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Gas chromatographic–mass spectrometric determination of α-phenylcinnamic acid isomers: practical and theoretical aspects

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Abstract

The Perkin condensation of benzaldehyde and phenylacetic acid was performed in the presence of acetic anhydride and triethylamine. After silylation, the resulting mixture was analysed with a Hewlett-Packard gas chromatograph equipped with a mass-selective detector. Excellent baseline separation was achieved for the α-phenylcinnamic acid trimethylsilyl ester (2,3-diphenylpropenoic acid trimethylsilyl ester) isomers. The fragments were identical in the mass spectra of the isomers; however, the relative intensities differed appreciably. The identification of most peaks was straightforward; nevertheless, non-trivial fragments were also formed via bond scissions and subsequent rearrangements of the McLafferty type. Semi-empirical quantum chemical calculations were also performed and bond orders were determined for the fully optimized geometries of various silyl ester derivatives of the neutral molecules and of the parent ions. These were aimed at mapping the strength of bonds expected to break in the mass-selective detector. It was found that the bond orders in the isomers do not differ significantly, hence the fragmentation patterns would be essentially the same, irrespective of the silylating agent. Major cleavage routes could be predicted, however. Predictions based on calculations were verified experimentally.

1. Introduction

Cinnamic acid derivatives are important building blocks in the production of lignins in higher plants. They derive from the shikimic acid metabolic pathway and their mechanism of formation is complex [1]. Nevertheless, the key reactions in this scheme are condensations (mostly of the Claisen type), just as in their laboratory-scale synthesis (mostly of the Perkin type). This latter reaction, a modified Perkin condensation leading to a mixture of (*E*)- and (*Z*)-α-phenylcinnamic acids (2,3-diphenylpropenoic acids) [2] was studied, with the aim of following and influencing isomeric distribution [3]. A high-resolution method was needed to monitor the accumulation products. GC–MS analysis was considered because of its simplicity, speed and the small amount of material required. It may be worth mentioning that *in situ* analysis was not performed in previous work on these compounds; the products were first isolated and their UV or IR spectra were recorded and evaluated [2,4,5] instead.

During this work several additional questions arose, such as (i) whether the mass spectra of the isomeric acid derivatives differ from each other, (ii) whether it is possible to identify fragments

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with significant abundances and suggest reaction pathways for non-trivial ones and (iii) whether there is any chance of obtaining differing mass spectra for the isomers by the application of various silylating agents. The results of this work, and possible answers to the above questions, are reported in this paper.

2. Experimental

Synthesis was based on the recipe of Fieser [6]. It involved heating a mixture of benzaldehyde (2 cm^3), phenylacetic acid (2.5 g), acetic anhydride (2 cm^3) and triethylamine (2 cm^3). Varying the duration of reflux varied the isomeric composition. To obtain reasonable amounts of both isomers, refluxing for 35 min was necessary.

2.1. Method

The following measurements were performed: (i) GC–MS analysis of the silyl esters of the pure acids, (ii) GC analysis of their mixture and (iii) GC analysis of silylated samples withdrawn from the reaction mixture. Silylation was necessary, as the pure acids were not volatile enough for GC measurements.

2.2. Silylation

The silylating agents were N,N-diethyltrimethylsilylamine and N-methyl-N-(*tert.*-butyldimethylsilyl)trifluoroacetamide (Fluka). Both compounds were applied to the pure isomers, but only N,N-diethyltrimethylsilylamine was used for silylating the mixture of the two pure acids and the withdrawn samples from the reaction mixture. The samples (10 mg) were dissolved in N,N-dimethylformamide (100 μl) and an excess of the silylating agent (500 μl) was added at room temperature. The reaction was complete within 5 min. A 5-μl volume of this mixture was used for analysis.

