VOLUME 20 NUMBER 10

ACCOUNTS OF CHEMICAL RESEARCH

OCTOBER, 1987



# Cobalt(III)-Promoted Hydrolysis of Amino Acid Esters and Peptides and the Synthesis of Small Peptides

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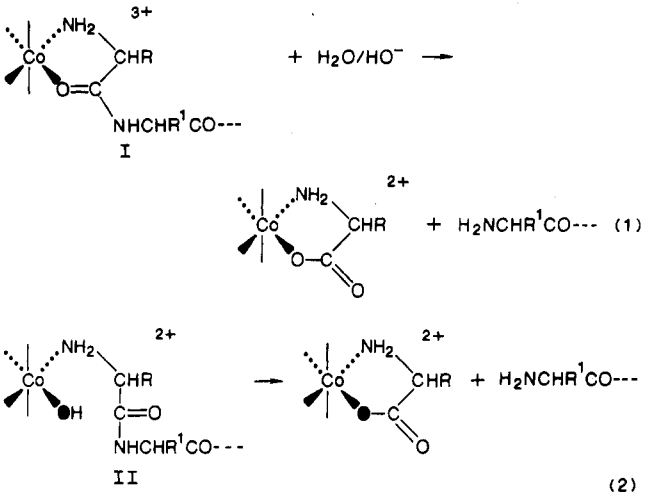
*Received August 4, 1986*

## Introduction

In 1951 Kroll discovered that $Cu^{2+}$ (and to a lesser extent $Co^{2+}$ and $Ni^{2+}$) catalyzed the hydrolysis of amino acid esters.[1] Li and co-workers[2] and Bender and Turnquest[3] soon followed with related studies, and today such catalysis is widely known for a variety of divalent metal ions and complexes. Two aims underscore such investigations: the evaluation of the mechanisms whereby metal ions might hydrolyze amino acid and peptide substrates in biological systems, and the extension in a more general way of our understanding of metal ion catalysis of ligand reactions.

Our investigations began in 1962 when one of us was starting his academic career as a postdoctoral associate with J. P. Collman at Chapel Hill. Collman had proposed that if octahedral Co(III) could be enticed to chelate to the N terminus of a peptide (as in I, eq 1) it might considerably enhance hydrolysis via direct polarization of the carbonyl function (just as Kroll had initially proposed). It was expected that Co(III) would strongly coordinate the amino group and that a five-membered chelate via *O*-carbonyl coordination would be favored at neutral pH. (*N*-amide coordination results in ionization and stabilization toward hydrolysis.) The alternative of cis attack by coordinated hydroxide (cf. II, eq 2) was also recognized but was considered less attractive since the carbonyl function is not especially activated.




The significance of using Co(III) in these studies is that, although being able to polarize and otherwise facilitate the reactions of coordinated ligands in the same way as divalent metals, the ligands are less labile in a kinetic sense so that reaction intermediates can often be identified and alternative mechanisms clearly differentiated. Even the hydrolyzed substrate often remains coordinated so that the reaction becomes stoichiometric (often termed "promoted") rather than catalytic.

Our early results demonstrated considerable enhancement in peptide hydrolysis using the $\beta$-[Co(trien)$(OH_2)$(OH)]$^{2+}$ ion[4] and to a lesser extent the

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David Buckingham, a Professor of Chemistry at the University of Otago, Dunedin, New Zealand, gained his undergraduate training (1955–1959) at Canterbury College of the University of New Zealand (now the University of Canterbury) and his Ph.D. at the Australian National University under Professor Frank Dwyer. Then followed postdoctoral experience at Chapel Hill with Jim Collman (1963–1964), an Assistant Professorship at Brown University (1964–1965), and Fellowships and Senior Fellowships at the Research School of Chemistry in Canberra (1965–1978).

(1) Kroll, H. *J. Am. Chem. Soc.* **1952**, *74*, 2036.
(2) Li, N. C.; Manning, R. A. *J. Am. Chem. Soc.* **1955**, *77*, 5225. White, J. M.; Manning, R. A.; Li, N. C. *J. Am. Chem. Soc.* **1956**, *78*, 2367. Li, N. C.; Doody, B. E.; White, J. M. *J. Am. Chem. Soc.* **1957**, *79*, 5859. Mathur, R.; Li, N. C. *J. Am. Chem. Soc.* **1964**, *86*, 1289.
(3) Bender, M. L.; Turnquest, B. W. *J. Am. Chem. Soc.* **1955**, *77*, 4271; **1957**, *79*, 1889.

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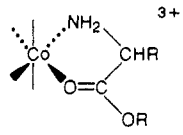
$[Co(en)_2(OH_2)(OH)]^{2+}$ ion.[5] Further experiments with the help of Marzilli and with the collaboration of Collman and Happer at Chapel Hill resulted in the first definitive publication in this area.[6] At that time experiments designed to differentiate the solvent and intramolecular paths (eq 1 and 2) were begun, and the possibilities of using chelated esters (III) for the N-



III

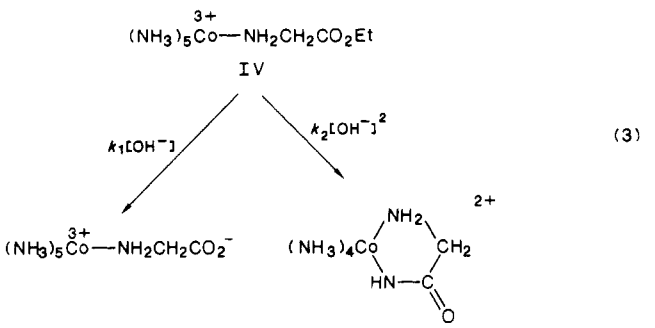
terminal synthesis of peptides and for probing the mechanisms of ester hydrolysis and exchange were initiated. This work was continued at the Australian National University in collaboration with Alan Sargeson (1965–1977), the emphasis now being toward understanding reaction mechanism.

About this time Alexander and Busch published their important paper identifying chelate III in solution,[7] and Collman in collaboration with Kimura continued his investigations into the use of Co(III) reagents to promote the hydrolysis and formation of peptide bonds.[8,9] By 1978 the essential features of the Co(III)-promoted hydrolysis of peptides and esters had been unravelled,[10] but a few uncertainties remained, and the whole area of peptide synthesis[11] had yet to be investigated in detail. This is now complete, and we now have a good understanding of the role played by Co(III) in such reactions.

