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THE ANALYSIS OF 2-ACETAMIDO-2-DEOXYALDOSE DERIVATIVES BY GAS–LIQUID CHROMATOGRAPHY AND MASS SPECTROMETRY

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ABSTRACT

The trimethylsilylated *O*-methyloximes of three naturally occurring 2-acetamido-2-deoxyaldoses were prepared. The mass spectra of these derivatives were recorded, and the conditions for resolving them by gas–liquid chromatography defined.

INTRODUCTION

During our studies of sugars in mammalian, cell-surface membranes by gas–liquid chromatography (g.l.c.), it became apparent that the alditol acetate derivatives commonly used to determine neutral sugars[1] are not suitable for amino sugar analysis, because of prolonged retention times, inadequate resolution, multiple peaks, and degradation. Other approaches to the derivatization of amino sugars include *O*-trimethylsilylation after *N*-acetylation[2], *N*,*O*-trimethylsilylation[3,4], simultaneous *N*-acetylation and *O*-trimethylsilylation[5], and trimethylsilylation after nitrous acid deamination[6]; these procedures, also, are limited by some or all of the problems just mentioned. Subsequently, we prepared the *O*-trimethylsilylated *O*-methyloxime derivatives of several 2-acetamido-2-deoxyaldoses by methods previously used to prepare trimethylsilyl derivatives of *O*-methyloximes of neutral sugars[6–8]. Because the *O*-methyloxime function effectively labels the carbonyl carbon atom and prevents the formation of anomers of ring forms, one of the major sources of ambiguity and multiple peaks is eliminated. The analysis of these derivatives by g.l.c., mass spectrometry (m.s.), and g.l.c.–m.s. is described.

RESULTS AND DISCUSSION

Chromatography. — Trimethylsilylated *O*-methyloxime derivatives were prepared from 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-mannose,

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and 2-acetamido-2-deoxy-D-galactose. The results of a preliminary investigation of trimethylsilylated derivatives of the *O*-methyloximes of the corresponding free amino sugars were not encouraging. As the strongly acidic conditions of hydrolysis needed to release amino sugars from their polysaccharides usually cleave naturally occurring *N*-acetyl groups, free amino sugars must be specifically re-*N*-acetylated[10] and the neutral products desalted by ion-exchange chromatography before our method of g.l.c. analysis can be applied.

TABLE I

RETENTION TIMES OF TRIMETHYLSILYLATED 2-ACETAMIDO-2-DEOXYALDOSE *O*-METHYLOXIMES ON 3% OF OV-225

Derivative of	*Retention times*[a]		
	Major peak	*Minor peak*	*Minor/Major*[b]
2-Acetamido-2-deoxy-D-glucose	5.70[c]	6.65	0.16
2-Acetamido-2-deoxy-D-mannose	6.10	7.30	0.07
2-Acetamido-2-deoxy-D-galactose	6.90	7.95	0.19

[a]In minutes, at a column temperature of 190°. Other chromatographic conditions as in Fig. 1. [b]Ratio of peak heights. [c]To nearest 0.05 minute.

When analyzed individually, each derivatized 2-acetamido-2-deoxyaldose gave a unique, major peak and a more-polar, minor peak or shoulder on 3% OV-225; this has been attributed to *syn* and *anti* forms of the *O*-methyloxime function[9]. Retention times and ratios of area for major to minor peaks are given in Table I. Fig. 1 shows the gas chromatogram after derivatization of an equimolar mixture of the three 2-acetamido-2-deoxyaldoses studied; the most polar peak, at 21.5 min, was identified as the minor isomer of the 2-acetamido-2-deoxymannose derivative. Conditions for g.l.c. are given in the legend. The major and minor components of the pertrimethylsilylated *O*-methyloxime of 2-acetamido-2-deoxy-D-glucose were resolved, and

