Mass Spectrometry of 2,4-Substituted Carcinogenic Thiazoles and Their Metabolites

Michael B. Mattammal*, Terry V. Zenser and Bernard Davis

Veterans Administration Medical Center, Geriatric Research, Education and Clinical Center, St Louis, Missouri 63125

and

St. Louis University School of Medicine, Department of Biochemistry and Internal Medicine, St. Louis, Missouri 63104

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The fragmentation pattern of some selected 2,4-substituted carcinogenic thiazoles and their metabolites are described. Multiple modes of cleavages are seen when the 2-amino group in thiazole is substituted.

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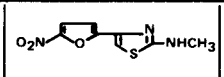
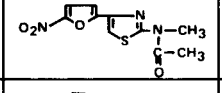
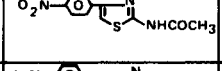
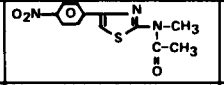
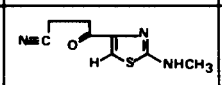
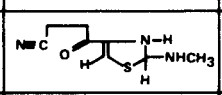
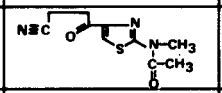
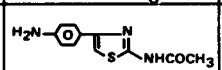
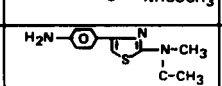
Introduction.

Nitro-aromatic compounds comprise a vast family of organic compounds currently used as antihelmintic, chemotherapeutic, antiparasitic, feed and food additives [1]. *In vivo* studies have shown that some of these agents especially the heterocyclic derivatives of 5-nitrofuryl-2-thiazolyl compounds possess mutagenic and carcinogenic properties. The primary pathway of their biotransformation leading to the cytotoxic, mutagenic and carcinogenic effects have been attributed to reductive metabolism of the nitro-group to the nitroso or the hydroxylamine derivatives and subsequent covalent binding of these metabolites to critical cellular macromolecules [2,3]. The isolation and identification of metabolite(s) are an integral part that provide

TABLE 1

Analytical data for the N-[4-(5-nitro-2-furyl) and 4-(4-nitrophenyl)]-2-substituted-thiazoles

Compound No.	Structure	mp,°C	Yield %	Formulae	GC R_t min	Analysis % Calcd C	Calcd H	Calcd N	Calcd S	Found C	Found H	Found N	Found S
1		220-221	63	$C_8H_7N_3O_3S$	6.43	42.65	3.13	18.66	14.22	42.64	3.13	18.63	14.16
2		234-236	60	$C_{10}H_9N_3O_4S$	9.27	44.93	3.40	15.73	12.00	44.86	3.38	15.62	11.87
3		276-277	70	$C_{11}H_9N_3O_3S$	14.55	50.18	3.45	15.96	12.16	50.14	3.44	15.90	12.00
4		230-231	65	$C_{12}H_{11}N_3O_3S$	7.95	51.96	4.00	15.16	11.55	51.94	3.88	15.10	11.39
5		188-189	–	$C_8H_9N_3OS$	9.72	49.21	4.65	21.53	16.40	49.11	4.64	21.43	16.35
6		Yellow oil [a]	–	$C_8H_{11}N_3OS$	8.44	48.71	5.62	21.31	16.23	48.70	5.60	21.22	16.12
7		200-201	–	$C_{10}H_{11}N_3O_2S$	10.36	50.61	4.68	17.71	13.50	50.59	4.67	17.68	13.40
8		190-191	–	$C_{11}H_{11}N_3OS$	7.32	56.63	4.76	18.02	13.73	56.61	4.64	18.02	13.59
9		210-211	–	$C_{12}H_{13}N_3OS$	9.11	58.28	5.30	16.99	12.95	58.24	5.28	16.94	12.88

[a] Decomboses on distillation

insight into biochemical reaction mechanisms. The diverse analytical capabilities of mass spectrometry makes it a unique tool in the identification of metabolite(s). In the present paper, we report the structural identification of metabolites obtained from some 5-nitrofuryl-2-substituted thiazoles and their isoelectronic 4-(4-nitrophenyl)-2-substituted thiazole derivatives by rat (Fischer 344) renal microsomal NADPH-cytochrome *c* reductase (E.C. 1.6.2.4). The electron ionization mass spectra of these compounds have never been reported hitherto. The structures of the compounds used in the present study are given in Table 1.

Mass Spectra of the Substrates (Compounds **1-4**).