## Hydrolysis of Directly Activated Esters

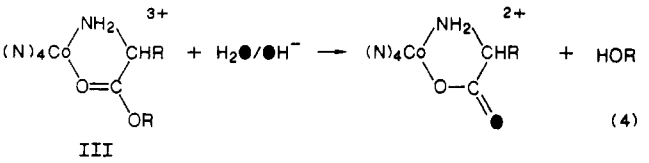
In the absence of direct coordination by the carbonyl group, little rate acceleration occurs. For example, hydrolysis of monodentate IV (eq 3) is accelerated about 100-fold over the uncoordinated molecule ($k_1$ = 50 vs. 0.6 $mol^{-1}$ $dm^3$ $s^{-1}$ for $H_2NCH_2CO_2Et + HO^-$);[12] this increase is similar to that provided by a proton in the same location (24 $mol^{-1}$ $dm^3$ $s^{-1}$ for $H_3N^+CH_2CO_2Et + HO^-$). In other complexes, Co(III) appears to be somewhat less effective than $H^+$ when two or three atoms removed from the carbonyl center, and smaller rate increases are observed.

Equation 3 demonstrates another important aspect: careful product analysis is essential before attributing an enhanced rate to a particular cause. In the above example, intramolecular condensation of a coordinated deprotonated ammine competes with hydrolysis, and an N,N-chelated amide is formed in addition to the monodentate acid anion.



IV (3)

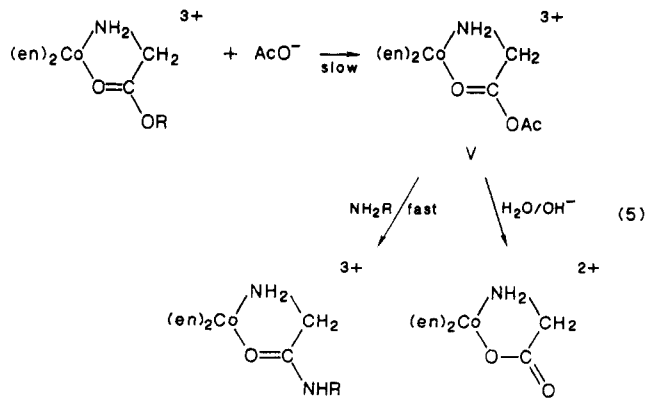
Hydrolysis of the directly activated ester III is accelerated some $10^6$ times by the metal. Table I gives rates for the water ($k_{H_2O}$) and hydroxide ($k_{OH}$) paths along with available activation parameters. Acceleration resides largely in positive $\Delta S^{\ddagger}$ components. $^{18}O$-tracer studies with the glycine and $\beta$-alanine chelates showed that the chelate ring remains intact during and subsequent to hydrolysis and that cleavage occurs in the (expected) alcohol part for primary and secondary esters (eq 4).[13,14] For R = *t*-Bu, however, O–R bond



III (4)

cleavage occurs,[15,16] and the smaller acceleration (~300 times[15]) must mean that the Co(III)–acyl species is a moderately good leaving group from the *t*-Bu carbocation.

Other bases in aqueous solution also catalyze hydrolysis,[7,17] and they do so by direct attack at the carbonyl center rather than by general base-catalyzed solvent addition.[17] This was convincingly demonstrated for acetate by trapping the resulting anhydride intermediate (V), eq 5, with an amine, which diverts the $AcO^-$-catalyzed reaction to an amide product.[18] Since $AcO^-$ is a weak O nucleophile, it is likely that all O nucleophiles behave similarly.

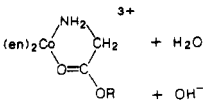
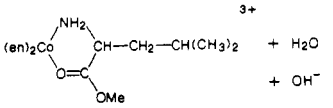
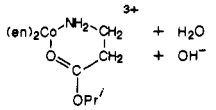
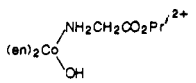
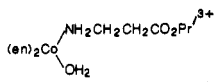
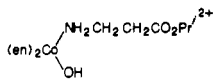
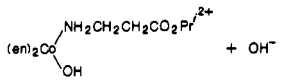
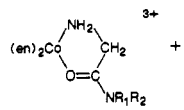
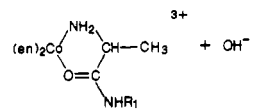
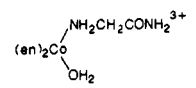
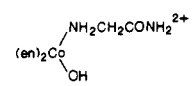
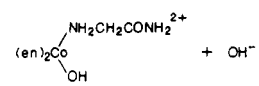


V (5)

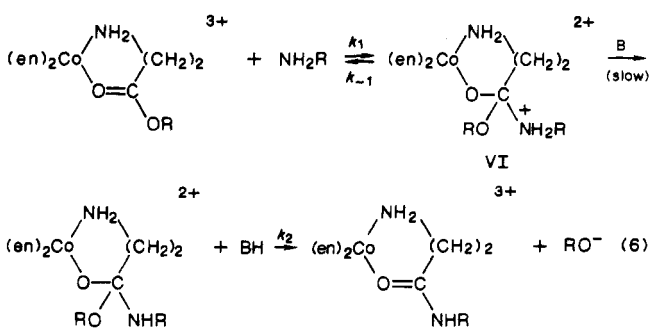
Amines only weakly catalyze hydrolysis in aqueous solution and likewise prefer to add directly to form the

(4) Collman, J. P.; Buckingham, D. A. *J. Am. Chem. Soc.* **1963**, *85*, 3039.
(5) Buckingham, D. A.; Collman, J. P. *Inorg. Chem.* **1967**, *6*, 1803.
(6) Buckingham, D. A.; Collman, J. P.; Happer, D. A. R.; Marzilli, L. G. *J. Am. Chem. Soc.* **1967**, *89*, 1082.
(7) Alexander, M. D.; Busch, D. H. *J. Am. Chem. Soc.* **1966**, *88*, 1130.
(8) Kimura, E.; Young, S.; Collman, J. P. *Inorg. Chem.* **1970**, *9*, 1183. Kimura, E. *Inorg. Chem.* **1974**, *13*, 951.
(9) Collman, J. P.; Kimura, E. *J. Am. Chem. Soc.* **1967**, *89*, 6096.
(10) Buckingham, D. A. In *Biological Aspects of Inorganic Chemistry*; Addison, A. W., Cullen, W. R., Dolphin, D., James, B. R., Eds.; Wiley: New York, 1976; Chapter 5.
(11) Buckingham, D. A.; Marzilli, L. G.; Sargeson, A. M. *J. Am. Chem. Soc.* **1967**, *89*, 2272, 4539.
(12) Buckingham, D. A.; Foster, D. M.; Sargeson, A. M. *J. Am. Chem. Soc.* **1969**, *91*, 3451.
(13) Buckingham, D. A.; Foster, D. M.; Sargeson, A. M. *J. Am. Chem. Soc.* **1968**, *90*, 6032.
(14) Baraniak, E.; Buckingham, D. A.; Clark, C. R.; Moyniham, B. H.; Sargeson, A. M. *Inorg. Chem.* **1986**, *25*, 3466.
(15) Wu, Y.; Busch, D. H. *J. Am. Chem. Soc.* **1970**, *92*, 3326.
(16) Buckingham, D. A.; Foster, D. M.; Sargeson, A. M. *Aust. J. Chem.* **1969**, *22*, 2479.
(17) Buckingham, D. A.; Foster, D. M.; Sargeson, A. M. *J. Am. Chem. Soc.* **1970**, *92*, 5701.
(18) Buckingham, D. A.; Dekkers, J.; Sargeson, A. M.; Wein, M. *J. Am. Chem. Soc.* **1972**, *94*, 4032.

