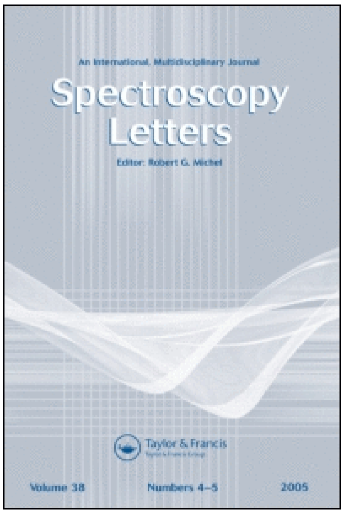

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Characterization of Some Isomeric Furocoumarinones Using Gas Chromatography Ion Trap Tandem Mass Spectrometry[#]

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ABSTRACT

A series of different furocoumarinones was submitted to electron impact (EI) and tandem MS experiments. The compounds examined showed decomposition patterns that allowed structural interpretation and the isomer pairs were characterised by collision experiments performed by an ion trap mass spectrometer. Electron impact spectra were partially

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ineffective for distinguishing between isomer pairs, while the breakdown curves of $M^{+\cdot}$ ions, obtained by varying the tickle voltage on an ion trap instrument, allowed their characterisation.

Key Words: Heterocycles; Furocoumarinones; Mass spectra; Ion trap MS/MS.

INTRODUCTION

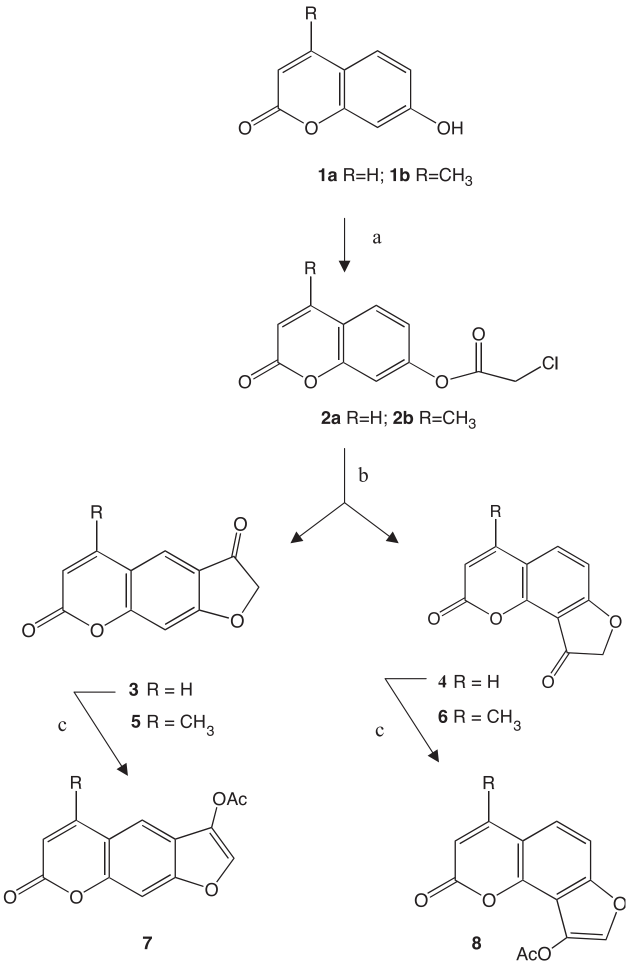
Furocoumarins are an important class of natural and synthetic compounds with properties of pharmacological and industrial interest.[1–3] In particular, both linear and angular furocoumarins are important as photochemotherapeutic agents that are used to treat a variety of skin diseases, thanks to their ability to intercalate in the double-stranded DNA, and to undergo photoaddition to thymine, thereby blocking cell growth and replication.[3,4] Since dihydrofurocoumarinones are tautomers of the furocoumarins, they possess similar properties, but they also have very interesting possibilities as versatile substrates for obtaining new furocoumarin derivatives via bromination, condensation with aldehydes and ketones,[5] oxime formation,[6] azacoupling with benzene diazonium salts,[7] and others. We are now preparing some simple derivatives. Our main interest is to use them as dienophiles of tetrazines in the Diels-Alder reaction of the inverse demand of electrons[8] to prepare aromatic furocoumarin isosters.[9,10]

Pursuing our research on the synthesis and mass spectrometric behaviour of heterocyclic compounds,[8–11] in this paper we focused our attention on the characterisation of a set of differently substituted furodihydrocoumarinones, by use of gas chromatography ion trap tandem mass spectrometry. All the synthesised compounds (**1–8**, see Scheme 1) have been characterized by IR, ^{1}H-NMR, and elemental analysis, and studies have been undertaken to investigate structure-activity relationships.

