Effects on forced and spontaneous motor activity were determined as previously reported (8). The ED_{50} values for effects on spontaneous activity were determined by the method of Litchfield and Wilcoxon (9).

RESULTS AND DISCUSSION

The alkylation of Va–Vc with various alkyl halides to yield the 1-alkyl derivatives was uneventful when R_2 was the sterically large phenyl or *tert*-butyl group. This alkylation was successful when 3-methylindeno[1,2-*c*]pyrazol-4(1*H*)-one was alkylated since VI*a*–VI*i* and VII*a*–VII*c* formed. The isomers were separated by chromatography, and the products were identified by proton NMR spectroscopy.

As indicated in Table III, the protons of the alkyl at position 2 in VII*a*–VII*c* are shielded with respect to the protons of the alkyl at position 1 in VI*a*–VI*i*. The 1-methyl protons appear at approximately δ 3.9 ppm while the 2-methyl in VII*a* appears at δ 3.8 ppm. A similar trend is seen for the methyl and methylene protons in VI*b*, VI*c*, and VI*h* with respect to VII*b* and the methyl and methine protons in VI*c*, VI*f*, and VI*i* with respect to VII*c*.

As shown in Table IV, LD_{50} values in mice ranged from 148.2 (VIII*a*) to 571.7 (XI) mg/kg. 2-Ethyl-3-methyl-4-(1-methyl-4-piperidyl)-4-hydroxyindeno[1,2-*c*]pyrazole (XI) was the only one of the series to cause a significant depression of spontaneous motor activity at all doses tested (Table V). The safety index (LD_{50}/ED_{50}) for this compound was 17.6, remarkably higher than any other compound in the series although lower than that of haloperidol. Of the remaining compounds, VIII*b* and IX*b* depressed spontaneous activity, but the effect was significant only at the highest dose. Compounds VIII*a* and VIII*c* also produced significant decreases in spontaneous activity, but the dose–response relationship was inconsistent.

Statistically significant depression of forced motor activity was seen with VIII*a*, XI, VIII*b*, and IX*b*, but only at the highest dose tested (Table VI). The series of compounds tended to decrease rectal temperature, with XI and VIII*b* producing a clear dose-related decrease statistically significant at the middle and high doses (Table VII).

Of the compounds in this series, XI is the most appropriate candidate for further evaluation. Its high safety index, along with its preferential effect on spontaneous motor activity relative to that on forced motor activity, suggests potential utility as a psychotropic agent. A positional isomer of XI, IX*c*, was without noticeable biological activity.

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Interactions of Caffeine and Theophylline with *p*-Cresol: UV Studies

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Abstract □ UV absorption studies demonstrated the formation of weakly bonded charge transfer complexes between caffeine and theophylline with *p*-cresol in chloroform. The transitions involved were detected at wavelengths longer than those of the single pure substances. Equilibrium constants from the Benesi–Hildebrand equation could be measured together with other thermodynamic constants and molar extinction coefficients. In general, the equilibrium constants were very small while the entropies of formation were quite high. Even though the equilibrium constants of caffeine–*p*-cresol were independent of wavelength over a narrow range, the apparent enthalpies of formation of both complexes indicated wavelength dependence.

Keyphrases □ Caffeine—formation of charge transfer complexes with *p*-cresol, UV absorption study □ Theophylline—formation of charge transfer complexes with *p*-cresol, UV absorption study □ *p*-Cresol—formation of charge transfer complexes with caffeine and theophylline, UV absorption study □ Complexes, charge transfer—formed by caffeine and theophylline with *p*-cresol, UV absorption study

Phenols (1–4) and the purine and pyrimidine bases (5, 6) form charge transfer complexes with many organic compounds. The formation of these complexes between nucleic acid bases and catechol or epinephrine in aqueous solutions containing 0.1 *N* HCl was demonstrated (7–9). Evidence for the formation of charge transfer complexes between isoproterenol and nucleic acid bases in different solvents was described[1] (10). It was hypothesized (7–10) that the charge transfer phenomenon between the nucleic acid bases and catechol or catechol-containing substances might be involved in the mechanism of action of the biogenic amines at the molecular level as well as in their storage in the storage granules at the adrenal medulla and other nerve endings (11).

The present study concerns interactions of caffeine and theophylline with *p*-cresol in chloroform. The contribution of the aromatic nucleus is important information in tracing the mechanism of the formation of complexes between drugs having this nucleus and their receptors and may help explain the mechanism of action of the phenol-containing drugs. Chloroform was chosen as the solvent because of the solubility of the substances studied and the desire to exclude solvent effects in the formation of the complexes.

