

GC-MS ANALYSIS OF LONG CHAIN MANNOFURANOSE DERIVATIVES AS BIOCOMPATIBLE SURFACTANT PRECURSORS. CORRELATION BETWEEN PEAK INTENSITIES AND STABILITY OF CORRESPONDING FRAGMENTS

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Abstract

A new method of screening between two mannose isomeric derivatives, based on mass spectra (EI-GC-MS) analysis and their correlation with heats of formation estimated from semi-empirical calculations, is presented. The heats of formation for some main fragments, selected from the analyzed compounds mass spectra, were calculated with HyperChem and MOPAC software using semi-empirical methods (AM1, MINDO3, MNDO, RM1, PM3 and PM7) in trying to explain the difference between peak intensities obtained experimentally. These glycoderivatives can find multiple applications as biocompatible and biodegradable surfactant precursors in fields like pharmacy, medicine and biotechnologies.

Rezumat

În această lucrare este prezentată o metodă de discriminare între doi derivați izomeri ai manozei, pe baza analizei spectrelor de masă (EI-GC-MS) și a corelării acestora cu călduri de formare estimate din calcule semiempirice. Căldurile de formare pentru câțiva ioni de fragmentare semnificativi, selectați din spectrele de masă ale compușilor analizați, au fost calculate cu programele HyperChem și MOPAC utilizând metode semi-

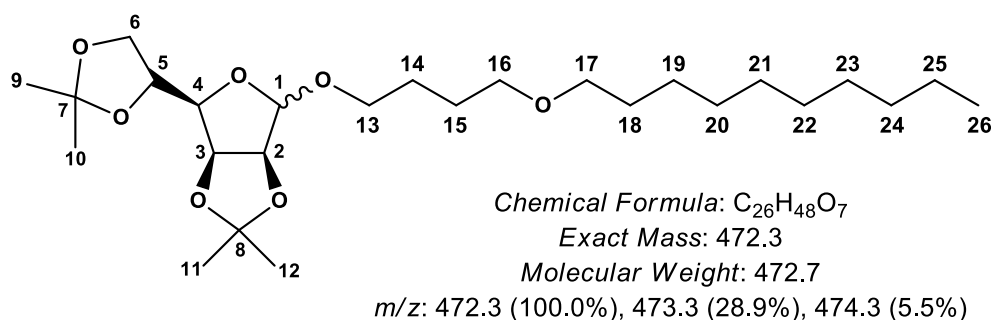
empirice (AM1, MINDO3, MNDO, RM1, PM3 și PM7) în încercarea de a explica diferența între intensitățile picurilor obținute experimental. Acești glicoderivați pot avea multiple aplicații ca precursori de surfactanți biocompatibili și biodegradabili în domenii ca farmacie, medicină și biotehnologii.

Keywords: glycoderivatives; mass spectrometry; computational chemistry; semi-empirical method.

Introduction

The distinction between diastereomers mass spectra is an old desiderate in analytical chemistry. Most of the time, researchers have to use spectral databases for comparison, which very often lead to poor results. In the last decade, Dincă et al. [1-11] elaborated a very attractive and styled alternative method: CSI-Diff-MS (Chemical structure identification - Differential mass spectrometry). The main idea is to use quantum chemical calculation (QCC) of thermodynamic values (heats of formation for compounds and fragmentation reactions) and expert software for the analysis of MS spectra in trying to explain and predict the observed differences in intensities for the same peak of structural isomers and diastereomers. This modern procedure has produced some very encouraging results in the past few years. As for evolving MS technology, the extraordinary development of soft ionization techniques (ESI and MALDI) has bestowed this analytical tool with remarkable findings in glycomics, lipidomics, proteomics and metabolomics [12-20].

