centration of the catalysts hydrogen chloride and *p*-toluenesulfonic acid is in accord with the predictions of the Debye–Hückel theory, but the behavior of methanesulfonic acid is anomalous. It appears that *p*-toluenesulfonic acid and methanesulfonic acids are incompletely dissociated in ethanol at concentrations of 1×10^{-2} to 1×10^{-1} mole/liter. The value calculated for the dissociation constant of trichloroacetic acid in ethanol is 6.9×10^{-6} and in reasonable agreement with previously published values.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, THE UNIVERSITY OF WISCONSIN]

Alkali-sensitive Glycosides of 3-Phenyl-4-hydroxycoumarin[1]

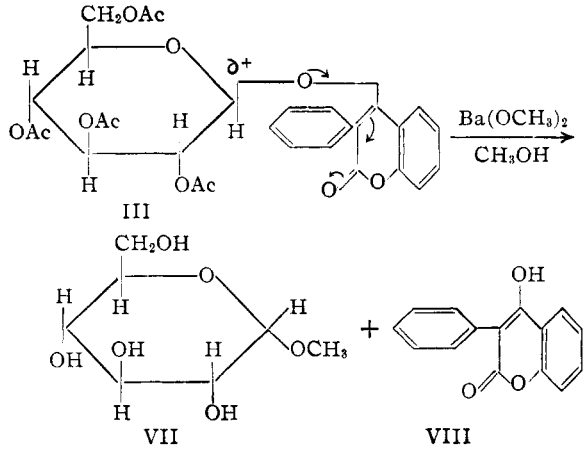
BY LEONARD SPERO,[2] CLINTON E. BALLOU AND KARL PAUL LINK

The synthesis and properties of some β-D-glucosides of 4-hydroxycoumarins were first reported from this Laboratory in 1944.[3] These compounds were made in connection with the biological studies on the hypoprothrombinemic effect of 3,3′-methylene-bis-(4-hydroxycoumarin) [Dicumarol] and related 4-hydroxycoumarins.[4] 4-Hydroxycoumarin D-glucoside tetraacetate (I), 4-hydroxy-6-methylcoumarin D-glucoside tetraacetate (II), 3-phenyl-4-hydroxycoumarin D-glucoside tetraacetate (III), and 3,3′-methylene-bis-(4-hydroxycoumarin) mono-D-glucoside tetraacetate (IV) were prepared by condensing the silver salt of the aglycon with tetraacetyl-D-glucosyl bromide. A modified Robertson method[5] was used in the preparation of 3,3′-methylene-bis-(4-hydroxycoumarin) diglucoside octaacetate (V), and 3-[6-oxo(1)-benzopyrano(4,3-b)(1)benzopyran-7-yl]-4-hydroxycoumarin D-glucoside tetraacetate (VI). Because of the method of preparation and the optical rotation of these glucosides the β-configuration was assigned.

As shown by Huebner, *et al.*,[3] these glucosides are extremely labile to alkali. Compounds I and II were deacetylated by the catalytic barium methoxide procedure. Attempts to deacetylate the glucosides in which there were substituents on position three of the coumarin residue resulted in cleavage of the glucoside linkage. This reaction is unique in that the starting compound is converted to the aglycon and methyl α-D-glucoside (VII). During attempted deacetylation of III, 80% of the starting material was converted to 3-phenyl-4-hydroxycoumarin (VIII) and VII.

Isbell's[6] interpretation of glycoside cleavage rationalizes the fact that the splitting may occur on either side of the glycosidic oxygen. An electrophilic aglycon promotes cleavage of the sugar–oxygen bond, while cleavage of the aglycon–oxygen bond occurs when the sugar residue is able to take electrons from the aglycon.

Cleavage of glycosides under anhydrous methanolic conditions could result in methanolysis, cyclization or unsaturation. This reaction demonstrates the methanolysis type. The formation of methyl α-D-glucoside (VII) indicates that a Walden inversion accompanies the cleavage of the glucosidic linkage, and this would be possible only if the sugar–oxygen bond were the one split. The following scheme, involving the indicated electronic shifts, may be used to rationalize this reaction.



A more involved mechanism is used to explain the products obtained during the alkaline cleavage of V [glucose, VII, and 3,3′-methylene-bis-(4-hydroxycoumarin) monomethyl ether]. For a detailed discussion see ref. 3.

