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SHORT COMMUNICATIONS

Hydrogen Bonding Interaction of Diphenylhydantoin and 9-Ethyladenine

Crystal Structure of a 2:1 Complex

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SUMMARY

A hydrogen-bonded complex of diphenylhydantoin (DPH) and 9-ethyladenine (EtAd) crystallizes from 2,4-pentanedione with the asymmetrical unit consisting of two DPH molecules, one EtAd molecule, and one solvent molecule. The crystal structure was solved by direct methods and refined to a residual of $R = 0.054$. Structure determination reveals that one DPH hydrogen-bonds to EtAd in a Watson-Crick scheme while the second DPH N(3)—H bonds to EtAd N(3) to form a 2:1 DPH-EtAd complex. Comparisons are made with barbiturate-adenine complexes and with an earlier postulation of a 1:1 DPH-EtAd complex derived from NMR and IR data. The 2,4-pentanedione molecule adopts the keto-enol configuration with an asymmetrical intramolecular hydrogen bond.

DPH[3] and phenobarbitol are the most widely used drugs in the treatment of grand mal, psychomotor, and focal epilepsies. Although certain stereochemical similarities have been shown to exist between these and other anticonvulsants (1), molecular mechanisms of action of antiepileptic drugs are not well understood.

The similarity of barbiturates to uracil has prompted several investigations into their hydrogen-bonding capabilities with adenine molecules. Since hydrogen bonds involving nucleic acids are important components of living systems, it has been suggested that some of the biological effects of barbiturates may derive from an ability to specifically bind and inactivate adenine-containing nucleic acids or coenzymes. Indeed, selective hydrogen bond formation between barbiturates and adenine derivatives has been firmly established *in vitro* by spectroscopic methods (2) as well as by the X-ray structure analysis of several crystalline, intermolecular complexes (3–6). However, it is still not clear whether such interactions are connected with barbiturates' pharmacological actions. The reported (7) selective accumulation of 5,5-DPH in nucleic acid-rich subcellular fractions of rat brain suggested that hydantoins may also interact with adenine derivatives, and a recent IR and NMR study (8) indicated that hydrogen bond formation between various substituted hydantoins and EtAd does occur. This study concluded that EtAd forms 1:1 complexes with 1,5,5-trimethylhydantoin and 3,5,5-trimethylhydantoin, but, because of solubility problems, could not determine with certainty the kind of association occurring between EtAd and DPH. From analogy with the trimethylhydantoins, the authors assumed that a 1:1 DPH-EtAd complex would also exist and then described three alternative hydrogen-bonding schemes that could hold such a complex together. We have crystallized DPH and EtAd with the aim of elucidating the structure of the complex and determining the exact nature of the intermolecular interactions. Comparisons of the complexes formed by both DPH and the various barbiturates with EtAd may prove helpful in understanding some modes of action of these anticonvulsant drugs. We report here the 3-dimensional structure determination of a 2:1 DPH-EtAd complex.

Approximately equimolar amounts of DPH and EtAd were dissolved in warm 2,4-pentanedione, and the solution was maintained at 45°. Solvent evaporation yielded large colorless crystals. A crystal measuring 0.58 × 0.39 × 0.17 mm was mounted along its long dimension on a glass fiber. Weissenberg and precession photographs revealed the space group to be $P\bar{1}$, with $a = 12.256 \pm 0.001$ Å, $b = 13.691 \pm 0.002$Å, $c = 14.835 \pm 0.002$ Å, $\alpha = 112.04 \pm 0.01°$, $\beta = 98.72 \pm 0.01°$, $\gamma = 110.99 \pm 0.01°$, and $V = 2033.19$ Å^3. The measured density obtained by flotation in a KI-water solution was 1.24 ± 0.01 g/ml.

Three-dimensional X-ray intensity data were collected on a Picker FACS-1 diffractometer using nickel-filtered

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[3] The abbreviations used are: DPH, diphenylhydantoin; EtAd, 9-ethyladenine.

