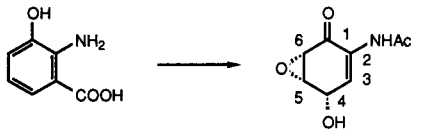
Biosynthesis of Antibiotic LL-C10037α: The Steps beyond 3-Hydroxyanthranilic Acid

Steven J. Gould,* Ben Shen, and Yvonne G. Whittle

Contribution from the Department of Chemistry, Oregon State University, Corvallis, Oregon 97331-4003. *Received September 22, 1988*

Abstract: The six steps from 3-hydroxyanthranilic acid to the epoxyquinol LL-C10037α, **1**, produced by *Streptomyces* LL-C10037 have been determined by whole-cell feedings with deuterated substrates and by cell-free studies. 3-Hydroxyanthranilic acid, **2**, is decarboxylated to 2-hydroxyaniline, **11**, and then oxidized to 2,5-dihydroxyaniline, **8**. Acetylation at nitrogen and oxidation afford acetamido-1,4-benzoquinone, **4**. A crude cell-free preparation has been found to epoxidize **4** to the epoxyquinone **16** in the presence of O_2 and either NaDH or NADPH. Reduction of **16** yields **1**. The relationship of this pathway to that of fungi that produce patulin via analogous intermediates from 6-methylsalicylic acid is discussed.

Antibiotic LL-C10037α, **1**, produced by *Streptomyces* LL-C10037,[1] is derived from 3-hydroxyanthranilic acid, **2**.[2,3] Thus, although it bears clear similarities to the polyketide-derived isoepoxydon, **3**,[4,5] in its peripheral functionalities, the carbocyclic ring is derived from a fundamentally different biogenesis. In this paper we detail the six biosynthetic steps from **2** to **1** (Scheme I).



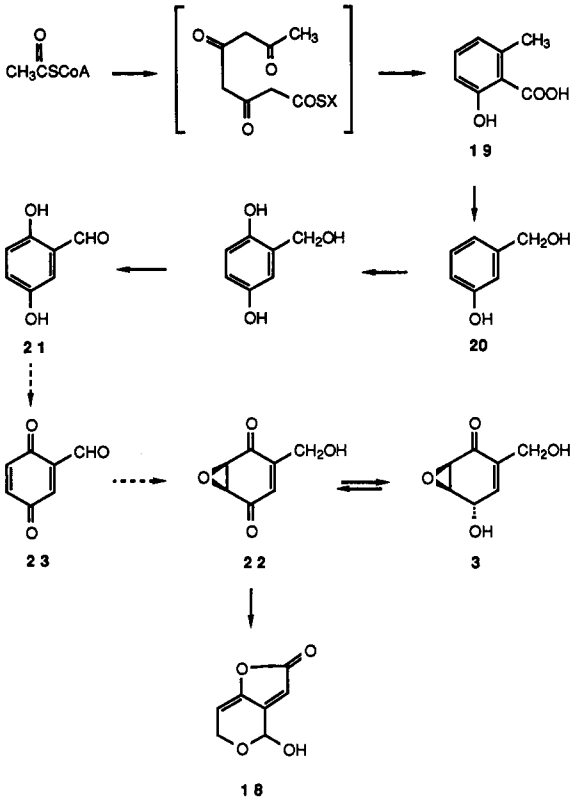
2 **1**

Results and Discussion

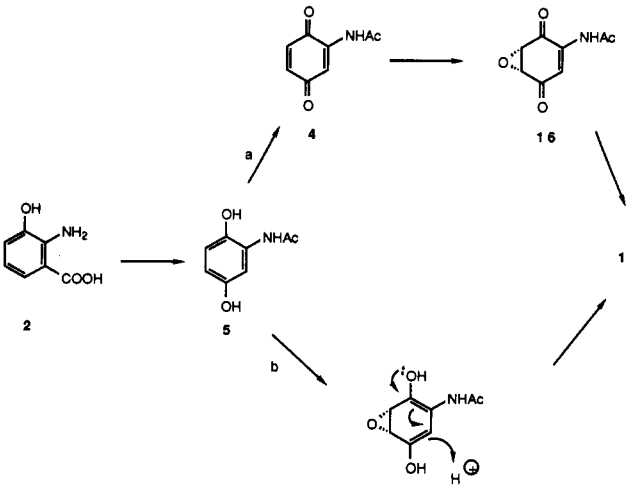
The conversion of **2** to **1** requires minimally oxidation at C-6, decarboxylation at C-1, acetylation at nitrogen, epoxidation at C-4/C-5, and tautomerization as shown in Scheme II (pathway a). The inclusion of the additional redox chemistry of pathway b would be consistent with the known[6]—but extremely inefficient—chemical oxidation of the quinone **4**. From this perspective, 2,5-dihydroxyacetanilide, **5**, was viewed as a likely key intermediate, without defining the sequence for the first steps.

2,5-Dihydroxyacetanilide. [2′,2′,2′,4,6-$^2H_5$]**5a** was synthesized from 2,5-dimethoxyaniline, **6**,[7] as shown in Scheme III. This approach was chosen after an investigation of the exchangeability of various nitrogen-substituted aromatic compounds under neutral and acidic conditions. Table I summarizes these results. In the event, exchange of **6** with deuterated trifluoroacetic acid at 80 °C led to 90% deuteration at C-4 and at C-6, with quantitative mass recovery. This was then acetylated with [$^2H_3$]acetyl chloride in the presence of triethylamine, in 92% yield. A variety of reagents were examined for the demethylation of **7a**. Neither trimethylsilyl iodide, purchased or generated in situ,[8] lithium thiomethoxide,[9] nor cerric ammonium nitrate[10] gave a clean reaction. However, boron tribromide gave **5a** in 83% yield; some loss of deuterium occurred in this step, and the final enrichments at H-4 and H-6 were 60 and 89%, respectively. This compound is prone to oxidation, but could be purified by careful recrystallization.