In past years different analytical approaches have been used extensively for the structural characterisation of both natural and synthetic coumarins.[12–14] The development of new analytical procedures capable of distinguishing an isomer pair by means of a low cost instrument has been of great interest.

In fact, the advent of ion trap mass spectrometry[15] has greatly contributed to the characterisation of isomeric compounds by tandem mass spectrometry experiments (MS/MS).[16]

The compounds that were examined, dihydrofuro[2,3-h]coumarin-9-one (**3**), 4-methyl–dihydrofuro[2,3-h]coumarin-9-one (**4**), dihydrofuro-[3,2-g]coumarin-6-one (**5**), 4-methyl-dihydrofuro[3,2-g]coumarin-6-one (**6**),



Scheme 1. Reagents and conditions: a) $(ClCH_2CO)_2CO$, DMAP, THF reflux; b) $AlCl_3$, 120°C; c) $(ClCH_2CO)_2CO$, DMAP, 60°C.

9-acetoxyfuro[2,3-h]coumarin (**7**), and 6-acetoxyfuro[3,2-g]coumarin (**8**) were studied under electron impact (EI) conditions, and their fragmentation pathways were assigned by performing tandem mass spectrometry experiments using collisional activation to induce ion fragmentation.

Collisionally-induced dissociation (CID) experiments were performed by irradiating the selected ion at its specific resonance frequency with a supplementary RF potential. The result is an increase in the ion kinetic energy and, through several collisions with the helium buffer gas, a 'step by step' increase in the ion internal energy which generally favours decomposition processes with lower critical energy.

EXPERIMENTAL

Synthesis

The compounds **3–8** were obtained following Scheme 1. Compounds **2a–b** and **3**, **4**, **5**, **6** were synthesized according to the method previously reported in literature.[17,18]

Compounds **7** and **8** were synthesized in good yields and identified by their analytical and spectral data as reported below.

General Procedure for the Acetylation of Dihydrofurocoumarinones

A mixture of dihydrofurocoumarinone (202 mg, 1 mmol), acetic anhydride (10 mL), and dimethylaminopyridine (DMAP) (20 mg) was stirred for 2 hours at 60°C.

It was then poured into water and the mixture was extracted with methylene chloride.

The extract was dried over sodium sulphate and the solvent was evaporated under reduced pressure.

The yellow residue was purified by fast chromatography using methylene chloride as eluent.

9-Acetoxyfuro[2,3-*h*]coumarin (**7**)

Yield 85%. Mp 152–4°C. IR (KBr) ν: 3165, 1786, 1722, 1620,1345, 1215,1154, 1104, 1083, 1055, 840 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 7.90 (s,1H, H-8), 7.76 (d, $J = 9.6$ Hz, 1H, H-4), 7.38 (s, 2H, H-5+H-6), 6.40 (d, $J = 9.6$ Hz, 1H, H-3), 2.48 (s,3H, CH_3).

6-Acetoxyfuro[2,3-*g*]coumarin (**8**)

