Interactions of Nucleic Acid Bases with Catechol: UV Studies

FAHAD A. AL-OBEIDI and HANNA N. BORAZAN ˣ

Abstract □ UV absorption studies on weak interactions of adenine, cytosine, thymine, and uracil with catechol in aqueous solutions containing 0.1 *N* HCl gave evidence for the formation of charge transfer complexes. The absorptions of these complexes were found in the UV region at longer wavelengths than those of the pure components. It was possible to calculate various thermodynamic parameters and molar extinction coefficients at different wavelengths. The biological significance of the results is discussed.

Keyphrases □ Nucleic acid bases—adenine, cytosine, thymine, and uracil, interaction with catechol, UV absorption studies □ Catechol—interaction with adenine, cytosine, thymine, and uracil, UV absorption studies □ Complex formation—adenine, cytosine, thymine, and uracil, with catechol, UV absorption studies □ UV spectrophotometry—absorption studies, interactions of adenine, cytosine, thymine, and uracil, with catechol

Catechols are widely distributed in nature and have many biological and biochemical actions (1–3). Catechol forms the aromatic nucleus of the sympathomimetics, the catecholamines.

Nucleic acids carry the genetic information required for the synthesis of different types of proteins and enzymes (4, 5). Therefore, nucleic acids seemed to be possible targets for the action of many drugs, either directly or indirectly. The interactions between nucleic acids and some drugs were studied previously (6). Indirect actions were attributed to the interference of drugs with protein synthesis; some induce and others inhibit protein synthesis (7, 8).

To shed some light upon the nature of the adrenergic receptors, the nature of the complexes between biopolymers and those compounds needs to be investigated. To be systematic in these studies, the interactions between nucleic acid bases and catechol in aqueous solutions containing 0.1 *N* HCl were investigated spectroscopically at different temperatures.

EXPERIMENTAL

Catechol[1], thymine[1], cytosine[1], a standard solution of hydrochloric acid[1], adenine[2], and uracil[2] were obtained commercially. All chemicals, except catechol, were used without further purification. Catechol was purified by sublimation under reduced pressure (9) and kept in dark-brown bottles in a refrigerator. The sublimed catechol had the correct melting point (104°).

The solutions studied, prepared by dilution, contained a fixed concentration of nucleic acid bases (0.02 *M*) with different concentrations of catechol (0.3–0.8 *M*) in 0.1 *N* HCl. All solutions were freshly prepared and their spectra were recorded in rectangular 1-cm pathlength cells within 12 hr. Most operations were performed in a room with subdued lighting, to minimize photooxidation of catechol, at 18–20°.

The spectrophotometer[3] was checked and calibrated by using oxide film[4] at the beginning of each experiment. A thermostated cell holder[4] was used to maintain constant temperatures throughout the measurements.

When working at temperatures below room temperature, the cell holder was connected to a constant-temperature circulator and refrigerator[5]. To avoid water condensations at the surfaces of the cells at low temperatures, the cell compartment was flushed with nitrogen gas.

The baseline was recorded before running the spectra of a given set of solutions, using 0.1 *N* HCl in the cells of sample and reference compartments. The absorbance was measured by fixing wavelengths to minimize errors arising from steepness of the absorption spectra.

RESULTS

At the wavelengths investigated, the UV absorbances of catechol and all nucleic acid bases, when measured separately at different concentrations, were temperature independent. The absorbances depended only on concentration, as one would expect if no form of interaction is taking place.

The representative spectra presented in Fig. 1 show that the UV absorptions of the mixed substances were shifted from the absorptions of the individual pure substances toward longer wavelengths. Similar spectra were obtained for the other combinations studied. In most cases, the absorption spectra of the mixed substances were outside the region of absorptions of the pure compounds.

To calculate the absorbances of a complex, the absorptivities of the reacting compounds were calculated at different wavelengths. The absorbance of a complex at a given wavelength was estimated from the difference between the absorbance of a mixture and the sum of the absorbances of the pure components at concentrations identical to those used in the mixture. The experimental data used to calculate equilibrium constants (Table I) were actually measured in regions where the absorption of the free components was as small as possible and that of the complex was appreciable. The accuracy of the measurements was ±0.005 absorbance unit.