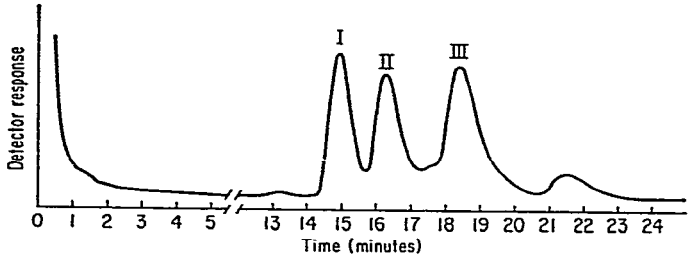
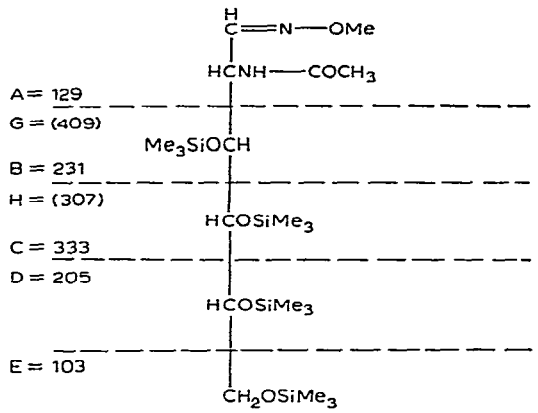


Fig. 1. Gas–liquid chromatography of the trimethylsilyl derivatives of the *O*-methyloximes of 2-acetamido-2-deoxy-D-glucose (I), 2-acetamido-2-deoxy-D-mannose (II), and 2-acetamido-2-deoxy-D-galactose (III), on a Hewlett–Packard Model 5700 gas chromatograph equipped with stainless-steel column (1.86 m × 3 mm) of 3% of OV-225 on Supelcoport (100–120 mesh) and an on-column, injection port. Temperatures: column, 170°; flame-ionization detector, 250°. Carrier gas: helium at a flow-rate of 30 ml.min^{-1}.

isolated, by preparative, thin-layer chromatography (t.l.c.). The two compounds have similar mass spectra, but, on the basis of physical measurements alone, it could not be proved that they were *syn* and *anti* forms. The retention times given in Table I, and comparative t.l.c. analysis, however, eliminated the possibility that the minor peak stems from partial epimerization at C-2 during derivatization. In practice, the preponderant isomer serves to identify the parent sugar.

Mass spectrometry. — The mass spectra of the three 2-acetamido-2-deoxyaldose derivatives, both the major and minor components, are very similar, and therefore, only the spectrum of 2-acetamido-2-deoxy-3,4,5,6-tetra-*O*-(trimethylsilyl)-D-glucose



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O-methyloxime (**1**) (major) is depicted in Fig. 2. The spectrum reveals a relatively simple fragmentation-pattern, with tentative identification of 17 major peaks possible. We have formulated all but two of the peaks as even-electron species. Ions L and N are designated as odd-electron species.

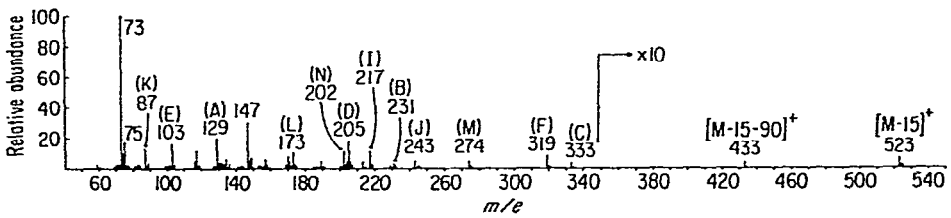
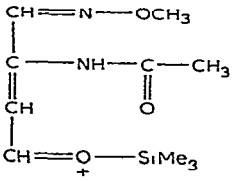
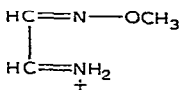


