

Gas Chromatography-Mass Spectrometry Studies of Several Allyl-substituted 6-Azauracil Derivatives

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Gas chromatography coupled with mass spectroscopy has been used to investigate three N-substituted 6-azauracils, alongside 6-azauracil and 5-bromo-6-azauracil. For each compound, the fragmentation patterns have been discussed and correlated with proposed fragmentation mechanisms. A loss of isocyanic acid or isocyanate has been noticed for all investigated azauracil compounds. This loss is similar to that found in substituted uracils and may be used for future identification of 6-azauracil derivatives.

Keywords: 6-azauracil derivatives, gas chromatography mass spectrometry, 1,2,4-triazine-3,5-dione

It is established that analogues of natural nucleobases and nucleosides, containing nitrogen atoms have good antimetabolite [1, 2], antiviral, antibacterial [3, 4] antitumor and antimetastatic properties [5]. One of the first compounds obtained, exhibiting good antitumor and antimetabolite activity is 6-azauridine, an analogue of 6-azauracil [2]. In recent years, a number of 6-azauracil analogues, N-substituted with alkyl groups containing a β -heteroatom (O,S) [6] or vicinal olefinic double bond [7] have been reported as having antiviral properties.

Beside some known compounds, we have reported the synthesis and characterization of new 5-substituted-6-azauracils [8, 9]. However, N-substituted 6-azauracils bear increased similarity to natural nucleosides, exhibiting good potential for useful biological properties.

The biological action and metabolism of 6-azauracil derivatives is fairly well known. This activity stems from impairment of nucleic acid synthesis caused by the inhibition of orotic acid metabolism by phosphorylated 6-azauridine [10]. The metabolites of 6-azauridine have been found to consist of 6-azauracil and phosphorylated 6-azauridine, along with untransformed 6-azauridine [11].

In both 6-azauracil and 6-azauridine derivatives, the chemical bond strengths and lengths may have a wide range. This fact, coupled with the diverse chemistry of such triazine derivatives (for example see the rearrangement described in [12]), should allow, at least in theory, for a number of possible decomposition patterns of the triazine heterocycle under electron ionization.

The present paper reports the GC-MS studies on a number of five 6-azauracil derivatives, consisting of 6-azauracil, 5-bromo-6-azauracil and three allyl N-substituted derivatives. The synthesis and characterization by other spectroscopic methods will be presented elsewhere. Starting from the fact that GC-MS investigations are more accessible than other characterization methods in organic chemistry (such as various NMR techniques), this study aims to find good correlation between MS data and the structure of investigated compounds.

Experimental part

Syntheses

All chemical materials used have been purchased from Fluka or Merck and used without further purification.

The syntheses of 6-azauracil and 5-bromo-6-azauracil have been performed by using the already described methodology [13, 14]. The syntheses of N-substituted 6-azauracils are based on established procedures [7] and will be presented in detail elsewhere. The investigated compounds, alongside with their structure are presented in table 1.

Analytical methods

The GC-MS spectra have been recorded using an Agilent 6890 gas chromatograph coupled with an Agilent 5975B mass spectrometer endowed with automatic injector module. The gas chromatography has been carried out on an Agilent 19091S-433 HP-5MS 5% phenyl methyl siloxane column with a 30 m length, 0.25 mm diameter and 0.25 μ m film thicknesses using 1.2 mL \cdot min⁻¹ hydrogen carrier.

