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SYNTHESIS OF 2-*O*-(2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYL)-D-MANNOSE, AND ITS INTERACTION WITH D-MANNOSE-SPECIFIC LECTINS*†

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ABSTRACT

Condensation of 3,4:5,6-di-*O*-isopropylidene-D-mannose dimethyl acetal with 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2′,1′:4,5]-2-oxazoline in the presence of a catalytic amount of *p*-toluenesulfonic acid afforded crystalline 2-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4:5,6-di-*O*-isopropylidene-D-mannose dimethyl acetal (**3**) in 25% yield. Catalytic deacetylation of **3** with sodium methoxide, followed by hydrolysis with dilute sulfuric acid, gave 2-*O*-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-D-mannose (**4**). Treatment of **3** with boiling 0.5% methanolic hydrogen chloride under reflux gave methyl 2-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranoside (**5**) and methyl 2-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannofuranoside (**6**). The inhibitory activities of **4**, **5**, and **6** against the hemagglutinating and mitogenic activities of *Lens culinaris* and *Pisum sativum* lectins and concanavalin A were assayed. From the results of these hapten inhibition studies, subtle differences of specificity between these D-mannose-specific lectins were confirmed.

*Dedicated to Professor Michael Heidelberger in honor of his 87th birthday.

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INTRODUCTION

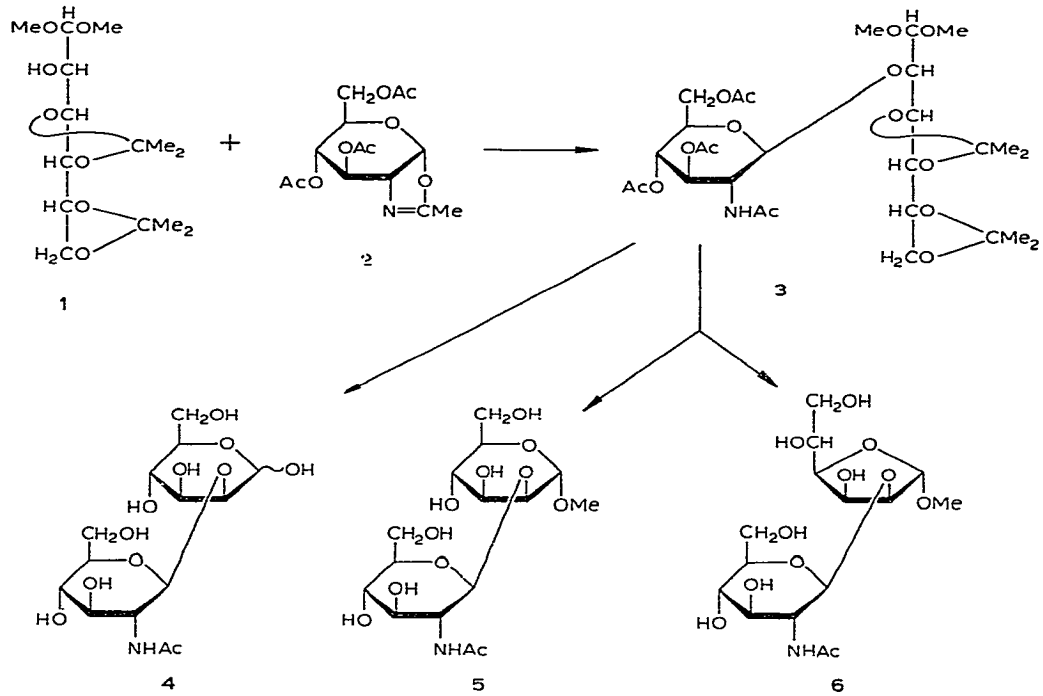
Recent investigations of the structure of carbohydrate chains of various glycoproteins[1] have shown that the sequence 2-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-mannose commonly occurs in the sialic acid-containing carbohydrate chains that are linked to the peptide backbone of the glycoprotein through a 2-acetamido-1-*N*-(L-aspart-4-oyl)-2-deoxy-D-glucosylamine linkage. Carbohydrate chains having this structure and located at the surface of erythrocytes and lymphocytes have been proposed to be specific receptor sites for certain lectins, *viz.*, concanavalin A[2] and lectins from *Phaseolus vulgaris*[2,3], *Wistaria floribunda*[2,4], *Lens culinaris*[2,5], and *Ricinus communis*[6]. Furthermore, it has been suggested that these lectins bind to different portions of the same carbohydrate chain on the cell surface[2,5]. For example, *P. vulgaris* lectin was found to recognize primarily the sequence D-galactosyl-(2-acetamido-2-deoxy-D-glucosyl)-D-mannose, and *L. culinaris* lectin was shown to bind preferentially to the sequence (2-acetamido-2-deoxy-D-glucosyl)-D-mannose, by hemagglutination inhibition assays with glycopeptides that were sequentially degraded by various glycosidases as hapten inhibitors[2,5]. The observation that the inhibitory activity of the glycopeptide against *L. culinaris* lectin is greatly decreased by removal of the 2-acetamido-2-deoxy-D-glucose residue that is bound to the penultimate D-mannose residue was surprising, because this lectin has been classified among the so-called "D-mannose-specific" lectins by inhibition assays with simple sugars as hapten inhibitors[7–9].

