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The MS-MS Determination of the Fragmentation Pathways of Some Substituted 1,2,3-Benzotriazin-4-ones with Possible Applications in Metabolic Studies

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Abstract

1,2,3-Benzotriazin-4-one derivatives are extensively used as herbicides, insecticides and nematicides. Incorporation of these compounds into the human food chain is a cause for concern, since their toxicity to man is well documented.

This work describes the development of a data-base of classical 1,2,3-benzotriazin-4-one fragmentation patterns and consequent sequential neutral mass losses which allows the prediction of the presence of a triazinone derived compound.

Positive and negative ion tandem mass spectrometry techniques were employed since they allow rapid and sensitive structural characterization of drugs and their metabolites in biological fluids through the unambiguous monitoring of daughter ions from pre-selected primary ions.

The herbicidal, insecticidal and nematicidal properties of 1,2,3-benzotriazin-4-ones are well documented and the toxicities of these compounds have been extensively studied (Neunhoeffer 1977). Incorporation of these compounds into the human food chain is a cause for concern and a fast and effective method for the detection of the metabolites of these compounds is essential.

The fragmentation pathway of 1,2,3-benzotriazin-4(3H)-one has previously been studied using high-resolution mass spectrometry (Tou et al 1969). This technique showed the initial loss of 28 mass units to be that of a nitrogen molecule. This and other fragmentation processes were supported by metastable ion studies. In the absence of any linked scan techniques metastable-ion studies are based on the assignment of plausible masses to the parent and daughter ions in order to satisfy the requirements of the position of the metastable peak according to the equation:

$$m^* = m_2^2/m_1 \qquad (1)$$

where m^* = metastable, m_2 = daughter and m_1 = parent.

The unambiguous identification of the daughter ions resulting from any pre-selected parent ion is a salient feature of the tandem mass spectrometer system. In such a system it is possible to identify both the daughters from a selected parent ion and also the parents of any pre-selected daughter ion. MS-MS experiments involve the use of two mass analyser regions within the same instrument. The first MS allows the mass selection of a characteristic ion and the second MS identifies the fragments produced by the collision-induced dissociation of this ion, thus providing a fingerprint pattern for the primary ion. The instrument shown in Fig. 1 is a VG TRIO 3 triple quadrupole system in which Q1 and Q3 are the two mass spectrometers (MS1 and MS2 respectively) and Q2 is the non-mass selective reaction region. The lower half of this figure shows the selection of a chosen primary ion in MS1 and a representation of the mass spectrum (MS2) resulting from the collision-induced fragmentation of this ion in the collision region.

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The fragmentation patterns cited in the literature for benzotriazinone are derived from metastable-ion studies data in combination with the high-resolution mass spectrometry assignment of either carbon monoxide or molecular nitrogen as the fragmentation product. This information was used to corroborate the data obtained from MS-MS results and thus validated the tandem technique for this study. This work was subsequently extended to include negative-ion fragmentation studies derived from MS-MS investigations.

Comparison of the fragmentation pattern of the starting compound with that derived from a possible parent ion present in a biological fluid may indicate the presence of a triazinone-based compound. Furthermore, the MS-MS analysis of the biological fluid should provide an insight into the metabolic decomposition pathway.

MS-MS techniques provide a method whereby a target analyte can be identified in the presence of an extremely complex mixture—such as a biological fluid extract. The primary ion, identified from the data base, is selected by MS1 and focussed into the collision region. It is likely that there will be several species contributing to this chosen m/z value and all of these ions will be focused into the collision cell. The collision-induced decompositions for these ions will be characteristic of each ion type and only those ions which produce the correct (ie previously cited m/z values in the data base) secondary ions are of interest. These are determined using selected ion monitoring where the second mass spectrometer (MS2) is controlled to search only for the ions of interest. Thus if no triazinone molecule is present in the sample, then no signal is observed at the detector since none of the anticipated secondary ions will be formed. In

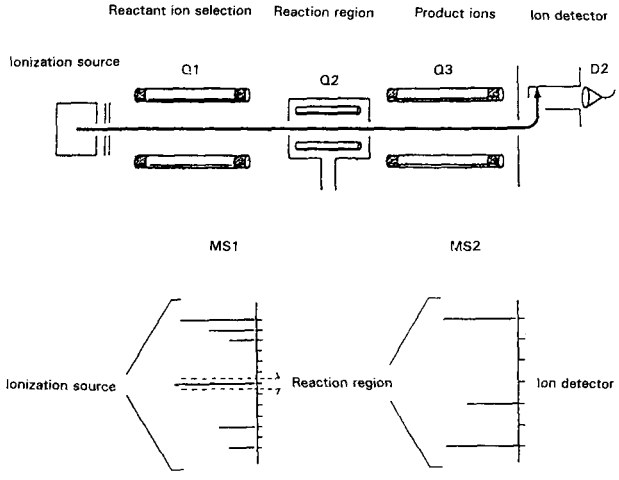


FIG. 1. Schematic representation of an MS-MS instrument.

