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Electron Ionization Mass Spectrometry of Curcumin Analogues: an Olefin Metathesis Reaction in the Fragmentation of Radical Cations

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The natural compound curcumin, used in cosmetics, traditional medicines and as a spice in food, is known as a multi-factorial anti-inflammatory agent. To study the anti-inflammatory activity of curcumin derivatives, 24 analogues were synthesized and their structures were confirmed by ^{1}H NMR and electron ionization (EI) mass spectrometry. Most signals in the EI mass spectra can be attributed to commonly known fragmentations, but the formation of ring-substituted 1,2-diphenylethene (stilbene)-type radical cations, observed in the spectra of all compounds investigated and resulting in the base peak for some compounds, requires a peculiar rearrangement. Metastable ion spectra and ^{13}C labelling studies show that the stilbene-type ions are formed directly from the molecular ions and contain the two original aryl groups and the 1 and 7 carbon atoms of the olefinic system. It is proposed that the formation of stilbene-type ions results from an intramolecular olefin metathesis reaction; this suggestion is supported by semi-empirical (MNDO/PM3) calculations. © **1998 John Wiley & Sons, Ltd.**

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INTRODUCTION

Curcumin, 1,7-bis(4-hydroxy-3-methoxyphenyl)hepta-1, 6-diene-3,5-dione (Fig. 1), may be obtained from various plant roots (among others from *Curcuma longa*). This orange–yellow phytochemical is often used as a colorant in cosmetics and food; it was first isolated in 1870 and its structure was elucidated in 1910.[1] The anti-inflammatory and possible anti-tumor activity[2,3] and the low toxicity of curcumin inspired us to investigate a wide variety of symmetric curcumin analogues. A synthetic route to curcumin, developed by Pavolini *et al.*[4,5] and by Pabon,[6] starts with 4-hydroxy,3-methoxybenzaldehyde (vanillin) and pentane-2,4-dione. Compounds with various substituents in the A, B and C ring positions or the aliphatic D position (Fig. 1) were synthesized from appropriately substituted benzaldehyde or pentane-3,5-dione, using Pabon's method.[7]

Although some curcumin analogues are even amenable to gas chromatography with electron ionization mass spectrometric (EIMS) detection and the EI mass spectrum of curcumin is included in common EI mass spectral libraries,[8] the fragmentation behaviour of these compounds has not been studied. Indeed, most fragmentations under EI conditions are straightforward and would not justify any further investigation, but one particular reaction appears strange. In the spectra of many of our synthetic curcumin analogues, this fragmentation reaction even gives rise to the base peak. Here, we report on the fragmentation of curcumin and its analogues, focusing attention on a new, peculiar rearrangement of the molecular ions.

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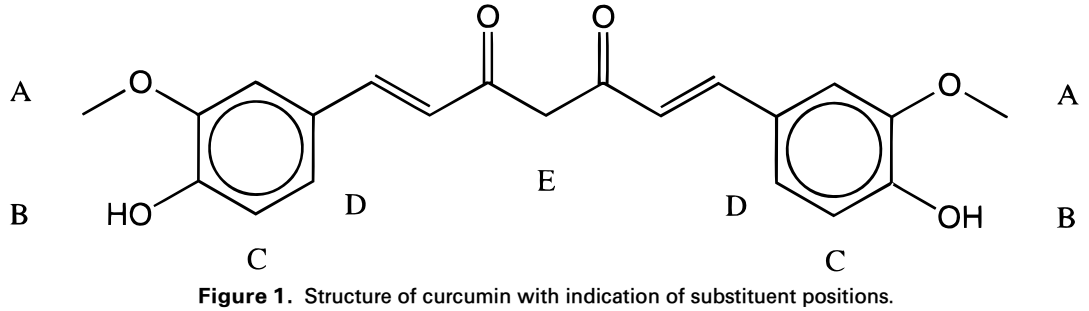

Figure 1. Structure of curcumin with indication of substituent positions.

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EXPERIMENTAL

Synthesis

All compounds were synthesized from the appropriately substituted benzaldehyde and pentane-2,4-dione following the procedure described by Pabon.[6] The reaction was performed at room temperature using tributyl borate, with 1-aminobutane as a catalyst. All compounds were characterized by their ^{1}H NMR and EI mass spectra and by exact mass measurement.[7] 1,7-$^{13}C_2$-labelled 1,7-diphenylhepta-1,6-diene-3,5-dione, **14**-$^{13}C_2$, was obtained from α-[^{13}C]benzaldehyde (99.9% ^{13}C; Campro Scientific, Veenendaal, The Netherlands) and pentane-2,4-dione, by scaling down the procedure to the mmole scale; in this case the final recrystallization step of Pabon's procedure was omitted and ^{1}H NMR was not applied.

