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An Infrared Study of the Interaction of Caffeine and Theophylline with 9-Ethyladenine in Chloroform Solution

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SUMMARY

In deuterochloroform solution theophylline hydrogen bonds strongly with 9-ethyladenine, the strength of the association being comparable with that of the hydrogen bonding between 1-cyclohexyluracil and 9-ethyladenine reported previously, while caffeine associates relatively weakly with the latter. In a hydrophobic medium caffeine has greater solubility while theophylline self-associates extensively. The alleged role of caffeine in interfering with a DNA repair mechanism is discussed.

INTRODUCTION

It is well known that caffeine (1,3,7-trimethylxanthine), theophylline (1,3-dimethylxanthine), and theobromine (3,7-dimethylxanthine) share several pharmacological actions of therapeutic interest (1). Recently it was reported that caffeine and, to some extent, theophylline might act as inhibitors of DNA polymerase activity (2). Since DNA polymerase is part of the enzyme system that routinely repairs DNA when it is damaged by various chemical and physical factors, the implication of the inhibition of the enzyme activity by caffeine is that this compound interferes with the repair mechanism (2). It was speculated that caffeine could interfere with normal DNA repair by combining with adenine sites on the undamaged strand opposite the damaged section of the other strand of the double helix, and, because of this template interference, repair of the damaged section could not take place (3). It is therefore of considerable interest to determine by the infrared method the ability of caffeine and theophylline to associate with monomeric adenine through hydrogen bonding.

The hydrogen bonding association between adenine and uracil derivatives was studied in chloroform-*d* solution in the infrared region (4). Specific hydrogen bonding of barbiturates to adenine derivatives has likewise been studied (5). By means of similar procedures, the interactions of caffeine and theophylline with 9-ethyladenine have been determined in chloroform-*d* solution. Theobromine could not be studied in this way, as it is practically insoluble in chloroform. A nonaqueous solvent was used in order to provide a medium that would permit a direct infrared spectroscopic study of the molecular interactions. It also minimized the effect of hydrophobic bonding, as individual purines and pyrimidines are believed to associate in aqueous solution through hydrophobic bonding (6).

METHODS

The method used by Kyogoku, Lord, and Rich (4) to study the self-dimerization of adenine and uracil and of the heterodimer has been applied to the caffeine–9-ethyladenine and theophylline–9-ethyladenine

systems. The theory of the procedure is discussed in ref. 4.

9-Ethyladenine was obtained from Cyclo Chemical Company, Los Angeles. Chloroform-*d*, obtained from Stohler Isotope Chemicals, Montreal, was distilled after being dried with phosphorus pentoxide overnight. Caffeine, theophylline, and theobromine were obtained from Sigma Chemical Company, St. Louis, Mo.

All infrared spectra were obtained with a Perkin-Elmer model 521 double-beam spectrophotometer. Fused silica cells (American Instrument Company) were employed, and the same cell was used for intensity measurements of both solvent and solution spectra. The absorbances of the solutions were calculated with the aid of the solvent curves as baselines.

RESULTS AND DISCUSSION

Equimolar mixture of caffeine and 9-ethyladenine. The infrared spectra of dilute solutions, 75 mM, of caffeine, 9-ethyladenine, and a mixture of the two in deuterochloroform are shown in Fig. 1. The spectrum of 9-ethyladenine shows two prominent bands, the symmetrical and antisymmetrical NH_2 stretching vibrations at 3416 and 3527 cm^{-1} respectively, and weak bands at 3482 and 3312 cm^{-1}, which are attributed to hydrogen bonding involving the NH_2 group between the adenine molecules. Caffeine has no strong absorption band in this region. In the spectrum of an equimolar mixture (75 mM each) of both compounds, the NH_2 stretching bands of 9-ethyladenine are decreased in intensity while the weak bands are increased in intensity. This is evidence that the 9-ethyladenine molecules associate through the NH_2 group with the caffeine molecules in addition to hydrogen bonding among themselves. From intensity measurements of the antisymmetrical stretching band of NH_2 at 3527 cm^{-1} on a series of equimolar mixtures in the concentration range 15–75 mM, an equilibrium constant $K = 1.2\ M^{-1}$ at 25° is estimated for the association between caffeine and 9-ethyladenine in deuterochloroform solution. The self-association of 9-ethyladenine is $K = 3.1\ M^{-1}$ at 25° (4).

