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Characteristics of the Hydrogen Bonding Interactions of Substituted Hydantoins with 9-Ethyladenine

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SUMMARY

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Specific binding of diphenylhydantoin and other substituted hydantoins to 9-ethyladenine was demonstrated using proton magnetic resonance and infrared spectroscopy. Downfield resonance shifts of the N-1 and N-3 protons of diphenylhydantoin occurred upon mixing with 9-ethyladenine in dimethyl sulfoxide, whereas no such resonance shift occurred upon the addition of 9-ethylguanine in this solvent. Chemical shifts of the exchangeable protons in deuterochloroform solutions of 9-ethyladenine with 1,5,5-trimethylhydantoin or 3,5,5-trimethylhydantoin are characteristic of a hydrogen-bonded cyclic interaction; this interaction involves one of the three —NH—CO— sites available on the hydantoin nucleus in a 1:1 complex with the acceptor-donor sites on] 9-ethyladenine. By measuring the change in intensity of the adenine NH (antisymmetrical) monomer band in the normal infrared region, association constants [K_{xy}, M^{-1}] were estimated for the interaction (in deuterochloroform) of 9-ethyladenine with the given hydantoin: 5,5-dimethylhydantoin, 63; 1,5,5-trimethylhydantoin, 16; 3,5,5-trimethylhydantoin, 2.7; 5,5-diphenylhydantoin, 178; 5-ethyl-1-methyl-5-phenylhydantoin, 179; 5-ethyl-3-methyl-5-phenylhydantoin, 5.4; 3-ethyl-5-phenylhydantoin, 2.6; and 3-methyl-5,5-diphenylhydantoin, 5.1.

INTRODUCTION

Recently it was reported that 5,5-diphenylhydantoin accumulates selectively in the nucleic acid-rich subcellular fractions of rat brain (1). Structural and electronic considerations, including molecular orbital theory (2), have suggested that this accumulation may be due in part to a specific hydrogen-bonded complex with the adenine components of certain nucleic acids. Furthermore, it has been observed that DPH[1] may cause a marked inhibition of leucine incorporation into nuclei, microsomes, and mitochondria, as well as into synaptosomes and myelin of rat brain (3). The time course of the inhibition of protein synthesis in the subcellular fractions corresponds well with the accumulation of DPH in those frac-

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[1] The abbreviations used are: DPH, 5,5-diphenylhydantoin; DMH, 5,5-dimethylhydantoin; 1,5,5-TMH, 1,5,5-trimethylhydantoin; 3,5,5-TMH, 3,5,5-trimethylhydantoin; DMSO, dimethyl sulfoxide; DMF, *N*,*N*-dimethylformamide.

tions. The foregoing information suggests that DPH may hydrogen bond sufficiently well to adenine to prevent normal protein synthesis. Although one could postulate a number of mechanisms whereby a DPH-adenine interaction could mediate the observed inhibition [e.g., disruption of energy-yielding mechanisms (see ref. 4), direct interference with the translation of a given messenger RNA into protein, etc.], it was of interest to measure this suggested interaction and to compare it quantitatively with that of the normal adenine-uracil or adenine-thymine interaction. In this way it could be determined whether or not competition may exist between DPH and uracil (or thymine) for adenine, wherever the site of action.

The specificity of the DPH–nucleic acid interaction for adenine, as suggested by theory, was confirmed in a series of experiments[2] in this laboratory, whereby solubility enhancement occurred upon the addition of RNA (yeast), denatured DNA (calf thymus), or polyadenylic acid to the strictly water-insoluble DPH. On the other hand, polymers not containing adenine had no effect on the aqueous solubility of DPH. In addition, this demonstrated that in order to affect solubility the nitrogenous bases had to be "exposed," since native DNA did not alter the solubility of DPH.

