JOURNAL OF MASS SPECTROMETRY, VOL. 33, 346–357 (1998)

Mass Spectrometric and Pyrolytic Behavior of Some Arylsubstituted Isoxazolines

P. Dallakian, H. Benoni, M. Schäfer and H. Budzikiewicz*
Institut für Organische Chemie, Universität zu Köln, Greinstrasse 4, D-50939 Köln, Germany

The synthesis and mass spectrometric (MS) and pyrolytic behavior of three 3,5-aryl-substituted isoxazolinyl acetophenones, 2-[4,5-dihydro-5-(4-methoxyphenyl)-3-phenyl-5-isoxazolyl]-1-phenylethanone (I), 2-[4,5-dihydro-5-(4-methoxyphenyl)-3-phenyl-5-isoxazolyl]-1-(4-methylphenyl)ethanone (II) and 2-[4,5-dihydro-5-(4-methoxyphenyl)-3-(4-methylphenyl)-5-isoxazolyl]-1-phenylethanone (III) are discussed. Compound I and the mixture of the isomeric compounds II and III were prepared by the reaction of 2,4,6-arylpyrylium salts with hydroxylamine. The substances were investigated using electron ionization, positive chemical ionization with methane and fast atom bombardment. The isomeric compounds II and III were separated by gas chromatography (GC)/mass spectrometry. MS/MS and high-resolution MS were used to establish the fragmentation mechanisms. The thermal decomposition products obtained by off-line pyrolysis/GC/MS of I–III were compared with the MS fragments. The results of the investigation will facilitate the detection and identification of 3,5-aryl-substituted isoxazolinyl acetophenones in plant extracts. © **1998 John Wiley & Sons, Ltd.**

J. Mass Spectrom. **33**, 346–357 (1998)

KEYWORDS: aryl-substituted isoxazolines; comparison of mass spectral techniques; pyrolysis

INTRODUCTION

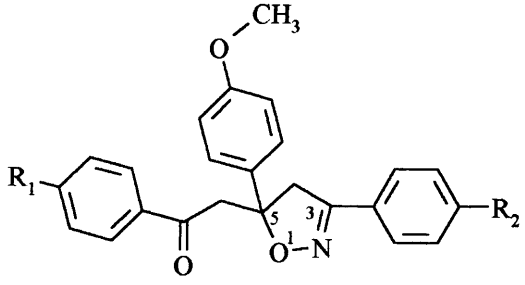
Isoxazoline rings are present in only a few pharmacologically interesting natural products, such as the antibiotic cycloserine,[1] dihydroibotenic acid/dihydromuscimol[2,3] from fly agaric (*Amanita muscaria*) and the 5-oxoisoxazoline derivatives isolated from the seeds of several Leguminosae (*Pisum sativum*, *Lathyrus odoratus*).[4] All these natural isoxazolines carry small substituents as alkyl or OH groups in positions 3 and 5. Isoxazolines such as 2-[4,5-dihydro-5-(4-methoxyphenyl)-3-phenyl-5-isoxazolyl]-1-phenylethanone (**I**), 2-[4,5-dihydro-5-(4-methoxyphenyl)-3-phenyl-5-isoxazolyl]-1-(4-methylphenyl)ethanone (**II**) and 2-[4,5-dihydro-5-(4-methoxyphenyl)-3-(4-methylphenyl)-5-isoxazolyl]-1-phenylethanone (**III**), which are aryl- or phenacyl-substituted in these positions, have so far not been found in nature. By analysing extracts of leaves from bog myrtle (*Myrica gale* L.) using fast atom bombardment (FAB) and high-resolution mass spectrometry (MS) we found a compound which probably is **II** or **III**. For identification purposes we synthesized **I–III** and investigated their mass spectrometric and pyrolytic behavior.

* Correspondence to: H. Budzikiewicz, Institut für Organische Chemie, Universität zu Köln, Greinstrasse 4, D-50939 Cologne, Germany

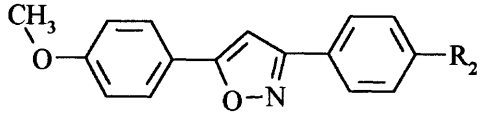
EXPERIMENTAL

Instrumentation

MS and gas chromatographic (GC)/MS measurements were performed with a Finnigan Incos 500 quadrupole mass spectrometer (Finnigan MAT, San Jose, CA, USA) coupled with a Varian Model 3400 gas chromatograph



