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(31) A dramatic example of the effect of inhomogeneous broadening on the absorption and emission envelopes can be seen in Figure 5 of ref 7b in which the spectra of undecapentaene and axerophtene are compared. Axerophtene, a retinyl polyene with a terminal β-ionylidene ring identical with that in *all-trans*-retinol, exhibits almost mirror image symmetry in its absorption and emission bands. The mirror image symmetry of *all-trans*-retinol absorption and emission spectra has been analyzed by Thomson, who observed a mismatch of roughly 900 cm^{-1} in the mirror-image superimposed spectra.[32]
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Nuclear Magnetic Resonance Studies of Catecholamines. Complex Formation with Adenosine 5′-Triphosphate in Aqueous Solution. Stoichiometry and Molecular Conformations

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Abstract: ^1H NMR investigation of the association between catecholamines (dopamine, norepinephrine, epinephrine) and ATP have been undertaken in order to elucidate the stoichiometry and the structure of their complexes in aqueous solution. Analysis of the changes in the chemical shifts upon complexation of the molecules involved confirm that the main catecholamine–ATP complexes formed have stoichiometries of 1:1 and 2:1, with stepwise formation constants of 16 M^{-1} and 10 M^{-1}, respectively. These complexes are stabilized by ring association, via vertical stacking and hydrogen bond formation between the catechol hydroxyls and the purine nitrogens, and electrostatic interaction between the protonated ammonium group (at pD 6.9) and the negative phosphate moiety. The hydrogen bonds are found to be weak, and it is suggested that they are solvent mediated. Concentrations of complexes of higher stoichiometries, in which the association involves chain interaction alone, are found to be practically negligible. A marked increase (of ~30%) in the population of the trans conformer about the C_α–C_β bond of dopamine due to complexation with ATP is inferred from changes in the vicinal proton spin coupling constants. The ribose ring of ATP, existing as a $^2E \leftrightharpoons {}^3E$ equilibrium with preference for the ^2E pucker, is found to display significant increase (of ~15%) in the population of the ^3E conformation upon complexation with catecholamines. Intermolecular geometries of the 1:1 and 2:1 complexes, compatible with the experimental results, are derived by means of a ring-current shift analysis.

The occurrence of ATP in storage sites of catecholamines, as well as its participation in the processes of uptake and release of catecholamines in biological organelles, indicate the importance of the association between these compounds. In a previous paper[1] a qualitative study of the interactions between catecholamines and adenine nucleotides has been reported. It has been shown that their association in aqueous solution (at pD ~7) involves mainly ring (purine and catechol) stacking. In addition the side chains also interact through electrostatic attraction between the positive ammonium group and the negative phosphate moiety. It has also been suggested that catecholamine nucleotide complexes of 1:1 and of 2:1 stoichiometries would be the prevalent complexes.

In the present work further, quantitative characterization of the catecholamine–ATP system is provided. The stoichiometries of the binary complexes have been established and the appropriate formation constants were derived. The structures of the complexes were determined through ring-current shift analysis. Changes in spin coupling constants were interpreted in terms of variations in the intramolecular conformations. The measurements were performed at pD 6.9. In the region of this pD the interactions between the catecholamines and ATP were found to be most pronounced.[1] Also this pD value is not close to the pK_as of either the adenine ring[2] or of the hydroxyl and ammonium groups of the amines;[3] thus, any complications due to acid–base reactions were avoided.

Experimental Section

Materials. Amines[4] and adenine nucleotides of highest purity were obtained from Sigma Chemical Co. Experimental solutions were made up by dissolving the materials in D_2O (99.7%).

NMR Spectra. ^1H NMR chemical shifts were measured on a Bruker HFX-10 spectrometer operating at 90 MHz (except for the shifts of the ATP ribose protons which were measured at 270 MHz to allow unambiguous assignment of the resonances and to avoid the considerable overlap occurring at 90 MHz). A trace of dioxane in the experimental solutions served as internal reference for for shift measurements. Upfield shifts (expressed in Hertz) are denoted by positive sign. Each measurement has been repeated three–four times to reduce random errors. The experimental uncertainty in the shift measurements is thus estimated as ±0.5 Hz. Spin coupling constants were measured on a Bruker WH-270 spectrometer, equipped with a Nicolet Model 1180 32K computer, operating at 270 MHz in the Fourier transform mode. All the measurements have been performed at an ambient probe temperature of 27 °C.

The notation of the proton resonances of the studied amines is as given in Table I of ref 1.

Calculation of Concentrations of Complexes. Analysis of NMR data (e.g., chemical shifts, relaxation times) for determination of stability constants requires expressions for the equilibrium concentrations of the various species present in the experimental solution in terms of these parameters. Referring to the case of binary complexes, a central compound (A) with a maximum complexation number N, would bind ligand molecules (L) to form a series of complexes AL, $AL_2 \cdots AL_N$ by N successive steps. The nth consecutive formation constant is thus defined by the mass law equation

$$AL_{n-1} + L \rightarrow AL_n \quad K_n = [AL_n]/[AL_{n-1}][L] \qquad (1)$$

If we let $A = [A]$ and $L = [L]$ denote the concentrations of the free components, and $L_n = [AL_n]$ for $n = 1, N$ denote the concentrations

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Table I. Formation Constants and Bound State Shifts for 1:1 Amine-Nucleotide Complexes

Complex	K_1, M^{-1}	δ_1-Amine protons, Hz					δ_1-ATP protons, Hz	
		6	5	2	β	α	8	2
PEA-ATP	14.2 ± 1.7[a]	27 ± 2[a]	27 ± 2	27 ± 2	17 ± 2	10 ± 2	9 ± 1	9 ± 1
TA-ATP	15.6 ± 1.7	29 ± 2	29 ± 2	29 ± 2	19 ± 2	11 ± 2	10 ± 1	10 ± 1
DA-ATP	16.3 ± 1.8	35 ± 2	31 ± 2	30 ± 2	22 ± 2	12 ± 2	11 ± 1	10 ± 1
DA-ADP	16.4 ± 1.8	36 ± 2	32 ± 2	30 ± 2	21 ± 2	12 ± 2	11 ± 1	10 ± 1
DA-AMP	15.5 ± 1.8	35 ± 2	30 ± 2	29 ± 2	20 ± 2	11 ± 2	9 ± 1	9 ± 1
NE-ATP	17.1 ± 2.0	23 ± 2	25 ± 2	14 ± 2	[b]	6 ± 1	8 ± 1	9 ± 1
E-ATP	17.0 ± 2.0	20 ± 2	22 ± 2	13 ± 1	-3 ± 1[c]	10 ± 1	9 ± 1	9 ± 1

[a] The errors in the parameters were calculated according to eq 6 and 7 of ref 5. [b] The β-hydrogen signals of NE and E were obscured by the HDO signal. [c] Refers to the methyl signal.

of the complexes, it follows that

$$L_n = AL^n \prod_{i=1}^{n} K_i \tag{2}$$

The total concentrations of the components are given by eq 3 and 4.

$$A_0 = A + \sum_{n=1}^{N} L_n = A\left(1 + \sum_{n=1}^{N} L^n \prod_{i=1}^{n} K_i\right) \tag{3}$$

$$L_0 = L + \sum_{n=1}^{N} nL_n = L + A \sum_{n=1}^{N} nL^n \prod_{i=1}^{n} K_i \tag{4}$$

Solving eq 3 for A and substituting in eq 4 we get eq 5.

$$L_0 = L + A_0\left(\sum_{n=1}^{N} nL^n \prod_{i=1}^{n} K_i\right) \Big/ \left(1 + \sum_{n=1}^{N} L^n \prod_{i=1}^{n} K_i\right) \tag{5}$$

In the last equation L is implicitly dependent on the total concentrations A_0 and L_0, which are known factors, and on the formation constants K_n. Therefore eq 5 can in principle be solved for L, with given values for K_n. The results can be used to calculate the complex concentrations via the relation shown in eq 6.

