Table I. Enzymatic Hydrolysis of 6-X-Substituted Purine Ribosides

X	λ, mμ	$\epsilon_M \times 10^{-3}$ RX	$\epsilon_M \times 10^{-3}$ Ino	K_m, M	V_{max}, μmole/min/ mg of protein	Error, %, V_{max} and K_m
NH₂	260	14.9	7.1	0.00025	16.4	2
NH₂NH	270	13.5	4.1	0.0067	15	20
Cl	264	9.0	4.5	0.050	19	50
CH₃NH	267	15.9	4.9	0.10	15	50
CH₃O	270 alk.	0.4	6.7	0.15	18	10

ties which are all very similar to that for adenosine itself.

6-Chloropurine riboside was purchased from Sigma Chemical Co., and the crystalline methylamino, methoxy, and hydrazino derivatives were prepared from it.[6] Each of these compounds, when incubated with the purified enzyme at pH 6.5, was completely converted to inosine, identified by ultraviolet absorption spectrum and paper chromatography. The dependence of initial rate (over the first 5% reaction or less) on substrate concentration was determined in 0.1 M potassium phosphate buffer at 25°. With the exception of the methoxy derivative, the reaction was directly followed by spectrophotometry in cuvettes of 1-cm and 1-mm light path with appropriate dilutions where necessary. For the methoxy derivative, whose spectrum is very similar to that of inosine at pH 6.5, the reaction was followed by the appearance of ultraviolet absorption in 1-ml aliquots removed at timed intervals and treated with 20 μl of 50% NaOH.
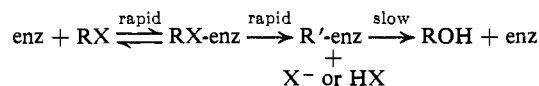
The wavelength at which each reaction was followed and the change in molar extinction coefficient at that wavelength for complete conversion of that substrate to inosine are indicated in Table I. Double reciprocal plots of the dependence of initial rate (corrected for the change in molar extinction for each substrate) on substrate concentration gave values for V_{max} and K_m as indicated. All substrates gave satisfactory linear plots, but the margin of error in the intercept is substantial in those cases where K_m is very high and the substrate absorbs strongly at wavelengths where the reaction may be followed.

It is clear that V_{max} is very similar, and may be identical, for all five substrates, whereas the apparent affinity of enzyme for the various substrates varies by a factor of approximately 600. A common rate of hydrolysis for such a variety of leaving groups strongly suggests that V_{max} represents the slow decomposition of an intermediate which is common to all five reactions, an intermediate from which the original 6-substituent has been completely displaced. The five substituents differ so widely in effectiveness as leaving groups, by any known mechanism, as to render it highly improbable that they are still present when the rate-controlling step occurs.

None of these reactions is detectably reversible at pH 6.5 or 9. Under more alkaline conditions the enzyme is rapidly and irreversibly inactivated. We have observed that neither K_m nor V_{max} for any of these reactions is detectably affected by the presence of ammonia, methylamine, hydrazine, methanol, or potassium chloride (each in 0.05 M total concentration) at pH 6.5 (0.1 M potassium phosphate buffer).

(6) J. A. Johnson, H. J. Thomas, and H. J. Schaeffer, J. Am. Chem. Soc., 80, 699 (1958).

A minimal mechanism is

$$\text{enz} + \text{RX} \underset{}{\overset{\text{rapid}}{\rightleftharpoons}} \text{RX-enz} \overset{\text{rapid}}{\longrightarrow} \underset{\substack{+ \\ \text{X}^- \text{ or HX}}}{\text{R}'\text{-enz}} \overset{\text{slow}}{\longrightarrow} \text{ROH} + \text{enz}$$

where K_m represents some combination of the rate constants for the first two steps, and V_{max} is proportional to the rate constant for the third step. This is consistent with the finding that V_{max} does not depend on the nature of X, and that the addition of HX has no effect on K_m or V_{max}. V_{max} varies when the nature of the R group is changed. Thus V_{max} for 3-β-(D-ribofuranosyl)adenine is about 25 times lower than that for adenosine.[2]

There is a clear formal analogy between this catalytic process and those which have been described for a number of proteolytic enzymes.[7] The point at which water enters the reaction, and the nature of the intermediate R′-enz, are under investigation.