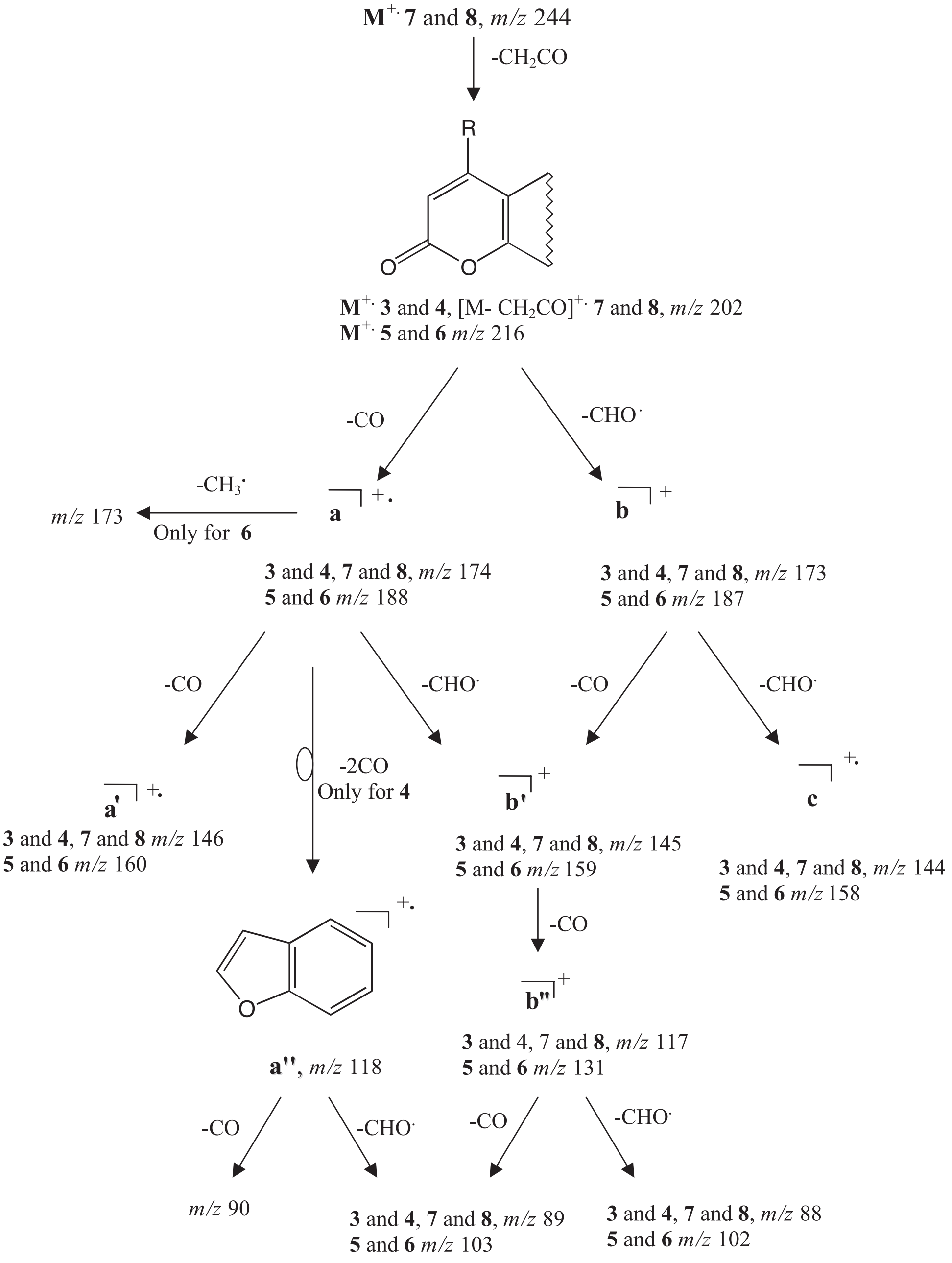
Yield 80%. Mp 177–9 °C. IR (KBr) ν: 3051, 1759, 1720, 1635, 1575, 1375, 1334, 1218, 1141, 884 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 8.10 (s, 1H, H-7), 7.80 (d, $J = 9.6$ Hz, 1H, H-4), 7.67 (s, 1H, H-5), 7.43 (s, 1H, H-6), 6.40 (d, $J = 9.6$ Hz, 1H, H-3), 2.41 (s, 3H, CH_3).

Instrumental

Melting points are uncorrected and were determined using a Reichert Kofler thermopan or in capillary tubes in a Buchi 510 apparatus. IR spectra were recorded using a Perkin-Elmer 1640FT spectrometer (ν in cm^{-1}). ^1H NMR spectra (300 MHZ) were recorded using a Bruker AMX spectrometer, using TMS as internal standard (Chemical shifts as δ values, J in Hz). Elemental analyses were performed with a Perkin-Elmer 240B micro-analyser. Analytical TLC was performed was on precoated silica gel plates (Merck 60 F254, 0.25mm).

Table 1. Main ions and their relative abundances (in parentheses) observed in the 70 eV EI mass spectra of compounds **3–8**.

Ionic species	**3**	**4**	**5**	**6**	**7**	**8**
$M^{+\cdot}$	202 (100)	202 (100)	216 (100)	216 (100)	244 (19)	244 (4)
$[M\text{-}CH_2CO]^{+\cdot}$	/	/	/	/	202 (100)	202 (100)
$[M\text{-}CO]^{+\cdot}$ (a)	174 (38)	174 (39)	188 (52)	188 (58)	174 (42)	174 (36)
$[a\text{-}CH_3]^{+}$	/	/	/	173 (5)	/	/
$[M\text{-}CHO]^{+}$ (b)	173 (24)	173 (20)	187 (15)	187 (9)	173 (16)	173 (6)
$[a\text{-}CO]^{+\cdot}$ (a^{I})	146 (7)	146 (12)	160 (9)	160 (14)	146 (10)	146 (17)
$[a\text{-}CHO]^{+}$ (b^{I})	145 (65)	145 (50)	159 (69)	159 (67)	145 (39)	145 (21)
$[b\text{-}CHO]^{+\cdot}$(c)	144 (12)	144 (5)	158 (1)	158 (2)	144 (2)	144 (2)
$[a\text{-}2CO]^{+\cdot}$ (a^{II})	118 (3)	118 (13)	132 (2)	132 (6)	118 (3)	118 (4)
$[b^{I}\text{-}CO]^{+}$ (b^{II})	117 (16)	117 (12)	131 (7)	131 (19)	117 (9)	117 (5)
$[a^{II}\text{-}CO]^{+}$	90 (4)	90 (7)	130 (4)	130 (6)	90 (2)	90 (6)
$[b^{II}\text{-}CO]^{+}$	89 (11)	89 (13)	103 (5)	103 (15)	89 (13)	89 (13)
$[b^{II}\text{-}CHO]^{+\cdot}$	88 (14)	88 (14)	102 (7)	102 (12)	88 (7)	88 (7)



