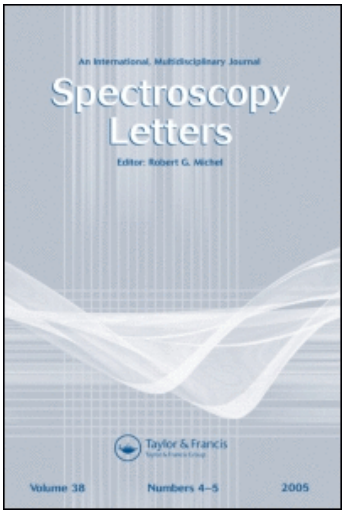


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# Mass Spectroscopy of the Antileukemic Drug MGBG and Related Bis(Amidinohydrazones) ['Bis(Guanylhydrazones)']

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# MASS SPECTROSCOPY OF THE ANTILEUKEMIC DRUG MGBG AND RELATED BIS(AMIDINOHYDRAZONES) ['BIS(GUANYLHYDRAZONES)']

Key Words: Electron-Impact Mass Spectra, Fragmentation Routes, Glyoxal Bis(guanylhydrazone), Methylglyoxal Bis(guanylhydrazone), Polyamine Antimetabolites

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## ABSTRACT

The first study on the mass spectroscopy of various bis(amidinohydrazones) is reported. The compounds studied included the investigational antileukemic drugs methylglyoxal bis(amidinohydrazone) ['methylglyoxal bis(guanylhydrazone)', MGBG] and glyoxal bis(amidinohydrazone), as well as seven mono- and dialkylglyoxal analogs thereof. The results indicate that the free bases of these high-melting compounds are volatilized well enough to allow a facile detection of the molecular ions and to make mass spectroscopy of the underivatized compounds a suitable method for the verification of the identity of the substances. This result is of importance considering the development of novel analogs and derivatives. A compilation of electron-impact mass spectra is reported and possible

fragmentation routes are outlined. The fragmentation of the various congeners appears to occur essentially similarly, the main paths involving breakage of the carbon-carbon single bond in the glyoxal moiety or breakage of either one of the nitrogen-nitrogen single bonds.

## INTRODUCTION

Thiele and Dralle reported glyoxal bis(amidinohydrazone) ['glyoxal bis(guanylhydrazone)', GBG][1] and some analogous compounds in 1898[2]. After those days, studies on the chemistry and spectroscopy of these compounds have, however, been almost totally neglected, in spite of the fact that GBG and its methylglyoxal analog MGBG were later found to be potent antileukemic and antiproliferative agents[3-6] and also potent specific inhibitors of adenosylmethionine decarboxylase, a key enzyme of polyamine biosynthesis[6,7]. In spite of intensive biochemical studies (for references, see[8,9]), the ultimate mechanism of the antileukemic action of GBG and MGBG, as well as the reasons for the unusually strict structural requirements for antileukemic activity[5,8-13] among this class of compounds, have remained unknown. Therefore, studies on the chemistry and spectroscopy of the agents are highly warranted, since the biochemical differences existing between the various congeners may well be due to some inherent chemical differences. Spectral studies are warranted also considering the development of rapid methods for the verification of the identity of the compounds and for the determination of the structures of novel analogs and derivatives.

Since the only previous report on the mass spectroscopy of bis(amidinohydrazones) appears to be that of Kourou-Daley et al.[14], describing the gas chromatography - mass spectroscopy of the trimethylsilyl derivatives of MGBG and one of its analogs, we considered it worthwhile to study the possibilities to obtain useful

structural information on bis(amidinohydrazones) with the aid of direct-inlet mass spectroscopy of the *underivatized* compounds. The results of those studies are reported here and indicate that in spite of their high melting points, the underivatized compounds are volatilized well enough to allow facile detection of the molecular ions and to make mass spectroscopy an attractive method in the identification of the compounds. The results also indicate that the fragmentation routes of various bis(amidinohydrazones) are very similar to each other.

