Note

Enzymic synthesis of 6-deoxy-α-D-glucopyranosyl α-D-glucopyranoside and α-D-xylopyranosyl α-D-glucopyranoside

E. BELOCOPITOW*, L. R. MARECHAL*,

Instituto de Investigaciones Bioquímicas "Fundación Campomar" and Departamento de Química Biológica,

AND E. G. GROS*

Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Perú 222, Buenos Aires (Argentina)

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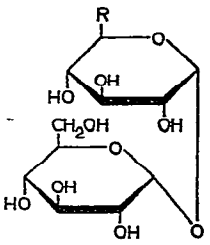
Several oligosaccharides have been synthesized enzymically by transglycosylation reactions in which enzymes catalyze the transfer of glycosyl groups from a glycosyl phosphate as the donor molecule to an acceptor molecule which may be a monosaccharide[1–3]. As a typical example, it has been shown that the maltose phosphorylase from *Neisseria meningiditis* catalyzes the formation of maltose-like disaccharides from β-D-glucopyranosyl phosphate and such sugars as 6-deoxy-D-glucose and D-xylose as acceptors for the D-glucopyranosyl group transferred from the phosphate donor[4].

We now report that, in the course of studies on the specificity of substrates for trehalose phosphorylase from *Euglena gracilis*[5], we have observed that 6-deoxy-D-glucose and D-xylose can replace D-glucose as the acceptor for the transglycosylation reaction, to yield the hitherto-unknown disaccharides now described.

For the synthesis of the title products, β-D-glucopyranosyl phosphate was incubated with the enzyme preparation in the presence of 6-deoxy-D-glucose or D-xylose, respectively. After incubation, the enzyme was inactivated, and the respective sugars were isolated by column chromatography on charcoal–Celite[6]. The chromatographically pure disaccharides were shown, by hydrolysis with acid, to have been formed from the monosaccharides used as substrates; they had the expected values for their molecular rotations[7], and the n.m.r. spectrum of each was in agreement with the respective structure proposed[8]. The ring sizes of the constituent units were assigned from the results obtained by oxidation with sodium periodate. According to previous results[8], they can be regarded as adopting the C_1^4 (D) conformation.

Each disaccharide was further identified as the respective peracetate; the physical properties of the crystalline hepta-*O*-acetyl derivatives of **1** and **2** confirmed the structures assigned for the parent disaccharides.

*Research member of the Consejo Nacional de Investigaciones Científicas y Técnicas.



1, R = CH_3
2, R = H

As far as we know, no nonreducing disaccharide formed by a D-glucopyranosyl and a 6-deoxy-D-glucopyranosyl moiety has been described previously. The only such nonreducing disaccharide known[9], a D-glucosyl D-xyloside, has $[\alpha]_D$ −36°, and therefore, at least one of its glycosyl residues presumably has the β-D configuration; neither the anomeric configurations nor the ring sizes were reported. In our work, the α,α configuration of both compounds **1** and **2** was proved.

Hence, the trehalose phosphorylase from *Euglena gracilis* constitutes a very useful enzymic system for the synthesis of new, nonreducing oligosaccharides.

EXPERIMENTAL

General. — Melting points were determined with a Fisher–Johns, hot-plate apparatus and are uncorrected. N.m.r. spectra were recorded with a Varian A-60 spectrometer for solutions in deuterium oxide or chloroform-*d*. β-D-Glucopyranosyl phosphate (dipotassium salt) was prepared according to Reithel[10], with slight modifications. 6-Deoxy-D-glucose was prepared by acid hydrolysis of methyl 6-deoxy-β-D-glucopyranoside (purchased from Pierce Chemical Co.). β-D-Glucopyranosyl phosphate was measured either by the labile P method (0.5M sulfuric acid, for 5 min at 100°) or with a specific phosphoglucomutase obtained from *E. gracilis* extracts, coupled with D-glucose-6-phosphate dehydrogenase and NADP[11]. Microanalyses were performed by the A. Bernhardt Laboratory, W. Germany. Solvents were removed under diminished pressure below 40°.

Preparation of the enzyme. — The scheme used was essentially that designed by Marechal and Belocopitow[5], and consisted in the culture of cells of *Euglena gracilis varbacillars* in a medium containing peptone (0.5%, w/v), yeast extract (0.2%), vitamin B_{12} (5×10^{-6}%), and ethanol (1%, v/v). For bulk growth, 15-liter batches were used, and sterile air was bubbled through the solution during the growing period. After 6 days, the cells were collected by means of an Alfa Laval centrifuge. The following steps were performed at 0–4°. The cells were washed repeatedly with water by centrifugation at 3,000 *g*. To prepare the enzyme extract, the cells were resuspended in 1.5 parts of a solution containing (ethylenedinitrilo)tetraacetate (EDTA; 2mM), phosphate buffer (2mM), and glycerol (25%), at pH 7.0, and disrupted in a Frech press at 12,000 lb.in.$^{-2}$ to give a "crude extract". This crude extract was centrifuged at 5,000 *g* for 15 min. The pellet was washed with the same solution as that used to

disrupt the cells. This process was repeated once more. The supernatant fluids were combined ("sp 5,000"), and treated with protamine sulfate; this mixture was kept for 15 min at 0°, and then centrifuged at 25,000 *g* for 15 min, and the pellet was discarded. In order to obtain the maximum amount of precipitate and the maximal trehalose phosphorylase activity in the supernatant liquor (about 150 μg/mg of protein), the final concentration of the protamine sulfate was determined for each batch of extract. The supernatant liquor from the treatment with protamine sulfate ("protamine sp") was centrifuged at 250,000 *g* for 3 h in a Spinco preparative ultracentrifuge. The pellet was resuspended in 0.1 part of the solution used to disrupt the cells ("250 pellet"), and used for the incubation process. The concentration of protein was 42 mg/ml, as determined according to Lowry *et al.*[12]. The enzyme activity was measured as indicated by Marechal and Belocopitow[13].