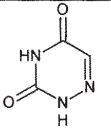
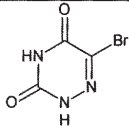
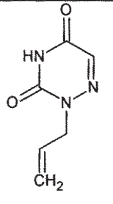
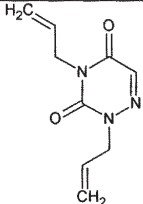
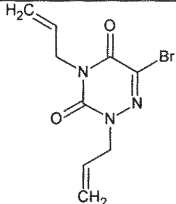
Compound	az1	az2	az3	az4	az5
Formula					
M ⁺ [u]	113.0220	190.9325	153.0533	193.0846	270.9951

Table 1
6-AZAUACIL DERIVATIVES
INVESTIGATED

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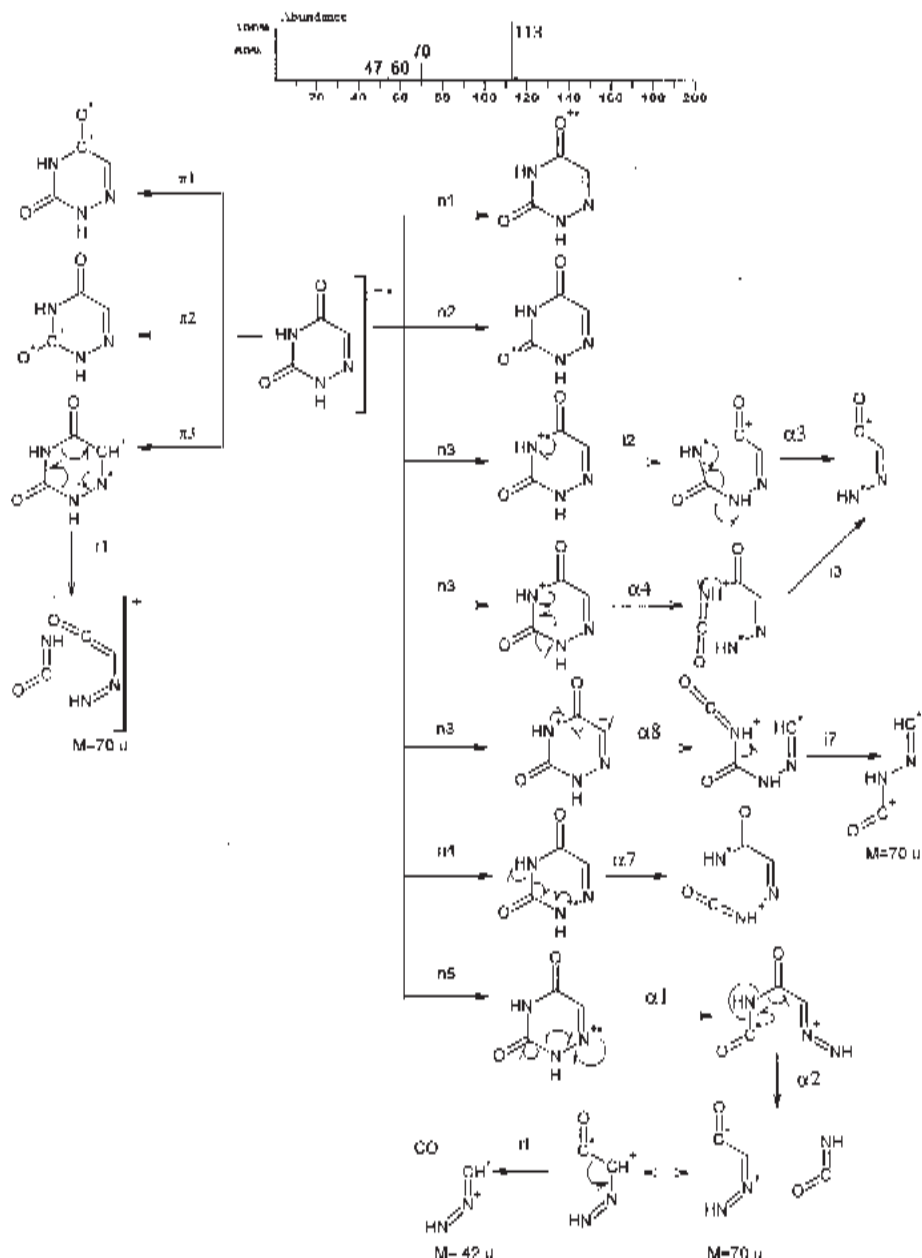


