tene values reported for A. I. V. silage. The extent to which the apparent carotene values differ from the true values is dependent on such factors as strength of acid used, lutein content of the forage, temperature of the silage and period of ensiling. While the usual method of analysis may give carotene figures that are nearly twice the true values for A. I. V. silage, too high results are also obtained in the analysis of silages made by normal fermentation processes. The need for a more reliable method of determining carotene in these silages is obvious.

Chromatographic data on butter indicate the presence of the same pigments as those found in silage. Although the amount of these new pigments has not been determined, their presence points to the need of more information regarding the value of acid-preserved silages for increasing the vitamin A potency of milk.

Summary

Five new carotenoids, designated as pigments A, B, C, E, and F, have been obtained from alfalfa silage and from acidified fresh alfalfa. They were not present in untreated forage. Dilute solutions of A, B, and E in benzine were greenish-yellow and those of C and F were reddish.

The pigments were partially fractionated by their phasic distribution between benzine and 85% ethanol; A, B, and C were epiphasic, E and F were hypophasic. Quantitative separation was effected by use of the magnesium oxide chromatogram and elution with benzine–alcohol mixtures. The bands of A, B, and C formed below that of lutein; those of E and F above it. The pigments were eluted from the column in the alphabetical order given by gradually increasing the percentage of alcohol in the solvent. Spectral absorption of the pigments was markedly less valuable for differentiation than chromatographic behavior. Absorption curves of A, B, and E were essentially the same as those of lutein; those of C and F showed no well-defined maxima.

A high order of solubility in the usual carotenoid solvents was shown by A, B, and C. Attempts to effect their crystallization were not successful.

A, B, and E were produced from lutein in large amounts by treatment with either 0.025 hydrochloric or sulfuric acid, and in smaller amounts with 0.025 *N* lactic and acetic acids. The stronger acids favored the production of B; weaker acids the production of A. The origin of C and F was not clearly established.

Inasmuch as the usual methods of carotene analysis fail to differentiate between carotene and pigments A, B, and C, values obtained by these methods on silages, especially those prepared with mineral acids, are obviously too high.

When fed to vitamin A deficient rats, A and B exhibited no biological activity. Both pigments were found in butters produced by cows on A. I. V. silage.

MADISON, WIS. RECEIVED AUGUST 27, 1938

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY]

Researches on Pyrimidines. CLIX. Synthesis of 6-Benzyl- and 5-Benzyluracils[1]

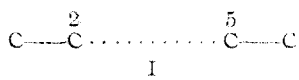
BY TREAT B. JOHNSON AND JOSEPH C. AMBELANG[2]

One conclusion predictable from an examination of the skeleton structure of the pyrimidine cycle as represented by formula II, is the existence of two interdependent zones of chemical activity which envelop the pyrimidine molecule. Each of these zones is characterized by its encompassment of a specific organic structure. Zone "A" includes the unsaturated amidine portion of the pyrimidine cycle, while zone "B" incloses the cyclic allyl structure of the molecule. Any alteration or tautomeric change in the specific, unsaturated structure embraced by one zone necessarily leads to a corresponding change in the constitution of the grouping in the other, and also its chemical reactivity. In other words, we are dealing with a dual system of reactions in our study and development of pyrimidine chemistry and if tautomeric structures be accepted for these unsaturated cyclic groupings in the different zones, as theory provides, positions 1 and 3 included in zone "A" and positions 4 and 6 in zone "B," respectively, are identical.

(1) Constructed from a portion of a dissertation presented by Joseph C. Ambelang, in June, 1938, to the Graduate Faculty of Yale University in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

(2) Sterling Professorship of Chemistry Research Assistant 1938–1939.

