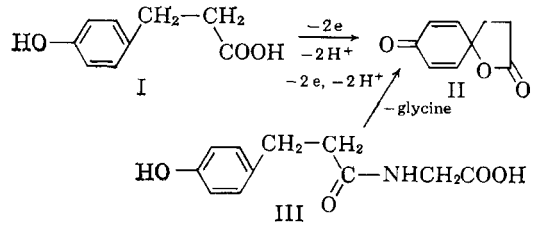


bridge. A supporting electrolyte of triethylammonium acetate–trifluoroacetate (0.4 *M*, pH 2.2) permitted a current flow of 1–2 ma. per cm.^{2} at a potential of 50 volts. While peptides such as alanylleucine were unaltered by these conditions,[3] alanine was released from N-benzoyl-3-nitrotyrosylalanine 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.

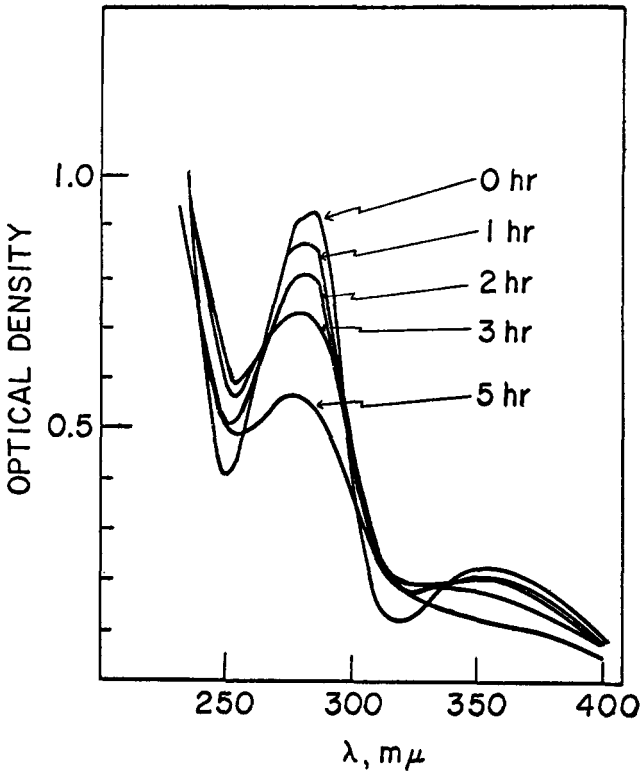


Fig. 2.—Changes in the ultraviolet spectrum of rufomycin A following electrolysis.

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.

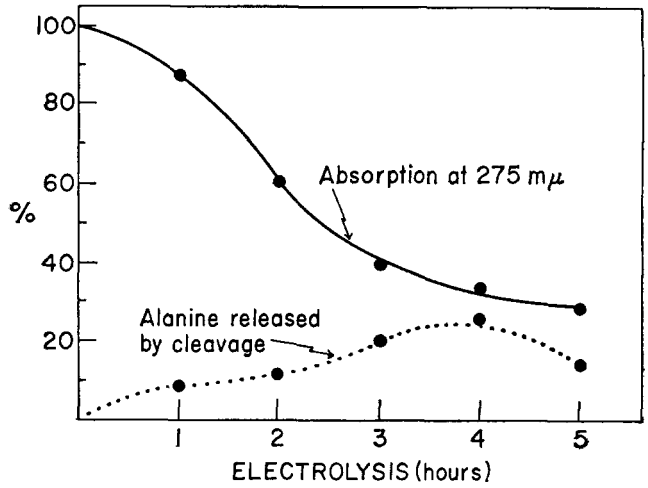


Fig. 1.—Relation between ultraviolet absorption and alanine released in the electrolysis of N-benzoyl-3-nitrotyrosylalanine.

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.

NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES
NATIONAL INSTITUTES OF HEALTH
BETHESDA 14, MARYLAND
H. IWASAKI
L. A. COHEN
B. WITKOP

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).

the presence of a second aromatic ring allows alternative mechanistic interpretation.

We now report experiments bearing directly on the mechanism of oxidative cyclization of simpler C_6–C_3 phenolic acids, which support our view[7] that such oxidations follow the general pathway of *para* substitution. Subsequent rearrangement can then be demonstrated to give the *gross* result of *meta* substitution.