Mass spectrometry

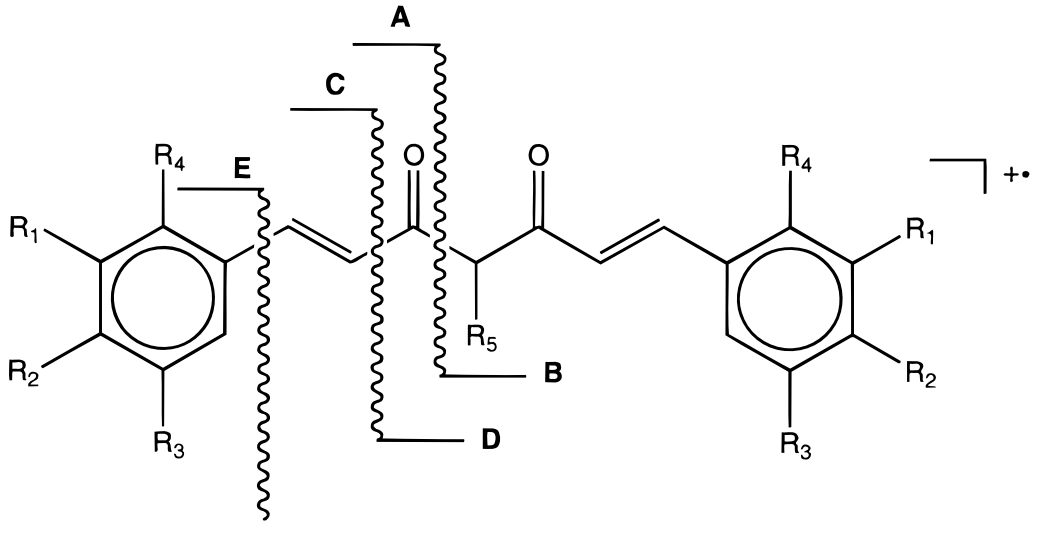
Common EI mass spectra and mass-analysed ion kinetic energy (MIKE) mass spectra were obtained on a Finnigan MAT 90 mass spectrometer (Finnigan MAT, Bremen, Germany), operated under EI conditions (70 eV ionization energy, 0.2 mA collector current, 250 °C source temperature) and using the standard direct inlet probe. Full MIKE spectra were recorded at an overall mass resolution of ~800 and metastable ion peak shapes for kinetic energy release deteminations were recorded at a main beam width at half-height, $w_{0.5}$, of <2.0 eV at an ion kinetic energy of ~4750 eV.

RESULTS AND DISCUSSION

The structures and partial mass spectra of the curcumin derivatives are given in Tables 1 and 2. These tables were obtained by first taking all signals with an intensity of more than 10%, relative to the base peak and then comparing them with similar masses and/or fragmentation products with an intensity of <10%. Table 1 shows the data for the analogues with a substituent on the 4-position of the heptadienedione moiety and Table 2 gives data for aryl-substituted analogues. Some important cleavage reactions are envisaged in Scheme 1; the characters refer to the product ions in Tables 1 and 2. For example, the cleavage of the C—CO bond, pathway **A**, specifically produces ene-acylium ions, [R—CH=CH—CO]$^+$. Many of the product ions formed can be explained by similar simple fragmentation reactions.

In contrast, most compounds also show open-and closed-shell product ions that can only result from extensive rearrangement reactions. The product ions of some of these rearrangements are given in Scheme 2, with characters again corresponding to Tables 1 and 2. The formation of ring-substituted styrene radical cations, **F**, can proceed either via a β-hydrogen shift (shown in Scheme 2) to the double bond or via a McLafferty rearrangement involving a hydroxy-hydrogen from the keto–enol-type molecules (not shown). The formation of ring-substituted benzyl cations, **G**, can only occur after the transfer of a hydrogen atom to the C-1 atom of the heptadienedione moiety, but the origin of the hydrogen atom involved is not speculated upon here. One particular rearrangement reaction, denoted **X** in Tables 1 and 2 (not shown in Scheme 2), becomes dominant in the fragmentation of curcumin analogues with bulky substituents. When the substituents at the E position (Fig. 1) become as large as phenyl, this rearrangement produces the base peak ions (*m/z* 272, Table 1) and the same holds for compounds with substituents in the aryl A and C positions becoming larger than ethyl groups. The prominence of the product ion signals of this particular rearrangement prompted us to investigate the formation of the product ions.

The MIKE mass spectra of the molecular ions all have a prominent signal for the formation of the **X** product ions, thus confirming that the product ions result directly from the molecular ions. The kinetic energy release (KER, specified by $T_{0.5}$), determined from the Gaussian-shaped peaks for this fragmentation and the intensity of the metastable ion peak relative to the precursor ion main beam are given in Table 3 for a



Scheme 1

Table 1. Partial EI mass spectra of curcumin (1) and some of its 4-substituted derivatives

