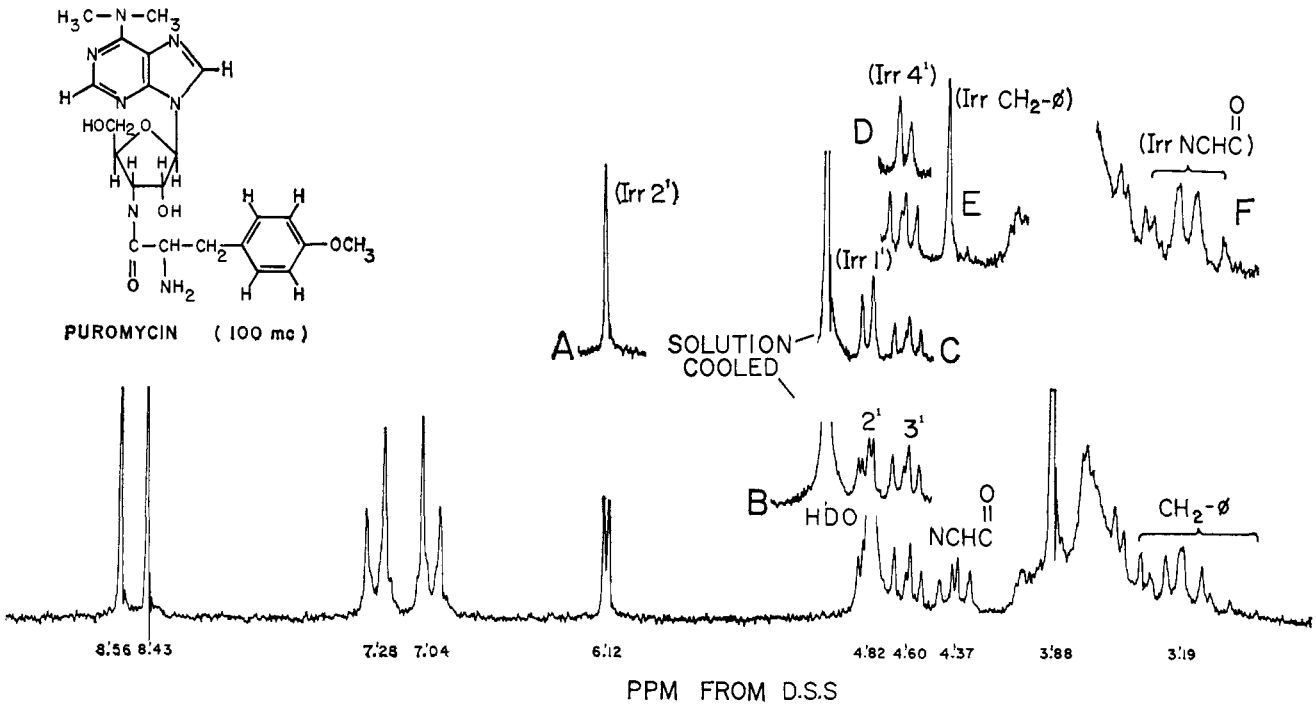

Figure 1.

signals in a different manner. Our interpretation of these new data does not require a folded conformation having the benzene ring over the $C_5'H_2$ group.

Figure 1 shows the 100-Mc. spectrum, including spin-decoupling data, of a 45-mg. sample of puromycin dihydrochloride[3] dissolved in 0.5 ml. of D_2O. Chemical shifts are measured relative to the methyl peak of 1.5 mg. of 2,2-dimethyl-2-silapentanesulfonic acid sodium salt[4,5] (DSS) added as an internal reference.