Hydrogen-bonded dimerizations (x:x) and heterodimerizations (x:y) have been studied for many biologically related chemical systems, using both proton magnetic resonance and infrared spectroscopy. The interactions of purines and pyrimidines in dimethyl sulfoxide and in mixtures of DMSO and dimethylformamide have been studied by measuring their PMR spectra (5, 6). Infrared spectroscopy has been used to study the interactions in deuterochloroform between adenine and uracil derivatives (7, 8); the interactions of adenine[3] with barbiturates (9) and with caffeine and theophylline (10) have been characterized in a similar manner. The interactions between 9-ethyladenine and DPH, DMH, 1,5,5-TMH, 3,5,5-TMH, ethotoin (3-ethyl-5-phenylhydantoin), mephenytoin (5-ethyl-3-methyl-5-phenylhydantoin), methetoin (5-ethyl-1-methyl-5-phenylhydantoin), and 3-methyl-5,5-diphenylhydantoin are reported in this paper. In addition to infrared quantification of the above interactions, PMR spectroscopy provided a structural characterization of the associations.

MATERIALS AND METHODS

DPH and DMH were obtained from Sigma Chemical Company. Ethotoin and mephenytoin were purchased as commercial tablets and were purified by ethanol extraction and recrystallization from chloroform. Methetoin was a gift from Sandoz Pharmaceuticals. 1,5,5-TMH and 3,5,5-TMH were synthesized according to Biltz and Slotta (11). 3-Methyl-5,5-diphenylhydantoin was synthesized by refluxing 5,5-diphenylhydantoin in ethanolic potassium hydroxide with methyl iodide. Chloroform-d and dimethyl-d_6 sulfoxide were purchased from ICN Chemical and Radioisotope Division, Irving, Calif. The deuterochloroform was distilled after being dried overnight with phosphorus pentoxide. The DMSO was dried for several days over a Linde 3A molecular sieve, followed by distillation. Nucleosides were obtained from Cyclo Chemical Company.

Nuclear magnetic resonance spectra were recorded on a JEOL C-60H high-resolution spectrometer operating at 60 Mc/sec. Probe temperature was controlled at 20° ± 2°, unless otherwise specified, with the JEOL JES-VT-3 temperature controller. Chemical shifts are expressed either in cycles per second or in parts per million from tetramethylsilane (for deuterochloroform solutions) or from 2,2-dimethyl-2-silapentane-5-sulfonate (for DMSO solutions) as internal standards.

[2] G. L. Jones and J. W. Kemp, unpublished results.

[3] When the nitrogenous bases referred to as adenine, guanine, cytosine, uracil, or thymine appear in an experimental context, it should be kept in mind that in order to achieve adequate solubility in deuterochloroform these bases are actually 9-ethyladenine, 9-ethylguanine, 1-methylcytosine, 1-cyclohexyluracil, and 1-cyclohexylthymine (or 1-methylthymine in PMR experiments), respectively.

Infrared spectra were recorded (deuterochloroform solutions, 25°) with a Perkin-Elmer model 521 double-beam spectrophotometer. Fused silica cells (Beckman), transparent in the 220–3500 nm range, were used; the same cell, oriented in the same direction in the cell holder, was used for both solvent and solution intensity measurements. Absorbances were calculated from solvent curves as baselines. The method used by Kyogoku, Lord, and Rich (7) for the determination of the association constant of the adenine-uracil heterodimer has been applied to the hydantoin–9-ethyladenine systems reported in this paper. Briefly, this method utilizes the regression of the absorbance, A, of the monomer NH frequency on C_0/A, where C_0 is the initial concentration of the mixture. Since the procedure was derived for molecules which contain both a C=O and an NH group, arranged so that two hydrogen bonds can be formed with the complementary groups of a second molecule, a straight line would be expected in the case of a cyclic interaction, the slope and intercept of which may be used to calculate an association constant. Significant deviation from linearity would be expected if the association were noncyclic, or linear in nature (one hydrogen bond). A least-squares fit of A against C_0/A was performed for these experiments with the aid of a Hewlett-Packard programmable calculator, model 9100B.

