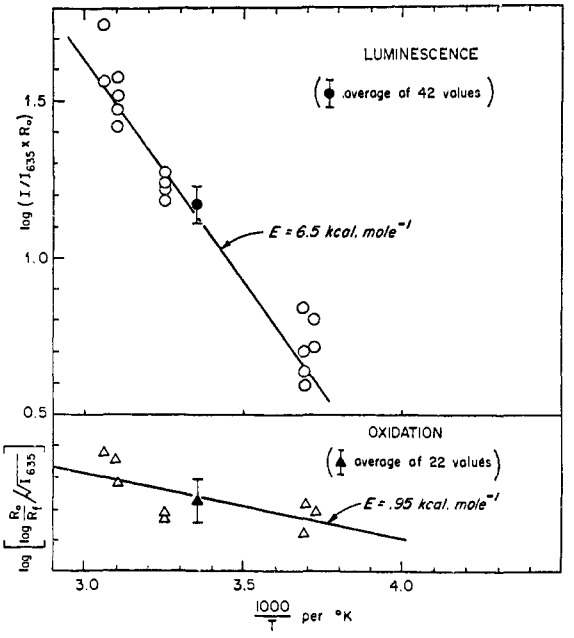


Figure 2. Temperature dependence of the light emission intensity and of the rate of rubrene oxidation.

the intermediacy of ^3R. Whereas the rate of oxidation and the temperature coefficient of the emission are little changed, in iodobenzene the absolute intensity is smaller by a factor of ~6 than in *o*-dichlorobenzene.[12] As the oxidation is unaffected,[13] deactivation of $^1O_2$ cannot be invoked: when the $^1O_2$ quencher DABCO[14] is added to the rubrene solution, both oxidation and luminescence are drastically reduced, thus confirming the common intermediacy of $^1O_2$. The intensity of rubrene (photo) fluorescence does not seem influenced by the solvent either.[15] Therefore, the effect of iodobenzene seemed best explained by the deactivation of triplet rubrene by increased spin-orbital coupling due to the heavy atom. Unfortunately, the position of triplet rubrene is not known. A low triplet ~22–29 kcal[16] would be compatible with its intermediacy. A high ^3R (~40 kcal[17]) would not fit with our observed activation energy, thus implying a mechanism *via* $[^1O_2]_2$.

Rubrene excitation (eq 1) and oxidation (eq 5) have been considered as alternate paths. From rough estimates, only one molecule fluoresces for 10^5 oxidized. Perhaps an unstable complex common to (1) and (5) is first formed between $^1O_2$ and R.

This reaction is an example of the light-emitting step proposed by Khan and Kasha[18] in their hypothesis about the role of $^1O_2$ in chemiluminescence.

Acknowledgments. Stimulating discussions with Professor P. D. Bartlett and support by the Petroleum Research Fund, administered by the American Chemical Society, are gratefully acknowledged.

(12) In 1-chloronaphthalene both the rate of oxidation and I are lower than in *o*-$C_6H_4Cl_2$, but I is still three times larger than in C_6H_5I although chloronaphthalene is twice as viscous.
(13) *Cf.* C. S. Foote, *Accounts Chem. Res.*, 1, 104 (1968).
(14) C. Ouannes and T. Wilson, *J. Amer. Chem. Soc.*, 90, 6527 (1968).
(15) In an Aminco spectrofluorimeter.
(16) Naphthacene, E_T = 29 kcal.
(17) See A. Yildiz, P. T. Kissinger, and C. N. Reilley, *J. Chem. Phys.*, 49, 1403 (1968).
(18) A. U. Khan and M. Kasha, *J. Amer. Chem. Soc.*, 88, 1574 (1966).

Thérèse Wilson
Converse Memorial Laboratory, Harvard University
Cambridge, Massachusetts 02138
Received October 21, 1968

The Cellulose Polymer Supported Sequential Analysis of Polyribonucleotides[1]

Sir:

Knowledge of the nucleotide sequence of polyribonucleotides of biological interest is a necessary prerequisite to understanding the process by which information coded in the nucleotide sequences of RNA molecules is translated into proteins. Any method for the sequential analysis of polyribonucleotides which proposes to be of practical value in gaining this knowledge must sequentially remove each nucleotide completely in a fast efficient step which can be repeated continuously without lengthy intermittent purifications. We wish to report such a procedure (Figure 1) accomplished by attaching polyribonucleotides to an insoluble cellulose support and sequentially removing the terminal base using an adaptation of the Whitfeld[2] periodate method.

