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CAPILLARY GAS CHROMATOGRAPHY–MASS SPECTROMETRY OF SEVERAL MACROCYCLIC TRICHOTHECENES

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SUMMARY

The mass spectra of verrucarin J and the trimethylsilyl derivatives of verrucarin A, roridins A and E, satratoxins G and H, and baccharin B_5 have, for the first time, been obtained after sample introduction by gas chromatography (GC). This has been accomplished by the use of a short-length fused-silica capillary column with on-column injection. The macrocyclic trichothecenes studied are separable by GC except for verrucarin J, derivatized verrucarin A and derivatized roridin E. However, these materials are distinguishable by selected ion monitoring. The fragmentation pathways for each of the compounds studied have been partially elucidated. Except for roridin E and satratoxin G, the macrocyclic trichothecenes give usably intense electron impact ions at high mass range for selected ion monitoring at 1–10 ng.

INTRODUCTION

The trichothecenes are a group of sesquiterpene alcohols and esters produced by various genera and species of fungi and can be divided into two broad classes: the simple trichothecenes such as T-2 toxin and deoxynivalenol and the macrocyclic trichothecenes such as those shown in Fig. 1. The macrocyclic trichothecenes are, in general, more toxic than the simple trichothecenes and some of them have been implicated in stachybotryotoxicosis[1,2], an often fatal disease in farm animals who have digested contaminated straw. In addition, there is considerable pharmaceutical interest in the macrocyclic trichothecenes because some of them have shown *in vivo* activity against P388 mouse leukemia[3].

While the simple trichothecenes can be separated on packed gas chromato-

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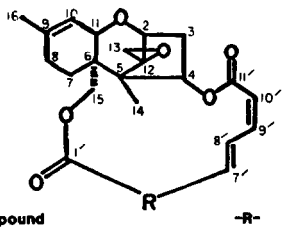


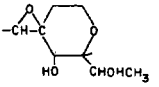
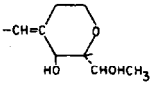
Compound	-R-
Verrucarin A	$-CHOHCHCH_3CH_2CH_2OC-$
Verrucarin J	$-CH=CCH_3CH_2CH_2OC-$
Roridin A	$-CHOHCHCH_3CH_2CH_2OCHCHOHCH_3$
Roridin E	$-CH=CCH_3CH_2CH_2OCHCHOHCH_3$
Satratoxin G	
Satratoxin H	
Baccharin B5	$-CH-CCH_3CHOHCH_2CHCHOHCH_3$ (In Baccharin B5 the 9.10 position has an epoxy group, not a double bond.)

Fig. 1. Structures of macrocyclic trichothecenes discussed in text.

graphy (GC) columns after conversion to their trimethylsilyl (TMS) ethers[4], heptafluorobutyrates[5] or trifluoroacetates[6], there have been no reports to date on GC or GC–mass spectrometry (MS) methodology for macrocyclic trichothecenes. Harrach *et al.*[2] separated and collected satratoxin G (S_G) and satratoxin H (S_H) from contaminated straw by high-performance liquid chromatography and subsequently confirmed their presence by direct-probe MS. Krishnamurthy and Sarver[7] hydrolyzed several macrocyclic trichothecenes to verrucarol A, converted the latter to its TMS derivative and analyzed by GC–MS. However, for some of the trichothecenes, losses during hydrolysis and derivatization amounted to more than 90%. This preliminary report demonstrates that it is possible to separate and identify some macrocyclic trichothecenes by GC–MS using a short capillary column with on-column injection.