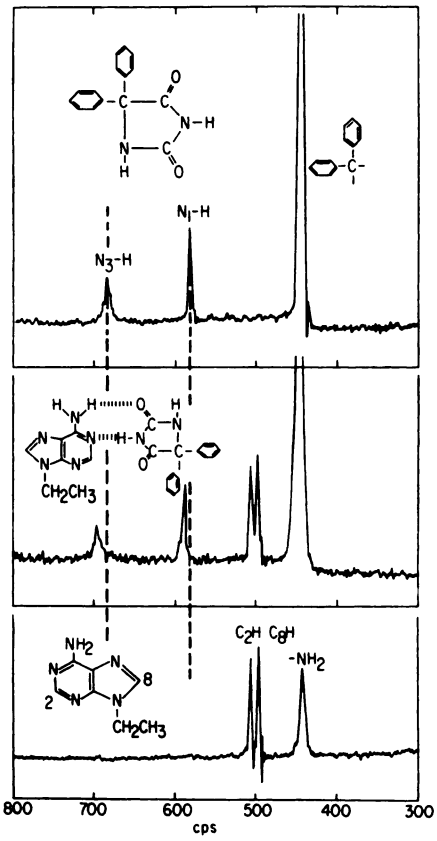


FIG. 1. *Proton magnetic resonance spectra showing effect of mixing diphenylhydantoin with 9-ethyladenine in DMSO*

Upper graph, diphenylhydantoin, 0.2 M; middle graph, diphenylhydantoin, 0.2 M, plus 9-ethyladenine, 0.2 M; lower graph, 9-ethyladenine, 0.2 M.

RESULTS

The nuclear magnetic resonance spectra for DPH, 9-ethyladenine, and an equimolar mixture of the two, using DMSO as solvent, are illustrated in Fig. 1. The downfield resonance shifts of the N-1 and N-3 protons of DPH upon the addition of 9-ethyladenine are delineated by the vertical dashed lines. Although these shifts are not as great as they would be with nonpolar solvents (see DISCUSSION), comparison with the data obtained in DMSO for other systems indicates a relatively high degree of association. The magnitude of the interaction shifts upon association in a number of common systems is shown in Table 1 (6). The guanine-cytosine interaction is by far the greatest (G-NH_2 = 0.41 ppm), while there is no apparent guanine-thymine interaction (G-NH_2 = 0.00 ppm); these observations are consistent with the Watson-Crick pairing scheme. Although the well-characterized interaction between adenine and uracil is too weak to be measured in DMSO (A-NH_2 = 0.00 ppm), the adenine-thymine association is clearly observed (A-NH_2 = 0.02 ppm). The reasons for this difference are described under DISCUSSION. The resonance shift of the N-3 proton of DPH exceeds slightly that of the N-3 proton of thymine upon mixing with 9-ethyladenine (D-NH = 0.10 ppm); no interaction is apparent between DPH and 9-ethylguanine. Resonance shifts were not observable in DMSO with any hydantoin–9-ethylade-

TABLE 1

Interaction shifts on association

Dimer	Concentration	Resonance shift						
		G-NH[a]	G-NH_2	C-NH	T-NH	A-NH_2	D-N_1H	D-N_3H
	M	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*	*ppm*
G-C[b]	0.2 + 0.2	0.94	0.41	0.43				
G-T[b]	0.2 + 0.2	0.00	0.00		0.00			
A-T[b]	0.2 + 0.2				0.08	0.02		
A-U	0.25 + 0.25					0.00	(U-NH = 0.00)	
A-D	0.2 + 0.2					—[c]	0.05	0.10
G-D	0.25 + 0.25	0.00	0.00				0.00	0.00

Data reported are for DMSO-d_6 solutions at 20°.

[a] G = 9-ethylguanine; C = 1-methylcytosine; T = 1-methylthymine; A = 9-ethyladenine; U = 1-cyclohexyluracil; D = diphenylhydantoin.

[b] Ref. 6.

[c] The A-NH_2 peak was obscured by the phenyl proton resonance.

nine system other than with DPH. Greater resonance shifts are obtainable with solvent mixtures such as equal volumes of DMSO and DMF.

