temp.). The hydrolyzate was neutralized ($BaCO_3$), filtered and evaporated to a sirup (24 mg.) which showed $[\alpha]^{25}D$ +38° in methanol (*c* 0.8) and +81° in water (*c* 0.8). Paper chromatographic analysis using solvents C and D revealed the presence of two main components, a and b, whose R_G values corresponded to those of 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3-di-*O*-methyl-L-arabinose (see Table I) and

TABLE I

Methylated sugar	R_G (solvent C)	R_G (solvent D)
Component **a**	0.49	0.22
Component **b**	.80	.86
2,4-Di-*O*-methyl-L-arabinose	.33	.16
2,5-Di-*O*-methyl-L-arabinose	.75	.30
2,3-Di-*O*-methyl-L-arabinose	.49	.21
2,3,4,6-Tetra-*O*-methyl-D-galactose	.80	.86

a small amount of a third component c which moved faster than 2,3,4,6-tetra-*O*-methyl-D-galactose. This component c was shown to be a reducing methylated disaccharide, since on rehydrolysis it afforded 2,3,4,6-tetra-*O*-methyl-D-galactose and 2,3-di-*O*-methyl-L-arabinose. The methylated fragments from the hydrolysis of II were isolated in the pure form by sheet paper chromatography using solvent D.

Identification of 2,3,4,6-Tetra-*O*-methyl-D-galactose.—The 2,3,4,6-tetra-*O*-methyl-D-galactose component, obtained as a sirup (6 mg.), had $[\alpha]^{25}D$ +54° in ethanol (*c* 1). It was boiled for 5 hr. with ethanol (2 ml.) containing aniline (0.1 ml.). Removal of the excess of the aniline and the solvent afforded a crystalline residue. Recrystallization from ethyl acetate gave N-phenyl-D-galactopyranosylamine 2,3,-4,6-tetramethyl ether, m.p. and mixed m.p. 196–197°, $[\alpha]^{20}D$ −73° in acetone (*c* 0.2); lit. values[8] m.p. 192°, $[\alpha]D$ −77° (acetone).

Identification of 2,3-Di-*O*-methyl-L-arabinose.—The 2,3-di-*O*-methyl-L-arabinose fraction obtained as a sirup (5 mg.) showed $[\alpha]^{26}D$ +84° in water (*c* 1.5). The sirup (3 mg.) was dissolved in dry pyridine (2 ml.) and after addition of *p*-nitrobenzoyl chloride (50 mg.) the solution was heated at 100–110° (bath temp.) for 2 hr. After keeping the reaction mixture overnight at room temperature, the excess of *p*-nitrobenzoyl chloride was destroyed by addition of a solution of sodium bicarbonate and the resulting solution diluted with 50 ml. of water. The *p*-nitrobenzoate, which separated as a pale yellow precipitate, was extracted with chloroform. The chloroform extract was dried (Na_2SO_4), and evaporated to give a sirup which crystallized on trituration with methanol. Recrystallization from ethanol gave 1,4-bis-*O*-*p*-nitrobenzoyl-2,3-di-*O*-methyl-L-arabinose, m.p. and mixed m.p. 150–153°.

ST. PAUL, MINNESOTA

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY]

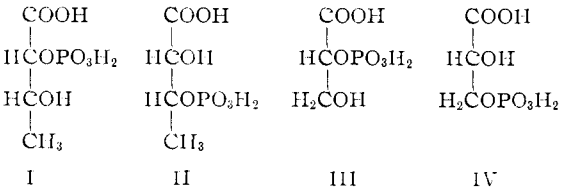
The D-*erythro*-2,3-Dihydroxybutyric Acid Monophosphates

BY CLINTON E. BALLOU

RECEIVED OCTOBER 15, 1956

The synthesis of D-*erythro*-2,3-dihydroxybutyric acid 2- and 3-phosphates is described. These compounds are analogs of the D-glyceric acid monophosphates and have been used in studies of the enzyme specificity of enolase and glyceric acid phosphate mutase.

This paper describes the synthesis of the 2-phosphate (I) and 3-phosphate (II) of D-*erythro*-2,3-dihydroxybutyric acid. These substances are analogs of the glycolytic intermediates D-glyceric acid 2-phosphate (III) and D-glyceric acid 3-phosphate (IV). As such they are useful for a study of the substrate specificity of the enzymes enolase and glyceric acid phosphate mutase.



