Studies in Organic Mass Spectrometry. IV [1]. Electron Impact Induced Fragmentation of 2-Substituted 3-(5-isoxazolyl)-4(3*H*)-quinazolinones of Pharmaceutical Interest

Leopoldo Ceraulo°, Salvatore Plescia* and Giuseppe Daidone

Istituto di Chimica Farmaceutica e Tossicologica della Università,
Via Archirafi, 32, 90123 Palermo, Italy

Maria Luisa Bajardi

Istituto di Tecnologie Farmaceutiche dell'Università,
Via Archirafi, 32, 90123 Palermo, Italy

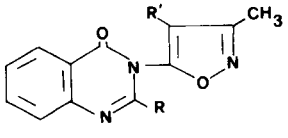
Received January 22, 1984

The fragmentation under electron impact of thirteen 2-substituted-3-(5-isoxazolyl)-4(3*H*)-quinazolinones has been investigated with the aid of metastable ion detection and high resolution measurements. Molecular ions are always abundant and the main primary fragmentation route involves acetonitrile elimination through isoxazole ring opening. The other common processes, particularly those leading to the abundant $[R\text{-}C_8H_4N_2]^+$ ion (**b** or **b'**), as well as those due to the nature of the 2-substituent are reported and discussed.

J. Heterocyclic Chem., **21**, 1209 (1984).

Introduction.

The well known hypnotic, anticonvulsant, sedative and analgesic activities of some 4(3*H*)-quinazolinones, like Methaqualone and Mecloqualone, prompted us to study the effect(s), if one of the homoaromatic 3-substituent replacement by an heteroaromatic one. In this line, we recently reported two simple syntheses [2,3] leading to the 2-*R*-3-(3-methyl-4-*R'*-5-isoxazolyl)-4(3*H*)-quinazolinones **1-12** (for compound **13** see Experimental).



1 - 13

	R	R'
1	H	H
2	CH_3	H
3	C_2H_5	H
4	$(CH_2)_2CH_3$	H
5	C_6H_5	H
6	o Cl C_6H_4	H
7	p Cl·C_6H_4	H
8	o NO_2·C_6H_4	H
9	p NO_2·C_6H_4	H
10	p CH_3·C_6H_4	H
11		H
12	CH_3	C_6H_5
13	C_6H_5	C_6H_5

This work was encouraged by the preliminary pharmacological screening of compounds **1, 2, 3** and **5** [3], which gave evidence, in addition to the expected sedative, hypnotic and analgesic activities, of also important antiinflammatory and hypothermic properties in the rat and mouse, in which low toxicity was observed, having LD > 400 mg/Kg by parenteral administration.

As the electron impact spectra of **1-13** showed both characteristic features, useful as analytical tool, and the "unusual" isoxazole ring breakdown, it seemed of interest to discuss our results and also compare them with those reported for Methaqualone and related compounds [4-10].

Results and Discussion.

The significant peaks of the 75 eV mass spectra of **1-13** are reported in Table 1, the elemental composition of all ions considered were determined by exact mass measurement and the metastable supported transitions were indicated by an asterisk on the arrows in the schemes.

The molecular ions are always abundant and undergo, as the main fragmentation process, the elimination of acetonitrile from the isoxazole moiety. Furthermore, as evidenced by the mass-analysed ion kinetic energy (MIKE) spectra of molecular ions of **2** and **12**, this is the only reaction occurring in the metastable energy window with the exception of compounds **4, 6** and **8** (see below). These results are independent of the nature of the 4-substituent of the isoxazole ring (R' = H or C_6H_5).

