METABOLISM OF NATURALLY OCCURRING PROPENYLBENZENE DERIVATIVES

II. SEPARATION AND IDENTIFICATION OF TERTIARY AMINOPROPIOPHENONES BY COMBINED GAS–LIQUID CHROMATOGRAPHY AND CHEMICAL IONIZATION MASS SPECTROMETRY

E. O. OSWALD, L. FISHBEIN, B. J. CORBETT AND M. P. WALKER

National Institute of Environmental Health Sciences, National Institutes of Health, Public Health Service and Department of Health, Education and Welfare, Research Triangle Park, N. C. 27709 (U.S.A.)

SUMMARY

A more simplified method of identification of the tertiary aminopropiophenones formed from various allylbenzene derivatives is reported. By use of combined gas–liquid chromatography and chemical ionization mass spectrometry, one is able to detect and verify the chemical structure of the tertiary aminopropiophenones of elemicin on nanogram quantities in the crude basic urine fraction. By use of this analytical method, it is concluded that upon oral administration of purified elemicin the rat excretes in urine the 3-N,N-dimethylamino-, the 3-piperidyl-, and the 3-pyrrolidinyl-1-(3′,4′,5′-trimethoxyphenyl)-propan-1-ones. These tertiary aminopropiophenones of elemicin seem to decompose more easily than those reported for safrole and myristicin.

INTRODUCTION

Substituted allyl- and propenylbenzene derivatives are widely distributed in the environment. These families of compounds have been identified in nutmeg[1], parsley[1], parsnip[2], carrots[3], bananas[4], black pepper[5], processed tobacco[6], and many other natural oils and flavoring agents with which individuals have daily contact.

One of these derivatives, safrole (1-allyl-3,4-methylenedioxybenzene), which at one time was widely used as a flavoring agent[7] in root beer, chewing gum, toothpaste and certain pharmaceutical preparations, has been shown to produce hepatic tumors in rats[8–10]. About 84 % of the aromatic fraction of nutmeg[11] is composed of myristicin (1-methoxy-2,3-methylenedioxy-5-allylbenzene), elemicin (1,2,3-trimethoxy-5-allylbenzene), and safrole. In general, myristicin has been considered in part to be responsible for the psychopharmacological activity of the total nutmeg extract[11].

An earlier report[12] from this laboratory described the production of basic ninhydrin-positive metabolites in the urine of rats and guinea-pigs after administration of myristicin, safrole, isosafrole, asarone (*trans*) or β-asarone (*cis*). Complete structural identification of the basic ninhydrin-positive metabolites of the allylbenzene derivatives, safrole[13], myristicin[14], and the presently reported elemicin and eugenol, indicate that these nitrogen-containing metabolites are tertiary aminopropiophenones and not amphetamines, as suggested earlier[1].

During the investigations concerning the production of these tertiary aminopropiophenones, this laboratory has reported the methodology as demanded by a particular research problem[12-14]. In most cases, a relatively large number of rats or guinea-pigs (twenty or more) were given specified quantities (5–500 mg/kg) of the allylbenzene derivatives for periods up to eight weeks. Once a sufficient amount of urine (1–3 l) was collected, the sample was extracted to yield the basic, acidic, and neutral fractions[12-14]. The basic fraction then was chromatographed by thin-layer chromatography (TLC) to verify the presence of the metabolites. After preparative TLC either prior to or in some cases followed by reduction with sodium borohydride, re-extraction and preparative TLC, the final metabolites and in most cases a major quantity of decomposition products were characterized. The chemical structures then were verified by UV and IR spectroscopy followed by direct probe electron impact mass spectrometry.

After these numerous manipulations, and if one has minimal decomposition, one is able to characterize fully these metabolites satisfactorily. On the contrary, as some of the research in this area dictates, more sensitive and desirable methodology is needed.

The present report describes the advantages of combined gas–liquid chromatography (GLC) and chemical ionization mass spectrometry (CIMS) as a qualitative tool for the metabolic characterization of the tertiary aminopropiophenones derived from elemicin and as discussed in a separate report for eugenol. The increased decomposition of these metabolites over those reported for safrole[13] and myristicin[14] demands that a more sensitive means be devised for these analyses.

EXPERIMENTAL

Materials

The synthetic elemicin was prepared from 0.30 moles of 4-allyl-2,6-dimethoxyphenol (Aldrich Chemical Co., Inc., Milwaukee, Wisc.), 0.30 moles of methyl iodide and 0.28 moles of anhydrous potassium carbonate in dry acetone (125 ml) under reflux for 24 h. Removal of the unreacted phenol by extraction of mixtures in ether with sodium hydroxide yielded 18–20 g of crude elemicin. This elemicin was purified by silicic acid column chromatography to a final purity of 99% or greater as determined by TLC, GLC, IR and NMR spectroscopy and MS.

The standard synthetic tertiary aminopropiophenone metabolites of elemicin were prepared from 3,4,5-trimethoxyacetophenone (Aldrich Chemical Co.), paraformaldehyde, and the appropriate secondary amine hydrochloride in ethanol by the Mannich reaction[15]: 3-N,N-dimethylamino-1-(3′,4′,5′-trimethoxyphenyl)-propan-1-one·HCl, m.p. 185–186°; 3-piperidyl-1-(3′,4′,5′-trimethoxyphenyl)-propan-1-one·HCl, m.p. 201–202°; and 3-pyrrolidinyl-1-(3′,4′,5′-trimethoxyphenyl)-propan-1-one·HCl, m.p. 189–191° in a 25–35% yield.

Methods

Purified elemicin was administered to male rats[18] orally (5–20 mg/kg). The urine was collected, stored, and extracted as described earlier for myristicin[14]. The basic urine fraction of elemicin was reduced immediately with sodium borohydride, re-extracted, and purified by preparative TLC in the methanol system[14].

Gas–liquid chromatography

Because of the general lability of all the previously reported metabolites[12-14], and especially because of the increased lability of the elemicin and eugenol metabolites, qualitative and only in a few instances semi-quantitative use will be made of the GLC methods.

