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FUNCTIONAL GROUP TRANSFORMATIONS IN THE GAS CHROMATOGRAPHIC DERIVATIZATION OF SEVERAL CARBAMATES AND URETHANES

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SUMMARY

Heptafluorobutyrylation and trimethylsilylation have been found with several carbamates and urethanes to result in the formation of unexpected products. Among the functional group transformations are conversion to the corresponding heptafluorobutyramide and trimethylsilyl ether. An on-column conversion of a trimethylsilylated carbamate to the corresponding isocyanate has been studied with respect to rate of trimethylsilylation and column temperature. By judicious choice of conditions the carbamate, its trimethylsilyl derivative, and the corresponding isocyanate can be chromatographed, allowing for an additional dimension of selective detection with a mass spectrometer.

INTRODUCTION

Use of derivatization techniques in the gas–liquid chromatography (GLC) of bioactive compounds is now widely accepted. The functional group transformations are usually straightforward reactions (*e.g.*, trimethylsilylation of hydroxyl groups[1] and heptafluorobutyrylation of amino groups[2]) carried out prior to injection into the chromatograph and which produce thermally stable (*i.e.*, to GLC conditions) compounds. Less frequently employed are approaches in which the derivatization step occurs when the sample and reagent are co-injected into the chromatograph[3,4], or those involving deliberate formation prior to GLC of a compound which will undergo a thermally-induced "on-column" conversion to a new species[5,6]. Workers in GLC generally desire "potent" reagents for rapid and quantitative derivatization. Under certain conditions, however, such reagents may form possibly unforeseen derivatives, *e.g.*, enol ethers[7] and enol esters[8]. When dealing with derivatization techniques, one must be alert to the possibility of the unexpected, both during derivatization and the subsequent GLC, as exemplified by the data reported in this paper.

EXPERIMENTAL

GLC and mass spectrometry (MS) conditions are as given in the legends to the

figures. Unless otherwise indicated, an LKB Model 9000 gas chromatograph–mass spectrometer was employed to obtain the chromatograms. Derivatization conditions are as follows:

(1) *p*-Amino-*p'*-ureidodiphenylsulfone (1 mg) was treated with heptafluorobutyric anhydride (0.2 ml) at 100° for 30 min. The reaction mixture was then treated with an excess of aqueous sodium bicarbonate solution, and the product extracted with ethyl acetate.

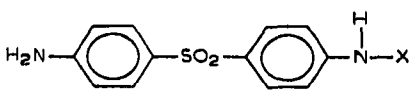
(2) Cambendazole (1 mg) was treated with heptafluorobutyric anhydride (0.2 ml) at (a) room temperature, (b) 100° for 60 min, and (c) 100° for 60 min in the presence of pyridine. The reaction mixtures were treated with an excess of aqueous sodium bicarbonate solution, and the products extracted with ether. Thin-layer chromatography (TLC) was carried out on silica gel GF plates (Analtech) with acetonitrile as the developing solvent. The heptafluorobutyryl derivatives of 5-aminothiabendazole were prepared by reaction of this compound with an excess of heptafluorobutyric anhydride at 100° for 1 h in the absence (monoamide) and presence (diamide) of pyridine.

(3) Ronidazole (50 μg) was treated with a 4:1 mixture of bis(trimethylsilyl)-trifluoroacetamide (BSTFA) and pyridine (50 μl) at 65° for 15 min.

(4) The di-trimethylsilyl(TMSi) derivative of IV was prepared by reaction with BSTFA–pyridine (4:1; 50 μl) at 65° for <2 min. Continued reaction (>30 min) led to the formation of the tri-TMSi derivative.

