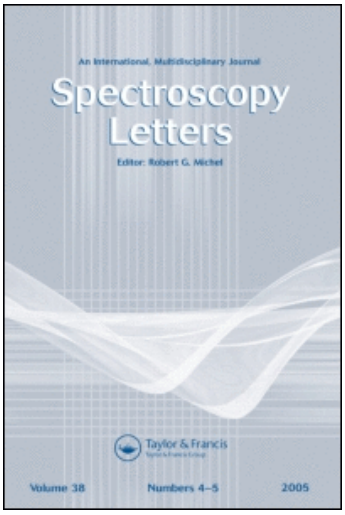

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Studies on the Spin-Lattice Relaxation Times of the Carbons of the Investigational Antileukemic Drug and Enzyme Inhibitor Methylglyoxal *Bis*(Amidino-Hydrazone) ['Methylglyoxal *BIS*(Guanylhydrazone)'] and Its Dialkylglyoxal Analogs

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STUDIES ON THE SPIN-LATTICE RELAXATION TIMES OF THE CARBONS OF THE INVESTIGATIONAL ANTILEUKEMIC DRUG AND ENZYME INHIBITOR METHYLGLYOXAL BIS(AMIDINO-HYDRAZONE) ['METHYLGLYOXAL BIS(GUANYLHYDRAZONE)'] AND ITS DIALKYLGLYOXAL ANALOGS

Key Words: Adenosylmethionine Decarboxylase Inhibitors, Antitumor Agents, Assignment of Carbon Resonances, Carbon NMR Spectroscopy, Enzyme Inhibitors, Polyamine Antimetabolites

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ABSTRACT

The first study on the ^{13}C relaxation times of bis(amidinohydrazones) is reported. The spin-lattice relaxation times (T_1) of the carbons of the free bases of methylglyoxal

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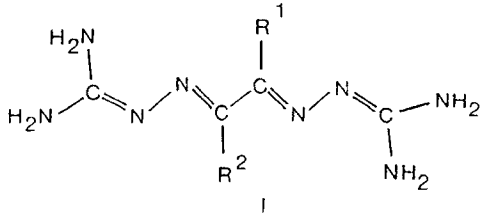
bis(amidinohydrazone) (MGBG) and of four dialkylglyoxal analogs thereof were determined with the aid of the inversion recovery method and using dimethyl sulfoxide as the solvent. In the series of compounds studied, one of the side chains was always a methyl group, while the other one was altered (hydrogen, methyl, ethyl, propyl, butyl). Remarkable differences were found to exist between the T_1 values of the various carbons within each molecule. The T_1 values were in the range 1.5 - 2 s for methyl carbons, 0.16 - 1.9 s for carbons of longer alkyl groups, 4.3 - 7.0 s for unprotonated carbons of the glyoxal moiety, 0.57 s for the protonated glyoxal carbon of MGBG, and 2.6 - 3.1 s for guanidino carbons. The bulk of the differences are explainable by assuming that the major relaxation mechanism for the protonated carbons is dipolar relaxation. In alkyl side chains, the T_1 values increased in a very regular fashion down the chain. This effect made possible the assignment of two previously unassigned carbon resonances of the butyl group of BMGBG. T_1 studies thus offer a facile and reliable method for the assignment of side-chain carbon resonances of bis(amidinohydrazones). Further, T_1 measurements were found to offer a very good method for the individual assignment of the glyoxal carbons of unsymmetrical congeners, whose assignment has so far constituted a problem. The method, based on the finding that the one of the carbons bonded to the shorter alkyl chain has a longer relaxation time than does the other one, made possible the unambiguous assignment of several previously unassigned carbon resonances. The results obtained also offer a reliable method for unambiguously distinguishing between the resonances of glyoxal carbons and guanidino carbons that have been difficult to distinguish from each other because the separation of their chemical shifts is often extremely small. Correlations observed between T_1 values and the degree of alkyl substitution in the molecule are discussed, as are also possible relaxation mechanisms. Somewhat unexpectedly, the results obtained suggest that dipolar relaxation through the hydrogens of neighboring carbon atoms may to a significant extent contribute to the relaxation of some unprotonated carbons in bis(amidinohydrazones).