(7) Cf. M. L. Bender, Chem. Rev., 60, 53 (1960).

Richard Wolfenden
Frick Chemical Laboratory, Princeton University
Princeton, New Jersey

The Molecular Structure of the Complex of Cyclooctatetraene and Iron Pentacarbonyl

Sir:

Cyclooctatetraene (COT) reacts with Fe₂(CO)₉ to give several products. The crystal structures of two of these [COTFe(CO)₃ and COTFe₂(CO)₆] have been determined.[1] We wish to report the determination of the structure of a third product, COTFe₂(CO)₅, for which a novel type of bonding must now be proposed. In an earlier report[2] this material was tentatively assigned the electronic structure I; to explain the single absorption in the nmr spectrum it was proposed that rapid degenerate valence tautomerism involving rotation of the Fe–Fe bond about the ring was occurring.

I
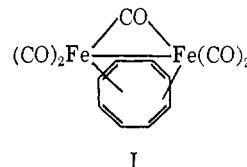
For the X-ray determination dark red prismatic crystals were prepared from benzene.[2] Precession photographs determined the space group as Pnma with $a = 7.71$, $b = 15.43$, and $c = 11.06$ A. Measurement of the density (1.86 g/cm³) showed that there are four molecules per unit cell; 602 reflections were examined

(1) B. Dickens and W. N. Lipscomb, J. Chem. Phys., 37, 2084 (1962).
(2) C. E. Keller, G. F. Emerson, and R. Pettit, J. Am. Chem. Soc., 87, 1390 (1965).

Figure 1.

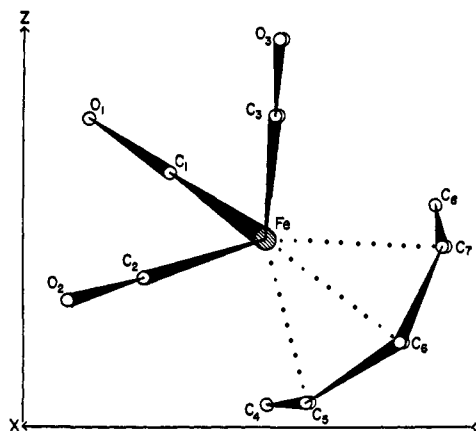

Figure 2.

using Mo Kα radiation with a General Electric XRD 5 diffractometer, employing stationary crystal–stationary counter techniques.[3] Background was measured as an average of minimum levels on either side of the peak.

The structure was solved using the Hauptmann and Karle direct-method approach.[4] A fully automatic computer program utilizing the symbolic addition technique[5] determined 127 phases, all subsequently determined to be correct, from which an E map showing the positions of the iron atom was calculated. Several cycles of least-squares refinement with anisotropic temperature factors produced a final R factor of 0.043 (with zeros) and 0.068 (for all reflections).

Figures 1 and 2 show the molecule as viewed parallel and perpendicular to the COT ring, and Table I gives some of the bond lengths. The molecule is bisected by a mirror plane passing through carbon atoms C_4 and C_8 of the COT ring and the bridging carbonyl group C_1O_1. This means that the bonding in the cyclooctatetraene ring must be symmetrical about the mirror plane, which rules out a structure with alternating single and double bonds.

