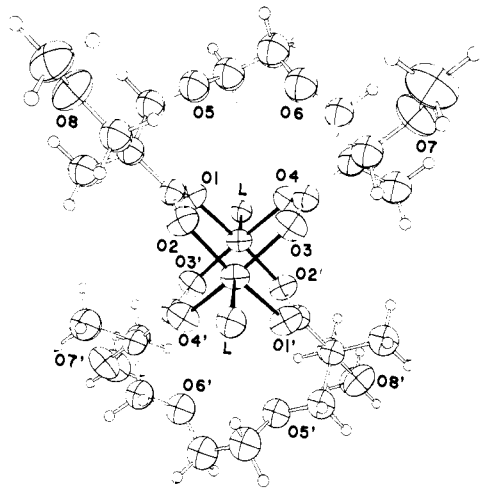
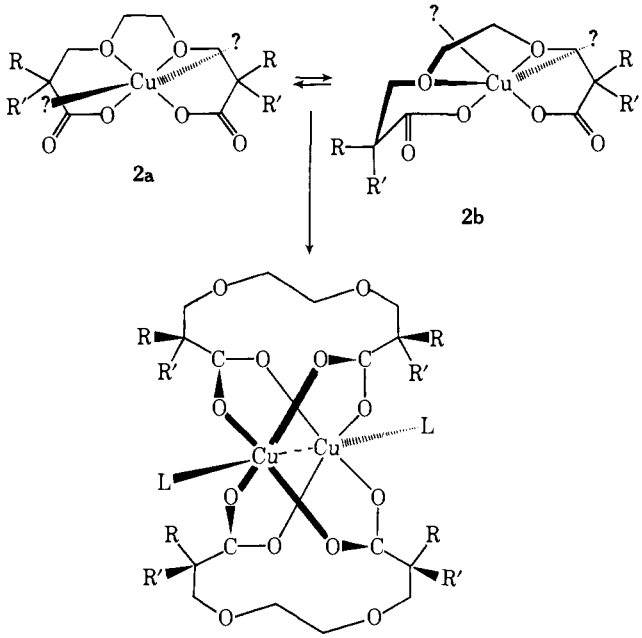


Figure 1. A view of the **3bii** molecule. For clarity, only the coordinated nitrogens of the pyridine moieties are shown.

formed than those obtained from aqueous acetone, and their structure was determined by x-ray analysis (vide infra); the crystals of the minor isomer **3aii** were unsuitable for structure determination. However, treatment of the benzene solutions obtained from **3ai** and **3aii** with pyridine or triphenylphosphine gave green solutions from which complexes of empirical formulae CuY·pyr (major, **3bi**, mp 132–133 °C *ex.* cyclohexane; minor, **3bii**, mp 156–158 °C *ex.* benzene–cyclohexane)[10] and CuY·PPh_3 (major, **3ci**, mp 131–132 °C; minor, **3cii**, mp 147.5–148 °C; both *ex.* cyclohexane)[10] were isolated. Recrystallization of both minor products (**3bii** and **3cii**) from ethanol gave crystals whose structures have been determined by x-ray crystallography.



3a,L = H_2O; 3b,L = pyridine; 3c,L = triphenylphosphine

(i) mixture of meso and *dl* ligands; R and R' are different and may be either CH_3 or CH_2OCH_3

(ii) meso ligand only; R = CH_3, R' = CH_2OCH_3

Compounds **3ai**, **3bii**, and **3cii** crystallize in the triclinic space group $P\bar{1}$ with one dimeric unit centered about the crystallographic center of symmetry. Three-dimensional intensity data were collected on a Hilger and Watts Y290 four-circle diffractometer using graphite monochromated Mo Kα radiation. The structures were solved by the Patterson-F_o Fourier method and refined by full-matrix least-squares calculations. Crystal data and refinement parameters are summarized in Table I. In **3ai**, the $-OCH_2CH_2O-$ segment of the ligand backbone is disordered over two equally populated conformations while the relative configurations at the asymmetric α carbon atoms are consistent with a mixture of *d*, *l*, and meso forms. Compounds **3bii** and **3cii** are both ordered, with the ligand present only in the meso form. Figure 1 shows a view of **3bii** and the meso configurations. In all three molecules the nature of the carboxylic cage about the copper atoms is very similar to that found in copper acetate dihydrate[12] with Cu···Cu distances 2.62 (1) in **3ai**, 2.654 (5) in **3bii**, 2.651 (5) Å in **3cii**, Cu–O (carboxylate) distances in the range 1.85–2.03 Å and Cu–O (water) 2.24 (3) Å; in the pyridine and triphenylphosphine complexes the Cu–N and Cu–P distances are respectively 2.175 (8) and 2.561 (8) Å.

