GC-MS Analysis of Pyrogallol and Purpurogallin

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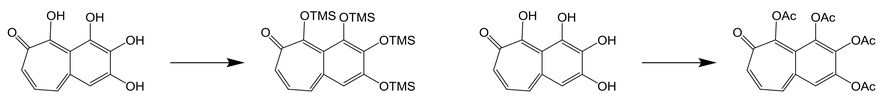
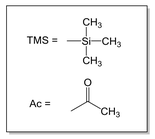
Introduction

Pyrogallol is a polyphenol formed from the decarboxylation of gallic acid found in tannins. Drinks like tea and coffee contain high levels of tannins that are responsible for staining of teeth and other surfaces. Purpurogallin is an oxidation product of pyrogallol. In this work, which was a collaboration with Professor Conrad Naleway's research group in the Department of Chemistry and Biochemistry, we developed a method to derivatize pyrogallol and purpurogallin and analyze the products by GC-MS. The analytical challenge was to derivatize a crowded arrangement of -OH groups on neighboring carbons and get all to accept a derivatizing group, quantitatively.

Pyrogallol:

Purpurogallin:





Objectives

The purpose of this research was to derivatize pyrogallol and purpurogallin and analyze via GC-MS. Derivatization was necessary to create analytes suitable for GC analysis. The research was also focused on method optimization to ensure quantitative derivatization.

Experimental

Sample Preparation: Pyrogallol was dissolved in $CHCl_3$ and derivatized using BSTFA/1% TMCS, BSTFA/10%TMCS, TMCS, MSTFA/1% TMCS, MSTFA/10% TMCS or acetic anhydride/triethylamine. GC vials were heated for a range of 45 minutes to 7 days with temperatures ranging from room temperature to 80C. The purpurogallin was prepared in a similar fashion.

Analysis: GC-MSD Agilent 7890A/5975C: Column HP-5ms, 20 m x 0.18 mm x 0.18 mcm load, helium carrier gas flow = 1mL/minute, split flow = 50:1.

Results

TMS Derivatization

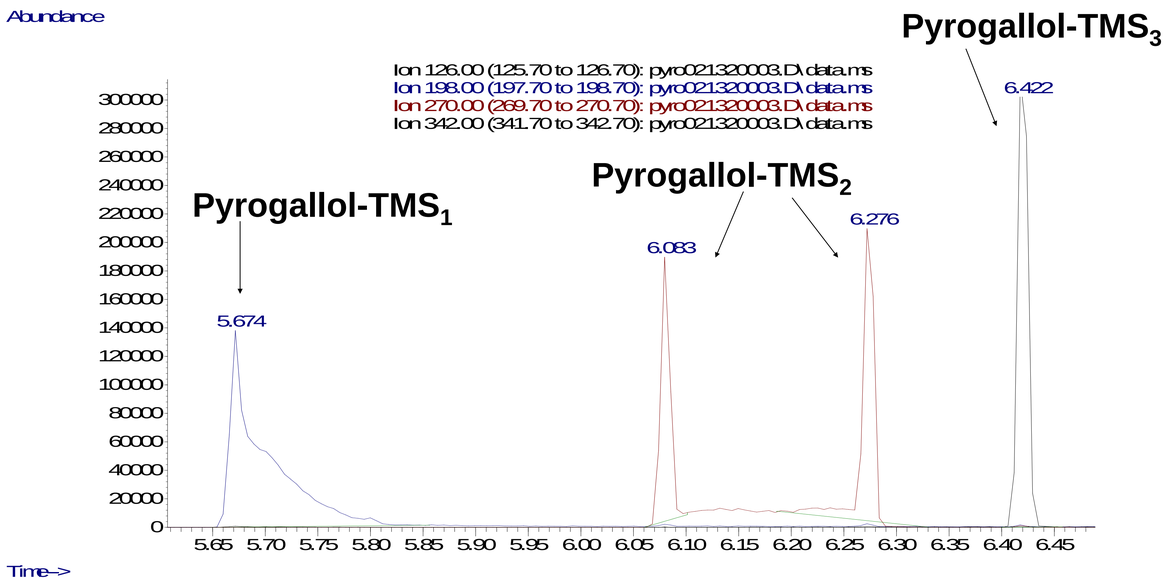


Figure 1: Extracted ion chromatogram showing the peaks for the molecular ions of the mono-substituted at 5.674 min, di- substituted at 6.038 min and 6.276 min and tri-substituted pyrogallol at 6.422 min. The pyrogallol-TMS_2 has two peaks because the TMS groups can either attach to the outside alcohol groups or middle and one outside group leading to two different compounds that resolve by GC.

