inductive effect. The net effect is the resonance stabilization of a partial positive charge on carbon atoms 7 and 6 and a weakening of the carbon–oxygen bond during reduction. The result is hydrogenolysis.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

4-Deoxy-3-oxo-D-*glycero*-2-hexulose, the Dicarbonyl Intermediate in the Formation of D-Isosaccharinic Acids[1]

BY ROY L. WHISTLER AND J. N. BEMILLER

RECEIVED NOVEMBER 20, 1959

An intermediate in the formation of D-isosaccharinic acids is isolated. Evidence that the isolated compound is 4-deoxy-3-oxo-D-*glycero*-2-hexulose is given by its oxidation to glycolic acid and 2-deoxy-D-*glycero*-tetronic acid and by the formation of the typical α-dioxo color reaction.

Knowledge of the mechanism by which saccharinic acids are produced through alkaline rearrangement of sugars has accumulated gradually over a period of time. Saccharinic acid formation by recombination of sugar fragments was discussed by Kiliani[2] and Windaus.[3] Nef[4] made the first extensive proposal of a reaction mechanism by suggesting an isomerization of sugar enediols and epoxy compounds to an α-dicarbonyl compound which would undergo a benzilic acid type of rearrangement to a saccharinic acid. Evans and co-workers[5] suggested that the initial isomerization might be effected through unsaturated oxide compounds but believed as others that the final step was a rearrangement of an α-dicarbonyl compound. Isbell[6] put the mechanism on a modern basis and showed that the α-dicarbonyl compound could be formed by consecutive electron-displacement reactions. Kenner and co-workers[7] demonstrated that the position of substitution on a sugar unit determined the acid which would be formed and that 4-*O*-substitution[7b] resulted in the formation of isosaccharinic acid through elimination of the substituent group as an alkoxy or glycosyloxy anion. This refinement of Isbell's proposal has been popularly known as the *β-alkoxy carbonyl mechanism* because elimination required a carbonyl group in the β-position to an etheric or glycosidic linkage. When a 4-*O*-substituted sugar is dissolved in an alkaline solution, this positioning occurs through a preliminary Lobry de Bruyn–Alberda van Ekenstein isomerization.[8]

Blears, Machell and Richards[9] obtained evidence of an intermediate in D-isosaccharinic acid formation by the appearance of a new spot on paper chromatograms when 4-*O*-substituted-D-glucose derivatives were degraded in 0.05 *N* sodium hydroxide solutions instead of lime water. This compound was removed from the reaction mixture by formation of the insoluble bis-2,4-dinitrophenylhydrazone (m.p. 249°). They suggested that it was the postulated dicarbonyl intermediate.

Two of these same authors have obtained indirect evidence for the formation of the dicarbonyl compound during the alkaline degradation of 4-*O*-substituted-D-glucose units by the detection of glycoaldehyde,[9] 2-deoxy-D-*glycero*-tetrose[9] and glycolic and 2-deoxy-tetronic acids[10] after the action of dilute sodium hydroxide solutions, both at boiling and room temperatures. It is suggested by these authors[9] that glycolic and 2-deoxy-D-tetronic acids are formed by an alkaline cleavage of the α-dicarbonyl system similar to that observed with benzil when the benzilic acid rearrangement is inhibited.[11]

In continuation of work in this Laboratory on the alkaline degradation of polysaccharides, a substance is now isolated from cellobiose (I) after the action of oxygen-free potassium hydroxide solution, which apparently is an intermediate in the formation of D-isosaccharinic acids and has properties leading us to believe it is the dicarbonyl compound, 4-deoxy-3-oxo-D-*glycero*-2-hexulose (II).[11a] Isolation of this compound is accomplished after stopping isomerization by neutralization. If lime water is used as the alkaline solution, the benzilic acid type of rearrangement to D-isosaccharinic acids occurs so rapidly that no intermediate can be isolated.

The combined information presented here gives support to the generally accepted belief that calcium ions specifically catalyze the formation of

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[1] Journal Paper No. 1528 of the Purdue Agricultural Experiment Station.

[2] H. Kiliani and S. Kleemann, *Ber.*, **17**, 1302 (1884).

