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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

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**UNAMBIGUOUS ASSIGNMENT OF THE ^{13}C NMR RESONANCES
OF THE SIDE-CHAIN CARBON ATOMS OF DIPROPYLGLYOXAL
BIS(AMIDINOHYDRAZONE) BY DEPT AND SELECTIVE HETERO-
NUCLEAR PROTON DECOUPLING TECHNIQUES**

Key Words: Dipropylglyoxal Bis(guanyldihydrazone),
Dimethyl Sulfoxide- d_6 , Chemical Shifts

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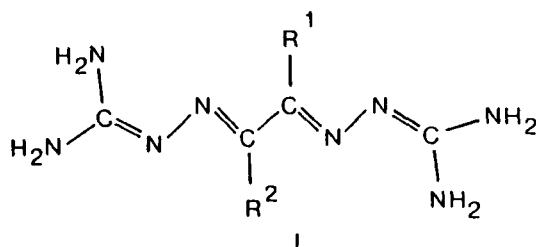
ABSTRACT

The previously unassigned carbon-13 NMR resonances of the side-chain carbon atoms of the enzyme inhibitor dipropylglyoxal bis(amidinohydrazone) (DPGBG) have been unambiguously assigned with the aid of DEPT measurements and experiments involving the selective decoupling of the protons of one of the methylene groups. The chemical shifts of the side-chain carbon atoms of DPGBG decrease in a nearly linear fashion as a function of the position of

the atom in the side chain, the terminal methyl groups having the lowest shift value. The carbon-13 shifts are positively correlated with the chemical shifts of the corresponding hydrogen atoms.

INTRODUCTION

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



inhibitors of adenosylmethionine decarboxylase, one of the two rate-limiting enzymes of polyamine biosynthesis¹⁻⁵, and because some of them are also potent antileukemic agents^{3,6,7}. Studies on them have, however, been mainly biochemical and pharmacological in nature, and therefore almost nothing is known about the chemical and spectroscopical properties of these potentially important compounds. Yet, in order to understand the biological effects and structure-activity relationships of these agents, knowledge of their chemistry is of course 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 ¹H and ¹³C spectra of a large number of bis(amidinohydrazones) were recently studied^{8,9}. Although most of the ¹³C 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. Therefore, we have now performed further studies on one such compound, di-

propylglyoxal bis(amidinohydrazone) (DPGBG; I, $R^1 = R^2 = \text{propyl}$), and report here an unambiguous assignment of all of its ^{13}C NMR signals. The results may be of value also for the assignment of carbon resonances of other congeners with propyl or even longer side chains.

EXPERIMENTAL

DPGBG free base was dissolved in dimethyl sulfoxide- d_6 ($\text{DMSO-}d_6$). The highest solvent peak was used as the internal standard.

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 10 mm dual $^1\text{H}/^{13}\text{C}$ probe. The tubes (diameter 10 mm) were rotated (ca. 20-30 rps). The measurements were carried out at an ambient temperature of $\text{ca. } 21.2 \pm 0.4^\circ\text{C}$ in a specially air-conditioned facility.

RESULTS AND DISCUSSION

Previous studies⁹ had indicated that the side-chain carbons of DPGBG base, dissolved in dimethyl sulfoxide, give rise to three signals (at 28.07, 21.30, and 15.72 ppm, as referenced to an internal 1,4-dioxan standard whose chemical shift is considered to be 67.4 ppm). We now measured the DEPT 135 spectrum of the compound in dimethyl sulfoxide- d_6 (Fig. 1), which clearly indicated that the resonance with the smallest chemical shift is due to a methyl group (i.e., side-chain carbon number 3) while the other two are due to methylene protons.

