Anal. Calcd. for $C_{21}H_{28}N_2O_{11}$: C, 52.06; H, 5.82; N, 5.78. Found: C, 52.43; H, 6.00; N, 5.97.

1-Ethyl-6-methyl-2(1),4(3)-pyrimidonedione.—Following isolation of the acetyl glucosides the sirupy residue was subjected to methanolysis with an excess of dry hydrogen chloride for three days at room temperature. Evaporation of the excess methanol, addition of water and cooling resulted in the precipitation of 2.5 g. of impure 6-methyluracil. Evaporation of the solvent a second time and the addition of ethanol brought down another 1.5 g. of 6-methyluracil. When the residual solution was cooled 0.4 g. of a compound was deposited which after treatment with Darco G-60 and recrystallization from ethanol melted at 197–198° and analyzed correctly for an ethyl-substituted 6-methyluracil. An additional 0.3 g. of this compound was extracted with chloroform from the impure 6-methyluracil. The melting point of the compound agreed with the literature value for 1-ethyl-6-methyluracil.[33] A sample of 1-ethyl-6-methyluracil prepared by treating V with ethyl iodide had the same ultraviolet absorption spectrum (Table I) and melting point, and the mixed melting point with the compound we isolated showed no depression.

Anal. Calcd. for $C_7H_{10}N_2O_2$: N, 18.17. Found: N, 18.09.

4-Ethoxy-6-methyl-2(1)-pyrimidone.—One gram of the α-form of IX was refluxed for 19 hr. in 40 ml. of 90% ethanolic 0.3 *N* sodium hydroxide. Excess solvent was evaporated and the residue was decolorized with Darco G-60 in ethanol. Evaporation of the solvent a second time and the addition of a few ml. of water resulted in a white crystalline precipitate (0.23 g.). After two recrystallizations the compound sintered at 190° and melted at 196°. It contained no sugar. Analysis confirmed the presence of an ethoxyl group. Hydrolysis of the compound in dilute hydrochloric acid yielded 6-methyluracil. Depression of mixed melting points with authentic samples of 2-ethoxy-6-methyl-4(3)-pyrimidone[34] and of 1-ethyl-6-methyl-2,4(1,3)-pyrimidonedione and comparisons of the ultraviolet absorption spectra of the three isomers (Table I) confirmed the assignment of structure.

With the exception of 6-methyluracil this compound was the only product isolated from the reaction of V with the tetra-O-acetyl-α-D-glycopyranosyl chlorides of galactose, arabinose and xylose.

(33) O. Hoebel, *Ann.*, **353**, 242 (1907).

(34) W. M. Bruce, THIS JOURNAL, **26**, 449 (1904).

Anal. Calcd. for $C_7H_{10}N_2O_2$: C, 54.53; H, 6.54; N, 18.17; ethoxyl, 29.14. Found: C, 54.07; H, 6.54; N, 18.05; ethoxyl, 34.55.

4-Tetra-O-acetyl-α-D-glucopyranosyloxy-2-ethylthio-6-methylpyrimidine.—Fifteen grams of VI and 15 g. of tetra-O-acetyl-α-D-glucopyranosyl bromide were heated at 80° for 26 hr. Upon the addition of ether there were precipitated 0.25 g. of 6-methyluracil and 0.9 g. of crude 2-ethylthio-6-methyl-4(3)-pyrimidone. After two more days of heating the remaining reaction mixture at 80°, the addition of ether resulted in the precipitation of 3.7 g. of a compound which melted at 118°. Decolorization with Norite and two recrystallizations from ethanol gave a product which melted at 125–126° and had an optical rotation $[\alpha]^{30}D$ +102.3 (*c* 0.5 in chloroform). Fehling solution was reduced after 5 minutes heating in a boiling water-bath. Recovery of an additional 0.3 g. of the α-isomer following the isolation of the β-isomer (see below) raised the yield to 4.0 g. (22%).

Anal. Calcd. for $C_{21}H_{28}N_2O_{10}S$: N, 5.59. Found: N, 5.44.

4-Tetra-O-acetyl-β-D-glucopyranosyloxy-2-ethylthio-6-methylpyrimidine.—The reaction mixture remaining after the α-glucoside had precipitated was heated at 80° for an additional two days. Addition of ether brought down a precipitate which was freed of traces of I by dissolving it in chloroform in which I is insoluble. Evaporation of the chloroform left a residue which when dissolved in ethanol, decolorized with Darco G-60 and placed in a refrigerator yielded 0.4 g. of the β-glucoside, m.p. 170°. After recrystallization the melting point was 176–177° and the optical rotation $[\alpha]^{30}D$ −3.0° (*c* 0.5 in chloroform). Both analysis and ultraviolet absorption spectrum (Table I) were like the α-isomer, but the reduction of Fehling solution required 15 minutes heating on a water-bath.