## EXPERIMENTAL



All of the bis(amidinohydrazones) studied were in the free base form). GBG free base was prepared via the corresponding nitrate and dimethylglyoxal bis(amidinohydrazone) (DMGBG) free base via DMGBG dihydrochloride essentially as described by Thiele and Dralle[2]. The syntheses of the sulfate salts of the bis(amidinohydrazones) of ethylglyoxal[12], ethylmethylglyoxal[13], diethylglyoxal[15], methylpropylglyoxal[16], butylmethylglyoxal[17] and dipropylglyoxal[17] (EGBG, EMGBG, DEGBG, MPGBG, BMGBG and DPGBG, respectively) have been described elsewhere. The identity of the sulfates was verified by elementary analyses and $^{1}H$ and $^{13}C$ NMR spectroscopy[18-20] and, in some cases, by X-ray diffraction[13,15,21]. With the exception of EGBG, the free bases of these compounds were prepared by treating heated aqueous suspensions or solutions of the sulfate salts with a large excess of ca. 25 % (w/v) aqueous NaOH and by collecting the slightly soluble free bases by filtration of the cooled reaction mixtures. Further details of the syntheses of the bases will be described elsewhere. The methods used for the preparation and purification of the readily water soluble free bases of MGBG and EGBG have been described[17]. The free bases were identified by $^{1}H$ and $^{13}C$ NMR[18-20]. Also the structure of GBG free base was verified by single-crystal X-ray analysis[22].

Electron-impact mass spectra were recorded with the aid of a JEOL JMS 01SG-2 mass spectrometer at 70 eV and 200 μA using direct inlet.

## RESULTS AND DISCUSSION

A generalized structural formula of the compounds studied is shown in Fig. 1. The compounds are shown in the form of the endiamine tautomer of which GBG free base was recently shown[22] to exclusively consist in the solid state, and of which all of the free bases also appear to consist at least in DMSO solutions[18] The results obtained are summarized in Tables 1-4. In all cases, one of the major peaks in the electron-impact mass spectrum was that of the molecular ion. In the case of symmetrical compounds ($R^1 = R^2$), a peak with an m/e ratio half that of the molecular ion (a), and thus consistent either with the formation ions (b) and (c) [see Fig. 1] or with the formation of the molecular dication, was observed. That the former is in question, is indicated by the fact that in the case of unsymmetrical compounds, peaks consistent with (b) and (c) were observed but peaks with an m/e ratio half that of the molecular ion were not. Thus, almost certainly, one (major) fragmentation route of bis(amidinohydrazones) (I) in electron-impact mass spectroscopy is based on the breakage of the carbon-carbon single bond in the glyoxal moiety. It is tempting to speculate that the resulting fragments (b) and (c) are perhaps stabilized by cyclization, e.g. to the five-ring structures (q) and (r), in which one of the nitrogen atoms is quaternary and bears the positive charge.

Another major fragmentation route also appears probable on the basis of the results: the breakage of either one of the nitrogen-nitrogen single bonds, leading to the formation of the ions (d) and (e) as well as the guanidine ions (j), (k), (l), and (m). This route is obviously very important, since in most cases, peaks with an m/e ratio consistent with (d) and (e) were predominant in the spectra.

