120°. After one further recrystallization the product melted at 120–121° either alone or in admixture with authentic β-D-ribofuranose tetrabenzoate.[11]

2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl Fluoride (XII) from 1-O-Acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (XI).—1-O-Acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose[10,12] (2.0 g.) was added to 40 ml. of 6 *N* hydrogen fluoride in ether. In the course of 30 min. at room temperature the ester dissolved; after 4 hr. the solvent and hydrogen fluoride was removed with a stream of air and the residue dissolved in methylene chloride. The solution was washed successively with cold aqueous sodium bicarbonate and water, dried with sodium sulfate, filtered through decolorizing carbon and evaporated *in vacuo* to give 1.92 g. of sirup. This material was dissolved in a mixture of 15 ml. of ether and 15 ml. of pentane. When cooled to +5° it gave 1.23 g. (67%) of prismatic needles melting at 81–83° and rotating $[\alpha]^{20}D$ +102° ($CHCl_3$, *c* 0.78). Two additional recrystallizations from ether–pentane afforded pure 2,3,5-tri-O-benzoyl-β-D-ribofuranosyl fluoride melting at 82–83° and rotating $[\alpha]^{20}D$ +105° ($CHCl_3$, *c* 0.61).[17]

Anal. Calcd. for $C_{26}H_{21}O_7F$: C, 67.24; H, 4.56. Found: C, 67.34; H, 4.78.

When the above reaction was attempted using liquid hydrogen fluoride at room temperature for varying periods of time, no crystalline product could be isolated.

β-D-Ribofuranose Tetrabenzoate (IX) from 2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl Fluoride (XII).—A mixture of 100 mg. of 2,3,5-tri-O-benzoyl-β-D-ribofuranosyl fluoride, 250 mg. of anhydrous calcium benzoate and 3.0 g. of benzoic acid was heated to 150° (bath) and stirred at that temperature for 1 hr. The product was isolated as described earlier for the preparation from the α-fluoride, 60 mg. (49%) of crude β-D-ribofuranose tetrabenzoate being obtained. Recrystallization gave pure β-D-ribofuranose tetrabenzoate melting at 120–121° either alone or in admixture with authentic material.

When 2,3,5-tri-O-benzoyl-β-D-ribofuranosyl fluoride, dissolved in benzene, was boiled with calcium benzoate for 24 hr. a yield of 38% of β-D-ribofuranose tetrabenzoate was obtained. In boiling acetonitrile or in benzene at room temperature no reaction was observed.

Methyl β-D-Ribofuranoside (XIII) from 2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl Fluoride (XII).—2,3,5-Tri-O-benzoyl-β-D-ribofuranosyl fluoride (400 mg.) was dissolved in 10 ml. of methanol and 1.0 ml. of 1.74 *N* sodium methoxide added to the solution which was then boiled under reflux for 1.5 hr. The cooled solution was neutralized with carbon dioxide, evaporated to dryness and the residue extracted with pentane (3 × 15 ml.) to remove methyl benzoate. The remainder was then extracted with hot ethyl acetate (4 × 5 ml.) and the combined extracts concentrated to a sirup which crystallized when seeded with methyl β-D-ribofuranoside. Recrystallization from a mixture of ether (10 ml.) and pentane (5 ml.) gave the pure product (60 mg., 42%) which rotated $[\alpha]^{20}D$ −48.5° (H_2O, *c* 0.41) and melted at 77–78° either alone or in admixture with authentic methyl β-D-ribofuranoside.

Acknowledgment.—We are indebted to the Institutes' Analytical Services Unit of this Laboratory, under the direction of Dr. W. C. Alford, for analyses and infrared spectra. We also wish to thank Dr. Norman E. Sharpless of this Institute for X-ray diffraction patterns and Dr. Edwin D. Becker of this Institute for proton magnetic resonance spectra.

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(17) Except for their rotations, the two anomeric 2,3,5-tri-O-benzoyl-D-ribofuranosyl fluorides are remarkably alike in their physical properties, the melting points, X-ray diffraction patterns and infrared spectra being essentially identical. A mixture of the two was found to melt at 80–82° and the proton magnetic resonance spectra of the two differed.