$$L_n = A_0 L^n \prod_{i=1}^{n} K_i \Big/ \left(1 + \sum_{n=1}^{N} nL^n \prod_{i=1}^{n} K_i\right) \tag{6}$$

Three cases are of interest. (1) $N = 1$. In this case a straightforward expression for L_1 is obtained.

$$L_1 = \{A_0K_1 + L_0K_1 + 1 - [(A_0K_1 + L_0K_1 + 1)^2 - 4A_0L_0K_1^2]^{1/2}\}/2K_1 \tag{7}$$

(2) $N = 2$. Rearranging eq 5 we get a cubic equation for L.

$$K_1K_2L^3 + K_1(2A_0K_2 - L_0K_2 + 1)L^2 + (A_0K_1 - L_0K_1 + 1)L - L_0 = 0 \tag{8}$$

This equation has three roots of which only one has a physical meaning (i.e., yielding a real positive value for L which is not larger than L_0), and will be used to calculate L_1 and L_2. (3) $N \geq 3$. In these cases the order of eq 5 is higher than three; hence it cannot be solved analytically. However numerical solutions can be obtained by using a simple computer program, and the complex concentrations may be derived with the desired level of accuracy.

In the present study A and L denote ATP and catecholamines, respectively. It is to be noted that the self-association of ATP was neglected in the calculations. By monitoring the change in the chemical shifts of ATP resonances as function of its concentration at pD 7, we obtained a formation constant of 1 M^{-1} for the self-association (assuming only dimerization of the ATP molecules). Calculation of the dimer concentration reveals that at low ATP concentrations (<60 mM) the self-association may indeed be assumed negligible. At higher concentrations the self-association would be more pronounced. However, at these concentrations mainly the 1:1 complex with catechalomines is formed (see below) and ATP dimerization would not significantly affect it. Actually additional types of complexes may be formed, i.e., amine-$(ATP)_2$ or amine-$(ATP)_2$-amine, where one or two catecholamine molecules are stacked at one or both sides of the ATP dimer. Insofar as the catecholamine molecules are concerned these complexes may reasonably be regarded as equivalent to the 1:1 complex.

Results and Discussion

A. The 1:1 Complex. (i) Formation Constants and Intrinsic Chemical Shifts.

Under conditions of fast chemical exchange (which is found to be the case in the amine-ATP system, as reflected in the unmodified time-averaged pattern of the measured spectra observed throughout the whole experimental range), the observed chemical shift ($\Delta\delta$), due to complexation of the amine molecules, is related to the intrinsic shift of the complex (δ_1) through the relation

$$\Delta\delta = \delta_1 L_1/L_0 \tag{9}$$

where all the shifts are relative to those of the uncomplexed molecules. By fitting eq 9 (together with eq 7) to the experimental shift data, obtained under appropriate conditions,[5] the values of K_1 and δ_1 can be determined. In the study of the catecholamines association with ATP, the formation of complexes of stoichiometries higher than 1:1 could not be ruled out.[1] However, on the basis of preliminary experiments, it was assumed that with low amine concentration and excess of ATP the 1:1 complex would predominate. Therefore titrations were carried out with amine concentrations of ~40 mM and ATP/amine molar ratio of 2-5. In order that suitable comparisons could be made, the association of ATP with PEA and TA and of DA with AMP and ADP were also investigated. Typical titration curves are shown in Figure 1. The calculated parameters are given in Table I. A constant trend is observed in the ATP complex parameters upon substitution of hydroxyl groups in the rings of the different amines, i.e., an increase of both the formation constants and the bound state shifts. The chemical shifts induced in the amine complexes with ATP can be considered to originate solely from ring-current effects.[1] Ring-current shifts vary with R^{-3}, R being the distance between the measured proton and the center of the ring inducing the shift.[6] Increases in the amine and ATP shifts indicate closer approach of the stacked rings in the binary complex. The double effect of the phenolic hydroxyl groups, as noted, may be due to the formation of hydrogen bonds between these groups and the purine nitrogens. The β-hydroxyl group substitution has further stabilizing effect on the association of NE and E with ATP. However an opposite effect on the intrinsic shifts is observed. This trend may also be attributed to hydrogen bond formation, this time with the P=O group[7] or the ribose oxygens of ATP. Such a bond may cause a displacement of the amine molecule in the complex in a manner which would decrease the ring-current effect (see also the discussion on the structure of the complex, below).

It is interesting to note the similarity of the catecholamine complexes with adenine nucleotides (see also ref 1), which is reflected both in the formation constants and the bound state shifts. This indicates that actually only the first phosphate of the nucleotide chain participates in the 1:1 association with catecholamines. Since the catecholamine-nucleotide complexes are found to be almost isostructural and of the same stability, the three adenine nucleotides might replace one another without affecting the association mode of the catecholamines.

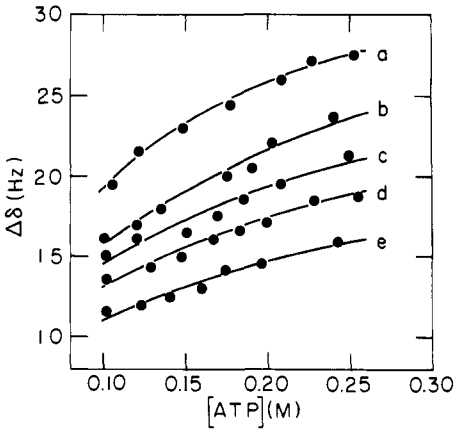

Figure 1. Chemical shifts induced by ATP in the H_6 proton of various amines in solution (pD 6.9): (a) DA (48 mM), (b) TA (49 mM), (c) PEA (52 mM), (d) NE (51 mM), (e) (50 mM).

(ii) Intramolecular Conformations. The effect on the intramolecular conformation of the side chain of DA upon complexation with ATP has been investigated by monitoring the changes in the spin coupling constant between the vicinal protons. (With NE and E this could not be applied with sufficient accuracy owing to marked broadening of the side-chain resonances upon addition of ATP.) DA has three possible conformers staggered about the C_α-C_β bond (Figure 2): a trans conformer and two mirror-image gauche conformers. Owing to rapid interconversion of the conformers, by rotation about the C-C bond, the geminal pairs of protons become chemically equivalent, i.e., have the same chemical shift. The vicinal coupling constants however may not be averaged, i.e., $J_{\alpha\beta} \neq J_{\alpha\beta'}$; hence the system should be described as AA'BB' (AA'XX').[8] The vicinal coupling constants depend mainly on the substituents on the bonded carbons and on the mutual orientation of the coupled protons. Assuming that for the three DA conformers all the trans couplings are equal to (J_t) and all the gauche couplings are equal (to J_g), the observed coupling constants, being the average of the three conformer contributions weighted according to their relative populations, are given by

$$J_{\alpha\beta} = P_t J_t + 0.5 P_g (J_g + J_t) \quad (10)$$

$$J_{\alpha\beta'} = P_t J_t + P_g J_g \quad (11)$$

where P_t and P_g are the fractional populations of the trans and the gauche rotamers respectively ($P_t + P_g = 1$). In cases of ambiguity in the assignment of the observed constants to either $J_{\alpha\beta}$ or $J_{\alpha\beta'}$ (which is indeed frequently the case), it seems that for rotamer population calculations the parameter $N = J_{\alpha\beta} + J_{\alpha\beta'}$ would be preferable. This parameter has an additional advantage since in many cases it is more directly obtained than the separate vicinal coupling constants. By adding the appropriate terms in eq 10 and 11 and rearranging, we get

$$P_t = (2N - 3J_g - J_t)/(J_t - J_g) \quad (12)$$

for the trans rotamer population. Equation 12 can be used to derive rotamer populations on condition that J_t and J_g are known.

