(±)-11,15-Dideoxyprostaglandin E₂ (2).—To a suspension of 393 mg (1 mmol) of tri-*n*-butylphosphine–copper(I) iodide (freshly prepared) in 5 ml of anhydrous ether cooled to −78° under an atmosphere of nitrogen was added with stirring a solution of 1-lithio-*trans*-oct-1-ene in anhydrous ether [prepared by treatment of 1-iodo-*trans*-oct-1-ene (476 mg, 2 mmol) in 3 ml of anhydrous ether cooled to −78° with 2.86 ml of *tert*-butyllithium (1.44 *M* in *n*-pentane) followed by stirring for an additional 2 hr]. The resulting mixture was stirred for approximately 45 min at −78° and then was treated with 220 mg (1 mmol) of cyclopentenone **7b** in 1 ml of ether. After the addition was complete, the reaction was warmed to 0° and stirring at that temperature was continued for 2 hr. The reaction was quenched by the addition of aqueous ammonium sulfate and the product was isolated by extraction with ether. Purification by preparative tlc afforded 154 mg (47%) of (±)-11,15-dideoxy-PGE₂ methyl ether [ν_max (CHCl₃) 1740, 970 cm⁻¹; nmr (CCl₄) δ 3.62 (s, 3 H, CH₃), 5.20–5.60 (m, 4 H, olefinic); *m/e* 334].

A solution of (±)-11,15-dideoxy-PGE₂ methyl ester (25 mg) in 1.2 ml of THF and 0.5 ml of water containing 1.0 ml of 0.1 *N* NaOH was stirred at room temperature for 24 hr. Work-up afforded 20 mg of pure (±)-11,15-dideoxy-PGE₂ (2) which was chromatographically identical in several solvent systems with a sample kindly provided by Dr. M. J. Weiss (Lederle Laboratories).

(±)-11-Deoxyprostaglandin E₂ Methyl Ester (1).—To a mixture of 393 mg (1 mmol) of tri-*n*-butylphosphine–copper(I) iodide in 7 ml of anhydrous ether cooled to −78° maintained under a nitrogen atmosphere was added with stirring a solution of 3-(α-ethoxy)ethoxy-1-lithio-*trans*-oct-1-ene in anhydrous ether [prepared by treatment of 3-(α-ethoxy)ethoxy-1-iodo-*trans*-oct-1-ene⁸ (652 mg, 2 mmol) in 5 ml of anhydrous ether cooled to −78° with 2.86 ml of *tert*-butyllithium (1.44 *M* in *n*-pentane); the solution was maintained at −78° for 1.75 hr]. The resulting mixture was stirred at −78° for 60 min and then was treated with 222 mg (1 mmol) of cyclopentenone (**7b**) in 5 ml of anhydrous ether. The reaction mixture was stirred at −78° for an additional 30 min and then warmed to 0°, where stirring was continued for 1.5 hr. The reaction was quenched by the addition of aqueous ammonium sulfate and the product was extracted with ether. The combined ether extracts were dried (MgSO₄), filtered, and concentrated. The residue was chromatographed on silica gel. There was obtained 90 mg (21%) of product plus 30 mg of recovered **7b**.

A solution of the above material (90 mg) in 0.2 ml of THF was added to 1.75 ml of acetic acid–water (65:35). The resulting solution was heated at 39° for 6 hr. After cooling, the product was isolated by extraction with ether. The combined ether layers were washed with saturated sodium bicarbonate solution and water and dried over anhydrous MgSO₄. Removal of the solvent under reduced pressure afforded 56 mg of (±)-11-deoxy-PGE₂ methyl ester (1) and (±)-11-deoxy-15-epi-PGE₂ methyl ester (8), which appeared to be present in equal amounts as indicated by tlc. Chromatography by preparative thin layer with ethyl acetate–hexane (1:2) afforded 18 mg of (±)-11-deoxy-PGE₂ methyl ester [ν_max CHCl₃ 3610, 3460, 1730, 970 cm⁻¹; nmr (CCl₄) δ 5.20–5.70 (m, 4 H, olefinic), 4.00 (m, 1 H), 3.62 (s, 3 H, OCH₃); *m/e* 350]. (±)-11-Deoxy-PGE₂ methyl ester was chromatographically identical in several solvent systems with a sample kindly provided by Dr. J. F. Bagli (Ayerst Laboratories).