Anal. Calcd. for $C_{21}H_{28}N_2O_{10}S$: N, 5.59. Found: N, 5.53.

Acknowledgments.—We are indebted to Drs. Stanley J. Cristol and John S. Meek of the Department of Chemistry, University of Colorado, for suggestions during this study. To Mrs. Patricia Ramey we are grateful for assistance in the preparation of intermediates and for nitrogen analyses.

LAWRENCE, KANSAS

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, WASHINGTON UNIVERSITY]

The Isomerization of C^{14}-Labeled Sugars to Saccharinic Acids[1]

BY JOHN C. SOWDEN, MARY GRACE BLAIR AND DOROTHY J. KUENNE

RECEIVED JULY 17, 1957

D-Galactose-1-C^{14}, lactose-1-C^{14} and D-mannose-1-C^{14} have been isomerized by treatment with lime water, respectively to "α"-D-galactometasaccharinic acid, "α"-D-isosaccharinic acid and "α"-D-glucosaccharinic acid. The observed distribution of label in the former two products is in agreement with the Nef–Isbell intramolecular mechanism for their formation. The pattern and relative distribution of the label found in the "α"-D-glucosaccharinic acid indicate that fragment recombination is a predominant feature in its formation from the monosaccharide.

Two general mechanisms have been proposed for the formation of saccharinic acids by the action of alkali on reducing sugars.[2] These are (1) recombination of appropriate fragments directly to the saccharinic acids[3,4] and (2) intramolecular isomerization involving, as the final step, the benzilic acid type of rearrangement of α-dicarbonyl intermediates to the saccharinic acids.[5–7] Obviously a mechanism involving fragment recombination followed by isomerization is also possible. Recent extensive studies[2] by Kenner and his associates on saccharinic acid formation from substituted monosaccharides (including oligo- and polysaccharides) have provided strong experimental support for the Isbell intramolecular isomerization mechanism.[7] The present study, in which certain aldose sugars labeled in C-1 with carbon-14 were isomerized to

(1) For a preliminary communication concerning part of this work, see J. C. Sowden and D. J. Kuenne, THIS JOURNAL, **75**, 2788 (1953).

(2) For a general review of the saccharinic acids, including theories of the mechanism of their formation, see J. C. Sowden, "Adv. in Carbohydrate Chem.," **12**, 35 (1957).

(3) H. Kiliani and S. Kleemann, *Ber.*, **17**, 1296 (1884).

(4) A. Windaus, *Chem. Ztg.*, **29**, 564 (1905).

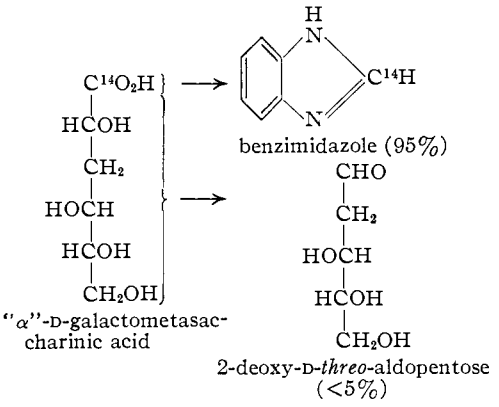
(5) J. U. Nef, *Ann.*, **357**, 214 (1907); **376**, 1 (1910).

(6) W. L. Evans and M. P. Benoy, cited in W. L. Evans, R. H. Edgar and G. P. Hoff, THIS JOURNAL, **48**, 2665 (1926).

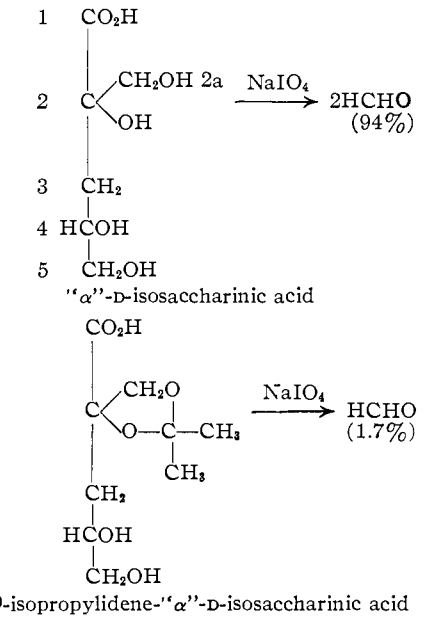
(7) H. S. Isbell, *J. Research Natl. Bur. Standards*, **32**, 45 (1944).

saccharinic acids and the relative distribution of the label in the products determined, was designed to provide direct experimental evidence on the mechanism or mechanisms involved.