Compound[a], R_5													
1 H	**2** Me	**3** Et	**4** n-Pr	**5** i-Pr	**6** n-Bu	**7** $PhCH_2$	**8** Ph	**9** 4-MePH	**10** 4-MeOPh	**11** 4-FPh	**12** 4-ClPh	**13** 4-CF_3Ph	Attribution[b]
368 (31)	382 (18)	396 (18)	410 (29)	410 (21)	424 (19)	458 (23)	444 (10)	458 (12)	474 (15)	462 (3)	478 (6)	512 (9)	$M^{+\cdot}$
350 (39)	364 (1)	—	—	—	—	—	426 (5)	440 (5)	456 (4)	444 (1)	460 (3)	494 (7)	$[M-H_2O]^{+\cdot}$
367 (1)	—	367 (1)	367 (1)	367 (2)	—	—	—	—	—	—	—	—	$[M-R_5]^{+}$
234 (2)	248 (1)	—	—	—	—	324 (15)	—	324 (5)	340 (6)	328 (6)	344 (4)	378 (4)	$[M-134]^{+\cdot}$
232 (12)	246 (2)	262 (2)	274 (3)	274 (2)	288 (2)	322 (4)	308 (6)	322 (8)	338 (6)	326 (3)	342 (3)	376 (4)	$[M-136]^{+\cdot}$
—	—	—	—	—	302 (7)	—	—	—	—	—	—	—	
298 (2)	298 (2)	298 (3)	298 (4)	298 (5)	—	—	—	—	—	—	—	—	
219 (3)	233 (1)	247 (1)	261 (1)	261 (1)	275 (2)	309 (1)	295 (7)	309 (10)	325 (5)	313 (1)	329 (3)	363 (2)	D
244 (4)	258 (6)	—	286 (6)	286 (5)	—	—	—	—	—	—	—	—	
—	206 (2)	220 (2)	234 (3)	234 (3)	248 (3)	282 (4)	268 (2)	282 (5)	298 (8)	286 (3)	302 (1)	336 (1)	
191 (32)	205 (2)	219 (1)	233 (1)	233 (2)	247 (2)	281 (19)	267 (2)	281 (3)	297 (2)	285 (1)	301 (1)	335 (3)	B
190 (39)	204 (2)	218 (1)	232 (1)	232 (2)	246 (1)	280 (2)	266 (2)	280 (2)	296 (2)	—	300 (1)	334 (1)	
272 (16)	272 (18)	272 (20)	272 (20)	272 (9)	272 (17)	272 (23)	272 (100)	272 (100)	272 (100)	272 (33)	272 (100)	272 (100)	X
—	—	—	—	219 (5)	—	—	—	—	—	—	—	—	
217 (14)	—	—	—	—	—	—	—	—	—	—	—	—	
—	—	—	205 (5)	—	—	—	—	—	—	—	—	—	
*	191 (3)	191 (5)	191 (5)	191 (5)	—	—	—	—	—	—	—	—	
*	190 (1)	190 (1)	190 (1)	190 (3)	—	—	—	—	190 (6)	—	—	—	
177 (100)	177 (100)	177 (100)	177 (100)	177 (100)	177 (100)	177 (100)	177 (42)	177 (56)	177 (74)	177 (100)	177 (49)	177 (35)	A
175 (12)	—	—	—	—	—	—	—	—	—	—	—	—	
174 (4)	174 (4)	174 (4)	174 (6)	174 (5)	174 (4)	—	—	—	—	—	—	—	
—	—	—	—	—	—	152 (40)	—	—	—	—	—	—	
—	—	—	—	—	—	151 (55)	—	—	—	—	—	—	
150 (24)	150 (9)	150 (6)	150 (7)	150 (7)	150 (9)	150 (16)	150 (26)	150 (29)	150 (22)	150 (39)	150 (39)	150 (33)	F
149 (13)	149 (7)	149 (7)	149 (8)	149 (9)	149 (7)	149 (7)	149 (5)	149 (5)	149 (7)	149 (7)	149 (6)	—	C
148 (6)	—	—	—	—	—	—	—	—	148 (15)	—	—	—	
147 (13)	—	—	—	—	—	—	—	—	—	—	—	—	
145 (39)	145 (30)	145 (29)	145 (28)	145 (30)	145 (25)	145 (21)	145 (16)	145 (19)	145 (23)	145 (26)	145 (16)	145 (14)	
137 (39)	137 (8)	137 (6)	137 (7)	137 (9)	137 (10)	137 (14)	137 (16)	137 (16)	137 (15)	137 (13)	137 (18)	137 (20)	G
135 (10)	135 (4)	135 (2)	135 (3)	135 (3)	135 (3)	—	135 (6)	135 (6)	135 (18)	135 (7)	135 (7)	135 (6)	
134 (13)	134 (6)	134 (6)	134 (6)	134 (6)	134 (5)	—	134 (5)	134 (5)	134 (5)	134 (4)	134 (4)	134 (4)	
131 (27)	131 (2)	131 (3)	131 (3)	131 (2)	131 (3)	131 (9)	131 (2)	131 (4)	131 (2)	131 (1)	131 (2)	131 (2)	
124 (2)	—	—	—	—	124 (1)	124 (1)	124 (3)	124 (2)	124 (1)	124 (1)	124 (2)	124 (2)	
123 (1)	—	—	—	—	123 (1)	123 (2)	—	—	—	123 (9)	—	—	E
—	—	—	—	—	—	—	—	119 (7)	—	—	—	—	
117 (22)	117 (15)	117 (14)	117 (12)	117 (13)	117 (11)	117 (11)	117 (10)	117 (10)	117 (10)	117 (10)	117 (8)	117 (7)	
—	—	115 (2)	—	—	115 (2)	—	115 (5)	115 (3)	115 (2)	115 (1)	115 (1)	115 (1)	
—	—	—	—	—	—	—	—	—	—	109 (11)	—	—	
105 (12)	105 (4)	105 (3)	105 (3)	105 (3)	105 (3)	105 (2)	105 (5)	105 (4)	105 (3)	105 (2)	105 (2)	105 (2)	
103 (15)	103 (1)	103 (1)	103 (1)	103 (2)	103 (1)	103 (2)	103 (1)	103 (3)	103 (1)	103 (1)	103 (1)	103 (1)	
91 (17)	91 (4)	91 (3)	91 (3)	91 (3)	91 (2)	91 (16)	91 (3)	91 (7)	91 (3)	91 (1)	91 (1)	91 (1)	
89 (27)	89 (10)	89 (12)	89 (9)	89 (11)	89 (8)	89 (10)	89 (13)	89 (10)	89 (9)	89 (8)	89 (9)	89 (6)	
—	—	—	—	—	—	86 (13)	—	—	—	—	—	—	
—	—	—	—	—	—	84 (23)	—	—	—	—	—	—	
78 (17)	78 (5)	78 (6)	78 (4)	78 (5)	77 (3)	77 (6)	78 (6)	78 (5)	78 (5)	78 (3)	78 (4)	78 (3)	
77 (22)	77 (5)	77 (5)	77 (3)	77 (4)	78 (4)	78 (6)	77 (8)	77 (6)	77 (7)	77 (4)	77 (4)	77 (2)	