The elimination of RCN from 3-*R*-substituted isoxazoles is an unusual process [11-19], being observed, even though in cases of minor relative abundance such as in the Stork intermediate **14** [13] and in a series of 5,3'-diisoxazolylmethane **15** [20] (Scheme 1). There is an evident relationship which is apparent between structures **1-13, 14** and **15**. This is the presence of a γ-carbonyl (**1-14**) or a γ-pseudocarbonyl (**15**) group in the 4-position of the isoxazole ring. It is well known that N-O bond of the isoxazole nucleus is the more labile one under electron impact [11-20] and that, at least in the unsubstituted isoxazole [21], its cleavage occurs before any reaction takes place, thus it seemed reasonable that the acetonitrile elimination should

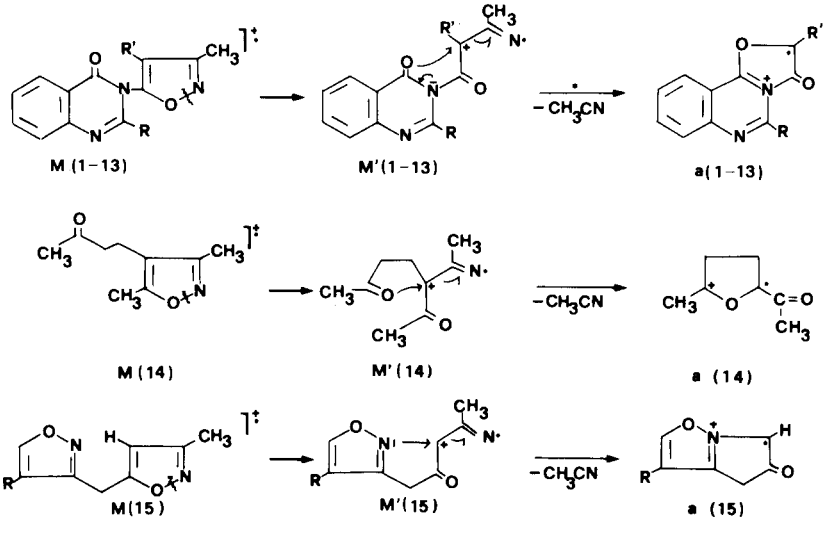
Table 1

m/z Values and Relative Abundances (in parenthesis) Referred to the Base Peak of the Characteristic Common Ions in the 75 eV Mass Spectra of Compounds **1-13** [a]

