[CONTRIBUTION FROM NATIONAL BUREAU OF STANDARDS, DIVISION OF CHEMISTRY]

# Reaction of Dimeric 5-Aldo-1,2-*O*-isopropylidene-D-*xylo*-pentofuranose with Cyanide and the Preparation of Calcium 1,2-*O*-Isopropylidene-L-idofururonate-6-$C^{14}$ Dihydrate[1]

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A new method is reported for the separation of 1,2-*O*-isopropylidene-L-idofururonic-6-$C^{14}$ acid from the reaction product of dimeric 5-aldo-1,2-*O*-isopropylidene-D-*xylo*-pentofuranose and $C^{14}$-labeled cyanide. After separation of barium 1,2-*O*-isopropylidene-D-glucofururonate-6-$C^{14}$ hydrate from the reaction mixture, crystalline calcium 1,2-*O*-isopropylidene-L-idofururonate-6-$C^{14}$ dihydrate was obtained in 27% yield. The combined radiochemical yield of the L-iduronic-6-$C^{14}$ and D-glucuronic-6-$C^{14}$ acid epimers was 85%. The high yield shows that under the conditions used the dimeric 5-aldo-1,2-*O*-isopropylidene-D-*xylo*-pentofuranose is cleaved, and the resulting monomer gives a normal cyanohydrin reaction.

By application of the cyanohydrin synthesis to 5-aldo-1,2-*O*-isopropylidene-D-*xylo*-pentofuranose, Sowden[2] obtained D-glucuronic-6-$C^{14}$ acid in about 20% yield. The cyanohydrin reaction mixture turned dark with formation of by-products of unknown character. Subsequently Shafizadeh and Wolfrom[3] as well as Schaffer and Isbell[4] found that by controlling the acidity of the reaction mixture the proportions of the epimeric acids could be altered.[5] By use of a bicarbonate–carbon dioxide buffer and chromatographic separation of the lactones, Shafizadeh and Wolfrom obtained crystalline 1,2-*O*-isopropylidene-D-glucurono-γ-lactone in 15.5% yield and 1,2-*O*-isopropylidene-L-idurono-γ-lactone in 16.1% yield. From an acetate-buffered cyanohydrin reaction mixture Schaffer and Isbell separated barium 1,2-*O*-isopropylidene-D-glucufururonate-6-$C^{14}$ monohydrate in 54% yield and a mother liquor containing an unknown amount of the 1,2-*O*-isopropylidene-L-idofururonic-6-$C^{14}$ epimer.

The continued interest in this synthesis in this Laboratory derives from a program for the development of methods for the preparation of position-labeled radioactive carbohydrates,[6] and from interest in the structure and properties of the crystalline dimer of 5-aldo-1,2-*O*-isopropylidene-D-*xylo*-pentofuranose.

In the present study a crystalline salt of 1,2-*O*-isopropylidene-L-idofururonic acid was sought because this would provide not only a simple means for purifying this acid-sensitive material but would also provide a means for its quantitative determination by use of the isotope dilution technique.

It was shown previously,[4] by isotope dilution analysis, that the D-glucuronate epimer was produced in 58% yield by the cyanohydrin reaction with an acetate buffer and in 38% yield with a bisulfite buffer. In view of these results it seemed probable that with the bisulfite buffer a larger proportion of the product went to the L-iduronate epimer. For this reason a reaction product obtained from a cyanohydrin reaction buffered with sodium bisulfite was used in a search for a crystalline salt of the 1,2-*O*-isopropylidene-L-idofururonic acid. After removal of barium 1,2-*O*-isopropylidene-D-glucofururonate from the reaction mixture, crystalline calcium 1,2-*O*-isopropylidene-L-idofururonate dihydrate was prepared. The new salt crystallizes readily and provides a convenient means for separation of the iduronate epimer in a pure state. In order to utilize the salt for separation of the radioactive L-iduronate from the mother liquor of the preparation of barium 1,2-*O*-isopropylidene-D-glucofururonate-6-$C^{14}$ hydrate it was necessary first to remove the barium acetate (by decationizing and freeze-drying), and the residual barium 1,2-*O*-isopropylidene-D-glucofururonate-6-$C^{14}$ hydrate (by crystallization). Barium in the purified mother liquor was replaced by calcium, and calcium 1,2-*O*-isopropylidene-L-idofururonate-6-$C^{14}$ dihydrate was separated in a radiochemical yield of 27%.[7] The combined yield of the D-glucuronate and L-iduronate epimers was 85%. The separation of the epimeric acids in high yield shows that under suitable conditions the dimer is cleaved and the resulting monomer gives a normal cyanohydrin reaction.

