p-NITROPHENYL α-MALTOSIDE

By P. M. BARNA*

[Manuscript received September 7, 1970]

Several recent reports mention the usefulness of *p*-nitrophenyl α-maltoside in assaying the activity of α-amylase[1–3] or maltosidase.[4] We undertook to synthesize this compound and examined its usefulness as substrate.

p-Nitrophenyl maltoside was first prepared by Babers and Goebel;[5] this was reportedly the β-anomer. Matsubara reported the synthesis of the α-anomer by two different routes but the results are contradictory. Nitration of phenyl α-maltoside heptaacetate with a nitrating mixture gives a product melting at 147–150° in one case[6] and at 176–178° in the other.[7] Furthermore, the *p*-nitrophenyl maltoside resulting from the deacetylation of the above heptaacetates is reported to have a melting point at 135–136° and a rotation of 281° (*c*, 6 in water) in one paper[6] and a melting point of 145–146° and a rotation of 265° (*c*, 1 water) in another paper.[7]

Several routes were open for us for the synthesis of this substrate. At the outset, we decided against the nitration procedure because we saw no easy way to prepare the *p*-nitrophenyl α-maltoside heptaacetate and foresaw difficulties in separating the *o*-nitrophenyl and *p*-nitrophenyl maltosides resulting from the nitration.

We could try, however, the method used by Jansen and Wydeveld, who fuse maltose octaacetate and *p*-nitrophenol in the presence of titanium(IV) chloride. No physical constants were available on their products.[1,8] Emil Fischer's high-temperature fusion of acetyl sugars and phenols in the presence of zinc(II) chloride was possible[9] and so was the anomerization of the easily prepared β-anomer by a Lewis acid.

In our hands, the fusion methods[1,8,9] produced only intractable tars. Anomerization of the β-anomer[5] by stirring it with concentrated sulphuric acid in acetic anhydride–acetic acid according to Lindberg[10] did not succeed; no change in rotation was observed in 6 hr. At this point we became intrigued by a report by Bose and Ingle[11] who found both anomers in the reaction mixtures of phenols, sugar acetates, tin(IV) chloride, and chloroform. It is known that the alkyl and aryl glycosides form in a two-step reaction in the presence of a Lewis acid; at first the halide ion replaces

* Calbiochem, P.O. Box 54282, Los Angeles, Cal. 90054, U.S.A.

[1] Jansen, A. P., and Wydeveld, P. G. A. B., *Nature*, 1958, **182**, 525.
[2] Matsubara, S., *J. Biochem., Tokyo*, 1961, **49**, 226.
[3] Matsubara, S., *J. Biochem., Tokyo*, 1961, **49**, 232.
[4] Tamaoki, H., Murase, Y., Minato, S., and Nakanishi, K., *J. Biochem., Tokyo*, 1967, **62**, 7.
[5] Babers, F. H., and Goebel, U. F., *J. biol. Chem.*, 1934, **105**, 473.
[6] Matsubara, S., Ikenada, T., and Adabori, S., *J. Biochem., Tokyo*, 1959, **46**, 425.
[7] Matsubara, S., *Bull. chem. Soc. Japan*, 1961, **34**, 718.
[8] Jansen, A. P., and Wydeveld, P. G. A. B., personal communication.
[9] Fischer, E., *Ber. dt. chem. Ges.*, 1916, **49**, 2813.
[10] Lindberg, B., *Acta chem. scand.*, 1950, **4**, 1386.
[11] Bose, J. L., and Ingle, T. R., *Chemy Ind.*, 1967, 1451.

the C1 acetyl group and forms an aceto halo sugar, which, under the reaction conditions, quickly reacts with the alcohol or phenol present. The second step proceeds with a Walden inversion: α-aceto halo sugars invariably produce the β-alkyl or aryl sugar acetates; 1-β-aceto halo sugars, therefore, would produce the desired α-anomers. β-Aceto halo sugars are difficult to prepare because they appear to be thermodynamically less stable than the α-anomers and anomerize to them under most circumstances.[12]

We found that the mild reaction conditions first used by Lemieux[13] and adopted by Bose[10] gave us the *p*-nitrophenyl α-maltoside heptaacetate in excellent yield as the only product. Deacetylation by ammonia afforded the *p*-nitrophenyl α-maltoside.