Thus, electrooxidation (see Table I for conditions) at

TABLE I
OXIDATION OF TYROSYL DERIVATIVES

Substrate	Method	Product(s)[c]	Yield, %
4	E[a]	**5**	20
Methyl ester of **4**	E	No reaction	
4a	E	**5a**	15
p-Hydroxy-*cis*-cinnamic acid	E	**8**	5
11	[b]NBS buffer	**13**	40
		14	15

[a] E: Electrolysis of 0.1 % aqueous solution at a smooth Pt anode, 70 v., 60°, cellophane cathodic membrane. [b] N-Bromosuccinimide buffer, see ref. 10. [c] Satisfactory analyses obtained for all new compounds.

a platinum anode of *p*-hydroxyphenylpropanoic acids leads to products corresponding to pairing of the derived phenol and carboxyl radicals. For example, phloretic acid (**4**) affords the dienone lactone (**5**), m.p. 106°; λ_{max}^{EtOH} 226 mμ (ε 17,600), 300 (ε 200); ν^{CHCl_3} 1760, 1680, 1620 cm.$^{-1}$; mol. wt. 164[8] in 20% yield. Using carboxyl-labeled **4** (25.2%^{18}O) the derived lactone (**5**) contained 24.3% of the heavy isotope,[8] a result in accord with the intramolecular mechanism and consistent with *in vivo* experiments on novobiocin.[3,4,8a]

Rearrangement of **5** to a mixture of 6- and 7-hydroxydihydrocoumarins (**6**, **7**) could be controlled to give either known coumarin predominantly (Table II).

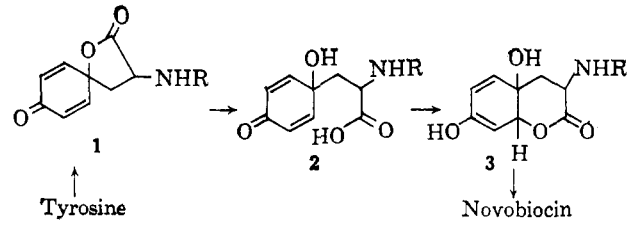
TABLE II
REARRANGEMENTS OF SPIROLACTONES IN REFLUXING ACID[a]

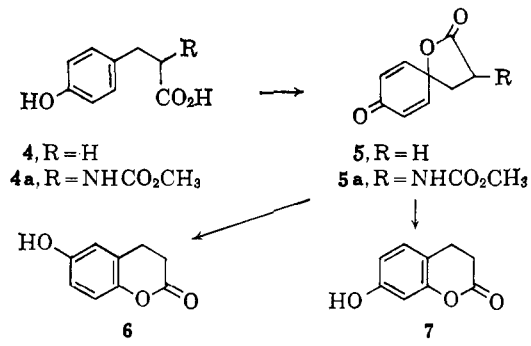
Lactone	Medium	Coumarin, % 7-OH	Coumarin, % 6-OH
5	1 *N* H_2SO_4	5	95
5	HOAc–H_2SO_4	90	10
8	6 *N* H_2SO_4	4	96
13	9 *N* H_2SO_4/CH_3CN	90	10

[a] Mixtures were analyzed by ultraviolet spectroscopy and preparative t.l.c. on silica gel [solvent, EtOAc–$CHCl_3$ (1:4)].

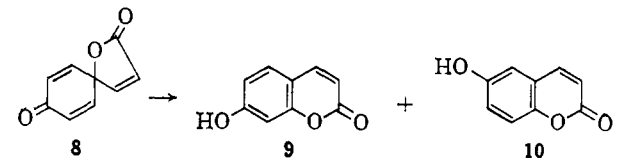
Still more compelling analogies for formation of natural coumarins by a cognate mechanism were observed in the electrooxidation of (1) N-carbomethoxytyrosine (**4a**) to the spirodienone[9] (**5a**) and (2) *p*-hydroxy-*cis*-cinnamic acid to the unsaturated spirolactone (**8**), m.p. 116°, λ_{max} 216 mμ (ε 22,000), λ_{infl} 223 mμ (ε 9000), ν^{CHCl_3} 1760, 1670, 1620 cm.$^{-1}$, mol. wt. 162. Rearrangement of the latter with mineral acid gave a separable mixture of umbelliferone (**9**) and 6-hydroxycoumarin (**10**).