In order to assign the two methylene carbon resonances, we selectively irradiated the protons bound to side-chain carbons number 2 while recording a ^{13}C spectrum. (The full assignment of the side-chain proton resonances of DPGBG has been previously reported; see Fig. 2). Thus, a spectrum was obtained, in which the carbon resonance

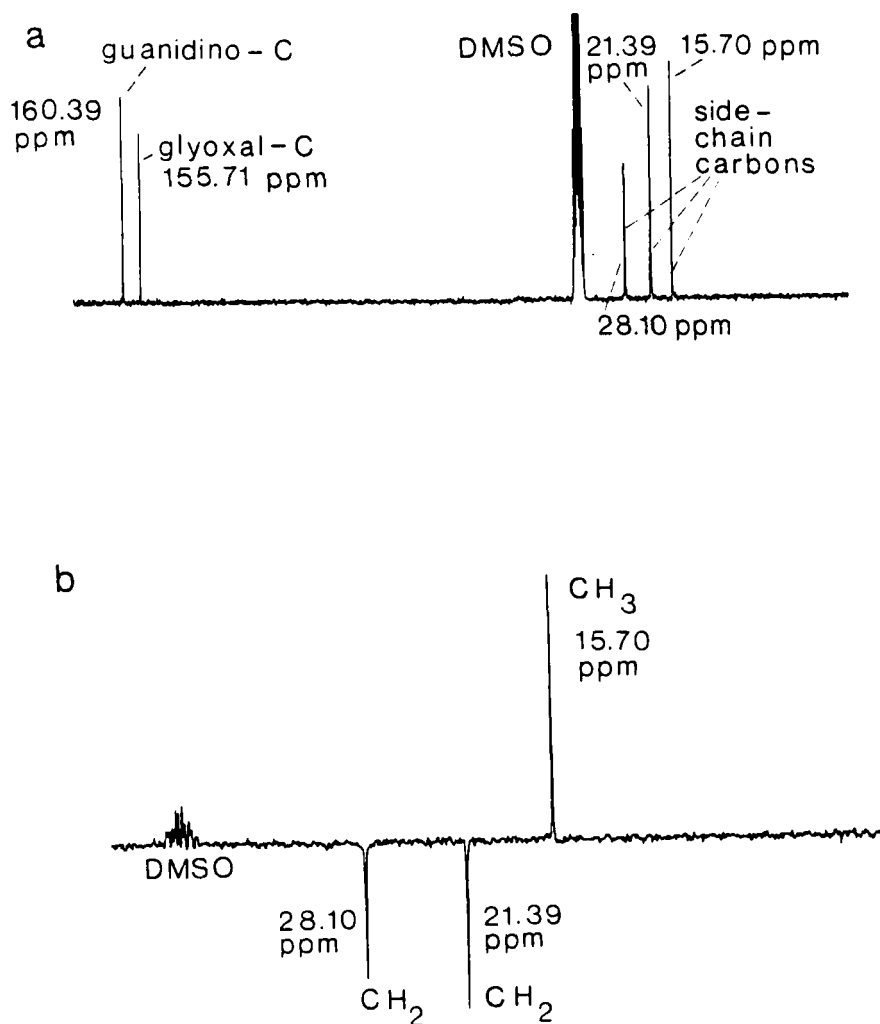


FIG. 1. (a) The completely proton noise decoupled carbon-13 NMR spectrum of DPGBG base dissolved in DMSO- d_6 . (b) Part of the DEPT 135 (DEPT = distortionless enhancement by polarization transfer) spectrum of the same solution. The chemical shifts are referenced to the highest solvent peak, whose δ value is considered to be 40.4. Thus, the chemical shift values differ numerically slightly from those previously reported⁸.