All trichothecenes studied are fungal metabolites except for baccharin B_5 (B_5), which is a metabolite of the plant *Baccharis megapotamica.*

EXPERIMENTAL

Instrumentation

A Finnigan MAT 212 mass spectrometer was interfaced to a Varian 3700 gas chromatograph via a 1 m × 0.17 mm I.D. fused-silica line that was fed through a standard heated line-of-sight (LOS) inlet. The GC column used for the analysis was a 7 m × 0.32 mm I.D. Foxboro Analabs GB1 gamma-bonded fused-silica capillary

column having a film thickness of 0.2 μm. The linear carrier velocity varied from 80 to 100 cm/s and the temperature was programmed from 200 to 340°C at 20°C/min and held at the final temperature for 5 min. Samples were injected with a J & W Scientific standard 0.15–0.17 mm O.D. fused-silica syringe needle through a J & W Scientific on-column injector. Mass spectrometer conditions were: filament voltage 100 eV, filament current 1 mA, interface and ion source temperatures 330°C, resolution 1000, multiplier voltage 2.1 kV (scanning mode) and 2.6 kV [selected ion monitoring (SIM) mode]. These voltages are equivalent to gains of *ca.* $1 \cdot 10^6$ and $5 \cdot 10^6$ respectively. SM was conducted at 1000 resolution and was controlled by an SS-200 data system.

Materials

All solvents were Burdick & Jackson Labs. (Muskegon, MI, U.S.A.) "high purity". N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) was purchased from Supelco (Bellefonte, PA, U.S.A.). The macrocyclic trichothecenes and methylene chloride extracts of fungal cultures were gifts from B. B. Jarvis, University of Maryland (College Park, MD, U.S.A.). Silica gel Sep-Paks were purchased from Waters Assoc. (Milford, MA, U.S.A.).

Clean-up procedure

Methylene chloride extracts (25 mg) of two fungal cultures were redissolved in 100 μl of methylene chloride and placed on the head of a Waters silica gel Sep-Pak. The cartridge contents were eluted with 10 ml of methylene chloride and then subsequently eluted with 10 ml of methanol–methylene chloride (1:9). The two eluates were combined and evaporated to dryness.

Derivative formation

A volume of 100 μl methylene chloride and 0.5 ml BSTFA were added to the concentrate and heated for 1 h at 90°C in a capped conical vial. The derivatized solution was then cooled and the excess reagent evaporated at room temperature in a stream of nitrogen. The resulting residue was reconstituted in *ca.* 1 ml of methylene chloride prior to analysis by GC–MS–SIM. For derivatization of standards, 100 μg of each trichothecene was dissolved in 100 μl methylene chloride and reacted with 0.5 ml BSTFA.

RESULTS AND DISCUSSION

The reconstructed ion chromatograms and the electron impact (EI) mass spectra of the macrocyclic trichothecenes studied are shown in Figs. 2–8. V_J*, (V_A)-TMS and (R_A)-TMS were chromatographically pure while impurities or decomposition products were observed for the other materials, especially S_H-TMS and B_5-TMS. Fig. 9 is a reconstructed ion chromatogram of a mixture of V_J, V_A-TMS, S_G-TMS and S_H-TMS while Fig. 10 shows the separation of R_A-TMS, R_E-TMS and B_5-TMS. The

* Abbreviations: V_J = verrucarin J, V_A = verrucarin A, R_A = roridin A, R_E = roridin E, S_G = satratoxin G, S_H = satratoxin H, B_5 = baccharin B_5. V_A-TMS, for example, is an abbreviation for the trimethylsilyl ether of verrucarin A.

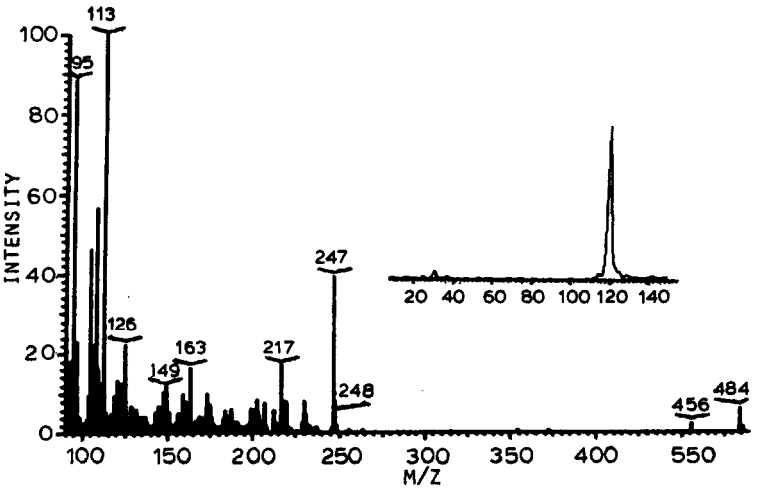


Fig. 2. Reconstructed ion chromatogram and EI mass spectrum of V_J standard.