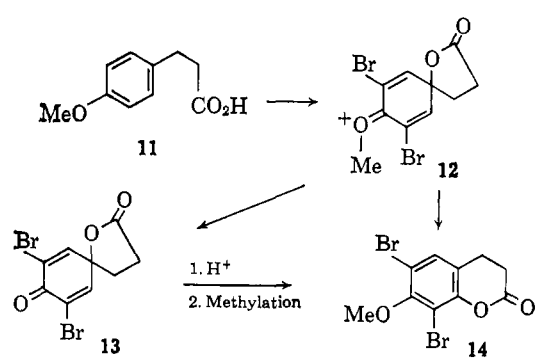
The consistent operation of Ar 1–5 oxidative participation was demonstrable even when the phenolic



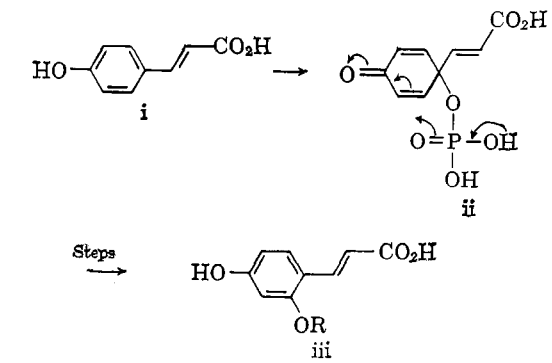


group was protected as its methyl ether. Reaction of *p*-methoxyphenylpropionic acid (**11**) with NBS in buffered (NaOAc–CH_3CN) solution[10] gave a mixture of the known spirodienone[10] (**13**) and **14** by way of intermediate **12**, of lifetime sufficient to generate **13** by hydrolysis.[11]





The mechanism of 7-hydroxycoumarin biosynthesis consistent with the observed feeding experiments[3,4,12] and with laboratory oxidations may now be summarized[13] as



(7) A. I. Scott, *Proc. Chem. Soc.*, 207 (1962). Some aspects of this work were reported at the Anniversary meeting of the Chemical Society, Sheffield, 1962.

(8) Mass spectrometric determination (Metropolitan-Vickers MS9 instrument). The technique will be described in a forthcoming paper (A. I. Scott, R. I. Reed, M. B. Meyers, and E. Clayton, in preparation).

(8a) NOTE ADDED IN PROOF.—A recent report by J. S. Davies, C. H. Hassall, and J. A. Schofield, *Chem. Ind.* (London), 740 (1963), describes an alternative preparation of **5**. A new synthesis of umbelliferone (**9**) [M. B. Meyers *Proc. Chem. Soc.*, 243 (1963)] although irrelevant to 7-hydroxycoumarin biosynthesis (ref. 4) indicates the low (0.1%) *meta* reactivity of these systems.

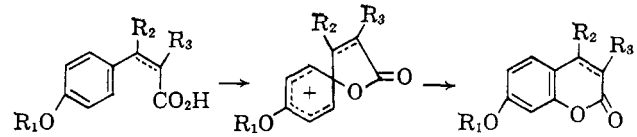
(9) This method holds promise as a selective procedure for determination of tyrosyl peptides since the conditions are relatively mild and reaction is carried out in aqueous solution: H. Iwasaki, L. A. Cohen, and B. Witkop, *J. Am. Chem. Soc.*, **85**, 3701 (1963).

(10) G. Schmir, L. A. Cohen, and B. Witkop, *ibid.*, **81**, 2228 (1959).

(11) *Cf.* R. Baird and S. Winstein, *ibid.*, **84**, 788 (1962). The conditions used were shown to effect no demethylation of phenyl ethers.

(12) S. A. Brown, *Science*, **137**, 977 (1962), and references cited therein.

(13) In the case of oxidation of *trans*-*p*-hydroxycinnamic acids (see ref. 12) a rather different mechanism must operate *in vivo*. We offer i ⟶ iii as a possible route, now under study.



Studies on the mechanism of these rearrangements are in hand.