We now report the synthesis of 2-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-mannose (**4**), and the use of this disaccharide and its derivatives as hapten inhibitors.

RESULTS AND DISCUSSION

The use of oxazoline derivatives of amino sugars[10–14] has greatly improved the yield in the synthesis of amino sugar-containing disaccharides, as compared to those from the unstable glycosyl halides previously used[15,16]. 2-Methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2′,1′:4,5]-2-oxazoline (**1**), prepared by the method of Khorlin *et al.*[17], was condensed with 3,4:5,6-di-*O*-isopropylidene-D-mannose dimethyl acetal[18] (**2**) in the presence of a catalytic amount of *p*-toluenesulfonic acid (pH 4), to give 2-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4:5,6-di-*O*-isopropylidene-D-mannose dimethyl acetal (**3**) in 25% yield. The β configuration of the 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranosyl group in **3** was confirmed by n.m.r. spectroscopy (doublet at τ 5.19, $J_{1,2}$ 8.5 Hz). The *O*-acetyl, and isopropylidene and methyl acetal, groups of **3** were removed by catalytic deacetylation with sodium methoxide, followed by hydrolysis with dilute sulfuric acid, to give amorphous 2-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-mannose (**4**). Treatment of **3** with methanolic hydrogen chloride afforded, after chromatography on activated charcoal, methyl 2-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranoside (**5**), methyl 2-*O*-(2-acetamido-2-deoxy-β-D-gluco-

pyranosyl)-α-D-mannofuranoside (**6**), and **4**. The presence of an α-D-glycosidic linkage in **5** was ascertained by a strong absorption at 815 cm^{-1} (Type 2a) in the i.r. spectrum. The furanoside structure of the D-mannoside residue of **6** was confirmed by (*a*) i.r. absorptions at 885 (Type B) and 783 cm^{-1}, and (*b*) periodate oxidation, which liberated one mole of formaldehyde per mole of **6**.



