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Note

Formation and controlled hydrolysis of *O*-acetylated 5-*tert*-butyloxycarbonylaminopentopyranosides

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We have recently developed chemically selective procedures for the fragmentation of methylated glycuronans wherein the cleavage of modified glycosiduronic acid linkages leads to oligosaccharides in which the alditol termini are those of former hexuronic acid residues[1–6]. The procedures are based on (*a*) Hofmann- or Curtius-type rearrangements of glycosiduronic acid derivatives[1,3,4], (*b*) decarboxylation–acetoxylation of glycosiduronic acids[2,4,5], and (*c*) zinc dust cleavage of 6-deoxy-6-iodoglycosidic linkages[6]. Selective cleavage has been achieved under mild conditions. Acid treatment, if employed, as in the cleavage of *tert*-butyloxycarbonylamino substituents, leads to negligible hydrolysis of normal glycosidic linkages. However, it may be desirable at times to be able to isolate the corresponding unsubstituted oligosaccharides, in which case it would be necessary, prior to depolymerization, to perform the structural modifications of hexuronic acid residues on polysaccharides bearing removable substituents for the protection of hydroxyl groups. To this end we report an exploration of the Hofmann–Curtius-type rearrangement of *O*-acetylated glycosiduronamides to the corresponding 5-*tert*-butyloxycarbonylaminopentopyranosides.

Treatment of acetylated glycosiduronamides **1** and **4** with lead tetraacetate in *tert*-butanol gave the corresponding *O*-acetylated *tert*-butyl carbamates **2** and **5** in high yield. Hydrolysis of methyl 2,3,4-tri-*O*-acetyl-(5*S*)-5-*tert*-butyloxycarbonylamino-β-L-arabinopyranoside (**2**) with formic acid, followed by reduction and acetylation, gave L-arabinitol pentaacetate in 51% isolated yield, whilst g.l.c. analysis (Table I) with an internal standard indicated its formation in 65% yield with no other detectable product. Low recoveries of methylated alditol acetates in our earlier studies[3] were attributed to inadvertent losses because of the volatility of these substances. Such an explanation in the case of acetylated alditols is unconvincing and product loss through the formation of volatile side-products, e.g. furan derivatives formed on elimination reactions of "pentodialdose" intermediates, appears more likely. A rather higher yield (85% by g.l.c. analysis) of arabinitol pentaacetate was obtained when careful *O*-deacetylation of **2** was performed before carbamate hydrolysis with formic acid. In the corresponding hydro-

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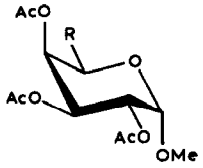
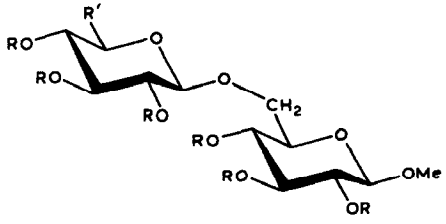
lysis of the disaccharide carbamate **5**, even lower conversion (22%, and 30% after *O*-deacetylation) of the uronic acid-derived carbamate residues to xylitol pentaacetate were obtained, although the aglycon component was liberated in better than 90% yield.



1 R = $CONH_2$

2 R = $NHCO_2Bu^t$



3 R = H, R' = $CONH_2$

4 R = Ac, R' = $CONH_2$

5 R = Ac, R' = $NHCO_2Bu^t$

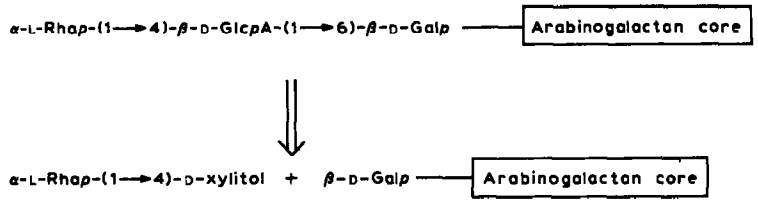
Despite the shortfall in yields of methylated pentitols from the corresponding carbamates in model compounds[3], studies on methylated glucuronans[3,4] have shown that selective hydrolysis of carbamates derived from uronic acid residues affords adequate yields of pentitol-terminated oligosaccharides from whose characterization new structural information on the polysaccharides could be obtained. Accordingly an acetylated gum arabic derivative was chosen as a suitable carbamate substrate with which to assess the modified procedure with removable protecting groups (see Scheme I for overall reaction).

