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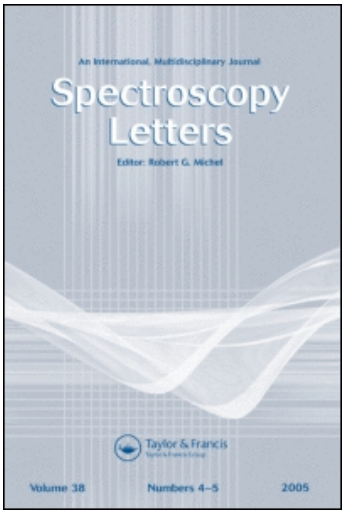

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Substituent Effects in the Carbon NMR of Aliphatic *bis*(Amidinohydrazones) ['*Bis*(Guanylhydrazones)']. Construction of a set of Quantitative Empirical Rules. Unambiguous Assignment of Several Previously Unassigned Carbon Resonances

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SUBSTITUENT EFFECTS IN THE CARBON NMR OF ALIPHATIC BIS(AMIDINOHYDRAZONES) ['BIS(GUANYLHYDRAZONES)']. CONSTRUCTION OF A SET OF QUANTITATIVE EMPIRICAL RULES. UNAMBIGUOUS ASSIGNMENT OF SEVERAL PREVIOUSLY UNASSIGNED CARBON RESONANCES

Key Words: Adenosylmethionine Decarboxylase Inhibitors, Antileukemic Agents, Assignment of Carbon Resonances, Carbon NMR Spectroscopy, Enzyme Inhibitors, Methylglyoxal Bis(guanylhydrazone), MGBG, Polyamine Antimetabolites, Substituent Parameters

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ABSTRACT

The effects of substituents on the ^{13}C chemical shifts of the various carbons of aliphatic 1,2-bis(amidinohydrazones) have been systematically studied using previously published experimental data as the basis. Mathematical formulae have been constructed that describe the effects of various structural features of the molecules on the chemical shifts of the carbons and that also make possible an accurate prediction of the spectra of compounds belonging to this class. It is

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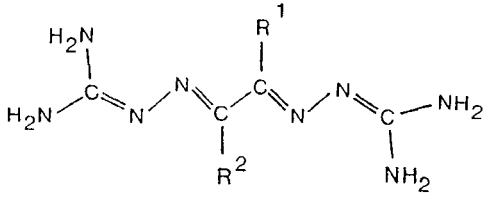
also shown that the effects of side chains on the chemical shifts of the two carbons of the glyoxal moiety are strictly additive. A mathematical model has been constructed that makes possible a very accurate prediction of the chemical shift of each one of the glyoxal carbons of symmetrical as well as unsymmetrical bis(amidinohydrazones). In the case of ethylmethylglyoxal bis(amidinohydrazone) free base dissolved in dimethyl sulfoxide, the theory predicts that the glyoxal carbons resonate at 157.45 ppm (the one connected to the ethyl group) and 151.21 ppm, while the experimental values are 157.30 and 151.29 ppm. This has, for the first time, made possible the unambiguous individual assignment of the resonances of the glyoxal carbons of unsymmetrical dialkylglyoxal bis(amidinohydrazones). The results also indicate that in all such compounds so far studied, that one of the glyoxal carbons that bears the longer alkyl side chain resonates more downfield than does the other one. This result is in total agreement with conclusions derived from relaxation time measurements.

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].



I

The biochemistry and pharmacology of these compounds and their congeners has been subject to intensive studies, but it has been impossible to determine the ultimate mechanism of the antitumor action of GBG and MGBG. Biologically oriented studies also have not been able to reveal the reasons for the finding that, among aliphatic congeners, only GBG and MGBG have antileukemic properties (for references, see[1-4]). Therefore, a couple of years ago, systematic physicochemical and spectral studies were initiated on this class of compounds in order to discover possible physicochemical reasons for the unusually strict structure-activity relationships among this group of agents. These studies, an integral part of which have been investigations on the NMR properties of the compounds, have already given very interesting and potentially important results[4,6-8,10-16]. For example, NMR studies, combined with crystallographic measurements, have indicated that there is no difference between the properties of GBG, MGBG and their inactive mono- and dialkylglyoxal analogs, as geometrical isomerism is concerned, and isomerism thus cannot lie behind the strict structure-activity relationships[4,6-8,10-14]. A striking correlation has also been discovered between the means of the chemical shift values of the two glyoxal carbons of bis(amidinohydrazones) and the ability of those compounds to inhibit adenosylmethionine decarboxylase[4].

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Because of their biochemical importance, further NMR studies on bis(amidinohydrazones) and their analogs are highly warranted. One further reason for continuous interest in the NMR of these agents is constituted by the fact that the chemistry and spectroscopy of these agents appears to be a challenging and nearly unexplored field, in which discoveries of chemical interest may await the investigator. All this has prompted me to re-examine previously published experimental data[4,13-15] on the carbon NMR of bis(amidinohydrazones) in order to construct mathematical formulae describing the effects of substituents. Such formulae were hoped, and have now been found, to be good tools that not only make possible an accurate prediction of chemical shifts in the spectra of

bis(amidinohydrazones) but also assist in explaining the origins of the effects of substituents. More importantly, they also make possible an unambiguous individual assignment of the previously unassigned resonances of the two glyoxal carbons of unsymmetrical bis(amidinohydrazones).

NOMENCLATURE OF BIS(AMIDINOHYDRAZONES). ABBREVIATIONS

The bis(amidinohydrazones) of various glyoxals (see formula I above) are commonly called 'bis(guanylhydrazones)'. Therefore, the commonly used abbreviations of their names contain the letters GBG (G for glyoxal, B for bis, and G for guanyl...), preceded by an indication of the substituents (e.g., DE for diethyl or EM for ethyl methyl). The term 'bis(amidinohydrazone)' is, however, more appropriate than the chemically incorrect 'bis(guanylhydrazone)'. 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.