Acknowledgment.—We are indebted to Dr. R. I. Reed and his colleagues for mass spectra and to Lederle Laboratories for generous financial support.

(14) I.C.I. Fellow, from 1961 to the present.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF BRITISH COLUMBIA
VANCOUVER, B. C., CANADA
A. I. SCOTT
P. A. DODSON
F. MCCAPRA

THE UNIVERSITY
GLASGOW, W. 2, SCOTLAND
M. B. MEYERS[14]

RECEIVED JULY 17, 1963

The Reaction of the $B_{20}H_{18}^{-2}$ Ion with Hydroxide Ion

Sir:

A previous report[1] of the reaction of hydroxide ion with the $B_{20}H_{18}^{-2}$ ion[1-3] indicated the initial product to be apically substituted $B_{10}H_9OH^{-2}$ which subsequently rearranged to equatorially substituted $B_{10}H_9OH^{-2}$. We have reinvestigated this reaction and have found compelling evidence for other products.

The reaction of one formula weight of triethylammonium $B_{20}H_{18}^{-2}$ with aqueous potassium hydroxide or anhydrous potassium methoxide in methanol consumed four equivalents of base and liberated two moles of triethylamine. The product of the potassium methoxide reaction (I) exhibited a methoxyl group in its H^1 n.m.r. and infrared spectra. *Anal.* Calcd. for $K_4B_{20}H_{17}OCH_3 \cdot 2H_2O$: B, 47.36; H, 5.29; C, 2.63; mol. wt., 457. Found: B, 47.88; H, 4.98; C, 2.83; mol. wt., 429. The product of the hydroxide ion reaction (II) was characterized as $K_4B_{20}H_{17}OH \cdot 3H_2O$. *Anal.* Calcd: B, 46.95; H_2O, 11.72; K/B, 0.200; mol. wt., 461. Found: B, 46.1; H_2O, 11.15; K/B, 0.198; mol. wt., 455. The salt II was converted on standing in neutral aqueous solution (25°, 12 hr.) to another salt (III) of apparently identical composition. *Anal.* Calcd. for $K_4B_{20}H_{17}OH \cdot 3H_2O$: B, 46.95; H_2O, 11.72; K/B, 0.200; mol. wt., 461. Found: B, 47.1; H_2O, 12.0; K/B, 0.202; mol. wt., 441. The salts II and III were easily distinguished by their characteristic B^{11} n.m.r. and infrared spectra. It is highly probable that the transformation of II to III accounts for the previously reported[1] $B_{10}H_9OH^{-2}$ rearrangement.

Potentiometric titration of I, II, and III with aqueous acid resulted in the consumption of one proton per formula weight. Equivalent weights and pK_a values were: I, 447 (theoretical 457), pK_a 6.1; II, 460 (theoretical 461), pK_a 5.8; III, 461 (theoretical 461), pK_a 5.8.

Protonation of II and III tetramethylammonium salts followed by rapid isolation produced the same tetramethylammonium salt (IV). *Anal.* Calcd. for $B_{20}H_{18}OH \cdot 3(CH_3)_4N$: B, 45.71. Found: B, 45.6. The salt IV gave an identical X-ray powder pattern and infrared spectrum (B–H–B bridge band near 5.60 μ) regardless of its source.

(1) A. Kaczmarczyk, R. D. Dobrott, and W. N. Lipscomb, *Proc. Natl. Acad. Sci. U. S.*, **48**, 729 (1962).

(2) W. N. Lipscomb, *ibid.*, **47**, 1791 (1961), proposed extensions of the valence theory which led to the satisfactory, although unproven, structure of $B_{20}H_{18}^{-2}$ shown in ref. 3. This structure is assumed throughout this discussion.

(3) A. R. Pitochelli, W. N. Lipscomb, and M. F. Hawthorne, *J. Am. Chem. Soc.*, **84**, 3026 (1962).

