

acid. Thus, the biosynthesis of the carbocyclic rings of phenazine pigments are, in all probability, additional cases of aromatic biosynthesis proceeding via the shikimic acid pathway.¹⁰

Anthranilic acid-1-¹⁴C was prepared by the method of Murray and Ronzio¹¹ and the labeled substrate was fed over a period of five days to a culture of *Pseudomonas chlororaphis* NRRL B-977^{11a} growing on a peptone-glycerol broth⁴ medium.

The pigment was isolated by ether extraction and purified by chromatography on alumina and crystallization from methanol. During this purification the original chlororaphin is entirely oxidized to phenazine-1-carboxamide, which is obtained as bright yellow needles, m.p. 241.5–243 (lit.¹² 241°).

The phenazine-1-carboxamide was hydrolyzed in base to the corresponding acid. The acid so obtained had, in all cases within the limits of experimental error, the same activity as the amide that had itself been purified to constant activity, thus rendering improbable the possibility that the observed activity was due to some highly active minor impurity.

Heating phenazine-1-carboxylic acid in diphenyl ether with copper powder at 260° for five hours resulted in decarboxylation and yielded phenazine, which was purified by chromatography over alumina followed by recrystallization from ethanol to give needles, m.p. 171–171.5° (lit. 171°).¹²

Similar degradations were carried out on chlororaphin obtained from a culture medium to which had been added sodium carbonate-¹⁴C, alanine-1-¹⁴C and alanine-2-¹⁴C. The specific activities of the various samples were determined either by combustion to carbon dioxide, which was counted in an ion chamber by means of a vibrating reed electrometer, or by counting the samples directly in a Nuclear Chicago gas-flow counter, model D47. The results of these experiments are shown in Table I.

TABLE I

Substrate	% Incorporation	% Activity in phenazine ring ^a
Anthranilic acid carboxyl- ¹⁴ C	0.002	21 ± 3%
Sodium carbonate- ¹⁴ C	0.0001	60 ± 10%
Alanine-1- ¹⁴ C	0.01	25 ± 4%
Alanine-2- ¹⁴ C	0.01	71 ± 2%

^a The remaining activity was in all cases found in the carbon dioxide, collected as barium carbonate from the decarboxylation.

Thus, the incorporation of anthranilic acid carboxyl-¹⁴C into chlororaphin has been demonstrated. The fact that all of the activity so incorporated does not reside in the carboxyl carbon of the phenazine pigment requires comment. It is known that anthranilic acid is very rapidly metabolized¹³ by *Pseudomonas* species with loss of the carboxyl carbon as carbon dioxide and formation of catechol.¹⁴ It is

(10) P. R. Srinivasan, *J. Am. Chem. Soc.*, **81**, 1772 (1959).

(11) A. Murray, III, and A. R. Ronzio, *Org. Synth. with Isotopes*, Part I, p. 316 (1958).

(11a) Kindly supplied by Dr. W. C. Haynes, Northern Regional Research Branch, USDA, Peoria, Illinois.

(12) B. F. Köggl and J. J. Postowsky, *Ann.*, **480**, 280 (1930).

(13) R. Y. Stanier, O. Hayaishi and M. Tsuchida, *J. Bact.*, **62**, 355 (1951).

(14) J. C. Yanofsky in "Amino Acid Metabolism," Editors W. D. McElroy and H. B. Glass, Johns Hopkins Press, Baltimore, Md., 1959, pp. 930–939.

likely that some of the carbon dioxide resulting from this metabolic decarboxylation is incorporated by diverse pathways into precursors of the phenazine pigments. We propose that ¹⁴C proceeding by such pathways accounts for the activity found in the aromatic ring system of chlororaphin biosynthesized from anthranilic acid carboxyl-¹⁴C as substrate; *i.e.*, the experimentally observed distribution of activity represents the sum of intact anthranilic acid incorporation and a more circuitous incorporation of the carboxyl carbon of anthranilic acid via a pathway that includes carbonate. The results with added sodium carbonate-¹⁴C support this suggestion.

The distributions of activity found in chlororaphin produced with alanine-1-¹⁴C and alanine-2-¹⁴C as labeled substrates are also in accord with their incorporation via a pathway involving shikimic acid and anthranilic acid.¹⁵

It is possible to write several detailed mechanisms for the dimerization reaction, some of which involve preliminary oxidation at nitrogen, or prior conversion of one of the rings to 3-hydroxyanthranilic acid. Since the precise structures of the species that couple is not defined by the experiments reported herein, the selection of one particular mechanism is premature. Further, it should be emphasized that possibilities are not limited to a dimerization of two identical units. These experiments do indicate, however, that the ring in the phenazine pigment bearing the carboxyl carbon has not proceeded via 3-hydroxyanthranilic acid as this pathway, which includes tryptophan, would result in loss of the carboxyl carbon originally present in the anthranilic acid substrate.¹⁶

(15) A. White, P. Handler, E. L. Smith and DeWitt Stetten, Jr., "Principles of Biochemistry," 2nd Ed., McGraw-Hill Book Co., New York, N. Y., 1959, pp. 400–413, 558.

