tained, but the reaction was complete after 6 hr. The solution was concentrated to about 20 ml. The remaining ammonia was frozen and removed by lyophilization. Homogeneity of the crude material (0.502 g, 100%) was confirmed by thin-layer chromatography, R_f 0.05 (B), 0.46 (C). This material was identical with IVA. Treatment with Z-Asn-ONph afforded the known protected pentapeptide V in almost quantitative yield.

Benzyloxycarbonyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (V). To a solution of IV (0.502 g) in ethyl acetate (3 ml) Z-Asn-ONph[30,34] (0.387 g, 1.0 mmol) was added and the suspension stirred at room temperature for 48 hr. The precipitate which formed was collected by filtration and washed with ethyl acetate (20 ml) and ethanol (5 ml), and then dried. The crude material was recrystallized from 40% methanol: yield, 0.718 g (99%); mp 212–213.5°; R_f 0.46 (B), 0.84 (C); $[\alpha]^{20}D$ −60.5° (*c* 1, dimethylformamide) (lit.[30] mp 212–213°; $[\alpha]^{22}D$ −59.4° (*c* 1, dimethylformamide); lit.[35] mp 210.5–211.5°; $[\alpha]^{20.5}D$ −59.5° (*c* 2, dimethylformamide)).

In the following, the peptide amide derivatives with a free α-amino group, obtained by catalytic hydrogenolysis in liquid ammonia, are described. These were subsequently converted into the next higher known protected peptides as described for V.

L-Asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (VI). V (5.37 g, 7.40 mmol) was hydrogenated for 8 hr in liquid ammonia (800 ml) using methanol–wet palladium black as described above to afford colorless crystals from methanol–ether; 4.36 g (99%); mp 102–104° with softening at 86°; R_f 0.02 (B), 0.55 (C); $[\alpha]^{21}D$ −59.1° (*c* 1, dimethylformamide).

Anal. Calcd for $C_{27}H_{41}N_7O_6S$ (591.7): C, 54.8; H, 6.98; N, 16.6; S, 5.42. Found: C, 54.6; H, 7.25; N, 16.4; S, 4.99.

Condensation with Z-Gln-ONph[30,45] afforded the protected hexapeptide (VII) in 89% yield: mp 209.5–210°; R_f 0.02 (B), 0.76 (C); $[\alpha]^{21}D$ −57.0° (*c* 1, dimethylformamide); lit.[30] mp 233–234° dec; $[\alpha]^{21}D$ −54° (*c* 1, dimethylformamide); lit.[46] mp 209°; lit.[45] mp 209–210°).

L-Glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (VIII). VII (3.82 g, 4.47 mmol) was hydrogenated in liquid ammonia (600 ml) for 6.5 hr to give a colorless powder from methanol–ether: 3.25 g (100%); mp 136–138°; R_f 0.55 (C); $[\alpha]^{21}D$ −62.6° (*c* 1, acetic acid); lit.[46] mp 145° dec; $[\alpha]D$ −67.3° (*c* 2.3, acetic acid); lit.[47] mp 135°).

The protected heptapeptide (IX) was prepared by the coupling of VIII with Z-Ile-ONph[30] in 100% yield: mp 232–233°; R_f 0.85 (C); $[\alpha]^{21}D$ −50.2° (*c* 1, dimethylformamide) (lit.[30] mp 233–235°; $[\alpha]^{20}D$ −50.0° (*c* 1, dimethylformamide); lit.[35] mp 230–231.5°; $[\alpha]^{21}D$ −48.8° (*c* 1, dimethylformamide)).

L-Isoleucyl-L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (X). IX (4.12 g, 4.26 mmol) was hydrogenated in liquid ammonia (600 ml) for 8.5 hr to produce colorless crystals from methanol–ether; 3.51 g (99%); mp 218–220° with softening at 168°; R_f 0.48 (C); $[\alpha]^{21}D$ −59.1° (*c* 1, dimethylformamide) (lit.[48] mp 211–213°; $[\alpha]^{20}D$ −57.1° (*c* 1, dimethylformamide).

This was coupled with Z-Tyr-ONph[49] to afford the protected octapeptide, Z-Tyr-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2 (XI), in 82.4% yield: mp 237.5–238.5°; R_f 0.02 (B), 0.66 (C): $[\alpha]^{21}D$ −43.0° (*c* 1, dimethylformamide).

