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Spectral Analysis of Diisopropylidenated Monosaccharides. Low Energy EI-MS Fragmentation Study

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Abstract: Five diisopropylidenated monosaccharides, namely 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose, 2,3:5,6-di-*O*-isopropylidene-α-D-mannofuranose, 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose, 2,3:4,5-di-*O*-isopropylidene-β-D-fruc-topyranose and 2,3:4,6-di-*O*-isopropylidene-α-L-sorbofuranose, have been investigated using spectroscopic techniques. These include GC-EI-MS at multiple ionization energies, FTIR, DSC, TG, ^{1}H and ^{13}C NMR, and also DEPT135, COSY, HMQC and HMBC measurements. A high instability towards electronic ionization has been found for this type of compounds.

Keywords: monosaccharide; isopropylidene; acetonide; gas chromatography - mass spectrometry; nuclear magnetic resonance

1. Introduction

Sugar based acetals are common substrates in carbohydrate chemistry. They are easily obtained and very useful for the generation of complex chirons. Among them, isopropylidenated monosaccharides are highly used particularly when simple one-step protection of all but one hydroxyl group is necessary [1-3].

These compounds were first systematically analyzed from mass spectrometry viewpoint by De Jongh and Biemann [4]. They presented the relationship between EI mass spectra of O-isopropylidene derivatives and the molecular weight, ring size and location, and stereochemistry of the parent monosaccharides, and hence the importance of information obtained by such analyses in structure elucidation and identification of monosaccharides. Although they made extensive research on the fragmentation pathways, they only explored the higher range of ionization energy (70 eV for standard MS, and 150 eV for HRMS). To fully understand the electron ionization behavior of such compounds we felt it necessary to shed some light on their low energy electron-impact-induced fragmentation.

As shown, by forming bi- or tricyclic ring systems depending on the availability of cis-1,2- or cis-1,3-diol systems, epimers may give isopropylidene derivatives which differ in actual bonding and are no longer merely configurational but structural isomers, to which mass spectrometry is very sensitive. These compounds are also particularly well suited to GC isolation [4-7].

GC-MS is a rapid and precise method for qualitative and quantitative analysis of complex mixtures, and has proven a useful complement to other methods for elucidating the structure of cyclic acetals [5-7]. It offers valuable information that sometimes cannot be easily uncovered using alternative methods. Using this tool along with other spectroscopic techniques, we investigated the following diisopropylidene derivatives (Fig. 1): 1,2:5,6-di-O-isopropyl-idene-α-d-glucofuranose (1), 2,3:5,6-di-O-isopropylidene-α-d-mannofuranose (2), 1,2:3,4-di-O-isopropylidene-α-d-ga-lactopyranose (3), 2,3:4,5-di-O-isopropylidene-β-d-fructo-pyranose (4) and 2,3:4,6-di-O-isopropylidene-α-l-sorbo-furanose (5).

2. Experimental

For GC-MS analysis a Hewlett Packard HP 6890 Series gas chromatograph coupled with a Hewlett Packard 5973 mass spectrometry detector system was used (calibration factor 1.0). A HP-5 MS capillary column (30 m length, 0.25 mm i.d., 0.25 µm film thickness) was used for the GC system. The temperature program was set up from 100°C to 250°C with 5 or 15°C/min; both the injector and detector temperatures were at 280°C and He was used as carrier gas. The injection volume was 1 µL. Ionization energies of 70, 20, 15, 10 and 5 eV were used for the mass spectrometry detector, with a source temperature of 150°C, a scan range of 34-400 amu, and a scan rate of 1 s^{-1}.

Unless specified otherwise, mass spectral data were collected from the highest point of the compound's signal. Relative intensities are referred to the base peak. Mass spectra taken at 70 eV using two different heating speeds (as stated above) and two different concentrations were practically identical (exception, molecular and [M+1]+ peaks, see below). Compound spectra were compared with Nist 0.2 (2002) database for identification purposes.

IR spectra were recorded in KBr pellets for crystals (compounds 1, 2, 4, 5), spectral range 4000-400 cm^{-1}, or neat between KBr plates for liquid samples (compound 3), spectral range 4000-530 cm^{-1}, on a Jasco FT/IR-430 spectrometer. Each spectrum was an average of 16 spectra measured at a resolution of 4 cm^{-1}. Wave numbers are given in cm^{-1}.

