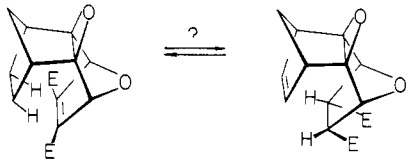
experiment are consistent with a concerted intramolecular mechanism involving the stereospecific 4,5-endo ⇄ 9,10-endo hydrogen double migration. To our knowledge, this is the first case of a thermoneutral [$_{\sigma}2_s + {}_{\sigma}2_s + {}_{\pi}2_s$] dyotropic transfer of hydrogen in a hydrocarbon.

The lack of a driving force is probably compensated by the compressed structures of **1** and **2**. The distance separating C(4) and C(10) is evaluated [assuming dihedral angles of 112° for C(2,1,10) and C(2,3,4) and 120° for C(1,2,3)[6]] to be a short as 1.8 Å. Consequently, the hydrogen transfer **1** ⇄ **2** must be a short motion. The adduct **6** reacted with air or *m*-chloroperbenzoic acid to yield the epoxide **9**.[6,14] When heated in benzene, **9** did not



9 10

rearrange into **10**. At 180 °C, it slowly decomposed. The apparently retarded dyotropic transfer **9** ⇄ **10** might be due to an unexpected lower stability of **10** compared with that of **9**. Another hypothesis is to invoke a higher activation energy for **9** ⇄ **10** than for **1** ⇄ **2** because of the larger C(4)–C(10) distance in **9**, **10** than in **1**, **2** (dihedral angle C(2,3,6,7)–C(1,2,7,8) being larger in **9**, **10** than in **1**, **2**).

Acknowledgment. We are grateful to Hoffmann-La Roche and Co., Basel, the Swiss National Science Foundation (FN. 2'456-0.79), and Fonds Herbette, Lausanne, for generous support.

Supplementary Material Available: Spectral data and elemental combustion analyses of compounds **2** and **7** (3 pages). Ordering information is given on any current masthead page.

(14) In contrast, the diimide reduction of the C(2,7) double bond in **6** was a very slow reaction and did not compete with the cycloreversion **6** → **5** + EC≡CE. See also: Paquette, L. A.; Carr, R. V. C. *J. Am. Chem. Soc.* **1980**, *102*, 7553.

The New Amino Acid β-Carboxyaspartic Acid (Asa). Laboratory Synthesis and Identification in the Ribosomal Proteins of *E. coli*

M. Robert Christy, Robert M. Barkley, and Tad H. Koch*

Department of Chemistry, University of Colorado Boulder, Colorado 80309

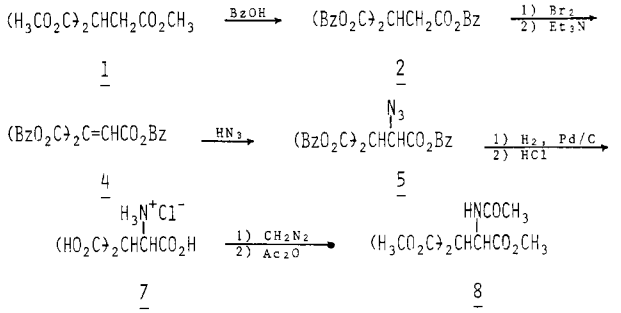
John J. Van Buskirk* and Wolff M. Kirsch

Division of Neurosurgery University of Colorado Health Sciences Center Denver, Colorado 80262

Received July 7, 1980

β-Carboxyaspartic acid (Asa) is the homologue of γ-carboxyglutamic acid (Gla), a biologically important amino acid. Gla is formed by the vitamin-K-mediated post-translational γ carboxylation of glutamyl residues in blood coagulation proteins (prothrombin, factors IX, X). The resulting γ-carboxyl groups are essential for calcium binding and blood coagulation.[1,2] Gla has also been identified in bone[3,4] and other tissues[5] and appears to be universally distributed in ribosomal proteins.[6,7] In this laboratory, comparison of alkaline and acid hydrolysates of *E. coli* ribosomal proteins revealed an excess of aspartic acid in the acid hydrolysate. Since Asa, like Gla, can be expected to be stable in alkali but readily decarboxylated in acid, it was theorized that the excess Asp resulted from decarboxylation of Asa, a previously unknown amino acid. We now report that amino acid analysis of alkaline hydrolysates of *E. coli* ribosomal proteins gives a ninhydrin-positive peak coinciding with that of racemic Asa, synthesized by addition of hydrazoic acid to 1,1,2-tris(carbobenzyloxy)ethylene followed by catalytic hydrogenation. Mass spectral studies have confirmed the identity of the synthetic and naturally occurring Asa.