Scheme 2. Collisionally-induced fragmentation pattern of compounds **3–8**.

Analyses were performed using a Saturn CP-3800 gas chromatograph directly connected to a Saturn 2000 ion-trap (Varian, Walnut Creek, CA, USA) operating under electron impact (EI) conditions (70 eV). The transfer line and the ion trap manifold were set to 250 and 150°C, respectively.

Full-scan data acquisition was made over the range *m/z* 40–400, at 0.56 s/scan under the following conditions: solvent delay 5 min, emission current 10 μA, automatic gain control (AGC) target 20000.

Collisional experiments were performed by application of a supplementary RF voltage (tickle voltage) in the range 0.1–1.4 V. In the MS/MS mode the AGC target was lowered to 7000 to avoid space charge effects and the AGC prescan ionisation time was changed from 100 to 1500 μs. Moreover, helium at a pressure of 1 mTorr was used for ITMS.

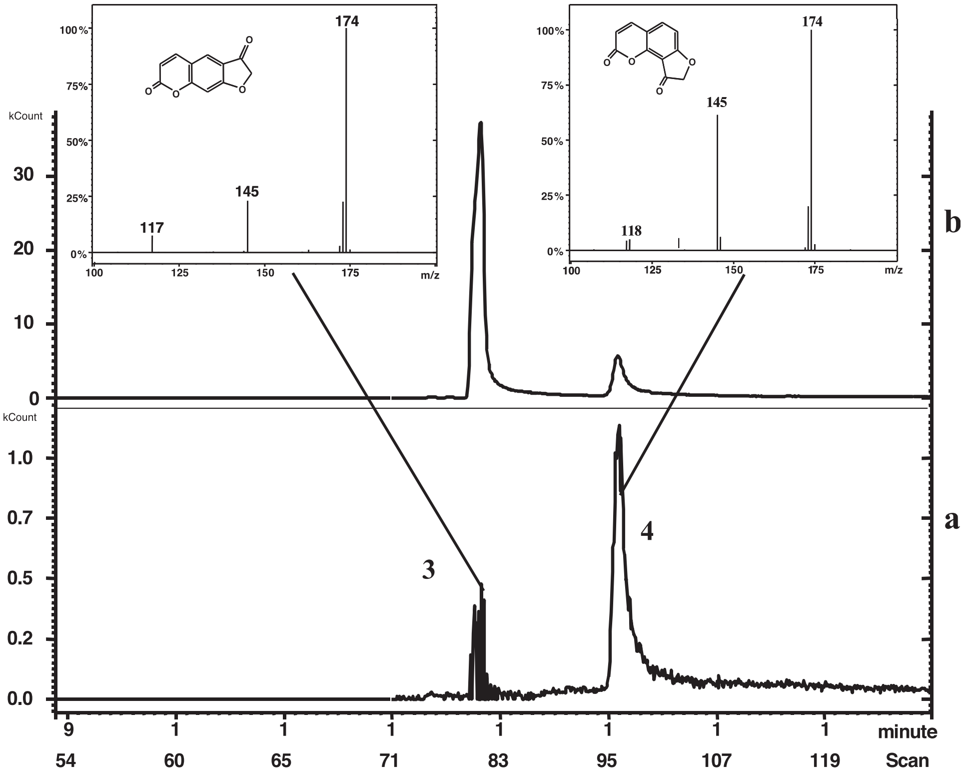


Figure 1. GC/MS chromatogram obtained from isomers **3** and **4** with the ion trap set to monitor the species generated by CID of the ions at *m/z* 174 (a = total ion current, b = reconstructed ion chromatogram for *m/z* 118).

Samples were introduced into the GC using a 1077 injector kept at 250°C. A CP-Sil 8 CB-MS capillary column (30 m × 0,25 mm, 0,25 μm film thickness) was used with helium as carrier gas with a flow-rate of 1 ml/min and the oven temperature was programmed as follows: 120°C for 1 min, followed by a temperature ramp at 10°C/ min up to 280°C.

