CHEMISTRY OF GLYCOSYL PHOSPHATES AND THEIR DERIVATIVES

COMMUNICATION 6. IMPROVED SYNTHESIS OF β-L-RHAMNOPYRANOSYL PHOSPHATE

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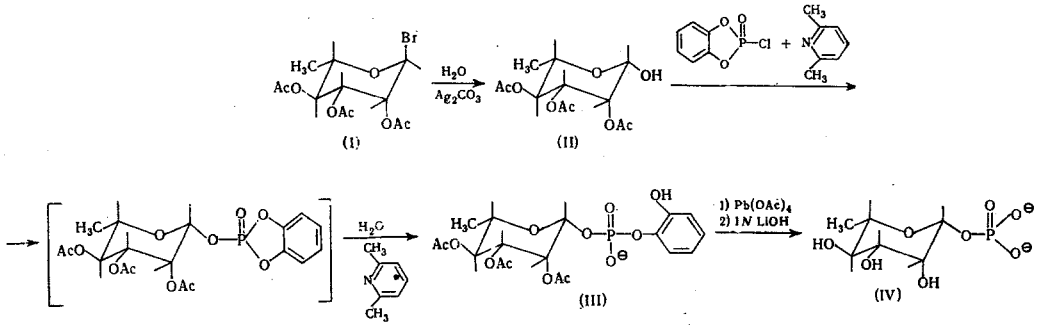
UDC 542.91:547.455:547.1'118

The methods for the synthesis of glycosyl phosphates, based on nucleophilic substitution at C-1 in the glycosyl halides or acetates [1, 2], are unsuitable for the preparation of 1,2-cis-glycosyl phosphates with the OH group at C-2 in the axial position. At the same time, the chemical synthesis of β-L-rhamnopyranosyl phosphate (IV) is a key step for the preparation of either thymidine or uridine diphosphate rhamnose, of the natural nucleoside diphosphate sugars that take part in the biosynthesis of a number of carbohydrate-containing biopolymers [3, 4]. A new approach to the synthesis of glycosyl phosphates was proposed recently, which proves to be suitable for solving this problem [5, 6]. This approach is based on the use of o-phenylene chlorophosphate to phosphorylate the polyacetal hydroxyl group, with a retention of the configuration at C-1 in the partially acetylated monosaccharide. Since we were interested in the synthesis of β-L-rhamnopyranosyl phosphate, we made a detailed study of this synthesis method after the appearance of communication [5], in which connection special attention was given to increasing the yield of the desired product and adapting the procedure for running the synthesis on a microscale. The synthesis scheme employed by us is given below:



The starting 2,3,4-tri-O-acetyl-β-L-rhamnopyranose (II) was obtained by the Fischer method [7] by the hydrolysis of 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl bromide (I) in the presence of Ag_2CO_3. In contrast to the data given in [6], the pure 2,3,4-tri-O-acetyl-L-rhamnopyranose β-anomer (II) was isolated in the crystalline state.

The phosphorylation of (II) by treatment with o-phenylene chlorophosphate in the presence of collidine under the conditions given in [5] gave unsatisfactory results when the synthesis was run on a microscale: product (III) was isolated in only 10% yield. In view of this a more detailed study was made of the effect of the reaction conditions on the yield of (III). It was found that in order to obtain high yields of (III) it is necessary to use a substantial excess of the phosphorylating agent; the reverse order of mixing the reactants is also essential, i.e. (II) is added to a mixture of o-phenylene chlorophosphate and 2,6-lutidine. Based on the TLC data, the starting compound disappears under these conditions and is converted to product (III). The latter was isolated in 70% yield by chromatographing on SiO_2. This compound,

N. D. Zelinskii Institute of Organic Chemistry, Academy of Sciences of the USSR. Translated from Izvestiya Akademii Nauk SSSR, Seriya Khimicheskaya, No. 8, pp. 1852-1855, August, 1974. Original article submitted December 25, 1973.