Scheme I



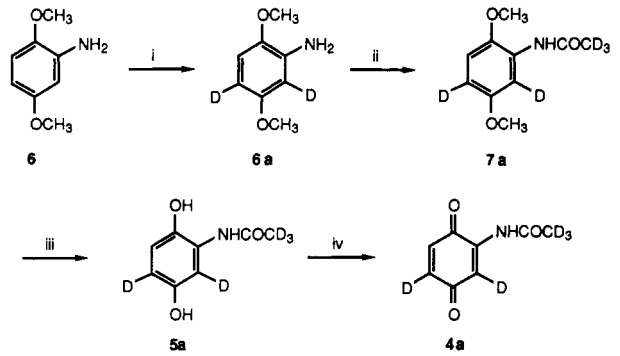
Scheme II



(1) Lee, M. D.; Fantini, A. A.; Morton, G. O.; James, J. C.; Borders, D. B.; Testa, R. T. *J. Antibiot.* **1984**, *37*, 1149.
(2) Whittle, Y. G. Gould, S. J. *J. Am. Chem. Soc.* **1987**, *109*, 5043.
(3) We have established the correct absolute stereochemistry as shown (unpublished results).
(4) Sekiguchi, J.; Gaucher, G. M. *Biochem. J.* **1979**, *182*, 445.
(5) Sekiguchi, J.; Gaucher, G. M. *Can. J. Microbiol.* **1979**, *25*, 881.
(6) Zeeck, A.; Schroder, K.; Frobel, K.; Grote, R.; Thiericke, R. *J. Antibiot.* **1987**, *40*, 1530.
(7) These compounds had previously been reported: Kehrmann, F.; Bahatrian, G. *Chem. Ber.* **1898**, *31*, 2399. However, the melting point of the material purported to be **5** was 80 °C lower than that of ours.
(8) (a) Morita, T.; Okamoto, Y.; Sakurai, H. *J. Chem. Soc., Chem. Commun.* **1978**, 874. (b) Olah, G. A.; Narang, S. C.; Gupta, B. G. B.; Malhotra, R. *J. Org. Chem.* **1979**, *44*, 1247. (c) Vickery, E. H.; Pahler, L. F.; Eisenbraun, E. J. *J. Org. Chem.* **1979**, *44*, 4444.
(9) Bartlett, P. A.; Johnson, W. S. *Tetrahedron Lett.* **1970**, 4459.
(10) Jacob, P.; Callery, P. S.; Shulgin, A. T.; Catagnoli, N. *J. Org. Chem.* **1976**, *41*, 3627.

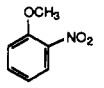
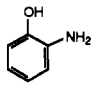
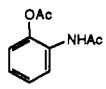
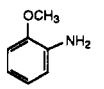
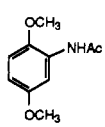
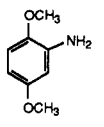
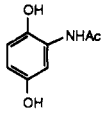
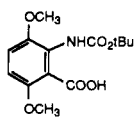
0002-7863/89/1511-7932$01.50/0

Scheme III[a]



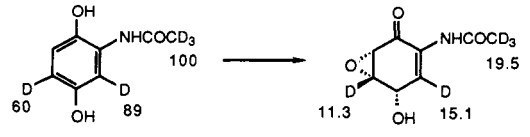
[a] (i) D_2O/TFA-*d*/MeOD/80 °C; (ii) $CD_3COCl/Et_3N/CH_2Cl_2$; (iii) BBr_3/CH_2Cl_2, −78 °C to room temperature; (iv) PbO_2/CH_2Cl_2–Et_2O.

Table I. Hydrogen Exchange of Some Nitrogen/Oxygen-Substituted Benzenes under Neutral and Acidic Conditions

substrate	CH_3OD/D_2O[a]	CH_3OD/D_2O/TFA-*d*	4 M DCl, 110 °C	3 M DCl, 80 °C
	NR	NR[a,b]	–	–
	NR	NR[a,b]	–	–
	NR	NR[a,b]	–	–
	NR	NR H-4, H-6[d]	H-4, H-6 0.5 h	
	NR	NR[a,d]		
	NR	H-4[a]; H-4, H-6[c]		
	–	–	–	H-4, H-6 5 h
	–	NR[d]		

[a] Room temperature for 24 h. [b] 60 °C for 24 h. [c] 80 °C for 12 h. [d] 80 °C for 48 h.

A portion of **5a** (100 mg) was fed to a 200-mL fermentation of *S.* LL-C10037 4 days after inoculation of the production broth. After an additional day the fermentation was worked up and the product, **1a**, was analyzed by ^2H NMR spectroscopy (Figure 1B).[11]



(11) All ^2H NMR spectra were obtained at 61.4 MHz, sweep width = 952 Hz, data points = 4K zero filled to 8K, pulse width = 90°, acquisition time = 2.15 s.

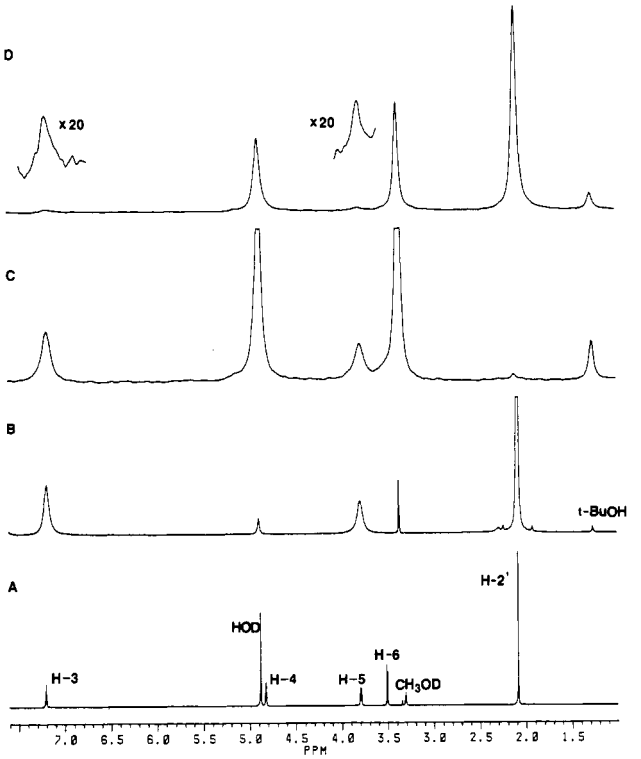
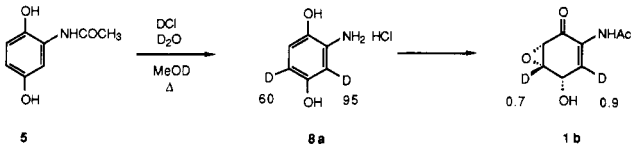
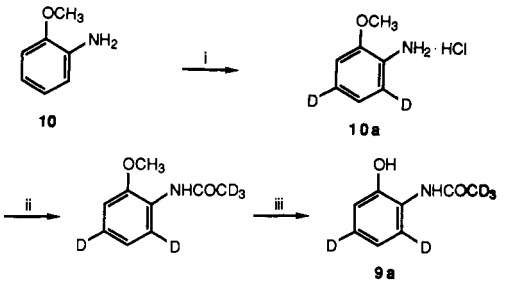


Figure 1. (A) 400-MHz ^1H NMR spectrum of **1** in methanol-d_4. (B) 61.4-MHz ^2H NMR spectrum of **1a** in methanol with *t*-BuOH for chemical shift reference. (C) ^2H NMR spectrum of **1b**. (D) ^2H NMR spectrum of **1c**. See ref 10 and the Experimental Section for the ^2H NMR acquisition parameters.

