Combined Gas-Liquid Chromatography–Mass Spectrometry Study of Cambendazole and Related Compounds

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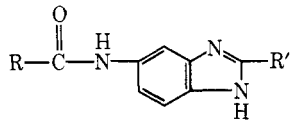
The behavior under gas–liquid chromatographic and mass spectrometric conditions of the anthelmintic cambendazole, 2-(4-thiazolyl)-5-isopropoxycarbonylamino-benzimidazole, and three of its urinary metabolites is reported. Data are also presented on the behavior of the trimethylsilyl derivatives. At column temperatures >200 °C, the isopropoxycarbonylamino group exhibits thermal instability, especially when transformed to its trimethylsilyl derivative, and undergoes an "on-column" transformation to the corresponding isocyanate. This thermally induced structural alteration parallels the electron-impact induced fragmentation observed in the mass spectra of these compounds. Structure determination based on combined gas-liquid chromatography–mass spectrometry of two urinary metabolites of cambendazole in the pig is discussed.

THE DEVELOPMENT of new drugs requires extensive metabolism studies, including the determination of structure of biotransformation products. Combined gas-liquid chromatography–mass spectrometry (GLC–MS) has proved to be a powerful method for obtaining such information (*1*). We now wish to discuss the behavior, under GLC conditions, of cambendazole [I, a new anthelmintic agent (*2*)] and related compounds. The structure determination of two urinary metabolites of I, studies in which combined GLC–MS proved to be especially valuable, is also reported.



I, R = $(CH_3)_2\overset{H}{C}-O$, R′ = 

II, R = $(CH_3)_2\overset{H}{C}-O$, R′ = H

III, R = $CH_3\overset{H}{C}(CH_2OH)-O$, R′ = 

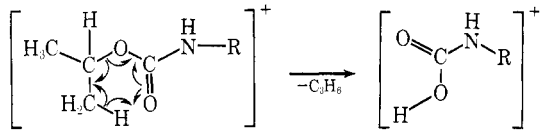
IV, R = CH_3, R′ = 

EXPERIMENTAL

The metabolites discussed in this paper are found, to varying extents, in each of the animal species investigated. Structure determination work was carried out, however, on urinary isolates from that species particularly rich in a given metabolite —i.e., II, calf; III and IV, pig. Urinary metabolite fractions were obtained via isolation procedures reported by Trenner *et al.* (*3*). Combined GLC–MS was effected with the LKB Model 9000. Column conditions were as follows: 2 ft × 2 mm i.d. glass spiral column; 1.8% OV-17 on 60–80 mesh acid-washed and silanized (*4*) Gas-Chrom P; flash heater 260 °C; column temperature 180, 200, or 240 °C; helium carrier gas (30 ml/min). Spectra were also obtained via a direct inlet (probe) technique. Spectrometer conditions included: source temperature, 270 °C; electron energy, 70 eV; accelerating voltage, 3.5 kV; trap current, 60 μA. Trimethylsilylation was carried out in tightly stoppered centrifuge tubes by treating the compound or isolate (10–20 μg) with pyridine and bis-trimethylsilylacetamide (BSA) (*5*) for 30 minutes at 60 °C.

RESULTS AND DISCUSSION

Two peaks are observed when cambendazole, I, is subjected to GLC (240 °C) (see Figure 1, left panel). The major, more slowly eluted component ("302") possesses a mass spectrum (Figure 2) fully compatible with that of the parent drug obtained by direct probe MS. Among the prominent signals are the molecular ion, *m/e* 302; M − 42, *m/e* 260, loss of C_3H_6 from the isopropoxycarbonylamino moiety probably via a McLafferty rearrangement as indicated below; M − 60, *m/e* 242, loss of the elements of isopropanol;



m/e 302 *m/e* 260

M − (42 + 44), *m/e* 216, loss of C_3H_6 and CO_2; M − (60 + 27), *m/e* 215, loss of C_3H_7OH and HCN (from the thiazole ring). Several of these fragment ions [*e.g.*, M − 42 and M − (42 + 44)] have been reported for isopropyl-*N*-phenylcarbamate (*6*). The minor, more rapidly eluted component ("242") has been shown by combined GLC–MS to possess a molecular ion of *m/e* 242 (see Figure 3). No M − 42 fragment ion is observed, and the fragment ion of greatest intensity is found at *m/e* 215, M − 27 (loss of HCN). The short retention time of "242" and the absence of fragmentations analogous to those found for "302" (involving the isopropoxycarbonylamino group) strongly suggest that this compound is a thermally-induced transformation product of I, the corresponding isocyanate, R—N=C=O. Zielinski