[3] A. Windaus, *Chem. Ztg.*, **29**, 564 (1905).

[4] J. U. Nef, *Ann.*, **357**, 214 (1907); **376**, 1 (1910).

[5] W. L. Evans, R. H. Edgar and G. P. Hoff, THIS JOURNAL, **48**, 2665 (1926).

[6] H. S. Isbell, *J. Research Natl. Bur. Standards*, **32**, 45 (1944).

[7] (a) W. M. Corbett and J. Kenner, *J. Chem. Soc.*, 2245 (1953); (b) J. Kenner and G. N. Richards, *ibid.*, 278 (1954).

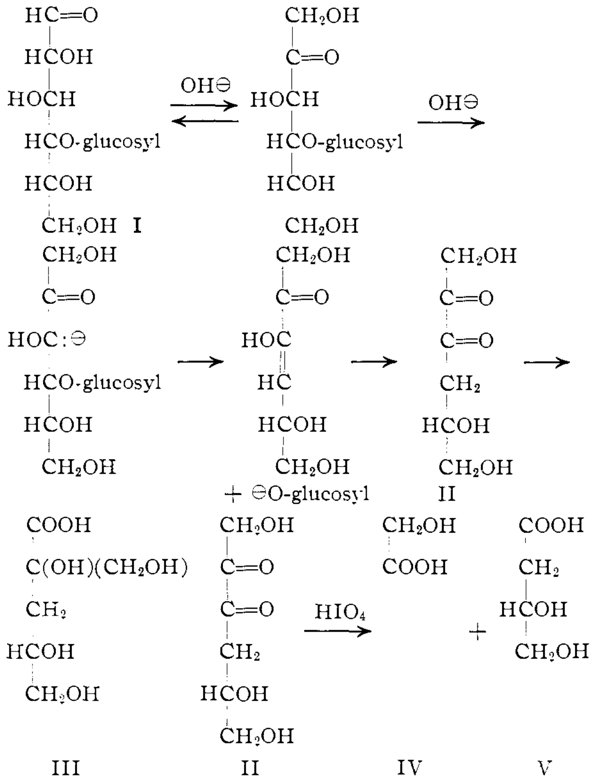
[8] C. A. Lobry de Bruyn and W. Alberda van Ekenstein, *Rec. trav. chim.*, **14**, 203 (1895); **16**, 262 (1897).

[9] M. J. Blears, G. Machell and G. N. Richards, *Chemistry & Industry*, 1150 (1957).

[10] G. Machell, G. N. Richards and H. H. Sephton, *ibid.*, 467 (1957); G. N. Richards and H. H. Sephton, *J. Chem. Soc.*, 4492 (1957); G. Machell and G. N. Richards, *ibid.*, 1199 (1958).

[11] E. Pfeil, G. Geissler, W. Jacquemin and F. Lömker, *Ber.*, **89** 1210 (1956).

[11a] Since this paper was submitted for publication, E. F. L J. Anet, THIS JOURNAL, **82**, 1502 (1960), has reported briefly the isolation of 3-deoxy-D-*erythro*-hexosone (3-deoxy-2-oxo-D-*erythro*-hexose); the dicarbonyl intermediate in the formation of D-glucometasaccharinic acids, and 3-deoxy-D-*threo*-hexosone (3-deoxy-2-oxo-D-*threo*-hexose), the dicarbonyl intermediate in the formation of D-galactometasaccharinic acids. The rapid conversion of each by lime water to the corresponding saccharinic acids further substantiates the accepted mechanism.



saccharinic acids[12] and that sodium and potassium hydroxide solutions afford more complex mixtures of acids than does lime water. This is likely because calcium ions rapidly catalyze the formation of saccharinic acids from the dicarbonyl compound before alkaline scission of the dicarbonyl can occur.

Maltose and lactose appear chromatographically to give the same intermediate, but, with both of these sugars, isomerization is so rapid that the intermediate is difficult to isolate.