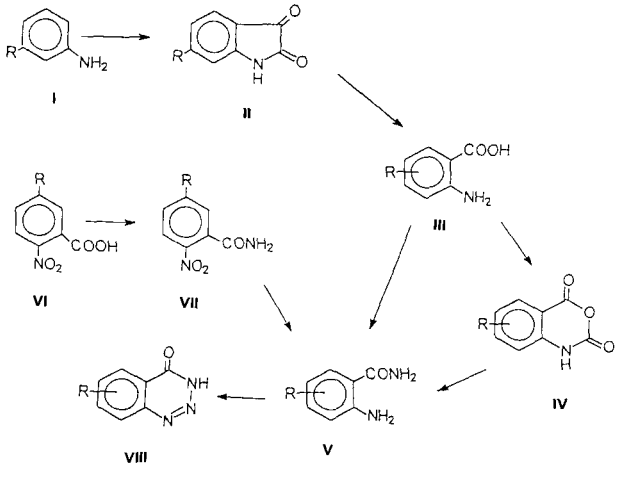
this mode of operation, both mass spectrometers in the MS-MS instrument are operating under conditions derived from a predetermined data base.

Initially, simple C-6 and C-7 substituted benzotriazinone derivatives were studied to enable the fragmentation process to be fully ascertained and to supply an adequate number of compounds to form a data-base. This was later expanded to include N-3 substituted benzotriazinone compounds such as *N*-benzoylbenzotriazinone.

Further work will apply the data base already developed to the examination of samples derived from biological extracts.

Materials and Methods

Simple C-6 and C-7 substituted benzotriazinones were used as reference compounds to develop the characteristic fragmentation pattern for this type of ring system. Synthesis of these compounds, summarised in Scheme 1, was based on the diazotization of the appropriately substituted 2-aminobenzamide. Where possible, the aminobenzamides (V) were obtained from commercially-available substituted aminobenzoic acids (III).



Scheme 1. Synthetic routes to benzotriazinone compounds.

The 4-nitro- and 5-nitro-aminobenzamides (V, R = 4-NO_2, 5-NO_2) were obtained by conversion of the appropriately substituted 2-aminonitrobenzoic acids (III, R = 4-NO_2, 5-NO_2) on reaction of the acid chloride with ammonia (Adamson et al 1971). Direct conversion of the aminobenzoic acid was not always possible and synthesis via the isatoic anhydride (IV, R = 6-Cl, 7-Cl, CH_3) was employed, subsequent treatment with ammonia gave the desired aminobenzamide. In cases where the aminobenzoic acids were not commercially-available, other routes to these compounds were employed.

7-Methyl- and 7-methoxy-benzotriazinone (VIII, R = 7-CH_3, 7-OCH_3) were prepared via the isonitrosoacetanilide derivatives which on cyclodehydration afforded the substituted isatin (II, R = 6-OCH_3, 6-CH_3). Oxidation with hydrogen peroxide gave the aminobenzoic acid (III, R = 4-OCH_3, 4-CH_3) in moderate yield. 3-Methyl-4-nitroanisole was oxidised under alkaline conditions to afford 5-methoxy-2-nitrobenzoic acid (VI, R = OCH_3), reaction of the acid chloride with ammonia gave the nitrobenzamide (VII, R = OCH_3). The reducing system of nickel (II) chloride-sodium borohydride (Nose & Kudo 1981) converted the nitroamide into 5-methoxy-2-aminobenzamide (V, R = OCH_3).

N-3-Benzoylbenzotriazinone was prepared by reaction of the parent triazinone under Schotten-Baumann conditions.

Instrument parameters

The MS-MS-VG TRIO 3 triple quadrupole used electron impact (70 eV) to produce positive and negative ions. The collision gas was argon, at a pressure of 5·0 mtorr, primary ion energy 5 eV and resolution 1000. MS2 scanned the range 50–500 Da, and the sample was introduced by direct insertion.