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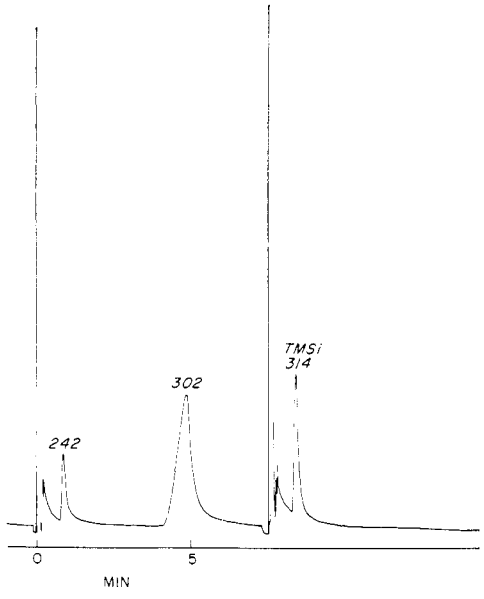
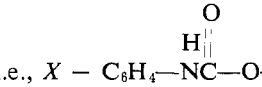


Figure 1. GLC behavior of cambendazole and its di-TMSi derivative at 240 °C

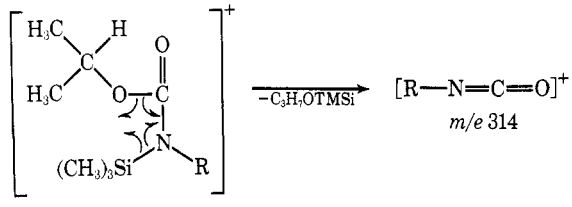
Peaks indicated as "242", "302", and "314" are identified in the text. Experimental conditions given in the Experimental section

et al. (*7*) have reported that aryl *N*-carbamoylaziridines are transformed during GLC to the corresponding aryl isocyanate (some of the aniline is also noted), and Spengler and Hamroll (*8*) have published on the GLC conversion of a number of carbamate herbicides to isocyanates by thermal elimination



of the alcohol—i.e., $X - C_6H_4-NC-O-R \rightarrow X-C_6H_4-N=C=O + ROH$). Fishbein and coworkers (*9*) have demonstrated that aryl-*N*-(*p*-tolyl)-carbamates undergo an "on-column" alteration with the elution of the respective phenols and *p*-toluidine; no isocyanates were observed. These workers attempted to stabilize the carbamates against thermal transformation by conversion to the trimethylsilyl (TMSi) derivative, but the derivatives were also observed to cleave.

GLC of the di-TMSi derivative of I (molecular ion of *m/e* 446 by direct probe MS) under the same column conditions as those for the parent drug resulted in the appearance of a single peak ("314," Figure 1, right panel). This component was shown by combined GLC–MS to possess a molecular ion of *m/e* 314 (Figure 4), not 446, a difference of 132 amu. M − 132, *m/e* 314, is an intense fragment ion (30%) in the direct probe mass spectrum of authentic di-TMSi-I, and can be ascribed to an electron impact induced loss of the elements of C_3H_7OTMSi to form the isocyanate ion:



The eluted compound resulting from the GLC of the di-TMSi of I was collected and subjected to IR spectrometric examination. No band was observed that could be ascribed to the presence of an isopropoxycarbonylamino group, but a strong band was present at 2260 cm^{-1}, entirely compatible with the presence of an isocyanate group. Rather than reducing the extent of GLC isocyanate formation, trimethylsilylation facilitates the thermally induced formation of the isocyanate. Rearrangement to the isocyanate is thus a preferred pathway, both under electron impact and GLC (thermal) conditions. [Elimination of ethanol from ethyl *N*-phenylcarbamate to form phenyl isocyanate has been reported to occur by electron bombardment and thermal degradation (in the heated inlet system of the mass spectrometer employed) (*10*).]