L-Tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (XII). XI (3.39 g, 3.0 mmol) was hydrogenated in liquid ammonia (500 ml) for 8 hr. Colorless crystals were obtained from methanol–ether: 2.98 g (100%); mp 179–180°; R_f 0.55 (C); $[\alpha]^{21}D$ −41.3° (*c* 1, dimethylformamide).

Anal. Calcd for $C_{47}H_{69}N_{11}O_{11}S$ (996.2): C, 56.7; H, 6.98; N, 15.5; S, 3.22. Found: C, 57.0; H, 6.62; N, 15.2; S, 2.88.

Condensation with Z-Cys(Bzl)-ONph[36,50] afforded the protected nonapeptide (XIII), Z-Cys(Bzl)-Tyr-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2, in 98% yield as colorless microscopic needles from dimethylformamide–formic acid (99:1): mp 235–236.5°; R_f 0.82 (C); $[\alpha]^{21}D$ −56.1° (*c* 1, dimethylformamide); $[\alpha]^{20}D$ −58.2° (*c* 2.5 acetic acid) (lit.[24] mp 243–245°; $[\alpha]^{22}D$ −43° (*c* 2, dimethylformamide); lit.[29] mp 224–225°; lit.[30] mp 245–248°; $[\alpha]^{20}D$ −50.5° (*c* 1, dimethylformamide); $[\alpha]^{20}D$ −64.5° (*c* 2.5, acetic acid); lit.[46] mp 241°; $[\alpha]^{20}D$ −51.5° (*c* 2.5, acetic acid)).

***S*-Benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-*S*-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (XIV).** Hydrogenation of XIII (52 mg) in liquid ammonia (20 ml) was carried out for 8 hr in the presence of palladium catalyst. Colorless crystals were obtained from ethanol: 44.1 mg (94.5%); mp 244–246° with softening at around 175°; R_f 0.28 (C); $[\alpha]^{21}D$ −48.8° (*c* 0.5, dimethylformamide).

Anal. Calcd for $C_{57}H_{80}N_{12}O_{12}S_2$ (1189.5): C, 57.6; H, 6.78; N, 14.1; S, 5.39. Found: C, 57.8; H, 6.62; N, 14.1; S, 5.83.

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Communications to the Editor

Facile Intramolecular Hydrolysis of Dipeptides and Glycinamide

Sir:

The enzymic hydrolysis of glycinamides and peptide derivatives continues to be of considerable interest, and some debate on the source of activation remains.[1] Previously, we have demonstrated activations of 10^4–10^6 when such substrates are directly coordinated *via* the carbonyl oxygen to octahedral metal ions such as Co(III)[2] and where solvolytic hydrolysis is involved. We now report the metal ion promoted *intramolecular* hydrolysis of a simple peptide which can give rise to accelerations comparable with those observed enzymically.

Treatment of *cis*-$[Co(en)_2Br(glyglyOC_3H_7)](NO_3)_2$ with *ca.* 1 *M* HOCl at 0° for 10 min, followed by chromatography on Sephadex (SP C-25, 0.5 *M* $NaClO_4$, pH 8, 2°) resulted in the separation of a violet-red 2+ band

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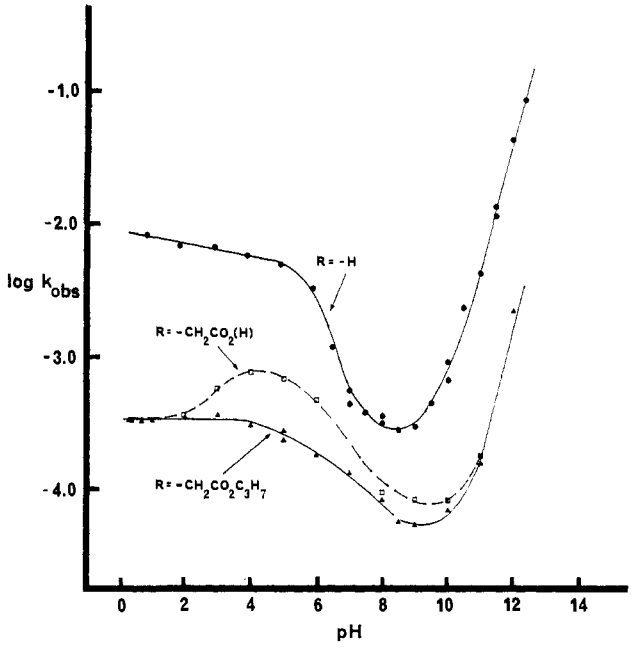