I - $R_1 = R_2 = H$
II - $R_1 = CH_3$, $R_2 = H$
III - $R_1 = H$, $R_2 = CH_3$



IV - $R_2 = H$
V - $R_2 = CH_3$

CCC 1076–5174/98/040346–12 $17.50

Received 5 November 1997
Accepted 19 December 1997

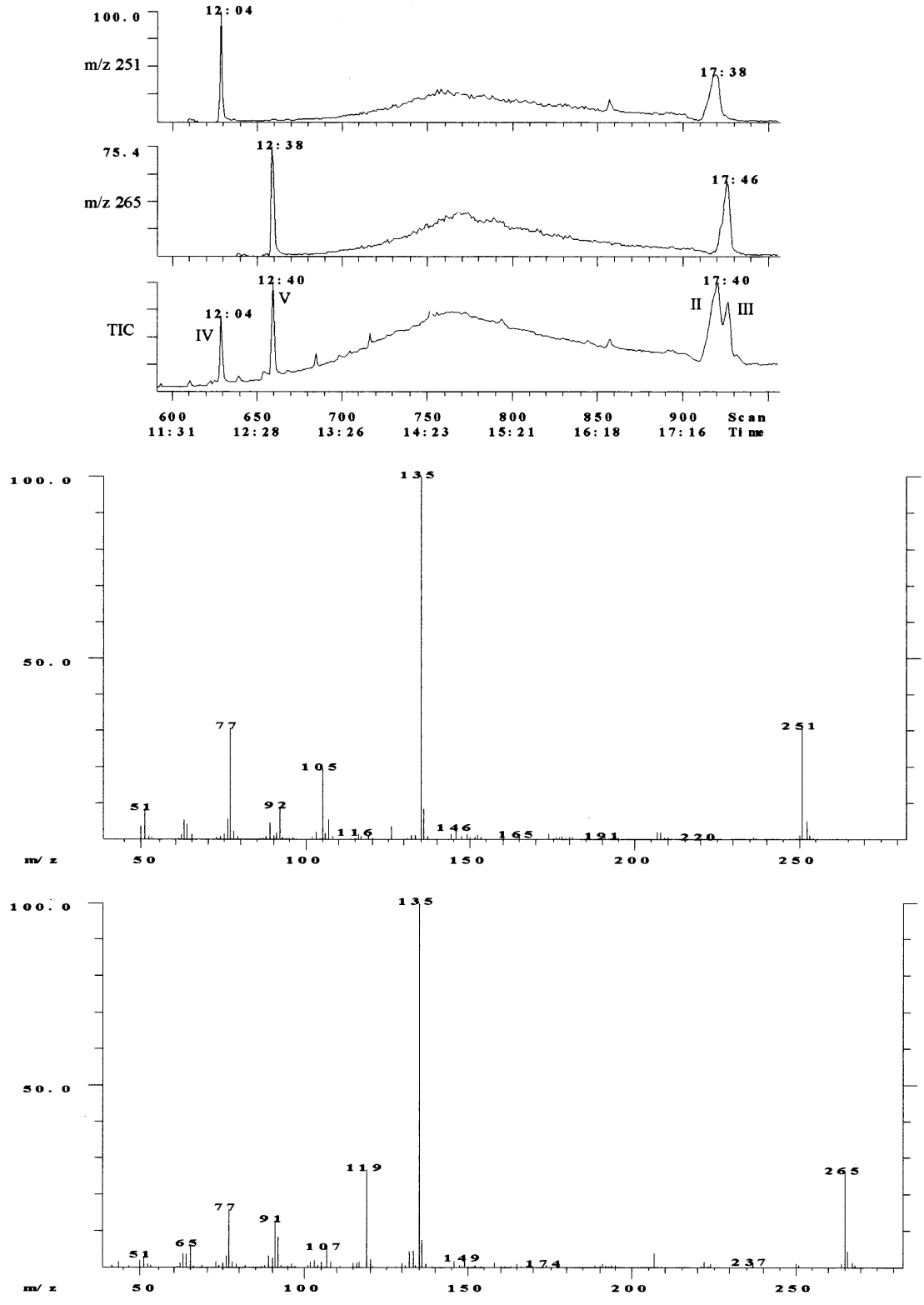


Figure 1. Mass chromatograms (*m/z* 251 and *m/z* 265) and total ion chromatogram (TIC) of **II** (t_R = 17 min 38 s) and **III** (t_R = 17 min 46 s) after keeping a CH_3OH solution for 1 week (top), EI mass spectra of the decomposition products **IV** (t_R = 12 min 4 s, M_r 251) and **V** (t_R = 12 min 40 s, M_r 265).

(Varian Analytical Instruments, Sunnyvale, CA, USA) with a split/splitless injector, and an Optima-5 capillary column (25 m × 0.25 mm i.d., 0.25 μm film thickness) (Macherey–Nagel, Düren, Germany). The He flow rate was 1 ml min^{-1} (head pressure 55 kPa). Injection (1 μl) was performed manually, split ratio 10:1; injection temperature, 280 °C; oven temperature program, initially 80 °C, held for 1 min; increased from 80 to 320 °C at 20 °C min^{-1}, held at 320 °C for 10 min; and transfer line temperature, 250 °C. The Incos 500 was equipped with ion sources for electron ionization (EI) (70 eV) and chemical ionization (CI). MS parameters were as follows: ion source set temperature, 180 °C for EI and 100 °C for CI; and electron multiplier voltage, 1150 V

for EI and 1400 V for CI. GC/MS data were acquired using a Data General DG-20 instrument.

Fast atom bombardment (FAB) and high-resolution mass spectra were obtained using an HSQ-30 hybrid (BEQQ) mass spectrometer (Finnigan MAT, Bremen, Germany) with a saddle-field FAB gun (Ion Tech, Teddington, UK). The FAB gas was Xe at 8 kV. Ar was used for collision-induced dissociation (CID) at a pressure of $\sim 5 \times 10^{-4}$ Pa; the pressure was adjusted to decrease the parent (precursor) ion abundance to 50%. The collision energy was 30 eV. FAB mass spectra were obtained with a thioglycerol–dithioethanol (1:1) matrix.

Pyrolysis

Pyrolysis of compound **I** and of a mixture of **II** and **III** was accomplished by heating the dry substances (100 mg) in closed Pyrex tubes to 250 °C and maintaining this temperature for 2 h. After cooling to room temperature, the pyrolysis products were dissolved in methanol and the solutions were investigated by GC/MS with a Chirasil-L-Val capillary column (25 m × 0.25 mm) (Macherey–Nagel) and the following oven temperature program: initially 60 °C, held for 1 min, then increased from 60 to 190 °C at 15 °C min^{-1}, held at 190 °C for 15 min. The transfer line temperature was 190 °C.

Decomposition in solution

When the mixture of **II** and **III** in CH_3OH was kept at room temperature for 1 week, two additional GC peaks appeared. The molecular masses of these compounds corresponded to (**II** – $CH_3C_6H_4COCH_3$) (*m/z* 251) and (**III** – $C_6H_5COCH_3$) (*m/z* 265) (Fig. 1). To these the structures **IV** and **V** were assigned. For a discussion of their mass spectra, see below.

Molecular modeling software

For structure optimization of **I** and **IV** the HyperChem molecular modeling system (Hypercube, Waterloo, Canada), Release 4 for Windows, was used, applying semi-empirical optimization by the Polak–Ribiere conjugate gradient method. The initial parameters were those of the free isoxazole ring.[5]

Syntheses

The arylpyrylium salts **VII** and **VIII** and the ketone **VI** were prepared according to published procedures[6,7] (see Scheme 1).

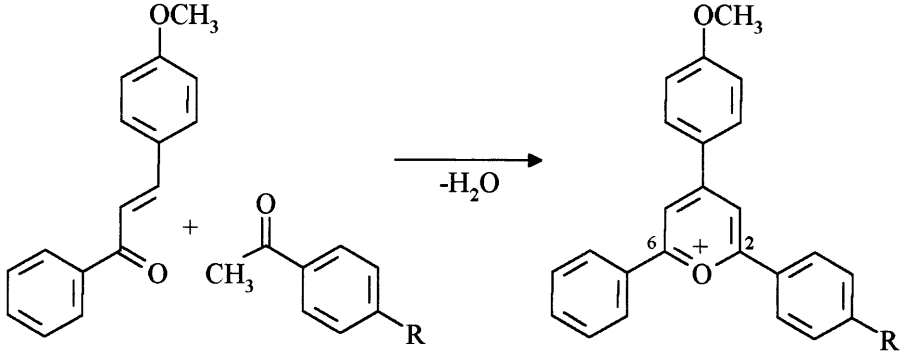
3-Phenyl-5-(4-methoxyphenyl)-5-(4-methylphenacyl)-isoxazoline (II) and 3-(4-methylphenyl)- 5-(4-methoxyphenyl)-5-phenacyl-isoxazoline (III). *Method according to Dorofeenko and co-workers*[8]. A mixture of 0.84 g (12 mmol) of hydroxylammonium chloride and 1.28 g (24 mmol) of sodium methylate (0.56 g of sodium in 40 ml of methanol) was prepared under reflux. A 2.82 g (8 mmol) amount of **VIII** was dissolved in the cold solution. After 2–3 h, yellowish crystals appeared in the orange solution. The crystallization process was completed by careful dilution with the same amount of water. The crystals were purified by recrystallization from hot ethanol, yielding 1.6 g of a mixture of **II** and **III** (m.p. 123 °C).

