

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, YALE UNIVERSITY, AND THE FERTILIZER AND FIXED NITROGEN DIVISION OF THE BUREAU OF CHEMISTRY AND SOILS, U. S. DEPARTMENT OF AGRICULTURE]

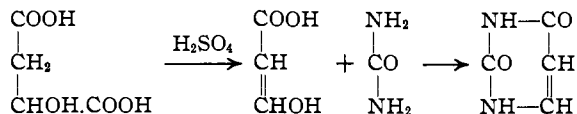
**PREPARATIONS OF URACIL-4-ACETIC AND OROTIC ACIDS.  
OROTIC ACID AS THE POSSIBLE INTERMEDIATE IN THE  
SYNTHESIS OF PURINES FROM HISTIDINE**

BY GUIDO E. HILBERT

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The excellent method of Davidson and Baudisch<sup>1</sup> for the synthesis of uracil consists in treating malic acid and urea with fuming sulfuric acid.



This synthesis is of considerable significance since it allows an easy mode of entrance into the pyrimidine series of compounds. With uracil as a starting material, many pyrimidines substituted in the 1, 2, 3, 5 and 6 positions can easily be made. Hilbert and Johnson<sup>2</sup> have taken advantage of this procedure and synthesized cytosine by a new method. More recently<sup>3</sup> it has been demonstrated that thymine can also be prepared directly from uracil in a manner analogous to Kircher's<sup>4</sup> synthesis of 4,5-dimethyluracil from 4-methyluracil. The reaction was carried out by treating uracil with formaldehyde and hydrochloric acid. The resulting uracil-5-methyl chloride was reduced with tin and hydrochloric acid and yielded thymine. Since the method of Davidson and Baudisch for the preparation of uracil is far superior to the older one of Wheeler and Merriam,<sup>5</sup> it was of interest to determine if it could also be applied for the preparation of other pyrimidines.

**The Syntheses of Uracil-4-Acetic and Orotic Acids.**—It is reasonable to expect that other  $\alpha$ -hydroxy- $\alpha,\beta$ -dicarboxylic acids will also break down with fuming sulfuric acid in a manner typical of  $\alpha$ -hydroxy acids to yield products which will combine with urea to form cyclic ureides. The easily available citric acid has such a configuration and should yield, with urea, uracil-4-acetic acid, a view which has now been confirmed.

As is well known, citric acid in the presence of fuming sulfuric acid breaks down to form acetonedicarboxylic acid. It was either this or more probably the enol form which condensed with the urea to form uracil-4-acetic acid. The structure of the pyrimidine was proved beyond reasonable

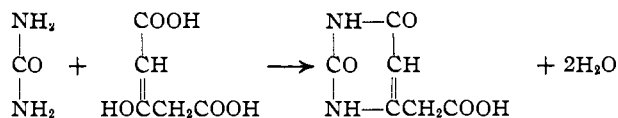
<sup>1</sup> Davidson and Baudisch, *THIS JOURNAL*, **48**, 2379 (1926).

<sup>2</sup> Hilbert and Johnson, *ibid.*, **52**, 1152 (1930).

<sup>3</sup> Unpublished results.

<sup>4</sup> Kircher, *Ann.*, **385**, 293 (1911).

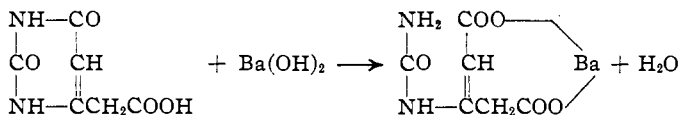
<sup>5</sup> Wheeler and Merriam, *Am. Chem. J.*, **29**, 478 (1903).



doubt since the properties of it and a number of derivatives were the same as those recorded by Wheeler and Liddle.<sup>6</sup> The method is recommended the best for the preparation of this substance.

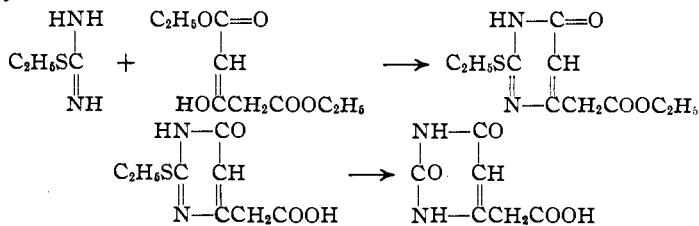
The limitations of this method for the synthesis of pyrimidines are well illustrated by the failure to isolate any products when ethyl acetoacetate and ethyl sodiumformylpropionate were used (for other examples see experimental portion) in place of malic or citric acids. Theoretically, one would have expected the synthesis of 4-methyluracil and thymine, respectively. The fact that these latter experiments were unsuccessful seems surprising, since these compounds are so similar in structure to formylacetic and acetonedicarboxylic acids which react so smoothly.