As in the earlier paper, the C_1'–H is assigned to the 2.5-c.p.s. doublet seen 6.12 p.p.m. from DSS. This doublet could be made to collapse to a singlet by simultaneously irradiating with a relatively large side-band component of the radiofrequency field 129 c.p.s. upfield from the C_1'–H resonance (see Fig. 1, insert A). This confirms the C_2'–H signal as the pair of doublets at 4.82 p.p.m. (see insert B where the otherwise interfering HDO resonance has been shifted toward lower field by cooling the solution slightly). Similarly, the 2.5 c.p.s. splitting in the C_2'–H pattern can be removed by irradiating at the C_1'–H resonant frequency while observing the C_2'–H signals (see insert C). The obvious tilt in the remaining doublet pattern suggests that the spin-coupled C_3'–H chemical shift is nearby. This is verified by removing one of the doublings in the adjacent pattern, 4.60 p.p.m., through another double resonance experiment (see insert D). Thus, inserts C and D show that the patterns at 4.82 and 4.60 p.p.m. have a mutual spin coupling of 5.5 c.p.s., and therefore C_3'–H must be assigned to the pattern at 4.60 p.p.m. The irradiating frequency which produced the pattern of insert D is 58 c.p.s. toward higher field, thus establishing the C_4'–H chemical shift at 4.02 p.p.m. The four-line pattern centered around 4.37 p.p.m. must therefore be due to phenylalanine α-CH proton split twice by spin coupling to the adjacent β-CH_2 group. The average chemical shift of these adjacent CH_2 protons, 3.19 p.p.m., is measured by determining the frequency of the large irradiating field which causes the CH pattern at 4.37 p.p.m. to collapse to a single peak (see insert E). Likewise, observation of the CH_2 signals, while irradiating the pattern of signals at 4.37 p.p.m., reveals the perturbed pair of doublets shown in insert F. The slight nonequivalence of these CH_2 protons is due to asymmetric substitution on the adjacent carbon atom.

This new assignment of the n.m.r. spectral data of puromycin means that the C_5'–H_2 signals must lie between 3.5 and 4.0 p.p.m., a value not unlike that found in other purine ribosides.[6] In addition, in phenylalanine the average of the CH_2 chemical shifts, 3.19 p.p.m., is in accord with that of other similar benzylic CH_2 groups.[7]

The folded conformation proposed by Jardetzky accounted for an apparently unusual $C_5'H_2$ chemical shift arising from magnetic anisotropy of the benzene ring. Having reassigned the spectrum as discussed above, the folded form is not necessary and in all probability the phenylalanine moiety exists as an ordinary extended chain.

(3) Obtained from Nutrition Biochemicals, Cleveland 28, Ohio.

(4) G. V. D. Tiers and R. I. Cook, *J. Org. Chem.*, **26**, 2097 (1961).

(5) Obtained from Eastman Organic Chemicals, Distillation Products Industries, Rochester 3, New York.

(6) Unpublished data, see also references given in footnote 1.

(7) For example, the average β-CH_2 shift in *p*-hydroxyphenylalanine in D_2O was measured to be 2.98 p.p.m. from DSS.

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RECEIVED AUGUST 19, 1963

The Cleavage of Tyrosyl–Peptide Bonds by Electrolytic Oxidation

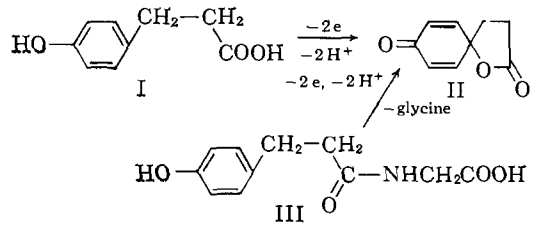
Sir:

Efforts to increase selectivity in the nonenzymatic cleavage of tyrosyl–peptide bonds[1] have stimulated an exploration of techniques such as electrolytic oxidation. At a platinum anode, phloretic acid (I) is converted into its dienone lactone (II) in 20% yield.[2] Under somewhat modified conditions, phloretylglycine (III) is cleaved to II and glycine to an extent of 30–50%.

The electrolysis is effected at 25° with catholyte and anolyte in separate compartments connected by an agar

(1) J. G. Wilson and L. A. Cohen, *J. Am. Chem. Soc.*, **85**, 564 (1963).