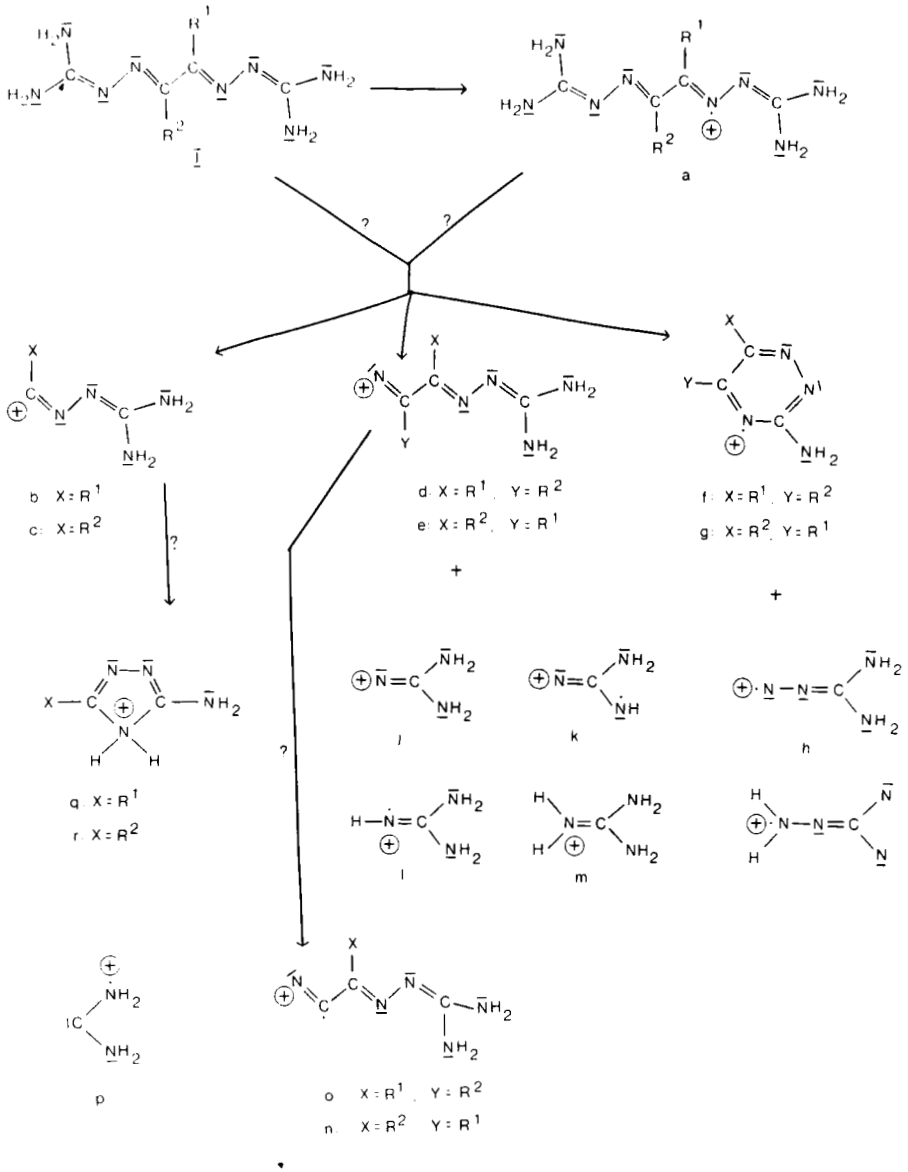

Fig. 1. A scheme showing the structures of the bis(amidinohydrazones) (I) studied, as well as the proposed fragments and fragmentation routes. Only one of the possible tautomers and resonance forms is shown in each case. It should be noticed that the bis(amidinohydrazones) studied consist of the *anti-anti* isomer only and, thus, any cyclization reactions must involve rearrangement of the double-bond system.

## TABLE 1.

### The Electron-Impact Mass Spectra of GBG, MGBG and EGBG.

I = Intensity of the peak, given in % of the intensity of the highest peak.

**GBG (I, $R^1 = R^2 = H$), 180°C**

| m/e | I | Interpretation |
|---|---|---|
| 42 | 35 | p minus two H |
| 43 | 100 | p minus H |
| 44 | 48 | p |
| 57 | 37 | k |
| 58 | 25 | j |
| 59 | 79 | l |
| 70 | 26 | h minus 2 H |
| 74 | 20 | i |
| 85 | 58 | b,c |
| 96 | 16 | f,g |
| 112 | 27 | d,e |
| 170 | 69 | a |

**MGBG (I, $R^1 = CH_3$, $R^2 = H$), 170°C**

| m/e | I | Interpretation |
|---|---|---|
| 42 | 25 | p minus two H |
| 43 | 100 | p minus H |
| 44 | 44 | p |
| 57 | 70 | k |
| 58 | 24 | j |
| 59 | 70 | l |
| 74 | 20 | i |
| 84 | 45 | c minus one H |
| 85 | 23 | c |
| 99 | 60 | b |
| 111 | 7 | f or g, plus one H; or n |
| 126 | 32 | d,e |
| 127 | 20 | d or e, plus one H, or with one C-13 |
| 184 | 86 | a |