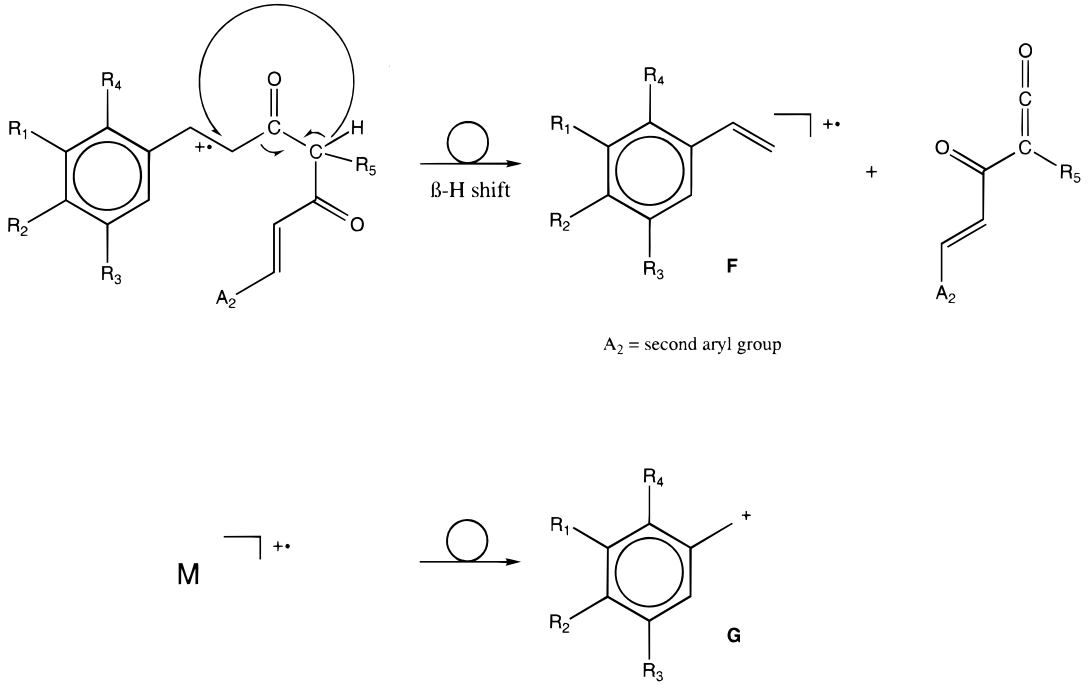
[a] Structure indication relates to Scheme 1, with R_1 = OMe, R_2 = OH, R_3 and R_4 = H.
[b] Attribution corresponding to Schemes 1 and 2.

Table 2. Partial EI mass spectra of aryl-substituted curcumin derivatives

	Compounds[a]											
	14	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	
R_1	H	H	H	H	H	H	OMe	Me	Et	i-Pr	t-Bu	
R_2	H	Me	H	H	OMe	OMe	OMe	OH	OH	OH	OH	
R_3	H	H	H	OMe	H	OMe	OMe	Me	Et	i-Pr	t-Bu	
R_4	H	H	OMe	H	H	H	H	H	H	H	H	Attribution[b]
	276 (88)	304 (33)	336 (44)	336 (85)	336 (29)	396 (30)	456 (69)	364 (12)	420 (18)	476 (21)	532 (24)	$M^{+\cdot}$
	275 (10)	303 (3)	335 (1)	335 (2)	335 (2)	—	—	363 (1)	419 (2)	475 (2)	531 (1)	$[M-H]^+$
	258 (16)	—	—	318 (12)	318 (30)	378 (39)	438 (83)	346 (20)	402 (40)	458 (44)	—	$[M-H_2O]^{+\cdot}$
	257 (18)	—	—	—	—	—	—	—	—	—	—	
	248 (13)	276 (1)	308 (1)	308 (1)	308 (3)	368 (1)	428 (3)	336 (1)	392 (3)	448 (2)	504 (1)	$[M-CO]^{+\cdot}$
	—	—	—	—	—	—	423 (17)	—	—	—	—	
	—	—	287 (13)	—	—	—	—	—	—	—	—	
	—	—	—	—	—	—	—	—	373 (19)	—	—	
	180 (1)	208 (5)	240 (5)	240 (2)	240 (22)	300 (13)	360 (8)	268 (12)	324 (44)	380 (100)	436 (100)	X
	199 (21)	213 (3)	229 (3)	229 (7)	229 (3)	259 (1)	289 (1)	—	271 (1)	299 (1)	—	F
	198 (1)	—	228 (4)	—	228 (7)	258 (3)	288 (2)	—	270 (1)	298 (1)	—	
	—	202 (5)	—	218 (17)	—	248 (10)	278 (3)	232 (14)	260 (4)	288 (3)	316 (28)	
	—	—	—	—	—	—	—	230 (5)	258 (16)	286 (25)	314 (17)	
	185 (5)	199 (5)	215 (8)	215 (10)	215 (7)	245 (2)	275 (2)	—	257 (2)	285 (1)	313 (1)	
	—	—	—	—	—	—	—	—	—	—	301 (19)	
	—	—	—	—	201 (11)	—	—	—	—	—	—	
	—	—	—	—	—	—	—	214 (12)	—	—	—	
	—	187 (10)	187 (4)	187 (10)	187 (6)	187 (1)	187 (1)	187 (1)	187 (6)	187 (11)	187 (7)	
	*	185 (11)	185 (1)	185 (10)	185 (2)	185 (2)	185 (1)	185 (1)	185 (5)	185 (3)	185 (3)	
	145 (23)	159 (34)	175 (19)	175 (44)	175 (25)	205 (32)	235 (33)	189 (30)	217 (19)	—	—	B
	144 (19)	158 (21)	174 (14)	174 (14)	174 (40)	204 (26)	234 (60)	188 (13)	216 (13)	—	—	
	131 (100)	145 (100)	161 (100)	161 (100)	161 (100)	191 (100)	221 (100)	175 (100)	203 (100)	231 (72)	259 (93)	A
	—	144 (10)	—	160 (19)	—	190 (11)	220 (15)	174 (14)	—	—	—	
	129 (10)	—	—	—	—	—	219 (18)	—	—	—	—	
	—	—	—	—	—	—	—	—	—	—	243 (10)	
	—	—	—	—	—	—	207 (11)	—	—	—	—	
	—	—	—	—	—	—	205 (17)	—	—	—	—	
	—	—	—	—	—	—	204 (33)	—	—	—	—	