Method according to Balaban[9]. A 1.80 g (4 mmol) amount of VIII suspended in 50 ml of ethanol was treated at 15 °C with a cold solution of 1.4 g of hydroxylammonium chloride and 0.8 g of NaOH in 6 ml H_2O. With mixing the suspension dissolved and after a short time yellowish crystals appeared. Dilution with water completed the crystallization process. After cooling with ice–water for 30 min, the precipitate was filtered off and dried under vacuum. The crystals were dissolved in 1000 ml of diethyl ether and filtered from the unreacted perchlorate **VIII**. The solvent was removed and the solid was dissolved in hot ethanol. Recrystallization afforded 2.1 g of a mixture of **II** and **III** (m.p. 123–124 °C). Compound **IV** was isolated from the mother liquor after removal of the crystals of **II** + **III**. Compound **I** was prepared in the same way from **VII** instead of **VIII**.

RESULTS AND DISCUSSION

Synthesis

The reaction between triphenylpyrylium perchlorate and hydroxylamine profits from the fact that the six-membered heterocyclic ring cations are readily acces-



(VI)

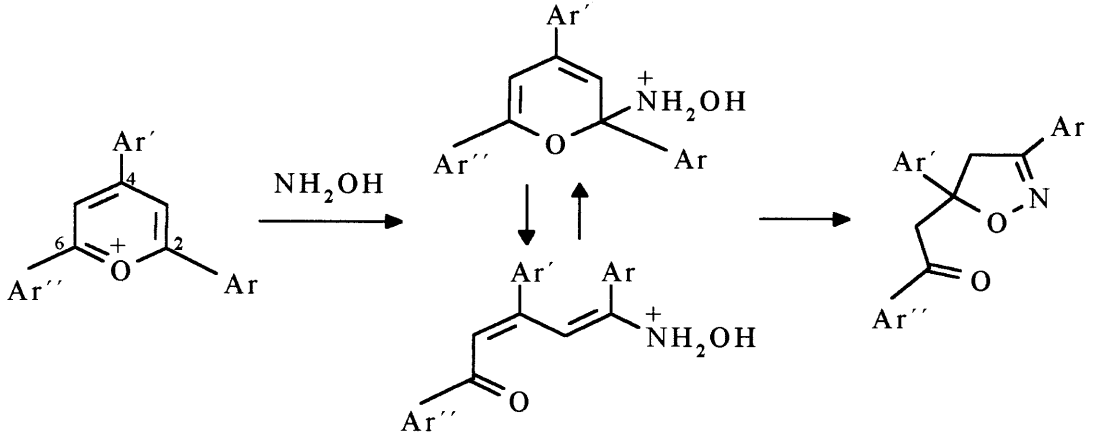
(VII) : R = H
(VIII) : R = CH_3

Scheme 1.

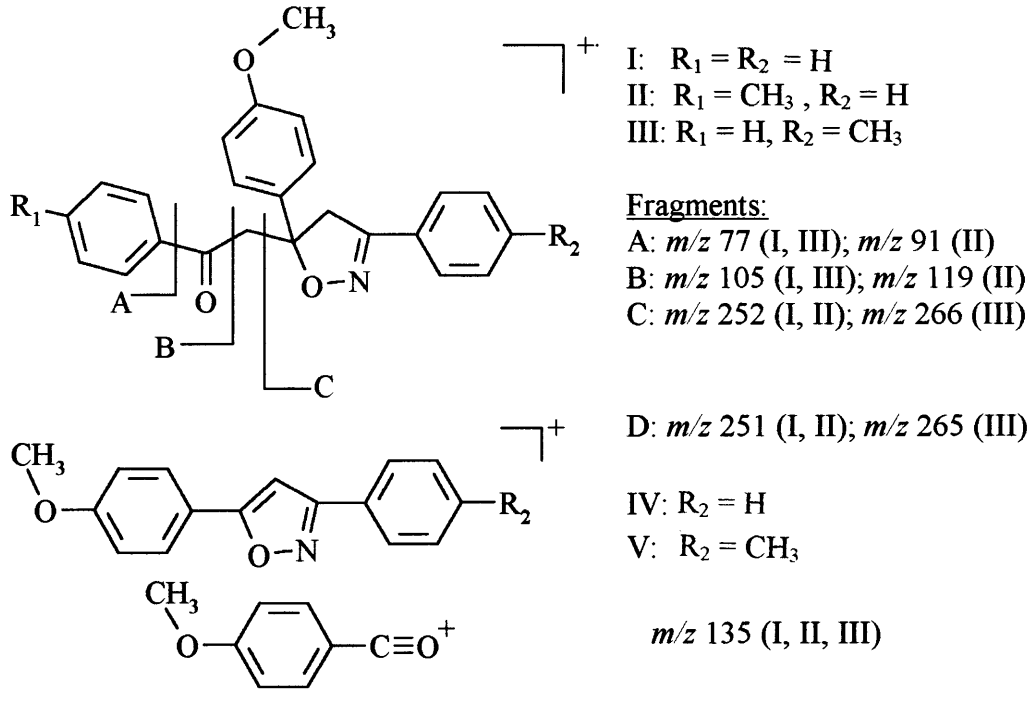
sible to nucleophilic additions in the 2-and 6-positions followed by ring opening and subsequent ring closure to form the five-membered isoxazoline ring with a phenacyl side-chain in position 5 (see Scheme 2). The nucleophilic addition of the hydroxylamine can take place either at C-2 or at C-6 of the pyrylium ring, resulting in a mixture of the two isomeric isoxazolines **II** and **III** from the asymmetric pyrylium salt **VIII**, whereas from the symmetrical pyrylium salt **VII** only **I** is obtained. (Owing to the asymmetric C-5 of the isoxazoline ring, **I**, **II** and **III** are racemates.)

EIMS and GC/MS investigation

The EI mass spectra of **I–III** were measured using a direct inlet system (Figs 2 and 3). They agree well with those[10] of analogous compounds[10,11] with Ar = Ar″. The molecular ions are of very low abundance, accompanied by $[M - H]^+$, $[M - H_2O]^{+\cdot}$ and $[M - H_2O_2]^{+\cdot}$. The formation of the last fragment was explained[10] by ring opening yielding an α,β-unsaturated oxime from which and from the enol form of the benzoyl unit two OH groups are eliminated with concomitant ring closure to give a 2,4,6-triarylpyridine. Elimination of the $Ar''COCH_2$ residue by α-cleavage next to the heterocyclic O as well as by McLafferty rearrangement yields abundant ions at *m/z* 252/251 for **I** and **II** and at *m/z* 266/265 for **III**. In the lower mass region $[Ar''CO]^+$ and $[Ar'']^+$ (*m/z* 119/105 and 91/77, respectively) can be found. After cleavage by McLafferty rearrangement the charge stays preferentially with the isoxazole portion (see above); ions $[Ar''C(OH)=CH_2]^{+\cdot}$ are essentially absent. In the spectra reported in ref. [10] the situation is reversed. This discrepancy can be explained by the influence of the electron-donating OCH_3 group whereas in ref. [10] the benzene rings carry electron-withdrawing substituents. The formation of the base peak (*m/z* 135), viz. $[CH_3OC_6H_4CO]^+$, can be explained by ring cleavage of ions formed by the loss of $Ar''COCH_2^{\cdot}$ (*m/z* 252 and 266). This could be confirmed by the mass spectra of IV and **V**, the main fragmentation of which leads to ions at *m/z* 135 (see Scheme 3).