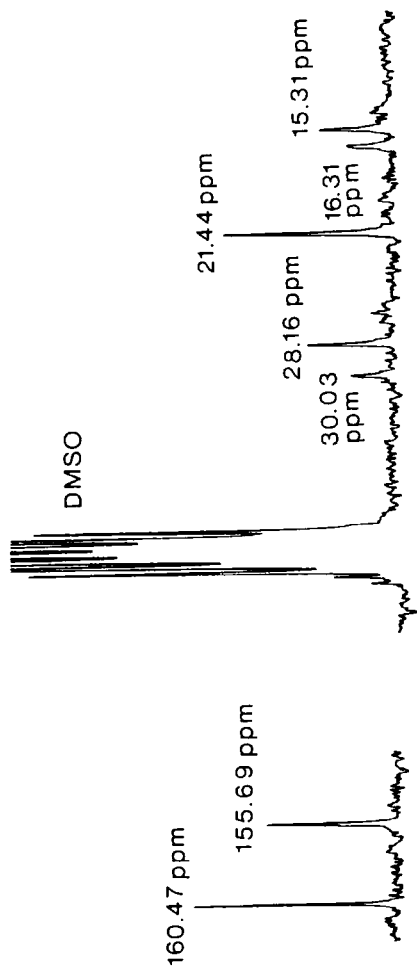


FIG. 2. A ^{13}C spectrum of DPGBG base, dissolved in DMSO-d_6 , recorded during simultaneous selective irradiation of the protons bound to side-chain carbons number 2. (The protons bound to side-chain carbons 1, 2, and 3 resonate at ca. 2.80, 1.50 and 0.95 ppm, respectively, as referenced to the highest residual proton peak of the solvent, the proton chemical shift of which is considered to be 2.64 ppm.) Several experiments were carried out, varying the experimental conditions, but the exact multiplicity of the non-singlet resonances could not be revealed in any case. The spectrum shown represents the optimal obtainable results.

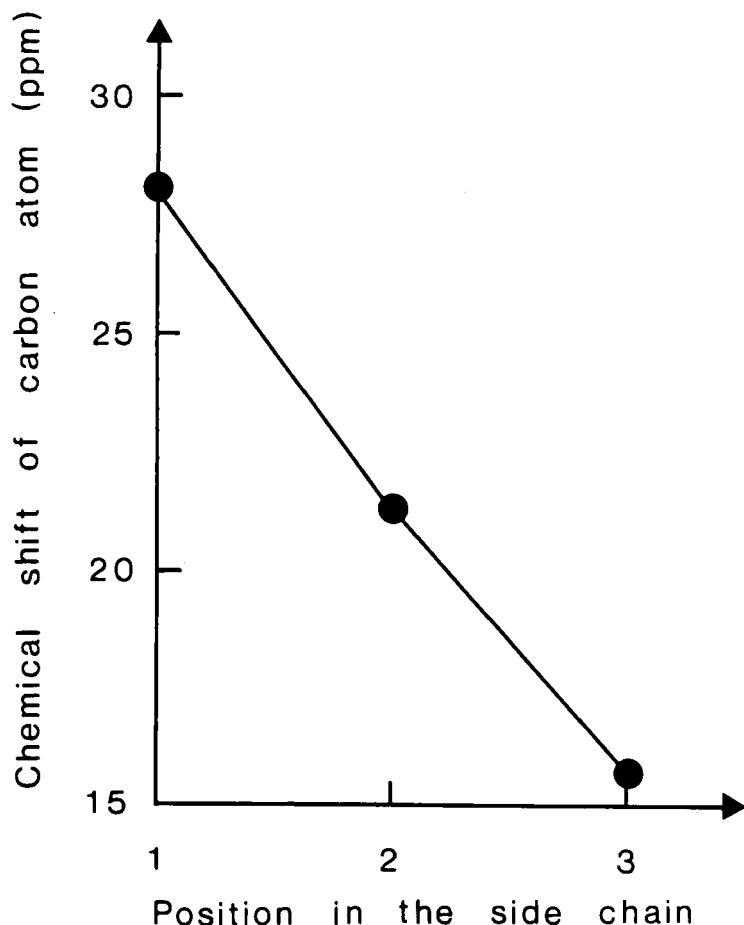


FIG. 3. The chemical shifts of the various side-chain carbons of DPGBG base as a function of the position of the atom in the chain. The shift values δ are referenced to an internal 1,4-dioxan standard ($\delta = 67.4$).

