RAPID COMMUNICATIONS IN MASS SPECTROMETRY, VOL. **11**, 1430–1434 (1997)

Differentiation of Two Geometric Isomers of the Pharmaceutical Eprosartan Using Atmospheric Pressure Chemical Ionization

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The utility of an atmospheric pressure chemical ionization interface for distinguishing stereoisomers has been demonstrated. Two geometrical isomers of the pharmaceutical eprosartan ((*E*,*Z*)-3-[butyl-1-(4-carboxybenzyl)-1*H*-imidazole-5-yl]-2-[2-thienyl)methyl]propenoic acid) were investigated in the positive- and negative-ion modes with in-source collision-induced dissociation (CID). In positive-ion mode, CID spectra display significant differences between the two isomers. Under identical collisional conditions several fragment ions present in the CID spectrum of the *E* isomer (SK&F 108566) are significantly suppressed in the spectrum of the *Z* isomer (SB 206328). Analysis of the fragmentation patterns of both isomers indicates that a pathway initiated by the loss of neutral thiophene from the *E* isomer is inhibited in the CID spectra of the *Z* isomer. In negative-ion mode, fragmentation and corresponding differences in spectra are not observed. Fragmentation is observed to result primarily from the ionization process. © 1997 by John Wiley & Sons, Ltd.

Received 17 June 1997; Accepted 30 June 1997
Rapid. Commun. Mass Spectrom. **11**, 1430–1434 (1997)
No. of Figures: 4 No. of Tables: 2 No. of Refs: 13

Problems involving stereochemistry continue to pose interesting questions in mass spectrometry.[1] In particular, structural analyses involving geometric isomers have been shown, in several cases, to be amenable to a variety of mass spectral techniques and remain an active area of research interest.[2] Maleic and fumaric acids offer a well-studied example of an isomeric pair which have produced distinctly different spectra under a variety of ionization and analysis procedures.[3] In other cases, clear differentiation of isomers is possible only with particular ionization conditions.[4] Techniques such as mass analyzed ion kinetic energy spectroscopy (MIKES)[5] and angle resolved mass spectrometry (ARMS)[6] have been shown to be useful in isomer differentiation in certain cases. The increased availability of a variety of 'soft' ionization sources offers additional potential techniques for these types of studies. For studies involving biologically active molecules the analytical utility of a simple combination of liquid chromatography and mass spectrometry (LC/MS) capable of distinguishing stereoisomers is clear.

In this paper we report on the mass spectrometry of two geometric isomers of the pharmaceutical eprosartan: (*E*,*Z*)-3-[butyl-1-(4-carboxybenzyl)-1*H*- imidazol-5-yl]-2-[2-thienyl)methyl]propenoic acid. This compound (SK&F-108566) is a nonpeptide angiotensin II receptor antagonist in development for the treatment of hypertension.[7] The compounds studied are shown in Fig. 1 as **1** and **2**. Atmospheric pressure chemical ionization (APCI) was employed in the positive- and negative-ion modes. In positive-ion mode in-source collision-induced dissociation (CID) generates extensive fragmentation for structural analysis. Under these experimental conditions significant differences between the CID spectra of **1** and **2** are observed. In negative-ion mode, fragmentation is less extensive and results primarily from the ionization process. Significant spectral differences are not noted in the negative-ion mode. Fragmentation pathways consistent with the observed spectra are proposed and the results discussed.

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EXPERIMENTAL

Mass spectra reported here were recorded on a Hewlett Packard (Palo Alto, CA, USA) 5989A spectrometer equipped with a Hewlett Packard G1075A APCI interface controlled through a Hewlett Packard 59987A electrospray cabinet. Mass spectra were obtained using both direct infusion and LC/MS techniques. On-column separation confirmed that observed results were not due to the presence of an impurity. Spectra discussed here were obtained from direct infusion with a syringe pump at a flow rate of 50μL/min teed to a 0.4 mL/min flow of 75% methanol and 25% water. Solutions of 300 mg/L of each test material were prepared in 75% methanol and 25% water. Drying gas (nitrogen) temperature was set at 325 °C and the nebulizer gas (nitrogen) temperature was set at 350 °C. In-source CID spectra were obtained by adjusting the potential between the exit of the transfer capillary and the first skimmer. This setting is referred to as the exit capillary voltage (ECV).