For routine characterization of the basic urinary fraction or TLC purified urine basic fraction, a Tracor MT-220 gas chromatograph was employed. With this particular unit using an 0.6 × 184 cm glass column packed with 3 % OV-1
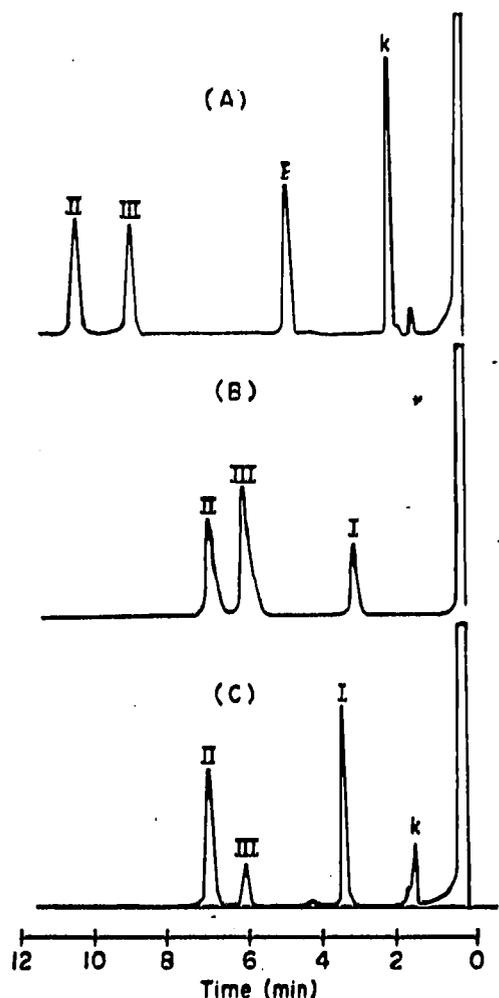

Fig. 1. (A) Gas chromatogram of tertiary aminopropiophenones formed from safrole. K = Allylic ketone; I = safrole I reduced; III = safrole III reduced; II = safrole II reduced. (B) Chromatogram of tertiary aminopropiophenones formed from myristicin. I = Myristicin I reduced; III = myristicin III reduced; II = myristicin II reduced. (C) Chromatogram of tertiary aminopropiophenones formed from elemicin. K = Allylic ketone; I = elemicin I reduced; III = elemicin III reduced; II = elemicin II reduced. All of the above chromatograms were obtained with the MT-220 gas chromatograph using the previously described standard conditions.

on Gas-Chrom Q (80–100 mesh) with a helium flow of 50 ml, injector temperature of 250°, and flame detector temperature of 250°, safrole and eugenol silylated metabolites were separated using the temperature program 150–180° with a temperature rate change of 5°/min. With the same column and flow-rate, the myristicin and elemicin metabolites were chromatographed at 180–210°, also with a temperature rate change of 5°/min (Fig. 1).

For combined GLC–CIMS, a Varian Aerograph Series 1400 gas chromatograph with a 152 × 0.2 cm I.D. glass column packed with 3 % OV-1 on Gas-Chrom Q (80–100 mesh) and interfaced to the Finnigan 1015C Quadrupole chemical ionization mass spectrometer with a measured methane flow of 15 ml/min through the GC column was used.

Routinely with the combined GC–MS system, the tertiary aminopropiophenones of elemicin after reduction with $NaBH_4$ were injected in an appropriate solvent (chloroform) volume (1–3 μl) on to the OV-1 column at 180° with the ionizer off. After 1 min the column temperature is programmed from 180–210° at a rate of 10°/min; simultaneously the ionizer is turned on and the computer control is initiated for collection of all desired spectral information.

The retention time of any of these derivatives is expressed as the time at which a particular peak reaches its maximum after zero time of injection.

Chemical ionization mass spectrometry

Unless otherwise stated, all of the mass spectra were obtained with a Finnigan 1015C chemical ionization mass spectrometer (Finnigan Instrument Co., Sunnyvale, Calif.) interfaced with a Varian Aerograph 1400 gas chromatograph. The mass spectrometer is controlled by a System 150 data collection system (System Industries, Sunnyvale, Calif.) composed of a PDP-8E, magnetic tape drive, plotter and interfacing hard and soft ware. The ion source conditions used are: 500 mA emission current; 70 V electron energy; 4 V ion accelerator voltage; 0–2 V ion repeller; and 10 V lens voltage with an applied 3000 V on the high-voltage power supply. Very important, as discussed later, the ionizer heater is off while maintaining a mass spectrometer manifold temperature of 175°, transfer line temperature of 210°, and an interface temperature of 225°. Under the above conditions, the ionizer temperature because of manifold heat transfer is only 50–75° maximally. With a maximum measured methane flow-rate of 15 ml/min, the ion source pressure is maintained at 1000–2000 μ with an overall pressure of 10^{-4} to 10^{-5} torr.

After injection of the desired sample in the specified solvent, the ionizer is maintained in the off position for a measured amount of time (1, 2, or 4 min as specified). At the given time intervals, the ionizer is turned on, and the data collection system is initiated to collect all desired information.

RESULTS AND DISCUSSION

Most of the earlier reports from this laboratory concerning the production of tertiary aminopropiophenones have been mainly concerned with structural identification of basic urinary metabolites with particular emphasis on the psychoactive properties of the initial natural products and/or metabolites. In addition, as reported for safrole and more recently as seen in this laboratory for other allyl-

benzene derivatives of this family, these allylbenzene derivatives produce tumors[8-10], general fibrosis, mass adhesions, liver degeneration and very abrupt pathological changes in experimental animals.

Methods have been developed as needed for the characterization of these nitrogen metabolites. Laborious as these earlier methods may seem, one can with sufficient samples fully characterize these urinary metabolites. On the contrary, the elemicin and especially the eugenol metabolites as discussed separately must be chemically exposed and manipulated through as few processes as possible in order to minimize decomposition. The small sample size requirement of GLC (nanograms to a few micrograms) combined with the greater capabilities of detecting the parent ion by CIMS in contrast to detection by electron impact MS makes the composite method a very satisfactory qualitative tool for characterization of the urinary nitrogen metabolites of elemicin and eugenol.

CIMS is a type of high-pressure MS in which the compound of interest interacts with the reactant or carrier gas ions. The reactant ions are formed by a combination of electron impact and ion-molecule recombination. The primary, secondary and tertiary reactant ions then combine with the desired sample and in this process transfer massive entities including protons (H^+), hydride ions (H^-) and alkylcarbonium ions (RCH_2^+) to the desired sample molecules. The reactant ions formed from methane which are mainly involved in this process are CH_5^+, $C_2H_5^+$ and $C_3H_5^+$. The amount of energy involved in chemical ionization reactions is relatively low, depending upon the reactant materials used. For a more detailed discussion on CIMS, the reader is referred to articles by MUNSON AND FIELD[16], MUNSON[17] and FALES et al. [18].

In order to simplify reference to the various tertiary aminopropiophenones in this discussion, the N,N-dimethyl derivative is designated I, the piperidyl derivatives II, and the pyrrolidinyl derivative III; therefore, 3-piperidyl-1-(3', 4', 5'-trimethoxyphenyl)-propan-1-one is elemicin II.

Prolonged exposure to air, alkali, silica gel or heat decomposes the tertiary aminopropiophenone to the allylic ketone plus the secondary amine[13,14]. The tertiary aminopropiophenones of elemicin and eugenol are much more labile than those of safrole or myristicin. Reduction of the carbonyl group of these metabolites decreases thermal decomposition.