In Figure 3 are shown the spectra of the DA side-chain protons at 90 and 270 MHz. At the lower frequency the multiplet exhibits an AA'BB' pattern, whereas at the higher frequency a more simplified AA'XX' pattern is revealed. This pattern, consisting of two nearly perfect triplets, implies very similar values of $J_{\alpha\beta}$ and $J_{\alpha\beta'}$. Previous analysis of the AA'BB' multiplet has yielded values of 6.8 and 7.6 Hz for the vicinal constants.[9] Although these values are quite close, in view of the 270-MHz spectrum (Figure 3b), it seems that closer values would be more compatible.

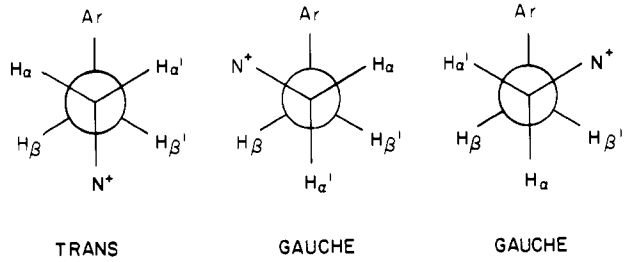

Figure 2. Staggered rotamers about the C_α-C_β bond of dopamine.

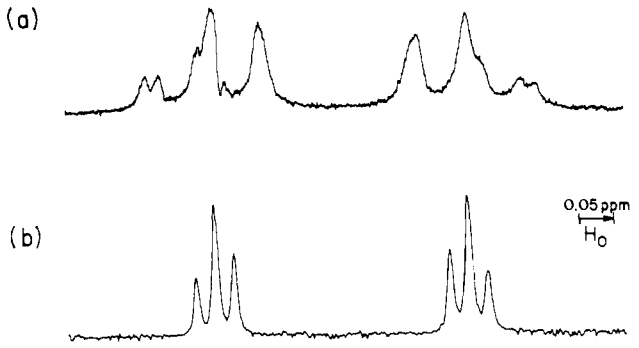

Figure 3. ^{1}H NMR spectra of the side-chain portion of dipamine at (a) 90 MHz, (b) 270 MHz.

It is interesting to note the selective broadening of the upfield multiplet (the H_β resonances) at 90 MHz which completely disappears at 270 MHz. Bustard and Egan[9] have ascribed this broadening to interactions with the nitrogen nucleus. The present observations confirm this assertion. A ^{14}N nucleus may affect proton spectra via two mechanisms: quadrupole relaxation (T_{1Q}) and spin coupling (J_{NH}). The broadening due to quadrupole relaxation would strongly affect protons closer to the nitrogen nucleus, i.e., the H_α resonances (the low-field multiplet) in the present case. Inspection of Figure 3a clearly shows that this is not the case. Moreover, the H_α resonances in themselves are only slightly broadened. This is due to the highly symmetrical environment of the nitrogen in the protonated ammonium group which causes considerable reduction of the electric field gradient at its nucleus, and consequently the shortening of the quadrupole relaxation rate. On the other hand, the N-H coupling through three bonds ($^3J_{NH}$) was found to be greater than the coupling through two bonds ($^2J_{NH}$);[10] hence the broadening of the H_β resonances is due to the long-range coupling with nitrogen. This coupling is modulated by the quadrupole relaxation.[11] When this relaxation rate is slow compared to the spin coupling (i.e., $T_{1Q}^{-1} \ll {}^3J_{NH}$), a distinct splitting of the proton resonances to 1:1:1 triplets would occur. With increasing relaxation rate the components of the triplet would broaden until coalescing to a single line (intermediate case). This broad line would then begin to narrow until a sharp line is obtained for very fast relaxation rate (i.e., $T_{1Q}^{-1} \gg {}^3J_{NH}$). From the expression for T_{1Q}[12]

$$T_{1Q}^{-1} = C\left(\frac{\tau_c}{1 + \omega^2\tau_c^2} + \frac{4\tau_c}{1 + 4\omega^2\tau_c^2}\right) \quad (13)$$

where C is a constant containing nuclear and molecular parameters, ω is the resonance frequency, and τ_c the reorientational correlation time, it follows that, as the resonance frequency is raised, the relaxation rate decreases. The lack of selective H_β broadening at 270 MHz indicates that at 90 MHz the intermediate state prevails; i.e., an incomplete washing out

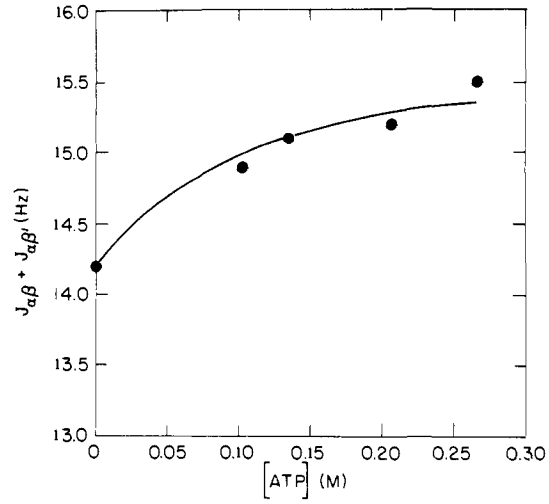

Figure 4. Variation of the dopamine side-chain vicinal coupling constants ($J_{\alpha\beta} + J_{\alpha\beta'}$) with the concentration of ATP ([DA] = 60 mM).

of the N-H_β coupling leads to the broadened proton signals. As for short correlation times (i.e., extreme narrowing case), no frequency dependence is expected; the above observations also indicate that the correlation time must be of the same order as magnitude of ω, i.e., $\tau_c \sim 10^{-9}$-10^{-10} s. A correlation time of this magnitude may be associated with the tumbling of the molecule as a whole.

The 270-MHz spectrum (Figure 3b) exhibits some asymmetry of the triplets indicating slight deviation from a "first-order" spectrum. Nevertheless the value of the parameter N can be obtained directly from the separation of the two side peaks of either triplet. The effect of ATP on the sum of the vicinal coupling constants of the DA side chain is shown in Figure 4. The data were analyzed with the equation

$$N = N_0 + (N_1 - N_0)L_1/L_0 \qquad (14)$$

where N_0 and N_1 are the spin couplings of DA, respectively uncomplexed or in 1:1 complex with ATP. Rotamer populations, given in Table II, were calculated with eq 12 using the average values J_t = 13.1 Hz and J_g = 3.6 Hz as calculated from the empirical relations of Abraham and Gatti[13] with the appropriate substituent electronegativities.[14] The free-energy differences between the trans and the gauche rotamers were also calculated by means of the relation

$$\Delta G_{tg} = -RT \ln (P_t/P_g) \qquad (15)$$

The results indicate that for DA in its complex with ATP there is significant preference for the trans conformation. This can be rationalized in terms of the electrostatic attraction between the ammonium group of DA and the phosphate group of ATP, which would draw these groups toward each other. Consequently, the DA side chain would tend to have a stretched form, compatible with a preferred trans conformation. The electrostatic energy involved can be estimated from a simple model consisting of two electronic point charges (one positive and one negative) separated by a distance of ~3 Å (which was obtained for the N^+···P—O^- distance in the DA-ATP complex by using atomic models on the basis of the complex structure given below) in aqueous medium (having a dielectric constant of 77 at 27 °C). The attraction energy was found to be −1.4 kcal/mol, which is of the order of magnitude of the energy needed to stabilize the trans conformer, as reflected in the change in ΔG_{tg} (Table II).