RESULTS AND DISCUSSION

Positive-ion APCI

At low ECV setting (ECV = 130 V) both isomers show definitive signals from the protonated parent molecule (*m/z* 425) with minimal fragmentation. In general, the signal from $[M+H]^+$ ions represents > 95%Σ (of reported features). Typical CID spectra (ECV = 250 V) for **1** and **2** showing extensive fragmentation are displayed in Fig. 2(a) and (b) respectively. It has been noted that in-source fragmentation can be more extensive than that of typical tandem mass spectrometric (MS/MS) experiments although overall the same pathways are accessed.[8] A summary of the observed fragment ions as relative abundances (RA) for a typical

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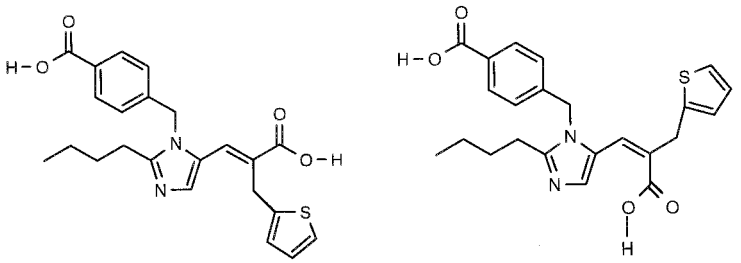


1 **2**

Figure 1. The two geometric isomers of the pharmaceutical eprosartan studied in this work.

data set is shown in Table 1. These spectra were obtained under identical instrumental conditions with exit capillary voltage at 250 V. In both cases the peak arising from the protonated parent ($[M + H]^+$ = 425.15 u) is still prominent, representing 73 and 100% RA for **1** and **2** respectively. Presumably, the initial site of protonation is in association with the imidazole ring. The gas-phase proton affinity of imidazole is 220 kcal/mol and this should be the most basic site of the molecule.[9] Both isomers show initial losses of H_2O followed by CO yielding the features at *m/z* 407 and 379 respectively, as well as direct loss of CO_2 (*m/z* 381). Inspection of Fig. 2(a) and (b) shows that fragment ions present at *m/z* = 341, 207 and 163 in the spectrum of **1**, the *E*-isomer (SK&F 108566), carry a significantly reduced intensity in the spectrum of **2**, the *Z*-isomer (SB 206328).

Scheme 1 shows a proposed fragmentation scheme

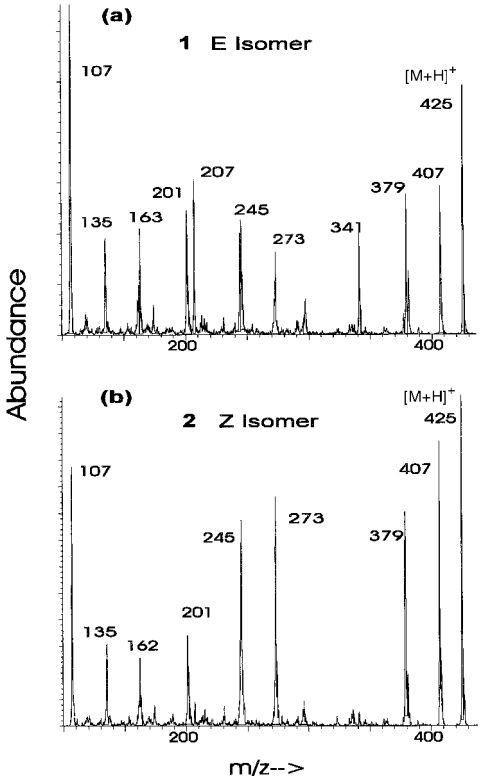


Figure 2. Representative positive ion CID spectra of **1** and **2** taken under identical experimental conditions (ECV = 250 V).