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The carbon spectra of the following compounds have so far been reported and are re-examined here:

GBG: $R^1 = R^2 =$ hydrogen
MGBG: $R^1 =$ hydrogen, $R^2 =$ methyl (or vice versa)
EGBG: $R^1 =$ hydrogen, $R^2 =$ ethyl (or vice versa)
DMGBG: $R^1 = R^2 =$ methyl
EMGBG: $R^1 =$ methyl, $R^2 =$ ethyl (or vice versa)
DEGBG: $R^1 = R^2 =$ ethyl
MPGBG: $R^1 =$ methyl, $R^2 =$ propyl (or vice versa)
DPGBG: $R^1 = R^2 =$ propyl
BMGBG: $R^1 =$ methyl, $R^2 =$ butyl (or vice versa)

Since 1,4-dioxane has served as the internal standard in all studies on the NMR spectroscopy of aliphatic bis(amidinohydrazones), all chemical

shifts of the carbons of this class of compounds cited in this paper are given in ppm versus 1,4-dioxane, whose carbons are considered to resonate at 67.40 ppm.

CONSTRUCTION OF EMPIRICAL RULES FOR THE CALCULATION OF THE CHEMICAL SHIFTS OF SIDE-CHAIN CARBONS

Various equations have previously been constructed for the calculation of ^{13}C chemical shifts in a variety of different compound types such as alkanes and alkenes, according to which the chemical shift of a particular carbon in the chain is obtained by summing up specific parameters for carbons one, two, three etc. bonds apart, each parameter being multiplied by the number of such carbon atoms (for some examples, see[17]). Thus, it was of interest to try to construct such equations also for bis(amidinohydrazones).

First Carbon Atom in a Chain

Suppose that we divide the structure of any compound of formula I bearing at least one alkyl group as a substituent, into fragments **A**, **B**, **C**, and **D** shown below in formula II:

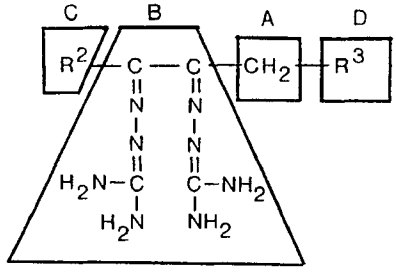

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TABLE 1.

Chemical Shifts of Carbon Atom(s) of the Methyl Side Chain(s) in Bis(amidinohydrazones) (I) Bearing at Least One Methyl Group.[a]

Compound	Solvent	δ Value of Methyl Carbon (ppm)
MGBG free base	DMSO	13.12
DMGBG free base	DMSO	13.24
EMGBG free base	DMSO	13.15
BMGBG free base	DMSO	13.17
mean of values	DMSO	**13.17**
MGBG free base	D_2O	12.30
MPGBG free base	D_2O	12.77
mean of values	D_2O	**12.54**
MGBG.2HCl	D_2O	11.28
DMGBG.2HCl	D_2O	11.81
mean of values	D_2O	**11.55**

[a]The values are taken from references 4, 13, and 15.

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All compounds of this structure can be considered as derivatives of MGBG (in which $R^2 = R^3 = H$). As is shown below, it is possible to calculate the δ value of the carbon in fragment **A** as a sum of additive effects:

(1) $$\delta(\mathbf{A}) = \mathbf{a} + \mathbf{b} + \mathbf{c} + \mathbf{d}$$

where parameters **a**, **b**, **c**, and **d** are the effects of fragments **A**, **B**, **C**, and **D**, respectively, the values of **c** and **d** being zero for the cases where R^2 and R^3, respectively, are hydrogen atoms.

Table 1 presents data on the effect of the other side chain (R^2) on the resonance of the methyl side 'chain' (R^1) for all methyl-substituted compounds II for which ^{13}C NMR spectra have been reported. Clearly, the other side chain has, taking the inaccuracy of measurements into account, almost zero effect on the resonance frequency of the methyl group, when the compounds are in the free

TABLE 2.

Chemical Shifts of First Carbon Atom(s) of the Ethyl or Propyl Side Chain(s) in Bis(amidinohydrazones) (I) Bearing at Least One Ethyl or Propyl Group.[a]

Compound	Solvent	δ Value of First Carbon of Ethyl Group (ppm)
EGBG free base	DMSO	19.60
EMGBG free base	DMSO	19.31
DEGBG free base	DMSO	19.25
mean of values	DMSO	**19.39**
EGBG free base	D_2O	19.49

Compound	Solvent	δ Value of First Carbon of Propyl Group (ppm)
DPGBG free base	DMSO	28.07
MPGBG free base	D_2O	28.27

[a]The values are taken from references 4, 13, 14, and 15.

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base form and are dissolved in dimethyl sulfoxide (DMSO). In the case of free bases or divalent salts dissolved in D_2O, the differences are slightly greater (ca. 0.5 ppm), but the number of congeners studied is too small for reliable conclusions to be drawn, and the discussion herein is restricted to the case of free bases in DMSO. In that case, parameter **c** thus has a value of essentially zero at least in those cases, where R^3 is hydrogen. A similar reasoning (see Table 2) indicates that **c** is essentially zero even if R^3 is an alkyl group. These results suggest that substituent effects, any gamma effects included, are not effectively mediated through the bis(amidinohydrazone) fragment **B**. This phenomenon is in striking contrast to the effects of carbons of fragment **D** equally many bonds removed that have a remarkable effect (several ppm) on the δ value of the carbon of fragment **A** (see below). Thus, fragment **B**, i.e. the moiety containing the conjugated C=N double bond system and the free electron pairs of the nitrogens, has an interesting 'isolating'

effect that prevents any substituent effect from being mediated from one side chain to the other, at least if the side chains are hydrogens or alkyl groups. This effect is seen in the case of divalent salts as well as in the case of free bases, although the bond system must be different in these two cases. Thus, the possibility must be considered that the 'isolating' property of fragment **B** is a result of the central fragment N=C-C=N. Whether **c** is essentially zero even for substituents other than alkyls (e.g., halogenated alkyls and aromatic groups) remains to be studied.

The effect of fragment **D** on the chemical shift of the carbon of fragment **A** (i.e., the effect of the lengthening of a side chain) is illustrated by the data in Tables 1 and 2. A comparison of the data in these tables indicates that the addition of further carbons to the chain has a prominent effect on the δ value of its first carbon atom. On the average, the effect of the addition of one more carbon to a methyl side chain increases the δ value of the first carbon by approximately (19.39 - 13.17) ppm = 6.22 ppm units. The addition of one further carbon again increases the value by approximately (28.07 - 19.39) ppm = 8.68 ppm. Thus, parameter d in equation (1) above is approximately 6.22 ppm for $R^3 = CH_3$ and 14.90 ppm for $R^3 = CH_2CH_3$.

