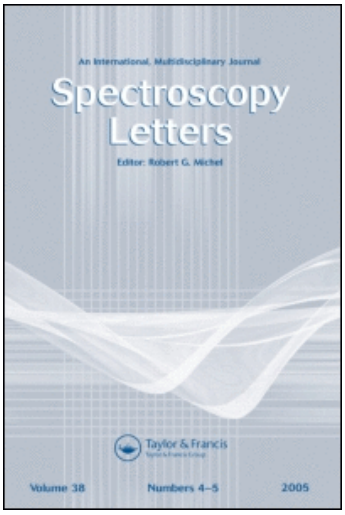

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UV/VIS SPECTROPHOTOMETRIC STUDIES ON THE ANTILEUKEMIC AGENT GLYOXAL BIS(AMIDINOHYDRAZONE) ['GLYOXAL BIS(GUANYLHYDRAZONE)']

Key Words: Adenosylmethionine Decarboxylase Inhibitors, Polyamine Antimetabolites, Solvent Effects, Ultraviolet Spectroscopy, Visible Spectroscopy

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ABSTRACT

Glyoxal bis(amidinohydrazone) (GBG) and several analogs thereof are compounds of considerable pharmacological interest, and a variety of HPLC and MECC methods have been developed for their analysis. In these methods, detection is invariably based on the strong UV absorption of the compound. Yet, almost nothing has been known of their UV and VIS spectral properties. In the present paper the UV and VIS spectroscopy of GBG has been studied in several

solvent systems (water, 0.03 M aqueous sodium acetate buffer, 0.1 mM aqueous NaOH and dimethylsulfoxide). In the case of solutions in bare water, the shape of the UV spectrum depends drastically on concentration, probably because of changes in the species distribution of GBG as a function of concentration. The spectrum comprises one maximum at ca. 200 nm, and between ca. 250 nm and 400 nm an absorption region with distinctly higher absorbance. In the case of aqueous sodium acetate as well as NaOH solutions, one strong maximum can be detected (at ca. 285-288 nm and 332-337 nm, respectively). In both cases, the maximum occurs at constant wavelength, being independent of concentration. In dimethylsulfoxide, the spectrum of GBG contains an absorption band at distinctly higher wavelengths (λ_{max} 354 nm) than in any one of the aqueous solvents studied, indicating that solvent effects are considerable in the UV spectrum of GBG. In no case, distinct absorption could be detected at wavelengths higher than 400 nm. The results indicate that if aqueous media are used as eluents in HPLC analyses of bis(amidinohydrazones) or as solvents in direct UV analysis, they must be buffered.

INTRODUCTION

The bis(amidinohydrazones)[1] of various glyoxals are important polyamine antimetabolites which makes these much-studied compounds important tools in the study of polyamine metabolism and functions.[2-13] Glyoxal bis(amidinohydrazone) (GBG) and methylglyoxal bis(amidinohydrazone) (MGBG) are also potent antileukemic agents. Detection in the HPLC and micellar electrokinetic capillary chromatography analyses[14-23] of this class of compounds are based on their strong UV absorption, but almost nothing is actually known of their UV spectroscopic properties. Also because of the fact that NMR studies[2, 24-29] have led to important novel findings on the tautomerism and isomerism of the bis(amidinohydrazones) as well as on other aspects of their chemistry, we considered also detailed UV/VIS studies on the compounds worthwhile. Thus, we now report an investigation on the UV and VIS spectral

properties of the parent compound of the class, glyoxal bis(amidinohydrazone) (GBG, Fig. 1).

EXPERIMENTAL

GBG free base[30] was used throughout the study, dissolved in four different solvents: water, 0.03 M aqueous sodium acetate buffer (pH 4.3), 1 mM aqueous NaOH solution, and dimethylsulfoxide [DMSO; E. Merck, Darmstadt, Germany, spectroscopy grade (Uvasol)]. In the case of each solvent, spectra were recorded using nine different concentrations (0.2 μM - 0.1 mM).

