Common stereochemical features in anti-epileptic drugs: a reinvestigation

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X-ray structure analysis of small drug molecules is a useful technique in attempting to establish the stereochemical requirements of drugs and drug receptor sites. Using this method it has been possible to identify common stereochemical features in chemically dissimilar drugs with common pharmacological action. The major difficulty in interpreting X-ray results is the uncertainty whether the conformation of the drug observed in the solid state is identical with that *in vivo* at the active site. However, solution studies (e.g. by nuclear magnetic resonance) often show that solid-state and solution conformations are similar.

We have recently determined the X-ray crystal structure of cannabidiol (CBD) (Jones, Falvello & others, 1977). This compound is a potent anti-epileptic in rats (Carlini, Leite & others, 1973; Izquierdo, Orsingher & Berardi, 1973). Our results have led us to re-examine earlier postulates concerning stereochemical requirements of anti-epileptic drugs. The structure determinations on which our discussions are based are of the following anti-epileptics: diphenylhydantoin (Camerman & Camerman, 1971a); diazepam (Camerman & Camerman, 1972a); procyclidine hydrochloride (Camerman & Camerman, 1971b); trihexyphenidyl (Camerman & Camerman, 1972b); phenacemide and ethylphenacemide (Camerman & Camerman, 1977); nitrazepam (Gilli, Bertolasi & others, 1977); and lorazepam (Bandoli & Clemente, 1976). The structure of CBD was also determined by Ottersen, Rosenquist, & others (1977). Structural formulae for these compounds are in Fig. 1.

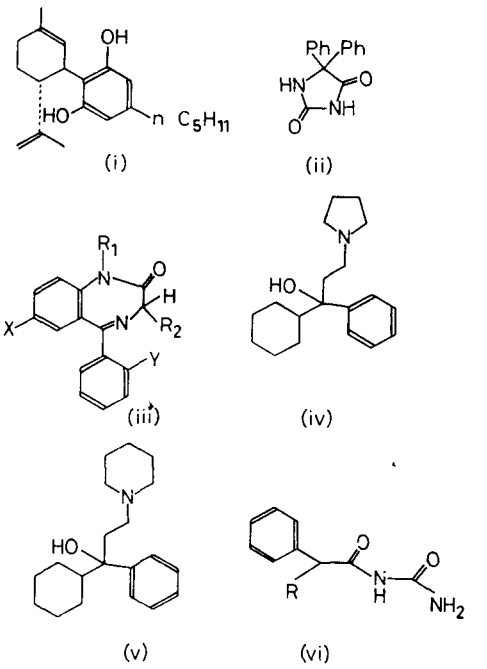

FIG. 1. Chemical structures of compounds discussed (i) cannabidiol (CBD); (ii) diphenylhydantoin (DPH); (iii) diazepam (R_1 = Me, R_2 = H, X = Cl, Y = H; DAP), nitrazepam (R_1 = R_2 = H, X = NO_2, Y = H; NAP), and lorazepam (R_1 = H, R_2 = OH, X = Y = Cl; LAP); (iv) procyclidine (PRO); (v) trihexyphenidyl (THP); and (vi) phenacemide (R = H; PA) and ethylphenacemide (R = Et; EPA).

Camerman & Camerman (1971a, b; 1972 a, b) studied space-filling models of diphenylhydantoin, diazepam, procyclidine and trihexyphenidyl (see Fig. 3 of Camerman & Camerman, 1971b) in an attempt to identify the stereochemical requirements for anti-epileptic activity. They noted that all these compounds have (a) atoms bearing lone pairs and (b) two hydrophobic rings, and suggested that both the presence and similar relative positions of these features were necessary for an active drug, and that 'the same class of receptor' was therefore involved. We have computed space-filling diagrams from published atomic coordinates using the program PLUTO (W. D. S. Motherwell, unpublished) (see Fig. 2). For diphenylhydantoin and diazepam it is seen that the hydrophobic rings and two electron donor atoms (O, O and N, O respectively) indeed occupy approximately the same relative positions. Procyclidine and trihexyphenidyl have the two hydrophobic rings and two electron donor atoms (the N of the former being protonated) and in the published Figure (see over) the space-filling models show the rings and electron donors in approximately the same orientation as in diphenylhydantoin and diazepam. However, we have been unable (using the authors' published coordinates) to compute diagrams in agreement with this; our diagrams (Fig. 2) show the rings in completely different orientations when the electron-donor atoms occupy the same relative positions.