With regard to NE and E, it has been mentioned above that, owing to line broadening of the side-chain resonances (and also interference of the solvent signal), conformational changes upon complexation with ATP could not be detected. These

Table II. Spin Coupling Constants, Rotamer Populations, and Free-Energy Differences between the Trans and Gauche Conformers of DA, Uncomplexed and in 1:1 Complex with ATP

DA formation	$J_{\alpha\beta} + J_{\alpha\beta'}$, Hz	P_t	P_g	ΔG_{tg}, kcal/mol
Uncomplexed	14.2 ± 0.2	0.47	0.53	0.07
In 1:1 complex with ATP	15.7 ± 0.2	0.79	0.21	−0.79

compounds however show preference for the trans conformer even in the uncomplexed state ($P_t \sim 0.80$[15]) and it is reasonable to assume that their interaction with ATP would give comparable effect as with DA.

(iii) Intermolecular Geometries. Determination of the intermolecular orientations of associating aromatic rings, from ^{1}H NMR shift data, can be implemented in systems in which the observed shifts can be considered to originate solely from anisotropic shielding due to ring-current effects. Theoretically computed shielding for benzene,[16] amino acids, purines, and pyrimidines[17] have been previously used in conformational studies, especially with systems involving nucleotide bases.[18] In the present work ring-current shift tables calculated[19] on the basis of the Johnson-Bovey equation[16a] were employed to construct isoshielding contours for the adenine and the catechol rings. For adenine, the ring-current intensities of the hexagonal and the pentagonal rings were taken as 0.90 and 0.66 relative to the respective six- and five-membered carbon rings.[20] No shielding data have been available for catechol. However, on the basis of the value of 0.94 calculated for the fractional ring-current intensity of phenol (relative to benzene),[20] a value of 0.90 was used as an approximation for the catechol ring current intensity.

To facilitate determination of the intermolecular geometries in the DA-ATP system, the molecular structures obtained by x-ray investigations of ATP[21] and DA[22] were used to draw these molecules. It was assumed that the time-average intramolecular conformations in aqueous solution are not appreciably changed relative to those in the solid state. Considering the adenine nucleotides, the above assumption follows the concept of the rigid nucleotide unit developed by Sundaralingam et al.[23] According to this concept, the mononucleotide unit maintains a rigid conformation in the series of its di- and triphosphates and in its di- and polynucleotide forms, whereas the P-O bonds are flexible. On the basis of x-ray results,[21,24] conformational energy calculations,[23,25] and NMR studies,[18,26] the preferred conformation of the adenine 5′-nucleotides both in solution and in the solid form was found to be anti about the glycosyl linkage and gauche-gauche about both the $C_{4'}$-$C_{5'}$ and $C_{5'}$-$O_{5'}$ bonds. The present results (cf. also ref 1), revealing similar interactions between catecholamines and the three adenine 5′-nucleotides, are also compatible with the rigid conformation concept. The structure of the mononucleotide unit can thus serve as the basic skeleton for the depiction of the structural features of the 1:1 catecholamine-nucleotide complexes. With regard to the catecholamines, energy calculations,[9,27,28] x-ray,[22,29] and NMR results[9,15] (as well as the present results), indicate that these compounds also show in solution preference for the solid-state conformation, i.e., trans about the C_α-C_β bond. Rotation about the C_1-C_β bond is also restricted by steric hindrance. Calculation of rotation energy barriers[28,30] yields minimum values for the conformation with the ethyl part of the side chain approximately perpendicular to the plane of the ring, in accordance with the solid-state results.

The molecules of DA and the mononucleotide unit of ATP were projected onto the planes of the catechol and the purine rings, respectively, and were plotted with the program ORTEP.[31] Isoshielding contours were superimposed on the molecular frames and intermolecular orientations were con-

Table III. Shielding Data for DA and ATP in Their Binary Complex Calculated According to the Models I and II

		DA protons					ATP protons	
		6	5	2	β^a	α^a	8	2
	I				3.0	5.4		
z^b		3.4	3.4	3.4			3.4	3.4
	II				3.8	1.4		
	I							
$\rho_5{}^c$		1.9	3.0	2.6	2.4	2.3		
	II							
	I							
$\rho_6{}^d$		3.5	2.7	2.95	4.4	4.1	3.35	3.45
	II							
	I				0.19	0.17		
$\delta_{calcd}{}^e$		0.39	0.35	0.35			0.12	0.11
	II				0.20	−0.41		
$\delta_{exptl}{}^f$		0.39	0.35	0.34	0.24	0.13	0.12	0.11

[a] The coordinates for the α and β protons were taken as the average for each pair. [b] Vertical distance from the ring inducing the shift (in Å). [c] In-plane distance from the center of the five-membered ring (in Å). [d] In-plane distance from the center of the six-membered ring (in Å). [e] Calculated shieldings (in parts per million). [f] Experimental shielding obtained from Table I (in parts per million).

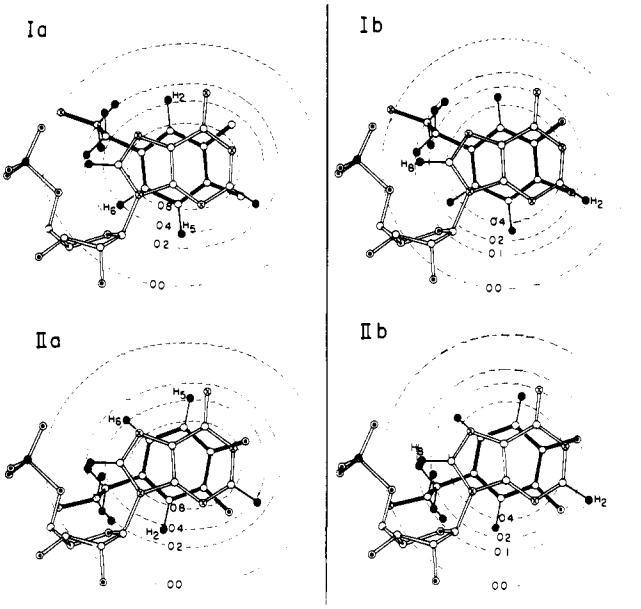

Figure 5. Preferred intermolecular geometries (I and II) between the DA and ATP molecules in the 1:1 complex. For ATP only the monocucleotide unit is shown, having the following geometry: sugar–base torsion = anti (χ = 69°), ribose = ^{3}E, $C_{4'}$–$C_{5'}$ = gauche–gauche (ψ = 67°), $C_{5'}$–$O_{5'}$ = gauche–gauche (ϕ = 224°).[21] The conformation of the DA molecule is trans about the C_α–C_β bond. The molecules are projected onto the planes of their respective rings, which are taken parallel to each other, at a vertical separation of 3.4 Å. Isoshielding contours are drawn for the adenine (Ia, IIa) and the catechol rings (Ib, IIb). The ring protons, experiencing the ring current shifts, are denoted H_2, H_5, and H_6 for DA and H_2 and H_8 for ATP.

structed in accordance with the intrinsic shifts (cf. Table I) of the three DA ring protons (H_2, H_5, and H_6) and the two ATP ring protons (H_2 and H_8), assuming that the stacked rings are parallel. Within the experimental error in the intrinsic shifts the interplanar distance could not be determined unequivocally, but was found to be in the range 3.3–3.5 Å. The rings were thus placed at a vertical distance of 3.4 Å, which has also been found in other systems involving association of aromatic biomolecules. Taking into consideration the formation of hydrogen bonds between the rings, this distance is somewhat larger than expected, which consequently implies that this interaction may be solvent mediated. This is compatible with the small effect of the catechol-ring hydroxyl substitutions on the formation constants in the amine–nucleotide system (cf. Table I).