Glucuronic acid residues in gum arabic were esterified by reaction with aqueous ethylene oxide[7], and amidation followed by acetylation gave acetylated gum arabic amide. That these reactions had proceeded to the desired extent without alteration in composition of the polysaccharide was shown by sugar analysis (Table II) and by their i.r. spectra. Hofmann-type rearrangement of the acetylated gum arabic amide on reaction with lead tetraacetate in *tert*-butanol proceeded

TABLE I

QUANTITATIVE ANALYSIS OF DEGRADATION PRODUCTS FROM *tert*-BUTYL CARBAMATES

Acetylated carbamate	*Internal standard*	*Method*	*G.l.c. column (°C)*	*Yields (%)*	
				Acetylated alditol	*Acetylated aglycon*
2	Mannitol hexaacetate	A	C (210)	65	
2	Me α-D-Gal*p*	B	B (210)	85	
5	Me α-D-Man*p*Ac_4	A	D (190)	22	90
5	Me α-D-Gal*p*	B	B (200)	30	90



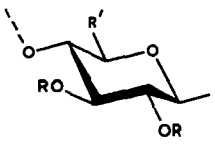
Scheme 1. Projected selective degradation of gum arabic with glycosiduronic acid cleavage.

smoothly with no significant change in neutral-sugar composition (Table II) and only a small proportion of the uronic acid residues remained unaltered. The acetylated gum arabic *tert*-butylcarbamate was then treated with formic acid under the previously established conditions, and processing of the mixture afforded as a precipitate a degraded polysaccharide containing only a small proportion of the original terminal rhamnose residues. However the fraction in which degradation products of low molecular weight were present contained much more material than would be expected from only selective cleavage of former glycosiduronic acid linkages. After treatment with sodium borohydride, the sugar constituents in the resulting mixture of alditols and oligosaccharide alditols were analyzed by g.l.c. (*a*) for alditols as their peracetates, and (*b*), after hydrolysis, for alditols (originally present and additionally liberated) and aldoses (as acetylated aldononitriles) simultaneously. The results (Table III) showed that during the supposedly mild acid hydrolysis of the *tert*-butylcarbamates, much more extensive hydrolysis had occurred.

The correctness of this latter conclusion was confirmed by methylation of the

TABLE II

SUGAR COMPOSITION OF NATIVE AND MODIFIED GUM ARABIC DERIVATIVES

Polysaccharide sample	*Status of glucuronic acid residues*[a]		*Rha*	*Ara*	*Gal*	*Uronic acid*[b]
	*R*	*R'*				
A. Gum acid	H	CO_2H	12	21	36	17
B. Acetylated gum amide	Ac	$CONH_2$	11	19	35	16
C. Acetylated *tert*-butyl carbamate	Ac	$NHCO_2Bu^t$	12	20	38	2
D. Acetylated degraded gum	Ac		2	14	28	n.d.

[a]Substituents in original or modified glucuronic acid residues are as indicated. R groups designate the status of hydroxyl groups in sugar residues. [b]Estimated as glucuronic acid with the 3-hydroxydiphenyl reagent[10].

TABLE III

COMPOSITION OF MIXTURE OF ALDITOLS AND OLIGOSACCHARIDE ALDITOLS FORMED ON REDUCTION OF DEGRADATION PRODUCTS FROM ACETYLATED GUM ARABIC *tert*-BUTYL CARBAMATE

Sugars/alditols	*Alditols*[a]	*Total aldoses + alditols after hydrolysis*[b]	*Aldoses and alditols liberated on hydrolysis*[c]
Rhamnitol	1	1	0
Arabinitol	1	9	8
Galactitol	0.7	4	3.3
Xylitol	1.3	5	3.7
Rhamnose		6	6
Arabinose		18	18
Galactose		17	17

[a]Alditols formed by direct reduction of degradation products and estimated as alditol acetates. [b]Total hydrolyzate treated with hydroxylamine in pyridine, followed by acetic anhydride, with estimation of alditols as acetates and aldoses as acetylated aldononitriles. Relative proportions are normalized with respect to rhamnitol. [c]Components (by difference) liberated on hydrolysis of oligosaccharide alditols.