trichothecenes have been separated into two groups for clarity of presentation. The retention time values observed under our experimental conditions were: V_J, V_A-TMS, and R_E-TMS, 267 s; S_G-TMS, 274 s; R_A-TMS, 298 s; S_H-TMS, 312 s; B_5-TMS, 332 s. An impurity or decomposition product present in the S_H standard co-eluted with R_A-TMS; but a mass spectrum of this material indicated it was not R_A-TMS. Although V_J, V_A-TMS and R_E-TMS co-elute, they can be distinguished because their mass spectra differ substantially.

High-resolution (10 000) mass spectra for some of the prominent ions in V_J, V_A-TMS, R_A-TMS, S_H-TMS and B_5-TMS was performed in order to assign ion identity.

All the TMS derivatives gave a base peak of $m/z = 73$ [$(CH_3)_3Si^+$]. An intense

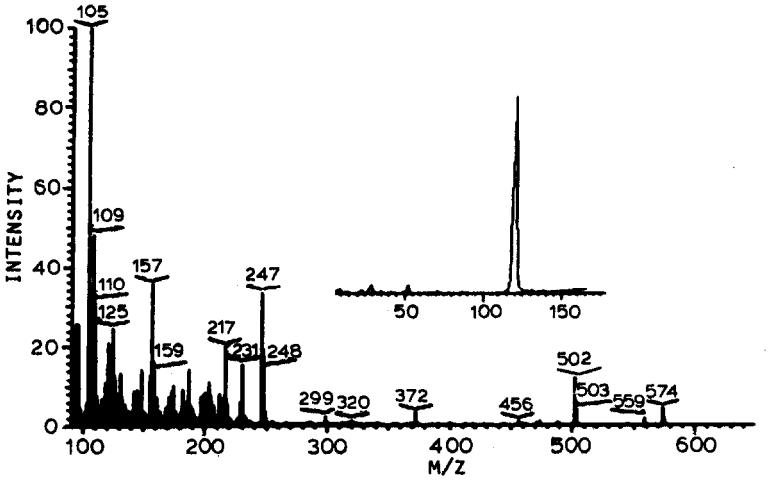


Fig. 3. Reconstructed ion chromatogram and EI mass spectrum of V_A-TMS standard.

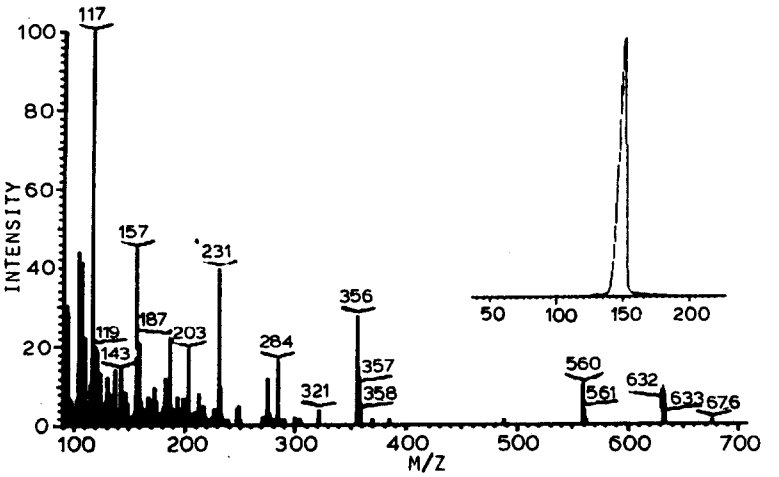


Fig. 4. Reconstructed ion chromatogram and EI mass spectrum of R_A-TMS standard.

peak at *m/z* 117 ($C_5H_{13}OSi^+$) was observed in the mass spectra of the TMS derivatives of R_A, R_E, S_G, S_H and B_5. This peak is formed by the mechanism depicted in Fig. 11 and involves the formation of a resonance-stabilized free radical.