The 2-methylamino-*N*-[4-(5-nitro-2-furyl)]thiazole (Compound **1**) and its 2-acetyl substituted derivative (Compound **2**, Fig. 1) show fragment ions arising from multiple

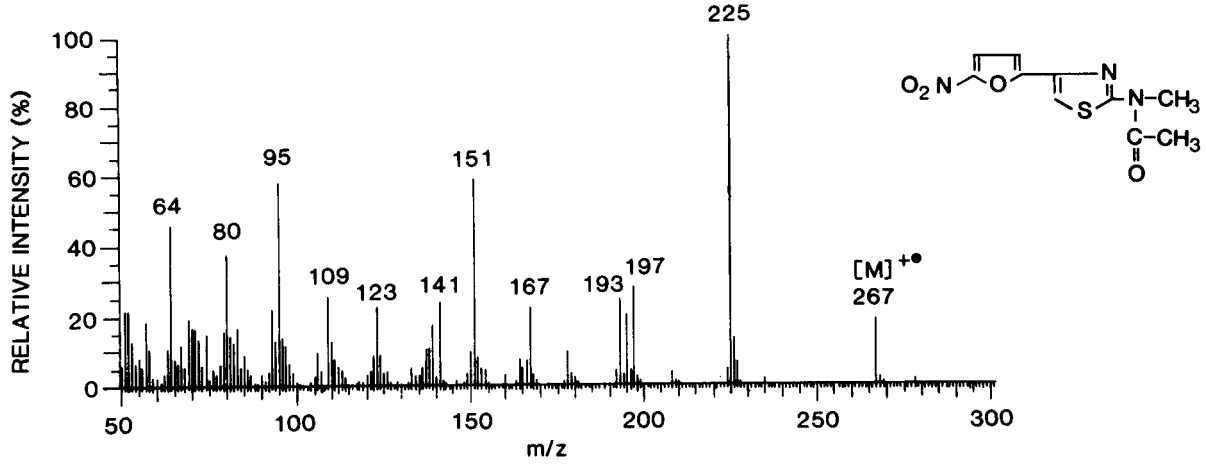
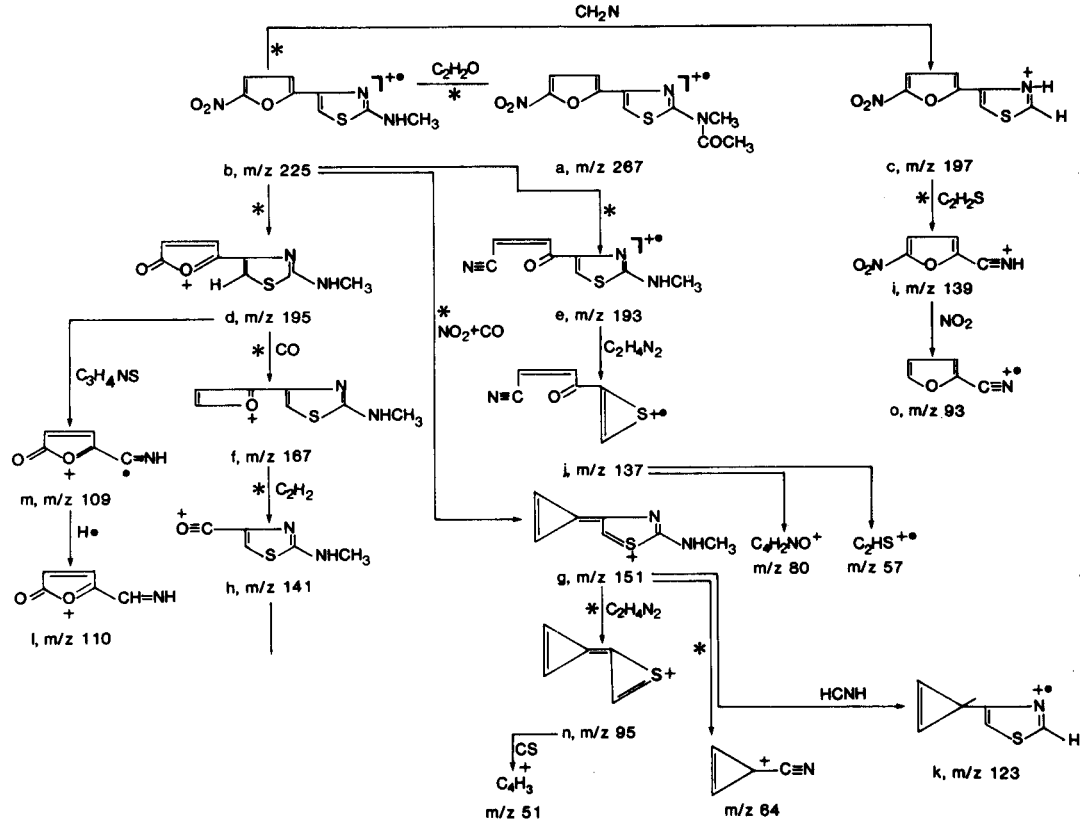


Figure 1. The low resolution mass spectrum of 2-acetyl-2-methylamino-4-(5-nitro-2-furyl)thiazole (Compound **2**).