α-Amylase at pH 6·5 was used to test the product enzymatically. Incubation at 30° for 15–60 min (100 mg substrate and 300 international units of α-amylase) produces *p*-nitrophenol, readable on an ultraviolet spectrophotometer at 410 nm.

We have found *p*-nitrophenyl α-maltoside an effective substrate for purified enzyme samples. An enzyme assay can be completed in 10–30 min.

Experimental

Analyses are by Shankman Laboratories, Los Angeles.

A mixture of 40 g (57 mmol) maltose β-octaacetate and 16·4 g (118 mmol) *p*-nitrophenol were dissolved in 250 ml dry chloroform. In a fume hood, 6 ml $SnCl_4$ was added carefully, and the mixture was refluxed with the exclusion of moisture for 1 hr. After cooling the solution was extracted with 3 × 200 ml water, washed with 5 × 100 ml saturated $NaHCO_3$ solution and with 7 × 200 ml water. The yellow organic layer was dried over Na_2SO_4. The solvent was evaporated and a small part of the residue was recrystallized from a large amount of boiling Skellysolve B for identification. The white crystals melt at 138–139°, $[\alpha]_D^{23}$ +103° (*c*, 1 in $CHCl_3$). *p*-Nitrophenyl β-maltoside heptaacetate melts at 175–176°, $[\alpha]_D^{25}$ +33·8° (*c*, 1·07 in $CHCl_3$).[5] Paper chromatography (in benzene containing 4% MeOH) shows a single spot on H_2SO_4 treatment: we concluded that the material on hand was *p*-nitrophenyl α-maltoside heptaacetate.

The bulk of the solid residue was deacetylated in methanol saturated with ammonia at 5° overnight. The solvent and the ammonia were then chased away and the residue was dissolved in warm absolute ethanol. The faintly yellow solution was treated with charcoal and filtered through a Celite bed. The nearly colourless solution would not yield crystals on concentration or cooling; nor did we get crystallization when fractional crystallization was attempted by seeding a small part of the solution with *p*-nitrophenyl β-maltoside. A slightly gummy white product was obtained when the alcoholic solution was added to stirred anhydrous ether dropwise; on trituration with 1:20 absolute ethanol–anhydrous ether the material soon became crystalline. *p*-Nitrophenyl α-maltoside melts at 105°, $[\alpha]_D^{25}$ is +134·7° (*c*, 1 in H_2O) (Found: C, 46·5; H, 5·3; N, 3·0. Calc. for $C_{18}H_{25}NO_3$: C, 46·6; H, 5·4; N, 3·0%). Paper chromatography showed a single spot on H_2SO_4 treatment. The mother liquor of the product on paper chromatography showed a single spot moving at the same speed with that of the crystals.

The Assay of α-Amylase

The method is similar to that published.[1] Potassium phosphate buffer (pH 6·5, 0·05M) was made as follows. Stock solutions of 0·87 g K_2HPO_4 in 100 ml H_2O and 0·68 g KH_2PO_4 in 100 ml H_2O were prepared. To 50 ml KH_2PO_4 solution K_2HPO_4 solution was added until pH 6·5 was obtained. *p*-Nitrophenyl α-maltoside was dissolved in water at a concentration of 200 mg/ml. Assays were run at 30° with a recording spectrophotometer (Beckman DU with a Gilford recording attachment) at 410 nm. To each cuvette 2·9 ml buffer and 0·1 g substrate were added. At zero time 100–1000 maltose international units of α amylase were added and A_{410} was recorded.

[12] Korytnyk, W., and Mills, J. A., *J. chem. Soc.*, 1959, 636.

[13] Lemieux, R. V., and Shyluk, W. P., *Can. J. Chem.*, 1953, **31**, 528.