The attempt to decarboxylate uracil-4-acetic acid by treatment with barium hydroxide solution was unsuccessful, since the pyrimidine ring was ruptured, yielding the insoluble barium salt of  $\beta$ -carbamidoglutaconic acid. This was readily decomposed by hydrochloric acid and regenerated uracil-4-acetic acid, indicating that the salt probably was the *cis*<sup>7</sup> modification. Although the rupture of a pyrimidine having a double bond in the 4,5 posi-



tion was odd, it was not new. Müller<sup>8</sup> condensed ethyl oxaloacetate with methylurea and obtained 3-methyluracil-4-ethylcarboxylate. When the sodium salt of this substance was treated with hydrochloric acid a  $\beta$ -methylcarbamidoacrylic acid,  $-\text{H}_2\text{NCON}(\text{CH}_3)\text{CH}=\text{CHCOOH}$ , was obtained. Neither acids nor alkali converted this substance into 3-methyluracil.

<sup>6</sup> Wheeler and Liddle [THIS JOURNAL, 30, 1156 (1908)] previously synthesized uracil-4-acetic acid. Acetonedithylcarboxylate was condensed with 2-ethylpseudothiourea in alkaline solution and yielded 2-ethylmercapto-6-oxypyrimidine-4-ethylacetate. This was saponified and the resulting mercapto acid digested with concentrated hydrochloric acid to form uracil-4-acetic acid.



<sup>7</sup> *Cis* with respect to the newly formed carboxyl and ureido groups.

<sup>8</sup> Müller, *J. prakt. Chem.*, 56, 498 (1897).

Johnson and Shepard<sup>9</sup> have reported a similar case. They isolated a by-product in the reaction of 2-thiouracil with sodium ethylate and ethyl chloroacetate which they believed to be  $\beta$ -thiocarbamidoacrylic acid, that was unaffected by acids. The fact that these acrylic acid derivatives did not yield pyrimidines on acid treatment was interpreted as indicating that they had the *trans* configuration.<sup>10</sup>

In order to obtain further information on the ease of hydrogenation of the 4,5 double bond in keto pyrimidines, uracil-4-ethylacetate and 3-methyluracil were hydrogenated using Adams and Shriner's platinum oxide as catalyst. The saturation of the double bond in both cases was found to be very slow.

Biscaro and Belloni<sup>11</sup> in 1905 isolated orotic acid from milk. Recently Bachstetz<sup>12</sup> showed that it was identical with uracil-4-carboxylic acid, which has been synthesized a number of times. Müller<sup>13</sup> prepared the ethyl ester by condensing urea with ethyl oxaloacetate in acetic acid. This was subsequently saponified to the acid by Wheeler.<sup>14</sup>

Later Behrend and Struve<sup>15</sup> synthesized the acid by the oxidation of 4-methyluracil in potassium hydroxide solution with potassium ferricyanide. More recently Johnson and Schroeder<sup>16</sup> have prepared it by the oxidation of uracil-4-aldehyde with chromic acid. Of these methods, that of Behrend and Struve appears to be the best since 4-methyluracil is readily available and the oxidation can easily be carried out, resulting in a good yield of orotic acid. As uracil-4-acetic acid can now be made as easily as 4-methyluracil, its smooth oxidation would be expected to lead to another convenient synthesis of orotic acid. The action of potassium ferricyanide on uracil-4-acetic acid in alkaline solution was studied. The oxidation did not go as smoothly as that of 4-methyluracil; the yield of orotic acid was somewhat less. It is possible that the ease in rupture of the ring was partly responsible for the decreased yield. Under the conditions operated, the method, as yet, cannot be recommended as being better than that of the oxidation of 4-methyl-uracil.

<sup>9</sup> Johnson and Shepard, *Am. Chem. J.*, **46**, 345 (1911).