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2,3,4-Tri-O-benzoyl-β-L-arabinopyranosyl Fluoride and a Transformation from the L-Arabinopyranose to the L-Ribopyranose Series Induced by Hydrogen Fluoride

BY CHRISTIAN PEDERSEN[1] AND HEWITT G. FLETCHER, JR.

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Brief treatment of β-L-arabinopyranose tetrabenzoate with anhydrous hydrogen fluoride affords a crystalline 2,3,4-tri-O-benzoyl-L-arabinopyranosyl fluoride which is probably the β-L-anomer. More prolonged treatment of either β-L-arabinopyranose tetrabenzoate or of the above-mentioned fluoride leads to the formation of 3,4-di-O-benzoyl-β-L-ribopyranosyl fluoride, the enantiomorph of a substance prepared earlier through the action of hydrogen fluoride on β-D-ribopyranose tetrabenzoate and on 2,3,4-tri-O-benzoyl-β-D-ribopyranosyl fluoride. 3,4-Di-O-benzoyl-β-L-ribopyranosyl fluoride was characterized through conversion to 2,3,4-tri-O-benzoyl-β-L-ribopyranosyl fluoride, methyl β-L-ribopyranoside and 3,4-di-O-benzoyl-2-O-methylsulfonyl-β-L-ribopyranosyl fluoride.

By way of extension of the work on benzoylated glycosyl fluorides described in the preceding paper,[2] we have now turned our attention to the L-arabinopyranose series.

When β-L-arabinopyranose tetrabenzoate (I)[3] is treated for a brief period (thirty minutes) at −15° with liquid hydrogen fluoride there is obtained in 38% yield a crystalline tri-O-benzoylpentosyl fluoride. Fusion of this halide with a mixture of benzoic acid and calcium benzoate converts it in good yield to α-L-arabinopyranose tetrabenzoate (III),[3] the *trans* - ester which would be expected from either of the anomeric tri-O-benzoyl-L-arabinopyranosyl fluorides. Attempts to remove the benzoyl groups without loss of the fluorine were unsuccessful.

The configuration at C_1 of the new crystalline fluoride cannot be stated with certainty. However, its molecular rotation, +48,800, is not very different from that of 2,3,4-tri-O-acetyl-β-L-arabinopyranosyl fluoride, +38,500,[4] and it may provisionally be assigned the β-L-configuration (II) normally encountered with such compounds in the arabinose series.

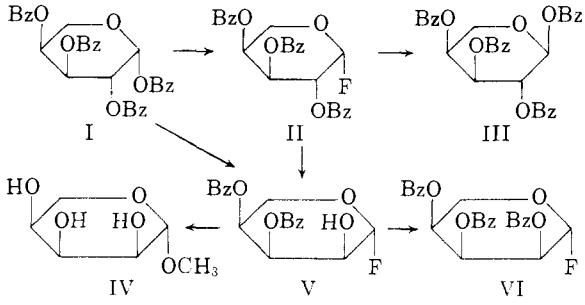
When β-L-arabinopyranose tetrabenzoate (I) was treated with liquid hydrogen fluoride at room temperature for six hours the fluoride mentioned above was not isolated. In its place was obtained in 36% yield a crystalline substance with the analysis of a di-O-benzoylpentosyl fluoride. The same compound was obtained in 44% yield by a

(1) Chemical Foundation Fellow 1958–1960.

(2) C. Pedersen and H. G. Fletcher, Jr., THIS JOURNAL, **82**, 941 (1960).

(3) H. G. Fletcher, Jr., and C. S. Hudson, *ibid.*, **69**, 1145 (1947).