INTRODUCTION

The bis(amidinohydrazones) (I) of various glyoxals have been subject to a great number of biochemical as well as pharmacological studies in recent years, since two of them (GBG, I: $R^1 = R^2 = H$, and MGBG, I: $R^1 = CH_3$, $R^2 = H$) are potent antileukemic agents and since several bis(amidinohydrazones) are also highly potent specific inhibitors of adenosylmethionine decarboxylase, a key enzyme of polyamine biosynthesis[1-9]. (For nomenclature of the compounds and for an explanation of the abbreviation system used, see footnote[10].) In spite of the intensive efforts, neither the mode of action of GBG and MGBG nor the reasons for the unusually strict structural requirements for antileukemic activity among this class of compounds have been discovered. Therefore, the elucidation of the chemical and physical properties of these agents has quite recently become a topic of great interest, since a better understanding of these properties may be the only way to an understanding of the behavior of the various congeners in biological systems. This approach has already given some very interesting results[4,6-8,11-16], such as the discovery of a close correlation between the acid-base properties of various bis(amidinohydrazones) and the antileukemic potency of the agents[4,11]. NMR studies[4,13-16] have also had a central role in the elucidation of the crucial chemical and physical properties of GBG, MGBG and their analogs, being of especially high value in studies on the isomerism and tautomerism of the compounds in solution[4,13-14]. An interesting correlation has also been discovered between the inhibitor



I

constants for adenosylmethionine decarboxylase and the mean of the chemical shifts of the two carbon atoms of the glyoxal moiety of the bis(amidinohydrazone)-type inhibitors[4]. During these studies, it has become increasingly evident that further chemical and spectroscopic studies on bis(amidinohydrazones) are highly warranted, NMR studies being of especially great interest.

In the NMR field, two topics are of special interest: the study of molecular motion in solution, and the development of novel methods for the assignment of the still unassigned resonances of some parts of bis(amidinohydrazones). Molecular motion may have considerable biochemical importance e.g. for binding of the molecules to enzymes or various receptor molecules, as well as for the ability of the molecules to penetrate cell membranes and other lipid-containing structures. Studies on the relaxation of the carbons and protons of the molecules might give valuable insights into the molecular motion of the compounds in solution, being actually the most promising method available for the study of this topic.

We report now the first study on the relaxation times of the carbons of bis(amidinohydrazones). The free bases of five compounds, including MGBG, have been studied and the spin-lattice relaxation times of all of their carbons have been determined. The results obtained indicate that studies on spin-lattice relaxation times offer a practical and reliable method for the assignment of the carbon resonances of bis(amidinohydrazones), being in some cases superior to other methods and making possible the assignment of some carbon signals, for which no methods except the incredible natural abundance double quantum transfer experiment (INADEQUATE) have been available.

EXPERIMENTAL

The bis(amidinohydrazones) studied are shown in Table 1 under 'Results and Discussion'. The substituents given in the table refer to

TABLE 1.

Spin-lattice Relaxation Times (T_1) of the ^{13}C Nuclei of the Free Bases of Various Bis(amidinohydrazones) in DMSO Solution.

Compound	Substituents		Spin-Lattice Relaxation Times (T_1) (s)								
			Side-Chain Carbons[a]				Carbons of the Glyoxal Moiety			Carbons of the Guanidino Groups[b]	
	R^1	R^2	1	2	3	4	That bound to H	Others[b]			
MGBG	CH_3	H	1.87	-	-	-	0.57	6.38		3.07	3.07
DMGBG	CH_3	CH_3	1.98	-	-	-	-	7.01		3.04	
EMGBG	CH_3	CH_3CH_2	0.17[d] h[c]	h	-	-	-	5.04	6.54	2.91	2.91
MPGBG	CH_3	$CH_3CH_2CH_2$	0.18[e] 1.54[c]	0.35	1.25	-	-	4.52	6.03	2.83	2.87
BMGBG	CH_3	$CH_3(CH_2)_3$	0.16[f] 1.51[c]	0.35[g]	0.81[g]	1.91	-	4.31	5.68	2.67	2.60

[a]Numbering begins from the carbon(s) directly bound to the glyoxal moiety. [b]The value given first is the relaxation time of that carbon, whose resonance occurs more downfield. [c]Methyl group. [d]Ethyl group. [e]Propyl group. [f]Butyl group. [g]Assigned on the basis of the present results. [h]The methyl and ethyl carbons have the same chemical shift. Their resonances and relaxations could not be separated. The 'T_1' value of the total intensity of the peak was 1.12 s.

the general structural formula (I). All of the compounds studied were in the free base form. The syntheses of the free bases of MGBG, DMGBG, EMGBG, and BMGBG were carried out according to previously published procedures[4]. The preparation of MPGBG free base will be described elsewhere.