All spectra were recorded using a Hitachi U-2000 double-beam UV/VIS spectrophotometer and employing 10.00 mm cuvettes (Quarzglas, Suprasil®). Hitachi Model U-2000 spectroscopy data station software, U-2000 manager (Revision 2) software was used. The wavelength range studied was 195 nm - 450 nm. The scan speed used was 200 nm/min and the wavelength interval 1.0 nm. First derivatives of the spectra were calculated using the formula $dA/d\lambda$. All calculations were performed by using the program Microsoft Excel Version 4.0.

RESULTS AND DISCUSSION

In the case of aqueous solutions, the shape of the UV spectrum of GBG was found to drastically depend on the concentration (Fig. 2). The spectrum comprises one maximum at approximately 200 nm and, at longer wavelengths (between ca. 250 and 400 nm), an absorption region with distinctly higher absorbance. The shape of the spectrum in this region and the changes observed on increasing concentration suggest that this region may actually contain two or perhaps even more overlapping absorption bands. At the lowest concentrations studied the highest absorption maximum resided at approximately 288-291 nm, while at higher concentrations it resided at markedly longer wavelengths (324-329 nm).

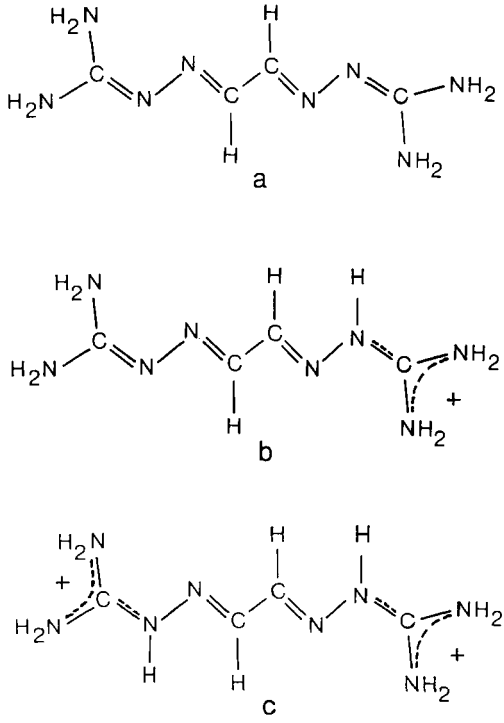

FIG. 1. The structures of GBG free base (a), monocation (b) and dication (c), as revealed by single-crystal X-ray diffraction and NMR spectroscopy.

This phenomenon might be due to a relative increase of the ε at the λ_{max} of a band at ca. 324-329 nm, as compared to the corresponding ε value at 288-291 nm. A more probable explanation, however, is that the spectral changes are only due to changes in the species (free base, monocation, dication) distribution of GBG as a function of concentration. On the basis of what is known about the solution equilibria of GBG[31], it is indeed obvious that, according to the law of mass action, the species distribution of the compound is drastically changed when going from 0.2 μM to 0.1 mM solutions. Thus, on increasing the concentration of the compound, the percentage of the free base species should increase and that of the cationic species should decrease. Further support for the species

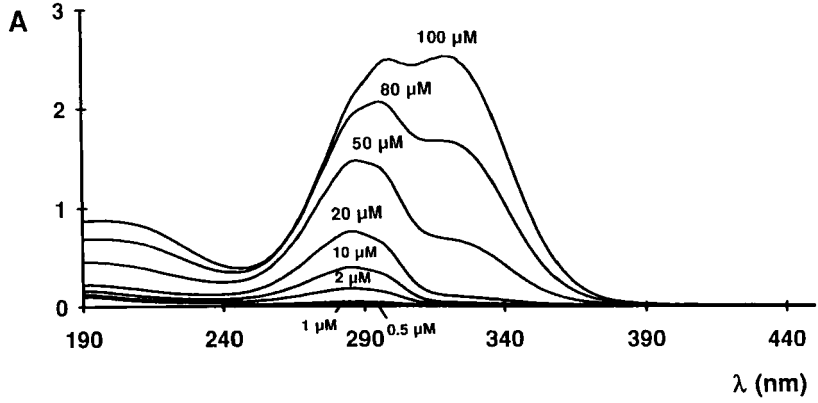

FIG. 2. UV spectra of different concentrations (0.5 - 100 µM) of GBG free base dissolved in water.

distribution theory comes from the finding that neither at 286 nm nor at 319 nm does the absorbance in aqueous solutions depend linearly on concentration (data not shown).