[1] Unpublished data.

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Solvation interactions are very weak in chloroform (12). Naturally, these interactions affect the equilibrium constant and heat of formation of complexes (13). Moreover, interactions between a drug and its receptor may occur in nonaqueous and low polar environments.

Preliminary IR studies revealed hydrogen-bonded complexes between caffeine and *p*-cresol and theophylline and *p*-cresol. Further UV absorption studies indicated the formation of charge transfer complexes between them. It was possible to calculate various thermodynamic parameters together with molar absorptivities of the complexes at various wavelengths of the charge transfer absorption regions.

EXPERIMENTAL

p-Cresol[2] was purified by distillation twice under reduced pressure in a nitrogen environment at 120–130° after being passed through anhydrous calcium chloride to remove moisture. The middle fraction of each distillate was used. *p*-Cresol is very sensitive to light, oxygen, and moisture. An increase in UV absorbance was observed at longer wavelengths after storage for a short time even when the proper precautions were taken, *e.g.*, storage in dark-brown bottles and under vacuum in a desiccator. Therefore, *p*-cresol was used on the day measurements were taken to avoid photooxidation and water contamination.

Caffeine USP[3] and theophylline[4] were dried under reduced pressure in a vacuum drying apparatus at the boiling point of absolute alcohol and water, respectively. Chloroform[5] was double distilled and dried over aluminum oxide. All chemicals were kept in dark-brown bottles in a refrigerator.

Concentrated solutions were prepared by weighing the chemicals. Solutions of the combinations studied contained a fixed concentration of caffeine or theophylline, 0.02 *M*, with varying concentrations of *p*-cresol, 0.4–1.0 *M*, in chloroform. Solutions were prepared by the dilution method from the concentrated standard solutions and kept in a refrigerator. The measurements were taken within 10 hr in an air-conditioned room (~22°) with subdued lighting.

Measurements were performed by fixing wavelengths to minimize errors attributed to the steepness of the absorption spectrum. Rectangular cells of 1-cm path length with stoppers were used.

The spectrophotometer[6] was calibrated by an oxide film[7] each day. When working at temperatures lower than 15°, the cell compartment of the instrument was flushed with nitrogen gas to avoid water condensation at the cell surface. A thermostated cell holder was connected to a constant-temperature circulator and refrigerator[8] to work at constant and different temperatures.

The baseline was recorded before taking the spectra of a given set of solutions with chloroform in the sample and the reference cells. The spectrum of a complex was detected at wavelengths longer than those of the single pure substances having identical concentrations as those in the mixture. A mixture was measured within approximately 10 min after mixing the solutions and placing the cell in the thermostated cell holder for equilibration.

RESULTS

The absorption spectra of *p*-cresol, caffeine, and theophylline in chloroform were temperature dependent. The absorbance increased as the temperature increased. Two opposing effects must be considered in explaining the hyperchromicity: the dilution resulting from the increase in the volume of solution and the dissociation of self-associated species.

Comparison of the absorption spectra of solutions containing single pure substances with those of the mixtures (Fig. 1) reveals that the absorption spectra of the mixtures shifted toward longer wavelengths with an increase in absorbance, indicating complex formation. Accordingly,

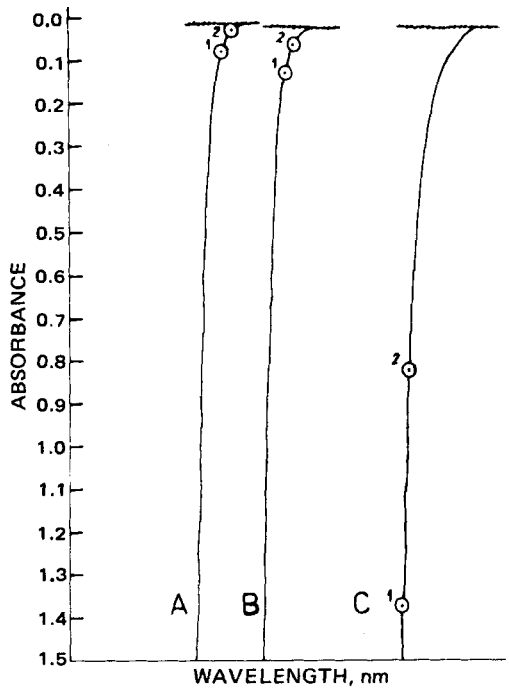

Figure 1—*Absorption spectra of chloroform solutions of* 2×10^{-2} M *caffeine (A), 1.0* M *p-cresol (B), and a mixture containing both concentrations of caffeine and* p*-cresol (C) at 7°. Points 1 and 2 represent the absorption at 308 and 310 nm, respectively, from the baseline (—).*