Scheme IV



Scheme V[a]



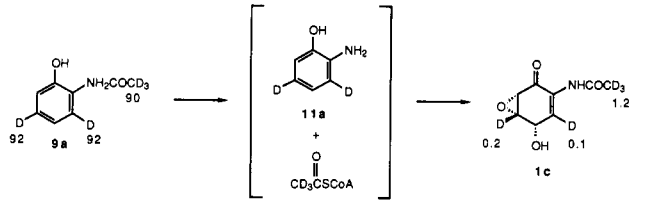
[a] (i) 3 M DCl/Δ; (ii) $CD_3COCl/Et_3N/CH_2Cl_2$; (iii) BBr_3/CH_2Cl_2/−78 °C to room temperature.

The resonances for deuterium at C-3, C-5, and the methyl group were readily observed, and the relative deuterium enrichments (indicated on the structures) reflected those of the material fed. Thus, the hydroquinone was specifically and efficiently (9.1%) incorporated into **1**.

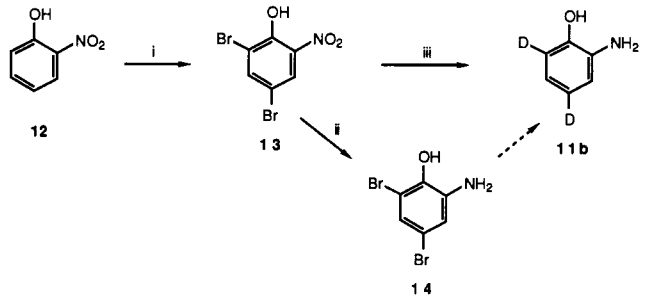
Conversion of 3-Hydroxyanthranilic Acid to 2,5-Dihydroxyacetanilide. Having established the role of **5**, it was necessary to determine the sequence of the first three reactions from **2**. When **5a** was hydrolyzed with aqueous HCl at reflux, 2,5-dihydroxyaniline, **8**, was obtained devoid of deuterium. Therefore, **5** was hydrolyzed in DCl/D_2O at reflux and yielded the hydrochloride salt [4,6-$^2H_2$]**8a**. Whereas the free base is very unstable,[12] the

(12) *Dictionary of Organic Compounds*, 5th ed.; Chapman and Hall: London, 1982; Vol. 1, p 146.

Scheme VI



Scheme VII[a]



[a] (i) Br_2/CH_3CO_2H; (ii) $SnCl_2/HCl$; (iii) D_2/Pd–C/NaOAc/EtOD.

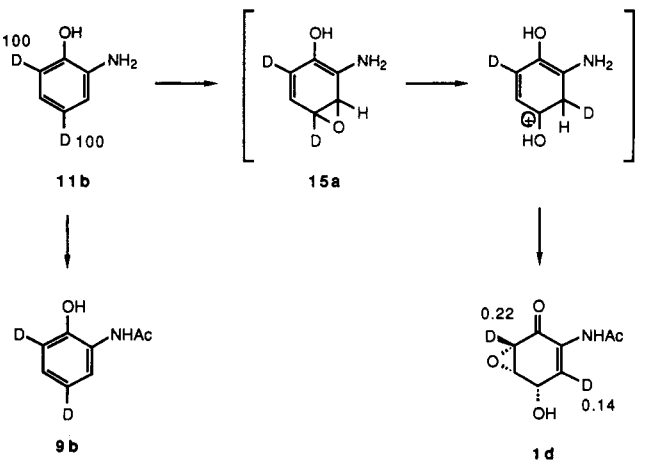
salt is quite stable. This was fed under standard conditions, and the sample obtained, **1b**, was enriched in deuterium at C-3 and C-5 to the extent of 0.9% and 0.7%, respectively, as determined by ^2H NMR (Scheme IV, Figure 1C). The incorporation of **8a** into **1b** had been 0.5%.

[2′,2′,2′,4,6-$^2H_5$]-2-Hydroxyacetanilide, **9a**, was next prepared as shown in Scheme V. An 82% recovery of exchanged **10a** was obtained from 2-methoxyaniline, **10**, and this was acetylated and demethylated in 71% overall yield. After feeding 100 mg of **9a** in the normal fashion, the bulk of this material (89 mg) was recovered during the workup, which also yielded a normal quantity of **1c** (45 mg). Examination of the ^2H NMR spectrum of this sample (Figure 1D) revealed deuterium enrichments at all three sites; however, the relative enrichments did not reflect those of the material fed. Thus, enrichment of H-3 was approximately half that of H-5, whereas the methyl group was at least 10 times as enriched. The much higher labeling of the methyl group was taken to indicate **9a** had not been incorporated intact but had first undergone deacetylation to [4,6-$^2H_2$]**11a** and—presumably—[$^2H_3$]acetylCoA (Scheme VI). The latter was apparently then more efficiently incorporated into **1**. The lower enrichment at H-3 of **1** was believed to reveal an NIH[13] shift in the hydroxylation of the aromatic ring. On the basis of unrecovered **9a**, a 0.54% incorporation of the ring and a 6.4% incorporation of the acetyl residue was obtained.