Results

The (EI) positive ion mass spectrum obtained from the parent benzotriazinone system was comparable with that published in the literature and the fragmentation pathways derived from the MS-MS experiments were the same as those based on the results of the high resolution data and metastable ion studies. The agreement between the three sets of results was taken as an indicator of the validity of the tandem MS approach.

Fig. 2 shows the derivation of the MS-MS data from two typical ions:- m/z 147 and m/z 119. The results for the positive and negative ions are summarized in Tables 1 and 2, respectively and the corresponding fragmentation processes are shown in Figs 3 and 4.

The fragmentation pattern derived from the MS-MS results for the *N*-benzoyl derivative is shown in Fig. 5.

Discussion

In the positive EI spectra, 1,2,3-benzotriazinones were found to fragment by two principal pathways, either by the loss of a nitrogen molecule from the molecular ion to produce the appropriate azetinone derivative, or by the loss of either N_3H or HNCO from the parent ion to produce a substituted benzene derivative.

Both pathways lead to further fragmentation via the elimination of neutral species such as N_2 or CO, both of

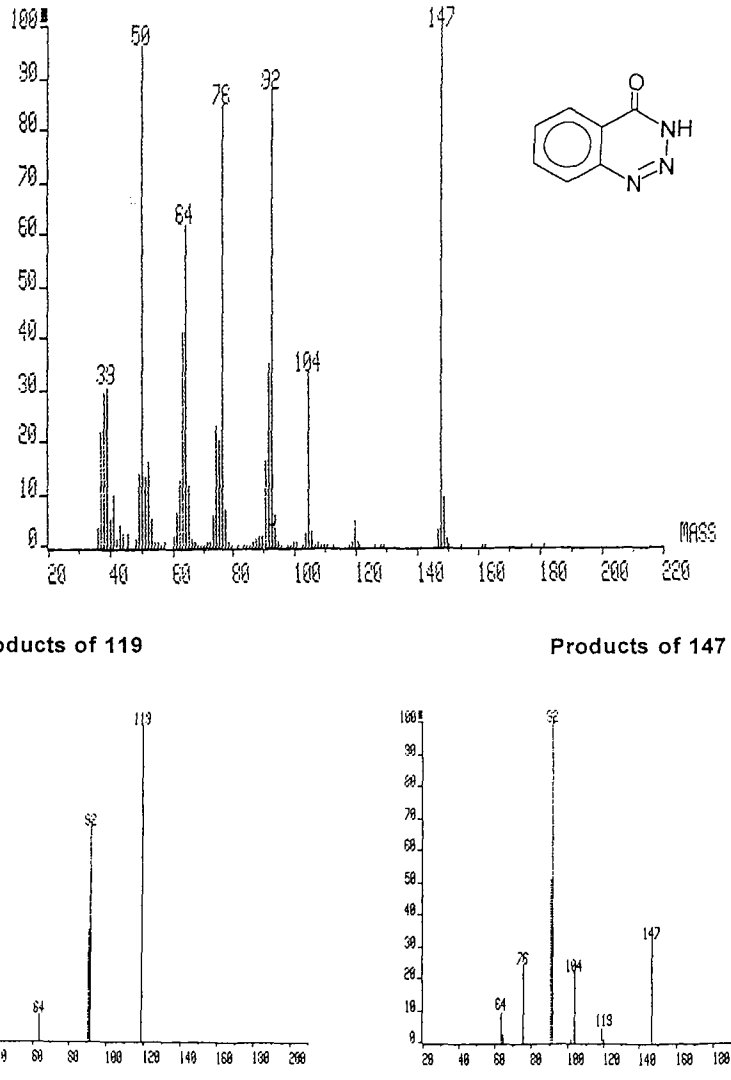


FIG. 2. EI positive spectrum for 1,2,3-benzotriazin-4(3H)-one.

Table 1. EI positive and MS-MS results.