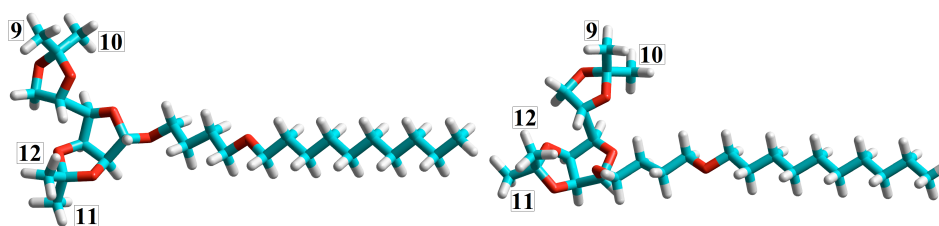
In this paper we present a particular application of the theory behind this method for two new mannose based surfactant precursors (Figure 1) which, based on their inherent biocompatibility and biodegradability [21], could very easily find applications in pharmacy, medicine, biotechnology and life-science research [22-24]. Sugar based surfactants, especially alkyl glycosides, are known to have good amphiphilic properties while they also possess very low toxicity, and this leads to their wide range of applications [21].

**Figure 1**

Structure and carbon atom numbering for the α and β anomers of the mannofuranose derivative

These long chain derivatives can be easily obtained with high yields from two renewable natural sources, sugars and fatty alcohols (from lipids) [20,25]. The two isopropylidene acetalic units from each molecule are remnants of the synthetic pathway employed. These were initially introduced as some of the most versatile and low-cost protecting groups [26-30] to provide regioselective attachment of the lateral chain. They also proved to be essential to the gas chromatographic (GC) analysis of this family of glycoderivatives, providing temperature stability and high-enough volatility to be transported through the chromatographic system by the carrier gas [31].

These two compounds, namely decyloxibutyl 2,3:5,6-di-*O*-isopropylidene-D-mannofuranoside (α/β), are basically diastereomers, so they produce very similar spectra in electron ionization - mass spectrometry (EI-MS). Because they differ in the configuration of only one asymmetric carbon atom (from the five totally present in the sugar residue), they can also be classified as epimers, or, more precisely, as anomers (because of the hemiacetal or anomeric carbon involved). Molecular models of the two anomers are presented in figure 2.

**Figure 2**

Tubes models for the two anomers (α left, β right), with methyl group numbering

To differentiate the two spectra obtained by EI-MS we took into consideration only the intensity of the peak resulted from losing one methyl group [31-35], which, when normalized to TIC, has clearly different values for the two anomers. As previously discussed [32], the molecular peak is absent or has a very low intensity for this type of compounds, so the $[M-CH_3]^+$ peak is usually used to find the molecular mass. The formation of this ion is illustrated in Figure 3 [31].

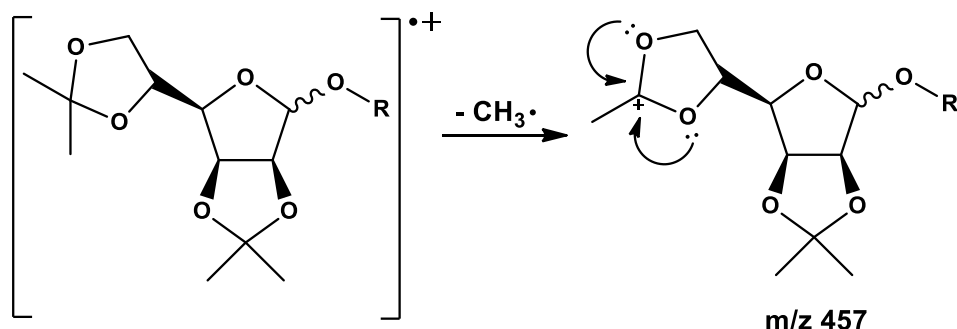


Figure 3

Formation of cations with m/z 457 (M-15); a similar process can take place for the other isopropylidene unit

Six semi-empirical methods were employed for calculating the heats of formation $\Delta_f H$ of such ions: AM1, MINDO3, MNDO, RM1, PM3 and PM7, and the results were related with the experimental values of the peak intensities for both anomers.

Materials and Methods

The two anomers were obtained starting from D-mannose, acetone, 1,4-butanediol and 1-bromodecane. The two step synthesis (bis-acetalation of D-mannose, followed by alkylation) of these O-alkylated mannofuranose derivatives (unknown in literature) and their spectroscopic analysis were presented alongside with those of other related compounds in a previous work [20].