Two models (denoted I and II) for the intermolecular orientations in the 1:1 DA–ATP complex which yield a best fit between the theoretical and the experimental intrinsic shifts (of the rings protons) are depicted in Figure 5. In both models the phosphate group and consequently (vide infra) the DA molecules lie below the plane of the purine. The two models are related by symmetry to each other, as is expected from the symmetry properties of the isoshielding contours of the rings involved. While the two models are equally probable as far as the rings protons alone are concerned, bringing the side-chain protons into consideration would yield discrepancies. Inspection of Figure 5 reveals that the DA moelcule is inverted in model II relative to I. Consequently the distance of the H_α protons from the purine plane would differ appreciably between the two models (to a lesser extent this would apply also to the H_β protons). In Table III are given the calculated shifts for the DA and the ATP protons. Evidently, an excellent agreement with the experimental values is obtained, except with the computed shift for the H_α protons in model II which is in complete disagreement with the experimental value. This indicates that model I better describes the DA–ATP association. The preferred intermolecular arrangement of the 1:1 DA–ATP complex thus involves significant ring overlap, with the DA side chain pointing toward the phosphate group, the β hydrogens being closer to the purine plane and the α hydrogens further away. Further support for the preference of model I is inferred from the following considerations. (a) The ammonium group in model I is closer to the phosphate group, a position which is favored in view of the electrostatic interaction between these groups. (b) Steric hindrance of the DA side chain as well as of the ribosyl group is reduced in model I. (c) The β-hydroxyl group of L-NE and L-E is found to be cis to the hydroxyl group in the meta position of the catechol ring.[29] Therefore, in model I, but not in II, this group would be directed toward the phosphate moiety enabling the formation of hydrogen bonds between these groups.[7] The deviations between the computed and the experimental shifts for the H_α and H_β protons (cf. Table III) can be rationalized in terms of the rotational flexibility of the side chain. Although the side chain assumes a preferred conformation in the complex with ATP, rotations about the C_1–C_β and the C_α–C_β bonds would populate other conformers as well. From Figure 5 it is seen that these would increase the average shielding of the β protons and decrease that of the α protons, in accordance with the experimental results.

Considering the complexes of NE and E with ATP, in view of model I, it appears that the hydrogen bond between the β-hydroxyl and the phosphate groups can account for the smaller intrinsic shifts observed in these complexes. This interaction would draw the NE molecule toward the P=O group. Consequently the overlap of the catechol ring with the five-membered ring of adenine would increase, whereas the overlap with the six-membered ring would decrease. This would result in deshielding of the ring protons relative to DA. The observation that $\delta_1(H_6) < \delta_1(H_5)$ in the complexes of NE and E is in accordance with the above suggestion. Further evidence is provided by the finding that the ribose protons of ATP are in general more shielded in the complexes with NE relative to those of DA (cf. Table IV). It is to be noted that the negative shifts associated with the methyl group of E in the ATP complex (cf. Table I) is also explicable on the basis of model I. Clearly, this group is found to be in the negative-shielding zone of the adenine ring.

Finally it must be stressed that the model for the preferred

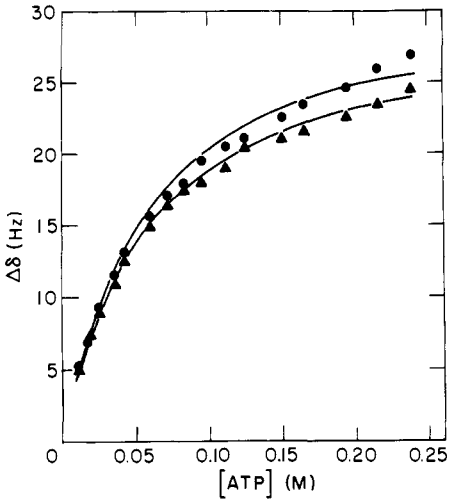

Figure 6. Chemical shifts induced by ATP in the H_5 (triangles) and H_6 (circles) protons of DA (62 mM).

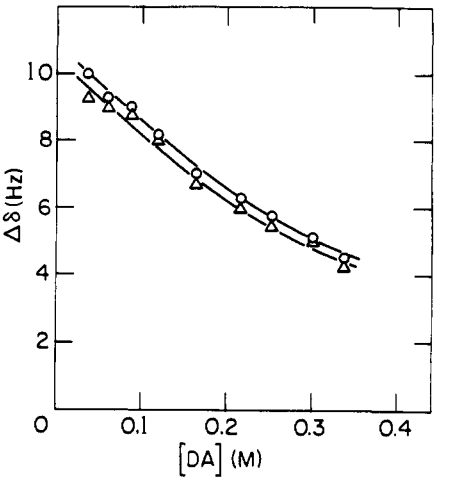

Figure 7. Chemical shifts induced by 26 mM, ATP in the H_5 (triangles) and H_6 (circles) protons of DA as a function of its concentration.

Table IV. Intrinsic Chemical Shifts[a] and Spin Couplings for the Ribose Moiety of ATP Bound in 1:1 and 1:2 Complexes with Catecholamines

	Chemical shifts, Hz					Spin couplings, Hz		
Complex	$H_{1'}$	$H_{2'}$	$H_{3'}$	$H_{4'}$	$H_{5'}(H_{5''})$	$J_{1'2'}$	$J_{2'3'}$	$J_{3'4'}$
1:1 ATP-DA	28	47	16	3	−18	4.3	4.8	5.2
1:2 ATP-DA	50	45	14	9	−15	4.7	4.8	4.7
1:1 ATP-NE	24	51	21	3	−16	4.1	4.8	5.2
1:2 ATP-NE	47	49	20	12	−13	4.8	4.8	4.7
ATP (free)						5.7	4.8	3.7

[a] Measured with a 270-MHz spectrometer.

intermolecular geometry is a time average over the various arrangements interrelated by the motions of the molecules and the rotations about single bonds which are rapid on the NMR time scale. In view of certain limitations and the assumptions encountered in the ring-current shifts calcultions,[18e] coupled with the experimental error in the intrinsic shifts, the above model can be regarded as an approximate description of the structure of the complex which is in good agreement with the experimental results.

B. The 2:1 Complex. Previous results[1] suggest that, in addition to the 1:1 complex, a 2:1 complex may be formed. The structure of the catecholamine-ATP complex described above would allow a second amine ring to stack on the free side of the purine ring of ATP, while the side chains would also interact as in the 1:1 complex. The observed chemical shift due to 1:1 and 2:1 complexation is given by

$$\Delta\delta = (\delta_1 L_1 + 2\delta_2 L_2)/L_0 \qquad (16)$$

where δ_1 and δ_2 are the intrinsic shifts of the 1:1 and 2:1 complexes. The data analysis employed in the present case follows the method outlined by Lenkinsi et al.[32] A brief description of the method is given below. Initial values for K_1 and K_2 are used to calculate the concentrations of the complexes by eq 10 and 8. The results for all of the data points are substituted in the following modified form of eq 16:

$$\frac{L_1}{L_0\Delta\delta} = \frac{1}{\delta_1} - \left(\frac{2L_2}{L_0\Delta\delta}\right)\left(\frac{\delta_2}{\delta_1}\right) \qquad (17)$$

A plot of $L_1/(L_0\Delta\delta)$ vs. $2L_2/(L_0\Delta\delta)$, being subjected to a linear least-squares analysis, allows the determination of $1/\delta_1$ and $1/\delta_2$ from the intercepts with the axes. Then eq 16 is used to derive the calculated shifts. This procedure is continued over a wide range of K_1 and K_2 values until a best fit (in a least-squares sense) between the calculated and the measured shifts is obtained.