(16) J. F. Nyc, H. K. Mitchell, E. Liefer, and W. H. Langham, *J. Biol. Chem.*, **179**, 783 (1949).

CONTRIBUTION NO. 2655
R. E. CARTER
GATES AND CRELLIN LABORATORIES OF CHEMISTRY
CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA
J. H. RICHARDS
RECEIVED DECEMBER 12, 1960

ON THE IONIZATION OF POLYSTYRENE SULFONIC ACID

Sir:

Raman spectroscopy has proven to be one of the most useful techniques for the determination of the extent of ionization of ordinary electrolytes.¹ In this note we communicate the results of preliminary Raman spectroscopic studies of the state of ionization of a strong polyelectrolyte with the intention of clarifying the nature of ion-binding, a phenomenon commonly encountered with these substances.

We have obtained the Raman spectra of polystyrenesulfonic acid (PSSA) and its monomeric unit, *p*-ethylbenzenesulfonic acid (EBSA) using a three prism Steinheil spectrograph with photoelectric recording.² The spectra were excited by the

(1) T. F. Young, L. F. Maranville and H. M. Smith, "The Structure of Electrolytic Solutions," W. J. Hamer, Editor, J. Wiley and Sons, Inc., New York, N. Y., 1959, Chapter 4 and the references contained therein.

(2) T. F. Young, *Record Chem. Prog.*, **12**, 81 (1951).

4358 Å. line from high intensity mercury lamps. PSSA was obtained from Dr. W. N. Vanderkoi of the Dow Chemical Co., Midland, Michigan. The molecular weight was approximately 10^6 . The original sample was purified by solution in water, precipitation with isopropyl alcohol in a Waring Blendor and thereafter passage through a column containing cation exchange resin (Dowex 50). EBSA was synthesized from ethylbenzene and sulfuric acid and purified by standard methods.

PSSA was studied in aqueous solutions at a concentration of 0.15 *N* and EBSA was studied at a concentration of 1.0 *N*. It would have been advantageous to study PSSA in more concentrated solution, but this proved impossible because of excessive light scattering. Because of the low concentration of the solutions of PSSA the number of Raman lines detected in these solutions is fewer than that observed in solutions of EBSA and the intensities are also lower. Because of intense light scattering, no lines closer to the exciting line than 965 cm.^{-1} could be observed in solutions of PSSA. The results are recorded in Table I.

TABLE I

RAMAN SPECTRA OF PSSA AND EBSA (cm.^{-1})		
EBSA	PSSA	
620 (w)	...	
780 (s)	...	
965 (w)	965 (vw)	
1035 (w)	1030 (vw)	
1060 (w)	1060 (vw)	
1125 (vs)	1125 (s)	w, weak
1205 (w)	...	s, strong
1450 (w)	1450 (vw)	vw, very weak
1590 (vs)	1590 (s)	vs, very strong

It is clear that the lines appearing in both spectra are identical. The 1125 cm.^{-1} line has been identified by Nisi³ as characteristic of the ionized sulfonic acid residue, whereas the other strong lines arise from the vibrational modes of the *p*-substituted benzene nucleus and are commonly observed in these compounds.⁴

Mock and Marshall have shown that solutions of PSSA have very low apparent degrees of ionization.^{5,6} They have found a degree of ionization of 0.38 which is independent of concentration. The absence of any new lines in the spectrum of PSSA and the observation that the relative intensities of the lines observed are the same as in EBSA strongly suggests that PSSA is completely ionized. This conclusion is in complete accord with nuclear magnetic resonance measurements of the proton shift in solutions of PSSA made in this laboratory.⁷ It is clear, then, that the low apparent degree of ionization of PSSA is due to electrostatic interaction between the hydrogen ions and the polyion. Although the counterions are localized in a small region near the charged polymer skeleton, they do not associate at the skeletal sites as if they were weak electrolytes. The counterions must be as-

(3) H. Nisi, *Jap. J. Phys.*, **6**, 1 (1930).(4) N. S. Ham and A. H. Hamby, *Aust. J. Chem.*, **6**, 135 (1953).(5) R. A. Mock and C. A. Marshall, *J. Polymer Sci.*, **13**, 263 (1955).(6) R. A. Mock, C. A. Marshall and T. E. Slykhouse, *J. Phys. Chem.*, **58**, 498 (1954).(7) L. Kotin and M. Nagasawa, *J. Am. Chem. Soc.*, in press.