**EGBG (I, $R^1 = CH_2CH_3$, $R^2 = H$), 170°C**

| m/e | I | Interpretation |
|---|---|---|
| 41 | 11 | p minus three H |
| 42 | 12 | p minus two H |
| 43 | 48 | p minus H |
| 44 | 20 | p |
| 58 | 37 | j |
| 59 | 16 | l |
| 60 | 29 | m |
| 69 | 4 | |
| 74 | 11 | i |
| 85 | 14 | c |
| 98 | 23 | |
| 113 | 30 | b |
| 124 | 7 | f,g |
| 125 | 4 | f or g, plus one H |
| 140 | 100 | d,e |
| 141 | 12 | d or e, plus one H or with one C-13 |
| 198 | 39 | a |

## TABLE 2.

### The Electron-Impact Mass Spectra of DMGBG, EMGBG and BMGBG.

I = Intensity of the peak, given in % of the intensity of the highest peak.

**DMGBG (I, $R^1 = R^2 = CH_3$), 180°C**

| m/e | I | Interpretation |
|---|---|---|
| 43 | 48 | p minus H |
| 44 | 21 | p |
| 57 | 41 | k |
| 59 | 11 | l |
| 60 | 11 | m |
| 68 | 11 | |
| 72 | 12 | h |
| 74 | 11 | i |
| 98 | 42 | b or c, minus one H |
| 99 | 22 | b,c |
| 124 | 13 | f,g |
| 125 | 14 | f or g, plus one H; or n,o |
| 140 | 100 | d,e |
| 141 | 13 | d or e, plus one H or with one C-13 |
| 198 | 31 | a |

**EMGBG (I, $R^1 = CH_2CH_3$, $R^2 = CH_3$), 160°C**

| m/e | I | Interpretation |
|---|---|---|
| 43 | 34 | p minus H |
| 44 | 19 | p |
| 57 | 16 | k |
| 58 | 21 | j |
| 59 | 11 | l |
| 60 | 12 | m |
| 67 | 11 | |
| 68 | 12 | |
| 86 | 11 | |
| 99 | 13 | c |
| 112 | 30 | b minus one H |
| 138 | 14 | f or g, minus 2 H |
| 139 | 12 | f or g , minus one H |
| 154 | 100 | d,e |
| 155 | 13 | d or e, plus one H or with one C-13 |
| 212 | 30 | a |

**BMGBG (I, $R^1 = CH_2CH_2CH_2CH_3$, $R^2 = CH_3$), 150°C**

| m/e | I | Interpretation |
|---|---|---|
| 41 | 14 | |
| 41 | 11 | |
| 43 | 30 | p minus H |
| 44 | 14 | p |
| 57 | 27 | k |
| 58 | 8 | j |
| 59 | 12 | l |
| 60 | 13 | m |
| 72 | 11 | h |
| 97 | 11 | c minus two H |
| 98 | 11 | c minus one H |
| 99 | 13 | c |
| 110 | 11 | |
| 124 | 20 | n minus one H |
| 140 | 31 | b minus one H |
| 141 | 12 | b |
| 167 | 11 | f or g, plus one H |
| 182 | 100 | d,e |
| 183 | 17 | d or e, plus one H or with one C-13 |
| 240 | 28 | a |

## TABLE 3.

### The Electron-Impact Mass Spectra of MPGBG and DEGBG.

I = Intensity of the peak, given in % of the intensity of the highest peak.