Earlier work on copper(II) complexes of hydroxy- or alkoxycarboxylic acids[9] has indicated that whereas α- or β-hydroxycarboxylates, or α-alkoxycarboxylates form chelates, β-alkoxycarboxylates in aqueous solution act only as monodentate ligands. This might lead one to predict that **1** should show little or no tendency to form chelates with Cu(II) involving the ether oxygens. However, formation from **1** of a chelate involving coordination of both carboxylate groups and the backbone ether oxygens (**2a** or **2b**) would result in a relatively favorable sequence of six-, five-, and six-membered rings.[13] The fact that the complexes obtained above are dimeric rather than polymeric (cf., e.g., copper(II) succinate dihydrate[14]) may indicate that the chelate **2a,b** is formed as an intermediate. Dimerization to **3** may then occur via displacement of the coordinated ether oxygens.

Acknowledgment. We thank the National Research Council of Canada for research awards (to G.F. and R.McC.).

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Preferential Inhibition of α-Chymotrypsin by the D Form of an Amino Acid Derivative, *N'*-Isobutyryl-*N*-benzyl-*N*-nitrosophenylalaninamide (Ia)[1]

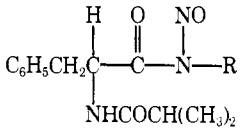
Sir:

We recently reported an irreversible inhibition of α-chymotrypsin by substrates (e.g., Ia and II) that produce carbonium ions.[2] *N'*-Isobutyryl-*N*-nitroso-*N*-benzylphenylala-

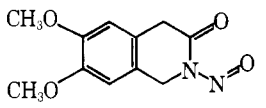
Table I. Inhibition of α-Chymotrypsin Activity[a]

Inhibitor	Moles of inhibitor:enzyme[b]	% inhibition
D-Ia	20:1	99
	10:1	93
	5:1	88
N-AT[c] plus D-Ia	3000:10:1	15
L-Ia	20:1	0
D-Ib	54:1	2
L-Ib	120:1	9

[a] *N*-Benzoyl-L-tryrosine ethyl ester (BTEE) assay method,[7] pH 7.9 (0.05 M) phosphate buffer (<1% CH_3CN), 25 °C. [b] Enzyme operational normality from cinnamoylimidazole titration.[8] Final enzyme concentration 1×10^{-5} M. [c] N-AT = *N*-acetyl-L-tryptophan.[6]



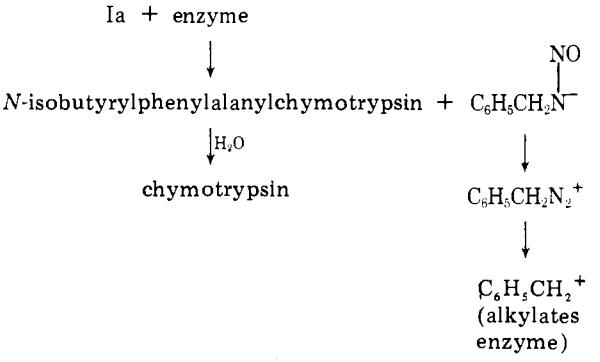
Ia, R = $CH_2C_6H_5$ and $^{14}CH_2C_6H_5$
b, R = CH_2CH_3



II

ninamide (Ia) was used with the expectation that at the acyl enzyme stage[3] nitrosobenzylamine anions would be produced, which ultimately would form benzyl carbonium ions capable of alkylating residues at the active site of the enzyme (Chart I). We assumed that the L form of phenylalanine should be

Chart I



used in the synthesis in view of the known specificity of α-chymotrypsin.[4] We have now found that contrary to expectations, the L form does not inhibit the enzyme,[5] whereas, surprisingly, the D form is an efficient inhibitor.