Scheme 1. Suggested fragmentation pathway of Compound **2**.

modes of cleavage. Loss of ketene molecule from Compound **2** ($M^{+\cdot}$, m/z 267) gives the ion m/z 225 as the base peak, whereas in Compound **1**, ion m/z 95 is the base peak. The ion b, m/z 225 undergoes four modes of cleavage as shown in Scheme I [4]. Ion h, m/z 141 arises from the loss of $NO + CO + C_2H_2$ while ion g, m/z 151 arises from the loss of $NO_2 + CO$. The fragmentation of ion h, m/z 141 is discussed with Compound **5**. Ion c, m/z 197 arises from the loss of 28 mass unit (CH_2N) from ion b. It's subsequent fragmentation gives rise to ions i, m/z 139 and o, m/z 93. Even though ion e, m/z 193 shows modest relative abundance, the transition from 225 to 193 was very weak (8-10%) and in some cases was totally absent. High resolution measurement of ions e and j corresponds to the proposed structure and composition.

The 2-acetylamino-4-(4-nitrophenyl)thiazole (Compound **3**, Fig. 2) shows a molecular ion at m/z 263. Loss of a ketene molecule gives rise to the 2-amino-4-(4-nitrophenyl)-thiazole ion radical, m/z 221. This ion radical shows characteristic fragmentation patterns of an aromatic nitro group giving rise to corresponding phenoxy and phenyl-2-aminothiazolyl cations, m/z 191 and m/z 175 by the elimination of NO and NO_2 groups. The 1,2-cleavage of the thiazole nucleus is not observed before the characteristic fragmentation of the nitro group [5-8]. Compound **4** shows base peak at m/z 235 (Fig. 3) arising from loss of ketene from the molecular ion m/z 277. The 2-methylamino-4-(4-nitrophenyl)thiazolyl cation radical also does not show any preferential 1,2-cleavage of the thiazole nucleus before the fragmentation of the nitrogroup. In addition two other intersting features seen in the spectrum are the formation of ions m/z 149 and m/z 148. High resolution measurement of ion m/z 149 corresponds to a protonated 4-nitrobenzonitrile. But no metastable peak was observed for the transition from the 4-(4-nitrophenyl)thiazolium ion m/z 207 (arising from ion m/z 235 by the loss of CH_2N) or any other transition leading to it. High resolution measurements and metastable transitions show ion m/z 148 arises from the loss of an isonitrile fragment from the 4-methylamino-phenyl cation m/z 189 (arising from the loss of NO_2

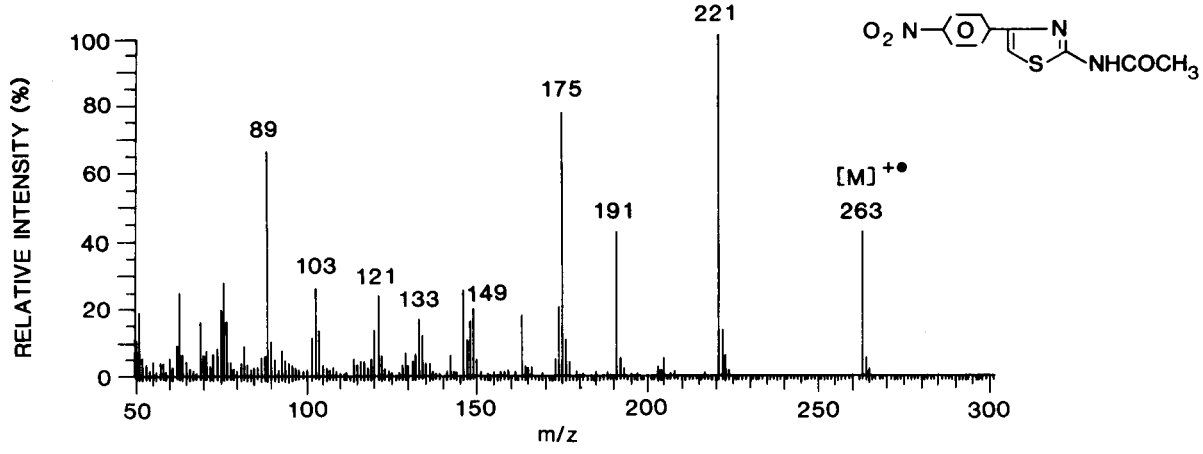


Figure 2. The low resolution mass spectrum of 2-acetylamino-4-(4-nitrophenyl)thiazole (Compound **3**).