NMR spectra were recorded with the aid of a 199.5 MHz/50.1 MHz (4.7 T) JEOL FX-200 FT NMR spectrometer using a dual $^{1}H/^{13}C$ probe. The tubes (diameter 10 mm) were rotated (20 Hz).

Spin-lattice relaxation times were measured by the inversion recovery method using the pulse sequence 180^{o} - τ - 90^{o}. The pulse delay time was 22 s. FID was recorded for 1.5 s. For each spectrum, the number of scans was 300. Peak heights were used as a measure of peak intensities.

The bis(amidinohydrazones) were dissolved in undeuterated synthetic grade dimethyl sulfoxide (DMSO) (E. Merck, Darmstadt, Germany) so that the amount of the compound was 40 - 50 mg, the volume of the solvent being 3 ml. Because of the low solubility of some compounds, higher concentrations could not be used. Each time, dissolution was performed at room temperature about 6 h before use. The solutions were not degassed. In order to achieve constant temperature, the tubes were kept in the probe for 30 min before starting the measurements. The temperature of the probe was $28^{o}C$.

RESULTS AND DISCUSSION

The bis(amidinohydrazones) studied were selected so that all of them contained one methyl group as a side chain (i.e., R^1 is methyl in all cases), while the other side chain R^2 was varied from hydrogen through methyl, ethyl and propyl to butyl. The ^{13}C spin-lattice relation times (T_1) of the various carbons of the compounds studied are shown in Table 1.

In all of the compounds studied, there are remarkably large differences between the spin-lattice relaxation times of the various carbons. As would be expected, the T_1 values of the carbons directly bound to one or more hydrogen atoms are distinctly smaller (0.16 - 1.98 s) than are those of the other carbons (2.60 - 7.01 s), indicating that dipolar relaxation indeed is the dominant relaxation mechanism for all bis(amidinohydrazone) carbons directly bonded to one or more hydrogen atoms. The relaxation of the carbons of the glyoxal moiety is in most cases especially slow (T_1 4.31 - 7.01 s). An exception is, however, constituted by that one of the glyoxal carbons of MGBG that is directly bonded to a hydrogen atom, its T_1 value being 0.57 s only.

As concerns the carbons of the guanidino groups, some interesting phenomena can be observed. All of the guanidino carbons appear to relax with approximately equal rates, their T_1 values falling within a range as narrow as 2.60 - 3.07 s. Thus, the differences between the various congeners are very small. Even more notably, the differences between the opposite ends of unsymmetrical (R^1 different from R^2) compounds are still smaller, both of the guanidino carbons apparently having nearly identical spin-lattice relaxation times even in those bis(amidinohydrazones) where the alkyl side chains are remarkably different (e.g. in the butylmethylglyoxal derivative BMGBG), suggesting that the opposite ends of the bis(amidinohydrazone) chain probably move in solution with essentially the same rate.

Although the differences between the T_1 values of the guanidino carbons of the different congeners are very small, a distinct tendency can yet be observed. Thus, the more highly substituted the bis(amidinohydrazone) is, the shorter is the spin-lattice relaxation time, relaxation rates thus increasing with increasing substitution. This phenomenon might be due to a less rapid movement of the more highly substituted molecules, giving rise to an increase of the effectiveness of the dipolar relaxation mechanism. This interpretation

is, however, questionable since the guanidino carbons do not bear any directly bonded hydrogen atoms. If spin-rotation relaxation were the dominant relaxation mechanism of the carbons of the guanidino groups, the observed tendency would obviously be very difficult to explain, since in that case the compounds bearing the largest substituents and thus obviously moving least rapidly in solution should have least effective relaxation and thus the longest relaxation times, which is not the case. If quadrupolar relaxation is the most important relaxation mechanism of the guanidino carbons, as might be expected, the reasons for the observed tendency of T_1 shortening on increasing substitution may simply result from a small contribution from dipolar relaxation. Further studies using ^{15}N labelled derivatives might shed further light on this topic.