<sup>10</sup> A number of examples have been reported in which the acrylic acid derivative was converted to a pyrimidine by treatment with acid. Behrend, *Ann.*, **229**, 8 (1885), noted that a carbamidocrotonic ester was converted to 4-methyluracil and Johnson and Clapp [*Am. Chem. J.*, **32**, 130 (1904)] found that  $\alpha$ -methyl- $\beta$ -guanidinoacrylic acid was changed to 2-amino-5-methyl-6-oxypyrimidine. In these cases the acid was assigned the *cis* structure.

<sup>11</sup> Biscaro and Belloni, *Estratto Annuario Soc. Chimica di Milano*, **11**, 1 (1905): *Chem. Centr.*, II, 63 (1905).

<sup>12</sup> Bachstetz, *Ber.*, **63**, 1000 (1930); *Giorn. chim. ind. applicata*, **12**, 174 (1930).

<sup>13</sup> Müller, *J. prakt. Chem.*, **56**, 488 (1897).

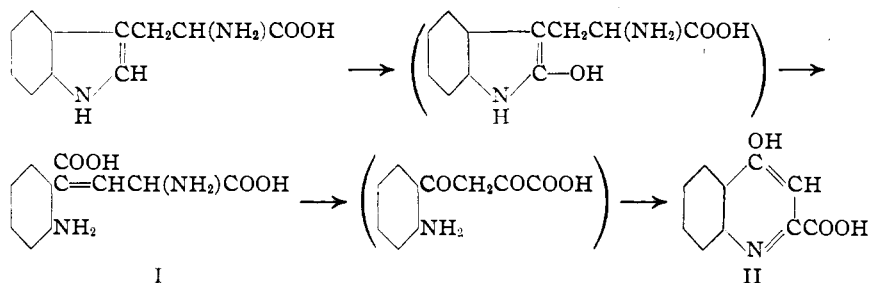
<sup>14</sup> Wheeler, *Am. Chem. J.*, **38**, 358 (1907).

<sup>15</sup> Behrend and Strive, *Ann.*, **378**, 153 (1910).

<sup>16</sup> Johnson and Schroeder, *THIS JOURNAL*, **53**, 1989 (1931).

**Orotic Acid as the Possible Intermediate in the Synthesis of Purines from Histidine.**—The occurrence of orotic acid in milk arouses interest as to the possible mechanism of its formation and the role that it may play in metabolism.<sup>17</sup> Because of the carbon grouping in position 4 it seems improbable that it is a degradation product of either purines or the pyrimidine nucleosides. The possibility that uracil might be the precursor of orotic acid is also unattractive. The conversion of uracil into orotic acid requires considerable energy as, in general, decarboxylations involve a decrease in free energy. One might thus expect that the synthesis of this product at the expense of considerable energy would necessitate its playing a rather important role in the organism regarding which, as yet, there is a lack of any positive information. A more plausible explanation is that which is suggested by the relationship of kynurenic acid to tryptophane.

Kynurenic acid (II) was first isolated from the urine of dogs by Liebig. Ellinger<sup>18</sup> in 1904 definitely proved that it was a metabolic product of tryptophane. A number of mechanisms<sup>19</sup> for the formation of kynurenic acid have been suggested; however, that proposed by Kotake and co-workers<sup>20</sup> is the most probable one. They have been able to isolate an intermediate—kynurenine (I)—that was converted either by an organism or barium hydroxide solution to kynurenic acid. They have offered the following scheme for the conversion of tryptophane to kynurenic acid.



An inspection of the structures of tryptophane and histidine shows that they have in common the grouping  $\begin{array}{c} \text{---CCH}_2\text{CH}(\text{NH}_2)\text{COOH} \\ || \\ \text{---NH---CH} \end{array}$  which, in the case of tryptophane, is directly involved in the conversion of the indole

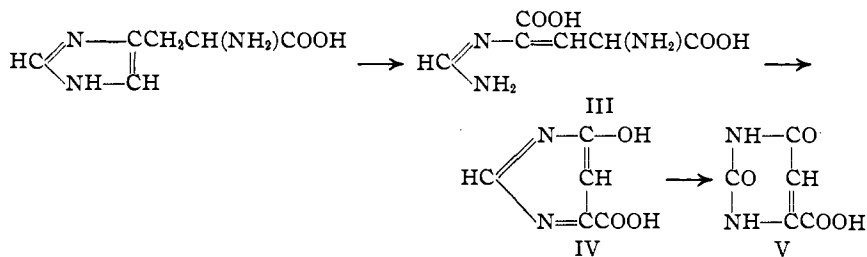
<sup>17</sup> It is possible that the orotic acid comes directly from the diet, that is, it might be a plant product. It has not as yet, however, been shown to occur in plants.