The following simple mathematical model:

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

is proposed for calculating the equilibrium constants from 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_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} may be obtained graphically from the slope and intercept of a plot of $(A_0)/A_\lambda^{AD}$ *versus* $1/(D_0)$, with (A_0) being kept constant. To minimize experimental errors, the slopes and intercepts were determined by the method of least squares. Plots of Eq. 2 for cytosine–catechol and uracil–catechol combinations are presented in Figs. 2 and 3, respectively. The intercepts calculated varied slightly with temperature for all combinations studied. By assuming the molar absorptivities to be constant over the temperature range studied, an average value was calculated from the values obtained when working at different temperatures. Thus, intercepts drawn in Figs. 2 and 3 actually represent the average.

Various thermodynamic parameters for different interactions between nucleic acid bases and catechol were calculated from the following equation:

$$\log K = -\frac{\Delta H^0}{2.303RT} + \text{constant} \qquad \text{(Eq. 3)}$$

[1] BDH Chemical Co., Poole, England.
[2] Hopkin and Williams Chemical Co., Chadwell Heath, Essex, England.
[3] Perkin-Elmer UV-visible model 402.
[4] Holmium.
[5] Model FK2, Haake Instrument Co.

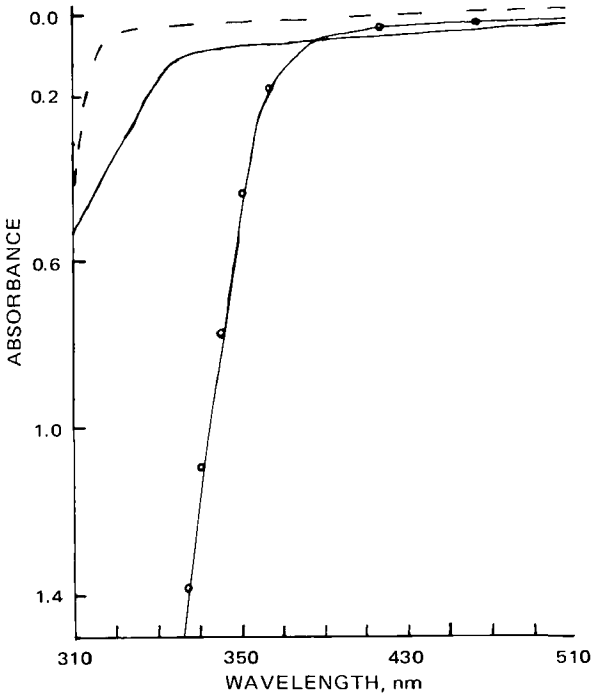

Figure 1—*Absorption spectra of 0.8* M *catechol* (——) *and* 2×10^{-2} M *cytosine* (- - -). *The* O–O–O *line represents a difference spectrum of 0.8* M *catechol plus* 2×10^{-2} M *cytosine* versus 2×10^{-2} M *cytosine. All solutions were in 0.1* N *HCl, and the measurements were at 18°.*

Therefore, a plot of log K *versus* the reciprocal of the absolute temperature, $1/T$, should give a linear slope of $-\Delta H^0/2.303R$ if the standard enthalpy change of the reaction, ΔH^0, does not depend on temperature; R is the gas constant. The Gibbs free energy change of a reaction, ΔG^0, can be related to changes in the standard enthalpy, entropy, ΔS^0, and equilibrium constant according to the following relationships:

$$\Delta G^0 = \Delta H^0 - T\,\Delta S^0 \qquad \text{(Eq. 4)}$$

$$\Delta G^0 = -RT \ln K \qquad \text{(Eq. 5)}$$

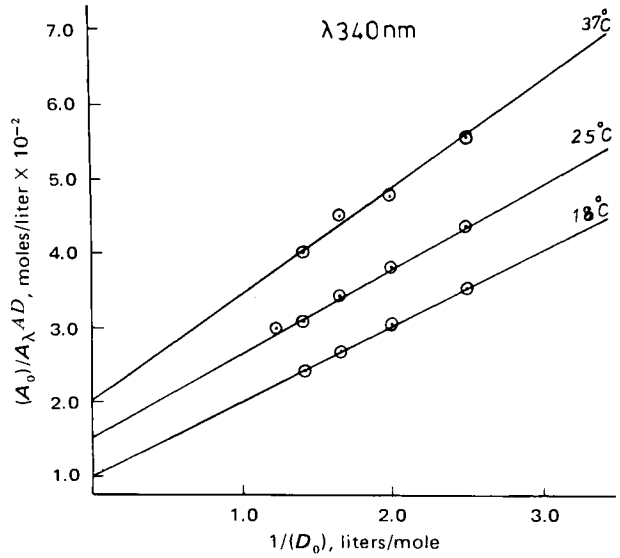

Figure 2—*Plots of* $(A_0)/A_\lambda^{AD}$ versus $1/(D_0)$ *of catechol solutions containing cytosine in 0.1* N *HCl at different temperatures. Cytosine total concentration,* (A_0), $= 2 \times 10^{-2}$ M, *and catechol total concentrations,* (D_0), $=$ *0.3–0.8* M; A_λ^{AD} *represents the absorbance of the complex at the wavelength. Curves have same intercepts as that of 18°. They were separated to avoid crowding.*

