

which has the flexibility of creating a diverse array of important substitution patterns, was developed. Other ring sizes may also be generated by simple modification of the conjunctive reagents. Thus, the  $[6 + m + n]$  bicycloannulation  $[(6 + 2 + 1)]$  for the case of equ 4 may offer useful strategies toward important synthetic targets.

**Acknowledgment.** We thank the National Science Foundation and the National Institutes of Health-General Medical Sciences for their generous support of our programs.

(11) Angus, R. O., Jr.; Johnson, R. P. *J. Org. Chem.* **1983**, *48*, 273.

## Allosteric Effects: Structural and Thermodynamic Origins of Binding Cooperativity in a Subunit Model

K. Onan\*

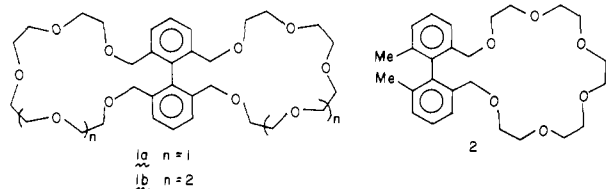
Department of Chemistry, Northeastern University  
Boston, Massachusetts 02115

J. Rebek, Jr.,\* T. Costello, and L. Marshall

Department of Chemistry, University of Pittsburgh  
Pittsburgh, Pennsylvania 15260  
Received August 18, 1983

We recently introduced the macrobicyclic ethers **1** as models for the allosteric effects shown by subunit enzyme systems.<sup>1</sup> The binding of organomercurials to **1a** showed *positive cooperativity*, while its homologue **1b** showed *noncooperativity* in comparable circumstances. This seemingly unpredictable behavior raised questions about the origins of the allosteric effects in these systems. These questions were approached through crystallographic and thermodynamic studies, and we report here our answers.

**Crystallography.** Crystals suitable for diffraction were obtained from EtOAc for **1a**·2Hg(CN)<sub>2</sub> and EtO<sub>2</sub> for **2**·Hg(CF<sub>3</sub>)<sub>2</sub>. Crystal



data for **1a**·2Hg(CN)<sub>2</sub>: C<sub>36</sub>H<sub>46</sub>Hg<sub>2</sub>N<sub>4</sub>O<sub>10</sub>, *M<sub>r</sub>*, 1096.0, space group *P2<sub>1</sub>/c*, *a* = 10.283 (1) Å, *b* = 25.263 (3) Å, *c* = 16.753 (2) Å,  $\beta$  = 110.10 (1)°, *Z* = 4. For **2**·Hg(CF<sub>3</sub>)<sub>2</sub>: C<sub>28</sub>H<sub>36</sub>HgF<sub>6</sub>O<sub>6</sub>, *M<sub>r</sub>*

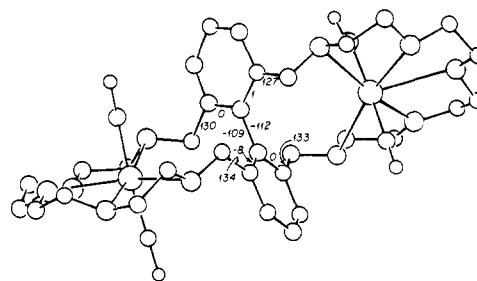


Figure 1. **1a**·2Hg(CN)<sub>2</sub>.

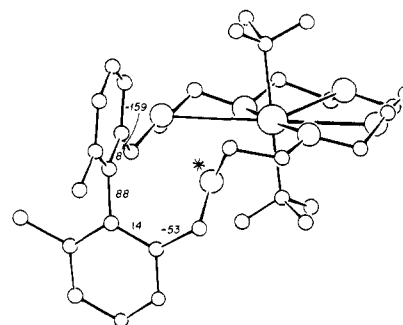


Figure 2. **2**·Hg(CF<sub>3</sub>)<sub>2</sub>. Computer-generated perspective drawings of the complexes; hydrogen atoms have been omitted for clarity. The uncoordinated benzyl oxygen in **2** is marked with an asterisk and selected endocyclic torsion angles are indicated on the figure.

