SOME CONSIDERATIONS CONCERNING THE MS ANALYSIS OF COMPLETELY PROTECTED THIOGLYCOSIDES WITH HETEROCYCLIC AGLICONE

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Introduction

The synthesis and investigation of the biological activity of 1,2,4-triazole glycosides [1] have been stimulated by the finding that Ribavirin (b-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is remarkable in its broad spectral activity against DNA and RNA viruses. Encouraged by these interesting structures and biological activities, we found that it would be of great interest to synthesize some novel 1,2,4-triazole glucosides to investigate their antibacterial and antifungal activities.

Along with the most commonly used magnetic resonance spectroscopy, modern mass spectrometers allow molecular mass determination, and the generation of fragmentation data that leads to structure elucidation, generally in tandem mass-spectrometric experiments [2].

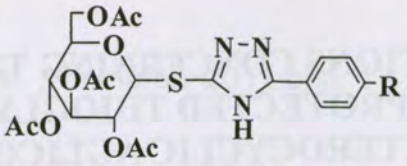
Methods

The proton spectra were recorded on a Varian 300 MHz spectrometer. Mass spectrometry was performed on a High Capacity Ion Trap (HCIT) Ultra PTM mass spectrometer (Bruker Daltonik, Bremen, Germany). The HCT mass spectrometer is interfaced to a PC running the Compass integrated software package under WindowsXP, which includes EsquireControl and Hystar modules for instrument tuning, control and spectrum acquisition, and DataAnalysis software for storing the ion chromatograms and processing the MS data. The samples were infused into MS by online syringe pump electrospray at a constant flow rate of 250 µl/h. Nitrogen at a flow rate of 10 l/min was employed at 300 °C for desolvation and as a nebulizer gas at 15 p.s.i. The instrument was set to operate in the positive ion mode under 3.0 kV ESI potential. For MS analysis the sample was dissolved to a concentration of about 5 pmol/µL, in MeOH/H_2O/HCOOH (1:1:0.001 v/v).

Discussion

The S-glycosides under investigation were synthesized by our group for the first time, as previously described [3,4] and were characterized by melting point, ^{1}H-NMR, ^{13}C-NMR and (+)ESI-IT-MS. The MS spectra of the above mentioned compounds were registered in positive ion mode and reasoned through pseudomolecular ions $[M+H]^+$ and $[M+Na]^+$ the presence of thioglycoside. MS^2 spectra of the isolated pseudomolecular ions are presented in Figures 1-3.

The compounds under investigation were of the general formula:



R = H, CH_3, OCH_3

The literature concerning the MS analysis of such compounds is scarce, most being performed with GC-MS methods, whereas the more sensitive ESI-IT-MS methods are mentioned by our group [5,6,7]. The fragmentations corresponding to the loss of ketene, acetyl and acetic acid, specific for peracetylated derivatives [8], were also identified.

In our study, we optimized our ESI-IT-MS and CID MS^2 methodology for the first mass spectrometric investigation of thioglycosides derived from 3-mercapto-5 substituted - 1,2,4 – triazole. The soft ionization techniques generate predominantly even-electron ions (whether $[M+H]^+$, $[M+Na]^+$, $[M-H]^-$, etc.) which fragment to generate even-electron fragments [9]. Most of the positive-ion fragments can be reasoned as having similar structures, whether the charge-bearing species is a proton or an alkali metal cation, most commonly a sodium ion. The weakest bond in a glycoconjugate and thus generally the easiest to fragment mass-spectrometrically is the glycosidic bond. Peracetylated glycosides are fragmented somewhat more complex than the deprotected thioglycosides, due to the fact that an acetoxyl group can be eliminated as four different radicals: CH_3COOH, $CH_3COO^{\cdot}$,$CH_3CO^{\cdot}$ and CH_2=C=O [10].