the absorbance of a complex at a given wavelength and temperature was estimated from the difference in absorbance between the mixture and the sum of the absorbances of the single pure substances, at identical concentrations as those in the mixture, at the same wavelength and temperature. The analytical wavelengths were chosen such that the absorbance of the complex was quite large and that of the pure components was as small as possible to minimize complications in measuring thermodynamic parameters arising from absorption of the free components.

The equilibrium constants of caffeine–*p*-cresol and theophylline–*p*-cresol complexes were calculated from a simple mathematical model by assuming the formation of a 1:1 complex:

$$A + D = AD \qquad \text{(Eq. 1)}$$

and by the application of the Benesi–Hildebrand equation:

$$\frac{[A_0]}{A_\lambda^{AD}} = \frac{1}{\epsilon_\lambda^{AD}} + \frac{1}{K_c^{AD}\epsilon_\lambda^{AD}[D_0]} \qquad \text{(Eq. 2)}$$

where $[A_0]$ and $[D_0]$ are the initial concentrations of the reactant species A and D, respectively; A_λ^{AD} is the absorbance of the complex at the wavelength λ; ϵ_λ^{AD} is the molar absorptivity; and K_c^{AD} is the equilibrium constant. The values of K_c^{AD} and ϵ_λ^{AD} can be obtained graphically from the slope and intercept of a plot of $[A_0]/A_\lambda^{AD}$ *versus* $1/[D_0]$, with $[A_0]$ kept constant and as small as possible compared to $[D_0]$ such that $[D_0] \simeq [D]$, where $[D]$ represents the concentration of free D.

The standard free energy, ΔG^0, standard enthalpy, ΔH^0, and standard entropy, ΔS^0, changes of the reactions can be calculated from the following well-known thermodynamic equations:

$$\Delta G^0 = -RT \ln K_c^{AD} \qquad \text{(Eq. 3)}$$

$$\log K_c^{AD} = -\frac{\Delta H^0}{2.303RT} + \text{constant} \qquad \text{(Eq. 4)}$$

$$\Delta G^0 = \Delta H^0 - T\,\Delta S^0 \qquad \text{(Eq. 5)}$$

where R is the gas constant and T is absolute temperature.

Representative plots of Eqs. 2 and 4 are presented in Figs. 2 and 3, respectively. To minimize experimental errors, the slopes and intercepts were calculated by the method of least squares.

The thermodynamic parameters together with molar absorptivities at the wavelengths are presented in Table I. In Table I and Fig. 2, the molar absorptivities represent average values obtained from working at different temperatures since only slight variations were observed that

[2] Searls Co., Hopkin and Williams Chemical Co., Chadwell Heath, Essex, England.
[3] Nutritional Biochemical Corporation (N.B.C.), Cleveland, Ohio.
[4] E. Merck, Darmstadt, West Germany.
[5] Carlo Erba, Milan, Italy.
[6] Model 402, Perkin-Elmer UV–visible spectrophotometer.
[7] Holmium.
[8] Model FK2, Haake Instrument Co.

