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Note

Synthesis and enzymic hydrolysis of some *p*-nitrophenyl 2-aroylamino-2-deoxy-β-D-glucopyranosides

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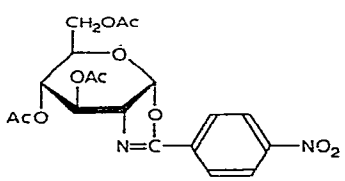
Recently, a novel β-D-hexosaminidase that hydrolyses *p*-nitrophenyl 2-benzamido-2-deoxy-β-D-glucopyranoside was found in the tissues of marine invertebrates and the fruiting bodies of certain mushrooms[1]. Various *N*-aroyl substituted *p*-nitrophenyl 2-amino-2-deoxy-β-D-glucosides were therefore synthesized in order to study the substrate specificity of the new enzyme.

Condensation of 2-amino-2-deoxy-D-glucose with 1.2 mol. of the appropriate, substituted benzoyl chloride in the presence of sodium hydrogen carbonate proved to be a suitable route to the *N*-aroyl derivatives shown in Table I (*cf.* ref. 2). The *p*-nitrophenyl β-D-glycosides in Table III were then obtained *via* the glycosyl chloride intermediates (Table II) by treatment with sodium or potassium *p*-nitrophenoxide[3] (see Experimental). The *N*-*p*-nitrobenzoyl glycoside in Table III was also obtained from the oxazoline **1** (prepared according to the methods of Pravdić *et al.*[4] and Khorlin *et al.*[5]) by treatment with *p*-nitrophenol in nitromethane in the presence of toluene-*p*-sulphonic acid.



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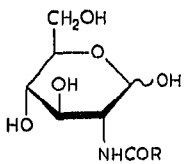
The β-configuration of the glycosides in Table III was confirmed by p.m.r. spectroscopy, and they were deacetylated using methanolic triethylamine[6] to give the compounds shown in Table IV.

The *p*-nitrophenyl 2-aroylamino-2-deoxy-β-D-glucosides shown in Table IV were used as substrates to study the specificity of a β-D-hexosaminidase, earlier termed an *N*-benzoyl-β-D-glucosaminidase[1]. An extract from the fruiting body of the mushroom *Hohenbuehelia serotina* (Fr.) Sing.[7] was used as the enzyme source. It was

TABLE I

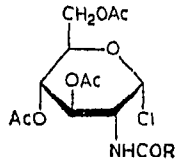
DATA ON SOME *N*-AROYL 2-AMINO-2-DEOXY-β-D-GLUCOPYRANOSIDES



R	*Yield* (%)	*M.p.* (*degrees*)	R_F (*p.c.*)	$[\alpha]_D^{20}$ (*methanol*) (*degrees*)	*Formula*	*Analysis*					
						Calc. (%)			*Found* (%)		
						C	*H*	*N*	*C*	*H*	*N*
p-Nitrophenyl	60	192–193	0.51	+66	$C_{13}H_{16}N_2O_8$	47.56	4.91	8.53	46.93	5.42	8.83
o-Nitrophenyl	63	224–225	0.40	+45					47.34	5.04	8.57
m-Nitrophenyl	56	223–224	0.50	+57					47.53	4.88	8.48
2,4-Dinitrophenyl	48	196–197	0.44	+42	$C_{13}H_{15}N_3O_{10}$	41.61	4.05	11.26	42.09	4.49	11.82
3,5-Dinitrophenyl	47	194–195	0.48	+78					41.02	4.71	11.25
p-Chlorophenyl	54	193–194	0.63	+73	$C_{13}H_{16}ClNO_6$	49.14	5.07	4.41	49.15	5.21	4.46
2,4-Dichlorophenyl	56	210–212	0.60	+66	$C_{13}H_{15}Cl_2NO_6$	44.34	4.29	3.98	44.23	4.64	4.64
p-Bromophenyl	61	208–210	0.62	+82	$C_{13}H_{16}BrNO_6$	43.11	4.45	3.87	42.59	4.60	3.56
o-Bromophenyl	57	212–213	0.42	+58					43.39	4.81	3.74