The species distribution theory lends strong support from the finding that in the case of the acetate buffer solution, at the pH of which (pH 4.3) GBG exists practically exclusively in the form of the dication species, there is a strong absorption maximum in the UV spectrum of GBG at 285-288 nm, but no detectable maximum and actually nearly no absorption at 324-329 nm (Fig. 3). In the case of GBG dissolved in 1 mM NaOH solution, there is a strong maximum in the spectrum at about 332-337 nm, but far less prominent absorption and no maximum at 285-290 nm (Fig. 4).

In the case of aqueous NaOH and sodium acetate buffer solutions, first derivative spectra indicate clearly that the maximum occurs at constant wavelength, being independent of concentration. When water alone is used as the

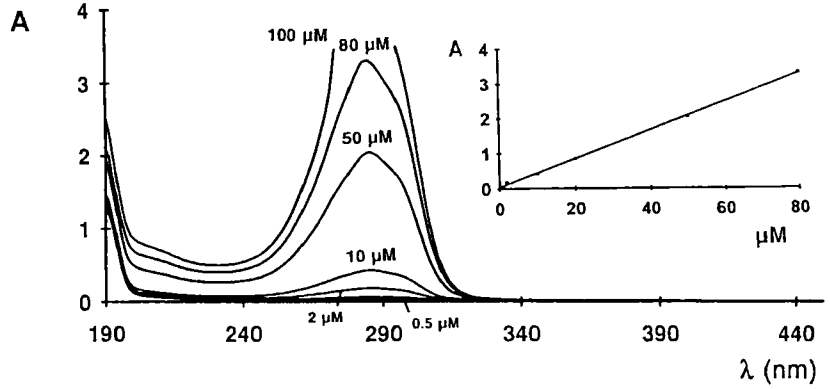

FIG. 3. UV spectra of different concentrations (0.5 - 100 µM) of GBG dissolved in 0.03 M aqueous sodium acetate buffer (pH 4.3). The linearity of the method is displayed in the insert (A = 0.041 C/µM + 0.039; correlation coefficient = 0.999).

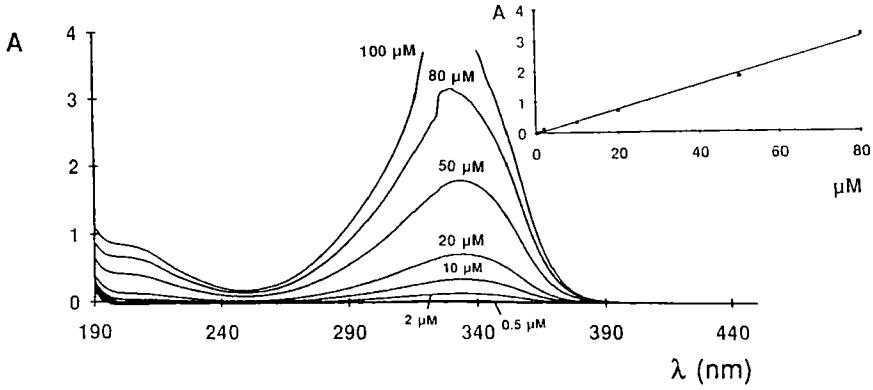

FIG. 4. UV spectra of different concentrations (0.5 - 100 µM) of GBG dissolved in 1 mM aqueous sodium hydroxide solution. The linearity of the method is displayed in the insert (A = 0.039 C/µM - 0.022; correlation coefficient = 0.997).