Scheme 2.



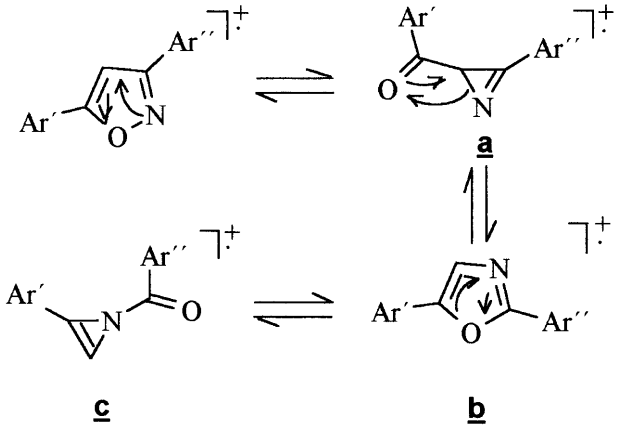
Scheme 3. EI fragmentation of **I–III**.

The fragmentation of 3,5-diarylisoxazoles had been studied by several groups and mechanisms for the various processes have been proposed.[12–15] Most important is the formation of $[CH_3OC_6H_4CO]^+$ (*m/z* 135), which can lose CO (*m/z* 107) and subsequently CH_2O (*m/z* 77). Rearrangement to a 3-benzoyl-2-phenylazirine structure (**a**; see Scheme 4) was invoked which can isomerize further to a 2,5-diaryloxazole (**b**) (for details and for minor fragments the original publications should be consulted). Of interest are the ions at *m/z* 105 from **IV** and *m/z* 119 from **V**. Analogous fragments can be found in the mass spectra of most 3,5-diaryloxazoles, but their mode of formation has not been commented on. As their elemental composition is C_7H_5O and C_8H_7O, respectively, their structures are most likely $[C_6H_5CO]^+$ and $[CH_3C_6H_4CO]^+$. Further rearrangement of **b** to the isomeric azirine **c** could explain the formation of $[Ar''CO]^+$.

The mixture of **II** and **III** could not be separated by gradient heating of the direct inlet probe. To obtain their individual mass spectra, the mixture of **II** and **III** as well as **I** were injected into GC/MS system, which resulted in partial decomposition, as can be seen from Fig. 4. The molecular ions (cf. Figs 2 and 3) cannot be detected but the major fragments correspond to those also found after direct insertion of the probe (Figs 4 and 5).

CI(CH_4)-MS investigation

The CI(CH_4) mass spectra of **I** and of the mixture of **II** and **III** (Figs 6 and 7) were obtained with a direct inlet system. From Figs 6 and 7, it can be seen that the most important ions in the molecular ion region are $[M - H_2O + H]^+$. Interestingly, $[M - 2OH + H]^+$ (cf. EI mass spectra) is also present. Protonated $[M - R_1C_6H_4CH(OH)=CH_2]$ (*m/z* 252/266) and their fragment at *m/z* 135 dominate the lower mass range. As expected, the fully aromatic **IV** and **V** show abundant $M^{+\bullet}$, $[M + H]^+$ and $[M + C_2H_5]^+$ ions as well as *m/z* 135.



Scheme 4.

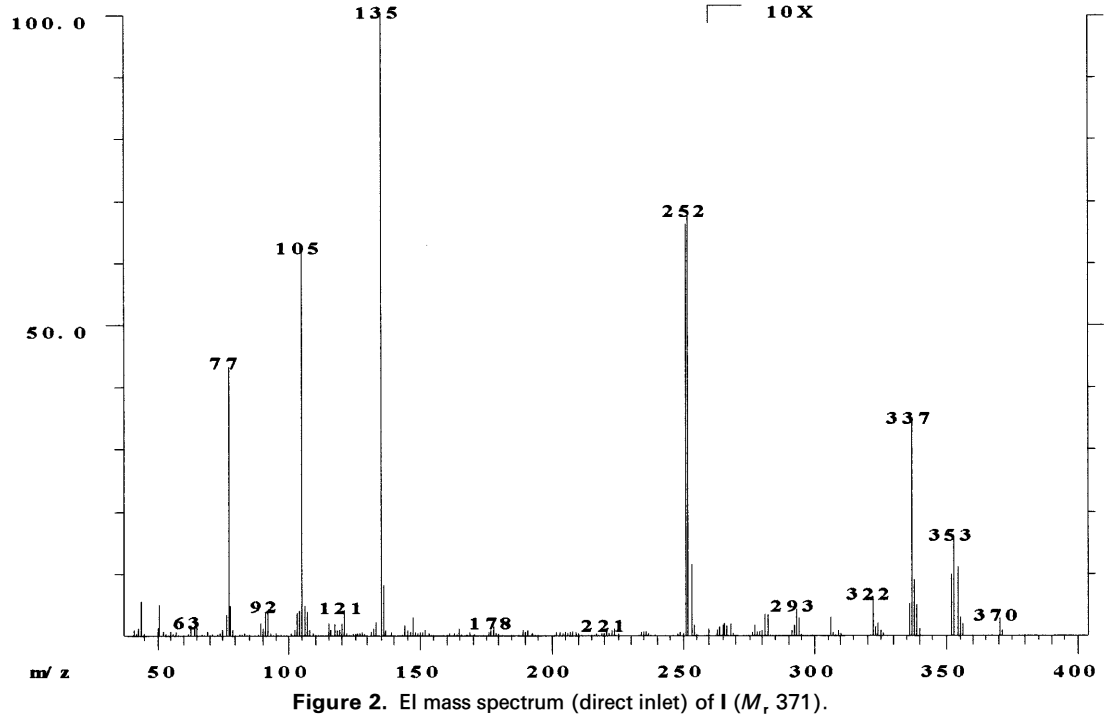


Figure 2. EI mass spectrum (direct inlet) of **I** (M_r 371).

