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(7) A solution (50 μL) of TLC-purified [4-^{14}C]cholesterol and 0.5 mL of diethyl ether were stirred with 0.2 mL of a 10% aqueous suspension of polystyrene latex beads (Dow Diagnostic, 0.82-μm average diameter) for 30 min; 2–3 mL of distilled H_2O was added; and the ether was removed by passing N_2 over the sample. The appropriate 0.1 M buffer (25 mL) (pH 4, acetate; 6, 7, and 8, phosphate; 10, carbonate) was then added.

(8) (a) Prepared by the method of A. D. McElroy and S. S. Hashman, *Inorg. Chem.*, **3**, 1798 (1964). *Caution:* explosions have been reported during this reaction. Extraction should be carried out in all-glass apparatus; avoid paper thimbles. (b) Material so prepared had mp 101 °C uncorr (lit.[8a] mp 97 °C) and assayed 96–98% $O_2^{-}\cdot$ by O_2 evolution. (c) A control showed that 60% of the original $O_2^{-}\cdot$ remained in the Me_2SO solution after 19 h. (Most of the losses occur during the first hour.) The figures in Table I are not corrected for these losses. The reduction of 10^{-3} M nitroblue tetrazolium in Me_2SO was used to assay $[O_2^{-}\cdot]$. Solutions were diluted 10-fold with Me_2SO and absorbance of the product (ϵ_{685} 85 000 M^{-1} cm^{-1}) was recorded.

(9) Using a scintillation counter; 10 mL of Biofluor (NEN) was added; the internal standard technique was used to correct for quenching.

(10) The aqueous layer was centrifuged and the amount of histidine reacted measured by the Pauly reagent;[14] unreacted controls were used for comparison. Controls also established that the extraction process did not remove histidine, and that buffer, histidine photooxidation products, or methylene blue did not interfere with the analysis.

(11) The fraction of $^1O_2$ trapped is $[A]/(\beta + [A])$ and was 1/8 at the 5×10^{-4} M concentration of histidine used.[16] Controls showed that the Ⓟ-chol did not trap a substantial fraction of the $^1O_2$ generated.

(12) Subsequent experiments with different preparations gave slightly lower efficiencies; however, the general conclusions are not affected.

(13) It is interesting to calculate the steady-state concentration of $[O_2^{-}\cdot]$ present. From the rate of addition of $O_2^{-}\cdot$ and the rate of decay at pH 7, calculated from Czapski's relationships[15] (6.36×10^5 M^{-1} s^{-1}), the steady-state concentration of $O_2^{-}\cdot$ is calculated to be 3.9×10^{-6} M and the fraction of $^1O_2$ quenched (from the $O_2^{-}\cdot$ quenching rate[1a]) to be ~1.6%. The steady-state concentration of $O_2^{-}\cdot$ increases with pH because of the slower dismutation of $O_2^{-}\cdot$;[15] however, even at pH 10, the amount of $^1O_2$ quenching is calculated to be no more than 20% under the conditions of the yield experiment at pH 10.

(14) H. T. McPherson, *Biochem. J.*, **40**, 470 (1946).

(15) D. Behar, G. Czapski, J. Rabani, L. M. Dorfman, and H. A. Schwartz, *J. Phys. Chem.*, **74**, 3209 (1970).

(16) For definitions of terms and values of constants, see C. S. Foote in "Free Radicals in Biology", Vol. II, W. A. Pryor, Ed., Academic Press, New York, 1976, p 94.

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Host–Guest Complex Formation between a Water-Soluble Polyparacyclophane and a Hydrophobic Guest Molecule

Sir:

The design of water-soluble host compounds which have a hydrophobic cavity of definite shape and size is of great interest in relation to substrate-specific binding in aqueous solution. Of these host compounds, cycloamyloses (native and modified) have been most widely studied and thoroughly reviewed.[1] Recently another class of compounds, water-soluble paracyclophanes, in which aromatic ring(s) and methylene units are expected to compose a hydrophobic cavity, have drawn attention as artificial host compounds.[2,3]

Although several spectral studies[2a,b,3a-c] have suggested that they form inclusion complexes with hydrophobic substrates in aqueous solution, there has not been *direct evidence for "inclusion"*. We report here the first example of a crystalline complex of a water-soluble paracyclophane with a hydrophobic substrate, which was isolated from an *aqueous solution* and characterized as an *inclusion complex* by the X-ray method.