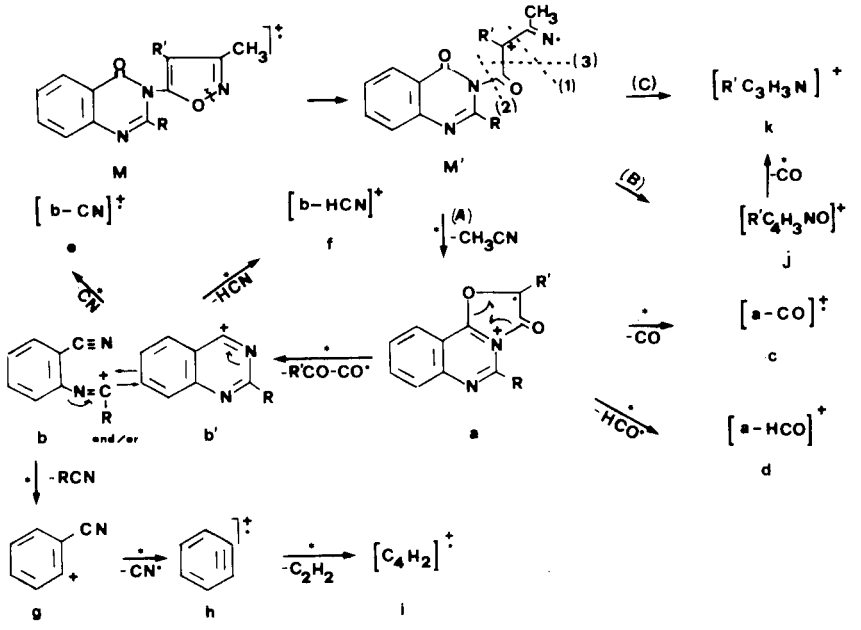
Compound Ion [b] assign.	**1**	**2**	**3**	**4** [c]	**5**	**6**	**7**	**8** [d]	**9**	**10**	**11**	**12**	**13**
M	227	241	255	269	303	337-339	337-339	348	348	317	293	317	379
	(44)	(21)	(36)	(18)	(55)	(29)	(51)	(58)	(44)	(65)	(68)	(29)	(51)
a	186	200	214	228	262	296-298	296-298	307	307	276	252	276	338
	(100)	(55)	(80)	(33)	(41)	(6)	(44)	(64)	(55)	(33)	(32)	(60)	(25)
b(b')	129	143	157	171	205	239-241	239-241	250	250	219	195	143	205
	(70)	(100)	(100)	(23)	(100)	(58)	(100)	(9)	(100)	(100)	(100)	(100)	(100)
c [e]	158	172	186	[f]	234	268-270	268-270	—	279	248	224	—	310
	(3)	(1)	(12)		(12)	(2)	(6)		(2)	(18)	(13)		(2)
d	157	171	185	199	233	267-269	267-269	278	278	247	223	247	309
	(2.5)	(5)	(31)	(14)	(7)	(3)	(5)	(5)	(6)	(5)	(13)	(2)	(2)
e	103	117	131	145	179	213-215	213-215	224	224	193	169	117	179
	(35)	(25)	(11)	(28)	(23)	(16)	(11)	(4)	(8)	(14)	(17)	(38)	(26)
f	102 [g]	116	130	144	178	212-214	212-214	223	—	192	168	116	178
	(25)	(8)	(44)	(9)	(14)	(3)	(1)	(3)		(14)	(10)	(6)	(10)
g	102 [g]	102	102	102	102	102	102	102	102	102	102	102	102
	(25)	(9)	(18)	(17)	(8)	(18)	(8)	(29)	(15)	(5)	(20)	(10)	(9)
h	76	76	76	76	76	76	76	76	76	76	76	76	76
	(41)	(30)	(30)	(28)	(25)	(42)	(19)	(45)	(60)	(17)	(50)	(23)	(22)
i	50	50	50	50	50	50	50	50	50	50	50	50	50
	(23)	(17)	(20)	(18)	(14)	(29)	(12)	(22)	(27)	(8)	(40)	(10)	(12)
j	82	82	82	82	82	82	82	82	82	82	82	158	158
	(43)	(15)	(24)	(40)	(12)	(17)	(13)	(34)	(28)	(12)	(40)	(5)	(5)
k	54	54	54	54	54	54	54	54	54	54	54	130	130
	(37)	(16)	(30)	(28)	(11)	(22)	(12)	(26)	(38)	(14)	(50)	(5)	(5)
l	65.5	72.5	79.5	86.5	103.5	120.5 121.5	120.5 121.5	126	126	110.5	98.5	72.5	103.5
	(0.5)	(8)	(3)	(8)	(27)	(47)	(44)	(11)	(15)	(35)	(38)	(1)	(3)

[a] The complete 75 eV mass spectra have been sent to the "Mass Spectrometry Data Center" Nettingham NGD 2RD, England. [b] For ion assignments see either Schemes 1, 2, 5 or the text. [c] For the base peak see Scheme 3. [d] For the base peak see Scheme 4. [e] The relative intensity was uncorrected from isotopic contribution of **d**. [f] For this compound the ion is isobaric to the ion $[a\text{-}C_2H_4]^{+\cdot}$ (base peak), see Scheme 3. [g] For this compound the ion **f** has identical composition and precursor as **g**.

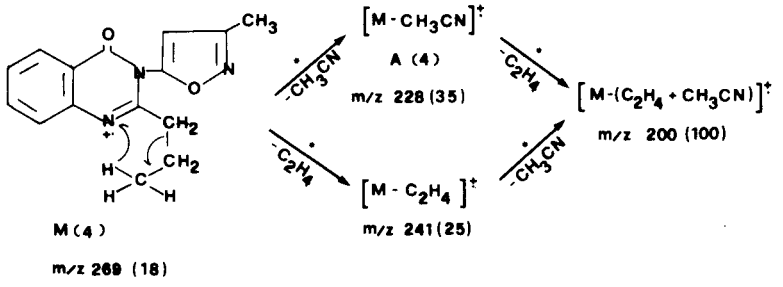
Scheme 1: Proposed mechanism for the CH_3CN elimination from 1-15 molecular ions.



