Anal. Calcd. for $C_{12}H_{17}NO_3$: C, 64.6; H, 7.6; N, 6.3. Found: C, 64.4; H, 7.5; N, 6.2.

The *picrate* melted at 203–204°.

Anal. Calcd. for $C_{20}H_{22}N_4O_{11}$: C, 47.8; H, 4.4. Found: C, 47.6; H, 4.5.

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The L-Glyceric Acid Monophosphates

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The syntheses of L-glyceric acid 2- and 3-phosphate from L-arabinose are described. The two new phosphate esters have been characterized by comparison with the previously synthesized D-isomers.

Studies of the effect of the unnatural isomers of substrates and substrate analogues on enzymatic reactions have given information about the configuration of the enzymes' catalytic sites.[1] With the intention of applying this kind of studies to the enzyme enolase, it was of interest to prepare the pure L-isomers of glyceric acid 2- and 3-phosphate. The D-isomers have been prepared previously from D-galactose,[2] but as L-galactose is not readily available as a starting material, a new synthetic route leading to the glyceric acid monophosphates was developed, starting with a more common L-sugar, L-arabinose.

The key intermediate, methyl 2-*O*-benzyl-L-glycerate, was obtained from L-arabinose by the following reaction sequence:

L-arabinose (I) → benzyl β-L-arabinopyranoside (II) → benzyl 3,4-*O*-isopropylidene-β-L-arabinopyranoside (III) → benzyl 2-*O*-benzyl-3,4-*O*-isopropylidene-β-L-arabinopyranoside (IV) → 2-*O*-benzyl-L-arabinose (V) → 2-*O*-benzyl-L-arabitol (VI) → 2-*O*-benzyl-L-glyceric acid (VII) → methyl 2-*O*-benzyl-L-glycerate (VIII).

VIII could be phosphorylated in the 3-position and unblocked in the usual manner[2-4] to give the 3-phosphate ester, or it could be benzoylated, debenzylated, phosphorylated, and unblocked again according to standard procedures[2-4] to give the 2-phosphate ester.

II, III, and IV were prepared in good yield, the first according to published methods,[5,6] and were obtained as readily characterizable crystalline products. The hydrolysis of IV to give V was not as easy to accomplish. The hydrolysis conditions must be chosen to give a minimum of hydrolysis of the benzyl ether, and yet be drastic enough to cleave the relatively stable benzyl glycoside. By refluxing for two to three hours with 1*N* hydrochloric acid moderately good yields of V could be obtained. During the first fifteen to twenty minutes of refluxing, the compound would slowly go into solution as the acetal was hydrolyzed. (If the reaction were cooled at this stage, a near quantitative yield of benzyl 2-*O*-benzyl-β-L-arabinoside would crystallize out of the aqueous solution.) After the removal of the acetal, the reducing power of the reaction mixture would slowly increase leveling off after two to three hours. At this time the acid was neutralized and the reaction mixture was taken to dryness. The product could be extracted into hot chloroform, leaving the inorganic salt and some free arabinose behind. A low and variable yield of crystals could be obtained from the chloroform solution upon concentration, and it was found that a drop of concentrated hydrochloric acid would increase the amount of crystalline material, indicating mutarotation and crystallization of one of the anomeric forms. This phenomenon was not investigated further. In practice the chloroform solution was taken to dryness, and if benzyl 2-*O*-benzylarabinoside and free arabinose were shown to be absent by paper chromatography, the sirup was used in the subsequent step without further purification. After reduction to VI and periodate cleavage to give 2-*O*-benzylglyceraldehyde, perpropionic acid oxidation[7] of the aldehyde to the acid was attempted; however, this led to cleavage of the benzyl ether. The iodine oxidation previously described[2] was therefore used giving variable yield.

The two monophosphate esters of L-glyceric acid were characterized by chromatography, titration, and optical rotation, in comparison with the known

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TABLE I

SPECIFIC ROTATION OF THE L- AND D-GLYCERIC ACID MONOPHOSPHATES

	1*N* HCl	10% Neutral Molybdate	10% Neutral Molybdate after 30 Min. at 100° in 1*N* HCl
L-Glyceric acid 2-P	−12.0 (c^a 0.7)	+9 (c^a 1)	+590 (c^a 0.06)
L-Glyceric acid 3-P	+12.0 (c^a 0.7)	+690 (c^a 0.06)	+580 (c^a 0.06)
D-Glyceric acid 2-P[2]	+13.0	+5	−600
D-Glyceric acid 3-P[2]	−14.5	−725	−610

[a] Concentration of the free acid.