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 Factor Four VF-35ms Capillary Column (30 m length, 0.25 mm i.d., 0.25 μ m film thickness) was

used for the GC system. The temperature range was set up from 100°C to 300°C with 6°C/min; the injector temperature was set up at 250°C and He was used as carrier gas with a flow rate of 1.0 mL/min. The injection volume was 1 µL and the sample was injected in the splitless mode. An ionization energy of 70 eV was used for the mass spectrometry detector, the electronic impact ion source temperature being 200°C while the quadrupole temperature was 100°C. A mass range of 34-800 amu and a scan rate of 1.6 scans/s were employed. Chromatograms and spectra were opened and investigated using Bruker Daltonics DataAnalysis v. 3.4. The two anomers were injected as they were obtained (mixture), without any previous separation.

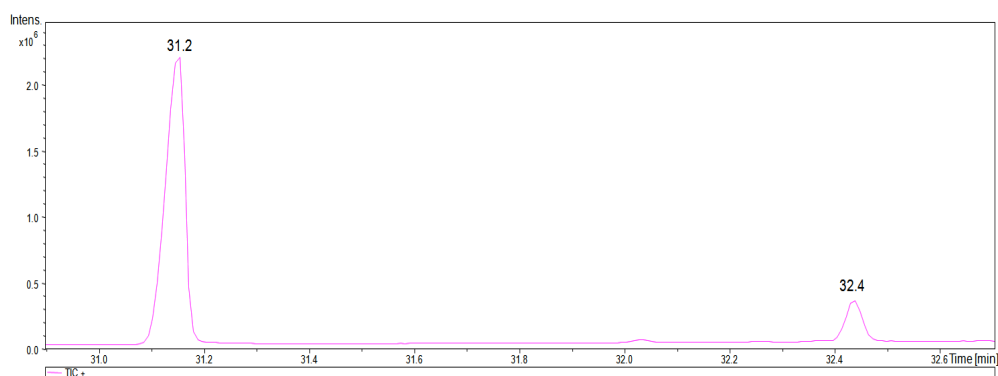
Before QCC, structures were drawn using HyperChem software [36]. For AM1, MINDO3, MNDO, RM1 and PM3, after the “Add H & Model Build” command, the molecules were optimized with each method and all the other species were obtained from these and optimized again with the same method. As for “Spin Pairing”, RHF operators were used for cations while UHF operators were employed for radicals and radical-cations. The SCF “Convergence limit” was set at 10^{-5} , without using the “Accelerate convergence” procedure. For geometry optimization and $\Delta_f H$ calculation, the “Polak-Ribiere (conjugate gradient)” algorithm was selected with a RMS gradient of 0.01 kcal/(Å mol), the molecules being considered in vacuum (conditions close with those inside the MS ion source and detector).

MOPAC 2012 software [37] was used for PM7 semi-empirical method, with CHARGE=+1 option for cations and UHF option for radicals. The same HyperChem starting structures were used (the ones obtained after the “Add H & Model Build” command, without other optimization). The corresponding .HIN files were then converted to .ZMT (MOPAC Z-matrix) files and run through the MOPAC interface for geometry optimization and $\Delta_f H$ calculation.

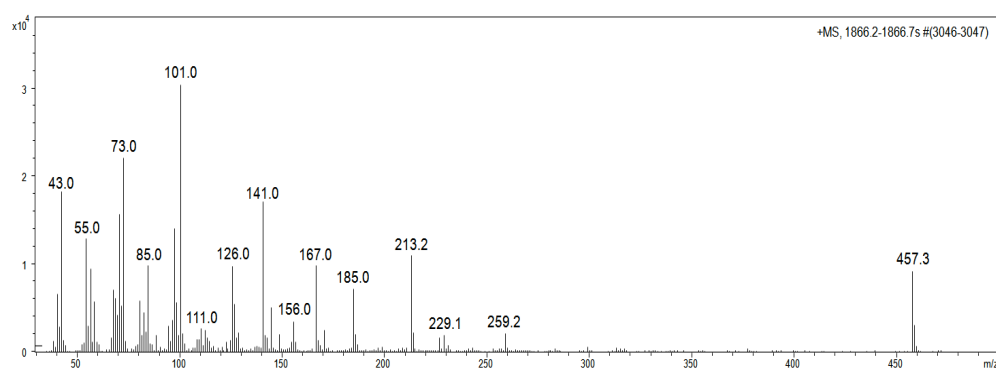
All computations were done on an Intel® Core™2 Quad CPU Q8400 @ 4x2.66 GHz, 2 GB of RAM system.