(4) D. H. Brauns, *ibid.*, **46**, 1484 (1924).



similar treatment of 2,3,4-tri-*O*-benzoyl-β-L-arabinopyranosyl fluoride (II). Comparison of the physical constants of this substance (Table I) with those of the isomeric compound made earlier[2] through the prolonged action of hydrogen fluoride on either β-D-ribopyranose tetrabenzoate or 2,3,4-tri-*O*-benzoyl-β-D-ribopyranosyl fluoride suggests that these may be enantiomorphs and, indeed, the infrared spectra of the two were found to be identical. That the new substance is actually a di-*O*-benzoyl-β-L-ribopyranosyl fluoride was shown in two ways. First, treatment with benzoyl chloride in 2,4,6-trimethylpyridine afforded a substance whose physical constants and infrared spectrum clearly showed it to be VI, the enantiomorph of 2,3,4-tri-*O*-benzoyl-β-D-ribopyranosyl fluoride reported earlier.[2] Second, sodium methoxide in methanol readily gave methyl β-L-ribopyranoside (IV). The di-*O*-benzoyl-β-L-ribopyranosyl fluoride was further characterized as its crystalline methanesulfonate.

TABLE I

THE ENANTIOMORPHIC 3,4-DI-*O*-BENZOYL-β-RIBOPYRANOSYL FLUORIDES

	M.p., °C.	$[\alpha]^{20}D$ ($CHCl_3$)
D-Isomer[a]	150–151	−50.6°
L-Isomer	150–151	+52.0

[a] Ref. 2.

This transformation of β-L-arabinopyranose tetrabenzoate (I) and of 2,3,4-tri-*O*-benzoyl-β-L-arabinopyranosyl fluoride (II) to 3,4-di-*O*-benzoyl-β-L-ribopyranosyl fluoride has an analog in a reaction discovered by Brauns[5] who observed that the prolonged action of hydrogen fluoride on cellobiose octaacetate led to the formation of a hexaacetate of 4-*O*-β-D-glucopyranosyl-α-D-mannopyranosyl fluoride, an acetyl group being lost and the configuration at C_2 of one of the glucose residues being inverted. Brauns and Frush[6] also showed that β-D-fructopyranose pentaacetate when similarly treated gave 3,4,5-tri-*O*-acetyl-β-D-fructopyranosyl fluoride. While unsupported by direct evidence, their conclusion that the product from cellobiose octaacetate is 3,6-di-*O*-acetyl-4-*O*-(tetra-*O*-acetyl-β-D-glucopyranosyl)-α-D-mannopyranosyl fluoride seems a reasonable one. An analogous structure here, 3,4-di-*O*-benzoyl-β-L-ribopyranosyl fluoride (V), seems highly probable. The isolation of a 3,5-di-*O*-benzoyl-α-D-ribofuranosyl fluoride in earlier work[2] also points to the apparent lability of 2-*O*-acyl groups toward hydrogen fluoride.

(5) D. H. Brauns, THIS JOURNAL, **48**, 2776 (1926).

(6) D. H. Brauns and H. L. Frush, *Bur. Standards J. Research*, **6**, 449 (1931).

The mechanism whereby 3,4-di-*O*-benzoyl-β-L-ribopyranosyl fluoride (V) is formed from I and II and the mechanism for the formation of its enantiomorph from β-D-ribopyranose tetrabenzoate can only be the subject of speculation in the present state of our knowledge. It seems probable that side reactions of this type may be responsible for the low yields of normal, fully acylated glycosyl fluorides which have been reported by various authors.

Experimental[7]

2,3,4-Tri-O-benzoyl-β-L-arabinopyranosyl Fluoride (II) from β-L-Arabinopyranose Tetrabenzoate (I).—Five grams of β-L-arabinopyranose tetrabenzoate[3] was dissolved in 15 ml. of liquid hydrogen fluoride at −15° and the solution kept at that temperature for 30 min. Methylene chloride (50 ml.) was then added and the solution poured into a mixture of 200 ml. of saturated aqueous sodium bicarbonate and ice. The methylene chloride layer was separated and washed successively with saturated aqueous sodium bicarbonate (2 × 200 ml.) and water (2 × 100 ml.). The solution was dried with sodium sulfate, filtered through a layer of decolorizing carbon and concentrated *in vacuo* to a sirup (4.28 g.) which crystallized when dissolved in 50 ml. of ether: 2.30 g. After two recrystallizations from ether, the product was obtained in two drops of colorless prisms (1.55 g., 38%) melting at 161–162° and rotating $[\alpha]^{20}D$ +106° ($CHCl_3$, *c* 0.33). One further recrystallization from ether raised the melting point to 162–163°, the product then showing $[\alpha]^{20}D$ +105° ($CHCl_3$, *c* 0.67).

