[CONTRIBUTION FROM THE WESTERN REGIONAL RESEARCH LABORATORY, BUREAU OF AGRICULTURAL AND INDUSTRIAL CHEMISTRY, AGRICULTURAL RESEARCH ADMINISTRATION, U. S. DEPARTMENT OF AGRICULTURE]

DERIVATIVES OF D-GALACTURONIC ACID-1-PHOSPHATE

E. L. PIPPEN AND R. M. McCREADY

Received October 2, 1950

In connection with biochemical studies of pectin at this laboratory, α-D-galacturonic acid-1-phosphate was desired. The first approach to the synthesis of this compound followed in general the scheme used by Cori, *et al.* (1) in their synthesis of α-D-glucose-1-phosphate. Since the acetylation of galacturonic acid does not lead to crystalline products (2), the methyl ester of α-D-galacturonic acid (I), prepared according to Jansen and Jang (3), was used as the starting material. This ester was converted to the necessary intermediate, methyl tetra-acetyl-D-galacturonate, by the routes shown in Figure 1.

Treatment of the ester (I) with pyridine and acetic anhydride at 0° yielded an acetate having the melting point 142° and the rotation[1] +138°. Acetylation of I with acetic anhydride in the presence of sodium acetate at 100° yielded an acetate having the melting point 142° and rotation +57.8°. Upon acetylation of I with acetic anhydride in the presence of zinc chloride, as described by the workers cited above (2), there was obtained invariably in five preparations by this method a mixture of the α- and β-isomers. In two of these runs a separation of the α- and β-isomers was accomplished by fractional crystallization. Of these, the more strongly-rotating isomer had the rotation +136° and melted at 142°, while the other lower-rotating isomer had the rotation +57.1° and melted at 142°. In conformance with Hudson's rules of nomenclature (4) the more strongly-rotating acetate was designated methyl 1,2,3,4-tetraacetyl-α-D-galacturonate (II). The properties of this compound, obtained by the two methods described above, agree well with the properties of the corresponding acetate obtained by Morell and Link (2). Although no one has unequivocally shown, to our knowledge, that this particular derivative of galacturonic acid has a six-membered ring, there seems little doubt that it has, since Morell and Link (5) have shown that α-D-galacturonic acid tends to form derivatives having the pyranose ring and Levene and co-workers (6, 7, 8) have shown that α-D-galacturonic acid forms acetyl and methyl ether derivatives in which the 2-, 3-, and 4-positions are substituted. The other isomer, of which no previous report was found, was designated methyl 1,2,3,4-tetraacetyl-β-D-galacturonate (III). This acetate was readily converted to the higher-rotating acetate (II) by heating it in acetic anhydride in the presence of zinc chloride. Husdon and co-workers (9–12) have shown that this behavior of the acetylated sugars is typical of a pair of *alpha*- and *beta*-isomers having the same ring structure. On the basis of the evidence presented above, the pyranose structure has been provisionally assigned to the derivatives described in this paper.

The next step in the synthesis required methyl 1-bromo-2,3,4-triacetyl-α-

[1] All rotations are constant specific rotations at 24° calculated from saccharimeter readings using sodium light. The concentration, *c*, is grams in 100 ml. of solution.

D-galacturonate (IV). This bromide appears to have been prepared by Morell, *et al.* (13); however, since no report of the β-isomer of (IV) could be found, a definitive α- or β-assignment to this bromide was impossible. To overcome this uncertainty we attempted to prepare the β-isomer of the bromide (IV). Bromination of either II or III (or a mixture of these) by different methods resulted in the isolation of a bromide whose properties agree well with those of the bromide