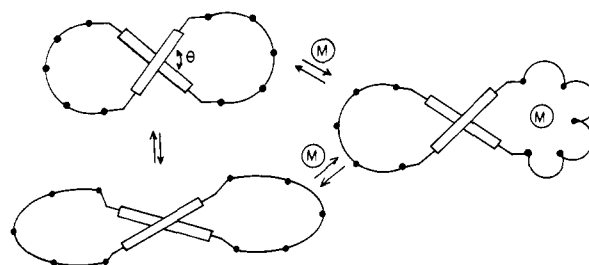


Figure 3.

783.2, space group *P1̄*, *a* = 11.532 (2) Å, *b* = 17.497 (3) Å, *c* = 8.245 (2) Å,  $\alpha$  = 98.4 (2)°,  $\beta$  = 107.0 (2)°,  $\gamma$  = 98.1 (2)°, *Z* = 2. Data for both were collected with a Syntex *P2*, diffractometer with CuK $\alpha$  radiation, and unique reflections with  $I > 2\sigma(I)$  [3500 for **1a**·2Hg(CN)<sub>2</sub>, 4275 for **2**·Hg(CF<sub>3</sub>)<sub>2</sub>] were used in the structure solutions (heavy atom method) and least-squares refinement (ultimately with anisotropic thermal parameters for all non-hydrogen atoms).<sup>2</sup> Convergence was reached at *R* = 0.067 for **1a**·2Hg(CN)<sub>2</sub> and 0.073 for **2**·Hg(CF<sub>3</sub>)<sub>2</sub>. Positional and thermal parameters for all atoms are in Tables I-VI (supplementary material).

Figures 1 and 2 reveal that while *all* of the ethereal oxygens are involved in binding Hg(CN)<sub>2</sub> to **1a**, only five of the six oxygens are involved in binding the Hg(CF<sub>3</sub>)<sub>2</sub> to the 22-membered **2**. The uncoordinated benzyl oxygen atom in **2** lies significantly out of the plane of the other oxygen atoms and at a distance of 3.45 Å from the Hg atom (as compared to the other Hg-O distances of 2.84-3.12 Å).

**Thermodynamic Origins.** In order to determine whether the cooperativity shown by **1a** to Hg(CN)<sub>2</sub> arises from entropic or enthalpic effects, we examined the binding as a function of temperature. The question reduces to the temperature dependence of the ratio *K*<sub>1</sub>/*K*<sub>2</sub>, a value that can be obtained directly from the NMR spectra as previously described.<sup>1</sup> This ratio was determined

(1) Rebek, J., Jr.; Wattlely, R. V.; Costello, T.; Gadwood, R.; Marshall, L. *J. Am. Chem. Soc.* **1980**, *102*, 7398-7400. Rebek, J., Jr.; Wattlely, R. V.; Costello, T.; Gadwood, R.; Marshall, L. *Angew. Chem.* **1981**, *93*, 584-585. For other models of subunit cooperativity introduced recently, see: Traylor, T. C.; Mitchell, M. J.; Ciccone, J. P.; Nelson, S. *J. Am. Chem. Soc.* **1982**, *104*, 4986-4989. Tabushi, I.; Sasaki, T. *Ibid.* **1983**, *105*, 2901-2902.

(2) All crystallographic calculations were carried out on a VAX 11/780 computer. The principal programs used were FMLS, anisotropic full-matrix least-squares refinement (Ganzel, P. L.; Sparks, R. A.; Trueblood, K. N.; UCLA; modified by McPhail, A. T., Duke University), and ORTEP, crystallographic illustration programs (Johnson, C. K., Oak Ridge, ORNL-3794).

at 5 °C intervals over the temperature range 240–290 °C and the populations of the free crown, its 1:1 complex, and its 2:1 complex were determined by total line-shape analysis of the spectra.<sup>3</sup> It was found that the  $K_1/K_2$  ratio is *invariant* with temperature. This requires that the enthalpies of binding be the same for both steps, i.e.,  $\Delta H_1 = \Delta H_2 = -7.3 \pm .3$  kcal/mol. The origins of cooperativity here must therefore be entropic ( $\Delta S_1 = -18.5 \pm 0.5$ ,  $\Delta S_2 = -16.5 \pm 1$  eu).

