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The influence of the ethyl group on the conformational flexibility of aminoglutethimide

Aminoglutethimide [AG, 3-(4-aminophenyl)-3-ethylpiperidine-2,6-dione] is extensively used for the treatment of oestrogen-dependent breast cancer [1]. Its principal action is thought to be inhibition of the enzyme aromatase (oestrogen synthetase) whereby the androgens androstenedione and testosterone are converted respectively into the oestrogens oestrone and oestradiol [2]. AG is a reversible, competitive inhibitor of the binding of these natural substrates to the enzyme binding site, and exhibits a type II binding spectrum [3] characteristic of nitrogenous inhibitors of cytochrome P-450 enzymes of which aromatase is an example, and indicating binding of the basic amino function of the drug to one of the haem coordination sites. Further details of the mode of binding of AG are unknown, in particular the disposition of the various functional groups in the enzyme binding site. Such information could help to guide the selection of analogues or related compounds having improved binding affinity for aromatase.

AG has a chiral centre at C-3 of the glutarimide (piperidine-dione) ring (here also C2). The enantiomers differ markedly in their inhibitory activity towards aromatase. The *R*-enantiomer has nearly 40-fold the activity of the *S*-enantiomer [3]. Such a marked dependence of inhibitory activity on stereochemistry indicates that the chiral centre and its immediate environment is intimately involved with the binding of the inhibitor to the enzyme [4]. The quaternary centre at C-3 is in a sterically crowded environment and it seems likely that replacement of the angular ethyl function by, for example, hydrogen could involve a substantial change in the ground state conformation (the authors thank Professor A. B. Foster for this suggestion). Such a change might well alter the affinity of the inhibitor for aromatase. Thus, replacement of ethyl by hydrogen leads to at least a 10-fold drop in inhibitory potency expressed as the IC_{50} value (concentration required to give 50% inhibition) as shown [5] in a comparative study of AG and of analogues having the ethyl substituent replaced by hydrogen or different alkyl groups. The methyl analogue had *ca.* 30% of the activity of AG.

No molecular structural information is as yet available for AG itself, or any of its derivatives (a full search has been conducted on the Cambridge molecular structure data base). In this study, the techniques of molecular modelling were used to investigate the major modes of conformational flexibility and low-energy conformers of AG and two analogues with the ethyl substituent replaced by methyl and hydrogen respectively.

Methods

The energy of a particular conformation was calculated as the sum of non-bonded and torsional contributions:

$$E_{TOT} = E_{N-B} + E_{TORS}$$

Parameters are as previously described [6].

The starting model for AG was constructed by means of the interactive computer graphics system MOLEC [7], using standard geometric considerations. The co-ordinates for the glutarimido ring were taken directly from the crystal structure of thalidomide [8], which resembles AG in having an aromatic group substituted at the equivalent position. The approximately half-chair pucker reported for this ring was kept fixed throughout the present study, and the phenyl group was attached equatorially for all derivatives examined.

Results

The effect of varying the two torsion angles in AG about the glutarimido-phenyl C2—C1 bond (α) and the angular ethyl group (C2–C21) (β) is shown in Fig. 1. Four approximately equi-energetic low-energy regions are discernible. Since the phenyl group can adopt equivalent orientations related by a 180° rotation about the C2—C1 bond, there are only two distinct energy minima in Fig. 1. These are at (60°, 195°) and (144°, 300°) for α and β respectively, and are very steep, non-interconvertible minima due to the severe requirements of the interactions between the ethyl group hydrogen atoms and those at the ortho positions on the phenyl ring. The (60°, 195°) conformer is slightly less favoured, by about 0.5 kcal mole^{-1}. Figure 2(a) shows that this conformer has the mean planes of the phenyl and glutarimido rings non-coplanar in a manner quite distinct from the other conformer (Fig. 2b). In this, the two planes are virtually at right angles.

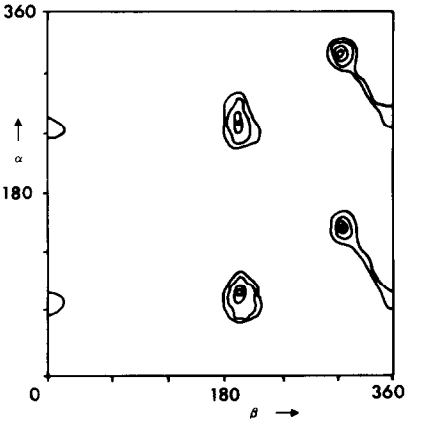

Fig. 1. Energy-contour plot for aminoglutethimide (AG). Torsion angle α has been varied along the vertical axis, and β along the horizontal. Contours have been drawn at intervals of 2.5 kcal mole^{-1}.