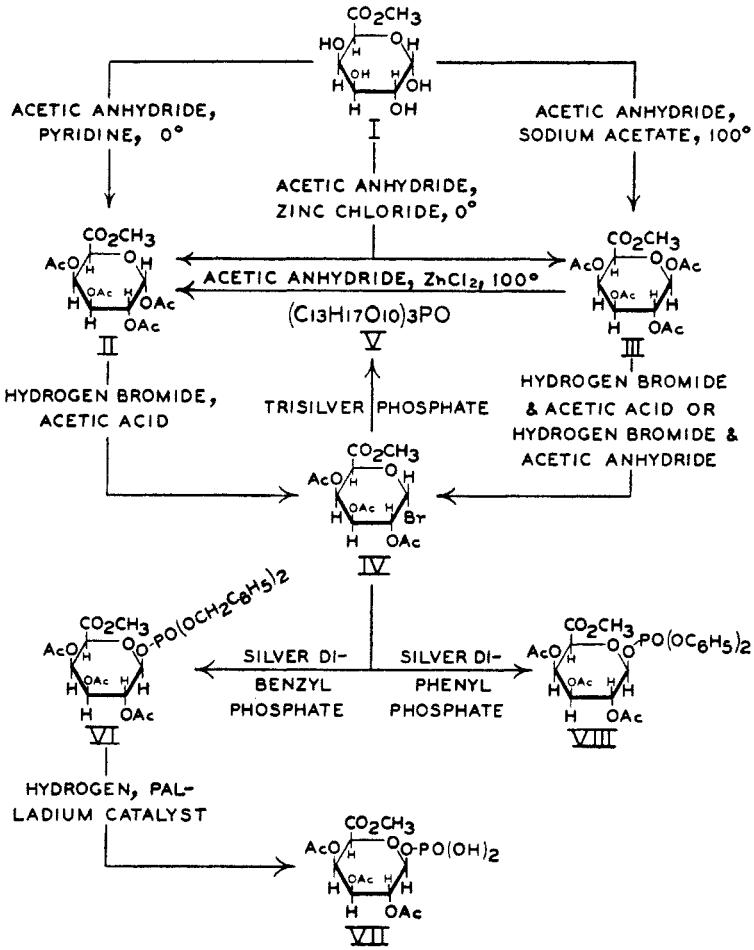

FIG. 1

obtained by the previous workers (13). All attempts to isolate the "β-isomer" from these reactions failed. As pointed out by Morell, *et al.* (13), it seems highly probable that this is the α-isomer; therefore, the next step in the synthesis, reaction of the bromide (IV) with silver phosphate, was carried out and the bromide was converted to an amorphous substance which is presumably the trisugar phosphate (V). The high rotation of this substance, +134°, indicates an α-type linkage and if this is true it is added evidence that the bromide (IV)

has the α-configuration, because the reaction of acetobromo sugars with silver phosphate is known to proceed without inversion of the configuration (1, 14).

Conversion of the trisugar phosphate (V) to α-D-galacturonic acid-1-phosphate requires deacetylation, the splitting off of two sugar residues, and deesterification. Several experiments to accomplish this, including the hydrochloric-acid-in-methanol method of Cori, *et al.* (1), were conducted but in no case could the barium salt of α-D-galacturonic acid-1-phosphate be isolated. These experiments indicated that the oxygen-phosphorus linkages in the trisugar phosphate (V) are more stable to acid than those linkages in the corresponding trisugar phosphate of glucose, and that once one of these linkages in V is cleaved the remaining two sugar residues are rapidly hydrolyzed.

Our efforts were then directed toward a method which might lead to the desired product by a path which would avoid a trisugar phosphate intermediate. Two methods were tried which could be expected to yield in one case the β-isomer and in the other the α-isomer of D-galacturonic acid-1-phosphate. The first was the phosphorylation procedure of Zervas (15) as described by Wolfrom, *et al.* (16). These workers have shown that this phosphorylation procedure resulted in a product having a definitive β-configuration when the reaction was carried out on α-acetobromoglucose. Reithel (17) also obtained the β-isomer on phosphorylation of 1-bromo-2,3,4,6-tetraacetyl-α-D-galactose by this method. Hence the product to be expected by this method if one uses the bromide (IV), would be the phosphorylated product (VI). Upon carrying out the phosphorylation there was indeed obtained a crystalline phosphorylated product which analyzed correctly for the expected methyl 1-(dibenzyl)phosphate-2,3,4-triacetyl-β-D-galacturonate (VI). It has been tentatively assigned the β-configuration because of its low rotation of +12.2° and because of its method of preparation. This material, when subjected to hydrogenation in the presence of a palladium oxide catalyst, gave an amorphous product whose properties indicated that it may have consisted mostly of methyl 1-phosphate-2,3,4-triacetyl-β-D-galacturonate (VII). Attempts to convert this to the deacetylated methyl 1-phosphate-D-galacturonate by acid hydrolysis in methanol (1) and by barium methoxide were unsuccessful.