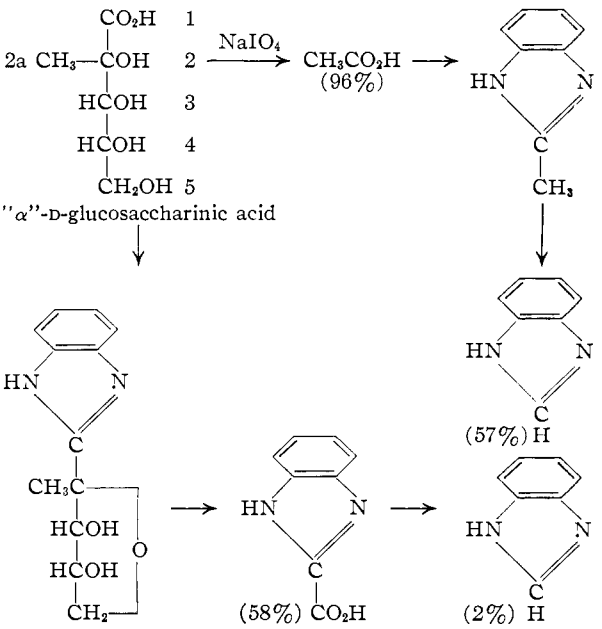
D-Galactose-1-C^{14} was isomerized by lime water at room temperature according to the directions of Kiliani and Naegeli.[8] The resulting "α"-D-galactometasaccharinic acid was degraded[8] by the Ruff method to 2-deoxy-D-*threo*-aldopentose and the latter was observed to contain less than 5% of the original radioactivity of the D-galactose-1-C^{14}. Condensation of the saccharinic acid with *o*-phenylenediamine, followed by oxidation to benzimidazole-2-carboxylic acid and decarboxylation of the latter, yielded benzimidazole containing approximately 95% of the original radioactivity of the D-galactose-1-C^{14}. Since this benzimidazole contained only C-1 of the saccharinic acid, the results are in essential agreement with the Nef–Isbell intramolecular mechanism.



Lactose-1-C^{14} was isomerized with lime water at room temperature[9] and the resulting "α"-D-isosaccharinic acid was isolated as the slightly soluble calcium salt. Oxidation of the saccharinic acid with sodium metaperiodate provided formaldehyde, from C-2a plus C-5, that contained approximately 94% of the original, total radioactivity. Acetonation of the "α"-D-isosaccharinic lactone yielded the 2,2a-*O*-isopropylidene derivative. Titration of the latter with sodium hydroxide to open the lactone ring, followed by oxidation with sodium metaperiodate, yielded formaldehyde from C-5 that was found to contain only 1.7% of the original radioactivity. The bulk of the radioactivity thus resides in C-2a of the isosaccharinic acid, in essential agreement with the prediction of the Nef–Isbell mechanism.



D-Mannose-1-C^{14} was isomerized by lime water at room temperature[10] to "α"-D-glucosaccharinic acid. Oxidation of the latter with sodium metaperiodate provided acetic acid from C-2 and C-2a containing 96% of the original, total radioactivity. The acetic acid was degraded, *via* 2-methylbenzimidazole, 2-styrylbenzimidazole and benzimidazole-2-carboxylic acid, to benzimidazole. The latter, which contained only C-2 of the saccharinic acid, showed 57% of the original radioactivity. Thus, the distribution of label between C-2 and C-2a, respectively, of the saccharinic acid is 57 and 39%. To confirm this result, the "α"-D-glucosaccharinic acid was converted *via* its anhydrobenzimidazole derivative to benzimidazole-2-carboxylic acid. This derivative, which contained C-1 and C-2 of the saccharinic acid, showed 58% of the original radioactivity. Decarboxylation of the benzimidazole-2-carboxylic acid yielded benzimidazole, which contained only C-1 of the saccharinic acid and showed only 2% of the original radioactivity.