RESULTS AND DISCUSSION

Need for an assay procedure to determine the levels of *p*-ureido-*p'*-aminodiphenylsulfone (Ia, a therapeutic agent effective against Marek's disease[9,10])



Ia, X = $CONH_2$
Ib, X = H

in chicken tissue led first to consideration of hydrolysis of Ia to *p,p'*-diaminodiphenylsulfone (Ib) and conversion of this compound to a derivative suitable for electron capture detection. The diheptafluorobutyramide of Ib possesses good GLC properties and <1 ng/injection can be readily detected by electron capture techniques. Derivatization of Ia with heptafluorobutyric anhydride was also investigated, and it was found that when the reaction was carried out at 100° for 30 min the product possessed the same retention time as the authentic diheptafluorobutyramide of Ib (Fig. 1). Combined GLC–MS demonstrated that in both cases the compound eluted from the column is the same, namely, the diheptafluorobutyramide of Ib. This remarkable conversion is not an "on-column" alteration, for the product isolated from the reaction mixture possesses the same infrared and direct probe mass spectra as the diheptafluorobutyramide of Ib. An interesting property of this compound is its base solubility: it can be extracted from benzene solution into aqueous sodium carbonate (pH 11.6) and then recovered from the aqueous phase by reduction of the pH to <4

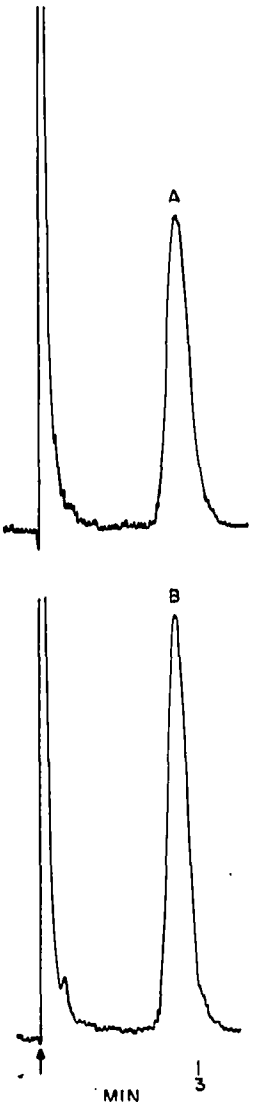
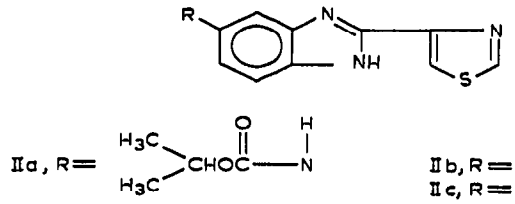


Fig. 1. Gas chromatogram resulting from analysis of the diheptafluorobutyramide of *p,p'*-diaminodiphenylsulfone (B) and the product resulting from treatment of *p*-ureido-*p'*-aminodiphenylsulfone with heptafluorobutyric anhydride (A). Column conditions: 1 ft. × 3 mm I.D. spiral glass column; SE-30–CHDMA two-component 3% stationary phase (2.5:0.5) on 80–100 mesh acid-washed and silanized Gas-Chrom P; 240°; 50 ml/min argon. Argon ionization detector: 8 V d.c.; Glowall instrument.

followed by re-extraction into benzene. This property facilitates the isolation and purification of the derivative prior to the final GLC step in the assay procedure. As Ib, also known as dapsone, is a widely used antileprotic agent[11], an incipient GLC assay for this drug at the nanogram level is thus available.

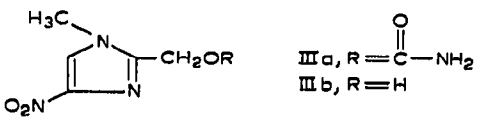
A somewhat related transformation has been observed to occur during the

heptafluorobutyrylation of the anthelmintic cambendazole[12], IIa. MS analysis suggested slight conversion of IIa to a monoheptafluorobutyryl derivative (molecular ion, *m/e* 498; 302 + 196) when