*Preparation of 6-deoxy-α-*D*-glucopyranosyl α-*D*-glucopyranoside* (**1**) *and of α-*D*-xylopyranosyl α-*D*-glucopyranoside* (**2**). *General procedure.* — For the preparation of **1**, the reaction mixture (20 ml) contained β-D-glucopyranosyl phosphate (100 mmoles), 6-deoxy-D-glucose (125 mmoles), enzyme preparation ("250 pellet") (1.7 ml), and imidazole hydrochloride buffer at pH 7.0 (50 mmoles).

For the preparation of **2**, the reaction mixture (33 ml) contained β-D-glucopyranosyl phosphate (60 mmoles), D-xylose (160 mmoles), enzyme ("250 pellet") (1.7 ml), and imidazole hydrochloride buffer at pH 7.0 (60 mmoles).

The mixtures were incubated under toluene, to prevent microbial contamination, for 8 h at 37°. After deproteinization by heating for 6 min at 100°, the precipitate was centrifuged off, and the supernatant liquor was passed through a column[6] of charcoal–Celite (2 × 6 cm). The column was washed first with water (2 liters), and then with 1% aqueous ethanol until no reducing power was detectable by the Somogyi–Nelson method[14,15]. The presumed disaccharide was then eluted with 10% ethanol, and the solvent was evaporated. The product was redissolved in water, and the amount of sugar present was determined by the phenol–sulfuric acid method[16].

Compound **1** (120 mg) was isolated as a syrup, $[\alpha]_D^{22}$ +176.1° (*c* 3.0, water); periodate oxidation resulted in the consumption of 4 moles of reagent per mole of sugar, and no liberation of formaldehyde was detected; for the acid hydrolysis, an aliquot (0.75 μmole) was dissolved in 0.5M hydrochloric acid (50 μl), and the solution was heated in a sealed tube for 1 h at 100°. The solution was cooled, pyridine was added, the solution was evaporated to dryness, and the residue was chromatographed on Whatman No. 1 paper, with 6:4:3 butyl alcohol–pyridine–water as the eluting solvent, for 48 h. An aliquot of the original disaccharide was also chromatographed. Sugars were detected according to Trevelyan *et al.*[17]. The pure disaccharide had R_G 0.80, and the hydrolysis products were identified as glucose and 6-deoxyglucose by comparison with authentic standards. N.m.r. data for **1**: τ 8.75 (doublet, 3 H, $J_{Me,H\text{-}5}$ 6 Hz, CH_3–CH), 6.0–6.8 (multiplet, 10 H, ring protons), and 4.80 (doublet, 2 H, $J_{1,2}$ 3.5 Hz, both anomeric protons).

Compound **2**, also, was isolated as a syrup (50 mg), $[\alpha]_D^{22}$ +164.4° (*c* 1.2, water); on acid hydrolysis, conducted as just described, it produced only glucose and xylose

(the disaccharide had R_G 0.67); on periodate oxidation it consumed 4 moles of reagent per mole of sugar; the n.m.r. spectrum showed between τ 5.80 and 6.80 a complex pattern for 11 protons, and the two anomeric protons resonated at τ 4.78 (doublet, 2 H, $J_{1,2}$ 3.5 Hz).

Hepta-O-acetyl derivative of **1**. — Compound **1** (60 mg) was dissolved in pyridine (2 ml), and treated with acetic anhydride (2 ml). After 18 h at room temperature, the mixture was poured onto ice–water, and the solid was filtered off, and dried. The crystalline product (94 mg) was recrystallized from ethanol, giving 82.5 mg of pure compound, m.p. 87–88°, $[\alpha]_D^{22}$ +177.5° (*c* 0.8, chloroform); the i.r. spectrum showed no hydroxyl absorption; n.m.r. data: τ 8.85 (doublet, 3 H, $J_{Me,H-5}$ 6 Hz, CH_3–CH); 7.92, 7.95, and 7.97 (three sharp singlets, 21 H, CH_3–CO); 5.70–5.90 (broad signal, 4 H, H-5, H-5′, CH_2–); the signal from the anomeric protons was overlapped by resonance signals due to other ring protons.

Anal. Calc. for $C_{26}H_{36}O_{17}$: C, 50.32; H, 5.85. Found: C, 50.12; H, 5.91.

Hepta-O-acetyl derivative of **2**. — Compound **2** (35 mg) was acetylated, as described for acetylation of **1**, with 1:1 pyridine–acetic anhydride. The crystalline product thus obtained was recrystallized from ethanol to give the pure heptaacetate (43 mg), m.p. 60–62°, $[\alpha]_D^{22}$ +151.8° (*c* 0.3, chloroform); the i.r. spectrum showed no bands for hydroxyl; n.m.r. data: τ 7.88, 7.90, 7.92 (three sharp singlets, 21 H, CH_3–CO); 5.80–6.20 (m, 6 H, H-5, H-5′, both CH_2–); the resonance due to both anomeric protons was overlapped by other ring-proton signals.

Anal. Calc. for $C_{25}H_{34}O_{17}$: C, 49.51; H, 5.65. Found: C, 49.85; H, 5.62.

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