Table I

	V	VI	VII	VIII	IX	X
$\theta_{13,14}$(D)[a]	−39° 50′	−49° 34′	−45° 16′	−37° 41′	−47° 57′	−46° 17′
$\theta_{14,15}$	+41° 51′	+34° 40′	+26° 40′	+37° 25′	+36° 10′	+38° 25′
$\theta_{15,16}$	−25° 04′	−6° 7′	+1° 19′	−15° 26′	−10° 45′	−13° 12′
$\theta_{16,17}$	+1° 52′	−24° 13′	−28° 25′	−5° 30′	−18° 2′	−15° 44′
$\theta_{13,17}$	+23° 33′	+44° 42′	+45° 31′	+22° 49′	+39° 3′	+37° 42′
$\theta_{13,14}$(C)[b]	+69° 36′	+64° 15′	+69° 35′	+51° 11′	+60° 2′	+62° 9′
$\Delta\theta$[18]	±3° 12′	±1° 25′	...	...	±2° 1′	±2° 41′

[a] Calculated using C_{15}, C_{14}, C_{13}, and C_{17}. [b] Calculated using C_8, C_{14}, C_{13}, and C_{12}.

the Norton, Kartha, and Lu coordinates[13] is −6° 7′. For the first time one can distinguish this slightly deformed β envelope (III) from the corresponding half-chair (II)[19] since for VI $\theta_{16,17}$ is −24° 13′, only slightly less than $\theta_{16,17}$ for 3β-chloro-7α-bromo-$\Delta^{5,6}$-cholestene (VII).[14]

The favoring of III for VI is not unexpected conformationally;[1] however, for lanostenyl iodoacetate (VIII),[15] the fact that $\theta_{16,17}$ is −5° 30′ and that a slightly deformed α envelope is preferred is unexpected since the C_{17}-β-side chain is driven into near eclipse with the C_{16}-β-hydrogen. Apparently the severity of the steric compression between the axial 14α-methyl and 17α-hydrogen forces this.

In interpreting X-ray crystallographic results some caution must be observed since these represent solid-state data and the packing forces of crystallization operating especially on the C_{17} side chain might deform ring D[20] whose α and β envelope energy differences are small and about 2–3 kcal/mole in magnitude.[1] Possible evidence on this point is seen in comparing $\theta_{15,16}$ calculated for VII ($\theta_{15,16}$ = +1° 19′)[14] and 2β,3α-dichloro-5-α-cholestane[16] (IX, $\theta_{15,16}$ = −10° 45′). Both have the long cholesterol side chain and differ conformationally only in rings A and B, yet VII is an envelope within experimental error while IX is a distorted half-chair. Another possible case in point is X, 4-bromo-9β,10α-pregna 4,6-diene-3,20-dione.[17] Since $\theta_{15,16}$ = $\theta_{16,17}$, ring D with a C_{17}-β-acetyl side chain is half-chair. In contrast to the others, however, X has a *cis* B/C junction and a C_{19}-α-methyl group.

Torsional angle data solve thoroughly the problem of the extent of puckering of ring D. $\theta_{13,14}$(D) varies between −38° (VIII) and −50° (VI) and averages out to −45°, a value close to the equilibrium value reported for cyclopentane itself by Pitzer and Donath.[21] Since ring D does not reach a maximally puckered value of $\theta_{13,14}$(D) equal to −60°, ring C must be detectably deformed. For VI, we calculate that $\theta_{13,14}$(C) is +64° 15′, $\theta_{12,13}$ is −55° 19′, $\theta_{11,12}$ is +53° 56′, $\theta_{9,11}$ is −56° 48′, $\theta_{8,9}$ is + 60° 18′, and $\theta_{8,14}$ is −65° 49′. Since all θ's for a perfect cyclohexane chair should be ±60°, ring C in VI is a slightly deformed chair.

Table I represents solid-state data; hence it is necessary to use the excellent nmr, infrared, ORD, and dipole moment data of Fishman, Djerassi, Brutcher, and Cross and their co-workers[1,5–8] to show that there is, as yet, for substituted 17-keto and 17β-hydroxy steroids no evidence that ring D conformational changes occur on solution. Solution data on VII and IX are not readily obtainable, but based on Table I a conformation between II and III might be anticipated.

