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Aminosugars. XVII. Synthesis of Benzyl 2-Acetamino-2-deoxy-α-D-glucopyranosiduronic Acidamides and *N*-(2-Acetamino-2-deoxy-D-glucuronyl)-aminoacids[1]*

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Benzyl 2-acetamino-2-deoxy-α-D-glucopyranosiduronic acid was obtained by direct oxidation of the corresponding glucoside, and converted to esters (methyl, ethyl and benzyl) and amides (methyl, ethyl, phenyl and benzyl). Condensation of the uronic acid with aminoacid benzyl-esters (gly, L-ala, L-asp, and L-glu) by dicyclohexylcarbodiimide gave the corresponding amides, some of which were hydrogenated to *N*-(2-acetamino-2-deoxy-D-glucuronyl)-aminoacids. Acid hydrolysis of the peptides were examined.

Hexosaminuronic acids which attract attentions as a polyfunctional sugar have been recently found in V_1-antigen of *Salmonella typhosa*[1] and other natural sources.[2] Heyns and Paulsen[3] first succeeded in the catalytic oxidation of methyl and benzyl 2-(benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranoside to the corresponding uronic acid in about 40% yield, using gaseous oxygen in the presence of platinum charcoal in a weakly alkaline solution, but had no success in the catalytic oxidation of the corresponding 2-acetamino-2-deoxy derivative. A few years later, Marsh and Levvy[4] obtained phenyl 2-acetamino-2-deoxy-α- and -β-D-glucopyranosiduronic acid in a yield of 29 and 49%, respectively, by a similar method using platinum oxide. Recently, Weidmann and Zimmerman[5] improved the method of Heyns and Paulsen,[3] and synthesized several derivatives of 2-acetamino-2-deoxy-D-glucuronic acid, starting from benzyl 2-(benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranosiduronic acid.

We synthesized several esters and amides of benzyl 2-acetamino-2-deoxy-α-D-glucopyranosiduronic acid (**1**) which was derived from the corresponding glucoside by direct oxidation, and in connection with our studies[6] on the carbohydrate-aminoacid linked compounds, carried out synthetic and hydrolytic works on *N*-(2-acetamino-2-deoxy-D-glucuronyl)aminoacids.

Results

Benzyl 2-acetamino-2-deoxy-α-D-glucopyranoside which was obtained in an excellent yield by an improved method of Kuhn *et al.*,[7] was oxidized by the method of Heyns and Paulsen[3] until the starting material was consumed, and the solution was filtered, deionized, and evaporated to give **1** in 70—90% yield. The use of secondary alcohols as an antiforming agent and antioveroxidant improved the yield.

Condensation of **1** with methanol, ethanol or benzyl alcohol in benzene by refluxing and azeotropic removal of water formed gave the corresponding esters (**2**, **3** and **4**) in about 50% yield. Treatment of **1** with saturated methanolic ammonia in a sealed tube at 90°C for 2 hr gave the corresponding amide (**5**) in a quantitative yield, which was identical with that synthesized by Weidmann and Zimmerman[8] from benzyl 2-(benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranosiduronic acid

*1 Part of this work was presented at the 21st Annual Meeting of The Chemical Society of Japan, April 1968. The main subject of this series of papers was revised from "Studies on D-Glucosamine Derivatives." Part XVI: *Carbohyd. Res.*, **4**, 435 (1967).

1) W. R. Clark, J. Mclaughlin and M. E. Webster, *J. Biol. Chem.*, **230**, 81 (1958).

2) T. H. Haskell and S. Hanessian, *Nature*, **199**, 1075 (1963); K. Heyns, G. Kiessling, W. Lindenberg, H. Paulsen and M. W. Webster, *Chem. Ber.*, **92**, 2435 (1959).

3) K. Heyns and H. Paulsen, *ibid.*, **88**, 188 (1955).

4) C. A. Marsh and G. A. Levvy, *Biochem. J.*, **68**, 617 (1958).

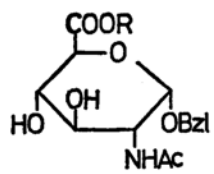
5) H. Weidmann and H. K. Zimmerman, *Ann.*, **639**, 198 (1961); *ibid.*, **684**, 226 (1965).

