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The Preparation of *p*-Nitrophenyl 2-Acetamido-3-*O*-methyl-2-deoxy-*β*-D-glucopyranoside

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The title compound is required for a mechanistic study on the *N*-acetyl-*β*-D-glucosaminidase [EC. 3.2.1.30] hydrolysis. The 3-*O*-substitution of 2-acetamido-2-deoxy-D-glucose is usually achieved by a reaction on the 4,6-*O*-benzylidene derivative as an intermediate.[1] However, in the case of *p*-nitrophenyl glycoside which is very acid-labile, the destruction of glycoside occurs when the 4,6-*O*-benzylidene group is removed. The cleavage of *p*-nitrophenyl glycoside under the conditions which had been used for debenzylidenation (in 66% acetic acid at 100°C for 30 min) was determined. The results (Table 1) indicate that *ca.* 30% of the *p*-nitrophenyl 2-acetamido-2-deoxy-*β*-D-glucopyranoside is damaged under those conditions. A more easily removable group than *O*-benzylidene was desirable, and *p*-methoxybenzylidene group was selected because it was found to be cleaved by the milder treatment.[2] Moreover, a further application of *O*-(*p*-methoxybenzylidene)-blocking and an improved procedure for *O*-benzylidenation was attempted in connection with the previous studies.[2,3] *O*-Benzylidene formation by acetal exchange reaction was recently reported as a convenient method.[3,4] Although *p*-methoxybenzaldehyde was less reactive than benzaldehyde, the preparation of 4,6-*O*-(*p*-methoxybenzylidene) derivative in good yield by the acetal exchange reaction of *p*-methoxybenzaldehyde dimethyl acetal was also successful.

Table 1. Destruction of *p*-nitrophenyl glycosides under the conditions used for debenzylidenations

	p-Nitrophenyl 2-acetamide 2-deoxy-*β*-D-glucopyranoside	*p*-Nitrophenyl-*β*-D-glucopyranoside
10 min	12%	1.3%
20 min	22%	1.7%
30 min	29%	2.1%

The treatment of I with *p*-methoxybenzaldehyde dimethyl acetal and a catalytic amount of *p*-toluenesulfonic acid under reduced pressure afforded an almost quantitative yield of II, which was then methylated by Kuhn's method. Then, the removal of 4,6-*O*-(*p*-methoxybenzylidene) group led to the title compound (IV). The conditions for the removal of the blocking group were successfully achieved by the treatment with 90% acetic acid at 100°C for 1 min.

The synthetic scheme and compounds prepared are shown in Fig. 1.

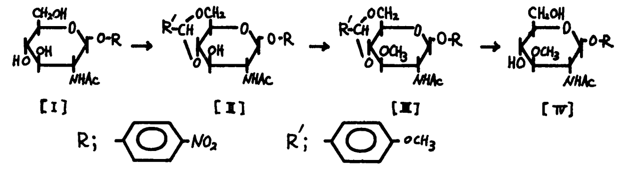

Fig. 1.

Experimental

The melting points were determined by means of the Yanagimoto-micro melting point apparatus and uncorrected. Optical rotations were measured by means of a recording spectropolarimeter (Yanagimoto ORD-185). Thin-layer chromatography was performed on Silica Gel H (Merck), and spots were detected with 5% sulfuric acid in methanol.

p-Methoxybenzaldehyde dimethyl acetal was prepared by the method of Claisen.[5] Methyl orthoformate was used in place of ethyl orthoformate. Bp 95—96°C/1.0 mmHg (lit,[6] bp 261—263°C/760 mmHg). (Found: C, 65.96; H, 7.76%. Calcd for $C_{10}H_{14}O_3$: C, 65.91; H, 7.74%).

p-*Nitrophenyl 2-Acetamido-4,6-*O-(p-*methoxybenzylidene)-2-deoxy, β-D-glucopyranoside (II).* *p*-Nitrophenyl 2-acetamido-2-deoxy-*β*-D-glucopyranoside (I)[7] (3.5 g) dissolved in 150 ml of dimethylformamide (DMF), was treated with 15 ml of *p*-methoxybenzaldehyde dimethyl acetal and 0.50 g of *p*-toluenesulfonic acid at 40°C for 8 hr under reduced pressure (15 mmHg). The reaction mixture was evaporated *in vacuo* to dryness, and then the residue, suspended in a cold saturated sodium bicarbonate solution, was collected by filtration. The precipitate dried *in vacuo* was crystallized from toluene and acetone; 4.5 g (96%) of (II) were thus obtained. Mp 239—240°C (dec.), $[\alpha]^{25}_{589}$ −14.5° (*c*, 0.813, DMF). Found: C, 57.25; H, 5.41; N, 5.93%. Calcd for $C_{22}H_{24}O_9N_2$: C, 57.39; H, 5.25; N, 6.08%.