1,6,20,25-Tetraaza[6.1.6.1]paracyclophane (**2b**) was designed as a host compound and synthesized employing the known method.[4] Equimolar amounts of *N,N'*-ditosyl-4,4'-diaminodiphenylmethane[5] (**1**) and tetramethylene bromide

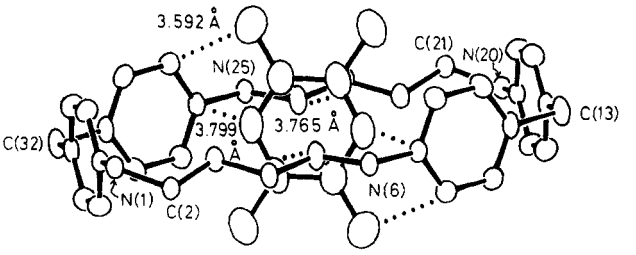
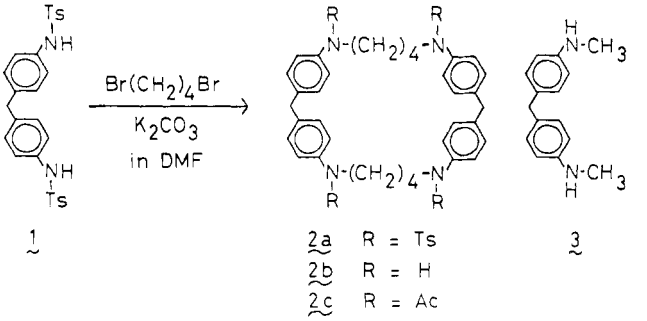


Figure 1. Perspective view of the host–guest complex of **2b**·4HCl with durene drawn by the ORTEP program.

were cyclized in DMF in the presence of potassium carbonate by high-dilution method to give **2a**,[6a] dec pt 305–306 °C, in 25% yield. Detosylation of **2a** gave **2b**,[6] mp 182.5–184 °C dec, in 67% yield after purification. The cyclic structure was confirmed on the basis of the mass spectra of **2b** and further of **2c**,[6a,c] mp 292–293 °C dec, which was obtained from **2b** in almost quantitative yield.



As **2b** was soluble in water below pH 2, the interactions of **2b** with various substrates having hydrophobic moieties were investigated in acidic aqueous solution. The fluorescence intensity of 1-anilinonaphthalene-8-sulfonate (1,8-ANS) was markedly enhanced in the presence of **2b**,[7] suggesting that 1,8-ANS was transferred into a nonpolar environment and/or subjected to a conformational change[8] by **2b**. The Benesi–Hildebrand plot[9] of the fluorescence intensity gave a straight line which indicated **2b** and 1,8-ANS formed a 1:1 complex with a dissociation constant of 1.6×10^{-4} M, comparable with other complexes from the known water-soluble paracyclophanes.[10] In the ^1H NMR spectrum the signals of 2,7-dihydroxynaphthalene moved upfield remarkably in the presence of **2b**;[11,12] this can be ascribed to a very strong shielding effect of the aromatic rings of **2b**. On the other hand the acyclic reference compound **3**[13] showed only a small effect in both the fluorescence and ^1H NMR spectra.[14] These spectral data suggest that **2b** and the substrates are in an intimate contact that does not occur without the cyclic structure of **2b**. Inclusion within the cavity of **2b** is considered to be a possible way of contact.