In order to confirm the NIH shift and simultaneously test 2-aminophenol, **11**, as an intermediate, [3,5-$^2H_2$]**11b** was prepared as shown in Scheme VII. Since exchange of **10** had yielded deuterium at H-4 and H-6, catalytic dehalogenation[14] of a 3,5-dibromide was now used. Thus, 2-nitrophenol, **12**, was brominated to **13** in 76% yield.[15] This was first reduced with stannous chloride[16] in HCl to give **14** in 56% yield, with plans to then hydrogenolyze the bromines catalytically. However, it was found that direct reduction of **13** using Pd–C/D_2 in the presence of sodium acetate gave **11b** in 64% yield. Again, 100 mg of substrate was fed to a 200-mL fermentation. In this case, 27 mg of labeled **1d** was isolated. In addition, 98 mg of the acetamide **9b** (corresponding to 71 mg of **11b**) was isolated from this experiment!

A 0.22% enrichment at H-6 and a 0.14% enrichment at H-3 were obtained in **1d**, confirming the NIH shift; thus, epoxidation to the arene oxide **15a** must have occurred (Scheme VIII). Although the second hydroxylation of an aromatic ring is usually

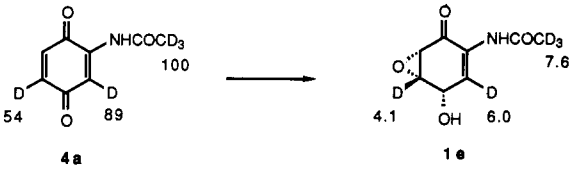
Scheme VIII



accompanied with complete loss of the ring hydrogen,[13,17] exceptions have been noted.[18,19] On the basis of the 29 mg of **11b** not recovered, a 0.13% incorporation was obtained.

The results obtained with **9a** and **11b** are remarkable. Apparently the acetamide of 2-hydroxyaniline is not an intermediate and is not readily taken up by this organism. However, what does get into the cell is efficiently deacetylated to the aniline, which is an intermediate. When cells are presented with a high concentration of the aniline, production of **1** seems to be substantially decreased and the bulk of the aniline is acetylated and excreted. This would be consistent with the aniline being toxic at high concentration; numerous *Streptomyces* protect themselves from their own[20-26] and from others'[27-31] antibiotics by transacetylation. Other microorganisms also use transacetylation for antibiotic resistance.[32-34]

From 2,5-Dihydroxyacetanilide to LL-C10037α. Labeled quinone **4a** was prepared from the hydroquinone **5a** (Scheme II) in 86% yield by oxidation with lead dioxide. A 100-mg portion of **4a** was fed to *S.* LL-C10037 and yielded **1e**. In this case, as



with the sample derived from **5a**, H-3, H-5, and the methyl group were labeled with deuterium in proportion to the material fed,

(13) Guroff, G.; Daly, J. W.; Jerina, D. M.; Renson, J.; Witkop, B.; Udenfriend, S. *Science* **1967**, *157*, 1524.
(14) Kohn, M.; Krasso, O. *J. Org. Chem.* **1946**, *11*, 641.
(15) Jacobs, W. A.; Heidelberger, M. *J. Am. Chem. Soc.* **1917**, *39*, 2188.
(16) Neilson, T.; Wood, H. C. S.; Wylie, A. G. *J. Chem. Soc.* **1962**, 371.
(17) Luckner, M. *Secondary Metabolism in Microorganisms, Plants, and Animals*; Springer-Verlag: New York, 1984; p 101.
(18) Auret, B. J.; Boyd, D. R.; Robinson, P. M.; Watson, C. G.; Daly, J. W.; Jerina, D. M. *J. Chem. Soc., Chem. Commun.* **1971**, 1585.
(19) Al-Ani, H. A. M.; Dewick, P. M. *J. Chem. Soc., Perkin Trans. 1* **1984**, 2831.
(20) Malik, V. S.; Vining, L. C. *Can. J. Microbiol.* **1972**, *18*, 583.
(21) Pérez-González, J. A.; Vara, J.; Jiménez, A. *Biochem. Biophys. Res. Commun.* **1983**, *113*, 772.
(22) Keeratipibut, S.; Sugiyama, M.; Nomi, R. *Biotechnol. Lett.* **1983**, *5*, 441.
(23) Paik, S.-Y.; Sugiyama, M.; Nomi, R. *J. Antibiot.* **1985**, *38*, 1761.
(24) Kobayashi, T.; Uozumi, T.; Beppu, T. *J. Antibiot.* **1986**, *39*, 688.
(25) Sugiyama, M.; Takeda, A.; Paik, S.-Y.; Nimi, O.; Nomi, R. *J. Antibiot.* **1986**, *39*, 827.
(26) Kobayashi, T.; Horinouchi, S.; Uozumi, T.; Beppu, T. *J. Antibiot.* **1987**, *40*, 1016.
(27) Argoudelis, A. D.; Coats, J. H. *J. Antibiot.* **1971**, *24*, 206.
(28) Benveniste, R.; Davies, J. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 2276.
(29) El-Kersh, T. A.; Plourde, J. R. *J. Antibiot.* **1976**, *29*, 292.
(30) Nakano, H.; Matsuhashi, Y.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1977**, *30*, 76.
(31) Tschäpe, H.; Tietze, E.; Prager, R.; Voigt, W.; Wolter, E.; Seltmann, G. *Plasmid* **1984**, *12*, 189.
(32) Shaw, W. V. *J. Biol. Chem.* **1967**, *242*, 687.
(33) Suzuki, Y.; Okamoto, S. *J. Biol. Chem.* **1967**, *242*, 4722.
(34) De Meester, C.; Rondelet, J. *J. Antibiot.* **1976**, *29*, 1297.

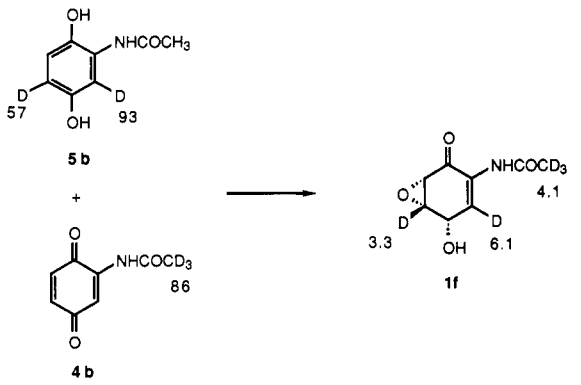
Table II. Whole-Cell Incorporations of Intermediates into Antibiotic LL-C100372 (**1**)