Anal. Calcd. for $C_{26}H_{21}O_7F$: C, 67.24; H, 4.56. Found: C, 67.38; H, 4.65.

α-L-Arabinopyranose Tetrabenzoate (III) from 2,3,4-Tri-O-benzoyl-β-L-arabinopyranosyl Fluoride (II).—A mixture of 200 mg. of 2,3,4-tri-*O*-benzoyl-β-L-arabinopyranosyl fluoride, 500 mg. of calcium benzoate and 6.0 g. of benzoic acid was heated, with good stirring, at 150–155° (bath) for 1.5 hr., darkening beginning toward the end of this period. The cooled mixture was dissolved in methylene chloride (50 ml.) and the solution washed successively with saturated aqueous sodium bicarbonate and water. The solution was then dried with sodium sulfate, filtered through decolorizing carbon and concentrated *in vacuo*, the residue crystallizing spontaneously. Recrystallization from a mixture of ether (5 ml.) and pentane (10 ml.) afforded 190 mg. (78%) of material which rotated $[\alpha]^{20}D$ +114.7° ($CHCl_3$, *c* 0.65) and melted at 163–164° either alone or in admixture with an authentic specimen of α-L-arabinopyranose tetrabenzoate. Fletcher and Hudson[3] reported m.p. 164–165° and $[\alpha]^{20}D$ +114.4° ($CHCl_3$, *c* 0.85) for α-L-arabinopyranose tetrabenzoate.

A suspension of calcium benzoate in boiling benzene was found to be without effect on 2,3,4-tri-*O*-benzoyl-β-L-arabinopyranosyl fluoride.

3,4-Di-O-benzoyl-β-L-ribopyranosyl Fluoride (V). (a) From β-L-Arabinopyranose Tetrabenzoate (I).—Twenty grams of β-L-arabinopyranose tetrabenzoate[3] was dissolved in 60 ml. of liquid hydrogen fluoride and the solution kept at room temperature for 6 hr. The hydrogen fluoride was then removed with a stream of air, the residue dissolved in methylene chloride and the solution freed of acid by successive washing with saturated aqueous sodium bicarbonate (3 × 200 ml.) and water (3 × 200 ml.). Moisture was removed with sodium sulfate, the solution filtered through decolorizing carbon and then concentrated *in vacuo* to give 13.8 g. of sirup which rotated $[\alpha]^{20}D$ +53° ($CHCl_3$, *c* 0.47). Cooling a solution of this sirup in 200 ml. of ether afforded 4.29 g. of prismatic crystals; a second crop (1.05 g.) was obtained by concentrating the filtrate. The two crops were combined and recrystallized from ether–pentane to give 4.64 g. (63%) of 3,4-di-*O*-benzoyl-β-L-ribopyranosyl fluoride melting at 148–149° dec. One additional crystallization from ether afforded a pure product melting at 150–151° and rotating $[\alpha]^{20}D$ +52.0° ($CHCl_3$, *c* 0.52). The infrared absorption spectrum of this substance was identical with that of the enantiomorph (m.p. 150–151°, $[\alpha]^{20}D$ −50.6° ($CHCl_3$, *c* 0.074)) obtained from β-D-ribopyranose tetrabenzoate and

(7) Melting points are corrected.

from 2,3,4-tri-*O*-benzoyl-β-D-ribopyranosyl fluoride as described in the preceding paper

Anal. Calcd. for $C_{19}H_{17}O_6F$: C, 63.33; H, 4.76. Found: C, 63.53; H, 4.82.