The second method was the phosphorylation procedure which has been described by Posternak (18). The phosphorylating agent, silver diphenylphosphate, reacted with 1-bromo-2,3,4,6-tetraacetyl-α-D-glucose to form the α-phosphate ester. Use of this phosphorylating agent with the bromide (IV) could be expected to yield methyl 1-(diphenyl)phosphate-2,3,4-triacetyl-α-D-galacturonate. A crystalline phosphate ester actually was obtained which analyzed correctly for the expected product. Because of the low yield (12%) and the low rotation (+24°), which indicates that the reaction proceeded with inversion resulting in the β-isomer (VIII), the product was not further investigated.

EXPERIMENTAL

All melting points reported are uncorrected. Methoxyl determinations were done by the Zeisel method as described by Shriner (19). Phosphorus analyses were performed by the method of Allen (20).

In the case of all derivatives, excepting the acetates, the assignment of an α- or β-configuration is based upon the evidence of rotation and method of preparation. These designations must remain tentative until the α- and β-isomers of these derivatives are prepared or until further work on this point is done.

METHYL 1,2,3,4-TETRAACETYL-D-GALACTURONATE

A. Preparation of the β-isomer (III). *Acetylation with acetic anhydride and sodium acetate.* To 108 ml. of acetic anhydride at 100° was added 8.8 g. of anhydrous sodium acetate. To this was added, with stirring, over a period of 30 minutes, 20.0 g. of thoroughly dry methyl α-D-galacturonate (I). After addition of the ester was complete, the mixture was heated at 100° for 52 minutes. The mixture was cooled to room temperature, mixed thoroughly with 400 ml. of ice-water, and stored for 3 days at 0°. A crop of the acetate was harvested from this aqueous solution and after recrystallization from warm 95% ethanol, had a dry weight of 4.95 g. The aqueous mother liquor was extracted with chloroform and after the chloroform extract had been dried with sodium sulfate it was concentrated *in vacuo* to a sirup. About an equal volume of ether was added and the mixture was cooled to 2°. After two hours a second crop of crystals was obtained, which when dry weighed 2.7 g. The total yield was 7.65 g. (21%). After recrystallization once from warm ethanol, and once from hot water, the acetate melted at 142–143°; rotation, +57.7° (*c*, 2.0 in chloroform).

Anal. Calc'd for $C_{15}H_{20}O_{11}$: CH_3O, 8.25. Found: CH_3O, 8.2.

It was observed that this β-acetate tended to crystallize in several different modifications. However, this seems to be a clear case of polymorphism, since a mixture of these crystalline modifications exhibit the same melting point and rotation as that of a single crystalline form. Furthermore, a mixture melting-point determination with the β-acetate obtained from another source showed no depression (m.p. 142–143°), whereas a melting-point determination of a mixture of α- and β-acetates melted over the range 109–141°. It was noticed that if the β-acetate was placed in a bath at 130°, immediate melting followed by solidification occurred and the acetate then remelted at 142°. However, when the β-acetate was placed in a bath at 90° or less this phenomenon did not occur; a normal melting-point behavior ensued.

B. Preparation of the α-isomer (II). *Acetylation with acetic anhydride and pyridine.* To a mixture of 44 ml. of pyridine and 29 ml. of acetic anhydride, cooled to 0°, was added, with vigorous stirring, 6.0 g. of thoroughly dry methyl α-D-galacturonate (I). Nearly all of the ester was in solution ten minutes after its addition. The mixture was then kept at 0°, with occasional stirring, for five hours (reaction time of 20 hours or less gave only the α-acetate, whereas a reaction time of 69 hours resulted in a mixture of α- and β-acetates) after which it was poured into a well-stirred mixture of 150 ml. of ice and water. The acetate was extracted from this mixture with 100 ml. of benzene, and the benzene extract, after having been washed once with 0.2 *M* sodium bicarbonate solution, four times with 0.1 *N* cupric sulphate solution, and twice with water, was dried with sodium sulphate. The dried solution was concentrated *in vacuo* to a sirup, which was taken up in 2 volumes of warm ethanol. After standing overnight at 2° a crop of crystalline methyl 1,2,3,4-tetraacetyl-α-D-galacturonate weighing 2.4 g. (yield, 22%) was obtained. After recrystallization from ethanol it melted at 142–144°; rotation +138° (*c*, 1.0 in chloroform).