compd fed	amt[a] fed, mg	positions labeled, % enrichment[b]	amt obtained,[c] mg	positions labeled,[d,e] % enrichment[f]	% incorp
5a	100.0	H-4, 60 H-6, 89 H-2′, 100	53.4	H-3, 15.1 H-5, 11.3 H-2′, 19.5	9.10
8a	100.0	H-4, 60 H-6, 95	47.6	H-3, 0.94 H-5, 0.67	0.47
9a	100.0	H-4, 92 H-6, 92 H-2′, 90	45.2 (**1c**) 89.0 (**9a**)	H-3, 0.12 H-5, 0.19 H-2′, 1.21	0.54 (based on H-5)
11b	100.0	H-3, 100 H-5, 100	26.6 (**1d**) 98.0 (**9b**)	H-3, 0.14 H-6, 0.22 H-2′, 0.09	0.13 (based on H-6)
4a	100.0	H-3, 89 H-5, 54 H-2′, 100	21.0	H-3, 6.0 H-5, 4.1 H-2′, 7.6	1.60
5b + **4b**	32.0 each	H-4, 57 H-6, 93 H-2′, 88	53.0	H-3, 6.07 H-5, 3.27 H-2′, 4.10	10.20 (**5b**) 7.90 (**4b**)

[a] Fed as an aqueous or aqueous ethanol solution to 200 mL of production broth 96 h after inoculation. [b] Determined from the ^{1}H NMR spectrum. [c] Worked up 24 h after feeding. [d] Determined by ^{2}H NMR analysis; see ref 11. [e] Compound (no. of scans): **1a** (6976), **1b** (16309), **1c** (15358), **1d** (39414), **1e** (22706), **1f** (14983). [f] Quantitated by comparison of integrals with that from 25 μL of *t*-BuOH added to each sample.

although the total incorporation was only 1.6%. The different incorporations for **4a** and **5a** (quantitated by comparing integrals with those from H-6) could not be taken as an indication of their relative positions in the pathway.

Since the incorporation of **4a** could have been due to the fortuitous action of an available dehydrogenase, a direct competition was established by feeding equimolar amounts of [4,6-$^2H_2$]**5b** and [2′,2′,2′-$^2H_3$]**4b** to the same fermentation. In this case **1f**, actually



representing two subpopulations of labeled **1**, was obtained with nearly equal enrichments at all three sites (10.2% incorporation of **5b** and 7.9% incorporation of **4b**). This established the ease of redox equilibration between the hydroquinone and quinone without, unfortunately, revealing its correct relationship to the pathway. Such experiments emphasize the variability in biological systems and the caution that must be exercised when analyzing incorporation figures from separate experiments. Table II summarizes the quantitative data from the six feeding experiments.

In order to resolve the last steps in the pathway and reconcile the incorporation of the quinone **4**, cell-free studies were required. The growth of *S.* LL-C10037 and production of **1** were monitored over time, and the results are presented in Figure 2. On this basis, cells were harvested at 96 h by centrifugation at 4 °C. They were then washed with 1 M KCl and with 0.8 M NaCl to remove any surface proteases, suspended in 0.01 M phosphate buffer (pH 7.0), and sonicated with cooling. Centrifugation then yielded a crude cell-free extract (CFE).

When either **4** or **5** was initially incubated with either NADH or NADPH in the presence of CFE (12 h old) for 5 h at 30 °C, **16**[35] was detected by TLC of the ethyl acetate extracts. For clear identification of the product, a 24-h-old CFE was added to a

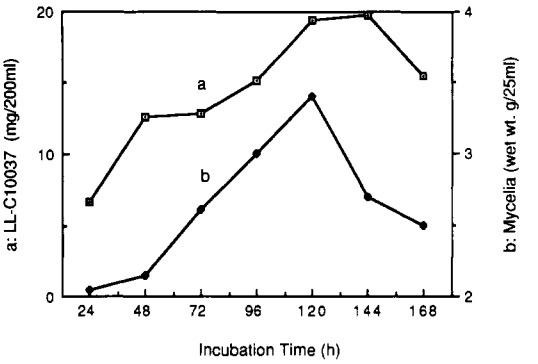
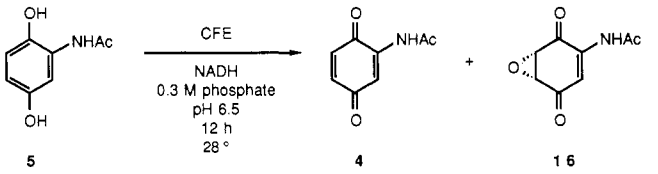


Figure 2. Growth of *S.* LL-C10037 and production of antibiotic LL-C10037α.

Scheme IX



solution of **5** and NADH. This was incubated at 28 °C and 250 rpm for 12 h (Scheme IX). Extractive workup and preparative TLC yielded quantities of two products that were confirmed to be **4** and **16** by ^{1}H NMR spectroscopy.

The oxidation of **5** to **4** may have been enzymatic and initially utilized NAD^+ present in the CFE or it may have been chemical: a stirred aqueous solution of **5** will oxidize to **4** over such a period of time. However, the formation of **16** clearly must have been enzymatic. Since the epoxide oxygen has previously been shown to come from molecular oxygen,[2] the enzyme is most likely a flavin monooxygenase.[36]

The cofactor requirements of this enzyme, acetamidobenzoquinone (ABQ) monooxygenase, were investigated with **4** as the substrate, and the results are presented in Table III. From entries 1 and 2 it is clear that O_2 and either NADH or NADPH can support epoxidation and from entries 3 and 4 that the reaction is clearly enzymatic. Some **5** was produced in all four experiments. We have previously isolated a very active NADH-dependent ABQ dehydrogenase[37] and have also found that either coenzyme will reduce **4** chemically although this is a slower reaction than the

(35) An authentic sample was prepared by oxidation of **1** with pyridinium chlorochromate: Box, S. J.; Gilpin, M. L.; Gwynn, M.; Hanscomb, G.; Spear, S. R.; Brown, A. G. *J. Antibiot.* **1983**, *36*, 1631.