It is conceivable that this reaction would be generally applicable to glycosides of this type.[7] One of the purposes of this study was to ascertain if the nature of the sugar residue played a role in determining the mechanism of the glycosidic cleavage. In order to study the reaction further,

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(2) Present address: Camp Detrick, Frederick, Maryland.

(3) Huebner, Karjala, Sullivan and Link, THIS JOURNAL, **66**, 906 (1944).

(4) Stahmann, Huebner and Link, *J. Biol. Chem.*, **138**, 513 (1941).

(5) Robertson and Waters, *J. Chem. Soc.*, 2729 (1930).

(6) Isbell, *Ann. Rev. Biochem.*, **XII**, 215 (1943).

(7) Dr. C. S. Hudson had originally suggested to one of us (K. P. L.) that this reaction might serve as the route through which certain inaccessible α-glycosides could be realized.

a series of glycosides of 3-phenyl-4-hydroxycoumarin (VIII) was prepared, and the methanolysis was carried out in a similar manner on each.

The silver salt of the aglycon is quite stable, and when treated with the acetylglycosyl bromide, forms the acetylated glycoside in yields of from 56–76%. The products were difficult to crystallize and purify.

The glucoside,[3] galactoside, mannoside and xyloside (all in the D series), which were assigned β-configurations because of the mode of synthesis, were cleaved to form methyl α-D-glycosides. The D-arabinoside, assigned an α-configuration by the same precedence, produced methyl β-D-arabinoside. These results corroborate the generalization of configurational assignment from mode of synthesis if a Walden inversion is assumed.

A study of the rotational changes[8] accompanying the cleavage showed a simple mutarotation curve (Figs. 1 and 2), and varying amounts of the catalytic agent increased the rate at which the reaction occurred (Fig. 3). The reactions were generally quite slow as determined by the length of time required for the rotation to stop changing. Cleavage of the glucoside required about two weeks, while cleavage of the arabinoside was not complete after six months. The concentration of the catalyst was the same in both reactions.

If concentrations of barium methoxide above 0.02 *N* were used, side reactions resulted and uncrystallizable sirups were obtained. In these cases the reaction went with extreme rapidity, the barium salt of the aglycon precipitated from the solution, and the final rotation indicated the formation of the free sugar residue. This may have been due to the presence of a small amount of barium hydroxide in the barium methoxide stock solution, and at higher concentrations of the latter, the former might have become critical.

Experimental

Preparation of the Silver Salt of 3-Phenyl-4-hydroxycoumarin[3] (IX).—Precautions were taken to work in a feeble light. VIII was dissolved in one equivalent of 1.5 *N* aqueous sodium hydroxide and one equivalent of silver nitrate plus 2% excess dissolved in a little water, was added while the solution was being vigorously stirred. The gelatinous, white silver salt which precipitated immediately, was filtered off. It was resuspended in water, collected on a funnel, pressed dry, and washed once with absolute ethanol. The salt was dried over calcium chloride *in vacuo*, finely pulverized, and dried again at 45° and 3 mm. pressure over phosphorus pentoxide. The product varied from a colorless to a light-gray powder; yield 96%.

Preparation of 3-Phenyl-4-hydroxycoumarin β-D-Galactoside Tetraacetate (X).—Pentaacetyl-β-D-galactose prepared according to Erwig and Koenigs[9] was converted to tetraacetyl-D-galactosyl bromide according to Ohle.[10] A mixture of 6.7 g. of IX, 7.2 g. of tetraacetyl-D-galactosyl bromide, 1.3 g. of Drierite and 65 ml. of dry benzene, was shaken in a dark bottle on a mechanical shaker. Upon completion of the reaction, as indicated by a negative free bromide test (no precipitate in a centrifuged sample when it was mixed with a drop of alcoholic silver nitrate and 3 volumes of absolute ethanol), the silver salts were centrifuged off and washed with hot benzene. The combined benzene solutions were concentrated *in vacuo* to a thick sirup. The sirup was taken up in hot methanol and the solution allowed to stand until crystallization was complete. Yield of the crude material was 6.9 g. Yield after one recrystallization was 76%, m. p. 91–

(8) The polarimetric studies were done with the use of a Franz Schmidt and Haensch polarimeter No. 52B with a monochromator attachment.

(9) Erwig and Koenigs, *Ber.*, **22**, 2207 (1889).

(10) Ohle, Marecek and Borjau, *ibid.*, **62**, 849 (1929).