sumed to form a "mobile monolayer" about the polyion skeleton with the very strong electrostatic interactions possible localizing individual counterions in what may be thought of as a quasi-cell in the vicinity of a fixed skeletal charge, but not "on the charge."⁸

We wish to thank the United States Public Health Service, the Air Force Research and Development Command and the National Science Foundation for financial support. We also wish to thank Prof. T. F. Young for the use of the Raman spectrograph and Mr. Don Irish for assistance with the measurements.

(8) S. Lapanje, J. Haebig, H. T. Davis and S. A. Rice, *ibid.*, in press.

(9) Department of Chemistry, University of Ljubljana, Ljubljana, Yugoslavia.

DEPARTMENT OF CHEMISTRY AND SAVO LAPANJE⁹
INSTITUTE FOR THE STUDY OF METALS
UNIVERSITY OF CHICAGO STUART A. RICE

RECEIVED DECEMBER 7, 1960

IRON TRICARBONYL COMPLEXES OF CYCLOHEPTATRIENE, CYCLOHEPTADIENE AND CYCLOHEPTADIENIUM ION¹

Sir:

Hydride ion abstraction from π -cycloheptatriene-metal-tricarbonyls (I) by triphenylcarbonium ion has been shown to provide a common route to the π -tropenium ion complexes (II), $(\pi\text{-C}_7\text{H}_7^+)\text{M}(\text{CO})_3\text{BF}_4^-$ where $\text{M} = \text{Mo}$,^{2a} Cr ^{2b,3} and W .^{2b} Recently, preliminary undetailed reports of extension of this method by Fischer and Palm⁴ and by Pauson⁵ to the π -cycloheptatriene-iron carbonyl complex, presumed to be $(\pi\text{-C}_7\text{H}_8)\text{Fe}(\text{CO})_2$ by Wilkinson and co-workers⁶ and by Manuel and Stone,⁷ have contended that the product is a π -tropenium ion complex, either $(\pi\text{-C}_7\text{H}_7^+)\text{Fe}(\text{CO})_2^4$ or $(\pi\text{-C}_7\text{H}_7^+)\text{Fe}(\text{CO})_3$.⁵ We wish to report our substantially different results which clearly demonstrate that cycloheptatriene reacts with iron pentacarbonyl to give iron tricarbonyl complexes of cycloheptatriene (I, $\text{M} = \text{Fe}$)⁵ and, more slowly, of cycloheptadiene (III), that triphenylcarbonium fluoroborate shows addition rather than hydride ion abstraction with the cycloheptatriene complex (I, $\text{M} = \text{Fe}$) to yield the trityl adduct (IV, $\text{R} = (\text{C}_6\text{H}_5)_3\text{C}-$), and that the stable cycloheptadienium ion complex (IV, $\text{R} = \text{H}$)⁸ is formed readily either by proton addition to the cycloheptatriene complex

(1) Partial support of this work by the Office of Ordnance Research, U. S. Army, is gratefully acknowledged.

(2) (a) H. J. Dauben, Jr. and L. R. Honnen, *J. Am. Chem. Soc.*, **80**, 5570 (1958); (b) H. J. Dauben, Jr., L. R. Honnen and D. J. Bertelli, Abstracts, 15th Southwest Regional Meeting, American Chemical Society, Baton Rouge, La., Dec. 3, 1959, p. 89.

(3) J. D. Munro and P. L. Pauson, *Proc. Chem. Soc.*, 267 (1959).

(4) Unpublished results of E. O. Fischer and C. Palm, quoted by R. D. Fischer, *Chem. Ber.*, **93**, 165 (1960).

(5) P. L. Pauson, *Proc. Chem. Soc.*, 297 (1960), originally proposed that cycloheptatriene and tropenium ion formed iron dicarbonyl complexes but later suggested that iron tricarbonyl complexes were produced.

(6) R. Burton, M. L. H. Green, E. W. Abel and G. Wilkinson, *Chemistry and Industry*, 1592 (1958).

(7) T. A. Manuel and F. G. A. Stone, *J. Am. Chem. Soc.*, **82**, 366 (1960).

(8) G. Winkhaus and G. Wilkinson, *Proc. Chem. Soc.*, 312 (1960), have mentioned that Burton, Pratt and Wilkinson have derived salts of $\text{C}_7\text{H}_8\text{Fe}(\text{CO})_3^+$ from cycloheptadiene by an unspecified method.