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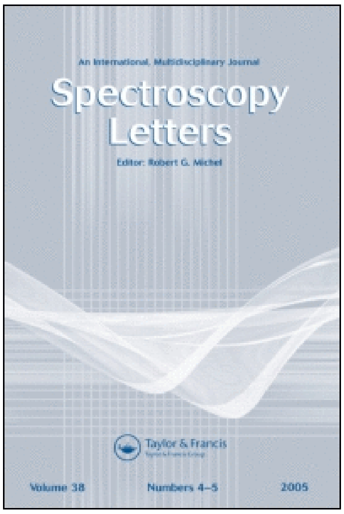

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2D-NMR Studies on Bis(Amidinohydrazones). I. A Proton-Carbon Heteronuclear Shift Correlation Study on the Enzyme Inhibitors Methylpropylglyoxal Bis(Amidinohydrazone) and Butylmethylglyoxal Bis(Amidinohydrazone)

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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)

Key Words: Adenosylmethionine Decarboxylase Inhibitors,
Bis(guanylhydrazones), Carbon-13 NMR Spectroscopy,
Chemical Shift Assignment, Polyamine Antimetabolites,
Substituent Effects

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ABSTRACT

The first 2D-NMR study on bis(amidinohydrazones) ['bis(guanylhydrazones)'] is reported. Heteronuclear shift correlation (HETCOR) experiments were performed on the enzyme inhibitors methylpropylglyoxal bis(amidinohydrazone) (MPGBG) and butylmethylglyoxal ·bis(amidinohydrazone) (BMGBG). The results obtained made possible the unambiguous assignment of the previously unassigned resonances of the side-chain carbon atoms of MPGBG. The results indicate that the chemical shifts of the protons of the propyl side chain of MPGBG are positively correlated to the ^{13}C chemical shifts of the corresponding carbon atoms. The chemical shifts of the carbon atoms

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of the propyl side chain decrease as a function of the position of the atom in the side chain, the terminal methyl group having the lowest shift value. These results are in full agreement with previous results on the analogous compound dipropylglyoxal bis(amidinohydrazone), whose side-chain carbon resonances were assigned using totally different techniques. In the case of BMGBG, however, HETCOR contour plots clearly indicate that there is no correlation between the chemical shifts of the protons of the butyl side chain and the ^{13}C chemical shifts of the corresponding carbons. Because the 200 MHz proton spectrum of BMGBG is not first-order, only the ^{13}C resonance of the methyl substituent and the resonances of carbons 1 and 4 (but not those of carbons 2 and 3) of the butyl side chain could be assigned on the basis of the HETCOR study. Yet, the results gave a rough estimate of the previously unknown chemical shifts of the protons bound to butyl carbon atoms 2 and 3.

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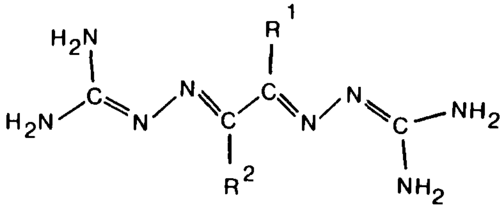
INTRODUCTION

The bis(amidinohydrazones) (I) of various glyoxals have been intensively studied in recent years because many of them are powerful



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inhibitors of adenosylmethionine decarboxylase, one of the two rate-limiting enzymes of polyamine biosynthesis[1-7], and because some of them are also potent antileukemic agents[3,8,9]. Previous studies have, however, mainly been concentrated on the biochemical and pharmacological properties of the compounds, and almost nothing has been known about the chemical and spectroscopical properties of these potentially important drugs. Yet, in order to understand the biological effects and structure-activity relationships of these agents, knowledge of their chemistry is of crucial importance. Since NMR studies may provide valuable information about the effects of substituents on the electron distribution in the bis(amidinohydrazone) chain and on other properties of the molecules, the ^{1}H and ^{13}C spectra of a large number of bis(amidinohydrazones) were recently studied[10,11]. Although most of the ^{13}C signals could be assigned on the basis of information derived from off-resonance proton noise decoupled spectra and with the aid of chemical shift correlations, some of the signals of the side-chain carbons could not be unambiguously assigned in cases where the molecule contained propyl or butyl side chains. In the case of one compound, dipropylglyoxal bis(amidinohydrazone) (DPGBG; I, R^1 = R^2 = propyl), the resonances of the carbons of the propyl side chains were assigned with the aid of DEPT measurements and selective heteronuclear proton decoupling[12]. I have now performed further studies on two bis(amidinohydrazones), namely methylpropylglyoxal bis(amidinohydrazone) (MPGBG; I, R^1 = methyl; R^2 = propyl) and butylmethylglyoxal bis(amidinohydrazone) (BMGBG; I, R^1 = methyl; R^2 = butyl), using proton-carbon heteronuclear shift correlation as the technique for the assignment of side-chain carbon resonances.