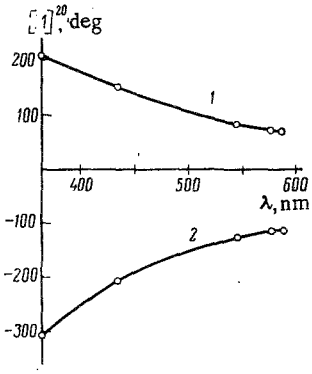


Fig. 1. Optical rotation dispersion curves of α- and β-L-rhamnopyranosyl phosphates (50 μmoles/ml, water): 1) β-anomer; 2) α-anomer.

which is homogeneous when based on the data of TLC on SiO_2 and paper electrophoresis,* had a UV spectrum (λ_{max} 273 and 233 nm, ε_{273} 2880, ε_{233} 450; λ_{min} 240 nm, ε_{min} 240) that is characteristic for o-hydroxyphenyl alkyl phosphates [8]. The acid hydrolysis of (III) led to the detection of o-hydroxyphenyl phosphate as the sole phosphorus-containing compound.

The next step of the process also required substantial modification. The cleavage of the protective group from the phosphate moiety by treatment with bromine water, as described in [5, 6], proved to be completely unsatisfactory in the microscale synthesis. We were able to detect phosphate (IV) only as traces. Much better results are obtained using $Pb(OCOCH_3)_4$, which was used previously to obtain alkyl and allyl phosphates [8], polyisoprenol phosphates [9], and the phosphatidic acid [10]. After oxidizing the o-hydroxyphenyl ester (III), and removal of the protective groups from the reaction product by treatment with 1 N LiOH solution, we detected β-L-rhamnopyranosyl phosphate (IV) as the main component of the reaction mixture, the purification of which was effected by ion-exchange chromatography on the anionite Dowex AG-1X8 (HCO_3^-), with elution using a linear gradient of triethylammonium bicarbonate (TEAB) solution ranging from 0 to 0.3 M. The product was isolated in 57% yield when based on (II).† When the values of the optical rotation in the fractions were measured it was found that the molecular rotation of the product, eluted from the column, remains constant on all sections of the peak within the limits of measurement error.

The obtained (IV) is homogeneous when based on the chromatography and paper electrophoresis data, in which connection in the chromatographic system, which effects a separation of the α- and β-anomers of rhamnopyranosyl phosphate [11], its mobility coincides with that described for the β-anomer. The acid-labile phosphorus : total phosphorus : rhamnose : reducing sugar ratio = 1 : 1.02 : 1.03 : 0.99. The configuration of the glycoside linkage in (IV) was confirmed by the NMR spectrum: the proton at C-1 gives a signal as a quadruplet ($J_{1,2}$ = 1.0 Hz, $J_{1,P}$ = 10 Hz) at δ 4.90 ppm, whereas in the authentic sample of α-L-rhamnopyranosyl phosphate, obtained as described in [12], the signal is observed at 5.08 ppm ($J_{1,2}$ = 1.5 Hz, $J_{1,P}$ = 10 Hz). The protons of the CH_3 group, both of the α- and the β-anomers, give distinct doublets at δ 1.17 ppm ($J_{5,6}$ = 6.0 Hz) in the NMR spectrum. The optical rotation dispersion curves of (IV) and α-L-rhamnopyranosyl phosphate (Fig. 1) also have characteristic differences, like, for example, the α- and β-glucopyranosyl phosphates [13].

The presented data do not leave any doubt as to the validity of the (IV) structure. The NMR spectra and the analytical data obtained by us for glycosyl phosphate (IV) practically coincide with the data given in [6]. At the same time, our compound has a noticeably higher positive rotation ($[M]_D^{20}$ +80°) than the previously described compound ($[M]_D$ +56° [6]). This is possibly due to the fact that the latter was contaminated with the α-anomer of the glycosyl phosphate, since it was obtained from the sirupy, and not from the crystalline 2,3,4-tri-O-acetyl-L-rhamnopyranose.

