

Mass spectra of N-substituted cantharidinimides

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The mass spectra of a series of *N*-substituted cantharidinimides were examined. The feature of this series compounds is a sequential double hydrogen transfer from the oxabicycloheptane unit to either the carbonyl group of the succinimide unit or the nitrogen atom of the pyridyl or thiazolyl substituent through space. The ability of the *N*-substituent to accept a hydrogen atom possibly leads to the different fragmentation pathway. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: cantharidin; N-substituted cantharidinimides; hydrogen transfer

INTRODUCTION

Cantharidin (1), isolated from *Mylabris caraganae* and various other insects,^{1,2} has an extremely high potency with antitumor and antihepatoma properties.^{3–5} However, it is rarely employed to the therapeutic treatment owing to the irritation and vesicating side-effects and as toxic properties.⁶ Employing simple chemical modification, a series of *N*-substituted cantharidinimides, **3–25**, were easily prepared starting from **1** with various amines,^{7–10} and the analogues exhibit less toxicity, no side-effects and potent biological activities.⁹ *N*-Substituted cantharidinimides possess a highly strained oxabicycloheptane,¹¹ succinimide¹² and *N*-substituted group units, and therefore are of interest with regard to their fragmentation patterns in mass spectrometry. So far as we are aware, this is the first report on the mass spectra of *N*-substituted cantharidinimides.

EXPERIMENTAL

Procedures for the preparation of *N*-substituted cantharidinimides 3-15,¹⁰ $16-19^9$ and $20-25^8$ (Fig. 1) have been described. Mass spectra were obtained on a Joel JMS-HX 110 and gas chromatography/mass spectrometry HPS989B with duplicate measurements. High-resolution mass spectra were measured on a JEOL JMS SX/SX 102A instrument at National Chung Hsien University, Taichung, Taiwan.

RESULTS AND DISCUSSION

Electron ionization mass spectral data are given in Table 1. The results indicate that, generally, three series of *N*-substituted cantharidinimides display weak molecular ions with common ions $[M - 43]^+ a$, $[M - 56]^{+\bullet} b$, $[M - 69]^+ c$, $[M - 70]^{+\bullet} d$, $[M - 151]^+ e$, $[M - 152]^{+\bullet} e'$, m/z 109 *f*, m/z

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Figure 1. Cantharidin (1) and N-substituted derivatives 3-25.

96 *g*, and m/z 81 *h*. In order to verify the possible fragment ions, the chemical compositions of selected molecular ions and some of common ions were confirmed by using high-resolution mass spectrometric analyses. The results are summarized in Table 2, and based on these the proposed fragmentation mechanisms described here are feasible.

Either the $[M - 69]^+$ ion or the m/z 96 ion as the base peak depends on the type of *N*-substituted group. The formation of $[M - 69]^+$ ions *c* must result from a hydrogen transfer occurring from the oxabicycloheptane