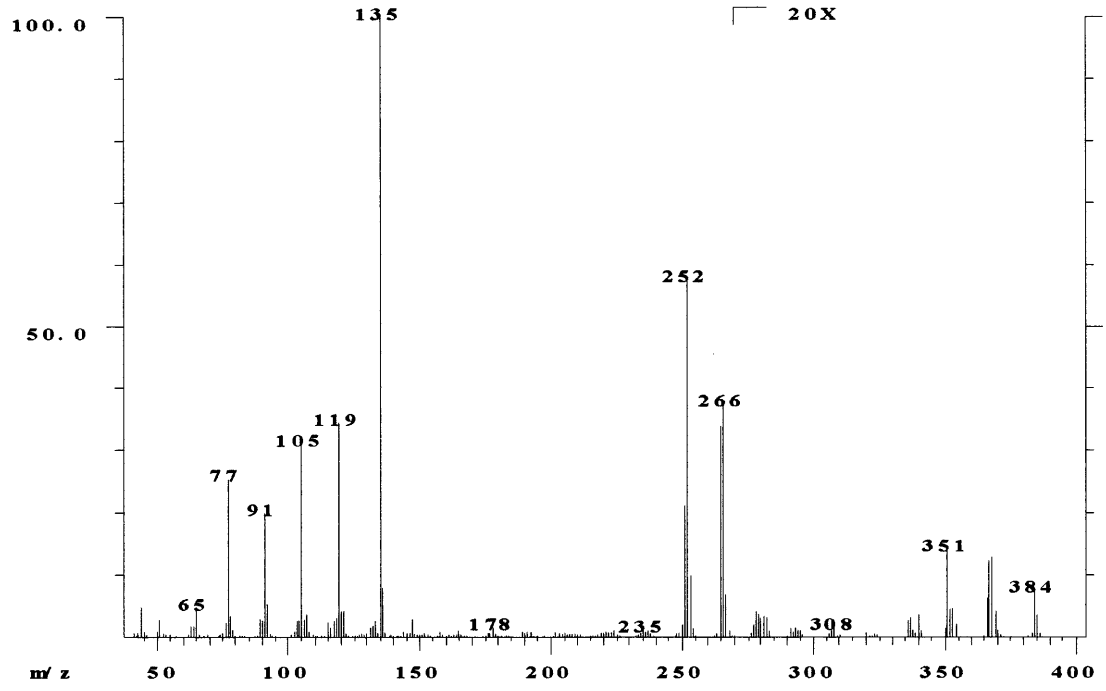


Figure 3. EI mass spectrum (direct inlet) of a mixture of **II** and **III** (M_r 385).

FABMS/MS investigation

The $[M + H]^+$ ions in the FAB mass spectra of **I–III** are not very prominent; they can, however, be used to clarify their fragmentation mechanisms. The main process is the formation of the fragment at *m/z* 252 from protonated **I** and **II** and at *m/z* 266 for **III** (see Fig. 8). The fragment ion scans of *m/z* 252 and 266 lead to *m/z* 135, which is also obtained from $[M + H]^+$ of **IV**. The elemental composition was determined by exact mass measurement in all cases as $C_8H_7O_2$; those of *m/z* 105 (C_7H_5O) and 119 (C_8H_7O) are in agreement with the benzoyl structures (B in Scheme 3).

Off-line pyrolysis/GC/MS

Compound **I** and the mixture of **II** and **III** were subjected to pyrolysis to determine whether ions observed in their mass spectra resulted (especially in GC/MS, which requires high temperatures) from pyrolytic decomposition rather than from ion fragmentation. The results are shown in Fig. 9. The major process is the elimination of acetophenone (**I**, **III**) or its *p*-methyl homolog (**II**), which also occurs at room temperature; hence it cannot be excluded that part of *m/z* 251 stems from a pre-ionization decomposition. The formation of the other pyrolysis products is self-evident. Corresponding ions are not found in the EI mass spectra. Pyrolysis may, however, be used for further identification of these compounds and especially for distinction of the isomers **II** and **III**.

Molecular modeling

Molecular modeling shows that **I** has a propeller-screw structure with a $\sim 19°$ torsion angle between the isoxazoline and C-3 phenyl ring and one of $\sim 52°$ between the isoxazoline and the C-5 phenyl ring. By elimination of acetophenone the essentially planar fully conjugated **IV** is formed. The energy gain resulting in the formation of **IV** is apparently the driving force for the facile elimination of acetophenone.

CONCLUSIONS

EI mass spectra of **I–III** show that the molecular ions are unstable. For identification with EIMS and CI(CH_4)-MS the characteristic fragments at *m/z* 252 for **I** and **II** and at *m/z* 266 for **III** can be used.

The protonated molecule ions in the positive FAB mass spectra also have relatively low abundances, but they are sufficient for obtaining fragment ion spectra which show that under FAB conditions the primary processes are the elimination of acetophenone or *p*-methylacetophenone and the formation of the 4-methoxybenzoyl ion (*m/z* 135). Positive FABMS in combination with fragment ion scans can be used for the identification of this type of isoxazolines in complex mixtures from plant extracts. Off-line pyrolysis/GC/MS can contribute to the identification of isomeric compounds.

Acknowledgements

Financial assistance from the Deutsche Forschungsgemeinschaft (DFG) and the Fonds der Chemischen Industrie is gratefully acknowledged.