2.3. Instruments and conditions

A Hewlett-Packard (HP) Model 5890 gas chromatograph equipped with a quadrupole mass-selective detector was used to measurements. Data analysis was performed on an HP 5997 Chemstation attached to the GC–MS apparatus. A 50-m HP-1 capillary column was used; the solvent delay was 7 min, the column temperature was programmed from 250 to 350°C at 4°C min^{-1} and the electron impact (EI) ionization energy was 70 eV.

3. Theoretical calculations

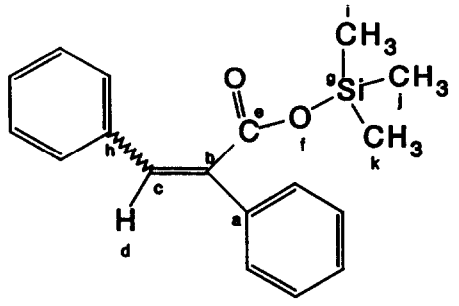
To explore the bond strengths in the isomers, semi-empirical quantum chemical calculations were carried out with the AM1 method, included in the PcMol package [7]. Full geometric optimization was performed for the neutral molecules and for the parent ions of the isomers of the trimethylsilyl (**1**) and *tert.*-butyldimethylsilyl (**2**) esters (Fig. 1). Bond orders were taken as a measure of bond strength, hence they were calculated for the optimized geometries and compared for the respective isomeric pairs.

4. Results and discussion

In the following, experimental results obtained with the trimethylsilyl esters are detailed, as preliminary measurements with the more expensive *tert.*-butyldimethylsilyl esters showed essentially identical behaviour.

4.1. GC–MS measurements

First, the trimethylsilyl ester derivatives of the pure isomers were analysed and the mass spectra of both isomers were recorded (Figs. 2 and 3). It can be seen that GC peaks corresponding to the isomers are distinct, allowing the convenient separation of the isomeric mixture. This was confirmed by the analysis of a mixture of isomers, and also a silylated sample withdrawn from the Perkin condensation mixture, showing



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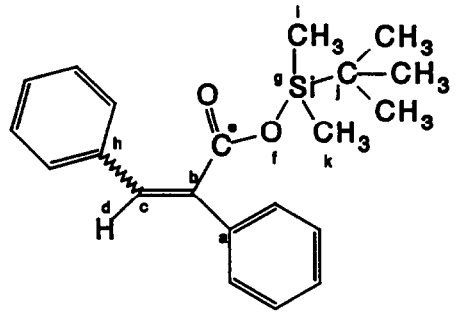

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Fig. 1. Structure **1**, α-phenylcinnamic acid trimethylsilyl esters; structure **2**, α-phenylcinnamic acid *tert.*-butyldimethylsilyl esters.

excellent baseline separation in a reasonable time (Figs. 4 and 5).

The ease of preparation of the silyl esters combined with the high resolution of GC gives

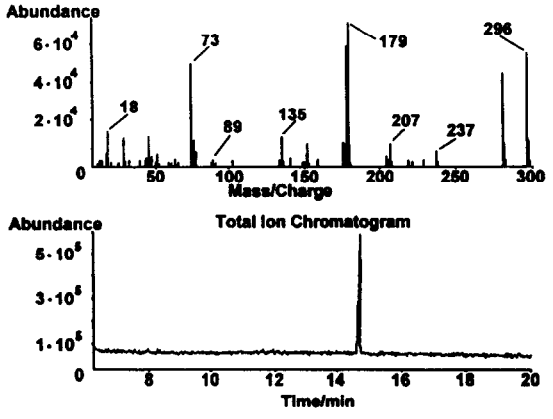

Fig. 2. Total ion chromatogram and mass spectrum of the *E* isomer of compound **1**.

