quinoline adduct.[8] Zink and Drago's model was originally proposed to explain base adduct behavior, but in their model they did not consider the possibility that the Cu(II) would be moved out of the equatorial plane by the base adduct (0.21 Å in the quinoline case). Thus, the effect of the more favorable relative energy positions of the metal and ligand atomic orbitals is opposed by the longer Cu-ligand distance and the less favorable overlaps of the orbitals. This point is discussed in more detail by Wayland and Garito.[9]

In conclusion the expectation that the $1/r^3$ dipolar anisotropic term gives a more straightforward indication of the covalency than the isotropic (Fermi contact) term is upheld. As reviewed elsewhere[10] there are still deficiencies in the theory of obtaining bonding parameters of transition metal complexes from ESR data, but Zink and Drago's mechanism does not appear to be the answer.

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Active Site Directed Inhibition of Enzymes Utilizing Deaminatively Produced Carbonium Ions. Application to Chymotrypsin

Sir:

Carbonium ions generated from diazonium ions in the deamination of aliphatic amines are exceptionally reactive species.

$$RNH_2 \longrightarrow RN_2^+ \longrightarrow R^+ + N_2 \qquad (1)$$

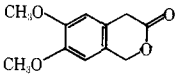
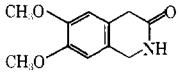
For example, they are capable of alkylating alcoholic and aromatic compounds and of abstracting halide ions from solvents such as chloroform.[1] Carbonium ions of this type if released at the active site of an enzyme should alkylate amide and other functional groups at the site, thus "labeling" them. We now report an application of this type of labeling to α-chymotrypsin.

Chymotrypsin catalyzes the hydrolysis of derivatives of carboxylic acid (proteins, amides, esters, etc.).[2] It is generally agreed that serine-195 of chymotrypsin attacks the carbonyl group of the substrate, displacing a leaving group X and generating a modified enzyme in which the serine hydroxyl group has been acylated.

$$RC(=O)X + HOEnz \longrightarrow HX + RC(=O)OEnz \xrightarrow{H_2O} RCO_2H + HOEnz \qquad (2)$$

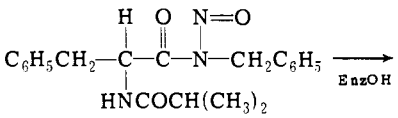
Hydrolysis of the acyl group then regenerates the enzyme (eq 2).[3]

Table I. Effect of Inhibitors and Related Compounds on Chymotrypsin Activity

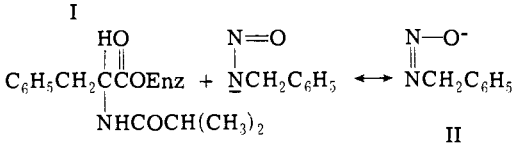
Inhibitor added[a]	% enzymatic activity[b]
None (control)[c]	100
Nitrosolactam III	6
Nitrosolactam III + hydrocinnamic acid[d]	12
Decomposition products of nitrosolactam III[e]	95
	101
	99
$NaNO_2$	100

[a] The compounds tested were added in 110-fold molar excess (final inhibitor concn = 2.5×10^{-3} *M* and chymotrypsin concn = 2.3×10^{-5} *M*). The enzyme–inhibitor solutions contained 9% acetonitrile in 0.08 *M* Tris buffer, 0.1 *M* in $CaCl_2$ at pH 7.8; they were incubated for 2 hr at 0°. See text for alternative conditions. [b] Determined by a rate assay using the *N*-benzoyl-L-tyrosine ethyl ester (BTEE) method.[11a] Enzyme concentrations in some of the runs were measured by titration with cinnamoylimidazole.[11b] [c] Acetonitrile (9%) added. [d] [Hydrocinnamic acid] = 1.2×10^{-2} *M* in the enzyme + III solution. [e] A completely decomposed solution (24 hr at 25°) of nitrosolactam (III) in the Tris buffer, pH 7.8. The chymotrypsin sample exposed to these products showed no incorporation of ^{14}C.

Utilizing the concepts outlined above, we have synthesized and tested as an irreversible inhibitor of chymotrypsin the *N*-nitroso-*N*-benzylamide of *N'*-isobutyrylphenylalanine (I).[4] As a derivative of the aromatic amino acid phenylalanine, this compound should be readily attacked by chymotrypsin, especially since a "good" leaving group (II) is formed.[5]



I

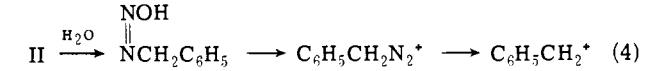


II

(3)

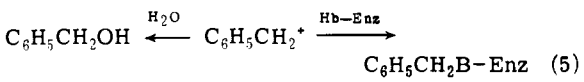
HOEnz = enzyme

The leaving group in this case is designed to yield carbonium ions (eq 4).[6] It is believed that the steps illustrated are



