$J_{4H,6\alpha F}$). This pattern should consist of four lines of relative intensity 0.95, 0.98, 1.05, and 1.02 with a line separation varying from 0.01, 1.7, and 0.01 c.p.s. when $J_{BX} = 0$ c.p.s. to 1.7, 0.3, and 1.7 c.p.s. when $J_{BX} = 2$ c.p.s. At maximum resolution with our instrument, tetramethylsilane exhibits a width at half-height of 1.2 c.p.s., which will lead to the appearance of the four lines as a doublet when $J = 0$–0.5 c.p.s., as a triplet when $J = 1.2$–2 c.p.s. and as merged peaks (at half-height) at intermediate J values. Since the C-4 proton actually appeared as an unsplit peak, the most probable value for the $6\alpha F$–4H coupling is between 0.5 and 1.2 c.p.s.

The axial 6β-fluoro C–F bond is approximately perpendicular to the plane passing through the C-4, 5, and 6 carbon atoms and parallels the π-orbitals of the double bond. Expressed in other terms, the dihedral angle of the projection of the C 4–4H bond and the C-6–6βF bond approximates 90° while the corresponding angle with the C-6–6αF bond is about 15°. Although the magnitude of $6\alpha F$–4H coupling cannot be determined with exactitude it is clearly minimal compared to the 5–5.5 c.p.s. of the axial 6β-fluoro atom. Thus, just as in the examples[4] of allylic 1,3-proton-proton coupling, this type of fluorine–hydrogen long-range coupling and transmission of spin information probably depends upon maximal overlap of the C–F σ-bond with the π-orbitals of the double bond. Caution should be exercised, however, in extrapolating these results to other long-range fluorine–proton coupling situations when the fluoro atom is adjacent to a π-electron system. For example, the C-4 proton of 2α-fluorotestosterone (Ic) is strongly 1,3-coupled (doublet at 342.5 and 347.5 c.p.s., $J_{2\alpha F,4H} = 5$ c.p.s.) to the equatorial 2α-fluorine, possibly via the π-electrons of the adjacent ketone function. In this case the dihedral angle of the projection of the pertinent C–F and C–H bonds is much closer to 0° than to 90°.

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N-Hydroxysuccinimide Esters in Peptide Synthesis

Sir:

The need for better methods of forming the peptide bond has been emphasized in a recent report of the synthesis of a 39-amino acid ACTH peptide,[1] although the p-nitrophenyl ester method[2,3] was a key to the synthesis. The recently reported esters of N-hydroxyphthalimide[4,5] appear to be more reactive than p-nitrophenyl esters, but have some disadvantages.[5] One disadvantage of both is the insolubility of by-products (p-nitrophenol and N-hydroxyphthalimide) in water except under alkaline conditions. In consideration of the ready water solubility of N-hydroxysuccinimide,[6] we have prepared esters of this compound with a number of N-acylamino acids; these are crystalline, highly reactive compounds which have given promising results in peptide synthesis. In several examples, yields in

(1) R. Schwyzer and P. Sieber, *Nature*, **199**, 172 (1963).
(2) M. Bodanszky, *ibid.*, **175**, 685 (1955).
(3) M. Bodanszky, *Ann. N.Y. Acad. Sci.*, **88**, 655 (1960).
(4) G. H. L. Nefkens and G. I. Tesser, *J. Am. Chem. Soc.*, **83**, 1263 (1961).
(5) G. H. L. Nefkens, G. I. Tesser, and R. J. F. Nivard, *Rec. trav. chim.*, **81**, 683 (1962).
(6) Beilstein, Vol. 21, p. 380; R. Wegler, F. Grewe, and K. Mehlhose, U. S. Patent, 2,816,111 (1957).

peptide synthesis averaged better than those with comparable literature methods, and purification was simplified. These new esters are particularly promising for the addition of an acylamino acid to the salt of a peptide in aqueous solution. Illustrative examples are given subsequently, and a more detailed paper is in process.