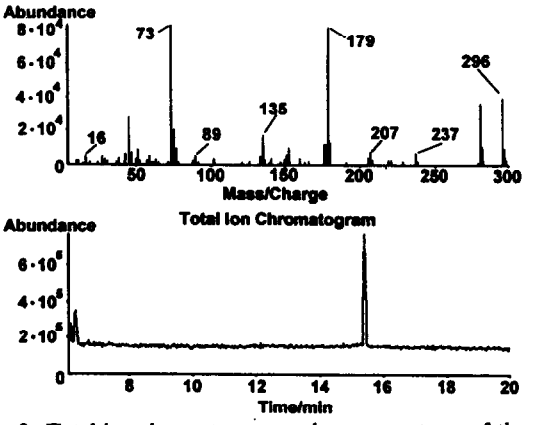

Fig. 3. Total ion chromatogram and mass spectrum of the *Z* isomer of compound **1**.

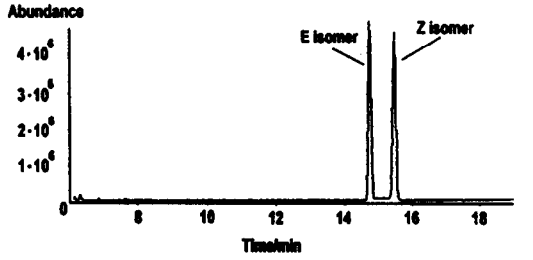

Fig. 4. Total ion chromatogram of the isomeric mixture of compound **1**.

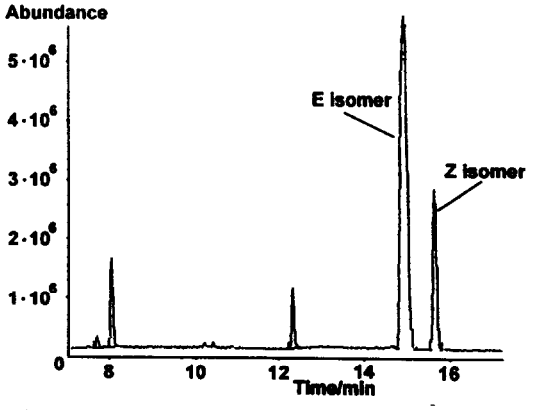

Fig. 5. Total ion chromatogram of the trimethylsilyl derivatives of the product mixture after a 35-min reflux of benzaldehyde, acetic anhydride, phenylacetic acid and triethylamine.

an excellent method for following the isomeric distribution in the Perkin reaction.

4.2. Analysis of mass spectra

Recording the EI mass spectra of the isomers, in addition to identifying the compounds, gives an insight into the bond strength distribution of the isomers. Moreover, secondary reactions, such as rearrangements after fragmentation, can also be studied [8,9].

Unfortunately, the mass spectra alone (Figs. 2 and 3) were not suitable for distinguishing between the isomers in an isomeric mixture, as the fragments were identical. Nevertheless, the relative intensities were significantly (an reproducibly) different (Table 1, columns 3 and 4), allowing the identification of the pure isomers without a known sample for comparison.

As far as the fragmentation pattern is concerned, the most abundant fragments and the molecular ion could be easily identified (Table 1). They were formed via the scission of the O–Si, Si–C and C–O bonds or decarboxylation occurring with the subsequent loss of the olefinic hydrogen. These last two processes were the most important fragmentation pathways.

However, there were fragments that underwent complicated secondary reactions in the mass spectrometer. These reactions involved the scission of several bonds and subsequent rearrangements of the McLafferty type [10,11]. For instance, the suggested route to the fragment of m/z 237 involved the scission of the olefinic hydrogen and then rearrangement to a coumarin or benzopyrilium derivative, resulting in ion **4**. This ion rearranged by losing the $Si(CH_3)_3$ but acquiring one of its CH_3 substituents, ending up with ion **5** (Fig. 6).

The formation of the fragment of m/z 135 may be explained by the rupture of the O–Si and, most interestingly, the olefinic double bond and the scission and subsequent rearrangement of a hydrogen from the α-phenyl group, ending up with a coumaran or benzofuran derivative (Fig. 7).

