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p-Nitrophenyl Formate as a Reagent for Preparing *N*-Formyl-hexosamines[1]

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In the course of a study of the substrate specificity of *N*-acetyl-β-D-glucosaminidase [EC. 3.2.1.30], *N*-formyl derivatives of *p*-nitrophenyl 2-amino-2-deoxy-β-D-glucopyranoside (I), 2-amino-2-deoxy-D-glucose (II), and 2-amino-2-deoxy-D-galactose (III) were required. The *N*-formylation of derivatives of II is usually achieved by a reaction in a mixture of formic acid and acetic anhydride,[2] by the pyrolysis of amine-formate,[3] or by the O→N acyl exchange of the formyl ester with sodium methoxide as the catalyst.[4] However, these known methods could not be applied to quite labile I because of their drastic conditions.

An attempt was made to use the active formyl ester for the *N*-formylation of I. *p*-Nitrophenyl formate, which had been used in the preparation of *N*-formyl amino acid,[5,6] was examined and found to be a suitable reagent. Further application of this reagent to II or III was also successful in the preparation of *N*-formyl-derivatives of II and III under milder conditions and by a simpler procedure.

After reporting[7] the use of *p*-nitrophenyl formate in the preparation of *p*-nitrophenyl 2-formylamino-2-deoxy-β-D-glucopyranoside, Pal and Mukerjee[8] reported the *N*-acylation of II using the *p*-nitrophenyl esters of acetic acid, benzoic acid, and stearic acid as the active esters. Active esters such as the *p*-nitrophenyl esters of organic acids seem to be very suitable for use in the *N*-acylation of D-hexosamines and their derivatives, as the reaction is performed under mild conditions. The further reaction of *p*-nitrophenyl formate with *p*-nitrophenyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranoside hydrochloride[9] yielded the corresponding *N*-formyl derivative.

1) A part of the results was presented at the 22nd Annual Meeting of the Chemical Society of Japan (Tokyo, April, 1969).

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3) A. Neuberger and R. U. Pitt Rivers, *Biochem. J.*, **33**, 1580 (1939).

4) W. Meyer zu Reckendorf and W. A. Bonner, *Chem. Ber.*, **94**, 3293 (1961).

5) M. Bodansky, *Nature*, **175**, 685 (1955).

6) K. Okawa and S. Hase, This Bulletin, **36**, 754 (1963).

7) K. Yamamoto, Preprint for the 22nd Annual Meeting of the Chemical Society of Japan, Part III, p. 1954 (1969).

8) H. Mukerjee and P. R. Pal, *J. Org. Chem.*, **35**, 2042 (1970).

9) E. L. May and E. Mosettig, *ibid.*, **15**, 890 (1950).

Experimental

The melting point was measured by means of a micro-melting-point apparatus (Yanagimoto Seisakusho) and was not corrected. The optical rotation was measured by means of a Rudolph (Model-200) photoelectric polarimeter at room temperature.

p-Nitrophenyl formate was prepared by the method of Okawa and Hase[6] and showed a mp of 74—76°C (lit, mp 75—77°C).

p-*Nitrophenyl 2-Formylamino-3,4,6-tri*-O-*acetyl-2-deoxy-β*-D-*glucopyranoside.* To a solution of *p*-nitrophenyl 2-amino-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranoside hydrochloride[7] (300 mg) in 30 m*l* of dimethylformamide, 0.1 m*l* of triethylamine was added, and then *p*-nitrophenyl formate (400 mg) in 15 m*l* of tetrahydrofuran was added, drop by drop. After the reaction mixture had been stirred in ice-bath for 3 hr, the slightly yellow solution was concentrated *in vacuo* (1—3 mmHg) to a syrup. The syrupy residue, dissolved in 100 m*l* of chloroform, was washed with a cold saturated sodium bicarbonate solution and finally with water. The chloroform layer which had been dried over anhydrous magnesium sulfate was evaporated *in vacuo* to dryness. The crystallization of the colorless residue from ethanol afforded 220 mg of *p*-nitrophenyl 2-formylamino-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranoside, which melted at 216—218°C. $[\alpha]_D^{19°} = -47.0$ (*c* 0.477, in acetone). Found: C, 50.05; H, 4.87; N, 6.16%. Calcd for $C_{19}H_{22}O_{11}N_2$: C, 50.22; H, 4.88; N, 6.16%.

2-Formylamino-2-deoxy-D-*glucose.* 2-Amino-2-deoxy-D-glucose hydrochloride (500 mg) was dissolved in 10 m*l* of water containing 10 m*l* of Dowex-1 (carbonate form) under cooling in an ice bath.[10] After the mixture had then been stirred for 1 hr, the ion exchange resin was removed by filtration. To the filtrate (10 m*l*) we then added 40 m*l* of tetrahydrofuran and 1.0 m*l* of pyridine, and then *p*-nitrophenyl formate (1000 mg) in 20 m*l* of tetrahydrofuran, drop by drop. After the reaction had been continued in an ice bath for 3 hr under stirring, the tetrahydrofuran was evaporated *in vacuo* and the residual aqueous solution, freed form the precipitate, was passed through a Dowex-50 (acidic from) column. The effluent was washed three times with chloroform and neutralized with Dowex-1 (carbonate form). The solution was then evaporated *in vacuo* to dryness, and the residual solid was crystallized from ethanol and ether to give 370 mg of 2-formylamino-2-deoxy-D-glucose, which melted at 112—118°C. $[\alpha]_D^{25°} = +30.9$ (Reading after 2 hr; *c* 0.905, in water). Found: C, 40.25; H, 6.33; N, 6.60%. Calcd for $C_7H_{13}O_6N$: C, 40.58; H, 6.33; N, 6.76%.

2-Formylamino-2-deoxy-D-*galactose.* A treatement of 2-amino-2-deoxy-D-galactose hydrochloride (500 mg) similar to that of 2-amino-2-deoxy-D-glucose gave 300 mg of 2-formylamino-2-deoxy-D-galactose, which melted at 162—164°C (dec.). $[\alpha]_D^{25°} = +80.8$ (Reading after 2 hr, *c* 1.06, in water). Found: C, 40.53; H, 6.45; N, 6.57%. Calcd for $C_7H_{13}O_6N$; C, 40.58; H, 6.33; N, 6.76%.

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10) S. Roseman and J. Ludowieg, *J. Amer. Chem. Soc.*, **76**, 301 (1954).