As indicated by the reconstructed gas chromatogram of the chemical ionization mass spectrum (Fig. 2A), injection of the free base of elemicin II unreduced yields the unsaturated ketone with a retention time of 1 min 30 sec, and only a very trace of the original material in the form of the tertiary aminopropiophenone with a retention time of 6 min as indicated by the limited mass search (Fig. 2C.).

Earlier reports from this laboratory have characterized the tertiary aminopropiophenone by electron impact MS. As seen for safrole[13] and myristicin[14] in the direct probe electron impact spectra of these metabolites, the parent ion (M^+) is present in a low but detectable abundance (5-15 %). On the contrary, for elemicin and especially for the silylated eugenol derivatives, the parent ion (M^+) is present only in a very low abundance (0-1.5 %) even by direct probe analysis using 70 eV or 20 eV electron energy with the Finnigan 1015C electron impact mass spectrometer. Using relatively large quantities (2-10 μg) of the elemicin II reduced metabolite on the electron impact unit with combined GC-MS, the

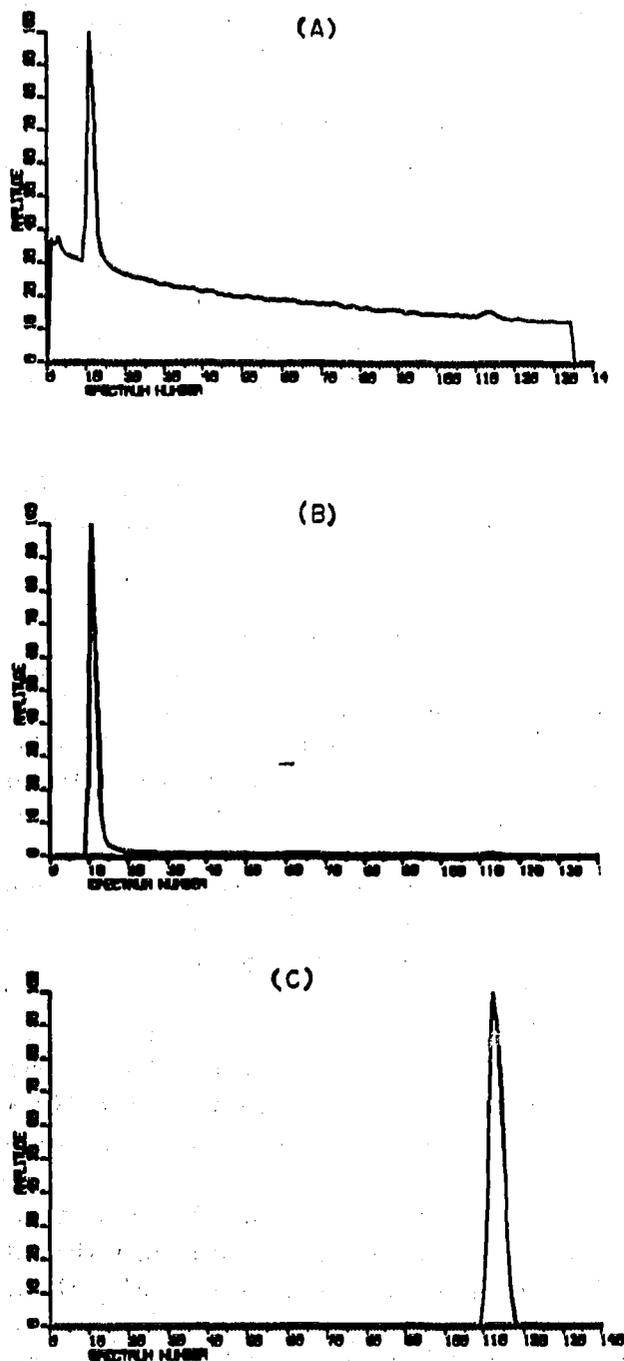Fig. 2. (A) Reconstructed chromatogram of the combined gas chromatograph–chemical ioniza-
tion mass spectrometer: 2 μg elemicin II unreduced (free base). (B) Reconstructed chromatogram
of 2A with a limited mass search m/e 222–224. Spectra 9–15 represent the major decomposition
product (3′,4′,5′-trimethoxyphenyl)-3-propen-1-one. (C) Reconstructed chromatogram of 2A
with a limited mass search m/e 337–339. Spectra 109–118 represent the intact unreduced
3-piperidyl-1-(3′,4′,5′-trimethoxyphenyl)-propan-1-one.

parent ion (M^+) in the region m/e 209 is detectable in a very low abundance
(0–1.0 %). Figs. 3 and 4 illustrate the characteristics of the piperidyl derivative
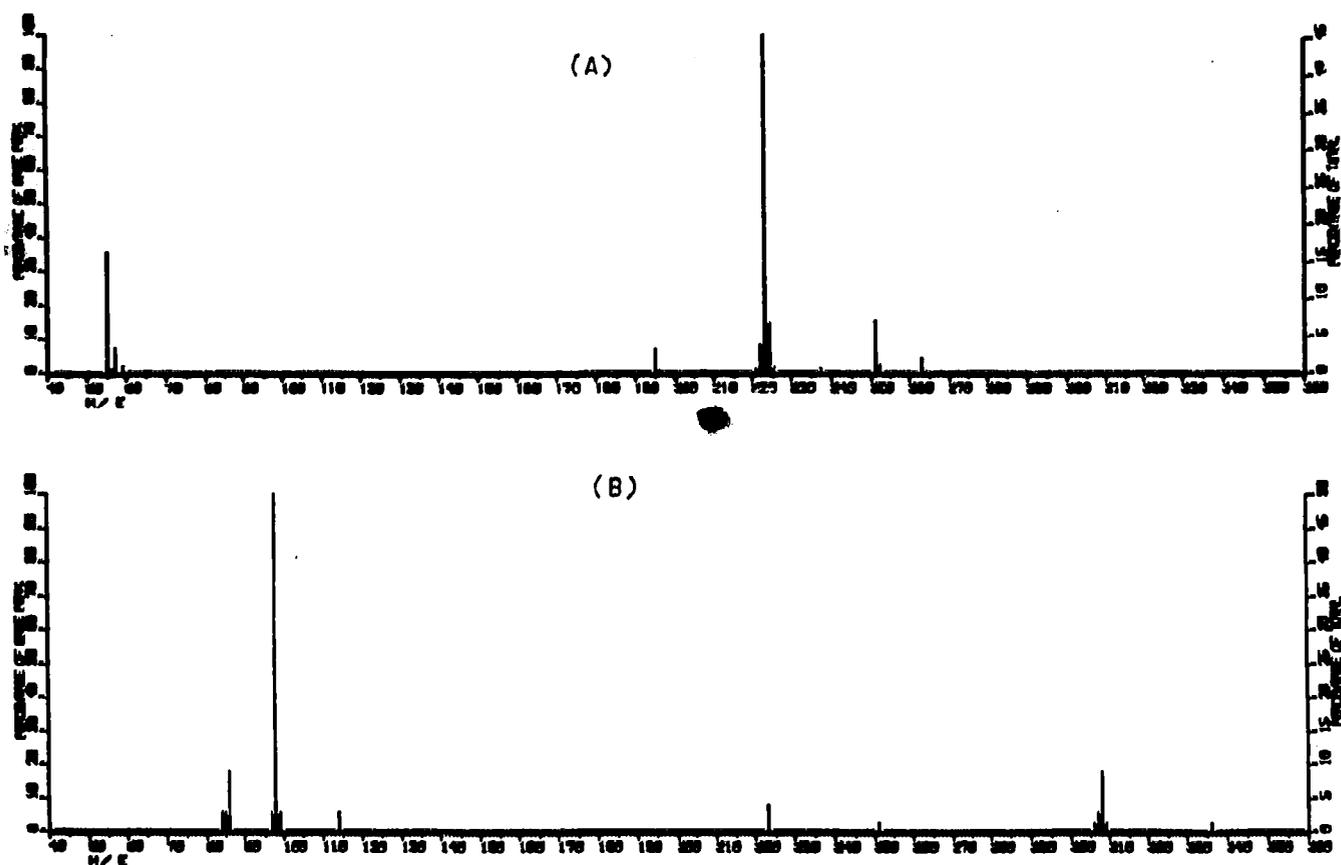of elemicin by CIMS and electron impact MS.