TABLE II

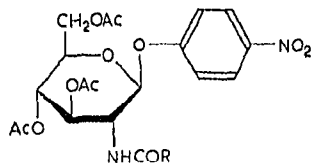
DATA ON SOME ACETYLATED 2-AROYLAMINO-2-DEOXY-α-D-GLUCOPYRANOSYL CHLORIDES



R	*Yield* (%)	*M.p.* (*degrees*)	R_F (*solvent* A)	$[\alpha]_D^{20}$ (*ethyl acetate*) (*degrees*)	*Formula*	*Analysis*	
						Calc. (%) *N*	*Found* (%) *N*
p-Nitrophenyl	86	93–94	0.54	+126	$C_{19}H_{21}ClN_2O_{10}$	5.92	5.62
o-Nitrophenyl	85	113	0.61	+63			6.42
m-Nitrophenyl	29	181–182	0.56	+78			6.15
2,4-Dinitrophenyl	30	183	0.76	+57	$C_{19}H_{20}ClN_3O_{12}$	8.11	7.76
3,5-Dinitrophenyl	70	145–146	0.53	+68			7.98
p-Chlorophenyl	50	148	0.74	+272.5	$C_{19}H_{21}Cl_2NO_8$	3.03	3.21
2,4-Dichlorophenyl	76	133	0.80	+135	$C_{19}H_{20}Cl_3NO_8$	2.83	3.06
p-Bromophenyl	39	158	0.48	+136	$C_{29}H_{21}BrClNO_8$	2.76	2.66
o-Bromophenyl	35	170–171	0.85	+131			3.43

TABLE III

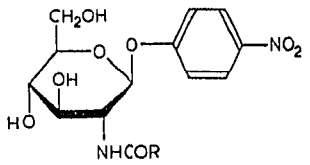
DATA ON SOME *p*-NITROPHENYL 3,4,6-TRI-*O*-ACETYL-2-AROYLAMINO-2-DEOXY-*β*-D-GLUCOPYRANOSIDES



R	*Yield* (%)	*M.p* (*degrees*)	R_F (*solvent* B)	$[\alpha]_D^{20}$ (*chloroform*) (*degrees*)	*Formula*	*Analysis*					
						Calc. (%)			*Found* (%)		
						C	*H*	*N*	*C*	*H*	*N*
p-Nitrophenyl	70·	244	0.33	+19	$C_{25}H_{25}N_3O_{13}\cdot H_2O$	50.59	4.58	7.08	50.46	4.44	6.87
o-Nitrophenyl	75	252	0.24	−48	$C_{25}H_{25}N_3O_{13}$	52.18	4.38	7.30	52.30	4.48	7.31
m-Nitrophenyl	52	241	0.28	+35					52.31	4.55	7.58
2,4-Dinitrophenyl	45	281	0.35	−42	$C_{25}H_{24}N_4O_{15}$	48.39	3.90	9.03	47.64	4.16	8.55
3,5-Dinitrophenyl	79	246–247	0.58	+44					48.84	4.15	9.44
p-Chlorophenyl	71	247	0.54	+31	$C_{25}H_{25}ClN_2O_{11}$	53.14	4.47	4.95	53.23	4.54	5.23
2,4-Dichlorophenyl	80	229	0.36	+18	$C_{25}H_{24}Cl_2N_2O_{11}$	50.09	4.07	4.69	49.98	4.17	4.71
p-Bromophenyl	47	239–240	0.51	+21	$C_{25}H_{25}BrN_2O_{11}$	49.27	4.13	4.60	49.42	4.51	4.71
o-Bromophenyl	45	249–250	0.45	−24					49.09	4.28	4.84