Figure 1. Plot of log k_{obsd} *vs.* pH for the intramolecular reaction of *cis*-$[Co(en)_2(NH_2CH_2CONHR)(OH)]^{2+}$ ions in the absence of buffers, 25°, μ = 1.0 *M* $NaClO_4$.

and an orange 3+ band. The 3+ species was identified as the N-O chelated dipeptide, $[Co(en)_2(glyglyOC_3H_7)]^{3+}$, by its physical properties (3+ ion; ϵ_{485} 97, pmr spectrum) and the rate and rate law

$$k_{obsd} = \frac{a[OH^-]}{b + [OH^-]} = \frac{7.5 \times 10^{-4}[OH^-]}{2.5 \times 10^{-4} + [OH^-]}$$

at 25°, (μ = 1.0 *M* $NaClO_4$), for its hydrolysis to $[Co(en)_2(glyO)]^{2+}$.[2a] The 2+ band (ϵ_{498} 103) has similar spectral properties to other *cis*-$[Co(en)_2OH(amine)]^{2+}$ ions[3,4] and has a pK_b of 7.74 (μ = 1.0 *M* $NaClO_4$). It hydrolyzes in the pH range 0–12 (pH stat) forming $[Co(en)_2(glyO)]^{2+}$ and $[Co(en)_2(glyglyOC_3H_7)]^{3+}$ (isolated by ion exchange chromatography) according to the rate law

$$k_{obsd} = k_{OH_2} + k_{OH} + k'_{OH}[OH^-]$$

(k_{OH_2} = 3.4 × 10^{-4} sec^{-1} (aquo complex), k_{OH} = 5.3 × 10^{-4} sec^{-1} (hydroxo), k'_{OH} = 0.10 M^{-1} sec^{-1} (hydroxo), μ = 1.0 *M* $NaClO_4$, 25°). The adjacent *cis* configuration of the hydroxo and dipeptide functions was established using optically pure $(+)_{589}$-*cis*-$[Co(en)_2Br(glyglyOC_3H_7)](NO_3)_2$ ($[M]_{440}$ = −1580°) which afforded $(+)_{589}$-*cis*-$[Co(en)_2(OH)(glyglyOC_3H_7)]^{2+}$ from whence optically pure $[Co(en)_2(glyO)]^{2+}$ ($[M]_{546}$ = +3400°) and $[Co(en)_2(glyglyOC_3H_7)]^{3+}$ ($[M]_{546}$ = +3270°) were obtained at pH 1. The $(+)_{589}$-*cis*-$[Co(en)_2(OH)(glyNHR)]^{2+}$ species (R = H, $CH_2CO_2^-$, $CH_2CO_2C_3H_7$) were also prepared by controlled treatment of $(+)_{589}$-*cis*-$[Co(en)_2X(glyNHR)](ClO_4)_2$ (X = Cl, Br) with alkali (pH 9–10) and chromatographic separation at 2° from $[Co(en)_2(glyNHR)]^{3+}$. However, by this method some racemic cis and trans hydroxo amide products are also formed.

Other *cis*-$[Co(en)_2(OH)(glyNHR)]^{2+}$ species (R = H, $CH_2CO_2^-$) formed in a similar manner behave similarly.

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Figure 1 gives the rate constants for the hydrolysis reactions as a function of pH (pH stat) and Table I

Table I. Product Distributions[a] for the Intramolecular Hydrolysis of *cis*-$[Co(en)_2(OH/OH_2)(glyNHR)]^{2+/3+}$ Expressed as the Ratio $[Co(en)_2(glyO)]^{2+}/[Co(en)_2(glyNHR)]^{3+}$

R	pH 1	pH 4	pH 8
H	100/0	100/0	100/0
CH_2CO_2H[b]	40/60		
$CH_2CO_2^-$[c]		77/23	76/24
$CH_2CO_2C_3H_7$[d]	46/54	44/56	45/55

[a] pH stat data in the absence of buffers. [b] pK_a = 2.9 (carboxylic acid residue). [c] pK_a = 6.3 (aquo ligand). [d] pK_a = 6.0 (aquo ligand); pK_w = 13.77 (μ = 1.0 *M* $NaClO_4$), 25°.

gives the product distributions. Above pH 9 hydrolysis of the chelated $[Co(en)_2(glyNHR)]^{3+}$ product proceeds more rapidly than does the intramolecular reaction, preventing analysis of the immediate reaction products under these conditions.