128 (14)	—	—	—	—	—	—	—	—	—	—	
127 (12)	141 (12)	—	—	—	—	—	—	—	—	—	
—	131 (20)	—	—	—	—	—	—	—	—	—	
—	128 (11)	—	—	—	—	—	—	—	—	—	
117 (20)	—	—	—	—	—	—	—	—	—	—	
—	—	—	147 (13)	147 (13)	—	—	—	—	—	—	
115 (29)	—	—	—	—	—	—	—	—	—	—	
—	119 (15)	135 (4)	135 (7)	135 (7)	165 (8)	195 (7)	149 (30)	—	—	—	
104 (10)	118 (13)	134 (4)	134 (9)	134 (24)	164 (10)	194 (11)	148 (16)	176 (15)	204 (10)	—	F
103 (62)	117 (37)	133 (2)	133 (20)	133 (32)	163 (14)	193 (11)	147 (15)	175 (18)	203 (11)	—	
102 (10)	116 (18)	132 (4)	132 (4)	132 (8)	162 (3)	192 (5)	146 (7)	—	—	—	
101 (2)	115 (77)	131 (17)	131 (8)	131 (11)	161 (8)	191 (21)	145 (8)	—	—	—	
—	—	—	—	—	160 (4)	190 (16)	144 (1)	—	—	—	
91 (27)	105 (31)	121 (29)	121 (14)	121 (52)	151 (39)	181 (53)	135 (24)	163 (41)	191 (42)	219 (36)	G
—	—	—	—	—	—	179 (18)	—	161 (15)	189 (59)	217 (11)	
—	—	—	—	—	—	177 (14)	—	—	—	—	
—	—	—	—	—	—	176 (13)	—	—	—	—	
—	—	—	—	—	—	—	—	—	—	203 (11)	
—	—	—	—	—	—	—	—	—	174 (12)	—	
—	—	—	—	—	—	—	—	—	173 (15)	—	
—	—	—	—	—	—	163 (14)	—	—	—	—	
—	—	—	—	—	—	161 (15)	—	—	161 (12)	—	
—	—	—	—	—	—	147 (11)	—	147 (13)	147 (11)	—	
—	—	—	—	—	—	—	131 (11)	145 (14)	—	—	
—	—	—	—	—	—	—	—	131 (10)	—	—	
—	—	—	—	—	—	—	—	128 (11)	—	—	
—	—	118 (23)	118 (18)	118 (11)	118 (6)	118 (6)	—	—	—	—	
—	—	115 (18)	115 (11)	115 (14)	115 (4)	115 (4)	115 (13)	115 (21)	115 (12)	115 (7)	
—	—	105 (21)	—	—	—	—	—	—	—	—	
—	—	103 (14)	—	—	—	—	—	—	—	—	
77 (37)	91 (48)	—	—	—	—	—	—	—	—	—	E
91 (27)	—	91 (22)	91 (6)	—	91 (8)	91 (6)	91 (24)	91 (16)	91 (6)	91 (4)	
—	90 (2)	90 (10)	90 (8)	90 (10)	90 (2)	90 (2)	—	—	—	—	
89 (1)	89 (8)	89 (13)	89 (6)	89 (10)	89 (5)	89 (3)	89 (2)	89 (1)	—	—	
—	77 (8)	77 (21)	77 (11)	77 (10)	77 (7)	77 (5)	77 (16)	77 (6)	77 (2)	77 (2)	

[a] Structure indication relates to Scheme 1, with R_5 = H.
[b] Attribution corresponding to Schemes 1 and 2.



Scheme 2

variety of compounds. The KER lies between 36 and 150 meV, has a common value for rearrangement or direct bond cleavages and does not give any indication of either a high reverse activation energy (large $T_{0.5}$) or the involvement of an ion–molecule complex (small $T_{0.5}$). The relative intensity indicates that in the normal EI mass spectra the particular fragmentation is enhanced by large substituents on the E position (Fig. 1) or on the aryl system. The MIKE data show that a rate-determining isomerization of the molecular ions, prior to fragmentation, would be possible.[9]

A simple calculation shows that the mass of the product ions consistently agrees with the mass of the two substituted aryl rings and an additional 26 u. This suggests, for all compounds, that the α- and ω-aryl groups of the molecular ion are somehow joined in the product ion. The minimum reorganization in a rearrangement reaction producing ions with the observed mass would give (aryl-substituted) 1,2-diphenylethene (stilbene)-type radical cations and a closed-shell neutral species. Two ways are conceivable of generating stilbene-type product ions, depicted in Scheme 3 for the fully unsubstituted compound, **14**. These pathways, either a formal 1,6-phenyl shift (I) or a formal intramolecular [2 + 2] cycloaddition with subsequent ring opening (an olefin metathesis reaction, II), are unconventional and, to our knowledge, unprecedented in cationic fragmentation.