The compounds phenacemide and ethylphenacemide (Camerman & Camerman, 1977) adopt pseudocyclic conformations in the solid state, due to intramolecular hydrogen bonding between the terminal -NH_2 and the CO farther from it. (There are no published atomic coordinates and so diagrams could not be computed from these compounds). The authors point out that

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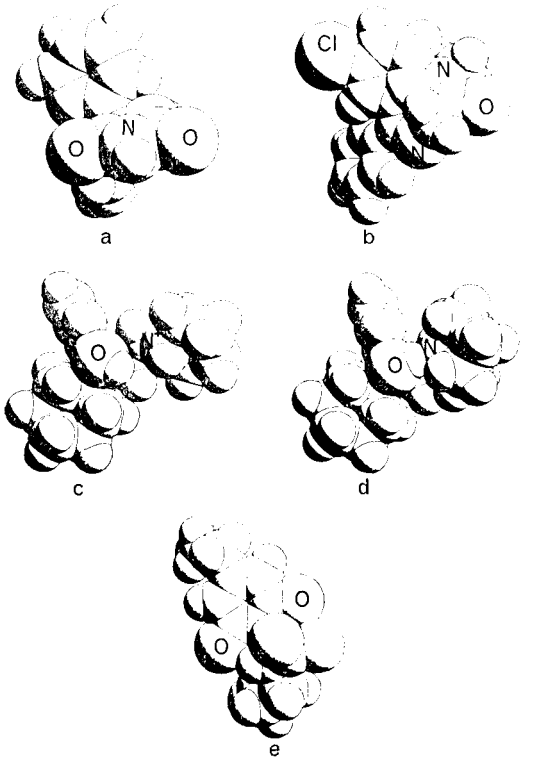

FIG. 2. Space-filling diagrams of (a) DPH: (b) DAP: (c) PRO (protonated): (d) THP: and (e) CBD. (a), (b), and (d) were computed from published coordinates, (c) from the opposite enantiomer to the published coordinates. All are arranged so that electron-donating atoms occupy approximately the same relative positions; these atoms are (a), two carbonyl O: (b) sp^2 N and carbonyl O: (c) and (d), ring N and hydroxyl O and (e) two hydroxyl O. The program used was PLUTO, written by Dr W. D. S. Motherwell.

this H bonding permits the oxygen atoms to occupy positions closely resembling those in diphenylhydantoin. However, both phenacemides have only *one* hydrophobic ring (the ethyl compound has an ethyl group in a position corresponding to one phenyl ring in diphenylhydantoin).

In CBD (Jones & others, 1977) the two electron donor atoms (-OH groups) are situated on the same phenyl ring; thus again there is effectively only one hydrophobic ring not bearing the donor atoms. We therefore conclude that previously postulated requirements concerning the number and orientation of hydrophobic rings may be too specific.

It is possible also to investigate the relative positions of the rings and donor atoms quantitatively. Table 1 shows donor-donor distances. Ignoring trihexyphenidyl as a special case (N-H . . . O hydrogen bonding in the solid state) it can be seen that these distances vary from 3·4 to 4·8 Å, with CBD displaying the largest

Table 1. *Distances between donor atoms in anti-epileptic drugs* (Å).

*DPH[a]	DAP[a,d]	PRO[a]	THP[a]	EPA[a]	PA[a]	CBD
4·6	3·4	3·6	2·8[b]	4·1	4·1	4·8, 4·8[c]

* See Fig. 1 for abbreviations.
[a] Quoted in the original papers.
[b] Hydrogen bonded atoms.
[c] Two crystallographically independent molecules.
[d] NAP and LAP are closely similar to DAP (Gilli & others, 1977)

Table 2. *Ring centroid to donor atom distances in anti-epileptic drugs* (Å).

*DPH[a,c]	DAP[a,d]	PRO[e,f]	THP[a,e]	EPA[a]	PA[a]	CBD[b]
5·5	5·4	5·4	4·7	5·9	6·4	4·3, 4·3
4·2	4·1	3·6	3·7	4·0	3·8	3·2, 3·3

* See Fig 1. for abbreviations.
[a] Quoted in the original papers.
[b] Two crystallographically independent molecules.
[c] Phenyl ring nearer to C=O group used in calculations.
[d] Chlorophenyl ring used in calculations.
[e] Phenyl ring used in calculations.
[f] In the original paper figures of 5·36, 3·47 were quoted; these correspond to N-phenyl and O-cyclohexyl distances respectively.

distance. In CBD the donor atoms are in fixed relative positions, both being on the same phenyl ring; thus, if a common receptor site is involved, 4·8 Å is the likely donor-donor distance required for *all* the drugs. This would involve changes in conformation between solid-state and *in vivo* structures; such changes are possible and must necessitate caution when discussing the significance of solid-state structures. Nevertheless, the wide range of observed distances casts doubt on the postulate that a common donor-donor distance is involved, unless indeed we are dealing with a very flexible receptor site.

