glycol analysis was obtained for dihydronarcissidine.[2,8] Chemical and spectral data for narcissidine are consistent with structure **2a** and will be reported in detail later. The double bond is now placed between C_{3a} and C_4 (1.32 ± 0.02 Å). The hydroxyl groups are placed in a *cis*-diaxial orientation on C_1 and C_3. The short O–O distance of these hydroxyls (2.83 ± 0.02 Å) is in good agreement with the infrared hydroxyl stretching frequencies[9] observed at 3544 and 3612 cm^{-1} in dilute solution.[10] The methoxyl group at C_2 is axial and *trans* to the adjacent hydroxyl groups at C_1 and C_3.

In view of this new structure, **2a**, for narcissidine, it should be necessary to revise the structures proposed for parkacine[4] from **1c** to **2b** and ungiminorine[5] from **1d** to **2c**.

Acknowledgment. Particular thanks are due James Benson and James R. Clark for technical assistance.

(8) This glycol analysis appears to have been fortuitous. A reexamination of several alkaloids containing the ring system of 2 has shown that even monohydroxy derivatives are cleaved by periodate.

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Design and Synthesis of Inhibitors for Crystallographic Studies on the Active Site of Chymotrypsin

Sir:

With the X-ray analysis of the structures of bovine α-chymotrypsin[1] and γ-chymotrypsin[2] now at high resolution, that is, at a Bragg spacing of 2.0–3.0 Å, it is possible to carry out detailed studies of the active site regions in these two forms of this serine protease. Difference Fourier maps of complexes between the protein and inhibitors will provide information about binding modes, recognition sites, and disposition of catalytic groups toward the peptide bond that is cleaved in a natural substrate.

We have designed and synthesized several peptide chloromethyl ketones which are effective inhibitors of γ-chymotrypsin crystals and have demonstrated that one of the crystalline derivatives is isomorphous with native protein crystals. Peptide chloromethyl ketones were chosen as the inhibitors to be investigated initially since tosyl-L-phenylalanyl chloromethyl ketone (TPCK) has been demonstrated to be an active site-specific reagent for α-chymotrypsin in solution, reacting only with His-57.[3]

The key intermediate for the synthesis of all the inhibitors was benzyloxycarbonyl-L-phenylalanyl chloromethyl ketone.[3] Deblocking with a saturated solution of HBr in acetic acid followed by the addition of ether gave the crystalline phenylalanyl chloromethyl ketone hydrobromide.[4] This could be acylated with simple anhydrides to yield, for example, Ac-PheCH_2Cl or could be coupled with blocked peptide acids using a mixed anhydride procedure.[5] Crystalline products were obtained in all cases in yields generally greater than 50%.

Chymotrypsin was prepared from bovine chymotrypsinogen by a 90-min rapid-activation procedure using acetylated trypsin. A chromatographically pure fraction (CM-cellulose, 2.0 × 60 cm column, linear gradient 0.075–0.225 *M* K^+ phosphate, pH 6.2) was concentrated and allowed to crystallize (after seeding with tetragonal chymotrypsin crystals) from a 2.0 *M* $(NH_4)_2SO_4$ solution at pH 5.6–5.9 and 20°. Well-formed tetragonal bipyramids formed within 2–4 days and grew to 0.5 mm in size during 1–3 weeks. The crystalline habit is characteristic for bovine chymotrypsin of the π, δ, and γ family.[6] End-group analysis has indicated that such a preparation is predominantly γ-chymotrypsin. α-Chymotrypsin crystals were prepared using the procedure of Sigler, *et al.*[7] The inhibition experiments with γ-chymotrypsin were performed by soaking the crystals in 2.4 *M* phosphate, pH 5.6, for 1 day to remove $(NH_4)_2SO_4$ and then placing them in a saturated solution of inhibitor in 2.4 *M* phosphate. The α-chymotrypsin crystals were treated in a similar fashion at pH 4.5. At the conclusion of the experiment, the crystals were washed with fresh salt solution and dissolved in 0.001 *M* HCl. The activity of the protein solution was then measured using a spectrophotometric assay with benzyloxycarbonyl-L-tyrosine *p*-nitrophenyl ester as substrate.[8]