<sup>18</sup> Ellinger, *Z. physiol. Chem.*, **43**, 325 (1904).

<sup>19</sup> Ellinger and Matsuoka. *ibid.*, **109**, 259 (1920); Robson, *Biochem. J.*, **22**, 1157 (1928).

<sup>20</sup> Kotake and Iwao, *Z. physiol. Chem.*, **195**, 139 (1931); Kotake and Kiyokawa, *ibid.*, **195**, 147 (1931); Kotake and Shichiri, *ibid.*, **195**, 152 (1931); Kotake, *ibid.*, **195**, 158 (1931); Kotake and Ichihara, *ibid.*, **195**, 171 (1931); Shichiri and Kiyokawa, *ibid.*, **195**, 166 (1931).

to the quinoline compound. The postulation of an analogous transformation with histidine results in the formation of a pyrimidine ring.



The intermediate (III) is the analog of kynurenine and (IV) of kynurenic acid. An additional oxidation of the 2 position of either the imidazole or the pyrimidine yields the 2-oxypyrimidine-otic acid (V). That such an oxidation is possible is indicated by the well-known conversion of purines in the organism to uric acid.

This set of equations represents not only the idea that histidine is the precursor of orotic acid but also suggests a mechanism of purine synthesis from histidine. The ease with which purines can be synthesized from pyrimidines *in vitro* has led a number of investigators to suggest that the latter may be the precursors of the former. The conversion of orotic acid to purines is quite easy to picture, since every step but one has already been reported in the literature. The decarboxylation of orotic acid yields uracil. This was converted by Wheeler and Johnson<sup>21</sup> into isodialuric acid, with which, upon heating with urea, Behrend and Roosen<sup>22</sup> synthesized uric acid.

In addition to the striking analogy that it bears to the intermediary metabolism of tryptophane, the above speculations are supported by the work of Ackroyd and Hopkins,<sup>23</sup> Rose and Cook,<sup>24</sup> and Stewart.<sup>25</sup> In general they found that in the absence of histidine in the diet, the allantoin content of the urine decreased. As allantoin is usually accounted for by the oxidation of uric acid, they interpreted this as indicating that histidine was the precursor of the purines, and hence essential for nuclear synthesis. A number of other investigators, however, have reported negative results. Mitchell and Hamilton<sup>26</sup> aptly summarized the status of the work in the following quotation, "Many negative results have been reported from experiments designed to establish a relationship between

<sup>21</sup> Wheeler and Johnson, *J. Biol. Chem.*, **3**, 183 (1907).

<sup>22</sup> Behrend and Roosen, *Ann.*, **251**, 235 (1888).

<sup>23</sup> Ackroyd and Hopkins, *Biochem. J.*, **10**, 551 (1916).

<sup>24</sup> Rose and Cook, *J. Biol. Chem.*, **64**, 325 (1925).

<sup>25</sup> Stewart, *Biochem. J.*, **19**, 1101 (1925).

<sup>26</sup> Mitchell and Hamilton, "The Biochemistry of the Amino Acids," New York, 1929, p. 360.

histidine and purines in animal metabolism but such results cannot be considered as invalidating the positive indications that have been observed."

In the absence of any experimental work on a relation between histidine and orotic acid, it must be emphasized that the above discussion is of a speculative nature but has been offered as it might prove of value in the elucidation of the intermediary metabolism of histidine and the anabolism of pyrimidines and purines.

I am greatly indebted to Dr. Reid T. Milner and Mrs. Mildred Sherman for carrying out the microanalyses recorded in this paper.

### Experimental

In the course of various investigations on pyrimidines large quantities of uracil were required, so attempts were made to improve upon the method of Davidson and Baudisch. The modification consisted of reversing the procedure of Davidson and Baudisch with regard to the addition of urea and malic acid to fuming sulfuric acid, and heating to 85°. This was an improvement inasmuch as it cut down to one-half the time devoted to the experiment and also increased the yield by 20%.

