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Note

Syn and *anti* forms of some monosaccharide *O*-methyl oximes: a ^{13}C-n.m.r. and g.l.c. study

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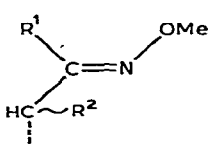
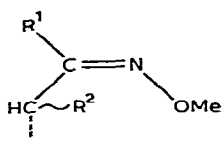
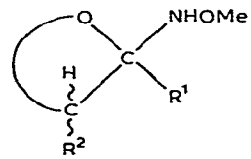
For the quantification of carbohydrates, g.l.c. of trimethylsilyl derivatives has been frequently used[1–3]. When a second carbonyl function is present, *e.g.*, in diuloses, it appears that the trimethylsilyl derivatives are not always volatile enough, and g.l.c. analysis may fail[4]. Borohydride reduction followed by trimethylsilylation can overcome this problem[4–6]. However, this convenient method cannot be applied if, for example, D-*gluco*-hexodialdose has to be determined in the presence of D-glucose[7] (both are reduced to D-glucitol). Methoximation[8,9] is then the method of choice. The number of methoximated, trimethylsilylated isomers observed by g.l.c. does not exceed two for aldoses and ketoses, and four for diuloses[7,8,10,11]. However, for quantification, one would like to ensure that only *syn*-(*E*) (**1**) and *anti*-(*Z*) (**2**) forms of the open-chain compounds are formed and that cyclic compounds (**3**) do not play a role. Because **3** contains an NH group, it might not be eluted from glass-capillary



1 *syn (E)*



2 *anti (Z)*



3 cyclic form

R^1 = H, CH_2OH, CH_2OSiMe_3

R^2 = H, OH, $OSiMe_3$

columns[12]. Compounds for which pure reference material is not available usually can be quantified with the help of an increment method[13] to calculate the g.l.c. response factors. However, this method yields low values if some of the derivative is not eluted. Therefore, we investigated the ^{13}C-chemical shifts of the *O*-methyl oximes of various aldoses, deoxyaldoses, and ketoses in order to find out whether or not the open-chain compounds are formed exclusively under silylating conditions; a cyclic form of D-glucose oxime is obtained on crystallisation[14].

TABLE I

^{13}C-CHEMICAL SHIFTS[a] FOR *O*-METHYL OXIMES AND UNREACTED METHOXYLAMINE IN PYRIDINE-d_5 AT 35°, AND THE *syn/anti* RATIOS

Parent sugar	syn-*Oxime*							anti-*Oxime*								syn/anti *Ratio*	
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*NOMe*	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*NOMe*	*NH_2OMe*	*N.m.r.*	*G.l.c.*
DL-Glyceraldehyde	152.3	70.9	65.6				61.8	154.1	67.7	64.6				62.0	63.6	3.5	3.6
D-Erythrose	152.2	70.8	75.0	63.8			61.5	153.3	66.8	74.8[b]	[c]			61.9	63.7	7.2	[d]
D-Arabinose	152.8	69.9	74.7[b]	72.7[b]	64.6		61.4	154.9	66.0	73.9[b]	[c]	64.7		61.9	63.8	4.0	3.5
D-Ribose	152.2	71.4	75.4[b]	73.6[b]	64.5		61.6	153.4	67.7	74.8[b]	73.0[b]	64.6		61.9	63.7	5.5	5.7
D-Lyxose	152.6	70.3	73.6[b]	71.8[b]	64.3		61.5	153.2	66.1	73.0[b]	72.1[b]	64.3		61.9	63.7	5.9	7.5
D-Glucose	151.5	71.4	72.6[b]	72.3[b]	71.8[b]	64.2	61.3	153.5	67.5	73.1[b]	[c]	[c]	64.2	61.8	63.3	7.6	7.2
D-Mannose	153.0	69.9	72.6[b]	72.4[b]	70.9[b]	64.7	61.4	153.7	65.5	[c]	71.7[b]	71.7[b]	64.7	61.7	63.6	8.2	7.9
D-Galactose	153.0	69.8	73.7[b]	71.7[b]	71.2[b]	64.8	61.4	155.2	66.0	72.9[b]	[c]	[c]	64.8	61.7	63.7	4.3	3.6
2-Deoxy-D-*erythro*-pentose	150.5	34.2	75.9[b]	71.1[b]	64.3		61.3	150.8	30.9	75.7[b]	70.5[b]	64.3		61.6	63.5	1.2	1.2[e]
2-Deoxy-D-*arabino*-hexose	150.4	35.0	74.5[b]	73.0[b]	69.4[b]	64.8	61.3	150.8	31.5	74.3[b]	73.0[b]	68.8[b]	64.8	61.7	63.7	1.2	1.2[e]
2-Deoxy-D-*lyxo*-hexose	150.7	34.9	74.7[b]	71.7[b]	69.6[b]	64.6	61.3	151.1	31.5	74.9[b]	71.7[b]	70.3[b]	64.6	61.7	63.6	1.2	1.1
D-Fructose	56.1	161.9	70.7	73.4[b]	72.6[b]	64.5	61.9	61.1	162.6	67.6	73.2[b]	72.3[b]	64.5	61.9	63.4	0.8	0.8
L-Sorbose	56.3	161.3	72.4	73.5[b]	73.1[b]	64.4	62.1	61.1	162.0	69.2	74.2[b]	72.7[b]	64.1	62.1	63.6	1.3	1.1