6) J. Yoshimura, M. Funabashi, S. Ishige and T. Sato, *Carbohyd. Res.*, **3**., 214 (1966); J. Yoshimura and M. Funabashi, This Bulletin, **39**, 2009 (1966); J. Yoshimura, H. Hashimoto and H. Ando, *Carbohyd. Res.*, **5**, 82 (1967).

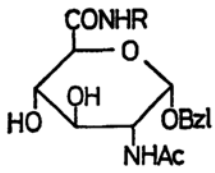
7) R. Kuhn, H. H. Baer and A. Seelinger, *Ann.*, **611**, 236 (1958).

8) H. Weidmann and H. K. Zimmerman, *ibid.*, **641**, 138 (1961).

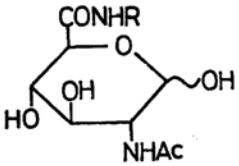
methylester by ammonolysis, *N*-debenzyloxycarbonylation and *N*-acetylation. **5** was also successfully converted to the corresponding nitrile by *O*-acetylation and dehydration with *p*-toluenesulfonyl chloride,[9] instead of triphenylphosphine dibromide.[8] Treatment of **1** with methylamine or ethylamine in methanol for 12 hr at room temperature, or with aniline or benzylamine and dicyclohexylcarbodiimide (DCC) gave the corresponding *N*-substituted amides (**6, 7, 8** and **9**) in about 50% yield, respectively.



1 : R=H
2 : R=CH_3
3 : R=C_2H_5
4 : R=$CH_2C_6H_5$



5 : R=H
6 : CH_3
7 : R=C_2H_5
8 : R=C_6H_5
9 : R=$CH_2C_6H_5$
10 : R=$CH_2COOC_7H_7$
11 : R=$CHCOOC_7H_7$ (CH_3)
12 : R=$CHCOOC_7H_7$ ($CH_2COOC_7H_7$)
13 : R=$CHCOOC_7H_7$ ($CH_2CH_2COOC_7H_7$)



14 : 2R=CH_2COOH
15 : R=$CHCOOH$ (CH_2CH_2COOH)

Comparison of the NMR spectra (Figs. 1 and 2) of 3,4-di-*O*-acetyl derivative of **6** and **9** (**6'** and **9'**) made the first-order analysis possible. Common signals to both figures, *i. e.*, the doublet at 4.28τ (J_{2,C_2-NH}=9.8 Hz), the quintet at *ca.* 4.81 τ (H_3: 4.72 τ; H_4: 4.90τ; $J_{2,3}=J_{3,4}=J_{4,5}$=10.0 Hz), the doublet at 5.01 τ ($J_{1,2}$=3.2 Hz), the AB-quartet at 5.30 and 5.53 τ ($J_{A,B}$=12 Hz), the sextet at 5.75 τ, and the doublet at *ca.* 5.80 τ in the former, and those at 4.25 τ ($J_{2,-C_2NH}$= 9.5 Hz), at *ca.* 4.69 τ (H_3: 4.61 τ; H_4: 4.76 τ; $J_{2,3}=J_{3,4}=J_{4,5}$=9.5 Hz), at 4.95 τ ($J_{1,2}$=3.5 Hz), at 5.23 and 5.46 τ ($J_{A,B}$=12 Hz), at *ca.* 5.66 τ, and at *ca.* 5.53 τ in the latter were respectively assigned to C_2-NH, partly superimposed H_3 and H_4, H_1, C_1-OCH_2, H_2, and H_5 protons from the intensity ratio and the aids of the double resonance technique. The broad signal at *ca.* 3.7 τ and the doublet at 7.22 τ in the former and the broadend-triplet at 3.30 τ in the latter were assigned to C_6-NH, N-CH_3 ($J_{C_6-NH,\ N-CH_3}$=6Hz), and C_6-NH protons, respectively.