**Uracil-4-acetic Acid.**—A large number of experiments was carried out under different experimental conditions. The success of the experiment was dependent upon the amount of sulfur trioxide in the fuming sulfuric acid and the temperature to which the reaction mixture was heated. Much variation of these two factors decreased the yield enormously. The following procedure was found to give the best results. To a 3-liter three-necked flask equipped with a stirrer was added 400 cc. of fuming sulfuric acid containing 15% sulfur trioxide. The acid was cooled to  $-10^{\circ}$  and 80 g. of finely ground citric acid added at such a rate that the temperature did not rise above  $5^{\circ}$ ; this usually required about fifteen minutes. If the temperature rose to  $10^{\circ}$ , an appreciable amount of the acetone dicarboxylic acid was formed. Since this reaction took place with the evolution of heat, considerable trouble was experienced in attempting to lower the temperature. The remainder of the citric acid, 80 g., and 100 g. of finely ground urea were then quickly added; a vigorous reaction took place with considerable foaming and the evolution of large quantities of carbon monoxide and carbon dioxide. The temperature of the reaction mixture immediately rose to  $55-60^{\circ}$  and then with the help of a burner was brought to  $75^{\circ}$  and held there for thirty minutes. The straw colored reaction mixture was cooled and poured on 1200 g. of ice. On inoculation, uracil-4-acetic acid started to separate out at once and was completely separated on standing in the ice box for two days, yield of crude dry product 42-43 g. It was decolorized with bone black and re-crystallized from water. When a hot water solution was rapidly cooled it separated in the anhydrous condition (fine needles); on very slow cooling, as the monohydrate (stout prisms); it did not melt at  $300^{\circ}$ .

*Anal.* Calcd. for  $C_8H_8N_2O_4 \cdot H_2O$ :  $H_2O$ , 9.58. Found:  $H_2O$ , 9.61. Calcd. for  $C_8H_8N_2O_4$ : C, 42.34; H, 3.56; N, 16.47. Found: C, 42.46, 42.40; H, 3.63, 3.80; N, 16.44.

The conditions (acidic) of the above reaction preclude the preparation of the analogous thiopyrimidines by the substitution of urea by thiourea. The preparation of 4-methyluracil and thymine from ethyl acetoacetate and ethyl sodiumformylpropionate, respectively, was unsuccessful; the conditions of the experiment were widely varied with respect to the strength of the sulfuric acid and the temperature at which the reaction was carried out. It was also impossible to synthesize quinolones by heating aniline with either malic or citric acid in fuming sulfuric acid.

**Uracil-4-methylacetate.**—Large blocky plates, m. p. 220°.

*Anal.* Calcd. for  $C_7H_8N_2O_4$ : N, 15.22. Found: N, 15.41, 15.23.

**Uracil-4-ethylacetate.**—It separated from a 50% alcohol-water solution as colorless plates containing one molecule of water of crystallization and melting at 191–192° (Wheeler and Liddle reported 187–188°).

*Anal.* Calcd. for  $C_8H_{12}N_2O_5$ :  $H_2O$ , 8.33. Found:  $H_2O$ , 8.55. Calcd. for  $C_8H_{10}N_2O_4$ : N, 14.14. Found: N, 14.24, 14.27.

**The Action of Barium Hydroxide on Uracil-4-acetic Acid.**—When a solution of uracil-4-acetic acid was mixed with a solution of barium hydroxide, there was no immediate precipitate. However, if this was warmed or allowed to stand for some time, an insoluble barium salt precipitated. This behavior would seem to indicate that the pyrimidine has been altered. The barium salt was best obtained by the following procedure. Two grams of uracil-4-acetic acid was dissolved in 25 cc. of hot water and treated with a hot solution of 15 g. of barium hydroxide hydrate in 75 cc. of water. A clear solution resulted which, on standing overnight, precipitated star-like clusters of prisms, yield 2.9 g. It was insoluble in boiling water. The analysis agreed best with that of the barium salt of  $\beta$ -carbamidoglutamic acid.

*Anal.* Calcd. for  $C_6H_5O_5N_2Ba$ : Ba, 42.47. Found: Ba, 42.25.

It dissolved readily in hot dilute hydrochloric acid and on cooling deposited uracil-4-acetic acid.

**Orotic Acid.**—A solution of 7.4 g. of uracil-4-acetic acid in 475 cc. of water was treated with 27.5 g. of potassium hydroxide and 78 g. of potassium ferricyanide. The reaction mixture was a dark reddish-brown, characteristic of this type of oxidation. It was allowed to stand at room temperature for twenty-two days; there was a slight decrease in the color of the solution at the end of this time. On acidification with acetic acid, considerable carbon dioxide was evolved and the brown potassium salt separated. This was allowed to stand for a few hours and filtered. The precipitate was recrystallized from boiling water and separated as star-like clusters of prisms; yield of potassium orotate 2.2 g. It was dissolved in water acidified with hydrochloric acid, cooled and filtered. The product after decolorization with bone black and recrystallization from water was compared optically with an authentic specimen. They were found to be identical. The crystals were biaxial negative, had a medium angle, and were strongly birefringent. The angle of extinction was either zero or very small. The low index was 1.66 and the high index was somewhat over 1.74.

