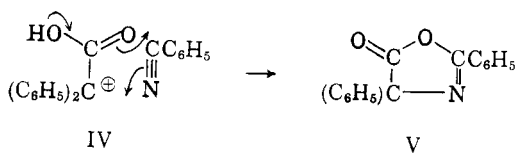
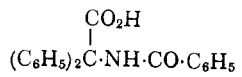
hydrolysis, as commented upon by the original authors, appeared remarkable for compounds having imino-ether structures as in II and III. Reinvestigation fully confirmed the reported transformations. It also was found that B was decarboxylated almost quantitatively to C by heating in quinoline. Consideration of the mode of formation of A suggested that the reaction was that of benzonitrile with the $(C_6H_5)_2{}^+CCO_2H$ carbonium ion, which is known[3] to be the main species present, as in IV leading to formulation of compound A as the azlactone V. Compound B then would be VI and



IV V



VI

compound C should be N-diphenylmethylbenzamide. This latter conclusion was confirmed by comparison of compound C with an authentic sample.[4] The formation of C from benzonitrile and benzhydrol must result from a reaction of the Ritter type.[5]

These revised structures obviously are equally compatible with the previously reported transformations, and also are supported by the infrared spectra in Nujol. Thus, in keeping with structure V, compound A shows a high frequency carbonyl band at 1820 cm.$^{-1}$ and a strong band at 1650 cm.$^{-1}$ characteristic of the —O—C=N— grouping. Compound B, apart from bands at 3350 (N—H), *ca.* 2450 and *ca.* 2600 cm.$^{-1}$ (carboxyl O—H), has two bands at 1705 cm.$^{-1}$ and 1625 cm.$^{-1}$ attributable to carbonyl groups.

Experimental

Compound A, namely 2,4,4-triphenyl-5-oxazolone, and compound B, α-benzamidodiphenylacetic acid, were obtained as previously described[1] and had the reported melting points.

Decarboxylation of α-Benzamidodiphenylacetic Acid.—The acid (0.9 g.) was heated under reflux in quinoline (5 ml.) for 1 hr. The cooled solution was poured into excess water and acidified. The solid product (0.7 g.) was isolated by chloroform extraction and crystallized from ethyl acetate m.p. 172–173°. This compound was shown by mixed melting point and infrared spectra to be identical to an authentic specimen of N-diphenylmethylbenzamide.

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Biosynthesis of Bacterial Pigments. II.[1] Chlororaphin

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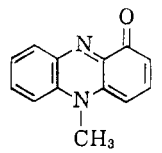
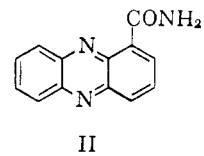
A review[2] of the chemistry of phenazines gives neglibible information on biosynthetic pathways of the bacterial phenazine pigments. However, several reports on the biosynthesis of two bacterial pigments, pyrocyanine[3] (I) and chlororaphin (a 3:1 molecular compound of phenazine-1-carboxamide (II) and its 5,10-dihydro derivative)[4] have been helpful in speculating on possible biosynthetic intermediates.



I



II

Recent observations that anthranilic acid, an intermediate in the biosynthesis of tryptophan in microörganisms,[5] has been incorporated into chlororaphin in trace amounts (0.002% incorporation) prompted us to study simulated biosyntheses of phenazine pigments through the dimerization of substituted anilines by symmetrical carbon–nitrogen pairing.

Biogenetic implications of phenol oxidation and the biosynthesis of dimeric and polymeric phenols are available in excellent laboratory analogies—*e.g.*, usnic acid,[6] gossypol,[7] griseofulvin,[8] and picrolichenic acid.[9] The oxidative dimerization examples can be regarded as either the pairing of radicals (C· → ·O and/or C· → ·C) or the substitution of one radical into a neutral phenol molecule followed by further oxidation. Coupling of radicals derived from amino phenols (C· → ·C and/or

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(3) M. V. Burton, J. J. R. Campbell, and V. A. Eagles, *Can. J. Res.*, **26C**, 15 (1948); A. C. Blackwood and A. C. Neish, *Can. J. Microbiol.*, **3**, 165 (1957); N. Grossowicz, P. Hayat, and Y. S. Halpern, *J. Gen. Microbiol.*, **16**, 567 (1957); L. H. Frank and R. D. DeMoss, *J. Bact.*, **77**, 776 (1959).