Scheme I



The synthetic approach to Asa shown in Scheme I was suggested by the recent report of Hall and co-workers[8] that methanol adds regiospecifically to 1,1,2-tris(carbomethoxy)ethylene to give 1,1,2-tris(carbomethoxy)-2-methoxyethane. We proposed that a nitrogen nucleophile would react similarly. The initial objective was the preparation of a protected DL-β-carboxyaspartic acid in which the protecting groups could be removed rapidly and simultaneously by catalytic hydrogenation, since the presumed Asa from natural sources was expected to be quite labile with respect to decarboxylation.

The starting material, 1,1,2-tris(carbomethoxy)ethane (**1**), was prepared as described by Hall and co-workers by the nucleophilic substitution reaction of sodium dimethylmalonate with methyl chloroacetate.[9] Transesterification of **1** in refluxing benzyl alcohol for 4 h at 60 °C under reduced pressure (5×10^{-2} torr) catalyzed with potassium hydroxide gave pure tris(carbobenzyloxy)ethane (**2**, 98%).[10] Bromination of **2** with 1.01 equiv. of bromine in refluxing carbon tetrachloride for 2 h with total exclusion of light yielded 1-bromo-1,2,2-tris(carbobenzyloxy)ethane (**3**, 88%).[11] When light was present the yield of **3** was substantially reduced by competitive bromination at the benzylic positions as indicated by the formation of benzaldehyde upon aqueous workup. Compound **3** was purified by TLC using Merck silica gel F 254 preparative layer plates eluting with 3:1 v/v methylene chloride–ether. Subsequent reaction of **3** in ether at 0 °C with 1 equiv of triethylamine for 3 h yielded pure tris(carbobenzyloxy)ethylene (**4**, 100%).[12] Compound **4** was reacted with hydrazoic acid by

(1) Stenflo, J.; Fernlund, P.; Egan, W.; Roepstorff, P. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, 71, 2730–2733.
(2) Nelsestuen, G. L.; Suttie, J. W. *Biochemistry.* **1972**, *11*, 4961–4964.
(3) Hauschka, P. V.; Lian, J. B.; Gallop, P. M. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 3925–3929.
(4) Price, P. A.; Otsuka, A. S.; Poser, J. W.; Kristaponis, J.; Raman, N. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 1447–1451.
(5) Hauschka, P. V.; Friedman, P. A.; Traverso, H. P.; Gallop, P. M. *Biochem. Biophys. Res. Commun.* **1976**, *71*, 1207–1213.
(6) Van Buskirk, J. J.; Kirsch, W. M. *Biochem. Biophys. Res. Commun.* **1978**, *80*, 1033–1038.
(7) Van Buskirk, J. J.; Kirsch, W. M. *Biochem. Biophys. Res. Commun.* **1978**, *82*, 1329–1331.
(8) Hall, H. K., Jr.; Dunn, L. C.; Padias, A. B. *J. Org. Chem.* **1980**, *45*, 835–838.
(9) Hall, H. K., Jr.; Ykman, P. *J. Am. Chem. Soc.* **1975**, *97*, 800–807.
(10) Compound **2** has the following physical properties: mp 40.5–42 °C; IR ($CHCl_3$) 5.78 μm; ^1H NMR ($CDCl_3$) δ 3.04 (d, J = 7.5 Hz, 2 H) 3.98, (t, J = 7.5 Hz, 1 H), 510 (s, 2 H), 5.15 (s, 4 H), 7.30 and 7.33 (2s, 15 H); mass spectrum (70 eV), *m/e* (relative intensity) 197 (45), 107 (74), 91 (base), 78 (10), and 65 (16).
(11) Compound **3** has the following spectroscopic properties: IR ($CHCl_3$) 5.73 and 12.70 μm; ^1H NMR ($CDCl_3$) δ 3.46 (s, 2 H), 5.02 (s, 2 H), 5.09 (s, 4 H), 7.23 and 7.27 (2s, 15 H); mass spectrum (70 eV), *m/e* (relative intensity) 107 (24), 91 (11), 90 (base), and 64 (13).
(12) Compound **4** has the following physical properties: mp 44–45 °C; IR ($CHCl_3$) 5.8 μm; ^1H NMR ($CDCl_3$) δ 5.11 (s, 4 H), 5.19 (s, 2 H), 6.89 (s, 1 H), and 7.19–7.29 (m, 15 H); mass spectrum (70 eV), *m/e* (relative intensity) 430 (M^+, 0.1), 107 (11), 91 (20), and 90 (base).