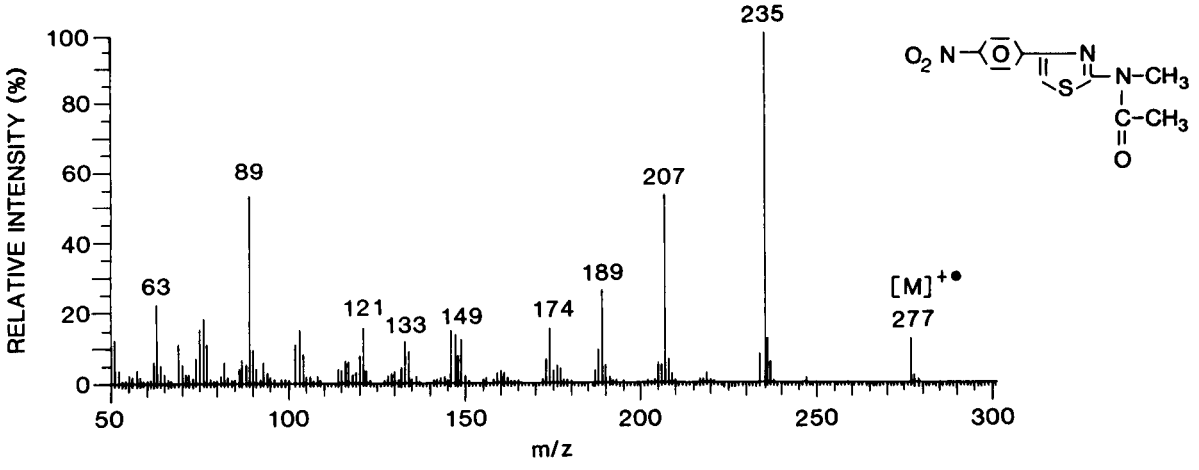


Figure 3. The low resolution mass spectrum of 2-acetyl-2-methylamino-4-(4-nitrophenyl)thiazole (Compound **4**).

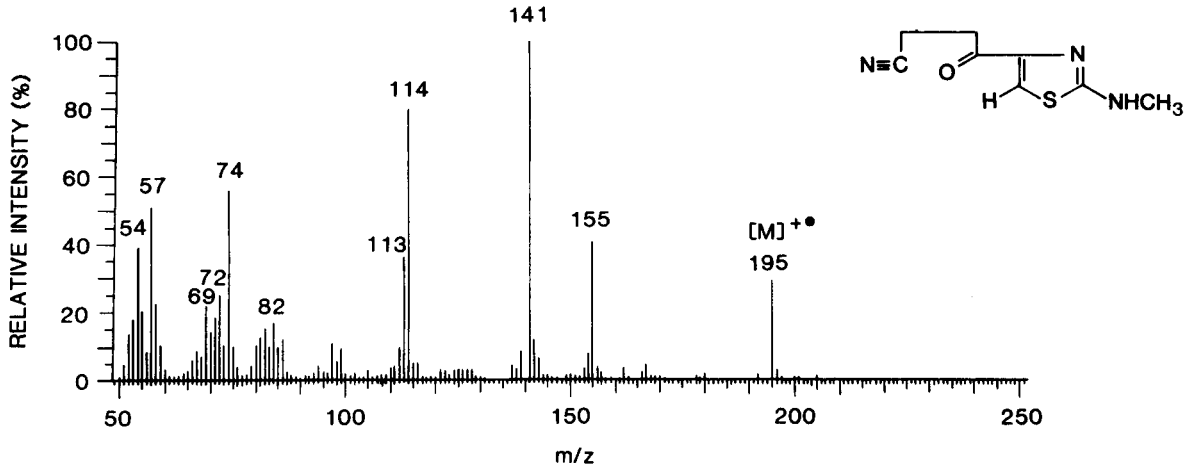
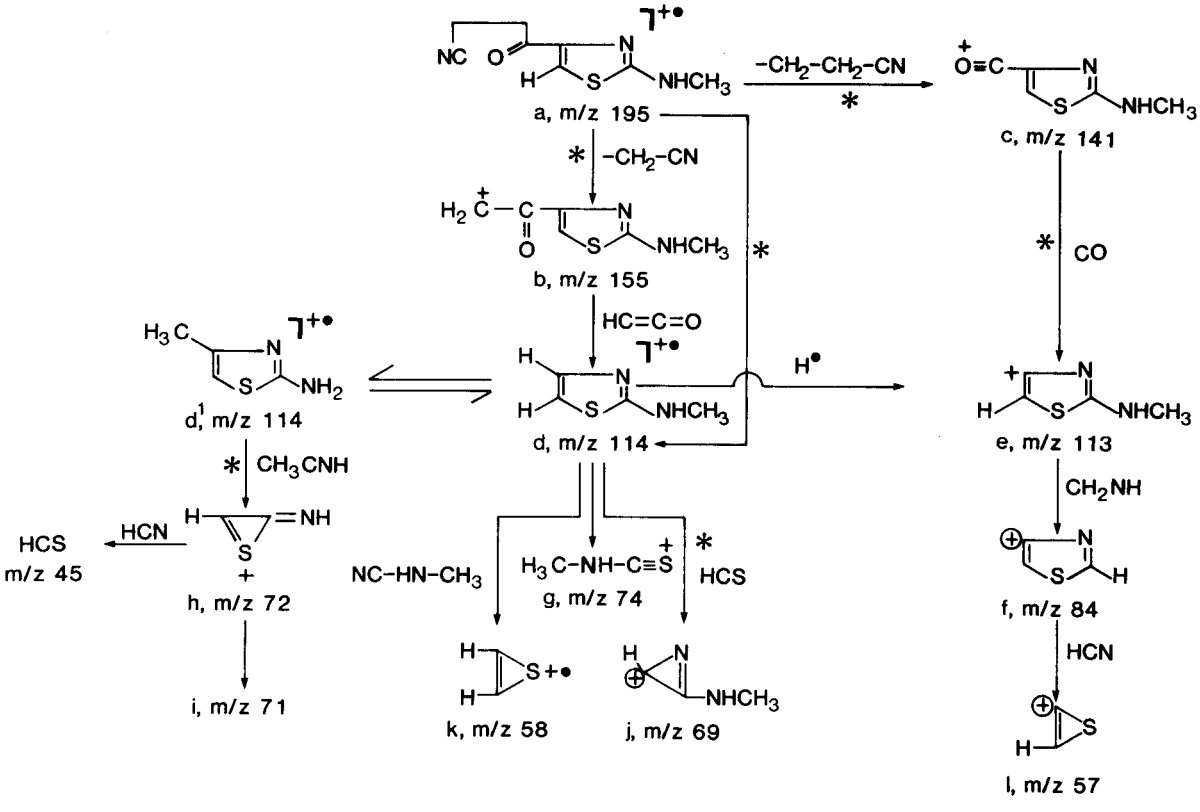