[a]P.p.m. downfield from that of Me_4Si. [b]Uncertain assignments. [c]Not separated from major signals. [d]Ratio of ~6 uncertain because of the presence of D-*glycero*-tetrulose derivatives. [e]No separation on OV-101, but separation on Dexsil.

Methoximation of solutions of 1 mol. of DL-glyceraldehyde, D-erythrose, D-arabinose, D-ribose, D-lyxose, D-glucose, D-mannose, D-galactose, 2-deoxy-D-*erythro*-pentose, 2-deoxy-D-*arabino*-hexose, 2-deoxy-D-*lyxo*-hexose, D-fructose, or L-sorbose severally in pyridine-d_5 containing 2 mol. of methoxylamine hydrochloride at room temperature was complete after a few minutes[15]. The ^{13}C-resonances of the *O*-methyl oximes, measured at 35°, were assigned as shown in Table I. For each sugar, the signals of two products were detected in addition to that of the OMe group of the excess of methoxylamine. Assignments to the *syn* and *anti* forms were made by comparison with simple aldoximes and ketoximes investigated previously by ^{13}C-n.m.r. spectroscopy[16].

As expected, the resonance of the sp^2-hybridized C-1 of aldose *O*-methyl oximes occurred in the range δ 150.4–155.2, whereas that of the sp^2-hybridized C-2 of ketose *O*-methyl oximes was in the range δ 161.3–162.6. The signals for C-1 or C-2 in the *syn* compounds were shifted upfield by up to 2 p.p.m. compared with those of the *anti* isomers.

The resonances of C-2 of the aldoses and of C-1 and C-3 of the ketoses shift upfield on formation of the *O*-methyl oxime. The effect of the carbon *cis* to the OMe group is greater than that for the *trans* carbon, an effect noted earlier with *cis* and *trans* alkenes[17]. The ^{13}C-chemical-shift difference for these two carbons is 3–4 p.p.m. This effect is the strongest argument for the assignments to the *syn* and *anti* forms.

The ^{13}C-signal for the =N-OMe group in each of the aldose *O*-methyl *syn*-oximes is at higher field than the corresponding signal of the *anti* derivatives.

The remaining signals at $\delta \sim 64$ and 70.3–75.9 have the shifts typical of primary and secondary alcohols, respectively. The signals, which were not unambiguously assignable, are indicated in Table I.

In the ^{13}C-spectra, there were no signals assignable to cyclic methoxime derivatives, indicating that, if present, their concentrations must be $<3\%$, as the noise level is very low.

