Biosynthesis of Phenazines

ULRICH HOLLSTEIN* AND LARRY G. MARSHALL

Department of Chemistry, The University of New Mexico, Albuquerque, New Mexico 87106

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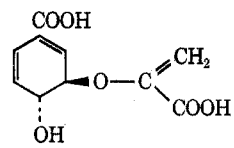
[G-^{14}C]- and [1,6-^{14}C]-DL-shikimic acid were fed to *Pseudomonas aureofaciens*, producer of phenazine-1-carboxylic acid, and to *Pseudomonas aeruginosa*, producer of pyocyanine. Incorporation was 16 and 9%, respectively, into phenazine-1-carboxylic acid and 12 and 7%, respectively, into pyocyanine. The products were degraded to phenazine + CO_2, 1-hydroxyphenazine, pyrazinetetracarboxylic acid, pyrazine, and carbon dioxide. The labeling data are in agreement with a pairing scheme of two shikimic acid molecules, whereby carbons 5 and 6 of shikimic acid become part of the center ring of the phenazine system.

During the past two decades about 30 phenazine (**1**) derivatives have been isolated from natural sources.[1,2] Microorganisms constitute the exclusive source of these compounds and there is considerable overlap in the production pattern: several microorganisms produce the same compound while also the same microorganism produces several phenazines. In addition, a dihydrophenazine derivative was found in several species of a green alga.[3] The isolated phenazines possess antibiotic properties, a feature which can be related to their interaction with deoxyribonucleic acid, presumably by intercalation of the planar aromatic ring system.[4]

Inspection of the presently known phenazine structures reveals a symmetrical pattern: C or O substituents are often found at the 1, 4, 6, and 9 positions with identical substituents often attached at the diagonally opposed positions 1, 6 (or 4, 9). This is also true for the algal dihydrophenazine derivative. The ring system as well as its unique substitution pattern has challenged several investigators to study the biosynthetic origin of the phenazine nucleus. The symmetrical element suggested strongly a generation of the phenazine ring system by dimerization of oppositely oriented aromatic precursors. The role of anthranilic acid as an attractive possibility for a coupling partner has been investigated.[5-9] Feeding phenazine producing microorganisms with ^{14}C-labeled anthranilic acid led to inconclusively low incorporation levels of label,[5,8] a result which may be interpreted in two opposite ways: either anthranilic acid is poorly transported through the cell wall or the low incorporation rates result from a nonspecific utilization of anthranilic acid or its breakdown products. Feeding of inactive anthranilic acid led invariably to inhibition of phenazine production.[6,7,9] This may indicate that the phenazines are formed from an anthranilic acid precursor whose formation is blocked through feedback inhibition by excess anthranilic acid.

We have presently reinvestigated the biosynthesis of phenazine-1-carboxylic acid (**2**), produced by *Pseudomonas aureofaciens*, and of pyocyanine (**3**), produced by *Pseudomonas aeruginosa*. If anthranilic acid causes indeed feedback inhibition it appeared worthwhile to test precursors of anthranilic acid for incorporation into the phenazine nucleus. We chose chorismic acid (**4**), intermediate between shikimic acid and anthranilic acid. This compound is an important branch point from which besides anthranilic acid a variety of other compounds is formed. [U-^{14}C]-Chorismic acid was prepared from [U-^{14}C]glucose using Gibson's mutant of *Aerobacter aerogenes*.[10] The compound was fed to *Ps. aureofaciens*. The low incorporation of label (0.23%) into phenazine-1-carboxylic acid left again unanswered the alternative whether chorismic acid or its degradation products are incorporated nonspecifically or whether there was a poor transport through the cell membrane. Indications that shikimic acid (see a) is incorporated had already been reported for iodinin, phenazine-1-carboxylic acid, chlororaphin (a complex of phenazine-1-carboxamide and its dihydro product), 2-hydroxyphenazine, and pyocyanine.[9,11-13] In our hands the incorporation of label from [G-^{14}C]-DL-shikimic acid into phenazine-1-carboxylic acid (**2**) amounted to 16%, substantially higher than previously reported for direct feeding experiments. The relative high activity made possible suitable dilution with inactive phenazine-1-carboxylic acid, obtained from 1-methylphenazine.[14,15]