Neutral loss of 44 was observed from the molecular ions of the trimethylsilyl derivatives of R_A, R_E, S_G and B_5 and possibly from the $M^+ - 72$ ion of S_H-TMS. This could result from loss of C_2H_4O in fragmentations involving either C-4′ and C-5′ or C-13′ and C-14′. As no such losses were observed in the mass spectra of V_J and V_A-TMS, we favor the mechanism proposed in Fig. 12.

Loss of $C_5H_{12}OSi$ from R_A-TMS, B_5-TMS and S_H-TMS to give *m/z* 560, 590 and 556, respectively, is visualized as occurring through the mechanisms shown in

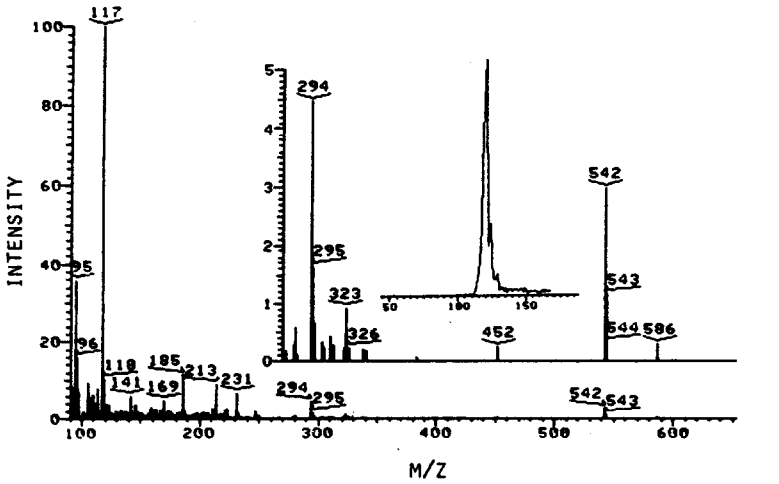


Fig. 5. Reconstructed ion chromatogram and EI mass spectrum of R_E-TMS standard. (Insert has a gain of 20.)

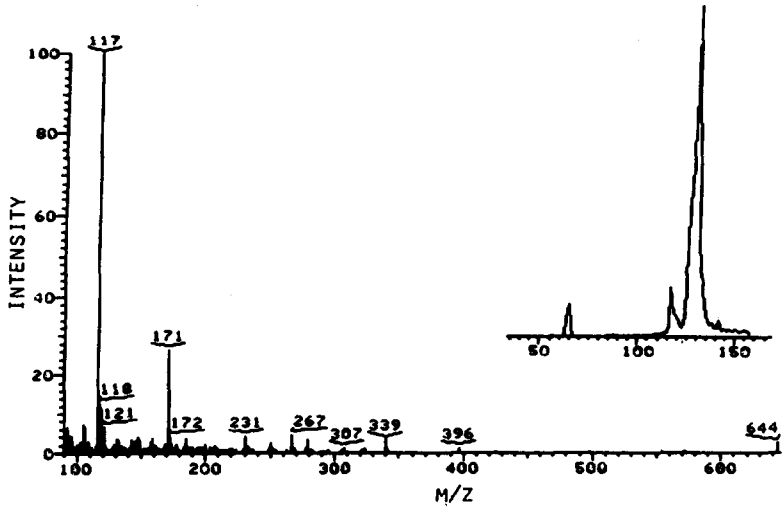


Fig. 6. Reconstructed ion chromatogram and EI mass spectrum of S_G-TMS standard.

