Carbohydrate Research, 152 (1986) 1–6
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

DERIVATIVES FOR THE ANALYSIS OF MONOSACCHARIDES BY CAPILLARY G.L.C.: TRIMETHYLSILYLATED DEOXY(METHOXYAMINO)ALDITOLS

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(Received August 16th, 1985; accepted for publication, December 17th, 1985)

ABSTRACT

A novel derivatisation method for g.l.c. of monosaccharides involves reduction of sugar methyloximes with sodium cyanoborohydride to the corresponding deoxy(methoxyamino)alditols, which are amenable to capillary g.l.c. after trimethylsilylation or acetylation. The reduction proceeds under mild conditions with high yields. Aldoses yield single peaks, but ketoses yield two peaks for diastereomers. The derivatives are stable for several weeks and are suitable for qualitative and quantitative determination of carbohydrates.

INTRODUCTION

Carbohydrates usually require derivatisation prior to g.l.c. Commonly used are the trimethylsilyl derivatives introduced by Bayer and Sweeley *et al.*[1,2]. The main drawback of this method is the formation of up to five derivatives (α- and β-furanoid, α- and β-pyranoid, and acyclic) which complicates the quantitative and qualitative analysis of mixtures. Acetylated[3] and trifluoroacetylated alditols[4], butaneboronates[5,6], aldononitriles[7], trifluoroacetylated methyl glycosides[8], trimethylsilylated oximes and methyloximes[9,10], and anhydrohexose dithioacetals[11] have been proposed in order to overcome these difficulties. Only one acyclic derivative is usually obtained from each sugar. However, the reduction of aldoses and ketoses may afford identical alditols. We have recently described[12] a new derivatisation method involving the reduction of methyloximes with borane to the corresponding aminodeoxyalditols, followed by *N*-ethoxycarbonylation and *O*-trimethylsilylation. However, on reduction with borane, oligosaccharides may be converted to some extent into monosaccharides. Thus, sucrose is extensively cleaved into methyl α- and β-D-fructofuranoside and D-glucose[13]. This can be avoided when sodium cyanoborohydride is used.

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0008-6215/86/$ 03.50

EXPERIMENTAL

General. — Sodium cyanoborohydride was recrystallised as its 1,4-dioxane complex and was liberated[14] from the complex by heating *in vacuo*. Pyridine was dried over potassium hydroxide for 48 h, refluxed over KOH, and distilled. Tetrahydrofuran and methanol were appropriately dried and distilled. G.l.c. was performed on a Pye Unicam PU 4500 instrument, equipped with split injector and flame-ionisation detector. Glass capillaries coated with SE-30, SE-54, OV-1, OV-101, OV-17, and Chirasil-Val were used. G.l.c.–m.s. was performed on a Shimadzu QP 1000 instrument. C.i.-mass spectra were obtained on a Finnigan 4021 instrument with an INCOS data system. Quantitative calculations were performed with a Shimadzu CR3-A computing integrator.

Derivatisation procedures. — An aliquot (200 μL) of an aqueous solution of the monosaccharides (each 1–2 mg/mL) containing 2 mg/mL of D-mannitol (internal standard) was transferred into a Reacty-Vial (Pierce) and concentrated in a light stream of nitrogen, and the residue was dried *in vacuo* over phosphorus pentaoxide. A portion (100 μL) of a solution of methoxyammonium chloride (250 mg) in dry pyridine (10 mL) was added, and the mixture was kept at 80° for 1 h and then concentrated in a stream of nitrogen. To the residue was added M $NaBH_3CN$ in methanol (200 μL), and the pH was adjusted to 3 by the addition of methanolic M HCl (50 μL). The solution was sonicated for 1 h with occasional addition of HCl/MeOH in order to keep the solution slightly acidic, and then concentrated in a stream of nitrogen. The residue was treated with methanol (150 μL) at 80° for 30 min, the solvent was evaporated under nitrogen, and the residue containing the deoxy(methoxyamino)alditols was dried *in vacuo* over P_2O_5.

The residue was treated with hexamethyldisilazane–pyridine–chlorotrimethylsilane (8:4:3, v/v) for 5 min at room temperature. The solution was centrifuged and aliquots (0.1–0.2 μL) of the supernatant liquid were used for g.l.c.