Results and Discussion

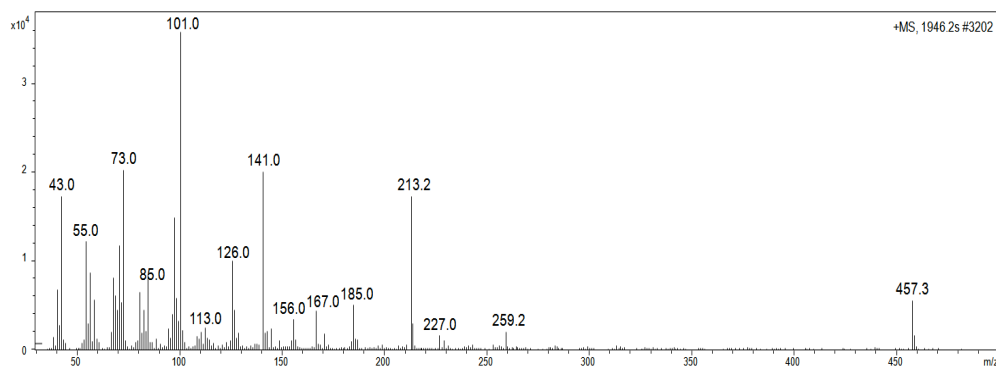
The GC-MS chromatogram obtained for the mixture of the two anomers is displayed in figure 4, while the EI-MS spectra corresponding to a similar value for the total ion current (TIC) are displayed at the same scale in figure 5 and figure 6.

**Figure 4**

GC-MS chromatogram presenting the two anomers (α has the lowest retention time) perfectly separated by the GC column

**Figure 5**

EI-MS spectra for the α anomer, taken from the ascending part of the peak at a TIC value very close to that corresponding to the highest point of the β anomer peak

**Figure 6**

EI-MS spectra for the β anomer, taken from the highest point of the peak

The following fragmentation reaction takes place for generating the m/z 457 cation:



The $[M-CH_3]^+$ cation was chosen for the study because it is formed directly from the initial radical-cation, its formation mechanism is clearly explained [31] and, very importantly, because it shows a significant difference in peak intensity between α and β anomers. For the same TIC, it has a double value of the intensity for the α anomer when compared with the β anomer, calculated from average values taken from the ascending part of the peak, where the effect of secondary fragmentations should be lower. In trying to compensate for the difference in concentration, only values above 20% of the highest TIC, where m/z 101 becomes the base peak, and only values below the highest TIC for the β anomer, which is present in lower concentration, were considered.

The fragmentation reaction enthalpy ΔH can be easily calculated from the formation enthalpies $\Delta_f H$ of the products and reactants according to the following formula:

$$\Delta H (\text{fragmentation}) = \Sigma \Delta_f H (\text{products}) - \Sigma \Delta_f H (\text{reactants})$$

The equation above then becomes:

$$\Delta H (\text{fragmentation}) = \Delta_f H ([M-CH_3]^+) + \Delta_f H (CH_3\cdot) - \Delta_f H (M^{\cdot+})$$

Considering that we have isobaric ions produced by similar processes and that peak intensities increase as reaction enthalpies decrease (the most exothermic or the least endothermic process is favored), we can write the following relations:

$$I_\alpha > I_\beta \leftrightarrow \Delta H_\alpha < \Delta H_\beta$$

All values obtained for both enthalpies of formation (of cations and radicals), taken as heat of formation from the software used, and the enthalpy of reactions for the two anomers are listed in table I. Although only few decimals are shown in the table, seven decimals were used in the calculations performed.