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(5) J. C. Yanofsky in "Amino Acid Metabolism," W. D. McElroy and H. B. Glass, ed., Johns Hopkins Press, Baltimore, Md., 1955, pp. 930–939; J. C. Yanofsky, *J. Biol. Chem.*, **224**, 783 (1957).

(6) D. H. R. Barton, A. M. Deflorin, and O. E. Edwards, *J. Chem. Soc.*, 530 (1956).

(7) J. D. Edwards, Jr., *J. Am. Chem. Soc.*, **80**, 3798 (1958).

(8) A. C. Day, J. Nabney, and A. I. Scott, *J. Chem. Soc.*, 4067 (1961).

(9) T. A. Davidson and A. I. Scott, *ibid.*, 4075 (1961).

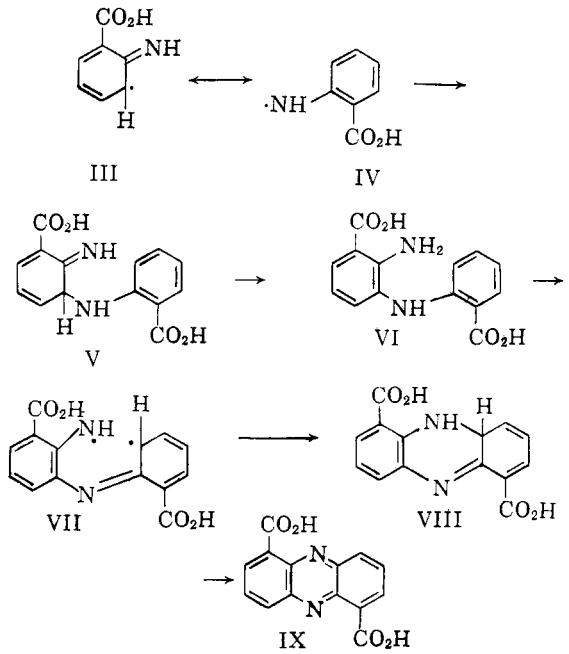
C· → ·N) can also be considered in the biogenesis of *Amaryllidaceae* alkaloids—*e.g.*, galanthamine.[10]

One possible explanation for biosynthesis of II involves dimerization of anthranilic acid with the loss of a carboxyl carbon.[4] Oxidative dimerizations of aromatic amines by pairing of radicals (C· → ·N) rather than substitution of one radical into a neutral molecule followed by further oxidation suggests a plausible biosynthetic pathway for phenazine-1-carboxamide (II) and its dihydro derivative from anthranilic acid (see structures III–IV). Presence for such a scheme is found in the radical coupling theory for oxidation of anilines to polymeric products—*e.g.*, nigraniline dyes[11] as well as concepts of the radical coupling theory of biogenesis.[10] To obtain II from IX would require two additional steps, decarboxylation and amination.

An interesting synthesis of phenazine through low temperature irradiation of aniline[12] gave support to the plan to investigate one-electron oxidizing agents on aniline and derivatives. We chose anthranilic acid for initial investigations with the goal in mind of finding pertinent information leading to a chemical model for the bacterial synthesis of phenazine pigments.

The effectiveness of manganese dioxide and lead dioxide as one-electron-transfer oxidizing agents for phenols has been attributed to coupling of radicals on a solid surface.[13] Presumably this explains the similar success now observed in the oxidation of anthranilic acid by each of these reagents. Active lead dioxide and manganese dioxide[13] were freshly prepared and used in dry benzene or chloroform under a nitrogen atmosphere. After shaking anthranilic acid in benzene or chloroform for several hours at 40° under nitrogen (oxygen-free) with either lead or manganese dioxide, phenazine-1,6-dicarboxylic acid (IX) was isolated (up to 16%). Apparently dihydro intermediates—*e.g.*, VIII—if produced are oxidized to IX. Extensive polymerization accompanying the formation of IX was not unexpected since intermolecular coupling of *para* isomers of III and IV leads to linear polymers incapable of forming IX. Oxidation of anthranilic acid with alkaline potassium ferricyanide resulted in unidentified polymers.