By isotope dilution analysis of the product from a bisulfite-buffered cyanohydrin reaction it was found earlier[4] that the D-glucuronate epimer had been formed in 38% yield; in the present study it was found that the L-iduronate epimer formed in 34% yield. The total radioactive yield of 72% for the two epimers, considerably below the 85% yield obtained in the acetate-buffered reaction, shows a substantial loss of labeled cyanide in the bisulfite-buffered reaction. A greater yield of the L-iduronate epimer (at the expense of the D-glucuronate) can be expected for more alkaline buffered systems, but the limit of alkalinity for an optimum yield of L-iduronate is restricted by the alkali-sensitivity of the 5-aldo-1,2-*O*-isopropylidene-D-*xylo*-pentofuranose. The behavior of this substance in alkaline solution is being studied and will be treated in a future publication.

## Experimental Procedure

**Calcium 1,2-*O*-Isopropylidene-L-idofururonate Dihydrate.**—An aqueous solution of 12.54 g. of 5-aldo-1,2-*O*-isopropyli-

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[1] This work was conducted as part of a project sponsored by the Division of Research of the Atomic Energy Commission.

[2] J. C. Sowden, THIS JOURNAL, **74**, 4377 (1952).

[3] F. Shafizadeh and M. L. Wolfrom, *ibid.*, **77**, 2568 (1955).

[4] R. Schaffer and H. S. Isbell, *J. Research Natl. Bur. Standards*, **56**, 191 (1956).

[5] H. S. Isbell, U. S. Patent 2,606,918 (1952).

[6] H. S. Isbell, J. V. Karabinos, H. L. Frush, N. B. Holt, A. Schwebel and T. T. Galkowski, *J. Research Natl. Bur. Standards*, **48**, 163 (1952); H. S. Isbell and J. V. Karabinos, *ibid.*, **48**, 438 (1952); H. L. Frush and H. S. Isbell, *ibid.*, **50**, 133 (1953); **51**, 167 (1953); **51**, 307 (1953); H. S. Isbell, H. L. Frush and N. B. Holt, *ibid.*, **53**, 217 (1954); **53**, 325 (1954); H. S. Isbell, H. L. Frush and R. Schaffer, *ibid.*, **54**, 201 (1955); H. S. Isbell, N. B. Holt and H. L. Frush, *ibid.*, **57**, 95 (1956); H. S. Isbell and R. Schaffer, THIS JOURNAL, **78**, 1888 (1956).

[7] Since the preparation of the labeled epimeric cyanohydrins was made two years prior to the isolation of the L-iduronate-6-$C^{14}$, a yield greater than 27% may have been produced originally.

dene-D-*xylo*-pentofuranose, 12.65 g. of sodium pyrosulfite and 3.4 g. of sodium cyanide was prepared at 0°, and allowed to warm to room temperature. After three days the solution was heated at 90° for one hour, then treated with 7 g. of sodium carbonate, and refluxed for three hours. The solution was cooled in an ice-bath, and passed through a column containing 350 ml. of cation exchange resin[8] at ice temperature. The effluent, including washings, was delivered into a flask immersed in an ice-bath, containing 41.6 g. of barium hydroxide octahydrate. After removal of excess barium hydroxide by carbonation and filtration, one-tenth of the filtrate was set aside for analysis by an isotope dilution technique. The remainder of the solution was concentrated under vacuum to a sirup that yielded 7.77 g. of crude crystalline barium 1,2-*O*-isopropylidene-D-glucofururonate monohydrate. After removal of the barium salt, the mother liquor was passed through a column containing 75 ml. of cation exchange resin[8] at ice temperature. The effluent, including washings, was delivered into a flask containing 5 g. of calcium carbonate. The mixture was filtered, and the filtrate concentrated under vacuum. Calcium 1,2-*O*-isopropylidene-L-idofururonate-6-$C^{14}$ dihydrate separated. The product, 4.2 g., was recrystallized from water with the addition of methanol.

*Anal.* Calcd. for Ca $C_{18}H_{26}O_{14}\cdot 2H_2O$: C, 39.8; H, 5.6; Ca, 7.4. Found: C, 39.8; H, 5.8; Ca, 7.2; $[\alpha]^{20}_{D}$ −11.8° (*c* 3, water).

**Preparation of Calcium 1,2-*O*-Isopropylidene-L-idofururonate-6-$C^{14}$ Dihydrate.**—The original synthesis began with 0.005 mole of sodium cyanide-$C^{14}$ (12.5 millicuries) and 1.11 g. of 5-aldo-1,2-*O*-isopropylidene-D-*xylo*-pentofuranose buffered with 0.005 mole of sodium hydroxide and 0.02 mole of acetic acid. The mother liquor that remained after separation of 6.75 millicuries of barium 1,2-*O*-isopropylidene-D-glucofururonate-6-$C^{14}$ monohydrate from the cyanohydrin synthesis previously reported had been set aside for approximately two years before the work reported here was resumed.