Furthermore **2b** formed crystalline complexes from aqueous solution with a variety of substrates having hydrophobic moieties, e.g., 1,3-dihydroxynaphthalene, 2,7-dihydroxynaphthalene, naphthalene, *p*-xylene, and durene.[15] When durene (1,2,4,5-tetramethylbenzene) was used as the substrate, the 1:1 crystalline complex which was characterized as **2b**·4HCl·durene·$4H_2O$[16] was successfully obtained, and its structure was determined by the X-ray method. Crystal data: monoclinic; space group $P2_1/n$; a = 14.552 (7), b = 22.582 (12), c = 7.238 (4) Å; β = 97.23 (4)°; V = 2359.6 Å^3; Z = 2. The crystal structure was solved by the direct method and refined by the method of block-diagonal least-squares to the final R factor of 0.065 for 3910 nonzero, independent reflections obtained by using graphite monochromated Cu Kα radiation.

As shown in Figure 1, **2b**·4HCl and durene form a *host–guest complex*[17] in which the guest molecule, durene, is *fully*

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included within the cavity of the host molecule **2b**·4HCl. The whole 1:1 complex sits on a center of symmetry, which means that the guest molecule is located exactly at the middle of the cavity.[18] The conformation of the host molecule is as follows. The four benzene rings are perpendicular to the mean plane of the macroring ("face" conformation[19,20]), and the bridging chain moieties take the trans-planar conformation except for the gauche conformation about the N(1)–C(2) and N(20)–C(21) bonds. As a result a cavity is formed which has rectangularly shaped open ends (~3.5 × 7.9 Å)[21] and a depth of 6.5 Å. The mode of inclusion of the guest molecule is as follows. As expected the benzene ring fits well with the cavity, being nearly parallel to the inner wall, and the methyl groups which are oriented to the outside protrude partly from the cavity. The closest contacts between the host and guest molecules (<3.80 Å) are shown in Figure 1 with dotted lines. Since durene is a nonpolar substrate and the complex was obtained from aqueous solution, it is indicated that hydrophobic interaction plays an important role and that polar interactions (i.e., electrostatic interaction and hydrogen bonding) do not participate in the complex formation between **2b**·4HCl and durene.

On the basis of the direct evidence of 1:1 inclusion described above, water-soluble paracyclophanes will be generally useful to trap and fix nonpolar substrates of definite shape and size in aqueous solution. Modification of the nature of the cavity and introduction of functional groups are now in progress.

Supplementary Material Available: Perspective view of host-guest complex with atomic numbering, positional parameters, thermal parameters, F(obsd)–F(calcd), bond distances, and bond angles of **2b**·4HCl·durene·4H_2O (25 pages). Ordering information is given on any current masthead page.

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(7) Measured in a KCl–HCl buffer of pH 1.95 at 25.0 ± 0.1 °C. Concentrations of 1,8-ANS and **2b** were 2.00 × 10^{-6} and 1.39–13.9 × 10^{-5} M, respectively. Excitation wavelength was 375 nm.
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(9) Benesi, H. A.; Hildebrand, J. H. *J. Am. Chem. Soc.* **1949,** *71,* 2703. Fluorescence intensity was measured at 505 nm.
(10) Dissociation constants of 1.8 × 10^{-3},[2a] 6.3 × 10^{-4},[2b] and 9.1 × 10^{-5} M[3a] have been reported for 1:1 complex formation between 1,8-ANS and a water-soluble paracyclophane in aqueous solution.
(11) Measured in a DCl–D_2O solution of pD 1.2 at ambient temperature of 28 ± 2 °C. Concentrations of 2,7-dihydroxynaphthalene and **2b** were 2.5 × 10^{-2} and 5.0 × 10^{-2} M, respectively. Me_4Si was used as external standard. pD was adjusted according to Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960,** *64,* 188.
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(15) In the cases of water-insoluble substrates, a HCl–H_2O solution of **2b** and a hexane solution of the substrate were shaken, and the resulting precipitates were crystallized from 0.1 N HCl.
(16) On the basis of the elemental analyses of C, H, N, Cl, and the LC determination using LiChrosorb RP-2 with acetonitrile–methanol–water–28% ammonium hydroxide (55:10:34:1).
(17) Here the term "host–guest complex" was used according to Cram, D. J.; Cram, J. M. *Acc. Chem. Res.* **1978,** *11,* 8.
(18) The chloride ions and water molecules are located outside the cavity.
(19) Tabushi, I.; Yamada, H.; Kuroda, Y. *J. Org. Chem.* **1975,** *40,* 1946. Predominance of "face" conformation in solution was suggested for polyparacyclophane system on the basis of ^{1}H NMR study.
(20) In **2b** the "face" conformation would be favored by the diphenylmethane skeletons. Cf.: (a) Cram, D. J.; Antar, M. F. *J. Am. Chem. Soc.* **1958,** *80,* 3103. (b) Kawato, T.; Inazu, T.; Yoshino, T. *Bull. Chem. Soc. Jpn.* **1971,** *44,* 200.
(21) The four corners of the rectangle are composed of the two methylene carbons of diphenylmethane skeletons [C(13) and C(32)] and the two N–C bonds having the gauche conformation [N(1)–C(2) and N(20)–C(21)]. The angle between the two benzene rings of diphenylmethane skeleton is 109.8°.