Analogous to the synthesis of p-nitrophenyl[7,8] and N-hydroxyphthalimide[4,5] esters, dicyclohexylcarbodiimide in dioxane or dimethoxyethane was used to make the N-hydroxysuccinimide esters; the products were obtained in yields of 50–90% after recrystallization from 2-propanol. Typical N-hydroxysuccinimide esters are those of carbobenzoxy-L-phenylalanine (I), m.p. 140–140.5°, carbobenzoxyglycine (II), m.p. 113–114°, and carbobenzoxy-L-proline (III), m.p. 90°. Reaction of I with an equivalent of ethyl L-tyrosinate[9] in dimethoxyethane for 40 min. at 25° followed by the addition of water yielded ethyl carbobenzoxy-L-phenylalanyl-L-tyrosinate, yield 85% after recrystallization from ethanol, m.p. 156–158°, $[\alpha]^{25}$D $-9.1°$ (c 10, EtOH); lit.[10] yield 46% by a mixed anhydride procedure. A solution of 1.53 g. of II in 10 ml. of dimethoxyethane was added to a solution of 0.87 g. of proline and 0.63 g. of sodium bicarbonate in 8 ml. of water; after an hour, acidification gave carbobenzoxy-glycyl-L-proline (IV), yield 75% after recrystallization from ethyl acetate, m.p. 157–158°; lit.[11] yield 68%, m.p. 155°, via a thiophenyl ester. The N-hydroxyphthalimide ester of carbobenzoxyglycine[5] was similarly treated with proline; slow acidification gave a poor fractional separation of N-hydroxyphthalimide, and IV was obtained in 45% yield, m.p. 145–150°. A solution of 3.46 g. of III in 30 ml. of ethanol was added to a solution of 2.79 g. of glycyl-L-phenylalanylglycine[12] and 1.68 g. of sodium bicarbonate in 50 ml. of water plus 25 ml. of ethanol; after standing 18 hr., acidification and removal of ethanol by vacuum distillation gave 4.53 g. (89%) of crystalline carbobenzoxy-L-prolylglycyl-L-phenylalanylglycine; recrystallization from water–ethanol yielded 4.12 g. (80%), m.p. 154–155°, $[\alpha]^{25}$D $-27.6°$ (c 2, dioxane). *Anal.* Calcd. for $C_{26}H_{30}N_4O_7$: C, 61.16; H, 5.92; N, 10.98. Found: C, 61.45; H, 6.11; N, 11.01.

(7) D. F. Elliot and D. W. Russell, *Biochem. J.*, **66**, 49P (1957).
(8) M. Rothe and F. W. Kunitz, *Ann.*, **609**, 88 (1957).
(9) E. Fischer, *Ber.*, **34**, 433 (1901).
(10) J. R. Vaughan, Jr., and R. Osato, *J. Am. Chem. Soc.*, **74**, 676 (1956).
(11) H. N. Rydon and P. W. G. Smith, *J. Chem. Soc.*, 1956, 3643.
(12) L. Zervas and D. M. Theodoropoulos, *J. Am. Chem. Soc.*, **78**, 1359 (1956).

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Hydrolytic Cleavage of N-Terminal Peptide Bonds by a Cobalt Chelate

Sir:

Metal bearing enzymes such as leucine amino peptidase[1] catalyze the hydrolysis of N-terminal peptide bonds through a process involving chelation between the enzyme, the substrate, and the metal ion. Divalent transition metal cations have also been shown to accelerate the hydrolysis of peptides.[2] Hydroxide gels of highly charged ions such as Ce(IV) and La(III) are

(1) E. L. Smith and R. L. Hill in "The Enzymes," Vol. 4, edited by P. D. Boyer, H. Lardy, and K. Myrback, Academic Press, New York, N. Y., 1960, p. 37.
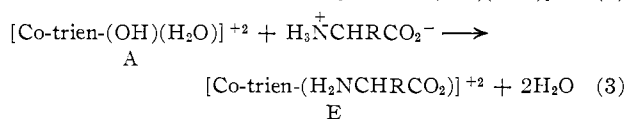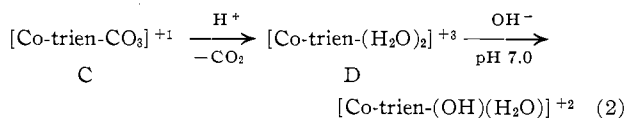(2) L. Meriwether and F. H. Westheimer, *J. Am. Chem. Soc.*, **78**, 5119 (1956).

even more effective in hydrolyzing peptide bonds,[3] but such hydrolyses[2,3] apparently take place in an indiscriminate manner.