Registry No.—1, 35120-22-0; 2, 40098-57-5; 4, 34638-26-1; 5a, 40899-59-0; 5b, 40899-60-3; 6a, 40899-61-4; 6b, 40899-62-5; 7a, 40899-63-6; 7b, 38698-54-3; triphenylphosphonioopentanoic acid, 39968-97-3; 1-lithio-*trans*-oct-1-ene, 37730-25-9; 3-(α-ethoxy)ethoxy-1-lithio-*trans*-oct-1-ene, 38380-59-5.

(8) Prepared by treatment of *trans*-1-iodo-1-octen-3-ol²ᵇ in methylene chloride containing a catalytic amount of *p*-toluenesulfonic acid at 0° with a slight excess of freshly distilled ethyl vinyl ether.

Biosynthesis of Phenazines. II. Incorporation of [6-¹⁴C]-D-Shikimic Acid into Phenazine-1-carboxylic Acid and Iodinin[1]

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Previously[1] we have shown that shikimic acid is incorporated into phenazine-1-carboxylic acid (**1a**) and into pyocyanine (**1b**). Degradation of these metabolites[1] from feeding with [G-¹⁴C]shikimic acid (**2**) was in agreement with incorporation of the intact shikimic acid molecule. Results from [1,6-¹⁴C]shikimic acid feedings narrowed the number of pairing possibilities of two shikimic acid molecules down to four.[1]
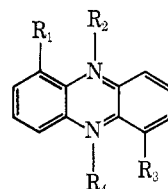
1a, R₁ = COOH; R₂ = R₄ = lone pair; R₃ = H
b, R₁ = O⁻; R₂ = lone pair; R₃ = H; R₄ = ⁺CH₃
c, R₁ = R₃ = OH; R₂ = R₄ = →O

We have now obtained a sample of [6-¹⁴C]-D-shikimic acid,[2] which allowed us to narrow down the number of possible pairing schemes. Feeding of this precursor to *Pseudomonas aureofaciens* led to a 36% incorporation into phenazine-1-carboxylic acid. The labeling data, as shown in Table I, further narrow the number of pairing schemes of two shikimic acid molecules from four to two, *viz.*, d and e. It was hoped that feeding with [¹⁴C₆]-D-shikimic acid would also allow us to distinguish between the pairing schemes proposed for iodinin by Gerber[3] (a), Holliman[4] (b), and us[1] (c),
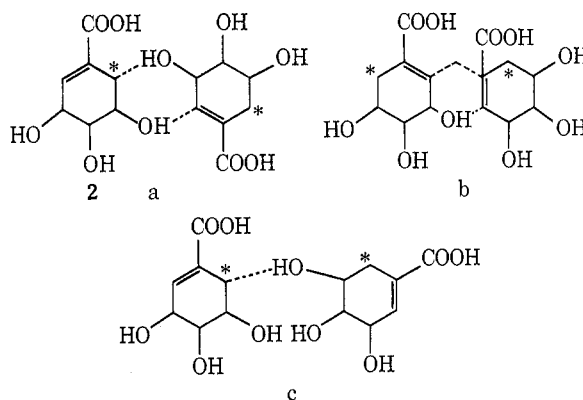
since our previous data[1] were not in agreement with the pairing schemes suggested in ref 3 and 4.

The three pairing schemes a–c can be distinguished by 6-monolabeled shikimic acid (Table II). This was

(1) Part I: U. Hollstein and L. G. Marshall, *J. Org. Chem.*, **37**, 3510 (1972).
(2) K. H. Scharf and M. H. Zenk, *J. Label. Compounds*, **7**, 525 (1971).
(3) M. Podojil and N. N. Gerber, *Biochemistry*, **9**, 4616 (1970).
(4) R. B. Herbert, F. G. Holliman, and D. N. Ibberson, *J. Chem. Soc., Chem. Commun.*, 355 (1972).