Fig. 1. Mass spectrum and proposed fragmentation pattern of **az1**

The experiments have been carried out using an ionization energy of 70 eV, MS source 250 °C, MS Quad 150 °C, with 1 μL splitless injection samples, 0.3 ssl time, 40 $\text{mL} \cdot \text{min}^{-1}$ vent, 3 min solvent delay, 46 – 650 mass scan interval, starting from initial oven temperature of 100 °C up to 280 °C with a rate of 4 °C $\cdot\text{min}^{-1}$.

Samples have been prepared as $10^{-3} \text{ mol} \cdot \text{L}^{-1}$ solutions in suitable solvents (acetonitrile, chloroform, ethyl acetate, dimethylformamide, dimethylsulfoxide and/or ethanol).

Results and discussions

The representative peaks in the mass spectra of 6-azauracil **az1** are the molecular ion peak at 113 m/z and a secondary peak at 70 m/z with relative abundance of 26.9%. Another low abundance peak can be found at 55 m/z , 4.5% relative abundance.

The electron loss in the 6-azauracil molecule can occur through π -type loss (from the carbonyl groups $-\pi1$, $\pi2$ or from the imine group $-\pi3$) or, more probably through n -type loss from the non-bonding orbitals of oxygen atoms ($n1$, $n2$) or nitrogen atoms ($n3$, $n4$, $n5$) (fig. 1). From the n -type electron loss mechanism, the $n5$ mechanism may be the most probable- as the N5 atom has the possibility for

conjugation stabilization and is the least electronegative heteroatom in the 6-azauracil molecule.

Considering the molecular formula of 6-azauracil, the peak corresponding to a positive fragment with mass of 70 u, representing a loss of 43 u from the molecular ion, could be explained by the loss of either CHNO , C_2OH_3 , N_3H or CN_2H_3 molecules. From these, only CHNO and N_3H can exist as neutral molecules; furthermore the hydrazoic acid is unlikely to be obtained as it would involve a large number of molecular rearrangements. In figure 2 it can be seen that the formation of such a fragment can be explained in several ways: a retro Diels Alder rearrangement of the radical cation obtained from $\pi3$ electron loss (r1); inductive cleaving (i2) of the radicals obtained through n electron loss mechanisms, followed by additional α cleavages leading to the formation of an isocyanic acid molecule or, more likely, by α cleavages followed by inductive ($\alpha4$ -i3, $\alpha8$ -i7) or α cleavage ($\alpha1$, $\alpha2$) loss of isocyanic acid. Electron ionization studies of various substituted uracil derivatives [15-17] have reported similar fragmentation patterns.

The same pattern involving the loss of isocyanic acid (43 u) can be seen for **az2** (peak 148/150 from molecular ion at 191/193). In the case of N,N-dialkyl substituted