at ca. 21 ppm clearly appeared as a singlet, while the resonances at ca. 28 and 15.7 ppm were split into multiplets (see Fig. 2). The exact multiplicity of the multiplets could not be determined, obviously because the proton resonances of the side-chain protons occur fairly close to each other, a rigorously selective irradiation of only one of their resonance frequencies thus being impossible to achieve in

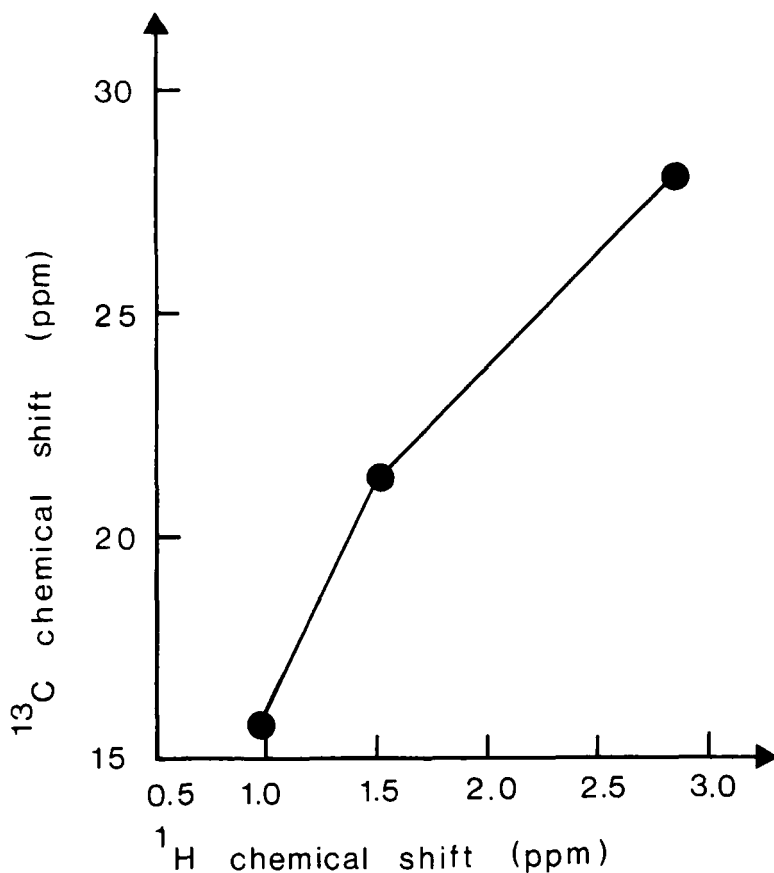


FIG. 4. The chemical shifts of the side-chain carbons of DPGBG base as a function of the chemical shifts of the corresponding hydrogens. The values of the shifts⁸ are referenced to an internal 1,4-dioxan standard whose carbon-13 and proton chemical shifts are considered to be 67.4 and 3.7 ppm, respectively.

practice. In any case, the results obtained clearly indicate that the carbon resonance occurring at ca. 21 ppm is due to side-chain carbons number 2. The results, combined with previous ones⁹ have thus made possible the unambiguous assignment of all of the carbon resonances of the DPGBG molecule.

The above results indicate that the nearer a side-chain carbon atom (in DPGBG) is to the bis(amidinohydrazone) chain of the

molecule, the more downfield occurs its resonance in ^{13}C NMR spectra. Whether this is true also in the case of other congeners with propyl or longer side chains, remains to be studied. In any case, this result is well in line with the results obtained in the case of compounds containing ethyl substituents⁹. As is evident from Fig. 3, there is a nearly linear relationship between the chemical shift value and the number of the carbon atom in the side chain. As is evident from Fig. 4, the chemical shifts of the side-chain carbons of DPGBG are positively correlated with the chemical shifts of the corresponding hydrogen atoms, suggesting that the electron densities at the various carbons are fairly well correlated with those at the corresponding hydrogens.

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