The only bis(amidinohydrazone) with a butyl side chain that has so far been subject to NMR studies is BMGBG[4,12,13,15]. The previously unassigned carbon resonances of carbons 2 and 3 of the butyl group of BMGBG free base in DMSO solution have recently been assigned with the aid of four independent methods (measurement of ^{13}C relaxation times[18], measurement of ^{1}H relaxation times and combination of the results with previous 2D-NMR data[19], ^{1}H spectral simulation combined with 2D-NMR data[19], and INADEQUATE measurements[20]), all of which indicate that the first carbon of the side chain resonates at higher field than does carbon number 2, this phenomenon being in striking contrast to the case of congeners with

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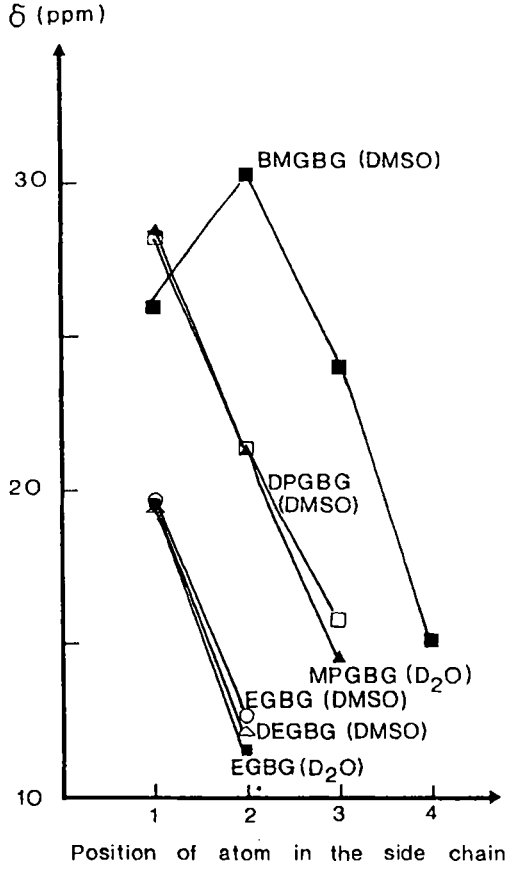

FIG. 1. Chemical shifts of side-chain carbon atoms in bis(amidinohydrazones) as a function of the position of the atom in the side chain. Numbering of side-chain carbons begins from the one directly bonded to the glyoxal moiety of the molecule.

propyl side chains (PGBG, MPGBG, DPGBG). [Numbering of side-chain carbons begins from the one directly bonded to the glyoxal moiety.] In the latter compounds, the chemical shifts of the carbons of the propyl group namely decrease in a nearly linear fashion when going from carbon 1 to carbon 3. These results (see Fig. 1) constituted an

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important impetus for performing the present study. They suggested that the lack of correlation between ^{13}C chemical shift and position of the atom in the chain might be caused by a gamma effect. The difference between the δ values of the first carbons of the butyl group of BMGBG base and the propyl groups of DPGBG base (in DMSO) is (25.88 - 28.07) ppm = - 2.19 ppm, this negative value indeed suggesting that the addition of a fourth carbon to the chain imposes a shielding gamma effect on the first carbon atom, this effect actually being of almost the same magnitude as has been reported for aliphatic hydrocarbons (-2.5 ppm)[17]. Considering equation (1), the δ value of the first butyl carbon of BMGBG [if it is typical for any butyl-substituted bis(amidinohydrazones), as would be expected] indicates that the value of parameter **d** for the case of R^3 = $CH_2CH_2CH_3$ is about 12.71 ppm.

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The above results indeed indicate that the chemical shift of the carbon of fragment **A** in (II) can be obtained by addition of the effects of substituents. Unfortunately, since the δ value of the carbon atom of methane in DMSO solution and under the conditions where bis(amidinohydrazone) spectra have been measured is not known, the values of the 'basic parameter' **a** and parameter **b** [i.e. the effect the bis(amidinohydrazone) fragment **B**] cannot be separated at present. In any case the equation can be presented in the following form:

(2) $$\delta(\mathbf{A}) = \mathbf{13.2\ ppm} + \mathbf{c} + \mathbf{d}$$

the value of **c** being approximately zero at least if substituent R^2 is hydrogen or alkyl. The equation may also be written in the following form:

(3) $$\delta(\mathbf{A}) = \mathbf{a} + \mathbf{b} + \mathbf{c} + \mathbf{n'}\,\mathbf{d'} + \mathbf{n''}\,\mathbf{d''} + \mathbf{n'''}\,\mathbf{d'''} \ldots$$

where **a**, **b**, and **c** are the same as before, and **d'** is the additive effect of a carbon atom contained in R^3 and directly bonded to fragment **A**, and **d''** and **d'''** are the additive effects of carbons

TABLE 3.

Chemical Shifts of Carbon Atom(s) Number 2 of the Alkyl Side Chain(s) in Bis(amidinohydrazones) (I).[a]

Compound		Solvent	δ Value of Side Chain Carbon Atom Number 2 (ppm)	Side Chain Under Consideration
EGBG	free base	DMSO	12.63	ethyl
EMGBG	free base	DMSO	13.15	ethyl
DEGBG	free base	DMSO	13.12	ethyl
DPGBG	free base	DMSO	21.30	propyl
BMGBG	free base	DMSO	30.23	butyl
EGBG	free base	D_2O	11.55	ethyl
MPGBG	free base	D_2O	21.18	propyl

[a]The values are taken from references 4, 13, 14, 15, and 18. Numbering of side-chain carbons begins from the one directly bonded to the glyoxal moiety.