Finally, we consider ring-donor distances (Table 2). In the compounds with more than one ring, it is necessary to specify the ring involved (the distances are similar whichever ring is considered). Again fairly wide ranges of distances are observed (4·3–6·4 and 3·2–4·2 Å); CBD is once more at one extreme of the range.

We thus conclude that the uncertainties concerning the number and orientation of rings, and the considerable variation in ring-donor and donor-donor distances, must lead to questioning of the earlier conclusions that the stereochemical features postulated (Camerman & Camerman, 1971a, b; 1972a, b) are responsible for anticonvulsant activity and that 'the same class of receptor' is involved for all the anti-epileptics. Two further points may be made in support of these conclusions. Firstly, Sternbach & others (1974) and Gilli & others (1977) noted that of five practically superimposable benzodiazepines, two were biologically inactive yet three were potent anticonvulsants; Gilli & others (1977) therefore stated that 'the problem of finding a relation between structure and activity . . . . . is far from being totally elucidated'.

Secondly, since diazepam is a well-known sedative and trihexyphenidyl is an antiparkinsonism agent, more than one anti-epileptic mechanism may be involved. Other techniques are needed for the further investigation of this topic.

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Note added at proof

(1) Cannabidiol has recently been demonstrated to possess anti-epileptic properties in man (Mechoulam, R. & Carlini, E. A. (1978). Naturwissenschaften **65**, 174–179).

(2) The structure of the antiepileptic diphenylsilanediol has recently been determined (Fawcett, J. K., Camerman, N. & Camerman, A. (1977). *Can. J. Chem.*, **55**, 3631–3635). The molecule possesses two hydrophobic rings (Ph) and two electron donor groups (-OH), and a space-filling model of it closely resembles diphenylhydantoin. The authors therefore state that this supports their postulate that the two rings and two electron donor groups are necessary for anticonvulsant activity. The fixed O . . . O separation of 2·66 Å is, however, the smallest donor–donor distance so far observed for an anti-epileptic; the ring to donor atom distances (all close to 4·1 Å) also represents one new limit (see Tables 1 & 2). This lends further support to our view that the wide range of observed values must lead to questioning of the stereochemical postulates for antiepileptic activity.

(3) The structure of the antiepileptic oxazepam (Fig. 1, iii; R_2 = H, R_2 = OH, X = Cl, Y = H) has recently been determined (Gilli, G., Bertolasi, V., Sacerdoti, M. & Borea, P. A. (1978). *Acta Cryst.* **B34**, 2826–2829). The authors state that "no correlation between molecular geometry and activity can be established within this class of drugs".

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Rauwolfia schueli as a potential source of ajmaline

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Ajmaline, first isolated by Siddiqui & Siddiqui (1931), is an alkaloid having antiarrhythmic activity, which has been reported to be present in a high yield in several African species of *Rauwolfia*, *R. mombasiana*, *R. vomitoria*, *R. caffra*, etc. (Court, Evans & Trease, 1958; Madati, Kayani & others, 1977).

Rauwolfia schueli Speg. (= *Rauwolfia boliviana* Mgf.) (*Apocynaceae*), is one of the species growing in Argentina together with *R. sellowii* and *R. mollis* (Xifreda, 1975). It is a small tree, with folk medicinal properties (Schulz, 1976), common name 'lecherón del monte' or 'lecherón negro', widespread in the northwestern region of Argentina (Provinces of Tucumán, Salta and Jujuy) and in the Andine regions of Bolivia (local name 'lecherón amarillo' or 'tinajero') in open woods and sandy hills.

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In a previous work Iacobucci & Deulofeu (1958) reported its root bark to contain the alkaloids aricine, reserpiline, isoreserpiline, reserpine and ajmaline.

In the present paper we report the ajmaline content of root and stem samples of *R. schueli* collected in Argentina and discuss several extraction methods.

Samples of plant material were collected at Departamento de General Güemes, Province of Salta, between September and November 1976. Root barks, roots without bark and stem bases of different diameters and height were separately analysed. Each sample was dried at 60° to constant weight and milled to a fine powder.

Several extraction procedures were tested, using quantitative operations.

Extraction procedure A. The sample (10·0 g) was macerated overnight with 100 ml methanol and refluxed for 3 h. The extract was filtered. The operation was re-