It is observed that 4-deoxy-3-oxo-D-*glycero*-2-hexulose (4-deoxy-2,3-dioxo-D-*glycero*-hexitol) (II), the dicarbonyl intermediate in the formation of D-isosaccharinic acids (III), forms a bis-2,4-dinitrophenylhydrazone, and yields an α-dioxime which forms a typical orange, water-insoluble, inner complex nickel salt.[13] It rearranges easily to D-isosaccharinic acids (III); crystalline "α"-D-isosaccharino-1,4-lactone has been isolated. It can be oxidized with an equivalent amount of periodic acid[14] to glycolic acid (IV) and 2-deoxy-D-*glycero*-tetronic acid (V), each of which have been identified by crystalline derivatives.

Its ready rearrangement to D-isosaccharinic acids suggests that the final step in saccharinic acid production is not the rate-controlling step.

Experimental

Chromatography.—Qualitative paper chromatography was done on Whatman No. 1 paper at 25° using irrigants A, 1-butanol–ethanol–water (40:11:19 v./v.), and B, ethyl acetate–acetic acid–water (100:13:11 v./v.). For preparative work Whatman No. 3 paper and irrigant A were used.

Detection methods were (1) the silver nitrate dip–ethanolic sodium hydroxide spray method of Trevelyan and coworkers[15] and (2) a spray method for α-dioxo compounds which was adapted from the spot test[13] and is dependent on the formation of an orange, water-insoluble, inner complex nickel salt of the α-dioxime prepared from the α-dioxo compound. The paper chromatogram was sprayed with a filtered solution of 50 g. of hydroxylamine hydrochloride and 50 g. of sodium acetate in 100 ml. of water. While wet, the paper was placed in a 110° oven for 5 min. The dry paper was then sprayed with a 5% solution of nickel nitrate. The dicarbonyl compound was indicated as an orange spot.

Preparation of 4-Deoxy-3-oxo-D-glycero-2-hexulose.—Five grams of cellobiose was dissolved in 500 ml. of oxygen-free 0.05 *N* potassium hydroxide solution at 25°. Aliquots from preliminary reactions at this temperature, when analyzed chromatographically, indicated that maximum content of dicarbonyl compound was reached in about 15 hr. Hence, after 15 hr. of reaction time, the reaction mixture was freed of potassium ions by passing the solution through Amberlite IR-120(H) cation exchange resin. The then slightly acidic solution was neutralized with barium carbonate and concentrated to dryness. The residue was extracted successively for 3 1-hr. periods by shaking with 100-ml. portions of 95% ethanol. The ethanol extracts were concentrated to dryness; the residue was dissolved in water, and the dicarbonyl compound was separated from other solution components by paper chromatography. The yield was about 85 mg. of a sirup with an $[\alpha]^{25}\text{D} + 11°$ (*c* 0.7, water).

When examined chromatographically with irrigant A, this compound had a R_{glucose} value of 1.8 and a R_f value of 0.5. The silver nitrate spray reagents[15] indicated that it is easily reduced.

The bis-2,4-dinitrophenylhydrazone was prepared by treating the sirup with a saturated solution of 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid. The crystals which separated were recrystallized from ethanol–water. The recrystallized material had a decomposition point of 240° and a $[\alpha]^{25}\text{D} + 26°$ (*c* 0.4, water).

Anal. Calcd. for $C_{18}H_{18}O_{11}N_8$: N, 21.4. Found: N, 21.1.

Isomerization to Isosaccharinic Acid.—When a water solution of the α-dicarbonyl compound was held at 35° overnight, "α"- and "β"-D-isosaccharinic acids were formed as indicated chromatographically. If 0.05 *N* potassium hydroxide solution was used to dissolve the sirup, no dicarbonyl compound could be detected after an hour.