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EXPERIMENTAL

The free bases of MPGBG and BMGBG were dissolved in dimethyl sulfoxide-d_6 (DMSO-d_6). In the case of BMGBG, 1,4-dioxane was used as an internal standard, the protons and carbons of which were considered to resonate at 3.700 ppm and 67.40 ppm, respectively. In order to maintain maximal solubility, no internal standard was used in the case of MPGBG.

NMR spectra were recorded with the aid of a Varian Gemini 200 NMR spectrometer using rotated tubes (diameter 5 mm). The measurements were carried out at an ambient temperature of ca. 24-26°C.

RESULTS AND DISCUSSION

The ^{1}H-^{13}C HETCOR contour plots obtained for the free bases of MPGBG and BMGBG are shown in Figs. 1 and 2, respectively. The resonances of the side-chain protons of MPGBG have been rigorously assigned in previous studies[10], and the contour plot now obtained for this compound makes possible the unambiguous assignment of all of the side-chain carbon resonances of the compound (see Fig. 1). Also, it is clearly evident from Fig. 1 that, in the case of MPGBG, the chemical shifts of the protons of the propyl side chain are positively correlated to the ^{13}C chemical shifts of the corresponding carbon atoms. In this respect, the compound behaves similarly to DPGBG[12]. Such a positive correlation between ^{1}H and ^{13}C chemical shifts may indeed be a general property of the propyl side chains of the bis(amidinohydrazones) of various glyoxals, at least in those cases where the other substituent is hydrogen or an n-alkyl group with no more than three carbon atoms, since the unpublished preliminary results of the present author suggest that also in the case of propylglyoxal bis(amidinohydrazone) (PGBG; I, R^1 = hydrogen, R^2 = propyl), a similar positive correlation exists. (It should, however, be noticed that, as is evident from Fig. 1, the correlation holds only *within* each side chain but not for atoms belonging to different side chains.) The conclusions are well in line with the results obtained in the case of compounds containing ethyl substituents[11]. A further similarity between MPGBG, DPGBG[12] and (according to unpublished preliminary results) PGBG is constituted by the fact that the ^{13}C chemical shifts of the carbon atoms of the propyl side chain decrease as a function of the position of the atom in the side chain, the terminal methyl group having the lowest shift value. A similar result was previously obtained for ethyl side chains[11].

Combined with previous ones, the results now obtained suggest that, in those n-alkyl side chains of bis(amidinohydrazones) that contain maximally three carbons, the electron densities at the various

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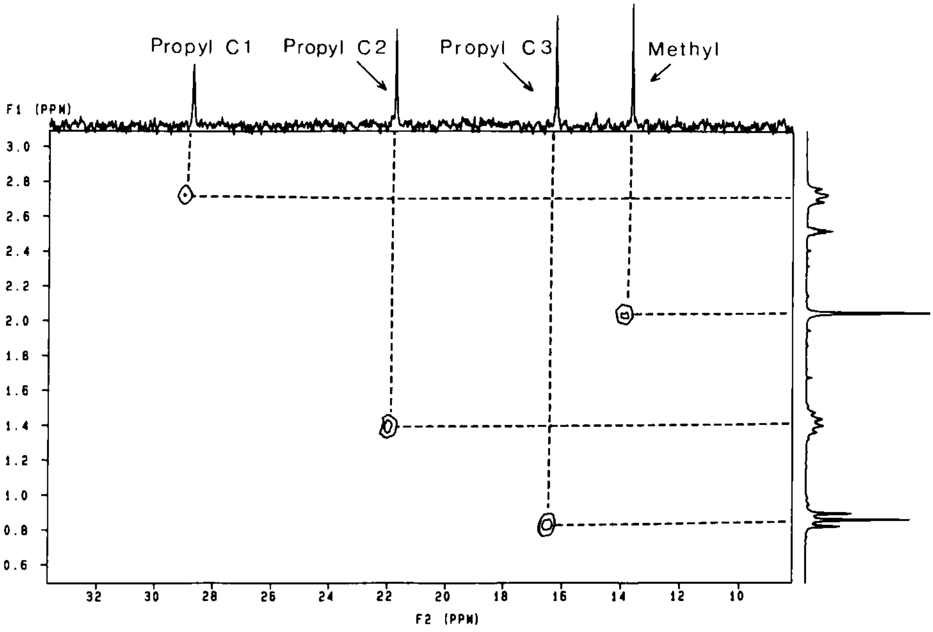

FIG. 1. The ^1H-^{13}C HETCOR contour plot obtained for MPGBG free base dissolved in DMSO-d_6. The HETCOR measurement was confined to those parts of the 1D-spectra that contain the signals of the side-chain protons and carbons. Since no internal standard was used, the arbitrary ppm scales suggested by the spectrometer are shown. Thus, the δ values deviate considerably from those measured against 1,4-dioxane. The results of previous studies[10] indicate that, at about 21.5°C, the protons of the methyl side chain of MPGBG base resonate at 2.163 ppm, and the protons bound to carbon atoms 1, 2 and 3 of the propyl side chain resonate at 2.844, 1.537 and 0.979 ppm, respectively, as referenced to 1,4-dioxane, whose protons are considered to resonate at 3.700 ppm. On the basis of the contour plot, it is thus evident that the carbon-13 signal with the lowest δ value must be due to the methyl side chain. Further, it is evident that the δ value of the carbon-13 resonance decreases when going from carbon 1 of the propyl side chain to carbon 2 and from carbon 2 to carbon 3.