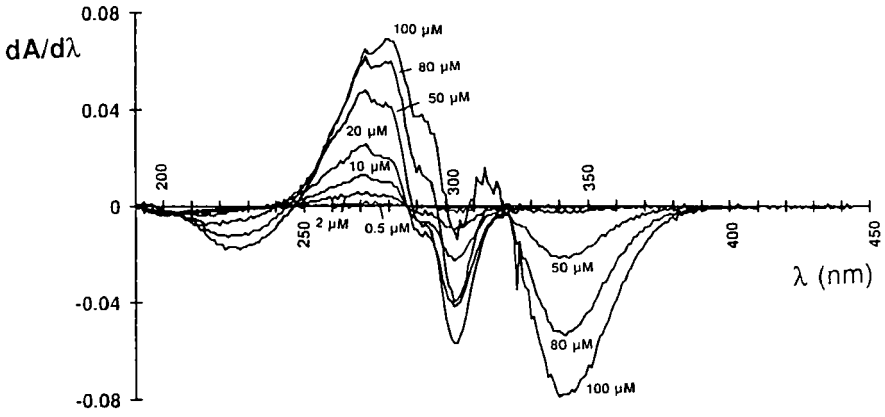

FIG. 5. First derivatives of the UV spectra of different concentrations (0.5 - 100 μM) of GBG free base dissolved in water.

solvent, derivative spectra clearly indicate a concentration dependence of the λ_{max} value (Fig. 5).

We also 'titrated' samples of GBG (20 ml of 50 μM aqueous solution) by adding to each sample a 5-ml volume of an aqueous solution containing varying amounts of HCl (600 μM), and determined in each case the UV spectrum of the mixture. The results are shown in Fig. 6. Isosbestic points can be observed.

In DMSO, the spectrum of GBG contains an absorption band at distinctly higher wavelengths (λ_{max} 354 nm) than in any one of the aqueous solvents studied (Fig 7). Thus, solvent effects are considerable in the UV spectra of GBG.

In the case of each solvent employed, one spectrum of GBG was also recorded between 195 and 900 nm, but no signals were detected above 400 nm. In order to study the applicability of UV spectroscopy for the quantitative analysis of GBG, we studied the linearity of absorbance versus concentration

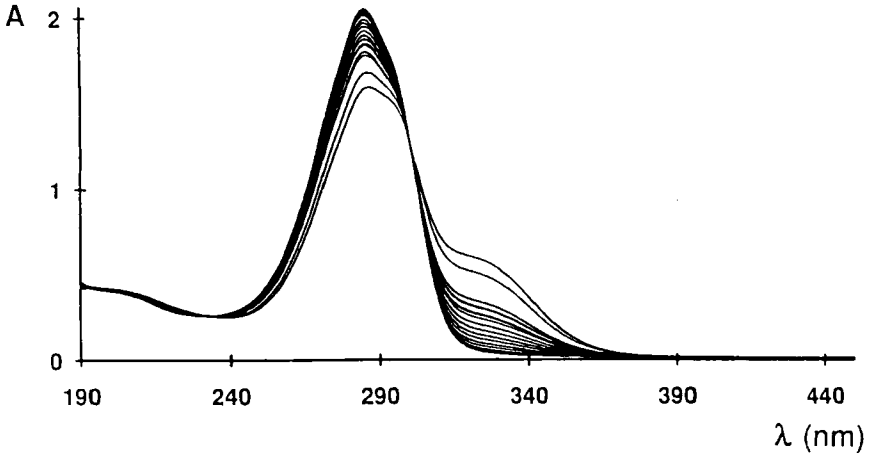

FIG. 6. 'Titration' of GBG free base with HCl in aqueous solution. Samples of GBG (20 ml of 50 μM aqueous solution) were titrated by adding to each sample a 5-ml volume of an aqueous solution containing varying amounts of HCl (600 μM).

