Carbohydrate Research, 100 (1982) C24–C26
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

Preliminary communication

A rapid, g.l.c.–m.s. method for identification of the *N*-acetyl group of amino sugars in complex carbohydrates

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(Received December 26th, 1981; accepted for publication, January 8th, 1982)

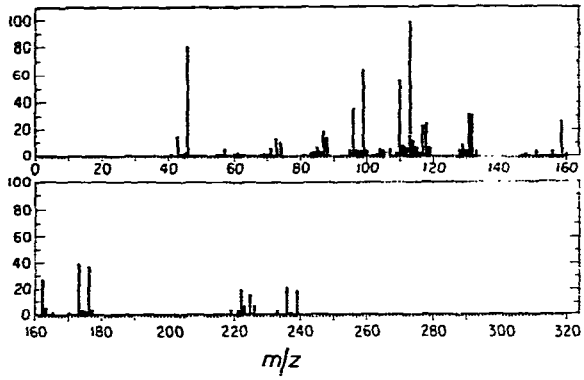
Complex carbohydrates are often subjected to different chemical modifications in order to reveal the nature of the glycosidic linkages, the unique sites of attachment, and the specific sequences of the various sugar units[1]. Common to all methods of determining the chemical composition of a polysaccharide, the total acid hydrolysis of the parent compound into its constituent monosaccharides is the primary step, followed by identification of specific sugars[2]. The reducing sugars may be converted into alditol acetate derivatives, in order to avoid formation of anomeric mixtures[3], and the acetates analyzed[4,5] by g.l.c.–m.s. However, such an analysis does not provide any information on the presence of *N*-acetyl groups in the original compound, or on the degree of *N*-deacetylation. The common constituents of complex polysaccharides, 2-amino-2-deoxy sugars and, sometimes, 2-amino-2,6-dideoxy, 3-amino-3,6-dideoxy, and 4-amino-4,6-dideoxy sugars, are usually *N*-acetylated[6]. After *N*-deacetylation, these sugar residues offer a starting point for specific degradation, such as partial hydrolysis to oligosaccharides, and deamination with nitrous acid[7].

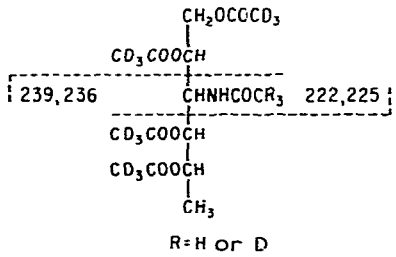
This communication describes a modified and rapid method, by g.l.c.–m.s. at the nanogram level, for the identification of the *N*-acetyl group, and for determination of the degree of *N*-deacetylation of the amino sugars obtained from complex carbohydrates. This method depends on the (trideuterioacetyl)ation of the alditols (formed by reduction of the monosaccharides obtained by acid hydrolysis) with trideuterioacetic anhydride in pyridine at room temperature.

We recently reported[8,9] the occurrence of the rare acetamido sugar 3-acetamido-3,6-dideoxy-L-glucose as an integral part of the core oligosaccharides obtained from the aquatic bacteria *Aeromonas hydrophila* and *Vibrio anguillarum*. The acetamido sugar was obtained by hydrolysis of the core oligosaccharide with 0.5M sulfuric acid for 4 h at 100°. The presence of the *N*-acetyl group, and the degree of *N*-deacetylation, were established by g.l.c.–m.s. analysis of the (trideuterioacetyl)ated aminoalditol obtained by successive borohydride reduction and (trideuterioacetyl)ation. The mass spectrum of the mixture of per(trideuterioacetyl)ated 3-amino- and 3-acetamido-3,6-dideoxy-L-glucitol gave, *inter alia*, peaks at the following *m/z* values: 239, 236, 225, 222, 176, 173, 162, 159, 132, 129, 118, 115, 113, 110, 99, 96, 46, and 43. The fission between C-2 and

0008-6215/82/0000–0000/$02.75

C-3, and C-3 and C-4, of the per(trideuterioacetyl)ated aminoalditol and acetamidoalditol gave the primary ions at *m/z* 239, 225 and 236, 222, respectively. These ions were degraded to secondary fragments by elimination of trideuterioacetic acid (63 m.u.) and dideuterioketene (44 m.u.) (see Fig. 1).