In any case, it appears probable that, as the carbons of the guanidino groups are concerned, the chemical properties of these groups as well as the environment in which they are in solution probably are very similar for all of the compounds studied. Thus, it is tempting to speculate that the differences between the relaxation times of the guanidino carbons of the various congeners either must be due to differences in the ability of the molecule to move in solution or to differences between the electron densities at the carbon atoms, the latter differences being suggested by the distinctly different pK_a values of the compounds[11] but not being supported by the finding that the chemical shifts of the guanidino carbons of the various congeners differ very little from each other[14].

One notable though not unexpected feature of the present results is constituted by the finding that the spin-lattice relaxation times of the carbons of the alkyl side chain (R^1 or R^2 in formula I) depend remarkably clearly on the position of the carbon atom in the side chain as well as on the total length of the side chain (see Table 1). The addition of one more carbon to the end of a side chain is enough to drastically decrease the T_1 value(s) of the pre-existing side-chain carbon(s). This effect is especially evident when

a one-carbon side chain is lengthened by addition of one or more carbons. Thus, in MGBG and DMGBG, i.e. the two compounds, neither of which contains longer than one-carbon side chains, the T_1 values of the ^{13}C nuclei of the side chains are 1.87 and 1.98 s, respectively. In the ethyl, propyl, and butyl side chains of EMGBG, MPGBG, and BMGBG, however, the spin-lattice relaxation time of the first carbon atom of the side chain is only 0.16 - 0.18 s. Very probably, this effect results from a decrease of the motion of the first side-chain carbon and the consequent increase of the effectiveness of the dipolar relaxation mechanism. (Numbering of the side-chain carbons begins from the one directly bonded to the glyoxal moiety.) When going down a side chain, the relaxation times of the carbons increase, obviously because of increasing rotational freedom (segmental motion). This aspect is illustrated graphically in Fig. 1.

When going from the dimethyl compound DMGBG through the methylpropyl analog MPGBG to BMGBG, the T_1 value of the ^{13}C nucleus of the methyl side chain decreases, obviously because the increase of the size of the other alkyl side chain leads to a decrease of the motion of the methyl group, too, and thus increases the effectiveness of dipolar relaxation. In contrast, when going from MGBG, in which the other 'side chain' is a hydrogen, to DMGBG, the T_1 value of the methyl group(s) increases. This phenomenon may suggest that the relaxation of the ^{13}C nucleus of the methyl group occurs through dipolar relaxation involving not only the protons bonded directly to that carbon but also, to a small extent, involving even the proton bonded to the next carbon atom (i.e., the glyoxal carbon). Another possible explanation for the observed phenomenon might perhaps comprise enhanced binding (as compared to DMGBG) of the MGBG molecule to solvent molecules by virtue of an interaction of the glyoxal proton with the solvent. [In this connection, it is worth noticing that the glyoxal proton is deshielded to such an extent (δ = 7.771 ppm versus 1,4-dioxane = 3.700 ppm) that it may perhaps be 'nearly acidic'.]

