1,2-dihydro-2-keto-4-ethoxypyrimidine was concentrated practically to dryness under diminished pressure. The resulting solid, after pressing on porous plate, possessed only a faint odor of 2,4-diethoxypyrimidine and consisted essentially of the sodium salt of 3,4-dihydro-4-keto-2-ethoxypyrimidine, contaminated with some sodium ethylate. It was very soluble in cold water and cold alcohol and was best purified by recrystallizing from a solution of 80% benzene and 20% ethyl alcohol. The salt was very hygroscopic and after drying at room temperature *in vacuo* melted at 225° (dec.). It gave the same type of Wheeler–Johnson color test as isocytosine.

Anal. Calcd. for $C_6H_7O_2N_2Na$: N, 17.29; Na, 14.19. Found: N, 16.84; Na, 14.18.

1,2-Dihydro-2-keto-4-ethoxypyrimidine.—The crude sodium salt of 1,2-dihydro-2-keto-4-ethoxypyrimidine (17.4 g.) was dissolved in 50 cc. of water at 60° and made slightly acid with acetic acid. On slow cooling large elongated prisms separated. The mixture was chilled in the ice

(5) Hilbert and Johnson, THIS JOURNAL, **52**, 1152 (1930).

(6) Hilbert and Johnson, *ibid.*, **52**, 2001 (1930).

(7) All temperatures corrected.

chest and filtered; yield 11.6 g. It was recrystallized thrice from a solution of 85% ethyl acetate and 15% ethyl alcohol; m. p. 167–167.5°. 1,2-Dihydro-2-keto-4-ethoxypyrimidine was insoluble in carbon tetrachloride and soluble in water, ethyl acetate, acetone and hot benzene. It gave the Wheeler–Johnson color test for cytosine.

Anal. Calcd. for $C_6H_8O_2N_2$: C, 51.40; H, 5.76; N, 20.00; OC_2H_5, 32.15. Found: C, 51.54; H, 5.74; N, 20.23; OC_2H_5, 32.15.

3,4-Dihydro-4-keto-2-ethoxypyrimidine.—A solution of the crude salt in 50 cc. of water at 60° was converted into the lactam in the same manner as that described above. A mass of prisms separated from the cold reaction mixture; yield 22.4 g. After recrystallizing twice from 100 cc. of water it melted at 127.5–129°. A microscopic examination showed that it still contained a little of the isomer. It responded to the Wheeler–Johnson color test for isocytosine and was soluble in benzene and ethyl acetate.

Anal. Calcd. for $C_6H_8O_2N_2$: C, 51.40; H, 5.76; N, 20.00; OC_2H_5, 32.15. Found: C, 51.66; H, 5.92; N, 20.06; OC_2H_5, 32.22.

Another procedure for obtaining 1,2-dihydro-2-keto-4-ethoxypyrimidine (I) and 3,4-dihydro-4-keto-2-ethoxypyrimidine (II) in the pure state consisted of converting the mixture of the sodium salts, obtained by concentrating the original alcoholic reaction products to dryness, into the mixed isomeric lactams. They could then be separated by taking advantage of the fact that (I) was less soluble in benzene and more soluble in water than (II). This process, however, is not recommended because of the poor yields of pure products.

Synthesis of Cytosine and Isocytosine

Cytosine.—To 20 cc. of absolute ethyl alcohol, saturated with dry ammonia at 0°, was added 1.22 g. of 1,2-dihydro-2-keto-4-ethoxypyrimidine. The mixture was placed in a glass container, which fitted into a steel bomb, and heated at 120° for eight hours in an oil thermostat. On cooling large plates separated which were filtered and dried; yield 0.93 g.; m. p. 308° (dec.). The melting point was unaltered by recrystallization from water, from which it separated with one molecule of water of crystallization. Its properties and the Wheeler–Johnson color test were the same as those of cytosine.

Anal. Calcd. for $C_4H_5N_3O$: C, 43.22; H, 4.54; N, 37.84. Found: C, 43.22; H, 4.56; N, 37.61.

Isocytosine.—This was prepared from 3,4-dihydro-4-keto-2-ethoxypyrimidine in the same manner as that described for the preparation of cytosine; yield 85% of the theoretical. It was recrystallized twice from water; m. p. 275–276° (dec.). It gave the isocytosine color test and a mixed melting point with an authentic specimen was unchanged.

Anal. Calcd. for $C_4H_5N_3O$: C, 43.22; H, 4.54; N, 37.84. Found: C, 43.34; H, 4.66; N, 37.81.

When the 3,4-dihydro-4-keto-2-ethoxypyrimidine was heated with ammoniacal alcohol at 80° for 48 hours, little if any isocytosine was formed. Under the same conditions approximately 50% of 1,2-dihydro-2-keto-4-ethoxypyrimidine was converted to cytosine.

OPTICAL CONSTANTS

	α	β	γ
Cytosine hydrate	1.445	1.747	1.782
Isocytosine	1.497	1.642	1.845
3,4-Dihydro-4-keto-2-ethoxypyrimidine	1.486	1.618	1.697
1,2-Dihydro-2-keto-4-ethoxypyrimidine	1.445	1.652	1.699
Sodium salt of 3,4-dihydro-4-keto-2-ethoxypyrimidine	1.489	1.584	1.650
Sodium salt of 1,2-dihydro-2-keto-4-ethoxypyrimidine	1.483	1.572	1.621

The values were determined by the microscopic immersion method using mercury yellow light.

Summary

The action of sodium hydroxide on 2,4-diethoxypyrimidine yields a mixture of 1,2-dihydro-2-keto-4-ethoxy and 3,4-dihydro-4-keto-2-ethoxypyrimidine. These lactams are easily separated and interact with ammonia to yield cytosine and isocytosine, respectively. The use of 1,2-dihydro-2-keto-4-ethoxypyrimidine as an intermediate in the synthesis of nucleosides and the application of the ammonia reaction to the synthesis of amino nucleosides are pointed out.

Optical measurements show that the birefringences, of these pyrimidine derivatives are extremely large, which suggests close structural similarity to benzene.

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