0002-7863/81/1503-3935$01.25/0

the dropwise addition of 8.3 equiv of a 0.37 M aqueous solution of sulfuric acid to a tetrahydrofuran solution of **4** and 8.0 equiv of sodium azide. After stirring for 2 h at ambient temperature and then cooling to 0 °C with ice, extraction with methylene chloride followed by solvent evaporation yielded a light yellow oil characterized as tribenzyl 1-azidoethane-1,2,2-tricarboxylate (**5**).[13] Attempts to purify the product further by silica gel or alumina chromatography or with activated charcoal led to quantitative elimination of hydrazoic acid giving the alkene **4**. DL-β-Carboxyaspartic acid (**6**) was then prepared by catalytic hydrogenation of **5** at 1 atm with 20% by weight of 10% palladium on charcoal catalyst in 5% acetic acid–absolute methanol at 0 °C. Rapid stirring of the reaction mixture was critical for optimum yield. Filtration through Celite followed by rotary evaporation of the solvent and toluene byproduct yielded crude **6**. The product was purified by cation exchange medium pressure chromatography at 35 psi with a column of Hamilton HC-X-4.00 resin eluting with 0.1 M hydrochloric acid at 12 mL/h. The white crystalline material obtained after solvent evaporation at reduced pressure was the monohydrate of the hydrochloride salt of DL-β-carboxyaspartic acid. The overall yield from alkene **4** was 46%.

The product of catalytic hydrogenation of **5** followed by cation exchange chromatography was characterized as the monohydrate of DL-β-carboxyaspartic acid hydrochloride (**7**) from spectroscopic and analytical data[14] and conversion to the stable derivative, 1-(acetylamino)-1,2,2-tris(carbomethoxy)ethane (**8**). The ^{1}H NMR spectrum of **7** in hexadeuteriodimethyl sulfoxide shows an AB pattern at δ 4.11 and 4.29 (J = 4.5 Hz) and a broad absorption from 8–10 ppm for the eight acidic protons relative to internal tetramethylsilane. In deuterium oxide the β proton was exchanged for deuterium and only a singlet at δ 4.68 was observed for the α proton relative to internal 3-(trimethylsilyl)propanesulfonic acid sodium salt. The γ proton of Gla exchanges with deuterium oxide, although less rapidly.[15] The ^{13}C NMR spectrum of **7** in hexadeuteriodimethyl sulfoxide shows singlets at δ 51.4, 52.22, 167.8, and 168.8 relative to tetramethylsilane.

The hydrochloride salt **7** was derivatized by reaction with etherial diazomethane in dry methanol at 0 °C followed by solvent evaporation and reaction with acetic anhydride in pyridine for 12 h at ambient temperature. The derivative was obtained as a white crystalline solid in 45% yield and characterized as 1-(acetylamino)-1,2,2-tris(carbomethoxy)ethane (**8**) from spectroscopic and analytical data.[16]