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Similarly [1,6-^{14}C]-DL-shikimic acid was incorporated to the extent of 9%. The incorporation of [G-^{14}C]- and [1,6-^{14}C]-DL-shikimic acid into pyocyanine (**3**), produced by *Ps. aeruginosa*, was 12 and 7%, respectively. The two pigments were degraded to phenazine (**1**) + CO_2, 1-hydroxyphenazine (**5**), pyrazinetetracarboxylic acid (**6**), pyrazine (**7**), and CO_2.[9b,16] The labeling data, presented in Table I, are in agreement with incorporation of two shikimic acid residues. They are also in agreement with the finding that **2** is a precursor of

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(2) C. D. Tipton and K. L. Rinehart, Jr., *J. Amer. Chem. Soc.*, **92**, 1425 (1970).
(3) G. Aguilar-Santos, *J. Chem. Soc. C*, 842 (1970).
(4) U. Hollstein and R. J. VanGemert, Jr., *Biochemistry*, **10**, 497 (1971); U. Hollstein and P. L. Butler, *ibid.*, **11**, 1345 (1972).
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(7) M. E. Levitch and E. R. Stadtman, *Arch. Biochem. Biophys.*, **106**, 194 (1964).
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(10) F. Gibson, *Biochem. J.*, **90**, 256 (1964).
(11) P. C. Chang and A. C. Blackwood, *Can. J. Biochem.*, **46**, 925 (1968).
(12) M. E. Levitch and P. Rietz, *Biochemistry*, **5**, 689 (1966).
(13) W. M. Ingledew and J. J. R. Campbell, *Can. J. Microbiol.*, **15**, 535 (1968).
(14) U. Hollstein, *J. Heterocycl. Chem.*, **5**, 299 (1968).
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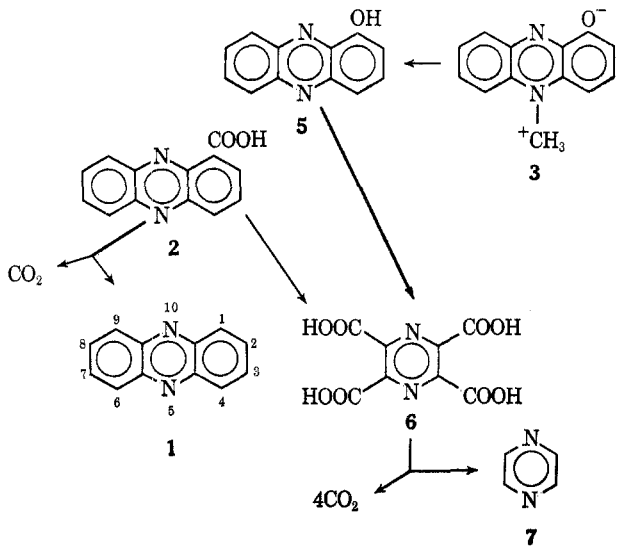
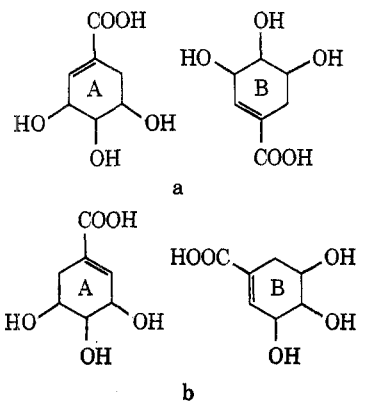
(16) D. L. Vivian, *Nature (London)*, **178**, 753 (1956).



3.[17,18] Our data are not in agreement with the pairing arrangements based on labeling data in studies on the biosynthesis of iodinin[9b,19] (a and b). A salient point