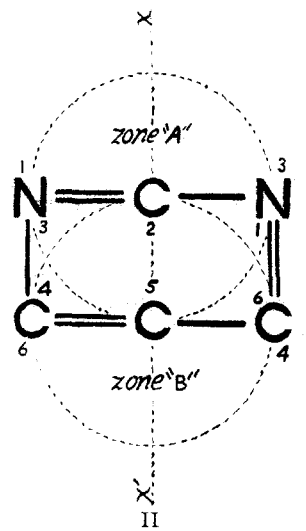
These conclusions lead to the recognition of an unique symmetry of the pyrimidine cycle characterized by an axis of symmetry (x-x') passing through the two permanent numerical positions of the ring, namely, 2 and 5. It is an interesting fact that in the pyrimidine portion of the constitutional formula of the vitamin B_1 molecule (thiamine), this axis of symmetry (x-x') prevails, and constitutes a chain of four carbon atoms as represented in formula I. This chain includes the two permanent positions of the pyrimidine moiety (2 and 5).



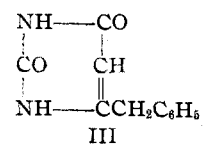
I

It might be predicted, therefore, on the basis of this knowledge and reasoning that the reactivity of a substituent grouping in position 5 of the pyrimidine ring would be dependent on, or influenced in some degree by, the character of the group or radical attached to the carbon in position 2. Such a postulation has already been supported by experimental data. In fact, we already have a knowledge of chemical reactions applicable to pyrimidine compounds, which are specific for the two zones of influence, "A" and "B," and permit us to confine our attack to either zone independently, without involving the grouping characteristic of the other. This dual system of reactions has also been revealed by researches on the chemistry of purines.[3]

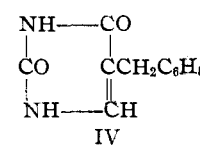
In the light of our previous knowledge from researches in this Laboratory, and under guidance of the above speculations, it became of great interest to the authors to learn more about structural differences influencing the degree of reactivity of organic groupings functioning in the pyrimidine ring and occupying particularly positions 5 and 6 or 5 and 4 in zone "B" (formula II) of the pyrimidine molecule. It was, therefore, decided to incorporate a new series into our researches, and to synthesize first for study the two isomeric benzyluracils, namely, 6-benzyluracil and 5-benzyluracil as represented by formulas III and IV, respectively. Neither compound has been described previously in the literature. The methods of synthesis and a description of several intermediates and new derivatives are presented in the experimental part of this paper. In a future publication the authors will discuss some interesting results obtained by catalytic reduction of these two isomeric uracil derivatives III and IV.



II



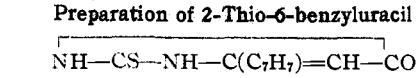
III



IV

Experimental Part

Preparation of 2-Thio-6-benzyluracil



Method 1.—Ten grams (0.05 mole) of ethyl phenylacetylacetate (ethyl γ-phenylacetoacetate) was mixed intimately with 3.7 g. (0.05 mole) of thiourea and 10 drops of concentrated hydrochloric acid. After standing for one week in a stoppered flask, the suspension was transferred to an open dish and dried *in vacuo* over concentrated sulfuric acid. The desiccated product was then refluxed for two hours with 9 g. of potassium hydroxide in 33 g. of absolute alcohol and the solution finally concentrated at 100°, until encrustation appeared. On acidifying with hydrochloric acid this pyrimidine separated as an oil which soon solidified. It was purified by crystallization from 95% alcohol and melted at 222–223°. The yield was only 9% of the theoretical. This method of synthesis has been applied previously by Sonn and Litten[4] but with no mention of yields obtained. They assigned a melting point of 216–219° to the compound. No attempt was made by them to desulfurize the pyrimidine. This reaction very probably involves first the formation of the intermediate ureide $C_6H_5CH_2COCH_2CONHCSNH_2$, which is then closed up to the pyrimidine by the action of the alkali.

Method 2.—Thiourea (52 g.) and ethyl phenylacetylacetate (140 g.) were dissolved in a cooled solution of sodium ethylate (0.68 mole of sodium in 495 g. of absolute alcohol) and the mixture heated on a steam-bath for four hours. The excess of alcohol was then removed by evaporation and the residue dissolved in 125 cc. of water. Acidification of the cooled solution with hydrochloric acid led to the precipitation of the desired thiopyrimidine in a

(3) Consult Gilman's "Organic Chemistry," Vol. II, Chapter 11, "Chemistry of Pyrimidines, Purines and Nucleic Acids," by T. B. Johnson, John Wiley and Sons, Inc., New York, N. Y., 1938.