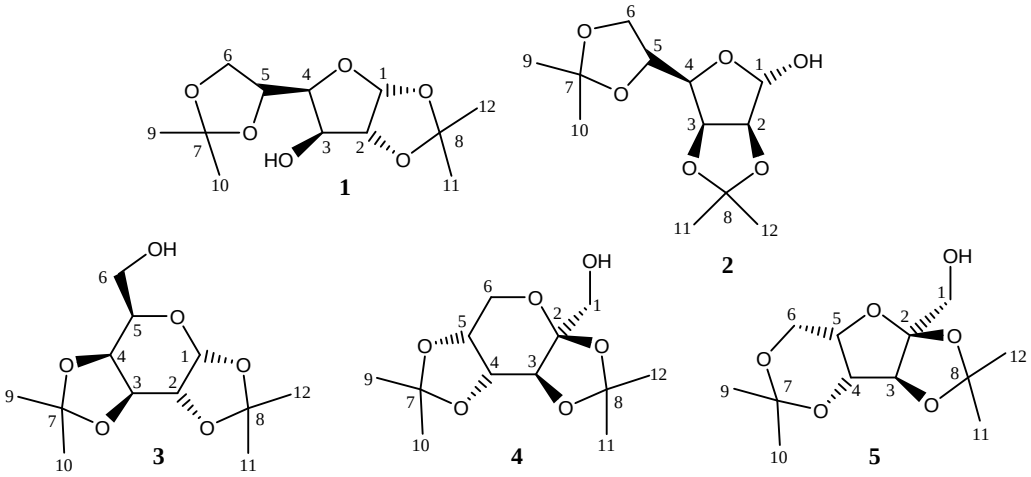

Figure 1.Structure of compounds 1-5

If not otherwise stated, 1H and 13C NMR data were recorded on a Bruker Avance DRX-400 spectrometer (1H 400 MHz, 100 MHz 13C) in deuterochloroform, referenced to TMS as an internal standard. Chemical shifts are given in ppm while coupling constants are in Hz.

Melting points were determined on a Böetius HMK apparatus (Veb Analytik Dresden) and also using differential scanning calorimetry carried out on a DSC 204 Netzsch apparatus under N_2 atmosphere, with a temperature program of 25÷300°C, the heating rate being 1 K/min. TG analyses were performed on a TG 209 Netzsch apparatus under N2 atmosphere, with a temperature program of 20÷500°C, the heating rate being 10 K/min. The acquisition and the processing of the data were performed with the DSC 204 / TG 209 Netzsch - Measurement and Netzsch Proteus - Thermal Analysis, ver. 4.0, respectively.

3. Results and Discussion

Compounds 1 (mp 109-110ºC, 109.1ºC DSC, lit. 110-111ºC), 2 (mp 121-123ºC, 119.8ºC DSC, lit. 122-123ºC) and 3 (syrup) were purchased and used as received, while 4 (mp 91-95ºC, lit. 97ºC) and 5 (mp 67-75ºC, 75.3ºC DSC, lit. 77-78ºC) were prepared according to classic protocols [3]. Besides the melting peak, the DSC curve also showed in each case a second endothermic peak above 160ºC, assigned to sample vaporization - as confirmed by TG analysis (Fig. 2).

Main FTIR absorption bands are given in Table 1. As can be seen from inspecting this data, most of the values are common to all five compounds. The ν C-H and the fingerprint regions are made from overlapped absorption bands that cannot be correlated with certainty to particular vibrations. Thus, infrared spectroscopy alone cannot offer reliable assignation criteria for acetals paternity, and more elaborated analytical methods are required.