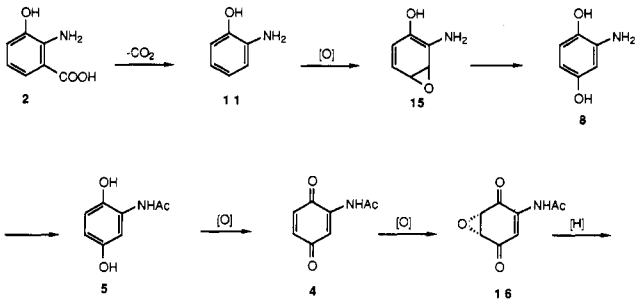
(36) Nozaki, M. *Top. Cur. Chem.* **1979**, *78*, 145.
(37) Shen, B.; Gould, S. J.; Whittle, Y. G. Unpublished results.

Table III. Epoxidation of Acetamidobenzoquinone (ABQ, **4**) by Cell-Free Preparation of *S.* LL-C10037

entry	assay components[a]	products (TLC)			% **16**[b]
	Aerobic (Phosphate, pH 5.0)				
1	**4**, NADH, CFE	**5**		**16**	3.8
2	**4**, NADPH, CFE	**5**		**16**	3.8
3	**4**, NADH	**5**	**4**		
4	**4**, NADPH	**5**	**4**		
5	**4**, CFE	**5**		**16**	11.6
6	**4**, **5**, NADH, CFE	**5**	**4**	**16**	10.1
	Anaerobic[c] (Phosphate, pH 5.0)				
7	**4**, NADH, CFE	**5**			
8	**4**, NADPH, CFE	**5**			

[a] Conditions: substrate, 0.1 mM; cofactors, 1.0 mM; buffer, 0.1 M; total volume, 10 mL; time, 20 h; temperature, 30 °C. [b] Determined by extactn with EtOAc after saturation with NaCl, preparative layer chromatography of extracts, and UV quantitation ($\epsilon_{310nm} = 7.2 \times 10^3$, MeOH). [c] Run under an N_2 atmosphere.

Scheme X

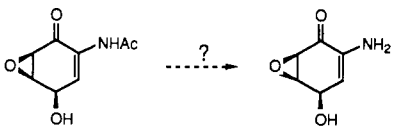


enzymatic reduction. On the assumption that the CFE might contain sufficient cofactor for the monooxygenase, the NADH was left out to decrease ABQ dehydrogenase activity (entry 5) and, indeed, substantially more **16** was produced. Addition of a quantity of **5** to the incubation mixture had the same effect (entry 6). Finally, as expected, under anaerobic conditions only **5** was produced from **4** (entries 7 and 8).

Conclusions

From the data presented here, it is clear that the biosynthesis of **1** from **2** follows the pathway shown in Scheme X. Detection of the ABQ monooxygenase activity establishes the quinone as the substrate of the reaction that generates the absolute stereochemistry for this series of compounds.

Remarkably, a different *Streptomyces* produces **17**,[35] the enantiomer of deacetyl **1**. The biosynthesis of **17** is currently under



17

study in our laboratory. In view of the obligatory acetylation in the pathway leading to **1**, as well as the unexpected additional transacetylase and acetamide hydrolase activities detected in *S.* LL-C10037, it is possible that the organism that produces **17** does so by deacetylation as the last step. If the monooxygenase of this pathway can be isolated, it may be possible to understand how these enzymes can each recognize a different face of the planar substrate and generate enantiomeric epoxides.

The biosynthesis of patulin, **18**, produced by the fungi *Penicillium patulum* and *P. urticae* is one of the most well studied of all secondary metabolites.[38] Of polyketide origin via 6-methylsalicylic acid, **19**, *m*-hydroxybenzyl alcohol, **20**, and gentisaldehyde, **21**, it was long believed that the aromatic ring of **21** was cleaved by a dioxygenase reaction. However, Gaucher et al. subsequently isolated isoepoxydon,[5] **3** (see Scheme I), and phyllostine,[39] **22**, from blocked mutants of *P. urticae* and demonstrated[5] that each was converted to **18**. Numerous enzymes in this pathway have been isolated,[40-50] including a dehydrogenase that interconverts **3** and **22**. While it has been assumed that **21** is epoxidized directly to **3**, the epoxidase has never been isolated.[51] No advanced studies on the biosynthesis of the polyketide-derived epoxyquinol terremutin and the related epoxyquinone terreic acid[52] have been carried out; the question of which is the first epoxide in this pathway was also raised here.

The parallel in structures between **2**, **11**, **8**, **(5)**, **4**, and **1** on the one hand and **19**, **20**, **21**, **22**, and **3** on the other is striking. It would now seem more likely that phyllostine is the first epoxide, perhaps with gentisaldehyde quinone, **23**, as a precursor, in the patulin pathway and isoepoxydon may actually be a shunt metabolite (Scheme I).

Experimental Section

General Procedures. ^1H NMR spectra were taken on a Varian FT 80 or Bruker AM 400 spectrometer; ^{13}C NMR spectra were taken at 20 and 100.6 MHz on Varian FT 80 and Bruker AM 400 spectrometers. ^2H NMR spectra were obtained at 61.4 MHz on a Bruker AM 400 spectrometer. All ^{13}C NMR spectra were broad-band decoupled and ^2H NMR spectra were proton decoupled and run unlocked. Five-millimeter NMR tubes were used for all NMR measurements. ^1H and ^{13}C NMR samples were referenced with TMS, CH_3CN, or *t*-BuOH. ^2H NMR samples were prepared in ^2H-depleted water or in methanol; with either one 25 μL of *t*-BuOH was used as reference for chemical shift (1.28 ppm) and quantification (0.38 μmol of ^2H in the methyl groups).

IR spectra were recorded on a Perkin-Elmer 727B or Nicolet 5DXB FTIR spectrometer. Low-resolution mass spectra were taken on a Varian MAT CH-7 spectrometer.

Melting points were taken on a Büchi melting point apparatus and are uncorrected. Flash chromatography was carried out on silica gel (EM Reagents, Keiselgel 60, 230–400 mesh). Analytical thin-layer chromatography (TLC) was carried out on precoated Keiselgel 60 F_{254} (either 0.2-mm aluminum sheets or 0.25-mm glass plates) and visualized by long- and/or shortwave UV.