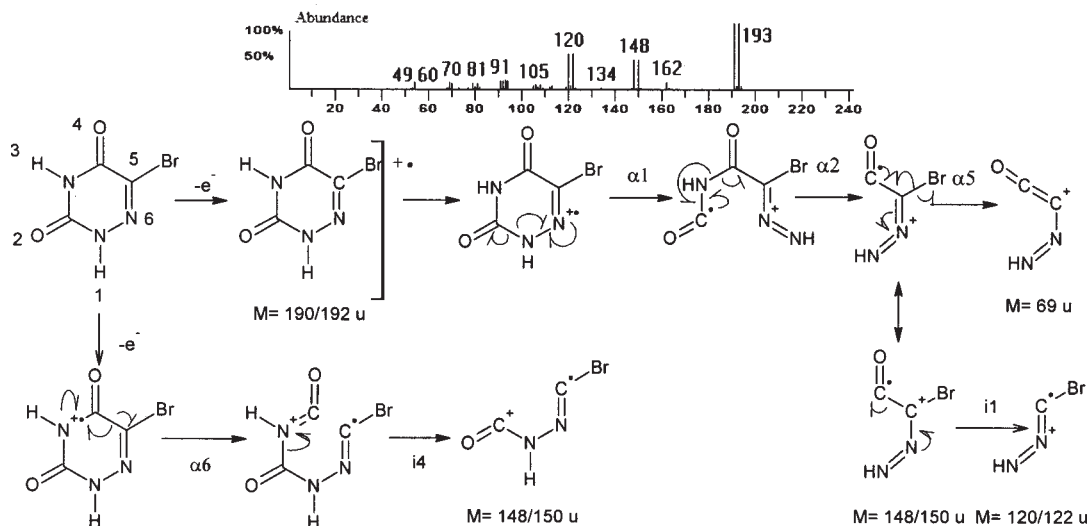


Fig. 2. Mass spectrum and possible fragmentation patterns for **az2**

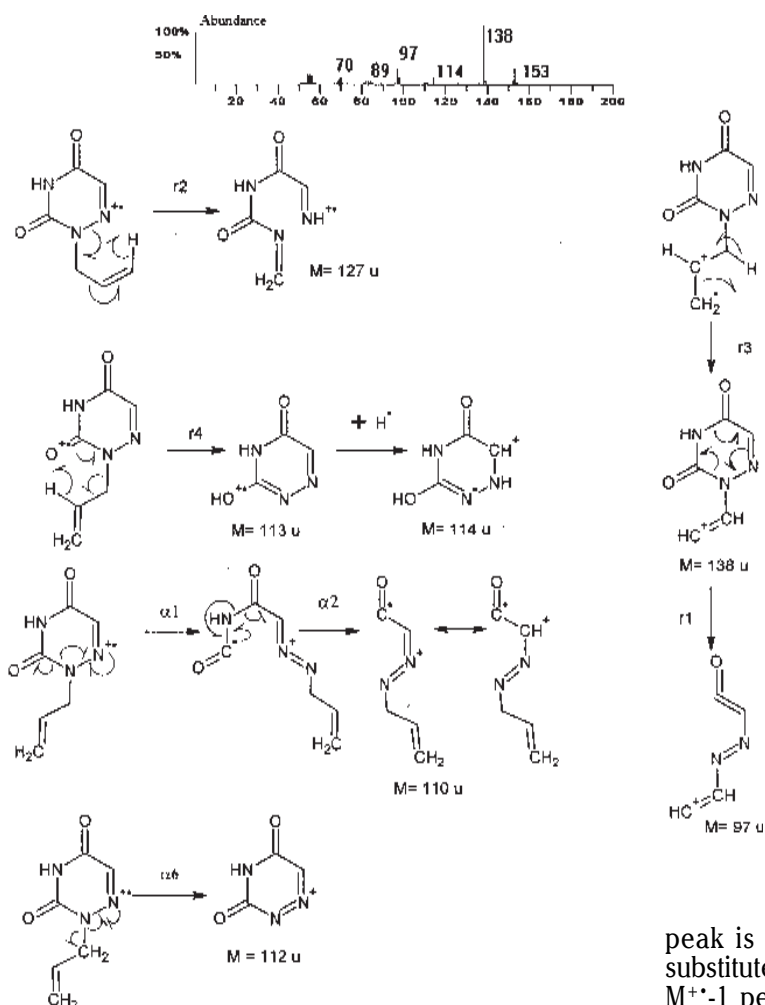


Fig. 3. Mass spectrum and possible fragmentation pattern of **az3**

compounds a loss of 83 u, corresponding to the loss of allyl isocyanate, can be seen for **az4** in the peaks 193/110; 178/95; 152/69 u. In the case of **az5** a loss of 82 u is seen, suggesting loss of an allyl isocyanate radical or proton transfer: 271/189; 256/174 and 231/149 u peaks.