TABLE 1. FTIR absorption bands (in cm^{-1}) for compounds 1-5

	ν O-H	ν C-H	Fingerprint (ν C-C, ν C-O, δ C-H, γ C-H, δ O-H, γ O-H, sk)
1	3429	2985, 2952, 2935, 2903, 2872	1457, 1376, 1318, 1288, 1249, 1222, 1162, 1120, 1092, 1069, 1031, 1007, 942, 885, 858, 847, 784, 639, 527, 508
2	3489, 3437	2988, 2979, 2949, 2900	1459, 1374, 1351, 1319, 1280, 1266, 1254, 1227, 1204, 1167, 1147, 1116, 1088, 1069, 1035, 1015, 992, 976, 955, 909, 891, 856, 839, 822, 775, 744, 684, 652, 531, 516, 419
3	3493	2987, 2937, 2905	1457, 1382, 1308, 1255, 1212, 1168, 1143, 1108, 1069, 1039, 1001, 962, 918, 899, 858, 803, 771, 648, 563, 552
4	3302	3006, 2985, 2938, 2898, 2884	1463, 1456, 1430, 1377, 1312, 1291, 1271, 1245, 1216, 1196, 1184, 1160, 1107, 1067, 1041, 1011, 980, 928, 899, 880, 865, 837, 758, 709, 664, 628, 574, 543, 523, 507, 451
5	3512	2988, 2941, 2893, 2877	1458, 1389, 1378, 1338, 1288, 1249, 1224, 1201, 1173, 1166, 1118, 1092, 1076, 1039, 977, 941, 898, 885, 874, 855, 845, 833, 809, 766, 687, 540, 526

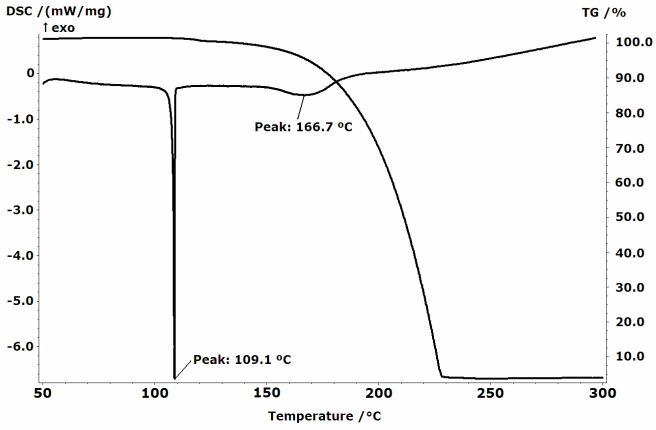

Figure 2. DSC / TG analysis of compound 1

On the other hand, NMR has proven a very useful tool in this respect. 400 MHz 1H and 100 MHz 13C NMR signals (except for compound 1 and 2, which are given at 200/50 MHz) are listed in Tables 2 and 3. Proton assignment for compounds 1 and 2 was made through decoupling experiments, while the rest of the assignments were made with DEPT135, COSY, HMQC and HMBC techniques. Shift values and coupling constants were in good agreement to those of acetylated or alkylated derivatives (400 MHz 1H, 100 MHz 13C NMR) [8,9]. NOESY experiments were not performed, so the spatial orientation of methyl groups 9-12 could not be determined.

While a very useful method for structure determination, NMR is often costly and time consuming. And probably even more important, the analysis of mixtures of similar compounds is difficult and ambiguous at best. In this respect, an alternative presents itself.

We subjected compounds 1-5 to GC-EI-MS at different ionization energies - 70, 20, 15, 10 and 5 eV - to investigate the effects of lowering the ionization energy on the mass spectra appearance. Peak retention times are given in Table 4, while EI-MS data at 70 eV is given in Table 5. These values are in general agreement with earlier findings [4]. Some differences were found for peaks at m/z 117 and 72, which, in contrast to what was stated previously, also appear in 1 - 5 (although usually faint) and thus are not typical for 1,2:4,5-di-O-isopropylidene-β-D-fructopyranose. Also, the peak at m/z 85 has almost the same intensity in 1 and 3, and is also well visible in 2, 4 and 5 so it cannot be used to distinguish between furanose and pyranose rings.

Besides a gradually decrease in all peak absolute intensities (Fig. 3) and the disappearance of some low-intensity peaks with the decrease of ionization energy, no major changes were noticed when moving between ionization steps for the same compound. This was somewhat surprising, and denoted a high instability for this family of compounds toward electronic ionization. Still, the spectra at 5 eV were distinct through the fact that they only contain a small number of peaks, between 11 and 20 at signal's highest point (Table 6), which was a promise for a much simpler interpretation. Also the disappearance of peaks is more abrupt from 10 to 5 eV when compared with the other steps.

