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Note

ω-Aldehydo 1-thioglycosides of methyl D-galactopyranuronate and related compounds*

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We have been studying the binding specificity of the Gal/GalNAc-binding lectin from rabbit liver by using neoglycoproteins bearing D-Gal and related analogs as inhibitors of the binding of ^{125}I-asialo-orosomucoid to the lectin. Our findings that the binding was little affected when the CH_2OH-5 group of the galactose moiety was absent[1], or was modified with another neutral monosaccharide[2], suggested that the CH_2OH group does not participate in the binding and that a large substituent on the CH_2OH group does not interfere sterically with the binding. However, many serum glycoproteins containing oligosaccharide chains terminating with α-NeuAc-(2→6)-Gal groups at the nonreducing end are poor inhibitors of the binding of asialo-orosomucoid. It was reasoned that the failure of these glycoproteins to be bound by the lectin was not caused by the size of the NeuAc moiety, but rather by the presence in NeuAc of a carboxylic acid group that is negatively charged under physiological conditions. To test this hypothesis, we decided to compare the binding of neoglycoproteins containing D-galacturonic acid (CO_2H at C-5) and those containing methyl D-galacturonate (CO_2Me at C-5). These two sugars were first converted into their ω-aldehydo 1-thioglycosides, to enable attachment to proteins *via* reductive alkylation[3,4].

We now report the preparation of (2,2-dimethoxyethyl)aminocarbonylmethyl 1-thioglycosides (**5** and **2**) of D-galacturonic acid and its methyl ester, starting from the readily available D-galacturonic acid monohydrate.

It is well documented that 4,5-unsaturated derivatives are produced from uronic acid derivatives by treatment with alkali. Neukom and co-workers reported[5,6] that alkali readily catalyzes β-elimination of galacturonate derivatives, leading to 4,5-unsaturation. The final step in our synthetic scheme for preparation of the 1-thioglycoside of methyl D-galactopyranuronate is an alkali-catalyzed *O*-deacetylation,

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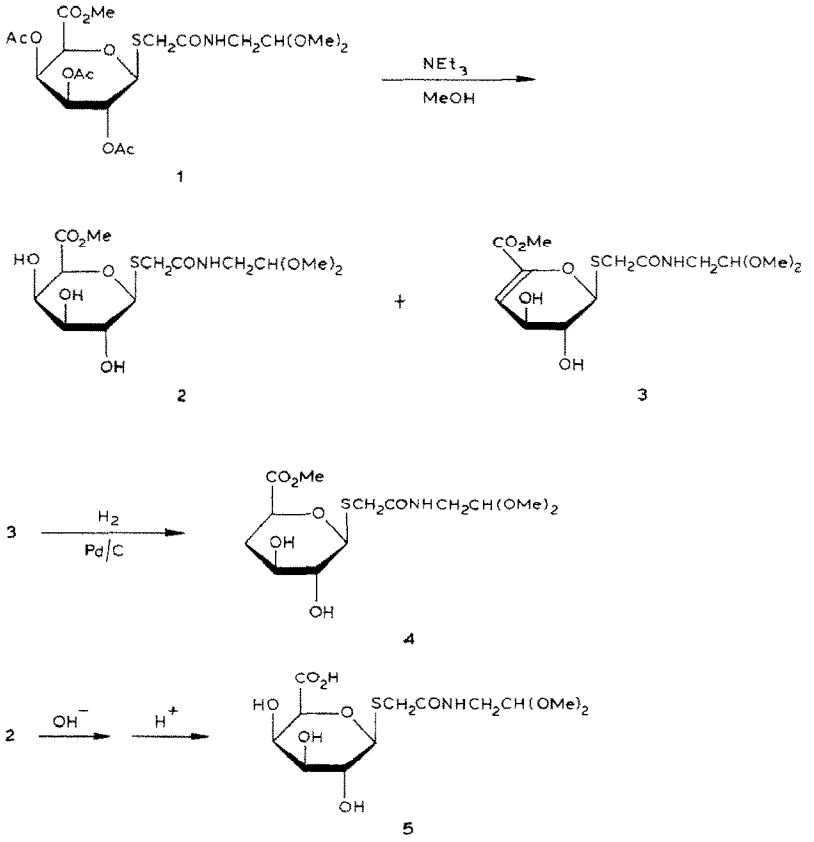
and, in this step, a 4,5-unsaturated by-product was indeed produced. *O*-Deacetylation of **1** by means of triethylamine in methanol produced the unsaturated product (**3**) and the desired deacetylation product (**2**) in the ratio of ~1:1, and an even higher proportion of the unsaturated product was obtained when 0.01M sodium methoxide in methanol was used. Owing to its increased hydrophobicity, this 4,5-unsaturated by-product was readily separable from the parent 1-thioglycoside by gel filtration through a column of Sephadex G-15. The 4,5-unsaturated product (**3**) was further converted by hydrogenation into methyl 4-deoxy-D-galactopyranuronate (**4**). Both the 4,5-unsaturated derivative (**3**) and the 4-deoxy derivative (**4**) of the D-galactosiduronic acid were found to be valuable additions to the specificity studies of the lectin.