Results (cont'd)

Acetyl Derivatization

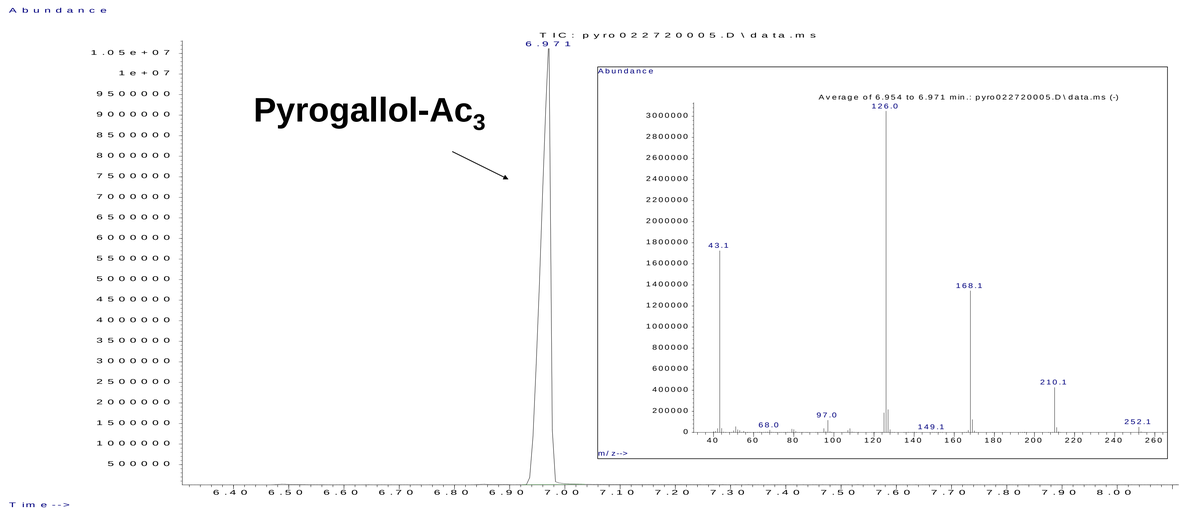


Figure 2: The chromatogram shows the peak at 6.971 minutes of the fully derivatized pyrogallol. No peak was observed for underivatized pyrogallol (expected at 5.67 min) which indicates quantitative derivatization. The inserted spectrum is of the derivatized pyrogallol with m/z of 252 for the M+.

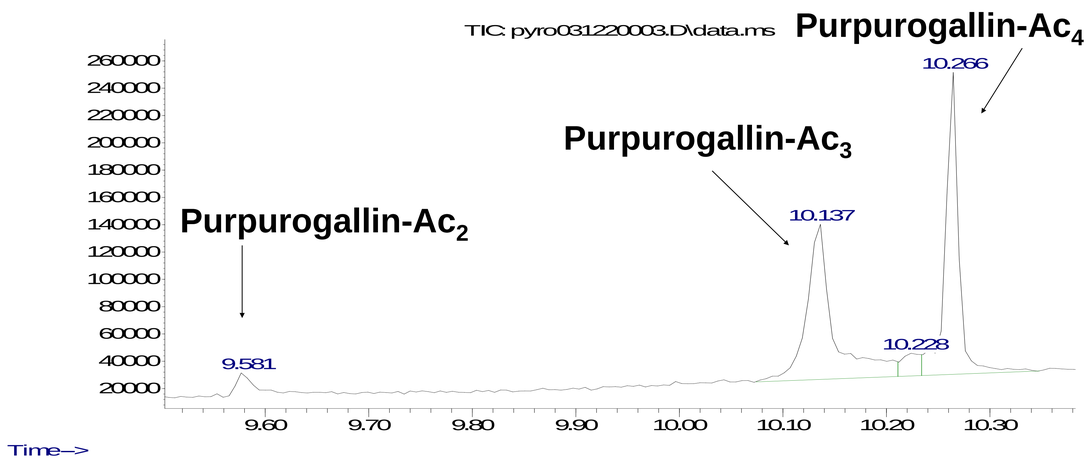


Figure 3: Chromatogram of Purpurogallin with peaks of the di-substituted at 9.581 min, tri-substituted at 10.137 min and per-substituted at 10.266 min (not optimized for quantitative derivatization).

Conclusions/Future Work

- Identification of the derivatized products was accomplished using library matches.
- Despite the existence of derivatization methods described in the literature, we were unable to achieve quantitative derivatization of pyrogallol using TMS.
- However, we were able to quantitatively derivatize pyrogallol using the acetic anhydride/triethylamine method.
- The derivatization of purpurogallin still must be optimized in the same manner as pyrogallol to achieve quantitative conversion.
- We are grateful to the Naleway group for analytical standards of pyrogallol and purpurogallin

References/Disclaimer

[1] Robin R. Shields-Cutler, Jan R. Crowley, Connelly D. Miller, Ann E. Stapleton; Human Metabolome-derived Cofactors Are Required for the Antibacterial Activity of Siderocalin in Urine, ***J. Biol. Chem.*** 2016, 291:25901-25910; doi: 10.1074/jbc.M116.759183.

[2] Elizabeth R. Tor,* Traci M. Francis, Dirk M. Holstege, and Francis D. Galey; GC/MS Determination of Pyrogallol and Gallic Acid in Biological Matrices as Diagnostic Indicators of Oak Exposure, ***J. Agric. Food Chem.*** 1996, 44, 1275-1279.