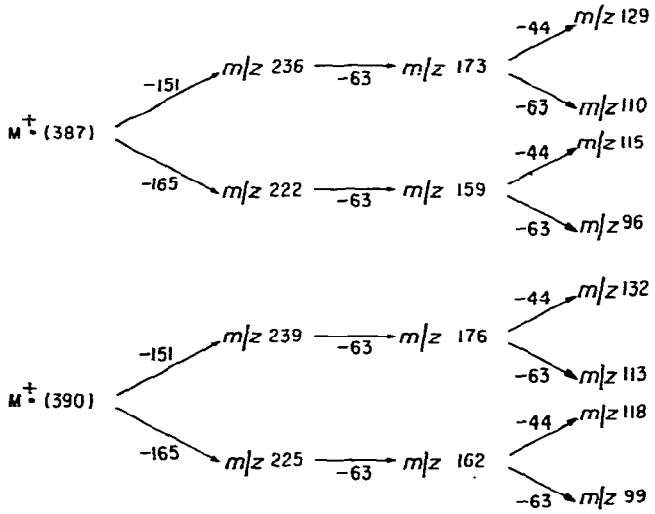


Fig. 1. Mass spectrum and fragmentation pattern of (trideuterioacetyl)ated 3-acetamido-3,6-dideoxy-L-glucitol. [Gas–liquid chromatography–mass spectrometry was performed in a Hewlett–Packard Model 5981A GC/MS instrument controlled by a 5939A data system, with a membrane separator, a source temperature of 160°, and an ionizing voltage of 70 eV, and gas–liquid chromatography was performed in columns (183 × 2 mm i.d.) packed with 1.5% of Silar 7CP on Gas Chrom Q (100–120 mesh) at 210°.]

The presence of the primary ions *m/z* 236 and 222 is diagnostic for the presence of the *N*-Ac group in this sugar (3-acetamido-3,6-dideoxy-L-glucose), and also for its position in the molecule, but the intensities of these two ions may vary, depending on the degree of *N*-deacetylation of the acetamido sugar during the initial hydrolysis with acid[2]. Classically, polysaccharides are hydrolyzed with sulfuric or hydrochloric acid, but Albersheim and coworkers[10] used trifluoroacetic acid for the hydrolysis of plant cell-walls, and this has also been suggested as an alternative to 6M hydrochloric acid in the determination of the amino sugars[11].

We have found that preliminary hydrolysis of the core oligosaccharide of *A. hydrophila* with either 2M trifluoroacetic acid for 4 h at 100°, or 90% acetic acid followed by 0.25M sulfuric acid[12], is extremely useful in avoiding *N*-deacetylation, thus increasing the intensities of the major ions *m/z* 236 and 222 in the mass spectrum of the (trideuterioacetyl)ated alditol from 3-acetamido-3,6-dideoxy-L-glucose.

When subjected to g.l.c.–m.s. analysis, the (trideuterioacetyl)ated aminoalditol obtained on hydrolysis of chitin [poly-(2-acetamido-2-deoxy-β-D-glucose)] with 6M hydrochloric acid for 6 h at 100° gave, *inter alia*, major peaks at the following *m/z* values: 150, 142, 116, 87, and 46. The major ion, *m/z* 150, and its derived secondary fragment, *m/z* 87, indicated that *N*-deacetylation had occurred during the hydrolysis (absence of major peaks at *m/z* 147 and 84).

It is noteworthy that g.l.c.–m.s. analysis of the (trideuterioacetyl)ated aminoalditols, in conjunction with that of the aminoalditol acetates, gives valuable information on the presence of the *N*-Ac group of the amino sugars, and on the chemical composition of the polysaccharide, prior to Fourier-transform n.m.r.-spectral studies and methylation analysis.

ACKNOWLEDGMENTS

Thanks are expressed to Mr. A. Dey for constructive criticism and for reviewing our original manuscript, and to Mr. Howard J. Hodder for technical assistance.

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