(2) A. I. Scott, P. A. Dodson, F. McCapra, and M. B. Meyers, *ibid.*, **85**, 3702 (1963). We are indebted to Prof. Scott for advising us of his findings prior to publication.



bridge. A supporting electrolyte of triethylammonium acetate–trifluoroacetate (0.4 *M*, pH 2.2) permitted a current flow of 1–2 ma. per cm.^{2} at a potential of 50 volts. While peptides such as alanylleucine were unaltered by these conditions,[3] alanine was released from N-benzoyl-3-nitrotyrosylalanine in a recovered yield of 25% after 4 hr. of electrolysis. Since the ultraviolet spectrum of the mixture showed only end absorption by that time, it was evident that electrolytic destruction of alanine had occurred competitively with peptide cleavage (Fig. 1). Addition of Dowex 50 resin to the electrolysis mixture (10% acetic acid as electrolyte) raised the recovered yield of alanine to 35% by removing it selectively from solution.

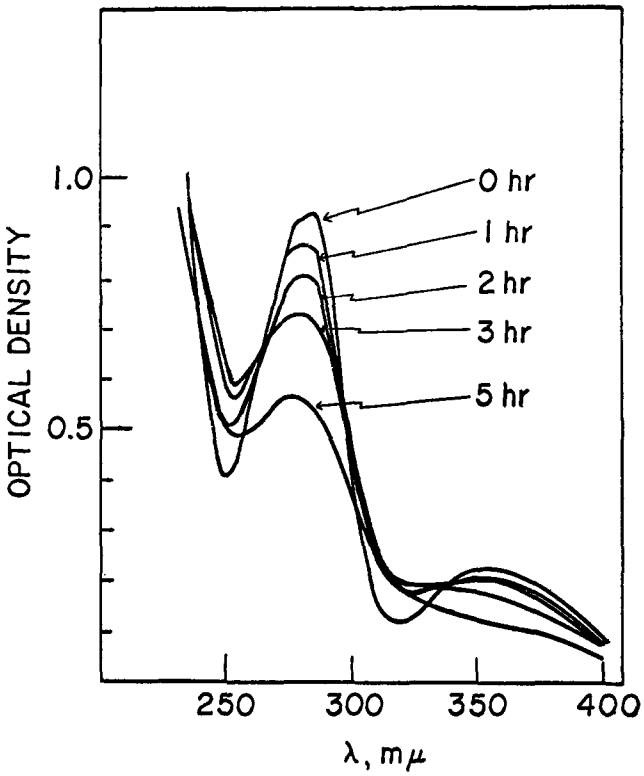


Fig. 2.—Changes in the ultraviolet spectrum of rufomycin A following electrolysis.

Although N-bromosuccinimide has been shown to cleave tryptophyl–peptide bonds in preference to those of tyrosine,[4] electrolytic oxidation has no discernible effect on tryptophyl peptides under the conditions cited above. The specificity was demonstrated by electrolysis of the cyclic polypeptide, rufomycin A, which contains one residue of 3-nitrotyrosine and one of tryptophan, as well as one each of alanine, leucine, N-methylleucine, 2-amino-4-hexenoic acid, and N,4-dimethyl-

(3) Electrolysis under more vigorous conditions leads to Kolbe decarboxylation and other degradations. See R. P. Linstead, B. R. Shepherd, and B. C. L. Weedon, *J. Chem. Soc.*, 2854 (1951); R. A. Boissonnas, *Nature*, **171**, 304 (1953); A. R. Thompson, *Biochim. Biophys. Acta*, **15**, 299 (1954).

(4) B. Witkop, "Advances in Protein Chemistry," C. B. Anfinsen, M. L. Anson, K. Bailey, and J. T. Edsall, Ed., Vol. 16, Academic Press, New York, N. Y., 1961, p. 272.