**Table I.**
**Rate Constants for the Co(III)-Promoted Hydrolysis of Coordinated Amino Acid Esters and Amides**

| reaction | $k$/mol$^{-1}$ dm$^3$ s$^{-1}$; $E_a$/kJ mol$^{-1}$; $\Delta S^{\ddagger}$/J K$^{-1}$ mol$^{-1}$ |
|---|---|
| | A. Esters |
|  | $6.9 \times 10^{-4}$ (R = Me)<br>$2.0 \times 10^{-6}$ (R = Pr$^i$); $E_a$, 67; $\Delta S^{\ddagger}$, −122<br>$2.3 \times 10^{-5}$ (R = $t$-Bu)<br>$8 \times 10^5$ (R = Pr$^i$); $E_a$, 73; $\Delta S^{\ddagger}$, 164 |
|  | $1.4 \times 10^{-4}$ (Λ($S$))<br>$5.8 \times 10^{-5}$ (Δ($S$))<br>$3 \times 10^6$ (Λ($S$))<br>$1.4 \times 10^6$ (Δ($S$)) |
|  | $8.3 \times 10^{-7}$<br>$4 \times 10^4$ (addition of OH$^-$ rate determining) |
|  | >$3 \times 10^{-3}$ s$^{-1}$ (at pH 9; rate not observed; intramolecular) |
|  | $1.8 \times 10^{-6}$ s$^{-1}$ (probably intramolecular) |
|  | $5.6 \times 10^{-6}$ s$^{-1}$ (intramolecular) |
|  | 0.13 (intramolecular) |
| | B. Amides |
|  | 14 (R$_1$ = R$_2$ = H); $E_a$, 60; $\Delta S^{\ddagger}$, −30 ± 10<br>0.87 (R$_1$ = R$_2$ = Me); $E_a$, 59; $\Delta S^{\ddagger}$, −59 ± 4 |
|  | 7 (R$_1$ = $CH_2CO_2Pr^i$)<br>4.1 (R$_1$ = $CH_2CH_2NH_2$)<br>112 (R$_1$ = $CH_2CH_2NH_3^+$) |
|  | $9.2 \times 10^{-3}$ s$^{-1}$; $E_a$, 77; $\Delta S^{\ddagger}$, −33 ± 9 (intramolecular) |
|  | $1.5 \times 10^{-4}$ s$^{-1}$; $E_a$, 52; $\Delta S^{\ddagger}$, −150 ± 20 (intramolecular) |
|  | 2.0; $E_a$, 37; $\Delta S^{\ddagger}$, −30 ± 10 (intramolecular) |

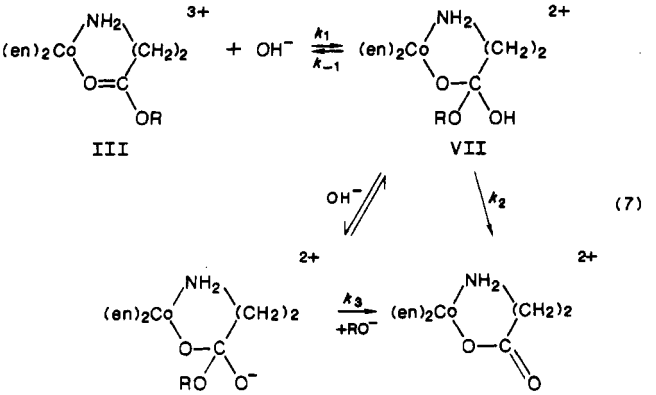
chelated amide (eq 6). Here addition is not rate determining, and another base molecule is required to form the product, $k_{obsd} = k_N[NH_2R][B]$.[19] It appears that almost any other nonsterically hindered base with a p$K_a$ above 7 will do ($AcO^-$ does not catalyze this reaction), which suggests that the amine–alcohol intermediate (VI) has a p$K_a$ of about 7.



VI

(19) Buckingham, D. A.; Clark, C. R. *Inorg. Chem.* **1986**, *25*, 3478.

For hydrolysis, the question of rate-controlling addition of $HO^-$ to the activated ester or rate-determining loss of $RO^-$ from the addition intermediate has been answered by using the six-membered β-alanine ester system.[14] The rate law takes the form $k_{obsd} = (k_1[HO^-] + k_2[HO^-]^2)/(1 + K[HO^-])$, which almost certainly implicates intermediate VII (eq 7). Below pH ~8.5,



III VII (7)

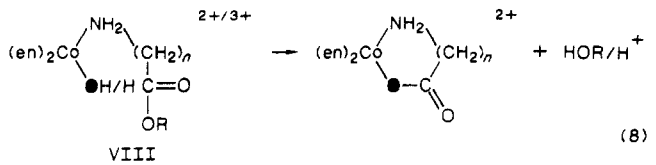
elimination of $RO^-$ is rate determining ($k_2$), but above this pH deprotonation of VII becomes important so that by pH 10 rapid loss of $RO^-$ from its conjugate base ($k_3/k_2$ is estimated at $\simeq 10^5$) is the preferred route and $k_1$ becomes rate controlling. This, in our view, is the first clear kinetic demonstration of a tetrahedral addition intermediate in amino acid ester hydrolysis. The more rapid hydrolysis of the five-membered glycine ester chelate (Table I) results from increased electron withdrawal via the amine function. This will influence both $k_1$ and the acidity of VII so that it is likely that a switch from rate-determining loss of $RO^-$ at low pH to rate-determining addition of $HO^-$ at high pH occurs here also.

The mild catalysis observed with nonnucleophilic buffers such as pyridine and $\alpha$-picoline is accounted for by aiding deprotonation of VII.[7,20] Such factors are, we believe, present in all metal-catalyzed ester hydrolyses, with chelate ring size playing an unimportant role. The requirement for deprotonation of the hydroxo addition intermediate VII to facilitate rapid loss of $RO^-$ has obvious parallels with the aminolysis reaction described above. The slowness of the water reaction ($k_{H_2O} = 8.3 \times 10^{-7}$ mol$^{-1}$ dm$^3$ s$^{-1}$ vs. $k_{OH} = 4 \times 10^4$ mol$^{-1}$ dm$^3$ s$^{-1}$ for the $\beta$-alanine chelate; Table I) suggests that here addition ($k_1$) is rate determining.