(4) Sonn and Litten, *Ber.*, **66**, 1518 (1933).

yield of 37%. It was purified by crystallization from 95% alcohol and melted at 222–223°. This pyrimidine is soluble in aqueous ammonia and hot glacial acetic acid; moderately soluble in hot alcohol and acetone, slightly soluble in water and insoluble in benzene, ether, chloroform, and carbon bisulfide.

Anal. Calcd. for $C_{11}H_{10}ON_2S$: N, 12.84. Found: N, 12.85, 12.95.

$NH—C(SC_2H_5)=N—C(C_7H_7)=CH—CO$, **2-Ethyl-thio-6-benzyl-4-oxypyrimidine.**—A solution of 9 g. of pseudoethylisothiourea hydrobromide in 10 cc. of water was mixed with 10 g. of ethyl phenylacetylacetate, and to the mixture was added 5.5 g. of potassium hydroxide dissolved in 8 cc. of water. After standing at ordinary temperature overnight the solution was acidified with acetic acid when the above mercaptopyrimidine separated in a yield of 28%. It was purified by crystallization from methyl alcohol and melted at 128–129°. This compound is soluble in ethyl alcohol, glacial acetic acid, acetone, benzene, chloroform and boiling carbon bisulfide; moderately soluble in ether, and insoluble in aqueous ammonia.

Anal. Calcd. for $C_{13}H_{14}ON_2S$: N, 11.38. Found: N, 11.52, 11.56.

6-Benzyluracil, $NH—CO—NH—C(C_7H_7)=CH—CO$. —This pyrimidine is prepared easily as follows: (1) by digestion of 2-thio-6-benzyluracil with 10% chloroacetic acid; and (2) by hydrolysis of the corresponding 2-ethylthio derivative with hydrochloric acid. The pyrimidine was purified by crystallization from glacial acetic acid or 95% alcohol and melted at 261–262°. It is soluble in warm acetic acid and in aqueous ammonia; moderately soluble in acetone, hot alcohol; slightly soluble in hot water, and insoluble in ether, benzene, chloroform and carbon bisulfide.

Anal. Calcd. for $C_{11}H_{10}O_2N_2$: C, 65.32; H, 4.98; N, 13.86. Found: C, 65.16, 65.34; H, 4.98, 4.90; N, 13.80, 13.85.

$NH—CO—NH—C(C_7H_7)=CBr—CO$, **6-Benzyl-5-bromouracil.**—6-Benzyluracil is attacked by bromine in glacial acetic acid at 40–50° with substitution in the pyrimidine ring giving this monobromo substitution product. The compound was purified easily by crystallization from glacial acetic acid and melted at 230–232°. The pyrimidine is soluble in warm glacial acetic acid, acetone, and aqueous ammonia, and insoluble in water, ether, benzene, and chloroform.

Anal. Calcd. for $C_{11}H_9O_2N_2Br$: N, 9.97. Found: N, 9.97, 10.00.

That the bromine is substituted in the pyrimidine ring and not in the aromatic nucleus of the benzyl group substituted in position-6 was shown as follows: 1 g. of the bromopyrimidine was oxidized by refluxing it in hot water with barium permanganate (0.0059 mole) and barium hydroxide. After complete discoloration of the permanganate the alkaline solution was filtered, and then concentrated to a volume of 20 cc. and cooled. On acidifying with hydrochloric acid colorless benzoic acid deposited and melted, after crystallization from hot water, at 121–122°.