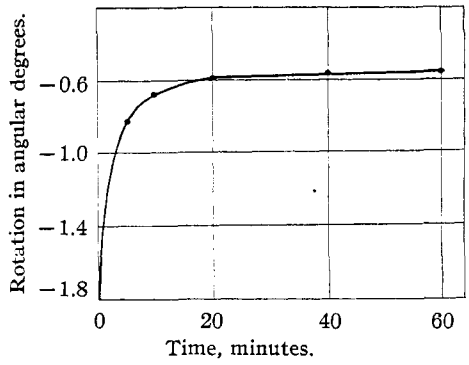

Fig. 1.—Mutarotation of 3-phenyl-4-hydroxycoumarin β-D-glucoside tetraacetate, 0.053 *M* in absolute methanol containing barium methoxide, 0.006 *M*.

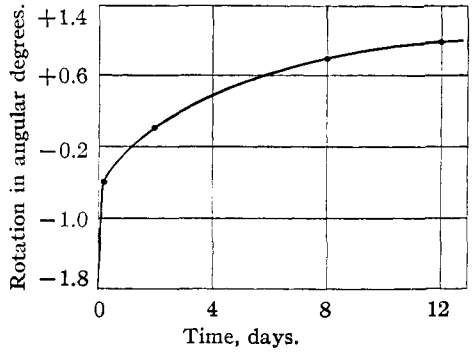

Fig. 2.—Mutarotation of 3-phenyl-4-hydroxycoumarin β-D-glucoside tetraacetate, 0.053 *M* in absolute methanol containing barium methoxide, 0.006 *M*.

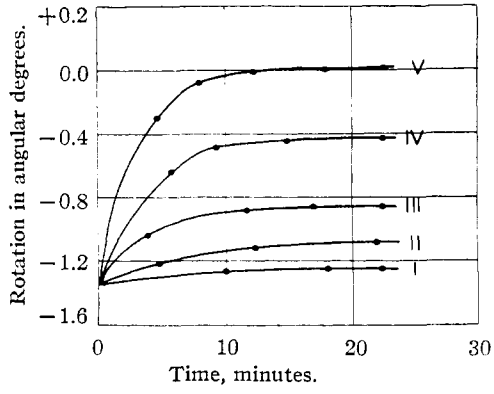

Fig. 3.—The effect of varying concentrations of barium methoxide (milliequivalents/liter of solution) on the methanolysis of 3-phenyl-4-hydroxycoumarin β-D-glucoside tetraacetate (0.058 *M*): I (1.14), II (2.28), III (4.56), IV(9.14), V(18.28).

93°; $[\alpha]^{25}D$ −39.6° (*c*, 1.2, benzene). *Anal.* Calcd. for $C_{29}H_{28}O_{12}$: C, 61.36; H, 4.94. Calcd. for $C_{29}H_{28}O_{12}\cdot{}^1/_2H_2O$: C, 60.31; H, 5.07. Found: C, 60.19; H, 5.27.

When dried over phosphorus pentoxide at 56.5° and 2 mm. pressure for fifteen hours, 96.12 mg. of substance lost 1.52 mg. of water. Calcd. for $C_{29}H_{28}O_{12}\cdot{}^1/_2H_2O$: H_2O, 1.57. Found: H_2O, 1.58.

Preparation of 3-Phenyl-4-hydroxycoumarin β-D-Xyloside Triacetate (XI).—D-Xylose was acetylated to tetraacetyl-β-D-xylose according to Stone.[11] The bromosugar was made by the method of Hudson and Johnson.[12] To 150 ml. of dry benzene in a dark bottle were added 18.7 g. of IX, 16.5 g. of triacetyl-D-xylosyl bromide and 3.0 g. of Drierite. The mixture was shaken for three days or until a centrifuged portion gave the negative test for free bromide as described above.

The silver salts were centrifuged off, washed with hot benzene, and the benzene solution concentrated *in vacuo* to a thick sirup which crystallized from a mixture of ethanol and water. The crude material amounted to 17.0 g. It was recrystallized from methanol; yield 60%, m. p. 120–122°; $[\alpha]^{25}D$ −98.9° (*c*, 0.95, benzene). *Anal.* Calcd. for $C_{26}H_{24}O_{10}$: C, 62.90; H, 4.87. Found: C, 62.79; H, 4.98.