Table I—Equilibrium Constants (K), Standard Free Energy (ΔG°), Standard Enthalpy (ΔH°), and Standard Entropy (ΔS°) Changes Associated with the Interactions of Nucleic Acid Bases with Catechol in Aqueous Solutions Containing 0.1 N HCl Together with Molar Absorptivities (ϵ) at Different Wavelengths (λ)

Temperature ±0.5°	K, M^{-1} ±0.01	ΔG°, kcal/mole	ΔH°, kcal/mole	ΔS°, cal/deg/mole ±0.1
		Adenine—Catechol		
9°	1.69	−0.294		
18°	1.59	−0.264	−1.015	−2.6
37°	1.44	−0.226		
λ = 340 nm; ϵ = 21				
		Cytosine—Catechol		
18°	1.00	0.000		
25°	0.93	0.027	−3.295	−11.3
37°	0.71	0.203		
λ = 340 nm; ϵ = 100			λ = 350 nm; ϵ = 43.5	
		Thymine—Catechol		
8°	1.64	−0.276		
18°	1.40	−0.194	−2.541	−8.1
25°	1.27	−0.139		
λ = 310 nm; ϵ = 71.4				
8°	1.30	−0.146		
18°	1.10	−0.055	−2.615	−8.8
25°	1.01	−0.003		
λ = 314 nm; ϵ = 47.2				
		Uracil—Catechol		
6°	0.64	0.246		
18°	0.51	0.374	−2.842	−11.0
25°	0.48	0.410		
37°	0.39	0.518		
λ = 306 nm; ϵ = 119.8				
6°	0.49	0.394		
18°	0.38	0.533	−3.564	−14.0
25°	0.32	0.625		
37°	0.26	0.745		
λ = 310 nm; ϵ = 96.1				

Representative plots of Eq. 3 are shown in Figs. 4 and 5. The slopes and intercepts were also calculated statistically. The thermodynamic parameters calculated from these equations are summarized in Table I.

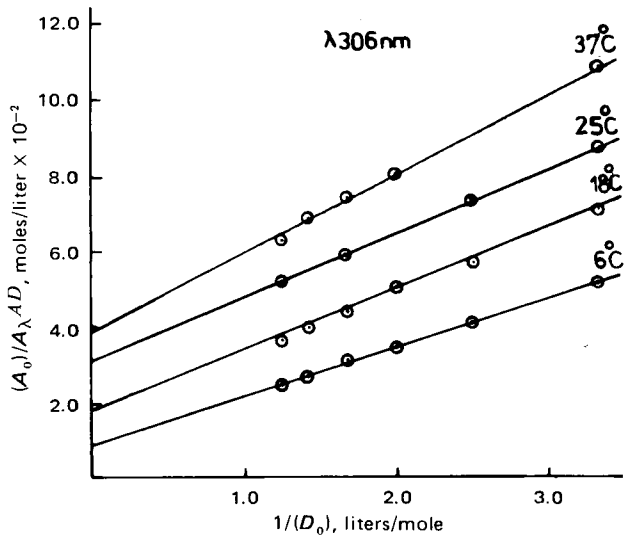

Figure 3—*Plots of* $(A_0)/A_\lambda^{AD}$ versus $1/(D_0)$ *of catechol solutions containing uracil in 0.1* N *HCl at different temperatures. Uracil total concentration,* (A_0), $= 2 \times 10^{-2}$ M, *and catechol total concentrations,* (D_0), $=$ *0.3–0.8* M; A_λ^{AD} *represents the absorbance of the complex at the wavelength. Curves have same intercepts as that of 6°. They were separated to avoid crowding.*

