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Note

Selective 6-*O*-acetylation of amylose*†

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Selective methods for protection of O-6 in amylose and other polysaccharides are of interest as part of a program, in this laboratory, concerned with synthesis of compounds having potential anticoagulant activity[1,2]. The direct, selective protection or primary hydroxyl groups in such polysaccharides as amylose has thus far only been achieved satisfactorily by tritylation[3–5] and, somewhat less selectively, by tosylation[4–7]. The trityl group introduced is normally removed by acid treatment, which may also cause some hydrolytic degradation of the polysaccharide chain. Removal of tosyl groups generally involves competing reactions[4,6,7]. Simple carboxylic ester groups would, therefore, afford good alternatives as base-labile protecting-groups.

Starting with per(trimethylsilyl)ated derivatives, carbohydrates of low molecular weight may be selectively acetylated at primary positions by using a mixture of pyridine, acetic anhydride, and a small proportion of acetic acid[8]. We now report that this simple method, in a modified form, may also be applied to amylose.

Potato amylose (Stein–Hall) was per(trimethylsilyl)ated to yield the fully protected derivative (**1**), which was soluble in chloroform and in carbon tetrachloride. Compound **1** was treated with pyridine–acetic anhydride–acetic acid in carbon tetrachloride to yield, according to the duration of the reaction, products having different degrees of acetylation. 6-*O*-Acetylation to give **2** was complete after ~5 days at 45–50°. Prolonged treatment caused a considerably slower, further acetylation at the secondary positions; thus, after 14 days, about a quarter of the secondary groups had also become acetylated. The degree and position of acetylation in crude mono-acetate **2** could be observed by integrating the separate ^{1}H-n.m.r. signals for the

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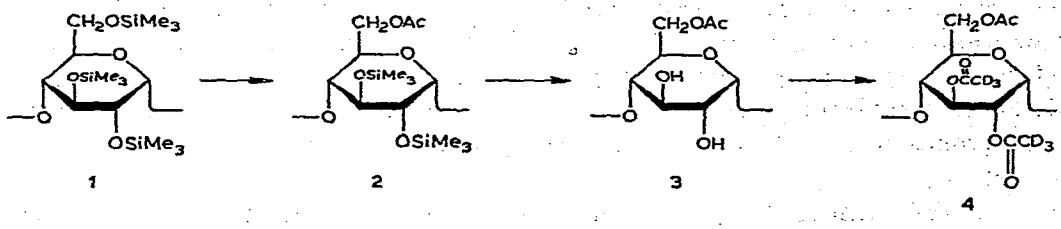
6-*O*-acetyl and the 2- and 3-*O*-acetyl groups. 6-*O*-Acetylamylose (3) was obtained by removing the *O*-trimethylsilyl groups from 6-*O*-acetyl-2,3-di-*O*-(trimethylsilyl)-amylose (2) in acetone by use of a mixture of methanol and aqueous acetic acid.

Further acetylation of 3 with acetic anhydride-d_6 under conditions where acetyl-group exchange or migration do not occur[9,10] gave the corresponding 2,3-di-*O*-(trideuterioacetyl) derivative 4, which had properties identical to those of tri-*O*-acetylamylose, except that its ^{1}H-n.m.r. spectrum[10,11] in chloroform-*d* showed only a 3-proton singlet at δ ~2.20 in the acetyl-group region; no signal was present at δ ~2.00, the region[10] for resonance of the 2- and 3-*O*-acetyl groups in tri-*O*-acetylamylose. The n.m.r.-spectral integrals give d.s. values of accuracy[10] approximately ±0.05 d.s.

Amylose may have quite varied chemical and physicochemical properties according to how, and from which source, it has been isolated, and how it is pretreated. Furthermore, the derivatives may behave differently, especially in their solubilities and reactivities. We found, however, that the procedure described here is applicable with various commercial samples of amylose without significant differences being observed in the d.s. values of the products.



EXPERIMENTAL

Materials. — The experiments described were performed with potato amylose (Stein–Hall, lot. no. 56457). Other lots were also used; no substantial differences in the results were observed, except for slight variations in the textures and handling characteristics of the products.

*2,3,6-Tri-*O*-(trimethylsilyl)amylose* (1). — A suspension of amylose (10 g) in pyridine (500 ml) was stirred for 24 h at 100°, and cooled to room temperature, and a mixture of hexamethyldisilazane (100 ml) and chlorotrimethylsilane (50 ml) was added with vigorous stirring. Stirring was continued for 24 h at 60–70°, and the mixture was then stirred (during 1 h) into cold methanol (2 l). Water (500 ml) was added after 1 h, and stirring was continued for a further 30 min. The precipitate was filtered off, and repeatedly washed with methanol and then with water. The product (1) was kept under vacuum over phosphorus pentaoxide; yield 18 g (77%). It was soluble in carbon tetrachloride and in chloroform. The i.r. spectrum (Nujol) did not show any hydroxyl band.