The caffeine molecule has no proton donor site but several proton acceptor sites at the 2- and 6-carbonyl oxygens and at N-9. Since the methyl group is a weak electron donor, the two methyl groups at the N-1 and N-3

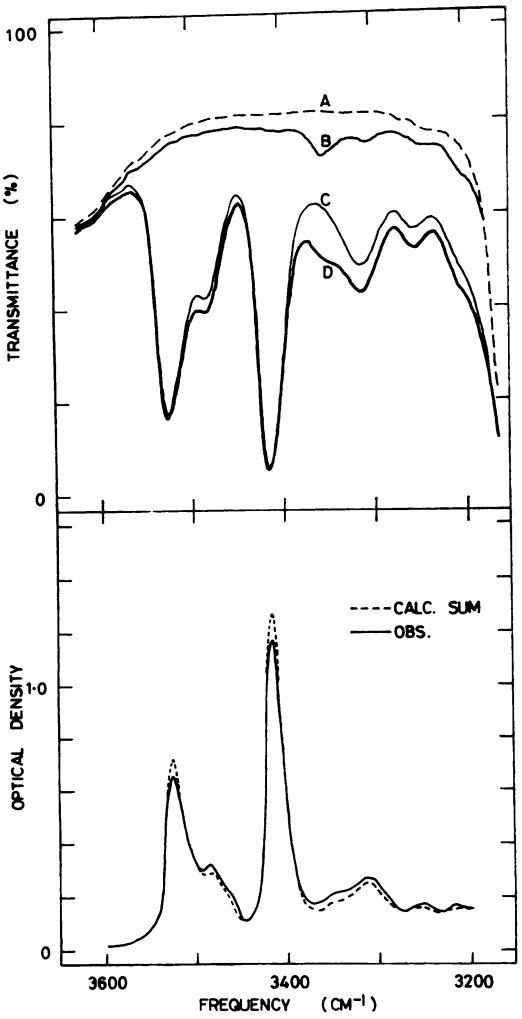
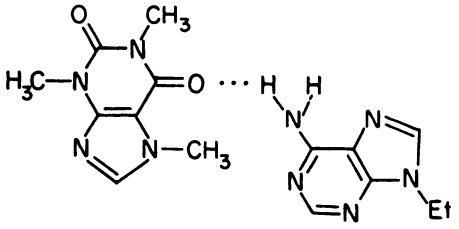


FIG. 1. *Infrared spectra of caffeine, 9-ethyladenine, and an equimolar mixture*

Upper graph: measurements were made in deuterochloroform from 3200 to 3600 cm^{-1}. A fused silica cell of 1-mm path length was used at 25°. Curve *A*, solvent and cell background; *B*, 75 mM caffeine; *C*, 75 mM 9-ethyladenine; *D*, equimolar mixture of 75 mM caffeine and 75 mM 9-ethyladenine.

Lower graph: by conversion of the measurements to optical density and by using solvent absorption as the baseline, the solute absorption is seen more clearly. The dashed curve represents the calculated sum of curves *B* and *C*, while the solid line is for curve *D*.

positions should slightly increase the basicities of the carbonyl oxygens, making them good proton acceptors. It is expected that 9-ethyladenine forms an open or linear dimer with caffeine involving an N—H···O hydrogen bond between the amino group and one of the carbonyl oxygens. It is not possible to distinguish between the two oxygen sites. The caffeine molecule has no suitable site at which to form a cyclic dimer with 9-ethyladenine. The low value of the association constant is consistent with the formation of a linear dimer, with the structure (I). In the dilute solutions used, it is not



(I)

expected that two 9-ethyladenine molecules would associate with a caffeine molecule to form a trimer, since entropic effects with small molecules make it very unlikely that the stabilizing energy of the hydrogen-bonded trimer would be large enough to permit formation of the trimer in detectable amounts.

Theophylline. The solubility of theophylline in deuterochloroform is about 14 mM. Theophylline is a stronger acid (pK_a 8.8) than uracil (pK_a 9.4). In deuterochloroform solution the infrared spectrum of theophylline (Fig. 2) shows a strong band at 3435 cm^{-1} and broad bands in the 3400–3200 cm^{-1} region. Upon deuteration at the N-7 position, all these bands disappear; the strong band at 3435 cm^{-1} is therefore assigned to the N—H stretch, and the broad bands in the 3400–3200 cm^{-1} region are due to the bonded N—H vibrations. As the intensity of these bands changes drastically upon dilution (see Fig. 2), it is concluded that theophylline molecules associate extensively through hydrogen bonding involving the N—H group. The relative low pK_a of the N—H group enables it to form very strong hydrogen bonds with a suitable