TABLE IV

DATA ON SOME *p*-NITROPHENYL 2-AROYLAMINO-2-DEOXY-β-D-GLUCOPYRANOSIDES



R	*Yield* (%)	*M.p.* (*degrees*)	R_F (*solvent* C)	$[\alpha]_D^{20}$ (N,N-*dimethyl-formamide*) (*degrees*)	*Formula*	*Analysis*						*Relative rate of enzymic hydrolysis*[a] (%)
						Calc. (%)			*Found* (%)			
						C	*H*	*N*	*C*	*H*	*N*	
p-Nitrophenyl	86	228–230	0.65	−7	$C_{19}H_{19}N_3O_{10}$	50.78	4.26	9.35	51.11	4.47	9.40	115
o-Nitrophenyl	49	227–228	0.39	−70	$C_{19}H_{19}N_3O_{10}\cdot H_2O$	48.82	4.52	8.99	48.64	4.78	8.95	0
m-Nitrophenyl	59	221	0.44	−9	$C_{19}H_{19}N_3O_{10}$	50.78	4.26	9.35	50.97	4.52	9.67	125
2,4-Dinitrophenyl	72	254	0.56	−47	$C_{19}H_{18}N_4O_{12}\cdot 0.5H_2O$	45.33	3.80	11.13	45.83	4.03	11.03	0
3,5-Dinitrophenyl	67	217–218	0.41	−2					45.55	4.12	11.24	15
p-Chlorophenyl	90	234	0.70	−4	$C_{19}H_{19}ClN_2O_8$	52.00	4.36	—	52.10	4.60	—	90
2,4-Dichlorophenyl	86	229–230	0.71	−17	$C_{19}H_{18}Cl_2N_2O_8$	48.22	3.83	—	48.44	4.01	—	0
p-Bromophenyl	68	224	0.75	+2	$C_{19}H_{19}BrN_2O_8$	45.52	4.20	5.58	45.45	4.09	5.53	100
o-Bromophenyl	89	224–225	0.67	−52	$C_{19}H_{19}BrN_2O_8\cdot H_2O$	47.22	3.96	—	47.42	4.09	—	0

[a]The rate for the reference compound *p*-nitrophenyl 2-benzamido-2-deoxy-β-D-glucopyranoside is taken as 100.

found that a *p*- or a *m*-substituent in the aroyl residue did not significantly affect the rate of hydrolysis, whereas two *m*-substituents caused a decrease. However, the presence of an *o*-substituent reduced the hydrolysis rate to zero. The relative rates of hydrolysis are shown in Table IV.

EXPERIMENTAL

General methods. — Melting points were determined on a Boethius table. Optical rotations were measured using a Perkin–Elmer Model 141 polarimeter. Paper chromatography (p.c.) was performed on Whatman No. 1 paper with 1-butanol–water–acetic acid (4:1:1). Thin-layer chromatography (t.l.c.) was performed on silica gel with ether (*A*), and on Siluphol with ether–benzene (17:3) (*B*) and ethyl acetate–alcohol (9:1) (*C*). Detection was effected with alkaline silver nitrate, ninhydrin, and chlorine–benzidine. Column chromatography was performed on silica gel.

*2-Deoxy-2-*p*-nitrobenzamido-*D*-glucose.* — To a solution of 0.1 mole of 2-amino-2-deoxy-D-glucose hydrochloride in water (90 ml), sodium hydrogen carbonate (30 g) in water was added followed by dropwise addition, with stirring and cooling in ice, of a solution of 0.12 mol of *p*-nitrobenzoyl chloride in *p*-dioxane (50 ml). The mixture was stirred for 1 h, acidified with hydrochloric acid, and stored at ~5° overnight. The title product was collected, washed with water and ether, dried in air, and then recrystallized from alcohol. The compounds shown in Table I were prepared by essentially the above procedure.

*3,4,6-Tri-*O*-acetyl-2-deoxy-2-*p*-nitrobenzamido-α-*D*-glucopyranosyl chloride.* — A suspension of 2-deoxy-2-*p*-nitrobenzamido-D-glucose (1 g) in freshly distilled acetyl chloride (20 ml) was saturated with dry hydrogen chloride at −15–−20° for ~15 min. The solution was kept in a tightly stoppered flask for 10–12 h at room temperature, and then concentrated *in vacuo* to dryness. Benzene was twice distilled from the residue, which was then dried over KOH *in vacuo* before elution from a column (2.5 × 10 cm) of silica gel with ether. The title compound crystallized from the appropriate fractions. The mother liquors were concentrated to dryness, and the residue was crystallized from ethyl acetate–ether to give more of the title compound. The compounds listed in Table II were prepared by essentially the above procedure.

