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Note

Synthesis of some *p*-nitrophenyl 2-acylamino-2-deoxy-D-glucosides and their hydrolysis with the β-D-hexosaminidase from *Hohenbuehelia serotina*

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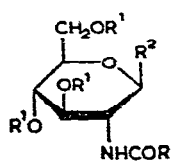
Two new β-D-hexosaminidases have been detected in marine-invertebrate tissues and the fruiting bodies of higher fungi. One enzyme hydrolysed *p*-nitrophenyl 2-deoxy-2-glycylamino-β-D-glucopyranoside[1–3] and the other hydrolysed *p*-nitrophenyl 2-benzamido-2-deoxy-β-D-glucopyranoside[4–5]. The usual β-D-acetylhexosaminidase (EC 3.2.1.30) had no effect on these substrates.

In order to investigate the specificity of the enzyme that hydrolyses *p*-nitrophenyl 2-benzamido-2-deoxy-β-D-glucopyranoside, we synthesized several related substrates in which the 2-acylamino group contained various fatty-acid residues. The 2-acylamino-2-deoxy-D-glucoses (Table I) were obtained by a variety of methods. Thus, reaction of the appropriate fatty-acid chloride with 2-amino-2-deoxy-D-glucose in aqueous alkali[6] gave **1h–k**. Compounds **1c** and **1d** were obtained by treating the appropriate fatty-acid anhydride with 2-amino-2-deoxy-D-glucose in methanol[7]. The derivatives **1a** and **1e–g** were obtained by reacting mixed anhydrides formed from methyl chloroformate and the appropriate acid with 2-amino-2-deoxy-D-glucose in aqueous solution. The last method was very convenient, especially when the products were only partly soluble in water.

Each of the 2-acylamino-2-deoxy-D-glucoses (**1a–k**) was treated with acetyl chloride saturated at −15° with dry hydrogen chloride. Each of the resulting, acetylated glycosyl chlorides, without purification, was treated with sodium *p*-nitro-



1 $R^1 = H$ $R^2 = OH$
2 $R^1 = Ac$ $R^2 = OC_6H_4NO_2$
3 $R^1 = H$ $R^2 = OC_6H_4NO_2$

a R = H **f** R = C_5H_{11}
b R = Me **g** R = C_6H_{13}
c R = Et **h** R = C_7H_{15}
d R = Pr **i** R = C_8H_{17}
e R = Bu **j** R = C_9H_{19}
k R = $C_{13}H_{27}$

TABLE I

DATA ON SOME 2-ACYLAMINO-2-DEOXY-D-GLUCOSES (1)

R in 1	*Yield* (%)	*Synthesis method*[a]	*M.p.* (*degrees*)	$[\alpha]_D^{20}$ (H_2O) (*degrees*)	*Formula*	*Analysis*					
						Found			*Calc.*		
						C	*H*	*N*	*C*	*H*	*N*
H (1a)	11	M	158	+31	$C_7H_{13}NO_6$[b]	38.75	6.78	6.93	38.92	6.52	6.47
Et (1c)	66	A	196	+37	$C_9H_{17}NO_6$	45.71	7.14	5.82	45.95	7.28	5.95
Pr (1d)	60	A	211	+42	$C_{10}H_{19}NO_6$	47.90	7.41	5.60	48.18	7.68	5.62
Bu (1e)	32	M	213	+36	$C_{11}H_{21}NO_6$	50.62	8.09	5.38	50.18	8.04	5.32
n-C_5H_{11} (1f)	33	M	216	+36	$C_{12}H_{23}NO_6$	52.23	8.20	4.95	51.97	8.36	5.05
n-C_6H_{13} (1g)	30	M	218	+72[c]	$C_{13}H_{25}NO_6$	53.93	8.69	4.70	53.59	8.65	4.81
n-C_7H_{15} (1h)	50	C	219	+55[c]	$C_{14}H_{27}NO_6$	54.90	8.96	4.60	55.06	8.91	4.58
n-C_8H_{17} (1i)	60	C	213	+50[c]	$C_{15}H_{29}NO_6$	56.24	9.19	4.15	56.41	9.15	4.38
n-C_9H_{19} (1j)	50	C	216	+52[c]	$C_{16}H_{31}NO_6$	57.82	9.26	4.24	57.63	9.39	4.20
n-$C_{13}H_{27}$ (1k)	54	C	205	+42[c]	$C_{20}H_{39}NO_6$	61.44	10.17	3.42	61.67	10.09	3.60