The mother liquor was dissolved in 100 ml. of water, treated with 700 ml. of ethanol, and passed through a carbon-coated filter. Under vacuum the filtrate was concentrated to a 50-ml. volume. The concentrate was passed, ice-cold, into a column containing 30 ml. of cation exchange resin,[8] washed through with ice-water, and collected in a flask containing 0.3 g. of barium carbonate. The partially neutralized effluent was freeze-dried to remove the acetic acid present. Addition of methanol to an aqueous solution of the residue yielded 0.18 g. of crude barium 1,2-*O*-isopropylidene-D-glucuronate-6-$C^{14}$ monohydrate.

The purified mother liquor was diluted with ice-water and passed through a column of 12 ml. of ice-cold cation exchange resin[8] into a flask containing 0.13 g. of calcium carbonate. Concentration of the solution under vacuum yielded calcium 1,2-*O*-isopropylidene-L-idofururonate-6-$C^{14}$ dihydrate, which was separated and washed with a mixture of ethanol and water (6:1). Recrystallization from water and ethanol yielded 2.81 millicuries of the salt. By use of non-radioactive carrier, an additional 0.53 millicurie of this product was obtained. The radiochemical yield based on the 12.5 millicuries of cyanide-$C^{14}$ originally used was 27%.

**Yield of the L-Iduronate Epimer in a Bisulfite-buffered Cyanohydrin Reaction.**—The portion of the solution from the preparation of non-radioactive calcium 1,2-*O*-isopropylidene-L-idofururonate dihydrate kept for the isotope dilution analysis was diluted with a tracer consisting of 95.2 microcuries of calcium 1,2-*O*-isopropylidene-L-idofururonate-6-$C^{14}$ dihydrate (10.68 mg.). After separation of the D-glucuronate epimer as the barium salt, the solution was passed through 15 ml. of cation exchange resin[8] into a flask containing 0.75 g. of calcium carbonate. The excess calcium carbonate was removed, and the calcium salt was crystallized as already described. After two recrystallizations, the air-dried salt had a specific radioactivity of 0.151 $\mu$c./mg. This activity corresponds to dilution of the tracer by 620 mg. of calcium 1,2-*O*-isopropylidene-L-idofururonate dihydrate or a 34.3% yield of this substance from the cyanohydrin reaction.

**Radioactivity Measurements.**—Determinations of $C^{14}$ were made by direct count of the materials in formamide or aqueous formamide solutions.[9] Samples were counted to a probable error of 1%, and the results converted to microcuries by a factor based on the $C^{14}$-standard issued by the National Bureau of Standards.

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[8] Amberlite IR-120 H, Resinous Products Division of Rohm and Haas Co., Philadelphia, Pa.

[9] A. Schwebel, H. S. Isbell and J. D. Moyer, *J. Research Natl. Bur. Standards*, **53**, 221 (1954).

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

# Acyl Migration in the D-Galactose Structure

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When 2,3,4-tri-*O*-acetyl-1,6-anhydro-$\beta$-D-galactose is treated successively with titanium tetrachloride and mercuric acetate, there are obtained two crystalline tetraacetates of D-galactose. The one (isomer A) is convertible to the other (isomer B) by traces of alkali. Both are convertible to $\beta$-D-galactopyranose pentaacetate on mild acetylation. Isomers A and B are resistant toward triphenylmethylation. Isomer A yields a *p*-toluenesulfonate which in turn forms a methyl tri-*O*-acetyl-*O*-*p*-toluenesulfonyl-$\beta$-D-galactopyranoside, both of which derivatives are different from known isomers believed to have the *p*-toluenesulfonyl group in the sixth position. Both isomers A and B yield tetra-*O*-acetyl-3-*O*-methyl-$\beta$-D-galactopyranose on methylation. Positions 2, 3 or 4 are assignable for the open hydroxyl group in each of the two D-galactopyranose tetraacetates, but the present evidence disallows definite allocations.

No tetraacetate of D-galactose is known in which the terminal primary hydroxyl group is unsubstituted. An attempt to prepare such a derivative by a procedure suitable for the synthesis of the analogous D-glucose structure, led to a crystalline D-galactose tetraacetate, m.p. 138–138.5°, $[\alpha]_D$ +37° (chloroform), herein designated isomer A.[2] This substance was considered to have its position six substituted by an acetate group since it was recovered unchanged on treatment with triphenylmethyl chloride and pyridine.[2] Isomer A had been prepared[2] by the successive action of titanium tetrachloride and mercuric acetate on 2,3,4-tri-*O*-acetyl-1,6-anhydro-$\beta$-D-galactopyranose. A second isomer has now been isolated, by chromatographic techniques, from the same reaction mixture. The substance, m.p. 140–141.5°, $[\alpha]_D$ +25.5° (chloroform), designated isomer B, possesses essentially the same melting point as isomer A but the mixed

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[1] Research Associate (A. T.) and Fellow (M. I.) of the Corn Industries Research Foundation.

[2] A. Thompson, M. L. Wolfrom and M. Inatome, THIS JOURNAL, **77**, 3160 (1955).