TABLE IV

G.L.C.–M.S. CHARACTERIZATION OF PERMETHYLATED DISACCHARIDE ALDITOLS

Disaccharide alditol	*Approx. relative proportions*	*Fragment ions* (m/z)	
		Glycosyl residue	*Alditol residue*
Rha*p*-(1→4)-xylitol	1	189, 157	191, 159, 133, 89, 45
Ara*p*-(1→3)-arabinitol	1	175, 143	191, 159, 89, 45
Gal*p*-(1→3)-arabinitol	5	219, 187	191, 159, 89, 45
Gal*p*-(1→3)-galactitol	1	219, 187	235, 203, 171, 89, 45
Gal*p*-(1→6)-galactitol	1	219, 187	235, 203, 177, 171, 133, 89, 45

alditol–oligosaccharide alditol mixture and g.l.c.–m.s. analysis of those components having retention times characteristic of permethylated disaccharide alditols. Table IV shows *m/z* values of characteristic fragment-ions from glycosyl and alditol residues of these components and the probable identities of the disaccharide derivatives which could be formed from known structural units in gum arabic[8]. The results clearly show that the selectivity of hydrolysis of *tert*-butyl carbamates observed for methylated polysaccharides has not been reproduced for this acetylated glycan. The evidence points to the presence of the desired disaccharide alditol [Rha*p*-(1→4)-xylitol] in the mixture, but other disaccharides from peripheral chains [Gal*p*-(1→3)-arabinitol and Ara*p*-(1→3)-arabinitol] and even from the inner galactan core [Gal*p*-(1→3)-galactitol and Gal*p*-(1→6)-galactitol] were present and had been liberated under surprisingly mild conditions. Although the necessary structural modifications of the polysaccharide were performed satisfactorily, lack of selectivity in the depolymerization step would seem to limit the usefulness of this variant of the carbamate degradation.

EXPERIMENTAL

General methods. — Solutions were concentrated under diminished pressure at 40° or less. Optical rotations were measured with a Perkin–Elmer model 141 polarimeter at 20 ±2°. The i.r. spectra were measured with a Unicam SP 200 spectrophotometer. The ^{1}H-n.m.r. spectra were recorded on Varian EM 360 and Bruker AM 300 spectrometers and, unless otherwise stated, are in deuteriochloroform with tetramethylsilane as internal standard. G.l.c. was performed on Tracor 560 and Perkin–Elmer Sigma 3B chromatographs using packed columns coated with (A) 3% of silicone gum OV-225, (B) 3% of silicone polyester copolymer ECNSS-M, or (C) 3% of silicone gum Silar 10 CP, (D) a S.C.O.T. column coated with silicone gum OV-225, (E) a glass capillary column coated with silicone DB5-15N, and (F) a fused-silica column coated with CBWX 20M 30 N. For g.l.c.–m.s., columns were attached by a jet separator to a VG Micromass 16F mass spectrometer operated with an inlet temperature of 250°, an ionization potential of 70 eV and an ion-source temperature of ~250°.

Polysaccharide samples were hydrolyzed with 2M trifluoroacetic acid for 2 h at 120°. D-Allose was added as the internal standard and the sugar mixtures were converted into alditol acetates[9] and examined by g.l.c. on column C at 210°. Uronic acid determinations were carried out spectrophotometrically with the 3-hydroxydiphenyl reagent[10]. Methyl 2,3,4-tri-*O*-acetyl-α-D-galactopyranosiduronamide[11] (**1**), not previously reported crystalline, was prepared by acetylation of methyl α-D-galactopyranosiduronamide[12] and had m.p. 152–154°, $[\alpha]_D$ +126° (*c* 0.53, chloroform).