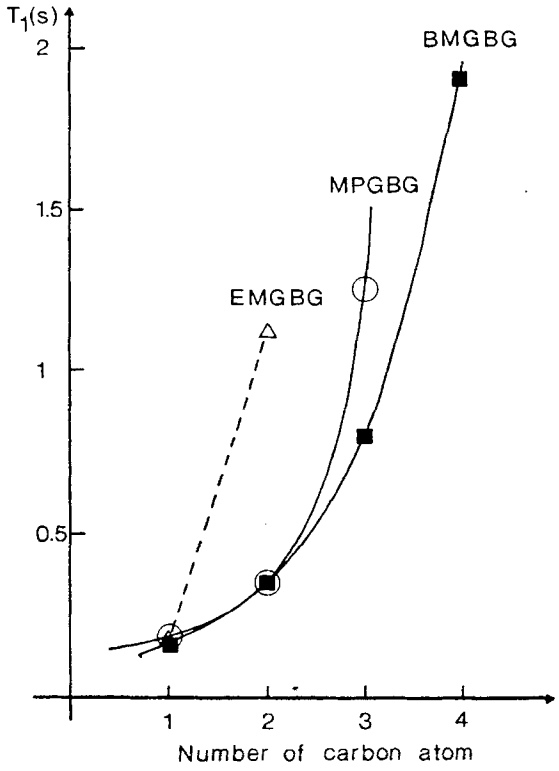


Fig. 1. Spin-lattice relaxation times of the side-chain carbon atoms of EMGBG, MPGBG, and BMGBG, shown as a function of the position of the atom in the side chain. Numbering of the carbon atoms begins from the one directly bonded to the glyoxal moiety of the molecule.

On the basis of the results obtained, one important conclusion can obviously be drawn concerning the assignment of the resonances of the carbons of the side chains of bis(amidinohydrazones). As can be seen from Fig. 1, the T_1 values of the carbons of the propyl side chain of MPGBG increase in a very regular fashion when going down the propyl chain. A similar effect would be expected also in the case of the butyl side chain of BMGBG, for which the resonances of carbon atoms 2 and 3 have so far not been assigned. Indeed, a similar

curve can be constructed also for the butyl side chain of BMGBG, if it is assumed that the further away from the bis(amidinohydrazone) chain a carbon atom is located in the alkyl side chain, the longer is its spin-lattice relaxation time. On this basis, it appears highly probable that carbon atom number 2 of the butyl side chain gives rise to that one of the carbon resonances of BMGBG, whose chemical shift in DMSO solution is 30.23 ppm (as measured against 1,4-dioxane, whose carbons are considered to resonate at 67.40 ppm), while carbon atom number 3 gives rise to the resonance at 23.87 ppm. (For details of the ^{13}C NMR spectrum of BMGBG, see[14,16].) The opposite assignment of carbons 2 and 3 would, in contrast, indicate that the relaxation times of the carbons of the butyl group would depend on their position in the chain in a totally irregular way that would be very difficult to explain. So, the measurement of spin-lattice relaxation times appears to be an effective tool for the assignment of side-chain carbon resonances of bis(amidinohydrazones).

The above assignment of the carbons of the butyl group of BMGBG is supported also by the results of our recent studies[17] on the relaxation times of the protons of BMGBG and other bis(amidinohydrazones).

The above method for the assignment of the resonances of the side-chain carbons of bis(amidinohydrazones) may find more general application, since it is relatively simple and does not require the availability of 2-D NMR facilities. Further, it is applicable also in those cases, where proton spectra are not first order or contain overlapping multiplets impossible to resolve and where assignment of carbon resonances with the aid of proton spectra and heteronuclear shift correlation is thus impossible. The experiences of the present authors indicate that the T_1 method is also superior to selective heteronuclear proton decoupling[15] in the assignment of bis(amidinohydrazone) side-chain resonances.

After completion of the present study, incredible natural abundance double quantum transfer experiments (INADEQUATE) have

been carried out that offer a rigorous verification of the present assignment of the carbon resonances of BMGBG (H. Elo and A. Pagelot, manuscript in preparation).

As is evident from Table I, the two glyoxal carbons of MGBG have remarkably different spin-lattice relaxation times, the proton-bound one relaxing far more rapidly. This not unexpected finding offers a means for the assignment of proton-bound and unprotonated glyoxal carbons, although the practical significance of this method may be limited, if methods such as DEPT or (possibly) heteronuclear shift correlation are available. Yet, a very rapid assignment may be possible without a full T_1 determination by simply recording the carbon spectrum of the appropriate monoalkylglyoxal bis(amidinohydrazone) using two or three suitably selected pulse delay times. In any case, assignment of proton-bound and unprotonated glyoxal carbons of bis(amidinohydrazones) with the aid of T_1 measurements is far more practical than the use of off-resonance proton-noise decoupled carbon NMR, especially as the separation of the chemical shifts of the two glyoxal carbons is often very small[14], making interpretation of off-resonance spectra difficult.