Preparation of 3-Phenyl-4-hydroxycoumarin α-D-Arabinoside Triacetate (XII).—D-Arabinose prepared by the method of Hockett and Hudson[13] was converted directly to the triacetyl-D-arabinosyl bromide according to Chavanne.[14] A mixture of 11.1 g. of the triacetyl-D-arabinosyl bromide, 12.6 g. of IX, 2.0 g. of Drierite and 110 ml. of dry benzene was shaken in a dark bottle for three days. The benzene solution obtained after centrifuging the silver salts off was concentrated to a thick sirup *in vacuo*. The product could not be obtained in a well-crystallized form. It was obtained as a white amorphous powder by pouring a methanolic solution of the sirup into a large volume of water; yield 56%, m. p. 76–96 (gradually softening); $[\alpha]^{25}D$ +43.3° (*c*, 0.9, benzene). *Anal.* Calcd. for $C_{26}H_{24}O_{10}$: C, 62.90; H, 4.87. Calcd. for $C_{26}H_{24}O_{10}\cdot{}^1/_2H_2O$: C, 61.8; H, 4.95. Found: C, 62.1; H, 5.07.

Preparation of 3-Phenyl-4-hydroxycoumarin β-D-Mannoside Tetraacetate (XIII).—Pentaacetyl-β-D-mannose was converted to the acetyl-D-mannosyl bromide according to Micheel.[15] The product was not crystallized, but was used as a sirup. To 100 ml. of dry benzene in a dark bottle were added 9.9 g. of the sirupy tetraacetyl-D-mannosyl bromide, 9.3 g. of IX and 2.0 g. of Drierite. After shaking for three and one-half days the reaction mixture gave a negative test with alcoholic silver nitrate. The silver salts were removed as above, and the benzene solution was concentrated *in vacuo*. The product could not be crystallized but turned to a white amorphous powder on standing. The same powder could be obtained by pouring a methanolic solution of the sirup into water; yield 72%, m. p. 68–72°; $[\alpha]^{25}D$ +29.6° (*c*, 1.4, benzene). *Anal.* Calcd. for $C_{29}H_{28}O_{12}$: C, 61.35; H, 4.94. Calcd. for $C_{29}H_{28}O_{12}\cdot{}^1/_2H_2O$: C, 60.3; H, 5.39. Found: C, 60.39; H, 5.39.

When dried over phosphorus pentoxide at 56.5° and 2 mm. pressure for four days, 78.46 mg. of substance lost 1.10 mg. of water. Calcd. for $C_{29}H_{28}O_{12}\cdot{}^1/_2H_2O$: H_2O, 1.57. Found: H_2O, 1.40.

Methanolysis of 3-Phenyl-4-hydroxycoumarin β-D-Galactoside Tetraacetate (X).—To 7.5 g. of the galactoside dissolved in 600 ml. of absolute methanol[16] was added 3.0 ml. of 0.573 *N* barium methoxide. An aliquot was taken to find the rotation at zero time, −0.98°, and the rotation was followed polarimetrically. Mutarotation ceased after ten days. The final value was +1.24°. Complete conversion to methyl α-D-galactoside would give a rotation of +1.68°.

The reaction mixture was treated with enough 0.1 *N* sulfuric acid to remove the barium. The barium sulfate was removed by filtration through an asbestos mat, and the filtrate concentrated to 50 ml. *in vacuo*. The aglycon that crystallized out during the concentration was filtered off. An equal volume of water was added and the concentration was repeated. A second crop of VIII was obtained. A total of 2.83 g. (90%) was isolated, m. p. 235°.

The filtrate was concentrated to a sirup and a small volume of ethanol added. On scratching, 1.2 g. (47%) of methyl α-D-galactoside crystallized; m. p. 109°, with no depression when mixed with an authentic sample, $[\alpha]^{25}D$ +175.3° (*c*, 1.05, water). Reported for methyl α-D-galactoside, m. p. 110°, $[\alpha]^{20}D$ +179.3° (*c*, 9.1, water). The product was acetylated to the tetraacetate with acetic anhydride and pyridine; m. p. 87°; $[\alpha]^{27}D$ +135.0° (*c*, 0.95, chloroform). Reported for methyl α-D-galactoside tetraacetate, m. p. 87°; $[\alpha]^{20}D$ +132.5° (*c*, 2.9, chloroform).