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copper radiation. A θ–2θ scan was used to collect a total of 5986 unique reflections to a maximum 2θ of 120°. A total of 5263 reflections had intensity (I) greater than $2\sigma(I)$, where $\sigma(I)$ is the standard deviation in the intensity obtained from counting statistics; these reflections were classified as "observed" and were used in the structure refinement. An empirical absorption correction based on the variation of the intensity of a reflection with rotation about its scattering vector at $\chi = 90°$ was applied. The maximal correction was 14%. The structure was determined with little difficulty by direct methods (9).

Coordinates and anisotropic temperature factors of all non-hydrogen atoms were refined by least-squares. The hydrogen atom coordinates were also refined, but their temperature factors were assumed to be isotropic and equal to the average of the three diagonal elements of the anisotropic tensor of the atom to which they are bonded. Each of the final cycles of refinement was done in two blocks; blocking of the variables was changed in each cycle to compensate for possible correlation effects. The final R factor was 0.054. Table 1 lists the atom fractional coordinates; the anisotropic thermal parameters, hydrogen positional parameters, and a list of observed and calculated structure factors are available from the authors on request. Line drawings of DPH and EtAd with the numbering scheme used in the discussion are shown in Fig. 1.

Elucidation of the crystal structure of the DPH-EtAd complex crystallized from 2,4-pentanedione reveals that the asymmetrical unit contains two molecules of DPH, one molecule of EtAd, and one molecule of 2,4-pentanedione. Both DPH molecules hydrogen-bond to EtAd, thus forming a 2:1 intermolecular complex. The hydrogen-bonding scheme is illustrated in Fig. 2. DPH(I) donates its N(1) hydrogen to N(1) of EtAd while its carbonyl oxygen O(6) accepts a hydrogen from the EtAd amino group, forming a Watson-Crick type pair of hydrogen bonds. DPH(II) forms just one hydrogen bond with EtAd by donating its N(3) hydrogen to N(3) of EtAd. All three molecules in the complex also form self-associated cyclic dimers through pairs of hydrogen bonds with like molecules related through crystallographic centers of symmetry. Carbonyl O(7) is not involved in any hydrogen bonds in either of the DPH molecules. Previous structural studies of crystalline complexes of adenine derivatives with barbiturates (3–6) have shown that the carbonyl groups adjacent to the tetrahedral carbon atom tend to be weak hydrogen bond acceptors. The 2-dimensional hydrogen-bonded layer in the present complex lies approximately in the (101) plane with the 2,4-pentanedione molecules positioned above and below this plane. The pentanedione molecules are in the *enol* configuration, with an asymmetrical intramolecular hydrogen bond between O(2) and O(4). They do not participate in any hydrogen bonding with EtAd and DPH atoms. Bond distances and angles are shown in Fig. 3, which clearly illustrates the pentanedione enol structure. Conformation of both DPH molecules in this complex are very similar to the conformation of uncomplexed DPH(10) in terms of both molecular bond lengths and angles and those stereochemical parameters considered to be related to the drug's anticonvulsant properties (see Table 2).

TABLE 1

Fractional atomic coordinates (× 10^4) of all non-hydrogen atoms[a]