Fig. 2. Mass spectrum of compound **1** (mol. wt. 538), recorded on a combined Hewlett–Packard 5700 gas Chromatograph–JEOL Model JMS-01SG-2 mass spectrometer system, equipped with the column described in the legend to Fig. 1. Ionizing electron energy, 70 eV.; temperatures of column, 170°, of molecular separator, 220–250°, and of ion source, 300°. Ionizing current 200 mA; ion-source vacuum, 9 μtorr. Carrier gas: helium at a flow rate of 30 ml.min^{-1}. When trideuteriomethoxyamine hydrochloride was used to prepare the deuterio derivative, the following *m/e* peaks were shifted three mass-units higher: 87, 129, 173, 202, 231, 243, 274, 333, 433, and 523.

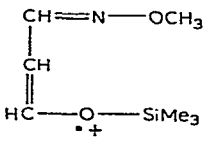
As would be expected[11], the molecular ion is too weak for practical detection in the spectrum measured in the g.l.c.–m.s. mode, although, in the spectrum of the solid-probe sample at 80°, it can be well identified at *m/e* 538. The ion at *m/e* 523, corresponding to the loss of CH_3 from the molecular ion, is readily recognizable, and can be used without difficulty for purposes of molecular-weight determination. Loss of trimethylsilanol[12] ($Me_3SiOH = 90$) from the M-15 fragment is envisaged to give *m/e* 433. Simple carbon–carbon cleavage results in fragments A (*m/e* 129), B (*m/e* 231), C (*m/e* 333), D (*m/e* 205), and E (*m/e* 103).



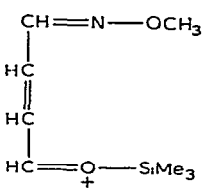
ion J, *m/e* 243



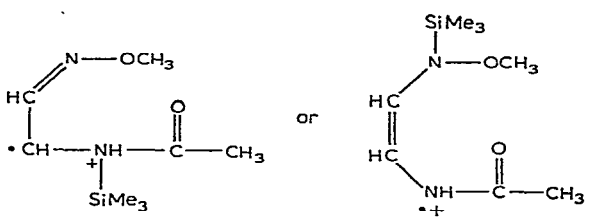
ion K, *m/e* 87



ion L, *m/e* 173



ion M, *m/e* 274



ion N, *m/e* 202

Fragment F (*m/e* 319) can be formally derived from ion G (*m/e* 409) by elimination of trimethylsilanol, even though ion G, a simple C-2–C-3 cleavage fragment, has no significant intensity at an ionizing energy of 70 eV in the g.l.c.–mass spectrum, but is discernible in the solid-probe mass spectrum. Similarly, formal loss of trimethylsilanol from ion H (*m/e* 307) could be expected to give fragment I (*m/e* 217). Loss of trimethylsilanol from ion C (*m/e* 333) results in fragment J (*m/e* 243). An expected loss[13] of ketene ($CH_2C{=}O$) from the acetamido group of ion A (*m/e* 129) gives significance

to fragment K (*m/e* 87), and the loss of the entire acetamido group (CH_3CONH) from ion B (*m/e* 231) is expected to give the odd-electron fragment L (*m/e* 173). On the other hand, formal elimination of acetamide (CH_3CONH_2) from ion C (*m/e* 333) will give the even-electron fragment M (*m/e* 274). The interesting ion N (*m/e* 202) is tentatively assigned to an odd-electron species, arising *via* the intramolecular transfer of a trimethylsilyl group to the amido nitrogen atom or to the nitrogen atom of the oxime, and cleavage of the C-2–C-3 bond. Intramolecular transfers of trimethylsilyl groups during mass-spectrometric fragmentation are not unprecedented. The *m/e* 202 fragment is relatively more intense when the spectrum is recorded for a sample introduced through the direct-inlet system, with the solid probe at 80°. Ions *m/e* 147, 75, and 73 are characteristic fragments of trimethylsilyl ethers of carbohydrates[14], and are not detailed here.