Fig. 3. (A) Chemical ionization mass spectrum of major decomposition product (3',4',5'-trimethoxyphenyl)-3-propen-1-one (Fig. 2B, spectrum 11). (B) Chemical ionization mass spectrum of intact unreduced 3-piperidyl-1-(3',4',5'-trimethoxyphenyl)-propan-1-one (Fig. 2C, Spectrum 113).

The allylic ketone (Fig. 3A) formed by the *in situ* thermal decomposition of the unreduced elemicin II on GC has a base peak quasi-molecular ion of m/e 223 ($M + 1$) on chemical ionization with the expected recombination fragments m/e 237 ($M + CH_3^+$), 251 ($M + C_2H_5^+$) and 263 ($M + C_3H^+_3$). As indicated in Figs. 3B, 4A and 4B, the intact elemicin II under all experimental conditions has a base peak of 98 as seen for the piperidyl derivatives of other allylbenzenes[13,14]. The trace of unreduced elemicin II which remained intact (Fig. 2C) has a base peak of 98, a very abundant (17%) quasi molecular ion of 308 ($M + 1$) (Fig. 3B) with a recombination fragment m/e 136 ($M + C_2H_5^+$) and less abundant m/e 251 and 223 fragments common to the allylic ketone.

In Fig. 4A, the electron impact spectrum of the elemicin II reduced metabolite has a base peak of m/e 98 and less abundant 112 and 198 fragment with the parent ion m/e 309 (M^+) present only in 0.33 % abundance. In most cases, the chemical ionization spectra are much more simplified. For the elemicin II reduced (Fig. 4B), the base peak is m/e 98 with a very abundant (22%) quasi-molecular ion m/e 310 ($M + 1$) with less abundant 338 ($M + C_2H_5^+$) and very weak 350 ($M + C_3H_5^+$) fragments.

Using a single preparation with serial dilutions, one is able to detect and identify using combined GC–CIMS a total quantity as injected on the gas chromato-
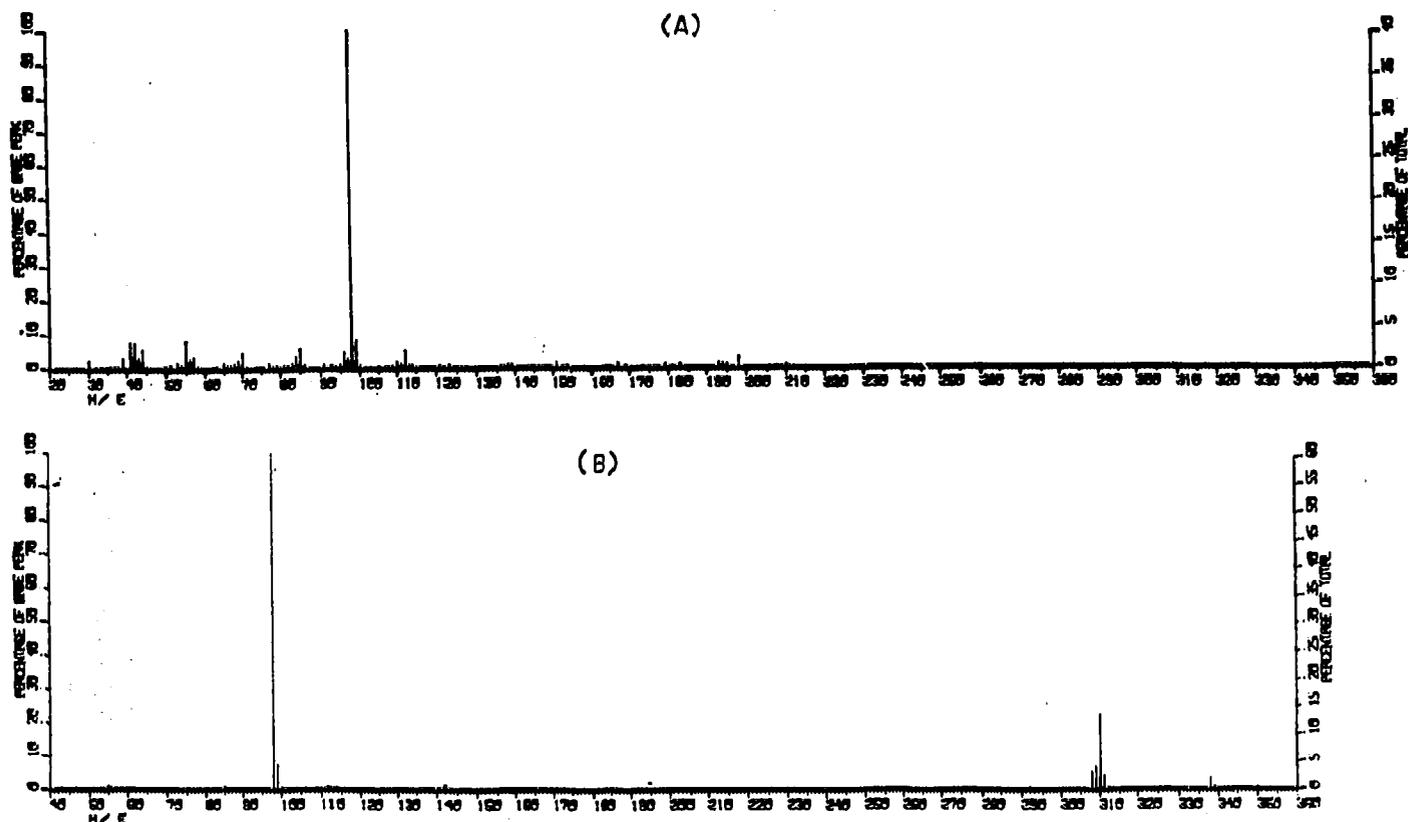
Fig. 4. (A) Electron impact mass spectrum of $NaBH_4$ reduced 3-piperidyl-1-(3',4',5'-trimethoxy-phenyl)-propan-1-one, 5 μg. (B) Chemical ionization mass spectrum of $NaBH_4$ reduced 3-piperidyl-1-(3',4',5'-trimethoxyphenyl)-propan-1-one, 5 μg.