Scheme 2 : Main fragmentation processes of 1-12.



Scheme 3 : Formation of m/z 200 ion (base peak) of 4.



be induced (or favoured), by the presence of the γ-heteroatom on the 4-position of the "open isoxazole ring" (**M'**), thus generating the new five membered ring **a**.

The greater stability of **a** in **1-13** in comparison to that of **a** in **14** or **15** should be responsible for the higher relative abundance of the former. Moreover, the presence of intense peaks attributable to ion **a** also in the case of **12** and **13**, which has a 4-phenyl-substituted isoxazole ring, is in agreement with the proposed mechanism which, being comparable to an SN_1 intramolecular reaction, should be almost uninfluenced by steric effects.

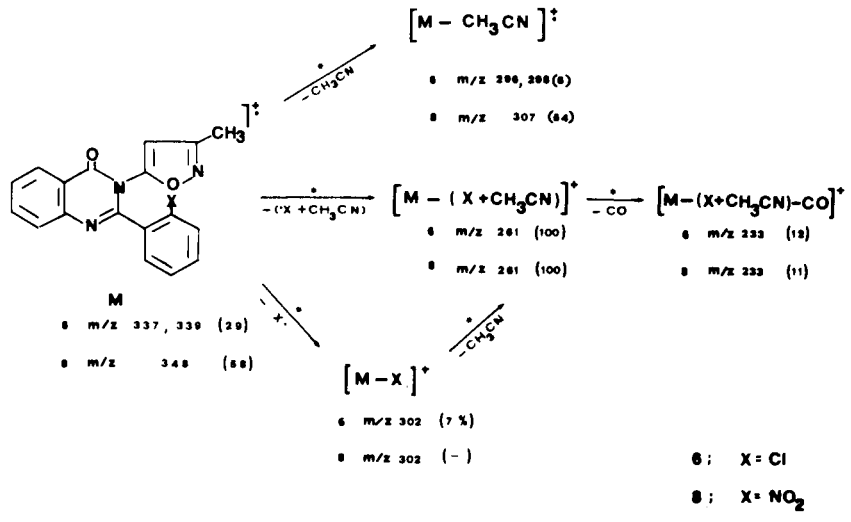
The only competing primary fragmentation of M^+ (**1-13**) involves the well known loss of the 5-substituent of the isoxazole ring [8,18-21], leading to ions **j** and **k** (Scheme 2, routes B and C respectively). The ion **a** generates, in addition to small amounts of ions **c** and **d**, the ion **b(b')**, which is responsible for the base peak of most of our compounds (Scheme 2, route A). Furthermore, the ions **c** to **i** arise from **b(b')** by a series of competitive and subsequent breakdown processes shown in Scheme 2.

The same ion **b(b')** is observed, even if with low relative abundance (≤7% of the base peak), in the electron impact mass spectrum of Methaqualone [5,6] and related compounds [7].

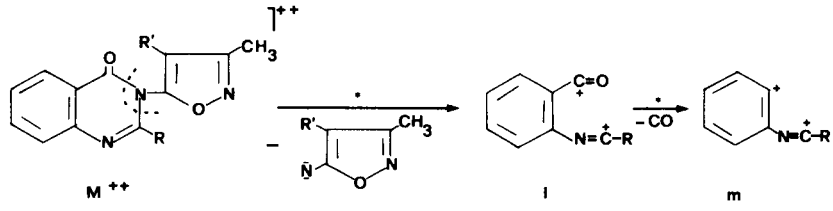
Only in the case of compounds **4, 6** and **8** is the base peak not due to ions **a** or **b**. In fact, the presence of the 2-*n*-propyl substituent in compound **4** makes competitive the McLafferty rearrangement with ethylene elimination, hence the base peak of **4** is due to the ion m/z 200, resulting from subsequent loss of acetonitrile and ethylene and *vice versa* (Scheme 3).