The present results also indicate that, in all of the unsymmetrical (R^1 different from R^2) bis(amidinohydrazones) now studied, the T_1 values of the two glyoxal carbons are distinctly different even in those cases, where neither carbon is directly bonded to a proton. One of the present authors has recently assigned the resonances of the glyoxal carbons of ethylmethylglyoxal bis(amidinohydrazone) (EMGBG) with the aid of a calculation method based on additivity of substituent effects and has shown that the one bearing the longer side chain (i.e., the ethyl group) is the one resonating downfield from the other one[18]. The results obtained also suggest that it may be a general rule that the glyoxal carbon bearing the longer side chain resonates more downfield than does the other one[18]. Combined with the present T_1 data, those results indicate that the one of the glyoxal carbons of EMGBG that relaxes

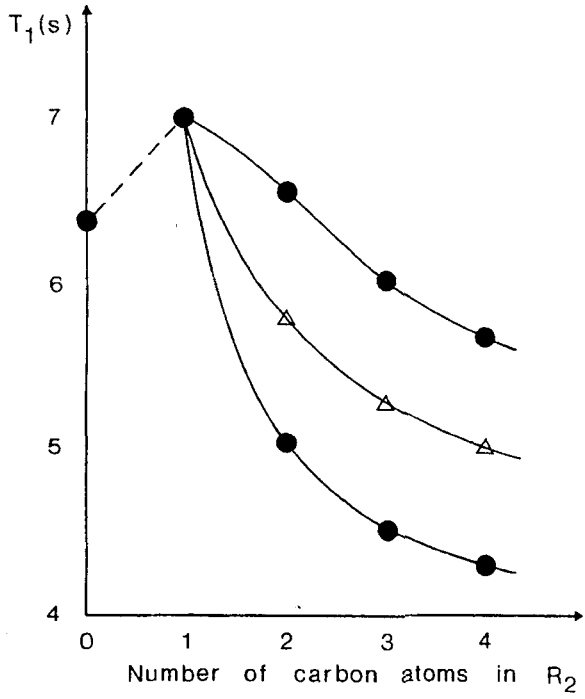


Fig. 2. The spin-lattice relaxation times of the carbons of the glyoxal moiety, shown as a function of the number of carbon atoms in side chain R^2. The relaxation time of the higher-field as well as the lower-field glyoxal carbon is given for each compound except MGBG, for which only the value of the unprotonated carbon is shown. The means of the higher-field and lower-field values are also shown.

more rapidly is the one that bears the ethyl group. In Fig. 2, the T_1 values of the glyoxal carbons of all of the compounds now studied are shown as a function of the length of the side chain. The figure strongly suggests that it is a general rule that the glyoxal carbon bearing the methyl group relaxes more slowly than the one bearing the longer alkyl side chain. Thus, it is possible to individually assign the resonances of both glyoxal carbon atoms of all of the unsymmetrical compounds now studied on the basis of T_1 data alone, i.e. it is possible to distinguish between the resonance of the glyoxal

carbon bonded to the shorter alkyl side chain and the carbon bonded to the longer alkyl side chain. For this purpose, only the above mentioned calculation method[18] has been available. So, the possibility to perform or confirm assignments with the aid T_1 measurements is a considerable advance. [In principle, it should be possible to assign the glyoxal carbons individually also with the aid of INADEQUATE, but no reports have appeared on the application of this method to the assignment of bis(amidinohydrazone) carbons.]

Why does the glyoxal carbon bearing the longer alkyl group relax more slowly than does its counterpart bearing the shorter chain? One possible explanation is constituted by the possibility that the relaxation of the glyoxal carbons to some extent occurs by the dipolar relaxation mechanism, in spite of the lack of directly bonded hydrogen atoms. If this is the case, the relaxation should become more efficient when the motion of the glyoxal carbon is decreased on increasing the length of the alkyl group. It is evident from Fig. 2 that the T_1 values of both glyoxal carbons decrease to a considerable extent when one of the side chains becomes longer. Also this finding is well in accord with and supports the theory that the dipolar mechanism plays a significant role in the relaxation of the glyoxal carbons even if they lack directly bonded hydrogens. This theory is further advocated by the finding (see Table 1 and Fig. 2) that both glyoxal carbons of MGBG (one of which is protonated) relax more rapidly than do those of DMGBG. The unprotonated glyoxal carbon of MGBG has two neighboring carbons bearing hydrogen(s) and should thus indeed experience a greater contribution from dipolar relaxation than do the glyoxal carbons of DMGBG. Studies on deuterated and ^{15}N labelled derivatives might shed more light on this topic.

