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.

$$\underset{\text{A}}{[\text{Co-trien-(OH)(H}_2\text{O)}]^{+2}} + \text{H}_2\text{NCHRC(=O)NHP} \longrightarrow \underset{\text{B}}{[\text{Co-trien-(H}_2\text{NCHRCO}_2)} \quad (1)$$

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.

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

$$\underset{\text{A}}{[\text{Co-trien-(OH)(H}_2\text{O)}]^{+2}} + \text{H}_3\overset{+}{\text{N}}\text{CHRCO}_2^- \longrightarrow \underset{\text{E}}{[\text{Co-trien-(H}_2\text{NCHRCO}_2)]^{+2}} + 2\text{H}_2\text{O} \quad (3)$$

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 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.

$$\underset{0.01\ M}{[\text{Co-trien-(OH)(H}_2\text{O)}]^{+2}} + \underset{0.02\ M}{\text{gly-gly}} \xrightarrow[\text{pH 7.5, 25 min.}]{60^\circ} [\text{Co-trien-gly}]^{+2} + \text{gly} \quad (4)$$

$$\underset{0.03\ M}{[\text{Co-trien-(OH)(H}_2\text{O)}]^{+2}} + \underset{0.03\ M}{\text{gly-gly-gly}} \xrightarrow[\text{pH 7.5, 12 min.}]{65^\circ} [\text{Co-trien-gly}]^{+2} + \text{gly-gly}^7 \quad (5)$$

$$4[\text{Co-trien-(OH)(H}_2\text{O)}]^{+2} + \text{gly-gly-gly-gly} \xrightarrow[\text{pH 7.5, 120 min.}]{65^\circ} 4[\text{Co-trien-gly}]^{+2} \quad (6)$$

$$\underset{0.01\ M}{[\text{Co-trien-(OH)(H}_2\text{O)}]^{+2}} + \underset{0.02\ M}{\text{gly-phe}} \xrightarrow[\text{pH 7.5, 25 min.}]{65^\circ} [\text{Co-trien-gly}]^{+2} + \text{phe} \quad (7)$$

$$\underset{0.01\ M}{[\text{Co-trien-(OH)(H}_2\text{O)}]^{+2}} + \underset{0.02\ M}{\text{phe-gly}} \xrightarrow[\text{pH 7.5, 25 min.}]{65^\circ} [\text{Co-trien-phe}]^{+2} + \text{gly} \quad (8)$$

$$[\text{Co-trien-(OH)(H}_2\text{O)}]^{+2} + \text{gly-L-phe(NH}_2) \xrightarrow[\text{pH 7.5}]{65^\circ} [\text{Co-trien-gly}]^{+2} + \text{L-phe(NH}_2)^8 \quad (9)$$

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.

Acknowledgment.—We are indebted to the National Institutes of Health for support of this work under the grant GM 08350-03.

(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(NH$_2$) = 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).

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

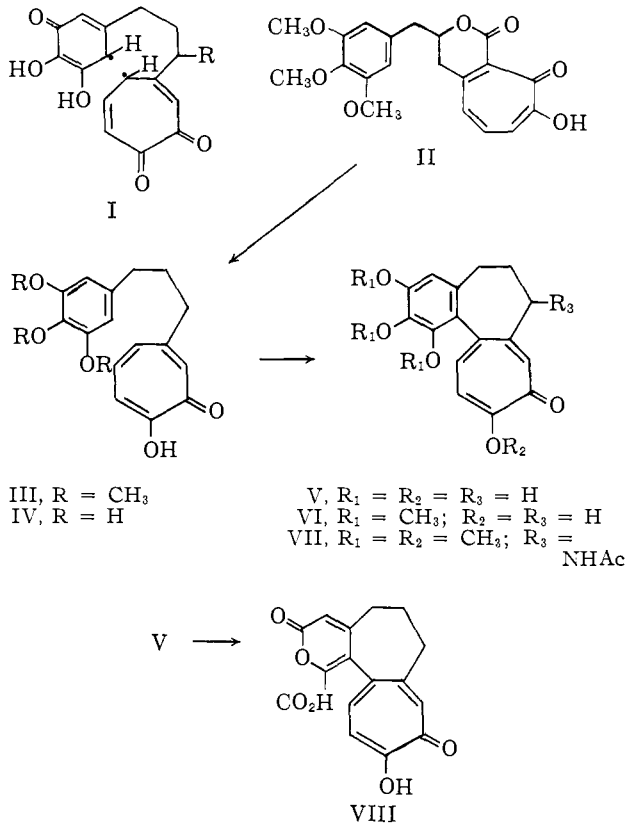
Sir:

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).

now illustrate our earlier hypothesis,[5] in which participation of the tropolone radical (as I) is invoked, by a convenient four-step total synthesis of the colchicine framework as shown in Chart I.