The results in Table I demonstrate that a variety of peptide chloromethyl ketones are able to inhibit γ-chymotrypsin crystals within a reasonable length of time. Our best inhibitor, BOC-Gly-PheCH_2Cl, is as effective as phenylmethanesulfonyl chloride (PMSF), a known crystal inhibitor.[9] The results obtained with Ac-Leu-PheCH_2Cl were surprising since Yamashita[10]

Table I. Per Cent Inhibition of γ-Chymotrypsin Crystals[a]

Inhibitor[b]	Series I, 2 weeks	Series II, 1 week
PMSF	100	
Z-PheCH_2Cl	30	
BOC-Gly-PheCH_2Cl	100	99
Ac-PheCH_2Cl		65
Ac-Gly-PheCH_2Cl		75
Ac-Ala-PheCH_2Cl		71
Ac-Leu-PheCH_2Cl		17

[a] Inhibitions were carried out with a saturated solution of inhibitor in 2.4 *M* phosphate containing 2% CH_2CN at pH 5.6. The amino acid residues Phe, Ala, and Leu are optically active and have the L configuration.

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had found that glycylleucyltyrosine amide is cleaved more rapidly by α-chymotrypsin and has a lower K_m value than peptides containing residues smaller than leucine in the second position. Our observation can be explained either by lack of space for the leucyl side chain in the γ-chymotrypsin crystal or by low solubility of the inhibitor. No peptide chloromethyl ketones have been found which inhibit α-chymotrypsin crystals although PMSF gives complete inhibition under the conditions of our experiments. We found it surprising that Ac-PheCH$_2$Cl and CHO-PheCH$_2$Cl would not react with α-chymotrypsin crystals since the Cambridge laboratory has obtained a Fourier difference map of the enzyme inhibited with formyltryptophan.[11] Possibly small peptide chloromethyl ketones are able to bind to α-chymotrypsin crystals and yet are unable to react due to geometric restraints imposed by the α-chymotrypsin dimer structure; crystallographic experiments designed to test this hypothesis are in process.

For the first crystallographic analysis we have chosen the inhibitor Ac-Ala-PheCH$_2$Cl because of its close structural resemblance to natural peptide substrates. Precession photographs of γ-chymotrypsin crystals (0.5 mm × 0.3 mm) inhibited with Ac-Ala-PheCH$_2$Cl in pure 2.4 *M* phosphate at pH 5.6 for 2–5 weeks (83% inhibition) were obtained with a Buerger camera using Cu Kα radiation and 15° precession. Control photographs of uninhibited crystals were obtained for comparison. The cell parameters are as follows: uninhibited $a = b = 70.1$ Å, $c = 97.4$ Å; inhibited $a = b = 69.3$ Å, $c = 98.4$ Å. A collaborative study with David R. Davies and David Segal of the National Institutes of Health on the difference Fourier maps of γ-chymotrypsin inhibited with Ac-Ala-PheCH$_2$Cl and related halomethyl ketones is in progress.

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(11) D. M. Blow, personal communication.

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On the Identity of the Anion of Mercury Cobalt Carbonyl. Characterization of a Complex Anion, $Hg[Co(CO)_4]_3^-$

Sir:

In a recent paper Vizi-Orosz, Papp, and Marko[1] described the formation of an anion of mercury cobalt carbonyl, $\{Hg[Co(CO)_4]_2\}^-$, by reaction of $Hg[Co(CO)_4]_2$ with sodium amalgam or with $NaCo(CO)_4$. While the presence of such an anionic intermediate in the sodium reduction of $Hg[Co(CO)_4]_2$ to $Co(CO)_4^-$ did not seem unreasonable to us, the "reduction" of $Hg[Co(CO)_4]_2$ to $Hg[Co(CO)_4]_2^-$ by $Co(CO)_4^-$ appeared to be quite unusual and worthy of further investigation.