Shortly after the initial report[1] of our results with "α"-D-glucosaccharinic acid, Kenner and Richards[11] offered the explanation that cleavage of D-mannose-1-C^{14} at C-3–C-4, to provide dihydroxyacetone (from C-1, C-2 and C-3) and D-glycerose (from C-4, C-5 and C-6), followed by realdolization, would provide hexose-1,3-C^{14}. The latter

(8) H. Kiliani and H. Naegeli, *Ber.*, **35**, 3528 (1902).
(9) H. Kiliani, *ibid.*, **18**, 631 (1885).
(10) H. Kiliani, *ibid.*, **15**, 2953 (1882).
(11) J. Kenner and G. N. Richards, *J. Chem. Soc.*, 1784 (1954).

would then yield, *via* the Nef–Isbell mechanism, "α"-D-glucosaccharinic acid labeled at C-2 and C-2a. This explanation is attractive, since it would account for the observed positions of labeling in our product. However, this suggested route could place a maximum of only 50% of the label in C-2 of the saccharinic acid. Moreover, any accompanying isomerization of hexose-1-C^{14} directly to the saccharinic acid, without prior cleavage and recombination, would be reflected in the appearance of more than 50% of the label in C-2a and a corresponding reduction below 50% of the label in C-2. Thus, the Kenner and Richards explanation cannot account for the appearance of considerably more label at C-2 than at C-2a of the saccharinic acid, an experimental result that we believe to be completely trustworthy. It can only be concluded at present that, although fragment recombination is a predominant feature of the formation of "α"-D-glucosaccharinic acid from unsubstituted hexoses, the precise nature of the recombination step is not yet known.

The monoisopropylidene derivative[12] of "α"-D-glucosaccharinic lactone, after titration with base to open the lactone ring, consumed one molecular equivalent of periodate with the production of formaldehyde. Hence, the derivative is 2,3-*O*-isopropylidene-"α"-D-glucosaccharinic lactone and the parent acid appears to have the D-*ribo* configuration,[2] rather than the D-*arabo* configuration assigned by Nef[5] on the basis of optical rotation studies.

Experimental

Radioactive Sugars and Assay Method.—D-Mannose-1-C^{14} was prepared from D-arabinose by the nitromethane synthesis.[13] D-Galactose-1-C^{14} and lactose-1-C^{14} were obtained from the National Bureau of Standards through the courtesy of Dr. Horace S. Isbell.

All radioassays were made directly on thin layers (75–100 μg./sq. cm.) of the individual substances, mounted by spreading with appropriate solvents on stainless steel planchettes, in an RCL Nucleometer.[14] The recorded values in each instance are averages of several successive determinations agreeing within ±3%.

D-Galactose-1-C^{14} and Lime Water. "α"-D-Galactometasaccharinic Lactone.—A solution of 75 g. of D-galactose, containing 91.3 μc. of D-galactose-1-C^{14}, in 750 ml. of water was added to 37.5 g. of calcium hydroxide. The resulting mixture was allowed to stand under nitrogen, with occasional swirling, in a stoppered flask at room temperature for 25 days. The reaction mixture was then filtered, the precipitate was washed with hot water, and the combined filtrate and washings were saturated with carbon dioxide. The resulting precipitate was removed by filtration and an aliquot of the filtrate was analyzed for calcium. The latter was precipitated from the main filtrate with a slight excess of oxalic acid, the calcium oxalate was removed by filtration, and the filtrate was concentrated at reduced pressure and a final temperature of 85° to a sirup. The sirup was dissolved in 95% ethanol, decolorized, and the solution again concentrated to a sirup. Seeding[15] with "α"-D-galactometasaccharinic lactone then produced 4.5 g., and successive smaller crops, of the crystalline lactone.[16] After recrystallization from 95% ethanol, the product showed m.p. 142–143° and $[\alpha]^{25}D$ −47.8° in water, *c* 1.

(12) L. M. Utkin and G. O. Grabilina, *Doklady Akad. Nauk* (*S. S. S. R.*), **93**, 301 (1953); *C. A.*, **48**, 12076 (1954).

(13) J. C. Sowden, *J. Biol. Chem.*, **180**, 55 (1949).

(14) A product of Radiation Counter Laboratories, Chicago, Ill.

(15) Seeding crystals were obtained by column chromatography of a sample of the crude lactone mixture, that had been thoroughly extracted with ether to remove lactic acid, on silicic acid using benzene–methanol (8:1) as the elution solvent.

(16) H. Kiliani, *Ber.*, **16**, 2625 (1883).

2-Deoxy-D-*threo*-pentose Benzylphenylhydrazone.—A solution of 0.5 g. of the above lactone in 10 ml. of water was boiled briefly with 0.13 g. of calcium hydroxide. After cooling, 80 mg. of barium acetate monohydrate and 50 mg. of hydrated ferric sulfate (Mallinckrodt) were added, and the solution was again boiled briefly. To the cooled (40°) solution was added 0.5 ml. of 30% hydrogen peroxide, followed after 45 minutes by an additional 0.5 ml. of the oxidant. After an additional 45 minutes, the solution was decolorized, deionized and concentrated at reduced pressure to a sirup. The latter was dissolved in 4 ml. of 95% ethanol and treated with 0.6 ml. of benzylphenylhydrazine and 0.5 ml. of water. After 24 hours at room temperature, the solution was concentrated in a stream of dry air. Trituration of the resulting residue with petroleum ether (b.p. 30–60°) yielded crystals of the benzylphenylhydrazone of 2-deoxy-D-*threo*-pentose. After recrystallization from ethanol–ether–petroleum ether, ethanol–water and, finally, acetone–petroleum ether, the hydrazone[17] showed m.p. 114–116° and $[\alpha]^{25}D$ +12.5° in pyridine, *c* 3.4.