[a]A, anhydride method; C, acid chloride method; M, mixed anhydride method. [b]With $0.5H_2O$ of crystallization. [c]In *N,N*-dimethylformamide.

TABLE II

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

R in 2	*Yield* (%)	*M.p.* (*degrees*)	$[\alpha]_D^{20}$ (*CHCl*$_3$) (*degrees*)	*Formula*	*Analysis*					
					Found			*Calc.*		
					C	*H*	*N*	*C*	*H*	*N*
H (2a)	39	198	−13	$C_{19}H_{22}N_2O_{11}$[a]	48.64	5.80	5.24	48.65	5.83	5.40
Et (2c)	42	214	−28	$C_{21}H_{26}N_2O_{11}$	52.60	5.57	5.74	52.28	5.43	5.81
Pr (2d)	44	210	−22	$C_{22}H_{28}N_2O_{11}$	53.42	5.92	5.45	53.22	5.68	5.64
Bu (2e)	46	186	−21	$C_{23}H_{30}N_2O_{11}$	54.24	5.97	5.72	54.11	5.92	5.49
n-C_5H_{11} (2f)	46	187	−17	$C_{24}H_{32}N_2O_{11}$	54.48	6.10	5.51	54.96	6.15	5.34
n-C_6H_{13} (2g)	40	170	−14.5	$C_{25}H_{34}N_2O_{11}$	55.54	6.58	5.20	55.76	6.36	5.20
n-C_7H_{15} (2h)	44	179	−19	$C_{26}H_{36}N_2O_{11}$	56.53	6.68	5.04	56.51	6.57	5.07
n-C_8H_{17} (2i)	35	168	−13	$C_{27}H_{38}N_2O_{11}$	57.37	6.91	4.88	57.23	6.76	4.94
n-C_9H_{19} (2j)	30	161	−12	$C_{28}H_{40}N_2O_{11}$	57.99	7.07	5.13	57.92	6.94	4.82
n-$C_{13}H_{27}$ (2k)	62	175	−10	$C_{32}H_{48}N_2O_{11}$	60.30	7.65	4.39	60.36	7.60	4.40

[a]With 2MeOH of crystallisation.

TABLE III

DATA ON SOME *p*-NITROPHENYL 2-ACYLAMINO-2-DEOXY-β-D-GLUCOPYRANOSIDES (3)

R in 3	*Yield* (%)	*M.p.* (*degrees*)	$[\alpha]_D^{20}$ (*HCONMe*$_2$) (*degrees*)	*Formula*	*Analysis*					
					Found			*Calc.*		
					C	*H*	*N*	*C*	*H*	*N*
H (3a)	70	211	−39	$C_{13}H_{16}N_2O_8$	47.34	4.88	8.62	47.56	4.91	8.53
Et (3c)	83	223	−36	$C_{15}H_{20}N_2O_8$	50.53	5.84	7.90	50.56	5.66	7.86
Pr (3d)	75	213	−24	$C_{16}H_{22}N_2O_8$	52.20	6.18	7.69	51.89	5.99	7.56
Bu (3e)	75	205	−29	$C_{17}H_{24}N_2O_8$	53.40	6.56	7.15	53.12	6.29	7.29
n-C_5H_{11} (3f)	70	202	−36.5	$C_{18}H_{26}N_2O_8$	53.91	6.56	7.31	54.26	6.58	7.03
n-C_6H_{13} (3g)	71	203	−29.5	$C_{19}H_{28}N_2O_8$	55.65	7.14	6.85	55.33	6.84	6.79
n-C_7H_{15} (3h)	80	199	−13	$C_{20}H_{30}N_2O_8$	56.36	7.46	6.14	56.32	7.09	6.57
n-C_8H_{17} (3l)	61	204	−24	$C_{21}H_{32}N_2O_8$	57.41	7.78	6.35	57.26	7.32	6.36
n-C_9H_{19} (3j)	60	204	−27.5	$C_{22}H_{34}N_2O_8$[a]	56.52	7.83	6.06	56.77	7.87	5.75
n-$C_{13}H_{27}$ (3k)	78	199	−23.5	$C_{26}H_{42}N_2O_8$	60.69	8.56	5.28	61.16	8.29	5.49