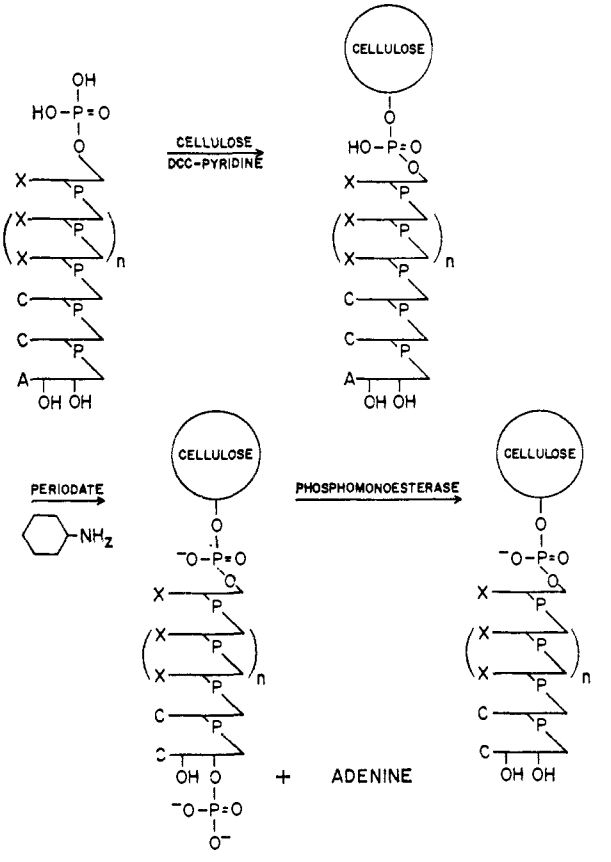


Figure 1. Schematic diagram of the attachment of sRNA to a cellulose support and subsequent removal of the 3′-terminal adenine base.

(1) This research was supported by the Petroleum Research Fund, Grant 3190-B, administered by the American Chemical Society.
(2) P. R. Whitfeld, *Biochem. J.*, 58, 390 (1959).

Yeast sRNA (100 mg, Calbiochem) was dissolved in 2 ml of water and heated to 85°. The denatured sRNA was added with rapid stirring to 10 ml of a 5% solution of tetradecyltrimethylammonium bromide. The resulting tetradecyltrimethylammonium salt of sRNA formed a thick precipitate which was removed by centrifugation, washed several times, and dried thoroughly *in vacuo* over P_2O_5. Attachment of the sRNA to cellulose (100 mg of Cellex N-1, Calbiochem) was accomplished by adding 100 mg of dicyclohexylcarbodiimide (Aldrich) to a suspension of the cellulose in 10 ml of an anhydrous pyridine solution of the tetradecyltrimethylammonium salt of sRNA (formation of this salt of sRNA renders it soluble in a number of anhydrous organic solvents) containing catalytic amounts of pyridinium hydrochloride and stirring the mixture for 10 days. After completion of the reaction the cellulose was removed by filtration, washed with 200 ml of pyridine, and treated with 50 ml of a 10% solution of sodium dodecyl sulfate to remove the tetradecyltrimethylammonium ions from the attached sRNA.[3] Further washings with water were carried out until no material absorbing at 260 mμ was observed in the washings. The resulting cellulose containing sRNA molecules linked by way of 5′-terminal phosphodiester bonds was dried, weighed (130 mg), and stored at −20°.

The sequential cleavage of the 3′-terminal bases from the cellulose-supported sRNA was carried out on 20-mg samples by repetition of the following procedure: (1) heat for 2 hr at 75° in the presence of 0.6 ml of a 1 *M* cyclohexylamine solution and 0.6 ml of a 0.1 *M* sodium periodate solution; (2) separate the cellulose-supported sRNA from the reaction solution and the cleaved terminal base by filtration; (3) exhaustively wash the cellulose-supported sRNA with water; (4) incubate the washed cellulose-supported sRNA in 1 ml of a 0.1 *M* NH_4HCO_3 solution containing 25 units of alkaline phosphatase (Calbiochem) at 37° for 1 hr; (5) exhaustively wash the cellulose-supported sRNA with 0.1 *M* NH_4HCO_3 solution to remove all traces of phosphomonesterase; (6) analyze the solution resulting from step 2 using thin-layer chromatography on cellulose plates developed with distilled water (adenine, R_f 0.61; cytidine, R_f 0.80) and paper chromatography developed with 2-propanol–acetic acid–water[4] (85:5:10, v/v).