(b) **From 2,3,4-Tri-*O*-benzoyl-β-L-arabinopyranosyl Fluoride (II).**—2,3,4-Tri-*O*-benzoyl-β-L-arabinopyranosyl fluoride (500 mg.) was dissolved in 5 ml. of liquid hydrogen fluoride and the solution kept at room temperature for 6 hr. The hydrogen fluoride was then removed with a stream of air, the residue dissolved in methylene chloride and washed successively with saturated aqueous sodium bicarbonate and water. Moisture was removed with sodium sulfate, the solution filtered through decolorizing carbon and then concentrated *in vacuo* to give 360 mg. of sirup. Dissolved in ether–pentane, seeded and cooled, the product, 170 mg. (44%), crystallized. It rotated $[\alpha]^{20}D$ +52.0° ($CHCl_3$, *c* 0.36) and melted at 147–149° dec., no depression in melting point being observed when mixed with the product obtained in (a) above.

2,3,4-Tri-*O*-benzoyl-β-L-ribopyranosyl Fluoride (VI) from 3,4-Di-O-benzoyl-β-L-ribopyranosyl Fluoride (V).—3,4-Di-*O*-benzoyl-β-L-ribopyranosyl fluoride (360 mg.) was added to an ice-cold solution of 0.230 ml. of benzoyl chloride in 3 ml. of anhydrous 2,4,6-trimethylpyridine and the mixture kept at room temperature for 18 hr. A few drops of water were then added and, after 15 min., the solution was diluted with methylene chloride (25 ml.) and washed successively with ice-cold 3 *N* sulfuric acid (3 × 25 ml.), saturated aqueous sodium bicarbonate (3 × 25 ml.) and water (3 × 25 ml.). The solution was dried with sodium sulfate, filtered through decolorizing carbon and concentrated *in vacuo* to a sirup which crystallized upon the addition of a little ether. Recrystallized from a mixture of ether (5 ml.) and pentane (15 ml.) the product (360 mg., 78%) melted at 138.5–140°. One further recrystallization from ether gave pure material which rotated $[\alpha]^{20}D$ +51.8° ($CHCl_3$, *c* 0.65) and melted at 139.5–140.5°. The infrared spectrum of the substance was identical with that of its enantiomorph, described in the preceding paper.

Anal. Calcd. for $C_{26}H_{21}O_7F$: C, 67.24; H, 4.56. Found: C, 67.48; H, 4.77.

Methyl β-L-Ribopyranoside (IV) from 3,4-Di-O-benzoyl-β-L-ribopyranosyl Fluoride (V).—3,4-Di-*O*-benzoyl-β-L-ribopyranosyl fluoride (400 mg.) was dissolved in a mixture of anhydrous methanol (5 ml.) and methylene chloride (5 ml.) in a 1-dm. all-glass polarimeter tube. A solution of sodium methoxide in methanol (1.74 *N*) was then added dropwise. The solution became neutral a few minutes after the addition of each drop, indicating the lability of the fluorine in the substance. After 0.60 ml. of the sodium methoxide had been, added the rotation of the solution was observed at 20° until it was constant (4 hr.). The solution was then evaporated *in vacuo* to dryness and the residue extracted with pentane (3 × 15 ml.) to remove the methyl benzoate. The remainder was then extracted with hot ethyl acetate (3 × 5 ml.) and the extracts concentrated *in vacuo* to a sirup which crystallized when dissolved in ether and cooled to −5°. Recrystallized from a mixture of ether (5 ml.) and pentane (5 ml.) the product (110 mg., 60%) melted at 75–78° and rotated $[\alpha]^{20}D$ +140° ($CHCl_3$, *c* 0.47). Two recrystallizations from ether–pentane gave 50 mg. of pure methyl β-L-ribopyranoside melting at 80–81° and showing $[\alpha]^{20}D$ +142° ($CHCl_3$, *c* 0.20). Methyl β-D-ribopyranoside melts at 83°[8] and we have found it to show $[\alpha]^{20}D$ −142.8° in chloroform (*c* 0.57). The infrared spectrum of the product obtained here was identical with that of its pure enantiomorph.