Nonnucleophilic buffers, especially those having a proton switch capability such as $HPO_4^{2-}$ and $HCO_3^-$, are extraordinarily effective in catalyzing this water reaction,[14] so much so that they cannot be accommodated by catalyzing the breakdown of VII and must be implicated from the start. $HPO_4^{2-}$ catalyzes only hydrolysis, but $HCO_3^-$ also catalyzes opening of the chelate by cleaving between O and C to form the hydroxo monodentate ester VIII ($n = 2$). This is very surprising for a metal chelate, but illustrates the intriguing features possible in such reactions. (O exchange in the ester has occurred in this instance.) Other factors, such as changing the remaining ligands attached to the metal or lowering the overall charge, have some effect on the rate, but these are rather minor by comparison with the effects of buffers.

## Intramolecular Hydrolysis by Coordinated $HO^-$ or $H_2O$

This possibility was first demonstrated in 1969 with VIII ($n = 1$).[21] Its synthesis required replacing coordinated Br (or Cl) by OH, and the rate of this replacement camouflaged in a kinetic sense the subsequent cyclization reaction, eq 8. However, $^{18}O$ tracers




VIII (8)

established that cyclization had occurred intramolecularly,[21] and more recently this has been followed kinetically in the $\beta$-alanine system where the rate is slowed by a factor of about $10^3$.[14] The importance of ring size in such intramolecular reactions, in contradistinction with the directly activated processes discussed above, is very important, and we will discuss this aspect below. However, many investigators have implicated a M–OH nucleophile in obvious bimolecular situations, and we believe that for esters, amides, and peptides such reactions are extremely unlikely.

M–OH is a good nucleophile in a bimolecular sense only when the substrate is very electrophilic such as with $SO_2$ or $CO_2$, or when a very good leaving group is involved, such as with acid chlorides, anhydrides, or reactive esters. Only then will M–OH compete with the aqueous solvent at pH 7. With $SO_2$, attack by Co–$OH^{2+}$ requires little activation, the rate being close to the diffusion limit ($k_{CoOH} = 4 \times 10^8$ mol$^{-1}$ dm$^3$ s$^{-1}$).[22] With $CO_2$ the process is much slower ($k_{CoOH} = (2\text{–}3) \times 10^2$ mol$^{-1}$ dm$^3$ s$^{-1}$),[23] but most M–OH nucleophiles can still react fast enough such that millimolar concentrations are sufficient to beat out the solvent reaction at pH 7.[10] With more discriminating electrophiles, M–OH is a poor substitute for $HO^-$(aq),[24,25] so much so that for biologically important esters or amides at near-neutral pH M–OH will be unimportant unless some concentrating factor which forces the reactants into juxtaposition is involved.[25]

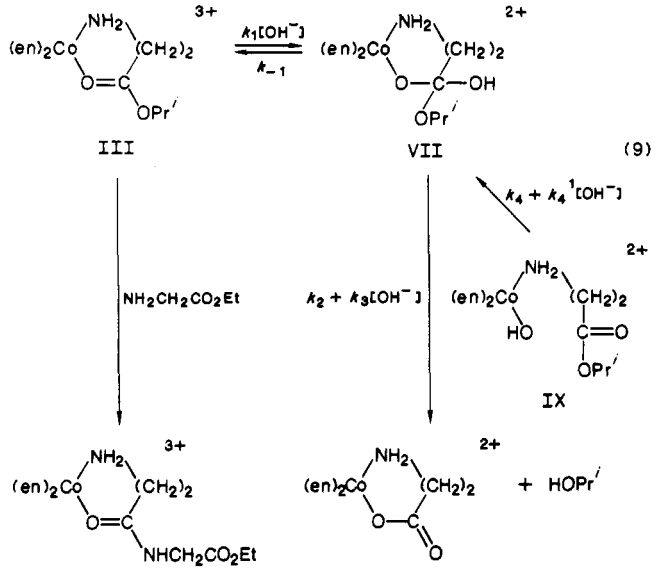
Coordinated water in M–$OH_2$ is, as expected, an even poorer nucleophile in a bimolecular sense. It only adds to species capable of expanding their valence such as unsaturated higher oxidation states of nonmetals ($H_2NO_2^+$, $SO_2$, $HIO_3$, $HSeO_3^-$, $HAsO_4^{2-}$) and possibly to $HMoO_4^-$ and $HCrO_4^-$. Then the bimolecular addition of Co–$OH_2^{3+}$ is extremely fast and in some instances seems to be even faster than solvolysis by the solvent itself. Toward lesser species such as $CO_2$, esters and amides, Co–$OH_2^{3+}$ is completely unreactive, and we believe that this will be true of all M–$OH_2$ species. But like M–OH, in intramolecular situations or when the two centers are otherwise held in close proximity, the behavior of M–$OH_2$ can be very different.

Such proximity is crucial in the reactions of Co–OH and Co–$OH_2$ with esters and amides. For the $\beta$-alanine ester system, the addition part of eq 8 appears to be rate determining even though the rate for the Co–$OH_2$ species ($k_{obsd} = 1.8 \times 10^{-6}$ s$^{-1}$) is similar to that for Co–OH ($k_{obsd} = 5.6 \times 10^{-6} + 0.13[OH^-]$ s$^{-1}$). Also, such hydrolyses are slower than those for the directly activated ester (Table I): 30 times for Co–$OH_2$ and $3 \times 10^5$ times for Co–OH. Such differences cancel in forming five-membered ring systems, with monodentate glycine ester now being hydrolyzed at least $10^3$ times faster than monodentate $\beta$-alanine ester (Table I). This presumably results from the proximity of the essentially unactivated ester to the coordinated solvent nucleophile.

Another useful feature of these intramolecular reactions is their marked catalysis by external buffers.

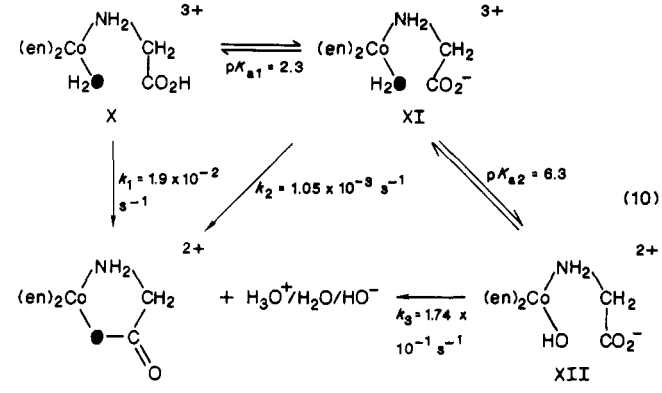
(20) Sutton, P. A., unpublished Ph.D. research.
(21) Buckingham, D. A.; Foster, D. M.; Sargeson, A. M. *J. Am. Chem. Soc.* **1969**, *91*, 4102.
(22) Van Eldik, R.; Harris, G. M. *Inorg. Chem.* **1980**, *19*, 880. El-Awody, A. A.; Harris, G. M. *Inorg. Chem.* **1981**, *20*, 1660. Dash, A. C.; El-Awody, A. A.; Harris, G. M. *Inorg. Chem.* **1981**, *20*, 3160.
(23) Chaffee, E.; Dasgupta, T. P.; Harris, G. M. *J. Am. Chem. Soc.* **1973**, *95*, 4169.
(24) For the substrates $SO_2$, $CO_2$, propionic anhydride, 2,4-dinitrophenyl acetate, and 4-nitrophenyl acetate an excellent correlation exists between $k_{CoOH}$ for $(NH_3)_5CoOH^{2+}$ and $k_{OH}$ for $HO^-$(aq). This takes the form $\log k_{CoOH} = 1.6 \log k_{OH} - 4.1$ and stretches over a $10^7$ variation in rate. For some of these data, cf. Buckingham, D. A.; Clark, C. R. *Aust. J. Chem.* **1982**, *35*, 431.
(25) Buckingham, D. A.; Clark, C. R. *Aust. J. Chem.* **1982**, *35*, 431.