graph in the range of 100 ng with the synthetic elemicin II reduced having a retention time of 6 min. Routinely 100–500 ng of material is used to confirm the recombination fragments ($M + C_2H_5^+$) and the less abundant ($M + C_3H_5^+$).

Preliminary characterization of the elemicin metabolites as initiated using a stainless-steel column. As this column became "aged", one would notice increased decomposition to the stage that only decomposition took place. At that time glass columns replaced the stainless-steel one and yielded only minimal decomposition.

In order to complete the structural verification of the basic urinary metabolites and also to improve the required methodology for this area of research, six male rats were given orally twice weekly for two weeks the synthetic purified elemicin (5 mg/kg). The total urine collected for this time period (about 700 ml) was extracted to yield the basic urinary fraction which was immediately reduced with $NaBH_4$. This elemicin rat basic fraction was then concentrated approximately 5000-fold. One half of this basic fraction was purified by preparative TLC[14] to yield purified fractions (1–3 and 2) which were further concentrated to a final 10,000-fold over the original urine. By TLC most of the desired metabolites had already decomposed. The remaining one-half of the crude basic urinary fractions was concentrated to a final 10,000-fold over the original urine. Aliquots (1–4 μl) of each of these preparations were then characterized by combined GLC–CIMS.

Semi-quantitative estimates of the above metabolite fractions indicate that the tertiary aminopropiophenone metabolites are present in the nanogram per
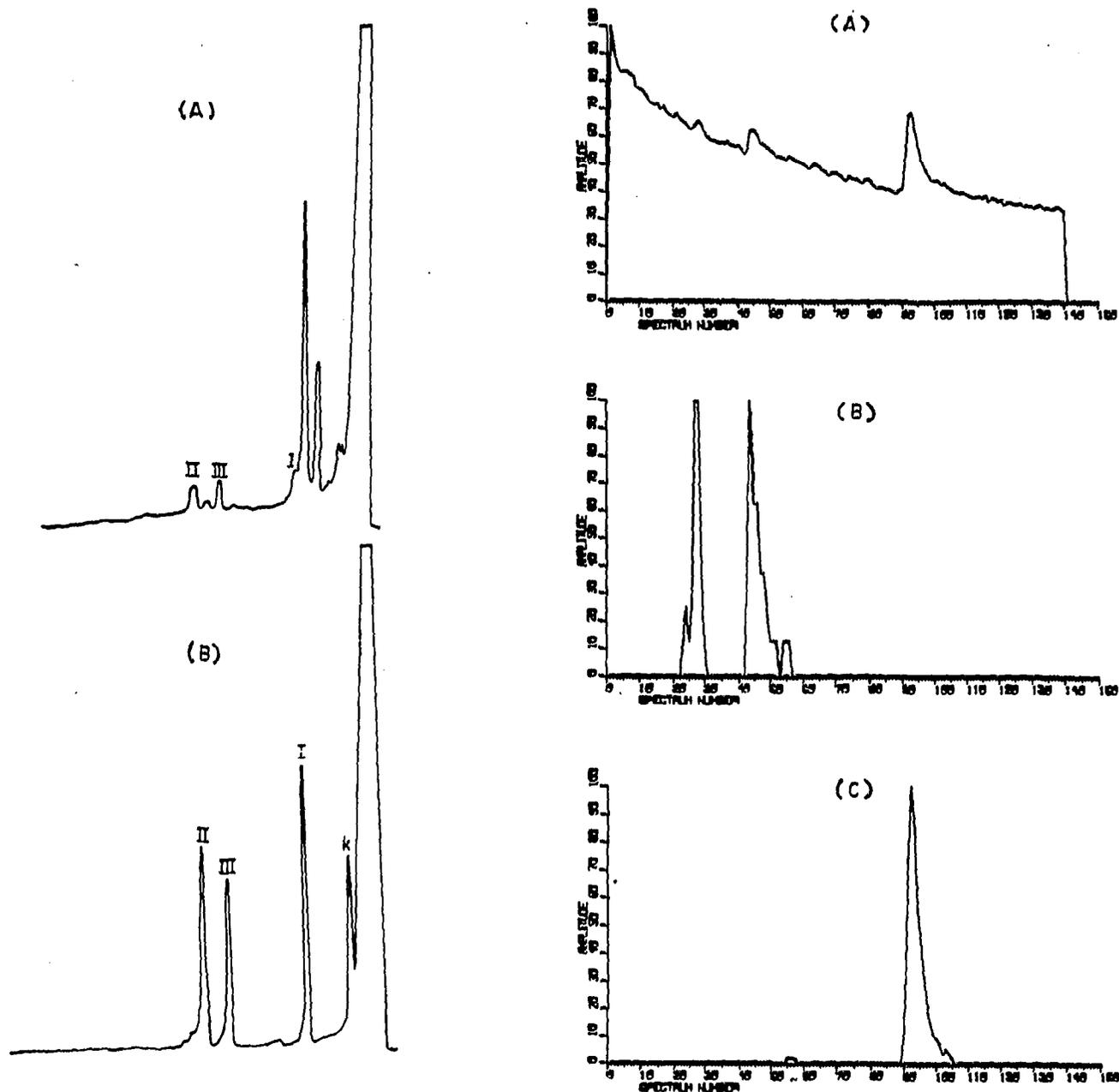
Fig. 5. (A) Gas chromatogram of crude rat urine basic fraction prior to GC–CIMS analyses using Tracor MT-220 unit (8 μg total sample). (B) Gas chromatogram of standard synthetic elemicin reduced mixture I, II and III using Tracor MT-220 (1.5 μg total). K = Allylic ketone; I = elemicin I reduced; III = elemicin III reduced; II = elemicin II reduced.