Methanolysis of 3-Phenyl-4-hydroxycoumarin β-D-Xyloside Triacetate (XI).—To 7.2 g. of the xyloside dissolved in 380 ml. of absolute methanol was added 3.0 ml. of 0.573 *N* barium methoxide. An aliquot was taken to find the rotation at zero time, −3.72°, and the reaction was followed polarimetrically. Mutarotation stopped after seven weeks. The final value was +1.21°. Some of the aglycon that crystallized in the reaction flask was filtered off. Enough 0.1 *N* sulfuric acid was added to remove the barium. The salt was removed by filtration through an asbestos mat, and the filtrate concentrated to 50 ml. *in vacuo*. The aglycon that crystallized out was collected on a funnel. An equal volume of water was added and the solution again concentrated. A third crop of the aglycon was removed to make a total of 2.2 g. (94%), m. p. 235°. The filtrate was concentrated to a sirup which did not crystallize. A small portion of the sirup was acetylated with acetic anhydride and sodium acetate. The product was crystallized from 95% ethanol; m. p. 84°, reported for methyl α-D-xyloside triacetate, m. p. 86°.

Methanolysis of 3-Phenyl-4-hydroxycoumarin α-D-Arabinoside Triacetate (XII).—One gram of the arabinoside was dissolved in 100 ml. of absolute methanol. To the solution was added 0.75 ml. of 0.387 *N* barium methoxide. The rotation at the start was +0.21°. It changed slowly over a period of six months. When it reached +0.92° the rate of mutarotation was about 0.03° per week.

To the solution was added 3.03 ml. of 0.096 *N* sulfuric acid and the barium sulfate removed as above. The filtrate was concentrated to dryness, and the white residue suspended in 5 ml. of water. The aglycon was filtered off; yield 0.5 g., after one recrystallization m. p. 235°.

The filtrate was concentrated to dryness, and the sirup taken up in absolute methanol. After three weeks in the cold, the crystals were collected by filtration; yield 30 mg., m. p. 165–169°. After one recrystallization from methanol, m. p. 167–169°; no depression with an authentic sample of methyl β-D-arabinoside; $[\alpha]^{26}D$ −239.0° (*c*, 1, water); reported for methyl β-D-arabinoside, m. p. 168°, $[\alpha]^{18}D$ −241.1° (*c*, 1, water).

Methanolysis of 3-Phenyl-4-hydroxycoumarin β-D-Mannoside Tetraacetate (XIII).—One gram of XIII was dissolved in 100 ml. of absolute methanol. The polarimetric reading at zero time was +0.36°. To this solution was added 0.75 ml. of 0.387 *N* barium methoxide. The rotation changed to +0.46° in twenty minutes. The rotation did not change for the next five days. Another milliliter of 0.387 *N* barium methoxide was added but the rotation remained constant for the next five days.

An amount of 0.1 *N* sulfuric acid equivalent to the barium was added. The barium sulfate was removed as above, and the filtrate concentrated to dryness. The white powder obtained was suspended in a few milliliters

(11) Stone, *Am. Chem. J.*, **15**, 653 (1893).

(12) Hudson and Johnson, THIS JOURNAL, **37**, 2748 (1915).

(13) Hockett and Hudson, *ibid.*, **56**, 1632 (1934).

(14) Chavanne, *Compt. rend.*, **134**, 661 (1902).

(15) Micheel and Micheel, *Ber.*, **63**, 386 (1930).

(16) The absolute methanol was obtained by refluxing commercial (99.9%) methanol with magnesium turnings (10 g./l.) until the magnesium had reacted completely. The methanol was then distilled and the center fraction was collected in small bottles and stored for use.

of water and the aglycon filtered off; yield 0.2 g., 50%; m. p. 235° after one recrystallization from ethanol and water.

The aqueous filtrate was concentrated to dryness. The sirup was taken up in 1 ml. of methanol. Upon standing in the cold for a week, crystals formed; yield 64 mg., m. p. 184–188°, after one recrystallization from methanol m. p. 188°; no depression when mixed with an authentic sample of methyl α-D-mannoside; $[\alpha]^{28}D$ +86.7° (*c*, 1, methanol). Reported for methyl α-D-mannoside, m. p. 193–194°; $[\alpha]^{20}D$ +87.5° (*c*, 1, methanol).

Effect of Varying Concentrations of Barium Methoxide on the Methanolysis.—A solution of glucoside tetraacetate of VIII was made up to contain 3.33 g. per 100 ml. of methanol. To 10-ml. aliquots, 0.02, 0.04, 0.08, 0.16 and 0.32 ml. of 0.573 *N* barium methoxide was added. The rotation was followed until the change in ten minutes was less than 0.02°. The results are plotted in Fig. 3.