[a]MeOH of crystallisation.

TABLE IV

RELATIVE EXTENTS OF ENZYMIC HYDROLYSIS OF *p*-NITROPHENYL 2-ACYLAMINO-2-DEOXY-β-D-GLUCOPYRANOSIDE (3)

Compound	*Extent of hydrolysis* (%)	*Compound*	*Extent of hydrolysis* (%)
3a	10	**3g**	23
3b	100	**3h**	14
3c	30	**3i**	2
3d	26	**3j**	3
3e	19	*p*-nitrophenyl 2-benzamido-2-deoxy-β-D-glucopyranoside	6
3f	18	*p*-nitrophenyl 2-acetamido-2-deoxy-β-D-galactopyranoside	13

phenoxide in *N,N*-dimethylformamide, and converted into the corresponding acylated *p*-nitrophenyl glycoside (**2a–k**)[8] (Table II). Deacetylation with triethylamine[9] then gave the *p*-nitrophenyl 2-acylamino-2-deoxy-D-glucosides **3a–k** (Table III).

Each of the compounds in Table III was hydrolysed (Table IV) by the β-D-hexosaminidase isolated from *H. serotina* (purified[10] by gel filtration on Sephadex G-150 and ion-exchange chromatography on carboxymethyl Sephadex C-50); the usual *N*-acetyl-β-D-hexosaminidase (EC 3.2.1.30) hydrolysed only **3a** and **3c**, and the rate was insignificant[11].

EXPERIMENTAL

General procedures. — T.l.c. was performed on Silufol plates (Chemapol, Czechoslovakia), using *A* ether–ethyl acetate (9:1) and *B* ethyl acetate–ethanol (9:1). P.c. was carried out on Whatman No. 1 paper, using 1-butanol–water–acetic acid (4:1:1). Optical rotations were determined using a Perkin–Elmer, Model 141 polarimeter. Melting points were determined on a Boethius table.

2-Deoxy-2-nonanamido-D-glucopyranose (**1i**). — To a solution of 2-amino-2-deoxy-D-glucose hydrochloride (10.75 g, 0.05 mol) and $NaHCO_3$ (15.5 g, 0.184 mol) in water (50 ml) at 0–4°, a solution of nonanoyl chloride (12.6 g, 0.079 mol) in *p*-dioxane (25 ml) was added slowly with stirring. Stirring was then continued for 1.5 h at 0–4°. The residue was collected, thoroughly washed with water and ether, and recrystallized from water to give **1i** (8.4 g), m.p. 213°, $[\alpha]_D^{20}$ +50° (*c* 0.5, *N,N*-dimethylformamide); **1h**, **1j**, and **1k** were obtained in a similar manner. Table I shows the properties of these compounds.