The results of hemagglutination inhibition assays of **4**, **5**, **6**, and other related, simple sugars against the D-mannose-specific lectins of *L. culinaris* and *P. sativum*, and against concanavalin A, are listed in Table I, and the effects of those sugars on [^{3}H-6]thymidine incorporation of human peripheral lymphocytes exposed to the three lectins just mentioned are given in Table II. Good agreement was observed between the results given in Tables I and II. The substitution at O-2 by a 2-acetamido-2-deoxy-D-glucopyranosyl group markedly increases the inhibitory activity of D-mannose and methyl α-D-mannopyranoside against *L. culinaris* lectin. These results clearly demonstrate the importance of the 2-acetamido-2-deoxy-D-glucosyl group in the hapten inhibitory activity against *L. culinaris* lectin, and suggest that the (2-acetamido-2-deoxy-β-D-glucopyranosyl)-D-mannose sequence of carbohydrate chains on the cell surface is the best receptor site for this lectin. On the other hand, among some simple sugars tested, methyl α-D-mannopyranoside was the most active inhibitor against concanavalin A, and substitution at O-2 by a 2-acetamido-2-deoxy-β-D-glucopyranosyl group did not significantly affect the inhibitory activity; this observation

TABLE I

HEMAGGLUTINATION INHIBITION OF LECTINS

Sugars	*Lectins*[a]		
	L. culinaris	*Concanavalin A*	P. sativum
4	0.8	0.8	26
5	0.8	0.4	12
6	>125	>125	>125
2-Acetamido-2-deoxy-D-glucose	45	90	90
D-Mannose	14	4	14
Methyl α-D-mannopyranoside	13	0.4	6

[a]Minimum concentration (μmol/ml) completely inhibiting 4 hemagglutinating doses.

TABLE II

EFFECT OF SUGARS ON [^{3}H-6]THYMIDINE INCORPORATION BY HUMAN PERIPHERAL LYMPHOCYTES EXPOSED TO LECTINS

Sugars	*Conc. of inhibitor (μmol/ml)*	*Incorporation (% of control experiment without inhibitor)*[a]		
		L. culinaris	*Concanavalin A*	P. sativum
None		100	100	100
4	2.5	15	15	30
	12.5	10	20	20
5	2.5	11	12	30
	12.5	6	7	24
6	12.5	>100	>100	>100
2-Acetamido-2-deoxy-D-glucose	25	100	90	[b]
	50	100	90	[b]
D-Mannose	25	88	96	25
	50	18	20	6
Methyl α-D-mannopyranoside	25	42	10	15
	50	9	5	4

[a]Average value of four experiments. [b]Not determined.

confirms the finding of Goldstein *et al.*[19] that α-D-mannopyranosyl groups, linked glycosidically at O-2 and located in the interior of the carbohydrate chains of the cell surface, can serve as receptor sites for concanavalin A. Also, the lectin of *P. sativum* was inhibited the most by methyl α-D-mannopyranoside, but, in this case, the substitution at O-2 by a 2-acetamido-2-deoxy-β-D-glycopyranosyl group decreased the inhibitory activity somewhat. Therefore, an α-D-mannopyranosyl group located at the nonreducing end of a carbohydrate chain may be the best receptor site for the *P. sativum* lectin. Furthermore, it is of interest that none of the three D-mannose-specific lectins tested were inhibited by **6**, in which the D-mannoside residue has the furanoside structure; this observation is in agreement with the suggestion[9,20,21] that unsubstituted hydroxyl groups at C-3, C-4, and C-6 of the D-*arabino*-hexopyranosyl

configuration are required for saccharide binding to these D-mannose-specific lectins. The results clearly indicate the existence of subtle differences of specificity between the so-called "D-mannose-specific" lectins.

EXPERIMENTAL

General. — Melting points were taken on a hot stage equipped with a microscope, and are not corrected. Specific rotations were determined in a semimicro polarimeter tube (length 1 dm) with a polarimeter Zeiss having a scale reading to 0.01°. I.r. spectra were recorded with a JASCO DS-402 G spectrophotometer, and n.m.r. spectra with a JEOL JNM-PS-100 spectrometer, with tetramethylsilane as the internal standard. The silicic acid used for chromatography was Wakogel C-100 (100 mesh; Wako Pure Chemical, Tokyo), used without pretreatment. The ratio of weight of substance to weight of silica gel was 1:100. The ratio of diameter of the column to its length was 1:20. The activated charcoal for column chromatography was Shirasagi activated charcoal (Wako Pure Chemical, Tokyo). T.l.c. was performed on precoated Silica Gel G plates (layer thickness 0.25 mm; E. Merck, Darmstadt, Germany); the solvent travel-distance was ~6 cm. The spots were detected by spraying the chromatogram with 1:1:18 (v/v) anisaldehyde–conc. sulfuric acid–ethanol. Evaporations were conducted *in vacuo*, with a bath temperature below 40°, unless stated otherwise. Microanalyses were performed by the Central Analyses Laboratory, Faculty of Pharmaceutical Sciences, University of Tokyo.