Although the cis hydroxo and aquo amide complexes are characterized here for the first time, a previous ^{18}O tracer experiment had supported their existence in one case (R = H) with subsequent hydrolysis occurring with retention of the Co–O bond.[5] The similarity in rate laws for all the hydrolyses, their rapidity, and the direct appearance of $[Co(en)_2(glyO)]^{2+}$ in the products, particularly in acid solution, is compelling evidence that they all involve the intramolecular attack of coordinated hydroxide, or water, at the adjacent carbonyl center.

Although hydrolysis is fast ($^+NH_3CH_2CONHCH_2CO_2^-$ + OH^-, k = 8 × 10^{13} sec^{-1} [6]; $[Co(en)_2(NH_2CH_2CONHCH_2CO_2)(OH)]^+$, k = 2 × 10^{-4} sec^{-1}, both at pH 7), it is accelerated further by added bases. For example, in the presence of phosphate, the rate law modifies to

$$k_{obsd} = k_{OH_2} + k_{OH} + k'[OH] + k_1[H_2PO_4^-] + k_2[HPO_4^{2-}] + k_3[PO_4^{3-}]$$

over the pH range 1–12 (k_1 = 5 × 10^{-4} M^{-1} sec^{-1}, k_2 = 0.07 M^{-1} sec^{-1}, k_3 = 0.07 M^{-1} sec^{-1} for *cis*-$[Co(en)_2OH(glyglyOC_3H_7)]^{2+}$) giving k_{obsd} = 5.5 × 10^{-3} sec^{-1} at pH 7 in 0.1 *M* phosphate (μ = 1.0 *M* $NaClO_4$, 25°). This represents an acceleration of 10^{10} over the uncatalyzed hydrolysis of glycylglycine[6] and compares favorably with the Zn^{2+}–carboxypeptidase A catalyzed hydrolysis of benzoylglycyl-L-phenylalanine (V_0 = 1.2 × 10^{-6} *M* sec^{-1} at [substrate] > 10^{-2} *M*, [ZnCPA] = 10^{-8} *M*, pH 7.5, 25°)[7] and with *N*-acetyl-L-phenylalanineamide as a substrate for α-chrymotrypsin (k_{cat} = 2 × 10^{-2} sec^{-1}, pH 7, 25°).[8] The present experiments demonstrate that specific conformational effects,[1] unusual coordination geometries or active site distortions of the amide bond,[1] are not necessary to produce rapid hydrolysis. *When correctly positioned a coordinated water molecule or*

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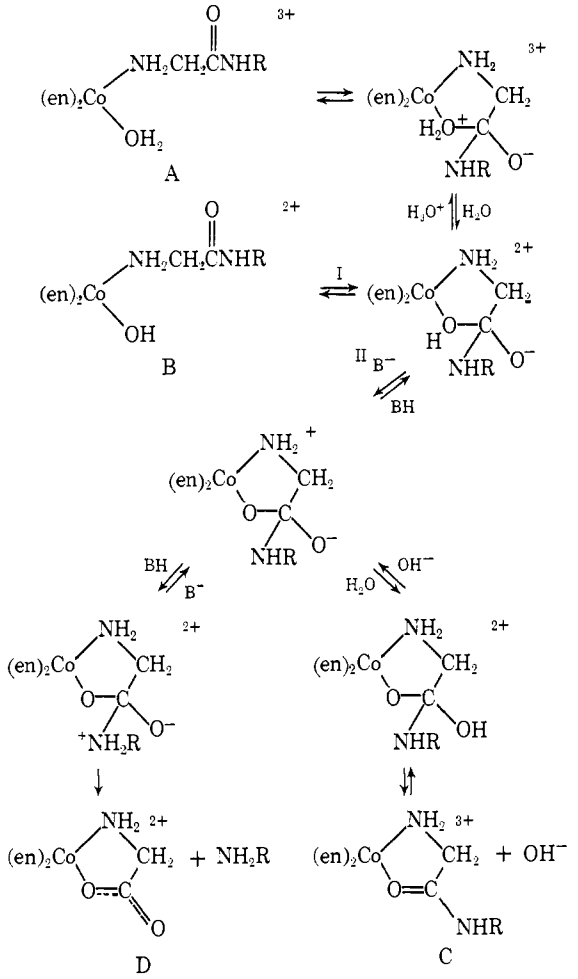
Scheme I