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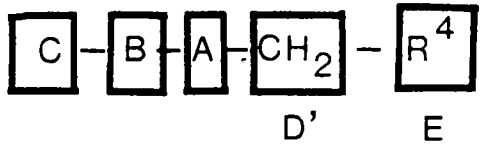
in alkyl group R^3 two and three bonds removed, while **n'**, **n''**, and **n'''** are the numbers of the carbons of R^3 one, two, and three bonds removed, and so on. With the aid of the parameter values that were obtained above, we can thus write for DMSO solutions of bis(amidinohydrazone) free bases:

(4) **δ (A) = 13.2 ppm + n' 6.2 ppm + n'' 8.7 ppm - n''' 2.2 ppm ..**

Of course, the above results prove this equation only for those cases where **n'**, **n''**, **n'''** etc. are either 1 or 0. This equation resembles to a considerable extent that one reported for the *i*th carbon in an aliphatic hydrocarbon chain[17]:

(5) $\delta_i = -2.6 + 9.1\, n_\alpha + 9.4\, n_\beta - 2.5\, n_\gamma + 0.3\, n_\delta$



III

The relative importance of the effects of carbons one bond removed and those two bonds removed is, however, slightly different. Further studies will be needed to find out, whether equation (4) is valid also for branched-chain compounds (those bearing several substituents in fragment **A**, or those with a branched-chain fragment **D**), just as equation (5) is known to be.

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Second Carbon Atom in a Chain

Data relevant for the study of the second carbon atoms in the side chain(s) of bis(amidinohydrazones) are shown in Table 3. Again, the discussion herein is limited to free bases in DMSO. Suppose that we again divide the molecule into the same fragments **A**, **B**, and **C** as before, and further divide fragment **D** into fragments **D'** (a CH_2 group) and **E**, as is shown in formula (III).

Assuming again additivity of substituent effects, we can write:

(6) $\delta(D') = a_2 + b_2 + c_2 + d_2 + e$

where d_2 is the 'basic parameter' of fragment **D'** as such, substituted with hydrogens only, and parameters a_2, b_2, c_2, and **e** are the additive effects of fragments **A**, **B**, **C**, and **E**, respectively, c_2 and **e** being zero, when **C** or R^4, respectively, is hydrogen.

Since $\mathbf{d_2}$ is actually the δ value of CH_4 in DMSO, it must be the same as **a** in equations (1), (3), and (4). Thus, we can write:

(7) $\delta\,(\mathbf{D'}) = \mathbf{a} + \mathbf{a_2} + \mathbf{b_2} + \mathbf{c_2} + \mathbf{e}$

The data shown in Table 3 indicate that, when going from EGBG to EMGBG or DEGBG, the chemical shift of carbon atom(s) number 2 of the ethyl side chain(s) increases by about 0.5 ppm. Thus, a (small) effect is mediated through the N=C-C=N fragment, the value of parameter $\mathbf{c_2}$ being 0.5 ppm for methyl and ethyl (and, possibly, for alkyl groups in general). Assuming that $\mathbf{c_2}$ is essentially the same for any alkyl group, the difference between the δ value of carbons number 2 of the ethyl groups of EMGBG and DEGBG, and that of carbon 2 of the propyl group of DPGBG, i.e. 8.2 ppm, must be the value of **e** for $R^4 = CH_3$. Likewise, the data for BMGBG indicate that the value of **e** for $R^4 = CH_2CH_3$ is 17.1 ppm. Further, on the basis of the data on EGBG, we can conclude that the sum $\mathbf{a} + \mathbf{a_2} + \mathbf{b_2}$ is 12.63 ppm. Thus, equation (7) can be rewritten in the form

(8) $\delta\,(\mathbf{D'}) = \mathbf{12.6\ ppm} + \mathbf{c_2} + \mathbf{e}$

Analogously with (4), we can write:

(9) $\delta\,(\mathbf{D'}) = \mathbf{12.6\ ppm} + \mathbf{c_2} + \mathbf{n'_2 e'} + \mathbf{n''_2 e''} + \ldots$

where $\mathbf{n'_2}$, $\mathbf{n''_2}$ etc. and $\mathbf{e'}$, $\mathbf{e''}$ etc. are defined analogously to $\mathbf{n'}$ and $\mathbf{d'}$ etc. Substituting the values of the known parameters in (9), we get:

(10) $\delta\,(\mathbf{D'}) = \mathbf{12.6\ ppm} + \mathbf{c_2} + \mathbf{n'_2}\ \mathbf{8.2\ ppm} + \mathbf{n''_2}\ \mathbf{8.9\ ppm} + \ldots$

and, for compounds with R^2 = alkyl (or, strictly, methyl or ethyl):

(11) $\delta\,(\mathbf{D'}) = \mathbf{13.1\ ppm} + \mathbf{n'_2}\ \mathbf{8.2\ ppm} + \mathbf{n''_2}\ \mathbf{8.9\ ppm} + \ldots$

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Again, we (in principle) cannot separate the effects of fragments **D'**, **A**, and **B**. Assuming, however, strict additivity of substituent effects (which may not be the case), we can further conclude that the effect of fragment **A**, i.e. $\mathbf{a_2}$, must be equal to 8.2 ppm, since also **A** is directly bonded to **D'**. Thus, we get:

(12) $\mathbf{12.6\ ppm = a + a_2 + b_2 = a + 8.2\ ppm + b_2}$

and

(13) $\mathbf{a + b_2 = 4.4\ ppm}$

Combined with the result that $\mathbf{a + b}$ = 13.2 ppm, equation (13) gives the result that $\mathbf{b - b_2}$ must be **very roughly** 8.8 ppm. In any case, it is evident that carbon(s) number 2 of any alkyl side chain(s) in bis(amidinohydrazones) (I) experience a far less deshielding (or more shielding) effect by the bis(amidinohydrazone) main fragment **B** than do carbons number 1.

The data available at present do not allow the derivation of equations describing the effects of carbons further away in the side chain on the δ value of carbon number 3. Yet, a comparison of the δ values of carbon 3 of the propyl group of DPGBG free base in DMSO (15.72 ppm) and that of the butyl group of BMGBG free base (23.87 ppm) indicates that carbon 3 experiences a downfield shift of 8.15 ppm when going from DPGBG to BMGBG. The similarity of the value with that in equation (11) suggests that the equation for the third carbon may be analogous to (6) and (11), i.e.