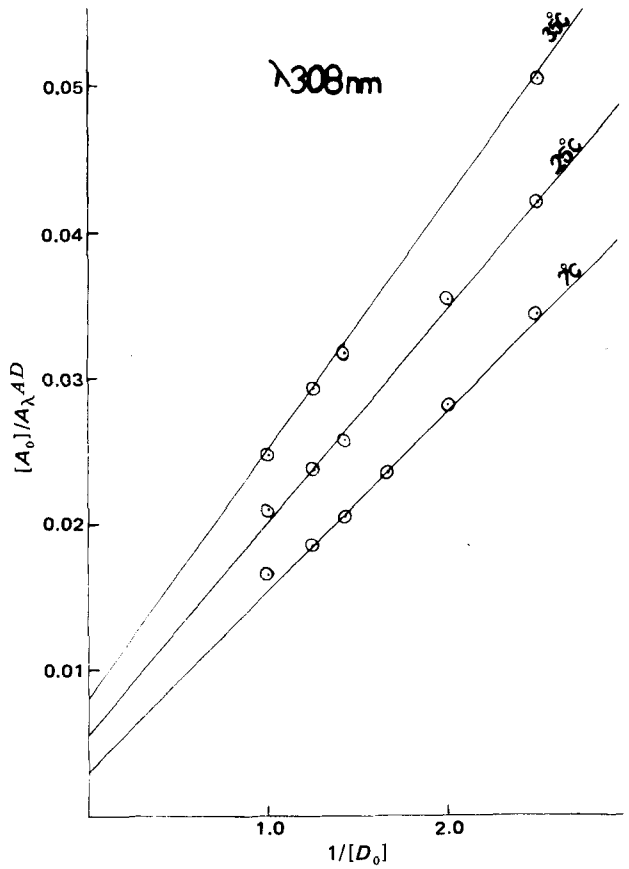

Figure 2—*Plots of* $[A_0]/A_\lambda^{AD}$ versus $1/[D_0]$ *of* p-*cresol solutions containing caffeine in chloroform at different temperatures. The total concentration of caffeine,* $[A_0]$, *was* 2×10^{-2} M; *the total concentration of* p-*cresol,* $[D_0]$, *was 0.4–1.0* M; A_λ^{AD} *represents the absorbance of the complex at the wavelength. Curves have the same intercepts as those of 7°, but they were separated to avoid crowding.*

could be attributed to experimental errors (indicating temperature independence). The equilibrium constants of the caffeine–*p*-cresol complex were wavelength independent; an increase of 2.4% was observed from 310 to 308 nm (Table I). However, with theophylline–*p*-cresol, the equilibrium constants decreased by about 35% over the same wavelength change, indicating wavelength dependence.

The greater dependence of the equilibrium constant of caffeine–*p*-cresol on temperature compared to theophylline–*p*-cresol (Table I) should reflect a greater change in its heat of formation. Although the limit of accuracy is relatively large in measuring enthalpies of formation of both complexes, the results (Table I) could indicate differences in heats of formation of both complexes as well as wavelength dependence.

Even though these reactions are exothermic, as evident from the sign of enthalpy, the free energy changes were observed to be positive since all equilibrium constants were less than unity. Thermodynamically, these reactions are not that favored. The high decrease in entropy of formation of these reactions accounts for the increase in ΔG^0 values and for the extent of complex formation and indicates a high degree of order and great specificity in these complexes as well as great changes in the structure of the solvent. In general, a correlation seems to exist between equilibrium constants and Gibbs free energy with the heat of formation and entropy changes of these complexes (Table I).

DISCUSSION

In forming charge transfer complexes, phenols (2–4) and the purine and pyrimidine bases (5, 6) were considered to act as electron donors. However, the nucleic acid bases were assumed to act as electron acceptors in their interactions with catechol or catechol-containing substances in aqueous or aqueous–alcoholic solutions containing 0.1 *N* HCl (7–10). Therefore, the experimental results presented here can be explained on the basis of formation of charge transfer complexes between caffeine and theophylline with *p*-cresol.

Table I—Equilibrium Constants, *K*, Standard Free Energy, ΔG^0, Standard Enthalpy, ΔH^0, and Standard Entropy, ΔS^0, Changes Associated with the Interactions of Caffeine and Theophylline with *p*-Cresol in Chloroform together with Molar Absorptivities, ϵ, at Different Wavelengths, λ

Temperature ±0.5°	K, M^{-1} ±0.01	ΔG^0, cal/mole	ΔH^0, cal/mole	ΔS^0, cal/degree/mole
		Caffeine–*p*-Cresol		
7°	0.25	774 ± 20		
25°	0.21	926 ± 30	−1966 ± 650	−9.8 ± 2.2
35°	0.18	1054 ± 30		
		λ = 308 nm; ε = 325.7		
7°	0.26	760 ± 20		
25°	0.20	945 ± 30	−2421 ± 500	−11.3 ± 1.7
35°	0.17	1082 ± 35		
		λ = 310 nm; ε = 188.3		
		Theophylline–*p*-Cresol		
6°	0.19	910 ± 30		
18°	0.17	1012 ± 34	−1281 ± 170	−7.9 ± 0.6
37°	0.15	1154 ± 40		
		λ = 308 nm; ε = 297.6		
6°	0.13	1149 ± 43		
18°	0.11	1261 ± 53	−1510 ± 70	−9.5 ± 0.3
37°	0.10	1444 ± 62		
		λ = 310 nm; ε = 260.4		

Quantum mechanical calculations, involving the π-system, of the highest occupied and lowest empty molecular orbitals of xanthine (14) and tyramine (15) indicated that xanthine could be a better electron donor and an acceptor. The electron-donor ability of xanthine seems to exceed its electron acceptability relative to tyramine. It is difficult to assign the donor or acceptor molecule since adjustments of all energy levels accompany electron transfer. Furthermore, the actual situation could be different, and other electrons might be involved in charge transfer, although the π-electrons have relatively lower ionization potentials than those of the lone-pair electrons of oxygen and nitrogen (16).