We wish to report the selective N-terminal hydrolysis of simple peptides by *cis*-hydroxyaquotriethylenetetraminecobalt(III) ions (A). The N-terminal amino acid residue is selectively hydrolyzed and simultaneously converted into an inert metal complex, B.[4] The reaction (1) takes place rapidly in aqueous solution at 65° and pH 7–8. Although stoichiometric rather than catalytic, this process is perhaps the best model to date for the *in vitro* action of *exo*metal peptidases. The reaction may prove useful as a method of sequential peptide analysis and for stepwise degradation of natural peptides.

$$[\text{Co-trien-(OH)}(H_2O)]^{+2} + H_2NCHRCNHP \longrightarrow$$
$$\text{A}$$
$$[\text{Co-trien-}(H_2NCHRCO_2) \quad (1)$$
$$\text{B}$$
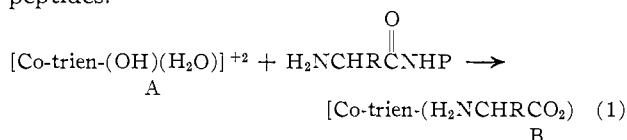
The chelate A was generated *in situ* by decomposing the carbonate,[5] C, in dilute acid and then adjusting the pH to 7.5. The diaquo chelate D acts as a dibasic acid, passing to the hydroxyaquo and dihydroxy forms as the pH is raised.[6] The glycine and phenylalanine chelates (E, R = H and $C_6H_5CH_2$) required for these studies were prepared, from A (eq. 3) and characterized as their chlorides by elemental analyses, infrared spectra, n.m.r. spectra, and paper chromatography.

$$[\text{Co-trien-CO}_3]^{+1} \xrightarrow[-CO_2]{H^+} [\text{Co-trien-}(H_2O)_2]^{+3} \xrightarrow[pH\ 7.0]{OH^-}$$
$$\text{C} \qquad\qquad\qquad \text{D}$$
$$[\text{Co-trien-(OH)}(H_2O)]^{+2} \quad (2)$$

$$[\text{Co-trien-(OH)}(H_2O)]^{+2} + H_3\overset{+}{N}CHRCO_2^- \longrightarrow$$
$$\text{A}$$
$$[\text{Co-trien-}(H_2NCHRCO_2)]^{+2} + 2H_2O \quad (3)$$
$$\text{E}$$

Typical examples of peptide hydrolyses are outlined below (eq. 4–9). In each case the products were analyzed by paper chromatography using known compounds as internal standards. In all of the reactions the product complex ions were isolated by column chromatography on cellulose and characterized by their infrared spectra and paper chromatograms. The course of the reactions was followed spectrophotometrically and minimum times required for completion are indicated. The yields were essentially quantitative.

The N-terminal specificity of this process is clearly demonstrated by the results of reactions 7–9. The requirement for a free N-terminal amino group is illustrated by the failure of reagent A to hydrolyze N-carbobenzoxyglycylphenylalanylamide, N-carbobenzoxyglycylphenylalanylamide, and 3-benzyl-2,5-diketopiperazine.
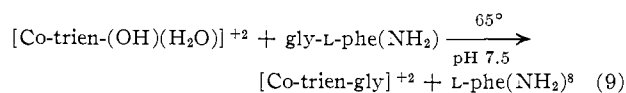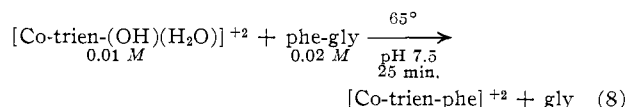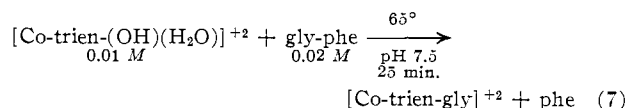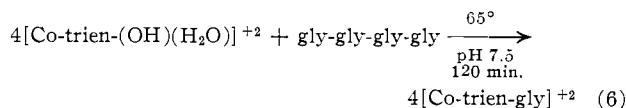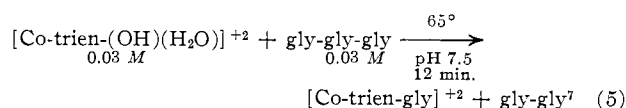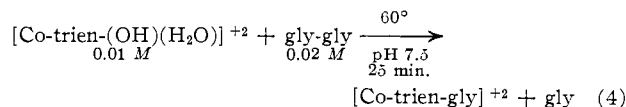
Temperature and pH sharply affect the rate of cleavage. Thus, at 65° reaction 7 required >2 hr. at pH 6 and 12 min. at pH 9. At 45° and pH 7.5 the reaction was complete in 3 hr. These rates are much

(3) E. Bauman, J. G. Hass, and H. Trapmann, *Arch. Pharm.*, **294**, 569 (1961); E. Bauman, A. Rother, and H. Trapmann, *Naturwiss.*, **43**, 326 (1956); E. Bauman, H. Trapmann, and A. Rother, *Chem. Ber.*, **91**, 1744 (1958).