	X	Y	Z
DPH(I)			
N(1)	6,621(3)	6,059(2)	3,695(3)
C(2)	5,946(3)	5,730(3)	4,248(2)
N(3)	6,101(3)	4,800(3)	4,319(2)
C(4)	6,853(3)	4,497(3)	3,805(3)
C(5)	7,271(3)	5,353(3)	3,333(2)
O(6)	5,292(2)	6,148(2)	4,624(2)
O(7)	7,131(2)	3,717(2)	3,726(2)
C(8)	6,793(3)	4,623(3)	2,151(2)
C(9)	5,738(3)	4,505(4)	1,544(3)
C(10)	5,346(4)	3,844(5)	483(4)
C(11)	6,000(4)	3,300(4)	17(3)
C(12)	7,051(5)	3,422(4)	617(3)
C(13)	7,454(4)	4,071(4)	1,678(3)
C(14)	8,662(3)	6,125(3)	3,746(2)
C(15)	9,151(4)	6,865(4)	3,331(3)
C(16)	10,403(4)	7,606(4)	3,681(4)
C(17)	11,190(4)	7,603(4)	4,447(4)
C(18)	10,726(4)	6,866(5)	4,850(4)
C(19)	9,457(4)	6,126(4)	4,508(3)
DPH(II)			
N(1)	10,408(3)	1,303(2)	9,839(2)
C(2)	11,077(3)	807(3)	9,396(3)
N(3)	11,810(3)	1,565(3)	9,080(2)
C(4)	11,652(3)	2,556(3)	9,323(2)
C(5)	10,670(3)	2,449(3)	9,873(2)
O(6)	11,075(2)	−126(2)	9,272(2)
O(7)	12,162(2)	3,349(2)	9,128(2)
C(8)	9,561(3)	2,448(3)	9,244(2)
C(9)	9,723(4)	3,470(3)	9,163(3)
C(10)	8,748(4)	3,483(4)	8,565(3)
C(11)	7,615(4)	2,499(4)	8,050(3)
C(12)	7,455(4)	1,503(4)	8,143(3)
C(13)	8,424(4)	1,470(3)	8,731(3)
C(14)	11,198(3)	3,419(3)	10,995(2)
C(15)	10,440(4)	3,353(4)	11,599(3)
C(16)	10,904(6)	4,192(5)	12,638(3)
C(17)	12,105(5)	5,077(5)	13,065(3)
C(18)	12,850(4)	5,151(4)	12,472(3)
C(19)	12,391(3)	4,323(3)	11,435(3)
EtAd			
N(1)	6,122(3)	7,722(2)	3,181(2)
C(2)	6,615(3)	7,926(3)	2,489(3)
N(3)	6,810(3)	8,808(2)	2,257(3)
C(4)	6,422(3)	9,557(3)	2,832(2)
C(5)	5,880(3)	9,453(3)	3,561(2)
C(6)	5,734(3)	8,490(3)	3,746(2)
N(6)	5,223(3)	8,265(3)	4,418(2)
N(7)	5,587(2)	10,378(2)	3,985(2)
C(8)	5,960(3)	11,003(3)	3,508(3)
N(9)	6,486(3)	10,566(2)	2,809(2)
C(9)	6,996(4)	11,056(4)	2,153(3)
C(10)	6,097(8)	10,524(7)	1,140(5)
2,4-Pentanedione			
C(1)	3,332(14)	2,319(9)	2,155(9)
C(2)	2,936(6)	1,783(4)	2,821(4)
O(2)	3,647(3)	1,496(3)	3,224(3)
C(3)	1,788(7)	1,579(6)	2,986(7)
C(4)	1,399(7)	1,037(7)	3,544(6)
O(4)	2,075(5)	678(5)	4,008(4)
C(5)	139(9)	752(11)	3,719(7)

[a] Standard deviations are shown in parentheses.

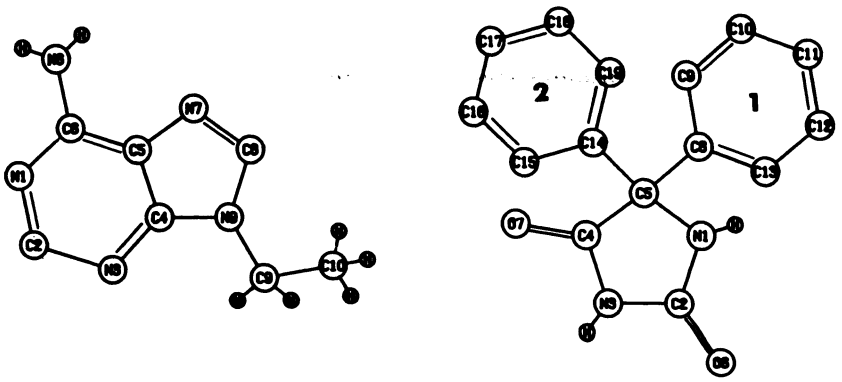

FIG. 1. *Line drawings of DPH and EtAd showing the chemical composition and numbering scheme*