$CO_3^{2-}$ and $PO_4^{3-}$ are outstanding in this regard, but several others are also useful.[14] Thus, the rate of reaction 8 is much influenced by the local environment, whereas hydrolysis of the directly activated ester is less affected and may even be indirect. (Nucleophilic addition of the buffer can occur.) The two direct processes do, however, occur via a common intermediate. Starting with the hydroxo ester IX (eq 9), the chelated ester III can be trapped by an amine, and it has been shown that the relative leaving abilities of $HO^-$ and $PrO^-$ from intermediate VII is about 7.[14]




(9)

Although cyclization in the monodentate glycine ester could not be directly observed it could in the related glycine acid X (reaction 10).[26] This process is very

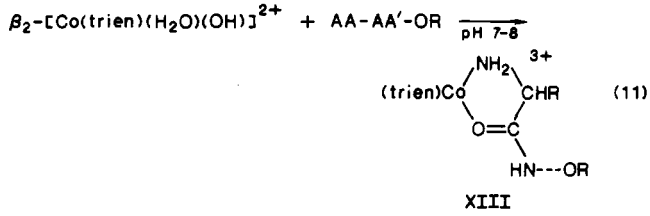



(10)

rapid in acidic solution ($t_{1/2} \simeq 40$ s) but is slow in alkali ($t_{1/2} \approx 11$ h). General acid catalysis is observed with XI at pH ~4. Such fast intramolecular lactonizations involving $M-OH_2$ species parallel in many ways those found with organic molecules when a hydroxy group is adjacent to a carboxylic acid residue.[27] Loss of water and cyclization may well occur in a concerted manner.

## Hydrolysis of Directly Activated Amides and Peptides

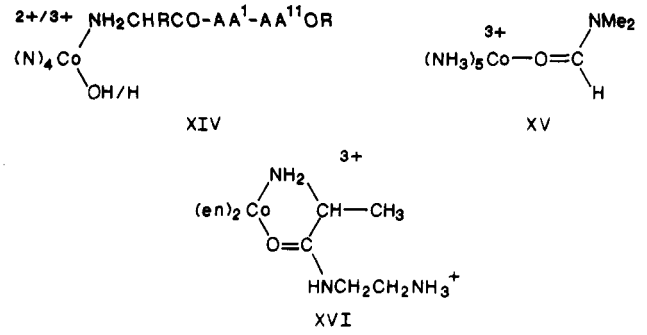
Co(III)-promoted hydrolysis of peptides using the $\beta$-$[Co(trien)(H_2O)(OH)]^{2+}$ ion[4,6] has been shown to occur via the N,O-chelated peptide intermediate XIII (eq 11). Such a species has been both detected in[9] and isolated



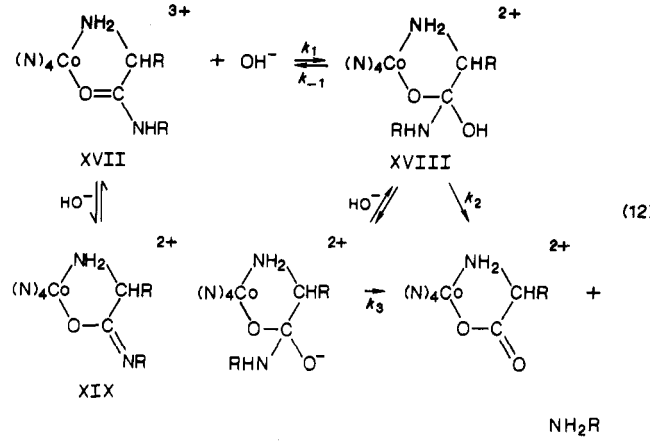

(11)

from[28] the reaction mixture. $\beta$-$[Co(trien)(H_2O)(OH)]^{2+}$ has subsequently been used for a variety of hydrolytic purposes.[29-33]

Some difficulties have been encountered,[32] and some comparative rate studies with other $[Co(N)_4(H_2O)(OH)]^{2+}$ reagents have been reported.[33] For reasonably dilute (millimolar) concentrations, the rate-determining step is the hydrolysis of XIII, which arises because displacement of coordinated water from the Co(III) reagent and from the aqua (or hydroxo) monodentate intermediate XIV is rapid in this case. However, such rapid displacements are not usual for Co(III) complexes, especially with species such as XIV, and it is more usual for the alternative hydrolysis pathway via intramolecular attack by Co–OH or $Co-OH_2$ to occur.




Hydrolysis of chelate XVII, eq 12, occurs by rate-determining attack of $HO^-$. This reaction is devoid of general acid or general base catalysis which would be expected if decomposition of intermediate XVIII were rate determining. Only at pH 7–8 does loss of $HO^-$ ($k_{-1}$)




(12)

compete with loss of amine ($k_2$).[34] Above pH ~11 the reaction loses its first order in $[HO^-]$ dependence due

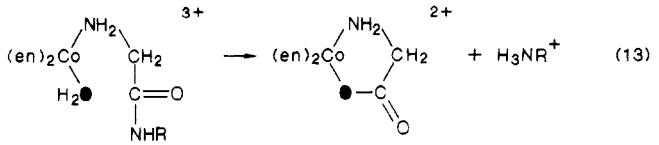
(26) Boreham, C. J.; Buckingham, D. A.; Francis, D. J.; Sargeson, A. M.; Warner, L. G. *J. Am. Chem. Soc.* **1981**, *103*, 1975.
(27) Hershfield, R.; Schmir, G. L. *J. Am. Chem. Soc.* **1973**, *95*, 8032.
(28) Buckingham, D. A.; Davis, C. E.; Foster, D. M.; Sargeson, A. M. *J. Am. Chem. Soc.* **1970**, *92*, 5571.
(29) Bentley, K. W.; Creaser, E. H. *Biochem. J.* **1973**, *135*, 507.
(30) Kimura, E. *Inorg. Chem.* **1974**, *13*, 951.
(31) Bentley, K. W.; Creaser, E. H. *Inorg. Chem.* **1974**, *13*, 1115.
(32) Fenn, M. D.; Bradbury, J. H. *Anal. Chem.* **1972**, *49*, 498.
(33) Rhee, M. J.; Storm, C. B. *J. Inorg. Biochem.* **1979**, *11*, 17.
(34) Boreham, C. J.; Buckingham, D. A.; Keene, F. R. *J. Am. Chem. Soc.* **1979**, *101*, 1409.

to deprotonation of the reactant to form unreactive XIX.[28] Protonation of the departing amine by the solvent or by another general acid must occur since the reverse aminolysis of the chelated ester requires general bases to catalyze the addition step (eq 6).