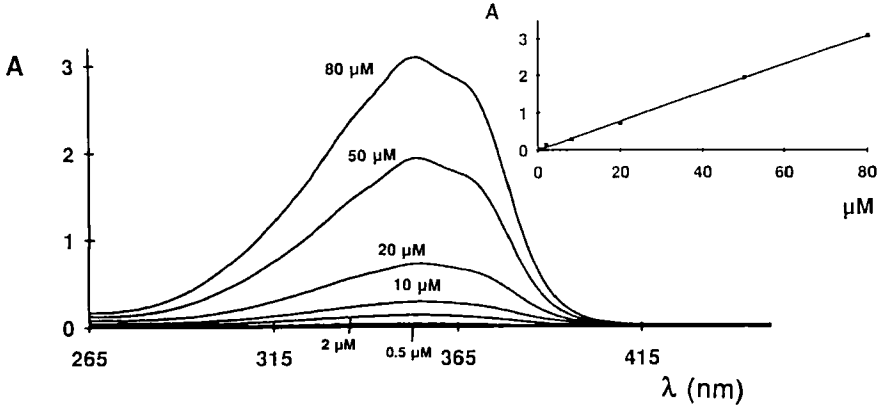

FIG. 7. UV spectra of different concentrations (0.5 - 80 μM) of GBG free base dissolved in DMSO. The linearity of the method is displayed in the insert (A = 0.039 C/μM + 0.003; correlation coefficient = 0.999).

TABLE 1.

λ_{max} and ε Values of GBG.

Solvent	λ_{max} (nm)	ε (l mol^{-1} cm^{-1})[a]
acetate buffer	285-288	b
NaOH	332-337	3.5×10^4
DMSO	354	3.7×10^4

[a] The values of ε given are the means of values obtained for three different concentrations (10 μM, 20 μM and 50 μM).

[b] In the case of the acetate buffer, the values of ε varied considerably and in an irregular fashion with concentration. Further studies are required on this point.

plots for GBG solutions in the aqueous sodium acetate buffer, in the aqueous NaOH solution and in DMSO (see figs. 3, 4 and 7). In each case, the method was linear in the range studied (0.5 - 80 μM). Values of λ_{max} and ε for GBG in various solvent systems are given in Table 1.

The results obtained have important consequences concerning the quantitative analysis of bis(amidinohydrazones) by HPLC using UV detection, or by direct UV methods: they indicate that if aqueous media are used as eluents or solvents, they must be buffered.

REFERENCES

1. 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 and EM for ethyl methyl). The term 'bis(amidinohydrazone)' is, however, more appropriate The Chemical Abstracts' systematic name for glyoxal bis(amidino-

hydrazone) is 2,2'-(1,2-ethanediylidene)bis(hydrazinecarboximidamide), and other congeners are named analogously.

2. 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.

3. 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.

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

5. 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.

6. Elo, H., Mutikainen, I., Alhonen-Hongisto, L., Laine, R., 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.

7. 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.

8. 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.

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

10. Williams-Ashman, HG., Schenone, A. Methyl Glyoxal Bis(guanylhydrazone) as a Potent Inhibitor of Mammalian and Yeast S-Adenosylmethionine Decarboxylases. Biochem. Biophys. Res. Commun. 1972; 46: 288.

11. Seppänen, P., Fagerström, R., Alhonen-Hongisto, L., Elo, H., Lumme, P., Jänne, J. Glyoxal Bis(guanylhydrazone) as an Inhibitor of Polyamine Biosynthesis in Tumour Cells. Biochem. J. 1984; 221: 483.

12. Corti, A., Dave, C., Williams-Ashman, HG., Mihich, E., Schenone, A. Specific Inhibition of the Enzymic Decarboxylation of S-Adenosylmethionine by Methylglyoxal Bis(guanylhydrazone) and Related Substances. Biochem. J. 1974; 139: 351.

13. Alhonen-Hongisto, L., Fagerström, R., Laine, R., Elo, H., Jänne, J. Different Efflux Rates May Determine the Cellular Accumulation of Various Bis(guanylhydrazones). Biochem. J. 1984; 221: 273.