(4) The following abbreviations are used: gly = glycine; phe = *d,l*-phenylalanine; L-phe(NH₂) = L-phenylalanineamide; gly-phe = glycyl-*d,l*-phenylalanine; trien = triethylenetettramine; P = a peptide chain. Amino acid groups in brackets are coordinated anions. It is not certain whether the tetradentate trien assumes α or β *cis* forms.

(5) A. Sargeson and G. H. Searle, private communication; R. D. Gilland and G. Wilkinson, *J. Chem. Soc.*, 3193 (1963).

(6) The situation here is very similar to the analogous bisethylenediamine complex: J. Bjerrum and S. E. Rasmussen, *Acta Chem. Scand.*, **6**, 1265 (1952).

$$[\text{Co-trien-(OH)}(H_2O)]^{+2} + \underset{0.01\ M}{\text{gly-gly}} \xrightarrow[\underset{25\ min.}{pH\ 7.5}]{60°}$$
$$[\text{Co-trien-gly}]^{+2} + \text{gly} \quad (4)$$

$$[\text{Co-trien-(OH)}(H_2O)]^{+2} + \underset{0.03\ M}{\text{gly-gly-gly}} \xrightarrow[\underset{12\ min.}{pH\ 7.5}]{65°}$$
$$[\text{Co-trien-gly}]^{+2} + \text{gly-gly}^7 \quad (5)$$

$$4[\text{Co-trien-(OH)}(H_2O)]^{+2} + \text{gly-gly-gly-gly} \xrightarrow[\underset{120\ min.}{pH\ 7.5}]{65°}$$
$$4[\text{Co-trien-gly}]^{+2} \quad (6)$$

$$[\text{Co-trien-(OH)}(H_2O)]^{+2} + \underset{0.01\ M}{\text{gly-phe}} \xrightarrow[\underset{25\ min.}{pH\ 7.5}]{65°}$$
$$[\text{Co-trien-gly}]^{+2} + \text{phe} \quad (7)$$

$$[\text{Co-trien-(OH)}(H_2O)]^{+2} + \underset{0.01\ M}{\text{phe-gly}} \xrightarrow[\underset{25\ min.}{pH\ 7.5}]{65°}$$
$$[\text{Co-trien-phe}]^{+2} + \text{gly} \quad (8)$$

$$[\text{Co-trien-(OH)}(H_2O)]^{+2} + \text{gly-L-phe}(NH_2) \xrightarrow[pH\ 7.5]{65°}$$
$$[\text{Co-trien-gly}]^{+2} + \text{L-phe}(NH_2)^8 \quad (9)$$

faster than those reported for divalent cations and at least as fast as the hydroxide gel reactions, although these are not directly comparable because of differences in substrates.

Since the stereochemistry and acid–base character of the hydrolytic chelate and chelate products are defined, a reasonable mechanism can be assigned to this process. There would seem to be two limiting cases. After the amino group combined with the cobalt ion by displacement of a water molecule, (a) either the adjacent coordinated hydroxyl group attacks the peptide carbonyl group through a five-ring intermediate or (b) the carbonyl becomes activated to attack by external hydroxide through prior coordination of the carbonyl oxygen with the cobalt atom. In the former mechanism the complex ion acts both as a template and a buffered source of hydroxide.

Work in progress is designed to evaluate the scope, mechanism, and utility of this reaction.

(7) Trace amounts of gly-gly-gly and gly were detected in the paper chromatogram.

(8) In another experiment using an 0.02 *M* of the reagent A the optically active L-phenylalanine chelate was also isolated.

(9) Alfred P. Sloan Foundation Fellow.

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RECEIVED JULY 22, 1963

Phenol Oxidation. IV.[1]
Simulation of the Biosynthesis of Colchicine by a Radical-Pairing Reaction of the Tropolone Ring

Sir:

Current speculation[2] regarding the biogenesis of the colchicine alkaloids awaits evaluation by tracer studies employing substrates of requisite complexity. We

(1) Part III: A. I. Scott and C. T. Bedford, *J. Am. Chem. Soc.*, **84**, 2271 (1962); parts I and II: A. I. Scott, *et al.*, *J. Chem. Soc.*, 4756 (1961).