Lectins. — *L. culinaris* hemagglutinin from lentils (kindly provided by Dr. H. Kanai, Department of Medicinal Plants, Katmandu, Nepal) was purified by the method previously described[9]. Concanavalin A from jack bean (Sigma Chemical Co., St. Louis, Mo. 63178) was purified according to the method of Agrawal and Goldstein[22]. *Pisum sativum* hemagglutinin, which was purified from commercial peas[21], was kindly provided by Dr. T. Shinohara, National Research Institute of Police Sciences, Tokyo.

Sugars. — D-Mannose, 2-acetamido-2-deoxy-D-glucose, and methyl α-D-mannopyranoside were purchased from Nakarai Chemical Co. (Tokyo).

Hemagglutination assays. — Titration and inhibition assays, using human erythrocytes freshly obtained from a donor, were performed according to the methods previously described[23].

Lymphocyte cultures for mitogenic assay. — Human peripheral lymphocytes were cultured by the method previously described[9]. Inhibition assays for [^{3}H-6]-thymidine incorporation into the lymphocytes with sugars were performed as described previously[4], each inhibitor usually being tested four times against the same lectin, and an average value of the incorporation calculated.

2-O-(2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-3,4:5,6-di-O-isopropylidene-D-mannose dimethyl acetal (**3**). — To a solution of 3,4:5,6-di-*O*-isopropylidene-D-mannose dimethyl acetal[18] (**1**, 6.6 g) and 2-methyl-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)-[2′,1′:4,5]-2-oxazoline[17] (**2**, 7 g) in dry, 1:1 (v/v) toluene–nitromethane (50 ml) was added sufficient *p*-toluenesulfonic acid to adjust the

pH of the solution to 4. The solution was heated for 5 h at 120°, and then evaporated under diminished pressure. The residue was dissolved in chloroform (21 ml), successively washed with cold, saturated sodium hydrogencarbonate solution and water, dried (sodium sulfate), and evaporated to give a residue that was dried *in vacuo* overnight. The brown syrup obtained was chromatographed on a column of silica gel with 97:3 (v/v) chloroform–ethanol; the fractions having R_F 0.14 in t.l.c. in ethyl acetate were combined and evaporated to a syrupy residue. This residue was rechromatographed on a column of silica gel with 2:1 (v/v) chloroform–ether, to give 3.4 g (25%) of pure **3**, which crystallized; it was recrystallized from ether–hexane to give needles containing one molecule of water of crystallization per molecule, m.p. 113–115°, $[\alpha]_D^{20}$ −20° (*c* 0.5, chloroform); n.m.r. data (100 MHz, chloroform-*d*): τ 4.39 (one-proton doublet, *J* 7.5 Hz, NH), 5.19 (one-proton doublet, $J_{1,2}$ 8.5 Hz, H-1 of GlcNAc), 5.45 (one-proton doublet, $J_{1',2'}$, 3.5 Hz, H-1′ of Man), 6.50 (6 protons, 2 OMe), ~8 (12 protons, acetates), 9.60 (12-proton triplet, 2 isopropylidene).

Anal. Calc. for $C_{28}H_{45}NO_{15}\cdot H_2O$: C, 51.45; H, 7.25; N, 2.14. Found: C, 51.78; H, 6.99; N, 1.97.