Preliminary evidence for Asa in *E. coli* ribosomal proteins was based upon amino acid analysis of the alkaline hydrolysate, with synthetic Asa as standard. Ribosomes were prepared as described by Staehelin and Maglott,[17] and then washed in 1 M ammonium chloride.[18] Protein was separated by the lithium chloride–urea method,[19] dialyzed against water at 4 °C, lyophilized, and hydrolyzed in 2 N potassium hydroxide under nitrogen for 24 h at 110 °C. Hydrolysates were neutralized with perchloric acid, cooled in ice, and centrifuged to remove potassium perchlorate. The clear supernatants were divested of the bulk of the common amino acids by chromatography on a 1.5 × 15-cm column of Dowex-2 (Sigma, 2×8-400) scaled up from the desalting method of Dreze et al.[20] Successive additions at ambient temperature were 2 N sodium hydroxide (40 mL), water (19 mL), supernatant (4 mL), water (13 mL), 1 N acetic acid (85 mL), 1 N hydrochloric acid (60 mL), and water (30 mL) at 45 mL/h. Fractions were collected at 6-min intervals, flash evaporated, and subjected to amino acid analysis. The common amino acids were found in the acetic acid eluate as expected,[20] but presumptive Asa and several unidentified compounds appeared in the first six fractions of the hydrochloric acid eluate beginning at an abrupt change from pH 2.5 to pH 1. These fractions were combined, flash evaporated twice at 30 °C, and dissolved in 0.2 N sodium citrate, pH 2.2, for amino acid analysis or derivatized for mass spectral analysis. Amino acid analyses were performed with a Beckman Model 120C analyzer at 52 °C using a 0.9- × 54-cm column of Benson BH-4 resin. Dwell time in the reaction coil was increased to 15 min by adjusting buffer and ninhydrin flow rates to 28.3 mL/h and 27.5 mL/h, respectively. At maximum recorder sensitivity this allowed estimation of as little as 0.5 nmol of Asa.

Table I. Influence of pH on the Elution Times of β-Carboxyaspartic Acid (Asa) and γ-Carboxyglutamic Acid (Gla)

elution buffer, pH	elution time of amino acid, min	
	Asa	Gla
3.25	36	45
2.70	46	97
2.40	56	166
2.10	73	246

Elution of the synthetic Asa from the column of the amino acid analyzer is delayed by decreasing the pH of the elution buffer. As shown in Table I, elution of Gla by the same buffers is also delayed, but to a much greater extent. Amino acid analyses of alkaline hydrolysates of *E coli* ribosomal proteins, previously eluted from Dowex-2 by 1 N hydrochloric acid as described, gave peaks corresponding to the positions of Asa at all pH's listed in Table I. These peaks, whether given by synthetic Asa or *E. coli* hydrolysates, were totally eliminated when the samples were heated at 110 °C for 18 h at pH 2.2. Asp was the only amino acid found upon analysis of the heated synthetic Asa, indicating complete decarboxylation under these conditions as well as the absence of significant amounts of ninhydrin-positive impurities.

E. coli hydrolysate material eluting at 46 min with pH 2.70 buffer (Table I) was isolated by fraction collecting before admixture of ninhydrin. The peak fractions were combined, adjusted to pH 2.2, and divided into equal portions, one of which was heated at 110 °C for 18 h. Amino acid analyses of the unheated portion indicated a total of 1.7 (±0.1) nmol of Asa, with no evidence of other compounds. The heated sample was found to contain 1.9 (±0.1) nmol of Asp, but no Asa or other significant peaks. This observed disappearance of presumptive Asa and generation of Asp, which took place under conditions known to decarboxylate the synthetic compound, is clear evidence of the decarboxylation of naturally occurring Asa. On the basis of analyses of the hydrolysate before and after Dowex-2 chromatography, a total of 22.6 nmol of Asa and 17.9 μmol of Asp (including deamidated Asn) were calculated for the *E. coli* ribosomal proteins used in this experiment, giving 1.26 mol of Asa/[1000 mol of (Asp + Asn)]. Assuming that Asp + Asn account for 8.5% of the amino acids in *E. coli* ribosomes,[21] the estimated minimum recovery of Asa was 1 mol of Asa/9300 mol of amino acids.