That the ^{13}C-signals of the cyclic forms would be easily detected was checked by using D-glucose oxime, which crystallises in the β-pyranoid form[14]. A fresh solution of this material in methyl sulphoxide-d_6 showed signals at δ 93.0 (C-1), 77.9–77.5 (C-3,5), 70.9–70.2 (C-2,4), and 61.8 (C-6) (*cf.* the ^{13}C-n.m.r. spectra of other glycosylamines[18]). In addition, major signals due to the two open-chain forms (arising by mutarotation) were observed. In pyridine-d_5, the mutarotation equilibrium is completely shifted in the acyclic forms.

The D-erythrose used was apparently contaminated with 6–10% of D-*glycero*-tetrulose, since the *O*-methyl oxime gave ^{13}C-signals typical of ketose *O*-methyl oximes at δ 161.3 (C-2 *syn*) and 161.5 (C-2 *anti*).

On raising the temperature from 35 to 60°, the signals for the OMe groups of methoxylamine and for the *syn* and *anti* forms of the *O*-methyl oximes broaden substantially. Surprisingly, no other ^{13}C-signals broadened. Therefore, rapid exchange must occur at the OMe group, whereas the C=N group is not affected.

Indeed, addition of ethanol (equimolar to methoxylamine) after the formation of the D-glucose *O*-methyl oximes immediately resulted in the formation of equal amounts of the respective methyl and ethyl derivatives. The ratio of the *syn* and *anti* forms of the D-glucose *O*-ethyl oximes [δ(C-1) 154.3 and 152.4, respectively] is nearly the same as that of the *O*-methyl oximes. On warming-up, no broadening of the OMe or OEt signals was observed.

The major *O*-methyl oximes formed from the aldoses are the *syn* forms (Table I), probably because of the steric hindrance exerted by HO-2; for the 2-deoxy sugars, almost equal proportions of *syn* and *anti* forms are present. Likewise, for the ketoses, the proportions of *syn* and *anti* forms are similar.

The ratios of *syn* and *anti* forms determined by ^{13}C-n.m.r. spectroscopy are similar to those obtained by g.l.c. after trimethylsilylation. The mass spectra of the *syn* and *anti* forms are generally quite similar, and reliable structural information cannot be drawn therefrom[8,11]. Trimethylsilylation is fast[1,19], and hence the equilibrium of *syn* and *anti* forms should be frozen. The ^{13}C-n.m.r. data allow assignment of the various g.l.c. peaks. This has been done in Table I.

EXPERIMENTAL

General. — The sugars studied were commercial materials.

G.l.c. of trimethylsilylated *O*-methyl oxime derivatives was performed on a Varian 1400 instrument equipped with a flame-ionization detector and a wall-coated[20] OV-101 glass-capillary column (80 m, 140→260°, 2°/min) or a Dexsil glass-capillary column (123 m, 170°) with hydrogen as the carrier gas.

Proton-decoupled ^{13}C-n.m.r. spectra were recorded on a Bruker WH 270 instrument (at 67.8 MHz with an internal deuterium lock) operating in the Fourier-transform mode. The spectrometer was equipped with a Nicolet BNC 12 computer with a 32K data memory and a Diablo Disk-System. All measurements given in Table I were carried out at 35° on solutions in pyridine-d_5 (10-mm sample tubes) spinning at ~30/sec. An average of 2000 scans with a pulse sequence of 10 sec and a pulse angle of 30° were used to record a spectrum. Internal standards were Me_4Si or the highest pyridine-d_5 signal (δ 148.4 relative to that of Me_4Si).

Sugar O-methyl oximes. — The sugar (2.25 mmol) was dissolved in 1.5 ml of pyridine-d_5 together with methoxylamine hydrochloride (4.5 mmol). All of this solution was used for the ^{13}C-n.m.r. measurements. The reaction was complete within a few minutes, and the mixture was stable for several days at room temperature.

Trimethylsilylation of sugar O-methyl oximes. — A portion (5 μl) of the mixture described above (0.9–1.6 mg of carbohydrate *O*-methyl oximes) was added to 0.1 ml of bis(trimethylsilyl)trifluoroacetamide together with 3 μl of chlorotrimethylsilane at room temperature.

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