D-isomers. The optical rotations determined for the L-isomers are given in Table I, together with the values reported for the D-glyceric acid monophosphates.[2]

EXPERIMENTAL

The melting points reported are all uncorrected. A flash evaporator operating at 45° and 15 mm. pressure was used for solvent removal unless other conditions are specified. The optical rotations were read in a Rudolf Polarimeter.

Benzyl β-L-arabinopyranoside. Fifty grams of L-arabinose C.P. (Pfanstiehl Laboratories, Inc.) was suspended in 250 ml. of freshly redistilled benzyl alcohol. The mixture was cooled in an ice bath, saturated with dry hydrochloric acid, and shaken for 24 hr. at room temperature. Four hundred milliliters of ethyl ether was added and after several hours at −10° the voluminous crystalline product was collected by filtration, washed with ether, and air dried. Recrystallization in two batches from 1 l. of boiling ethanol gave 71 g. (88%) of product, m.p. 168–171°, ($[\alpha]_D^{25}$ +206° (*C* 0.3, water)) in good agreement with the literature values.[6] The compound showed a rapid periodate consumption of 1.85 moles of periodate, confirming the pyranose structure.

Benzyl 3,4-O-isopropylidene-β-L-arabinopyranoside. Thirty grams of the benzyl arabinoside was dissolved in 1.5 l. of acetone (C.P. acetone from a previously unopened bottle was used without further drying) and the solution was shaken with 1 ml. of concd. sulfuric acid and 150 g. of cupric sulfate (dried at 90° for 24 hr.) for 25 hr. The acid was neutralized with dry ammonia and the inorganic salts were removed by filtration through Celite. The filtrate was taken to dryness, redissolved in ether, and a purple color removed by extraction with water. The ether phase was dried over sodium sulfate and upon removal of the ether, 34.99 g. (86%) of a colorless sirup which crystallized slowly was obtained. The product did not react with periodate, but after a short exposure to hot aqueous acid, a positive periodate test was obtained. Recrystallization from ether–petroleum ether (b.p. 30–60°) at −10° gave large rosettes melting at 59–59.5°; $[\alpha]_D^{25}$ +222 (*c* 1, chloroform).

Anal. Calcd. for $C_{15}H_{20}O_5$ (280.3): C, 64.3; H, 7.15. Found: C, 64.28; H, 7.31.

Benzyl 2-O-benzyl-3,4-O-isopropylidene-β-L-arabinopyranoside. To a solution of 30 g. of the crystalline mass obtained above in 120 ml. of toluene in a three neck flask equipped with a mechanical stirrer was added 50 g. of powdered potassium hydroxide and 175 ml. of freshly redistilled benzyl chloride. The reaction mixture was left on a steam bath with rapid stirring for 5 hr. and allowed to cool. The solution was transferred to a separatory funnel with 200 ml. of benzene and extracted with water to remove the base. The organic phase was dried over sodium sulfate and the excess benzyl chloride was distilled off at 150° and 15 mm. (water aspirator). The product was collected by distillation (160–190° at 0.3 mm.) and crystallized from a small volume of ethanol giving 33.5 g. (80%) of crystalline material. It was later found that the residue after the unchanged benzyl chloride has been removed, can be crystallized directly and that the distillation step thus can be eliminated. Recrystallization from hot ethanol gave prisms melting at 77°; $[\alpha]_D^{25}$ +199 (*c* 2, chloroform).

Anal. Calcd. for $C_{22}H_{26}O_5$ (370): C, 71.4; H, 7.03. Found: C, 71.46; H, 7.15.