Condensation of **1** with *p*-toluenesulfonates of amino acid benzylester (amino acid: glycine, L-alanine, L-aspartic acid, and L-glutamic acid) in pyridine by DCC method gave the corresponding amides (**10, 11, 12** and **13**) in 20–30% yield, of which **10** and **13** were successfully hydrogenated in the presence of palladium-charcoal and a small amount of hydrochloric acid in methanol to give *N*-(2-acetamino-2-deoxy-D-glucuronyl)-glycine and L-glutamic acid (**14** and **15**), but the corresponding compounds for **11** and **12** could not be purely isolated.

The physical constants, analyses and yield of new compounds obtained here are summarized in

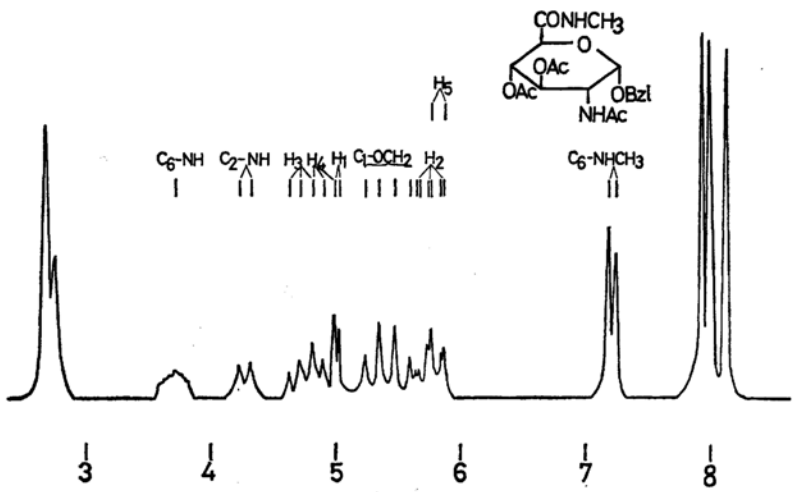


Fig. 1. MNR spectra of benzyl-2-acetamino-2-deoxy-3,4-di-*O*-acetyl-α-D-glucopyranosiduronic acid methylamide (100 Mc, $CDCl_3$).

9) H. Weidmann, *ibid.*, **679**, 178 (1964).

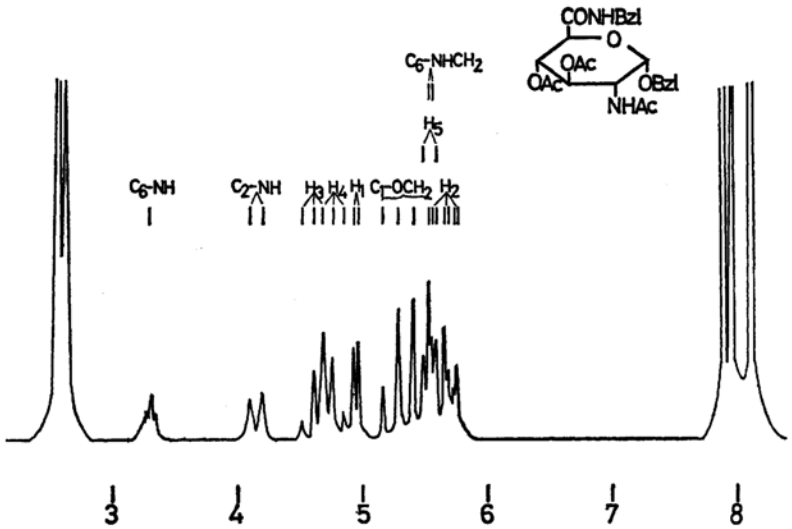


Fig. 2. NMR spectra of benzyl 2-acetamino-2-deoxy-3,4-di-*O*-acetyl-α-D-glucopyranosiduronic acid benzylamide (100 Mc, $CDCl_3$).