Standard Culture Conditions. *S.* LL-C10037 was maintained at 5 °C as spores on sterile soil. A loopful of this material was used to inoculate 50 mL of seed medium containing 1.0% glucose, 2.0% soluble potato starch, 0.5% yeast, 0.5% N-Z Amine A 59027, and 0.1% $CaCO_3$ in glass-distilled water, the whole adjusted to pH 7.2 with 2% KOH. The culture, contained in a 250-mL Erlenmeyer flask, was incubated for 3 days at 28 °C, 200 rpm. Production broths (200 mL in 1-L Erlenmeyer flasks), consisting of 1.0% glucose, 0.4% bactopeptone, 2.0% molasses (Grandma's Famous light unsulfured), and 0.1% $CaCO_3$ in glass-distilled water and adjusted to pH 7.2 with 10% HCl prior to sterilization, were subsequently inoculated to 5% v/v with vegetative inoculum from seed broths. The production broths were incubated for 120 h. For precursor feedings labeled compounds were dissolved in 5 mL of an appropriate H_2O/ethanol mixture and added in a sterile manner through micropore filters after ca. 96 h.

(38) For comprehensive papers, see: (a) Neway, J.; Gaucher, G. M. *Can. J. Microbiol.* **1981**, *27*, 206. (b) Scott, A. I.; Zamir, L.; Phillips, G. T.; Yalpani, M. *Bioorg. Chem.* **1973**, *2*, 124.

(39) Sekiguchi, J.; Gaucher, G. M. *Biochemistry* **1978**, *17*, 1785.
(40) Light, R. J. *Biochim. Biophys. Acta* **1969**, *191*, 430.
(41) Dimroth, P.; Walter, H.; Lynen, F. *Eur. J. Biochem.* **1970**, *13*, 98.
(42) Forrester, P. I.; Gaucher, G. M. *Biochemistry* **1972**, *11*, 1108.
(43) Scott, A. I.; Beadling, L. C.; Georgopapadakou, N. H.; Subbarayan, C. R. *Bioorg. Chem.* **1974**, *3*, 238.
(44) Scott, A. I.; Beadling, L. *Bioorg. Chem.* **1974**, *3*, 281.
(45) Murphy, G.; Vogel, G.; Krippahl, G.; Lynen, F. *Eur. J. Biochem.* **1974**, *49*, 443.
(46) Gaucher, G. M. *Methods Enzymol.* **1975**, *43*, 540.
(47) Murphy, G.; Lynen, F. *Eur. J. Biochem.* **1975**, *58*, 467.
(48) Grootwassink, J. W. D.; Gaucher, G. M. *J. Bacteriol.* **1980**, *141*, 443.
(49) Sekiguchi, J.; Shimamoto, T.; Yamada, Y.; Gaucher, G. M. *Appl. Environ. Microbiol.* **1983**, *45*, 1939.
(50) Sekiguchi, J.; Gaucher, G. M.; Yamada, Y. *Adv. Biotechnol.*, [*Proc. Int. Ferment. Symp.*], *6th* **1980**, *3*, 107–112.
(51) Neither [^{14}C]gentisyl alcohol nor [^{14}C]gentisylquinone was incorporated into whole wild-type *P. urticae* cells. Neither toluquinone nor gentisaldehyde quinone has been fed to fermentations or cell-free extracts of the mutant that accumulates isoepoxydon and phyllostine. Gaucher, G. M. Personal communication.
(52) Read, G.; Westlake, D. W. S.; Vining, L. C. *Can. J. Biochem.* **1969**, *47*, 1071.

Isolation. The cultures were filtered through cheesecloth and Celite, and the filtrate was adjusted to pH 4.7 with solid KH_2PO_4. This was then saturated with $(NH_4)_2SO_4$ and extracted repeatedly with EtOAc (typically eight times). After concentration in vacuo the residue was dissolved in a minimum volume of methanol and adsorbed onto a small quantity of silica gel. This was applied to the top of a column of flash-grade silica gel (25 g/200 mL fermentation) prepared in 40% hexane/EtOAc. After low-polarity colored impurities had been eluted, the solvent was changed to 20% hexane/EtOAc and elution yielded **1**, which was recrystallized from methanol.

[4,6-$^2H_2$]-2,5-Methoxyaniline (6a). 2,5-Dimethoxyaniline (600 mg, 3.92 mmol), MeOD (20 mL), D_2O (2.0 mL), and TFA-*d* (1.5 mL) in a flame-dried 50-mL round-bottomed flask were heated at reflux for 12 h. All solvents were removed by vacuum distillation. ^1H NMR analysis (MeOH-d_4, 80 MHz) of a portion of the residue (560 mg, 93%) indicated 90% exchange at H-4 (δ 6.20) and H-6 (δ 6.25).

2,5-Dimethoxyacetanilide (7). 2,5-Dimethoxyaniline (154 mg, 1 mmol) was dissolved in CH_2Cl_2 (5 mL) and Et_3N (122 mg, 1.2 mmol), and acetyl chloride (95 mg, 1.2 mmol) was added at 0 °C. After stirring at room temperature for 1 h, the mixture was diluted with CH_2Cl_2 (20 mL) and extracted with H_2O (2 × 5 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo to give 2,5-dimethoxyacetanilide in quantitative yield as gray crystals: mp 91–92 °C (lit.[53] mp 91 °C); ^1H NMR (acetone-d_6, 80 MHz) δ 8.80 (1 H, brs), 8.00 (1 H, d, *J* = 3.8 Hz), 6.82 (1 H, d, *J* = 8.2 Hz), 6.48 (1 H, dd, *J* = 8.2, 3.8 Hz), 3.81 (3 H, s), 3.78 (3 H, s), 2.10 (3 H, s).

[2′,2′,2′,4,6-$^2H_5$]-2,5-Dimethoxyacetanilide (7a). With use of the above procedure, **6a** (560 mg, 3.6 mmol), acetyl-d_3-chloride (381 mg, 4.7 mmol), and Et_3N (473 mg, 4.7 mmol) in CH_2Cl_2 (20 mL) yielded 672 mg (92%) of **7a**. ^1H NMR analysis (acetone-d_6, 80 MHz) indicated 70% enrichment at H-4 (δ 6.48), 90% enrichment at H-6 (δ 8.00), and 100% enrichment in the methyl group (δ 2.10).