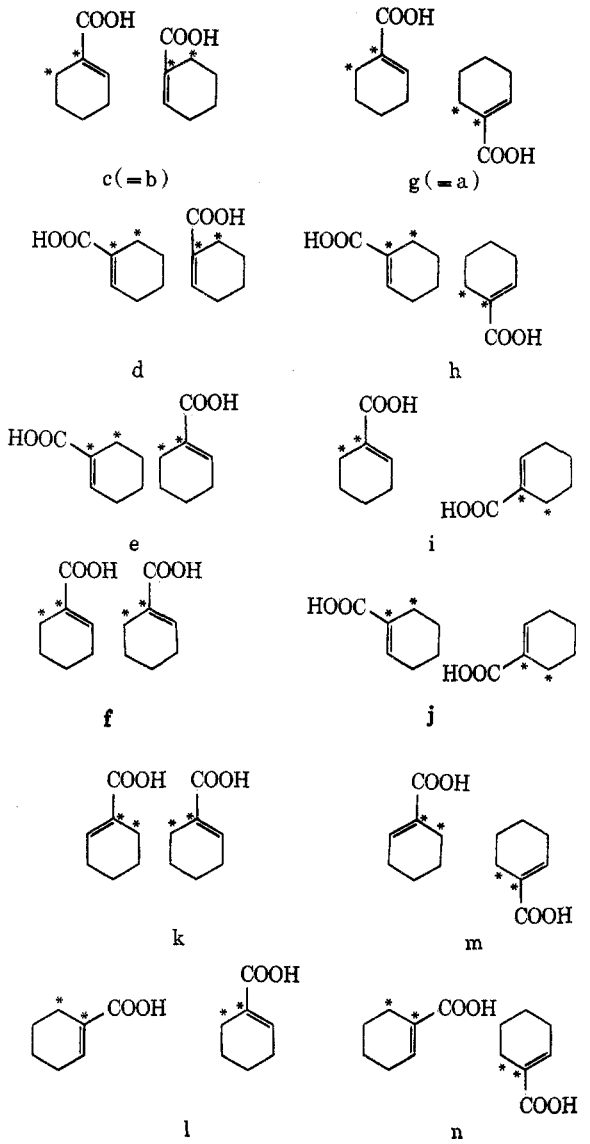


in the iodinin studies is that one of the hydroxyls is present before formation of the phenazine system.

A detailed analysis[20] of the problem shows that there are eight possible pairing schemes of two shikimic acid molecules which accommodate the labeling data in iodinin[9b] (c–j; hydroxyls have been omitted for clarity).

There are four pairing schemes of two shikimic acid molecules which accommodate the labeling data for **2** and **3** in the present work (k–n).

For kinetic reasons the two nitrogen bridges are formed in different reaction steps. After formation of the first nitrogen bridge, either ring can rotate 180° around the N–C bond prior to locking through the second nitrogen bridge. Thus, there is a correlation between the schemes of the set c–j with those of the set k–n. Furthermore, the labeling data indicate strongly that the phenazine system is formed from two identical units so that no dilution in specific activity occurs between the one and the other. It is significant that only e, which correlates with m, satisfies this condition.



On this basis, the biosynthetic pathway shown on page 3512 is proposed.

Nitrogen enters shikimic acid at the 5 position. The first nitrogen bridge is formed by attack of the nitrogen in one unit on the 6 position of the other unit. Rotation of one ring around the N–C bond and cyclization between N and C_4 leads, after aromatization, to 6-hydroxyphenazine-1-carboxylic acid and subsequently to iodinin.[19] Cyclization between N and C_6 leads to phenazine-1,6-dicarboxylic acid and subsequently to **2** and **3**.[17,18] The latter ring closure would also be required to explain the formation of several other 1,6-dicarbon substituted natural phenazines, such as griseolutin A and B,[21] phenazine-1,6-dicarboxylic acid,[1] lomofungin,[2] and caulerpin.[3]

It has been shown that ^{2}H-labeled phenazine-1,6-dicarboxylic acid is not incorporated into pyocyanin,[18] as would be required by our proposed pathway. Also, phenazine-1,8-dicarboxylic acid, whose formation is suggested by our pathway, has not so far been isolated. These negative results must, however, be interpreted with caution.

Although the original idea of a coupling of two aromatic precursors, such as anthranilic acid, must now

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(18) M. E. Flood, R. B. Herbert, and F. G. Holliman, *J. Chem. Soc., Perkin Trans. 1*, **1**, 622 (1972).

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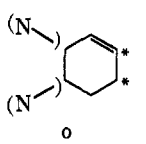
(20) We gratefully acknowledge valuable suggestions made by Professor F. G. Holliman, which led to this analysis.

(21) K. Yakishita, *J. Antibiot. (Tokyo)*, **13A**, 83 (1960).