heptafluorobutyric anhydride was employed at room temperature. Application of heat increased conversion to this product, but two other derivatives (very minor components) also resulted, with apparent molecular weights of 412 and 608. These values correspond to the molecular weights of the mono- and diheptafluorobutyramides of IIb. In order to ascertain whether the isopropoxycarbonylamino moiety had been replaced by a heptafluorobutyramide group, two samples of IIa, one labeled with ^{14}C in the benzene ring, and the other labeled in the carbonyl group, were allowed to react with heptafluorobutyric anhydride (pyridine, 100°, 1 h). Both the reaction products were shown by TLC to be mixtures, with the two broad major zones exhibiting R_F values (determined by UV inspection) of 0.67 and 0.83, respectively. The slower moving component from both experiments was identified by its mass spectrum and R_F as IIa*. The more mobile zone, which on the basis of UV absorption was present in greater quantity, was subjected to MS and found to be a mixture of the two compounds with molecular weights 412 and 608. Further, analysis of the zones of R_F 0.83 by liquid scintillation spectrometry indicated radioactivity was present in only the zone from the ring-labeled precursor; no radioactivity was found in the corresponding zone from the carbonyl-labeled IIa. These data demonstrate the cleavage of the carbamate group during derivatization with the formation of the corresponding heptafluorobutyramide. This was confirmed by preparation of and comparison with the mono- and di-heptafluorobutyramides of IIb.

The antiparasitic compound ronidazole[13,14], IIIa, possesses a nitro group which should permit detection of this drug by electron capture techniques at the ng level. Initial experiments employing combined GLC–MS disclosed that injection of IIIa results in the elution of the corresponding alcohol, IIIb.



This thermally-induced transformation with elimination of isocyanic acid is not surprising, for carbamates are known to undergo a GLC conversion to the corresponding isocyanate and alcohol[15]. Quantitative aspects of the GLC of alcohols are often improved by analysis as their trimethylsilyl (TMSi) ethers[16], and thus it was decided to

* A heptafluorobutyryl group on the imidazole N of IIa is labile, and this system tends to exhibit loss of the acyl group and revert to the imidazole NH functional group during isolation TLC and GLC.