1,5,5-TMH and 3,5,5-TMH were soluble enough in deuterochloroform to allow their PMR characterization; the use of such a nonpolar solvent yielded much larger resonance shifts than those obtainable with DMSO or DMF. This made it possible to observe separately the N-1 and the N-3 protons on the hydantoin nucleus upon increasing the mole fraction of 9-ethyladenine, and vice versa. As the ratio of 1,5,5-TMH to 9-ethyladenine is increased, an increasing downfield shift, characteristic of hydrogen bonding, is observed for the amino protons of the nucleoside and is depicted graphically in Fig. 2. Similar results were obtained with 3,5,5-TMH, although the relative independence of the amino proton resonant frequency with 9-ethyladenine concentration is indicative of a lower degree of association. The increasing downfield shift with increasing TMH concentration results from a greater opportunity for the adenine nucleoside to participate in complex formation. Likewise, the N-1 or the N-3 proton resonance of the particular TMH shifts downfield as the mole fraction of 9-ethyladenine is increased. The association shift of each component is linearly dependent upon the concentration of the other; this suggests a 1:1 stoichiometry of complexation. Furthermore, since all the exchangeable protons are affected by mixing, it appears that the complex formed is a cyclic heterodimer involving two hydrogen bonds.

The infrared spectra of DMH, 9-ethyladenine (each 50 mM), and a 1:1 mixture of the two (100 mM total concentration) in deuterochloroform are depicted in Fig. 3. The spectrum of DMH shows only a single broad band at 3446 cm^{-1}, which is due to the free NH stretching vibration. The spectrum of 9-ethyladenine shows two relatively sharp bands at 3526 and 3414 cm^{-1}, the antisymmetrical and the symmetrical stretching vibrations of the non-hydrogen-bonded amino group. Additional bands may occur at 3481 and 3312 cm^{-1} (easily visible in spectra of more concentrated adenine solutions) and are due to the NH_2 involvement in hydrogen bonding between adenine molecules. In the spectrum of the equimolar mixture the dashed line represents the calculated spectrum (the sum of the upper two spectra) which would be obtained if the molecules did not interact. The solid line shows the actual spectrum obtained. In addition to an observed decrease in intensity of the stretching bands of the non-hydrogen-bonded NH_2 and NH of 9-ethyladenine and DMH, respectively, the intensity of the weak bands at 3481 and 3312 cm^{-1} becomes much more pronounced. The latter are association bands of the nucleo-

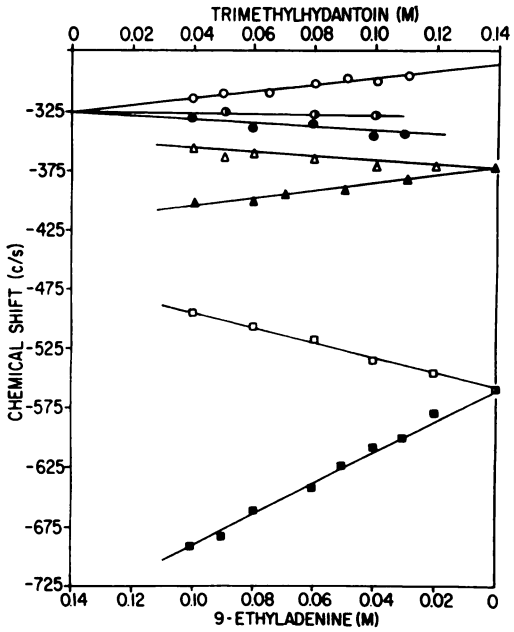


FIG. 2. *Chemical shifts of adenine amino protons* (○, ◑, ●), *N-1 proton of 3,5,5-trimethylhydantoin* (△, ▲), *and N-3 proton of 1,5,5-trimethylhydantoin* (□, ■) *at various concentrations in the absence (open symbol) and presence (closed and half-closed symbols) of complementary species.*

Spectra were recorded in deuterochloroform at 25°. ○, △, and □, dilution curves for 9-ethyladenine, 3,5,5-trimethylhydantoin, and 1,5,5-trimethylhydantoin, respectively; ●, ◑, ▲, and ■, chemical shifts obtained when enough complementary component was added to yield a total concentration of 0.14 M. Chemical shifts of the adenine amino protons are shown for the addition of 1,5,5-trimethylhydantoin (●) and 3,5,5-trimethylhydantoin (◑).