EXPERIMENTAL METHOD

For the chromatography and the paper (FN 11) electrophoresis, and also for the TLC on SiO_2 (KSK grade) we used the following solvent systems: A) methanol–HCOOH–water (80 : 15 : 5); B) chloroform–methanol (9 : 1); C) 0.05 M triethylammonium bicarbonate (TEAB) solution, pH 7.5. The sugar and

* The electrophoretic mobility of (III) corresponds to the mobility of the diesters of phosphoric acid.
†In order to obtain a high yield of (IV) it is important to thoroughly wash the precipitates of basic lead salts that is formed in the alkaline treatment of the reaction mixture.

phosphorus-containing derivatives were detected on the paper chromatograms and electrophoregrams by, respectively, spraying with alkaline $AgNO_3$ solution [14] and Haines–Isherwood reagent [15]. In the case of TLC the compound was detected with 50% H_2SO_4 solution by heating.

The paper electrophoresis was run using a PVEF-1 instrument and a voltage gradient of 31 V/cm. The solvents were evaporated in vacuo below 37°. The NMR spectra were taken at 60 MHz on a Varian DA-60-IL instrument, in $CDCl_3$ relative to TMS, or in D_2O relative to dioxane (δ 3.62 ppm) in the case of the glycosyl phosphates. The optical rotation was measured on a Perkin–Elmer Model 141 instrument.

The quantitative determination of the reducing sugar after hydrolysis (15 min, 100°, 0.05 M HCl solution) was run as described in [16], while the rhamnose was determined by the Dishe method [17].

The acid-labile phosphate was determined as described in [18]. The total phosphorus was determined in a similar manner after prior combustion of the sample. For the combustion a mixture of the analyzed solution and 1.2 ml of 2 N H_2SO_4 solution was heated at 200° for 1 h, followed by the addition of several drops of H_2O_2 until colorless, and the mixture was heated again at 200° for 1 h. To the cooled mixture were added 0.6 ml of the reagent for determining phosphate [18] and water up to 5 ml, and the phosphate was determined the same as in the case of the acid-labile phosphate.

2,3,4-Tri-O-acetyl-β-L-rhamnopyranose (II). To 15 g of 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl bromide (I) [7], dissolved at 0° in 80 ml of acetone plus 2.08 ml of water, was added 12 g of freshly prepared anhydrous Ag_2CO_3 and the mixture was stirred for 30 min, after which the precipitate was washed with chilled acetone (0°). The mother liquor and rinse solution were combined and evaporated to a sirup, which was dried using the vacuum of an oil pump. Crystalline (II) was obtained by dissolving the sirup in ether and then gradually adding heptane under cooling. The pure β-anomer was isolated by fractional recrystallization from ether to give 8 g (67% yield) of product, mp 98–100°, $[\alpha]_D^{20}$ +31° (C 1.0; absolute alcohol) $\xrightarrow{10\ \text{days}}$ −18°; R_f 0.61 (TLC, system B). From [7]: mp 96–98°, $[\alpha]_D$ +28° (alcohol).