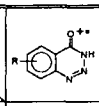
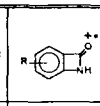
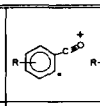
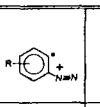
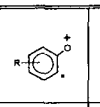
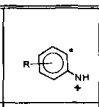
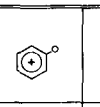
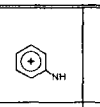
Ions / R									Others
H	✓	✓	✓	✓	✓	✓	-	-	
6-Me	✓	✓	✓	✓	✓	✓	-	-	-
7-Me	✓	✓	✓	✓	✓	✓	-	-	-
6-OMe	✓	✓	✓	✓	✓	✓	-	-	✓
7-OMe	✓	✓	✓	✓	✓	✓	-		✓
6-Cl	✓	✓	✓	✓	✓	✓	✓	✓	-
7-Cl	✓	✓	✓	✓	✓	✓	✓	✓	-
6-NO_2	✓	✓	✓	✓	-	✓	-	✓	✓
7-NO_2	✓	✓	✓	✓	-	✓	-	✓	✓

Table 2. EI negative and MS-MS results.

Ions / R					$^{-\bullet}_{\bullet}$N=C=O	Others
H	✓	✓	✓	-	✓	-
6-Me	✓	✓	✓	✓	✓	-
7-Me	✓	✓	✓	✓	✓	-
6-OMe	✓	-	✓	-	✓	✓
7-OMe	✓	-	✓	-	✓	✓
6-Cl	✓	✓	✓	✓	✓	-
7-Cl	✓	✓	✓	✓	✓	-
6-NO_2	✓	✓	✓	-	-	✓
7-NO_2	✓	✓	✓	-	-	✓

which have the same nominal mass ie 28 Daltons. The identification of the specific group eliminated requires the use of high-resolution mass spectrometry techniques.

The MS-MS data obtained from the range of C-6 and C-7 substituted benzotriazinones indicated that the nature of the substituent had only limited effect on the fragmentation pattern. Loss of the substituent occurred only in the cases of the nitro- and chloro- compounds; where both these substituents are good leaving groups.

The negative EI spectra of the same compounds showed a much reduced level of fragmentation. The principal fragmentation to afford the azetinone derivative occurred either by loss of a proton followed by elimination of nitrogen or by direct loss of a nitrogen molecule. Further decomposition was limited to those compounds containing a good leaving group, where loss of the substituent was observed as the subsequent fragmentation step.

The EI positive spectrum of the N-benzoyl derivative followed two pathways. The loss of a nitrogen molecule led to the formation of the substituted azetinone, which further fragmented through loss of a neutral species, thus complementing the fragmentation pattern seen for the C-6/C-7 substituted derivatives. Alternatively the benzoyl moiety was eliminated to afford the deprotonated, parent benzotriazinone ring system which fragmented to the azetinone by loss of a nitrogen molecule. A similar loss of the N-3 substituent to produce the parent ring system was described by Tou et al (1969) in the case of the chloromethyl derivative. The elimination of N-3 substituents to give the parent triazinone ring system appears to be a characteristic feature of the fragmentation pattern of these compounds.

Elimination of the *N*-benzoyl moiety was the principal fragmentation shown in the EI negative spectrum of *N*-benzoylbenzotriazinone. Further fragmentation leads to the formation of the azetinone via loss of a nitrogen molecule.

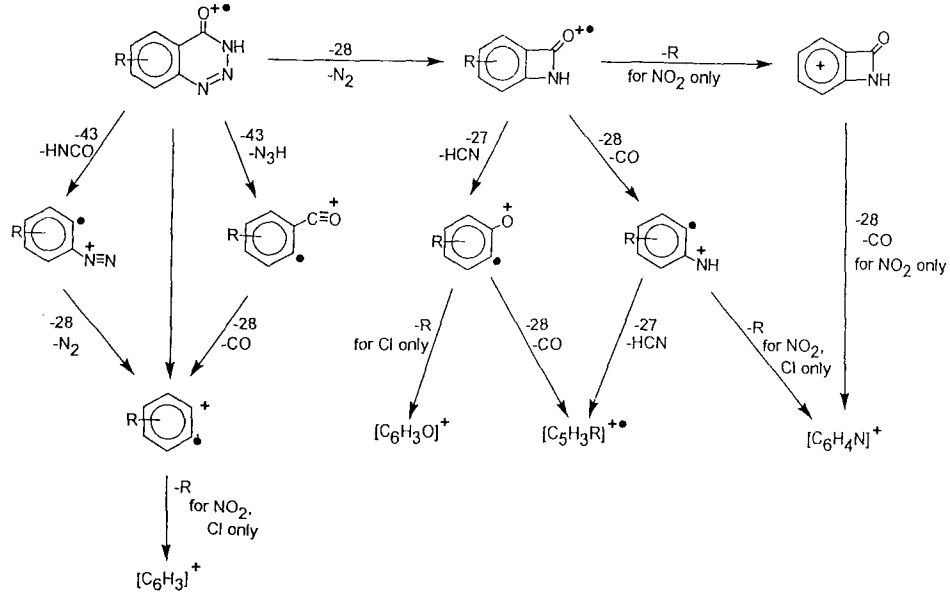


FIG. 3. Fragmentation pattern for substituted benzotriazin-4-ones (EI positive).