(4)

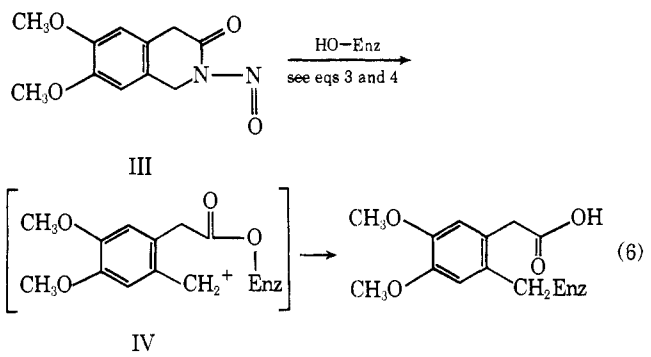
fast processes,[7] and therefore the carbonium ion should be formed at or near the active site of the attacking enzyme molecule—in a position to alkylate basic functional groups (HB) on the enzyme.



(5)

Compound I is, in fact, attacked by α-chymotrypsin. The rate of decomposition of a 5.7×10^{-4} *M* solution of I is accelerated (ca. six times) by 6.7×10^{-7} *M* enzyme in Tris buffer,[8] and, in addition, the enzyme becomes irreversibly inhibited to the extent of ~20%. The products of the reaction are benzyl alcohol and *N*-isobutyrylphenylalanine. Neither these products nor the *N*-benzylamide of isobutyrylphenylalanine irreversibly inhibit the enzyme; the amide, further, is only slowly hydrolyzed by the enzyme.[5]

The low extent of inhibition observed is probably a result of diffusion of the carbonium ions away from the active site. To circumvent this difficulty, we turned to a cyclic analog (III),[4] in which the carbonium ion formed is prevented from diffusing away by its temporary attachment to the enzyme (through the acyl bond to the serine OH of chymotrypsin; IV, eq 6). Nitrosolactam III reacts with, and irreversibly inhibits, chymotrypsin. In the presence of 4.6×10^{-5} M enzyme, a 1.2×10^{-3} M solution of III in 0.05 sodium phosphate buffer (containing 4% acetonitrile) at pH 7.9 and 20° is hydrolyzed >22 times faster than in the absence of the enzyme. The enzyme turns over 12 molar equiv of III within 5 min, and in this process becomes inhibited to the extent of ~98%. The inhibition is decreased in the presence of the competitive inhibitor hydrocinnamic acid (Table I), suggesting that the action of III is exerted at the active site.[9] The products of hydrolysis of III do not inhibit the enzyme (Table I). Treatment of the inhibited enzyme with hydroxylamine and/or dialysis does not regenerate activity, suggesting that the inhibition observed is not due to acylation.[10]



Inhibition of α-chymotrypsin with ^{14}C labeled III (at the NCH_2 position; activity 1.1×10^{10} (counts/min)/mol) and dialysis of the products led to labeled enzyme, the ^{14}C content of which showed that 1.6 mol of inhibitor had become bonded to 1 mol of the enzyme. Degradation of the inhibited enzyme to identify the labeled amino acid(s) is in progress.

In the inhibition of chymotrypsin outlined above, the substrate (nitrosolactam III) has little or no intrinsic derivatizing power for the enzyme; the active species for the inhibition ($C_6H_5CH_2^+$) is formed as a result of the enzyme "modifying" the substrate in the exercise of its normal catalytic function.[12,13] Inhibitor systems of this type favor derivatization of the functional groups at the active site relative to those elsewhere on the enzyme since the reactive groups are unmasked only at the active site.[13] Substrates analogous to I and III should function with trypsin, pepsin, papain, and other proteolytic enzymes. Further, oxidases could be labeled by this technique with substrates such as hydrazine derivatives that yield diazonium salts on oxidation. Reductases and other enzymes could presumably be derivatized by other suitable substrates and active species.

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Lithium 1,1-Dicyclopentadienyl-1-bromo-2,3,4,5-tetraphenylstannole, a Five-Coordinated Tin(IV) Heterocycle with Pseudorotating Axial- and Equitorial-Fluxional η^1-Cyclopentadienyl Groups in an $[R_4SnBr]^-$ Anion

Sir:

1,1-Dibromo-2,3,4,5-tetraphenylstannole [1] which is formed in 95% yield from phenyltin cleavage of hexaphenylstannole by elemental bromine at −40° in CCl_4 undergoes alkylation and complexation reactions typical of a diorganotin dibromide.[2] The expected dimethylstannole[3] results, for example, from treatment with methyllithium.

Treatment with excess lithium cyclopentadiene (from *n*-