Biochemical Characterization of Propylglyoxal Bis(guanylhydrazone). Facile Synthesis of Monoalkylglyoxal Bis(guanylhydrazones)


* Division of Inorganic Chemistry, Department of Chemistry, University of Helsinki, Vuorikatu 20, SF-00100 Helsinki 10, Finland
** Department of Biochemistry, University of Helsinki, Unioninkatu 35, SF-00170 Helsinki 17, Finland

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Propylglyoxal Bis(guanylhydrazone). Ethylglyoxal Bis(guanylhydrazone). Adenosylmethionine Decarboxylase Inhibition, Tumor Cells, Cellular Uptake

Propylglyoxal bis(guanylhydrazone) sulfate, a novel analog of the well-known antileukemic drug methylglyoxal bis(guanylhydrazone), has been prepared from 2,2-dibromopentanal, and the compound has been characterized biochemically. Although it is a powerful inhibitor of S-adenosylmethionine decarboxylase, its $K_v$ value (0.2 $\mu$M) is considerably higher than that of ethylglyoxal bis(guanylhydrazone) (0.06 $\mu$M). The compound is only poorly taken up by tumor cells, and its accumulation is not stimulated by a prior exposure of the tumor cells to difluoromethylornithine, a compound that causes polyamine depletion. Thus, the uptake characteristics of the compound are similar to those of ethylglyoxal bis(guanylhydrazone), but in striking contrast to those of methylglyoxal and glyoxal bis(guanylhydrazones). Since the configuration of the double bonds in glyoxal, methylglyoxal and propylglyoxal bis(guanylhydrazones) has been shown to be identical, the different uptake characteristics are probably only due to differences in side chain size and/or hydrophobicity.

Introduction

Since the discovery of the antileukemic activity of certain bis(guanylhydrazones) by Freedlander and French [1], a great number of derivatives were screened for their antiproliferative activity (for references see [2] and [3]). In contrast to MGBG [4] and GBG [4], which displayed a distinct antileukemic activity, minor modifications, such as dialkylation of the glyoxal portion resulted in the loss of the anti-tumor activity [2, 3]. The finding that MGBG powerfully inhibited S-adenosymethylimine decarboxylase and hence the synthesis of spermidine and spermine [5], renewed the interest in this class of substances. It soon became evident that no relationship existed between the inhibition of S-adenosymethylimine decarboxylase and the antileukemic activity. Thus, ethyl and dimethyl derivatives of GBG were much more effective inhibitors of the enzyme [6, 7] but were reported to be devoid of antitumor activity [2]. The lack of antileukemic activity may be attributable to the fact that EGBG [4] and dimethylglyoxal bis(guanylhydrazone) are poorly taken up by mammalian cells [7, 8] via the putative polyamine carrier [9] inducible by polyamine depletion [10]. In any event, it appears that compounds such as EGBG are much more specific inhibitors of polyamine biosynthesis than the parent compound GBG and do not display the profound antimitochondrial activity typical of MGBG [8, 11–13].

Thus, when going from GBG to MGBG, or from MGBG to EGBG, the addition of one single methylene group to the side chain of the molecule drastically changes the biochemical characteristics of the compound. Therefore, we considered it worthwhile to study the biochemical properties of the next compound of the series, namely PGBG [4]. PGBG has not been previously reported. In this paper, we describe a facile synthesis that was developed for PGBG and its analogs (including EGBG), as well as the results of enzyme inhibition and cellular uptake studies on PGBG.

Abbreviations: GBG, glyoxal bis(guanylhydrazone); MGBG, methylglyoxal bis(guanylhydrazone); EGBG, ethylglyoxal bis(guanylhydrazone); PGBG, propylglyoxal bis(guanylhydrazone).

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Materials and Methods

Biochemical measurements

S-adenosylmethionine decarboxylase inhibition was studied according to previously published procedures [7, 14].

Mouse L1210 leukemia cells were grown in Gibco’s medium RPMI 1640 supplemented with 5% (v/v) of pooled human serum (Finnish Red Cross Transfusion Service), 2 mM glutamine, and 50 mg of the sodium salt of penicillin G and 50 mg of streptomycin per litre.

Determination of intracellular PGBG concentrations was based on the inhibition of S-adenosylmethionine decarboxylase and was performed according to the method originally developed for MGBG by Seppänen et al. [15].

Melting points

Melting points of the products were measured using an Electrothermal melting point apparatus. Obviously because of decomposition, they were greatly dependent on the rapidity of heating and the amount of substance in the capillary.

Synthesis of starting materials

2,2-Dibromobutanal (la) was made from butyraldehyde and 2,2-dibromopentanal (lb) from valeraldehyde according to the method of Verhe et al. [16], with the exception that the dibromoaldehydes appeared to be powerful irritants and lachrymators, and great care should be exercised in handling them. Their smell is not strong enough to act as a warning signal. All handling of them should be performed in a good hood.