Oxidation of II or III with hydrogen peroxide, ferric ion, or ceric ion in aqueous acid solution produced the same yellow $B_{20}H_{17}OH^{-2}$ anion (V) in 30–55% yield, λ_{max} 230 mμ (ϵ 12,000) and 300 mμ (ϵ 16,000). *Anal.* Calcd. for $B_{20}H_{17}OH \cdot 2(CH_3)_4N$: B, 54.26. Found: B, 54.0. The B^{11} n.m.r. spectrum of V clearly indicates the presence of equatorial –OH substitution on $B_{20}H_{18}^{-2}$, since the low field (apex) region of the hydroxyl derivative is identical with that found in $B_{20}H_{18}^{-2}$. A small difference is observed in the high field (equatorial) region. The infrared spectrum of V contains a sharp –OH band at 2.80. Similar oxidation of I produced $B_{20}H_{17}OCH_3^{-2}$.

These results suggest that the hydroxide ion cleavage of $B_{20}H_{18}^{-2}$ ion[2,3] results in the cleavage of one B–H–B three-center bond and abstraction of a proton from the second bridge position. The resulting product is a substituted $B_{20}H_{18}^{-4}$ ion[2,4] which gives reactions characteristic of $B_{20}H_{18}^{-4}$.[4]

The kinetics of the hydroxide ion cleavage and certain structural considerations will be presented at a later date.

Acknowledgments.—The research reported in this publication was generously supported by the Advanced Research Projects Agency through Army Research Office (Durham) and the Naval Ordnance Laboratory (Corona). The authors thank Mr. Donald Young for several boron analyses and thermogravimetric water determinations.

(4) M. F. Hawthorne, R. L. Pilling, P. F. Stokely, and P. M. Garrett, *ibid.*, **85**, 3704 (1963).

(5) (a) Alfred P. Sloan Foundation Research Fellow; (b) National Science Foundation Undergraduate Research Assistant, Summer, 1962.

DEPARTMENT OF CHEMISTRY
THE UNIVERSITY OF CALIFORNIA
RIVERSIDE, CALIFORNIA
M. FREDERICK HAWTHORNE[5a]
RICHARD L. PILLING
PETER F. STOKELY[5b]
PHILIP M. GARRETT

RECEIVED AUGUST 21, 1963

The Isolation and Characterization of $B_{20}H_{19}^{-3}$ and $B_{20}H_{18}^{-4}$ Ions

Sir:

The ceric ion oxidation of $B_{10}H_{10}^{-2}$ was previously reported[1] to produce a second isomer of the $B_{20}H_{18}^{-2}$ ion.[2] Reinvestigation of this reaction has shown the product to be a double salt (I), $B_{20}H_{18}^{-2} \cdot B_{20}H_{19}^{-3} \cdot 5(C_2H_5)_3\overset{+}{N}H$, in which the $B_{20}H_{18}^{-2}$ ion is identical with that produced by the ferric ion oxidation[2] of $B_{10}H_{10}^{-2}$.[3] *Anal.* Calcd. for $B_{20}H_{18} \cdot B_{20}H_{19} \cdot 5(C_2H_5)_3NH$: B, 44.12; C, 36.73; H, 12.01; N, 7.14. Found: B, 45.27; C, 37.17; H, 12.02; N, 6.41.

Treatment of I (m.p. 199–200°) with aqueous tetramethylammonium chloride precipitated a white tetramethylammonium salt which was converted by ion exchange to a triethylammonium salt (II), m.p. 163°. *Anal.* Calcd. for $B_{20}H_{19} \cdot 3(C_2H_5)_3NH$: B, 39.93. Found: B, 39.4. Crystallization of a 1:1 mixture of II and authentic[2] triethylammonium $B_{20}H_{18}^{-2}$ from water afforded I in 70% yield. The reaction of one formula weight of II with aqueous potassium hydroxide liberated 2.98 moles of triethylamine and consumed 4.05 moles of hydroxide ion.

$$B_{20}H_{19}^{-3} + 3(C_2H_5)_3\overset{+}{N}H + 4OH^- \longrightarrow B_{20}H_{18}^{-4} + 3(C_2H_5)_3N + 4H_2O$$

(1) A. R. Pitochelli, W. N. Lipscomb, and M. F. Hawthorne, *J. Am. Chem. Soc.*, **84**, 3026 (1962).

(2) A. Kaczmarczyk, R. D. Dobrott, and W. N. Lipscomb, *Proc. Natl Acad. Sci. U. S.*, **48**, 729 (1962)

(3) This same conclusion was independently obtained by A. Kaczmarczyk, private communication, September, 1963.