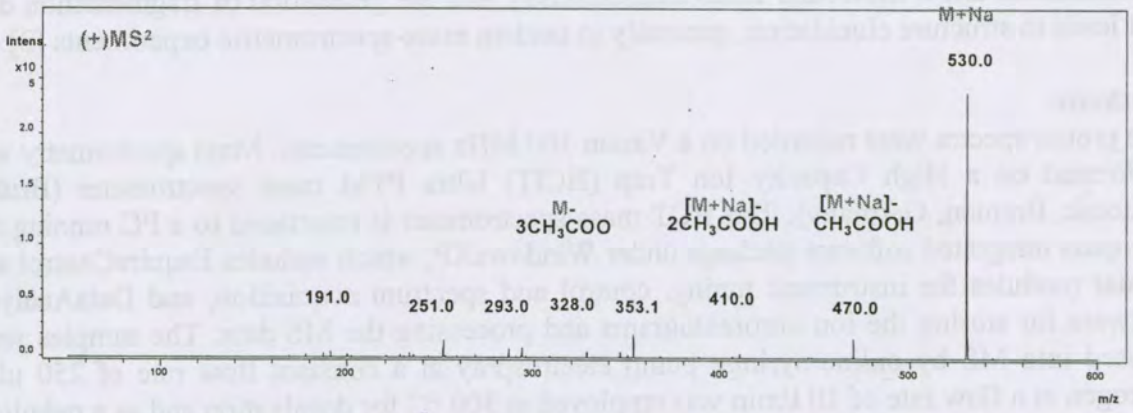


Figure 1: (+)MS^2 spectrum for compound where R = H

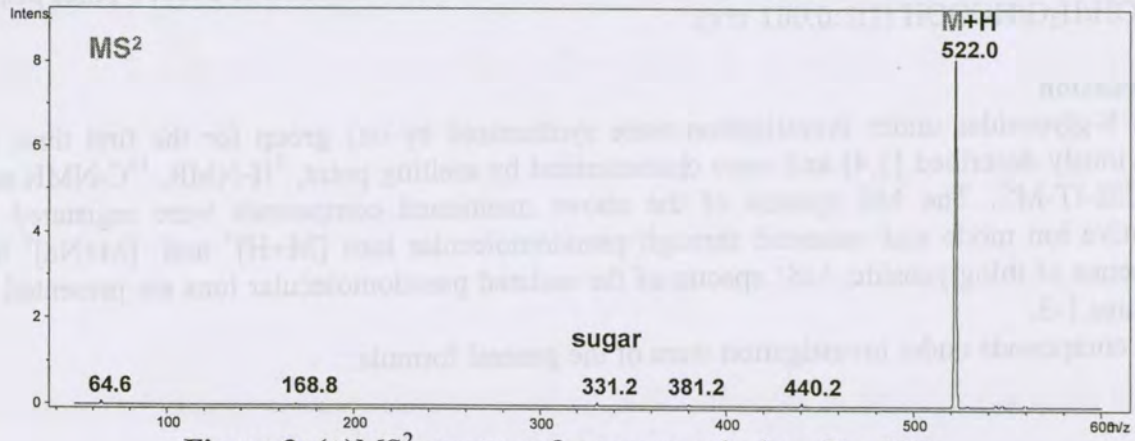


Figure 2: (+)MS^2 spectrum for compound where R = CH_3

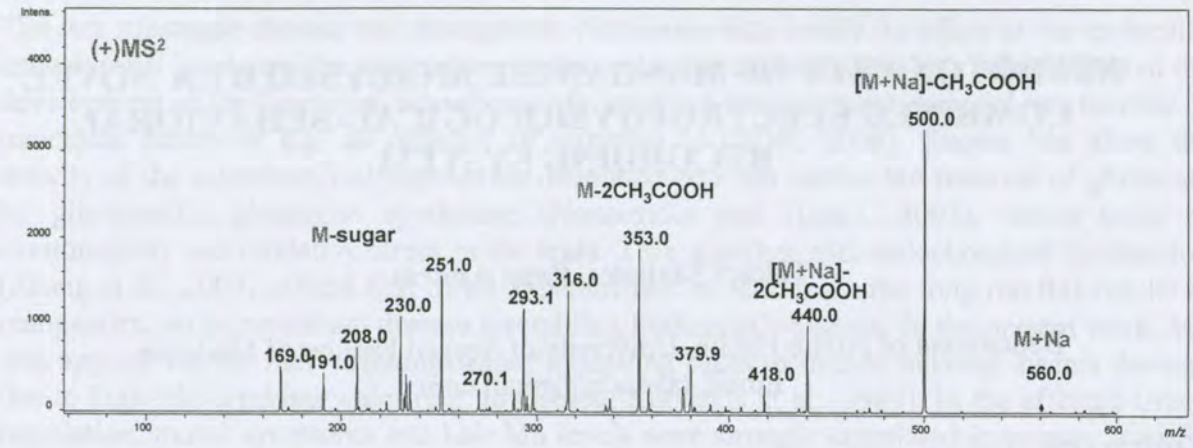


Figure 3: (+)MS^2 spectrum for compound where R = OCH_3

Conclusions

Both (+)MS^2 spectra of the sodiated species present the characteristic ions for the elimination of the acetate protecting groups. The MS^2 spectra in both cases reinforce and confirm without doubt the proposed structure, that was also reasoned by ^{1}H-NMR and ^{13}C-NMR analysis. The (+)MS^2 spectrum of the compound where R = CH_3 presents the characteristic fragment resulting from the breaking of the glycosidic bond, which represents the weakest bond in the molecule.

References

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