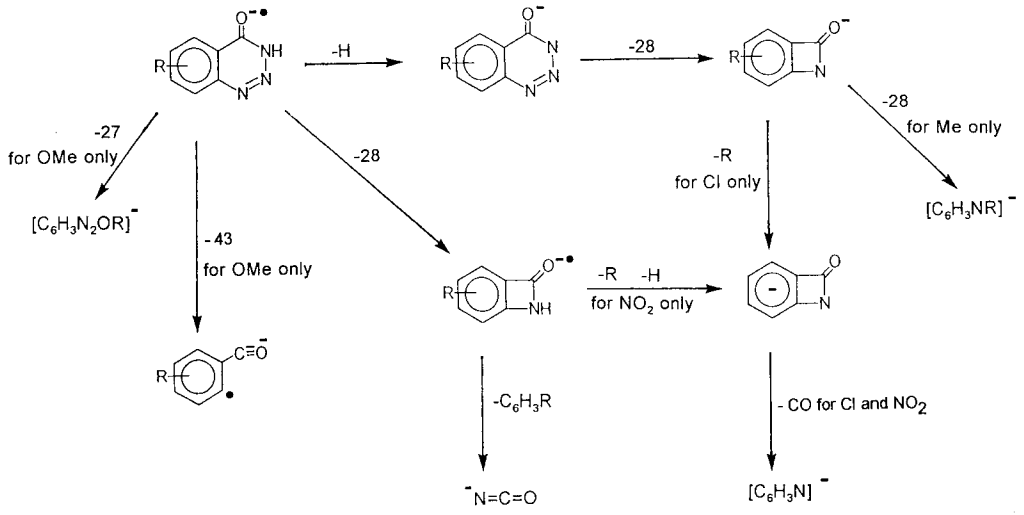


FIG. 4. Fragmentation pattern for substituted benzotriazin-4-ones (EI negative).

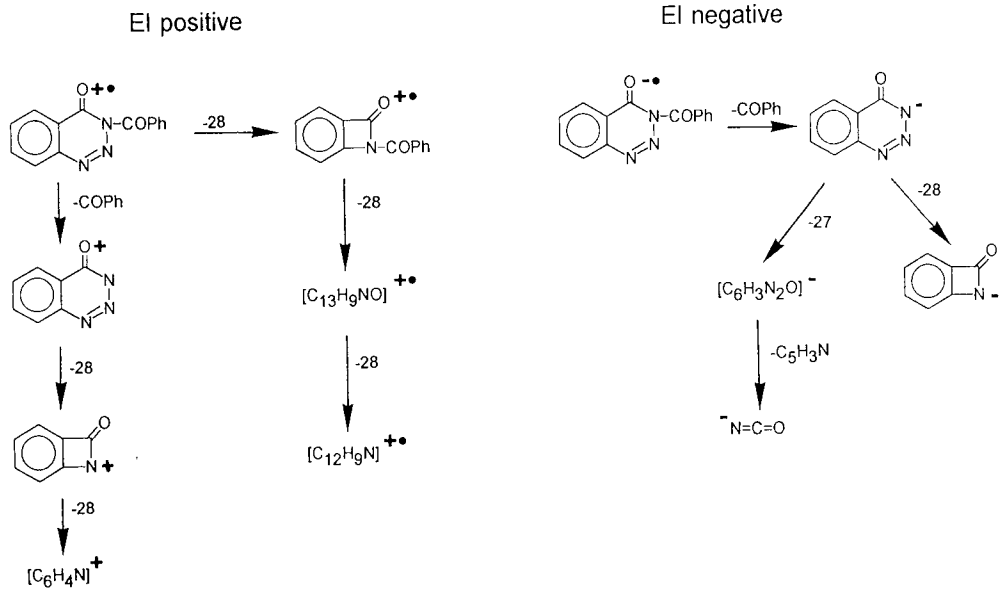


FIG. 5. Fragmentation patterns for *N*-benzoyl-1,2,3-benzotriazin-4-one.

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