Figure 4. The low resolution mass spectrum of 1-(2-methylamino-4-thiazolyl)-3-cyano-1-propanone (Compound **5**).



Scheme 2. Suggested fragmentation pathway of nitro reduction product Compound **5**.

from ion m/z 235). This fragmentation pattern is not usually seen in 2-substituted-1,3-thiazoles [8,9].

Mass Spectra of Microsomal Metabolites.

In the 1-[4-(2-methylaminothiazolyl)]-3-cyano-1-propanone (Compound **5**, Fig. 4) the base peak, ion c (Scheme 2) m/z 141 arises from the typical α- and β-cleavage, [5] of an aliphatic nitrile by the loss of $[(CH_2)_nCH)]^+$ ions (n = 1,2). Interestingly, while ion c, m/z 141 arises from the β-cleavage of the nitrile, in Scheme 1, ion h, m/z 141, arises from the loss of acetylene from the oxonium ion f, m/z 167. The transition leading to ion d, m/z 114 from ion a, m/z 195 also indicates α-cleavage of the carbonyl group in ion a, with the back transfer of hydrogen atom. Another notewor-

thy feature in the mass spectra of compound **1** and compound **5** is the formation of the fairly abundant ions m/z 72 and 71. High resolution data shows both ions contain a nitrogen atom. These ions can arise only if the methyl group of the 2-methylamino group undergoes rearrangement to the isomeric 4-methyl-2-aminothiazole ion radical d^1, followed by N-C(4) bond cleavage as shown in Scheme 2. Both these isomeric compounds were prepared by the Hantzsch cyclization method and their fragmentation were studied [10].

Synthesis of Isometric 4-Methyl-2-Aminothiazole and 2-Methylaminothiazole.

Both these isomeric compounds were prepared from dimethyl chloroacetal and the corresponding thiourea by the Hantzsch cyclization method [10]. The 2-amino-4-methylthiazole showed molecular ion at m/z 114 (100%) and ions m/z 72 (55%), m/z 71 (77%) with no M-I ion at m/z 113. The 2-methylaminothiazole on the other hand showed molecular ion at m/z 114 (60%), M-I ion at m/z 113 (26%), base peak at m/z 86, m/z 74 (25%) and m/z 72 (5%) and m/z 71 (7%). These differences in fragmentation pattern shows that methyl scrambling also occur in the fragmentation of ion m/z 141 in Compound **5**.