Preparation of tert-*butyl carbamates from acetylated glycosiduronamides.* — A mixture of peracetylated glycosiduronamide (0.5 g), pyridine (0.2 mL), and lead tetraacetate (1 g) in *tert*-butanol (17 mL) was heated under reflux for 4 h. A further quantity (0.5 g) of lead tetraacetate was added and heating was continued overnight. The mixture was filtered, the insoluble residue was washed with *tert*-butanol, and the combined filtrate and washings were evaporated. The residue was extracted with chloroform, and the extract was washed with water, dried and evaporated. The resulting syrup was chromatographed on silica gel with chloroform–acetone mixtures to yield *tert*-butyl carbamate which was recrystallized from ether–light petroleum. In this manner methyl 2,3,4-tri-*O*-acetyl-α-D-galactopyranosiduronamide (**1**) furnished methyl 2,3,4-tri-*O*-acetyl-(5*S*)-5-*tert*-butyloxycarbonylamino-β-L-arabinopyranoside (**2**), (83%), m.p. 184–185°, $[\alpha]_D$ +139° (*c* 0.17, chloroform); n.m.r. δ 1.49 (s, 9 H, *tert*-Bu), 2.15 (m, 9 H, 3 OAc), 3.55 (s, 3 H, OMe), 4.95 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 5.58 (d, 1 H, $J_{5,NH}$ 10.5 Hz).

Anal. Calc. for $C_{17}H_{27}NO_{10}$: C, 50.36; H, 6.71; N, 3.45. Found: C, 50.17; H, 6.88; N, 3.57.

Methyl (methyl β-gentiobiosid)uronate[2] was kept in methanolic ammonia overnight and afforded methyl β-gentiobiosiduronamide (**3**), m.p. 248°, $[\alpha]_D$ −52° (*c* 0.12, water); n.m.r. (D_2O with *tert*-BuOH as internal reference): δ 3.59 (s, 3 H, OMe), 4.40 and 4.60 (2 d, 2 H, $J_{1,2}$ 8.0 and 7.9 respectively, H-1 and H-1′).

Anal. Calc. for $C_{13}H_{13}NO_{11}$: N, 3.79. Found: N, 3.69.

Acetylation of **3** with acetic anhydride–pyridine gave the hexa-*O*-acetyl derivative (**4**), m.p. 173–174°, $[\alpha]_D$ −64° (*c* 2.8, chloroform); n.m.r. δ 2.01 (18 H, 6 × OAc), 3.52 (s, 3 H, OMe), 4.42 and 4.63 (2 d, 2 H, $J_{1,2}$ 7.95 and 7.5 Hz respectively, H-1 and H-1′).

Anal. Calc. for $C_{26}H_{37}NO_{17}$: C, 48.31; H, 5.67; N, 2.21. Found: C, 48.72; H, 5.81; N, 1.96.

The acetylated glycosiduronamide **4** afforded methyl 2,3,4-tri-*O*-acetyl-6-*O*-[(5*S*)-5-*tert*-butyloxycarbonylamino-2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl]-β-D-glucopyranoside (**5**) (81%), m.p. 132–133°, $[\alpha]_D$ −12° (*c* 0.3, chloroform); n.m.r. δ 1.43 (s, 9 H, *tert*-Bu), 2.05 (18 H, 6 × OAc), 3.51 (s, 3 H, OMe), 4.39 and 4.65 (2 d, 2 H, $J_{1,2}$ 7.95 and 7.5 Hz, respectively, H-1 and H-1′).

Anal. Calc. for $C_{29}H_{43}NO_{18}$: C, 50.22; H, 6.25; N, 2.02. Found: C, 50.06; H, 6.28; N, 2.02.

Degradations of tert-*butyl carbamates.* — Carbamate **2** (100 mg) was kept in 98% formic acid for 1 h at room temperature. The solution was diluted with water and concentrated under diminished pressure at room temperature with distillation of toluene. The residue in 1:1 oxolane–water was treated with sodium borohydride (45 mg) for 2.5 h, excess hydride was decomposed, and sodium ions were removed with Amberlite resin IR-120 (H^+), and the filtered solution was concentrated with methanol. Treatment of the resulting syrup with 1:1 acetic anhydride–pyridine (1 mL), followed by chromatography on silica gel with chloroform, afforded L-arabinitol pentaacetate (46 mg, 51%), m.p. and mixed m.p. 74–75°, $[\alpha]_D$ −36° (*c* 0.5, chloroform).