The AG derivative where the angular ethyl group is replaced by methyl, has also been examined. Conformational calculations were performed with variation of torsion angles about the C2—C1 bond (as defined above), and about the C2—C21 bond that here defines the orientation of the hydrogen atoms in the methyl group. The energy map (Fig. 3) shows multiple minima that differ at most by 0.9 kcal mole^{-1}. These twelve minima fall into two classes; half have the C2—C1 torsion angle at values of 60° or 310°, the other six have values of 130° or 240°. The last is the highest energy set. As for AG itself, rotation about the C2—C1 bond (i.e. the phenyl group) demonstrates the equivalence of conformers related by 180°, and the energy map (Fig. 3) has twofold symmetry about the 180° value for the C2–C1 torsion angle. In addition, a threefold repetition around the methyl group torsion angle is found. The energy barriers between these sets of equivalent positions that have the same α torsion angle, are only 6–7 kcal mole^{-1}. This indicates relatively easy interconvertibility, in contrast to AG itself. It is noteworthy that the positions of the minima in the AG energy map, all correspond to low energies in the methyl derivative.

The AG derivative with a hydrogen atom in the angular position rather than the sterically more bulky ethyl or methyl groups, only requires a one-angle (C2–C1) torsion angle energy scan in order to assess its conformational flexibility. Figure 4 shows that there are two very broad energy minima, centred at 115° and 295°, which are equivalent because of the twofold rotational symmetry of the phenyl group. The minima found for AG itself and its

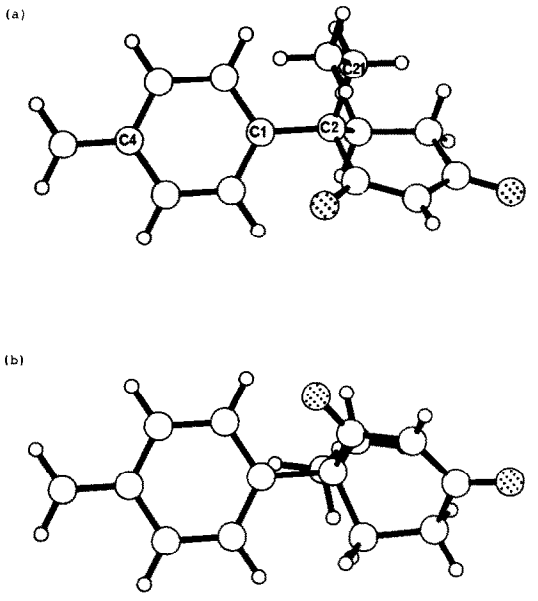

Fig. 2. (a) Computer-drawn plot of the lowest-energy conformer for the AG molecule. (b) Computer-drawn plot of the alternative, slightly higher energy conformer for the AG molecule.

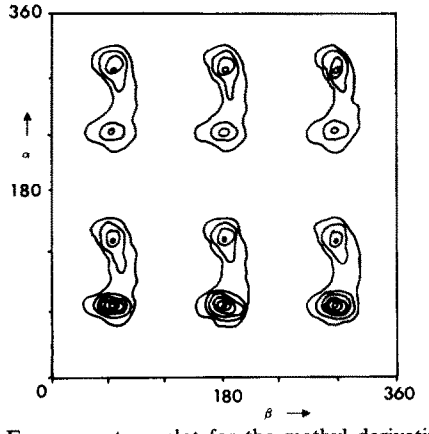

Fig. 3. Energy-contour plot for the methyl derivative of AG. Torsion angle α has been varied along the vertical axis, and β along the horizontal. Contours have been drawn at intervals of 1 kcal mole^{-1}.

methyl deriative fall within the range of this low energy region. Thus, this derivative is able to accommodate a range of conformers without having to overcome a significant energy barrier.

Discussion

This study has shown that AG adopts a small number of energetically non-interconvertible conformers, and is thus a rather rigid molecule. By contrast, its methyl and hydrogen-substituted derivatives have much greater flexibility. This implies that these latter compounds can fit into differing receptor sites without undue energy expenditure. For all these compounds, substitution of the aminiophenyl ring by 4-pyridyl would not alter the overall conformations and energies. The terminal amino group at the 4-position in

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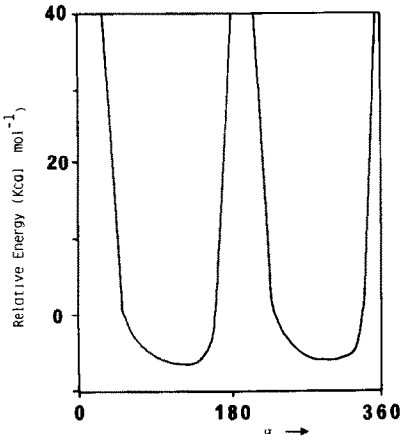

Fig. 4. One-dimensional energy plot for the derivatives of AG that has a hydrogen atom replacing the angular methyl group. The plot has relative energies, in kcal mole^{-1}, along the vertical axis, for values of the torsion angle α along the horizontal.

AG plays no role in the conformational properties. Thus conclusions derived from molecular modelling studies on AG and its analogues should apply equally to their 4-pyridyl counterparts which are currently under study [9, 10] as selective inhibitors of aromatase.