TABLE 2. Assignment of ^{1}H chemical shifts (in ppm) for compounds 1-5; coupling constants (in Hz) are given in parentheses

	*1**		*2*	*3*	*4*	*5*	*6**		*9*	*10*	*11*	*12*	*OH*
	a	*b*					*a*	*b*					
1	5.94 d (3.5)		4.53 d (3.5)	4.34 m	4.05 dd (6.1, 0.9)	4.34 m	4.17 dd (8.4, 6.3)	4.01 dd (8.4, 6.1)	1.45 s	1.37 s	1.50 s	1.32 s	3.01 d (3.9)
2	5.37 d (2.4)		4.61 d (5.9)	4.81 dd (5.6, 3.7)	4.17 dd (7.1, 3.6)	4.41 ddd (6.2, 6.2, 6.0)	4.07 d (5.4)		1.46 s	1.38 s	1.46 s	1.33 s	3.83 d (2.5)
3	5.57 d (5.2)		4.34 dd (5.2, 2.4)	4.62 dd (7.8, 2.2)	4.28 dd (8.0, 1.6)	3.87 m	3.87 m	3.75 dd (9,0, 1,4)	1.46 s	1.34 s	1.54 s	1.34 s	2.45 bs
4	3.70 d (12,0)	3.66 d (11.6)	-	4.35 d (2.4)	4.62 dd (7.8, 2.6)	4.25 dd (8.0, 0.8)	3.92 dd (13.0, 1.8)	3.78 d (12.8)	1.48 s	1.35 s	1.55 s	1.41 s	2.29 bs
5	3.87 dd (11.8, 6.2)	3.80 dd (11.8, 4.6)	-	4.49 s	4.11 s	4.33 d (1.2)	4.09 dd (13.4, 1.0)	4.06 dd (13.4, 1.0)	1.45 s	1.38 s	1.52 s	1.38 s	2.36 dd (5.8, 5.8)

* *a* refers to the geminal proton with the highest magnetic shift

TABLE 3. Assignment of ^{13}C chemical shifts (in ppm) for compounds 1-5

	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
1	105.3	85.1	74.9	81.2	73.2	67.6	109.6	111.8	26.8	25.2	26.8	26.2
2	101.2	85.6	79.7	80.1	73.4	66.5	109.2	112.7	26.8	25.2	25.9	24.5
3	96.3	70.6	70.8	71.6	68.2	62.2	109.5	108.7	25.9	24.3	26.0	25.0
4	65.4	103.1	70.8	70.1	70.9	61.3	109.1	108.5	25.8	24.0	26.5	25.4
5	63.6	114.3	84.9	72.3	73.3	60.3	97.6	112.0	18.6	28.9	27.4	26.5

TABLE 4. Peak retention times for compounds 1-5

Heating rate (*°C/min*)	Peak retention time (*min*)				
	1	**2**	**3**	**4**	**5**
5	22.5	23.5	23.0	22.5	23.1
15	5.1	5.3	5.1	5.1	5.2

A quick look at Table 6 reveals the base peak at 245 (M-15) in all five cases, along with its two isotopic peaks in the predicted ratios (100:13:2, ChemBioDraw Ultra v. 12.0). This abundant fragment (Fig. 4) results from the molecular ion through the loss of a methyl radical from the 2,2-dimethyl-1,3-dioxolane or 2,2-dimethyl-1,3-dioxane rings. This peak is useful for the determination of molecular weight of a carbohydrate through its O-isopropylidene derivative [4].

The molecular peak at 260 and its companion [M+1]+ are not observed at these low energies. They only appear above 15 eV and only if the sample concentration in the mass analyzer is sufficiently high. Their intensities are very low with respect to normalization to the base peak (below 0.1%). For 1, 4 and 5, [M+1]+ is slightly more intense, being visible even when M+ is missing.

Other intense peaks are: 229 (M-31) from the loss of CH2OH group situated at a ketal-carbon atom in 4 and 5; as expected, this fragment completely lacks in the other three cases; 187 (M-15-58) from the loss of one methyl and one acetone from the opposite acetal group (except for 4, for which it doesn't appear); 159 (M-101) which is well visible for 5 where it corresponds to a triple breaking (at C-2, O-5, at C-4, C-5 and at C-7, O-4); 101 (M-159) for 1 and 2 (cleavage of the C-4, C-5 bond); 100 (M-160) for 3 (breaking at C-1, O-5 and C-2, C-3, and/or at C-2, C-3 and C-4, C-5)[4].