2-O-Benzyl-L-arabinose. Fourteen grams of the fully blocked arabinoside from above was refluxed with 210 ml. of 1*N* hydrochloric acid. During the first 30 min. the reaction mixture was shaken frequently to disperse the oil of the melted starting material, which slowly disappeared as the isopropylidene group hydrolyzed and acetone distilled out. [Upon cooling at this stage, a near quantitative yield of benzyl 2-*O*-benzyl-β-L-arabinopyranoside crystallized from the aqueous solution. It could be recrystallized from chloroform–petroleum ether (b.p. 60–90°) melted at 127–128°, $[\alpha]_D^{25}$ +207° (*c* 1, chloroform).]

Anal. Calcd. for $C_{19}H_{22}O_5$ (330): C, 69.1; H, 6.67. Found: C, 67.8; H, 6.60.

As the compound dissolved, the reducing power, as tested by the Willstätter-Schudel method,[8] increased, and reached a constant value after about 2.5 hr. After cooling, the solution was brought to *p*H 7 with concd. potassium hydroxide (bromthymol blue end point) and taken to dryness. None of the characteristic very voluminous precipitate of benzyl 2-*O*-benzyl-β-L-arabinopyranoside appeared during the concentration. The dry semisolid sirup was extracted twice with hot chloroform, and after removal of the chloroform, 8 g. of a semicrystalline, colorless sirup was obtained (88%). Ascending paper chromatography in butanol-water (3:2) containing enough acetic acid to give a single phase, gave a spot with R_f = 0.85. In this solvent system arabinose, benzyl arabinopyranoside and benzyl 2-*O*-benzyl-arabinopyranoside have R_f values of 0.35, 0.85, and 0.95 respectively, but the reaction product could be distinguished from benzyl arabinoside by the latter's negative reducing test (benzidine spray[9]).

It may be of interest to note that hydrochloric acid gave a nice compact spot with R_f = 0.5 in this system. It gave a yellowish-brown spot with the periodate-benzidine spray reagent.[10]

The above sirup was dissolved in hot chloroform containing a drop of concd. hydrochloric acid, and upon cooling, 3.7 g. of crystals were obtained melting at 109–112°, $[\alpha]_D^{25}$ +87° after 5 min., and +75 after 90 min. (*c* 1, ethanol).

Anal. Calcd. for $C_{12}H_{16}O_5$ (240): C, 60.0; H, 6.67. Found: C, 59.6; H, 6.65.

Continuous boiling and concentration of the mother liquors would give additional crops of crystals, but the total yield of crystalline product was never over 60% of the original sirup.

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2-O-Benzyl-L-glyceric acid. Six grams of crystalline 2-*O*-benzyl-L-arabinose was dissolved in 100 ml. of water and the *p*H was adjusted to 10 with potassium hydroxide. Sodium borohydride (500 mg.) in about 2 ml. of water, adjusted to *p*H 10, was added and the solution was left for 10 hr. After treatment with Dowex 50 H^+ to remove the excess borohydride, the solution was nonreducing.

After concentration to a small volume, the borate was removed as the methyl ester by taking the product to dryness repeatedly from methanol. The final semicrystalline colorless sirup weighed 6 g. (100%) and was oxidized without further purification. For the oxidation 10.64 g. of sodium periodate was dissolved in 100 ml. of water and the solution was cooled in an ice bath. A concentrated aqueous solution of 6 g. of benzyl arabitol was then added slowly with rapid stirring, the addition being completed in about 30 min. Ten minutes later a couple of drops of glycerol were added and the reaction mixture was extracted three times with 100 ml. of ether. The dry ether solution was concentrated and gave 4.6 g. (100%) of a reducing, colorless sirup. This sirup (2-*O*-benzyl-L-glyceraldehyde) was dissolved in 100 ml. of water and a solution of 9.6 g. of iodine and 11.7 g. of potassium iodide in 15 ml. of water was added followed immediately by a solution of 8.9 g. of potassium carbonate and 6.9 g. of potassium bicarbonate in 80 ml. of water. After 2 hr. in the dark, the reaction mixture was acidified with 5*N* sulfuric acid, and the excess iodine was destroyed with thiosulfate. The product was extracted into ether, and after drying the ether solution over sodium sulfate, the ether was removed, yielding 2.7 g. (55%) of a colorless sirup, having a neutralization equivalent corresponding to that of 2-*O*-benzyl-glyceric acid. The cyclohexylammonium salt could be crystallized from absolute ethanol and gave a melting point of 156–157°.