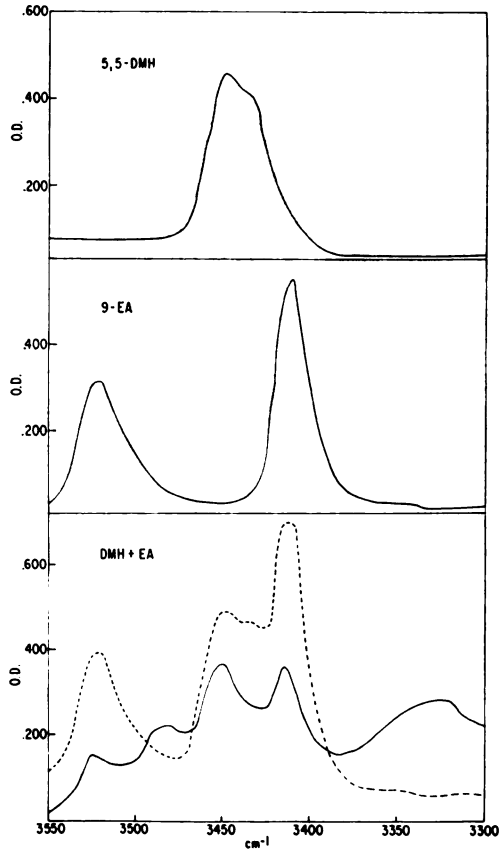


FIG. 3. *Infrared spectra of 5,5-dimethylhydantion, 9-ethyladenine, and an equimolar mixture.*

Spectra were recorded in deuterochloroform from 3300 to 3550 cm^{-1} for 5,5-dimethylhydantoin, 50 mM (upper), 9-ethyladenine (EA), 50 mM (middle), and an equimolar mixture, 100 mM (lower). Original spectra were replotted using the solvent curves as baselines. Path length was 0.5 mm. The dashed line represents the sum of the two individual spectra (which would be obtained if there were no interaction), and the solid line (lower graph) is the spectrum observed.

side amino group (see above), whose source is the amino NH bond that remains free during the association (3481 cm^{-1}) and the amino NH which is bonded to the 2-carbonyl of DMH (3312 cm^{-1}). Therefore, in addition to the self-dimerization of 9-ethyladenine molecules, there is a significant heterodimerization between 9-ethyladenine and DMH involving the NH_2 of the former and the NH of the latter compound. The association constant for the above interaction in deuterochloroform at 25° was calculated from intensity measurements of the NH_2 stretching band at 3526 cm^{-1} on a series of equimolar mixtures in the concentration range 10–50 mM: $K = 63\ M^{-1}$.

Original spectra are presented in Fig. 4 for the interactions of 9-ethyladenine with DPH (*a*), 1,5,5-TMH (*b*), ethotoin (*c*), and mephenytoin (*d*). (Notice that in Fig. 3 the data were replotted using the solvent absorption as the baseline, whereas the original spectra are presented in Fig. 4.) 9-Ethyladenine and the respective hydantoin were each present in 50 mM concentration, except for the 9-ethyladenine–DPH system, whose concentration was 5.4 mM in each component. In each spectrum there appears an

association band between 3526 cm^{-1} and the hydantoin NH monomer stretching band (3428–3453 cm^{-1}), and also below 3350 cm^{-1}. The intensity of the association bands, indicative of the degree of molecular association, is seen to decrease from DPH (*a*) to mephenytoin (*d*). The association constants representative of the systems described here are presented in Table 2. Also shown are the NH absorption frequency and pK of each hydantoin derivative.