Fig. 6. (A) Reconstructed chromatogram of the combined gas chromatograph–chemical ionization mass spectrometer for TLC purified elemicin rat urinary metabolite 1–3. (B) Reconstructed chromatogram of 6A with limited mass search m/e 269–271. (C) Reconstructed chromatogram of 6A with limited mass search m/e 295–297.

microliter range. It must be realized that this estimate is very crude. There is no satisfactory way of estimation of the decomposition that has taken place during the extraction, the reduction, and during other manipulations prior to analyses.

In the case of the TLC purified fractions, the metabolites are almost entirely decomposed and existed in the low nanogram range (10 to 100 ng/μl). On the contrary, the crude reduced basic fraction has not decomposed because of the minimized manipulation; therefore, the tertiary aminopropiophenones existing at the time of analyses seem to be present in the 100 to 1000 ng range. Fig. 5A represents the crude rat basic fraction prior to analyses by MS with the appropriate synthetic reduced elemicins I, II and III (5B). Fig. 6A is the reconstructed chromatogram of the TLC purified urinary metabolite fractions 1–3 of elemicin by combined GC–CIMS. As indicated earlier, very little intact metabolite is present after preparative TLC. With the assistance of the computer, a limited mass search indicates that there are two materials with a mass fragment m/e 269–271 (Fig. 6B). One of these materials has a retention time of about 2 min; the second material with a mass 269–271 has a retention time of 2 min 45 sec. The standard elemicin I — (N,N-dimethylamino-) — derivative has a retention time of 2 min 45 sec. The material from rat urine with a retention time of 2 min 45 sec (spectrum 44) has a base peak of 58, a quasi-molecular ion $(M + 1)$ of m/e 270 and the appropriate $(M + C_2H_5^+)$ ion of 299; this spectrum is identical to that of the synthetic reduced 3-N,N-dimethylamino-1-(3',4',5'-trimethoxyphenyl)-propan-1-one. In addition, with the limited mass search m/e 295–297 (Fig. 6C), the material present in the rat urine having a retention time of 4 min 45 sec, base peak of 84, an $(M + 1)$ fragment of 296 and a recombination fragment $(M + C_2H_5^+)$ of 324 and $(M + C_3H_5^+)$ of 336 is concluded to be chemically equivalent to elemicin III reduced.

Fig. 7A is the reconstructed chromatogram of the GC–CIMS system for the elemicin rat TLC purified fraction 2. The metabolite has a retention time of 5 min 45 sec. As illustrated also by Fig. 7B of the limited mass search m/e 309–311, base peak of m/e 98, $(M + 1)$ fragment 310, and recombination fragment $(M + C_2H_5^+)$ of 338, the elemicin rat TLC fraction 2 is identical to the synthetic elemicin II reduced (see Fig. 4B).
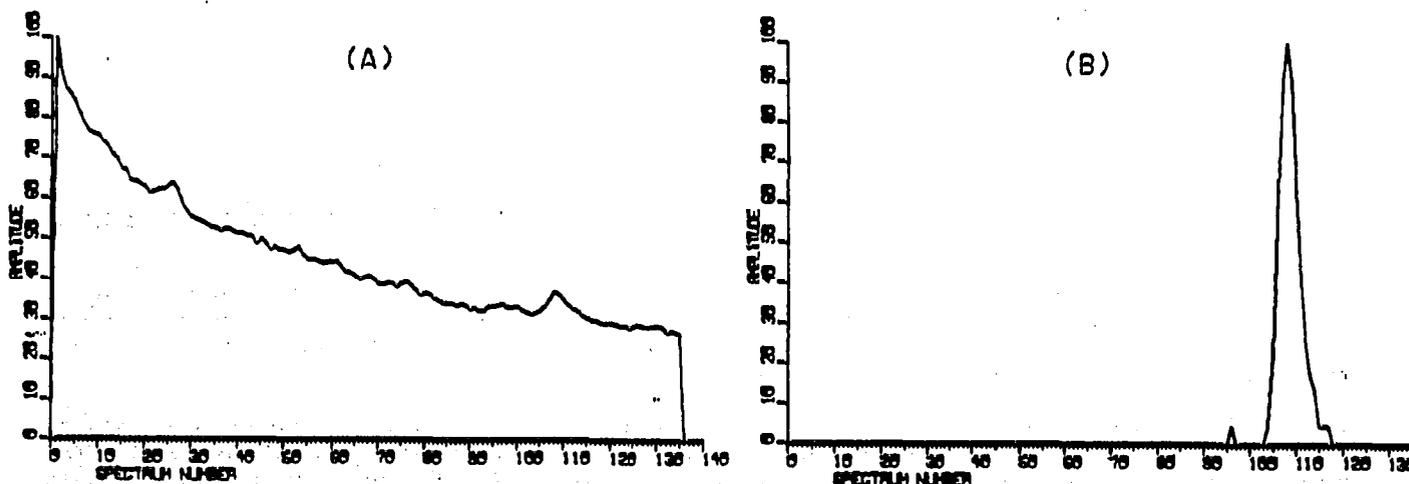

Fig. 7. (A) Reconstructed chromatogram of the combined gas chromatograph-chemical ionization mass spectrometer of TLC purified elemicin rat urinary metabolite fraction 2. (B) Reconstructed chromatogram of 7A with a limited mass search m/e 309–311.

With the capabilities of the System 150 data acquisition system coupled with the combined gas chromatograph–chemical ionization mass spectrometer,

one is able to analyze very satisfactorily the crude reduced urinary basic fraction. Fig. 8A represents the reconstructed gas chromatogram of the chemical ionization mass spectrometer. The sample was injected at zero time (180°) with the ionizer off; after 1 min the ionizer is turned on, the temperature program is begun and the computerized spectral collection is initiated. The largest peak in this chromato-
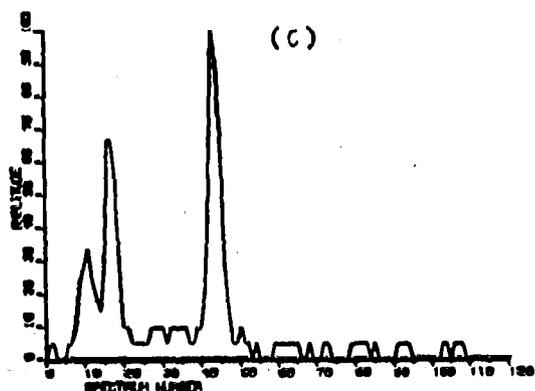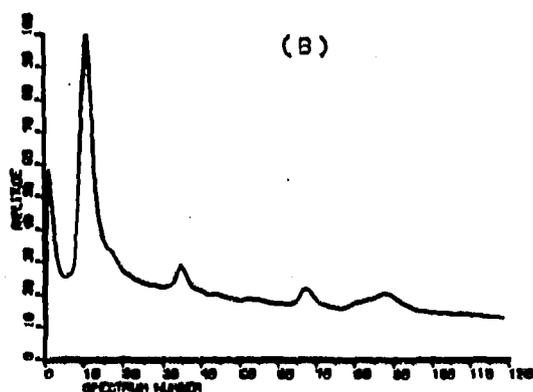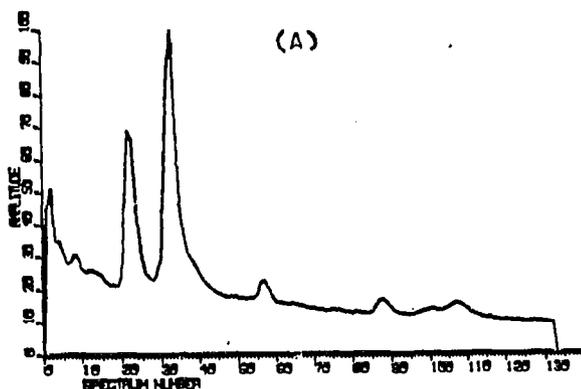