Attachment of these 1-thioglycosides to bovine serum albumin, and use of such neoglycoproteins in the study of the Gal/GalNAc-binding lectin, have already been reported[2].



EXPERIMENTAL

Materials. — D-Galacturonic acid monohydrate was obtained from Pfanstiehl Laboratory (Waukegan, IL), and 10% palladium-on-carbon from Aldrich Chem. Co.

Methods. — All evaporations were conducted under diminished pressure below 40°. Melting points (uncorrected) were measured with a Fisher–Johns apparatus. Elemental analyses were performed by Galbraith Labs., Inc. (Knoxville, TN). Thin-layer chromatography (t.l.c.) was performed on aluminum plates coated with a layer (0.2 mm) of silica gel F-254 (Merck). Solvent systems used were: (*A*) 1:4 (v/v) toluene–ethyl acetate, (*B*) 9:4:2 (v/v) ethyl acetate–isopropyl alcohol–water, (*C*) 3:2:1 (v/v) ethyl acetate–isopropyl alcohol–water, (*D*) 3:2:1 (v/v) ethyl acetate–acetic acid–water, and (*E*) 5:5:1:3 (v/v) ethyl acetate–pyridine–acetic acid–water. Carbohydrates were made visible on the t.l.c. plates by spraying with 15% sulfuric acid in 50% ethanol and heating at ~140°. Aldehydes and acetals were made visible by spraying with[7] 0.4% (2,4-dinitrophenyl)hydrazine in 2M HCl, after which aldehydes appeared yellow without heating, whereas acetals produced a yellow color only after heating. Proton magnetic resonance (p.m.r.) spectra were obtained with a Bruker WM-300 wide-bore n.m.r. spectrometer.

The following colorimetric methods were used for characterization of products: a modification of the phenol–sulfuric acid method[8] for carbohydrates; the neocuproine method[9] for aldehydes; the carbazole method[10] for uronic acids; and the hydroxylamine–ferric chloride method[11] for esters. Acetals were hydrolyzed to the corresponding aldehydes by heating in 0.05M HCl for 15 min at 100° before assay by the neocuproine method.

Methyl D-galacturonate was prepared from D-galacturonic acid monohydrate according to the method of Jansen and Jang[12]. The ester was per-*O*-acetylated with acetic anhydride–dry pyridine, and the product was converted into the 1-bromide (81% yield) by treating it with 30% HBr in glacial acetic acid, using a method analogous to that for the preparation of methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate[13]. The 1-bromide was converted into the thiopseudourea derivative by using a method similar to that employed for neutral sugar derivatives[14]. The yield of crystalline, thiopseudourea derivative was 73%; m.p. 177–178.5°.

*Methyl [(2,2-dimethoxyethyl)aminocarbonylmethyl 2,3,4-tri-*O*-acetyl-1-thio-β-*D*-galactopyranosid]uronate* (**1**). — The thiopseudourea derivative was converted into the title compound *via* the 1-thio derivative according to a published method[3]. The product (**1**) crystallized from 95% ethanol, yield 78%; m.p. 93–97°; homogeneous by t.l.c. in solvent *A* (R_F 0.20).