Over-all, the hydrogen bonding between DPH and EtAd is very similar to that found in barbiturate-adenine complexes. Pairs of bonds formed between adenine N(1) and the amino group with an N—H and C=O of the drug are common to all. There are some differences between DPH and the barbiturate complexes, however. All of the reported barbiturate crystal structures have stoichiometries of either 1:1 or 1:2 barbiturate to adenine molecules, where the barbiturate molecule, in most cases, forms four hydrogen bonds with the adenine derivatives. This scheme is not a favorable one for the hydantoins as they possess one less carbonyl oxygen atom. As mentioned previously, the oxygen atom of one carbonyl group in both barbiturates and hydantoins is a weak hydrogen bond receptor. Thus hydantoins are left with only one site for base-pairing types of intermolecular interactions and do not readily form complexes with two adenine molecules, as is possible for the barbiturates. In none of their complexes do the barbiturates form self-associated cyclic dimers, indicating that the complex formation is energetically favored over self-association. Indeed, IR studies (2) show the association constants for various barbiturate-adenine complexes to be approximately 150 times greater than the self-association constants of the barbiturates. The association constants of the complexes also increase as the acidity of the barbiturates increases. The reported (8) association constants of various hydantoin-adenine complexes are a factor of 10 smaller than the barbiturate-adenine association constants—which is not surprising, since the hydantoins are less acidic. Evidently the hydantoin self-association is able to compete successfully with the hydantoin-adenine association, resulting in the present "mixed complex" structure. Another major difference between the barbiturate-adenine complexes and the DPH-EtAd structure is the hydrogen bond to the adenine N(3) atom. In only one of the barbiturate complexes (6) is there an interaction between a barbiturate N—H and the adenine N(3), and in that case the hydrogen bonds of that type are not strong: the reported N---N distances are 2.97 and 3.03 Å, significantly longer than the N---N distance of 2.816 Å for the comparable bond in the DPH-EtAd complex.