A dihydrothiazole derivative (compound **6**, Fig. 5) was also isolated from the microsomal reduction of compound **1**. Its mass spectrum showed molecular ion at m/z 197 and base peak at m/z 143 [M-(CH_2-CH_2-CN)]. Subsequent loss of carbon monoxide gives the fairly abundant ion m/z 115. Transitions from ion m/z 115 to the thiirene ions m/z 57 and m/z 58, and the presence of *N*-methylthiourea ion at m/z 90 show that reduction of the 2,3-position of the thiazole ring has occurred. Interestingly, the acetyl derivative (compound **2**) on reduction gave only an open chain nitrile product compound **7**, with molecular ion at m/z 237 and base peak at m/z 195 ($M^{+\cdot}$-(ketene)].

The isoelectronic 4-(4-nitrophenyl)thiazoles on the other hand gave only the 4-(4-aminophenyl)thiazoles as the reduction products showing a different biochemical transformation pathway. The mass spectrum of compound **8**

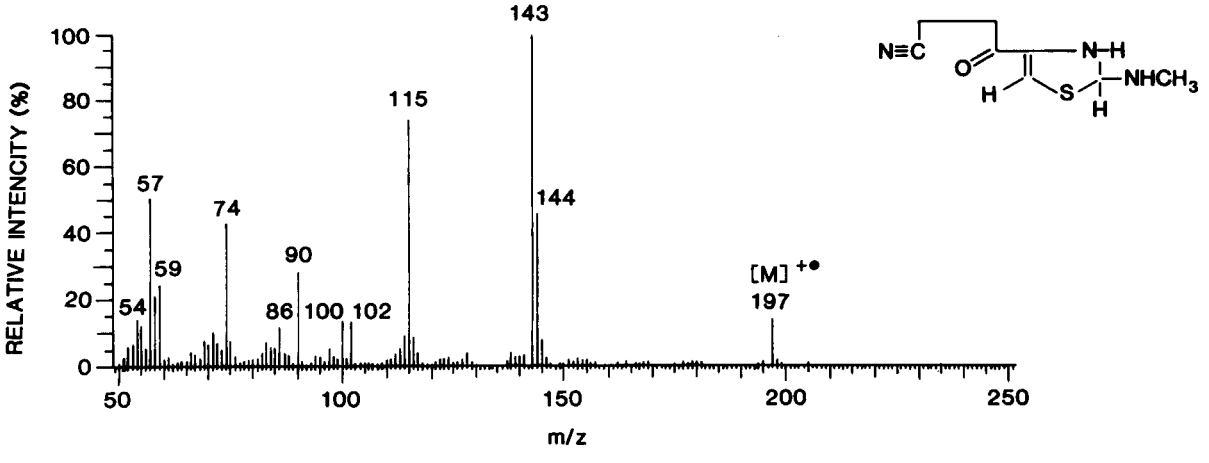


Figure 5. The low resolution mass spectrum of 1-(2-methylamino-2,3-dihydro-4-thiazolyl)-3-cyano-1-propanone (Compound **6**).

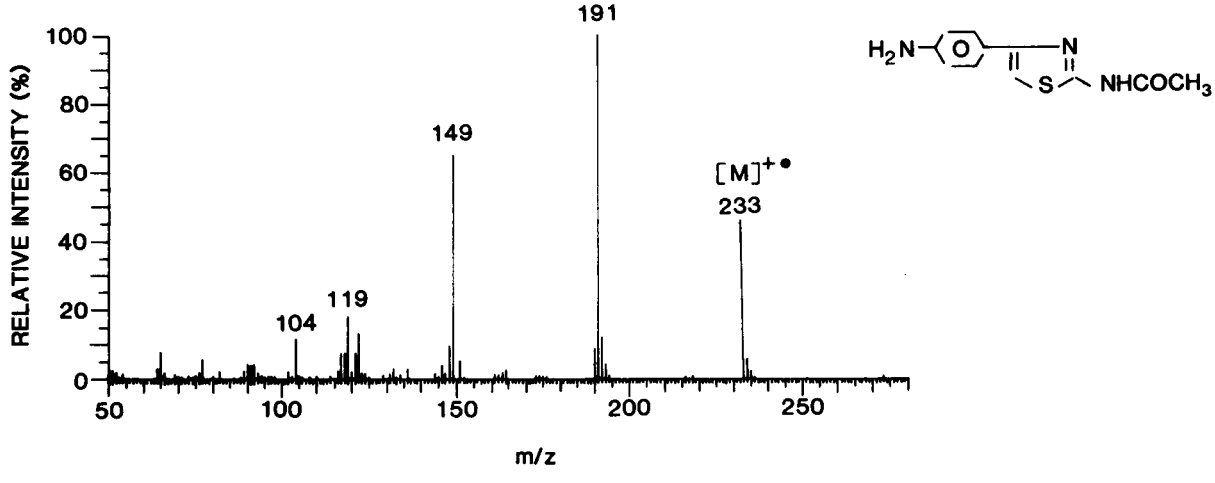


Figure 6. The low resolution mass spectrum of 2-acetylamino-4-(4-amino phenyl)thiazole (Compound **8**).