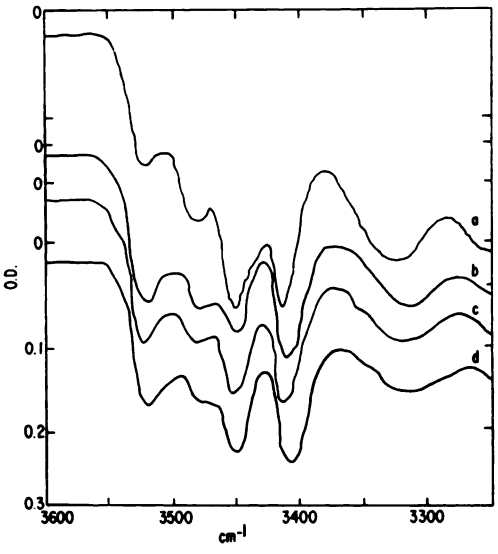


FIG. 4. *Infrared spectra of equimolar mixtures of 9-ethyladenine with various substituted hydantoins.*

Spectra were recorded in deuterochloroform from 3250 to 3600 cm^{-1} for mixtures of 9-ethyladenine with diphenylhydantoin (*a*), 1,5,5-trimethylhydantoin (*b*), ethotoin (*c*), and mephenytoin (*d*). The concentration of each component was 50 mM except for the 9-ethyladenine–DPH system, whose concentration was 5.4 mM in each component. The spectra were not replotted using the solvent curve baseline. Path length was 1.0 mm.

DISCUSSION

All measurements were performed in nonaqueous solvents because of the extremely low aqueous solubility of most of the hydantoin derivatives. However, the use of nonaqueous solvents confers distinct advantages to spectroscopic techniques of hydrogen bond characterization, regardless of solubility properties. Nucleosides are found to associate in an aqueous environment by a stacking interaction; this is observed as small shifts in the nonexchangeable ring resonances, which vary with temperature and concentration (15). Nonaqueous solvents avoid the stacking interactions by removing the driving force (a positive entropy change) for hydrophobic bond formation. Deuterochloroform, in addition to its "quenching" effect on stacking energy, also minimizes the hydrogen bonding interactions between solutes and solvent. Therefore this solvent was employed consistently except in those systems (involving DPH) where PMR measurements required the greater solubility afforded by DMSO. DMSO, because of its strong hydrogen bonding capacity, is an inferior solvent in

TABLE 2

Constants for association of 9-ethyladenine with hydantoins

Hydantoin added	pK[a]	Wave No.	Association constant, K_{xy}
		cm^{-1}	M^{-1}
5,5-Dimethylhydantoin	9.19 (N_3–H)	3446	63
1,5,5-Trimethylhydantoin	9.20 (N_3–H)	3432	16
3,5,5-Trimethylhydantoin	>12.00 (N_1–H)	3451	2.7
5,5-Diphenylhydantoin	8.31 (N_3–H)	3428	178
5-Ethyl-1-methyl-5-phenylhydantoin	8.27 (N_3–H)	3425	179
5-Ethyl-3-methyl-5-phenylhydantoin	>12.00 (N_1–H)	3451	5.4
3-Ethyl-5-phenylhydantoin	>12.00 (N_1–H)	3453	2.6
3-Methyl-5,5-diphenylhydantoin	>12.00 (N_1–H)	3445	5.1

[a] pK values are literature values (12, 13) except for that of 5-ethyl-1-methyl-5-phenylhydantoin (methetoin), which was evaluated in this laboratory by the spectrophotometric method described by Albert and Sargeant (14).

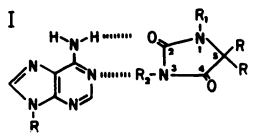
the sense that it can be expected to interact to an extent with any solute which has a proton-donating capacity. Temperature dependence profiles of the protons in DMSO solutions of 9-ethyladenine and of 9-ethylguanine have verified this expectation (5). It was necessary with this solvent to consider as a reference the state in which the purine or the hydantoin is bonded to the solvent and to observe further downfield shifts of the NH_2 or NH protons upon admixture with the appropriate hydrogen bonding complement.