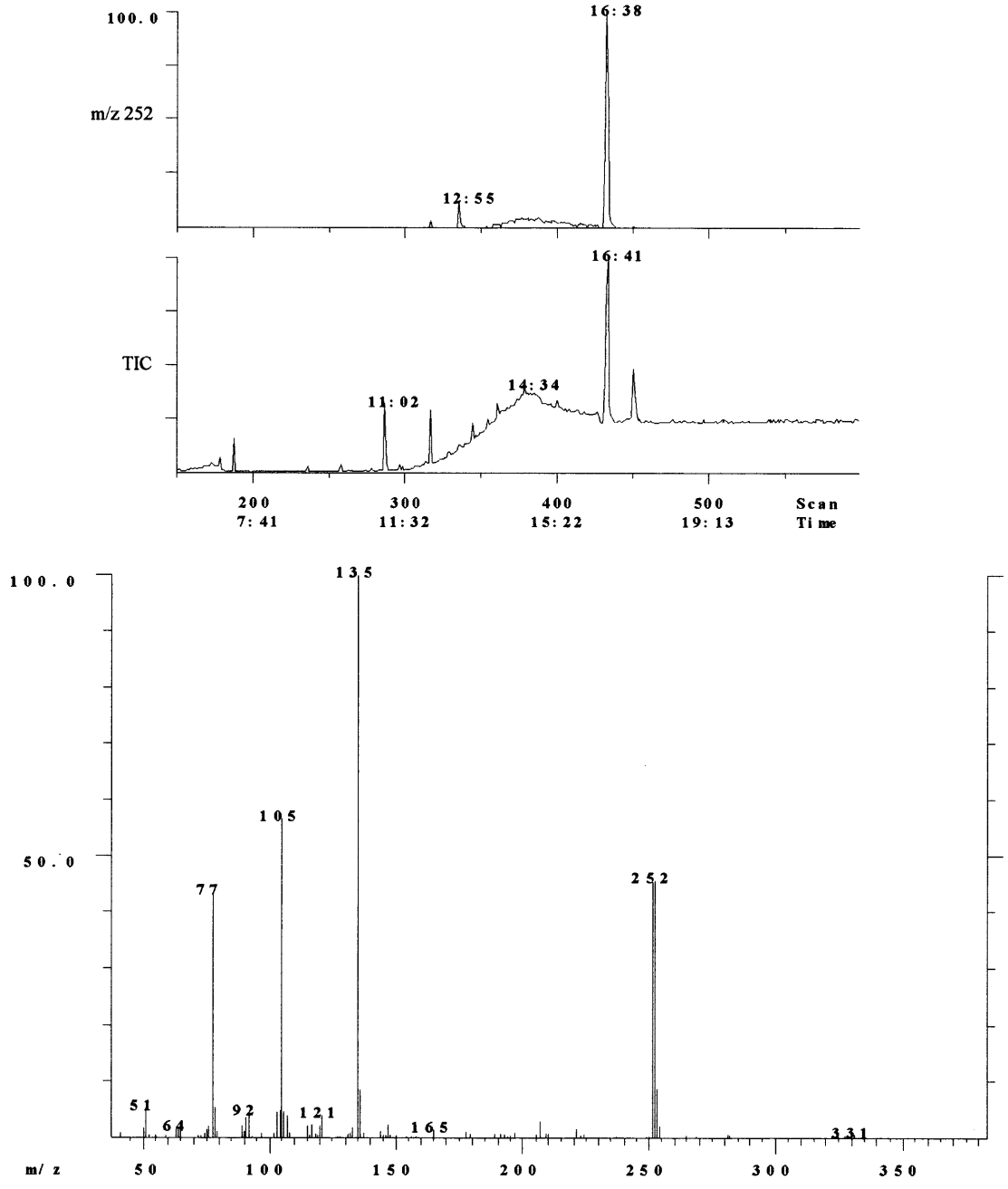


Figure 4. Mass chromatogram (*m/z* 252) and TIC of **I** (with decomposition, top) and EI mass spectrum of the main peak (t_R = 16 min 38 s, bottom).

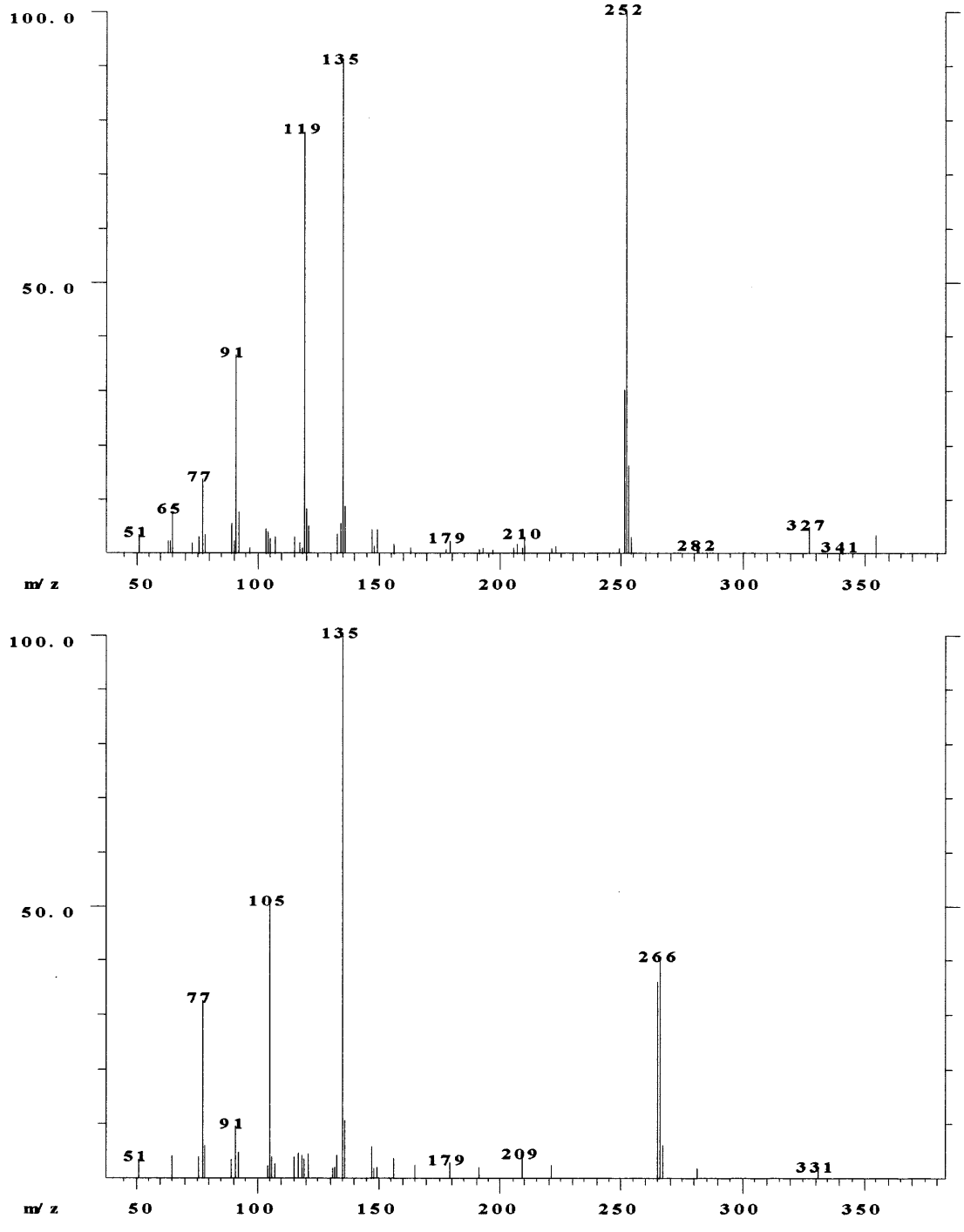


Figure 5. EI mass spectra of **II** (top) and **III** (bottom) after GC separation.