Decarboxylation of phenazine-1,6-dicarboxylic acid in refluxing diphenyl ether containing copper powder under nitrogen (oxygen-free) affords a mixture of phenazine and phenazine-1-carboxylic acid from which the amide, II, was prepared by standard procedure with thionyl chloride and ammonia.[14]



Experimental[15]

Oxidation of Anthranilic Acid with Manganese and Lead Dioxide.—A solution of 5.0 g. (0.036 mole) of anthranilic acid in chloroform or benzene (50 ml.) was shaken with active manganese dioxide[16] at 40° for 3 hr. under nitrogen (oxygen-free). The manganese dioxide was removed by filtration and the dark solution extracted with 50 ml. of 5 *N* potassium hydroxide. The dark alkaline extract was concentrated to a volume of about 25 ml. and allowed to stand at 0° for several days. The resulting brown microcrystals of the dipotassium salt of phenazine-1,6-dicarboxylic acid were collected. Neutralization of the salt with 0.5 *N* hydrochloric acid afforded phenazine-1,6-dicarboxylic acid, charring without melting 300–320°, 16% yield.

Anal. Calcd. for $C_{14}H_8N_2O_4$: C, 62.69; H, 3.01; N, 10.44. Found: C, 62.77; H, 3.21; N, 10.29.

A similar experiment with active lead dioxide[17] gave phenazine-1,6-dicarboxylic acid (5%) which proved difficult to purify.

Ethyl Ester of Phenazine-1,6-dicarboxylic Acid.—A solution of 1.5 g. (0.0056 mole) of phenazine-1,6-dicarboxylic acid in 20 ml. of concentrated sulfuric acid was quickly added to 125 ml. of absolute ethanol. The solution was allowed to cool slowly to room temperature and made basic (pH 9) with 1 *N* sodium hydroxide. Several extractions with moist ether afforded a 63% yield of the diethyl ester of phenazine-1,6-dicarboxylic acid, recrystallized from ethanol as greenish yellow needles, m.p. and mixture m.p. 143°.[18]

Pyrolysis of Phenazine-1,6-dicarboxylic Acid.—Phenazine 1,6-dicarboxylic acid (2 g., 0.0075 mole) was dissolved in 500 ml. of hot diphenyl ether and refluxed at 260° for 3 hr. with 25 g. of copper powder under nitrogen (oxygen-free). After cooling to room temperature, the dark solution was filtered

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(11) T. W. J. Taylor and W. Baker, "Sidewick's Organic Chemistry of Nitrogen," Oxford University Press, London, England, 1942, p. 52.

(12) B. K. Malaviya and S. Dutt, *Proc. Acad. Sci. United Provinces Agra and Oudh, India*, **4**, 319 (1935); *Chem. Abstr.*, **30**, 1956 (1936).

(13) For previous uses of solid oxidants for effecting phenol coupling see ref. 6, 8, 9, and 10.

(14) F. Kögl and B. Tönnis, *Ann.*, **486**, 497 (1932).

(15) Semimicro analyses by Alfred Bernhardt, Max Planck Institute Microanalytisches Laboratorium, Mülheim (Ruhr), Germany. Melting points are uncorrected.

(16) S. Ball, T. W. Goodwin, and R. A. Marton, *Biochem. J.*, **42**, 576 (1948).

(17) R. Kuhn and I. Hammer, *Ber.*, **83**, 413 (1950).

(18) L. Birkofer and A. Widmann, *ibid.*, **86**, 1295 (1953).

and extracted with 0.1 *N* sodium hydroxide. The organic layer was neutralized with 0.1 *N* hydrochloric acid, and chromatographed over alumina (grade III). Elution of the column with methanol afforded brownish needles of phenazine (33%) which recrystallized from ethanol as yellow needles, m.p. and mixture m.p. 171–171.5°.

The alkaline fraction was neutralized with 0.1 *N* hydrochloric acid and the precipitate isolated and recrystallized from acetone–water as yellow needles of phenazine-1-carboxylic acid (25%), m.p. and mixture m.p. 242–244°.

Phenazine-1-carboxamide (II) was prepared in 42% yield from phenazine-1-carboxylic acid according to the procedure of Kögl and Postowsky[19] employing thionyl chloride and ammonium hydroxide.