A similar alkaline hydrolysate of *E. coli* ribosomal proteins eluted from Dowex-2 by 1 N hydrochloric acid, hence essentially free of common amino acids, was derivatized with diazomethane and acetic anhydride as described for the synthetic Asa. The

(13) Compound **5** has the following spectroscopic properties: IR ($CHCl_3$) 4.74 and 5.73 μm; ^{1}H NMR ($CDCl_3$) δ 3.92 (d, J = 9 Hz, 1 H), 4.60 (d, J = 9 Hz, 1 H), 5.02 (s, 4 H), 5.05 (s, 2 H), and 7.19–7.23 (m, 15 H); mass spectrum (70 eV), m/e (relative intensity) 197 (14), 107 (34), 91 (35), 90 (base), and 65 (12).

(14) *Anal.* Calcd for $C_5H_{10}ClNO_7$: C, 25.92; H, 4.52; N, 6.05. Found: C, 25.85; H, 4.52; N, 6.43.

(15) Märki, W; Oppliger, M.; Thanei, P.; Schwyzer, R. *Helv. Chim. Acta* **1977**, *60*, 798–806.

(16) Compound **8** has the following physical and analytical properties: mp 77–78 °C; IR ($CHCl_3$) 2.90, 5.73, 5.94, 6.64, and 6.93 μm; ^{1}H NMR ($CDCl_3$) δ 1.98 (s, 3 H), 3.67 (s, 6 H), 3.69 (s, 3 H), 4.12 (d, J = 4 Hz, 1 H), 5.24 (dd, J = 4 Hz and 9 Hz, 1H), and 6.53 (br d, J = 9 Hz, 1 H); mass spectrum (CI), m/e 262 (M + 1); (70 eV) m/e (relative intensity) 261 (M^+, 0.2), 202 (61), 171 (13), 160 (100), 128 (18), 101 (13), 88 (11), and 70 (23). *Anal.* Calcd for $C_{10}H_{15}NO_7$: C, 45.98; H, 5.79; N, 5.36. Found: C, 46.02; H, 5.79; N, 5.35.

(17) Staehelin, T.; Maglott, D. R. *Methods Enzymol.* **1971**, *20C*, 449–456.

(18) Subramanian, A. R.; Haase, C.; Giesen, M. *Eur. J. Biochem.* **1976**, *67*, 591–601.

(19) Leboy, P. S.; Cox, E. C.; Flaks, J. G. *Proc. Natl. Acad. Sci. U.S.A.* **1964**, *52*, 1367–1371.

(20) Dreze, A.; Moore, S.; Bigwood, E. J. *Anal. Chim. Acta* **1954**, *11*, 554–558.

(21) Published analyses of *E. coli* ribosomal proteins, using Beckman Model 120 amino acid analyzer: Spahr, P. F. *J. Mol. Biol.* **1962**, *4*, 395–406.

presence of the derivative of natural Asa was demonstrated by electron-impact GC–mass spectrometry at 70 eV. The retention time and mass spectrum of the natural Asa derivative were consistent with those of the synthetic material in all respects. The retention times of the derivatives of synthetic Asa and natural Asa and a mixture of the derivatives of synthetic and natural Asa were all 5.7 min. The major fragment ions in the mass spectrum of the derivative of the natural Asa and their relative intensities were *m/e* 202 (45), 171 (23), 160 (100), 128 (17), 101 (10), 88 (11), and 70 (30). The intensities of the major fragment ions are the same as the intensities of the major fragment ions in the mass spectrum of the derivative of synthetic Asa, as reported in ref 16, within the experimental error of the background corrections. GC–mass spectral data were obtained by using an SE-52 coated fused silica capillary column (0.3-mm i.d. × 30 m) in a Hewlett Packard 5982A GC/mass spec/data system modified for direct connection of the fused silica column to the mass spectrometer ion source.

The distribution and biological significance of this new amino acid remain to be determined and are subjects of current investigation in our laboratories. In view of the divalent cation chelation effects associated with the malonic acid moiety of Gla,[1,2] a systematic study of proteins known to bind divalent metals may detect the presence of Asa in other biological systems.