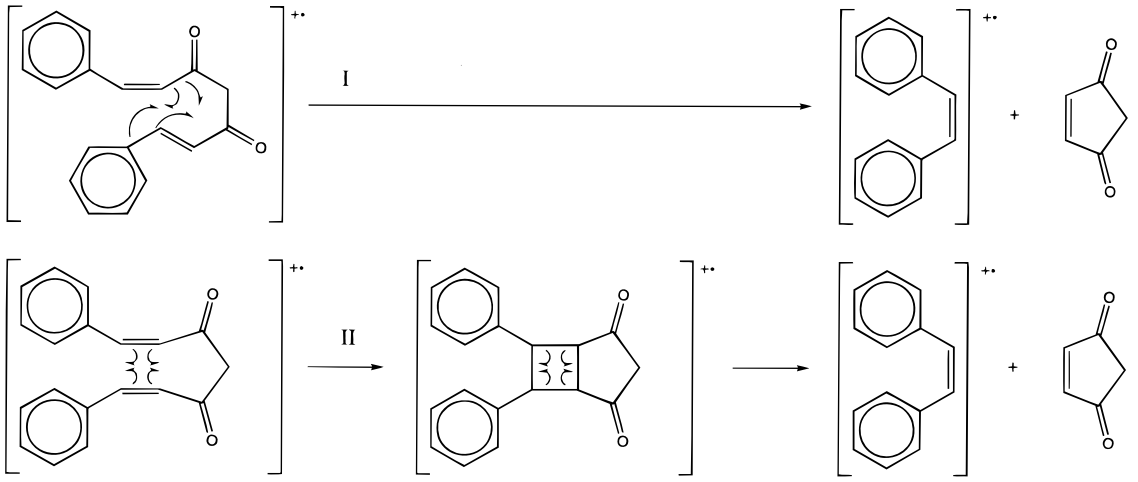
It is noted that a considerable part of the molecules may have one of the keto groups in the tautomeric enol form at the time of ionization; the enolone–dione equilibrium is a general feature for neutral β-diketones, even in the gas phase.[10–13] The enolone form would, however, not lead to markedly different product ions but to a different neutral species (i.e. 3-hydroxycyclopenta-2,4-dien-1-one instead of cyclopent-2-ene-1,4-dione in the case of **14**). Although it is known for simple β-diketones that some of the product ion relative abundances reflect the amount of dione and enolone molecules,[12] there is no way of obtaining spectra of either pure form. As far as the reaction mechanism for the formation of ions **X** is concerned, the possible involvement of the enolone will be considered along with that of the dione.

The most straightforward test for distinguishing between the two mechanisms depicted in Scheme 3 is provided by ^{13}C labelling at the 1- and 7-positions of the heptadienedione moiety. These positions can in principle be labelled by using the ^{13}C-labelled (substituted) arylaldehyde, i.e. with the ^{13}C in the carbonyl function, in the synthesis of a curcumin analogue.

Table 3. Kinetic energy release ($T_{0.5}$ values) and relative peak intensities for the proposed formation of aryl-substituted stilbene-type radical cations

Compound	KER[a,b] (meV)	Intensity ratio[c] (×10⁴)	Compound	KER[a,b] (meV)	Intensity ratio[c] (×10⁴)
1	110	1.9	**13**	33	15
2	96	3.9	**14**	38	1.2
3	50	6.6	**15**	68	0.64
4	93	4.2	**16**	89	0.31
5	130	1.1	**17**	130	0.38
6	76	3.0	**18**	62	1.3
7	56	1.3	**19**	92	0.68
8	38	15	**20**	77	0.92
9	36	23	**21**	85	0.90
10	37	25	**22**	120	1.4
11	46	20	**23**	62	5.9
12	36	18	**24**	52	50

[a] $T_{0.5}$ obtained at a main beam width, $w_{0.5}$, of 1.5–2 eV and a main beam energy of 4750–4800 eV.
[b] 1 meV ≈ 96 J mol^{-1}.
[c] Intensity determined from peak height, with ratio = /[fragment]//[main beam].



Scheme 3

Here **14**-$^{13}C_2$ was synthesized, because the labelled precursor, α-[^{13}C]benzaldehyde, was commercially available. The disadvantage of the low relative product ion abundance in the EI mass spectrum (*m/z* 180, 1%) is partly compensated for by the relative simplicity of the mass spectrum of **14**. The EI mass spectrum shows an exclusive mass shift of 2 u (to *m/z* 182) for the product ions of ionized **14**-$^{13}C_2$. Figure 2 shows the relevant part of the MIKE mass spectra of **14** and **14**-$^{13}C_2$ radical cations. The most prominent signals in this part of the spectrum represent the loss of a benzyl radical (*m/z* 185 and 186 for $\mathbf{14}^{+\cdot}$ and $\mathbf{14}^{+\cdot}$-$^{13}C_2$, respectively). From the signals at *m/z* 180 ($\mathbf{14}^{+\cdot}$) and 182 ($\mathbf{14}^{+\cdot}$-$^{13}C_2$), it is clear that both ^{13}C atoms are present in the product ions and that no ^{13}C–^{12}C scrambling occurs in the process of fragmentation. This experiment readily affords evidence for the formal breaking of two double bonds in the course of a rearrangement reaction; moreover, it shows that the rearrangement can only be an intramolecular olefin metathesis-type reaction (Scheme 3, pathway II).