Table 1. Ion intensities as relative abundances for most prominent fragments in typical positive ion APCI-CID spectra of 1 and 2

Ion (*m/z*)	1 (*E Isomer*)	2 (*Z Isomer*)
425 $[M+H]^+$	73	100
407	43	84
381	19	15
379	41	63
341	30	4
297	10	6
273	24	68
272	10	8
245	33	61
244	28	25
207	43	6
201	36	26
163	32	8
162	14	19
135	29	23
107	100	78

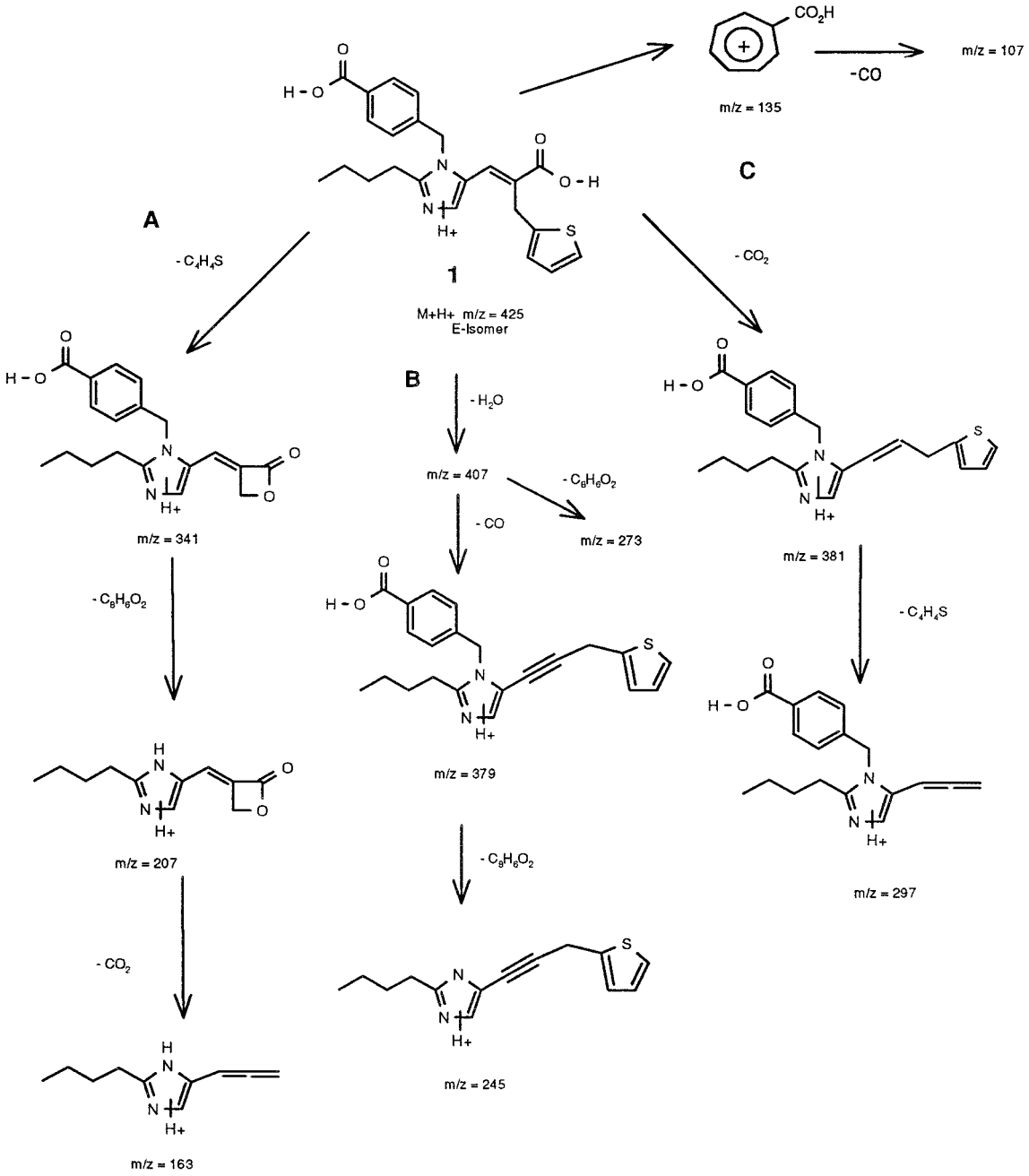
which is consistent with the observed ions for **1**. Three primary routes are assigned for production of the majority of observed ions. Route A is initiated by the loss of thiophene yielding *m/z* 341. Further neutral losses of $C_8H_6O_2$ and CO_2 yield ions at *m/z* 207 and 163. Route B proceeds with loss of H_2O (*m/z* 407), then CO (*m/z* 379) followed by loss of $C_8H_6O_2$ yielding the ion at *m/z* 245. The $C_8H_6O_2$ fragment may also be lost after the initial expulsion of H_2O yielding the ion at *m/z* 273. Features at *m/z* 272 and 244 show that loss of the substituted phenyl moiety may proceed without hydrogen transfer to the imidazole ring indicating the competitive formation of open-shell products.[10] Route C proceeds with loss of CO_2 (*m/z* 381) followed by loss of thiophene (*m/z* 297). The fragment at *m/z* 135 is assigned as the substituted tropylium ion shown in Scheme 1, although the point(s) of formation in the fragmentation pattern cannot be discerned from these data. The feature at *m/z* 107 arises from loss of mass 28 from *m/z* 135 (presumably as CO) as supported by the analysis of CID spectra of lower molecular weight congeners (e.g. $NH_2CH_2(C_6H_4)CO_2H$) which produce the same features at *m/z* 135 and 107. Consistent with this assignment, there is no indication of ions that would be expected from substituted protonated imidazole ring systems as 'terminal' fragments. There is also no observation of the 27 mass unit neutral loss (as