TABLE 1. THE PHYSICAL CONSTANTS AND ANALYSES OF NEW COMPOUNDS

Compd.	Formula	Mp, °C	Optical rotation			Found			Calcd			Yield %
			$[\alpha]_D^{23}$	Solvent	c	C%	H%	N%	C%	H%	N%	
1	$C_{15}H_{19}O_7N$	198	133	H_2O	1	55.30	7.18	4.49	55.38	5.89	4.31	90
2	$C_{16}H_{21}O_7N$	186—187	159	MeOH	1	56.69	6.22	4.12	56.63	6.24	4.13	52
3	$C_{17}H_{23}O_7N$	154—155	159	MeOH	1.13	57.57	6.44	3.99	57.78	6.56	3.96	49
4	$C_{22}H_{25}O_7N$	183—184	158	MeOH	0.98	63.42	6.02	3.57	63.60	6.07	3.37	51
6	$C_{16}H_{22}O_6N_2 \cdot 3/4H_2O$	203—204	140	EtOH	0.54	54.76	6.57	7.85	54.56	6.68	7.98	52
7	$C_{17}H_{24}O_6N_2 \cdot 3/4H_2O$	178—179	127	EtOH	0.45	55.50	7.13	7.72	55.75	6.97	7.65	54
8	$C_{21}H_{24}O_6N_2 \cdot 3/4H_2O$	204—205	70.1	$CHCl_3$	0.44	60.90	6.01	6.61	60.88	6.16	6.76	51
9	$C_{22}H_{26}O_6N_2 \cdot 3/4H_2O$	207—208	99.7	$CHCl_3$	0.47	61.32	6.23	6.77	61.23	6.45	6.54	50
6'	$C_{20}H_{26}O_8N_2$	198—199	120	MeOH	0.47	56.72	6.02	6.66	56.86	6.20	6.63	92
9'	$C_{26}H_{30}O_8N_2$	200—201	110	MeOH	1.08	62.73	5.95	5.87	62.64	6.07	5.67	90
10	$C_{24}H_{28}O_8N_2 \cdot H_2O$	170—171	140.7	MeOH	1	58.54	6.50	5.61	58.76	6.17	5.71	22
11	$C_{25}H_{30}O_8N_2 \cdot H_2O$	181—182	83.0	MeOH	1	59.67	6.82	5.52	59.51	6.39	5.55	21
12	$C_{33}H_{36}O_{10}N_2 \cdot H_2O$	125—126	35.8	MeCOMe	1	62.13	6.11	4.61	62.06	6.00	4.39	30
13	$C_{34}H_{38}O_{10}N_2$	186—187	60.5	$HCON(Me)_2$	1.17	64.66	6.14	4.69	64.34	6.04	4.41	20
14	$C_{10}H_{16}O_8N_2 \cdot H_2O$	sirup	57.3	MeOH	1.15	38.98	5.93	9.11	38.71	5.85	9.03	85
15	$C_{13}H_{20}O_{10}N_2 \cdot H_2O$	sirup	30.6	MeOH	1.21	40.83	6.12	7.19	40.85	5.80	7.33	88

Table 1.

In order to compare the stability of *N*-(2-acetamino-2-deoxy-D-glucuronyl)-amino acid with that of other carbohydrate-amino acid compounds having peptide bond, **14** and **15** was hydrolysed in 2 N hydrochloric acid at 100°C, and glycine and L-glutamic acid liberated was estimated at suitable intervals with an amino acid analyser. The first-order rate constant of **14** and **15** was 4.45 and 4.25×10^{-3} min^{-1}, respectively. These values indicate that the stability of the peptide bond between the uronic acid and amino acid is the same order as that of *N*-aminoacyl-D-glucosylamine and *N*-aminoacyl-D-glucosamine,[10] and consequently, the oxo-structure of sugar moiety has no effect on the stability of the peptide bond in the same molecule.

Experimental

All melting points were uncorrected. The solutions were evaporated under diminished pressure at a bath temperature usually not exceeding 45°C. Optical rotations were measured in a 0.5-dm tube at 578 mμ with a Carl-Zeiss Polarimeter. The content of water of crystallization was calculated from the analytical

10) G. S. Marks, R. D. Marshall and A. Neuberger, *Biochem. J.*, **87**, 274 (1963); J. Yoshimura and H. Hashimoto, *Carbohyd. Res.*, **4**, 435 (1967).

values.