Acknowledgment. We thank Tom Wenzel for performing some of the NMR analyses and the Solar Energy Research Institute (SERI) for the use of their JEOL FX-90Q NMR spectrometer. We also thank Professor Robert Sievers for the use of his HP 5982A CG/mass spec/data system. Acknowledgment is made to the National Cancer Institute (CA-24665, CA-19137) as well as the General Medical Institute (GM-24965) of the NIH and the VA Research Organization (4964) for financial assistance.

Polymer Films on Electrodes. 6. Biconductive Polymers Produced by Incorporation of Tetrathiafulvalenium in a Polyelectrolyte (Nafion) Matrix

Timothy P. Henning, Henry S. White, and Allen J. Bard*

Department of Chemistry, The University of Texas Austin, Texas 78712

Received February 9, 1981

We describe the preparation of a polymer layer on an electrode [based on the perfluorinated ion exchange polymer, Nafion[1] and tetrathiafulvalene (TTF)] which exhibits both ionic and electronic conductivity. Most "polymer electrodes"[2] involve charge transport by electron exchange between incorporated electroactive centers and ionic migration; the overall redox process in such polymers is diffusional in nature.[3] Other purely electronically conductive polymer layers, e.g., polypyrrole, have also been described.[4] We have found that when TTF^+ is incorporated into Nafion, the behavior approaches that of an "organic or one-dimensional conductor"[5] and allows significantly more rapid redox processes of solution species compared to the same matrix with other cations.

The electrodes were prepared, as previously described,[6] by covering a Pt disk with a drop of an EtOH solution of Nafion (equiv wt ~970) and allowing the EtOH to evaporate. The dry thickness of the resulting film determined with a Sloan Dektak surface profile measuring system was 0.7 μm. TTF^+ was incorporated into the film by immersing the electrode in an aqueous solution of ~1 mM TTFCl for 20 min. The behavior of this electrode, denoted Pt/Nafion,TTF^+, will be contrasted with others incorporating other ions, e.g., $Ru(bpy)_3^{2+}$[6] or Cp_2FeTMA^+ ([(trimethylammonio)methyl]ferrocene). The cyclic voltammogram (CV) of the Pt/Nafion,TTF^+ electrode (Figure 1a) in an aqueous 1 M KBr solution is remarkably similar to the CV of a film of TTF alone (prepared by evaporation of a benzene solution of TTF on a Pt electrode) (Figure 1b). The anodic peak corresponds to TTF^+ formation and the sharp cathodic peak to reduction to TTF. The nature and shape of the CV's suggest major structural changes, e.g., as described for TTF–TCNQ electrodes.[7] The redox processes in the electrode are clearly indicated by the accompanying color changes (colorless in the TTF form and purple in the TTF^+ form). At scan rates (v) up to 50 mV/s, the peak currents (i_p) vary directly with v, and even at v = 20 V/s little diffusional tailing is observed on the CV waves. Contrast this to the Pt/Nafion,Cp_2FeTMA^+ electrode (Figure 1c) where the waves resemble diffusion controlled reactions and for $v > 25$ mV/s i_p varies as $v^{1/2}$.[8] For this latter electrode the effective diffusion coefficient (D) determined from electrochemical measurements[3] is $\sim 8.5 \times 10^{-11}$ cm^2/s; similarly D for $Ru(bpy)_3^{2+}$ in Nafion is $\sim 10^{-10}$ cm^2/s.[6b] Note that the "effective diffusion coefficient" actually represents the rate of charge transfer through the film through electron hopping, counter ion movement, and actual diffusion.[3] The effective diffusion coefficient determined from electrochemical measurements for Pt/Nafion,TTF^+ electrodes is about 1000 times higher.