The metal promotes hydrolysis by about $10^4$ (Table I), and this again resides entirely in $\Delta S^{\ddagger}$. Unlike O-bound esters, O-bound amides are stable as monodentates in aqueous solution, and the accelerated hydrolysis found for monodentate XV, $\sim 10^4$ times,[35] is similar to that found for the chelated amides, so that chelation is not a necessary feature. Also, protonation as in XVI causes an additional acceleration of $\sim 30$ times, but this is thought to result from facilitated addition of $OH^-$ ($k_1$) rather than from intramolecular involvement of the protonated leaving group.[36]

## Intramolecular Hydrolysis of Amides and Peptides

Intramolecular hydrolysis by coordinated $H_2O$ is very efficient, with reaction 13 being nearly $10^2$ times faster

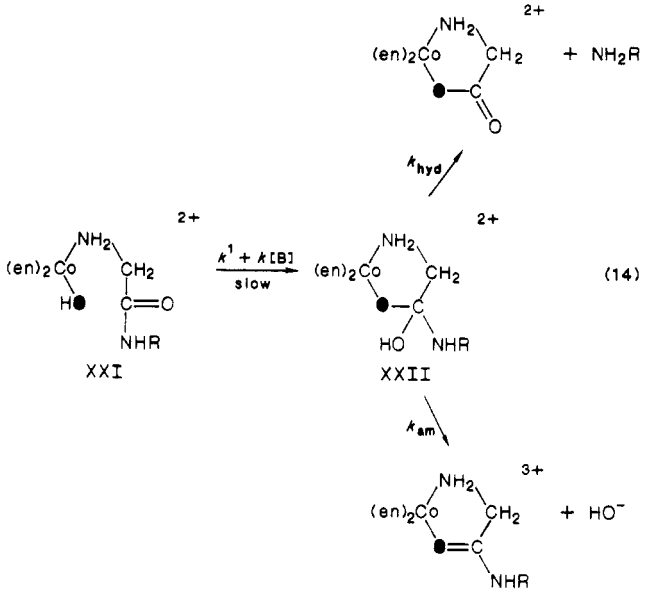



XX

than the same process involving coordinated $HO^-$.[34,37] There is no parallel in the reaction of the uncoordinated molecule, and its efficiency must result in some way from cyclization and concerted loss of protonated amine. Intermediate XVIII is probably avoided, with the reaction resembling in many ways that found for the glycine acid X (reaction 10).

Acceleration resides entirely in $\Delta S^{\ddagger}$ (Table I), and there is no doubt as to its intramolecular nature.[38] A similar process involving coordinated $H_2O$ is not found with the otherwise identical monodentate aminoacetonitrile complex where a leaving group is not involved,[39] which implies that elimination of amine is an important feature. When deprotonated, the coordinated water molecule becomes less reactive. But at pH 7 reaction 14 is still some $10^2$ times faster than that via the chelated amide (reaction 12). Hydrolysis is now, however, catalyzed by other bases B (including $HO^-$), and cyclization appears to be rate determining.[34] However, some chelated amide is also formed, and the $^{18}O$ tracer results in the pH range 6–8 require the hydroxo amine intermediate XXII to lose both $NH_2R$ and $HO^-$.[37] Only at neutral pH can the chelated amide product be trapped since at higher pH its own hydrolysis becomes commensurate with, and then exceeds in rate, that of the hydroxo amide.

General bases divert the products mostly toward hydrolysis ($k_{hyd}$) presumably because deprotonation of XXII is helpful. $HCO_3^-$, $H_2PO_4^-$, and $HPO_4^{2-}$ are




particularly useful in this respect, and they can also protonate the leaving amine (bifunctional catalysis). Amine loss (compared to $HO^-$ loss) follows the order $NH_3 > NH_2CH_2CO_2^- > NH_2CH_2CO_2Pr^i$, consistent with protonation being a requirement. No similar reaction is observed with the β-alaninamide monodentate where a larger chelate ring would be required.

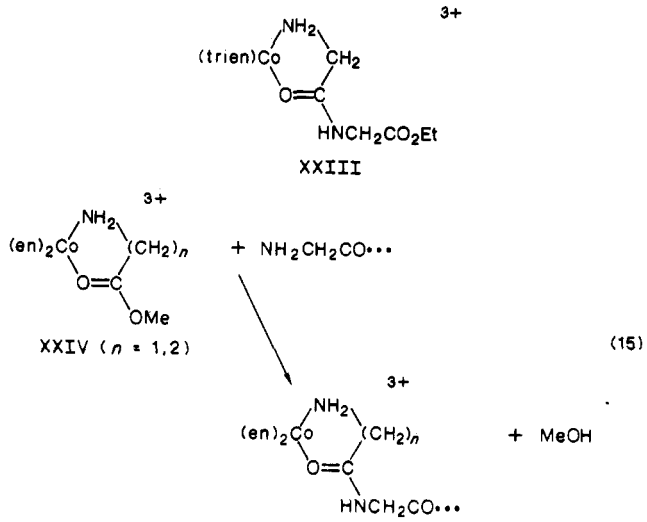
Such studies require that peptide hydrolysis using $[Co(en)_2(H_2O)(OH)]^{2+}$ occur via the intramolecular mechanism.[5] This arises because of the reluctance of coordinated $H_2O$ in XX (or $HO^-$ in XXI) to be displaced by the carbonyl function of the peptide. The intramolecular pathway is also faster than that via the directly activated amide in neutral or acidic solution. But above pH $\sim 9$ the $HO^-$-induced reaction of the latter is some 10–$10^2$ times more efficient. Clearly, the choice adopted by a particular metal will depend on its ability to coordinate strongly the carbonyl functionality. When this is not possible, the intramolecular process should prevail.

## Co(III) Active Esters in Peptide Synthesis

Peptide synthesis on the metal was discovered when $\beta_2$-$[Co(trien)(glyOEt)Cl](ClO_4)_2$ was treated with glyOEt in a nonaqueous environment.[41] The dipeptide product XXIII remains chelated to the metal, and the reaction is complete within minutes at room temperature. An even more rapid condensation occurs with $[Co((trien)(BuO)_3PO)_4](ClO_4)_3$,[41] and Collman and Kimura have used β-$[Co(trien)Cl_2]Cl$.[9] The ester chelate XXIV is the active ingredient in all these reactions, and following its isolation in 1967 it was shown to condense readily with amino acid esters and small peptides, eq 15 ($n = 1$).[11] Such condensations were the beginning of peptide synthesis in our laboratory.