Benzimidazole from "α"-D-Galactometasaccharinic Lactone.—A mixture of 300 mg. of the saccharinic lactone, 350 mg. of *o*-phenylenediamine dihydrochloride, 1.3 ml. of water and 0.3 ml. of ethanol was heated in an open test-tube, in an oil-bath, at 135° for 2 hours. The resulting product was isolated and purified by way of the copper salt, as recommended for water-soluble benzimidazole derivatives,[18] to provide 2-(D-*xylo*-1,3,4,5-tetrahydroxypentyl)-benzimidazole. After recrystallization from absolute ethanol, the latter showed m.p. 186–187° and $[\alpha]^{25}D$ +36° in water, *c* 2.

Anal. Calcd. for $C_{12}H_{16}O_4N_2$: C, 57.2; H, 6.40. Found: C, 57.1; H, 6.53.

A solution of 200 mg. of the above benzimidazole derivative, 70 mg. of sodium carbonate and 1 g. of potassium permanganate in 40 ml. of water was heated for 30 minutes at 100°. Excess permanganate was destroyed with a few drops of ethanol and the solution was decolorized, filtered, and concentrated to a volume of about 5 ml. Acidification with acetic acid then precipitated 45 mg. of benzimidazole-2-carboxylic acid dihydrate, m.p. 174° dec. The acid was decarboxylated to benzimidazole by heating it gradually in an open test-tube, in an oil-bath, from 140 to 195°. The benzimidazole, which sublimed to the upper surfaces of the test-tube during the heating operation, showed m.p. 171–172°.

Radioassay data for the various products described above are given in Table I.

TABLE I

DISTRIBUTION OF RADIOACTIVITY IN THE SACCHARINIC ACIDS FROM THE ACTION OF LIME WATER ON D-GALACTOSE-1-C^{14}, LACTOSE-1-C^{14} AND D-MANNOSE-1-C^{14}

Sample	Carbon atoms	Radioactivity, cts./min./mM × 10^{-3}
"α"-D-Galactometasaccharinic lactone	All	305
Benzimidazole	1	291
2-Deoxy-D-*threo*-pentose benzylphenylhydrazone	2-6	10
"α"-D-Isosaccharinic lactone	All	290
Formaldehyde dimedon (from lactone)	2a,5	136
Formaldehyde dimedon (from acetonated lactone)	5	5
"α"-D-Glucosaccharinic lactone	All	106
Sodium acetate	2,2a	103
2-Methylbenzimidazole	2,2a	101
Benzimidazole	2	60
Benzimidazole 2-carboxylic acid dihydrate	1,2	61
Benzimidazole	1	2
Sodium formate	3,4	3
Benzimidazole	3,4	2
Formaldehyde dimedon	5	0

(17) P. A. Levene and T. Mori, *J. Biol. Chem.*, **83**, 803 (1929).

(18) *Cf.* S. Moore and K. P. Link, *ibid.*, **133**, 293 (1940); N. K. Richtmyer, *Adv. in Carbohydrate Chem.*, **6**, 184 (1951).

Lactose-1-C^{14} and Lime Water. "α"-D-Isosaccharinic Lactone.—A solution of 150 g. of lactose, containing 100 μc. of lactose-1-C^{14}, in 1500 ml. of water was added to 75 g. of calcium hydroxide. The mixture was allowed to stand under nitrogen, with occasional swirling, in a stoppered flask at room temperature for 8 days. After filtration, the solution was saturated with carbon dioxide, heated to boiling, decolorized, and again filtered. Concentration of the resulting solution at 100° to a volume of about 300 ml., followed by cooling, resulted in the precipitation of 28.7 g. of calcium "α"-D-isosaccharinate. The latter was suspended in 500 ml. of water containing 9.08 g. of oxalic acid dihydrate, and the mixture was boiled for several minutes. The calcium oxalate was removed by filtration and the solution was concentrated, finally at 100° on the water aspirator, to a thick sirup. The latter crystallized spontaneously to yield 19.7 g. of "α"-D-isosaccharinic lactone. After recrystallization from ethanol–ethyl acetate, the lactone[19] showed m.p. 93–95° and $[\alpha]^{25}D$ +62° in water, *c* 4.