Alternatively, the residue was treated with pyridine (100 μL) and acetic anhydride (100 μL) at 100° for 20 min. Aliquots (0.1–0.2 μL) of the resulting solution were used for g.l.c.

RESULTS AND DISCUSSION

The reduction of sugar methyloximes with borane affords the corresponding aminodeoxyalditols which may then be trimethylsilylated[13]. Although these derivatives have good g.l.c. properties, deactivated capillaries are required. Also, *N*-trimethylsilyl groups are relatively sensitive towards weakly basic groups or humidity, and analysis must always be performed with freshly derivatised samples. Furthermore, two chromatographic peaks for each monosaccharide have often been found, due to formation of the respective *N*-trimethylsilyl and *N*-bis(trimethylsilyl) derivatives. The relative proportions are unpredictable, but seem to depend upon the origin and nature of the sample and the age of the reagent (Fig. 1). Another disadvantage is that the treatment with methanolic hydrogen chloride, required for

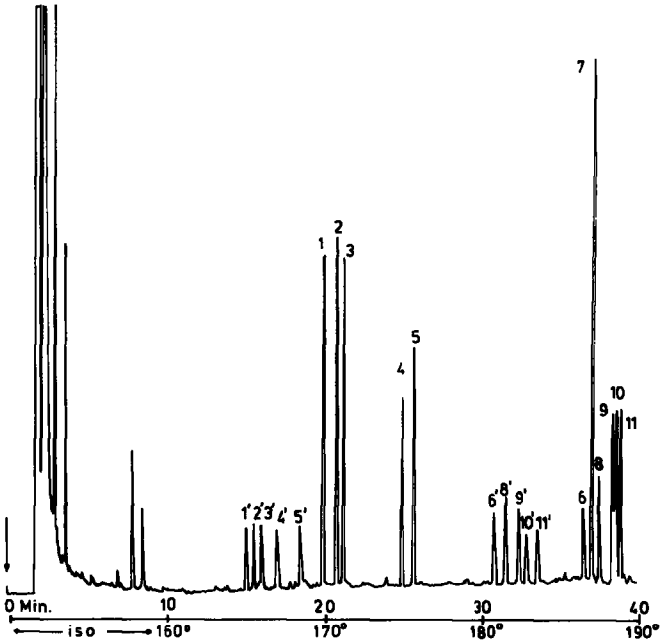

Fig. 1. Gas chromatogram of a mixture of monosaccharides after trimethylsilylation of the corresponding aminodeoxyalditols on a glass capillary column (25 m × 0.25 mm i.d.) coated with OV-101; carrier gas, hydrogen (90 kPa); split ratio, 1:50; injector and FID temperature, 300°. Peaks [n, *N*-bis(trimethylsilyl) derivatives; n', *N*-trimethylsilyl derivatives]: 1,1', xylose; 2,2', ribose; 3,3', arabinose; 4,4', rhamnose; 5,5', fucose; 6,6', fructose 1; 7, mannitol (internal standard); 8,8', fructose 2; 9,9', glucose; 10,10', mannose; 11,11', galactose.

liberation of the derivative from the boron–nitrogen complexes, may cleave glycoside linkages.

The reduction of sugar methyloximes with sodium cyanoborohydride circumvents these problems and yields the corresponding deoxy(methoxyamino)alditols at room temperature and pH ~3. Treatment with methanol is sufficient to cleave the boric esters. Thus, one derivatisation step, namely *N*-ethoxycarbonylation, is eliminated. As the nitrogen functions remain blocked, the reduction step can be followed immediately by trimethylsilylation or methylation; with either reaction, the presence of a free amino group would lead to complications.

The identity of the deoxy(methoxyamino)alditols (RCH_2NHOCH_3) was confirmed by g.l.c.–m.s. of the trimethylsilylated or acetylated derivatives.