**MPGBG (I, $R^1 = CH_2CH_2CH_3$, $R^2 = CH_3$), 120°C**

| m/e | I | Interpretation |
|---|---|---|
| 43 | 28 | p minus H |
| 44 | 13 | p |
| 55 | 9 | k minus two H |
| 58 | 38 | j |
| 59 | 10 | l |
| 60 | 12 | m |
| 72 | 9 | i |
| 83 | 6 | |
| 98 | 9 | c minus one H |
| 100 | 9 | c plus one H, or with one C-13 |
| 124 | 9 | n minus one H |
| 126 | 32 | b minus one H |
| 127 | 11 | b |
| 151 | 9 | f or g, minus one H |
| 152 | 9 | f,g |
| 153 | 10 | f or g, plus one H or with one C-13; or o |
| 168 | 100 | d,e |

**MPGBG, 120°C, continued**

| m/e | I | Interpretation |
|---|---|---|
| 169 | 14 | d or e, plus one H or with one C-13 |
| 226 | 34 | a |

**MPGBG, 130°C**

| m/e | I | Interpretation |
|---|---|---|
| 41 | 18 | p minus three H |
| 43 | 34 | p minus H |
| 44 | 20 | p |
| 58 | 27 | j |
| 126 | 35 | b minus one H; or n plus one H |
| 168 | 100 | d,e |
| 169 | 19 | d or e, plus one H or with one C-13 |
| 226 | 34 | a |

**DEGBG (I, $R^1 = R^2 = CH_2CH_3$), 130°C**

| m/e | I | Interpretation |
|---|---|---|
| 43 | 27 | p minus H |
| 44 | 15 | p |
| 56 | 12 | $CN_3H_2^+$ |
| 58 | 34 | j |
| 59 | 10 | l |
| 60 | 10 | m |
| 67 | 13 | |
| 69 | 10 | |
| 86 | 16 | |
| 113 | 14 | b,c |
| 126 | 22 | |
| 152 | 12 | f,g |
| 168 | 100 | d,e |
| 169 | 14 | d or e, plus one H or with one C-13 |
| 226 | 27 | a |

**TABLE 4.**

**The Electron-Impact Mass Spectrum of DPGBG (I, $R^1 = R^2 = CH_2CH_2CH_3$).**

I = Intensity of the peak, given in % of the intensity of the highest peak. Temperature: 140°C.

| m/e | I | Interpretation | m/e | I | Interpretation |
|---|---|---|---|---|---|
| 43 | 57 | p minus H | 154 | 53 | n or o, plus one H |
| 44 | 26 | p | | | |
| 54 | 13 | $CN_3^+$ | 180 | 17 | f,g |
| 57 | 82 | k | 181 | 13 | f or g, plus one H or with one C-13 |
| 58 | 26 | j | | | |
| 59 | 25 | l | | | |
| 70 | 15 | h minus two H | 196 | 100 | d,e |
| 83 | 15 | | 197 | 36 | d or e, plus one H or with one C-13 |
| 100 | 30 | | | | |
| 111 | 17 | | | | |
| 127 | 30 | b,c | 254 | 67 | a |
| 152 | 18 | n or o, minus one H | 255 | 13 | M+1 (one C-13) |



An intramolecular cyclization reaction, giving (f) and (g) as well as the aminoguanidine type ions (h) and (i) as the products, may perhaps also occur, since this postulated route can easily explain the formation of several ions whose formation is difficult to explain otherwise, and would indeed give a six-membered ring structure with considerable resonance stabilization. The verification of this putative route, however, requires further studies.

In some cases, some minor peaks observed are most easily explained by assuming the formation of the radical ions (n) and (o), possibly by loss of one of the alkyl side chains from (d) and (e).

A typical property of all of the spectra was the presence of peaks with an m/e ratio of 44, 43, 42, and/or 41, these peaks being

most probably due to fragments formed from the terminal guanidino groups of the molecule [(p) and deprotonated forms thereof].

The above results indicate that useful structural information may be derived from the electron-impact mass spectra of bis(amidinohydrazones) in spite of the relatively high melting points of the compounds. This result is of obvious practical value since the development of different kinds of novel analogs and derivatives of GBG and MGBG is currently actively ongoing in various laboratories. The extensive possibilities for resonance stabilization of the degradation products may well be an important factor that facilitates the detection of the fragments observed.



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