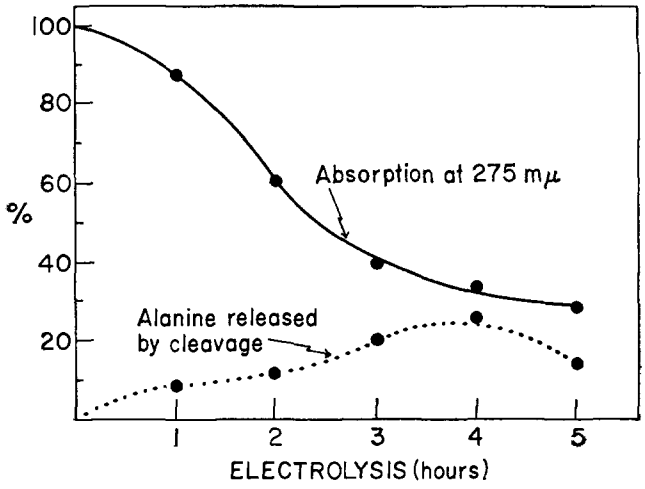


Fig. 1.—Relation between ultraviolet absorption and alanine released in the electrolysis of N-benzoyl-3-nitrotyrosylalanine.

glutamic-γ-semialdehyde.[5] As shown in Fig. 2, the phenolic bands at 275 and at 350 mμ disappear gradually over 4–5 hr., the residual spectrum being due, primarily, to the intact tryptophan chromophore. The cyclic polypeptide is cleaved to a single open-chain polypeptide with alanine as N-terminal. The cleavage yield, based on trinitrophenylation of the peptide,[6] was 38%; the recovery of DNP-alanine, following acid hydrolysis of the DNP-peptide, was 8%.[7]

The nitrodienone analog of II is not observed in the ultraviolet spectrum following cleavage; the possibility that it is destroyed due to unusually high reactivity is being investigated. Also in progress are efforts to improve reaction conditions and yields in the specific electrolytic cleavage reaction.

(5) J. Ueyanagi, H. Iwasaki, M. Fujino, T. Kamiya, A. Miyake, and S. Tatsuoka, presented before the Organic Division, 142nd National Meeting of the American Chemical Society, Atlantic City, N. J., September, 1962, Abstracts, p. 10.

(6) K. Satake, T. Okuyama, M. Ohashi, and T. Shinoda, *J. Biochem.* (Tokyo), **47**, 654 (1960).

(7) Small amounts of DNP-leucine, probably arising from contamination of rufomycin by other polypeptides, were also found.

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RECEIVED SEPTEMBER 19, 1963

Electrooxidation of Tyrosyl Derivatives: A Model for Coumarin Biosynthesis

Sir:

Diverse proposals regarding the mechanism of formation of 7-hydroxycoumarins *in vivo* from C_6–C_3 precursors have been made.[1–5] The observation that both oxygen atoms of the carboxyl group of tyrosine are utilized in lactone formation in novobiocin can be interpreted either in terms of *direct* oxidative attachment of carboxyl ion (or radical) *meta* to the phenolic hydroxyl group,[3,4] or indirectly *via* spirolactone formation[5] (*cf.* **1**) followed by rearrangement (as **1** → **2** → **3**). Analogy for the direct *meta* reaction was based on the oxidative cyclization of 4,4′-dimethoxydiphenyl-2-carboxylic acid to the corresponding benzocoumarin[6] where, however,

(1) R. D. Haworth, *J. Chem. Soc.*, 448 (1942).

(2) F. Weygand and H. Wendt, *Z. Naturforsch.*, **14b**, 421 (1959).

(3) K. Chambers, G. W. Kenner, M. J. T. Robinson, and B. R. Webster, *Proc. Chem. Soc.*, 291 (1960).

(4) C. A. Bunton, K. Chambers, G. W. Kenner, M. J. T. Robinson, and B. R. Webster, *Tetrahedron*, **19**, 1001 (1963).

(5) W. D. Ollis and H. Grisebach, *Experientia*, **17**, 4 (1961).

(6) G. W. Kenner, M. A. Murray, and C. B. Tylor, *Tetrahedron*, **1**, 259 (1957).