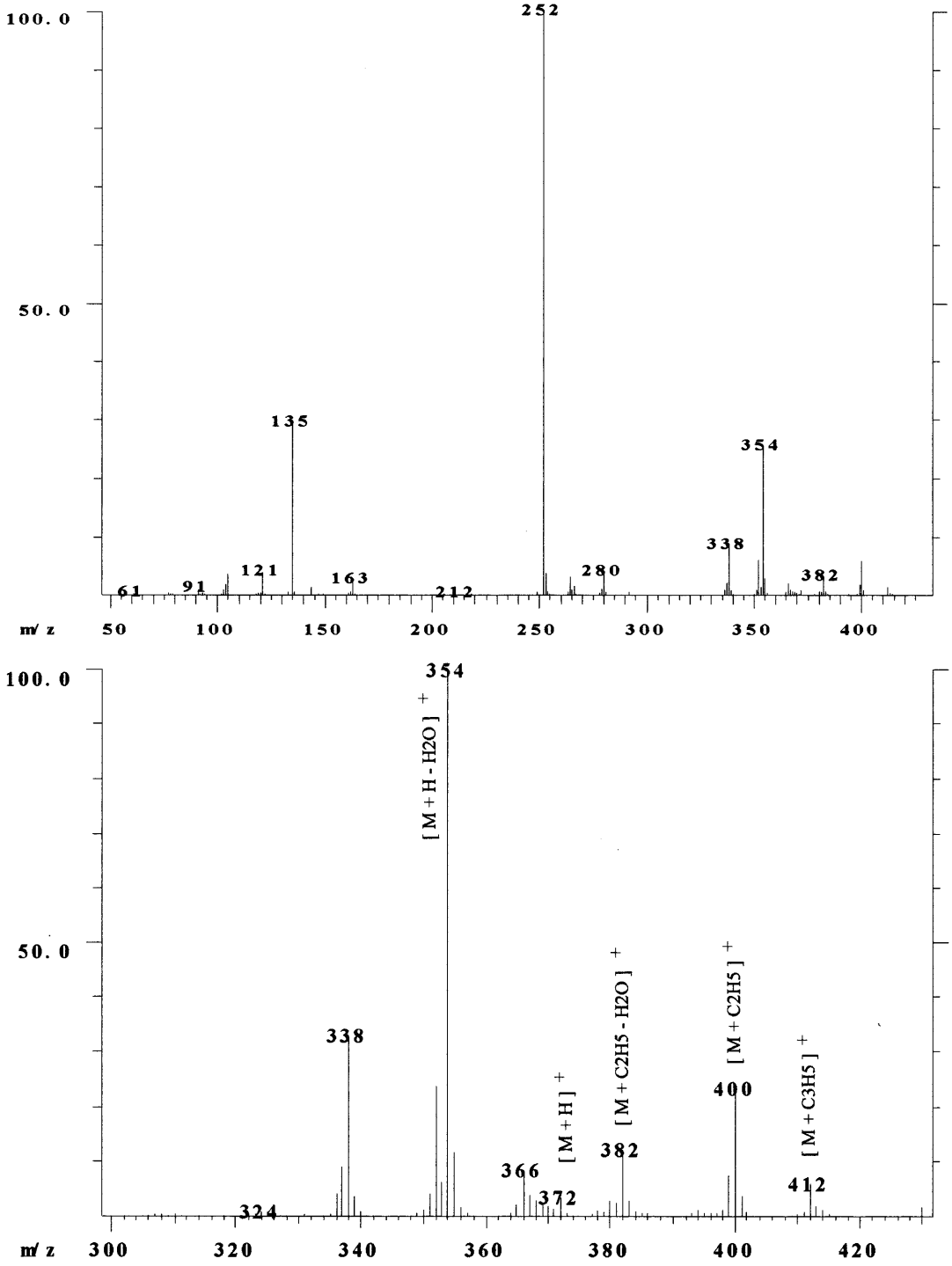


Figure 6. $CI(CH_4)$ mass spectrum of **I** (top) and mass range *m/z* 300–430 (bottom).

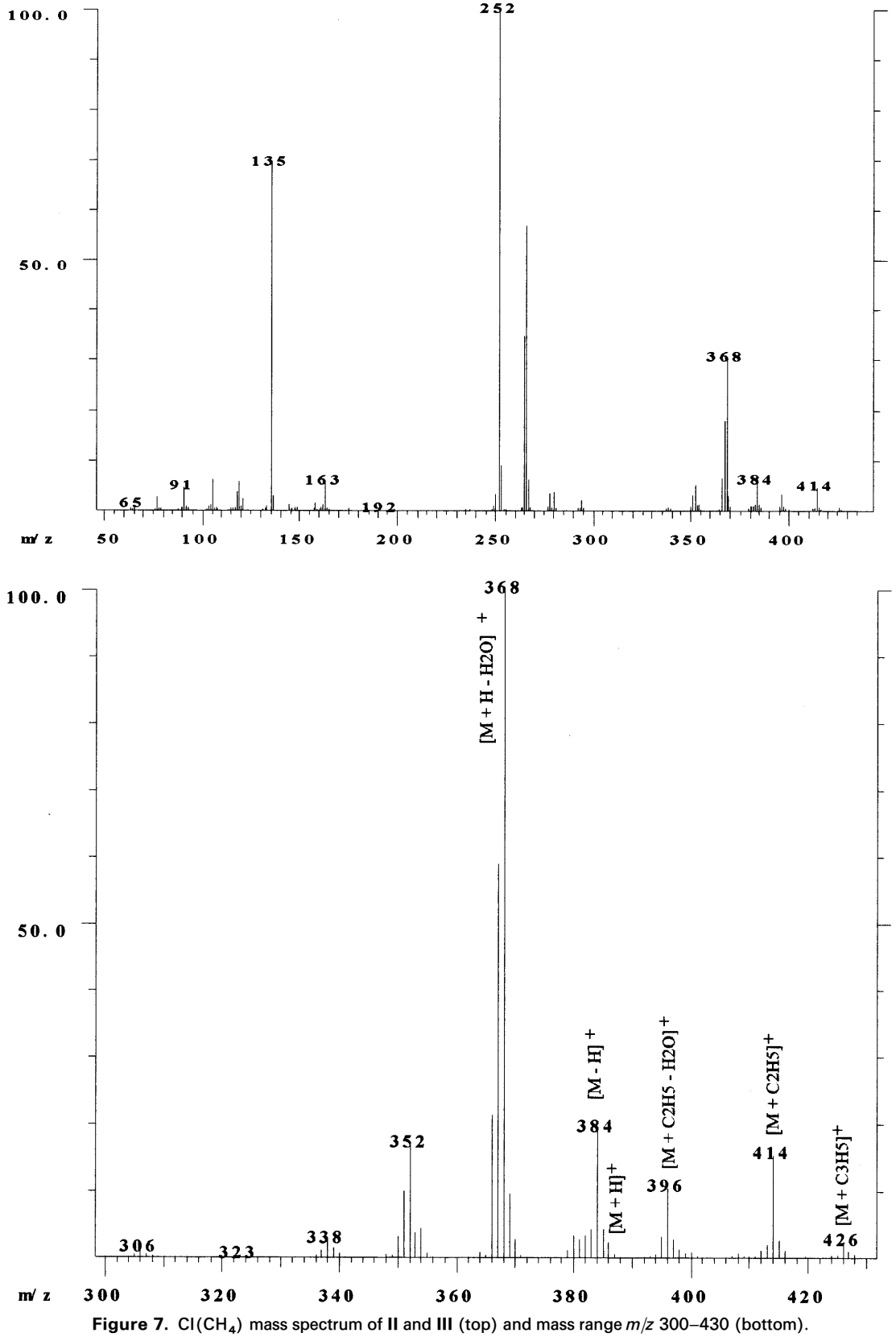


Figure 7. $CI(CH_4)$ mass spectrum of **II** and **III** (top) and mass range *m/z* 300–430 (bottom).