*2-*p*-Nitrophenyl-4,5-(3,4,6-tri-*O*-acetyl-2-deoxy-*D*-glucopyrano)-Δ²-oxazoline* (**1**). — (*a*) A solution of 2-deoxy-2-*p*-nitrobenzamido-D-glucose (1 g) and freshly fused zinc chloride (2.9 g) in freshly distilled acetic anhydride (9.2 ml) was stirred for 20 min at 85–90°. The cooled mixture was diluted with chloroform (50 ml), washed with saturated, aqueous sodium hydrogen carbonate and water, dried (Na_2SO_4), and concentrated. The residue was eluted from a column of alumina with benzene, benzene–ether, and ether. Fractions containing the substance with R_F 0.6 (solvent *B*) were combined and concentrated to give **1** (0.11 g, 10%), m.p. 145–146° (from ether), $[\alpha]_D^{20}$ +50° (*c* 0.5, benzene), ν_{max} 1681 cm^{-1}.

Anal. Calc. for $C_{19}H_{20}N_2O_{10}$: C, 52.34; H, 4.62; N, 6.42. Found: C, 52.36; H, 4.65; N, 6.63.

(*b*) To a suspension of silver nitrate (5 mmol) in acetone (14.5 ml) and collidine (2 ml), a solution of 3,4,6-tri-*O*-acetyl-2-deoxy-2-*p*-nitrobenzamido-α-D-glucopyranosyl chloride (3 mmol) in acetone (8.5 ml) was added. The mixture was stirred for 2 h at room temperature, diluted with ether (85 ml), and then filtered through a column (3 × 5 cm) of alumina. The column was washed with ether (300 ml), the washings were concentrated to dryness, and the residue was eluted from a column (2.5 × 8 cm) of alumina to give the title product (0.9 g, 70%).

p-*Nitrophenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-*p*-nitrobenzamido-β-D-glucopyranoside.* — (*a*) To a solution of 0.8 mmol of the foregoing oxazoline in nitromethane (20 ml) were added 0.8 mmol of *p*-nitrophenol, and toluene-*p*-sulphonic acid until an acid reaction was obtained. The mixture was boiled under reflux for 30 min and then concentrated to dryness. The residue was eluted from a column (2.5 × 8 cm) of silica gel with ethyl acetate (0, 5, 10, 15, and 20%) in ether. Fractions containing a substance with R_F 0.33 (solvent *B*) were concentrated to dryness, and the residue was crystallized from alcohol to yield the title compound (0.08 g, 20%).

(*b*) 3,4,6-Tri-*O*-acetyl-2-deoxy-2-*p*-nitrobenzamido-α-D-glucopyranosyl chloride (2.1 mmol) was mixed with potassium or sodium *p*-nitrophenoxide (4.2 mmol) in *N*,*N*-dimethylformamide (5 ml). After 14–16 h, the mixture was poured into ice–water (100 ml), and the product was collected, washed with water, and crystallized from alcohol. Additional amounts were obtained from the aqueous solution by extraction with chloroform (total yield, 0.85 g, 70%). The compounds listed in Table III were obtained by essentially the above procedure.

p-*Nitrophenyl 2-deoxy-2-p-nitrobenzamido-β-D-glucopyranoside.* — To a solution of the foregoing triacetate (1.7 mmol) in methanol (50 ml), a 10% solution (34.5 ml) of triethylamine in methanol was added. The mixture was stirred for 8–10 h and then concentrated, and methanol was twice evaporated from the residue, which was then dried over P_2O_5 and crystallized from alcohol to give the title compound (0.8 g, 67%). The compounds listed in Table IV were obtained by essentially the above procedure.

Enzymic hydrolyses. — To a 0.5-μM solution of each *p*-nitrophenyl 2-aroylamino-2-deoxy-β-D-glucoside (Table IV), in 0.2 ml of 0.1M citrate–phosphate buffer (pH 4.0) containing 0.5M NaCl, was added an extract from the fruiting body of *H. serotina* (0.1 ml) or an extract partially purified by chromatography on CM-Sephadex. The mixture was incubated for 30 min at 50°, with subsequent addition thereto of M sodium carbonate (1 ml). The released *p*-nitrophenol was estimated spectrophotometrically at 400 nm. The controls and blanks were incubated and treated in a similar manner.

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