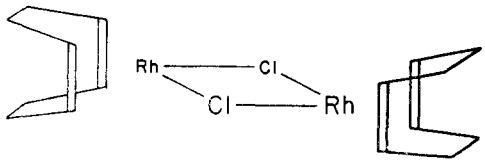

Fig. 1.—Sketch of the $(RhCl\text{-}1,5\text{-cycloöctadiene})_2$ molecule. The rhodiums, chlorines, and double-bond centers are coplanar.

are in an octahedral configuration in the carbonyl they are in a square planar configuration in the cycloöctadiene complex. Thus the close parallelism of bonding often inferred from non-structural studies to hold between olefin complexes and carbonyl halides of low-valence transition metals (*e.g.*[2]) does not exist in this case.

The deep yellow crystals of $(RhClC_8H_{12})_2$ were prepared by D. W. Barnum of these laboratories by the reaction of rhodium chloride trihydrate with the diene, as described by Chatt and Venanzi.[2] (Chatt and Venanzi also note that $(RhClC_8H_{12})_2$ is produced in the reaction of $(RhCl(CO)_2)_2$ with C_8H_{12}.) On the basis of precession and Weissenberg photographs prepared with Mo Kα radiation the crystals are found to belong to the monoclinic system with $a = 9.05$, $b = 25.4$, $c = 7.28$ Å., $\beta = 91.6°$. The calculated density of 1.95 g./cm.[3] for four dimer molecules in the unit cell is in excellent agreement with the density of 1.93 g./cm.[3] obtained pycnometrically. The systematic absences $0k0$, k odd; $h0l$, $h + l$ odd, are consistent with the space group C_{2h}^5-$P2_1/n$. Since the general positions of this space group are fourfold, the dimeric molecules are not required to possess symmetry, and the solution of the crystal structure involves the location of all atoms in a dimer.

Intensity data were collected at room temperature with the use of Mo Kα radiation from a very small crystal (average dimension, 0.2 mm.), and were estimated visually. The rhodium and chlorine atoms were located without difficulty on a three-dimensional Patterson map and the carbon atoms were located subsequently on a difference Fourier. The structure has been refined by least-squares techniques. The present reliability factor R_1 is 9.7% for the 800 observed reflections; it is 16% if the carbon atom contributions to the structure factors are omitted. The limits of error at this stage of the refinement are approximately 0.004, 0.014, and 0.07 Å. for Rh, Cl, and C.

The structure of the molecule is sketched in Fig. 1; some of the bond distances and angles are shown in Fig. 2. All distances and angles are normal. There are no significant differences among bond lengths of a given type. The C–C distances in the ring average 1.52 ± 0.10 Å., the C=C distances 1.44 ± 0.06. The closest atoms in other molecules to a given rhodium are carbons at about 3.8 Å. away. Thus a given rhodium atom is bonded only to two chlorine atoms and to two double-bond centers of a cycloöctadiene ring (boat configuration). Within the limits of error of this study the four double-bond centers, the two rhodiums, and the two chlorine atoms in a given molecule are coplanar. Accordingly a given rhodium atom is in

(2) J. Chatt and L. M. Venanzi, *J. Chem. Soc.*, 4735 (1957).

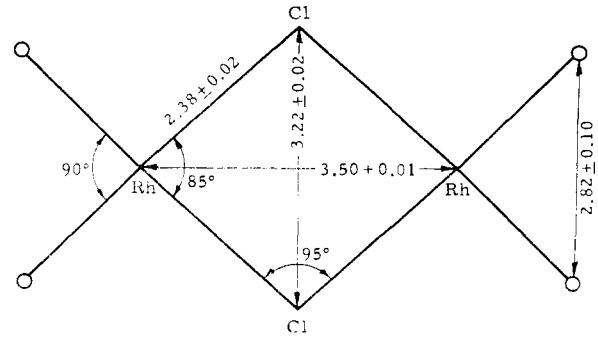

Fig. 2.—Principal bond distances and angles in the plane of the Rh, Cl, and double-bond centers (symbolized by O)

a square-planar configuration and the structure of the molecule is that envisioned by Chatt and Venanzi.[2] In the rhodium chloride carbonyl dimer the two planar $Rh(CO)_2Cl$ groups intersect at an angle of 124° and Dahl, *et al.*,[1] describe the configuration of a given rhodium as octahedral and the bonding to involve bent metal–metal bonds. Thus there are striking differences between the bonding in the carbonyl and in the cycloöctadiene complexes of rhodium(I) chloride.