*Methyl [(2,2-dimethoxyethyl)aminocarbonylmethyl 1-thio-β-*D*-galactopyranosid]uronate* (**2**). — Treatment of **1** with 17:83 (v/v) triethylamine–dry methanol overnight at room temperature yielded two products, which gave a positive response both to charring and to the aldehyde reagent (R_F 0.75 and 0.48 in t.l.c. with solvent *B*) in the ratio of ~1:1. The two products were separated from each other by gel filtration through a column (2 × 150 cm) of Sephadex G-15, using 0.1M acetic acid as the eluant, and collecting 6-mL fractions. The material of lower R_F (0.48) appeared in fractions No. 47–52, and was identified as **2**, and the material of higher R_F (0.75) appeared in fractions 56–61, and was identified as the 4,5-unsaturated derivative of **2** (see the following section). The fractions containing the material (**2**) of low R_F

were evaporated to dryness, and the residue was crystallized from 95% ethanol; m.p. 148–150°.

Anal. Calc. for $C_{13}H_{23}NO_9S$ (369.39): C, 42.27; H, 6.28; N, 3.79; S, 8.68. Found: C, 42.49; H, 6.42; N, 3.81; S, 8.90.

A small portion of compound **2** was reacetylated with acetic anhydride–pyridine. A single product having R_F 0.20 in solvent *A*, and thus matching that of **1**, resulted. Another portion was treated with a 2-fold excess of mercuric acetate under slightly acidic conditions, to hydrolyze the 1-thioglycoside[15]. Mercuric ion was removed by passing the hydrolyzate through a small column (0.5 × 4 cm) of Dowex-50 X-8 (H^+) ion-exchange resin, and the eluate was analyzed by t.l.c. in solvents *B* and *D*. The eluate gave a single, charrable spot which matched that for methyl D-galacturonate in both solvent systems (R_F 0.45, solvent *B*; 0.52, solvent *D*).

Methyl [(2,2-dimethoxyethyl)aminocarbonylmethyl 4-deoxy-1-thio-α-L-threo-hex-4-enopyranosid]uronate (**3**). — The material of high R_F from the deacetylation mixture had, in addition to the properties already described, the following characteristics. It was highly u.v.-absorbing, and the chromogen produced in the ester-determination method (hydroxylamine–ferric chloride) had an absorbance maximum at 515 (instead of 500) nm. This bathochromic effect is consistent with the presence of a conjugated double-bond in the chromogen formed between the hydroxamate of **3** and the ferric ion. On acetylation, it gave a material having an R_F (0.27) higher than that (0.20) of **1** in solvent *A*. The ^{1}H-n.m.r. spectrum of acetylated **3** showed the presence of a single, vinylic H. These data led to the conclusion that **3** is the title compound, resulting from β-elimination and *O*-deacetylation of **1**. For acetylated **3**, ^{1}H-n.m.r. data ($CDCl_3$): δ 2.092 and 2.115 (s, 6 H, C-CH_3), 3.393 [s, 6 H, CH-(OCH_3)$_2$], 3.842 (s, 3 H, CO-O-CH_3), 4.423 [t, 1 H, C-C*H*(OMe)$_2$], 5.16–5.18 (m, 2 H, H-2,3), 5.603 (d, 1 H, *J* 3.68 Hz, H-1), and 6.27–6.29 (m, 1 H, H-4).

Methyl [(2,2-dimethoxyethyl)aminocarbonylmethyl 4-deoxy-1-thio-β-D-xylo-hexopyranosid]uronate (**4**). — Compound **3** (~0.3 g) was dissolved in 10% acetic acid (6 mL), and hydrogenated under atmospheric pressure in a Brown micro-hydrogenator, using palladium-on-carbon (0.6 g). After treatment overnight, the mixture was filtered through a layer of Celite, and the residue was washed with 50% ethanol. The filtrate and washings were combined, and evaporated to a syrup that was dried in a vacuum desiccator over NaOH pellets; yield 0.26 g. This material (R_F 0.70, solvent *B*) was not u.v.-absorbing, and produced only a negligible amount of color in the uronic acid determination. The ^{1}H-n.m.r. spectrum of D_2O-exchanged **4** in Me_2SO-d_6 indicated the presence of a new methylene group, absent from the spectra of either **2** (data not shown) or peracetylated **3**. The chemical shifts of the new methylene protons were much smaller than those for the two methylene groups present in the aglycon. By a series of decoupling experiments starting from H-1, all of the ring protons were located, and the methylene group was found to be at C-4, as expected. For **4**, ^{1}H-n.m.r. data: δ 1.653–1.716 (m, 1 H, H-4*a*), 2.011–2.096 (m, 1 H, H-4*e*), 3.11–3.27 (m, S-CH_2 and N-CH_2), 3.266 and 3.268 (s, 3 H each, CH-OCH_3), 3.313–3.341 (t, 1 H, H-2), 3.570–3.614 (m, 1 H, H-3), 3.668 (s, 3 H, CO-