Figs. 13 and 14. The loss of 72 a.m.u. from V_A-TMS probably proceeds through the McLafferty-type rearrangement shown in Fig. 15. V_J contains no hydroxyl group and cannot form a TMS derivative. Nor does it posses a methylcarbinol group. Hence, V_J is the only macrocyclic trichothecene in this study whose mass spectrum is not dominated by fragmentations involving the TMS or methylcarbinol groups. At the high mass end of the spectrum, the ion at *m/z* 456 ($C_{25}H_{28}O_8$) is due to loss of ethylene via a retro Diels-Alder mechanism involving C-7 and C-8. The peaks at *m/z* 113 and 95 could not be determined precisely by high-resolution MS because they were multiplets. We propose $^{+}O{\equiv}C{-}CH{=}C(CH_3){-}CH_2{-}CH_2OH$ and $^{+}O{\equiv}C{-}CH{=}C(CH_3){-}CH{=}CH_2$, respectively, as reasonable structures for these

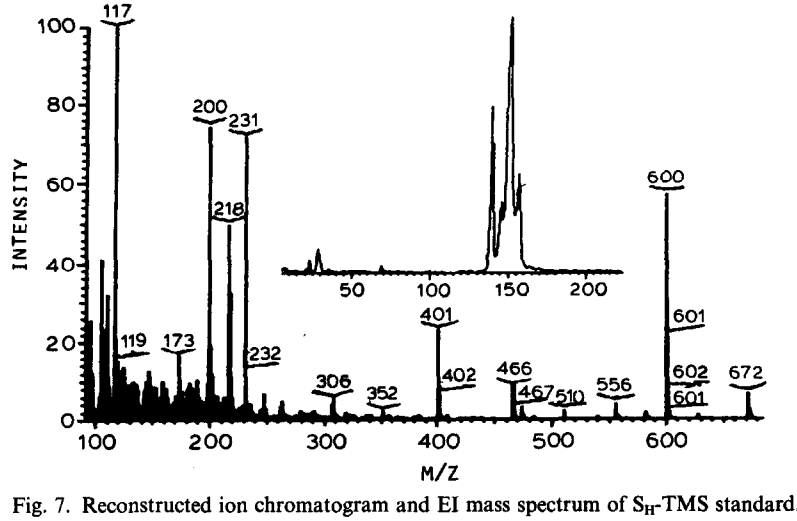


Fig. 7. Reconstructed ion chromatogram and EI mass spectrum of S_H-TMS standard.

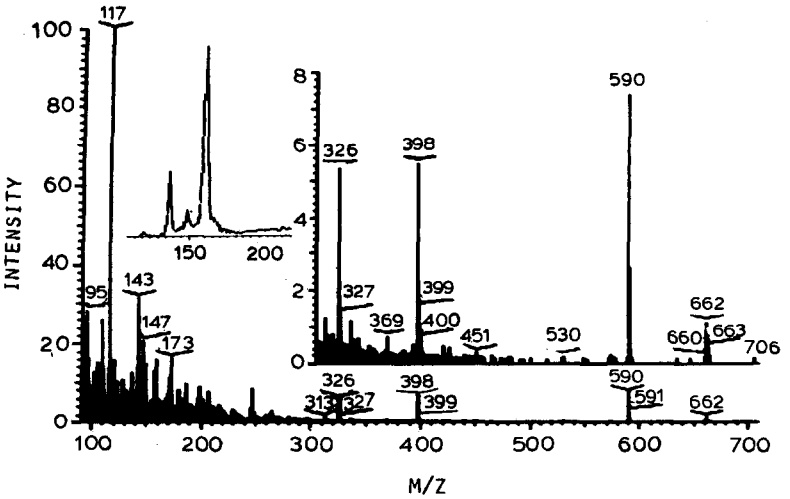


Fig. 8. Reconstructed ion chromatogram and EI mass spectrum of B_5-TMS standard. (Insert has a gain of 12.5.)