Other Co(III) reagents (e.g., *cis*-$[Co(en)_2(glyOMe)Br](ClO_4)_2$ and *cis*-$[Co(en)_2(\beta\text{-alaOMe})Br](ClO_4)_2$) have since been used to add glycine or β-alanine to the N terminus of amino acid esters or peptide fragments,[42] and their chelated intermediates (XXIV, eq 15) have also been isolated.[14,43] The difficulty for many years

(35) Buckingham, D. A.; Harrowfield, J. MacB.; Sargeson, A. M. *J. Am. Chem. Soc.* **1974**, *96*, 1726.
(36) Buckingham, D. A.; Binney, G. S.; Clark, C. R.; Garnham, B.; Simpson, J. *Inorg. Chem.* **1985**, *24*, 135.
(37) Buckingham, D. A.; Keene, F. R.; Sargeson, A. M. *J. Am. Chem. Soc.* **1974**, *96*, 4981.
(38) Boreham, C. J.; Buckingham, D. A.; Keene, F. R. *Inorg. Chem.* **1979**, *18*, 28.
(39) Buckingham, D. A.; Morris, P. J.; Sargeson, A. M.; Zanella, A. *Inorg. Chem.* **1977**, *16*, 910.
(40) Wautier, H.; Marchal, D.; Fastrez, J. *J. Chem. Soc., Dalton Trans.* **1981**, 2484.
(41) Buckingham, D. A. Marzilli, L. G.; Sargeson, A. M. *J. Am. Chem. Soc.* **1967**, *89*, 2772.
(42) These reagents are easily prepared from *trans*-$[Co(en)_2Br_2]Br$ (Alexander, M. D.; Busch, D. A. *Inorg. Chem.* **1966**, *5*, 602; ref 17).
(43) Buckingham, D. A.; Wein, M. *Inorg. Chem.* **1974**, *13*, 3027.

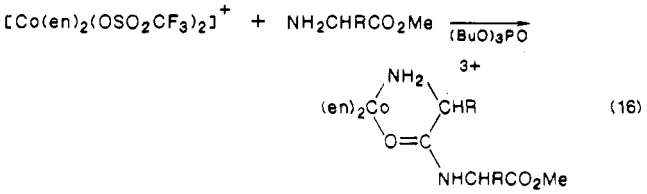


XXIII



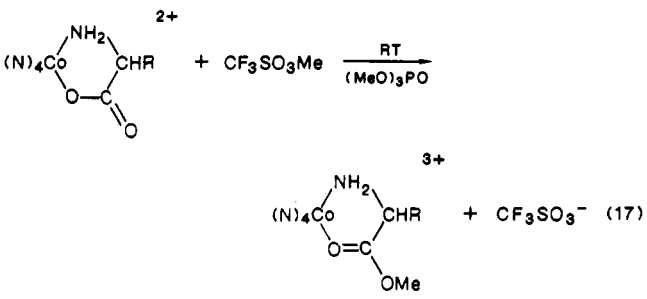
XXIV ($n$ = 1,2) (15)

was to generalize this to include all amino acids, the problem being to find an easy route to the generalized active ester chelate III. However, *symmetrical* dipeptides can be prepared with $[Co(en)_2((n\text{-}BuO)_3PO)](ClO_4)_3$ and more recently with *cis*-$[Co(en)_2(OSO_2CF_3)_2](CF_3SO_3)$[44] (eq 16). This method is an excellent way for making such dipeptides by persons with little laboratory experience. Condensations are complete within minutes at, or just above, room temperature.



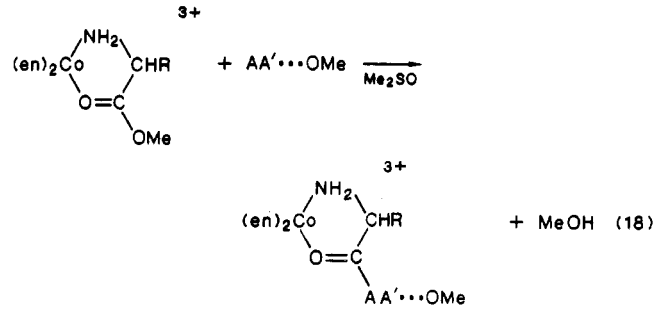
(16)

The breakthrough in the generalized synthesis came in 1980 via esterification of the chelated amino acid using methyl triflate (eq 17).[45,46] Earlier attempts at



(17)

esterification using $SOCl_2$, $PCl_3$, $PBr_3$, AcCl, HCl(g), and $BF_3$ in MeOH or EtOH were partly successful, but $Et_3OBF_4$, $CH(OEt)_3$, and $Me_2C(OMe)_2$ in $CH_2Cl_2$/$Me_2SO$ failed.[47] Now the Co(III) active ester can be made in gram quantities, and it is stable for long periods once reprecipitated (or recrystallized) from anhydrous MeOH. Condensations with amino acid or peptide esters in $Me_2SO$, MeOH, MeCN, or $MeNO_2$ as solvent are rapid, taking only a few seconds in most cases at the millimolar level (eq 18). Routinely, we add the Co ester



(18)

dissolved in $Me_2SO$ to a buffered AAOMe/$HAAOMe^+Cl^-$ solution (or the $Et_3N$-neutralized peptide ester HCl) in $Me_2SO$ to give a final Co ester concentration of $\sim 5 \times 10^{-2}$ mol dm$^{-3}$ with a [Co ester]/[AAOMe]/[$HAAOMe^+Cl^-$] ratio of 1:3:2. Provided no moisture is present, the reaction is quantitative.

The peptide is easily released by reducing Co(III) to Co(II) or the metal, and we routinely do this electrolytically using a Hg-pool cathode at −1.0 V (vs. SCE). The peptide is then recovered by SP-C25 cation exchange or Bio-Gel P2 gel filtration chromatography.[48] The coupling rate is largely independent of the chirality of the AA'OMe nucleophile but is of course very dependent on what the chelated AAOMe and nucleophile AA'OMe are. One interesting facet which has relevance to epimerization is that coupling of the (Λ)Co(*S*) ester diastereomer is often much faster than coupling of the (Δ)Co(*S*) ester, but this effect is somewhat solvent dependent. Thus, large discriminations are found with Ala–PheOMe and Leu–LeuOMe couplings, smaller discriminations with Ala–ValOMe and Phe–PheOMe, and an inverse discrimination with Val–PheOMe (i.e., (Δ)Co(*S*)ValOMe reacts more rapidly than does (Λ)-Co(*S*)ValOMe). These factors mean that couplings of unwanted (R) amino acid chelates can often be discriminated against or avoided.