Table I
 $\Delta_f H$ values (in kcal/mol) for chemical species and ΔH for the fragmentation reaction

Anomer	[M-CH ₃] ⁺				CH ₃ ·	M ^{·+}	ΔH (fragmentation)*			
	9	10	11	12			9	10	11	12
AM1										
α	- 208.6	- 208.6	- 199.4	- 199.4	29.91	- 181.8	3.099	3.105	12.359	12.359
β	- 207.4	- 207.4	- 198.6	- 198.6		- 180.8	3.316	3.339	12.106	12.101
MINDO3										
α	- 249.8	- 249.8	- 248.0	- 248.0	40.97	- 207.9	-0.889	-0.891	0.937	0.930
β	- 247.2	- 247.2	- 246.2	- 246.2		- 206.9	0.601	0.589	1.628	1.615
MNDO										
α	- 174.7	- 174.7	- 166.9	- 166.9	24.59	- 131.3	-	-	-	-
β	- 173.8	- 173.8	- 167.7	- 167.7		- 131.9	-	18.775	-	-
RM1										
α	- 192.2	- 192.2	- 184.8	- 184.7	24.87	- 172.4	5.071	5.077	12.490	12.577
β	- 190.8	- 190.4	- 185.0	- 185.0		- 171.1	5.196	5.594	11.024	11.032
PM3										
α	- 171.1	- 171.9	- 163.5	- 163.5	27.92	- 148.0	4.793	4.026	12.350	12.351
β	- 171.1	- 171.1	- 163.0	- 163.0		- 149.2	5.986	5.993	14.112	14.087
PM7										
α	- 195.8	- 195.8	- 190.4	- 190.4	27.97	- 178.7	10.880	10.899	16.309	16.289
β	- 210.4	- 210.4	- 201.7	- 201.7		- 202.5	20.085	20.071	28.764	28.747

*shaded table cells contain the chosen values (see text below)

As can be seen from table I, the removal of each of the four methyl groups from 9 to 12 (numbering depicted in figure 2) results in different heat of formation values. The carbocations formed in this manner are exceptionally stable because of the two neighboring acetal oxygen atoms that stabilize the positive charge, so intense peaks are produced. By comparison, the contribution of the terminal methyl from the decyl chain to the intensity of $[M-CH_3]^+$ peak should be insignificant [38], so this was not taken into consideration. Notable enthalpy differences can be observed between methyl groups from lateral dioxolane (9 and 10) and those attached at the condensed dioxolane (11 and 12), breaking of the former groups being energetically favored. Because parallel reactions are involved, we can

consider only the smaller or the most negative ΔH values [11], which are marked as shaded cells in table I.

All semi-empirical methods gave smaller values for ΔH (fragmentation) in the case of α anomer, which would imply higher values for the intensity of $[M-CH_3]^+$ peak for the same compound. This is obvious from the experimental data; as previously mentioned, after normalization to TIC, the intensity for this peak has a double value of intensity in the case of the α anomer when compared with the same peak for the β anomer.

The results obtained in the present work are in good agreement with the general principles stated by CSI-Diff-MS analysis method for diastereomers (and other types of isomers) which was widely illustrated in literature [1-11].

Conclusions

The relationship between MS peak intensity and the enthalpy of reaction for the generation of the $[M-CH_3]^+$ cation was presented for the two anomers considered. Lower enthalpies calculated with semi-empirical methods (AM1, MINDO3, MNDO, RM1, PM3 and PM7) for the α anomer correlate well with higher $[M-CH_3]^+$ peak intensity for this anomer when compared with the β anomer, so this allows for an easy distinction by GC-EI-MS between the two species, when both spectra are available.

Acknowledgements

This work was partially supported by the strategic grant POSDRU/88/1.5/S/50783, Project ID50783 (2009), co-financed by the European Social Fund – Investing in People, within the Sectoral Operational Programme Human Resources Development 2007 – 2013.

M.C.P. wishes to thank prof. dr. eng. Nicolae Dincă for great advice. Also, the authors thank prof. dr. Mircea Mracec for HyperChem software support.

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Manuscript received: December 3rd 2012