TABLE 5. Overview of key peaks at 70 eV for compounds 1-5

m/z	Relative intensity (%)				
	1	2	3	4	5
245	85.7	100.0	100.0	100.0	100.0
243	~0	1.6	0.2	0.2	0.3
229	0.2	0.5	0.8	24.9	12.7
187	39.0	25.5	19.0	5.4	19.6
185	1.1	2.6	4.7	1.1	0.6
171	0.1	0.2	4.9	37.9	32.8
169	1.0	0.5	1.3	12.9	1.6
159	6.5	1.7	0.2	0.1	22.6
144	1.3	1.5	1.0	2.4	0.6
139	0.5	0.4	2.4	0.5	11.5
131	10.4	1.9	0.5	1.7	2.4
130	0.8	1.5	0.3	9.1	17.1
129	9.2	16.4	1.5	1.2	1.5
127	36.6	17.8	23.2	40.7	15.4
117	0.4	0.6	0.5	0.9	0.5
115	7.8	7.9	2.5	14.0	17.5
113	9.2	2.4	26.6	12.8	31.2
101	100.0	80.5	7.2	6.4	18.5
100	8.5	3.4	38.8	1.2	3.6
99	8.4	13.6	16.0	8.0	7.1
85	28.9	22.5	33.6	21.4	14.0
81	5.9	12.9	27.3	4.3	5.5
72	12.1	14.1	1.0	0.9	2.4
71	10.9	10.0	18.8	5.1	6.0
69	15.9	8.7	26.3	36.6	27.7
59	60.4	38.3	29.5	28.6	39.0
43	70.0	64.7	47.9	45.7	59.1

Fingerprint ions are also present, although at reduced intensity. These ions could be used in the rapid automatic qualitative analysis of such compounds. Because the spectra at 70 eV are almost continuous if we were to magnify each domain, their interpretation is somewhat subjective. This seems to be no problem at 5 eV. Peaks at 72, 99, 141, 174, 202 and 243 are unique to 2. Compound 1 also has a series of unique peaks at 129, 131, 142 and 143. These two compounds are together characterized by the detachment of the dioxolane ring (C-5, C-6, C-7, O-5 and O-6) which generates the ion peer with m/z 101 and 159 [4]. The 101 peak has high intensity only in these two cases; although it is found also in 3 and 5, it is only 1% from the base peak. On the other hand, the 159 peak appears in low intensity for 1 and 2, but it is pronounced for 5 (13%), where it appears due to other mechanism, as stated above.

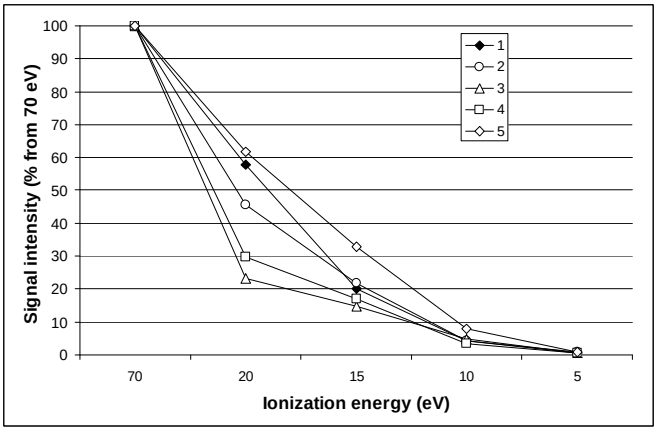

Figure 3. Dropping in signal intensity for *m/z* = 245

3, 4 and 5 share a unique structural feature between these five diisopropylidenated sugars. They are all condensed tricyclic systems with cis/cis configuration. We have established the peak at m/z 171 as fingerprint ion for this kind of systems. With its intensity increasing, this ion remains a good fingerprint even at higher ionization energies. Its intensity is below 1% at 70 eV for 1 and 2. This ion results from its parent ion at m/z 229 that easily loses 58 mass units in the form of acetone [4]. Unique signals for the previous three compounds are: 81 and 185 for 3, 68 and 169 for 4, and 97, 130, 139 and 160 for 5.

If we were to make cross-sections trough the GS-MS signal areas for each of these compounds at 5 eV, this is what we would find. For 1 the first ion that appears has m/z 245, followed shortly by m/z 101; then 187, 113, 131 and 159 appear in this particular order. For 2, the priority is: 245, 101, 187, 126, 141 and 98, and then 202. For 3: 245, 187, 100, 98, 171, 113 and 81. For compound 4: 245, 229, 169, 171, 127, 69, and 144. For 5: 245, 159, then 113, 130, 171 and 229, 172, 187 and 101. As can be seen, the peaks appear roughly following their intensities (the highest appear first), along with the increase in the effective concentration corresponding to the position in the chromatographic area.