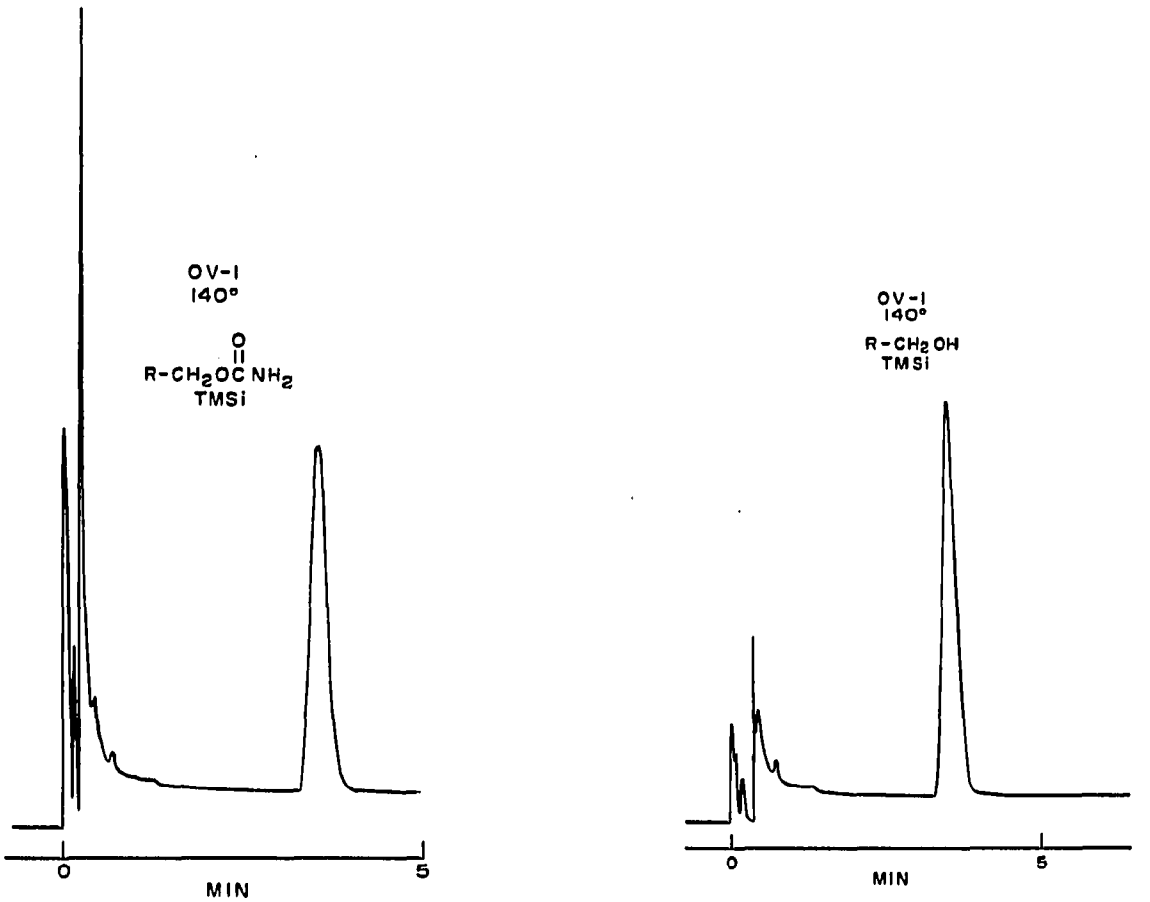


Fig. 2. Gas chromatogram resulting from analysis of the product arising from trimethylsilylation of ronidazole. Column conditions: 4 ft. × 3 mm I.D. spiral glass column; 3% OV-1 on 80–100 mesh acid-washed and silanized Gas-Chrom P; 140°; 30 ml/min helium.

Fig. 3. Gas chromatogram resulting from analysis of the trimethylsilyl ether of the alcohol corresponding to ronidazole. Column conditions same as for Fig. 2.

subject IIIa to trimethylsilylation conditions prior to GLC. The retention time of the peak resulting from GLC of an aliquot of such a reaction mixture (Fig. 2) was found to be the same as that of the TMSi ether of IIIb (Fig. 3), and GLC–MS demonstrated that both gave the same mass spectrum (Fig. 4). These data were initially interpreted as indicating the on-column conversion of trimethylsilylated IIIa to the TMSi ether of IIIb. It was noted during experiments designed to establish an assay for IIIa (less than 1 ng of trimethylsilylated IIIa gives an electron capture response satisfactory for quantitative analysis) that a normal tissue component formed a TMSi derivative with a retention time very close to that of the IIIb TMSi ether. This potentially interfering compound was shown to be trimethylsilylated nicotinamide. Addition of the appropriate amount of ethanol to a trimethylsilylated mixture of IIIa and nicotinamide resulted in elimination of the nicotinamide TMSi peak. The ability of ethanol to react selectively with nicotinamide TMSi was puzzling, as one might

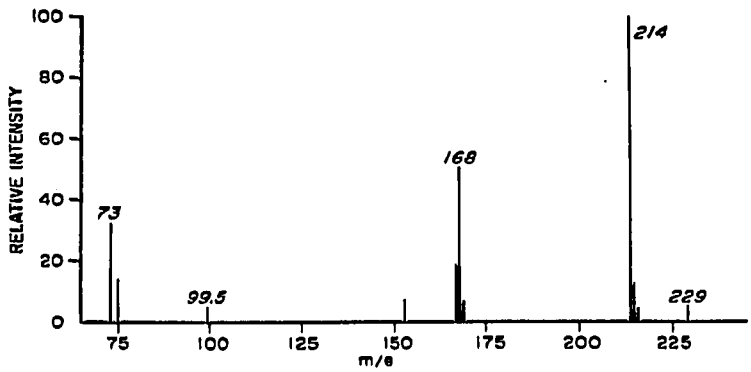
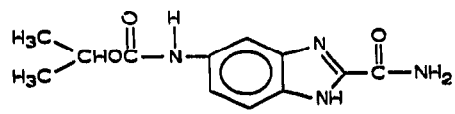