CHART I



III, R = CH_3
IV, R = H

V, $R_1 = R_2 = R_3 = H$
VI, $R_1 = CH_3$; $R_2 = R_3 = H$
VII, $R_1 = R_2 = CH_3$; R_3 = NHAc

VIII

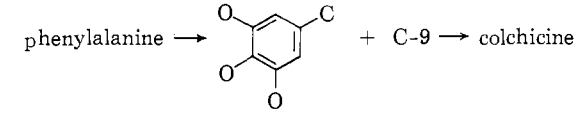
Condensation of 3,4,5-trimethoxyphenylacetaldehyde[6] with the anhydride of 3-carboxy-4-carboxymethyltropolone[7] at 95° afforded the lactone II, m.p. 172°; ν^{CHCl_3} 1720, 1615 cm.$^{-1}$; λ_{max}^{EtOH} 255 (ϵ 20,000), 332 (ϵ 5000), and 377 mμ (ϵ 7000) in 78% yield.[8] Decarboxylation of II with copper bronze at 260° (10^{-2} mm.) now gave β-(3,4,5-trimethoxyphenyl)prop-2-enyltropolone[10] (70%), m.p. 115°; λ_{max}^{EtOH} 270 (ϵ 23,000), 330 (ϵ 5000), 380 mμ (ϵ 7000). Controlled reduction (10% Pd–C; EtOAc) of the olefinic linkage led quantitatively to III, m.p. 113–115°; λ_{max}^{EtOH} 245 (ϵ 15,000), 320 (ϵ 10,000), 350 mμ (ϵ 5000). Demethylation of III (HBr, 48%; 30-min. reflux) yielded the pyrogallol IV (50%), m.p. 128–132°. Control of oxidation of IV was maintained by ascending paper chromatography [Whatman No. 1 paper; HOAc–HCl–H_2O (20:10:1)]. In this system IV had R_f 0.81 (carmine fluorescence). After 2 hr. at 10° a solution of IV in sodium iodate (5 *M*; pH 6) showed a single identifiable spot (R_f 0.75) corresponding in color (yellow-green nonfluorescent) and R_f (0.75) to V, m.p. 238°, λ_{max} 242 (ϵ 17,000) and 361 mμ (ϵ 12,000), prepared by demethylation (HBr, 48%) of desacetylaminocolchiceine[11] (VI).

For isolation, advantage was taken of the facile conversion (0.1 *M*; $NaHCO_3$–$K_3Fe(CN)_6$; 90 sec.; 50%) of V to the pyrone VIII, m.p. 288°, λ_{max}^{EtOH} 233 (ϵ 12,000) and 365 mμ (ϵ 9000). Oxidation of IV with the latter reagent (1 hr.) now gave VIII (10%), identical in every respect (m.m.p., mass, infrared, n.m.r. spectra) with the material from V. Methylation of V (MeI–K_2CO_3–acetone; 3 hr.) gave back VI which has been converted to colchicine (VII).[11,12] Since prolonged treatment (1 hr.) of VIII with alkaline ferricyanide leads to considerable degradation, the radical pairing reaction (I; R = H) must proceed in at least 20% yield.[13]

The availability of the various substrates described in this synthesis sets the stage for transfer of laboratory analogy to biological experiment.

Acknowledgments.—We are grateful to Glaxo Laboratories Ltd. for generous financial support and to Miss P. Mackenzie and P. Tremaine for skilled technical assistance. J. N. thanks N.R.C. for a fellowship.

(2) For a comprehensive summary see K. Mothes, *Angew. Chem.*, **57**, 357 (1963). The results of administering labeled C-1–C-9 units may be portrayed[3] as



The "missing" C-9 unit appears to be derived from tyrosine.[4]

(3) A. R. Battersby, *Quart. Rev.* (London), **17**, 259 (1961).

(4) E. Leete, IUPAC Meeting, London, 1963, Abstracts A, p. 284.

(5) A. I. Scott, *Nature*, **186**, 556 (1960).

(6) Prepared most conveniently by Rosenmund reduction of the corresponding acid chloride in 90% yield.

(7) R. D. Haworth and J. D. Hobson, *J. Chem. Soc.*, 561 (1951).

(8) *Cf.* T. Nozoe, *Proc. Japan Acad.*, **29**, 203 (1953), for a preparation of the phenyl analog. For the reported failure of this condensation in the case of substituted phenylacetaldehydes see ref. 9.

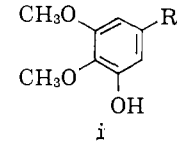
(9) T. Nozoe, "Nonbenzenoid Aromatic Compounds," D. Ginsberg, Ed., Interscience Publishers, Inc., New York, N. Y., 1959, p. 339.

(10) This and all subsequent new compounds showed requisite mass, infrared, and n.m.r. spectra, which will be detailed in our full publication.

(11) J. Schreiber, W. Leimgruber, M. Pesaro, P. Schudel, T. Threlfall and A. Eschenmoser, *Helv. Chim. Acta*, **44**, 540 (1961).

(12) E. E. van Tamelen, T. A. Spenser, D. S. Allen, and R. L. Orvis, *Tetrahedron*, **14**, 8 (1961).

(13) Other pathways involving protected (but both less reactive and biologically relevant) pyrogallols (as i) have been investigated and will be reported elsewhere.



i

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Cycloheptatrienide (Tropenide) Anion

Sir:

The unsuccessful attempts[1] to prepare cycloheptatrienide (tropenide) anion, $C_7H_7^-$, usually have been ascribed[2] to unfavorable properties predicted for it by the Hückel molecular orbital method (8 π-electrons, two of which are unpaired and occupy a pair of doubly-degenerate, antibonding molecular orbitals; relatively low *DE* ($C_7H_7^+$, 2.99 > $C_7H_7\cdot$, 2.54 > $C_7H_7^-$, 2.10 β)).[2a,c]

(1) (a) J. Thiele, *Ann.*, **319**, 226 (1900); (b) G. Wittig and E. Hahn, *Angew. Chem.*, **72**, 918 (1960); *Cf.* K. Hafner and W. Rellensman, *ibid.*, **72**, 781 (1960).

(2) (a) E. Hückel, *Z. Physik*, **70**, 204 (1931); **76**, 628 (1932); *Z. Elektrochem.*, **41**, 752 (1937); (b) G. W. Wheland, *J. Chem. Phys.*, **2**, 474 (1960); (c) J. D. Roberts, A. Streitwieser, Jr., and Clare M. Regan, *J. Am. Chem. Soc.*, **74**, 4579 (1952); (d) A. Streitwieser, Jr., *Tetrahedron Letters*, 23 (1960).