Synthesis of PGBG sulfate

(a). 4.54 g (33.4 mmol) of aminoguanidine bicarbonate (EGA-Chemie) was dissolved in 10.6 ml of 2.5 M H2SO4 and 20 ml of H2O. This solution was added to 4.07 g (16.7 mmol) of 2,2-dibromopentanal and, with continuous stirring, the mixture was warmed in a water bath up to about 55 °C. A white precipitate was initially formed, but it was dissolved on heating while a yellow color developed. The solution was allowed to cool to room temperature and was stirred further for about 4 hours. It was left overnight at room temperature and then cooled in an ice/water bath. The thin white needles formed were filtered off, washed with diethylether and dried in vacuo. The yield was 3.00 g (52%). Recrystallization of 2.23 g of the product from boiling water gave a yield of 1.92 g (86% recovery). M.p. 222—224 °C (decomp.). IR (in KBr): broad bands at 1000—1200(s), 1460(w), 1590(s), 1675(s), 2600—3700(s) cm⁻¹. Crystal water content was determined at 150 °C and was consistent with the formula C7H16N8'H2SO₄·2H2O. This crop was used for crystal growing for the X-ray study.

(b). Another batch was prepared so that the 2,2-dibromopentanal was first dissolved in a mixture of ethanol and water. The reaction was performed essentially as above, but no precipitate was formed (even after standing overnight) before the solution was cooled in an ice/water bath.

Synthesis of EGBG sulfate

4.85 g (35.6 mmol) of aminoguanidine bicarbonate (EGA-Chemie) was dissolved in 71.1 ml of 0.5 M aqueous H2SO4, and this solution was added to 4.10 g (17.8 mmol) of 2,2-dibromobutanal. 95 ml of ethanol was then added, and the solution was heated for 2 h at 40—60 °C. After standing overnight at room temperature, the mixture was cooled in an ice/water bath. The precipitate formed was filtered off, washed with water and dried in vacuo. Thus, 3.88 g (66%) of a white powder were obtained. The product was recrystallized from water. M.p. 225—228 °C (decomp.). Found: C, 22.03%; H, 5.99%; N, 34.0%. Calculated for C6H14N8H2SO₄·1.75H2O: C, 21.98%; H, 5.99%; N, 34.2%.

Results and Discussion

EGBG and higher alkylglyoxal bis(guanylhydrazones) are commonly prepared either from the corresponding alkylglyoxals or their acetals or from the corresponding dichloromethyl alkyl ketones. The syntheses of these starting materials in pure form are, however, usually tedious [17]. The problems encountered in these syntheses have been briefly reviewed by Podrebarac et al. [17].

Verhe et al. [16] reported a facile one-pot synthesis of 2,2-dihaloaldehydes (I). We presumed that those
compounds could possibly be used as starting materials of alkylglyoxal bis(guanylhydrazones) (II):

\[
R - C\text{H}_2\text{CHO} + 2\text{NH}_2\text{NH-C} = N\text{-NH-C} + \text{H}_2\text{O} \rightarrow R = \text{CH}_3(\text{CH}_2)_2, X = \text{Br}
\]

The sulfates of PGBG and EGBG were made according to this sequence. Although the 2,2-dibromoaldehydes employed are not soluble in water, water was found to be a good medium for the reaction. Alternatively, the aldehydes can be dissolved in mixtures of water and ethanol.

Fig. 1 shows that both EGBG and PGBG act as competitive inhibitors of S-adenosylmethionine decarboxylase in respect to the substrate. The \(K_i\) value for PGBG was 0.2 \(\mu\)M and that for EGBG about 0.06 \(\mu\)M. In comparison to MGBG and GBG [11], PGBG was relatively poorly taken up by tumor cells (Table I) and just like in the case of EGBG [8] its accumulation was not stimulated by a prior exposure of the tumor cells to difluoromethylornithine (Table I).

The identity of the PGBG sulfate dihydrate synthesized was confirmed by a single crystal X-ray crystallographic study, the results of which will be published elsewhere [18]. The carbon-nitrogen double bonds between the aminoguanidine moieties and the propylglyoxal part of the molecule were found to have the same configuration as the corresponding bonds in GBG dihydrochloride [19] and MGBG dihydrochloride dihydrate [20] are known to have.

Thus, the poor uptake of PGBG as compared to MGBG and GBG cannot be due to any configurational differences between PGBG and MGBG or GBG. Instead, it may be due to the bulk size and/or the hydrophobicity of the propyl group of PGBG. The bulk size of the propyl side chain most probably also lies behind the fact that PGBG is a considerably weaker inhibitor of S-adenosylmethionine decarboxylase than is EGBG.

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The systematic names of the compounds are: GBG = 2,2'-(1,2-ethanediylene)bis(hydrazinecarboximidamide), MGBG = 2,2'-(1-methyl-1,2-ethanediylene)bis(hydrazinecarboximidamide), EGBG = 2,2'-(1-ethyl-1,2-ethanediylene)bis(hydrazinecarboximidamide), and PGBG = 2,2'-(1-propyl-1,2-ethanediylene)bis(hydrazinecarboximidamide).