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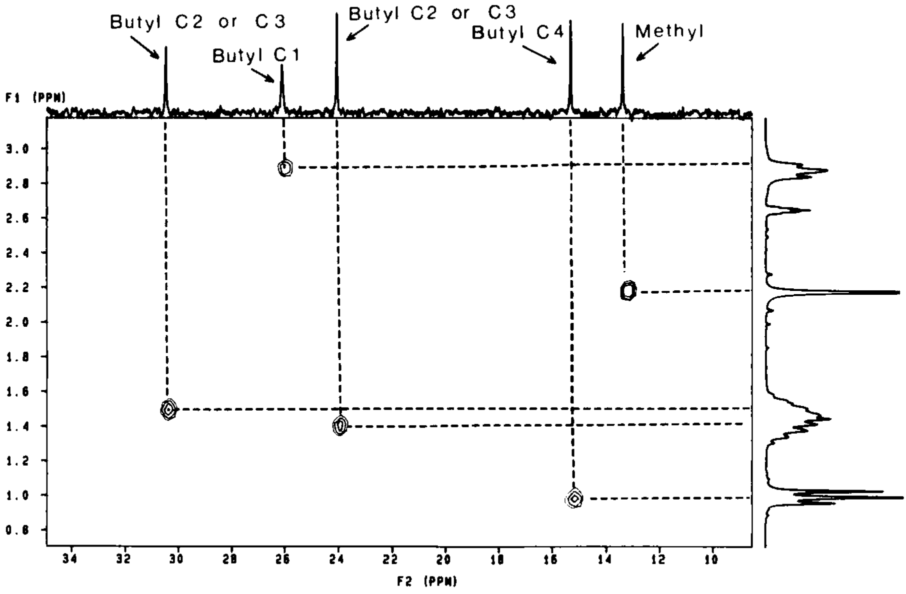

FIG. 2. The ^{1}H-^{13}C HETCOR contour plot obtained for BMGBG free base dissolved in DMSO-d$_6$. The HETCOR measurement was confined to those parts of the 1D-spectra that contain the signals of the side-chain protons and carbons. All chemical shifts are referenced to 1,4-dioxane (3.700 ppm/67.40 ppm). The results of previous studies[10] indicate that, at about 21.5°C, the protons of the methyl side chain of BMGBG free base resonate at 2.158 ppm, and the protons bound to carbon atoms 1 and 4 of the propyl side chain resonate at 2.864 and 0.985 ppm, respectively, and that the protons bound to carbons 2 and 3 of the butyl side chain give rise to an unresolved multiplet with its highest maximum at ca. 1.43 ppm. On the basis of the contour plot, it is thus evident that the carbon resonating at ca. 13 ppm is that of the methyl substituent, and the carbons resonating at ca. 15 ppm and ca. 26 ppm are carbons 4 and 1 of the butyl side chain, respectively, and the carbons resonating at ca. 24 and 30 ppm are carbons 2 and 3, or 3 and 2, of the butyl side chain. The contour plot also indicates that the chemical shifts of the protons bound to carbons 2 and 3 of the butyl side chain are roughly 1.4 and 1.5 ppm, in either order.

carbons are fairly well correlated with those at the corresponding hydrogens.

In the case of BMGBG, the HETCOR contour plot obtained (Fig. 2) clearly indicates that no correlation exists between the chemical shifts of the protons of the butyl side chain and the ^{13}C chemical shifts of the corresponding carbons. Thus, in this respect, the NMR properties of the butyl side chain differ drastically from those of all two- and three-carbon side chains of bis(amidinohydrazones) (I) so far studied. The reasons for this difference are worth further studies. It would be especially interesting to find out, whether the differential NMR properties of BMGBG (as compared to congeners with maximally three-carbon side chains) reflect differences between electron densities at corresponding atoms of BMGBG and its congeners with shorter side chains, since any such differences may be of considerable biochemical significance. The results obtained also indicate that, in contrast to the case of congeners with shorter side chains, the ^{13}C chemical shifts of the carbon atoms of the butyl side chain of BMGBG are not correlated in any straightforward way to the positions of the carbon atoms in the side chain.

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Because the 200 MHz proton spectrum of BMGBG is not first-order[10], only the ^{13}C shifts of carbons 1 and 4, but not those of carbons 2 and 3, of the butyl side chain could be assigned on the basis of the HETCOR study. Yet, the results gave a rough estimate of the previously unknown chemical shifts of the protons bound to carbon atoms 2 and 3 of the butyl group (see Fig. 2). Further studies are underway in order to assign the resonances of carbons 2 and 3 of the butyl side chain.

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