Benzyl 2-Acetamino-2-deoxy-α-D-glucopyranoside. A suspended solution of 2-acetamino-2-deoxy-D-glucose (80 g, 0.36mol) in benzyl alcohol (900 m*l*) and boron trifluoride etherate (10 m*l*) was heated for 2 hr at 95—100°C, and after the addition of hydrogen chloride (2 g) in benzyl alcohol (100 m*l*) the solution was again heated for 1 hr at the same temperature. After cooling, the reaction mixture was poured into ether (500 m*l*) and filtered. The precipitate was dissolved in water (1 *l*) and the solution was extracted with ether to remove benzyl alcohol. The water layer was evaporated, and the residue was treated with charcoal in ethanol, crystallized by the addition of ether to give 80 g (67%) of fine crystals. Mp 182—183°C, $[\alpha]_D^{23}$ 178° (*c* 1, water) (Lit,[7] mp 183—184°C).

When the reaction mixture was directly evaporated without being poured into ether, and treated by the same procedure, the crystals weighed 97 g (85%). In the course of evaporation, the deposition of a small amount of benzyl 2-acetamino-2-deoxy-4,6-*O*-benzylidene-α-D-glucopyranoside[11] was always observed.

Benzyl 2-Acetamino-2-deoxy-α-D-glucopyranosiduronic Acid (1). In a four-necked flask equipped with stirrer, reflux condenser, dropping funnel, and in-let capillary was placed benzyl 2-acetamino-2-deoxy-α-D-glucopyranoside (25 g, 80 mmol) and platinum-charcoal (10%, 15 g) in water (500 m*l*), and air or oxygen was blown into the solution with violent stirring at 95°C, under the adjustment of pH at 6.8—7.7 with sodium bicarbonate solution. A secondary alcohol such as isopropyl alcohol or isobutyl alcohol was added at a suitable interval as an antifoaming agent and antioveroxidant, and the reaction was continued until the starting material had disappeared on TLC. After cooling, the catalyser was filtered off, and the solution was evaporated. The residue was extracted with ethanol to remove the starting material and dissolved in water. The solution was deionised with Amberlite IR-120 and evaporated to give a sirup which was decolorized and crystallized from ethanol. Yield, 19 g (77.3%). The second crop (3.7 g, 15.6%) was obtained from the mother liquid.

Benzyl 2-Acetamino-2-deoxy-α-D-glucopyranosiduronic Acid Esters (2, 3 and 4). The solution of benzyl 2-acetamino-2-deoxy-α-D-glucopyranosiduronic acid (2 g, 6.2 mmol) in an alcohol (20 m*l* of methanol, ethanol or benzyl alcohol) and benzene (200 m*l*) was refluxed on a boiling water bath for 3 hr, and the water formed was removed azeotropically. The solution was evaporated and the sirup obtained was crystallized from a small amount of hot ethanol and ether. The physical constants, analytical data and yields of these compounds are shown in Table 1.

Benzyl 2-Acetamino-2-deoxy-α-D-glucopyranosiduronic Acidamide (5). The uronic acid (28 g, 86 mmol) was heated with methanolic ammonia (200 m*l*) at 90°C for 2 hr in a sealed tube, and the solution was evaporated. Crystallization of the residual sirup from ethanol (20 m*l*) gave the amide in a quantitative yield. Mp 242°C, $[\alpha]_D^{17}$ 178° (*c* 1, water) (Lit,[9] mp 243°C)

Found: C, 55.86; H, 6.43; N, 8.57%. Calcd for $C_{15}H_{20}O_6N_2$: C, 55.55; H, 6.22; N, 8.64%.

Benzyl 2-Acetamino-2-deoxy-α-D-glucopyranosiduronic Acid Methyl- and Ethylamide (6 and 7). A solution of the uronic acid (2 g, 6.2 mmol) and methylamine or ethylamine (2 m*l* of 40% aqueous solution) in a large amount of methanol was stirred at room temperature for 12 hr, and evaporated to give a sirup which was crystallized from a small amount of hot acetone and ether. The physical constants, analytical data and yields are shown in Table 1.

Benzyl 2-Acetamino-2-deoxy-α-D-glucopyranosiduronic Acid Anilide and Benzylamide (8 and 9). A solution of the uronic acid (3.2 g, 9.9 mmol), dicyclo-hexylcarbodiimide (2.5 g, 12 mmol) and aniline (1.1 g, 12 mmol) or benzylamine (1.3 g, 12 mmol) in pyridine was agitated for 2 days, and dicyclohexylurea deposited was filtered off. The filtrate was evaporated, and the sirup obtained was crystallized from a small amount of methylene chloride and ether. Several data on these compounds are shown in Table 1.