Cambendazole undergoes metabolic transformation on both the thiazole ring and the isopropoxycarbonylamino group (*3*). The metabolite 5-isopropoxycarbonylaminobenzimidazole, II, is devoid of the thiazole ring substituent.

Figure 3. Mass spectrum (GLC–MS) of the more rapidly eluted component ("242") in left panel of Figure 1

Experimental conditions given in the Experimental section

(7) W. L. Zielinski, Jr., L. Fishbein, R. O. Thomas, and T. E. Wilsko, *J. Chromatogr.*, **29,** 58 (1967).
(8) D. Spengler and B. Hamroll, *ibid.*, **49,** 205 (1970).
(9) L. Fishbein, W. L. Zielinski, Jr., and R. O. Thomas, *ibid.*, **30,** 596 (1967).

(10) C. P. Lewis, ANAL. CHEM., **36,** 1582 (1964).

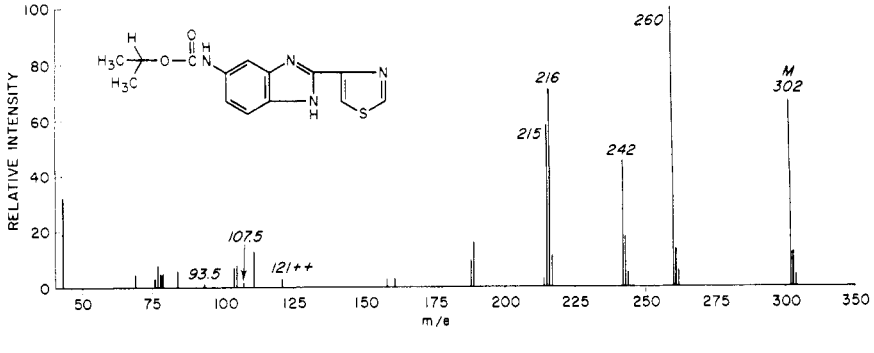


Figure 2. Mass spectrum (GLC–MS) of the more slowly eluted component ("302") in left panel of Figure 1

Experimental conditions given in the Experimental section

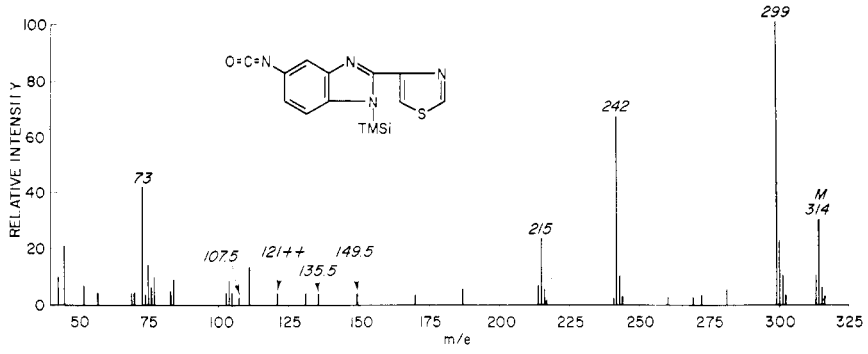
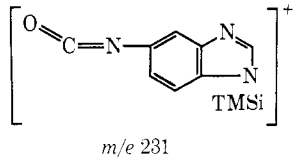


Figure 4. Mass spectrum (GLC–MS) of the component ("314") eluted in the right panel of Figure 1