TABLE I

LABELING RESULTS

	Feeding of [6-^{14}C]-D-shikimic acid to					
	Ps. aureofaciens			*Chr. iodinum*		
Compd	dpm/mmol	Found, %	Calcd,[a] %	dpm/mmol	Found, %	Calcd,[b] %
Phenazine-1-carboxylic acid	303,000	100	100			
CO$_2$ (from C$_1$-COOH)	680	0.2	0			
Phenazine	291,000	96.1	100			
Iodinin				448,000	100	100
1,6-Dihydroxyphenazine				443,000	96.7	100
Pyrazinetetracarboxylic acid	302,000	99.6	100	455,000	101.6	100
Pyrazine	309,000	101.6	100	464,000	103.6	100
CO$_2$	3,560	1.2	0	55,680	12.4	0

[a] For pairing schemes d and e. [b] For pairing schemes d–i.

TABLE II

DIFFERENT PAIRING SCHEMES FOR IODININ

	Specific activity (%) for		
	a (Gerber)	b (Holliman)	c (Hollstein)
Iodinin	100	100	100
Pyrazinetetra- carboxylic acid	50	50	100
Pyrazine	50	0	50
CO$_2$	0	50	50

not possible with the 1,6-dilabeled precursor. Our data (Table I) clearly show that the pairing scheme cannot be a, b, or c. Six pairing schemes (d–i) can be written to accommodate our data. Of these, only d and c are identical with those deduced for phenazine-1-carboxylic acid. Assuming that the hydroxyls in iodinin are generated in an identical manner in both rings, schemes d, f, g, h, and i must be excluded. It


d   e   f   g   h   i
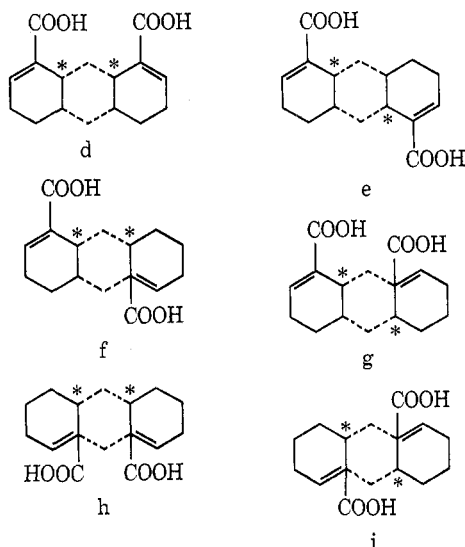
is to be noted that none of the set d–i can accommodate Gerber's data for the [1,6-^{14}C]shikimic acid generated iodinin.[3]

Recently it was demonstrated that chorismic acid[5] (3) is an intermediate in the pathway leading to pyocyanine and that it represents also the point in the general aromatic pathway at which the route to pyocyanine branches off.[6] It may be inferred that
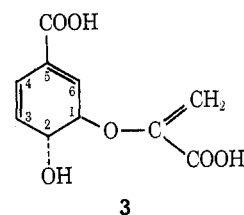
3

chorismic acid is also the precursor for phenazine-1-carboxylic acid since pyocyanine is generated from phenazine-1-carboxylic acid[7] and possibly for iodinin. Thus, the proposed schemes for iodinin where one of the hydroxyls comes from the C$_3$–OH in shikimic acid[1,4] are no longer valid. Chorismic acid has no hydroxyl at the position corresponding to C$_3$ of shikimic acid.

Based on the foregoing we propose that the biosynthesis of the phenazine skeleton proceeds *via* two identical C$_6$- or C$_1$-N-substituted chorismic acids. Introduction of nitrogen at C$_6$, either initially into chorismic acid or by a C$_1$-N-substituted chorismic acid during the formation of the tricyclic ring system, would be analogous to the formation of anthranilic acid from chorismic acid. Further work on these aspects is in progress.