(14) δ **(E')** $= \mathbf{c_3 + b_3 + a_3 + d'_2 + e' + n'_3\ 8.2\ ppm + ...}$
$= \mathbf{c_3 + b_3 + a_3 + d'_2 + a + n'_3\ 8.2\ ppm + ...}$
$\approx \mathbf{15.7\ ppm + n'_3\ 8.2\ ppm + ...}$

Thus, assuming further that the effects of the two carbons between carbon 3 and **B** (i.e., $\mathbf{d'_2}$ **and** $\mathbf{a_3}$) are equal to 8.2 ppm + 8.9 ppm, and that **C** has nearly no effect, we would conclude that

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(15) $\mathbf{a + 8.2\ ppm + 8.9\ ppm + b_3 + 0\ ppm = 15.7\ ppm}$

where $\mathbf{b_3}$ is the effect of **B** over the three bonds. Thus, $\mathbf{a + b_3}$ would be - 1.4 ppm, and (because $\mathbf{a + b}$ = 13.2 ppm), $\mathbf{b - b_3}$ would be roughly equal to 14.6 ppm and $\mathbf{b_2 - b_3}$ roughly equal to 5.8 ppm. Thus, carbon atom number 3 in the side chain appears to experience an even lesser deshielding effect by the bis(amidinohydrazone) main fragment **B** than do carbons number 2.

Since the addition one more carbon atom to the end of an ethyl side chain appears to have practically the same effect on the δ value of carbon 2 as the addition of one more carbon to end of a propyl group has on the δ value of carbon 3, it is tempting to speculate that also carbon number 4 in a bis(amidinohydrazone) side chain would experience an essentially similar effect on addition of one more carbon, and that the effects of carbons that are one, two and three bonds removed from carbon 4 actually are the same as in the case of carbons number 2 and 3. If this is so, we can further **speculate** that, for carbon 4 of the butyl side chain of BMGBG (whose δ value is 15.10 ppm[13,15]), the following equation is valid:

(16) $\mathbf{15.10\ ppm = a + 8.2\ ppm + 8.9\ ppm - 2.2\ ppm + b_4 + 0\ ppm}$

Thus, if carbon 4 experiences (by carbon 1) a gamma effect equal to that caused by carbon 4 and experienced by carbon 1, and if the other side chain has a negligible effect, we conclude that:

(17) $\mathbf{a + b_4 = 0.2\ ppm}$

Since $\mathbf{a + b}$ = 13.2 ppm, we get:

(18) $\mathbf{b - b_4 = 13\ ppm}$

which suggests that the deshielding effect of the bis(amidinohydrazone) fragment **B** on carbon 4 probably is distinctly smaller than that on carbon 2 but somewhat greater than that on carbon 3.

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Assuming that **a**, i.e. the δ value of CH_4 in DMSO, is **roughly** -2.6 ppm[17], we may further conclude that the effects of **B** on carbons one, two, three, and four bonds removed, are roughly 15.8 ppm, 7.0 ppm, 1.2 ppm, and 2.8 ppm, respectively. More accurate values could be obtained if the exact δ value of CH_4 in DMSO were available. These results are based on several assumptions, whose validity has not been strictly proved, and probably are subject to considerable inaccuracy even if the basic assumptions are valid. In spite of that, they are of considerable interest. The results are illustrated in Fig. 2. At a first glance, they might suggest that the relative deshielding effect of fragment B [i.e. of the bis(amidinohydrazone) skeleton of the molecule], weakens very steadily and in a nearly linear fashion when going down the aliphatic side chain, and that at carbon atom number 4, the deshielding effect for some reason again increases somewhat, carbon number 4 thus constituting either an exception to a general tendency, or a point of inflection. This straightforward interpretation, however, may not be valid, since it is very difficult to explain, why the deshielding effect should either begin to increase at carbon 4 or why carbon 4 should be an exception (if the effect no longer increases after carbon 4). Further, it is obvious that if the deshielding effect again decreases in a linear fashion (after an "exception" at carbon 4), carbon atoms from carbon 5 on will actually experience a considerable shielding effect very difficult to explain. Therefore, another interpretation appears more probable, according to which the deshielding effect decreases in a **non-linear** fashion, carbon atom number 4 departing in no way from the curve, but carbon atom number 3 actually constituting an exception. This would be very easy to explain by assuming that a gamma effect is responsible for the exceptional behavior of carbon 3. The fact that, as compared to carbons 1, 2 and 4, carbon 3 apparently experiences a relative shielding effect, supports the gamma effect theory. If a gamma effect indeed is responsible for the results obtained, it obviously either must be due to the nearest one of the two glyoxal carbons only, since it is not experienced by carbon 2, or it must somehow be caused by the glyoxal

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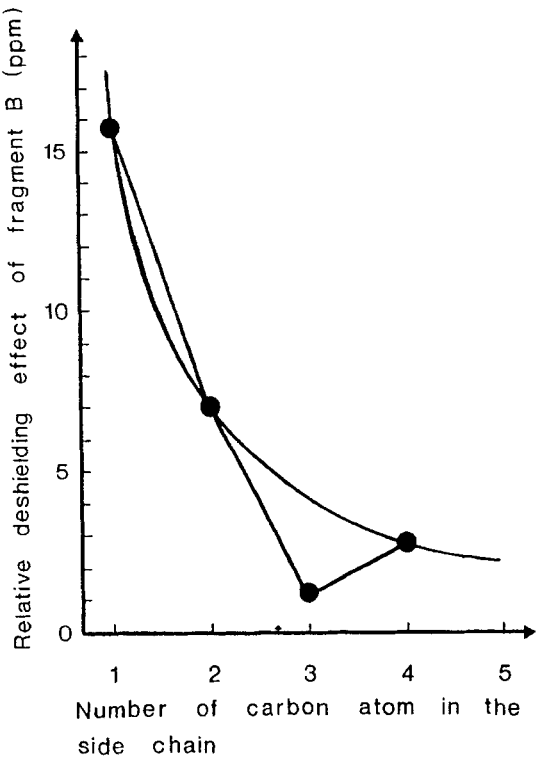

FIG. 2. Relative deshielding effect of fragment **B** experienced by side-chain carbon atoms, shown as a function of the number of the carbon atom in the side chain. The non-linear curve has been constructed by assuming that the effect of **B** on carbon atom number 3 (and on it only) deviates from the general tendency.

moiety as a whole. The result is, in all of its inaccuracy, very interesting, since it has been shown that addition of more carbons to the side chain(s) of a bis(amidinohydrazone) increases the arithmetic mean of the δ values of the carbons of the glyoxal moiety in a linear fashion up to the stage of the diethyl compound DEGBG. Thereafter, the remarkable correlation (r = 0.997), however, breaks down, the mean of the δ values of the glyoxal carbons of BMGBG being roughly 4 and that of the glyoxal carbons of DPGBG roughly 5

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ppm lower than predicted. This phenomenon, whose origin (the presence of more than two carbons in one chain, or the presence of a total of five carbons or more?) has not been elucidated, might indeed result from a gamma effect that would be a counterpart of the gamma effect caused by the glyoxal carbon(s) and experienced by carbon number 3 in a side chain. The gamma effect theory deserves further studies. Unfortunately, bis(amidinohydrazones) with five-carbon or longer side chains are not available for study at present.