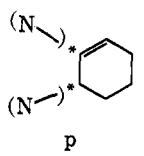
TABLE I
LABELING RESULTS

	Feeding with [G-^{14}C]Shikimic acid			Feeding with [1,6-^{14}C]Shikimic acid							
						% calcd					
Compd	dpm/mmol	% found	% calcd[a]	dpm/mmol	% found	k–n	a, b	*b*	*c*	*d*	*e*
Phenazine-1-carboxylic acid	230,000	100	100	64,000	100	100	100	100	100	100	100
CO_2 (from C_1-COOH)	25,100	10.9	7.7	1,690	2.6	0	0	0	0	0	0
Phenazine	205,000	88.4	92.3	59,000	92.2	100	100	100	100	100	100
Pyrazine-tetracarboxylic acid	151,000	65.7	61.5	62,000	96.9	100	75	100	50	0	100
CO_2	73,500	32.0	30.8	29,000	45.3	50	50	50	50	0	0
Pyrazine	80,050	34.8	30.8	33,000	51.6	50	25	50	0	0	100
Pyocyanine	23,100	100	100	16,800	100	100	100	100	100	100	100
1-Phenazinol	21,900	94.9	100	16,100	95.8	100	100	100	100	100	100
Pyrazine-tetracarboxylic acid	13,300	57.6	66.7	14,900	88.7	100	75	100	50	0	100
CO_2	6,250	27.0	33.3	7,170	42.7	50	50	50	50	0	0
Pyrazine	7,340	31.7	33.3	8,440	50.2	50	25	50	0	0	100

[a] For pairing of one or two shikimic acid molecules. [b] For incorporation of one shikimic acid molecule positioned as ring A in a. [c] For incorporation of one shikimic acid molecule positioned as ring B in a. [d] For incorporation of one shikimic acid molecule positioned as o.



o



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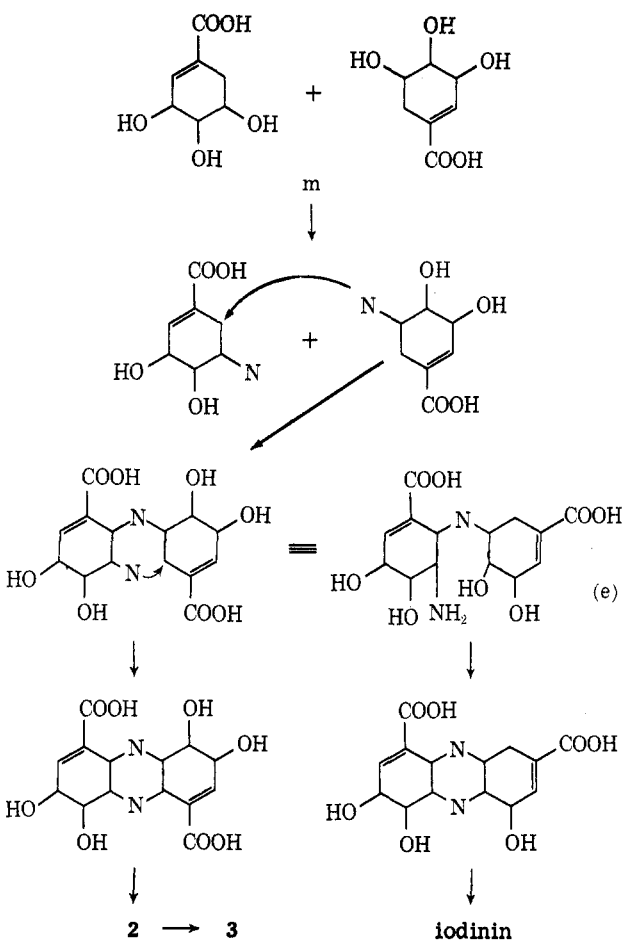
[e] For incorporation of one shikimic acid molecule positioned as p.



be abandoned, the possibility of an aromatic oxidative coupling between N and C is still an open question. The mechanism of phenazine formation is contrasted by the pathway leading to microbial phenoxazines, which are generated by aromatic oxidative coupling of two *o*-hydroxyaniline derivatives.[22]

Experimental Section

Melting points were obtained on a Kofler hot stage apparatus. Counting was done with a Beckman liquid scintillation spectrometer. Ultraviolet determinations were made with a Cary recording spectrophotometer, Model 14. Infrared spectra were determined with a Perkin-Elmer Model 621 grating spectrophotometer. Vapor phase chromatography was done with a Varian Aerograph Model 700.

Microorganisms.—With the single substitution of Tryptone (Difco) for Peptone (Difco), the storage slants, inoculum medium, and production medium were taken from the literature for *Pseudomonas aureofaciens*[23] and for *Pseudomonas aeruginosa*.[24] *Ps. aureofaciens*, ATCC 13985, and *Ps. aeruginosa*, ATCC 10145, were started from lyophilized cultures on storage slants. The integrity and viability of the cultures was preserved by monthly transfers to new slants. In both cases, loop inoculations from these slants were used to start several 250-ml inoculum flasks, each containing 100 ml of inoculum medium, which were grown on an Eberbach shaker rotating through an orbit of 2 in., 60 times per minute. Growth was allowed to continue until an arbitrarily specified optical density could be visually determined. With *Ps. aeruginosa*, the optical density was allowed to become twice that of *Ps. aureofaciens*. Two milliliters of *Ps. aeruginosa* inoculum medium were used to inoculate 1 l. of the production medium in a 2800-ml Fernbach flask.