Fig. 8. (A) Reconstructed chromatogram of the combined gas chromatograph–chemical ionization mass spectrometer of the crude elemicin rat basic urine fraction — ionizer initiated 1 min after injection (8 μg total material). (B) Reconstructed chromatogram of the combined gas chromatograph–chemical ionization mass spectrometer of the crude elemicin rat basic fraction — ionizer initiated 2 min after injection (8 μg total material). (C) Reconstructed chromatogram of 8B with limited mass search m/e 269–271.

gram (Fig. 8A), spectrum number 30–45, is made up of at least two components with a retention time of 2 min 15 sec to 3 min. The elemicin I metabolite makes up the later shoulder of this peak with a retention time of 2 min 45 sec. By injection of the sample at zero time (180°), then after 1 min by beginning the temperature program, and finally after 2 min by initiating the ionizer and computer, one is able to intensify the presence of the elemicin I shoulder with a retention time of 2 min 45 sec (Fig. 8B); however, mass spectra taken in this region will contain fragments of the larger peak. A limited mass search of the spectra collected after 2 min with the ionizer off indicates that the shoulder with a retention time of 2 min 45 sec does have a mass fragment m/e 269–271 (Fig. 8C). On the contrary, a very trace contaminant with a retention time of 4 min 15 sec (spectrum number 37–47) also has a mass fragment m/e 269–271.

The above-described results indicate that because of normal contaminants of rat urine, the presence of elemicin I reduced cannot be established solely by use of GC using the crude reduced basic urine fraction. For this particular metabolite, elemicin I, some preliminary purification must be used to remove the normal existing contaminants prior to analysis. Separation of the normal substituents from the elemicin I metabolite is possible by use of more polar GC columns (OV-17 or OV-225); however, under these new conditions an increased temperature requirement is demanded (> 230° to chromatograph all three metabolites). The increased temperature requirement also increases thermal decomposition without net advantage. As indicated earlier, the contaminating material which chromatographs in the region of elemicin I (Fig. 8A) can be removed by TLC or micro-column chromatography; during this purification procedure, decomposition is also increased. In addition, partial removal of this normal urinary contaminant of elemicin I can be accomplished by re-extraction through the acid, neutral and basic sequence; however, complete removal by any one means without undesirable major decomposition has not been accomplished.

Additional means of intensifying the spectra collected with a retention time of 4 min or greater are also possible. Injection of the sample at zero time, 180°, followed after 1 min by the temperature program sequence, and finally by initiation of the ionizer and computer after 4 min will intensify the region of elution of elemicin III and elemicin II metabolite (Fig. 9). Spectra 17–24 (Fig. 9A) represent the elemicin metabolite III and spectra 36–44 represent the elution profile elemicin II metabolite. Care should be taken as to the choice and subtraction of background spectra and choice of other computer manipulations as one approaches the low nanogram range (Fig. 9A). At this concentration range the limited mass search becomes very desirable. Figs. 9B and 9C illustrate the computer analyses of the spectra collected from the crude basic elemicin rat urine — with the ionizer off for 4 min after injection.

By controlling the time of initiation of the ionizer one can obtain representative spectra of these materials in the 10–100 ng range, depending upon the complexity of the mixture. It is conceivable that one may be able to detect picogram quantities of these materials with a controlled ionizer time and careful GC conditions coupled with CIMS. In most cases, uncontrollable parameters governing decomposition of the tertiary aminopropiophenones will limit the detection capabilities to nanogram quantities.
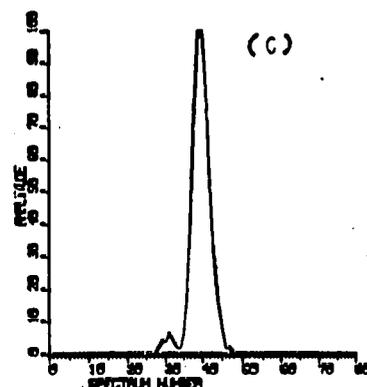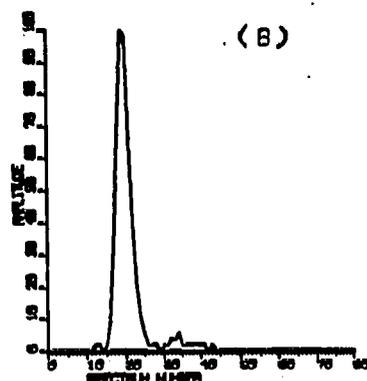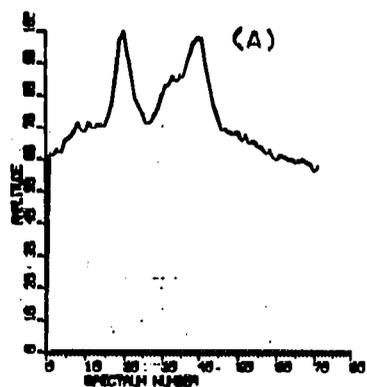
Fig. 9. (A) Reconstructed chromatogram of the combined gas chromatograph-chemical ionization mass spectrometer of the crude elemicin rat basic fraction — ionizer initiated 4 min after injection (8 μg total material). (B) Reconstructed chromatogram of 9A with a limited mass search m/e 295–297. (C) Reconstructed chromatogram of 9A with a limited mass search m/e 309–311.

Representative chemical ionization spectra collected 4 min after injection (Fig. 10) indicate that one can verify the presence of elemicin III and elemicin II metabolites in the crude rat urine reduced basic fraction (Figs. 8 and 9) by combined GC–CIMS on nanogram levels of these metabolites.