CONSTRUCTION OF AN EMPIRICAL RULE FOR THE CALCULATION OF THE CHEMICAL SHIFTS OF THE CARBONS OF THE GLYOXAL MOIETY

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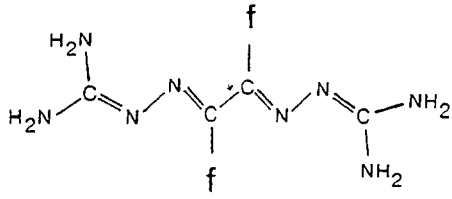
The most important and most interesting part of the present study concerns the chemical shifts of the carbons of the glyoxal moiety of the molecules (= those carbons that were the carbonyl carbons of the glyoxal used as the starting material in the synthesis of the compounds, i.e. the carbons of the central N=C-C=N fragment).

For analyzing the effects of substituents on the glyoxal carbons, a theoretical model is now devised, based on the assumption of a 'theoretical compound' with formula (IV), in which **f** is a 'formal substituent' that is defined so that in the presence of two substituents **f** in the bis(amidinohydrazone) molecule, the chemical shift of both glyoxal carbons of the molecule is 150 ppm. [150 ppm was chosen instead of 0 ppm as the 'basic value' since in all bis(amidinohydrazones) studied, the glyoxal carbons resonate between 145 and 161 ppm.] Further, the present theoretical model assumes that, when a substituent (e.g. a methyl group) replaces **f** in compound (IV), the chemical shift of the glyoxal carbon atom to which the new substituent is directly bonded, is changed by an amount **l** (the 'near-parameter' of the substituent) that is a function of the substituent only, being constant for each substituent, and is independent on the nature of the substituent attached to the other glyoxal carbon. Likewise, the model assumes that, simultaneously, the chemical shift



IV

of the other glyoxal carbon is changed by an amount **k** (the 'other-side-parameter' of the substituent), that likewise is a function of the substituent only. Further, the model assumes that the effects of any two substituents are additive, so that in a compound with two substituents, say a methyl group and a hydrogen atom, the chemical shift of one of the glyoxal carbons is **150 ppm + k(Me) + l(H)**, and that of the other carbon is **150 ppm + k(H) + l(Me)**. As will be shown below, the model indeed is consistent with experimental data and gives very accurate estimates of chemical shift values, the assumed independence of any substituent effect on the other substituent as well as the additivity of substituent effects thus becoming proved.

The chemical shifts of the glyoxal carbons of several bis(amidinohydrazones) have been reported in[13]. On the basis of those data, a set of linear equations can be constructed that are given, alongside with the experimental δ values, in Table 4. From this set of linear equations, the **sum** of any two parameters can be obtained. For example, the sum of the near-parameter of a methyl group and the other-side-parameter of an ethyl group can be solved using equations II, IV and VII.

Thus, from (II) we get:

(X) **l(Me) + k(H) = 1.15 ppm**

By combining this result with equation (IV), we can conclude that

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TABLE 4.

Chemical Shifts of the Carbons of the Glyoxal Moiety in a Variety of Bis(amidinohydrazone) Free Bases, and a Set of Linear Equations Constructed with the Aid of the Chemical Shift Values and Assuming Strict Additivity and Constancy of Substituent Effects[a]

Compound	δ Value(s) of Carbons (ppm)	Linear Equation (Number of Equation in Parentheses)	
MGBG	148.91[b]	150 ppm + l(H) + k(Me) = 148.91 ppm	(I)
	151.15	150 ppm + l(Me) + k(H) = 151.15 ppm	(II)
EGBG	148.05[b]	150 ppm + l(H) + k(Et) = 148.05 ppm	(III)
	156.11	150 ppm + l(Et) + k(H) = 156.11 ppm	(IV)
DMGBG	152.49	150 ppm + l(Me) + k(Me) = 152.49 ppm	(V)
EMGBG	151.29[c]	300 ppm + l(Me) + l(Et) + k(Me) + k(Et)	
	157.30[c]	= 151.29 ppm + 157.30 ppm	(VI)
DEGBG	156.17	150 ppm + l(Et) + k(Et) = 156.17 ppm	(VII)
DPGBG	155.41	150 ppm + l(Pr) + k(Pr) = 155.41 ppm	(VIII)
BMGBG	151.70[c]	300 ppm + l(Me) + k(Me) + l(Bu) + k(Bu)	
	156.22[c]	= 151.70 ppm + 156.22 ppm	(IX)

[a]Only results obtained for free bases in DMSO solution are considered in the table. The chemical shift values are taken from references 4, 13, 14, and 15. The following abbreviations are used: Me = methyl, Et = ethyl, Pr = propyl, Bu =butyl.

[b]The one directly connected to a proton.

[c]Individual assignment has not been reported. The results of reference 18 that were obtained with the aid of some results of the present study, however, provide a means for the individual assignment of the resonances of the glyoxal carbons of BMGBG and thus make possible the construction of two independent linear equations for BMGBG instead of equation (IX) (see text for details).

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(XI) **l(Me) + 6.11 ppm - l(Et) = 1.15 ppm**

Further, with the aid of this result and equation (VII), we get:

(XII) **l(Me) + k(Et) = 1.21 ppm**

The sum of the near-parameter of an ethyl group and the other-side parameter of a methyl group is likewise easily obtained. Thus, from (V) we get:

(XIII) **k(Me) + l(Me) = 2.49 ppm**

which in turn, after substitution of (II) and (IV), gives:

(XIV) **k(Me) + l(Et) = 7.45 ppm**

Thus, we can predict that, if the model presented is valid, the chemical shift of that one of the glyoxal carbons of EMGBG that is directly connected to the ethyl side chain and therefore, according to our model, experiences the near-effect of the ethyl group, i.e. **l(Et)**, and the other-side-effect of the methyl group, i.e. **k(Me)**, should be 150 ppm + 7.45 ppm = 157.45 ppm. Similarly, we can calculate that the other glyoxal carbon should resonate at 151.21 ppm. These results are very interesting, since the two glyoxal carbons of EMGBG free base (in DMSO) that have never been individually assigned indeed resonate at 151.29 ppm and 157.30 ppm. Thus, the present model indeed can predict the chemical shifts of the two glyoxal carbons with astonishingly great accuracy, the deviations being of the order of the experimental inaccuracy only. Further, the model constructed obviously not only works well and has a sound basis, but also provides the first means for the individual assignment of the resonances of the glyoxal carbons of dialkylglyoxal bis(amidinohydrazones). These carbons have resisted assignment mainly because they cannot be assigned with the aid of methods such as DEPT, off-resonance proton-noise decoupled carbon NMR,

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heteronuclear shift correlation or selective proton decoupling techniques, since both of them are unprotonated. Thus, the present model is clearly a considerable advancement considering the individual assignment of the glyoxal carbons, making the incredible natural abundance double quantum transfer experiment (INADEQUATE) unnecessary.

One disadvantage of the present model is constituted by the fact that, in the form presented above, it does not allow one to calculate individual substituent parameters such as **k(Me)** but only the sums of two parameters. Although the former actually are not required for assignment purposes, they would be far more practical. Therefore, a further modification of the present model might be of interest, in which the model is not fixed to any theoretical substituents (i.e. to **f**), but to a real compound such as GBG (I: $R^1 = R^2 = H$). Unfortunately, the chemical shift of the glyoxal carbons of the highly insoluble GBG free base has never been reported. Therefore, the dimethyl compound DMGBG is now taken as the 'zero effect level' in the modified model to be given below. The same postulates are accepted as before, yet with the following changes: (a) a methyl group, whether directly bonded to the glyoxal carbon under consideration or to the other glyoxal carbon, has no effect on the chemical shift of the carbon, both **l(Me)** and **k(Me)** thus being exactly zero; and (b) the 'basic level' of glyoxal carbon chemical shifts is the chemical shift value of the glyoxal carbons of DMGBG free base in DMSO, i.e. 152.49 ppm. Now, we can calculate the near-parameters and other-side-parameters individually for each real substituent. In order to avoid confusion with the model given above, capital letters are now used: **K** and **L**. The values of the parameters obtained this way are given in Table 5. (The reason for the fact that the former model that employs **f** as a theoretical substituent cannot be used to give values for individual parameters is that the sets of equations obtained in that model are underdetermined because no measurements can be performed on compounds containing one **f** as a substituent.)

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TABLE 5.

The Values of Parameters K and L for Some Substituents.[a]

L(H)	- 3.58 ppm	K(H)	- 1.34 ppm
L(Me)	0	K(Me)	0
L(Et)	+ 4.96 ppm	K(Et)	- 0.86 ppm
L(Pr)	+ 4.18 ppm	K(Pr)	- 1.48 ppm
L(Bu)	+ 3.73 ppm	K(Bu)	- 0.79 ppm

[a]The values are applicable in the case of compounds (free bases) dissolved in DMSO. They were calculated according to the model in which the chemical shift of DMGBG free base in DMSO is the basic parameter, methyl groups having no effect on the chemical shift values of glyoxal carbons. For details of the model, see text. The table is based on the experimental data presented in Table 4. The values of **K(Bu)** and **L(Bu)** are based on the assignment of the glyoxal carbons of BMGBG with the aid of relaxation time measurements (reference 18). The values of **L(Pr)** and **K(Pr)** are based on the correction of the chemical shift values obtained for the free base in D_2O solution (see text) and on the use of relaxation time measurements for assignment purposes.

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The calculation of some parameters is very straightforward. For example, the values of **K(H)** and **L(H)** are easily obtained from the experimental data on MGBG (in which one of the substituents is methyl). Others are obtained by using the results obtained this way, and employing the experimental data on the chemical shifts in compounds that lack methyls (e.g., EGBG).

With the aid of the data of Tables 4 and 5, one can easily find out that the **K** and **L** parameters indeed are strictly additive and that the values of the parameters are quite constant irrespective of the compound studied. Thus, for example, the data of Table 5 indicate that the chemical shift of the glyoxal carbons of DEGBG should be 152.49 ppm + 4.96 ppm - 0.86 ppm = 156.59 ppm, this result being in good agreement with the experimental value 156.17 ppm. (The latter value was not used for calculating the parameters given in Table 4.)

The **K** and **L** parameters of the propyl group cannot be calculated at present because the only compound containing a propyl side chain, for which measurements have been carried out in DMSO, is DPGBG that does not contain any other substituent except propyl. Thus, only the sum of the near-parameter and the other-side-parameter can be calculated for the propyl group.

Combined with the results of studies on the ^{13}C spin-lattice relaxation times of the carbons of a variety of bis(amidinohydrazones)[18], the present results have also led to the conclusion that in the case of the glyoxal carbons of various dialkylglyoxal bis(amidinohydrazones), the one of the glyoxal carbons that is directly bonded to the shorter alkyl chain has a longer relaxation time than does the other one, also this conclusion making the individual assignment of the glyoxal carbons facile.

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The carbon spectra of MGBG and EGBG have been reported for both D_2O and DMSO solutions. In the case of MGBG, the chemical shift of the unprotonated glyoxal carbon decreases by 2.503 % when going from D_2O to DMSO, and the corresponding value of EGBG decreases likewise by 2.498 %. Assuming that also the chemical shifts of the glyoxal carbons of MPGBG (both of which are unprotonated) are decreased by approximately 2.50 % on going from D_2O to DMSO, the chemical shifts in DMSO solution should be approximately 151.01 and 156.67 ppm. These values, combined with the results of the spin-lattice relaxation study[18], make possible the calculation of approximate **K** and **L** values for the propyl group. Thus, the value obtained for **K(Pr)** is about - 1.48 ppm and that for **L(Pr)** about + 4.18 ppm. The sum of **K(Pr)** and **L(Pr)** obtained this way is 2.70 ppm, while the value obtained with the aid of the chemical shift of DPGBG glyoxal carbons in DMSO solution is 2.92 ppm. With the aid of the **K(Pr)** and **L(Pr)** values obtained by correction of D_2O spectral data, the chemical shift of the glyoxal carbons of DPGBG is predicted to be 155.19 ppm, this result deviating from the experimental value 155.41 ppm by only - 0.14 %. Thus, the values obtained for **K(Pr)** and **L(Pr)**

apparently are accurate enough for spectral interpretation and prediction. (In this connection, it deserves to be mentioned that the conclusions[18] concerning the correlation between spin-lattice relaxation times and the length of the side chain directly bonded to the carbon in question actually were critically dependent on the results of the present study concerning the unambiguous assignment of the glyoxal carbons of EMGBG, since no other data were then available on the unambiguous assignment of the glyoxal carbons of any dialkylglyoxal bis(amidinohydrazones). Thus, the present results on the **K** and **L** values of the propyl and butyl groups actually are partially based on a 'feed-back' of the results of the present study itself. Without relaxation studies, only data on further derivatives with propyl and butyl side chains could have provided means for obtaining the values of **K** and **L** for these substituents, but such data are so far not available.)