14. Rosenblum, M.G., Loo, T.L. High-Performance Liquid Chromatographic Analysis of the Anticancer Agent Methylglyoxal Bis(guanylhydrazone) (MGBG, NSC-32946) in Biological Fluids. J. Chromatogr. 1980; 183: 363.

15. Marsh, K.C., Repta, A.J., Sternson, L.A. High-Performance Liquid Chromatographic Assay for Methylglyoxal Bis(guanylhydrazone) (Methyl GAG) in Plasma and Urine. J. Chromatogr. 1980; 187: 101.

16. Roboz, J., Wu, K.T., Hart, R.D. Determination of Methylglyoxal-Bis(guanylhydrazone) in Body Fluids by Ion-Pair Chromatography. J. Anal. Toxicol. 1980; 4: 127.

17. Gildersleeve, D.L., Tobes, M.C., Natale, R.B. Rapid Analysis for Methylglyoxal Bis(guanylhydrazone) by Reversed-Phase Ion-Pair Liquid Chromatography. Clin. Chem. 1985; 31: 1979.

18. Wallace, H.M., Cameron, G.A. Determination of Methylglyoxal Bis(guanylhydrazone) in Cells in Culture Using High-Performance Liquid Chromatography. J. Chromatogr. 1986; 380: 122.

19. Negro, A., Fernández, A.E., Ortiz, A., Balaña, R., Ordóñez, D. Ion-Paired Liquid Chromatographic Determination of Phenylglyoxal Bis(guanylhydrazone) in Serum and Urine. J. Liq. Chromatogr. 1990; 13: 3849.

20. Negro, A., Méndez, R., Martin-Villacorta, J., Ortiz, A.I., Ordóñez, D. A Simplified Method for the Determination of Methylglyoxal Bis(guanylhydrazone), MGBG, in Biological Fluids by Reversed-Phase Ion-Pair HPLC. J. Liq. chromatogr. 1991; 14: 2409.

21. Lukkari, P., Jumppanen, J., Jinno, K., Elo, H., Riekkola M.-L. Determination of Bis(amidinohydrazones) by Micellar Electrokinetic Capillary Chromatography. J. Pharm. Biomed. Anal. 1992; 10: 561.

22. Koskinen, M.V., Elo, H.O., Lukkari, P., Riekkola, M.-L. Separation of the Antileukemic Drug Mitoguazone and Seven Closely Related

Bis(amidinohydrazones) ['Bis(guanylhydrazones)'] by High-Performance Liquid Chromatography in the Abstract Book of the 'XII Helsinki University Course of Drug Research' (Espoo, June 17-18, 1993), p.47.

23. Koskinen, M., Elo, H., Lukkari, P., Riekkola, M.-L. HPLC Analysis of Aliphatic bis(guanylhydrazones) in human blood serum in the Abstract Book of the 'Finnish Chemical Congress 1993' (Helsinki, November 2-4, 1993), p. 41.

24. 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.

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

26. 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.

27. 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.

28. Soljamo, K. and Elo, H. Studies on the Spin-Lattice Relaxation Times of the Carbons of the Investigational Antileukemic Drug and Enzyme Inhibitor Methylglyoxal Bis(amidinohydrazone) ['Methylglyoxal Bis(guanylhydrazones)'] and its Dialkylglyoxal Analogs. Spectroscopy Lett. 1992; 25: 1315-1332.

29. Elo, H. Substituent Effects in the Carbon NMR of Aliphatic Bis(amidinohydrazones) ['Bis(guanylhydrazones)']. Construction of a set of Quantitative Empirical Rules. Unambiguous Assingment of Several Previously Unassinged Carbon Resonances. Spectroscopy Lett. 1992; 25: 1267-1296.

30. Thiele, J., Dralle, E. Zur Kenntniss des Amidoguanidins. I. Condensationsproducte des Amidoguanidins mit Aldehyden und Ketonen der Fettreihe. Liebig's Ann. Chem. 1898; 302: 275.

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

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