By use of combined GLC–CIMS one can very satisfactorily characterize the tertiary aminopropiophenones formed from the various allylbenzene derivatives.
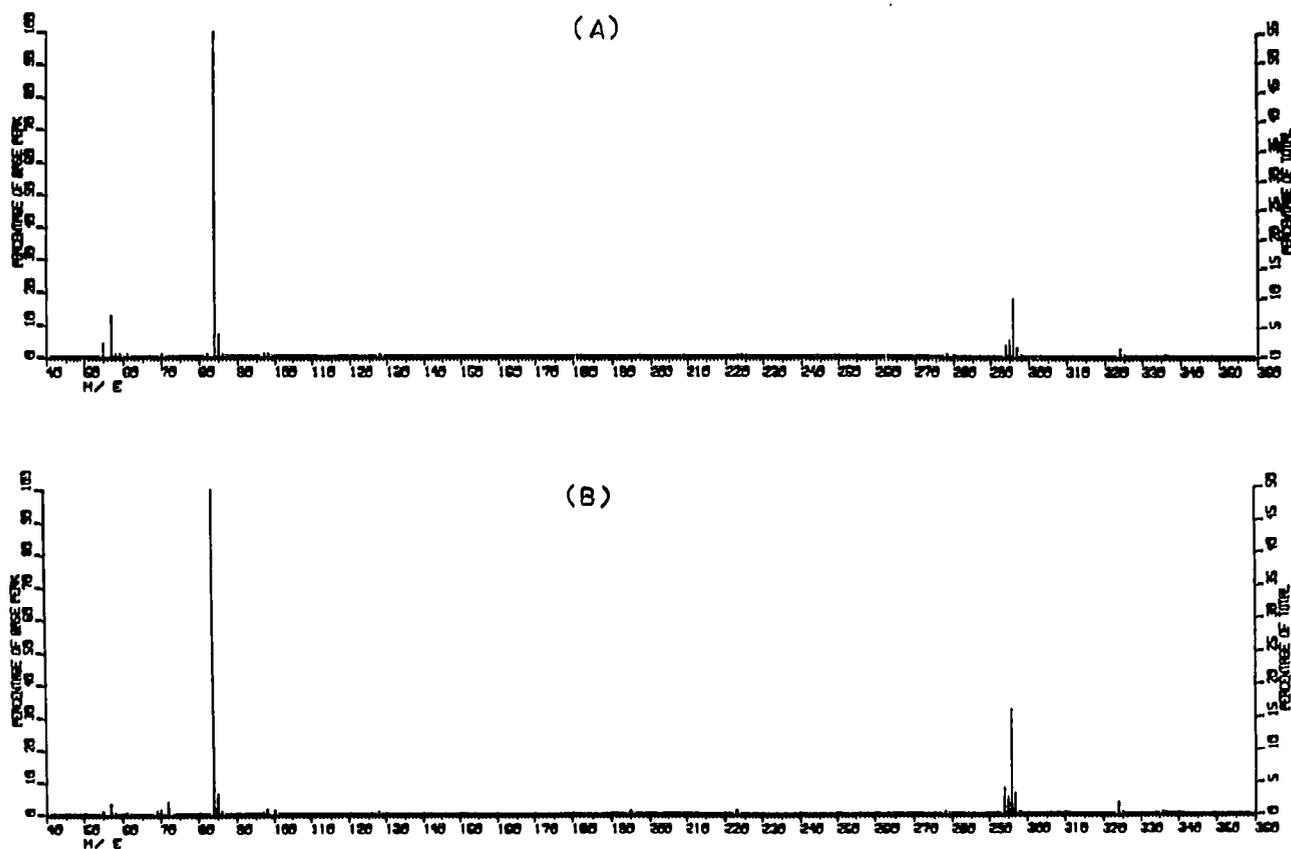
Fig. 10. (A) Chemical ionization mass spectrum from the crude rat elemicin reduced basic fraction (Fig. 9A Spectrum 19) — 3-pyrrolidinyl-1-(3', 4', 5'-trimethoxyphenyl)-propan-1-one. (B) Chemical ionization mass spectrum of synthetic standard elemicin III reduced — 3 pyrrolidinyl-1-(3', 4', 5'-trimethoxyphenyl)-propan-1-one.

The varying degree of decomposition of these nitrogen metabolites seems to be the major limiting factor in the analyses of these metabolites.

REFERENCES

1 A. T. SHULGIN, Nature, 210 (1966) 380.
2 E. P. LICHTENSTEIN AND J. E. CASADA, J. Agr. Food Chem., 11 (1963) 410.
3 R. G. BUTTERY, R. M. SEIFERT, D. G. GUADAGNI, D. R. BLACK AND L. C. LING, J. Agr. Food Chem., 16 (1968) 1009.
4 P. ISSENBERG, H. E. NURSTEN AND E. L. WICK, 1st Int. Congr. Food, Sci. Technol., Gordon and Breach, New York, 1964, p. 467.
5 G. F. RUSSEL AND W. G. JENNINGS, J. Agr. Food Chem., 17 (1969) 1107.
6 I. SCHMELTZ, C. J. DOOLEY, R. L. STEDMAN AND W. J. CHAMBERLAIN, Phytochemistry, 6 (1967) 33.
7 L. FISHBEIN AND H. L. FALK, Environ. Res. 2 (1969) 297.
8 F. HOMBURGER, T. KELLEY, JR., G. FRIEDLER AND A. B. RUSSFIELD, Med. Exp., 4 (1961) 4.
9 E. L. LONG, A. A. NELSON, O. G. FITZHUGH AND W. H. HANSON, Arch. Pathol., 75 (1963) 595.
10 E. C. HAGAN, P. M. JENNER, W. I. JONES, O. G. FITZHUGH, E. L. LONG, J. G. BROUWER AND W. K. WEBB, Toxicol. Appl. Pharmacol., 7 (1965) 18.
11 D. H. EFRON, B. HOLMSTEDT AND N. S. KLINE, Ethanopharmacologic Search for Psychoactive Drugs (Proc. Symp. San Francisco, Calif., 1967), Public Health Service, Publ. No. 1645 (1967) p. 185.
12 E. O. OSWALD, L. FISHBEIN AND B. J. CORBETT, J. Chromatogr., 45 (1969) 437.

13 E. O. Oswald, L. Fishbein, B. J. Corbett and M. P. Walker, *Biochim. Biophys. Acta*, 230 (1971) 237.
14 E. O. Oswald, L. Fishbein, B. J. Corbett and M. P. Walker, *Biochim. Biophys. Acta*, 244 (1971) 322.
15 E. C. Horning (Editor), *Organic Syntheses, Collective*, Vol. III, Wiley, New York, 1955, p. 305.
16 M. S. B. Munson and F. H. Field, *J. Amer. Chem. Soc.*, 88 (1966) 2621.
17 M. S. B. Munson, *Anal. Chem.*, 43 (1971) 29A.
18 H. M. Fales, G. W. A. Milne and T. Axenrod, *Anal. Chem.*, 42 (1970) 1432.