Anal. Calc'd for $C_{15}H_{20}O_{11}$: CH_3O, 8.25. Found: CH_3O, 8.24.

Additional acetate can be obtained from the mother liquor but we have found that it may be contaminated with the β-isomer.

C. Acetylation with acetic anhydride in the presence of zinc chloride. Methyl α-D-galacturonate (I) (50 g.) was acetylated with acetic anhydride in the presence of zinc chloride according to the procedure of Morell and Link (2). Six crops of crystalline acetate having a total weight of 50 g. (yield, 54%) were obtained. Crop one had the rotation +123.8° (*c*, 1.0 in chloroform), melted over the range 110–142°, and analyzed for 8.3% methoxyl. Subsequent crops of the crystalline acetate also had rotations intermediate between that of the pure α- or β-isomer. In one run the isomeric acetates were separated by fractional

crystallization into the two pure α- and β-compounds. Of these, one acetate had m.p. 141–142°, and rotation +136° (*c*, 1.0 in chloroform) and is therefore the α-isomer, while the other melted at 141–142° and rotated +57.1° (*c*, 1.0 in chloroform) and is the β-isomer. Five duplicate preparations by this method gave invariably a mixture of the α- and β-isomers.

Conversion of methyl 1,2,3,4-tetraacetyl-β-D-galacturonate to methyl 1,2,3,4-tetraacetyl-α-D-galacturonate. Methyl tetraacetyl-β-D-galacturonate (III) (2.5 g.) rotating at +57.8° and melting at 141–142° was treated with 7.5 ml. of acetic anhydride and the tube was shaken until all of the acetate had dissolved. There was then added 0.5 g. of fused zinc chloride after which the tube was heated in a steam-bath, with occasional shaking, for 15 minutes. The reaction mixture was poured into 25 g. of cracked ice and the mixture brought to *p*H 6 by the addition of sodium bicarbonate solution. The acetate was extracted with chloroform, and the extract, after having been washed twice with water and dried over sodium sulphate, was concentrated *in vacuo* to a sirup. This was taken up in 95% ethanol, whereupon a few crystals of the unchanged β-acetate appeared immediately. These were removed and the filtrate was stored overnight at 2°; from it there was harvested the following morning a crop of well-formed crystals weighing 1.2 g. (yield, 48%); m.p. 141–142°, rotation, +136.8° (*c*, 1.0 in chloroform).

Methyl 1-bromo-2,3,4-triacetyl-α-D-galacturonate (IV). *A. Bromination of methyl tetraacetyl-β-D-galacturonate* (III). Two grams of III were converted to the bromo derivative in a mixture of acetic acid and hydrogen bromide according to the procedure described by Bates, *et al.* (21). There was obtained 1.54 g. of bromide (yield 73%) having m.p. 128°, and rotation +241° (*c*, 1.0 in chloroform). These properties as well as an x-ray powder pattern show that this bromide is identical with the bromide obtained by acetylating methyl tetraacetyl-α-D-galacturonate according to Morell, *et al.* (13).

When 1.2 g. of III was brominated with hydrogen bromide in acetic anhydride by the procedure described by Morell, *et al.* (13) for the bromination of methyl tetraacetyl-α-D-galacturonate there was obtained 0.72 g. of bromide (yield 57%) with m.p. 129° and a rotation of +239° (*c*, 1.0 in chloroform). An x-ray powder pattern of this bromide was also identical with the x-ray powder pattern of the bromide described by Morell, *et al.* (13).

B. Bromination of a mixture of α- and β-isomers of methyl tetraacetyl-D-galacturonate. Methyl tetraacetyl-D-galacturonate, 16.0 g. melting over the range 110–142°, having the rotation +124° (*c*, 1.0 in chloroform) and 8.3% methoxyl was brominated with hydrogen bromide in acetic acid as described by Bates, *et al.* (21). There was obtained 13.7 g. of bromide (yield 81%) with m.p. 130° and rotation +242° (*c*, 1.0 in chloroform).

Tri(methyltriacetyl-α-D-galacturonate) phosphate (V). Methyl 1-bromo-2,3,4-triacetyl-α-D-galacturonate (IV) (25.2 g.) was reacted with silver phosphate (22) in the same manner that Cori, *et al.* (1) reacted acetobromoglucose with silver phosphate. There was obtained 22.9 g. of amorphous material, which is presumably mostly tri(methyltriacetyl-α-D-galacturonate) phosphate (V). This material had the rotation +134° (*c*, 1.0 in chloroform).