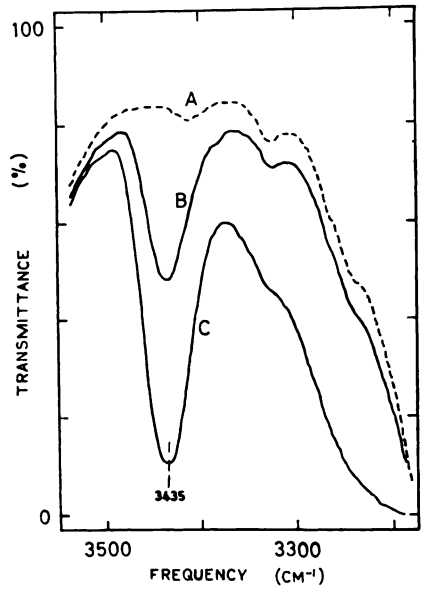
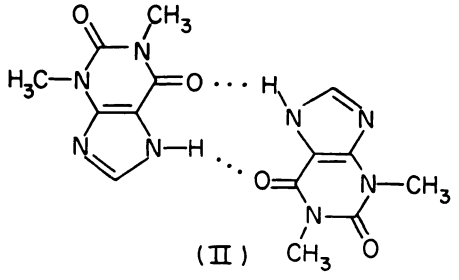


FIG. 2. *Infrared spectrum of theophylline*
Measurements were made in deuterochloroform from 3200 to 3500 cm^{-1}. A fused silica cell of 10-mm path length was used at 25°. Curve *A*, solvent and cell background; *B*, 1.8 mM theophylline; *C*, 14 mM theophylline.

proton acceptor. As in the case of caffeine, the 2- and 6-carbonyl oxygens of theophylline are good proton acceptor sites. The 6-carbonyl oxygen and the 7-amino group form suitable sites for cyclic dimer formation with another theophylline molecule or with an adenine molecule. Thermodynamic considerations indicate that formation of the cyclic dimer is much more likely than the linear dimer.

Intensity measurements on the N—H stretching band at 3435 cm^{-1} in the concentration range 0.9–14 mM in deuterochloroform solution yield a self-association constant K = 313 M^{-1} at 25° for theophylline. An equilibrium constant of this magnitude indicates very strong hydrogen bonding in a



(II)

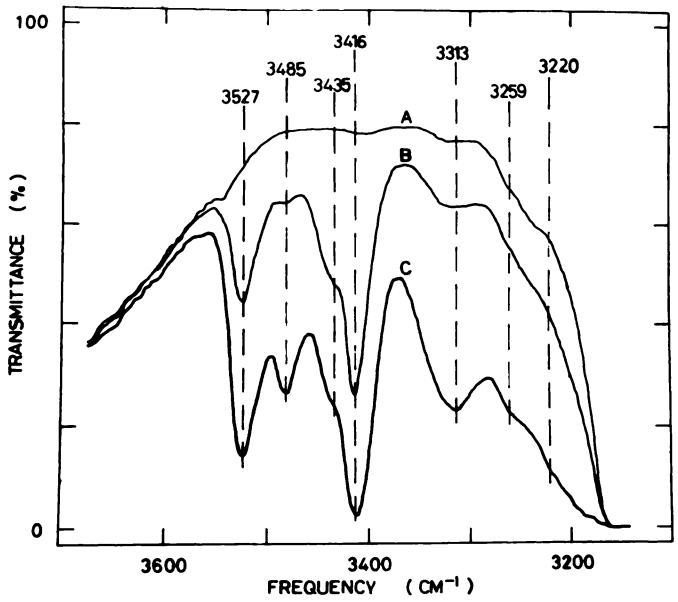
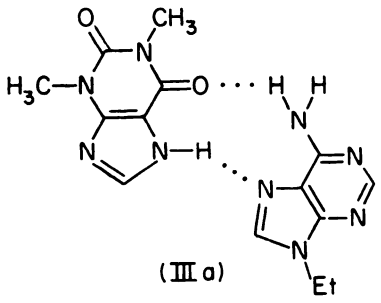


FIG. 3. *Infrared spectrum of an equimolar mixture of 9-ethyladenine and theophylline*
Measurements were made in deuterochloroform from 3150 to 3600 cm^{-1}. A fused silica cell of 5-mm path length was used at 25°. Curve *A*, solvent and cell background. The total concentrations of the two components were either 7.5 mM (curve *B*) or 30 mM (curve *C*).