2,2a-*O*-Isopropylidene-"α"-D-isosaccharinic Lactone.—To a solution of 1 g. of the above lactone in 50 ml. of dry acetone was added 0.5 ml. of sulfuric acid. After 3 hours at room temperature, the solution was passed over a column of 25 ml. of Duolite A-4[20] in acetone, and concentrated to a crystalline mass. Recrystallization from ether–petroleum ether yielded 710 mg. (56%) of the acetonated lactone. After an additional recrystallization from the same solvents, the product showed m.p. 56–57°. The position of the isopropylidene group in this compound is established by its behavior with periodate, as described below.

Anal. Calcd. for $C_9H_{14}O_5$: C, 53.4; H, 6.98. Found: C, 53.1; H, 6.88.

Formaldehyde Dimedon from "α"-D-Isosaccharinic Lactone.—Oxidation of the lactone with excess 0.1 *N* sodium metaperiodate for 2 days at 4°, followed by precipitation of formaldehyde dimedon as described by Reeves,[21] yielded 1.93 moles of the formaldehyde derivative (m.p. 189–190°) per mole of lactone. For radioassay the formaldehyde dimedon was recrystallized from ethanol–water, and then showed m.p. 190.5–191.5°.

Formaldehyde Dimedon from Sodium 2,2a-*O*-Isopropylidene-"α"-D-isosaccharinate.—To the acetonated lactone was added a slight excess of 0.1 *N* sodium hydroxide and, after 2 hours at room temperature, the solution was back-titrated to the phenolphthalein end-point with 0.1 *N* hydrochloric acid. The consumption of base was 1.0 equiv. per mole. Excess 0.2 *N* sodium metaperiodate then was added and the solution was allowed to stand for 7 hours at room temperature. At this time, oxidation had ceased with the consumption of 1.0 equiv. of periodate per mole of acetonated lactone. Precipitation of formaldehyde dimedon as described by Reeves[21] then yielded 1.0 mole of the formaldehyde derivative (m.p. 189–190°) per mole of acetonated lactone. Recrystallization from ethanol–water raised the m.p. to 190.5–191.5°.

Radioassay data for the above compounds are recorded in Table I.

D-Mannose-1-C^{14} and Lime Water. "α"-D-Glucosaccharinic Lactone.—A solution of 50 g. of D-mannose, containing 22 μc of D-mannose-1-C^{14}, in 400 ml. of water was shaken with 9.6 g. of calcium hydroxide for 5 minutes, filtered, and diluted to 500 ml. The resulting solution was kept under nitrogen at room temperature for 52 days. Removal of calcium ions from an aliquot of the solution by ion-exchange, followed by titration with base, then indicated that 192 meq. of acids and 19 meq. of lactones were present in the reaction mixture. The latter was passed over appropriate columns of Amberlite IR-100[22] and Duolite A-4, to separate calcium ions and acidic materials. Acids were removed from the Duolite A-4 column with 1 *N* sodium hydroxide, and sodium ions were removed from this effluent with Amberlite IR-100. Volatile acids (31 meq.) then were removed from the solution by continuous distillation with water at reduced pressure and the solution was concentrated, finally at 55° on the water-aspirator, to a sirup containing 82 meq. of lactones and 67 meq. of acids. A solution of this sirup again was passed over Duolite A-4, and the effluent was concentrated to a sirup containing 55 meq. of lactones and 8 meq. of acids. An additional 35 meq. of lactones and 14 meq. of acids were obtained by re-working (ion-exchange, lactonization, ether extraction to remove lactic acid) the first effluent from the initial column of Duolite A-4, and material displaced by sodium hydroxide from the final column of Duolite A-4. Seeding[15] of the combined sirups yielded crystalline "α"-D-glucosaccharinic lactone. Several recrystallizations from water provided 1.5 g. of the pure lactone,[23] m.p. 162–163° and $[\alpha]^{25}D$ +93.6° in water, *c* 3.2.

Formic and Acetic Acids from "α"-D-Glucosaccharinic Lactone.—A solution of the above lactone at room temperature consumed 3.0 molecular equivalents of sodium metaperiodate in 10 minutes, and 4.0 molecular equivalents in 24 hours, indicating rapid oxidation to formaldehyde, formic acid and pyruvic acid, followed by slow oxidation of the latter to acetic acid and carbon dioxide.