Anal. Calc'd for $C_{39}H_{51}O_{31}P$: P, 2.96; CH_3O, 8.9.

Found: P, 2.5; CH_3O, 7.8.

The material slowly reduced Fehling solution, but the reduction was more rapid and pronounced after the substance was first hydrolyzed at 100° for 3 minutes in 1 *N* aqueous sulfuric acid.

Methyl 1-(dibenzyl)phosphate-2,3,4-triacetyl-β-D-galacturonate (VI). In this experiment the phosphorylation procedure of Wolfrom, *et al.* (16) was used. The silver dibenzylphosphate was prepared from dibenzylphosphoric acid (23) and silver nitrate by the procedure of Reithel (17).

Methyl 1-bromotriacetyl-α-D-galacturonate (IV) (20 g.), 29.1 g. of silver dibenzylphosphate, 10 g. of anhydrous calcium sulphate, and 110 ml. of dry benzene were introduced into a flask equipped with a mercury-sealed stirrer, and a reflux condenser carrying a drying-tube. The mixture was heated with stirring at 45–50° for 30 minutes and then heated under reflux for 70 minutes. After cooling, the solid material was filtered off and the clear

benzene solution was concentrated *in vacuo* to a sirup. The sirup was transferred to a beaker with ether rinses and stored at 2° for several hours to permit unreacted silver salt to crystallize. After filtering and evaporating off the ether, the crude product was obtained from the solvent pair ethanol-water. Recrystallized twice from ethanol-water and once from ether, it gave 5 g. (yield 17%) of crystalline methyl 1-(dibenzyl)phosphate-2,3,4-triacetyl-β-D-galacturonate, m.p. 84.5–86°, with the rotation +12.2° (*c*, 1.0 in chloroform).

Anal. Calc'd for $C_{27}H_{31}O_{13}P$: P, 5.2; CH_3O, 5.2.

Found: P, 5.0; CH_3O, 5.2.

This substance reduced Fehling solution about equally well whether tested before or after acid hydrolysis in 1 *N* H_2SO_4 at 100° for 3 minutes.

Methyl 1-phosphate-2,3,4-triacetyl-β-D-galacturonate (VII). One gram of methyl 1-(dibenzyl)phosphate-2,3,4-triacetyl-β-D-galacturonate (VI) dissolved in 20 ml. of absolute ethanol was hydrogenated at atmospheric pressure in the presence of 0.2 g. of palladium oxide catalyst (24). Within 15 minutes hydrogenation was complete and the filtrate, which smelled noticeably of toluene, was concentrated *in vacuo* to a white, amorphous solid having an acrid odor. After drying overnight at room temperature *in vacuo* over phosphorus pentoxide, the material weighed 0.4 g. (yield 57%). Although the following analyses do not check perfectly with the theoretical values, they strongly indicate that the product obtained was chiefly the expected methyl 1-phosphate-2,3,4-triacetyl-β-D-galacturonate.

Anal. Calc'd for $C_{13}H_{19}O_{13}P$: Equiv. wt., 207.15; P, 7.5; CH_3O, 7.5.

Found: Eq. wt., 224; P, 6.9; CH_3O, 7.2.

An attempt was made to purify this compound by way of its dipotassium salt, but the salt failed to crystallize. This material slightly reduced Fehling solution. After hydrolyzing for 3 minutes in 1 *N* sulfuric acid at 100°, the reduction of Fehling solution was more rapid and pronounced.

Methyl 1-(diphenyl)phosphate-2,3,4-triacetyl-β-D-galacturonate (VIII). The phosphorylating agent, silver diphenylphosphate, was prepared as described by Posternak (18). In the usual apparatus, a mixture of 28 ml. of benzene, 6.3 g. of methyl 1-bromo-2,3,4-triacetyl-α-D-galacturonate (IV), and 5.7 g. of silver diphenylphosphate was refluxed with stirring for 15 minutes. Then an additional 2 g. of silver diphenylphosphate was added and refluxing was continued for 30 minutes. After cooling, the silver salts were centrifuged off and washed exhaustively with benzene. The benzene solution was concentrated *in vacuo* (40–50°) to a mushy white solid. This was taken up in warm methanol from which the product readily crystallized on cooling. After repeated recrystallizations from methanol the melting point remained unchanged. Additional product was obtained by extracting the silver salts with hot methanol. A total of 1.1 g. (yield 12%) of crystalline product was obtained; m.p. 115–116° with a rotation of +24.2° (*c*, 1.0 in chloroform). Like the corresponding dibenzyl derivative, this material reduced Fehling solution about equally well both before and after acid hydrolysis.