Fig. 4. Mass spectrum obtained when trimethylsilylated ronidazole is subjected to combined GLC–MS as in Fig. 2. Spectrometer settings: 70 eV ionizing potential; 270° source temperature; 3.5 kV accelerating potential; 60 μA trap current.

expect the TMSi derivative of IIIa to also act as a good "silyl donor". Additional data obtained on trimethylsilylated IIIa (direct probe mass and infrared spectra, and R_F values in two TLC systems), however, demonstrated its identity with IIIb TMSi. Thus the trimethylsilyl mediated conversion of IIIa to IIIb must occur during the derivatization step rather than in the chromatograph. Indeed, Klebe[17] reports that in some cases N-silylated esters of carbamic acids may be converted to the corresponding isocyanates at temperatures as low as −20°.

Successful GLC of the carboxamide IV necessitates derivatization of the polar functional groups.



IV

Trimethylsilylation appeared to be a possible choice for this, and IV was allowed to react with BSTFA–pyridine at 65°; the reaction was monitored by GLC. Fig. 5 shows the two-component chromatograms obtained by analysis on a 7% SE-30 column at 240° of aliquots of the reaction mixture at reaction times of 3 and 15 min, respectively. The component with the shorter retention time was found by GLC–MS (Fig. 6) to possess a molecular weight of 346, and does not exhibit a fragmentation involving 42 a.m.u., loss of C_3H_6, seen with compounds containing the isopropoxycarbonylamino side chain[18]. The component with the longer retention time was found to exhibit a molecular ion of 406 with an intense fragment ion at *m/e* 349 [M−(15 + 42)] (see Fig. 7), and thus possesses the isopropoxycarbonylamino group. Direct probe MS of the derivatization mixture at reaction time 3 min indicated that the major product is the species with a molecular weight of 406, whereas at 15 min an intense molecular ion is observed at *m/e* 478, 132 mass units higher than 346. The initially formed TMSi derivative of IV is di-TMSi in nature [262 + (2 × 72); confirmed by use of BSA-d_{18} (ref. 19)]. As thiabendazole, IIc, forms a mono-TMSi derivative within several minutes exposure to these reaction conditions, and the carboxamide

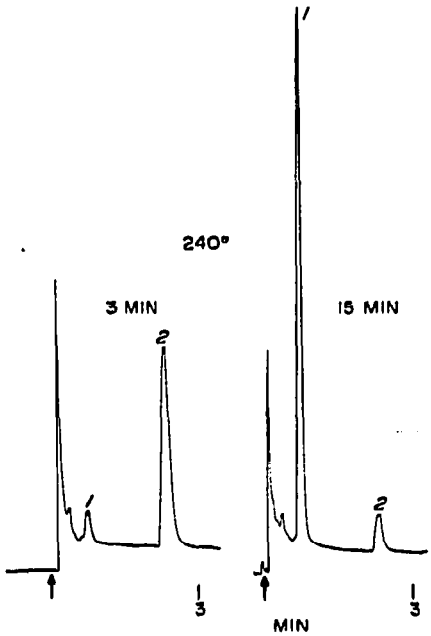


Fig. 5. Gas chromatograms resulting from the analysis of the trimethylsilylation reaction mixture of compound IV at 3 min and 15 min reaction times. Column conditions: 2 ft. × 1.5 mm I.D. spiral glass column; 7% SE-30 on acid-washed and silanized Gas-Chrom P; 240°; 30 ml/min helium.

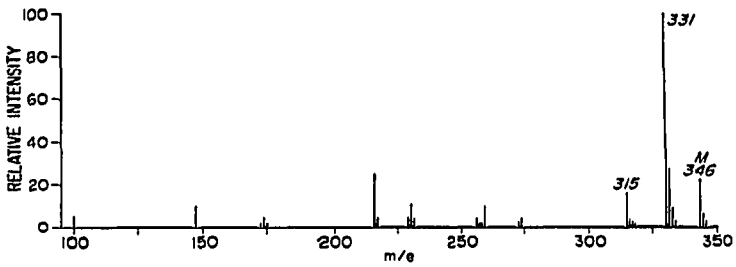


Fig. 6. Mass spectrum obtained by combined GLC–MS of the component in Fig. 5 with the shorter retention time. Spectrometer settings same as in Fig. 4.