Table V. Formation Constants and Bound State Shifts for 1:1 and 2:1 Catecholamine-ATP Complexes

Catecholamine	K_1, M^{-1}	K_2, M^{-1}	H_5 δ_1	H_5 δ_2	H_6 δ_1	H_6 δ_2
DA	16 ± 3	10 ± 2	29 ± 3	33 ± 4	32 ± 3	35 ± 4
NE	17 ± 4	10 ± 2	26 ± 3	25 ± 3	24 ± 3	23 ± 3

The association of DA and NE with ATP was monitored by three types of titrations: (a) titration of dilute amine (~60 mM) by ATP (0.02–0.3 M), (b) titration of concentrated amine (~0.3 M) by ATP (0.02–0.4 M), and (c) titration of dilute ATP (~20 mM) by amine (0.02–0.4 M). These titrations were found to enable very good differentiation between the 1:1 and 2:1 complexes, thus leading to reliable calculated parameters for both species. The data obtained in the three titrations were analyzed simultaneously with the same set of parameters (K_1, K_2, δ_1, δ_2). Only the shifts for the ring protons of DA and NE were used in the computation since these were found to experience the largest values, hence subjected to the lowest relative errors. The results of the fitting procedure are shown in Figures 6–11. The calculated parameters are given in Table V. The results unequivocally demonstrate that both 1:1 and 2:1 catecholamine-ATP complexes exist in aqueous solution. Within the experimental error the present values obtained for δ_1 are in agreement with those obtained above assuming the presence of 1:1 complexes alone. The significant similarity of the bound shifts found for the 1:1 and 2:1 complexes is in agreement with the model for the 2:1 complex suggested above, indicating that the two amine molecules associating at both sides of the purine ring are approximately equivalent. A structural model for the 2:1 complex, compatible with the experimental results, can be directly constructed from those shown in Figure 5. Clearly, a parallel translation of the DA molecule in model II to a vertical separation of 3.4 Å at the other side of the purine ring would bring the side-chain protons to a position equivalent to that in model I. Thus the structure of the complex would be as depicted in Figure 12. The representation of the electrostatic interaction as involving the P_α and P_γ phosphate groups of ATP is tentatively based on the folding of the phosphate moiety as obtained by x-ray studies.[21] The actual sites of the interaction can be determined by a ^{31}P NMR investigation. This study is now being carried out in our laboratory. The lower value of K_2 relative to K_1 reflects the

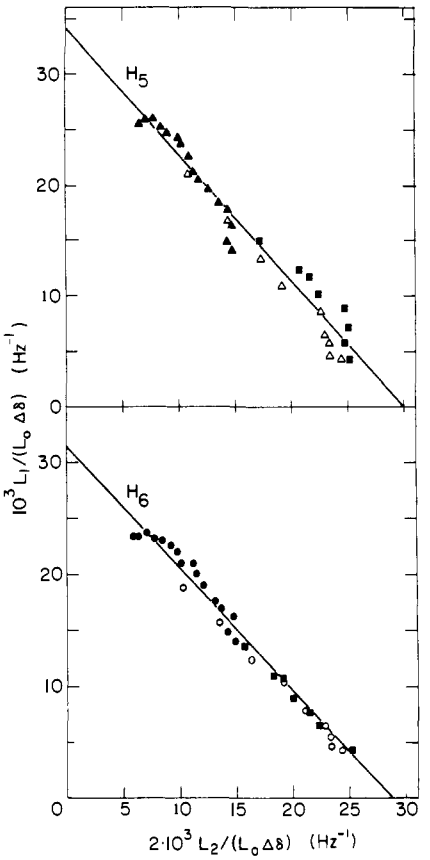

Figure 8. Chemical shift data of Figures 6 and 7 plotted according to eq 17 (see text). The data obtained by titrating 0.35 M DA by ATP (0.03–0.36 M) are also included (filled squares).

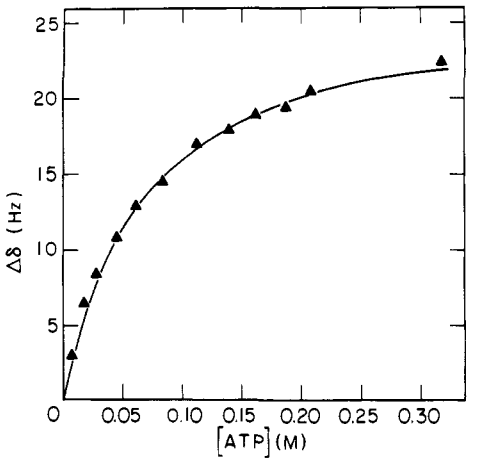

Figure 9. Chemical shifts induced by ATP in the H_5 protons of NE (66 mM).

decrease in the electrostatic interaction of the side chains due to the presence of an additional positively charged group when a second amine molecule binds to the 1:1 complex. In addition the hydrogen bonds between the adenine and the two DA rings would be weaker than with only one DA molecule.

Figure 13 depicts the variation of the fractional concentrations (relative to the total concentration) of the various species present in a DA-ATP solution, as a function of [ATP]/[DA] for DA concentrations of 0.05 and 0.4 M. Clearly for [ATP]/[DA] < 1 the 2:1 complex is formed in appreciable amounts, whereas for [ATP]/[DA] > 2 the assumption that the 1:1 complex predominates is reasonably valid, especially for lower DA total concentrations. It is,

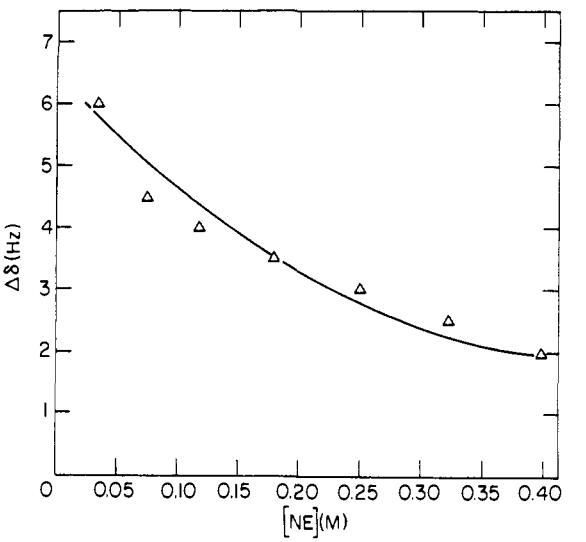

Figure 10. Chemical shifts induced by 18 mM ATP in the H_5 protons of NE as a function of its concentration.

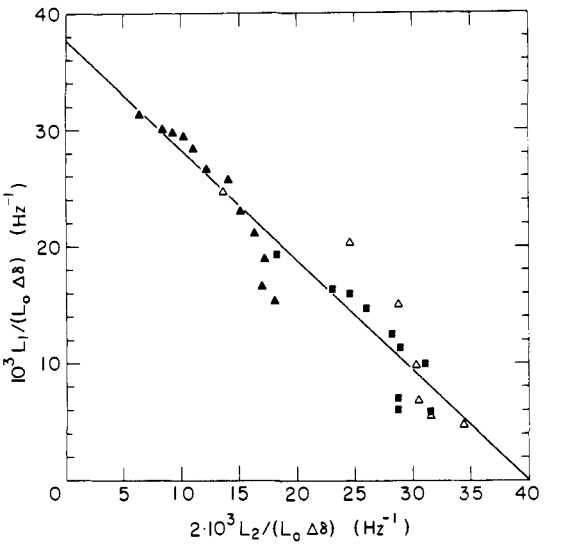

Figure 11. Chemical shift data of Figures 9 and 10 plotted according to eq 17 (see text). The data obtained by titrating 0.31 M NE by ATP (0.02–0.36 M) are also included (filled squares).