One further result that may be of considerable significance for resonance assignment is constituted by the discovery that, for all of the bis(amidinohydrazones) now studied, the carbons of the guanidino groups relax distinctly more rapidly (T_1 2.60 - 3.07 s) than do the

non-proton-bound carbons of the glyoxal moiety (T_1 4.31 - 7.01). Because the separation of the chemical shifts of the resonances of these two types of carbons in bis(amidinohydrazones) is sometimes very small, there may be considerable difficulty in determining, whether a resonance actually results from a guanidino carbon or from a glyoxal carbon. Thus, for example, the non-proton-bound glyoxal carbon of ethylglyoxal bis(amidinohydrazone) free base in D_2O solution resonates only 0.44 ppm upfield from the resonance of one of the guanidino carbons of the compound[14]. In the case of MPGBG free base, dissolved in D_2O, a separation of 0.53 ppm is likewise observed. Since neither of the carbons are directly bonded to protons, it is not possible to distinguish between them with the aid of DEPT, off-resonance proton-noise decoupled carbon spectra, selective proton decoupling, or proton-carbon heteronuclear shift correlation. The distinct difference between the T_1 values of the two types of carbons, in contrast, offers a facile and obviously very reliable method for distinguishing between the two types. In this connection, it is also worth noticing that the spin-lattice relaxation times of the guanidino carbons of the compounds now studied fall within a very narrow range, this further facilitating a reliable assignment of these carbons. Further studies are needed to find out, whether the T_1 values of the guanidino carbons of more remote congeners of the present bis(amidinohydrazones) [e.g., bis(amidinohydrazones) of cyclic 1,2-diketones] also fall within or near the very narrow range now observed for guanidino carbons. If this is the case, studies on the NMR spectroscopy of such compounds will be much simplified.

In the light of the present results, it is worth remembering that, in any quantitative work on bis(amidinohydrazones), and also when novel analogs of these compounds are studied, fairly long pulse delay times may be necessary.

REFERENCES

1. Alhonen-Hongisto, L., Seppänen, P., Nikula, P., Elo, H., Jänne, J. Structure-Activity Relationship of Bis(guanylhydrazones). Recent Progress in Polyamine Research 1985; 261-270, and references therein.

2. Williams-Ashman, H.G., Seidenfeld, J. Aspects of the Biochemical Pharmacology of Methylglyoxal Bisguanylhydrazone. Biochem. Pharmacol. 1986; 35:1217.

3. Jänne, J., Alhonen-Hongisto, L., Nikula, P., Elo, H. S-Adenosylmethionine Decarboxylase as Target of Chemotherapy. Adv. Enz. Regul. 1986; 24: 125, and references therein.

4. Elo, H. *Bis(amidinohydrazones) ['Bis(guanylhydrazones)'] as Antineoplastic Agents. Chemical and Biochemical Studies*, Ph.D. Thesis, Department of Biochemistry, University of Helsinki, Helsinki, Finland, 1989, and references therein.

5. Elo, H., Laine, R., Alhonen-Hongisto, L., Jänne, J., Mutikainen, I., Lumme, P. Biochemical Characterization of Propylglyoxal Bis(guanylhydrazone). Facile Synthesis of Monoalkylglyoxal Bis-(guanylhydrazones). Z. Naturforsch. 1985; 40c: 839.

6. Elo, H., Mutikainen, I., Alhonen-Hongisto, L, Laine, R., Jänne, J., Lumme, P. Biochemical Properties and Crystal Structure of Ethylmethylglyoxal Bis(guanylhydrazone) Sulfate - an Extremely Powerful Novel Inhibitor of Adenosylmethionine Decarboxylase. Z. Naturforsch. 1986; 41c: 851.