RESULTS AND DISCUSSION

Our study was devoted to the characterisation of three different sets of isomeric compounds by using ion trap GC/MS with electron impact ionisation (EI).

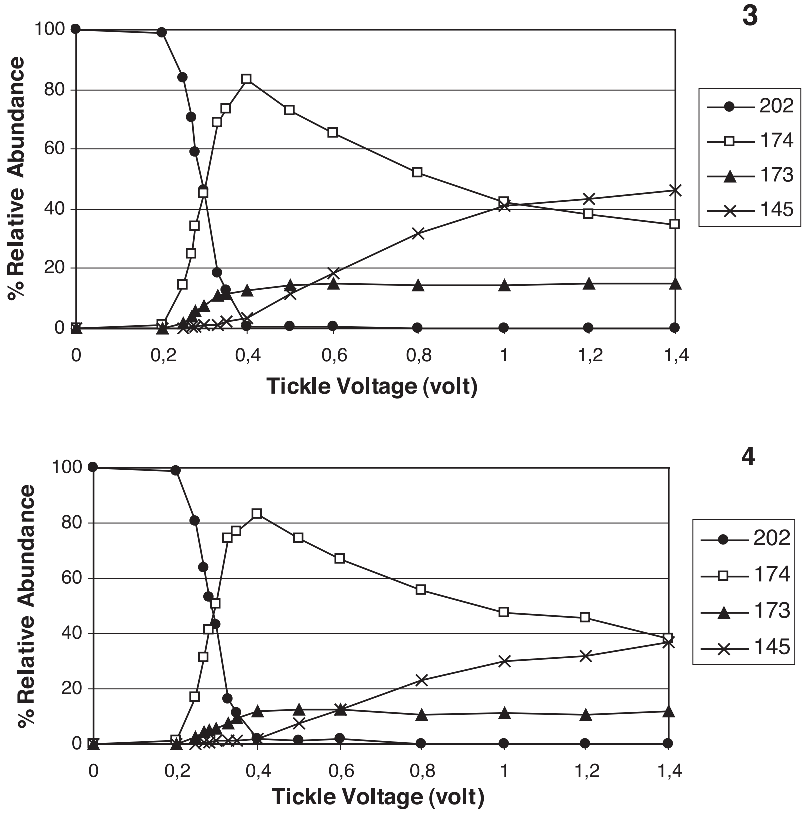


Figure 2. Breakdown curves of $M^{+\cdot}$ ion of compounds **3** and **4** at *m/z* 202.

The EI spectra of compounds **3–8** are reported in Table 1 and the related fragmentation pattern is shown in Scheme 2, as obtained by MS^n experiments.

The samples of isomers (**3** and **4**; **5** and **6**; **7** and **8**) show similar electron impact(EI)-induced fragmentation patterns. In fact, each pair of isomers displays nearly the same set of fragment ions and the same base peak, namely isomers **3** and **4** and isomers **5** and **6** have the molecular ion at *m/z* 202 and *m/z* 216 respectively as their base peak, while isomers **7** and **8** have their base peak at *m/z* 202 corresponding to the loss of CH_2CO from the molecular ion. In any case, there are differences in the relative abundances of some of their fragment ions.

The EI mass spectrum of isomer **4** shows an abundant fragment ion at *m/z* 118 (13%) for the sequential 2CO losses from the precursor *m/z* 174