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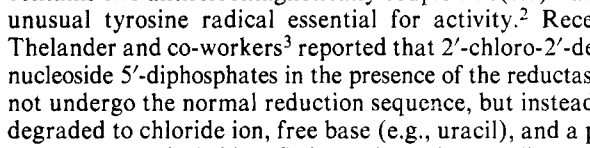
Inorganic Pyrophosphate Is Released from 2′-Chloro-2′-deoxyuridine 5′-Diphosphate by Ribonucleoside Diphosphate Reductase

Sir:

Ribonucleoside diphosphate reductase (RDPR) (E.C. 1.17.4.1) catalyzes the reduction of ribonucleoside 5′-diphosphates to the corresponding 2′-deoxyribonucleotides (eq 1). This enzyme has been purified to homogeneity by Eriksson



(1)

and co-workers[1] and consists of two nonidentical subunits B1 and B2 which form an active (1:1) complex in the presence of magnesium ions.[2] Protein B1 (mol wt, 160 000 daltons), a dimer of the general structure $\alpha\alpha'$,[1] contains active thiols and the binding sites for the nucleoside diphosphate substrates and the nucleoside triphosphate allosteric regulators. Protein B2 (mol wt, 78 000 daltons), a dimer of general structure $\beta\beta$, contains two antiferromagnetically coupled Fe(III)'s and an unusual tyrosine radical essential for activity.[2] Recently, Thelander and co-workers[3] reported that 2′-chloro-2′-deoxynucleoside 5′-diphosphates in the presence of the reductase did not undergo the normal reduction sequence, but instead was degraded to chloride ion, free base (e.g., uracil), and a phosphosugar tentatively identified as 2-deoxyribose 5-diphosphate. In addition, inactivation of B1 was observed accompanied by modification of several thiol groups. We felt that the elucidation of the mechanism of this enzyme-catalyzed degradation required the absolute identification of this phosphosugar. We report that in our hands *inorganic pyrophosphate* is quantitatively liberated from 2′-chloro-2′-deoxyuridine 5′-diphosphate by the action of the reductase. This finding demonstrates a remarkable loss of all substituents from the ribose moiety and has important mechanistic implications.

Incubation of 2.6 μmol of [β-^{32}P]-2′-chloro-2′-deoxyuridine 5′-diphosphate (12 000 cpm/μmol) with RDPR in the presence of the positive effector dTTP afforded >80% uracil formation.[4] Chromatography on DEAE Sephadex resulted in the isolation of 2.2 μmol of an unknown diphosphate.[5] ^{1}H NMR analysis of this material using a Brüker 270-MHz Fourier transform spectrometer revealed small amounts of contaminants which were present in the starting material. The amazing feature of this spectrum was the lack of any new protons in the anomeric sugar region, the 2-deoxy region, or the 5-hydroxymethylphosphate region. These findings suggested the possibility that inorganic pyrophosphate was the product. Analysis by ^{31}P NMR (Figure 1) revealed a singlet at −7.7 ppm which was in agreement with a known sample of tetrasodium pyrophosphate in the same buffer.

Since the assignment of the presumed phosphosugar by Thelander and co-workers[3] was based on chromatography on polyethyleneimine (PEI) and Whatman 3 MM paper, we compared our unknown diphosphate with authentic [^{32}P]-

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