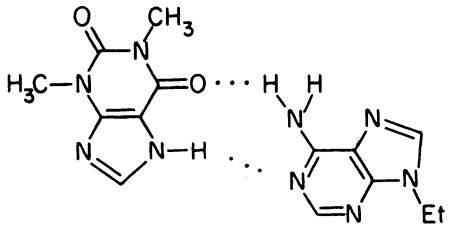
cyclic dimer with Structure II. This structure is consistent with the crystal structure of theophylline, which shows that molecules are linked by two hydrogen bonds of length 2.76 A between the 6-carbonyl oxygen and N-7 of related molecules (7). These bonds are somewhat shorter than the usual N—H···O hydrogen bond distance, and this of course reflects the great strength of the hydrogen bonds in the theophylline dimer.

Equimolar mixture of theophylline and 9-ethyladenine. The infrared spectrum of a mixture of theophylline and ethyladenine in deuterochloroform (Fig. 3) resembles that of a mixture of 1-cyclohexyluracil and 9-ethyladenine (4). Besides the NH_2 vibrations at 3416 and 3527 cm^{-1} of monomeric 9-ethyladenine and the N—H stretch at 3435 cm^{-1} of monomeric theophylline, there are relatively strong bands at 3485 and 3313 cm^{-1}. The latter bands rapidly diminish in intensity with dilution of the solution. This is evidence for hydrogen bonding between theophylline and 9-ethyladenine. The band at 3485 cm^{-1} is the association band of the amino group, and arises from the NH band that remains free when the other NH is bonded to the 6-carbonyl oxygen of theophylline, forming the N—H···O hydrogen bond having the vibrational frequency centered at 3313 cm^{-1}. From intensity measurements on the 3527 cm^{-1} band of monomeric 9-ethyladenine in the concentration range 2–8 mM in deuterochloroform solution, an association constant $K = 90$ M^{-1} at 25° is estimated for the interaction between theophylline and 9-ethyladenine. This value may be compared with that of $K = 100$ M^{-1} reported for the interaction between 1-cyclohexyluracil and 9-ethyladenine (4). Two structures (IIIa and b) are readily drawn for the ethyladenine–theophylline cyclic dimer.

The main conclusion of this study is that in chloroform solution caffeine associates with 9-ethyladenine relatively weakly while theophylline, in spite of its strong tendency to self-associate, associates strongly with 9-ethyladenine. As judged from the association constants, the strength of the theophylline–ethyladenine interaction is comparable with that of the uracil–ethyladenine interaction (4). However, the total insolu-



(III a)



(III b)

bility of theobromine in chloroform makes a corresponding determination of its interaction with 9-ethyladenine impossible. Theobromine has an —NH—CO—NH— site like that in uracil, which can potentially form strong cyclic dimers with adenine.

The relevance of the results of the present study to the physiological activities of these substances has yet to be established. The derivatives of adenine are found in a large number of biochemically important molecules, including coenzymes and ATP. It may not be possible to relate directly the studies in nonaqueous solution to the interactions in aqueous solution. However, if these substances are active in nonpolar regions, as in membranes, then studies in chloroform solution may not be entirely unrelated to their physiological activity. From this point of view it is not unreasonable to assume that should the theophylline molecule gain access to the interior of the DNA molecule, it would interact strongly with the DNA adenine base through hydrogen bonding. The interior of the DNA molecule is believed to be largely hydrophobic. In contrast, the caffeine molecule is expected to interact only weakly with the adenine base under similar conditions. On the other hand, caffeine is considerably more soluble than theophylline in a hydrophobic medium, and is not expected to self-associate extensively, as does theophylline. Furthermore, there is a possibility that the caffeine molecule suffers demethylation *in vivo*, being converted to either a theophylline or theobromine molecule. This assumption is drawn from the fact that the *N*-methylated barbiturates are demethylated *in vivo* and that their effectiveness is probably related to this process (8). Therefore the potency of caffeine may not be obvious from its interaction with monomeric 9-ethyladenine as revealed in this study; its effectiveness might lie in its greater solubility and the possibility of demethylation in the body. It should be pointed out that if the methylated xanthines can bind to the adenine sites in adenine derivatives *in vivo*, barbiturates may be bound even more effectively to these adenine sites (5).

Since caffeine cannot form a strong cyclic dimer with a DNA base, it is very doubtful that in aqueous solution a single hydrogen bond is sufficiently strong to maintain the association of caffeine with the base. Such a lack of pairing ability was tacitly presumed in studies of the binding of caffeine to nucleic acids in aqueous solution (9, 10). It has been shown that in aqueous solution the binding of caffeine to nucleic acids probably involves intercalation via hydrophobic stacking interaction (9, 11).

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