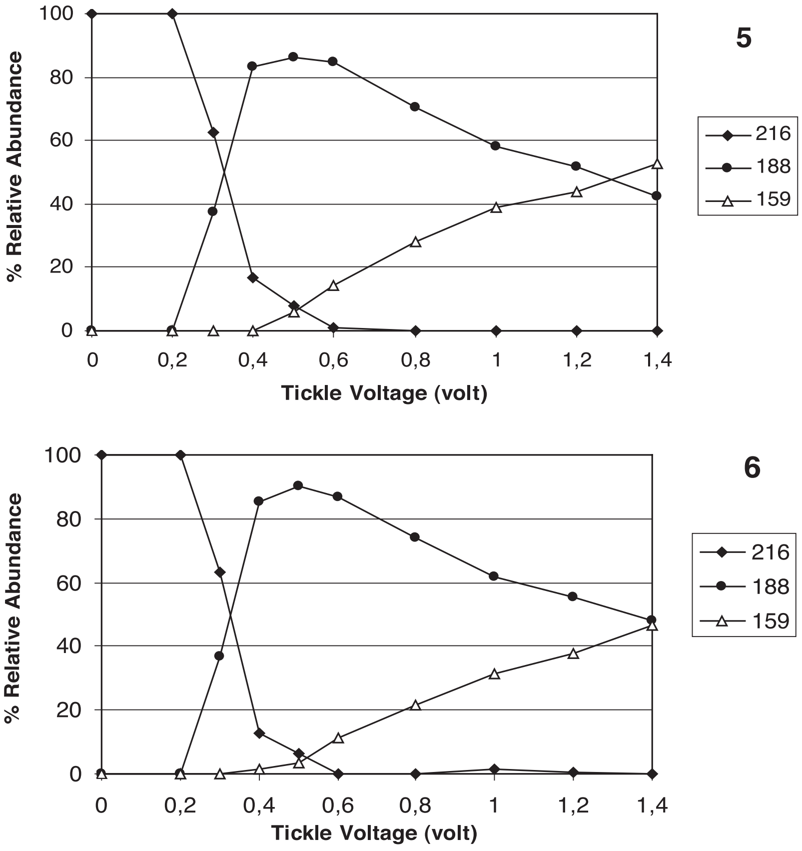


Figure 3. Breakdown curves of $M^{+\cdot}$ ion of compounds **5** and **6** at *m/z* 216.

compared to the linear isomer **3** (3%). This difference is confirmed by CID-MS/MS between the EI-induced fragment ion at *m/z* 174 of **3** and **4** with a collision energy of 0.4 V. Only in the case of **4** the ionic species at *m/z* 118 is detected (see Figure 1a and 1b). Isomers **3** and **4** can be also distinguished, on the basis of the EI spectra, by comparing the fragment ions at *m/z* 144 and *m/z* 146. The angular isomer **4** yields a 2.4 ratio of *m/z* 146:144 ions while the linear isomer **3** yields a ratio of 0.6.

Isomer **6** differs from the linear **5**, particularly in displaying a scarcely abundant ion (5%) at *m/z* 173, assigned to the loss of $^{\cdot}CH_3$ from the $[M\text{-}CO]^{+\cdot}$ ion (*m/z* 188). Compounds **5** and **6** can be further distinguished by comparing the abundance of the ions at *m/z* 131 and m/z 103 originated from the precursor ion at *m/z* 159 through the sequential losses of CO molecules.

Both linear and angular furocoumarinones, **3** vs **4** and **5** vs **6**, can be also distinguished by comparing the sequential losses of CO molecules from the respective molecular ion, yielding ionic species at *m/z* 146 and m/z 160, respectively for each pair. In fact the angular isomers **4** and **6** show a higher relative abundance compared to linear isomers **3** and **5**.

The last pair of isomers, compounds **7** and **8**, which carry an acetoxy substituent on position 9 of the furano ring, show practically superimposable mass spectra. A little differentiation between the two isomers can be obtained only on the basis of the different relative abundance of the fragment ions at *m/z* 173 and *m/z* 146.

Further experiments were performed by studying the variation of the relative abundances of collisionally generated product ions. The breakdown curves, obtained by varying the resonant excitation voltage of $M^{+\cdot}$ in the range 0.2–1.4 V, and measuring the relative abundance of the precursor and the predominant product ions, were plotted for two pairs of isomers, **3**,**4** and **5**,**6**. They are reported in Figures 2 and 3 respectively. For compounds

Table 2. Crossing point (V) for energy dependences of relative abundances of the main decomposition pathways observed in the CID-MS/MS spectra of $M^{+\cdot}$ of **3–6** (see Figures 2 and 3).

	Compounds under investigation			
Ionic species	**3**	**4**	**5**	**6**
$M^{+\cdot} \rightarrow [M\text{-}CO]^{+\cdot}$	0.30	0.29	0.33	0.33
$M^{+\cdot} \rightarrow [M\text{-}CHO]^{+}$	0.33	0.34	–	–
$M^{+\cdot} \rightarrow [M\text{-}CO_2H]^{+}$	0.39	0.4	0.51	0.53
$M^{+\cdot} \rightarrow [M\text{-}CO\text{-}CHO]^{+}$	1.15	1.4	1.3	1.4

3 and **4** a clear difference is present between the crossing point of the curves related to the ions at m/z 174 ($[\text{M-CO}]^{+\cdot}$) and 145 ($[\text{M-CO-CHO}]^{+}$) (see Figure 2 and Table 2).