Quantitative analysis of products from degradations of tert-*butyl carbamates.* — *Method A.* Weighed quantities of acetylated *tert*-butyl carbamates and an internal standard in approximately equimolar proportions were treated with formic acid, and the products were reduced and then acetylated as just described to give derivatives for g.l.c. analysis.

Method B. Weighed quantities of acetylated *tert*-butyl carbamate were *O*-deacetylated by treatment with methanolic 0.1% barium methoxide for 5 min. Carbon dioxide was passed through the resulting solutions to precipitate barium ions as barium carbonate which was removed by centrifugation, and the supernatant solutions were concentrated. The residues were treated with formic acid, reduced and acetylated as before to give products for g.l.c. analysis.

Acetylated gum arabic tert-*butyl carbamate.* — Arabic acid (1.5 g) was kept in water (150 mL) containing ethylene oxide (30 mL) until the solution attained a constant pH of 6.8 (3 days). The solution was dialyzed, concentrated, and freeze-dried to give the 2-hydroxyethyl ester of arabic acid (1.2 g). Polysaccharide ester (0.5 g) in dry methyl sulfoxide (30 mL) was treated under pressure with liquid ammonia (20 mL) for 48 h at room temperature, and precipitation with ethanol, followed by dissolution in water and freeze-drying afforded gum arabic amide (420 mg), ν_{max} 1640 cm^{-1} (amide C=O) but no residual absorption at 1740 cm^{-1} (ester

C=O). Acetylation of the polysaccharide amide (350 mg) by the method of Carson and Maclay[13] gave acetylated gum arabic amide (290 mg) (no hydroxyl absorption at 3300 cm^{-1}). Acetylated polysaccharide amide (150 mg) in *tert*-butanol (8 mL) containing pyridine (0.5 mL) and lead tetraacetate (500 mg) was heated under reflux for 6 h. A further quantity (200 mg) of lead tetraacetate was added and heating was continued for another 16 h. The mixture was filtered, the insoluble residue was washed with water, and the aqueous washings were extracted with dichloromethane. The combined organic extracts were dried and evaporated, and the residue was purified by chromatography on Sephadex LH-20 with 10:1 dichloromethane–acetone to give acetylated gum arabic *tert*-butyl carbamate (135 mg), ν_{max} 3400 cm^{-1} (NH) with no detectable absorption at 3480 and 3430 cm^{-1} (NH_2). Compositional analysis (Table II) showed substantial diminution in uronic acid content with no alteration in neutral-sugar content.

Degradation of acetylated gum arabic tert-*butyl carbamate.* — Acetylated polysaccharide carbamate (150 mg) was kept in 98% formic acid (5 mL) for 1 h at room temperature. Concentration of the solution (to 0.5 mL) followed by addition of water (2 mL) gave a precipitate which was washed with water and then chromatographed in dichloromethane on Sephadex LH-20 with removal of products of low molecular weight to give degraded, acetylated polysaccharide (76 mg), whose sugar composition is shown in Table II. The supernatant liquid combined with aqueous washings was concentrated by distillation of toluene to a syrup (60 mg) which was treated with sodium borohydride and processed conventionally to give a mixture of alditols and oligosaccharide alditols. A portion of the mixture was acetylated and the resulting alditol acetates were analyzed by g.l.c. on column D at 190°. A second portion of the mixture was hydrolyzed with 2M trifluoroacetic acid for 2 h at 120° (sealed tube), and the resulting mixture of alditols and reducing sugars was heated with hydroxylamine in pyridine, and again after addition of acetic anhydride to furnish the corresponding mixture of alditol acetates and acetylated aldononitriles[14] for analysis by g.l.c. on column D at 190°. A third portion of the mixture of alditols and oligosaccharide alditols was methylated by the Hakomori procedure[15] and the permethylated disaccharide alditol components in the resulting mixture were analyzed by g.l.c.–m.s. using column E (140°, 4°/min to 220° and hold).

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