Interpretation of these results is facilitated by reference to Figure 3. The free crown ethers enjoy a number of conformations or effective sizes defined by the dihedral angle  $\theta$ . Binding of mercuric cyanide at one site of **1a** fixes this angle at the value optimal for complexation because both the benzyl oxygens are involved. The molecular rigidity of the biaryl system requires that this angle be reproduced at the remote site. To the extent that this second site is now more organized for complexation, binding of the second metal is enhanced;  $\Delta S_2$  is less negative than  $\Delta S_1$ .

In the binding of mercuric trifluoromethyl to **1b**, no such restriction of  $\theta$  occurs since only one of the benzyl oxygens is involved. The sites then act independently, and the result  $\Delta H_1 = \Delta H_2$  and  $\Delta S_1 = \Delta S_2$  leads to noncooperativity. Apparently in either case, initial binding and its attendant conformational restrictions do not alter the potential energy of the remote site.

**Conclusion.** In this study we have shown that allosteric cooperativity can arise from purely entropic effects in a model system. The simplicity of the model leads to interpretable data, but its relevance to biochemical systems may be remote.<sup>4</sup> Whether potential energy changes can result in cooperativity is the focus of our current research.

**Acknowledgment.** We thank the National Institutes of Health for financial support of this research. The purchase of the diffractometer was supported in part by NSF Grant CHE 76-06503.

**Supplementary Material Available:** Numbered drawings of complexes and tables of positional and thermal parameters for all atoms (13 pages). Ordering information is given on any current masthead page.

(3) Stephenson, D. S.; Binsch, G. *J. Magn. Reson.* **1978**, *32*, 145–152. available as DNMRs: *QCPE*, **1978**, *10*, 365.

(4) For a discussion of problems in assigning the cooperativity of hemoglobin to entropic or enthalpic effects, see: Imai, K. *J. Mol. Biol.* **1979**, *133*, 233–247.

## Proton-Transfer Dynamics Involving the Intramolecular Charge-Transfer State of Photoexcited 1-(*p*-Aminophenyl)pyrene<sup>1</sup>

Sair Hagopian and Lawrence A. Singer\*

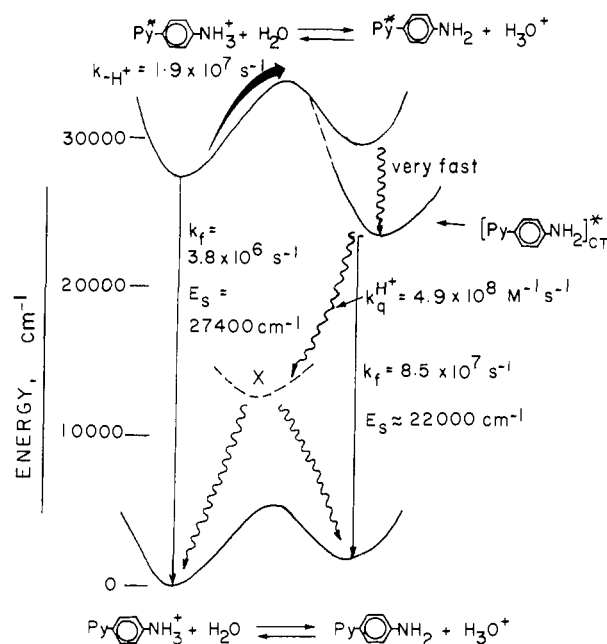
Department of Chemistry  
University of Southern California  
Los Angeles, California 90089-1062

Received June 3, 1983

The relationship between molecular structure and photophysics has been investigated with a new probe system, 1-(*p*-aminophenyl)pyrene (**1**). In particular, we have studied proton-transfer reactions in the excited state of **1** including deprotonation of the anilino function in a locally excited  $\pi, \pi^*$  state and proton transfer quenching of the charge-transfer (CT) state. Rate parameters have now been measured for these processes.

In most solvents, a single fluorescence is observed from **1** that is highly solvatochromic (near 400 nm in nonpolar and near 500 nm in polar media). In alcohol–water (50:50), at acidic pH's, dual fluorescences are observed, which are assigned to emission from excited protonated (near 400 nm,  $35 \pm 2$  ns) and free-base

(1) Singer, L. A.; Hagopian, S. "Abstracts of Papers", Presented at the 185th ACS National Meeting of the American Chemical Society, Seattle, WA, March 20–25, 1983; American Chemical Society: Washington, DC, 1983; ORGN 49.