When a saturated calcium hydroxide solution was added to the sirup, the rearrangement took place almost instantly, and calcium "α"-D-isosaccharinate crystallized from the solution kept at 4° for 24 hr. It was removed by filtration and redissolved in hot water. Calcium ions were removed from the solution with Amberlite IR-120(H) cation exchange resin. The acidic solution was concentrated to dryness and heated for lactonization. The residue was dissolved in hot ethyl acetate from which crystals of "α"-D-isosaccharino-1,4-lactone separated upon cooling, m.p. and mixed m.p. 96°.[4,16]

Oxidation of 4-Deoxy-3-oxo-D-*glycero*-2-hexulose.—Fifty mg. of the sirup was oxidized with an equivalent amount of periodic acid[14] at 4° for 3 hr. At the end of this period, the small amount of unused periodic acid was destroyed and the mixture was examined chromatographically with irrigant B. Three components were detected by brom thymol blue and permanganate–periodate[17] spray reagents: one which had a R_l[18] value of 0.7 and a R_f value of 0.4 (values identical to those of glycolic acid), a second with a R_l[18] value of 1 and a R_f value of 0.6 (thought to be the 1,4-lactone of 2-deoxy-D-*glycero*-tetronic acid), and a third minor component with an R_l[18] value of 0.6 and a R_f value of 0.3 (thought to be the free 2-deoxy-D-*glycero*-tetronic acid).

Glycolic Acid.—Glycolic acid was isolated by paper chromatography and identified as its 4-bromophenacyl ester, m.p. and mixed m.p. 138°.[19]

2-Deoxy-D-*glycero*-tetronic acid was isolated by paper chromatography as its lactone. An aqueous solution equili-

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[12] See for example, J. Kenner and G. N. Richards, *J. Chem. Soc.*, 3019 (1957).

[13] F. Feigl, "Spot Tests in Organic Analysis," Elsevier Publishing Co., London, 5th English Ed., 1956, p. 200.

[14] P. W. Clutterbuck and F. Reuter, *J. Chem. Soc.*, 1467 (1935).

[15] W. E. Trevelyan, D. P. Parker and J. S. Harrison, *Nature*, **166**, 444 (1950).

[16] L. Cuisinier, *Monit. sci. Docteur Quesneville* [3] **12**, 521 (1882), *Bull. soc. chim.* (*France*), [2] **38**, 512 (1882).

[17] R. U. Lemeiux and H. F. Bauer, *Anal. Chem.*, **26**, 920 (1954).

[18] R_l is the rate of movement relative to lactic acid.

[19] W. L. Judefind and E. E. Reid, THIS JOURNAL, **42**, 1043 (1920).

brated at 24° had an $[\alpha]^{25}D$ −8° (*c* 3.1, water).[20] The sirupy acid was identified by conversion to its crystalline phenyl hydrazide, m.p. 98°.[20]

Acknowledgment.—The authors gratefully acknowledge a grant from the Department of Health, Education, and Welfare which supported a part of this work.

LAFAYETTE, IND.

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(20) J. W. E. Glattfeld and G. E. Miller, THIS JOURNAL, **42**, 2314 (1920).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, WASHINGTON UNIVERSITY]

The Configuration of "α"-D-Glucosaccharinic Acid: 2-C-Methyl-D-*ribo*-pentonic Acid

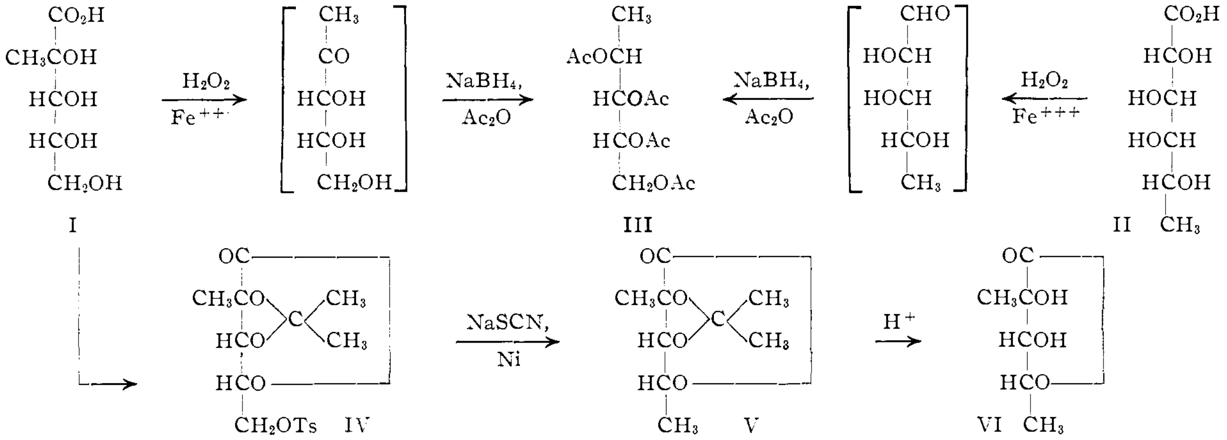
BY JOHN C. SOWDEN AND DONALD R. STROBACH

