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LOSS OF O-METHYL GROUPS FROM METHYLATED 2-AMINO-2-DEOXY-D-GLUCITOL AND -D-GALACTITOL DERIVATIVES UNDER ACIDIC CONDITIONS USED DURING THE ANALYSIS OF GLYCOPROTEINS

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SUMMARY

In the acetolysis/hydrolysis procedure (1), usually employed for the cleavage of permethylated oligosaccharides isolated from glycoproteins, O-demethylation of C-1 of the substituted, terminal hexosaminitol derivative occurs. Upon acetylation of the hydrolysate this yields a 1-O-acetyl-2-(N-methylacetamido)-2-deoxy-hexitol derivative and not, as suggested (2), a 2-acetylacetamido-2-deoxy-hexitol derivative.

INTRODUCTION

To overcome the difficulties associated with the acid catalysed hydrolysis of methylated polysaccharide chains obtained after treatment of glycoproteins first with base and borohydride and then with dimethylsulfinyl sodium/methyl iodide (3), Stellner et al. (1) elaborated a very efficient acetolysis/hydrolysis procedure which gave excellent recoveries of the constituent sugars. N-acetylated aminosugars are transformed into N-methylacetamido [-N(Me)COMe] derivatives during this procedure. Examination of the mass spectrum of permethylated 2-methylacetamido-2-deoxy-D-glucitol [1] treated according to Stellner et al. (1) led Hase and Rietschel (2) followed by Finne and Rauvala (4) to the conclusion that during the acetolysis/hydrolysis procedure loss of the N-methyl group occurred and that during the final acetylation procedure two MeCO-radicals were introduced on the amino group to give, as the final product 2-(N-acetylacetamido)-2-deoxy-1, 3, 4, 5, 6-penta-O-methyl-D-glucitol [2].

As in the conditions of acetylation used the formation of N-acetylacetamido-glucitol derivatives have never been observed previously and as loss of an N-methyl group in the acetolysis/hydrolysis procedure of Stellner et al. is not expected, the above mentioned reactions were

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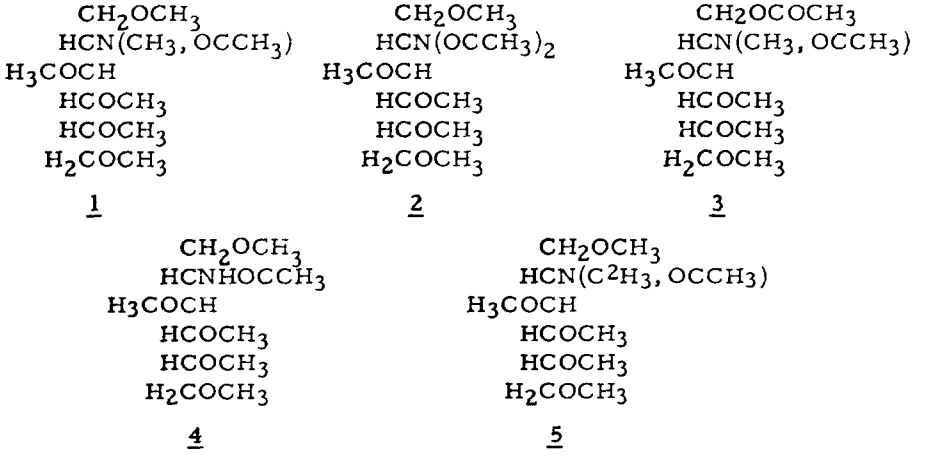
reinvestigated. This was deemed necessary in view of the importance of the knowledge of exact structures of methylated alditol acetates for structural determinations of glycoproteins. The results obtained establish that while no N-demethylation occurs during the acetolysis procedure of Stellner et al. the O-methyl group of position 1 is entirely or partially lost upon acetolysis, strong acidic hydrolysis or even methanolysis. The compound to which the N-acetylacetamido structure was assigned is, in fact, 1-O-acetyl-2-(N-methylacetamido)-3, 4, 5, 6-tetra-O-methyl-D-glucitol [3].

MATERIALS AND METHODS

Gas-liquid chromatography (1500 x 3. 2 mm stainless steel column packed with 3 % SE 30 methyl silicone on Varaport 30, 100-120 mesh; 140-220°C at 2°C/min; carrier: 40 ml/min He; injector: 220°C; detector:250°C) was performed on a Varian Aerograph, Model 2700, instrument coupled to a DuPont 21-492B, double focusing, medium resolution mass spectrometer (He separator:glass, jet type at 220°C; electron beam energy: 75 eV ; ionising current: 250-300 μA ; ion source temp. :240°C). All samples were introduced into the mass spectrometer through the gas chromatograph. Spectra were taken manually.