2-Deoxy-2-butyramido-D-glucopyranose (**1d**). — A solution of sodium methoxide (from 2.3 g of sodium and 200 ml of methanol) was added with cooling to 2-amino-2-deoxy-D-glucose hydrochloride (21.5 g, 0.1 mol). The sediment was removed by centrifugation, butyric anhydride (16 ml, 0.1 mol) was added to the supernatant with cooling and stirring, and the mixture was stored overnight at room temperature. The

product was collected and thoroughly washed with ether to give **1d** (17.3 g), m.p. 205°. Additional **1d** (3 g) was isolated from the mother liquor. Recrystallization from methanol gave material (17 g) having m.p. 211°, $[\alpha]_D^{20}$ +42° (*c* 1, water). Compound **1c** was obtained in a similar manner. The properties of these compounds are shown in Table I.

*2-Deoxy-2-hexanamido-*D*-glucopyranose* (**1f**). — Triethylamine (14 ml, 0.128 mol) was added with stirring and cooling to a mixture of hexanoic acid (14 ml, 0.128 mol) and toluene (150 ml). To the stirred mixture at −5°, methyl chloroformate (10 ml, 0.128 mol) was added at such a rate that the temperature of the reaction mixture did not exceed −5°. The mixture was kept at −5° for 20 min. A solution of 2-amino-2-deoxy-D-glucose hydrochloride (27.5 g, 0.128 mol) and triethylamine (18 ml) in water (50 ml) was then added with stirring. When the mixture had attained room temperature, it was stirred for 3 h at 20°. The residue was collected, washed with water and chloroform, and crystallized from methanol to give **1f**.

The mother liquor was concentrated, the residue was dried, thoroughly extracted with chloroform, and then dissolved in 50% aqueous methanol, and the solution was treated for 1 h with Cationite KU-2 (H^+) resin (100 ml). The filtrate was neutralized with Amberlite IRA-400 (HCO_3^-) resin and concentrated to give more (4.5 g) of crystalline **1f** (total yield, 33%). Compound **1d** was obtained in a similar way, and **1a** was isolated from the reaction mixture in the same manner as **1f** was obtained from the mother liquor.

Each compound in Table I was homogeneous in p.c.

p-*Nitrophenyl 3,4,6-tri-*O*-acetyl-2-acylamino-2-deoxy-β-*D*-glucopyranosides* (**2**). — A solution of each 2-acylamino-2-deoxy-D-glucose (**1**, 10 mmol) in acetyl chloride (60 ml) was saturated with dry HCl at −15°. After 15 h in a sealed vessel at room temperature, each solution was concentrated *in vacuo* and the residue was dried for 2 h *in vacuo* over KOH. Each resulting, crude, acetylated glycosyl chloride was dissolved in *N*,*N*-dimethylformamide, and a two-fold excess of sodium *p*-nitrophenoxide was added. Each mixture was stirred for 5 h at room temperature, then poured into cold water (150 ml), and extracted with chloroform (3 × 100 ml). The extract was washed carefully with aqueous Na_2CO_3 and water to remove *p*-nitrophenol, then dried (Na_2SO_4), and concentrated. Each residue was quickly dissolved in the minimum amount of ethanol, and the solution was diluted with a five-fold volume of ether and refrigerated. Each product was recrystallized from ethanol or methanol. The properties of the resulting compounds (**2**) are shown in Table II.

Each compound was homogeneous in t.l.c. (solvent *A*).

p-*Nitrophenyl 2-acylamino-2-deoxy-β-*D*-glucopyranosides* (**3**). — A solution of each acetate **2** (1 g) in 10% methanolic triethylamine (100 ml) was kept overnight at room temperature and then concentrated. Each residue was crystallized from alcohol or methanol. The resulting products are shown in Table III.

Each compound was homogeneous in t.l.c. (solvent *B*).

Enzyme hydrolyses. — A 0.5mM solution (0.2 ml) of each compound in Table III, in 0.1M phosphate buffer (pH 4) containing 0.5M NaCl, was incubated with a solution

(0.1 ml) of β-D-hexosaminidase for 30 min at 50°. The reaction was terminated by adding M Na_2CO_3 (1 ml); the amount of *p*-nitrophenol released was determined spectrophotometrically at 400 nm.

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