TABLE 2

Major Fragment Ions (70 ev, Electron Ionization) and Chemical Ionization Mass Spectra of the Compounds 1-9, m/z (% Relative Intensities)

1. 225(88) $M^{+\cdot}$, 197 (25), 195(33), 193(16), 167(35), 164(13), 151(70), 141(30), 124(16), 97(18), 95(100), 93(36), 80(39), 74(25), 71(16), 70(20), 69(25), 57(37), 55(29), 52(35), 51(30). CI, $[M + H]^+$ 226(100), $[M + C_2H_5]^+$, 254(12), $[M + C_3H_5]^+$ 266(45).

2. 267(19) $M^{+\cdot}$, 225(100), 197(28), 195(20), 193(25), 167(23), 151(59), 141(25), 123(23), 109(26), 95(58), 93(23), 80(38), 69(20), 64(47), 52(22), CI, $[M + H]^+$, 268(100), $[M + C_2H_5]^+$, 296(23), $[M + C_3H_5]^+$, 308(12).

3. 263(43) $M^{+\cdot}$, 221(100), 191(42), 175(77), 174(21), 149(20), 146(25), 133(17), 121(24), 103(27), 89(67), 77(16), 76(28), 75(20), 63(25).

4. 277(13) $M^{+\cdot}$, 235(100), 207(54), 189(26), 174(16), 146(15), 121(16), 103(15), 89(54), 76(18), 75(16), 63(23). CI, $[M + H]^+$ 278(100) $[M + C_2H_5]^+$, 306(40), $[M + C_3H_5]^+$, 318(20).

5. 195(30) $M^{+\cdot}$, 155(41), 141(100), 114(82), 113(36), 84(17), 74(56), 72(55), 71(19), 69(21), 58(23), 57(51), CI, $[M + H]^+$, 196(100), $[M + C_2H_5]^+$ 224(25), $[M + C_3H_5]^+$, 236(14).

6. 197(14) $M^{+\cdot}$, 144(46), 143(100), 115(74), 102(14), 90(30), 86(13), 74(43), 59(25), 58(21), 57(51).

7. 237(8) $M^{+\cdot}$, 195(71), 155(55), 141(76), 114(100), 113(24), 85(17), 82(22), 74(23), 72(37), 71(34), 70(20), 57(25), 54(57). CI, $[M + H]^+$ 238(100), $[M + C_2H_5]^+$, 226(20), $[M + C_3H_5]^+$, 278(15).

8. 233(46) $M^{+\cdot}$, 191(100), 149(65), 122(14), 119(19), 104(12), CI, $[M + H]^+$, 234(100), $[M + C_2H_5]^+$, 262(30), $[M + C_3H_5]^+$, 274(14).

9. 247(39) $M^{+\cdot}$, 205(100), 197(31), 177(25), 176(18), 149(80), 135(21), 122(20), 119(29), 118(21), 117(39), 104(20), 87(18), 65(20).

(Fig. 6) shows molecular ion at m/z 233 and base peak at m/z 191 ($M^{+\cdot}$-(ketene)]. The major fragment 4-aminophenylthiirene ion m/z 149 arises from the typical 1,2-cleavage of the thiazole ring and shows a fragmentation pattern characteristic of an aromatic amine [5]. The compound **9**, the acetyl derivative, shows molecular ion at m/z 247 and a base peak at m/z 205 ($M^{+\cdot}$-(ketene)]. A complete analyses of this 2-methylamino-4-(4-aminophenyl)thiazole ion radical has been recently published [11].

The electron impact mass spectral study of the carcinogenic 4-(5-nitro-2-furyl) and 4-(4-nitrophenyl)-2-(*N,N*-disubstituted)thiazoles and their metabolites (Table 2) shows some subtle differences in their fragmentation patterns. Multiple modes of cleavages are seen when the 2-amino group in thiazole is substituted. Even though they showed complex fragmentation pattern, their electron impact mass spectra contained some characteristic diagnostic fragment ions for the determination of the metabolites' structure. The structural information of the metabolites also indicate the differences in biochemical transformation of these two classes of carcinogens.