HCN) observed in both even- and odd-electron imidazole ring systems (J. L. Brum, unpublished results).[11,12] We note that some contribution to the signal at *m/z* 107 via the loss of 56 mass units from *m/z* 163 (as C_4H_8) is possible in route A for **1**. However the data do not support this as the primary route to the origin of this signal. The presence of *m/z* 107 in the spectrum of **2**, where route A is suppressed, is further evidence that this ion forms as described above.

The routes outlined above are supported by the analysis of isotope structure resulting from the presence of a sulfur atom in the parent molecule. The natural abundance of ^{34}S is 4.4% and fragments bearing the S atom are easily identified. Analysis of spectral features for ^{34}S was consistent with the fragmentation scheme outlined. Experiments were conducted in deuterated solvents where exchangeable hydrogens were substituted with deuterium and the samples subsequently run via direct infusion. Results from these studies also gave results consistent with the pathways outlined above.

For **2**, the viability of the pathway initiated by loss of neutral thiophene (route A from Scheme 1) is greatly reduced under the same fragmentation conditions. The fragments at *m/z* 341, 207 and 163 lack any significant intensity in the CID spectrum of **2**. For example the %Σ for signals at *m/z* 341, 207 and 163 is reduced by factors of 7.7, and 7.3 and 4.6 respectively compared to the CID spectrum of **1**. The fragment ions associated with routes B and C are present with intensities similar to those of the spectrum of **1**.

The data reflect some significant differences between



Scheme 1. Proposed positive ion fragmentation pathways consistent with the features observed in the CID spectrum of **1**, the *E*-isomer of eprosartan.

the potential energy surfaces for **1** and **2** with regard to the loss of SC_4H_4. It is clear that under the moderate nebulization/ionization conditions used in this study, the isomers do not interconvert to a great extent. Further, the rate of interconversion upon collisional excitation is not highly competitive with the observed fragmentation pathways. The number of molecular conformations energetically accessible under these experimental conditions is significant. Given the number of degrees of freedom in the molecule as well as placement of the functional groups relative to the double bond, it is difficult to infer the nature[13] of the observed ibhibition of route A for the *Z*-isomer from these data. Semi-empirical molecular orbital calculations may be of interest for investigating these questions further.

Nebulizer temperature was investigated for its effect on the ratio of ion intensities $[M + H–C_4H_4S]^+/[M + H]^+$ ([*m/z* 341]/[*m/z* 425]) for both **1** and **2** (i.e. loss of thiophene). The temperature was varied from 250 °C to 450 °C with the ECV set at 250 V and was found to produce no trend for **1** for which an average ratio of 0.17 (s_x = 0.04) was observed. For **2**, an increase of this ratio was observed which spanned from << 0.04 at 250 °C to 0.17 at 450 °C. Whereas this increased thermal energy increases the branching ratio for route A in **2** it does not appear to result from isomerization prior to fragmentation, since a corresponding decrease in the ratio for **1** is not observed. Such a decrease would be expected, assuming similar energies for both protonated isomers. (Preliminary semi-empirical quantum calculations yield values for ΔH_f of 66.4 and 68.2 kcal/mol for the protonated forms of **1** and **2** respectively using the AM1 parameterization.) For isomer differentiation, an optimal nebulizer temperature exists which minimizes route A fragments and still allows for reasonable sensitivity (T = 325 °C).