Table 1
The most abundant fragments, their m/z values and relative peak intensities in the EI mass spectra of (*E*)- and (*Z*)-α-phenylcinnamic acid trimethylsilyl ester isomers (structure **1** in Fig. 1)

Fragment[a]	m/z	Relative intensity (%)	
		E	*Z*
$M^{+\cdot}$	296	78.2	48.6
$[M - CH_3]^+$	281	64.1	43.2
5[b]	237	11.5	8.1
$[M - OSi(CH_3)_3]^+$	207	16.7	9.5
$[M - CO_2Si(CH_3)_3]^+$	179	100.0	100.0
$[M - H - CO_2Si(CH_3)_3]^+$	178	82.1	81.1
Unidentified	152	15.4	12.2
6[c]	135	20.5	21.6
$[(CH_3)_3SiO]^+$	89	5.1	8.1
$[(CH_3)_2SiOH]^+$	75	19.2	24.3
$[(CH_3)_3SI]^+$	73	70.5	100.0
$[SiOH]^+$	45	20.5	33.8

[a] $M^{+\cdot}$ represents the parent ion, *i.e.*, the ion which is formed with the loss of an electron.
[b] see Fig. 6.
[c] see Fig. 7.

4.3. Theoretical aspects

AM1 semi-empirical quantum chemical calculations with full geometric optimization resulted in the minimum energy structures in the gas phase of the two pairs of neutral molecules (**1** and **2**) and their (positively charged) parent ions.

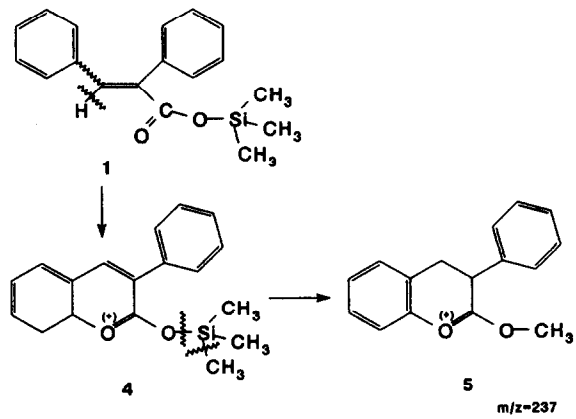

Fig. 6. Route to fragment of m/z 237.

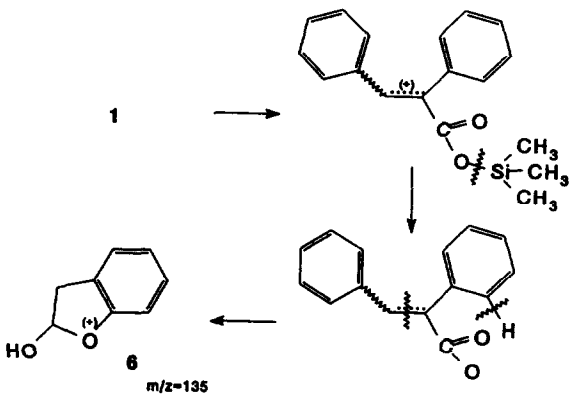

Fig. 7. Route to fragment of m/z 135.

Table 2
Enthalpy of formation for the E and Z isomers of the parent ions and the neutral molecules of α-phenylcinnamic acid silyl ester derivatives (structures **1** and **2** in Fig. 1) calculated by AM1 semi-empirical quantum chemical method after full geometric optimization

Compound	$\Delta H_{f,298}$ (kJ mol^{-1})			
	Parent ion		Neutral molecule	
	E	*Z*	*E*	*Z*
1	647.7	650.2	−320.7	−319.2
2	581.6	584.9	−360.9	−355.9

The calculated standard enthalpies of formation are given in Table 2.