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As is evident from Table 5, the absolute value of **L** is greater than that of **K**, this being true for all of the substituents studied (except the zero-effect methyls). (This would be the case even if the **K** and **L** values of hydrogen and not those of the methyl group were considered to be exactly zero.) In Fig. 3, the values of **L** and **K** are shown as a function of the number of carbon atoms in the substituent (R).

The value of **L** increases in an almost linear fashion up to the two-carbon-side-chain level, after which it abruptly begins to decrease. The decrease of **L** between the two-carbon and three-carbon stages is easily accounted for by assuming that a gamma effect lies behind the phenomenon. The decrease between the three-carbon and four-carbon stages is, however, far more difficult to explain. The other-side parameter **K** behaves in much the same way as **L**. Thus, after an initial increase between zero and one carbons, the value decreases when going from two to four side-chain carbons. The decrease is nearly linear and the slope is almost the same as that of the **L** function between carbons 2 and 4. The decreased value of **K** at two

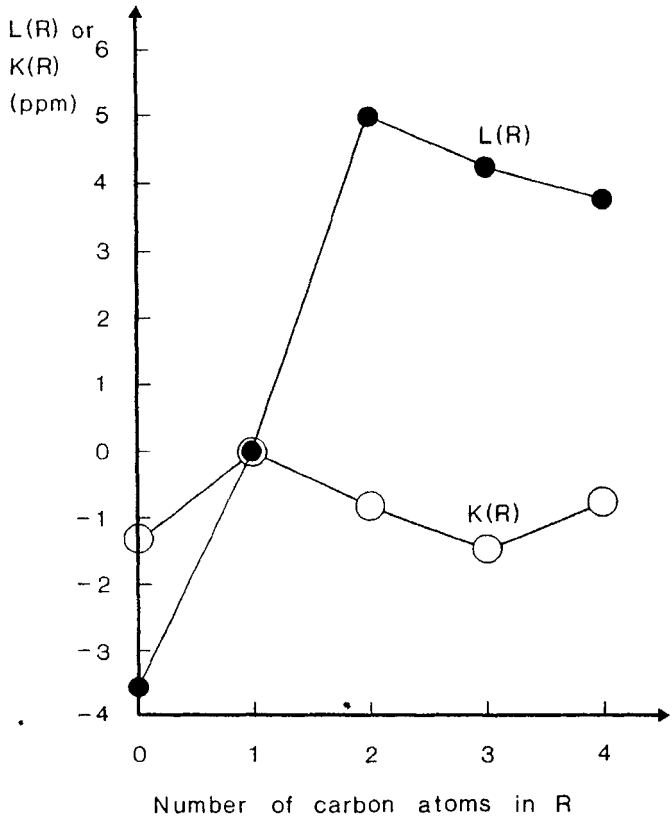

FIG. 3. Parameters **K(R)** and **L(R)** shown as a function of the number of carbon atoms in the substituent (R).

carbons can again be explained by assuming the occurrence of a gamma effect. What causes the decrease of the value when going from the two-carbon case to the three-carbon case, however, remains to be studied (perhaps a steric effect?). After the three-carbon level, the value again begins to increase, **K(Bu)** having almost the same value as **K(Et)**. Further studies on longer-chain analogs (if they were available) would without doubt shed more light on these intrinsically interesting aspects. It would be especially interesting to

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find out, whether also the value of **L** begins to increase after the four-carbon-side-chain level, since if this is the case, it is tempting to speculate that a 'delta effect' occurs that is nearly similar to the gamma effect and is responsible for the decrease of **L** between the three- and four-carbon levels as well as the decrease of **K** between the two- and three-carbon levels.

At least for all bis(amidinohydrazones) so far studied by NMR, the present results indicate that the one of the glyoxal carbons that resonates more downfield is the one that is directly bonded to the longer alkyl side chain. This result is valid even in those cases where one of the 'side chains' is a hydrogen atom. The general validity of this result is, however, uncertain as the values of **K** and **L** are at present not known for longer than four-carbon side chains. Combined with the results of relaxation time studies[18], the results of the present study also indicate that there is an intimate but simple correlation between side chain length and the chemical shift value of the glyoxal carbon and the spin-lattice relaxation time of the carbon, a maximum of the former two parameters indicating a minimum of the third parameter, and vice versa.

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The results now obtained concerning the individual assignment of the resonances of the glyoxal carbons and the chemical shifts of individual carbons of the glyoxal part of the bis(amidinohydrazone) molecules are of special interest because a distinct correlation has been discovered between the means of the chemical shifts of the two glyoxal carbons of various bis(amidinohydrazones) and the ability of the compounds to inhibit adenosylmethionine decarboxylase[4].

With the aid of the present results, it can be predicted that the glyoxal carbons of GBG free base dissolved in DMSO resonate at ca. 147.6 ppm, while those of the propylglyoxal derivative PGBG resonate at 147.4 ppm (proton-bound C) and 155.3 ppm (unprotonated C). Experimental work to test these predictions is currently underway. The prediction concerning PGBG, if valid, indicates that the compound

does not obey the remarkable linear correlation that has previously been observed between the mean of the chemical shifts of the glyoxal carbons and the total number of side-chain carbon atoms and that has been found to be valid for MGBG, EGBG, DMGBG, EMGBG, and DEGBG but not for BMGBG and DPGBG. This result strongly suggests that the breakage of the excellent correlation after the diethyl stage is not due to the increase of the total number of side-chain carbons over four, but instead is due to gamma effects (and obviously also some kind of delta effects) caused by three-carbon or longer side chains.

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