RECEIVED DECEMBER 14, 1959

"α"-D-Glucosaccharinic acid has been converted, by Ruff degradation followed by reduction and acetylation, to 1-deoxy-D-arabinitol tetraacetate. Thus, the partial configuration of the acid at C-3 and C-4 is D-*erythro* and, since the partial configuration at C-2 and C-3 had been previously established as *erythro*, the acid is 2-*C*-methyl-D-*ribo*-pentonic acid.

Although "α"-D-glucosaccharinic acid was shown many years ago by Kiliani to be a 2-*C*-methyl-pentonic acid,[1] its configuration has not been rigorously established up to the present time.

Prior to the present work, the accumulated evidence bearing on the configuration of "α"-D-glucosaccharinic acid included the following. Nef[2] concluded, on the basis of his theory of the mechanism of saccharinic acid formation, that the acid was limited to the D-*arabino* or D-*ribo* configurations. Of these, he chose the former because of qualitative similarities between certain derivatives of the saccharinic acid and the corresponding derivatives of D-arabinonic acid. Isbell's modification[3] of the Nef mechanism did not change the conclusion that the saccharinic acid must possess either the D-*arabino* or D-*ribo* configuration, but the recent observation[4] that "α"-D-glucosaccharinic lactone forms a 2,3-*O*-isopropylidene derivative supports the assignment of the D-*ribo* configuration rather than the D-*arabino* configuration chosen by Nef. Adding uncertainty to either choice, however, was the fact that tracer experiments[4b] have revealed that fragment recombination plays an important role in the formation of "α"-D-glucosaccharinic acid and, hence, that the Nef–Isbell mechanism is not a safe basis for assigning configuration in this instance. Accordingly, a direct proof of the configuration at C-3 and C-4 of the acid was undertaken.



"α"-D-Glucosaccharinic acid (I) was converted by a Ruff degradation[5] to a sirupy product which, after reduction with sodium borohydride and acetylation, provided 1-deoxy-D-arabinitol tetraacetate (III). For comparison, the latter compound also was prepared unequivocally by applying the same sequence of reactions to D-fuconic acid (II). The products from the two sources were identical in melting point, optical rotation and X-ray diffraction pattern. Thus, the partial configuration of "α"-D-glucosaccharinic acid at C-3 and C-4 is D-*erythro* and, in view of the structure of its acetonated lactone, the acid is 2-*C*-methyl-D-*ribo*-pentonic acid.

The assignment of the *erythro* configuration to C-2 and C-3 of "α"-D-glucosaccharinic acid is based on the assumption that the hydroxyl groups at these positions must be *cis* to each other in the lactone to allow the formation of a cyclic ketal. This assumption is particularly acceptable if 2,3-*O*-isopropylidene-D-glucosaccharin is a γ-lactone. Accordingly,

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(1) H. Kiliani, *Ber.*, **15**, 701, 2953 (1882); *Ann.*, **218**, 361 (1883). For a review of saccharinic acid chemistry, see J. C. Sowden, *Adv. in Carbohydrate Chem.*, **12**, 35 (1957).

(2) J. U. Nef, *Ann.*, **376**, 1 (1910).

(3) H. S. Isbell, *J. Research Natl. Bur. Standards*, **32**, 45 (1944).

(4) (a) L. M. Utkin and G. O. Grabilina, *Doklady Akad. Nauk (S.S.S.R.)*, **93**, 301 (1953); *C. A.*, **48**, 12676 (1954); (b) J. C. Sowden, M. G. Blair and D. J. Kuenne, THIS JOURNAL, **79**, 6450 (1957).

(5) O. Ruff, *Ber.*, **31**, 1573 (1898).