The major disadvantage of nonaqueous solvents is the difficulty of interpreting data obtained in such solvents relative to events which may occur *in vivo*. However, since it is impossible at present to define the precise molecular environment in which the hydantoins may have access to the various adenine derivatives, it is likewise impossible to conclude that hydrophobic regions containing an adenine "receptor" do not exist *in vivo*. Evidence that DPH is extensively bound to brain proteins (13), although quite conclusive, does not detract from the possibility that many of these very proteins provide an apolar site for the attachment of DPH (by virtue of its diphenylmethane moiety), whence secondary hydrogen bonding interactions with a particular adenine compound may occur. This likelihood is readily visualized in membranous regions, which, because of their hydrophobic character, may exclude water sufficiently to permit the complexation described here. Thus, even though the physiological relevance of such a complexation has yet to be established, it is very likely that regions do exist intracellularly where the association may occur.

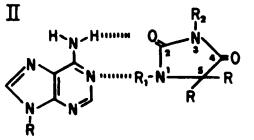
While the adenine-uracil interaction, $K_{xy} = 100\ M^{-1}$ (7, 8), is too weak to be measured in DMSO solution, the adenine-thymine interaction, $K_{xy} = 130\ M^{-1}$ (8), is readily detected (Table 1) (6). The resonance shifts of the protons involved in the 9-ethyladenine–DPH interaction are slightly greater than those for the adenine-thymine interaction. Since these observations involve comparisons of the N-3 proton resonance shifts of two different compounds, 1-methylthymine and DPH, when mixed with 9-ethyladenine, it is not possible to conclude from these data that either one of the two associations is greater than the other. However, infrared characterization clearly reveals the greater hydrogen bonding affinity of 9-ethyladenine for DPH ($K_{xy} = 178\ M^{-1}$) than for any of the nucleotide complements discussed. This suggests that the adenine-DPH interaction may exert a pharmacological action (based upon such a physical property) distinct from the other hydantoins, whose interactions with adenine were not detectable in DMSO. This unique action may reside in the recently reported ability of diphenylhydantoin to inhibit protein synthesis, as already described (3). However, many commonly used barbiturates have the hydrogen bonding —NH—CO— group common to the hydantoin nucleus and interact with adenine to a degree significantly greater than do the hydantoins (9), an observation corroborated in our laboratory. Therefore the suggestion that the inhibition of protein synthesis is due to the effective competition of DPH for adenine on the messenger RNA (or to any other metabolic consequence of a DPH–9-ethyladenine interaction) would be strengthened by evidence confirming a similar activity for certain barbiturates.

Since all the exchangebable protons are affected by mixing (Figs. 1 and 2), any of three cyclic dimers are possible, whose proposed structures are I, II, III, where R = phenyl or alkyl, and R_1 and R_2 = methyl or hydrogen.

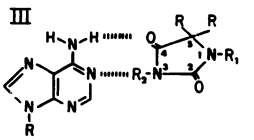
I



II



III



The resonance shift of the N-3 proton of DPH upon mixing with 9-ethyladenine is twice that of the DPH N-1 proton (Fig. 1 and Table 1). However, the nature of the solvent (DMSO, see above) made it impossible to observe clearly the concentration dependence of the resonance frequencies. Furthermore, the nucleoside amino proton resonance was obscured by the phenyl proton resonance of DPH, making it impossible to observe comparative amino proton frequency shifts. These difficulties do not exist with 1,5,5-TMH and 3,5,5-TMH, which are adequately soluble in deuterochloroform.

The linear dependence of the downfield resonance shift of the 9-ethyladenine amino protons on 1,5,5-TMH and 3,5,5-TMH concentrations illustrated in Fig. 2 clarifies the relationship (e.g., at 50 mole %) whereby the ratio between the amino proton shift and the N-3 proton shift of 1,5,5-TMH approximates the corresponding ratio involving the N-1 proton shift of 3,5,5-TMH. Thus it appears likely that for a given amount of association with 9-ethyladenine both the N-1 and the N-3 proton resonances of 3,5,5-TMH and 1,5,5-TMH, respectively, shift by an equal amount, and that the greater dependence of the N-3 proton of 1,5,5-TMH on 9-ethyladenine concentration reflects the greater participation of this proton in hydrogen bonding. Somewhat striking is the virtually linear dependence of the association shifts of each hydantoin on the concentration of 9-ethyladenine, and vice versa, suggestive of 1:1 complexation. It is known that the adenine amino group can simultaneously participate in hydrogen bond formation to the carbonyl oxygens of two pyridmidine molecules (17, 18). If such were the case for the 9-ethyladenine–hydantoin interactions, Fig. 2 should display marked deviation from linearity at high molar ratios, characteristic of a concentration dependence of an order greater than one. Furthermore, as described in MATERIALS AND METHODS, the infrared procedure for evaluating association constants would detect such a higher-order association by virtue of a significant deviation from linearity of a plot of A against C_0/A.