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THE ISOLATION AND CHARACTERIZATION OF A FULLY DEUTERIATED PROTEIN[1]

Sir:

The successful culturing of fully deuteriated micro-organisms has been reported by Katz, Crespi and co-workers,[2] and it now has been possible for the first time to isolate, purify and characterize a fully deuteriated protein containing deuterium in both exchangeable and non-exchangeable positions from one of these organisms. An algal chromoprotein, phycocyanin, has been isolated from the blue-green alga *Plectonema calothricoides* now cultured in D_2O for two years. The harvested algae were frozen and thawed twice, and the water-soluble protein then was extracted at 5° in aqueous acetate buffer (pH 4.7, $\mu = 0.1$) for a period of a week. The extracted protein then was purified by ammonium sulfate reprecipitation and fractionation in ordinary water. An identical purification procedure was used to isolate phycocyanin from ordinary water-grown *P. calothricoides*. Consistent with cytological observations of the deuterio-algal cell which indicate a difference in cell walls,[2] cell lysis and extraction of the deuterio-protein is considerably faster than for the H_2O-grown organism.

To establish unambiguously the fully-deuteriated nature of the protein, the deuterium content has been ascertained by direct analysis. The protein isolated from the completely deuteriated algae was dialyzed against D_2O for three days with daily

(1) Based on work performed under the auspices of the U. S. Atomic Energy Commission.

(2) H. L. Crespi, S. M. Conrad, R. G. Uphaus and J. J. Katz, *Ann. New York Acad. Sci.*, **84**, 648 (1960).

changes of D_2O in order to re-exchange any hydrogen introduced during the isolation procedure. The protein then was thermally denaturated by allowing the sample to stand in an 80° bath for ten minutes. The denatured protein was centrifuged, washed with D_2O, centrifuged again, and carefully dried in vacuum. The dried protein was burned, the D_2O collected and analyzed spectrophotometrically.[3] The analysis showed 98.4 atom % D_2O in the water of combustion. Although this is sufficient to establish the completely deuteriated nature of the protein, an infrared examination of the material was carried out. A small amount of the protein was mixed in a mull with Perkin–Elmer fluorocarbon oil, and a search was made for the characteristic C–H absorption at 2900 to 3000 cm.$^{-1}$. No detectable C–H or N–H absorption was observed, but absorptions in the 2220 to 2240 cm.$^{-1}$ and 2450 cm.$^{-1}$ regions characteristic of C–D and N–D bonds, respectively, were present.

To establish the homogeneity and purity of the isolated protein, the visible and ultraviolet absorption spectrum, ultracentrifuge pattern, and electrophoretic properties of the deuterio- and hydrogen-protein preparations have been investigated. The absorption spectra in the visible region of both protein preparations are quite similar. In aqueous acetate buffer, pH 4.7, maximum in absorption occurs at 622 mμ in both cases, and the specific extinction coefficient (for a 0.1% solution in a 1-cm. cell) is 7.9 for the deuterio and 8.1 for the hydrogen preparation. Another maximum is observed in both preparations at 350 mμ. The absorption maximum at 622 mμ indicates that the phycocyanin isolated from the deuterio and hydrogen *P. calothricoides* are definitely of the C-phycocyanin type.[4] Further absorption spectra studies showed that the ratio of optical density at 622 mμ to that in the 280 mμ region was approximately the same in both protein preparations. This indicates that the number of chromophores in both the deuterio- and hydrogen-phycocyanin molecules is very likely the same. Electrophoresis on cellulose acetate strips (acetate buffered, pH 4.70) showed a single band for both deuterio and hydrogen preparations of purified phycocyanin. The deuterio band appeared to be somewhat broader than for the hydrogen compound. Since the pK values for the charged groups in the two proteins are different,[5] changes in pH will affect electrophoretic behavior of the two proteins differently. The deuterio-protein definitely exhibits a lower mobility in cellulose acetate electrophoresis, and a mixture of deuterio- and hydrogen-protein is resolved into two bands on electrophoresis at pH 4.70.