α-Chymotrypsin (10^{-5} M) is inhibited to the extent of 99% by a 20 molar amount of D-Ia (Table I). The inhibition is slowed by the competitive inhibitor *N*-acetyl-L-tryptophan[6] (Table I), indicating that the action of Ia occurs at the active site. Inhibition by ^{14}C labeled Ia and purification of the modified enzyme showed that about one benzyl group had been incorporated per inhibited enzyme molecule. Acid hydrolysis of the product afforded principally three labeled amino acids; identification of the tagged amino acids is in progress.

Both the D and L forms of Ia are rapidly hydrolyzed by α-chymotrypsin (Table II). Thus the ability of only the D form to irreversibly inhibit the enzyme is not a rate phenomenon; instead, it indicates that differentiation occurs at the stage of carbonium ion formation and reaction.

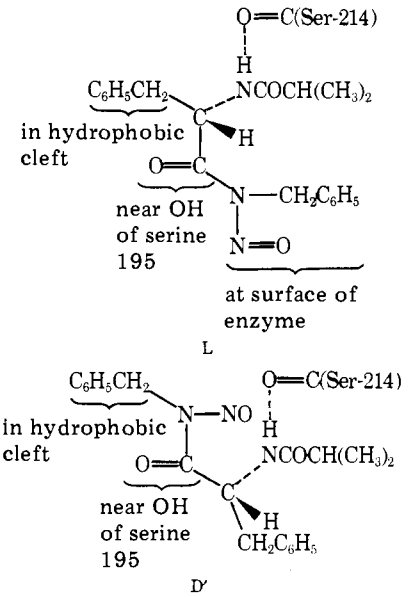
The stereospecificity of chymotrypsin has been interpreted in terms of a matching of four groups attached to the chiral center of the substrate with corresponding subsites on the enzyme.[10] A molecular model of chymotrypsin shows that the L isomer of substrate Ia can be positioned in the active site with binding at the subsites in a manner typical of L amino acid derivatives. The phenylalanine aromatic ring fits inside the

Table II. Reaction Rates of α-Chymotrypsin with Substrates Ia and Ib[a]

Substrate	Half-life (s)[b]	Relative rate[c]
D-Ia	~17	0.14
L-Ia	1.5	0.8[d]
D-Ib	~54	0.008
L-Ib	1.4	1

[a] From the decrease in absorption of the *N*-nitroso chromophore[9] at 243 nm using stopped-flow techniques. [b] 10:1 substrate:enzyme molar ratio; 4.5×10^{-6} M enzyme in 0.05 M pH 7.9 phosphate buffer, 9% in CH_3CN. [c] Determined for the first 0.5 s of reaction; 1:1 substrate:enzyme molar ratio; 4.5×10^{-6} M in 0.05 M pH 7.9 phosphate buffer, 9% in CH_3CN. [d] $V_{max} \sim 6 \times 10^{-6}$ M s^{-1}.

Chart II



hydrophobic cleft of the active site (Chart II), the isobutyrylamino group forms a hydrogen bond to the carbonyl group of serine-214, and the reactive carbonyl group is in close proximity to the OH group of serine-195. The nitrosoamino function is positioned essentially on the surface of the enzyme and the benzyl carbonium ions formed are in excellent position to be scavenged by water molecules in the medium.

Bonding the D isomer of Ia in the same way (except that the positions of H and $NHCOCH(CH_3)_2$ are reversed) is not possible, largely because the isobutrylamide group approaches too close to methionine-192 (in this mode, the H bond to serine-214 is also lost). On the other hand, the D isomer will fit if the molecule is rotated so that the positions of the two aryl groups relative to the enzyme are reversed; the stronger interactions of the D isomer bound in this way (D′, Chart II) appear to be the same as those for the L isomer. The consequence of this interchange is that the nitrosoamino function in D′ is imbedded in the hydrophobic cleft of the active site; the benzyl carbonium ions formed after the attack by serine-195 are positioned well for reaction with the amino acids making up the walls of the cleft.