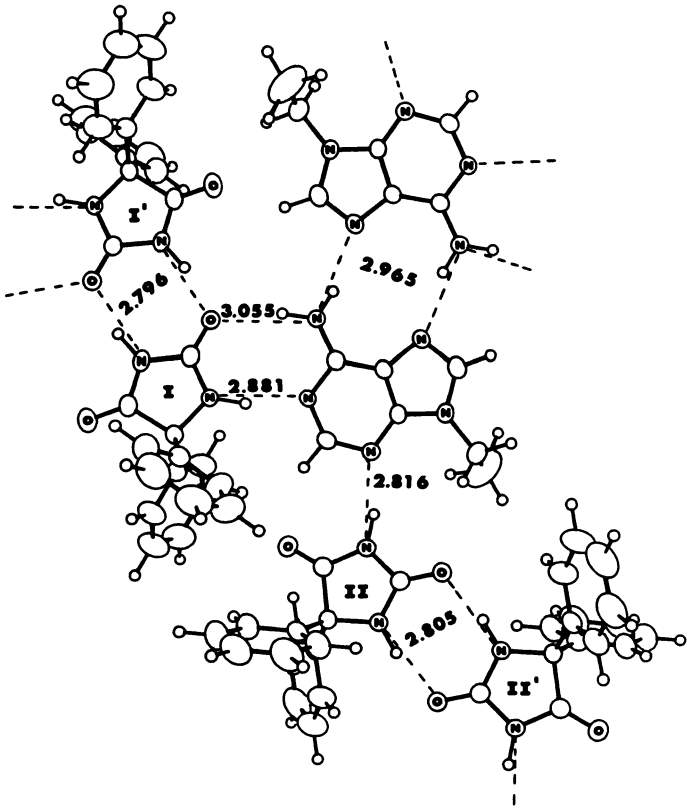

FIG. 2. *Perspective drawing showing the intermolecular hydrogen bonding*

The NMR investigation of the interaction of DPH with EtAd (8) was severely hindered by the relative insolubility of DPH in a large number of solvents. Adequate solubility was attained with dimethyl sulfoxide, but interference between the dimethyl sulfoxide proton and the DPH N—H signals and between the DPH phenyl and adenine amino hydrogen atoms made it impossible to observe concentration effects on the NMR resonance shifts. By mixing 1,5,5- and 3,5,5-trimethylhydantoins with EtAd in deuterochloroform and measuring the chemical shifts of the adenine amino and hydantoin nitrogen protons as a function of concentration, the investigators concluded that the trimethylhydantoins form 1:1 complexes with EtAd. They then suggested that, if the same relationship holds for DPH and EtAd, three different arrangements of a 1:1 DPH-EtAd complex can be postulated, depending on which DPH carbonyl oxygen atoms and nitrogen protons are utilized in the hydrogen-bonding scheme.

