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Note

Determination of the position of the *O*-acetyl group in a β-(1 → 4)-mannan (acemannan) from *Aloe barbardensis* Miller

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Acemannan, a β-(1 → 4)-linked acetylated mannan[1] (mannose content, 97%) isolated from the leaves of *Aloe barbardensis* Miller, was subjected to methylation analysis according to Arnarp et al.[2] in order to determine the position of the *O*-acetyl groups[3]. Acemannan was treated with methyl trifluoromethanesulfonate in trimethyl phosphate in the presence of 2,6-di-*tert*-butylpyridine[2,4]. The resultant partially methylated acemannan was subjected to alditol acetate formation[5] and subsequently analyzed by GLC–MS[6].

The peak at 10.61 min in the GLC–MS shows major fragments at *m/z* 43, 102, 118, 162, 201, 231, 261, and 305. Primary fragments at *m/z* 118 and 261 are due to the cleavage of two adjacent methoxy-bearing carbons (C-2 and C-3). Secondary fragments are due to the loss of formaldehyde (*m/z* 30), ketene (*m/z* 42), acetic acid (*m/z* 60), and methanol (*m/z* 32). The *m/z* 118 peak indicates that C-2 must be methoxylated and that C-1 is bonded to a deuterium atom. These data conclusively prove that the peak at 10.61 min is 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methyl-D-(1-^{2}H)mannitol. The peak at 10.42 min shows fragmentations at *m/z* 43, 45, 87, 129, 140, 185, 218, 260, 290, and 362. The higher mass fragmentation pattern is similar to that of an acetylated hexitol. The major peak at *m/z* 45 signifies the presence of a terminal methoxy group in the molecule. These data prove that the peak at 10.42 min is 1,2,3,4,5-penta-*O*-acetyl-6-*O*-methyl-D-(1-^{2}H)mannitol. The presence of this compound can be explained by reference to a molecular model, which shows that an acetyl group in the C-2 or C-3 position of the sugar crowds the hydroxyl group at the C-3 or C-2 position for two reasons: the C-2- and C-3-oxygen bonds are in the *cis* configuration and the acetyl group

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TABLE I

Position of the *O*-acetyl group in acemannan

D-(1-^{2}H)Mannitol acetate methylated at positions	Retention time (min)	Mol ratio	Position of *O*-Ac group
2,3,6	8.4	0.10	
2,6	9.5	0.20	C-3
3,6	10.3	0.20	C-2
6	10.4	1.0	C-2/C-3
2,3	10.6	0.95	C-6
3	11.1	0.05	
2	11.9	0.10	
D-Mannitol hexaacetate	13.1	2.20	

rotates along the single bond. Thus the approach of the bulky reagents to the hydroxyl group is sterically restricted. However, the OH-6 group is readily methylated producing the 6-*O*-methyl compound. The presence of a large amount of D-(1-^{2}H)mannitol hexaacetate suggested incompleteness of the reaction due to lack of solubility of the polysaccharide. All the peak assignments in this analysis are given in Table I. From the data it is apparent that the endogenous acetyl groups in acemannan were located at the C-2/C-3, and C-6 in a ratio of ~ 50:50.

This observation was also verified by the method of de Belder and Norrman[7] where the free hydroxyl groups were protected with methyl vinyl ether and the *O*-acetyl groups were replaced with *O*-methyl groups. In this process, a semi-solution of acemannan was prepared in Me_2SO and reacted with methyl vinyl ether in the presence of *p*-toluenesulfonic acid, whereby the unhindered alcohol and *cis*-diol formed a 1-(methoxy)-ethyl ether and cyclic acetal, respectively. The crude product, after removal of the gum by dissolving in benzene, was methylated (dimsyl anion–methyl iodide) and converted to the alditol acetates for GLC–MS analysis. The results of this analysis are similar to those already described in Table I. The major peaks are 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methyl-D-(1-^{2}H)mannitol (10.61 min) and 1,2,3,4,5-penta-*O*-acetyl-6-*O*-methyl-D-(1-^{2}H)mannitol (10.42 min) were almost in a 1:1 ratio, along with a large amount of D-(1-^{2}H)mannitol hexaacetrate as seen in the previous procedure.

From these data it is concluded that the *O*-acetyl groups in acemannan are located at C-2/C-3, and C-6 in a ratio of ~ 50:50.

EXPERIMENTAL

*Methylation of acemannan with methyl trifluoromethanesulfonate in the presence of 2,6-di-*tert*-butylpyridine.*—A sample of acemannan (6 mg) was dried under vacuum, suspended in trimethyl phosphate (2 mL, Aldrich) under N_2 and sonicated for 2 h. To the semi-solution 2,6-di-*tert*-butylpyridine (0.35 mL, Aldrich) was added and stirred well. Methyl trifluoromethanesulfonate (0.15 mL, Aldrich) was then added

and allowed to react for 2–3 h at 60–65°C. After cooling to room temperature, the mixture was diluted with cold, deionized water (5 mL) and dialyzed (MW cut off 3500) against deionized water for 2–3 days. The contents of the tube were freeze-dried and subjected to conventional methylation analysis (hydrolysis, reduction with sodium bododeuteride and acetylation).

Reaction of acemannan with methyl vinyl ether.—Acemannan (10 mg) was suspended in Me_2SO (5 mL) under N_2 and *p*-toluenesulfonic acid monohydrate (8 mg) was added at 15°C. To the resulting semi-solution was added methyl vinyl ether (4 mL, Aldrich, collected from a lecture bottle with the aid of a cold finger). The yellow semi-solution was kept, with stirring, at room temperature for 20 h. The dark-colored mixture was transferred to dialysis tubing (MW cut off 3500) and dialyzed against deionized water for 48 h. The resulting brown globule was collected and suspended in benzene, and white flaky material was deposited at the bottom. The white material (protected acemannan) was collected and washed alternately with benzene and ether and finally dried under vacuum. This was methylated with dimsyl anion–MeI and the converted to the alditol acetates for GLC–MS analysis.

GLC–MS analysis.—Each acemannan derivative (3 mg) was hydrolyzed in 2 M trifluoroacetic acid (2 mL) for 1 h at 110°C. After evaporation of solvents, the partially methylated monosaccharides were reduced with $NaBD_4$ (20 mg in 1 mL of 1 M NH_4OH, 1 h) and acetylated with acetic anhydride (2 mL) in pyridine (2 mL) for 30 min at 110°C. The partially methylated alditol acetates were analyzed by GLC–MS on a Hewlett–Packard 5970 MSD instrument using a DB-23 column (J & W Scientific, temperature program: holding 2 min at 80°C, increasing to 170°C at 30°C min, and then to 240°C at 4°C min).

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