2-O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-D-mannopyranose (**4**). — To a suspension of **3** (158 mg) in dry methanol (2 ml) was added 0.1M sodium methoxide (0.5 ml), and the mixture was kept for 15 h at room temperature. After the addition of 0.05M sulfuric acid (20 ml), the solution was heated for 90 min at 80°, the acid neutralized with barium carbonate, the suspension filtered, and the filtrate evaporated under diminished pressure. The residue was chromatographed on a column of activated charcoal (6 g) with 2% aqueous ethanol; the fractions having R_F 0.20 in t.l.c. in 7:5:2 (v/v) 2-propanol–ethyl acetate–water were combined and evaporated. The residue was dissolved in a small amount of absolute ethanol, and precipitated with ether, to give 57 mg (54%) of pure **4** as a hygroscopic, amorphous powder showing [on examination by t.l.c. on silica gel with 4:5:3 (v/v) butanol–acetone–water] only one spot (R_F 0.53), $[\alpha]_D^{20}$ −13 (8 min) → −17° (at equilibrium, *c* 0.5, water).

Anal. Calc. for $C_{14}H_{25}NO_{11}\cdot 2H_2O$: C, 40.09; H, 6.97; N, 3.34. Found: C, 39.80; H, 6.28; N, 3.42.

Methyl 2-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannopyranoside (**5**) *and methyl 2-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-α-D-mannofuranoside* (**6**). — A solution of **3** (665 mg) in 0.5% methanolic hydrogen chloride (4 ml) was boiled under reflux for 15 min, neutralized with Dowex-1 (OH^-) ion-exchange resin, and evaporated. The residue was chromatographed on a column of activated charcoal with water (500 ml), and then with 2% aqueous ethanol. The effluents from 600–1,200 ml, from 1,200–1,400 ml, and from 1,400–2,200 ml, respectively, gave 52 mg (13%) of **5**, 30 mg (7%) of **4**, and 180 mg (44%) of **6**.

Compound **5** was dissolved in a small amount of ethanol, and precipitated with absolute ether, to afford a hygroscopic, amorphous powder showing [on examination by t.l.c. on silica gel with 7:5:2 (v/v) propanol–ethyl acetate–water] only one spot (R_F 0.27), $[\alpha]_D^{20}$ −11° (*c* 0.80, water); i.r. data: ν_{max}^{KBr} 1645 (Amide I), 1550 (Amide II), and 815 cm^{-1} (Type 2a).

Anal. Calc. for $C_{15}H_{27}NO_{11}\cdot 2H_2O$: C, 41.57; H, 7.21; N, 3.23. Found: C, 41.23; H, 7.71; N, 3.30.

Compound **6** was crystallized and recrystallized from ethanol–ether to give needles, m.p. 234–235°, $[\alpha]_D^{20}$ +29° (*c* 0.9, water); i.r. data: ν_{max}^{KBr} 1655 (Amide I), 1550 (Amide II), 885 (Type B), and 783 cm^{-1} (Type D); t.l.c. in 7:5:2 (v/v) propanol–ethyl acetate–water: R_F 0.27.

Anal. Calc. for $C_{15}H_{27}NO_{11}\cdot 0.5H_2O$: C, 44.33; H, 6.95; N, 3.45. Found: C, 43.97; H, 6.85; N, 3.48.

Samples of **5** and **6** (6 mg each) were separately dissolved in sodium metaperiodate (5 ml), the solutions were incubated in the dark for 20 h at 25°, and the consumption of periodate was measured by the spectrophotometric method of Aspinall and Ferrier[24]. Both **5** and **6** were found to consume 2 moles of periodate per mole. The formaldehyde produced on periodate oxidation of **5** and **6** was determined by the chromotropic acid method of O'Dea and Gibbons[25]: compound **6** released 0.9 mole of formaldehyde per mole, whereas no formaldehyde was released from **5**.

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