I II III IV

The key intermediate for the synthesis of these butyric acid derivatives is 2-*O*-benzyl D-*erythro*-2,3-dihydroxybutyric acid. This was prepared by benzylation in the 4-position of an appropriately blocked D-rhamnoside (V–VII); followed by hydrolysis of the acid labile blocking groups, and oxidative cleavage to remove C1 and C2 of the D-rhamnose portion of the molecule (VIII–X). The remaining steps are similar to those used in this Laboratory for syntheses of D-glyceric acid phosphates.[1] X was esterified to XI, which was phosphorylated and the product XII then unblocked by hydrogenation and saponification to give II. Benzoylation of XI, followed by debenzylation, gave XIII which was phosphorylated to XIV. Unblocking of XIV by hydrogenation and saponification gave I.

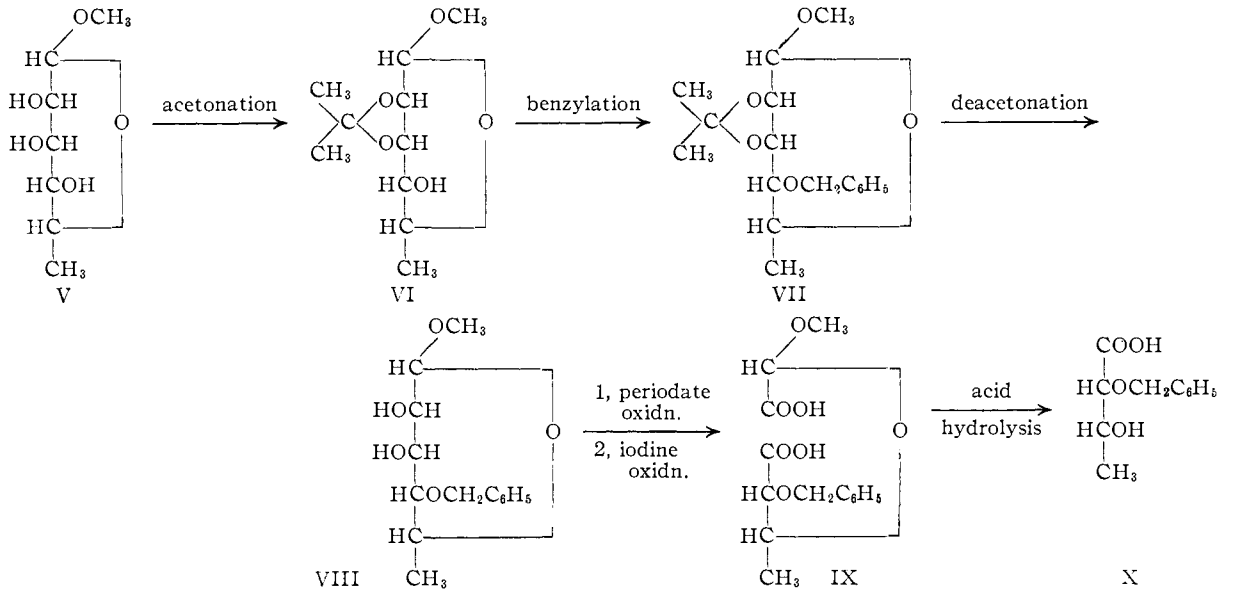
These two new organic phosphates parallel in chemical properties the glyceric acid phosphates; they show approximately the same optical rotations; and the 3-isomer gives the same exaltation of the rotation in the presence of molybdate ions. Acid-catalyzed phosphate migration occurs with the same ease, and approaches about the same equilibrium mixture of 2- and 3-phosphates obtained by similar treatment of the glyceric acid phosphates.

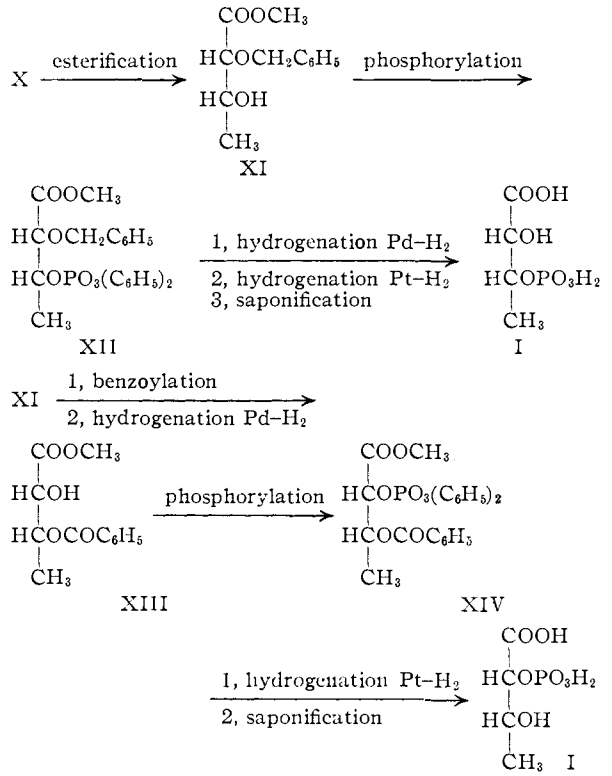
Biochemical studies carried out with these new analogs have shown that, (1) they are substrates for *glyceric acid phosphate mutase*,[2] being interconverted by the mutase, however, at rates several thousandths less rapidly than the D-glyceric acid phosphates; and (2) the D-*erythro*-2,3-dihydroxybutyric acid 2-phosphate does not act as a substrate for *enolase*, but is a very effective competitive inhibitor of this enzyme.[3] Additional experiments are under way designed for the more complete delineation of the substrate specificity of the latter enzyme.