TABLE 6. List of all peaks at 5 eV for compounds 1-5

m/z	Relative intensity (%)					m/z	Relative intensity (%)				
	1	2	3	4	5		1	2	3	4	5
260						*141*		3.2			
247	1.3	1.9	2.0	1.8	1.8	*139*					1.6
246	11.5	13.0	12.8	12.5	12.4	*131*	4.3				
245	100.0	100.0	100.0	100.0	100.0	*130*					3.8
243		0.1				*129*	1.9				
230				2.4	1.4	*127*	1.3	0.5	0.4	2.0	
229				20.7	10.1	*126*		3.6			0.4
202		1.4				*114*	2.7		0.3		1.4
188	0.7	0.3				*113*	5.2		1.7		5.8
187	14.6	10.0	7.5		1.7	*102*	2.8	1.2			
185			0.7			*101*	46.6	26.9	0.6		0.6
174		0.5				*100*	2.8	1.1	9.6		
172					4.2	*99*		0.4			
171			1.9	3.9	4.2	*98*		4.2	4.4		
169				4.8		*97*					0.1
160					1.1	*81*			0.5		
159	2.8	1.3			13.1	*72*		0.3			
144		0.7		1.5		*69*				1.3	0.3
143	1.0					*68*				0.4	
142	0.9					*59*	0.6	0.5			

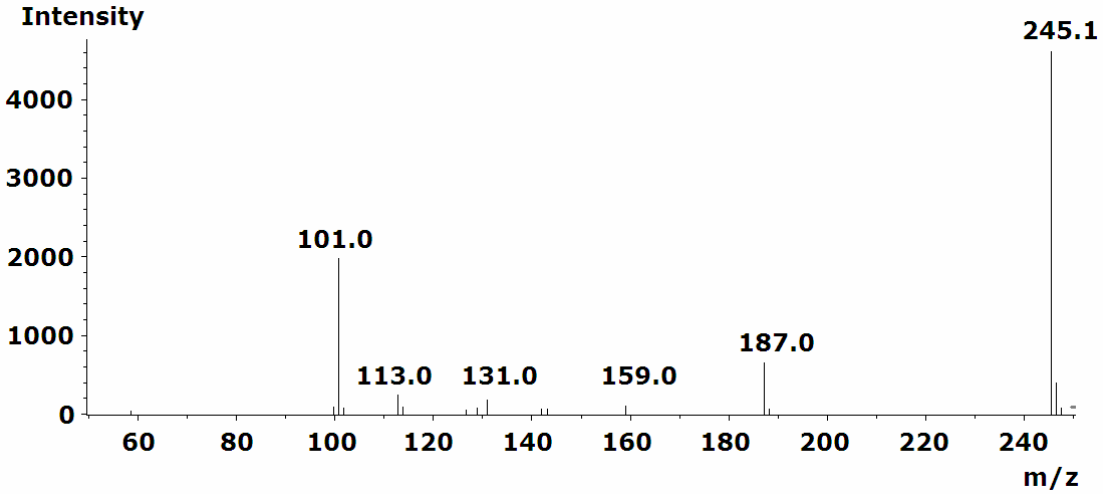

Figure 4. EI-MS spectrum of compound 1 taken at 5 eV

It is worth mentioning that poor results were obtained when interpreting our spectra with the help of Nist 02 database. The only compound identified with certainty was compound 1 (90 % match at 70 eV and 91 % match at 20 eV, lower accuracy at other ionization energies). On the other hand, MS spectra for compounds 1 and 3 were identical with those found in the Spectral Database for Organic Compounds (SDBS) of National Institute of Advanced Industrial Science and Technology (AIST), Japan [10].

4. Conclusions

As discussed above and shown earlier [4-7], isopropylidenation and subsequent GC-MS analysis may be a useful technique for the characterization or structure determination of carbohydrates and their derivatives, either from natural products or of synthetic origins. Working at low energy levels for molecule ionization may offer the advantage of simplifying the mass spectra while at the same time providing for a quick examination and

interpretation. This could be an advantage where the analysis of more complex structures with multiple fragmentation pathways is involved. We plan to analyze in the future some derivatives of 1-5, to further test the capacity of this method.

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