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On the Crystallography and Stereochemistry of Antiepileptic Drugs

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Abstract

Prompted by investigations by the present authors into the stereochemistry of antiepileptic drugs, papers have appeared which attempt to link structural differences in benzodiazepines with differences in their anticonvulsant activities. Some of these papers have not made reference to important pharmacological data in this area and have somewhat misinterpreted the crystallographic conclusions drawn by the present authors. The present authors wish to clarify the relationship of molecular stereochemistry to anticonvulsant properties.

In their papers on the crystal structures of nitrazepam and oxazepam, Gilli, Bertolasi, Sacerdoti & Borea (1977, 1978 respectively) (hereinafter referred to as GBSB) address themselves to two questions: (1) the validity of the 'hypothesis of Camerman & Camerman (1974) of a common steric pattern of most anticonvulsant agents such as benzodiazepines, hydantoins and barbiturates' and (2) whether small geometrical changes observed in the molecular frame of benzodiazepines of known structure are relevant to biological activity.

With regard to the first question, GBSB state that the hypothesis 'needs to be supported by neurophysiological data; that is by evidence of the fact that the different anticonvulsants are acting on the same part of the central nervous system. Such evidence is lacking.'

Neurophysiological evidence of the kind that GBSB refer to does exist and lends strong support to our hypothesis. Since publication of the crystal structures of diphenylhydantoin and diazepam (Camerman & Camerman, 1970), there have been four sets of experiments undertaken to investigate whether the similarities found in the two drugs' stereochemistries, and which were proposed to be responsible for their one similar pharmacological property, antiepileptic activity, would be maintained under physiological conditions.

In the first of these experiments Schussler (1971) measured the binding of diazepam to thyroxine-binding globulin (TBG). Since diphenylhydantoin is well known to be a competitive inhibitor for thyroxine binding to TBG, Schussler reasoned that if the crystal conformational similarities are maintained in solution, then diazepam should also compete with thyroxine in binding to TBG. His results showed that, although previously unsuspected, diazepam is an even stronger competitive inhibitor than diphenylhydantoin. Although binding to TBG is not directly related to antiepileptic activity, this result strongly supports, in a physiological environment, the stereochemical conclusions drawn from the crystal structures of the two drugs.

The other three experiments are completely neurophysiological in nature and all demonstrate the close similarities between effects of diphenylhydantoin and diazepam on specific regions of the brain. It had been shown that diphenylhydantoin augments inhibitory cerebellar Purkinje-cell discharge rates (Julien & Halpern, 1971), and because the stereochemical results suggested a possible common receptor for these drugs, Julien (1972) tested the effect of diazepam on the cat cerebellum. He found that diazepam causes the same cerebellar response as did diphenylhydantoin.

In another study, after it had been observed that diphenylhydantoin increases post-synaptic inhibition in the cerebral cortex (Raabe & Ayala, 1976), Raabe & Gumnit (1977) investigated the action of diazepam on the same brain area. They found that diazepam also increases cerebral cortical post-synaptic inhibition and, in fact, suggested that this activity may be the anticonvulsant mechanism of action for both drugs.

The fourth and latest experiment was made possible by the recent discovery of specific benzodiazepine receptors in the brain. The marked similarities in diphenylhydantoin and diazepam molecular conformations led Mimaki, Deshmukh & Yamamura (1980) to study the effects of diphenylhydantoin both *in vivo* and *in vitro* on benzodiazepine receptors in rat brain. Their biochemical and ultrastructural data reveal that diphenylhydantoin is a competitive inhibitor of

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benzodiazepine receptor binding, and suggest that it acts at the same sites as benzodiazepines both in the cerebral cortex and cerebellum.

These experiments lend very powerful support to the concept of common sites of action for the chemically different anticonvulsant drugs. They also demonstrate that, in this research area at least, stereochemical results obtained from crystal-structure analysis are still relevant under physiological conditions.

The second question has been addressed by GBSB in terms of their own crystal-structure determinations of benzodiazepines and by an analysis of the conformational similarities and differences of those benzodiazepines, active and inactive as anticonvulsants, whose structures have been reported. They conclude 'that no correlation between molecular geometry and activity can be established within this class of drugs'. Because GBSB report that the impetus for their work in this area derives from our correlation of antiepileptic activity with specific stereochemical parameters in a number of anticonvulsant drugs, contrasted with the report of close geometric resemblance between diazepam and two inactive benzodiazepines (Sternbach, Sancilio & Blount, 1974, hereinafter referred to as SSB), we would like to add some observations on this topic.

The rationale behind our crystal-structure analysis program on antiepileptic drugs has been to examine chemically different compounds and to correlate common three-dimensional stereochemical features with common pharmacological activity. Such correlations, if any are possible, can then be used to identify stereochemical parameters which can form the basis for design of new drugs. Once these new drugs have been designed with retention of the apparently activity-determining stereochemical features, all possible changes in substituents attached to the framework can and should be made in order to maximize the factors which govern the ability of the drug to get to the receptor (solubility, transport properties, *etc.*) and to minimize unwanted side effects. The focus of both SSB and GBSB's crystallographic investigations were somewhat different. SSB compared the molecular geometry of two inactive benzodiazepines with that of diazepam, found all three to have similar overall conformations, and presented their results as rebuttal to our conclusions. GBSB compared the geometries of the molecular frame of benzodiazepines which differ from diazepam in the nature of one or two substituents and which are qualitatively similar to diazepam in anticonvulsant activity, and also found them to be similar. The small differences in overall conformation between diazepam, the inactive molecules studied by SSB, and nitrazepam and oxazepam have led GBSB to the conclusion quoted above.