Experimental conditions given in the Experimental section

This structure was established without the aid of combined GLC–MS, but the GLC behavior of II is worthy of consideration. As II is much lower in molecular weight than the parent drug, the GLC column temperature employed for its elution is approximately 60 °C lower than that used for I. Figure 5 shows that GLC of II results in the appearance of only one peak, characterized as unchanged II by combined GLC–MS—none of the corresponding isocyanate is observed. The base peak in the mass spectrum is M − (42 + 44), *m/e* 133; intense signals are also observed corresponding to M − 42 (*m/e* 177, 77%), the molecular ion (*m/e* 219, 55%), and M − 60 (*m/e* 159, 33%). The spectrum is very similar to that of I, except, of course, for no loss of HCN. GLC of the di-TMSi derivative of II (molecular ion *m/e* 363; characterized by direct probe MS), as with the di-TMSi of I, produces one peak (Figure 5), but in this case the compound eluted is the unaltered derivative, and not the isocyanate. Prominent ions include the molecular ion (*m/e* 363, 10%), M − 42 (*m/e* 321, 11%), M − (42 + 44) (*m/e* 277, 14%) and M − (42 + 44 + 15) (*m/e* 262, 16%). The second most intense fragment ion (the base peak is $(CH_3)_3Si$, *m/e* 73) is found at *m/e* 231, M − 132 (20%); this corresponds to the isocyanate



m/e 231

resulting from the loss of the elements of C_3H_7OTMSi. This transformation is still highly preferred under electron impact conditions, although at the lower column temperature, the thermally induced elimination fails to occur.

MS of cambendazole and metabolite II has disclosed that each of these compounds undergoes loss of C_3H_6 from the isopropoxycarbonylamino group. If this group is not present—e.g., in the isocyanate resulting from the thermally-induced GLC rearrangement—no loss of 42 amu is observed. We have isolated recently from the pig a previously unknown urinary metabolite of cambendazole—III. Combined GLC–MS disclosed only one component which possessed the same retention time and mass spectrum (molecular ion *m/e* 242) as the early eluted component from cambendazole. When the metabolite was trimethylsilylated and subjected to combined GLC–MS, a single peak was observed with the same retention time and mass spectrum (molecular ion *m/e* 314) as the isocyanate resulting from GLC of di-

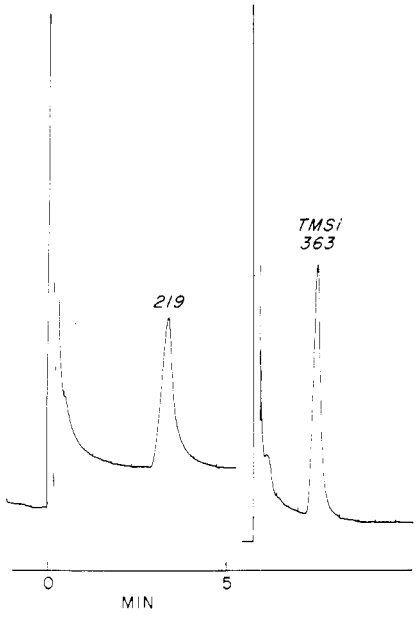


Figure 5. GLC behavior of metabolite II and its di-TMSi derivative at 180 °C

Experimental conditions given in the Experimental section

TMSi cambendazole. Direct probe MS of III disclosed a molecular ion of 318 amu, but no loss of 42 amu. Rather than M − 42, a strong signal was observed at M − 58 (*m/e* 260), and an intense signal was also found at M − 76 (*m/e* 242), but not M − 60. The *m/e* values for the fragmentation products of III are identical to those of cambendazole—the only difference is in the *m/e* of the molecular ion. Direct probe MS of the trimethylsilylated III indicates a molecular ion of *m/e* 534, demonstrating the presence of the three active hydrogen atoms in III. Rather than the M − 132 fragment (loss of C_3H_7OTMSi) noted with di-TMSi cambendazole, a fragment of M − 220 is observed (*m/e* 314). I and III differ by 16 amu, and high resolution MS has demonstrated conclusively that the difference is caused by an oxygen atom. The metabolite and its tri-TMSi derivative are transformed during GLC to compounds also formed by cambendazole and its di-TMSi derivative. As these transformations involve loss of the isopropyl group, the difference between I and II (an oxygen atom) must be associated with this group, and the structure shown at the end of the first paragraph was proposed for metabolite III. Comparison of the metabolite