Acknowledgment.—This work was supported in part by Grant CA 06566 from the National Cancer Institute, U. S. Public Health Service.

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The Nef Reaction of 1,2,3,4,5,6,7,8,9,10,11,14-Dodecahydro-9-nitrophenanthrene

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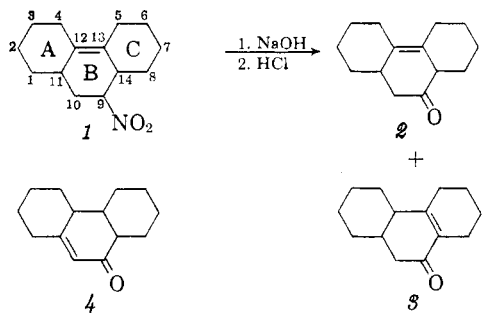
Recently it was reported that 1,2,3,4,5,6,7,8,9,-10,11,14 - dodecahydro - 9 - nitrophenanthrene (*1*) under Nef reaction conditions afforded a pair of isomeric ketones, 1,2,3,4,5,6,7,8,9,10,11,14-dodecahydro - 9 - ketophenanthrene (*2* and *3*, respectively, Fig. 1).[1] The conjugated ketone *3* also was



1 2 3 4

Figure 1

obtained by heating the Nef ketone *2* in an acidic medium. The assignment of the position of the olefinic linkage in the conjugated ketone *3* was based on the ultraviolet spectral characteristics of both the α,β-unsaturated ketone and the 2,4-dinitrophenylhydrazone derivative. The absorption contants of the ketone *3* were reported to consist of maxima at 235 mμ (ϵ 3880) and 280 mμ (ϵ 940) in isoöctane.

Interest in this problem was aroused when it was noted that the above maxima and extinction values were not consistent with structure *3*. In particular, the application of the usual correction factor for solvent change (isoöctane → ethanol, + 7 mμ) implied that the K-band for the ketone *3* was in the area of 242 mμ. A trisubstituted α,β-unsaturated ketone similar to *3* would be expected to absorb at about 247 mμ (Woodward's rules).[2] This discrepancy in the K-band's observed absorption maxima, as compared to the calculated value and the unusually low extinction sum, led to a reconsideration of the structure previously formulated for compound *3*. An interesting alternative to *3* was ketone *4* which was expected to possess an absorption around 240 mμ. If *4* was the correct structure for the conjugated ketone, then an abnormal reaction pathway must be invoked to rationalize the rearrangement of the double bond into the $\Delta^{10,11}$ position.

The nitro olefin *1* was resynthesized by a modification in the literature route. Specifically, bi-1-cyclohexen-1-yl was condensed with nitroethylene which was generated simultaneously *in situ* from 2-nitroethyl acetate and sodium acetate.[3] The well known disadvantages of pure nitroethylene were avoided by this indirect sequence. The presence of the double bond at $\Delta^{12,13}$ in compound *1* was assigned in the original work by ozonolysis experiments as well as by infrared arguments.

The n.m.r. spectrum of *1* revealed the proton on C-9 at 4.60 δ. This signal appeared to consist of a pair of doublets with couplings of 7 and 12 c.p.s., presumably due to spin-spin interaction with the two protons at C-10 as well as the proton at C-14. This information was used in an attempt to ascertain the preferred state of ring B and whether the nitro group existed in either the axial or equatorial configuration. If one assumed that the hydrogens C-11 and C-14 were *cis* to each other (*cis* addition *via* a Diels–Alder reaction) and were in the axial position, then the nitro group must be either in the axial or equatorial conformation. First, it may be said that any boat or half-boat form for ring B as visualized by Dreiding models appeared to be unstable and would collapse into the more rigid chair or half-chair form. More importantly, if the dihedral angles for the couplings between $J_{9,10}$ and $J_{9,14}$ were measured for the various axial or equatorial nitro configurations, then reference to the literature curve relating dihedral angle to $J_{H,H'}$ provided theoretical coupling values which were incompatible with the observed pattern.[4] Thus, the boat form of ring B was eliminated from further consideration.

Four possible chair or half-chair representations of ring B must be considered now. Number one of

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