$$CH_2{=}CH{-}CH_2MgBr \rightleftharpoons BrMgCH_2{-}CH{=}CH_2$$
IV V

rium lies fairly far on the side of I. The extent of the contribution of II to the spectrum determines the chemical shift of the $-CH_2-$ resonance; if II were the dominant form this resonance would appear in the vinyl region. Since it actually appears in or near the $-CH_2-Mg$ region, we can safely infer that I is the dominant form. A comparison[8] of the effects of vinyl and methyl groups (as R) on the chemical shift of the methylene resonances in RCH_2X, X = Br, and MgBr suggests that the $-CH_2-$ resonance of the Grignard reagent in Fig. 1 may actually be some 50 cps. (at 60 Mc) downfield from the position it would have if the Grignard reagent were exclusively crotylmagnesium bromide. This figure corresponds to about 84% of I and 16% of II. A rather strong argument against the validity of the composition so calculated is provided by the fact that the position of the $-CH_2-$ resonance changes by only 1 cps. between $+30°$ and $-20°$, corresponding to an increase of but 0.3% in the proportion of the crotyl isomer. It could be argued that through coincidence ΔH is virtually zero for the equilibrium, but it seems more reasonable to conclude that I is more than 99% of the mixture. If so, then even a several-fold temperature effect on the concentration of II would give only a very small change in spectrum.

As with allylmagnesium bromide,[8] the spectrum of butenylmagnesium bromide shows no evidence of "freezing out"[9] of the separate allylic isomers at temperatures down to $-60°$. Interestingly, dibutenylmagnesium shows the same chemical shifts as does the ordinary Grignard reagent. This fact can be taken as an argument against the Grignard formula RMgBr since the bromine ought to have some influence on the chemical shift of $-CH_2-$ resonance. The n.m.r. evidence gives no support for structure III for the Grignard reagent. Bridged structures are favored for crotyl palladium chloride complex[10] and crotyl cobalt tricarbonyl,[11] which have very different n.m.r. spectra from the Grignard reagent.

If the Grignard reagent is accepted to be crotylmagnesium bromide (I) with the possibility of very rapid equilibration with a small proportion of α-methylallylmagnesium bromide (II), then the remaining problem is to account for the high yields of products in which it reacts, even with highly sterically-hindered carbonyl compounds, to insert α-methylallyl groups. Presumably if equilibration were rapid, formation of the less-crowded crotyl addition products would be favored. This dilemma is resolved if we assume that coördination of the carbonyl oxygen with the magnesium of I diminishes the electrophilic character of the magnesium sufficiently to cause the rate of allylic isomerization to be much less than the rate of addition. In these circumstances, only the α-methylallyl group would be inserted by the cyclic addition mechanism.[2a] If insertion of the α-methylallyl group were very highly retarded by steric hindrance, as with di-*t*-butyl ketone,[12] then allylic isomerization could lead to the crotyl-addition product.

The n.m.r. spectra of other allylic Grignard reagents will be reported in later papers.

(8) J. E. Nordlander, Ph.D. Thesis, California Institute of Technology, 1960, pp. 41–43.

(9) Ref. 6, Chap. IV.

(10) H. C. Dehn and J. C. W. Chien, *J. Am. Chem. Soc.*, **82**, 4429 (1960).

(11) D. W. Moore, H. B. Jonassen, T. B. Joyner and A. J. Bertrand, *Chem. and Ind.*, 1304 (1960).

(12) K. W. Wilson, J. D. Roberts and W. G. Young, THIS JOURNAL, **72**, 218 (1950).

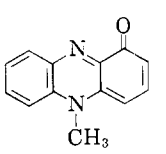
CONTRIBUTION NO. 2649 J. ERIC NORDLANDER
GATES AND CRELLIN LABORATORIES OF CHEMISTRY
CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA WILLIAM G. YOUNG
DEPARTMENT OF CHEMISTRY JOHN D. ROBERTS
UNIVERSITY OF CALIFORNIA AT LOS ANGELES
LOS ANGELES, CALIFORNIA

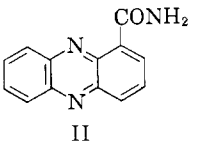
RECEIVED DECEMBER 12, 1960

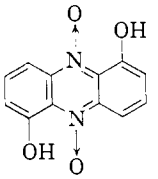
THE BIOGENESIS OF PHENAZINE PIGMENTS[1]

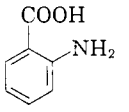
Sir:

Pigments that contain the aromatic phenazine nucleus such a spyocyanine (I),[1a] chlororaphin (a 3:1 molecular compound of phenazine-1-carboxamide (II) and its 9,10 dihydro derivative),[2] iodinin (III)[3] and phenazine-1-carboxylic acid[4] present an interesting biogenetic problem for which no definitive proposals have yet been made though a significant amount of work on the biosynthesis of pyocyanine (I) has been reported.[5,6,7,8]


I


II


III


IV

We were attacted by the possibility that natural products of this type may arise by an oxidative dimerization of anthranilic acid (IV) with loss of the carboxyl carbon where appropriate. A somewhat tenuously analogous mechanism apparently produces the phenoxazine ring system of the actinomycins.[9] We wish to report results which we interpret as indicating that one ring, at least, of phenazine-1-carboxamide (II) originates in anthranilic

(1) Supported in part by Grant E-2775 from the U. S. Public Health Service.

(1a) F. Wrede and E. Strack, *Z. physiol. Chem.*, **181**, 58 (1929).

(2) C. Dufraisse, A. Etienne and E. Toromanoff, *Compt. rend.*, **235**, 920 (1952).

(3) G. R. Clemo and A. F. Daglish, *J. Chem. Soc.*, 1481 (1950).

(4) W. C. Haynes, F. H. Stodola, J. M. Locke, T. G. Pridham, H. F. Conway, V. E. Sohns and R. W. Jackson, *J. Bact.*, **72**, 412 (1956).

(5) M. V. Burton, J. J. R. Campbell and B. A. Eagles, *Can. J. Res.*, **26C**, 15 (1948).

(6) A. C. Blackwood and A. C. Neish, *Can. J. Microbiol.*, **3**, 165 (1957).

(7) N. Grossowicz, P. Hayat and Y. S. Halpern, *J. Gen. Microbiol.*, **16**, 576 (1957).

(8) L. H. Frank and R. D. DeMoss, *J. Bact.*, **77**, 776 (1959).

(9) E. Bullock and A. W. Johnson, *J. Chem. Soc.*, 1602 (1957).

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-^{14}C was prepared by the method of Murray and Ronzio[11] 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 broth[4] 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-^{14}C, alanine-1-^{14}C and alanine-2-^{14}C. 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-^{14}C 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 metabolized[13] by *Pseudomonas* species with loss of the carboxyl carbon as carbon dioxide and formation of catechol.[14] It is 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 ^{14}C proceeding by such pathways accounts for the activity found in the aromatic ring system of chlororaphin biosynthesized from anthranilic acid carboxyl-^{14}C 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-^{14}C support this suggestion.

The distributions of activity found in chlororaphin produced with alanine-1-^{14}C and alanine-2-^{14}C 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]

(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. Kögl 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.

(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).