Yeast sRNA carried through the above procedure three times yielded the sequence ···CpCpA. Each step appeared to be quantative since no observable adenine spot was found on the thin layer or paper chromatographic analysis of the solution resulting from step 2 in the second or third cycle through the procedure. When a fourth step was carried out, paper chromatographic analysis showed that all four bases were present as products of the cleavage reaction. Although Whitfeld[4] and Heppel[5] observe breakdown of RNA at high temperatures in the periodate–amine step, we were unable to detect the release of any nucleotide material from the cellulose-supported sRNA upon heating the material from step 3 with additional periodate–amine solution at 75° for 24 hr. As well as facilitating the recovery of reacted RNA, attachment to cellulose apparently also serves to stabilize the RNA to the high temperatures required for complete reaction in the periodate–amine step. Thus, the use of a modified Whitfeld procedure on a cellulose-supported polyribonucleotide seems to provide a fast, efficient method for the sequential analysis of RNA molecules which should lend itself to automation with little difficulty.

(3) R. L. Letsinger and T. E. Wagner, *J. Am. Chem. Soc.*, **88**, 2026 (1966).
(4) P. R. Whitfeld, *Biochim. Biophys. Acta*, 108, 202 (1965).
(5) H. C. Neu and L. A. Heppel, *J. Biol. Chem.*, 239, 2927 (1964).

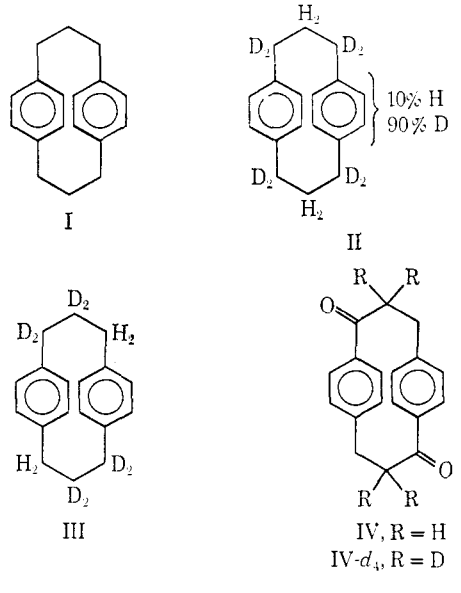
Thomas E. Wagner, Huali G. Chai, Anna S. Warfield
Department of Chemistry, Wellesley College
Wellesley, Massachusetts
Received January 13, 1969

Nuclear Magnetic Resonance Studies of Conformation and Ring Inversion in the [3.3]Paracyclophane System

Sir:

In the crystalline state [3.3]paracyclophane (I) has been shown to exist in the chair conformation[1] (Figure 1). We now report[2] variable-temperature nmr data on I and partially deuterated I. The results show that, in solution, I exists as a mixture of boat and chair conformations in the ratio of about 2:1, and that the free-energy barrier for the boat–chair conversion is about 11.7 kcal/mol.

At room temperature, the aromatic protons of I (4% solution in 1:1 $CDCl_3$–$CDCl_2F$) appeared as a singlet (τ 3.33). At −88°, the aromatic region showed four partially resolved broad bands (Figure 1). This complex and unsymmetrical spectrum could not have arisen from a single A_2B_2 system and suggested the presence of both chair and boat conformations. To



(1) P. K. Gantzel and K. N. Trueblood, *Acta Cryst.*, **18**, 958 (1965).
(2) Variable-temperature nmr studies have been done on: [3.2]-metacyclophane (R. W. Griffin, Jr., and R. A. Coburn, *Tetrahedron Letters*, 2571 (1964)); [8]paracyclophane (G. M. Whitesides, B. A. Pawson, and A. C. Cope, *J. Am. Chem. Soc.*, 90, 639 (1968)); various heterophanes (I. Gault, B. J. Price, and I. O. Sutherland, *Chem. Commun.*, 540 (1967); H. Nozaki, T. Koyama, T. Mori, and R. Noyori, *Tetrahedron Letters*, 2181 (1968); F. Vögtle, *ibid.*, 3623 (1968)); [2.3]-, [3.4]-, and [4.4]paracyclophanes (F. A. L. Anet, unpublished work); [2.4]-paracyclophane derivatives (D. J. Cram and H. Reich, unpublished work).