Given the stability of the parent, positive-ion APCI in effect represents a relatively 'soft' ionization for these isomers. The amount of internal energy deposited as a result of ionization and nebulization is effectively dispersed through the molecule with minimal bond cleavage. The results reported here are very similar to those obtained using electrospray ionization with CID (J. L. Brum, unpublished results). As has been noted in other studies, soft forms of ionization may carry distinct advantages in the differentiation of some isomeric pairs.[6]

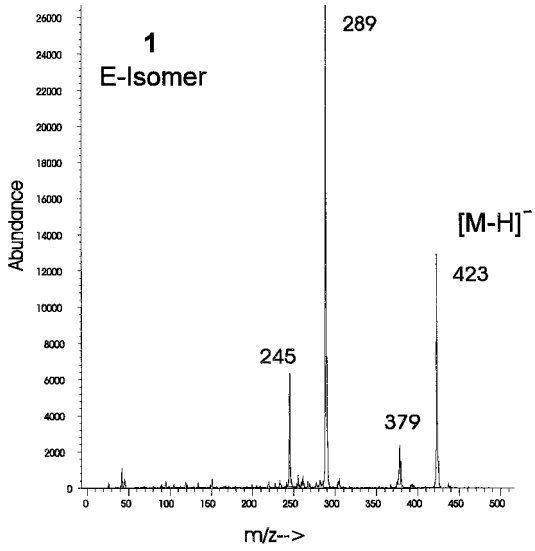


Figure 3. A typical CID spectrum from the negative ion APCI of **1**. The ECV is set at 125 V.

Table 2. Ion intensities as relative abundances for the most prominent fragments in typical negative ion APCI spectra of 1 and 2

Ion (*m/z*)	1 (*E Isomer*)	2 (*Z Isomer*)
423 $[M–H]^-$	50	48
379 $[M–H – CO_2]^-$	6	10
378 $[M–H – HCO_2]^-$	9	8
289 $[M–H–C_8H_6O_2]^-$	100	100
245 $[M–H–C_8H_6O_2–CO_2]^-$	24	19

Negative-ion APCI

Isomers **1** and **2** were also investigated by negative-ion APCI. (A representative CID spectrum is shown in Fig. 3 for **1**.) No significant differences are noted between the spectra for the two isomers. Far fewer fragments are observed for both isomers as compared to the positive-ion mode and they appear even under minimal CID conditions, indicating that primary fragmentation processes occur upon ionization. For reference ECV = 125 V is used for the spectra reported here. Consistent with fragmentation occurring upon ionization (i.e. that the molecular ion is less stable) the $[M–H]^-$ parent peaks carry significantly less total ion intensity as compared to positive-ion spectra for both **1** and **2**. In both cases *m/z* 289 represents the base peak in the spectra carrying > 50%Σ for each isomer. Observed fragment losses are related to loss of CO_2 and the phenyl moiety. Fragment ion intensities as relative abundances and observed neutral losses are summarized in Table 2.

The ratio of ion intensities $[M–H–CO_2]^-/[M–H]^-$ ([*m/z* 379]/[*m/z* 423]) was investigated as a function of nebulizer temperatures from 300 °C to 500 °C for each isomer. Both **1** and **2** displayed significant increases of this ratio with temperature. For **1** the range was < 0.1 to 0.4. For **2** the range spanned 0.15 to 0.5. No diagnostic value is currently ascribed to the observed difference in the ratio at elevated nebulizer temperature. The ratio for $[M–H–C_8H_6O_2]^-/[M–H]^-$ (*m/z* 289/*m/z* 423) did not show a similar thermal trend indicating that the route to *m/z* 289 is via direct loss of $C_8H_6O_2$ and not sequential loss initiated by loss of CO_2. Given the much more significant spectral differences displayed in positive-ion mode it is clearly the superior choice for isomer differentiation. As such, negative ion spectra are not discussed further.

CONCLUSIONS

Two geometrical isomers of the pharmaceutical eprosartan are easily distinguished by the use of positive-ion APCI. A prominent fragmentation route for the *E* isomer initiated by the loss of thiophene from the protonated parent molecule is nearly totally suppressed in the CID spectrum of the *Z* isomer. Higher nebulizer temperatures appear to enhance signal from the suppressed route for the *Z* isomer. This fact may be of significance when investigating isomers by APCI where the spectral differences may be more subtle. The

isomers were not readily distinguishable in negative-ion mode.

Acknowledgement

The authors thank Mr. L. Killmer for his review of the manuscript and comments.

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