Growth Curves.—With sterile technique, 10-ml aliquots were regularly withdrawn from the *Ps. aureofaciens* production medium for determination of cell density. The relative number of cells present in the aliquot was determined using a spectrophotometer by measuring the absorbance at 500 nm. Production of **1** was measured after extraction of a 5-ml aliquot of the bacterial suspension with chloroform as a change in absorbance at the $\lambda_{max}^{CHCl_3}$ of 370 nm. The maximum rate of phenazine-1-carboxylic acid production per cell was then determined by plotting the phenazine-1-carboxylic acid production rate per cell *vs.* time. This

(22) E. Katz and H. Weissbach, *J. Biol. Chem.*, **237**, 882 (1962).
(23) W. C. Haynes, F. H. Stodola, J. M. Locke, T. G. Pridham, H. F. Conway, V. E. Sohns, and R. W. Jackson, *J. Bacteriol.*, **72**, 415 (1956).
(24) L. H. Frank and R. D. DeMoss, *ibid.*, **77**, 776 (1959).

occurred at 16 hr at 28.5°. The beginning of pyocyanine production in the flasks of *Ps. aeruginosa* was visually determined to coincide with a sudden increase in optical density at 14 hr of growth at 33°. The intensity of blue color in the flasks reached a plateau at approximately 40 hr.

Labeled Feeding.—[G-^{14}C]-DL-Shikimic acid (2 μCi) (New England Nuclear, 1.86 mCi/mmol) or 1 μCi of [1,6-^{14}C]-DL-shikimic acid[25] were added under sterile conditions to each production flask of *Ps. aureofaciens*, which was grown for 12 hr at 28.5°. Growth was continued for 12 hr.

G-^{14}C-Labeled shikimic acid (2 μCi) or 1 μCi of [1,6-^{14}C]-shikimic acid were fed to each flask of *Ps. aeruginosa* after 14 hr of growth at 33°. Growth was continued for another 26 hr.

Pigment Extraction and Purification.—With *Ps. aureofaciens* each liter of bacterial medium was extracted three times with 500 ml of chloroform after the pH was adjusted to 5 with hydrochloric acid. Crude **2** (100 mg) was obtained after evaporation of chloroform and chromatographed on a 20 × 400 mm Florisil column. Elution was started with chloroform and gradually changed to 100% methanol. **2** comes down as a yellow-green band in the 50–100% methanol fractions. The fractions were determined spectrophotometrically, combined, and evaporated to dryness. This material, which did not melt below 360°, was presumably the Mg salt of phenazine-1-carboxylic acid. It was dissolved in 6 *N* HCl and, after adjustment of the pH to 3–5 with alkali, phenazine-1-carboxylic acid, mp 242° (reported[26] mp 243°), could be extracted with chloroform. The yield per liter of bacterial medium was between 25 and 50 mg. This material was radioactive. It was diluted 5–20 times with inactive phenazine-1-carboxylic acid and further purified by recrystallization from isopropyl alcohol to constant specific activity.

From *Ps. aeruginosa* pyocyanine was obtained by three extractions with chloroform at pH 7. The extract was evaporated below 30° in order to prevent decomposition. Crude pyocyanine (80 mg) was purified on a 20 × 300 mm silica column (G. F. Smith, 50–200 mesh). Elution with 200 ml of chloroform brought down one yellow band. Through gradual increase to 25% methanol another yellow band and blue pyocyanine were eluted successively. The yield per liter of bacterial medium was 11 mg. This material was radioactive. It was diluted five times with inactive pyocyanine and recrystallized from water to constant specific activity. The melting point was 131° (reported[27] mp 133°).