Unlike as in the case of benzaldehyde dimethyl acetal,[3] the formation of mixed acetal at 3-hydroxyl could not be observed when the experiment was conducted under reduced pressure.

p-*Nitrophenyl 2-Acetamido-3-*O-*methyl-4,6-*O-(p-*methoxybenzylidene)-2-deoxy-β-D-glucopyranoside (III).* To a solution of II (0.50 g) in 20 ml of DMF, we added 30 ml of methyl iodide and 5.0 g of barium oxide. The reaction mixture was then allowed to stand at room temperature for 20 hr. The filtrate, freed from insoluble materials, was evaporated *in vacuo* to dryness. The subsequent crystallization of the residue form toluene and chloroform afforded 0.32 g (62%) of III. Mp 250—251°C (dec), $[\alpha]^{25°}_{589}$ −5.59° (*c*, 0.760, DMF). Found C, 58.12; H, 5.51; N, 5.90%. Calcd for $C_{23}H_{26}O_9N_2$: C, 58.22; H, 5.52; N, 5.90%.

A second crop of crystalline III (0.12 g, 23%) was obtained from the evaporation of the mother liquor. Mp 249—251°C (dec.).

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1) *Cf.* for example; A. Neuberger, *J. Chem. Soc.*, **1941**, 50.
2) K. Yamamoto, This Bulletin, **46**, 290 (1973).
3) K. Yamamoto and Taku Hayashi, *ibid.*, **46**, 656 (1973).
4) M. E. Evans, *Carbohyd. Res.*, **21**, 473 (1972).
5) L. Claisen, *Ber.*, **40**, 3903 (1907).
6) L. Claisen, *ibid.*, **31**, 1010 (1898).
7) D. H. Leaback, "Biochemical Preparation," **10**, 118 (1963).

p-Nitrophenyl 2-Acetamido-3-O-methyl-2-deoxy-β-D-glucopyranoside (IV). Two hundred mg of (III) in 20 ml of 90% acetic acid (v/v) were heated to 100°C for 1 min, and then the acetic acid solution was evaporated *in vacuo* to dryness. The subsequent crystallization of the residue from ethanol gave 60 mg (40%) of a crystalline IV. Mp 186—187°C (dec.), $[\alpha]^{25}_{589}$ −34.8° (*c*, 0.467, DMF). Found: C, 50.32; H, 5.70; N, 7.71%; Calcd for $C_{15}H_{20}O_8N_2$: C, 50.56; H, 5.56; N, 7.86%.

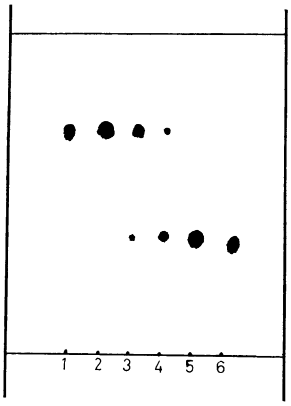

Fig. 2. Thin layer chromatogram of (III) treated with 90% acetic acid at 50°C.
1: Compound (III), 2: Reaction mixture of III, Sampling at 0 min, 3: Reaction mixture of III, Sampling after 2 min, 4: Reaction mixture of III, Sampling after 5 min, 5: Reaction mixture of III, Sampling after 10 min, 6: Compound (IV).

The further crystallization of the mother liquor from ethanol and ether afforded a second crop of IV (70 mg, 47%). Mp 184—187°C (dec.).

Treatment of III with 90% Acetic Acid. The reaction of III (1.0 mg) in 1.0 ml of 90% acetic acid (v/v) was followed by sampling the aliquots of 0.2 ml at 2, 5, and 10 min intervals. Each sample, after it had been neutralized with 0.5 ml of pyridine, was applied to a tlc plate and developed in toluene: pyridine: acetone (2: 1: 1). A thin-layer chromatogram of the reaction mixture is shown in Fig. 2.

The removal of the *O*-(*p*-methoxybenzylidene) group was completed within 10 min at 50°C. Then, the conditions (at 100°C for 1 min) were good enough for the removal because the reaction rate was about 32 (2^5) times faster at 100°C than at 50°C.

Acid Hydrolysis of p-Nitrophenyl-β-D-glucopyranoside and p-Nitrophenyl-2-acetamido-2-deoxy-β-D-glucopyranoside. A solution of each *p*-nitrophenyl glycoside (1 mg) in 1.0 ml of 66% acetic acid was heated at 100°C, and 0.2 ml aliquots were removed at 10, 20, and 30 min intervals. Three ml of 0.5M-sodium cabonate were then added to it, and the absorbance was measured at 400 nm. The destruction of glycosides was estimated from the amount of *p*-nitrophenol which was liberated from the glycosides by acid hydrolysis. The results are shown in Table 1.

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