The e.i.-mass spectra of the trimethylsilylated derivatives contain no molecular ions. For pentoses and deoxyhexoses, $(M - 15)^+$ ions (*m/z* 454 and 468, respectively) are present; for hexoses, $(M - 32)^+$ ions (*m/z* 539) are present. All spectra contain several chain-cleavage fragments (*m/z* 103, 205, 307, 366) together with some cascades of ions from sequential elimination of trimethylsilanol. The mass spectrum of 1-deoxy-1-methoxyamino-2,3,4,5,6-penta-*O*-trimethylsilyl-D-mannitol (Fig. 2), for example, contains ions at *m/z* 450 − 360 − 270, 435 − 345,

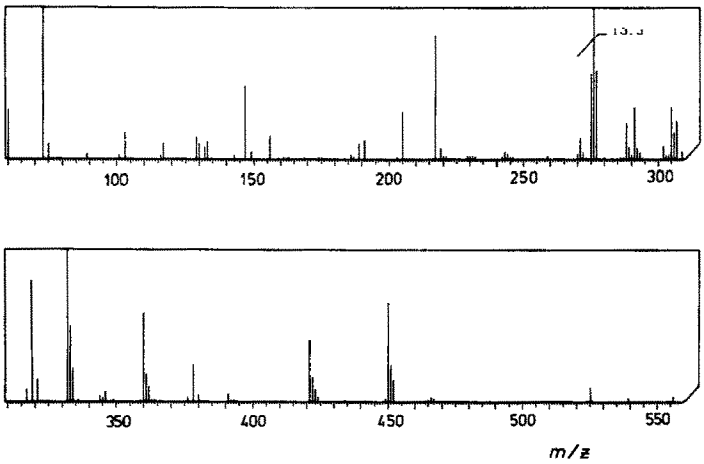

Fig. 2. E.i. (70 eV) mass spectrum of 1-deoxy-1-methoxyamino-2,3,4,5,6-penta-*O*-trimethylsilyl-D-mannitol. G.l.c.–m.s. interface and ion source at 250°.

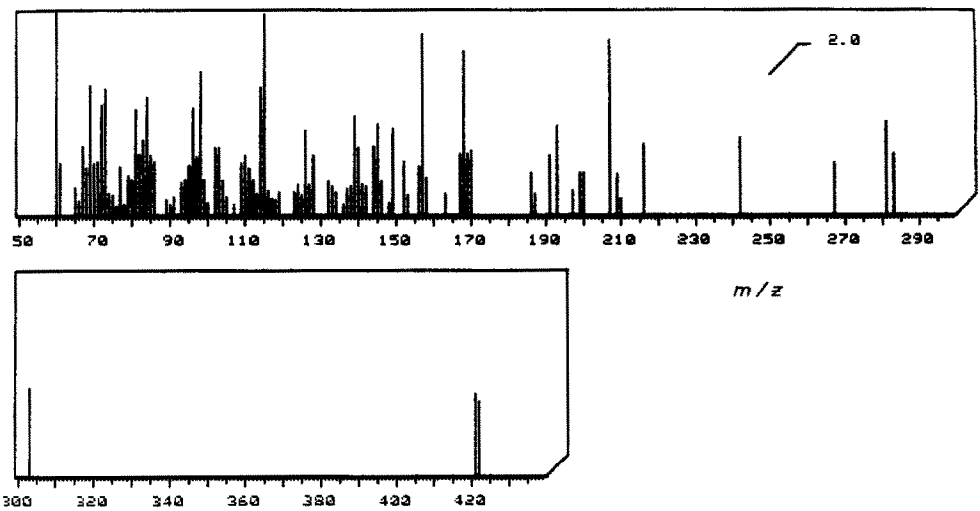

Fig. 3. E.i. (70 eV) mass spectrum of 2,3,4,5,6-penta-*O*-acetyl-1-deoxy-1-methoxyamino-D-mannitol. G.l.c. interface, 250°; ion source, 300°.

391 (M − 90 − 90)$^+$, and 217 (307 − 90). Other diagnostic ions are *m/z* 556 (M − 15)$^+$, 539 (M − 32)$^+$, 525 (M − 46)$^+$, 421 (M − 60 − 90)$^+$, 306 (M − 60 − 205)$^+$, 320 (M − 46 − 205)$^+$, and 102 (162 − 60). The mass spectra show that the nitrogen is not trimethylsilylated and this is confirmed by the c.i.(ammonia)-mass spectra which contained only one abundant ion at *m/z* 572, the quasimolecular ion.

For the acetylated deoxy(methoxyamino)alditols. M$^+$ or MH$^+$ ions were present. In some instances, fragments corresponding to the loss of 15 or 32 a.m.u.