For **az3** a loss of 83 u is observed for the 153/70 m/z peaks; a loss of 43 u can be observed for the 138/97 and 153/110 m/z peaks (fig. 3).

The peak of 82 m/z, presenting large abundance for **az4** and **az5** (but not for **az3**) could be explained as an allyl isocyanate cation. Such cation could be obtained through hydride radical loss or proton transfer. The fact that this

peak is present in large abundance only for the di-substituted compounds, coupled with the absence of a $M^{+•}-1$ peak suggests that it is formed after the initial fragmentation of the molecule. One possible way for such a mechanism is presented in figure 5.

In the case of allyl substituted compounds, a series of common fragmentations can be observed. Firstly, a loss of 15 u (r3) from the molecular ion and from the peaks of mono-substituted species, in the case of **az4** and **az5**, can be observed. This loss could be attributed to a methyl radical loss. The methyl loss mechanism is appreciable for all three allyl compounds, as it can be seen from the relative abundances of demethylated peaks.

Another fragmentation possibility consists in the McLafferty rearrangement involving the N-6 atom (r2) and

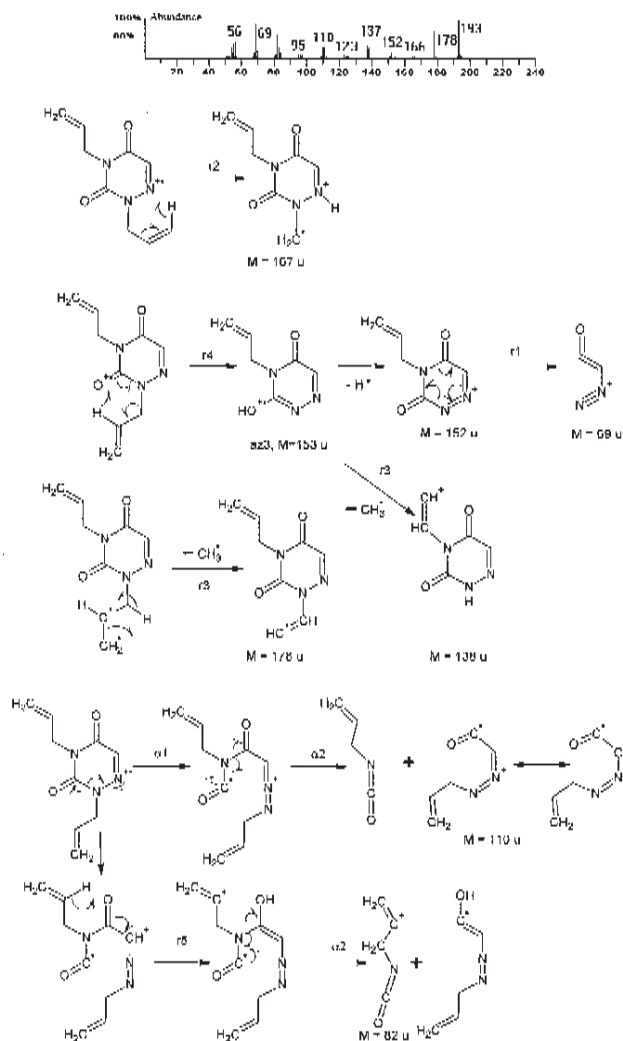


Fig. 4. Mass spectrum and proposed fragmentation pattern of **az4**

resulting in the loss of C_2H_2 ; $m=26$ u. This behavior could explain the peaks at 127, 167 and 245 m/z for **az3**, **az4** and **az5**, respectively. The peaks corresponding to this rearrangement have low relative abundances for all the investigated compounds.

A final fragmentation possibility consists of the total loss of allyl substituent. The possible mechanism could involve a McLafferty rearrangement involving a neighboring carbonyl oxygen atom ($r4 - 40$ u loss), an α cleavage resulting in an allyl radical ($\alpha5 - 41$ u loss) or a mechanism involving subsequent losses of methyl and acetylene.