The mediated oxidation of solution species, such as FeY^{2-} (Y^{4-} is the tetraanion of ethylenediaminetetraacetic acid), is also very different with Nafion,TTF electrodes. The CV of a Pt/Nafion,TTF^+ electrode prepared as above and cycled several times in 1 M KBr, when immersed in a 0.08 M FeY^{2-} solution (Figure 1d) shows redox processes for the FeY^{2-}/FeY^- system which occur at essentially the same potentials as on Pt (Figure 1e) (i.e., oxidation of FeY^{2-} occurs *before* the TTF/TTF^+ wave), with the currents about one-third those of a bare Pt electrode of the same area. The redox waves for the Nafion,TTF^+ itself are essentially unchanged. With the Pt/Nafion,Cp_2FeTMA^+ electrode in an identical experiment (Figure 1f), the mediated current is much smaller and is found only where the polymer oxidation occurs. Similar contrasting behavior between the Nafion,TTF^+ and Nafion,Cp_2FeTMA^+ is found with the $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ couple as the solution species. The highly conductive nature of the Pt/Nafion,TTF^+ electrode is also illustrated by the following experiment. If the CV sweep is stopped at a potential where the polymer is in the oxidized (TTF^+) form (0.0 V in Figure 1d), the current for FeY^{2-} oxidation remains steady. If the electrode is now disconnected, the purple TTF^+ is immediately reduced by FeY^{2-} to the colorless TTF form, and on a new CV scan the peak for TTF^+ reduction has disappeared.

The following experiments demonstrate that the described effects cannot be attributed to TTF^+-enhanced transport of solution species through the polymer layer or porosity of the layer. If, after the experiments with the Pt/Nafion,TTF^+ electrode in FeY^{2-} or $Fe(CN)_6^{3-}$ solutions, the electrode was immersed in a supporting electrolyte solution not containing electroactive species and cyclic voltammetry carried out immediately, no waves for the Fe species were observed. Thus the film does not absorb appreciable amounts of these. Moreover CV at a Pt electrode

(1) (a) Produced by E. I. duPont de Nemours and Co., Wilmington, DE. (b) Grot, W. *Chem.-Ing.-Tech.* **1978**, *50*, 299.

(2) (a) Peerce, P. J.; Bard, A. J. *J. Electroanal. Chem. Interfacial Electrochem.* **1980**, *112*, 97. (b) Merz, A.; Bard, A. J. *J. Am. Chem. Soc.* **1978**, *100*, 3222. (c) Itaya, K.; Bard, A. J. *Anal. Chem.* **1978**, *50*, 1487. (d) Van de Mark, M. R.; Miller L. L. *J. Am. Chem. Soc.* **1978**, *100*, 639, 3223. (e) Daum, P.; Murray, R. W. *J. Electroanal. Chem. Interfacial. Electrochem.* **1979**, *103*, 289. (f) Kaufman, F. B.; Engler, E. M. *J. Am. Chem. Soc.* **1979**, *101*, 547. (g) Brown, A. P.; Anson, F. C. *Anal. Chem.* **1977**, *49*, 1589. (h) Oyama, N.; Anson, F. C. *J. Electrochem. Soc.* **1980**, *127*, 640. (i) *Ibid.* **1980**, *127*, 247 and references therein.

(3) (a) Peerce, P. J.; Bard, A. J. *J. Electroanal. Chem.* **1980**, *114*, 89. (b) Laviron, E. *Ibid.* **1980**, *112*, 1. (c) Andrieux, C. P.; Saveant, J. M. *Ibid.* **1980**, *111*, 377.

(4) (a) Diaz, A. F.; Kanazawa, K. K.; Gardin, G. P. *J. Chem. Soc., Chem. Commun.* **1979**, 635. (b) Noufi, R.; Tench, D.; Warren, L. F. *J. Electrochem. Soc.* **1980**, *127*, 2310.

(5) See, e.g.: (a) Engler, E. *Chem. Tech.* **1976**, 274. (b) Perlstein, J. *Angew. Chem., Int. Ed. Engl.* **1977**, *16*, 519 and references therein.

(6) (a) Rubinstein, I.; Bard, A. J. *J. Am. Chem. Soc.* **1980**, *102*, 6641. (b) *Ibid.*, submitted for publication.

(7) (a) Jaeger, C. D.; Bard, A. J. *J. Am. Chem. Soc.* **1979**, *101*, 1690. (b) *Ibid.* **1980**, *102*, 5435.

(8) At very low scan rates (~1 mV/s), however, the Nafion,Cp_2FeTMA^+ electrode shows i_p proportional to v.

0002-7863/81/1503-3937$01.25/0