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(19) In the half-chair, $\theta_{15,16}$ will equal $\theta_{16,17}$ and be approximately −16°.

(20) Ethylene carbonate is nonplanar (half-chair) in the solid state (X-ray, C. J. Brown, *Acta Cryst.*, 7, 92 (1954)) and also in the vapor phase (microwave, I. Wang, C. O. Britt, and J. E. Boggs, *J. Am. Chem. Soc.*, 87, 4950 (1965).

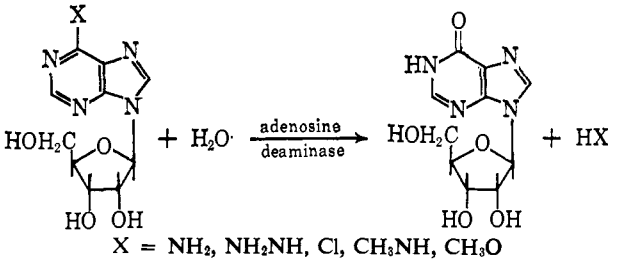
(21) K. S. Pitzer and W. Donath, *ibid.*, 81, 3213 (1959).

Enzymatic Hydrolysis of 6-Substituents on Purine Ribosides[1]

Sir:

The insensitivity of the adenosine deaminase of *Aspergillus oryzae* to carbonyl group reagents and to dialysis suggests the absence of groups appropriate for Schiff base formation with the substrate.[2] Enzymatic deamination of nucleosides may thus differ sharply in mechanism from the deamination of amino acids.[3] Alternative mechanisms, involving specific enzymatic protonation of the nucleoside at N_1 or N_3 to force it over to a potentially hydrolyzable 6-imino tautomer, are rendered doubtful by the fact that 3-β-(D-ribofuranosyl)adenine is also a substrate.[2]

Prompted by recent findings that mammalian adenosine deaminase preparations catalyze rather slow hydrolysis of chloride ion from 6-chloropurine riboside,[4] we have tested the enzyme from *Aspergillus* on a variety of substrates. We wish to report that this enzyme, which has been purified approximately 5000-fold,[5] catalyzes hydrolysis of 6-hydrazinopurine riboside, 6-chloropurine riboside, 6-methylaminopurine riboside, and 6-methoxypurine riboside, at limiting veloci-



X = NH_2, NH_2NH, Cl, CH_3NH, CH_3O

(1) This work was supported by Research Grant USPHS-GM-12725 from the National Institutes of Health, U. S. Public Health Service.

(2) R. Wolfenden, T. K. Sharpless, I. S. Ragade, and N. J. Leonard, *J. Am. Chem. Soc.*, 88, 185 (1966).

(3) *Cf.* A. E. Braunstein, *Enzymes*, 2, 113 (1960).

(4) J. G. Cory and R. J. Suhadolnik, *Biochemistry*, 4, 1733 (1965); S. Frederiksen, *Arch. Biochem. Biophys.*, 113, 383 (1966); H. P. Baer, G. T. Drummond, and E. L. Duncan, *Federation Proc.*, 25, 786 (1966).

(5) T. K. Sharpless and R. Wolfenden, *Methods Enzymol.*, 8, in press.

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
NH_2	260	14.9	7.1	0.00025	16.4	2
NH_2NH	270	13.5	4.1	0.0067	15	20
Cl	264	9.0	4.5	0.050	19	50
CH_3NH	267	15.9	4.9	0.10	15	50
CH_3O	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

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

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).

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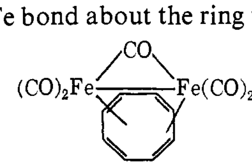
The Molecular Structure of the Complex of Cyclooctatetraene and Iron Pentacarbonyl

Sir:

Cyclooctatetraene (COT) reacts with $Fe_2(CO)_9$ to give several products. The crystal structures of two of these [$COTFe(CO)_3$ and $COTFe_2(CO)_6$] have been determined.[1] We wish to report the determination of the structure of a third product, $COTFe_2(CO)_5$, 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).