 272 (M⁺, 15), 243 (H₂), 229 (H₂, 203 (100)^c, 202 (15)^d, 121 (7)^c, 109 (4)^d, 96 (26)^d, 81 (4)^b, 79 (5), 67 (6), 55 (1), 53 (8) 286 (M⁺⁺, 5), 27 (2), 243 (6)^d, 217 (100)^c, 216 (7)^d, 135 (4)^c, 124 (2), 109 (4)^d, 96 (21)^b, 72 (5), 67 (6), 65 (4), 55 (4), 55 (4), 53 (6) 286 (M⁺⁺, 4), 257 (3), 243 (6)^b, 217 (100)^c, 203 (3), 135 (6)^d, 109 (5)^d, 96 (21)^b, 92 (5), 81 (4)^b, 79 (6), 65 (6), 55 (9), 53 (8) 300 (M⁺⁺, 4), 257 (3), 243 (6)^b, 217 (100)^c, 203 (3), 135 (6)^d, 100 (5)^d, 79 (8), 96 (18)^b, 81 (6)^b, 67 (9), 65 (6), 55 (9), 53 (8) 300 (M⁺⁺, 4), 253 (4), 271 (3), 257 (24)^b, 233 (15), 232 (15), 231 (100)^c, 230 (8)^d, 217 (9), 201 (4), 157 (10), 174 (5), 173 (6), 150 (5), 149 (30)^d, 148 (12), 109 (3)^d, 168 (9), 105 (5), 96 (20)^b, 81 (4)^b, 79 (3), 67 (5), 55 (3), 53 (5) 308 (5), 306 (M⁺⁺, 10), 323 (1), 321 (2), 300 (7), 307 (7)^b, 233 (2), 232 (44), 231 (24)^c, 230 (44)^d, 257 (1)), 55 (7), 53 (15) 315 (1) 317 (M⁺⁺, 5), 274 (2)^b, 261 (1)^b, 256 (1), 248 (100)^c, 133 (12)^b, 130 (7), 100 (7)^d, 96 (20)^b, 81 (7)^b, 67 (11), 55 (8), 53 (7) 317 (M⁺⁺, 5), 274 (2)^b, 261 (1)^b, 230 (100); 202 (47)^d, 189 (5), 124 (6), 121 (5)^a, 109 (13)^d, 96 (49)^b, 95 (7), 81 (8)^b, 79 (7), 67 (11), 55 (8), 53 (10) 272 (M⁺⁺, 13), 229 (6)^b, 216 (3)^b, 203 (100)^c, 202 (47)^d, 189 (5), 124 (6), 121 (5)^a, 109 (13)^d, 96 (49)^b, 95 (11), 81 (14)^b, 79 (13), 67 (13), 57 (13), 67 (13), 57 (13), 67 (13), 57 (13), 51 (14) 308 (11), 306 (M^{++,3}, 31), 226 (4), 223 (100)^c, 302 (47)^d, 189 (4), 172 (3), 77 (3), 77 (3), 70 (18), 67 (55), 52 (24), 33 (55) (19), 33 (24) 272 (M^{++,1}, 13), 229 (6)^b, 216 (3)^b, 233 (100)^c, 202 (47)^d, 189 (4), 172 (2), 124 (5), 121 (5)^c, 109 (10)^d, 96 (45)^b, 95 (11), 81 (14)^b, 79 (13), 77 (33), 70 (13), 67 (3)), 79 (13), 77 (33), 70 (13), 67 (3	Compound	m/z (relative abundance, %)					
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 $^{a}[M - CH_{3} - CO]^{+}.$

 $^{b}[M - 2CO]^{+\bullet}.$

 $^{c}[M-C_{4}H_{5}O]^{+}.$

 $^{d}[M - C_{4}H_{6}O]^{+\bullet}.$

 $e [M - C_9 H_{11} O_2]^+.$

 $e' [M - C_9 H_{12} O_2]^{+\bullet}.$

 ${}^{f}[C_{5}H_{3}NO_{2}]^{+}.$

 $^{g}[C_{5}H_{6}NO]^{+\bullet}.$

 ${}^{h}[C_{5}H_{5}O]^{+}.$



Compound	Mol. formula mass units (Δ)	$[M - C_4H_5O]^+ c$ mass units (Δ)	$[M - C_4 H_6 O]^{+\bullet} d$ mass units (Δ)	$[M - C_9 H_{11} O_2]^+ e$ mass units (Δ)	$[C_7H_9O]^+ f$ mass units (Δ)	$[C_4H_8O]^{+\bullet}$ g mass units (Δ)
3	$C_{15}H_{16}N_2O_3$ 272.1157 (0.5)	$C_{11}H_{11}N_2O_2$ 203.0819 (0.2)	$C_{11}H_{10}N_2O_2$ 202.0744 (-0.1)	$C_6H_5N_2O$ 121.0403 (-0.1)	109.0657 (-0.4)	96.0573 (0.2)
4	$C_{16}H_{18}N_2O_3$	$C_{12}H_{13}N_2O_2$	$C_{12}H_{12}N_2O_2$	$C_7H_7N_2O$	100.0658 (.0.5)	96.0580 (. 0.5)
8	$C_{15}H_{15}ClN_2O_3$	$C_{11}H_{10}ClN_2O_2$	$C_{11}H_9ClN_2O_2$	$C_6H_4ClN_2O$	109.0038 (-0.3)	96.0360 (-0.3)
15	306.0774 (-0.1) C ₁₅ H ₁₆ N ₂ O ₃	237.0430 (-0.2) C ₁₁ H ₁₁ N ₂ O ₂	236.0354 (-0.4) C ₁₁ H ₁₀ N ₂ O ₂	155.0021 (–0.8) C ₆ H ₅ N ₂ O	109.0647 (0.6)	96.0574 (0.1)
16	272.1160 (0.2) C ₁₃ H ₁₄ N ₂ O ₃ S	203.0829 (-0.8) C ₉ H ₉ N ₂ O ₂ S	202.0745 (-0.2) C ₉ H ₈ N ₂ O ₂ S	121.0402 (-0.1) C ₄ H ₃ N ₂ OS	109.0650 (0.3)	96.0577 (-0.2)
20	278.0730 (-0.4)	209.0383 (0.3)	208.0305 (0.2)	126.9969 (-0.2)	109.0655 (-0.2)	96.0579 (-0.4)
20	271.1211 (-0.1)	202.0872 (-0.2)	201.0790 (0.0)	120.0447 (0.2)	109.0660 (-0.7)	96.0570 (0.5)