The base peak of compounds **6** and **8** is due to the ion m/z 261, arising by loss of both acetonitrile and the *ortho* substituent of the phenyl ring (Scheme 4). The low intensity (≤3%) of the fragment ion m/z 261 in the mass spectra of the corresponding *para*-substituted isomers **7** and **9** indicates that the above processes must be attributable to an "*ortho*" interaction between the 2-substituent and the isoxazole ring. Important "*ortho*" effects involving the 2- and 3-substituents of 4(3*H*)-quinazolinones have been pre-

Scheme 4 : Formation of m/z 261 ion (base peak) of 6 and 8.



Scheme 5: Nitrene elimination from doubly charged molecular ion (M^{++}) of 1-13.



viously reported [22].

At last, another common fragmentation arises from the doubly charged molecular ions (M^{++}), which undergo, formally, nitrene elimination to give ion **l** followed by expulsion of carbon monoxide to form ion **m** (Scheme 5). In contrast with the low intensity of M^{++} and **m** peaks ($\leq 1\%$), the ion **l** is very important, particularly in the 2-aryl-substituted derivatives **5-10** and the 2-furyl-substituted derivative **11**.

Conclusions.

In conclusion the mass spectra of these compounds **1-13** evidence that the isoxazole ring lability upon electron impact is responsible for the major breakdown process.

It is worthy of mention that the several fragmentation routes often observed in the mass spectra of 4(3*H*)-quinazolinones, like the carbon monoxide elimination [23] and the loss of the 2-substituent [10] are not observed in our case. On the contrary, the peak due to **b(b')** which is of little importance in the mass spectra of Methaqualone and other 3-aryl-substituted-4(3*H*)-quinazolinones, is the base peak of the most of the compounds examined. Moreover, these spectra should be useful as an analytical tool, as their examination leads to an immediate identification of the compounds. These data are also useful in the case of the isomeric pairs **6**, **7** and **8**, **9**.

EXPERIMENTAL

Low resolution mass spectra were run on Jeol JMS-01-SG-2 mass spectrometer, with an electron beam energy of 75 eV, an accelerating voltage of 5 KV and an electron current of 100 μA. The samples were introduced by a direct inlet system (with a probe) into the ion source at about 200°. Exact mass measurements were performed at 15000, resolving power, using a photo-plate detection technique and were carried out to an accuracy of ± 10 ppm of the theoretical values. First field-free region metastable ions were detected by the accelerating voltage scan technique.

The mass-analysed ion kinetic energy (MIKE) spectra of the molecular ion of **2** and **12** were taken in the usual manner with an accelerating voltage of 7 KV on a VG ZAB 2F instrument. The title compounds **1**, **2**, **3**, **5** and **4**, **6-12** were prepared as in ref [2] and [3] respectively.

Compound **13** was obtained according to the procedure in ref [2], by the reaction of ethylorthobenzoate with *N*-(4-phenyl-3-methylisoxazol-5-yl)-2-aminobenzamide, mp 130° (ethanol). Its structure was supported by satisfactory elemental analysis and spectral data (see Table 1 and Table 2).

Acknowledgements.

The authors thank Dr. P. Traldi (C.N.R. Padova) for the MIKE spectra of **2** and **12**. Financial support (C.N.R. and Ministero P.I.) is gratefully acknowledged.

Table 2

2-Substituted-3-(5-isoxazolyl)-4(3*H*)-quinazolinones **1-13**

Compound	Formula	Analyses Calcd./Found C	H	N
1	$C_{12}H_9N_3O_2$	63.43	3.99	18.49
		63.54	3.78	18.42
2	$C_{13}H_{11}N_3O_2$	64.72	4.60	17.42
		64.81	4.74	17.47
3	$C_{14}H_{13}N_3O_2$	65.87	5.13	16.46
		65.96	5.43	16.43
4	$C_{15}H_{15}N_3O_2$	66.90	5.61	15.61
		66.81	5.69	15.69
5	$C_{18}H_{13}N_3O_2$	71.27	4.32	13.86
		71.37	4.25	13.81
6	$C_{18}H_{12}N_3O_2Cl$	64.00	3.55	12.44
		64.11	3.61	12.40
7	$C_{18}H_{12}N_3O_2Cl$	64.00	3.55	12.44
		64.09	3.65	12.37
8	$C_{18}H_{12}N_4O_4$	62.07	3.47	16.09
		62.18	3.54	16.01
9	$C_{18}H_{12}N_4O_4$	62.07	3.47	16.09
		62.13	3.51	16.11
10	$C_{19}H_{15}N_3O_2$	71.91	4.76	13.24
		71.98	4.75	13.36
11	$C_{16}H_{11}N_3O_3$	65.52	3.78	14.33
		65.62	3.81	14.38
12	$C_{19}H_{15}N_3O_2$	71.91	4.76	13.24
		71.86	4.79	13.32
13	$C_{24}H_{17}N_3O_2$	75.97	4.52	11.08
		75.99	4.50	11.15