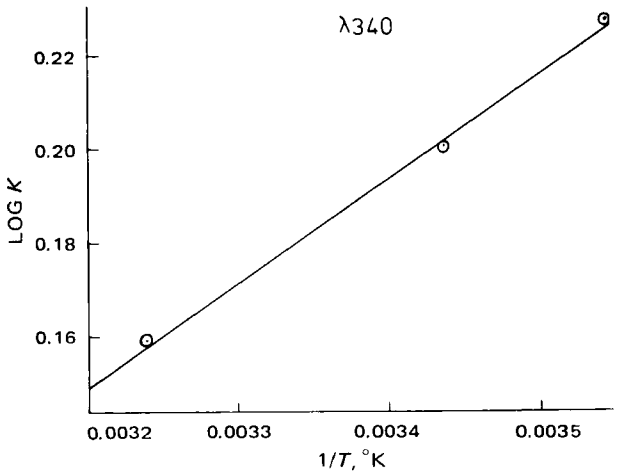

Figure 4—*Log* K *of the adenine–catechol interaction* versus *1*/T.

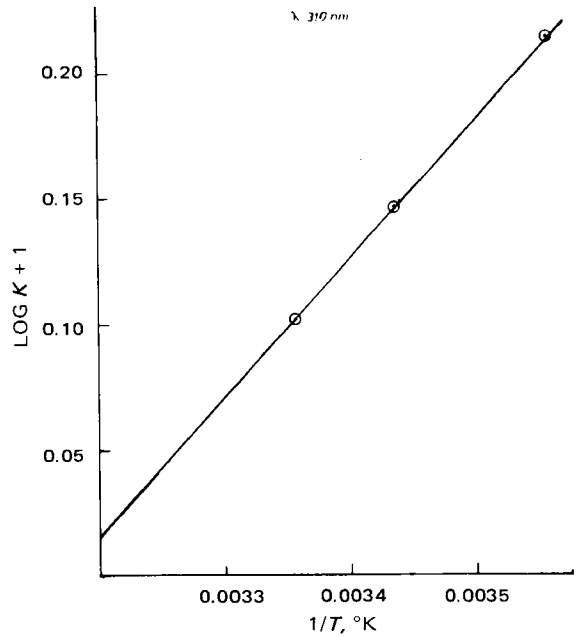

Figure 5—*Log* K *of the thymine–catechol interaction* versus *1*/T.

DISCUSSION

The differences in electronegativity and charge density existing between the molecules concerned account for the differences in their electronic properties. A charge transfer interaction can occur between different molecules or different regions within the same molecule as a result of inequalities in the sharing of electrons. The absorption of light by such weakly bonded donor–acceptor complexes is attributed to the so-called charge transfer transition.

Catechols are known to have strong electron donor properties when forming complexes with many substances (10). When catechol is mixed in solution with hydroquinone, the spectrum changes rapidly to that of a semiquinone. This effect is enhanced when oxygen is bubbled into the solution and can be attributed to charge transfer complex formation between catechol and oxygen; oxygen acts as the electron acceptor and catechol acts as the electron donor. As a result of the formation of such a complex, the oxidative power of oxygen is increased. Indeed, it was also observed that dehydrogenation of the coenzyme model 1-benzyl-1,4-dihydronicotinamide pyridinium salt is catalyzed by catechol in the presence of oxygen (11).

Nucleic acid bases also have been shown to form charge transfer complexes with many compounds (12). In those studies, nucleic acid bases were considered as the electron donors.

The electron donor–acceptor property of a molecule varies according to the environment. Thus, positively charged molecules are more likely to act as electron acceptors relative to neutral or negatively charged ones when forming complexes. The experimental results obtained here can be explained on a similar basis, *i.e.*, charge transfer complexes are formed between nucleic acid bases and catechol.

The electron donor ability of a molecule or an atom is usually measured by the ionization potential. The magnitude of the ionization potential is essentially equal to that of the binding energy of the highest occupied molecular orbital. In an analogous manner, the electron acceptor ability of a molecule or an atom can be measured by its electron affinity. However, the electron affinity is not equal to the binding energy of the lowest empty molecular orbital, because adding an electron to the lowest empty molecular orbital will alter the positions of all energy levels in the molecular or atomic orbitals.

The greater the electron affinity, the greater is the tendency of the molecule to act as an electron acceptor; the smaller the value of the ionization potential, the greater is the ability of a molecule or an atom to act as an electron donor. At present, no data are available on the ionization potential and electron affinity of catechol. Therefore, the electron donor or acceptor ability of catechol relative to various nucleic acid bases cannot be assigned.