(1) C. E. Ballou and H. O. L. Fischer, THIS JOURNAL, **76**, 3188 (1954).

(2) R. W. Cowgill and L. Pizer, *Federation Proc.*, **14**, 198 (1955).

(3) F. Wold and C. E. Ballou, unpublished.





Experimental

Methyl 2,3-*O*-Isopropylidene-α-D-rhamnopyranoside.—Methyl α-D-rhamnopyranoside (15.0 g.), prepared according to Haskins, Hahn and Hudson,[4] was acetonated by the procedure of Levene and Muskat[5] for the L-isomer, with the modification that the acid catalyst was neutralized with ammonia gas. The yield of acetonated product was 17.5 g. with b.p. 78–82° (0.2 mm.). The colorless sirup showed $[\alpha]_D$ +16.8° (*c* 2, ethanol), and contained 25.4% acetone (theory 26.6%).

Methyl 4-*O*-Benzyl α-D-rhamnopyranoside.—Methyl 2,3-*O*-isopropylidene-α-D-rhamnopyranoside (12.0 g.) was benzylated by stirring it at 100° with 50 ml. of toluene, 20 ml. of benzyl chloride and 15 g. of powdered potassium hydroxide for 5 hours. The mixture was cooled, mixed with water, and the organic layer was washed free of alkali with water. The toluene and excess benzyl chloride were distilled off *in vacuo*, and the residue distilled at 0.1 mm. of pressure. The fraction distilling at 100–120° was collected. It weighed 16 g.

This crude methyl 2,3-*O*-isopropylidene-4-*O*-benzyl-α-D-rhamnopyranoside was deacetoned by heating it for 1 hr. on a steam-bath with 50 ml. of 70% acetic acid. The solution was then concentrated to a sirup which was dissolved in ethanol and again concentrated. The residue crystallized when stirred with a little ether. The solid was collected and recrystallized by dissolving it in 400 ml. of warm ether, and concentrating the solution to 100 ml. When left at 5° overnight, the product crystallized in heavy needles. The yield was 10 g., the purest sample melting at 105–107° and showing $[\alpha]_D$ +72° (*c* 4, chloroform).

Anal. Calcd. for $C_{14}H_{20}O_5$ (268): C, 62.7; H, 7.5; OCH_3, 11.5. Found: C, 62.9; H, 7.7; OCH_3, 11.3.

The methyl 4-*O*-benzyl α-D-rhamnopyranoside consumed one mole of periodate per mole of compound, a result consistent with the expected structure.

2-*O*-Benzyl-D-*erythro*-2,3-dihydroxybutyric Acid.—To a solution of 10 g. of methyl 4-*O*-benzyl α-D-rhamnopyranoside in 50 ml. of ethanol, was added a solution of 10 g. of sodium metaperiodate in 200 ml. of water. After three hr. at room temperature, during which crystalline material separated, another 200 ml. of water was added and the reaction mixture was left at 5° overnight. The crystalline dialdehyde was collected by suction filtration and washed with water on the funnel. When dry it weighed 7.5 g. and melted at 110–112°.

An oxidizing solution was prepared by dissolving 28 g. of iodine and 30 g. of potassium iodide in 50 ml. of water. This was mixed with a buffer of 32 g. of potassium carbonate and 24 g. of potassium bicarbonate in 1 l. of water. The dialdehyde (7.5 g.) was shaken in a closed vessel with this oxidizing solution, and it dissolved in about an hour. The solution was then left in the dark for 3 hr., after which time the oxidation to the dicarboxylic acid was complete. It was then acidified with 70 ml. of 10 *N* sulfuric acid, and the excess iodine was destroyed by adding solid sodium thiosulfate. The clear solution was extracted three times with 500-ml. portions of ether, and the combined ether extracts were concentrated to a sirup. The sirup was readily soluble in water, and was heated at 100° in 200 ml. of water for 3 hr. to hydrolyze the acetal structure.