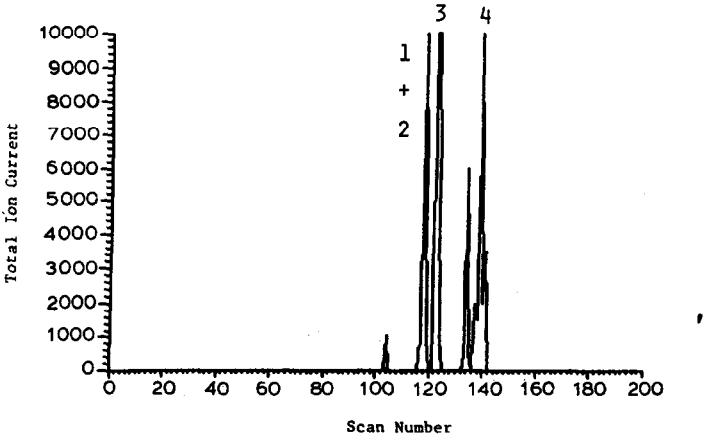


Fig. 9. Reconstructed ion chromatogram of (1) V_J, (2) V_A-TMS, (3) S_G-TMS and (4) S_H-TMS standards.

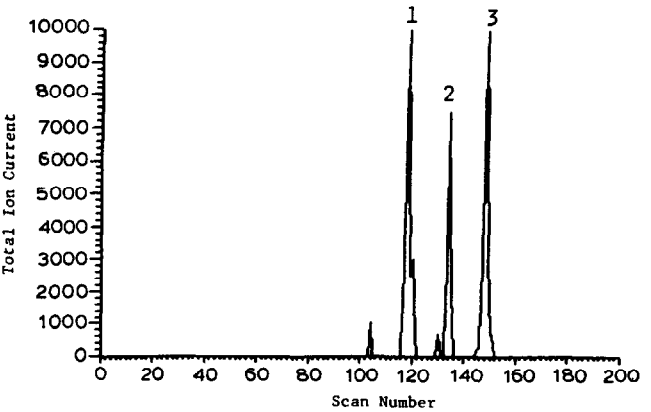


Fig. 10. Reconstructed ion chromatogram of (1) R_E-TMS, (2) R_A-TMS and (3) B_5-TMS standards.

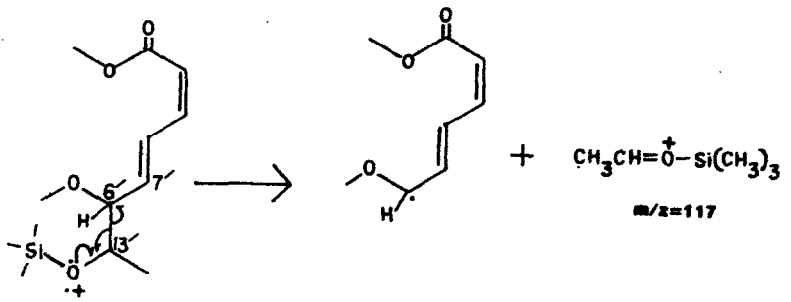


Fig. 11. Proposed mechanism for the formation of m/z = 117 in TMS derivatives of R_A, R_E, S_G, S_H and B_5.

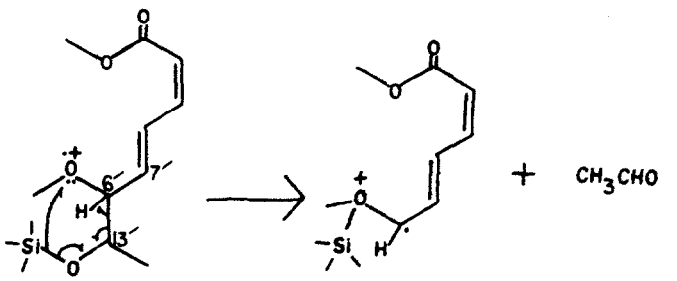


Fig. 12. Proposed mechanism for loss of 44 a.m.u. in the TMS derivatives of R_A, R_E, S_G, S_H and B_5.