REFERENCES AND NOTES

[1] For Part III see: V. Sprio, P. Agozzino, L. Ceraulo, M. Ferrugia and F. Filizzola, *Farmaco, Ed. Sci.*, **36**, 159 (1981).

[2] S. Plescia, M. L. Bajardi and G. Daidone, *Boll. Chim. Farm.*, **121**, 563 (1982).

[3] S. Plescia, G. Daidone, L. Ceraulo, M. L. Bajardi and R. Arrigo Reina, *Farmaco, Ed. Sci.*, **39**, 120 (1984).

[4] C. Bogentoft and B. Danielsson, *Acta Pharm. Suecica*, **6**, 589 (1969).

[5] Fr. R. Preuss, H. Hoffmann-Pinther, H. Achenbach and H. Friebolin, *Pharmazie*, **25**, 752 (1970).

[6] S. Pfeifer, H. Polmann and R. Kraft, *Pharmazie*, **29**, 765 (1974).

[7] C. Bogentoft, O. Ericsson, B. Danielsson, J. E. Lindgren and B. Holmsted, *Acta Pharm. Suecica*, **9**, 151 (1972).

[8] C. Bogentoft, *ibid.*, **9**, 1 (1972).

[9] P. Daenens and M. van Boven, *Azneim.-Forsch.*, **24**, 195 (1974).

[10] M. Z. Kirmani and K. Sethi, *Muslim Sci.*, **8**, 45 (1979).

[11] M. Ohashi, H. Kamachi, H. Kakisawa, A. Tatematsu, H. Yoshizumi and H. Nakato, *Tetrahedron Letters*, 379 (1968).

[12] H. Nakata, H. Sakuzai, H. Yoshizumi and A. Tatematsu, *Org. Mass Spectrom.*, **1**, 199 (1968).

[13] M. Ohashi, H. Kamachi, H. Kakisawa, A. Tatematsu, H. Yoshizumi, H. Kano and H. Nakata, *Org. Mass Spectrom.*, **2**, 195 (1969).

[14] T. Nishiwaki, *Tetrahedron*, **25**, 747 (1969).

[15] J. H. Bowie, R. K. M. R. Kallury and R. G. Cooks, *Aust. J. Chem.*, **22**, 563 (1969).

[16] B. K. Simons, R. K. M. R. Kallury and J. H. Bowie, *Org. Mass Spectrom.*, **2**, 739 (1969).

[17] G. L. Aldous and J. H. Bowie, *ibid.*, **10**, 64 (1975).

[18] D. C. Nonhebel, *Org. Mass Spectrom.*, **3**, 1519 (1970).

[19] J. L. Auabagnac and D. Bourgeon, *Org. Mass Spectrom.*, **12**, 65 (1977).

[20] A. Selva, R. Stoyanova, V. Vettori and S. Auricchio, *Ann. Chim. (Rome)*, **69**, 81 (1979).

[21] G. Bouchoux and Y. Hoppiliard, *Org. Mass Spectrom.*, **16**, 459 (1981).

[22] C. Bogentoft, V. Bondesson, O. Ericsson and B. Danielsson, *Acta Chem. Scand.*, **B28**, 479 (1974).

[23] T. J. Batterham, A. C. K. Triffett and J. A. Wunderlich, *J. Chem. Soc. (B)*, 892 (1967).