Unfortunately compounds **5** and **6** are difficult to distinguish on the basis of the breakdown curves. In fact, on inspection of the plots, it appears that these isomers behave nearly identically over the entire range of collision energies examined (Table 2). On the basis of the breakdown curves related to fragmentation processes less favoured than those mentioned previously, some further, small differences were detected, as shown by Figures 4 and 5. Again a clear distinction for isomers **3** and **4** can be obtained: the formation of fragments at *m/z* 146 and *m/z* 118 (6% and 5.3% at 1.4 V, respectively) was only observed in the case of the angular isomer **4**, while the relative abundance of fragment at *m/z* 117 of isomer **3** is

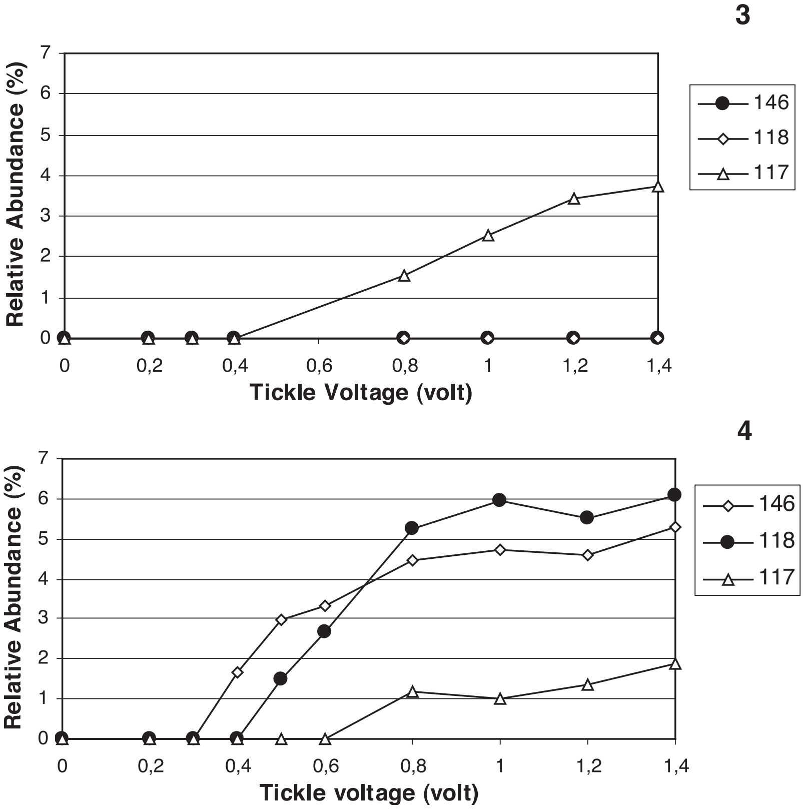
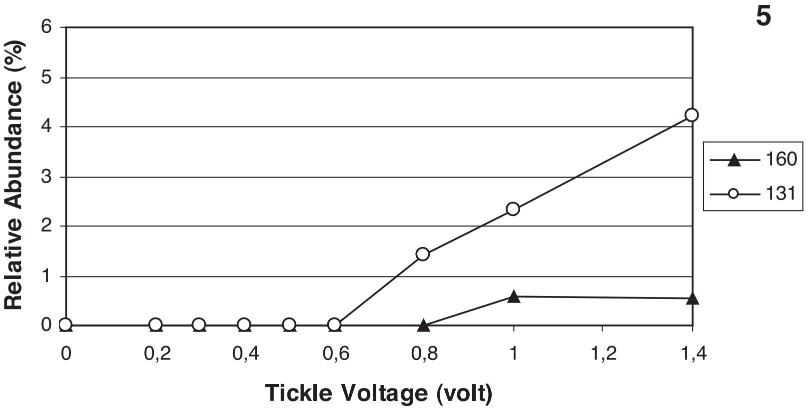


Figure 4. Breakdown curves of $\text{M}^{+\cdot}$ ion of compounds **3** and **4** at *m/z* 202.

slightly higher than that of **4** (3.8% vs 2% at 1.4V) (see Figure 4). Similarly the other sets of isomers **5** and **6** can be differentiated considering the relative abundances of ions at *m*/*z* 160 and *m*/*z* 131 (see Figure 5).

These data suggest that angular furocoumarinone isomers require a lower activation energy for the analogous decomposition processes than the linear ones. This result can be justified by a higher stability of the linear structure, which is also in agreement with the heat of formation of compounds **3–6**, performed using the AM1 semi-empirical method. Infact these results show that the energy of angular furocoumarinones (**4** and **6**) is 3 Kcal/mol higher than that of the related linear furocoumarinones (**3** and **5**).