*Anal.* Calcd. for  $C_5H_4O_4N_2 \cdot H_2O$ :  $H_2O$ , 10.35. Found:  $H_2O$ , 10.38. Calcd. for  $C_5H_4O_4N_2$ : C, 38.45; H, 2.58; N, 17.95. Found: C, 38.54, 38.60; H, 2.53, 2.56; N, 17.95, 17.99.

**4,5-Dihydrouracil-4-ethylacetate.**—A solution of 2.2 g. of ethyluracil-4-acetate in 100 cc. of warm alcohol was treated with 0.2 g. of platinum oxide and subjected to 42 pounds' pressure of hydrogen for twenty-four hours. The reaction at the end of this time was complete and the reduction product had crystallized out. It was brought into solution by heating and the platinum black removed by filtration. The filtrate was concentrated to 50 cc. and cooled. The 4,5-dihydrouracil-4-ethylacetate that separated was recrystallized from 25 cc. of ethyl alcohol, from which it separated as a mass of colorless prisms melting at 155–156°, yield 1.5 g.

*Anal.* Calcd. for  $C_8H_{12}N_2O_4$ : C, 47.98; H, 6.04; N, 14.00. Found: C, 47.92, 48.14; H, 6.16, 6.31; N, 14.08, 14.12.

**3-Methyl-4,5-dihydrouracil.**—This was prepared by reducing 6 g. of 3-methyluracil in the same manner as described above. The filtrate from the reduction mixture was

concentrated to 25 cc. and cooled; long thick needles separated. It was recrystallized from 25 cc. of hot alcohol, in which it was very soluble; m. p. 175–176°; the yield was very good.

*Anal.* Calcd. for  $C_5H_8O_2N_2$ : C, 46.84; H, 6.30; N, 21.87. Found: C, 47.15, 46.93; H, 6.27, 6.21; N, 21.89.

### Summary

1. Uracil-4-acetic acid was prepared by treating urea and citric acid with fuming sulfuric acid. This pyrimidine ring was easily ruptured by barium hydroxide solution and yielded the barium salt of  $\beta$ -carbamido-glutaconic acid.

2. Orotic acid was synthesized by oxidizing uracil-4-acetic acid in alkaline solution with potassium ferricyanide.

3. The possibility that orotic acid may be an intermediate in the synthesis of purines from histidine has been discussed.

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## THE ACTION OF ACETIC ACID UPON CERTAIN CARBOHYDRATES<sup>1</sup>

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It has been shown<sup>2</sup> that native cellulose is esterifiable by the action of boiling acetic acid to a limited extent represented in the formula  $C_{24}H_{39}O_{20}(COCH_3)_3$ , whereas hydrated cellulose under the same conditions can yield an ester of the limiting composition  $C_{24}H_{36}O_{20}(COCH_3)_4$ . A study of the behavior of other carbohydrates toward acetic acid was therefore undertaken in the hope of finding a clue to the nature of the factors which tend to restrict the esterifiability of cellulosic hydroxyl groups.

Reducing carbohydrates, such as glucose and fructose, caramelize under the influence of boiling acetic acid alone or in the presence of sodium acetate. This applies also to the two non-reducing carbohydrates of the furanose type which have been examined, namely, sucrose and inulin—a finding which need occasion no surprise, in view of the well-established ease with which the five-membered ring of sugars may be opened by acid reagents. The cyclic structure of non-reducing glycosides of the pyranose type, on the other hand, withstands the action of boiling acetic acid, and such compounds ultimately yield fully esterified products. Thus  $\alpha$ -methylglucoside,  $\beta$ -methylglucoside and  $\alpha$ -methylmannoside are converted into the corresponding tetraacetates, while potato starch yields a "tri-acetate." Similarly, mannitol is converted into its hexaacetate.

<sup>1</sup> Work supported by a research grant from The Chemical Foundation.

<sup>2</sup> C. J. Malm and H. T. Clarke, *THIS JOURNAL*, 51, 274 (1929).