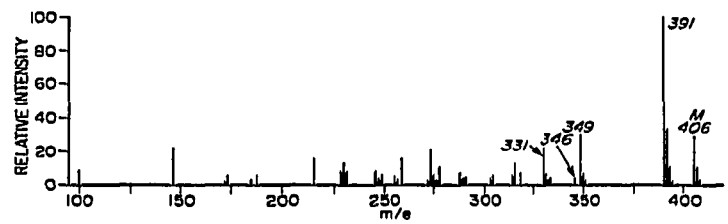
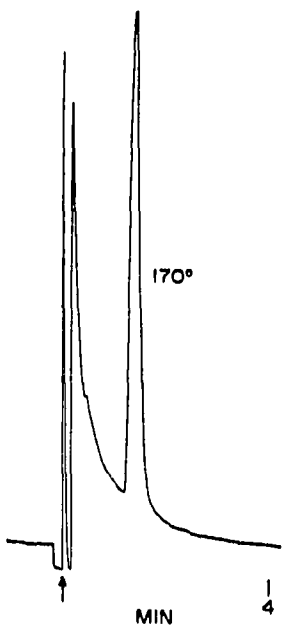


Fig. 7. Mass spectrum obtained by combined GLC–MS of the component in Fig. 5 with the longer retention time. Spectrometer settings same as in Fig. 4.

from IIc forms a di-TMSi derivative, it would appear that the isopropoxycarbonylamino group in di-TMSi IV is underivatized, with the TMSi groups on the imidazole and carboxamide group. This proposal is supported by the GLC–MS behavior of di-TMSi IV. The presence of the fragment ion involving loss of 42 mass units requires an underivatized isopropoxycarbonylamino group, as it is known that once the isopropoxycarbonylamino moiety is trimethylsilylated (conversion to the tri-TMSi derivative is complete within 45 min) it becomes thermally labile and rearranges during GLC via elimination of isopropyl TMSi ether (mol. wt. 132) to the corresponding isocyanate[18]. The compound of shorter retention time arises from the tri-TMSi derivative of IV [262 + (3 × 72); confirmed by use of BSA-d_{18}] via this thermal elimination of isopropyl TMSi ether. It should be possible by judicious choice of GLC conditions (*i.e.*, lower column and flash heater temperature) to reduce or avoid altogether this transformation, and with a 1 % OV-1 column operated at 170° (flash heater temperature of 200°) the tri-TMSi derivative of IV can be eluted intact (see Figs. 8 and 9).



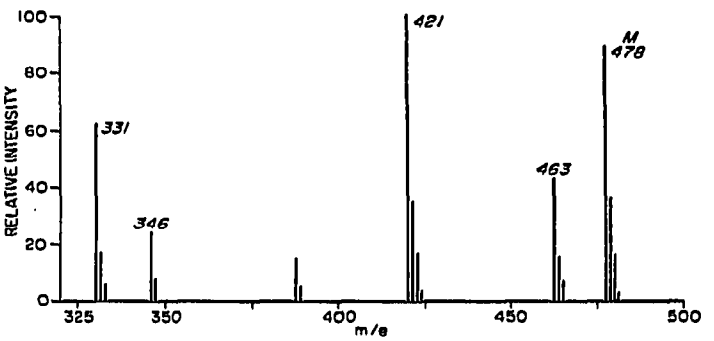


Fig. 8. Gas chromatogram resulting from the analysis of the trimethylsilylation reaction product of compound IV at 45 min reaction time. Column conditions: 1.5 ft. × 1.5 mm I.D. spiral glass column; 1 % OV-1 on 80–100 mesh Supelcoport; 170°; 30 ml/min helium.