Scintillation Solutions.—Pyocyanine, phenazine-1-carboxylic acid, and their degradation products 1-phenazinol, phenazine, pyrazinetetracarboxylic acid, and pyrazine were counted in 10 ml of a solution containing 3 g of PPO (Amersham-Searle, Scintillation Grade), 0.25 g of POPOP (Nuclear-Chicago, Scintillation Grade), 500 ml of methanol (A. R.), and 500 ml of toluene (A. R.). Carbon dioxide was counted in a mixture of 3 ml [0.75 ml of methanol (A. R.), 0.75 ml of phenethylamine (Packard), and 1.5 ml of toluene (A. R.)] and 7 ml (6 g of PPO, 0.5 g of POPOP, 1000 ml of toluene).[28] Counting efficiency in each of these scintillation solutions was determined with standards. They were 78 and 80%, respectively.

Determination of Specific Activity.—Specific activities in dpm/mmol were determined in all cases by extrapolating to zero concentration the logarithm of the specific activity as a function of concentration. The effect of quenching, which was pronounced for phenazine-1-carboxylic acid, phenazine, and 1-phenazinol, could thus be eliminated. Phenazine-1-carboxylic acid from [U-^{14}C]- and [1,6-^{14}C]shikimic acid showed an incorporation of label of 16 and 9%, respectively. For pyocyanin the corresponding values were 12 and 7%, respectively.

[U-^{14}C]Chorismic acid (4).—The labeled compound was prepared with *Aerobacter aerogenes* mutant 62-1.[29] Gibson's[13] procedure for inactive **4** was generally followed. To 6 l. of broth, 108 g of glucose, containing 48 μCi of [U-^{14}C]glucose, was fed. ^{14}C-Labeled **4** (3.35 g) with a spectral purity of 98% [$\lambda_{max}^{H_2O}$ 275 nm (ϵ 2630) for the hemihydrate, mol wt 235], mp 114–116° (reported[30] mp 112° dec for the hemihydrate), was obtained. The specific activity, 3.05 × 10^4 dpm/mmol of carbon, showed that all its activity was derived from [U-^{14}C]glucose.

Phenazine (1) + CO_2.—Phenazine-1-carboxylic acid (10 mg) in admixture with an equal amount of copper(II) chromite[31] was heated to 290° in a porcelain boat placed in a 10-mm-diameter tube. A stream of CO_2-free nitrogen was passed through the tube and through five successive traps each containing 7 ml of a 0.2 *N* barium chloride and a 0.2 *N* sodium hydroxide solution. **1** sublimed into the cold region of the tube and was removed with methanol (2.5 ml). The concentration was determined spectrophotometrically, using λ_{max}^{MeOH} 248 nm (ϵ 124,000), 362 (13,200),[32] an aliquot was dried, mp 171° (reported[27] mp 171°), and the methanol solution was, after proper dilution with scintillator solution, used for counting. Barium carbonate was filtered, dried at 110°, and weighed, giving 95% of the theoretical amount. An aliquot of $BaCO_3$ was suspended in 5 ml of CO_2-free water in a three-neck 50-ml flask closed by a septum. The second neck carried a tube and valve and the third neck was connected to a 50-ml flask containing 8 ml of a 1:1:2 mixture of phenethylamine (Packard), methanol (A. R.), and toluene (A. R.).[28] The entire system was purged with nitrogen and closed off. Carbon dioxide was generated by addition through the septum of a fivefold excess of hydrochloric acid; 24 hr was allowed for absorption of carbon dioxide by the magnetically stirred phenethylamine solution. The efficiency of the entire system had previously been determined by using a known activity of barium carbonate and found to be 99.1%. The phenylethylamine solution was, after proper dilution with scintillator solution, used for counting.

Pyrazinetetracarboxylic Acid (6) from Phenazine-1-carboxylic Acid.—Phenazine-1-carboxylic acid (100 mg) was dissolved in 2 ml of 1% aqueous potassium hydroxide. Hot 17% potassium permanganate (5 ml) was added with stirring over a period of 5 min. After additional heating at 90° for 2 hr, excess permanganate was destroyed by dropwise addition of ethanol. Manganese dioxide was removed by filtration and washed with hot water to give a total filtrate of 4 ml, which was passed through an ion exchange column (IR-120-CP, H^+ form, 35-ml bed volume) and eluted with distilled water. Elution of **6** was followed in the ultraviolet at its λ_{max} of 295 nm. Fractions containing the oxidation product were stored at 5° or were immediately evaporated to dryness, yielding 80% of the crude product. After hot filtration and several recrystallizations from 20% hydrochloric acid to constant specific activity, the melting point was 205–208° (reported[33] mp 205° dec), $\lambda_{max}^{H_2O}$ 291 nm (ϵ 8100) [reported[34] 291 nm(ϵ 8310).