The lactone (298 mg. in 16 ml. of water) was treated with 4 molecular equivalents of sodium metaperiodate for 1 hour at 0°, followed by 23 hours at room temperature. The solution then was extracted continuously with ether for 9 hours, and the extracted, mixed acids were titrated with sodium hydroxide to the phenolphthalein end-point. Following concentration to about 1.5 ml., the sodium salts were converted at 0° to the free acids with 1 *N* sulfuric acid, and the mixture was adsorbed at 0° on about 2 g. of silicic acid.[24] This was added to a column containing 24 g. of silicic acid[24] and developed by the gradient elution method[25] using 1-butanol–chloroform (15:85, v./v.) passed through a reservoir of chloroform (465 ml.). Complete separation of the acids was accomplished, with a recovery of about 85%. The peak volumes observed under the above conditions were 225 and 385 ml., respectively, for acetic and formic acids.

For radioassay, the acetic acid was converted to both sodium acetate and 2-methylbenzimidazole. For the preparation of the latter, 100 mg. of sodium acetate, 275 mg. of *o*-phenylenediamine dihydrochloride, 1.8 ml. of water and 0.28 ml. of 90% phosphoric acid were heated as directed by Roseman.[26] The cooled mixture was dissolved in 3 ml. of water, decolorized, and made just alkaline with ammonium hydroxide. The precipitated 2-methylbenzimidazole (110 mg.), recrystallized from 0.1 *N* ammonium hydroxide, melted at 175–176°.

The 2-methylbenzimidazole was degraded,[26,27] by way of 2-styrylbenzimidazole and benzimidazole-2-carboxylic acid, to benzimidazole. For radioassay, the latter (m.p. 171–172°) was recrystallized from 0.1 *N* ammonium hydroxide.

The formic acid was radioassayed after conversion both to sodium formate and to benzimidazole. The latter (m.p. 171–172°) was prepared by condensing sodium formate with *o*-phenylenediamine under the conditions indicated above for the preparation of 2-methylbenzimidazole from sodium acetate.

Formaldehyde from Sodium "α"-D-Glucosaccharinate.—After neutralization of 77 mg. of the lactone, in 10 ml. of water, with 0.1 *N* sodium hydroxide, 4 molecular equivalents of sodium metaperiodate were added. The oxidation mixture was titrated with base to the phenolphthalein end-point after 30 minutes, and formaldehyde dimedon was precipitated in the usual way. For radioassay, the derivative (95 mg., m.p. 190–191°) was recrystallized from 95% ethanol.

2-(1,4-Anhydro-1-*C*-methyl-D-*ribo*(?)-tetrahydroxybutyl)-benzimidazole.—All attempts to prepare the normal benzimidazole derivative of "α"-D-glucosaccharinic acid failed to give a crystalline product. Accordingly, the lactone was condensed with *o*-phenylenediamine under conditions (zinc chloride, hydrochloric acid, 180°)[18] designed to give the anhydro derivative. The product, obtained in 94%

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

(20) A product of Chemical Process Co., Redwood City, Calif.

(21) R. E. Reeves, THIS JOURNAL, **63**, 1476 (1941).

(22) A product of Rohm and Haas Co., Philadelphia, Pa.

(23) C. Scheibler, *Ber.*, **13**, 2212 (1880); E. Peligot, *Compt. rend.* **90**, 1141 (1880).

(24) Prepared according to W. A. Bulen, J. E. Varner and R. C. Burrell, *Anal. Chem.*, **24**, 187 (1952).

(25) K. O. Donaldson, V. J. Tulane and L. M. Marshall, *ibid.*, **24**, 185 (1952).

(26) S. Roseman, THIS JOURNAL, **75**, 3854 (1953).

(27) To establish that there was no exchange of carbon atoms during the series of reactions in the degradation of the acetic acid, known sodium acetate-2-C^{14} was converted in the same manner to benzimidazole. The latter, in this instance, was devoid of radioactivity.

yield, showed m.p. 240–241° after recrystallization from water.

Anal. Calcd. for $C_{12}H_{14}O_3N_2$: C, 61.5; H, 6.02. Found: C, 61.3; H, 6.00.

Since "α"-D-glucosaccharinic acid is believed to have the D-*ribo* configuration,[2] and since the anhydrobenzimidazoles normally possess the hydrofuran ring with unchanged configuration,[18] the product may be assigned the tentative structure shown above. In agreement with this assignment, the derivative was observed to consume 1.0 molecular equivalent of periodate in 30 minutes (1.2 molecular equivalents in 70 hours) with no production of formaldehyde.

The anhydrobenzimidazole derivative was oxidized to benzimidazole-2-carboxylic acid, and the latter was decarboxylated to benzimidazole, as described above for 2-(D-*xylo*-1,3,4,5-tetrahydroxypentyl)-benzimidazole.

Radioassay data for "α"-D-glucosaccharinic lactone and its degradation products are recorded in Table I.