Chlorination of 6-Benzyluracil in Methyl Alcohol

$NH—CO—NH—C(OCH_3)(C_7H_7)—C(Cl_2)—CO$,[5] **Formation of 2,4-Diketo-6-benzyl-6-methoxy-5,5-dichlorohexahydropyrimidine.**—Chlorine gas was passed into an ice-cooled suspension of 0.5 g. of 6-benzyluracil in 30 cc. of methyl alcohol until the pyrimidine dissolved. The cold reaction mixture was then exposed to a blast of air to remove the alcohol and excess of chlorine and the residue allowed to dry. We obtained 0.6 g. of the above hexahydropyrimidine, which was purified by crystallization from methyl alcohol, or a mixture of chloroform and carbon tetrachloride. It melted at 157–159° with decomposition. This pyrimidine is soluble in glacial acetic acid, acetone, ether, and chloroform; and slightly soluble in warm benzene, hot water, and insoluble in petroleum ether.

Anal. Calcd. for $C_{12}H_{12}O_3N_2Cl_2$: N, 9.24. Found: N, 9.20, 9.17.

This pyrimidine is not changed by exposure to pyridine. Two grams was dissolved in 20 cc. of pyridine and the solution allowed to stand for one week. It had assumed a greenish fluorescence. After volatilizing the pyridine a sirup was obtained which deposited crystals on treatment with water. This solid was identified as the original hexahydropyrimidine, and after crystallization from dilute methyl alcohol melted at 160–162°.

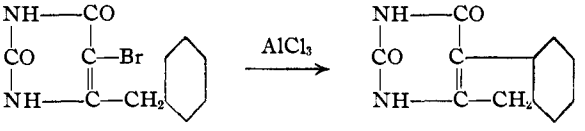
Anal. Calcd. for $C_{12}H_{12}O_3N_2Cl_2$: C, 47.54; H, 3.99; N, 9.24. Found: C 47.62; H, 3.64; N, 9.57.

$NH—CO—NH—C(C_7H_7)=C(Cl)—CO$, **Action of Hydrobromic Acid on the above Hexahydropyrimidine. Formation of 6-Benzyl-5-chlorouracil.**—This change is brought about by digesting the hexahydropyrimidine (1 g.) in glacial acetic acid (25 cc.) with constant boiling hydrobromic acid (2 cc.). After boiling for two hours the acid solution was poured into water when the above chlorouracil derivative separated. It was purified by crystallization from dilute acetic acid and melted at 266–267°.

Anal. Calcd. for $C_{11}H_9O_2N_2Cl$: N, 11.84. Found: N, 12.02.

This same chloropyrimidine is formed in poor yield by passing chlorine gas into a suspension of 6-benzyluracil in 10% acetic acid.

Attempted Cyclization of 6-Benzyl-5-bromouracil:



one and five-tenths grams of 6-benzyl-5-bromouracil and 1.5 g. of aluminum chloride were dissolved by stirring in 50 cc. of nitrobenzene. After standing for four hours at room temperature and two hours at 50° the solution was then poured into ice water. The nitrobenzene was removed by steam distillation and the insoluble residue left behind purified by crystallization from dilute acetic acid. We recovered 1.4 g. of the unaltered bromopyrimidine melting at 232–233°.

Preparation of $C_6H_5CH_2CH_2COOC_2H_5$, Ethyl Hydrocinnamate.—A quantitative yield of this ester was pre-

(5) See Johnson and Sprague, THIS JOURNAL, **60**, 1622 (1938).

pared for our research by reduction of ethyl cinnamate in the presence of Raney nickel catalyst.[6] Eighty grams of the cinnamic ester was diluted with ethyl alcohol to a volume of 100 cc., the mixture transferred to a copper liner of an autoclave, and after addition of 3 g. of the nickel catalyst was subjected to 120 atmospheres of hydrogen at room temperature. After twenty minutes of shaking the observed drop in pressure corresponded to the absorption of 0.5 mole of hydrogen. The purified ester boiled at 104–105° at 3 mm. and at 245° under atmospheric pressure.

Formation of the Sodium Salt of Ethyl-formyl 3-Phenylpropionate.[7]—A mixture of 178 g. (1 mole) of ethyl hydrocinnamate and 81 g. (1.1 mole) of ethyl formate was added slowly over a period of twenty hours to 23 g. of sodium wire suspended in 500 cc. of anhydrous ether. The excess of ether was then distilled off *in vacuo* at room temperature and the crude salt of the resulting formyl derivative used for the following reaction.