**Figure 1.** Comprehensive photophysical scheme for **1**. Values given are for solution in 50:50 ethanol–water and refer to the following quantities: energy of radiative singlet state ( $E_s$ ), radiative rate constant ( $k_f$ ), proton-transfer quenching constant ( $k_q^{H^+}$ ), deprotonation rate constant ( $k_{-H^+}$ ). X = metastable ground state of products resulting from proton-transfer quenching of CT state.

(near 500 nm, limiting lifetime  $3.9 \pm 0.1$  ns)<sup>2</sup> fluorophors. We assign the shorter wavelength, structured, fluorescence to a  $\pi, \pi^*$  state wherein the excitation is localized in the pyrene moiety and the longer wavelength, structureless, fluorescence to a CT state with the aniline as donor and the pyrene as acceptor.<sup>3</sup> From low-temperature, time-resolved fluorescence studies in our own laboratory (to be published separately) and the work of others on closely related systems,<sup>4</sup> we conclude that the anilino moiety is twisted out of the plane of the pyrene ring in the CT state.

The ground-state  $pK_a$  of **1** in ethanol–water (50:50) appears in Table I along with other relevant values. The measured  $pK_a$  in alcohol–water (4.05) indicates no unusual ground-state acid-base properties for **1**.

The excited state  $pK_a$  values ( $pK_a^*$ ) for **1**, obtained by Förster cycle calculations<sup>5</sup> and fluorescence titrations,<sup>9</sup> are in excellent agreement and indicate only a modest enhancement in acidity of the excited state over the ground state. In contrast, systems with the amine function directly attached to an excited aromatic moiety show enhancements in acidity in the excited singlet state of many orders of magnitude (Table I).

The similarity of the  $pK_a$  and  $pK_a^*$  values for **1** most likely results from similar rates of deprotonation for the conjugate acids in the ground and excited states, respectively (assuming similar and diffusion-limited protonation in both cases),<sup>10</sup> indicating little

(2) Determined by the phase-plane deconvolution method. Demas, J. N. *J. Chem. Ed.* **1976**, *53*, 657.

(3) On the basis of one-electron redox potentials of pyrene and aniline, we estimate that the CT state will be 2.7 eV above the ground state in polar media compared to a locally excited  $\pi, \pi^*$  state at 3.4 eV.

(4) (a) Grabowski, Z. R.; Rotkiewicz, K.; Siemiarz, A.; Cowley, D.; Baumann, W. *Nouv. J. Chim.* **1979**, *3*, 443 and references therein. (b) Grabowski, Z. R.; Rotkiewicz, K.; Siemiarz, A. *J. Lumin.* **1979**, *18*, 420. (c) Rotkiewicz, K.; Rubaszewska, W. *Chem. Phys. Lett.* **1980**, *70*, 444. (d) Lippert, E.; Ayak, A. A.; Rettig, W.; Wermuth, G. *J. Photochem.* **1981**, *17*, 237. (e) Rettig, W. *J. Phys. Chem.* **1982**, *86*, 1970. (f) Kolos, R.; Grabowski, Z. R. *J. Mol. Struct.* **1982**, *84*, 251. (g) Kosower, E. M. *Acc. Chem. Res.* **1982**, *15*, 259 and references therein.

(5) Perrin, D. D. "Dissociation Constants of Organic Bases in Aqueous Solution"; Butterworths: London, 1965.

(6) Donck, E. V.; Dramaix, R.; Nasielski, J.; Vogels, C. *Trans. Faraday Soc.* **1969**, *65*, 3258.

(7) Shizuka, H.; Tsutsumi, K.; Takeuchi, H.; Tanaka, I. *Chem. Phys. Lett.* **1979**, *62*, 408.

(8) Tsutsumi, K.; Shizuka, H. *Chem. Phys. Lett.* **1977**, *52*, 485.

(9) Ireland, J. F.; Wyatt, P. A. H. *Adv. Phys. Org. Chem.* **1976**, *12*.