Helene *et al.* (13), when studying the fluorescence of tyrosine or tyramine in mixed aggregates with nucleic acid bases, demonstrated fluorescence quenching of the phenol nucleus. They ascribed this phenomenon to electron transfer from the excited phenol ring to the purine or pyrimidine rings. One would expect that the electron-donating ability of catechol is greater than that of phenol, since it contains an extra electron-donating group. When these observations are applied to the present studies, catechol is considered as an electron donor and the nucleic acid bases are considered as electron acceptors.

It is observed from Table I that the order of the equilibrium constants of the interactions between nucleic acid bases and catechol is as follows: adenine–catechol > thymine–catechol > cytosine–catechol > uracil–catechol. Table I also makes clear that the ΔH^0 values of these complexes cannot be correlated with their equilibrium constants or their ΔG^0 values. The order of their enthalpy of formation is the reverse of that of the standard entropy change associated with such reactions. Higher values are observed for those of the uracil–catechol combination at a wavelength of 310 nm.

To explain the differences in the various thermodynamic parameters in terms of the specificity of these interactions, the degree and sites of protonation of the interacting molecules must be considered. Pullman and Pullman (14) classified the types of nitrogen present in nucleic acid bases and concluded that they all carry formal negative charges. Therefore, these atoms were considered to be the sites of protonation and alkylation. Spectrophotometric studies also indicated the ring nitrogens to be the principal site of attack by protons. However, the electronic charge on ring nitrogens cannot be taken solely as the measure of basicity of the nitrogen-containing heterocyclic compounds such as purine and pyrimidine derivatives (15).

Nakajima and Pullman (16) pointed out the significance of a coulombic integral between the lone pair of electrons on nitrogens and other π-electrons. The pKa's of the various nucleic acid bases and the positions of the most basic nitrogens were calculated (16). The net electronic charges on the carbonyl oxygens of uracil and thymine were found to be more negative compared to other atoms in the two molecules, ranging from −0.45 to −0.46. It may be concluded that the sites of initial protonation are nitrogen numbers 1 and 3 of adenine and cytosine, respectively, and the carbonyl oxygens of uracil and thymine.

The pKa of catechol at 25° and at an ionic strength of 0.18 was found to be 9.173 ± 0.002 (17). Therefore, all of the nucleic acid bases and catechol are protonated at the working pH.

The complex formation between all nucleic acid bases and catechol presumably occurs through parallel stacking. This mechanism of interaction in aqueous solutions has been suggested by many investigators for both self-association and complexation of nucleic acid bases, nucleosides, and similar systems (18–21).

Structural changes in nucleic acid bases seem to play a role in determining ΔH^0 and ΔS^0 values for the various interactions; for example, introducing a methyl group into uracil to give a thymine molecule leads to an appreciable change in these two parameters. This change can be explained on the basis of the net effect of two opposing factors, the steric and electronic effects imparted by the methyl group. A great change in entropy also reflects great changes in the structure of the solvent. As shown in Table I, a high degree of entropy change seems to be the determining factor for the magnitude of ΔG^0 and the formation of these complexes at the expense of ΔH^0.

No relationship was found between the magnitude of the equilibrium constants and the dipole moments of the nucleic acid bases. A fairly good correlation can be observed between equilibrium constants and the polarizability of the bases, consistent with the observations made by Broom *et al.* (19).

Table I shows the wavelength independence of the equilibrium constant of the reaction between cytosine and catechol that could indicate the formation of one type of complex (22). In the case of thymine–catechol and uracil–catechol interactions, the equilibrium constants were, however, wavelength dependent due to the formation of more than one type of complex in solution (22). The equilibrium constants decreased with an increase in wavelength.

Furthermore, Borazan (23), in explaining experimental results on complexation of the naturally occurring nucleosides and related compounds in dilute aqueous solutions (24–27), pointed out that multiple consecutive equilibria possibly can exist. Thus, for similar systems, such as the present case, plots of Eq. 2 that lead to linear slopes do not necessarily mean simple equilibrium situations as presented in Eq. 1. It may very well be that extinction coefficients of various associated forms and combinations of equilibrium constants for the multiple consecutive processes follow certain patterns such that linear slopes can be generated from the equation.

Finally, it is hoped that the discovery of the charge transfer phenomenon between nucleic acid bases and catechol will help in understanding the mechanism of action of catechol-containing substances in living systems at a more fundamental level.