In summary, the present study predicts that the ethyl substituent will limit to two only the number of stable minimum energy conformations which the substituted phenyl and glutarimido rings can adopt relative to each other, whilst such restriction does not apply to the lower homologues. If one of these conformations coincides with that which would fit most favourably into the enzyme binding site, this structural constraint could in part explain the favourable aromatase-inhibitory properties of AG and its greater activity compared with these lower homologues though of course other factors, such as lipophilicity, could contribute. Conversely, a more bulky angular substituent could further limit conformational flexibility and produce a conformation unfavourable to strong binding to aromatase. This possibility will be explored in future extensions of this work.

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Enantiomers of cyclophosphamide and iphosphamide

Cyclophosphamide (CP) (**I**) and iphosphamide (IP) (**II**) are two of the most widely used alkylating agents in cancer chemotherapy. Both compounds are activated by metabolism via their conversion (largely in the liver) by cytochrome P-450 to 4-hydroxy derivatives (**III**, **IV**). These spontaneously decompose to yield the potent alkylating species, phosphoramide mustard (**V**) from CP and iphosphoramide mustard (**VI**) from IP. The 4-hydroxy derivatives **III** and **IV** are also detoxified by oxidation to the 4-keto derivatives **VII** and **VIII** or, via their acyclic tautomers, to the carboxylic acids **IX** and **X**. An additional detoxification pathway is dechloroethylation of the parent drug molecules which yields the monochloroethyl derivatives **XI** from CP or **XI** and **XII** from IP.

The molecules of CP and IP contain a chiral centre at the phosphorus atom, but both drugs were initially administered clinically as their racemic forms. Interest in the possibility that one of the enantiomers of CP might show higher anti-cancer activity and/or lower toxicity was aroused in 1975–1976 both by the first synthesis of the individual enantiomers by Kinas *et al.* [1] and by the observation by Cox *et al.* [2, 3] that patients administered the racemic drug excreted optically active CP in their urine. In fact the latter observation was later shown by a more rigorous method of determining enantiomeric composition (using NMR and chiral shift reagents) to be incorrect [4], but nevertheless studies of the anticancer effectiveness and metabolism of the enantiomers had by that time been initiated and were further stimulated by the discovery that the (−)-enantiomer of CP had *ca.* twice the therapeutic index (LD_{50}/ID_{90}) of the (+)-enantiomer against the ADJ/PC6 plasma cell tumour in mice ((+)-CP 68.9, (−)-CP 128.1, (±)-CP 93.0) [3]. Chemical, crystallographic and pharmacological studies of the CP-enantiomers, and shortly afterwards of IP-enantiomers, rapidly followed and eventually led to limited use of the enantiomers in man.

(a) *Chemical studies of enantiomeric CP and IP*

The first enantiomeric preparation of CP was carried out via the synthesis of diastereoisomeric derivatives bearing α-phenylethyl groups on N-3 [1–5]. Hydrogenolysis of these led to (+)- and (−)-CP with optical rotation α_D 2.3 ± 0.2° [1, 6]. The enantiomeric homogeneity of these was checked by NMR with the use of an optically active shift reagent [6]. Several other approaches to the synthesis of these enantiomers have been reported or patented; the procedure of Sato *et al.* [7] is notable in view of the high stereoselective efficiency of the synthesis. The absolute configuration of the laevorotatory form as *S*- and that of the dextrorotatory form as *R*- was independently determined by X-ray diffraction methods by Adamiak *et al.* [8, 9] and by Karle *et al.* [10]. The oxazaphosphorine ring exists in a chair form with the P=O bond axial.

The resolution of racemic CP has also been used as a source of the enantiomers. To achieve this the racemate is reacted with an optically active derivatising reagent. The resulting diastereoisomers are separated chromatographically or by crystallisation and deprotected to yield the enantiomeric CPs. The derivatising agents used have included optically active α-naphthylphenylmethylsilyl chloride (reaction with the lithium salt of CP) [11] and α-phenylbutyric acid (reaction with the N-3 alkylol produced by the reaction of CP with chloral) [12]. The reaction of CP with the optical isomers of 1-phenylethyl alcohol has also been used as the basis of a method using TLC for monitoring enantiomeric composition of CP [13], and resolution of the enantiomers is also possible by chromatography on polyamides with optically active substituents [14].

The synthesis of optically active IP (α_D −39.4°, +39.0°) was achieved by Kinas *et al.* [15] shortly after that of CP, by an analogous route involving diastereoisomeric *N*-α-phenylethyl intermediates. Several modifications of this approach were subsequently published [16, 17]. Racemic IP has also been resolved by chromatography on optically active phases [14], or by forming diastereoisomeric Pt (II) complexes which may be separated and then reconverted to (+) and (−)-IP [18]. The absolute configuration of (−)-IP was determined to be *S*- at the phosphorus atom by X-ray diffraction methods [19] and by stereochemical correlation with CP-enantiomers.

(b) *Metabolism of enantiomers of CP* (in vitro *and in animals)*

Initial metabolic studies on the purified enantiomers of CP, carried out *in vitro* using rat liver microsomes, indicated that *R*- and *S*-CP were metabolised at essentially the same rate [3]. However, later investigations revealed a fascinating species difference in enantiomeric selectivity in metabolism. The nature and rates of metabolism of the enantiomers, while present in a racemic mixture, were studied simultaneously by the use of "pseudoracemates",