7. Elo, H., Mutikainen I., Alhonen-Hongisto L., Laine R., and Jänne J. Diethylglyoxal Bis(guanylhydrazone): a Novel Highly Potent Inhibitor of S-Adenosylmethionine Decarboxylase with Promising Properties for Potential Chemotherapeutic use. Cancer Lett. 1988; 41: 21.

8. Elo, H., Mutikainen, I. Biochemical and Chemical Characterization of Trifluoromethylglyoxal Bis(guanylhydrazone), a Close Analog of the Antileukemic Drug Mitoguazone. Z. Naturforsch. 1988; 43c: 601.

9. Elo, H. Adenosylmethionine Decarboxylase Inhibitors - Lack of Activity Against Cytopathic Effects of HIV. Chemotherapy 1990; 36: 373.

10. Bis(amidinohydrazones) are commonly called 'bis(guanylhydrazones)'. Therefore, their established abbreviations contain the letters GBG, preceded by an indication of the substituents (e.g., DE for diethyl or EM for ethyl methyl). The term 'bis(amidino-

hydrazone)' is, however, more appropriate. The Chemical Abstracts' systematic name for methylglyoxal bis(amidinohydrazone) is 2,2'-(1-methyl-1,2-ethanediylidene)bis(hydrazinecarboximidamide), and other congeners are named analogously.

11. Elo, H.O., Tilus, P.T.E., Mutikainen, I.P., Heikkinen, I., Riekkola, M.-L. Remarkable Differences Between the Species Distributions of Various Bis(guanylhydrazones) at Physiological Conditions, and Their Possible Involvement in the Strict Structural Requirements for Antileukemic Activity. Anti-Cancer Drug Des. 1989; 4: 303.

12. Lumme, P.O., Mutikainen, I., Elo, H.O. Structure of Propylglyoxal Bis(amidinohydrazone) Sulfate Dihydrate. Acta Cryst. 1986; C42: 1209.

13. Elo, H. Proton Nuclear Magnetic Resonance Spectroscopy of Bis(amidinohydrazones) (Bis(guanylhydrazones)), and Its Use for Studies on the Isomerism and Tautomerism of the Compounds. Spectroscopy Letters 1989; 22: 123.

14. Elo, H. Carbon-13 NMR Spectroscopy of the Antileukemic Drug MGBG and Related Bis(amidinohydrazones) ['Bis(guanylhydrazones)']. Spectroscopy Letters 1989; 22: 161.

15. Elo, H., Soljamo K. Unambiguous Assignment of the ^{13}C NMR Resonances of the Side-Chain Carbon Atoms of Dipropylglyoxal Bis(amidinohydrazone) by DEPT and Selective Heteronuclear Proton Decoupling Techniques. Spectroscopy Lett. 1989; 22: 1141.

16. Elo, H. 2D-NMR Studies on Bis(amidinohydrazones). I. A Proton-Carbon Heteronuclear Shift Correlation Study on the Enzyme Inhibitors Methylpropylglyoxal Bis(amidinohydrazone) and Butylmethylglyoxal Bis(amidinohydrazone). Spectroscopy Lett. 1990; 23: 877.

17. Elo, H., Soljamo, K. Manuscript to be submitted for publication.

18. Elo, H. Manuscript to be submitted for publication.

19. Mutikainen, I., Elo, H, Lumme, P. Crystal and molecular structure of glyoxal bis(amidinohydrazone) dihydrochloride; biochemical aspects. J. Chem. Soc., Perkin Trans. II 1986; 291.

20. Hamilton, W.C., La Placa, S.J. The Crystal and Molecular Structure of an Anti-Leukemia Drug: Methylglyoxal Bisguanylhydrazone Dihydrochloride Monohydrate, $C_5N_8H_{12}.2HCl.H_2O$. Neutron and X-Ray Diffraction Studies. Acta Cryst. 1968; B24: 1147.

21. Edmonds, J.W., Hamilton, W.C. The Crystal and Molecular Structure of Dimethylglyoxal Bisguanylhydrazone Dihydrochloride Dihydrate, $C_6H_{14}N_8.2HCl.2H_2O$. Acta Cryst. 1972; B28: 1362.

22. Mutikainen, I., Elo, H., Tilus, P. Manuscript in preparation.

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