CONCLUSION

Evidence was obtained from UV absorption studies for the existence of charge transfer complexes between nucleic acid bases and catechol in aqueous solutions containing 0.1 *N* HCl. It was possible to measure the absorbances of these complexes at different wavelengths in the UV region and to calculate thermodynamic parameters together with molar extinction coefficients from a simple mathematical model.

The results obtained indicate that these reactions are relatively specific and that they have, in most cases, a high heat of formation. In general, the equilibrium constants were found to be less than 2. Various thermodynamic parameters calculated from working at different wavelengths gave different values in many cases, indicating the formation of complexes having different stoichiometries.

REFERENCES

(1) B. Pullman and A. Pullman, "Quantum Biochemistry," Wiley, New York, N.Y., 1963, p. 471.

(2) H. R. Mahler and E. H. Cordes, "Biological Chemistry," Harper International Ed., Harper & Row, New York, N.Y., and John Weatherhill, Inc., Tokyo, Japan, 1971, p. 641.

(3) *Ibid.*, p. 646.

(4) J. D. Watson, "Molecular Biology of the Gene," 2nd ed., Benjamin, Menlo Park, Calif., 1970, p. 327.

(5) *Ibid.*, p. 355.

(6) M. J. Waring, *Nature*, **219**, 1320(1968).

(7) J. D. Watson, "Molecular Biology of the Gene," 2nd ed., Benjamin, Menlo Park, Calif., 1970, p. 553.

(8) V. M. Ingram, "Biosynthesis of Macromolecules," 2nd ed., Benjamin, Menlo Park, Calif., 1972, p. 212.

(9) A. I. Vogel, "Practical Organic Chemistry," 3rd ed., Longmans, Green and Co., London, England, 1964, p. 156.

(10) M. A. Slifkin, "Charge Transfer Interactions of Biomolecules," Academic, London, England, 1971, p. 214.

(11) G. Cilento and K. Zinner, *Biochim. Biophys. Acta*, **120**, 84(1966).

(12) M. A. Slifkin, "Charge Transfer Interactions of Biomolecules," Academic, London, England, 1971, pp. 76–95.

(13) C. Helene, T. Montenay-Garestier, and J. L. Dimicoli, *Biochim. Biophys. Acta*, **254**, 349(1971).

(14) B. Pullman and A. Pullman, "Quantum Biochemistry," Wiley, New York, N.Y., 1963, pp. 230–238.

(15) *Ibid.*, p. 231.

(16) T. Nakajima and A. Pullman, *J. Chim. Phys.*, **1958**, 793.

(17) R. F. Jameson and M. F. Wilson, *J. Chem. Soc., Dalton Trans.*, **23**, 2610(1972).

(18) P. O. P. Ts'o, I. S. Melvin, and A. C. Olson, *J. Am. Chem. Soc.*, **85**, 1289(1963).

(19) A. D. Broom, M. P. Schweizer, and P. O. P. Ts'o, *ibid*, **89**, 3612 (1967).

(20) J. L. Dimicoli and C. Helene, *ibid.*, **95**, 1036(1973).

(21) M. P. Schweizer, A. D. Broom, P. O. P. Ts'o, and D. P. Hollis, *ibid.*, **90**, 1042(1968).

(22) G. D. Johnson and R. E. Bowen, *ibid.*, **87**, 1655(1965).

(23) H. N. Borazan, *J. Pharm. Sci.*, **64**, 770(1975).

(24) F. M. Goyan and H. N. Borazan, *ibid*, **57**, 861(1968).

(25) H. N. Borazan, Ph.D. thesis, University of California, San Francisco, Calif., 1969.

(26) H. N. Borazan and F. M. Goyan, *J. Pharm. Sci.*, **62**, 923(1973).

(27) H. N. Borazan, *ibid.*, **62**, 1982(1973).

ACKNOWLEDGMENTS AND ADDRESSES

Received March 18, 1975, from the *Department of Pharmaceutical Chemistry, College of Pharmacy, University of Baghdad, Baghdad, Republic of Iraq.*

Accepted for publication September 4, 1975.

Abstracted in part from a dissertation submitted by F. A. Al-Obeidi to the University of Baghdad in partial fulfillment of the Master of Science degree requirements.

The authors thank Professor Hatif H. Al-Jalil for his encouragement during the investigation. They also thank the University of Baghdad for the financial support of this work.

[x] To whom inquiries should be directed.