Bond orders were calculated for the optimized geometries. Data for bonds expected to break in the mass spectrometer are displayed in Table 3. It is obvious that bond orders of the parent ions are of relevance to mass spectrometry, as fragmentation and other side-reactions are derived from the parent ion. Nevertheless, bond orders for the neutral molecules are included in Table 3 in italics in order to examine the changes in bond strength due to ionization.

It can be seen that for compound **1**, the experimentally found major routes of bond scission coincide with theoretical calculations. Bond order calculations predict that the most abundant fragments should form via the cleavage of C_b–C_e, O–Si, Si–C or the olefinic C–H bonds and, indeed, this was found experimentally. The loss of the β-phenyl group only occurred in the more complicated scission, the subsequent rearrangement of which was a remarkably important pathway with relative intensities above 20% (fragment **6**; for the mechanism, see Fig. 7).

Comparison of the bond orders of the neutral molecules and their cations revealed that the olefinic double bonds (b–c) became significantly weaker in the parent ions than they were in the neutral molecules. It is highly probable that an electron form the olefinic π-bond was removed

Table 3
Bond orders in the parent ions (roman type) and the neutral molecules (italic type) of the silyl ester derivatives of stereoisomeric α-phenylcinnamic acids (structures **1** and **2** in Fig. 1) determined for geometries optimized by the AM1 semi-empirical quantum chemical method

Bond	**1**				**2**			
	E		*Z*		*E*		*Z*	
a–b	1.14	*0.99*	1.15	*0.99*	1.13	*0.99*	1.16	*0.99*
b–c	1.45	*1.82*	1.44	*1.84*	1.46	*1.82*	1.46	*1.85*
b–e	0.94	*0.94*	0.94	*0.92*	0.95	*0.94*	0.95	*0.92*
c–d	0.93	*0.94*	0.93	*0.94*	0.93	*0.94*	0.93	*0.94*
c–h	1.20	*1.02*	1.20	*1.02*	1.19	*1.02*	1.18	*1.02*
e–f	1.04	*1.12*	1.04	*1.11*	1.04	*1.11*	1.04	*1.11*
f–g	0.67	*0.65*	0.67	*0.65*	0.68	*0.65*	0.67	*0.66*
g–i	0.88	*0.87*	0.89	*0.87*	0.91	*0.88*	0.90	*0.87*
g–j	0.88	*0.88*	0.88	*0.88*	0.87	*0.83*	0.87	*0.84*
g–k	0.91	*0.88*	0.91	*0.88*	0.88	*0.88*	0.89	*0.88*

by electron bombardment in the mass-selective detector and the resulting three-electron bond grew appreciably weaker, facilitating the rupture of the bond in further reactions. The C–O bond (e–f) also weakened, accounting for the relative importance of fragment of m/z 207. Bonds a–b and c–h (the bonds between the olefinic carbons and the phenyl groups) grew stronger, however, explaining the experimental finding that the relative intensity of **5** (direct removal of the α-phenyl group) was lower than that of **6** (indirect removal of the β-phenyl group) in both isomers.

Calculated bond orders for the two pairs of different silyl esters revealed that the major cleavage routes remained the same irrespective of the silylating agent. Experiments with compound **2** verified this finding. It was also predicted that routine EI mass spectrometry could not differentiate between the isomeric esters, again irrespective of the silylating agent. This was also verified experimentally with compounds **1** and **2**.

5. Conclusions

A convenient and rapid GC–MS method was developed for the analysis of the complex reaction mixture leading to an α-phenylcinnamic acid isomeric mixture. Analysis of the mass spectra resulted in the identification of the major cleavage pathways. Bond scission and subsequent rearrangement reaction sequences were suggested to explain the formation of some unusual fragments. Bond order calculations for the optimized geometries of the parent ions were found to be able to predict major cleavage routes. Finally, calculations and experiments revealed that a routine mass spectrometer with an EI source cannot distinguish between the isomers, irrespective of the silylating agent.

6. Acknowledgements

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