If the relationship described above for the trimethylhydantoins prevails for the N-1 and N-3 protons of DPH (i.e., equal frequency shifts for a given amount of association), the data in Table 1 for the 9-ethyladenine–DPH interaction would allow the assumption of equal participation of forms I, II, and III (above); with equal participation, forms I and III may collectively exhibit twice the resonance shift (N-3 proton) as form II (N-1 proton), exactly as the data indicate. Steric and electronic features of the DPH molecule (19, 2) suggest, however, that forms II and III should contribute relatively little to the total association. The association constant measured for the complexation of 9-ethyladenine with 3-methyl-5,5-diphenylhydantoin ($K_{xy} = 5.1$ M^{-1}) provides experimental evidence that form II probably contributes relatively little to the total association ($K_{xy} = 178$ M^{-1}).

Although no direct experimental evidence exists to distinguish the relative occurrence of forms II and III, certain general features can now be recognized by which one may reasonably predict the nature of a given adenine-hydantoin interaction. For DPH, the strongest hydrogen bonds will involve the more acidic (between two carbonyl groups) N-3 proton (pK = 8.3) (12) and the carbonyl at position 2, which (because of its position between the two NH groups) is more basic than the C-4 carbonyl. The nonacidic N-1 proton (pK > 12), the lower basicity of the C-4 carbonyl, and the steric hindrance provided by the diphenyl moiety are all responsible for the diminished participation of form II and presumably form III in the above dimerization. The empirical considerations correlate with molecular orbital calculations of the atomic charges at each of the terminal atoms involved in hydrogen bonding (2).

That the above analysis is valid and can be extended to other adenine-hydantoin systems is suggested by the association constants presented in Table 2. Hydantoin derivatives which are substituted at position N-3 (3,5,5-TMH, ethotoin, mephenytoin, 3-methyl-5,5-diphenylhydantoin) interact less with adenine than do those hydantoins with a free N-3 proton (1,5,5-TMH, DMH, DPH, methetoin). Furthermore, the presence of electron donors (alkyl groups) or

electron acceptors (phenyl groups) will alter the affinity for adenine, depending on where the groups are located. These inductive effects may be evaluated relative to their influence on the acidities of the imino protons: DMH, whose N-3 proton is slightly less acidic (pK = 9.19) (20) than the corresponding proton of unsubstituted hydantoin (pK = 9.12) (13), hydrogen bonds to adenine less than half as strongly as does DPH (pK = 8.3). Thus it appears that the unusually large association constant for the adenine-DPH interaction (K_{xy} = 178 M^{-1}) is due to the presence of the two phenyl groups at C-5, whose negative inductive effects are adequate to increase substantially the acidity of the N-3 proton. Interesting is the magnitude of the 9-ethyladenine–methetoin association constant, which is of the same order as that for DPH. One may expect an increased participation of form III because of the decreased steric hindrance at the adjacent C-5 position. That steric hindrance is important is apparent from considering two 3-methyl-substituted analogues. When a 5-phenyl moiety (3-methyl-5,5-diphenylhydantoin) is replaced with an ethyl group (mesantoin), a slight increase is observed in the association constant (Table 2). However, the surprisingly low pK (8.27) for the N-3 hydrogen is probably most responsible for the binding efficacy of methetoin.

The results reported here suggest that the basic adenine-hydantoin interaction is qualitatively similar to the normal adenine-uracil interaction. The magnitude of the association can be explained in terms of electronic and steric effects, as described above. The relatively high degree of association between adenine and DPH, explained on the basis of such effects, may be responsible for the inhibition of protein synthesis (3) by interfering with the normal adenine-uracil or adenine-thymine interactions.

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