Experimental Section

Melting points were obtained on a Kofler hot stage apparatus. Counting was done with a Beckman liquid scintillation spectrometer. Electronic spectral determinations were made with a Cary recording spectrophotometer. The degradation of phenazine-1-carboxylic acid, the determination of specific activities and various scintillation solutions have been previously described.[1]

Microorganisms and Pigment Production.—*Pseudomonas aureofaciens*, ATCC 13985, was maintained, grown, and extracted as described.[1] *Chromobacterium iodinum* was obtained from the collection of Dr. Waksman, strain 26, Rutgers University. From the same strain ATCC maintains *Ps. iodinum* 15728 (IMRU 26). The integrity and viability of the culture was preserved by monthly transfers to new slants. Storage slants were made of an aqueous solution of 1% glucose (Difco), 1% yeast extract (Difco), and 1.5% Agar (Difco). The production and inoculum medium consisted of the same solution without the Agar. Fifty milliliters of inoculum medium was autoclaved in a 250-ml flask, which was loop inoculated from the storage slant and placed on an Eberbach shaker, rotating through an orbit of 2 in., 60 times/min, at 26°. After growing for 24 hr, 2 ml of the inoculum solution was added to 650 ml of production medium of the same nutrient composition in a 2800-ml Fernbach flask. The characteristic purple color of iodinin appeared after about 50–60 hr. After a total production period of about 70 hr, the suspension was extracted with an equal

(5) The numbering conforms to the formulation α-(5-carboxyl-1,2-dihydro-2-hydroxyphenoxy)acrylic acid.

(6) R. P. Longley, J. E. Halliwell, J. J. R. Campbell, and W. M. Ingledew, *Can. J. Microbiol.*, **18**, 1357 (1972).

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

volume of chloroform, and the resulting emulsions were broken up by centrifuging. Per 650 ml of production medium about 110 mg of iodinin was obtained, $\lambda_{max}^{CHCl_3}$ 530 nm (ϵ 6300) [reported[8] 530 nm (ϵ 6340)] and mp 236° dec (reported[9] 224–225° dec).

Labeled Feeding and Extraction of Active Pigments.—[6-^{14}C]-D-Shikimic acid (1 μCi, 35.6 μCi/μmol) was fed in two equal portions under sterile conditions to two 1-l. production media of *Ps. aureofaciens* each in a 2800-ml Fernbach flask, which had been grown for 12 hr at 28.5°. Growth was continued for 12 hr and phenazine-1-carboxylic acid extracted and purified as described.[1] The yield was 151 mg. The material showed an incorporation of 36% (100 × total activity isolated over total activity fed). It was diluted 3.56 times in chloroform with inactive phenazine-1-carboxylic acid.

After 44 hr of growth 1 μCi of [6-^{14}C]-D-shikimic acid was added under sterile conditions in two equal portions to each of two 650-ml production media of *Chr. iodinum*, when the characteristic purple color of iodinin was not yet apparent. The color appeared at 46 hr. Growth was continued for another 32 hr and the pigment was extracted after a total of 78 hr: yield 206 mg, 34% incorporation of fed activity. The compound was diluted 2.00 times in pyridine with inactive iodinin, obtained from previous inactive productions.

1,6-Dihydroxyphenazine from Iodinin.—Iodinin (200 mg) in 100 ml of dioxane (AR) were added to 200 mg of reduced PtO$_2$ in 50 ml of dioxane. Reduction at atmospheric pressure and room temperature was complete in 30 min after an uptake of 3 mol of H$_2$. The colorless solution, presumably of 1,6-dihydroxy-5,10-dihydrophenazine, was filtered whereupon it rapidly turned yellow. Upon passing O$_2$ through the solution a golden yellow color was soon attained. Evaporation yielded 171 mg (98%) of gold-brown crystals of 1,6-dihydroxyphenazine, mp 271–278° (reported[10] 274°).

Pyrazinetetracarboxylic Acid from 1,6-Dihydroxyphenazine.—A 109-mg sample was oxidized in 2 ml of 1% KOH with 7.7 ml of 17% hot KMnO$_4$ as described,[1] in 45% yield.

Registry No.—1a, 2538-68-3; 1c, 68-81-5; 2, 138-59-0.

(8) N. Gerber and M. P. LeChevalier, *Biochemistry*, **3**, 598 (1964).

(9) H. P. Sigg, *Helv. Chim. Acta*, **50**, 716 (1967).

(10) H. Akabori and M. Nakamura, *J. Antibiotics (Tokyo)* Ser. A, **12**, 17 (1959).