Pyrazine (7).—The method described in the literature[9b] could not be repeated. Extremely volatile **7** is difficult to condense and its high water solubility precludes recrystallization of small quantities from this solvent. A modification of the isolation procedure is described here. To a 10-ml pear-shaped flask, 100 mg of **6** and 5 ml of diethyl phthalate [Fisher Scientific, pure, redistilled at 148° (2 mm)] were added. Upon heating at 190° for 1 hr, **7** distilled into the neck of the flask, from which it was carried through a connected tube by a gentle stream of dry nitrogen into a 10-mm-diameter U-shaped tube. The latter contained 5 ml of methanol and was cooled in Dry Ice. The pyrazine solution was rinsed out with additional methanol and diluted to a known volume. The yield was 17% as determined spectrophotometrically using the reported[35] values λ_{max}^{EtOH} 261 nm (ϵ 6030), 310 (850). Due to the volatility of **7**, the purity of small samples could not be efficiently determined by the melting point. However, in addition to spectrophotometric determination the identity of the trapped compound was established by its infrared spectrum ($CHCl_3$) and its gas chromatographic behavior (20% SF 96, 100°) in comparison with an authentic sample (Aldrich analyzed).

Carbon Dioxide from Phenazinetetracarboxylic Acid (6).—**6** (10 mg) was decarboxylated with copper chromite as described for **2**. The specific activity was multiplied by four prior to comparison with the other degradation products because four molecules of carbon dioxide are obtained from each molecule of **6**.

1-Hydroxyphenazine (5).—**3** (100 mg) was dissolved in 120 ml of water, 8.4 ml of 8 *N* sodium hydroxide was added to the solution, and the mixture was allowed to stand for 16 hr at room

(25) We are indebted to Dr. H. G. Floss for a generous sample of 1,6-^{14}C-shikimic acid.

(26) K. Isono, K. Anzai, and S. Suzuki, *J. Antibiot. (Tokyo)*, **11A**, 264 (1959).

(27) "The Merck Index," 8th ed, Merck and Co., Rahway, N. J., 1968.

(28) F. H. Woeller, *Anal. Biochem.*, **2**, 508 (1961).

(29) We are indebted to Dr. T. I. Baker for this organism.

(30) J. M. Edwards and L. M. Jackman, *Aust. J. Chem.*, **18**, 1227 (1965).

(31) "Organic Syntheses," Collect. Vol. II, Wiley, New York, N. Y., 1959, p 142.

(32) "UV Atlas of Organic Compounds," Vol. I, Butterworths, London, 1966, p H21/1.

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(35) S. F. Mason, *J. Chem. Soc.*, 1247 (1959).

temperature. The maroon solution was filtered, and the filtrate was extracted with an equal volume of ether. The organic layer was discarded. After the aqueous layer was acidified with glacial acetic acid, it was extracted twice with equal volumes of ether. The ether layers were combined to yield crude **5**. Purification was achieved by sublimation at 115° (0.1 mm). The yield varied from 50 to 70% and the compound melted at 155–158° (reported[16] mp 159–160°).

Pyrazinetetracarboxylic Acid (6) from 1-Hydroxyphenazine (5).—**5** (100 mg) was oxidized to **6** as described for **2**, in 70% yield.

Registry No.—**2**, 2538-68-3; **3**, 85-66-5; DL-shikimic acid, 138-59-0.

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δ-Chloro Alcohols and Tetrahydrofurans from Primary and Secondary Alkyl Hypochlorites[1]

CHEVES WALLING* AND DOUGLAS BRISTOL

Department of Chemistry, University of Utah, Salt Lake City, Utah 84112

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The photodecomposition of primary and secondary alkyl hypochlorites in the presence of *cis*- or *trans*-dichloroethylene and similar chloro olefins which act as chlorine atom traps leads to greatly improved yields of δ-chloro alcohols (or tetrahydrofurans after treatment with base). Yields of 50–90% are obtained from a number of hypochlorites, and the method appears to offer substantial advantages over lead tetraacetate oxidations and other more complex techniques for carrying out intramolecular alkoxy radical reactions.

