Notes

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Adventitious trimethylsilylation during combined gas–liquid chromatography–mass spectrometry

Combined gas–liquid chromatography–mass spectrometry (GLC–MS) is now recognized as a powerful method for the identification of a variety of substances from both natural and synthetic sources[1–12]. A technique which does not harbor pitfalls for the unwary practitioner is most rare, however, and combined GLC–MS offers no exception. For example, FOLTZ *et al.*[13] recently reported that they obtained the mass spectrum of trimethylfluorosilane when a sample of trimethylchlorosilane was analyzed on their instrument. The transformation was evidently caused by the presence of a fluorocarbon contaminant in the coupling system between the gas chromatograph and the molecular separator (which preferentially removes the carrier gas from the column effluent before it enters the mass spectrometer). We wish to report another anomalous finding, one which appears to be column related—trimethylsilylation of phenols during analysis using a GLC system which had previously come in contact with *bis*-trimethylsilylacetamide (BSA).

Experimental

All experiments were carried out with an LKB Model 9000 gas chromatograph–mass spectrometer equipped with the stainless steel molecular separator system of Ryhage[1,2]. Unless otherwise specified the mass spectrum of an eluted compound (5–10 μg samples were employed) was obtained at the time of maximum peak height on the chromatogram (scan time 6 sec). Gas chromatography conditions: 4 ft. × 3 mm. I.D. glass spiral or "cobra" column[14]; 5 % F-60 (DC-560), a non-polar methylpolysiloxane containing a few percent of *p*-chlorophenyl groups (Dow-Corning) on 80–100 mesh acid-washed and silanized[15] Gas-Chrom P; column, 210°; flash heater, 240°; molecular separator, 245°; helium carrier gas 30 ml/min. The mass spectrometer operating conditions were as follows: ion source, 290°; accelerating voltage, 3.5 kV; electron energy, 70 eV.

Results and discussion

A phenolic compound, the structure of which was "known" with considerable certainty (based on synthetic route, infrared, ultraviolet and NMR spectra) was submitted for combined GLC–MS analysis. The assumed molecular weight of this substance was 294, and it was expected that a signal for the molecular ion would be found at the appropriate *m/e*. The mass spectrum did contain a signal at this position, but there was a considerably stronger signal (3 times) at *m/e* 366 which appeared to be the molecular ion. The difference between 294 and 366 is 72 mass units, a number well known to those who work with trimethylsilyl (TMSi) derivatives—it is the difference in molecular weight between an alcohol and the corresponding TMSi ether. When a sample of the compound was allowed to react with BSA (a powerful trimethyl-

silylation reagent[16]) and then applied to the instrument the mass spectrum obtained showed no signal at *m/e* 294, and that at *m/e* 366 was the base peak (most abundant ion). This was clearly the spectrum of the TMSi ether derivative. (The phenol and the TMSi ether exhibit very similar retention behavior; the retention time of the latter is only 1.03 times that of the parent compound.) Determination of the mass spectrum of the phenol via a direct inlet or probe technique (*i.e.*, the sample was not introduced into the ion source through the gas chromatography system), however, produced a spectrum entirely compatible with the proposed structure, with no suggestion of trimethylsilylation. Comparison of the three spectra disclosed that the one resulting from analysis of the phenol using the combination technique was actually composite of the two "pure" spectra, and the inescapable conclusion was that this spectrum represents a mixture of phenol and TMSi ether. A plausible explanation for this "on column" derivatization is given below.

For several days previous to these observations, and actually until about an hour prior to combined GLC–MS analysis of the phenol, numerous such analyses were carried out on substances (not related to the compounds used in this study) dissolved in BSA. This liquid was used both as solvent and reagent, a not uncommon practice[17–19]. We assumed that sufficient BSA remained in the gas chromatography system to effect partial trimethylsilylation of the phenol as it passed through the column. (Deliberate "on column" derivative formation has been studied by ANDERS AND MANNERING[20].) To test this hypothesis, the flash heater and column temperatures were increased 20° above normal operating values for 15 h (overnight) and the carrier gas flow rate maintained at 30 ml/min in an attempt to "condition" the system and remove the source of TMSi groups. Samples of the phenol were then run by the combination technique under the normal operating conditions immediately before and 15 min after the application of 10 μl of BSA to the column (with the dual inlet valve, located between the molecular separator and the mass spectrometer, closed). The mass spectrum of the sample run prior to this BSA-treatment of the column was again a composite; however, it exhibited *m/e* values of 294 and 366 in the intensity ratio of 3 : 1, respectively, in marked contrast to the relative intensities of these signals observed before the "baking out" of the column. (One should not assume that the 294/366 amplitude ratio accurately represents the relative proportion of parent phenol and TMSi ether in the mixture, but it is a convenient approximation.) The mass spectrum of the eluted peak resulting from analysis of the phenol after application of BSA to the column was very similar to the composite spectrum first obtained for this compound, with a 294/366 ratio of 1 : 2.

In order to investigate this phenomenon further, the overnight column deactivation treatment was repeated and tested with the phenolic steroid estrone. The mass spectrum obtained from the mid-point of this peak indicated the presence of both estrone and its TMSi ether, but the signal at *m/e* 270 (molecular ion of estrone) was ten times the intensity of that of *m/e* 342, the molecular ion of estrone TMSi ether. Although an authentic mixture of estrone and its TMSi ether was resolved into two peaks (relative retention times of 1.0 and 1.1, respectively) a second peak was not observed in the initial analysis of estrone. Analysis of estrone 15 min after the application of 10 μl of BSA to the column produced a chromatogram in which the single peak gave mass spectrometric evidence for the presence of both estrone and its TMSi ether. Mass spectra of the ascending, center, and descending portions of the peak were taken.

Each of the spectra was a composite of those of authentic estrone and estrone TMSi ether; the 270/342 amplitude ratios for the three spectra were 5:1 for the front, 3:1 for the middle, and 1:1 for the rear of the peak. One must conclude that estrone TMSi ether is formed *in situ* during the analysis of the parent steroid*.

Demonstration of the presence of the TMSi ether in the front portion of the peak and the fact that two gas chromatographic peaks are not seen suggests that the functional group alteration does not occur "instantaneously" at the top of the column, but probably continuously as the estrone moves through the column. This effect may persist even after "conditioning" of the column system, and the conclusion to be drawn is that partial "on column" trimethylsilylation is a definite possibility when phenolic compounds are analyzed on a column previously exposed to BSA. Although BSA may be considered to be "innocuous" under certain conditions[21], this is certainly not always true. A more detailed investigation of this effect is beyond the scope of this note. We hope we have drawn attention to an interesting but potentially misleading phenomenon.

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* When androstane-3β-ol-17-one was employed as a test compound in the same manner as estrone only a very slight conversion was noted for this non-phenolic alcohol.