Benzyl 2-Acetamino-2-deoxy-3,4-di-*O*-acetyl-α-D-glucopyranosiduronic Acidnitrile. Benzyl 2-acetamino-2-deoxy-3,4-di-*O*-acetyl-α-D-glucopyranosiduronic acidamide (0.5 g, 1.2 mmol) prepared from **5** by the procedure of Zimmerman *et al.*[9] was heated with *p*-toluenesulfonyl chloride (1.17 g, 6 mmol) in pyridine (7 m*l*) for 3 hr at 80—90°C. After cooling, the solution was poured into ice-water (100 m*l*) and extracted three times with chloroform (50 m*l*). The combined extracts was washed in turn with 1 N hydrochloric acid, sodium bicarbonate and water, dried and then evaporated. The sirup (0.5 g) obtained was crystallized from ethanol and ether. Yield, 0.3 g (63%), mp 165—165.5 °C (Lit,[9] mp 159°C).

Found: C, 58.15; H, 5.33; N, 6.98%. Calcd for $C_{19}H_{22}O_7N_2$: C, 58.45; H, 5.68; N, 7.18%.

Benzyl 2-Acetamino-2-deoxy-3,4-di-*O*-acetyl-α-D-glucopyranosiduronic Acid Methylamide and Anilide (6′ and 9′). Acetylation of **6** and **9** with 4 mol equivalent of acetic anhydride in the same amount of pyridine was carried out in the usual manner. The physical constants, analyses and yield are shown in Table 1.

***N*-(Benzyl 2-Acetamino-2-deoxy-α-D-glucopyranosiduronyl)-aminoacid Benzyl Esters (10, 11, 12 and 13).** A solutions of the uronic acid (6.5 g, 20 mmol) and DCC (5 g, 24 mmol) in pyridine was added to a chilled solution of amino acid benzyl ester *p*-toluene sulfonate (each 20 mmol of gly, L-ala, L-asp and L-glu) and triethylamine (2 g, 20 mmol) in pyridine with stirring. After standing the solution at room temperature for 2 days, dicyclohexylurea separated was filtered off, and the filtrate was evaporated to give a sirup which was crystallized from a small amount of hot ethanol, and recrystallized from methylene chloride. The physical constants, analytical data and yield are listed in Table 1.

***N*-(2-Acetamino-2-deoxy-D-glucuronyl)-glycine and -L-glutamic Acid (14 and 15).** A solution of **10** or **13** (0.5 g) and palladium-charcoal (0.5 g) in aqueous ethanol was adjusted to pH 2 with diluted hydrochloric acid and hydrogenated until the theoretical amount of hydrogen was taken up. The catalyst was filtered off, and the filtrate was evaporated to give a sirup which was purified from ethanol and ether. Several data on these compounds are listed in Table 1.

Hydrolysis of *N*-(2-Acetamino-2-deoxy-D-glucu-

11) J. Yoshimura, M. Funabashi, S. Ishige and T. Sato, This Bulletin, **39**, 1760 (1966).

ronyl)-glycine and -L-Glutamic Acid. Each sample (22.5 μmol) was dissolved in 2 m*l* of 2 N hydrochloric acid, and the solution was heated at 100°C in a sealed tube. After 40, 60 and 80 min, the hydrolysate was analyzed with an amino acid analyzer Hitachi Model KLA-2, using a column packed with Amberlite CG-120 Type III, 1×50 cm; the eluent was 0.2 N citric buffer (pH 3.25) percolating at 30 m*l*/hr at 30°C. Glycine and glutamic acid were eluted with an elution time of 3.8 and 5.3 hr, and the amount was determined by comparison with standard. The first-order rate constant of **14** and **15** was 4.45 and 4.25×10^{-3} min^{-1}, respectively.

The authors are indebted to Mr. Kiyofumi Fukukawa of our Laboratory for the NMR measurements.