OCH_3), 4.329–4.366 [t, 1 H, $CH(OCH_3)_2$], 4.731–4.772 (q, 1 H, H-5), and 5.037–5.049 (d, 1 H, *J* 3.52 Hz, H-1).

(2,2-Dimethoxyethyl)aminocarbonylmethyl 1-thio-β-D-galactopyranosiduronic acid (**5**). — The ester **2** was hydrolyzed in aqueous sodium hydroxide at pH 11.5 for 0.5 h at room temperature; all of compound **2** (R_F 0.48, solvent *B*) had then been converted into a material of R_F 0.02 in solvent *B*, and 0.27 in solvent *C*. The mixture was acidified with Dowex-50 X-8 (H^+) ion-exchange resin to pH 7.5, filtered, and the filtrate evaporated, yielding the sodium salt of **5** as a colorless solid. Compound **5** can be obtained as the free acid by adjusting the pH to 2.5 (instead of 7.5) with Dowex-50 resin after basic hydrolysis, filtration, and evaporation. Because the acetal group is readily hydrolyzed on storage, once the free carboxyl group has been generated, compound **5** was usually stored as the sodium salt. On treatment with mercuric acetate, and analysis by t.l.c. (solvent *E*) as described for **2**, compound **5** produced a single, charrable material whose R_F (0.60) was identical to that of galacturonic acid.

Colorimetric characterization of the compounds. — As these compounds contain many functional groups for which colorimetric assays are available, the four colorimetric assays described in *Methods* were applied to solutions of **2**, **3**, **4**, and **5** (see Table I). The neocuproine assay applied to the (2,2-dimethoxyethyl)aminocarbonylmethyl 1-thioglycosides of some neutral and amino (*N*-acetyl) sugars produced[3] millimolar absorbances in the range of 20 to 22 at 460 nm. In the present study, the millimolar absorbances of **2** and **5** were found to lie in the same range, 21.4 and 22, respectively. It appears that the aglycon, the (2,2-dimethoxyethyl)aminocarbonylmethyl group, of all of the 1-thioglycosides gives a millimolar absorbance of ~21.5, regardless of the glycosyl group. As **3** and **4** were not obtained solid, the concentration of solutions containing **3**, or **4**, was determined on the basis of the millimolar absorbance of 21.5 at 460 nm in the neocuproine assay. Compound **4** did not give the

TABLE I

MILLIMOLAR ABSORBANCE OF THE SYNTHETIC PRODUCTS IN SOME COLORIMETRIC ASSAYS[a]

Compound	*Millimolar absorbance*			
	Aldehyde (460 nm)	*Carbohydrate (480 nm)*	*Uronic acid (530 nm)*	*Ester (520 nm)*
2	21.4	4.0	17.3	0.39
3	21.5[b]	3.1	17.3	0.40
4	21.5[b]	1.8	1.7	0.32
5	22.0	4.6	17.4	0.01

[a]These assays are: the neocuproine method for aldehyde, the phenol–sulfuric acid method for carbohydrate, the carbazole method for uronic acid, and the hydroxylamine–ferric chloride method for ester, as described in *Methods*. [b]For compounds **3** and **4**, a millimolar extinction coefficient of 21.5 was assumed (see text).

characteristic, uronic acid reaction, and also had a decreased millimolar absorbance in the phenol–sulfuric acid reaction; both phenomena may be caused by the absence of a hydroxyl group at C-4.

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