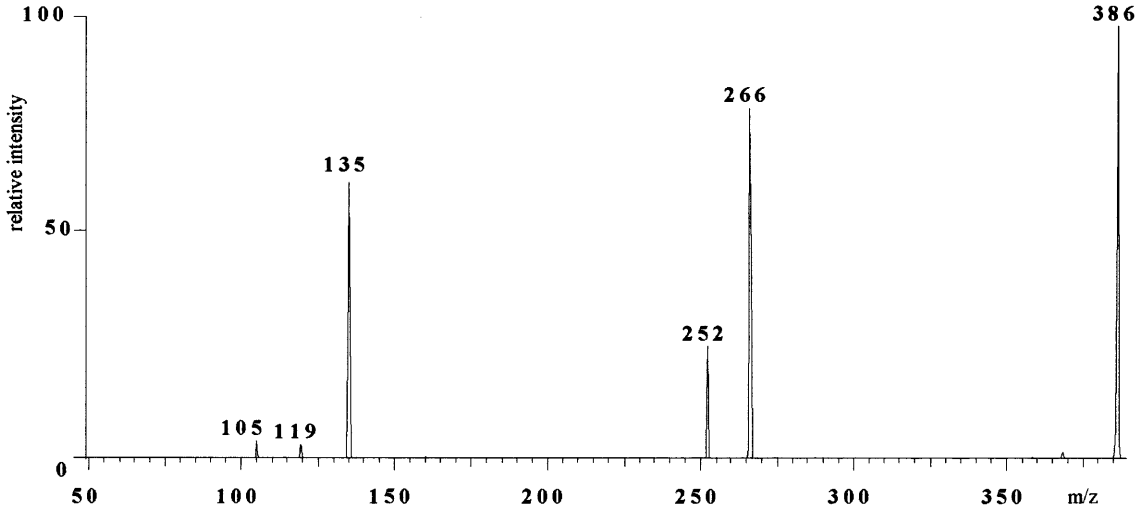


Figure 8. FAB fragment ion scan mass spectrum of $[M + H]^+$ (*m/z* 386) of **II** and **III**.

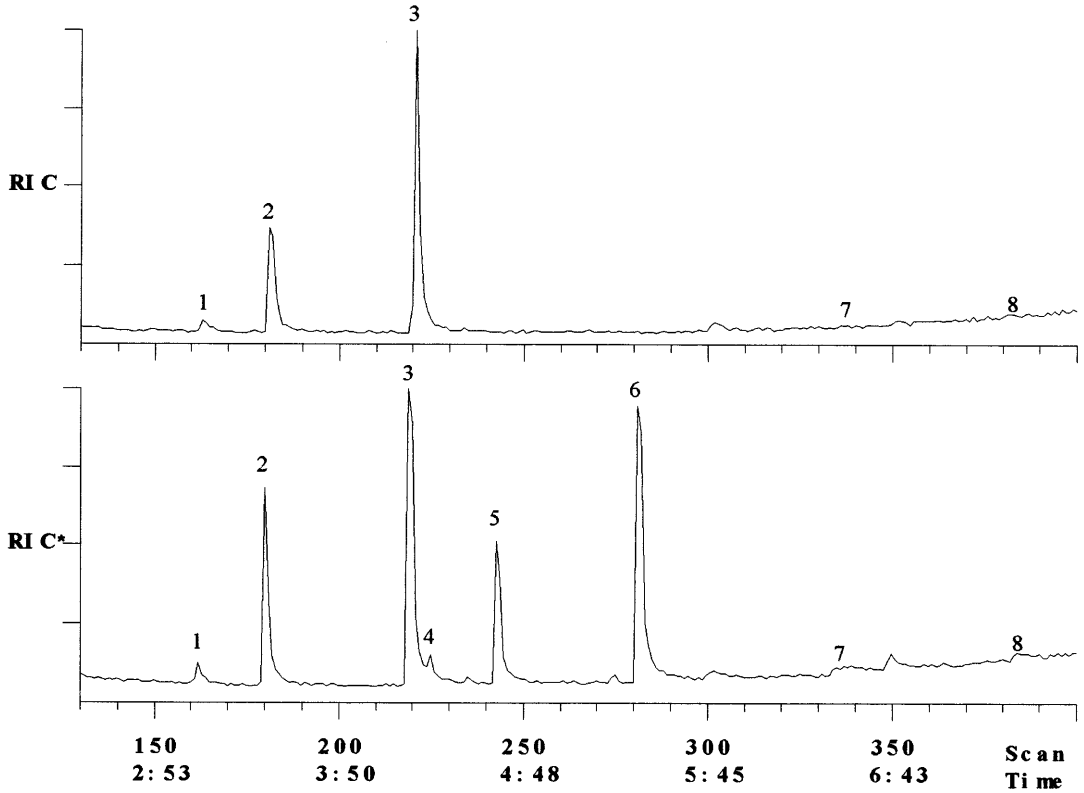


Figure 9. GC/MS profiles of the pyrolysis products of **I** (top) and **II** + **III** (bottom): 1, benzaldehyde (**I**, **III**); 2, benzonitrile (**I**, **II**); 3, acetophenone (**I**, **III**); 4, 4-methylbenzaldehyde (**II**); 5, 4-methylbenzonitrile (**III**); 6, *p*-methylacetophenone (**II**); 7, 4-methoxybenzaldehyde (**I**–**III**); 8, *p*-methoxyacetophenone (**I**–**III**).

REFERENCES

1. D. G. Martin, D. J. Duchamp and C. G. Chidester, *Tetrahedron Lett.* 2549 (1973).
2. T. Takemoto and T. Nakajima, *Yakugaku Zasshi* **84**, 1183 (1964) (*Chem. Abstr.* **62**, 8121c).
3. K. Krogsgaard-Larsen, L. Brehm and K. Schaumburg, *Acta Chem. Scand., Ser. B* **35**, 311 (1981).
4. F. Ikegami, F. Lambein, Y.-H. Kuo and I. Murakoshi, *Phytochemistry* **23**, 1567 (1984).
5. B. J. Wakefield, in *Houben-Weyl, Methoden der Organischen Chemie*, edited by E. Schaumann, Vol. E8a, p. 45. Georg Thieme, Stuttgart (1993).
6. W. Dilthey, *J. Prakt. Chem.* **94**, 53 (1916).
7. K. Dimroth, *Angew. Chem.* **72**, 331 (1960).
8. E. A. Zvezdina, A. N. Popova, A. I. Pyshchev and G. N. Dorofeenko, *Khim. Geterotsikl. Soedin.* 461 (1982).
9. A. T. Balaban, *Tetrahedron* **24**, 5059 (1968).
10. A. M. Duffield and O. Buchardt, *Org. Mass Spectrom.* **3**, 1043 (1970).
11. P. L. Kumler, C. L. Pedersen and O. Buchardt, *Acta Chem. Scand.* **22**, 2719 (1968).
12. J. H. Bowie, R. K. M. R. Kallury and R. G. Cooks, *Aust. J. Chem.* **22**, 563 (1969).
13. K. K. Zhigulev, S. D. Sokolov and R. A. Khmelnitsky, *Khim. Geterotsikl. Soedin.* 755 (1974).
14. D. C. Nonhebel, *Org. Mass Spectrom.* **3**, 1519 (1970).
15. G. L. Aldous and J. H. Bowie, *Org. Mass Spectrom.* **10**, 64 (1975).