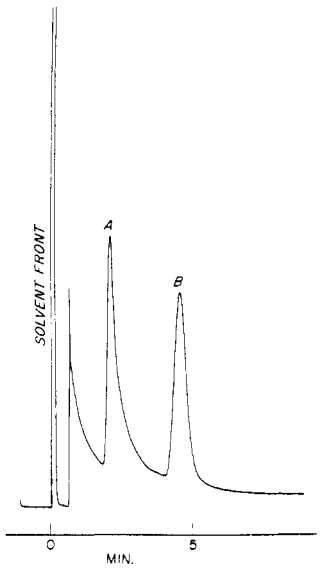


Figure 6. Comparison of GLC behavior (200 °C) of (in order of elution) the tri- and di-TMSi derivatives of metabolites III and IV (*A* and *B*, respectively)

The latter is eluted unaltered, whereas the former is converted to the corresponding isocyanate. Experimental conditions given in the Experimental section

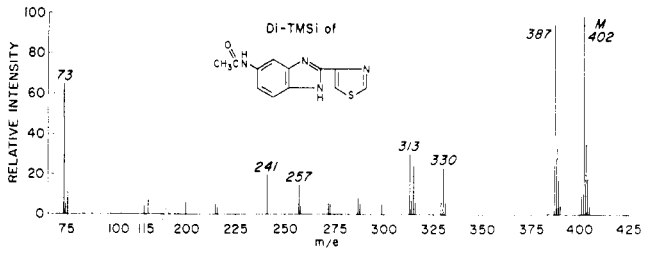


Figure 7. Mass spectrum (GLC–MS) of the di-TMSi derivative of metabolite IV

Experimental conditions given in the Experimental section

with authentic synthetic alcohol has conclusively proved the validity of the proposed structure.

A second unknown metabolite (IV) isolated from pig urine was found, both by combined GLC–MS and direct probe MS, to exhibit a molecular ion of *m/e* 258. The mass spectrum indicates ions resulting from familiar fragmentation—e.g., losses of 42 and 27 amu—suggesting at first the presence of both the isopropyl group and the thiazole ring, respectively. Because of the apparent molecular weight of the metabolite, however, this is unlikely. Further, no losses of 60 (C_3H_7OH) or 86 ($C_3H_6 + CO_2$) are observed in the mass spectrum. Under GLC conditions where the TMSi derivatives of I and III (di- and tri-, respectively) undergo the thermal elimination reaction to form the same isocyanate, the compound resulting from trimethylsilylation of IV is eluted (see Figure 6) without change—i.e., combined GLC–MS and direct probe MS give the same result. None of the mass spectral fragmentations (e.g., M − 132) expected for a trimethylsilylated isopropoxycarbonylamino group are observed (see Figure 7). This mass spectrum clearly shows that IV possesses two reactive groups (258 + (2 × 72) = 402). The above observations compel one to propose a metabolite structure not containing the isopropoxycarbonylamino moiety; however, a functional group which would lose 42 amu under electron impact, and also form a TMSi derivative, must be present. Exact mass measurement on the molecular ion of IV indicated an empirical formula of $C_{12}H_{14}ON_4S$, and a structure which fits all data is 2-(4-thiazolyl)-5-*N*-acetylamino-benzimidazole. This compound was prepared and found to exhibit GLC and MS (free and TMSi) properties corresponding exactly to those of metabolite IV.

The complementary nature of GLC and MS data, and the special synergism found when these two techniques are combined directly, is well recognized (*4*, *11*, *12*). This is especially true for studying compounds which are thermally labile and undergo transformation when subjected to GLC at elevated temperatures (*13*). Such molecular alterations are often looked upon as undesirable phenomena; however, behavior of this type, if recognized and understood, may actually contribute useful structural information.

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(12) J. A. McCloskey, in "Methods in Enzymology," Vol. XIV, "Lipids," J. M. Lowenstein, Ed., Academic Press, New York, N. Y., 1969.
(13) W. J. A. VandenHeuvel, *J. Chromatogr.*, **43**, 215 (1969).