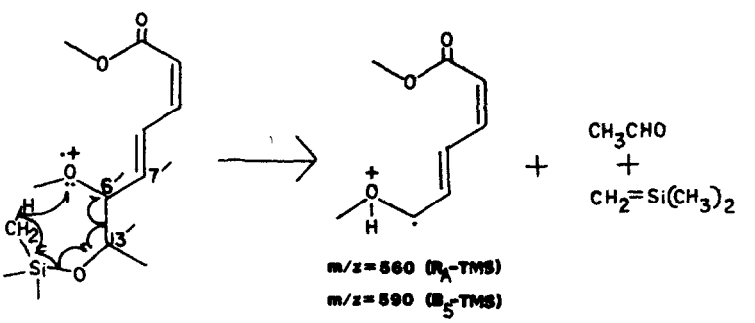


Fig. 13. Proposed mechanism for loss of 116 a.m.u. from molecular ions of R_A-TMS and B_5-TMS.

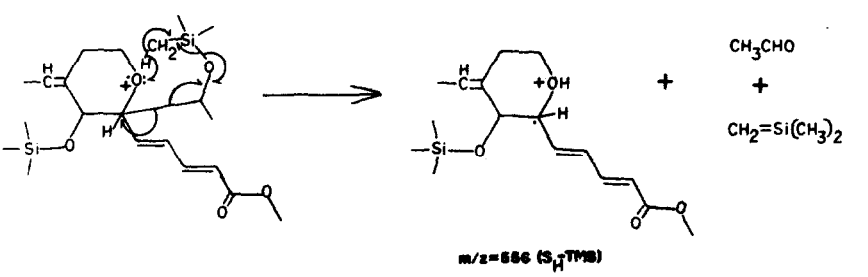


Fig. 14. Proposed mechanism for loss of 116 a.m.u. from molecular ion of S_H-TMS.

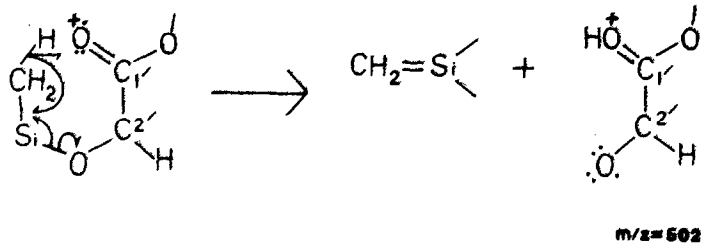


Fig. 15. Proposed mechanism for loss of 72 a.m.u. from molecular ion of V_A-TMS.

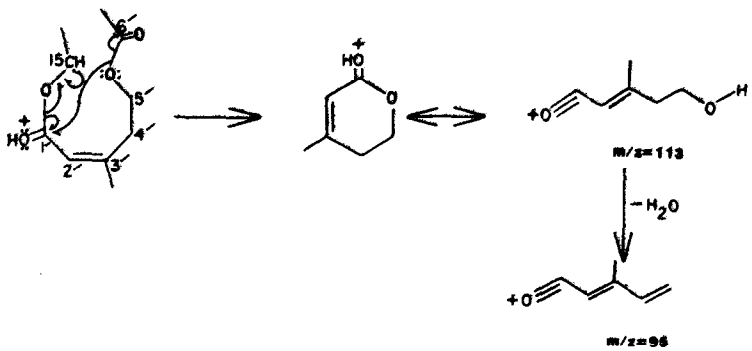


Fig. 16. Proposed mechanism for formation of m/z = 113 and m/z = 95 in V_J.

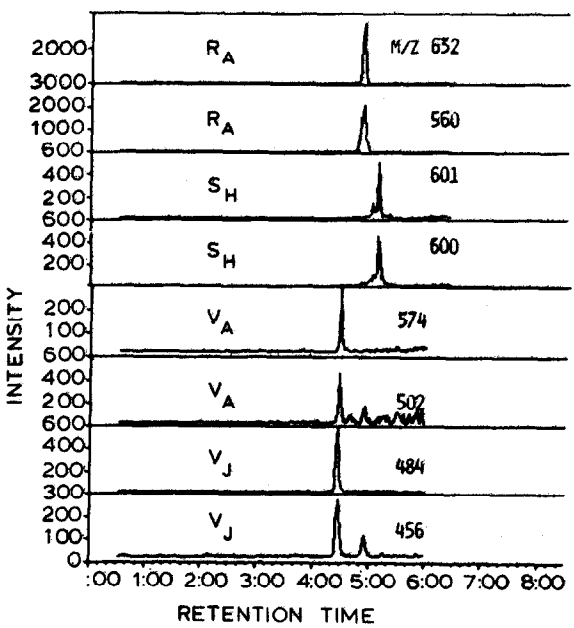


Fig. 17. Selected ion monitoring chromatogram of V_j, V_A-TMS, R_A-TMS and S_H-TMS standards. (The second set of two was performed on a different data than the first set of two.)