The sedimentation coefficients of both protein preparations in aqueous acetate buffer (pH 4.7, $\mu = 0.02$) were measured with Schlieren optics in the analytical ultracentrifuge. In both cases four peaks were observed. S_{20}^0 values listed for the deuterio protein are approximately 1.3 times the S_{20}^0 values for the hydrogen protein. The 14.16 S component in the deuterio and the 11.15 S in the hydrogen protein are present in the largest amount and are responsible for the major portion of the light absorption. These observations for the ordinary hydrogen-phyocyanin from *P. calothicoides* are consistent with the S_{20}^0 values originally determined by Svedberg and co-workers[6] for phycocyanin from the algae *Porphyra tenera*, *Ceramium rubrum* and *Aphanizomenon flos aquae*, and are consistent in part with the observations of Brody.[7] The pH dependence of fluorescence depolarization shown by Goedheer[8] is consistent with the kind of dissociation equilibria that probably are responsible for the observed sedimentation properties of both the hydrogen and deuterium preparations.

S_{20}^0 for the Deuterio-phycocyanin	S_{20}^0 for the Hydrogen-phycocyanin
4.80×10^{-13}	3.73×10^{-13}
8.74×10^{-13}	7.17×10^{-13}
14.16×10^{-13}	11.15×10^{-13}
22.67×10^{-13}	15.84×10^{-13}

To make meaningful comparisons possible, it is clearly essential to ascertain whether the hydrogen- and deuterio-proteins are chemically identical and differ only in isotopic composition, or whether the deuterio-protein arises from a D_2O tolerant mutant organism that synthesizes phycocyanin differing in amino acid composition and sequence from the hydrogen prototype. Preliminary results obtained by Moore–Stein analysis with a Beckman Spinco amino acid analyzer indicate that the number of amino acid residues of each of the amino acids listed is identical within experimental error for both the hydrogen- and deuterio-phycocyanin: lysine, histidine, arginine, threonine, serine, glutamic acid, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine and phenylalanine. Cystine and proline are present in both the deuterio- and hydrogen-proteins, but the number of residues found differs in the two proteins by more than the experimental error. We believe that these differences result primarily from difficulties in the hydrolysis procedure, but the possibility does exist that the amino acid compositions are different. Further studies to clarify this situation are in progress.

(3) H. L. Crespi and J. J. Katz, *Anal. Biochem.*, **2**, 274 (1961).

(4) F. T. Haxo and C. O'H Eocha, "Handbuch der Pflanzenphysiologie," Vol. V, Springer, Berlin, p. 497.

(5) H. H. Hyman, A. Kaganove and J. J. Katz, *J. Phys. Chem.*, **64**, 1653 (1960).

(6) T. Svedberg and I. Erickson, *J. Am. Chem. Soc.*, **54**, 3998 (1932); T. Svedberg and T. Katsumai, *ibid.*, **50**, 525 (1928); I. G. Eriksson-Quensel, *Biochem. J.*, **32**, 585 (1938).

(7) S. S. Brody and M. Brody, *Biochim. Biophys. Acta*, **50**, 348 (1961).

(8) J. C. Goedheer, Doctoral Thesis, Rijksuniversiteit, Utrecht, 1957.

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ADDITION OF TETRAFLUOROETHYLENE TO DICYCLOPENTADIENYLNICKEL

Sir:

Since the preparation of π-allylcobalt tricarbonyl,[1] several π-allyl complexes of transition metals

(1) R. F. Heck and D. S. Breslow, *J. Am. Chem. Soc.*, **82**, 750 (1960); see also R. F. Heck and D. S. Breslow, *ibid.*, **83**, 1097 (1961), and references cited therein.