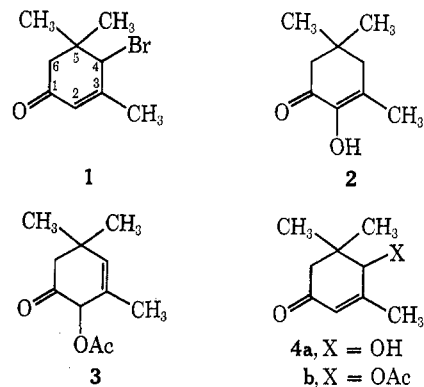# Nucleophilic Displacement Reactions on 4-Bromoisophorone

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Marx and coworkers[1] have recently reported their work of nucleophilic displacements on 4-bromoisophorone (1) with NaOH and silver acetate. They obtained the 2-substituted products (2 and 3) in addition to other materials and no 4-substituted derivatives (4a and 4b) as earlier reported.[2] Based upon the results of the earlier workers, we had hoped to prepare several 4-thio and 4-sulfonyl derivatives of isophorone

(1) J. N. Marx, A. W. Carnrick, and J. H. Cox, *J. Org. Chem.*, **37**, 2308 (1972).

(2) A. J. B. Edgar, S. H. Harper, and M. A. Kazi, *J. Chem. Soc.*, 1083 (1957).

1   2

3   4a, X = OH
           b, X = OAc

(5 and 6) *via* nucleophilic displacement upon 4-bromoisophorone as shown in Scheme I. The products obtained, however, were the 2-substituted materials 7 and 8.

SCHEME 1

1
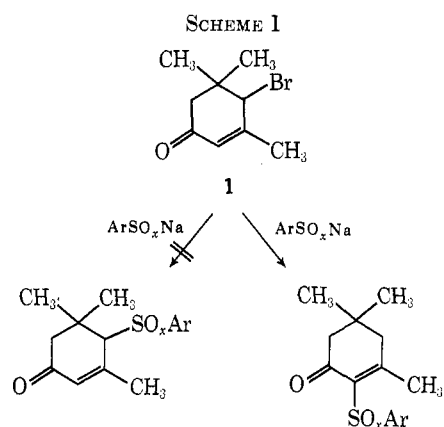
5, Ar = p-CH$_3$C$_6$H$_4$; x = 0   7, Ar = p-CH$_3$C$_6$H$_4$; x = 0
6, Ar = p-CH$_3$C$_6$H$_4$; x = 2   8, Ar = p-CH$_3$C$_6$H$_4$; x = 2

The structural assignments of these products were based on nmr and ir analyses and alternate synthesis. The nmr data given in the Experimental Section support the assignments made. The ir spectra of the 2-thio and 2-sulfonyl derivatives exhibited a carbonyl band at 1675–1680 cm^{-1} characteristic of a conjugated cyclohexenone.[3] The carbonyl band in 2-ethylsulfinylisophorone (11) appeared at 1650 cm^{-1}.

2-*p*-Toluenethioisophorone (7) was alternatively synthesized by reaction of sodium *p*-toluenethiolate with 2,3-isophorone oxide[4] (9) (Scheme II). Oxidation of 7 with *m*-chloroperbenzoic acid yielded 8. Tomoeda and coworkers[5] have published the synthesis and nmr spectra of 2-ethylthioisophorone (10) and therefore the preparation was repeated as shown in Scheme II for comparison of spectrum. The 2-ethylsulfinylisophorone (11) and 2-ethylsulfonylisophorone (12) were formed from 10 and the nmr spectra of these derivatives compared well with those of the corresponding *p*-tolyl analogs. Treatment of 4-bromoisophorone with sodium ethylthiolate gave isophorone and ethyl disulfide and no ethylthio-substituted isophorone (Scheme III), with displacement apparently occurring on the bromine and not on a carbon atom. Our work

(3) L. J. Bellamy, "The Infra-red Spectra of Complex Molecules," 2nd ed, Wiley, New York, N. Y., 1958, p 148.

(4) G. B. Payne, *J. Org. Chem.*, **24**, 719 (1959).

(5) M. Tomoeda, M. Inuzuka, T. Furuta, M. Shinozuka, and T. Takahashi, *Tetrahedron*, **24**, 959 (1968).