Table I. Bond Lengths in $Fe_2(CO)_5COT$

Bond	Length, A	Std dev
$Fe-C_1$	1.968	(0.017)
$Fe-C_2$	1.786	(0.015)
$Fe-C_3$	1.802	(0.014)
$Fe-C_4$	2.494	(0.015)
$Fe-C_8$	2.496	(0.016)
$Fe-C_5$	2.136	(0.013)
$Fe-C_6$	2.113	(0.013)
$Fe-C_7$	2.123	(0.013)
C_4-C_5	1.392	(0.018)
C_5-C_6	1.398	(0.021)
C_6-C_7	1.410	(0.022)
C_7-C_8	1.432	(0.022)
$Fe-Fe$	2.742	(0.003)

Examination of the iron–ring carbon distances shows that the carbon atoms of the COT may be divided up into two groups, one of three carbon atoms (C_5, C_6, and C_7) at a distance of 2.1 A from the nearest iron atom, and one of two carbon atoms on the mirror plane (C_4 and C_8) at a distance of 2.5 A from the iron atoms.

(3) T. C. Furnas, "Single Crystal Orienter Instruction Manual," General Electric Co., Schnectady, N. Y., 1957.

(4) See I. L. Karle, K. S. Dragonette, and S. A. Brenner, *Acta Cryst.*, 19, 713 (1965), and references therein.
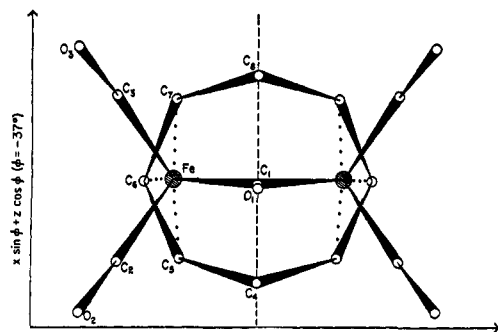
(5) R. Dewar and A. Stone, to be published.

The group of three carbon atoms equidistant from the iron atom suggests a π-allyl type of bonding. This leaves two carbon atoms on the mirror plane, each with a single electron available for bonding. Since the molecule is diamagnetic,[2] these electrons must be involved in bonding, and the structure is consistent with two three-center bonds each containing two electrons, extending over the two iron atoms and a carbon atom on the mirror plane, much as in the electron-deficient boron hydrides. Each iron atom is bonded to two terminal carbonyl groups, and an iron–iron bond is then proposed in order that each Fe attain the effective atomic number of krypton.

The structure shown in Figure 1 contains three nonequivalent hydrogen atoms; the single peak which is seen in the nmr is then explained on the basis of rapid degenerate valence tautomerism as shown. The facility of this isomerism is indicated by the fact that the spec-

IIa   IIb   IIc
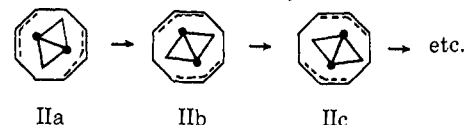
trum remains as a single peak even at −80°. Structure I could well be a stable intermediate involved in this rearrangement and could offer a low-energy pathway for the extensive electronic reorganization associated with the conversion of IIa to IIb.[6,7]

(6) The infrared absorption spectrum of the complex in the solid state shows the same major regions of absorption as those when taken in solution. This indicates that no new species having a different mode of metal–ligand bonding in which eight chemically equivalent hydrogen atoms are present is produced in solution.

(7) This work was supported by grants from the National Institutes of Health and Advanced Research Projects Agency.

(8) Alfred P. Sloan Fellow.

(9) U. S. Rubber Fellow.

(10) Shell Fellow.

E. B. Fleischer,[8] A. L. Stone[9]
R. B. K. Dewar,[10] J. D. Wright
Department of Chemistry, University of Chicago
Chicago, Illinois

C. E. Keller, R. Pettit
Department of Chemistry, University of Texas
Austin, Texas

A New Method for Determining the Stereochemistry of Substitution Reactions

Sir:

There are three main methods for determining the stereochemistry of substitution reactions at carbon:[1]

(1) E. Eliel, "Stereochemistry of Carbon Compounds," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, pp 116–119.