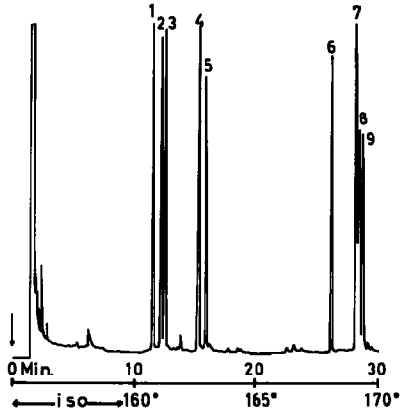

Fig. 4. Gas chromatogram of trimethylsilylated 1-deoxy-1-methoxyaminoalditols on a glass capillary column (25 m × 0.18 mm i.d.) coated with SE-30; carrier gas, hydrogen (90 kPa), split ratio, 1:40; injector and FID temperature, 300°. Peaks: 1, xylose; 2, arabinose; 3, ribose; 4, rhamnose; 5, fucose; 6, mannitol (internal standard); 7, mannose; 8, glucose; 9, galactose.

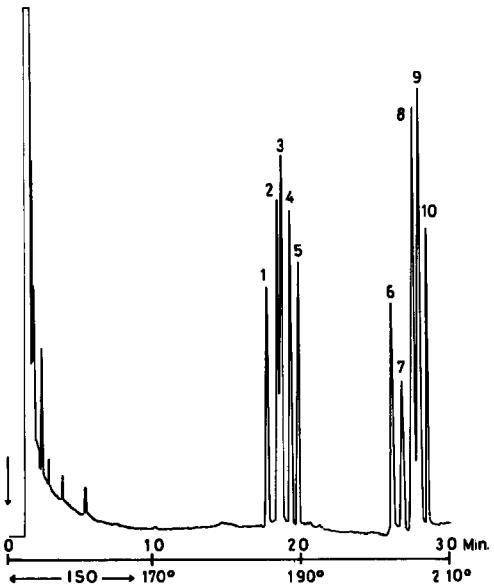

Fig. 5. Gas chromatogram of acetylated 1-deoxy-1-methoxyaminoalditols on a glass capillary column (25 m × 0.25 mm i.d.) coated with OV-101; carrier gas, hydrogen (50 kPa); split ratio, 1:40; injector temperature, 250°; FID temperature, 300°. Peaks: 1, rhamnose; 2, arabinose; 3, fucose; 4, xylose; 5, ribose; 6 and 7, fructose; 8, mannose; 9, glucose; 10, galactose.

from M^+ were observed, especially for the deoxyhexose derivatives. For hexoses, the loss of 119 a.m.u. (73 + 74) from the quasimolecular ion is characteristic (Fig. 3). Chain fragments are present at *m/z* 60, 73, and 145 (*m/z* 86 for deoxyhexoses), as expected for the proposed structure of the derivatives.

Separation of the components of a mixture of arabinose, xylose, ribose,

fucose, rhamnose, mannose, glucose, and galactose as the trimethylsilylated deoxy(methoxyamino)alditols has been accomplished on glass capillaries coated with SE-30 (Fig. 4). Only one derivative was formed from each aldose. Another stationary phase well suited for separation of monosaccharide isomers[15] is Chirasil-Val[16]. Chromatography on capillaries coated with this phase is possible at a temperature about 20–30° lower. Increase of the programming rate or the initial temperature still maintained an acceptable resolution and increased the speed of analysis significantly. Thus, at 150°, a complete separation of the eight monosaccharides was achieved in <15 min. On capillaries coated with OV-17, the arabinose and xylose derivatives were co-eluted, as were those of mannose and glucose on OV-101 and SE-54; OV-101 was suitable for acetylated derivatives (Fig. 5).

Relative response factors were calculated from mixtures containing various amounts of each sugar and a fixed amount of D-mannitol as internal standard. Good linearity was found.

Trimethylsilylated deoxy(methoxyamino)alditol derivatives are stable for at least three weeks if kept in a refrigerator. Ketoses yield two diastereomers, but their derivatives are different from those of the aldoses. The formation of side products is negligible and the amount of sample required for analysis is small (2 μmol). The present method is well suited for sensitive g.l.c. analysis of carbohydrates in biological samples.

ACKNOWLEDGMENT

The authors thank the Stiftung Volkwagen Werk for financial support (grant Az. I/37812).

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