Anal. Calc. for $(C_{15}H_{34}O_5Si_3)_n$: C, 47.58; H, 9.05. Found: C, 47.50; H, 8.88.

*6-*O*-Acetyl-2,3-di-*O*-(trimethylsilyl)amylose* (2). — To a solution of freshly

prepared compound **1*** (10 g) in carbon tetrachloride (1 liter) was added a mixture of pyridine (400 ml), acetic anhydride (250 ml), and acetic acid (100 ml). If gelatinous material precipitated, more carbon tetrachloride was added, but no more than a further 500 ml; an absolutely clear solution was never obtained. The mixture was kept, with stirring, for 5–6 days at 45–50°, and then cooled in an ice bath, and methanol (200 ml) was added to decompose the excess of acetic anhydride. The mixture was next kept for 10 h at 25°, and methanol, carbon tetrachloride, and part of the pyridine and acetic acid were evaporated off under diminished pressure to afford a concentrate (300 ml) which was stirred into a mixture of methanol (700 ml) and water (300 ml). The precipitate was washed successively with methanol, water, and methanol, and then dried; yield 7 g (75%); ν_{max}^{Nujol} 1750 cm^{-1} (OAc, strong), OH band very weak; ^{1}H-n.m.r. (100 MHz, chloroform-*d*, δ values relative to the $OSiMe_3$ signal): δ 1.95 (3 H, s, fully acetylated O-6), no resonance observed at δ ~1.83 (2,3-OAc).

When the time of reaction was extended to 14 days, the ^{1}H-n.m.r. spectrum of the product showed the same 3-proton singlet (6-OAc) at δ 1.95 (relative to the $OSiMe_3$-group signal) as before, but a signal at δ 1.83, absent from the previous spectrum, had an intensity ~25% that of the 6-OAc signal. If the reaction time was shortened to 2 days, the signal at δ 1.95 (6-OAc) had only ~1 H intensity (~30% acetylation at O-6); no signal was present near δ 1.83 (2,3-OAc). Reaction for 10 days caused complete acetylation of O-6 (δ 1.95, 3 H) and traces of acetylation of O-2 and O-3 (very weak peak at δ 1.83).

6-O-Acetylamylose (**3**). — Compound **2** (5 g) was mixed with acetone (100 ml) to give an opalescent solution; this was stirred vigorously, methanol (50 ml), acetic acid (25 ml), and water (25 ml) were added, and the mixture was stirred or shaken for 24 h. More water (100 ml) was added, and the mixture was concentrated to ~100 ml. Most of the acid was removed by adding water and concentrating under diminished pressure to ~100 ml (3–4 times). The concentrate, which contained some insoluble material, was freeze-dried. The product was almost insoluble in water and in chloroform, but was soluble in pyridine and in tetrahydrofuran; yield 2.8 g (97%).

Anal. Calc. for $(C_8H_{12}O_6)_n$: C, 47.06; H, 5.92. Found: C, 46.92; H, 5.77 (average of 2 determinations).

6-O-Acetyl-2,3-di-O-(trideuterioacetyl)amylose (**4**). — Compound **3** (100 mg) was dissolved in a mixture of pyridine (2 ml) and acetic anhydride-d_6 (0.5 ml), and the viscous solution was kept for 7 days at ~25°. The mixture was diluted with pyridine (2 ml), and poured into vigorously stirred methanol (100 ml). The suspension was stirred for 1 h, and the precipitate was filtered off, and dried; yield 110 mg. The 100-MHz, ^{1}H-n.m.r. spectrum (chloroform-*d*, tetramethylsilane reference) of the product was identical to that[10,11] of a sample of tri-*O*-acetylamylose prepared conventionally, except that the spectrum showed essentially only a 3-proton singlet in the acetyl region, at δ 2.19 (6-OAc)[10], and the integrated intensity in the region δ 1.97–1.99 (2,3-OAc)[10] was negligible.

*On being kept, compound **1** appears to become less soluble.

Repetition of the experiment, but with material derived from the 14-day reaction at the conversion stage **1** → **2**, gave a product whose ^{1}H-n.m.r. spectrum was identical to that of **4**, except for a signal at δ 1.97–1.99 (~0.9 H, 2,3-OAc[10]) whose intensity indicated ~30% incorporation of protioacetate at secondary hydroxyl groups (O-2 and O-3).

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