Formation of $\overset{\frown}{\text{NH—CS—NH—CH=C(C}_7\text{H}_7\text{)—CO}}$, **2-Thio-5-benzyluracil.**—The crude sodium salt from the preceding preparation and 50 g. of pulverized thiourea were added to 350 g. of absolute alcohol and the mixture then refluxed at 100° for four hours. The excess of alcohol was then evaporated, the crude reaction product dissolved in 500 cc. of cold water and the aqueous solution acidified with hydrochloric acid. Sixty-eight grams of the above pyrimidine separated. It was purified by crystallization from ethyl alcohol and melted at 210–211°. This pyrimidine is soluble in aqueous ammonia, glacial acetic acid, and acetone, moderately soluble in hot ethyl alcohol; slightly soluble in hot water and insoluble in ether, chloroform and benzene.

Anal. Calcd. for $C_{11}H_{10}ON_2S$: N, 12.84. Found: N, 12.83, 12.76.

5-Benzyluracil, $\overset{\frown}{\text{NH—CO—NH—CH—C(C}_7\text{H}_7\text{)—CO}}_2$.—This pyrimidine is formed by digesting the above 2-thiopyrimidine with 10% chloroacetic acid. It was purified by crystallization from glacial acetic acid and melted at 294–295°. The pyrimidine is soluble in aqueous ammonia, slightly soluble in dioxane, acetone, ethyl alcohol and hot water, and insoluble in chloroform, ether and benzene.

Anal. Calcd. for $C_{11}H_{10}O_2N_2$: C, 65.32, H, 4.98; N, 13.86. Found: C, 65.18, 65.61; H, 4.90, 4.95; N, 13.77, 13.82.

$\overset{\frown}{\text{NH—CO—NH—CH(OCH}_3\text{)—C(Cl)(C}_7\text{H}_7\text{)—CO}}$, **2,4-Diketo-6-methoxy-5-benzyl-5-chlorohexahydropyrimidine.**—Chlorine gas was bubbled into an ice-cooled suspension of 5-benzyluracil (1 g.) in methyl alcohol (50 cc.) until the pyrimidine practically dissolved. The alcohol solution was then exposed to a blast of air to remove the excess of solvent and chlorine gas when we obtained 1 g. of this hexahydropyrimidine. It was purified by crystallization from methyl alcohol. This pyrimidine showed a characteristic double melting point, melting first at 217–218°, then solidifying and melting finally at 232–234°. It was soluble in acetone and glacial acetic acid, and insoluble in ether and benzene.

Anal. Calcd. for $C_{12}H_{13}O_3N_2Cl$: N, 10.34. Found: N, 10.59, 10.70.

When this pyrimidine was digested with hydrobromic acid in glacial acetic acid it was converted into 5-benzyluracil melting at 292–294°.

Summary

1. 2-Thio-6-benzyluracil and 2-thio-5-benzyluracil have been synthesized.

2. These two isomeric pyrimidines are desulfurized by digestion with chloroacetic acid giving smoothly 6-benzyluracil and 5-benzyluracil.

3. 6-Benzyluracil is attacked by bromine in glacial acetic acid solution with formation of the corresponding 5-bromo derivative.

4. Both 6-benzyl- and 5-benzyluracil are attacked normally by chlorine in wood alcohol giving hexahydropyrimidines. The chlorination is confined entirely to zone "B" of the pyrimidine molecule.

NEW HAVEN, CONN. RECEIVED OCTOBER 4, 1938

(6) Adkins, *Ind. Eng. Chem., Anal. Ed.*, **4**, 342 (1932); Covert and Adkins, THIS JOURNAL, **54**, 4116 (1932).

(7) Phalnikar and Nargund, *J. Univ. Bombay*, **4**, 106 (1935); *Chem. Zentr.*, **107**, 1, 4556 (1936).