2,3,4-Tri-O-acetyl-β-L-rhamnopyranosyl o-Hydroxyphenyl Phosphate (III). To a solution of 286 mg of o-phenylene chlorophosphate [8] in 5 ml of absolute THF was added 0.23 ml of 2,6-lutidine, the mixture was cooled to 0° and, with stirring, a solution of 145 mg of (II) in 4 ml of THF was added in drops. After stirring at 20° for 25 min the precipitate was separated by centrifuging, washed (4 × 1 ml) with absolute THF, and to the obtained solution were added 0.28 ml of 2,6-lutidine and 12 ml of THF containing 75 μl of water. After stirring at 20° for 30 min the reaction mixture was evaporated, and the residue was dried in vacuo, dissolved in the minimum amount of THF, and transferred as a mixture with SiO_2 to a column (1.8 × 17 cm) containing SiO_2. The compounds were eluted from the column with $CHCl_3$ (100 ml) (traces of unreacted (II) are eluted here), and then with a $CHCl_3$–methanol mixture (9:1, 150 ml). The fractions (15 ml each) were analyzed by TLC in system B, and also by paper electrophoresis in system C. The fractions that contained (III) were evaporated to dryness and dried in vacuo. We obtained 200 mg (70%) of (III) as an amorphous hygroscopic powder, $[\alpha]_D^{20}$ +1.3° (C 1.34; THF). In order to obtain its UV spectrum, (III) was converted to the K salt by passing a methanol solution through a column (1.5 × 10 cm) containing Dowex 50 (K^+ form); the compound was obtained as fine crystals that deliquesce in the air. λ_{max} 273 and 233 nm, ε_{273} 2880, ε_{233} 450; λ_{min} 240 nm, ε_{min} 250; R_f 0.21 (TLC, system B). M_2 0.61* (electrophoresis, system C).

β-L-Rhamnopyranosyl Phosphate (IV). To a solution of 158 mg of o-phenylene chlorophosphate in 4 ml of absolute p-dioxane was added 130 μl of 2,6-lutidine. The solution was cooled strongly and 70 mg of (II) was added. Then the temperature was raised gradually up to 20° in 20 min and the solution was stirred vigorously for 45 min, after which the precipitate was separated by centrifuging and washed with dioxane (3 × 2 ml). To the supernatant liquor were added 160 μl of 2,6-lutidine and 10 μl of water. After stirring at 20° for 30 min the small amount of precipitate was filtered, the filtrate was evaporated in vacuo to a sirup, and then 10 ml of dioxane was added. The insoluble residue was filtered, washed with dioxane, the filtrate was evaporated, and the residual sirup was dried by azeotropic distillation with benzene (10 × 1 ml), and then evacuated for several hours using an oil pump. The obtained dry residue was dissolved in 4 ml of absolute dioxane, the solution was cooled strongly, and 250 mg of $Pb(OCOCH_3)_4$ was added. After vigorous stirring of the mixture at 20° for 30 min the solution was added in drops, at 0°, to 10 ml of 1 N LiOH solution, and the mixture was stirred for 14 h at 20°. The pH of the solution was adjusted to ~3 by the gradual addition of the cationite Dowex 50 (H^+ form) at 0°, the precipitate and cationite were separated by centrifuging, the supernatant liquor was quickly neutralized to pH 7 with 1 N LiOH solution, while 20

*The mobility is expressed relative to α-D-glucopyranosyl phosphate.

ml of chilled water was added to the precipitate, and the treatment with the cationite was repeated. After a sevenfold treatment of the precipitate the combined supernatant liquors were diluted with water to 300 ml and transferred to a column (1.7 × 18 cm) containing Dowex AG-1X8 (HCO_3^- form). The column was washed with water (100 ml), and then with a linear gradient of TEAB solution ranging from 0 to 0.3 M (300 ml each). The acid-labile phosphorus and the optical rotation values were determined in the fractions (9 ml). The fractions, containing phosphate (IV) (eluted with 0.18 M TEAB solution) were combined, evaporated to dryness, and water (5 × 3 ml) was distilled from the residue in order to remove the TEAB. The yield of (IV) was 137 μmoles (57%), $[M]_D^{20}$ +80° (water). M_1 0.73* (system A), M_2 1.0† (buffer C). NMR spectrum (δ, ppm): 4.90 q (1H), $J_{1,2}$ = 1.0 Hz, $J_{1,P}$ = 10 Hz; 1.17 d (3H), $J_{5,6}$ = 6 Hz.

CONCLUSIONS

A modified synthesis of β-L-rhamnopyranosyl phosphate was proposed, which makes it possible to obtain high yields of the product and is suitable for microscale operation.

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*The mobility is expressed relative to α-L-rhamnopyranosyl phosphate.
†See footnote on previous page.