The cooled solution was extracted twice with ether (200-ml. portions), and the extract was concentrated *in vacuo* to give 4.5 g. of crude 2-*O*-benzyl 2,3-dihydroxybutyric acid. This was taken up in 50 ml. of water and the solution brought to *p*H 8–9 with cyclohexylamine. On concentration, crystals were obtained. This product was stirred with ether and filtered off to give 7.7 g. (67%) with m.p.

(4) W. T. Haskins, R. M. Hahn and C. S. Hudson, THIS JOURNAL, **68**, 628 (1946).

(5) P. A. Levene and I. E. Muskat, *J. Biol. Chem.*, **105**, 431 (1934).

174–179°. It was purified by crystallization from absolute ethanol by addition of ether. The m.p. was 175–179°, and the compound showed $[\alpha]_D$ +51.0° (*c* 2, water).

Anal. Calcd. for $C_{17}H_{27}O_4N$ (309): C, 66.1; H, 8.8; N, 4.5. Found: C, 66.3; H, 9.1; N, 4.9.

Methyl 2-*O*-Benzyl-D-*erythro*-2,3-dihydroxybutyrate.—Two grams of the cyclohexylammonium salt above was dissolved in 10 ml. of 1 *N* hydrochloric acid and 10 ml. of water. The solution was extracted three times with 50-ml. portions of ether, the combined ether extract was dried over anhydrous sodium sulfate, and then filtered. An ether solution containing 0.4 g. of diazomethane was added to the dry ether solution of the carboxylic acid. Esterification took place instantaneously. When a slight excess of diazomethane remained, the solution was concentrated to a sirup *in vacuo*, then freed of traces of solvent in a high vacuum. The product weighed 1.4 g. (97%), and showed $[\alpha]^{25}_D$ +76° (*c* 1.5, ethanol).

Anal. Calcd. for $C_{12}H_{16}O_4$ (224): C, 64.4; H, 7.15; OCH_3, 13.8. Found: C, 64.5; H, 7.26; OCH_3, 13.5.

D-*erythro*-2,3-Dihydroxybutyric Acid 3-Phosphate.—A solution of 1.4 g. of the methyl ester above in 10 ml. of dry pyridine was cooled to 5° in ice-water, 1.7 g. of diphenylphosphorochloridate was added dropwise, and the mixture was left at 5° overnight. A few drops of water were added to destroy the excess phosphorylating reagent, and most of the pyridine was distilled off *in vacuo*. The residue was taken up in chloroform, the solution washed with water, ice-cold 1 *N* hydrochloric acid, ice-cold 1 *M* potassium bicarbonate and finally with water. The chloroform layer was dried over sodium sulfate and concentrated to a sirup of crude methyl 2-*O*-benzyl 3-diphenylphosphonyl D-*erythro*-2,3-dihydroxybutyrate that weighed 2.4 g. (86%).

This intermediate was unblocked by reductive cleavage with hydrogen and palladium in absolute ethanol (hydrogen uptake 150 ml. in 2 hr.), followed by hydrogen and platinum (hydrogen uptake 1450 ml. in 1 hr.).[1] The catalyst was removed and 20 ml. of 1 *N* sodium hydroxide was added to the ethanol solution. The mixture was concentrated *in vacuo* to remove the alcohol, 25 ml. of water was added and the solution was left overnight to complete saponification of the methyl ester.

The alkaline solution was treated with Dowex-50 in the hydrogen form to remove all cations, and after removal of the resin, was brought to *p*H 8–9 with cyclohexylamine. Concentration of the solution to dryness left a crystalline residue that was recrystallized from absolute ethanol. The pure tricyclohexylammonium D-*erythro*-2,3-dihydroxybutyrate 3-phosphate showed $[\alpha]_D$ −14.5° (*c* 1, free acid in 1 *N* hydrochloric acid), and $[\alpha]_D$ −737° (*c* 0.2, free acid in neutral molybdate).

Anal. Calcd. for $C_{22}H_{48}O_7N_3P$ (497): N, 8.47; P, 6.24. Found: N, 8.54; P, 6.13.