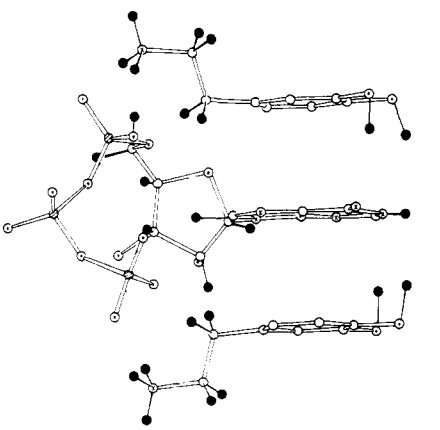

Figure 12. Perspective model for the structure of the 2:1 DA-ATP complex. Intermolecular conformations are as in Figure 5. The molecules are projected onto planes perpendicular to those of the rings. These planes are tilted by 5° to facilitate observation of the ring atoms. The two catechol rings are at a vertical distance of 3.4 Å from the adenine.

however, to be noted that in any case the total fraction of bound amine does not exceed 0.75 for [ATP]/[DA] ~ 1, and falls

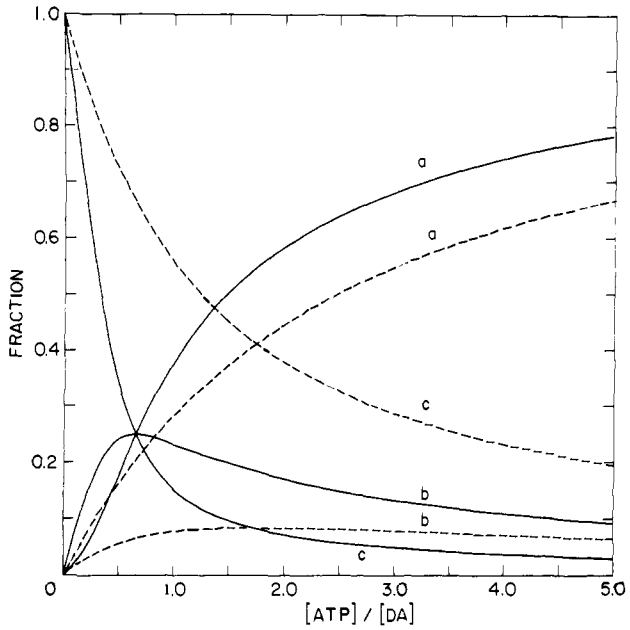

Figure 13. Calculated fractional concentrations of the various species present in 0.05 M (dashed lines) and 0.4 M (solid lines) solutions of DA as a function of the ATP/DA molar ratios: (a) L_1/L_0, (b) L_2/L_0, (c) L/L_0.

quickly as the concentration ratio decreases. Hence, with an excess of catecholamines over ATP, in aqueous solution, a large fraction of the catecholamines would be in an uncomplexed state.

C. The 3:1 Complex. For a large excess of amine molecules over ATP, complexes of stoichiometries higher than 2:1 may be formed in which the association with the additional ligand molecules takes place only via electrostatic interaction between the side chains.[1] This association is much weaker than the one which also involves ring stacking. Since catecholamines do not self-associate,[1] the ring of a third molecule, which binds to the 2:1 complex, would not be in the vicinity of the other aromatic rings; therefore its protons would not experience ring-current shifts. Assuming $N = 3$, i.e., formation of complexes up to 3:1 stoichiometry, the observed chemical shifts would be given by the relation

$$\Delta\delta = [\delta_1 L_1 + 2\delta_2(L_2 + L_3)]/L_0 \qquad (18)$$

To evaluate the formation constant for the 3:1 complex, highly concentrated catecholamine solutions were used (~0.7 M). Shifts induced by ATP titrations are shown in Figure 14. The data were first analyzed assuming the formation of only 1:1 and 2:1 complexes (with eq 6, 8, and 16). The results are depicted by the dashed lines in Figure 14. Next, the data were analyzed assuming the formation also of the 3:1 complex (with eq 5, 6, and 18), yielding the solid lines in Figure 14. In both analyses the parameters of Table V were used in the computations. The average formation constants which were obtained for the 3:1 complex (K_3) are 0.37 ± 0.10 and 0.34 ± 0.10 M^{-1} for the ATP complexes with DA and NE, respectively. These relative low values are compatible with the association in the absence of ring interactions. Calculation of the 3:1 complex concentration reveals that practically it may be considered negligible (e.g., <1% for catecholamine concentration <0.4 M).

D. Effect of Complexation on the Ribose Protons of ATP. Structural Implications. spin couplings and chemical shifts for the ribose moiety of ATP (49 mM) were measured as a function of increasing concentrations of catecholamines (30-350 mM). Coupling constants were calculated from the spectra under the assumption that there is no coupling between non-

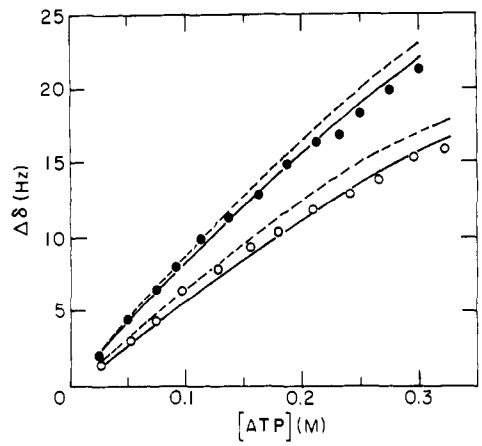

Figure 14. Chemical shifts induced by ATP in the H_5 protons of DA (0.72 M, filled circles) and NE (0.69 M, empty circles). The dashed lines were obtained by least-squares analysis of the data assuming the formation of 1:1 and 2:1 complexes only. The solid lines were obtained by assuming the formation of a 3:1 complex as well.

vicinal protons. The data were analyzed taking into account the possible formation of 1:1 and 2:1 catecholamine-ATP complexes. The results are summarized in Table IV.

(i) Spin Couplings. The ribofuranose ring of nucleotides can assume in aqueous solution a large variety of conformations which rapidly interconvert between one another. A method for quantitative analysis of sugar-ring conformations from vicinal couplings constants has been outlined by Altona and Sundaralingam.[33,34] Essentially their method was based on a pseudorotation model by which the conformational dynamics of the ring were described in terms of two parameters: the angle of pseudorotation and the amplitude of the pucker. An error analysis of this method, undertaken by Evans and Sarma,[35] has shown that the pseudorotation approach yields practically no improvement over the traditional Karplus approach.[35-38] It has been suggested that the conformation of the ribose ring might be treated as a simple $C_{2'}$-endo (^2E) ⇄ $C_{3'}$-endo (^3E) equilibrium. The fractional populations of the ^2E and ^3E conformations (P_{2E} and P_{3E}, respectively) would thus relate directly to the vicinal coupling constants through eq 19.[26f]

$$J_{1'2'} = (J_{1'2'} + J_{3'4'})P_{2E} \qquad (19a)$$

$$J_{3'4'} = (J_{1'2'} + J_{3'4'})P_{3E} \qquad (19b)$$

Inspection of Table IV indicates that upon complexation of ATP with catecholamines the value of $J_{1'2'}$ decreases whereas that of $J_{3'4'}$ increases, but their sum remains essentially constant. This implies that the observed changes in the vicinal coupling constants do not result from alterations in the dihedral relations in the pure conformations of the ribose (the average contribution of which is measured under conditions of rapid interconversion), but are due to changes in the populations of these conformations. The average value of 9.4 Hz obtained for $J_{1'2'} + J_{3'4'}$ in the present study is in accordance with values obtained for ribose moieties of other nucleotides.[26e] Using this value, the populations of the ^2E and ^3E conformations were calculated for ATP free and complexed with catecholamines. The results, given in Table VI, indicate that complex formation

Table VI. Populations of the Ribose Ring Conformations in 1:1 and 1:2 Complexes of ATP with Catecholamines

Complex	P_{2E}	P_{3E}
1:1 ATP-DA	0.45	0.55
1:2 ATP-DA	0.50	0.50
1:1 ATP-NE	0.44	0.56
1:2 ATP-NE	0.50	0.50
ATP (free)	0.61	0.39

Table VII. Shielding Data for the Ribose Protons of ATP Calculated for Association with a DA Molecule Either on Side A or Side B of the Adenine[a]