The last two isomers, **7** and **8**, were distinguished by comparing the relative losses of CHO$^{\cdot}$ and CO through MS/MS experiments performed on



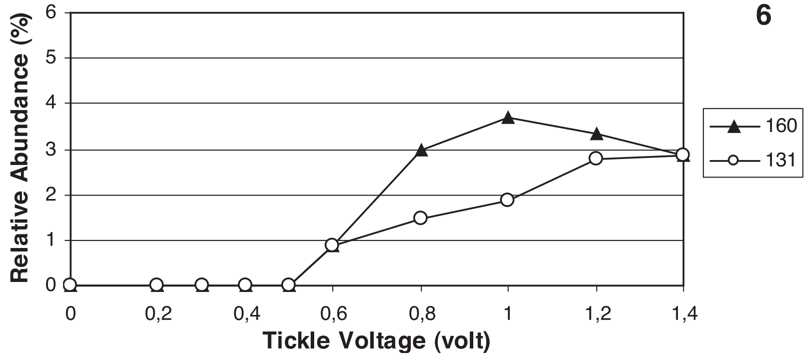


Figure 5. Breakdown curves of $M^{+\cdot}$ ion of compounds **5** and **6** at *m*/*z* 216.

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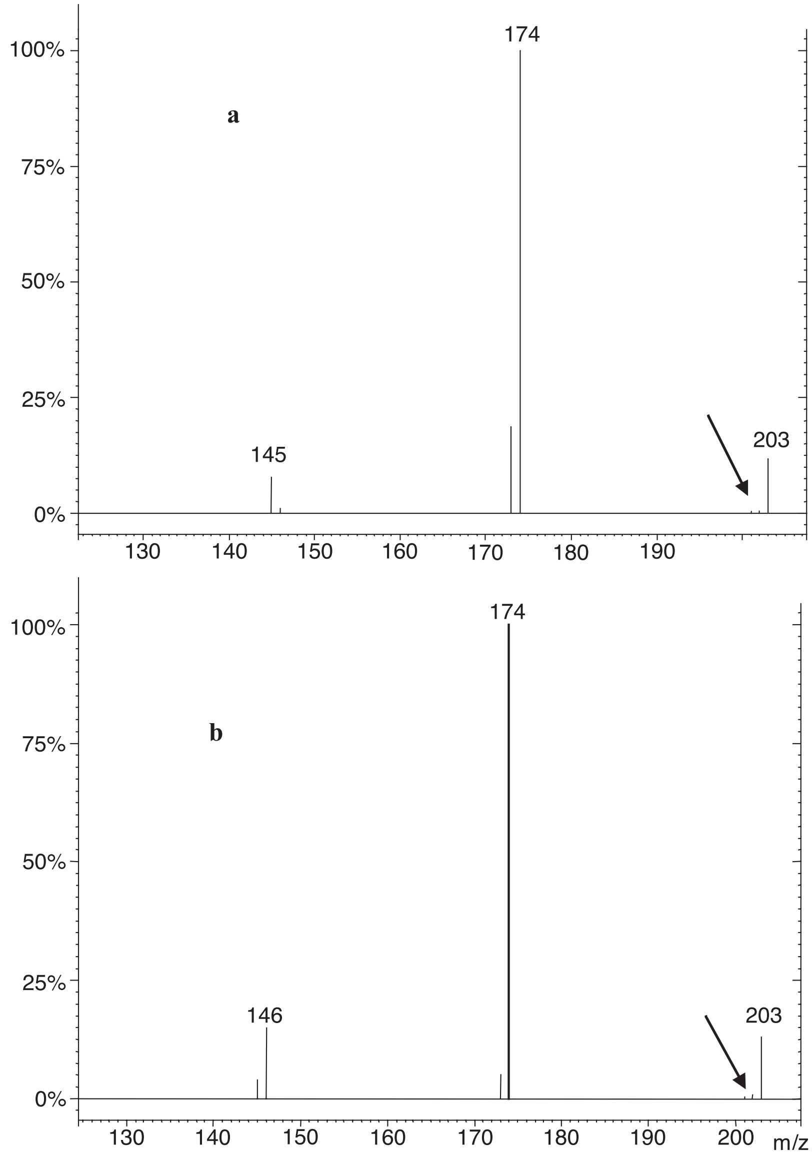

Figure 6. MS/MS spectra of isolated ions *m*/*z* 202 at 0.5 V for compounds (a) **7** and (b) **8**.

the EI-induced fragment ion at *m/z* 202. As can be seen in the two spectra reported in Figure 6a and 6b, angular isomer **8** differs from the linear **7** by displaying a large abundance of the ion at *m/z* 146.

CONCLUSIONS

In this paper, the compounds studied showed that EI spectra are partially ineffective to distinguish isomer pairs, while the breakdown curves of $M^{+\cdot}$ ions obtained by varying the tickle voltage on an ion trap instrument allowed their characterisation.

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