Consistent with the view that both the L and D forms of aryl derivatives of aromatic amino acids bind to the enzyme is our observation that both forms of Ia are rapidly hydrolyzed by the enzyme (Table II), the L/D rate ratio being merely ~5 (comparative kinetic analysis of the hydrolyses is difficult because of the concurrent irreversible inhibition of the enzyme during the hydrolysis of the D form). The *N*-ethyl analogues (Ib), in contrast, are hydrolyzed at appreciably different rates with the rate ratio favoring the L form by a factor of ~125 (Table II). For the *N*-ethyl derivatives, binding of the lone aromatic ring to the hydrophobic cleft presumably occurs with both the L and

D forms, leading to poor binding interactions for hydrolysis in the D case. Neither the D nor the L *N*-ethyl derivative effectively inhibits the enzyme (Table I), a result most probably of the formation of diazoethane from the ethyl diazonium ion rather than the ethyl carbonium ion,[11] although the position of the group released may be involved as discussed in the case of L-Ia.

Our results indicate that mapping studies of enzyme active sites should be pursued with suitable derivatives of all stereoisomers.

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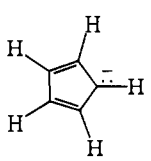
Synthesis, Nuclear Magnetic Resonance Observation, Basicity, and Reactions of Bicyclo[3.2.0]heptatrienideiron Complexes

Sir:

Because it may be viewed either as a perturbed cyclopentadienide (**1**) or cycloheptatrienide (**3**), bicyclo[3.2.0]heptatrienide[1] (**2**) occupies an important position on the borderline between conjugative stabilization and destabilization[2] of cyclic π-conjugated carbon anions. Although very dilute solutions of **2** have been prepared, its UV spectrum recorded and an approximate pK_a estimated, solutions concentrated enough for NMR or IR observation or extensive chemical study apparently cannot be obtained.[1b,d]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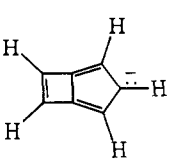
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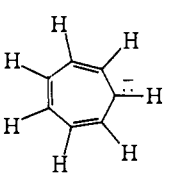
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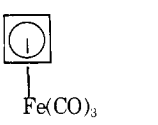
It seemed reasonable to us that complexation of **2** to an appropriate transition metal might provide a molecule stable enough for NMR observation and possibly isolation.[3] In addition, such complexation raises the interesting possibility of using the number of other ligands on the metal to determine whether it coordinates to the (4π electron) four-membered ring (analogy: cyclobutadiene(tricarbonyl)iron[3] (**4**)) or to the (6π electron) five-membered ring (analogy: η^5-cyclopentadienyl-(dicarbonyl) ferrate[4] (**5**)). We now wish to report the preparation of surprisingly stable, spectroscopically observable solutions of two iron complexes of anion **2**, NMR and IR spectra of these materials, and certain of their chemical reactions. We also discuss the chemistry and pK_a's of their neutral precursors, the latter providing a quantitative evaluation of the effect of changing the metal-bound dative ligands upon the acidity of the complexed triene.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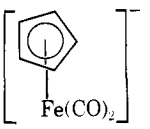
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King and his co-workers recently found[5] that treatment of air-sensitive bicyclo[3.2.0]hepta-1,4-6-triene[1d,6] (**6**) with $Fe_3(CO)_{12}$ in refluxing hexane for 20 h led to substituted cyclobutadiene complex **7**. We have found that the presence of CH_3OH (a stabilizer normally added to commercial $Fe_3(CO)_{12}$ by the supplier) is required to make this reaction proceed efficiently. In fact, it is best carried out in CH_3OH as solvent; under these conditions either $Fe_3(CO)_{12}$ or $Fe_2(CO)_9$ may be used as the source of metal. The complex is isolated as a yellow oil by short-path distillation: NMR (THF-d_8) H_a δ 6.24 (d of t, J_d = 5 Hz; J_t = 2 Hz), H_b δ 6.02 (d of t, J_d = 5 Hz; J_t = 2 Hz), H_c, H_d δ 4.20 (s), H_e, H_n δ 3.06 (m); IR (hexane) 2050, 1980 cm^{-1}; mass spectral parent peak at *m/e* 229.967 (calcd: 229.967).

Complex **7** readily undergoes ligand substitution reactions, including nitrosation,[7] and the complexes shown in Scheme

Scheme I