Tertiary alkyl hypochlorites containing a side chain of at least four carbons are readily converted to δ-chloro alcohols on irradiation or treatment with a free-radical source[2,3] *via* a sequence involving intramolecular hydrogen abstraction. The reaction has been

$$\underset{\text{A}}{\text{RCH}_2\text{CH}_2\text{CH}_2\text{CR}_1\text{R}_2\text{O}\cdot} \longrightarrow \underset{\text{B}}{\text{RCHCH}_2\text{CH}_2\text{CR}_1\text{R}_2\text{OH}} \quad (1)$$

$$\text{B} + \text{RCH}_2\text{CH}_2\text{CH}_2\text{CR}_1\text{R}_2\text{OCl} \longrightarrow \text{RCHCH}_2\text{CH}_2\text{CR}_1\text{R}_2\text{Cl} + \text{A} \quad (2)$$

much less successful with primary and secondary hypochlorites, presumably because of competing steps initiated by α-hydrogen attack.

$$\text{X}\cdot + \text{RCH}_2\text{OCl} \longrightarrow \text{HX} + \text{R}\dot{\text{C}}\text{HOCl} \quad (3)$$
$$(\text{X} = \text{Cl}, \text{RCH}_2\text{O}\cdot)$$

$$\text{R}\dot{\text{C}}\text{HOCl} \longrightarrow \text{RCHO} + \text{Cl}\cdot \quad (4)$$

$$\text{HCl} + \text{RCH}_2\text{OCl} \longrightarrow \text{RCH}_2\text{OH} + \text{Cl}_2 \quad (5)$$

$$\text{Cl}_2 + 2\text{RCH}_2\text{OH} \longrightarrow 2\text{RCHO} + 2\text{HCl} \quad (6)$$

$$\text{Cl}_2 + \text{RH} \longrightarrow \text{RCl} + \text{HCl} \quad (7)$$

In short, molecular chlorine is introduced into the system, and is continually regenerated *via* eq 5–7, making the reaction no longer intramolecular and specific. In fact, the aldehydes produced are chlorinated further to acid chloride, which ends up largely as ester.

$$\text{RCHO} + \text{Cl}_2 \longrightarrow \text{RCOCl} + \text{HCl} \quad (8)$$

$$\text{RCOCl} + \text{RCH}_2\text{OH} \longrightarrow \text{RCOOCH}_2\text{R} + \text{HCl} \quad (9)$$

Modest yields of δ-chloro alcohols have only been obtained under special conditions (the presence of $NaHCO_3$ and reflux under reduced pressure) designed to remove HCl and thus prevent eq 5.[2,4]

Adventitious chlorine sometimes complicates the reactions of tertiary hypochlorites by similarly introducing chlorine atom chains, but here the effect can be largely eliminated[5] by adding olefins with strong electron-withdrawing groups which are inert toward alkoxy radicals but act as chlorine atom traps.

$$\text{Cl}\cdot + \text{CHCl}{=}\text{CCl}_2 \longrightarrow \text{CHCl}_2\dot{\text{C}}\text{Cl}_2 \quad (10)$$

$$\text{CHCl}_2\dot{\text{C}}\text{Cl}_2 + \text{ROCl} \longrightarrow \text{CHCl}_2\text{CCl}_3 + \text{RO}\cdot \quad (11)$$

This paper describes application of the same technique to the intramolecular chlorination of long-chain primary and secondary hypochlorites, and demonstrates that the reaction can be made to give high yields of δ-chloro alcohols (or, subsequently, tetrahydrofurans), making it a remarkably simple and specific synthetic procedure.

Results

Initial experiments were carried out with *n*-butyl hypochlorite, irradiated under N_2 or in sealed, degassed tubes, and are summarized in Table I. In the absence of a chlorine atom trap yields are low and erratic and a wide spectrum of products is produced. In the presence of trichloroethylene, yields of 4-chloro-1-butanol rise to 50–60%, and the remaining products are quite cleanly 1-butanol, butyl butyrate, and pentachloroethane, giving overall material balances of 90–100%. Since the stoichiometry of the ester-forming reaction should be eq 12, a small amount of random chlorination

$$4\text{RCH}_2\text{OCl} + 2\text{C}_2\text{HCl}_3 \longrightarrow \text{RCOOCH}_2\text{R} + 2\text{ROH} + 2\text{C}_2\text{HCl}_3 \quad (12)$$

must be taking place as well. In the presence of olefin, base appears to have no further beneficial effect, and yields decrease only slightly when the temperature is raised from 0 to 50°.

In Table II the reaction is extended to 1-pentyl hypochlorite and several radical traps investigated. Yields are higher than in Table I, presumably because of the greater reactivity of secondary hydrogens in the intramolecular process, and *cis*- or *trans*-dichloroethylene appear to be the most efficient chlorine atom

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