L-Glyceric acid 2-phosphate. One and seven tenths grams of the above sirup of 2-*O*-benzyl-L-glyceric acid in 20 ml. of ether was treated with an excess of etheral diazomethane, and upon concentration 1.8 g. of the methyl ester was obtained as a sirup (100%). The sirup was dissolved in 15 ml. of dry pyridine, and 2 ml. of benzoyl chloride (10% excess) was added slowly to the ice cold pyridine solution. After 20 hr. at 4°, a few drops of water was added, followed by 50 ml. of chloroform. The chloroform phase was washed with 50 ml. portions of 1*N* hydrochloric acid, 1*M* potassium bicarbonate and water, and the pyridine free, neutral chloroform solution was dried over sodium sulfate. Upon removal of solvent (high vacuum at 50°), 2.8 g. of methyl 3-*O*-benzoyl-2-*O*-benzyl-L-glycerate (100%) was obtained as a sirup. The benzyl group was next removed by catalytic hydrogenation with palladium on carbon in ethanol solution. The theoretical uptake of hydrogen (210 ml.) was completed in 2 hr., and after removal of catalyst and solvent, 2 g. of a semicrystalline residue of methyl 3-*O*-benzoyl-L-glycerate was obtained (100%). This was phosphorylated directly in 20 ml. of dry pyridine with 2 g. of diphenyl phosphorochloridate at ice bath temperature. After 10 hr. at 4°, the reaction mixture was freed of pyridine hydrochloride and excess reagent as indicated in the benzoylation step, yielding finally 2.5 g. of a sirupy product (66%) of methyl 3-*O*-benzoyl-2-*O*-diphenyl phosphonyl-L-glycerate. The phenyl groups were removed by hydrogenation with 500 mg. of platinum oxide catalyst and the theoretical uptake of 1430 ml. of hydrogen was completed in 90 min. The ethanol solution was freed of catalyst and 20 ml. of 1*N* sodium hydroxide was added to saponify the methyl and benzoyl esters. The ethanol was removed and another 5 ml. of base was added to complete the saponification. Attempts to obtain the crystalline sodium salt of L-glyceric acid 2-phosphate failed, and the product was converted to the tricyclohexylammonium salt which crystallized from water-acetone. One and three tenths grams of crystals was collected (50%). The product was indistinguishable from authentic D-glyceric acid 2-phosphate[2] on paper chromatography in several solvents. After conversion to the free acid it titrated with 3 equivalents of base, and its optical rotation was numerically very similar to that of the D-isomer (Table I).

L-Glyceric acid 3-phosphate. One and eight tenths grams of methyl 2-*O*-benzyl-L-glycerate was phosphorylated as above with 1.9 ml. of diphenyl phosphorochloridate in 10 ml. of pyridine. The product after the workup (3.05 g. of methyl 2-*O*-benzyl-3-*O*-diphenylphosphonylglycerate) was reduced with palladium and hydrogen (170 ml. in 1 hr.) and platinum and hydrogen (1400 ml. in 2 hr.) and after saponification with 20 ml. of 1*M* sodium hydroxide, the tricyclohexylammonium salt of L-glyceric acid 3-phosphate (1.8 g., 71% yield) was collected. Again the product was indistinguishable from the authentic D-isomer by its titration and chromatographic properties, and numerically the optical rotation checked well with that of the D-isomer (Table I).

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF CREIGHTON UNIVERSITY]

Long Carbon-Chain Sugars. Condensation of Diethyl Acetonedicarboxylate with Aldoses in Concentrated Hydrochloric Acid at 0°

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Diethyl acetonedicarboxylate condenses with D-glucose, L-arabinose, and D-xylose, respectively, in concentrated hydrochloric acid at 0°, producing carbethoxy derivatives of long chain unsaturated keto sugars.

The molar proportion of the ester to the aldose condensed was 1:1 or 1:2 depending on the relative concentration of the reagents and the reaction time.

The products were converted and characterized as phenylhydrazine or 2,4-dinitrophenylhydrazine derivatives.

In a previous publication[1] the condensation of diethyl acetonedicarboxylate with 1,2-*O*-isopropylidene-D-xylopentadialdose, using piperidine as a catalyst was described. It was pointed out then, that this method favored the formation of long chain sugars. In view of the method used in the

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