From the above – proposed mechanisms, it may be seen that all the investigated compounds present common features; they include the loss of one isocyanate molecule as well as a common fragmentation pattern leading to the loss of allyl moiety.

Conclusions

Five 6-azauracil compounds have been investigated by means of GC-MS. From the data obtained, it can be seen that two fragmentation patterns occur in all compounds. Firstly, the main fragmentation occurring in the 6-azauracil heterocycle is the loss of isocyanic acid or allyl isocyanate. This pattern can be noticed in all compounds.

Secondly, the allyl substituents are lost through three possible mechanisms: the initial loss of a methyl radical, the loss of ethyne through McLafferty rearrangement involving N6 or involving a neighboring carbonyl oxygen atom. The di-substituted compounds show an 82 m/z peak,

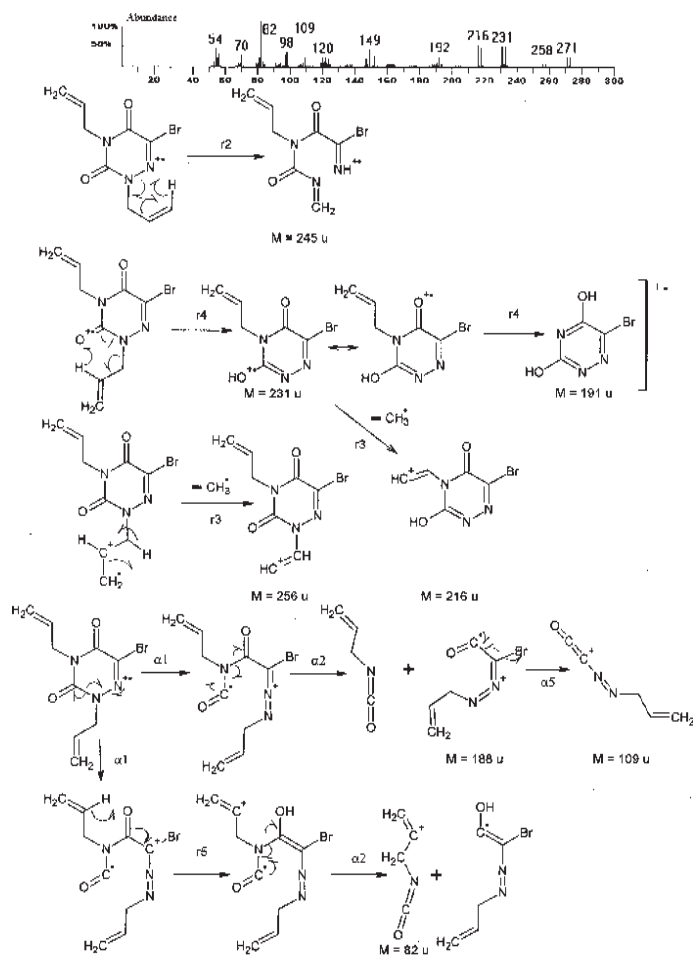


Fig. 5. Mass spectrum and proposed fragmentation mechanisms for **az5**

corresponding to an allyl isocyanate cation, which is not significantly present in the mono-substituted compound. These fragmentation patterns can form the basis for future facile identification of substituted 6-azauracils through mass spectroscopy.

Furthermore, a correlation between behaviour in living systems and in the conditions of mass spectroscopy could be seen in the similar loss of N-substituents from the 6-azauracil heterocycle. While this loss is similar with the formation *in vivo* of 6-azauracil from 6-azauridine, to the best of our knowledge there is no equivalent case described in the literature for the fragmentation mechanism leading to the formation of isocyanic acid or isocyanate from 6-azauracil derivatives, *in vivo*. This behaviour, similar to that of uracil derivatives used as radio sensitizers incorporated into cancer cells [15] could make 6-azauracil derivatives suitable for such a role.

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