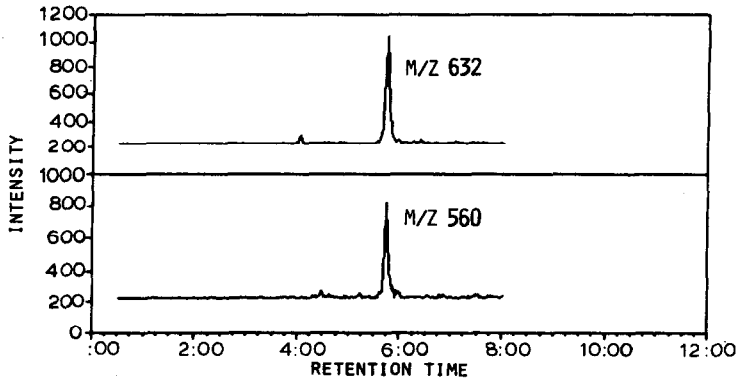


Fig. 18. Selected ion monitoring chromatogram obtained from *M. verrucaria* culture MV26148.

ions arising by the mechanism depicted in Fig. 16. The fragment at *m/z* 247 ($C_{15}H_{19}O_3$) probably arises by a similar mechanism.

A preliminary investigation was made into the use of SIM for the analysis of fungal cultures for the presence of macrocyclic trichothecenes. Fig. 17 shows SIM traces for standards of R_A-TMS, S_H-TMS V_A-TMS and V_J. Although V_A-TMS and V_J have identical retention times under our GC conditions, they are easily differentiated by SIM. Limits of detection for V_J, V_A-TMS, R_A-TMS, S_H-TMS and B_5-TMS standards were 1–10 ng by SIM. R_E-TMS and S_G-TMS could not be seen unless 100 ng of material was injected due to the very low ion intensities of the high mass fragments of these materials.

Two cultures of *Myrothecium verrucaria* were qualitatively analyzed after a simple clean-up procedure. Fig. 18 is the SIM chromatogram obtained from the first culture (MV26148) and shows the presence of R_A. The second culture (MV26146) contained R_A and V_A, as shown in Fig. 19. The presence of V_J in the sample is questionable because the 484/456 intensity ratio is not in accord with that of standard material.

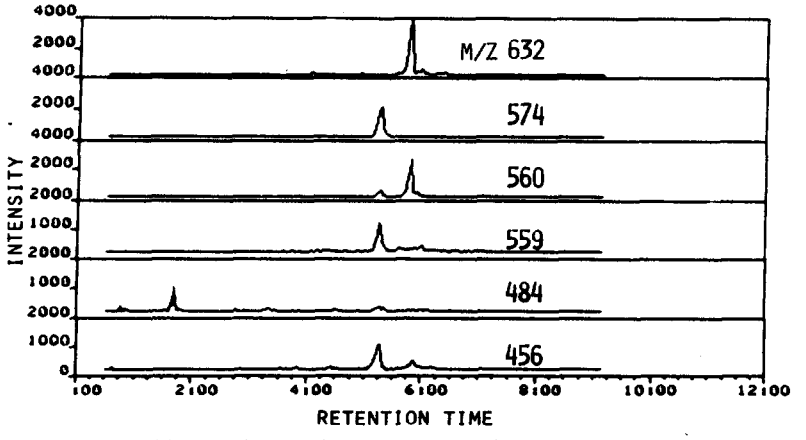


Fig. 19. Selected ion monitoring chromatogram obtained from *M. verrucaria* culture MV26146.

ACKNOWLEDGEMENTS

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