Table 2. High-resolution mass units for selected molecular ions and some common ions^a

^a Mass units, observed mass; Δ , error (mmu) = (calculated mass – observed mass) × 1000.

unit to either the succinimide or the N-substituted group prior to the fragmentation of the oxabicycloheptane unit. Thus, a hydrogen transfer occurs through space to form the corresponding intermediates I, sequentially followed by a second transfer to the nitrogen atom on the pyridyl or thiazolyl substituent through a six-membered transition state leading to the corresponding intermediates II as shown in Scheme 1. If the nitrogen atom of the succinimide unit behaves as a receptor to abstract a hydrogen atom for this series of compounds, and distance will be too great to reach and all compounds will give the same $[M - 69]^+$ ion *c* as the base or main peak. However, only N-pyridyl and Nthiazolyl derivatives yielded $[M - 69]^+$ as the base peak. The presence of the $[M - 151]^+$ ion *e* also proved that the corresponding protonated isocyanates produced from *c* via the loss of 1,2-dimethylcyclopropenone must be derived from a hydrogen transfer process. For the N-pyridyl and N-thiazolyl derivatives, a nitrogen or sulfur atom on the ring acts as an ionization center, receiving a hydrogen for further fragmentation leading to $[M - 69]^+$ as the base peak.

The thiazolyl ring has a lower ionization energies and a high efficiency for the formation a cation radical, and $[M - 70]^{+\bullet}$ ions are not observed from this series of compounds.^{13–15} The fragment of RC₂HS derived from the thiazolyl ring became an important product as observed from 2-substituted thiazoles. Halogen (Cl, Br)-containing pyridine derivatives are the exception owing to the lower ionization potential of halogen to compete for ionization.

For the *N*-aryl series, the nitrogen of succinimide could be a better ionization center than the aryl ring. Formation of the corresponding $[M - 69]^+$ ions with relatively weak intensity in comparison with those of the *N*-pyridyl and *N*thiazolyl series is ascribed to the efficiency of intramolecular hydrogen transfer being relatively low. Thus, the simple cleavage of oxabicycloheptane to evolve divinyl ether neutral species leads to the corresponding $[M - 70]^{+\bullet}$ radical cations *c* with relatively high abundance as shown in Scheme 2. Subsequently, the corresponding ions *c* undergo a maleimide ring cleavage to form the corresponding $[M - 152]^{+\bullet}$ arylisocyanate radical cations *e'* instead of their



protonated counterparts *e* generated from the *N*-pyridyl and *N*-thiazolyl series. Maleimide is also a strain unit, and leads to a lactam ion by loss of a CO molecule.^{16,17} This fragmentation

process along with the loss of a methyl radical or another CO leads to the corresponding $[M - 43]^+$ ions *a* and $[M - 56]^+$ radical cations *b*, respectively, as shown in Scheme 3.







Scheme 4

It is noteworthy that ions of m/z 109 f, m/z 96 g, and m/z 81 h with fairly constant relative abundance are always observed, and become the significant fragments for *N*-substituted cantharidinimides. The formation of those ions is independent of the *N*-substituted groups. It is postulated that the formation of the m/z109 f ion comes from the loss of isocyanates from aas shown in Scheme 3, and the ion of m/z 81 h is derived from the fragmentation of the corresponding *N*substituted maleimides d as illustrated in Scheme 2. For JMS

the *N*-arylcantharidinimides, the fragment ion of m/z 96 is always the base peak and consists of a C₆H₈O unit on the basis of high-resolution mass unit analysis. Since there is no heteroatom on the *N*-substituted groups, a sequential double hydrogen transfer does not occur as shown in Scheme 1. Thus, another proposed mechanism is illustrated in Scheme 4.

CONCLUSION

Although cantharidinimiodes contain highly strained oxabicycloheptane and succinimide units, their fragmentation patterns in mass spectrometry are strongly dependent on the type of *N*-substituted groups. The main feature in this series of compounds is a sequential double hydrogen transfer from the oxabicycloheptane unit to either the carbonyl group of the succinimide unit or the nitrogen atom of the *N*pyridyl or *N*-thiazolyl substituent through a relatively short distance.

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