D-*erythro*-2,3-Dihydroxybutyric Acid 2-Phosphate.—Two grams of methyl 2-*O*-benzyl-D-*erythro*-2,3-dihydroxybutyrate was benzoylated in 10 ml. of dry pyridine with 2.0 g. of benzoyl chloride. The reaction was left overnight at room temperature, and was then worked up in the manner of the phosphorylation described above, to give 2.9 g. (99%) of methyl 2-*O*-benzyl-3-*O*-benzoyl-D-*erythro*-2,3-dihydroxybutyrate.

The benzyl group was removed from this compound, without further purification, by reductive cleavage in absolute ethanol with hydrogen and palladium.[1] The hydrogen uptake was 200 ml. in 2 hr. The catalyst was removed, and the alcohol solution was concentrated to a dry sirup. The yield of crude methyl 3-*O*-benzoyl-D-*erythro*-2,3-dihydroxybutyrate was 2.6 g. (95%).

This substance (2.0 g.) was phosphorylated, without further purification, in 10 ml. of dry pyridine at 5° with 2.75 g. of diphenylphosphorochloridate added dropwise. The reaction mixture was left overnight at 5°, and was then worked up as described for the phosphorylation of the 3-isomer. The yield of methyl 2-*O*-diphenylphosphonyl 3-*O*-benzoyl-D-*erythro*-2,3-dihydroxybutyrate was 3.9 g. (99%).

This intermediate was unblocked by reductive cleavage with platinum oxide (1.0 g.) and hydrogen at atmospheric pressure in absolute ethanol.[1] The hydrogen uptake was 2450 ml. in one hr. The catalyst was removed, and 40 ml. of 1 *N* sodium hydroxide was added to the alcoholic solution. The mixture was concentrated *in vacuo* to remove the alcohol, and water was added to a volume of 25 ml. After 24 hr. at room temperature to complete saponification, the solution was freed of cations by treatment with Dowex-50 in the hydrogen form. The resin was removed, and the aqueous layer was extracted with ether to remove the benzoic acid. The aqueous layer was then brought to *p*H 8–9 with cyclohexylamine, and the solution was concentrated to dryness *in vacuo*. The solid residue was stirred up with ethyl acetate and filtered. It was reprecipitated from solution in absolute ethanol by addition of ethyl acetate. The tricyclohexylammonium D-*erythro*-2,3-dihydroxybutyrate 2-phosphate (2.5 g.) was dried in a vacuum desiccator at room temperature. It showed $[\alpha]_D$ +15° (*c* 1 free acid, 1 *N* hydrochloric acid), and had a very slight positive rotation in the presence of molybdate.

Anal. Calcd. for $C_{22}H_{48}O_7N_3P$ (497): N, 8.47; P, 6.24. Found: N, 8.50; P, 6.22.

Acid-catalyzed Isomerization of the Phosphates of 2,3-Dihydroxybutyric Acid.—Approximately 1% solutions of the compounds in 1 *N* hydrochloric acid were heated at 100° for two hr. After neutralization and addition of ammonium molybdate to a final concentration of 15%, the specific rotations were determined. The 2-isomer gave $[\alpha]_D$ −560° while the 3-isomer gave $[\alpha]_D$ −630°.

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[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

Cyclic Dimercaptals and Dimercaptols from Alkylene Dithiols[1]

BY C. S. MARVEL AND RALPH C. FARRAR, JR.

RECEIVED SEPTEMBER 10, 1956

The reaction of decamethylene dithiol with several aldehydes and one ketone has given further examples of 26-membered ring compounds containing 4 atoms of sulfur and 22 atoms of carbon. These cyclic dimercaptals and dimercaptols have been oxidized to tetrasulfones. Nonamethylene dithiol has also given a cyclic dimercaptal which is a 24-membered ring compound. Heptamethylene dithiol and vanillin did not give a crystalline cyclic material, but the 20-membered ring compound is formed with acetone. The cyclic dimercaptal from decamethylene dithiol and vanillin is converted to a polymer of about 13,000 mol. wt. on heating.

It has been found that a higher alkylene dithiol may react with an aldehyde or ketone to produce a cyclic dimercaptal or dimercaptol I or a linear polymer II.[2–5] The present study was under-

(1) The work discussed herein was performed as a part of the synthetic rubber research project sponsored by the National Science Foundation.

(2) W. Autenrieth and A. Geyer, *Ber.*, **41**, 4249 (1908).

(3) W. Autenrieth and F. Beuttel, *ibid.*, **42**, 4346, 4357 (1909).

(4) C. S. Marvel, E. H. H. Shen and R. R. Chambers, THIS JOURNAL, **72**, 2106 (1950).

(5) C. S. Marvel, E. A. Sienicki, M. Passer and C. N. Robinson, *ibid.*, **76**, 933 (1954).