Summary

1. A series of glycosides of 3-phenyl-4-hydroxycoumarin (VIII) has been prepared by condensing the enol silver salt of the aglycon with the respective acetylglycosyl bromides.

2. The glycosides all undergo methanolysis when treated with catalytic amounts of barium methoxide in dry methanol. The mechanism appears to be the same in each case. A Walden inversion accompanies the cleavage. From the β-D-glycosides of VIII are formed methyl α-D-glycosides, and from the α-D-arabinoside of VIII methyl β-D-arabinoside is obtained.

3. An electronic mechanism, in which the electrophilic nature of VIII promotes electronic shifts which labilize the sugar–oxygen bond, is offered as a rationalization of the products of the alkaline cleavage.

MADISON, WISCONSIN RECEIVED JUNE 2, 1949

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF AGRICULTURE, UNIVERSITY OF WISCONSIN]

Alkaline Methanolysis of Theobromine β-D-Glucoside Tetraacetate[1]

BY CLINTON E. BALLOU AND KARL PAUL LINK

The observations of Huebner, *et al.*,[2] and Spero, *et al.*,[3] concerning the cleavage products formed during the attempted deacetylation of 3-phenyl-4-hydroxycoumarin D-glycoside tetraacetates, have stimulated an investigation of various other alkali labile glycosides. This paper is on the alkaline methanolysis of theobromine D-glucoside tetraacetate (I).

Fischer[4] first described the difficulty of deacetylating certain purine glucoside tetraacetates without cleaving the glucosidic linkage. From theobromine D-glucoside tetraacetate (I) in aqueous alkali, Fischer obtained theobromine and glucose (II). Due to the sensitivity of this glucoside to aqueous acid and alkali, he postulated the glucosidic linkage as being to position 2 or 6 of the enol form of the purine residue.

In our studies of the cleavage of alkali sensitive glycosides the reactions are run in dry methanol[5] in the presence of catalytic amounts of barium methoxide. Under these conditions cleavage involves a methanolysis of the glycosidic linkage, and in those cases reported[2,3] a methyl glycoside and the free aglycon are formed. The cleavage is accompanied by a Walden inversion, and the products formed suggest a mechanism for the cleavage.

According to the mechanism advanced by Isbell,[6] the cleavage anion may add to either the sugar residue or the aglycon, depending upon the shift of electrons between these two parts of the molecule. During the cleavage of the glycosidic linkage under the influence of basic catalysts, a cationoid center is derived from either the sugar residue or the aglycon. If the cation comes from the sugar residue it may be attacked by an anion such as hydroxyl or methoxyl, to give the free sugar or a methyl glycoside. This sugar cation may also be attacked by an anion present within the sugar molecule with the formation of an inner anhydride. If the cationoid center derives from the aglycon it likewise may be attacked by an anion present, again hydroxyl or methoxyl, to give the free aglycon or a methoxyl derivative, or it may coördinate with an adjacent carbon atom to form a carbon–carbon double bond, a proton being expelled during the reaction.

The cleavage of 3-phenyl-4-hydroxycoumarin β-D-glucoside tetraacetate[2] with the formation of methyl α-D-glucoside represents a case in which the oxygen–sugar bond is split. This is indicated because the anion, in this reaction the methoxyl group, becomes attached to the sugar residue, and the configuration of the anomeric carbon atom changes from the β to the α form. Peat[7] has thoroughly reviewed this type of inversion as encountered in alkaline scission of anhydro sugar rings.

(1) Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation. This paper was presented before the Division of Sugar Chemistry and Technology at the 116th Meeting of the American Chemical Society, Atlantic City, September, 1949.

(2) Huebner, Karjala, Sullivan and Link, THIS JOURNAL, **66**, 906 (1944).

(3) Spero, Ballou and Link, *ibid.*, **71**, 3740 (1949).

(4) Fischer and Helferich, *Ber.*, **47**, 210 (1914).

(5) The dry methanol was obtained by refluxing commercial (99.9%) methanol with magnesium turnings (10 g./liter) until the magnesium had completely reacted. The methanol was then distilled and the center fraction was collected in small bottles and stored for use.

(6) Isbell, *Ann. Rev. Biochem.*, **XII**, 215 (1943).

(7) Peat, *Adv. in Carbohydrate Chem.*, Vol. 2, 45 (1946).