The increase in equilibrium constants by methylation can be explained on the basis of the inductive effect of the methyl group, which increases the availability of the π-electrons, under the assumption that caffeine and theophylline act as electron donors. If it is assumed that caffeine and theophylline are electron acceptors, then the presence of an extra methyl group in caffeine will increase its basicity relative to theophylline, resulting in a decrease in association with *p*-cresol, a result opposite to what is observed experimentally.

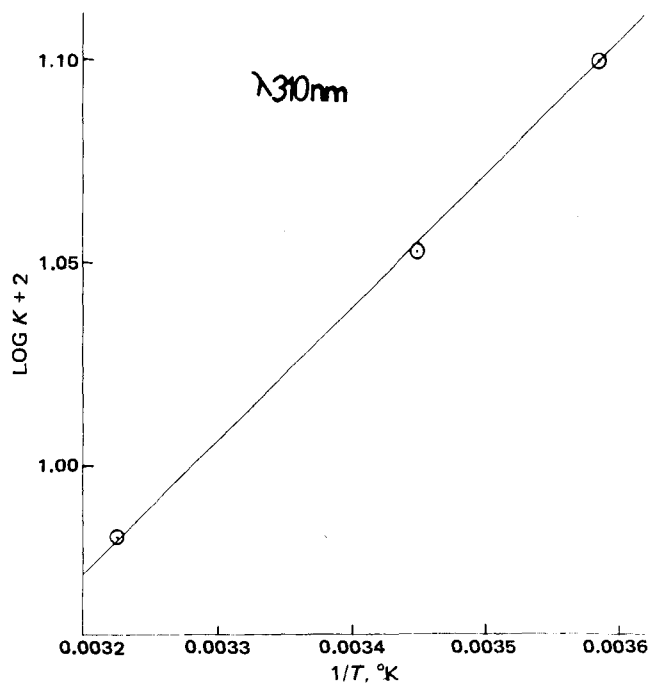

Figure 3—*Log* K *of the theophylline–*p*-cresol interaction* versus 1/T.

The correlation observed in the present work between the heat and entropy of formation of the complexes is consistent with previous observations (7–9, 17, 18) in that a higher heat of a reaction usually accompanies a greater loss in entropy (greater order in the final complex). No correlations were observed, however, between the equilibrium constant and ΔG^0 values with ΔH^0 or ΔS^0 (7–9).

The possible wavelength dependence of ΔH^0 (Table I) can be explained as being due to the formation of complexes having different stoichiometries (19) since the apparent ΔH^0 values determined experimentally are actually functions of ΔH^0 values of different associated forms. Furthermore, the wavelength dependence of the equilibrium constant can be explained by the formation of more than one complex, while wavelength independence was attributed to formation of one type of complex (20, 21). The thermodynamic parameters presented here were measured over a narrow range of wavelengths, and further experimental data can clarify the problem of the wavelength dependence of the equilibrium constant and ΔH^0.

Caffeine and theophylline form complexes and self-associate in different solvents (17, 22–28). It is difficult, however, to propose geometries for the associated forms from UV data alone. Based on IR measurements, Sellini *et al.* (29) presented evidence, supported by PMR studies, for the formation of a hydrogen-bonded, cyclic, and planar dimer between 9-ethyladenine and *p*-cresol in chloroform. Moreover, IR measurements in the present work revealed hydrogen bonds between caffeine and *p*-cresol, theophylline and *p*-cresol, and the self-associated species. Several models for geometries of the associated forms, having predominantly planar structures, were proposed[9]. The electron donor–acceptor theory of hydrogen bonding was proposed previously (30). Thus, the experimental results are consistent.

It may be concluded that the discovery of a charge transfer phenomenon among these systems should help in understanding the mechanism of action of drugs having the phenolic nucleus in the living system at the molecular level.

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[9] To be published.