Epimerization at the chelated α-carbon center was recognized as a potential problem at the time of the first couplings,[9,11] but the speed and mild experimental conditions suggested this might be minimal. Certainly the lower charged amino acid chelates $[Co(en)_2(AAO)]^{2+}$ do not H exchange or epimerize at neutral pH in water, and a similar property was known to hold for the coupled product, $[Co(en)_2(AA\text{–}AAOMe)]^{3+}$. To investigate this problem, we initially used $^3H$ tracers in the synthesis; this allows very low incorporations (at the <1% level) to be measured. Early results[45] gave very small incorporations (~0.20%), and there was the possibility that this came about by incorporation into the coordinated amine functions of other ligands rather than into the α-carbon of the dipeptide.

Recent studies[20] using the separate diastereomers have confirmed small $^3H$ incorporations into the α-carbon center of the chelated amino acid ester (e.g., 3.0% for (Λ)Co(*S*)AlaOMe + AlaOMe; 5.0% for (Δ)-Co(*S*)AlaOMe + AlaOMe) with some of this resulting in an inverted α-carbon product. However, $^1H$ NMR and HPLC results show larger amounts of the inverted product, and the net result is an appreciable $^1H/^3H$ discrimination in favor of the lighter isotope. This

(44) Dixon, N. E.; Jackson, W. G.; Lancaster, M. J.; Lawrence, G. A.; Sargeson, A. M. *Inorg. Chem.* **1981**, *20*, 470.
(45) Tasker, R. F. Ph.D. Thesis, University of Otago, June 1982.
(46) Clark, C. R.; Tasker, R. F.; Buckingham, D. A.; Knighton, D. R.; Holding, D. R. K.; Hancock, W. S. *J. Am. Chem. Soc.* **1981**, *103*, 7023.
(47) Dekkers, J. Ph.D. Thesis, The Australian National University, Jan 1972.
(48) Knighton, D. R.; Harding, D. R. K.; Friar, M. J.; Hancock, W. S.; Reynolds, G. D.; Clark, C. R.; Tasker, R. F.; Buckingham, D. A. *J. Am. Chem. Soc.* **1981**, *103*, 7025.

means that the $^3H$-labeling experiments are not especially useful as a measure of H exchange or inversion, a result we perhaps should have foreseen.

While this work was in progress, Fastrez and coworkers[40] and Mensi and Isied[49] have published results which suggest complete or major epimerization in the chelated amino acid during coupling. Our recent results show that this can be minimized. For example, the (Δ)Co(*S*)LeuOMe + AlaOMe coupling in $Me_2SO$ gives 40% epimerization under certain conditions whereas the (Λ)Co(*S*)LeuOMe diasteromer gives <1%. Similar discriminations are shown by other amino acid combinations.

### Summary and Prognosis

Where do we go from here? The ability of coordinated $H_2O$ and $HO^-$ to promote the hydrolysis of esters and amides over and above that available to the directly activated substrate has obvious implications to metalloenzymes. This has already been suggested for some, but it will be difficult to demonstrate unequivocally in the in vivo situation. Modern spectroscopic methods together with low-temperature X-ray investigations offer some promise, but in the absence of direct verification it is important to establish with certainty such alternatives in labile "model" systems. So far, such studies have lacked originality and leave much in doubt; kinetic information alone is not sufficient. The Co(III) ester method for synthesizing small peptides offers some new opportunities, not the least of which is the orange color imparted to the coupled product, but the accompanying epimerization at asymmetric C centers will need to be carefully documented before the method can be considered alongside the more conventional organic routes.

(49) Mensi, N.; Isied, S. S. *Inorg. Chem.* 1986, *25*, 147.

**Registry No.** Cobalt(III), 22541-63-5.

# Unusual Reactivity of Prostacyclin: Rational Drug Design through Physical Organic Chemistry†

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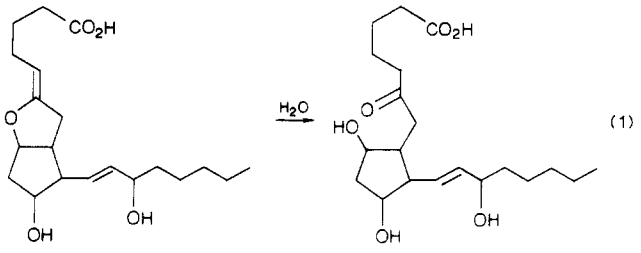
*Received March 2, 1987 (Revised Manuscript Received June 3, 1987)*

Prostacyclin, 1, a naturally occurring bioregulator discovered just over 10 years ago,[1] has remarkable physiological properties: it is the most potent inhibitor of blood-clot formation known.[2] This gives it tremendous potential as a therapeutic agent for the treatment of heart attack and stroke and also as an anticlotting factor to confer noncoagulant properties upon polymeric materials used to manufacture vascular prosthetic devices such as heart valves and blood vessel replacements. Unfortunately, prostacyclin is also very unstable: its lifetime at physiological pH is only 3 min, which drastically limits its biomedical applications.

It was determined early in the short history of prostacyclin that this instability is due to hydrolysis of the molecule's vinyl ether functional group (eq 1).[3]




1 (1)

Jerry Kresge received his undergraduate education at Cornell University and did graduate work at the University of Illinois. After postdoctoral research at University College, London, Purdue, and M.I.T., he joined the staff of Brookhaven National Laboratory. In 1960 he moved to the Illinois Institute of Technology and in 1974 took up his present position at the University of Toronto.

That discovery aroused our attention, for we had been studying the hydrolysis of vinyl ethers in considerable detail. Our interest in vinyl ether hydrolysis originally, and for some time thereafter, was purely theoretical: the process provided a good example of what was then a rare reaction type, rate-determining proton transfer from catalyzing acid to substrate, and we were using it to learn as much as possible about the proton-transfer process. We never thought that our work might have a practical application, but it has. Through our knowledge of vinyl ether hydrolysis, we have been able to contribute to the chemistry of prostacyclin and to suggest ways in which its instability might be overcome. This Account describes that work.

### Vinyl Ether Hydrolysis

The hydrolysis of vinyl ethers (eq 2) bears some similarity to the hydrolysis of saturated ethers (eq 3). Both

$$CH_2{=}CHOR \xrightarrow[HA]{H_2O} CH_3CHO + ROH \qquad (2)$$

$$CH_3CH_2OR \xrightarrow[HA]{H_2O} CH_3CH_2OH + ROH \qquad (3)$$

† Dedicated to Prof. Nelson J. Leonard on the occasion of his 70th birthday.

(1) Moncada, S.; Gryglewski, R.; Bunting, S.; Vane, J. R. *Nature (London)* 1976, *263*, 663-665.

(2) For recent reviews of the physiological and chemical properties of prostacyclin, see: Bartmann, W.; Beck, G. *Angew. Chem., Int. Ed. Engl.* 1982, *21*, 751-764. Nelson, N. A.; Kelly, R. C.; Johnson, R. A. *Chem. Eng. News* 1982, *60*(30), 30-44.

(3) Cho, M. J.; Allen, M. A. *Prostaglandins* 1978, *15*, 943-954.

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