2-Deoxy-2-(N-methylacetamido)-1, 3, 4, 5, 6-penta-O-methyl-D-glucitol [1] and 2-acetamido-2-deoxy-1, 3, 4, 5, 6-penta-O-methyl-D-glucitol [4] were obtained from 2-acetamido-2-deoxy-D-glucitol by methylation according to Hakomori (3) and Kuhn et al., (5) respectively. 2-Deoxy-2-(N-trideuterio-methylacetamido)-1, 3, 4, 5, 6-penta-O-methyl-D-glucitol [5] was obtained from compound 4 by methylation with C^2H_3I by Hakomori's method (3). Detailed description of the syntheses and analysis of the mass spectra will be published in Carbohydrate Research.



RESULTS AND DISCUSSION

When a sample of compound 1 was submitted to the acetolysis/hydrolysis procedure of Stellner et al. (1) the acetylated material gave a single peak on gas-liquid chromatography which had a retention time (R = 1.26) different from that of compound 1 (R = 1). In the mass spectrum of this material (base peak: m/e 88, 100 %) M+1 at m/e 336 (1.3%) was clearly visible and in agreement with a molecular weight calculated for a 2-amino-2-deoxy-hexitol carrying 5 methyl groups and 2 acetyl radicals. However, a fragment at m/e 275 (7.8 %) indicating a formal loss of acetic acid (M-60), incompatible with the location of the 2 acetyl radicals as an N-acetylacetamido group, suggested the presence of at least one O-acetyl group within the molecule. The presence of the latter was demonstrated by treating the material first with methanolic ammonia solution to remove any O-acetyl groups and then with C^2H_3I and Ag_2O so as to introduce a trideuteriomethyl group on the hydroxyl group(s) set free during the alkaline treatment. Upon gas-liquid chromatography/mass spectrometry of the re-acetylated material a single peak emerged which had the same retention time as 2-deoxy-2-(N-methylacetamido)-1, 3, 4, 5, 6-penta-O-methyl-D-glucitol (compound 1). That it was in fact 2-deoxy-2-(N-methylacetamido)-1-O-trideuteriomethyl-3, 4, 5, 6-tetra-O-methyl-D-glucitol was unequivocally established by comparison of its mass spectrum with that of the unlabeled compound 1: the M+1 peak at m/e 311 proved the introduction of a single trideuteriomethyl group into the molecule (M+1 of compound 1 at m/e 308), while the fragments at m/e 177 ($^2H_3COCH_2$-CHN(Me)Ac-$CHOCH_3^+$; 74%) and m/e 133 ($^2H_3COCH_2$-$CHN(Me)Ac^+$; 98.7%) analogs of m/e 174 (57%) and 130 (100 %) observed for the non deuterated compound 1, proved that the trideuteriomethyl group was located on C-1.

The retention of the N-methyl group during the acetolysis/hydrolysis procedure was rigorously proven by first submitting the N-trideuteriomethylated compound 5 to it and then acetylating the product obtained. A single peak emerged upon gas-liquid chromatography: in its spectrum - which was that of a 1-O-acetyl-2-deoxy-2-(N-methylacetamido)-3, 4, 5, 6-tetra-O-methyl-hexitol containing one trideuteriomethyl group - the fragments $AcOCH_2$-$CHN(C^2H_3)Ac^+$ (m/e 161; 46 %) and CH_2=$CN(C^2H_3)Ac^+$ (m/e 101; 100 %) appeared 3 a. m. u. higher than in the non-deuterated

compound: this proved that the $-C^2H_3$ radical introduced on the N atom had been retained during the acetolysis.

Identical results, i. e. quantitative O-demethylation of C-1, were obtained when 2-amino-2-deoxy-D-galactitol derivatives were substituted for glucosaminitol, and also when acid catalysed hydrolysis (4M HCl/4 h/ 100°C) was employed instead of the acetolysis/hydrolysis procedure; upon methanolysis (6M HCl in MeOH/2 h/80°C) 50 % of O-demethylation occurred

While the loss of a methyl group from C-1 of 2-acetamido-2-deoxy-hexitol derivatives is, per se, not likely to introduce ambiguity in the interpretation of the substitution pattern of monosubstituted hexosaminitol derivatives, this may not be the case if more than one substituent were present: indeed, it is well known (6) that interpretation of mass spectra of amino sugars is not as straightforward as that of partially methylated alditol acetates.

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REFERENCES

1. Stellner, K. , Saito, H. , and Hakomori, S. I. (1973) Arch. Biochem. Biophys. 155, 464-472.
2. Hase, S. , and Rietschel, E. T. (1976) Eur. J. Biochem. 63, 93-99.
3. Hakomori, S. I. (1964) J. Biochem. 55, 205-208.
4. Finne, J. , and Rauvala, H. (1977) Carbohydr. Res. 58, 57-64.
5. Kuhn, R. , and Trischmann, H. (1963) Chem. Ber. 96, 284-287.