Anal. Calc'd for $C_{25}H_{27}O_{13}P$: P, 5.48; CH_3O, 5.47.

Found: P, 5.3; CH_3O, 5.43.

Acknowledgment. We express our appreciation to Dr. K. J. Palmer for preparation of x-ray powder patterns of some of the compounds, to Dr. F. T. Jones for helpful suggestions concerning the crystalline properties of the acetates, and to Miss E. A. McComb for phosphorus determinations.

SUMMARY

Three new crystalline derivatives of α-D-galacturonic acid are described. These include methyl 1,2,3,4-tetraacetyl-β-D-galacturonate and two derivatives of galacturonic acid-1-phosphate which, since their α- or β-isomer is unknown, have been tentatively designated as methyl 1-(dibenzyl)phosphate-2,3,4-tri-

acetyl-β-D-galacturonate and methyl 1-(diphenyl)phosphate-2,3,4-triacetyl-β-D-galacturonate. The preparation of amorphous tri(methyltriacetyl-α-D-galacturonate) phosphate and of amorphous methyl 1-phosphate-2,3,4-triacetyl-β-D-galacturonate is described. Attempts to convert these phosphate esters to either galacturonic acid-1-phosphate or its methyl ester were unsuccessful.

ALBANY 6, CALIFORNIA

REFERENCES

(1) CORI, COLOWICK, AND CORI, *J. Biol. Chem.*, **121**, 465 (1937).
(2) MORELL AND LINK, *J. Biol. Chem.*, **108**, 763 (1935).
(3) JANSEN AND JANG, *J. Am. Chem. Soc.*, **68**, 1475 (1946).
(4) HUDSON, *J. Am. Chem. Soc.*, **31**, 66 (1909).
(5) MORELL AND LINK, *J. Biol. Chem.*, **104**, 183 (1934).
(6) LEVENE AND KREIDER, *J. Biol. Chem.*, **121**, 155 (1937).
(7) LEVENE, TIPSON, AND KREIDER, *J. Biol. Chem.*, **122**, 199 (1937).
(8) LEVENE AND CHRISTMAN, *J. Biol. Chem.*, **122**, 203 (1937).
(9) HUDSON AND DALE, *J. Am. Chem. Soc.*, **37**, 1280 (1915).
(10) HUDSON AND PARKER, *J. Am. Chem. Soc.*, **37**, 1589 (1915).
(11) HUDSON, *J. Am. Chem. Soc.*, **37**, 1591 (1915).
(12) HUDSON AND JOHNSON, *J. Am. Chem. Soc.*, **38**, 1223 (1916).
(13) MORELL, BAUR, AND LINK, *J. Biol. Chem.*, **110**, 719 (1935).
(14) MEAGHER AND HASSID, *J. Am. Chem. Soc.*, **68**, 2135 (1946).
(15) ZERVAS, *Naturwissenschaften*, **27**, 317 (1939).
(16) WOLFROM, SMITH, PLETCHER, AND BROWN, *J. Am. Chem. Soc.*, **64**, 23 (1942).
(17) REITHEL, *J. Am. Chem. Soc.*, **67**, 1056 (1945).
(18) POSTERNAK, *J. Biol. Chem.*, **180**, 1269 (1949).
(19) SHRINER, *Quantitative Analysis of Organic Compounds*, Edward Brothers, Inc., Ann Arbor, Michigan, 1940, p. 35.
(20) ALLEN, *Biochem. J.*, **34**, 858 (1940).
(21) BATES AND ASSOCIATES, *Polarimetry, Saccharimetry and the Sugars*, U. S. National Bureau of Standards, Circular C440, United States Government Printing Office, Washington, 1942, p. 500.
(22) LIPMANN AND TUTTLE, *J. Biol. Chem.*, **153**, 571 (1944).
(23) LOSSEN AND KÖHLER, *Ann.*, **262**, 212 (1891).
(24) SHRINER AND ADAMS, *J. Am. Chem. Soc.*, **46**, 1683 (1924).