However, the crystal structure results show that DPH

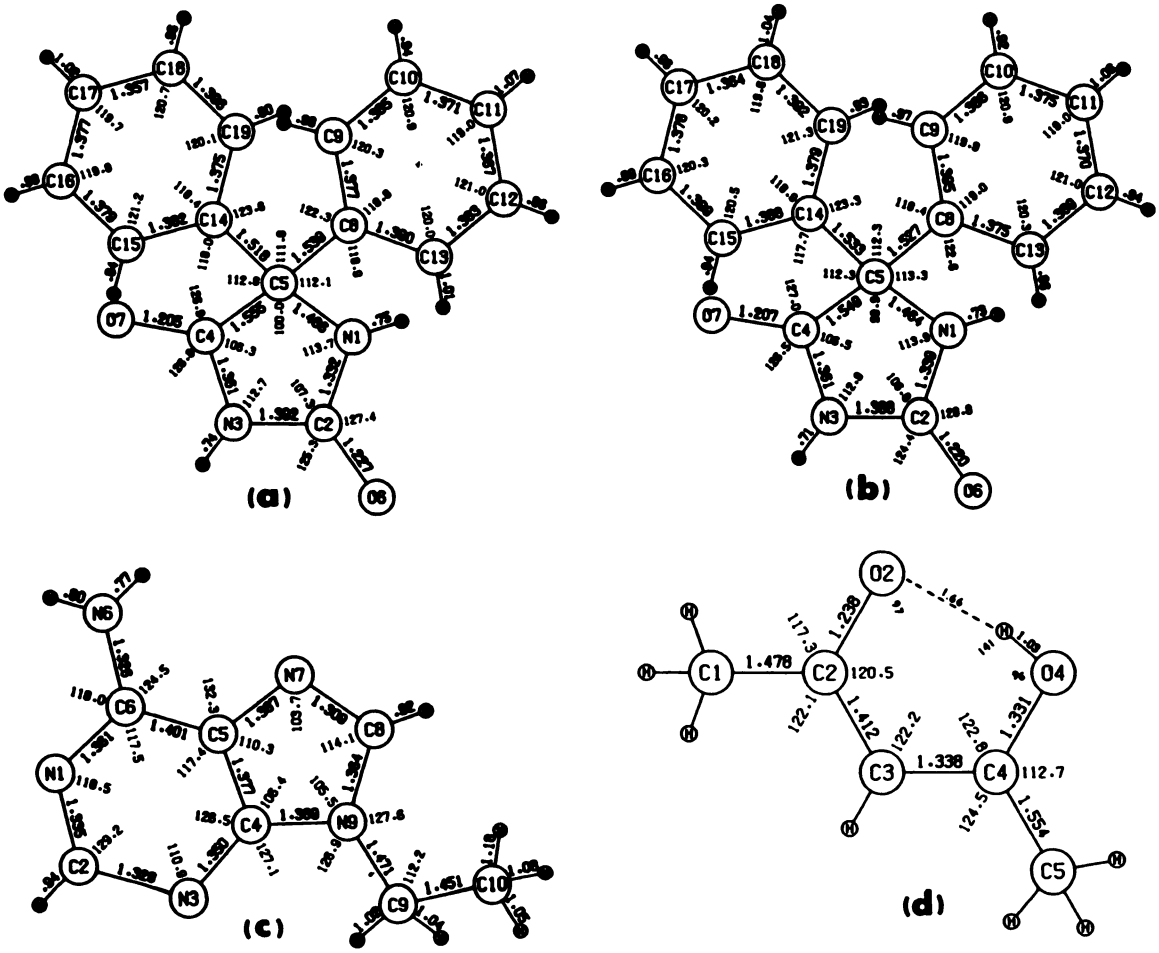

FIG. 3. *Bond distances and angles for DPH(I) (a), DPH(II) (b), EtAd (c), and 2,4-pentanedione (d)*
Additional angles not given are as follows: N(1)—C(5)—C(14) = 111.1° for DPH(I) and 110.0° for DPH(II); C(4)—C(5)—C(8) = 108.7° for DPH(I) and 108.5° for DPH(II).

TABLE 2

Comparison of selected molecular distances and angles in DPH uncomplexed and when hydrogen-bonded to EtAd

	DPH[a]	DPH(I)[b]	DPH(II)[b]
Molecular distance[c]			
O(6)–Ring 1	5.68	5.627	5.681
O(7)–Ring 1	3.97	4.054	3.975
O(6)–Ring 2	5.51	5.643	5.559
O(7)–Ring 2	4.23	4.170	4.230
O(6)–O(7)	4.56	4.571	4.557
Ring 1–Ring 2	4.84	4.796	4.878
Dihedral angle			
Hydantoin–Ring 1	114	110.6	114.9
Hydantoin–Ring 2	113	116.3	115.1
Ring 1–Ring 2	90	76.9	84.1

[a] Camerman and Camerman (10).
[b] Present study.
[c] Involving oxygen atoms and phenyl ring centroids.

can form a 2:1 hydrogen-bonded complex with EtAd in a mode that is not available to either of the trimethylhydantoins. As described above and illustrated in Fig. 2, DPH(I) interacts with EtAd using N(1) and O(6) atoms and with another DPH(I) molecule using N(3) and O(6). DPH(II) bonds to EtAd with N(3) as proton donor to adenine N(3) and with another DPH(II) by means of N(1) and O(6) hydrogen-bonding pairs. Thus all DPH N—H pairs take part in hydrogen bond formation, and EtAd accepts one H bond from one DPH molecule's N(1) and another from the second DPH molecule's N(3). Obviously, since the trimethylhydantoins have only one N—H available for hydrogen bonding, they cannot form the 2:1 complex as found for DPH. Thus extension of the NMR concentration-dependence findings from the trimethylhydantoin studies to the DPH-EtAd system is shown to be inappropriate. All of the limited NMR and IR data obtained for the DPH-EtAd complex (8) are consistent with the 2:1 complex; together with the fact that the DPH N(3)-EtAd N(3) hydrogen bond is strong, these observations suggest that the 2:1 DPH-EtAd complex is the most likely species in solution as well as in the solid state.

It is of interest to note that, even though 2,4 pentanedione (which has both hydrogen bond donor and acceptor potential) is present in the crystal lattice, there is no interaction between the solvent and DPH or EtAd. This adds to the accumulated evidence (8) that hydrogen bond formation between adenine and DPH is uniquely strong and specific. Whether this capacity to form hydrogen-bonded complexes with adenine is responsible for any of

DPH's pharmacological actions is not known and is not likely to be established until the drug's sites of action are more clearly identified.

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