(1) A. Vizi-Orosz, L. Papp, and L. Markó, *Inorg. Chim. Acta*, 3, 103 (1969).

The infrared spectrum of a deep orange-brown solution of equimolar quantities of $Hg[Co(CO)_4]_2$ and $NaCo(CO)_4$ in THF, prepared and observed under an argon atmosphere, showed very strong absorptions at 2036 and 1986–1958 (broad) cm^{-1}.[2] In addition there were weak absorptions at 2068, 1895 (sh), and 1862 cm^{-1}. The first of these may be attributed to unreacted $Hg[Co(CO)_4]_2$, while the latter two are characteristic of $Co(CO)_4^-$ in THF.[3] A 1:1 stoichiometry was indicated for the reaction by infrared analysis of various mixtures of $Hg[Co(CO)_4]_2$ and $Co(CO)_4^-$. If $Hg[Co(CO)_4]_2$ were actually reduced by $Co(CO)_4^-$, a substantial quantity of $Co_2(CO)_8$ should have been found as the oxidized species.

$$Hg[Co(CO)_4]_2 + Co(CO)_4^- \longrightarrow Hg[Co(CO)_4]_2^- + Co(CO)_4 \quad (1)$$

$$Co(CO)_4 \longrightarrow {}^1/_2Co_2(CO)_8 \quad (2)$$

No evidence of $Co_2(CO)_8$ was obtained. Furthermore solutions prepared as above or with stoichiometric quantities of sodium amalgam and $Hg[Co(CO)_4]_2$ exhibit no esr signal and are diamagnetic when examined by the nmr technique of Evans.[4]

The identity of the dark-colored species in these solutions responsible for the observed spectrum has been shown to be $Hg[Co(CO)_4]_3^-$. This anion has been isolated as the tris(1,10-phenanthroline)iron(II), tetraphenylarsonium and tetramethylammonium salts. In addition to the analytical results[5] a number of other observations on these derivatives lead us to believe we have isolated $Hg[Co(CO)_4]_3^-$ and to question the existence of $Hg[Co(CO)_4]_2^-$ under these conditions. The first of these, $[Fe(1,10\text{-phen})_3][HgCo_3(CO)_{12}]_2$ (**1**), obtained only as a red powder by Markó and coworkers,[1] crystallizes with a monoclinic lattice in the space group $P2_1/c$. The unit cell parameters, $a = 14.41 \pm 0.02$ Å, $b = 27.34 \pm 0.05$ Å, $c = 17.94 \pm 0.06$ Å, and $\beta = 72° 40' \pm 5'$, with the observed density of 2.00 g/cc, give an observed molecular weight of 2038 for the molecule assuming that it occupies the general positions of the unit cell. The calculated molecular weights of $[Fe(1,10\text{-phen})_3][HgCo_3(CO)_{12}]_2$ and $[Fe(1,10\text{-phen})_3][HgCo_2(CO)_8]_2$ are 2023 and 1681, respectively.

Treatment of the diamagnetic $(CH_3)_4N^+Hg[Co(CO)_4]_3^-$ (**2**) with excess triphenylphosphine in methanol resulted in rapid precipitation of 98% of the expected quantity of $Hg[Co(CO)_3P(C_6H_5)_3]_2$.[6] The infrared spectrum of the resulting light yellow solution contained only the carbonyl absorptions corresponding to $Co(CO)_4^-$. This anion was precipitated as the insoluble $[Ni(1,10\text{-phen})_3][Co(CO)_4]_2$[7] in 90% yield by addition of an aqueous solution of $[Ni(1,10\text{-phen})_3]Cl_2$ to the aqueous extracts of the residue left on evaporation of the methanol. These results, summarized in the following scheme, are consistent only with a $Co(CO)_4/Hg$ ratio of 3:1.

(2) These values may be compared with 2035 and 1969 cm^{-1} reported for $[Hg[Co(CO)_4]_2]^-$ in ref 1.
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