3,4-Di-O-benzoyl-2-O-methylsulfonyl-β-L-ribopyranosyl Fluoride.—3,4-Di-*O*-benzoyl-β-L-ribopyranosyl fluoride (360 mg.) was added to an ice-cold mixture of 0.16 ml. of methanesulfonyl chloride and 5 ml. of anhydrous 2,4,6-trimethylpyridine and the reaction mixture kept at room temperature for 2 hr. when some darkening was observed. A little water was then added and, 15 min. later, 25 ml. of methylene chloride. The solution was then washed successively with cold 3 *N* sulfuric acid (3 × 25 ml.), cold, saturated aqueous sodium bicarbonate (2 × 25 ml.) and water (2 × 25 ml.). Moisture was removed with sodium sulfate, the solution filtered through decolorizing carbon and concentrated *in vacuo* to give a sirup which crystallized on treatment with a little ether. The ether was evaporated and the product recrystallized from a mixture of ethyl acetate (5 ml.) and pentane (15 ml.) to give 380 mg. (87%) of colorless prisms melting at 157–158° and rotating $[\alpha]^{20}D$ +41.3° ($CHCl_3$, *c* 0.59). One further crystallization from ether–pentane gave 300 mg. of pure 3,4-di-*O*-benzoyl-2-*O*-methylsulfonyl-β-L-ribopyranosyl fluoride melting at 157–158° and rotating $[\alpha]^{20}D$ +41.8° in chloroform (*c* 0.98).

Anal. Calcd. for $C_{20}H_{19}O_8FS$: C, 54.79: H, 4.37; S, 7.31. Found: C, 54.55; H, 4.46; S, 7.34.

Acknowledgment.—We are indebted to the Analytical Services Unit of this Laboratory, under the direction of Dr. W. C. Alford, for analyses and infrared absorption measurements.

(8) E. L. Jackson and C. S. Hudson, THIS JOURNAL, **63**, 1229 (1941).

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, DUKE UNIVERSITY MEDICAL CENTER]

The Mechanism of Glucose-6-phosphatase[1]

BY LOUIS F. HASS AND WILLIAM L. BYRNE

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Hepatic microsomal glucose-6-phosphatase, prepared from normal and alloxan-diabetic rats, was found to catalyze the following exchange reaction: G-6-P + C^{14}-glucose ⇄ C^{14}-G-6-P + Glucose. When glucose inhibition of glucose 6-phosphatase was correlated with the exchange reaction, it was found that the number of μmoles of orthophosphate whose liberation was prevented by glucose was approximately equal to the number of μmoles of C^{14}-glucose incorporated into G-6-P. Under identical conditions, P^{32}-orthophosphate was not incorporated; however, C^{14}-fructose was an acceptor at approximately one-twentieth the rate of glucose. These observations were found to be consistent with the mechanism: (1) Enz + G-6-P ⇄ E(G-6-P) ⇄ E-P + Glucose, (2) E-P + H_2O → Enz + Orthophosphate. Lineweaver–Burk plots of enzymatic activity at different concentrations of glucose yielded a series of lines with increasing ordinate intercepts and slightly increasing slopes. Steady-state kinetic treatment of the proposed mechanism gave a reciprocal velocity expression which was in good agreement with the described plots. No difference was found between the kinetic parameters (K_m, K_s and K'_I for glucose) of the enzymes prepared from normal and diabetic animals. Orthophosphate was found to be a competitive inhibitor of glucose-6-phosphatase activity.

In contrast to previously studied non-specific phosphatases[2,3] phosphoserine phosphatase specifically catalyzes the exchange of C^{14}- serine with the serine moiety of phosphoserine.[4,5] The purpose of the present publication is to extend these observa-

(1) Supported in part by a grant from the National Institutes of Health, U. S. Public Health Service. This work is part of a dissertation presented by L. F. Hass for the degree of Doctor of Philosophy at Duke University.

(2) B. Axelrod, *Adv. Enz.*, **17**, 159 (1956).

(3) R. K. Morton, *Biochem. J.*, **70**, 139 (1958).

(4) L. F. Borkenhagen and E. P. Kennedy, *J. Biol. Chem.*, **234**, 849 (1959).

(5) F. C. Neuhaus and W. L. Byrne, *ibid.* **234**, 113 (1959).