Fig. 9. Mass spectrum obtained by GLC–MS of the compound eluted in Fig. 8.

Many compounds, including those which do not possess high electron capture affinity, can often be determined with high sensitivity and selectivity by use of a mass spectrometer as a GLC detector[20]. With a mass spectrometer equipped with an accelerating voltage alternator[21] or multiple ion detector[22] a heavy atom labeled analog of the compound of interest can be employed as carrier and internal standard in an isotope dilution assay[23–26]. A sample of IV was prepared in which the three hydrogen

atoms of the benzene ring were replaced by deuterium atoms. The partial mass spectrum of a mixture of this labeled compound and IV obtained by GLC–MS of the tri-TMSi derivatives analyzed at 240° is presented in Fig. 10; the M—15 ions (*m/e* 331 and 334) of the isocyanates possess high relative intensities and are good candidates for multiple ion detection and internal standard monitoring. Fig. 11 illustrates this quantitative MID approach for the fragment ions of *m/e* 331 (H) and 334 (D) from a 20 (H)/80 (D) mixture of the two compounds (240°); the total sample injected was 50 ng. Similarly the M, M—15 and M—(15 + 42) ions from the intact tri-TMSi are attractive for monitoring in combination with the corresponding deuterium-containing ions. The ability to follow the isotope ratio for the same injected compounds, but as two radically different eluted species, increases the selectivity of this approach. A third species could also be followed if desired: the di-TMSi of IV.

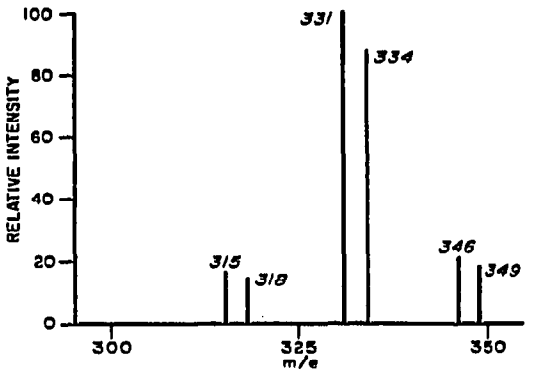


Fig. 10. Partial mass spectrum obtained by GLC–MS (240°, 7% SE-30 column) of a trimethylsilylated (45 min reaction time) mixture of IV and trideutero-IV.

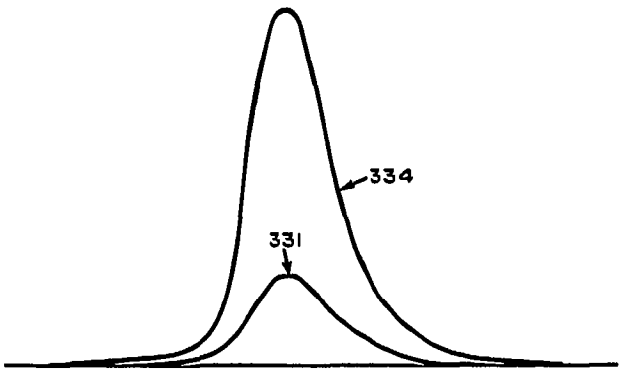


Fig. 11. Multiple ion detector monitoring of the ions *m/e* 331 and 334 from a 20:80 mixture of the compounds mentioned in Fig. 10 (same operating conditions).

The importance of a careful investigation into the chemistry occurring during a derivatization step and during the subsequent GLC is evident. Indeed, a better understanding of these may lead to a more simplified and/or rational approach to analysis. For example, knowledge that the ureido moiety of *p*-amino-*p'*-ureidodiphenylsulfone

is cleaved during heptafluorobutyrylation with concomitant conversion to the heptafluorobutyramide eliminates the necessity for a separate hydrolysis step. An understanding of the trimethylsilylation of IV and the thermal stability and GLC behavior of the resulting derivatives allows an increased latitude in choosing the preferred species to be monitored by MS detection. That derivatization plays a key role in GLC cannot be denied, and it occasionally plays a greater role than may be initially appreciated.

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