α-L-Rhamnopyranosyl phosphate*

The preparation of glycosyl phosphates by the reaction of a fully acetylated, reducing monosaccharide with anhydrous phosphoric acid[1] provides, in general, an easy route to these compounds. The reaction normally leads to production of the thermodynamically more-stable anomer (having the phosphate group axially attached), although, in certain instances, both anomers have been isolated[2]. Recent work has shown that, under suitable kinetic control, the thermodynamically less-stable anomer (having the phosphate group equatorially attached) can be obtained as the major product; for example, brief fusion of β-D-glucopyranose pentaacetate with anhydrous phosphoric acid gives, by neighboring-group participation, β-D-glucopyranosyl phosphate[3], whereas prolonged reaction affords the α-D anomer.

In the D-*manno* series, neighboring-group participation leads to the formation of the stable anomer, so that, in this case, a good yield of the α-D (axial) anomer[4] is invariably obtained. We have had occasion to prepare an L-rhamnopyranosyl phosphate from the syrupy mixture of L-rhamnose tetraacetates obtained by low-temperature acetylation of L-rhamnose with acetic anhydride and pyridine. By analogy with the reaction for D-mannose, it would be expected that the product of the fusion reaction would be the stable α-L-rhamnopyranosyl phosphate. Evidence now presented suggests that this is, indeed, the product.

It has been shown that, in the n.m.r. spectrum of α-D-glucopyranosyl phosphate, which has H-1 and H-2 in the *e-a* relationship, $J_{1,2}$ is 3.0 Hz. For α-D-mannopyranosyl phosphate, which has these protons in the *e-e* relationship, $J_{1,2}$ is[4d,5] 1.5 Hz. We have confirmed these results, and, furthermore, have found for the L-rhamnopyranosyl phosphate described herein a value of 1.5 Hz, indicating that the compound is α-L-rhamnopyranosyl phosphate in the *1C* conformation (having an axially attached phosphate group). The spectra were recorded for solutions in D_2O, with a Varian A-60 spectrometer.

The optical rotation of the cyclohexylammonium salt isolated is in agreement with this finding. Hudson pointed out[6] that the conversion of methyl α-D-mannopyranoside into methyl α-D-rhamnopyranoside results in a diminution of *ca.* 4,000 in the molecular rotation. The cyclohexylammonium salt of α-D-mannopyranosyl phosphate (mol. wt. 458) has $[\alpha]_D$ +28.7° and [4b] [M] +13,200. If Hudson's observation may be extended to the glycosyl phosphates, α-D-rhamnopyranosyl phosphate should have [M] +9,200, and its enantiomorph, −9,200. The α-L-rhamnopyranosyl (cyclohexylammonium phosphate) hemihydrate (mol. wt. 451) should show $[\alpha]_D$ −20°. The value actually observed for the phosphate reported herein is −21.5°, strongly suggesting that it is, indeed, the α-L anomer. On the basis of a 2A value of 25,400 for the

*Note added in proof. G. A. BARBER [*Biochim. Biophys. Acta*, 141 (1967) 174] has prepared an L-rhamnopyranosyl phosphate by the fusion method. This compound, with $[\alpha]_D$ −6.7°, is presumed to be the α-L anomer based on the method of synthesis employed and the optical rotation observed.

phosphate group, as determined by Putman and Hassid[7], the molecular rotation of the β-L anomer should be +15,700, giving $[\alpha]_D$ +35° for a hydrated cyclohexylammonium salt of β-L-rhamnopyranosyl phosphate.

EXPERIMENTAL

L-Rhamnose tetraacetate[8]. — A solution of L-rhamnose (10 g) in a mixture of dry pyridine (40 ml) and acetic anhydride (40 ml) was kept for 3 days at 0°. The solution was poured into ice–water, the mixture was extracted with chloroform, and the extract was washed (repeatedly) with 1% hydrochloric acid, and then successively with saturated aqueous sodium hydrogen carbonate solution and water, and dried (sodium sulfate). Removal of the solvent gave 18.6 g (92%) of a syrupy mixture of acetates having $[\alpha]_D^{20}$ −45.6° (*c* 1.1, chloroform); lit.[9] $[\alpha]_D^{25}$ −61.7° (*c* 2.7, chloroform) for syrupy α-L-rhamnopyranose tetraacetate.

α-L-Rhamnopyranosyl (dicyclohexylammonium phosphate). — A mixture of crystalline anhydrous, phosphoric acid (11.5 g) and L-rhamnopyranose tetraacetate (5.3 g) was stirred *in vacuo* for 2 h at 50°. The melt was cooled, 225 ml of cold *ca.* 2M lithium hydroxide was added, and the mixture was shaken to disperse the solids and kept overnight. Lithium phosphate was removed by filtration and washed with dilute lithium hydroxide, and the resulting solution [containing 10.4 mmole (65%) of labile phosphate] was cooled and passed through an ice-cold column (3 × 22 mm) of Dowex 50 (H^+) ion-exchange resin. The column was then eluted with *ca.* 500 ml of water, the eluate and water washings being collected in an excess of cyclohexylamine in water. This basic solution was evaporated to dryness, and the resulting syrup was dissolved in water (*ca.* 30 ml). The product crystallized on gradual addition of 300 ml of acetone. The colorless, crystalline salt was air-dried; wt. 4.5 g (62%), m.p. 195°, $[\alpha]_D^{20}$ −21.5° (*c* 1, water).

Anal. Calc. for $C_{18}H_{39}N_2O_8P \cdot 0.5\ H_2O$: C, 47.88; H, 8.93; N, 6.21; P, 6.86. Found*: C, 47.56; H, 8.68; N, 6.24; P, 6.96.

ACKNOWLEDGMENTS

This work was supported by grants from the U. S. Public Health Service (GM 09756) and the Corn Industries Research Foundation. One of the authors (A.K.C.) thanks the Fulbright Committee for the award of a Travel Grant. Our thanks are due Dr. J. Seubert for recording the n.m.r. spectra.

Department of Biochemistry and Biophysics,
Oregon State University,
Corvallis, Oregon 97331 (U. S. A.)

ASISH K. CHATTERJEE
DONALD L. MACDONALD**

*Analyses by Elek Microanalytical Laboratories, Torrance California.
**Research Career Development Awardee, U. S. Public Health Service.

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(Received November 6th, 1967)