Both the procedures and criticisms of these groups are open to question. Firstly, it is obvious that small substituent changes on the relatively rigid benzodiazepine molecular frame will not dramatically alter the gross conformation. Secondly, it is not the overall conformation of diazepam which was found to be common to several chemically different anticonvulsants, but the possession of certain hydrophobic areas and electrophilic functions which occupy relatively similar positions in space, regardless of the gross conformational features of the molecules. Molecular alterations which result in the loss or transformation of these specific stereochemical features may be expected to affect activity; small changes in molecular framework which leave the aforementioned features relatively intact may not.

What has been overlooked by GBSB (and SSB) is that the two inactive benzodiazepine compounds they cite differ from diazepam in aspects of the stereochemical framework which we had suggested to be necessary for anticonvulsant activity. One compound lacked the chlorine atom substituted at C(7), a factor which may be necessary to make diazepam's ring system similar in space-filling characteristics to that of diphenylhydantoin (or possibly prevent oxidation at this site). The second compound had a fluorine atom substituted on the other phenyl ring, drastically altering the lipophilic nature of that part of the molecule. On the other hand, the active benzodiazepines investigated by GBSB all maintain the essential stereochemical framework and differ only by substitution of atoms on that frame.

In light of these facts, GBSB's conclusions that 'these small conformational changes are not correlated in any way with their anticonvulsant activity,' and further, that 'no correlation between molecular geometry and activity can be established within this class of drugs,' are somewhat misleading. Since there are specific stereochemical features which correlate well with anticonvulsant properties for the benzodiazepines and many other drugs and are implicated in drug–receptor interaction (Camerman & Camerman, 1980), we feel that there are two specific conclusions that can be drawn from crystallographic investigations of the benzodiazepines. First, substitutions on the benzodiazepine molecular framework which affect the chemical properties of the features that are correlated with activity are likely to drastically alter anticonvulsant action. (The 'chemical' half of the notion of 'stereochemistry' is just as important as the 'steric' half.) Secondly, alterations to other parts of the molecule are not likely to affect drug–receptor binding but may markedly alter chemical properties of the drug such as solubility, hydrophobicity, polarizability and reactivity with respect to enzymatic or chemical inactivation. Chemical substitutions may thus greatly influence absorption, transport and distribution of the drug and affect its measurable pharmacological activity by altering the ability of the drug to get to its site of action.

All of the above chemical factors, as well as geometric considerations, must be taken into account when attempting to relate pharmacological activity to stereochemical parameters.

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The Structure of 2,6-Dimethylpiperidinium Dithiosalicylate. X-ray Diffraction Study at 200 K and Neutron Diffraction Study at 20 K

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Abstract

2,6-Dimethylpiperidinium dithiosalicylate, $C_7H_{16}N^+.C_7H_5OS_2^-$, orthorhombic, $Pna2_1$, $Z = 4$, $a = 13{\cdot}966$ (4), $b = 10{\cdot}894$ (3), $c = 10{\cdot}252$ (3) Å, $d_c = 1{\cdot}207$, $d_m = 1{\cdot}227$ Mg m^{-3}, $V = 1559{\cdot}8$ Å^3 at 293 K. The X-ray structure at 200 K ($R = 0{\cdot}0439$, $R_w = 0{\cdot}0474$ for 1809 reflections) is compared with the neutron diffraction results at 20 K ($R = 0{\cdot}080$, $R_w = 0{\cdot}077$ for 1214 reflections). The ions are linked by one normal NH···S hydrogen bond and one symmetrically bifurcated NH···S hydrogen bond. The dithiosalicylate ion contains a very short OH···S hydrogen bond with an O···S distance of 2·848 (4) Å (X-ray). This is the shortest hydrogen bond of this class so far observed.

Introduction

In the course of their spectroscopic studies of thioamides, Fulea & Krueger (1977) used the Wilgerodt–Kindler reaction to prepare a number of compounds. The reaction mixture contains the appropriate aldehyde and amine, and elemental sulphur. The reaction of 2,6-dimethylpiperidine with salicylaldehyde in the presence of elemental sulphur produced a dark-red compound with a yield of about 5%. The compound was thought to be 1-(2′-hydroxythiobenzoyl)-2,6-dimethylpiperidine. It had been anticipated that the steric hindrance introduced by the two methyl groups would rotate the piperidine ring out of the plane of the *o*-hydroxythiobenzoyl group. This would be the first reported example of a thioamide with no conjugation between the nitrogen lone pair and the thiocarbonyl and would provide a model for the transition state for rotation about the C–N bond in compounds of this type.

Spectroscopic data seemed consistent with this interpretation. Both IR and NMR data showed the presence of a strong intramolecular hydrogen bond. NMR signals from the N–CH protons remained coalesced even at low temperatures. However, crystal structure analysis has shown the compound to be the 2,6-dimethylpiperidinium salt of dithiosalicylic acid. Although xanthic ($ROCS_2H$) and dithiocarbamic (R_2NCS_2H) acids are commonly used as ligands in organic chemistry, dithio acids are seldom encountered; however, known examples are dark red in colour. A standard method of synthesis (Reid, 1962) involves

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