The structural assignments of fragments given are supported by an analysis of the mass spectrum of deuterated **1** (major) prepared with trideuteriomethoxyamine hydrochloride (CD_3ONH_2; Regis P48-118-2). For this compound, all fragments containing the trideuteriomethyl group of the *O*-(trideuteriomethyl)oxime are shifted three mass-units higher than for **1**; the peaks shifted are listed in the legend of Fig. 2. The data indicate that the peak at *m/e* 129 in the undeuterated sample is composite.

The fragmentation pattern supports the structure assignment postulated for the trimethylsilyl derivative of the *O*-methyloxime. The details of this pattern gain significance when materials from natural sources are analyzed. The gas chromatograms of sugars from membrane hydrolyzates usually contain some noncarbohydrate peaks, even when preliminary carbohydrate purification is extensive. Analysis by g.l.c.–m.s. minimizes the possibility of misinterpreting peaks due to artifacts having retention times close to those of carbohydrate derivatives of interest.

Although the mass spectra of the three 2-acetamido-2-deoxyaldose derivatives are qualitatively the same, a significant and potentially useful variation in the intensity ratios of *m/e* 319 to *m/e* 333 was observed. This ratio was 2.9, 22.0, and 6.7, respectively, for the derivatives having the *gluco*, *manno*, and *galacto* configurations. This observation is valid for spectra collected in the g.l.c.–m.s. mode, as well as for solid-probe samples. The fact that solid-probe mass-spectra were, in all cases, similar to spectra taken in the g.l.c.–m.s. mode proved that on-column degradation did not occur.

EXPERIMENTAL

Preparation of trimethylsilyl derivatives of O*-methyloximes.* — The 2-acetamido-2-deoxyhexoses (Grade A) were obtained from Calbiochem (San Diego, California) and were used without further purification. A solution of the 2-acetamido-2-deoxyhexose (1 mg) in pyridine (0.5 ml) was added to a Teflon-capped, conical vial containing a solution of methoxyamine hydrochloride (10 mg; Eastman, Rochester, N.Y.) in pyridine (0.5 ml). The vial was sealed, heated for 2 h at 80°, and cooled. A 9:1 (v/v) mixture of hexamethyldisilazane–chlorotrimethylsilane (0.25 ml; Supelco, Bellefonte,

Pa.) was then added, the vial and contents were heated for 30 min at 80°, and cooled, and the vial was centrifuged to sediment the salts as a pellet. The supernatant liquor was removed with a Pasteur pipet, the salt pellet was washed with chloroform (5 ml), and the supernatant liquors were combined and evaporated *in vacuo*. The residue was applied to a column of Unisil (1 g; Clarkson Chem. Co., Williamsport, Pa.) in chloroform, and eluted with chloroform (15 ml). The derivatives recovered were used for solid-probe mass-spectral analysis, g.l.c.–m.s. analysis, and t.l.c.

Preparative thin-layer chromatography. — The trimethylsilyl derivative **1** was resolved on plates of Silica Gel HF-254 (Merck, Darmstadt, Germany) into two zones having R_F 0.44 and 0.22 by developing with 1:1 (v/v) diethyl ether–petroleum ether (b.p. 30–60°); the zones were made visible with a permanganate–sulfuric acid spray[15]. The zone having R_F 0.44 corresponded to the major peak in the gas chromatogram.

Instrumentation. — The specifications for the g.l.c. analyses are given in the legend to Fig. 1. For the mass-spectrometric analyses, column B of the same gas chromatograph was directly connected to the ion source of the mass spectrometer through a double-stage slit (jet) type of interface (made by JEOL). All mass spectra were recorded at an ionizing voltage of 70 eV on the double-focusing, Mattauch–Herzog geometry instrument specified in the legend to Fig. 2.

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