		$H_{2'}$[b]		$H_{3'}$[b]				
	$H_{1'}$	^2E	^3E	^2E	^3E	$H_{4'}$	$H_{5'}$	$H_{5''}$
ρ[c]	3.6	2.8	3.3	4.5	4.0	5.8	6.0	6.5
$z(A)$[d]	3.7	2.3	1.4	4.3	2.1	3.8	5.4	3.7
$z(B)$[d]	3.1	4.5	5.4	5.5	4.7	3.0	1.4	3.1
$\delta_{calcd}(A)$[e]	0.10	−0.09[f]		−0.03[f]		0.00	0.00[g]	
$\delta_{calcd}(B)$[e]	0.07	0.15[f]		0.07[f]		−0.03	−0.06[g]	
δ_1[h]	0.10	0.17		0.06		0.01	−0.07	
$\delta_2-\delta_1$[i]	0.08	−0.01		−0.01		0.02	0.01	

[a] The sugar-base torsion of ATP was fixed at 40°, as an average value for an anti conformation. [b] The spatial coordinates for $H_{2'}$ and $H_{3'}$ are given for the ^2E and ^3E conformations of the ribose. [c] In plane distance from the center of the catechol ring of DA (in Å). [d] Vertical distance (in Å) from the plane of the catechol ring placed 3.4 Å from the adenine, either on side A or side B (see text). [e] Calculated shieldings (in parts per million). [f] Average shieldings calculated under the assumption of equal contribution from the ^2E and ^3E conformations. [g] Average shieldings for the $H_{5'}$ and $H_{5''}$ protons. The shielding values for $H_{5''}$ were obtained by extrapolation since theoretical shieldings for $\rho > 6$ Å were not available. [h] Experimental shieldings (in parts per million) for the 1:1 complex, obtained from Table IV. [i] Difference in the shieldings (in parts per million) between the 2:1 and 1:1 complexes, obtained from Table IV.

between ATP and catecholamines introduces redistribution in the ribose-ring preferred conformations. While the ribose of free ATP shows high preference for ^2E pucker, the formation of 1:1 catecholamine-ATP complex causes significant increase, of ~16%, in the ^3E population. Association of a second catecholamine molecule has a reverse effect, i.e., to decrease slightly (~5%) the population of the ^3E pucker. It should be noted that, as pointed out by Sarma et al.,[18d,35,39] although the error in the calculated populations might be as much as 10% (or more), the differences in relative populations are far more accurate. A similar trend in the change of the conformation populations, i.e., increased preference for ^3E pucker, has also been observed upon self-association of AMP[35] and ATP,[40] association of AMP with purine[41] and tryptamine,[42] and dimerization of dinucleotide monophosphates.[26e] These $^2E \rightarrow {}^3E$ transitions can be rationalized in terms of slight changes in the sugar-base torsion due to association involving ring stacking.[26e] In the system presently under investigation additional effects may be introduced via changes in the conformation of the exocyclic group due to the electrostatic interaction between the phosphate moiety of ATP and the ammonium group of the catecholamines.

(ii) Chemical Shifts. Inspection of Figure 12 reveals that the ribose protons show marked differences in their oritentations with respect to the rings of the catecholamine molecules associated on either sides of ATP. For example, $H_{2'}$ is much closer to the center of the catechol ring of one molecule (which associates on side A of the adenine[18c,e]) than to that of the other molecule (which associates on side B of the adenine[18c,e]). Such differences are expected to be reflected appreciably in the proton shieldings. Therefore ring-current shift analysis of the chemical shifts induced in the ribose protons can be used to determine which side of the adenine is preferred for association with catecholamines.

The spatial positions of the ribose protons relative to the center of the catechol ring of a DA molecule associated either on side A or side B of the adenine ring of ATP were determined for both the ^2E and ^3E conformations of the ribose. Actually it was found that only the positions of $H_{2'}$ and $H_{3'}$ are significantly affected by the change in the ribose pucker. Calculated shieldings are given in Table VII. Comparing the theoretical and the experimental shielding data, a clear correlation is observed between the values of $\delta_{calcd}(B)$ and δ_1 and between $\delta_{calcd}(A)$ and $\delta_2-\delta_1$ (cf. Table VII), where $\delta_{calcd}(A)$ and $\delta_{calcd}(B)$ denote shieldings induced by a DA molecule associated on side A or B of the adenine, respectively, and δ_1 and δ_2 are the respective intrinsic shifts of the ribose protons in the 1:1 and 2:1 catecholamine-ATP complexes. It should be noted that the high flexibility of the ribose moiety renders prediction of accurate shieldings somewhat difficult. While shielding data are computed on the basis of static model orientations, changes in the ribose pucker and in the sugar-base torsion upon complexation would alter the positions of the ribose protons relative to both the adenine and the catechol rings. This would consequently affect the shielding of these protons.[43] In view of this argument, coupled with other uncertainties in the computations (vide supra), the agreement between the calculated and the observed shieldings can be considered satisfactory. Yet the correlations found between these shieldings allow unambiguous determination of the preferred side of adenine for association with catecholamines. Evidently the first DA molecule would associate on side B of the adenine to form the 1:1 complex with ATP. Ribose chemical shifts for the complexes of ATP and NE comparable with the corresponding complexes of DA (cf. Table IV) imply that the above conclusion holds true also for this amine. The preference of side B for association is compatible with the findings that all three adenine nucleotides form similar 1:1 complexes with catecholamines (see above). From Figure 12 it appears that the α-phosphate and the ammonium group, which are involved in electrostatic interaction, would be in close proximity when the catecholamine molecule associates on side B of the adenine. Consequently this side would be energetically more favored than side A of the adenine.

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Approaches to the Synthesis of Masked *p*-Quinone Methides. Applications to the Total Synthesis of (±)-Cherylline

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Abstract: The synthesis and chemistry of *p*-quinone methide ketals, prepared from *p*-quinone monoketals **9a–c** and α-trimethylsilylamides or phosphoranes, is discussed within the context of the total synthesis of Amaryllidaceae alkaloid cherylline (**3**).

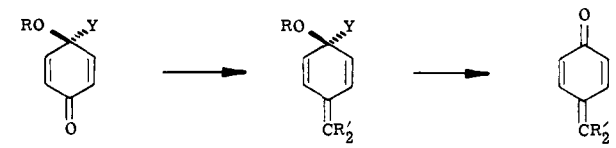
Introduction

o- and *p*-quinone methides constitute a class of highly electrophilic molecules that are frequently encountered in natural products chemistry.[1-3] A large number of quinone methides have been isolated as fungal metabolites,[2] wood pigments,[2] and insect pigments.[4] In addition, quinone methides have been implicated as intermediates in oxidative phosphorylation[2] and in the biosynthesis of chromans,[2,5] lignin,[2,6] and alkaloids.[7] It has also been suggested that some quinoid substances that exhibit antitumor properties may be activated in vivo by conversion to quinone methides.[8]

A recent survey indicates that there are no general methods for preparing *p*-quinone methides such as **2** from quinoid precursors.[9] In principle, olefination of a quinone carbonyl group offers the most direct route from a quinone to a quinone methide.[10] Although several Wittig reactions on quinone substrates have been reported, this method has yet to be established as a generally effective approach to the synthesis of quinone methides.[10d-f]

Recently, research in this laboratory has been directed toward exploiting "blocked" quinones such as **1a, 1b,** and **1c** as intermediates in the synthesis of naturally occurring quinones,[11] *p*-quinols,[12] and alkaloids. A strategy for generating *p*-quinone methides which is conceptually similar to direct olefination of quinones, but operationally more attractive, is outlined in Scheme I. This report describes the investigation of this reaction sequence within the context of the total synthesis of the unique Amaryllidaceae alkaloid, cherylline (**3**).[13-15]

Scheme I



1, a; Y = CN, R = $SiMe_3$
b; Y = CN, R = $SiEt_3$
c; Y = OR, R = alkyl

2

Synthesis and Reactions of *p*-Quinone Methide Ketals

The general approach which was conceived for the synthesis of cherylline is outlined in Scheme II. The critical feature in

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