The proposed involvement of a bicyclo[3.2.0]heptane type intermediate (Scheme 3, II) has a notable analogy in organic synthesis. Many reactions have been described either for the formation of substituted bicyclo[3.2.0]heptane from the appropriate diene[14–19] or for the dissociation of substituted bicyclo[3.2.0]heptane into two olefins.[19–23] Some attention has been devoted to the formation of the four-membered ring via a concerted [2 + 2] addition or via a (non-concerted) biradical mechanism.[15] Only in the work of Salomon *et al.*[19] has the intramolecular olefin metathesis been completed, albeit in three steps (Scheme 4). These examples show that the proposed fragmentation mechanism in ionized curcumin analogues is at least feasible. Moreover, the symmetry restrictions that might apply to a concerted [2 + 2] cycloaddition in neutral molecules are broken by the charge and, therefore, the reaction should be thermodynamically more favourable in ions than in neutral species.

MNDO/PM3[24] calculations were conducted in order to investigate this peculiar rearrangement in more detail. Although it is known that the MNDO formalism does not give correct predictions of the heat of formation of open-shell ions in general,[25] a comparison of calculation results within the set of the present test compounds allows a reasonable approximation, because a systematic error is nearly eliminated. The complementary reaction with respect to charge retention was considered, but the enthalpy of formation of a stilbene molecule and the radical cation of cyclopent-2-ene-1,4-dione (calculated: ΔH_f^{298}[stilbene] = 310,

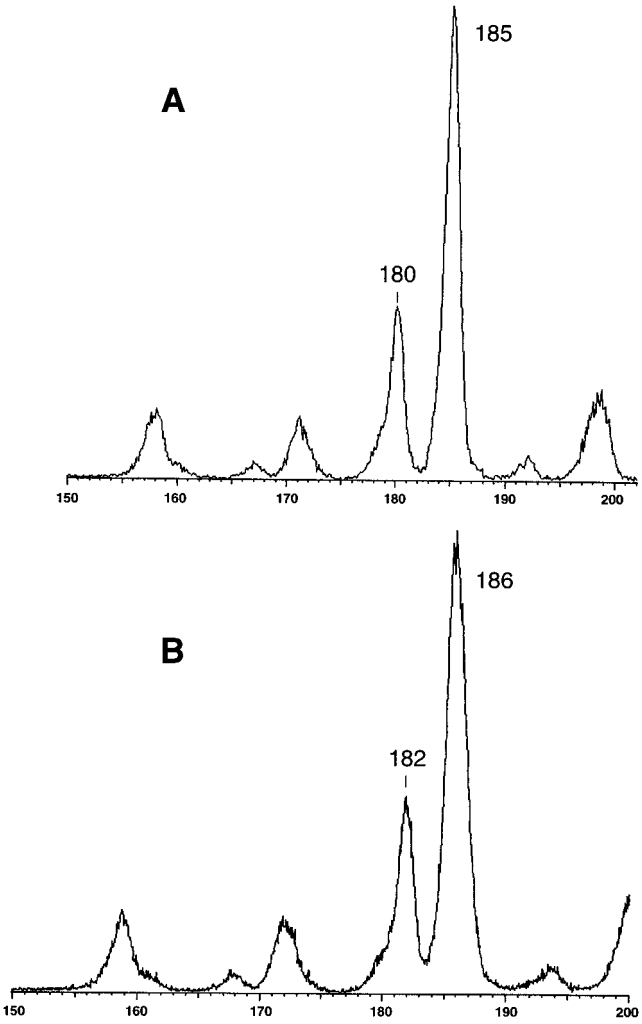
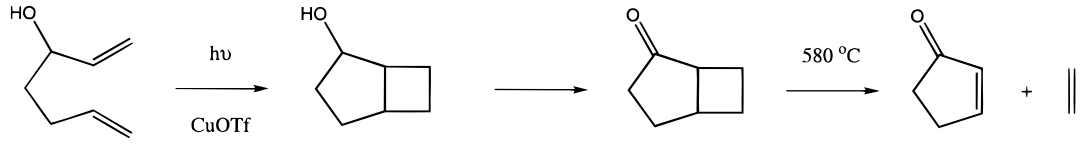


Figure 2. Partial MIKE mass spectra of the molecular ions of (A) 14 and (B) 14-$^{13}C_2$.



Scheme 4

Table 4. MNDO/PM3-calculated enthalpies of formation[a] and of reaction[b] for the formation of (substituted) stilbene-type ions from ionized curumin analogues