EXPERIMENTAL

All melting points are uncorrected and were obtained with a Fisher-Jones melting point apparatus. Gas chromatography of compounds **1-9** was carried out on a Hewlett-Packard Model 5830A instrument equipped with a flame ionization detector and a Model 18850 data terminal. A glass column (2m × 2mm) packed with 3% OV-17 on 80/100 mesh Chromosorb W (Applied Science Laboratories) was used. The column was temperature programmed from 200° to 250° at 10°/minute.

Compounds **5-9** were prepared from anaerobic renal microsomal incubation mixture by the method described previously [12]. After incubation, enzyme activity was terminated by the addition of sodium hydrixide (0.5 mℓ, 1.0 mole/ℓ) followed by 1 mℓ of cold ethyl acetate. The ethyl acetate extracts from several incubations were pooled, concentrated to 100-200 $\mu\ell$ under nitrogen and purified by high pressure liquid chromatography. A Beckman Model 332 gradient liquid chromatograph equipped with a 25 × 10 mm (i.d.), 5 μ particle size reversed phase ultra sphere - ODS, semipreparative column was used. The chromatograph was operated isocratically using methanol:water (45:55) at a flow rate of 1.0 ml/minute. The low resolution mass spectra were run at 70 eV on a Finnigan 3200 gas chromatograph/mass spectrometer. A Varian Model MAT 731 or Kratos MS-50-S mass spectrometer was used to measure exact masses by scanning at a resolution of 10,000-12,000 and to observe metastable peaks by scanning the accelerating voltage or linked scanning of the electric vector voltage and magnetic field at constant B/E. Samples were introduced into the mass spectrometer *via* a gas chromatographic column comparable to that described above and with similar conditions. Elemental analyses were performed with a Perkin-Elmer Model 240 analyzer. The elemental composition of all ions reported in the Schemes 1 and 2 and the molecular ions of compounds of **1-9** were confirmed to within 2.0 mmu by high resolution measurements.

The (4-(4-nitrophenyl) and 4-(5-nitro-2-furyl)-2-methylaminothiazoles were synthesized from their corresponding 2-bromoacetyl-5-nitrofuran or 2-bromo-4'-nitroacetophenone and *N*-methylthiourea or thiourea by the

Hantzsch cyclization method [10]. For the acetyl derivatives, the hydrobromide salts obtained were treated with base, extracted, evaporated and acetylated with acetic anhydride/pyridine at room temperature overnight. The reaction mixture was added to water and the precipitated acetyl derivative was collected, repeatedly washed with water, and crystallized from methanol to a constant melting point. All the compounds used in the studies were checked chromatographically (thin-layer chromatography, high pressure liquid chromatography and gas chromatography) for purity and was found to be greater than 99.5%.

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REFERENCES AND NOTES

[1] G. T. Bryan, "Carcinogenesis, A Comprehensive Survey", Vol 4, Plenum Press, New York, NY, 1978, p 1.

[2] R. Kato, A. Takahashi and T. Oshima, *Biochem. Pharmacol.*, **19**, 45 (1970).

[3] D. R. MaCalla and D. Voutsinos, *Mutat. Res.*, **26**, 3 (1974).

[4] It is to be noted that structures of fragment ions in the schemes have been used to facilitate understanding of the fragmentation, but only limited studies have been done to distinguish possible isomeric structures. The elemental composition of all the ions in the schemes were confirmed to within 2.0 mmu by high resolution measurements. Most, if not all, transitions in the schemes are supported by the observation of metastable peaks.

[5] H. Budzikiewicz, C. Djerassi and D. H. Williams, "Mass Spectrometry of Organic Compounds", Holden Day, San Francisco, 1967, p 326.

[6] A. N. Porter and J. Baldas, in "Mass Spectrometry of Heterocyclic Compounds", A. Weissberger and E. C. Taylor, eds, John Wiley and Sons, Inc., New York, NY, 1971, p 532.

[7] J. V. Metzger, in "Thiazole and Its Derivatives", Part I, J. V. Metzger, ed, John Wiley and Sons, Inc., New York, NY, 1979, pp 81-84.

[8] J. P. Anne and J. V. Metzger, *Bull. Soc. Chim. France*, 3536 (1972).

[9] R. Tabacchi, *Helv. Chim. Acta.*, **57**, 324 (1974).

[10] A. Hantzsch and H. J. Weber, *Ber.*, **16**, 345 (1883).

[11] M. B. Mattammal, E. White V, T. V. Zenser and B. B. Davis, *Biomed. Mass Spectrom.*, **11**, 149 (1984).

[12] M. B. Mattammal, T. V. Zenser and B. B. Davis, *Carcinogenesis*, **3**, 1339 (1982).