Formic, Acetic and Lactic Acids from the Isomerization Reaction.—A sample (4.1 meq.) of the volatile acids from the preparation of "α"-D-glucosaccharinic lactone was chromatographed on silicic acid, as described above, to yield fractions of pure formic (1.46 meq.) and acetic (0.90 meq.) acids. A sample (5.74 meq.) of the crude lactic acid, obtained by ether extraction during the preparation of "α"-D-glucosaccharinic lactone, was chromatographed similarly to yield a fraction (2.98 meq., peak volume 600 ml.) of pure lactic acid.

The formic and acetic acids were further treated exactly as described above for the formic and acetic acids from the periodate oxidation of "α"-D-glucosaccharinic lactone. The lactic acid was degraded by way of 2-(α-hydroxyethyl)-benzimidazole and benzimidazole-2-carboxylic acid to benzimidazole as described by Roseman.[26] Radioassay data for the various products are given in Table II.

Acetonation of "α"-D-Glucosaccharinic Lactone.—Treatment of 1 g. of the lactone in 50 ml. of dry acetone with 0.5 ml. of sulfuric acid for 3 hours at room temperature, followed by removal of acid (ion-exchange) and concentration, gave the monoacetone derivative[12] in 83% yield. After recrystallization from benzene–petroleum ether, the product showed m.p. 62–63° and $[\alpha]^{25}D$ −38.4° in chloroform, *c* 3.4.

TABLE II

DISTRIBUTION OF RADIOACTIVITY IN THE FORMIC, ACETIC AND LACTIC ACIDS FROM THE ACTION OF LIME WATER ON D-MANNOSE-1-C^{14}

Sample	Carbon atoms	Radioactivity, cts./min./m$M \times 10^{-3}$
Formic acid		
Sodium formate	...	21.9
Benzimidazole	...	23.7
Acetic acid		
Sodium acetate	1,2	46.8
2-Methylbenzimidazole	1,2	48.3
Benzimidazole	1	32.5
Lactic acid		
2-(α-Hydroxyethyl)-benzimidazole	1,2,3	57.0
Benzimidazole-2-carboxylic acid dihydrate	1,2	40.5
Benzimidazole	1	13.5

Titration of the acetonated lactone with sodium hydroxide, followed by oxidation with sodium metaperiodate, resulted in the consumption of 1.0 molecular equivalent of the oxidant (30 minutes or 20 hours). Formaldehyde dimedon (0.6 mol. equiv., m.p. 189–190°) was isolated in the usual manner from the oxidation mixture. Thus, the product may be assigned the structure 2,3-*O*-isopropylidene-2-*C*-methyl-D-*ribo*(?)-pentonic lactone.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

The Action of Diazomethane on the Pentaacetates of *aldehydo*-D-Glucose and *aldehydo*-D-Galactose[1]

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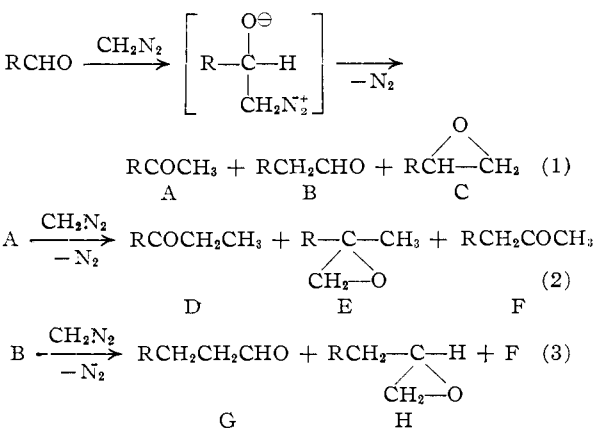
The action of diazomethane on the pentaacetates of *aldehydo*-D-glucose and *aldehydo*-D-galactose has given the corresponding 1,2-dideoxy-3-*keto*-octulose pentaacetates. These structures have been established by functional group tests and by syntheses from the penta-*O*-acetylaldonyl chlorides and diazoethane. The intermediate 1-deoxy-*keto*-D-heptulose pentaacetate was isolated in the D-glucose structure and its nature was proved by group tests and by synthesis through reduction of the diazomethyl ketone.

The action of diazomethane on aldehydes and ketones is known to yield homologous epoxides or carbonyl compounds.[3,4] If the initial product is itself an aldehyde or ketone, then further reaction may occur as indicated below for the aldehydes. It is well known that electronegative substituents promote epoxide formation in this reaction.[3,4] Thus, the reaction of diazomethane with CH_3-



(1) Paper No. 17 in the series entitled "The Action of Diazomethane upon Acyclic Sugar Derivatives"; previous communication: M. L. Wolfrom, J. B. Miller, D. I. Weisblat and A. R. Hanze, THIS JOURNAL, **79**, 6299 (1957).

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