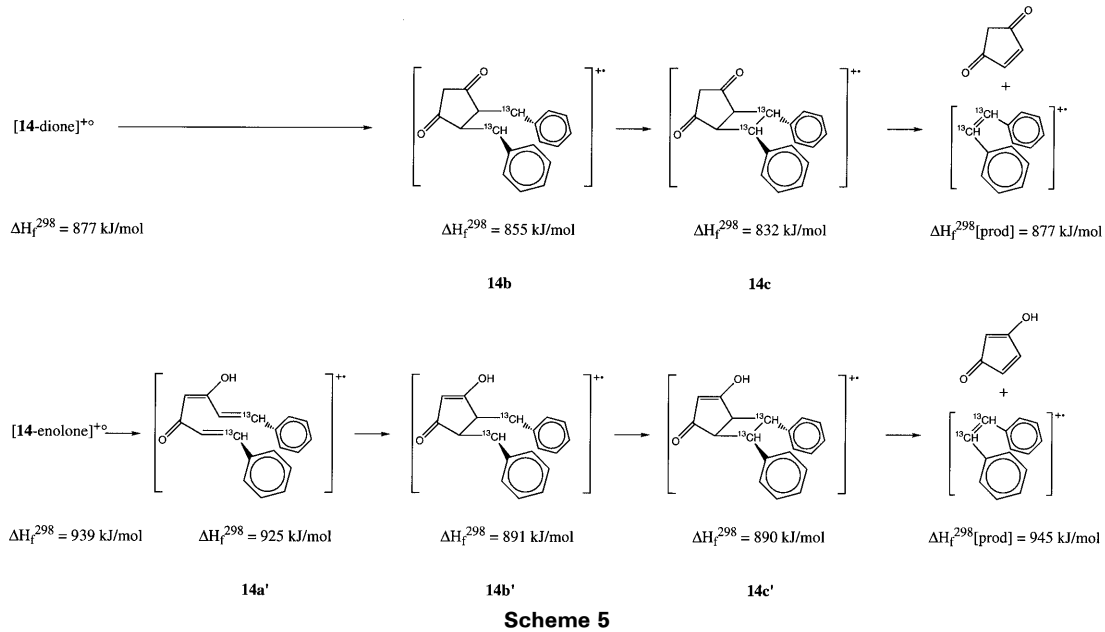
Compound	$\Delta H_f^{298}[M^{+\cdot}]$ (kJ mol^{-1})	$\Delta H_f^{298}[Stilb^{+\cdot}]^c$ (kJ mol^{-1})	$\Delta H_f^{298}[cpd]^c$ (kJ mol^{-1})	ΔH_r^{298} (kJ mol^{-1})
1	218	355	−220	−83
2	161	355	−238	−44
3	147	355	−257	−49
4	128	355	−279	−52
5	134	355	−231	−10
6	108	355	−302	−55
7	284	355	−109	−38
8	334	355	−82	−61
9	300	355	−119	−64
10	156	355	−241	−42
11	158	355	−261	−64
12	315	355	−107	−67
13	−309	355	−741	−77
14	877	1097	−220	0
15	779	999	−220	0
16	536	760	−220	4
17	504	757	−220	33
18	532	735	−220	−17
19	227	456	−220	−9
20	−68	155	−220	3
21	303	532	−220	9
22	236	476	−220	20
23	176	389	−220	−7
24	152	406	−220	34

[a] Stretched configuration of molecular ions, *cis*-configuration for stilbenes.
[b] Assuming formation of the cyclopentenedione neutral.
[c] Stilb=stilbene; cpd=cyclopentenedione.

ΔH_f^{298}[cyclopent-2-ene-1,4-dione] = 812, ΔH_f^{298}[prod] = 1122 kJ mol^{-1}) lies well above that of ionized stilbene and the neutral cyclopentenedione (ΔH_f^{298}[prod] = 877 kJ mol^{-1}). This implies that cyclopentenedione-type ions need not be present in the mass spectrum as a complementary fragmentation to any mechanism forming neutral cyclopentenedione and ionized stilbene. Table 4 gives the calculated enthalpies of reaction, ΔH_r^{298}, and the enthalpies of formation, ΔH_f^{298}, of the species involved in the fragmentation according to the mechanism of Scheme 3 (II).

The calculated ΔH_f^{298} of the molecular radical cations, $\mathbf{1}^{+\cdot}$–$\mathbf{24}^{+\cdot}$, is strongly influenced (±50 kJ mol^{-1}) by the molecular configuration chosen; the values specified in Table 4 were obtained for the configurations of lower energy, i.e. with two E double bonds in stretched molecules. The calculations show that the reaction is nearly thermoneutral in all cases. However, the calculated values for stilbene and its radical cation (310 and 1097 kJ mol^{-1}, respectively) lie well above the experimental values (252 and 1005 kJ mol^{-1}, respectively[26]). If this is taken to be a general trend for the substituted stilbenes and if it is assumed (no experimental data available) that MNDO/PM3 closely predicts the heats of formation of the neutrals and of the molecular ions, the reaction is exothermic rather than close to thermoneutral.

In order to obtain some information on a possible intermediate in the reaction and on variations in the reaction pathway, e.g. with the enolone-type precursor cations, MNDO/PM3 calculations were performed for the fully unsubstituted compound, **14**, and fragments



Scheme 5

derived therefrom. The calculations were limited to the case of the [2 + 2] cycloaddition with the bicyclo[3.2.0]heptane-type intermediate. The calculation results are given in Scheme 5 (results obtained for the fully ^{12}C compound), which also shows the approximate configuration (**14b**) of the reactant **14**$^{+\bullet}$, the possible intermediates and the products. First the 'stretched' configuration (cf. Table 4, for the dione) was brought to the possible configuration preceding ring closure. In the case of the dione, this reacting configuration was not a local minimum and collapsed to give the distonic five-membered ring intermediate, **14b**, whereas the enolone has a distinct local minimum for both structures **14a′** and **14b′**. The relative stability of the distonic **14b** may imply that it can also act as an intermediate in other fragmentations, e.g. in the loss of CO or in the net loss of the mass of [aryl + CH]; however, given the complexity of the system, it would be difficult to prove that this intermediate is the key in some rate determining isomerization. The two bicyclo[3.2.0]heptane-type intermediates, **14c** and **14c′**, are both local minima. The overall reaction is thermoneutral and the envisaged intermediates have an enthalpy of formation below that of the reactant ions and that of the products. Hence it is clear that the intermediates required for the olefin metathesis reaction are accessible once ionization of **14**, be it in the enolone or the dione form, has taken place. The calculations show that the overall olefin metathesis reaction can proceed once the neutral molecules, be they of dione or enolone type, are ionized.

CONCLUSION

Although much of the fragmentation behaviour of substituted curcumins can be explained by conventional fragmentation mechanisms, all 24 curcumin-type compounds investigated in this study after ionization undergo a rearrangement to form substituted stilbenes. ^{13}C labelling shows that the stilbene product ions are probably formed via a [2 + 2] cycloaddition and a subsequent retro-[2 + 2] cycloaddition, formally an olefin metathesis-type reaction. The possible involvement of a bicyclo[3.2.0]heptane-type intermediate, analogous to particular organic synthetic pathways, was confirmed by MNDO/PM3 calculations.

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