hydroxide ion can be a potent nucleophile for a carbonyl substrate even though the metal significantly reduces their basicity (~10⁹ for OH⁻).

The interrelation of the intramolecular hydrolysis of *cis*-$[Co(en)_2OH(glyNHR)]^{2+}$ and the intermolecular hydrolysis of $[Co(en)_2(glyNHR)]^{3+}$ by OH^- [2] is supported by the fact that the former reaction produces $[Co(en)_2(glyNHR)]^{3+}$ as well as the hydrolyzed product. A common intermediate, or set of intermediates, appears to be required. The observation of a similar Co(III) aminocarbinol intermediate which divides to chelated ester and chelated amide in the aminolysis of an amino acid ester[9] supports this claim. Amine loss follows the order $NH_3 > NH_2CH_2CO_2^- > NH_2CH_2CO_2C_3H_7$ (Table I), the order of decreasing basicity, and supports the contention that the amine leaving group is protonated. The same product ratio is found with both the aquo and hydroxo reactants in the absence of added buffer (Table I, pH 4 and 8) which supports the involvement of a single common intermediate leading to products. Also, for the hydrolysis of *cis*-$[Co(en)_2OH(glyglyOC_3H_7)]^{2+}$ in 0.1 *M* phosphate buffer at pH 7.4, 87% of $[Co(en)_2glyO]^{2+}$ is formed, compared with 45% at the same pH in the absence of buffer. This is consistent with general acid catalysis for the loss of amine and its relative absence for loss of OH. These observations are accommodated in the mechanism shown in Scheme I.

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It is proposed that the rate determining step for the intramolecular hydrolysis occurs in the proton abstraction step, II. This may be concerted accompanying I, but, since most of the bases used have pK_a's outside the range spanned by the substrate hydroxo ($pK_a \sim 20$ est) and the immediate addition product ($pK_a \sim 6$ est), we contend that a stepwise process will be preferred by all but very strong bases.[10] For bases of $pK_a > 6$ proton abstraction II becomes diffusion controlled, $k = 10^9$–10^{10}[B] sec^{-1}, with every contact leading to deprotonation. Thus, HPO_4^{2-}, PO_4^{3-}, and OH^- are equally effective ($k_{obsd}/[B] \simeq 0.1\ M^{-1}\ sec^{-1}$, Brønsted $\beta = 0$) whereas for maleate, succinate, acetate, tartrate, and furoate the second-order rate constant decreases linearly with decreasing pK_a, $\beta = 0.75$. Reprotonation by BH is unfavorable and the chelated amide C hydrolyzes to D with no B being formed. This latter reaction is not general base or acid catalyzed consistent with intermolecular attack of OH^- being rate determining.

We are at present extending these mechanistic aspects and are continuing our study of the source of the large activation.

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Stereochemistry of Oxidative Addition of Alkyl Halides to Palladium(0) Complexes

Sir:

The stereochemistry of oxidative addition[1] of alkyl halides to the transition metals of group VIII can provide information as to which of the many possible mechanisms is operative. The addition of alkyl halides to d^8 iridium complexes has been reported to proceed with retention,[2] inversion,[3] and racemization.[4,5] The racemization was proposed to proceed *via* a free-radical mechanism at the asymmetric carbon center. The kinetics of this reaction are consistent with nucleophilic displacement by iridium on carbon.[6] Similar oxidative additions of alkyl halides to d^8 cobalt have been reported to occur with inversion of configuration at carbon.[7] In the oxidative addition of silicon compounds to d^8 and d^{10} platinum,[8–10] d^8 iridium,[10] and cobalt,[10] however, retention of configuration at silicon was exclusively observed.

Oxidative addition reactions[11] of certain alkyl and

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