2,5-Dihydroxyacetanilide (5). 2,5-Dimethoxyacetanilide (98 mg, 0.5 mmol) was dissolved in CH_2Cl_2 (25 mL) in a dried 100-mL round-bottomed flask which was then flushed with Ar, cooled to −78 °C, and treated with a solution (1 M) of BBr_3 in CH_2Cl_2 (1.2 mL, 1.20 mmol). After 1 h, the stirred mixture was warmed to room temperature and stirred for an additional 6 h. The reaction was quenched with water (20 mL), extracted with EtOAc (5 × 20 mL), and washed with saturated brine. The combined organic fractions were dried (Na_2SO_4) and concentrated in vacuo to give 83 mg (100%) of nearly pure **5** as white crystals. Recrystallization from EtOAc gave 55 mg (65%) of **5** as a gray powder: mp 165–168 °C (sealed capillary) (lit.[7] mp 100 °C); ^1H NMR (acetone-d_6, 400 MHz) δ 9.10 (1H, br s, exch D_2O), 8.57 (1 H, s, exch D_2O), 7.89 (1 H, s, exch D_2O), 7.03 (1 H, d, *J* = 2.6 Hz), 6.72 (1 H, d, *J* = 8.7 Hz), 6.52 (1 H, dd, *J* = 8.6, 2.7 Hz), 2.18 (3 H, s); ^{13}C NMR (acetone-d_6, 100.6 MHz) δ 170.90, 151.24, 141.89, 127.94, 118.81, 112.96, 109.35, 29.85.

[2′,2′,2′,4,6-$^2H_5$]-2,5-Dihydroxyacetanilide (5a). With use of the above procedure, **7a** (640 mg, 3.26 mmol) in CH_2Cl_2 (75 mL) was treated with a 1 M BBr_3 solution (7.83 mL, 7.83 mmol) in CH_2Cl_2. Workup gave a brown solid that was recrystallized from EtOAc/MeOH to yield 460 mg (83%) of **5a** as tan crystals (mp 171–172 °C). ^1H NMR analysis (acetone-d_6, 400 MHz) indicated 60% deuterium enrichment at H-4 (δ 6.5), 89% at H-6 (δ 7.0), and 100% at the methyl group (δ 2.2).

[4,6-$^2H_2$]-2,5-Dihydroxyaniline Hydrochloride (8a). 2,5-Dihydroxyacetanilide (300 mg, 1.80 mmol), MeOD (10 mL), D_2O (5 mL), and 3 M DCl (5 mL) in a 50-mL round-bottomed flask were heated at reflux under an N_2 atmosphere for 5 h. After cooling of the mixture, the solvents were removed in vacuo and the light green powder recrystallized from EtOAc/MeOH to give 260 mg (90%) of **8a**: ^1H NMR (MeOH-d_4, 400 MHz) δ 6.84 (0.4 H, d, *J* = 8.8 Hz), 6.77 (0.05 H, d, *J* = 2.4 Hz), 6.73 (1 H, d, *J* = 8.8, 2.9 Hz).

[4,6-$^2H_2$]-2-Methoxyaniline Hydrochloride (10a). 2-Hydroxyaniline (500 mg, 4.06 mmol) and 4 M DCl (6 mL) were sealed in a heavy-walled 25-mL flask and heated at 100 °C for 30 min in an oil bath. After cooling, the solvent was removed in vacuo and the black solid was recrystallized from EtOH/EtOAc to give beige needles (538 mg, 82%) of **10a** (mp 232–233 °C). ^1H NMR analysis (methanol-d_4, 80 MHz) indicated >90% exchange at H-4 and H-6 (δ 6.7 and 6.5).

2-Methoxyacetanilide. 2-Methoxyaniline (493 mg, 4.0 mmol) was treated with acetyl chloride (379 mg, 4.8 mmol) and Et_3N (486 mg, 4.8 mmol) in CH_2Cl_2 (35 mL) for 2 h. Additional CH_2Cl_2 (20 mL) was added and the mixture washed with water (2 × 10 mL). After drying over $MgSO_4$ and filtration, the filtrate was concentrated in vacuo to give a white solid. Recrystallization from EtOH yielded 660 mg (100%) of 2-methoxyacetanilide: mp 84–85 °C (lit.[53] mp 84 °C); ^1H NMR ($CDCl_3$, 80 MHz) δ 8.30 (1 H, brdd, *J* = 8, 3 Hz), 7.70 (1 H, brs), 6.90 (3 H, m), 3.85 (3 H, s), 2.17 (3 H, s).

[2′,2′,2′,4,6-$^2H_5$]-2-Methoxyacetanilide. With use of the above procedure, a mixture of **10a** (360 mg, 2.3 mmol), acetyl-d_3 chloride (217 mg, 2.8 mmol), and Et_3N (542 mg, 5.6 mmol) in CH_2Cl_2 (30 mL) gave deuterated 2-methoxyacetanilide (280 mg, 74%).

2-Hydroxyacetanilide (9). 2-Methoxyacetanilide (41 mg, 0.25 mmol) in dry CH_2Cl_2 (15 mL) under an Ar atmosphere was treated at −78 °C with 1 M BBr_3/CH_2Cl_2 (300 μL, 0.30 mmol). After 1 h the mixture was warmed to room temperature and after an additional 3 h was worked up as described for **5** to yield 35 mg (93%) of **9** after recrystallization from EtOH: mp 208–209 °C (lit.[55] mp 209 °C); ^1H NMR (acetone-d_6, 400 MHz) δ 9.49 (1 H, s, exch D_2O), 9.40 (1 H, brs, exch D_2O), 7.39 (1 H, dd, *J* = 8.1, 1.3 Hz), 7.03 (1 H, m), 6.89 (1 H, dd, *J* = 8.1, 1.3 Hz), 6.80 (1 H, m), 2.21 (3 H, s); ^{13}C NMR (acetone-d_6, 100.6 MHz) δ 171.22, 149.42, 127.72, 126.61, 122.84, 120.51, 118.96, 23.49.

[2′,2′,2′,4,6-$^2H_5$]-2-Hydroxyacetanilide (9a). As described above, [2′,2′,2′,4,6-$^2H_5$]-2-methoxyacetanilide (280 mg, 1.65 mmol) was demethylated with 1 M BBr_3/CH_2Cl_2 (2.0 mL, 2.00 mmol) to yield 247 mg (96%) of **9a** (mp 206–207 °C). ^1H NMR analysis (acetone-d_6, 400 MHz) indicated 92% deuteration at H-4 (δ 7.03) and H-6 (δ 7.39) and 90% deuteration at the methyl group (δ 2.18).

2-Nitro-4,6-dibromophenol (13). 2-Nitrophenol (6.95 g, 0.05 mol) was dissolved in glacial HOAc (30 mL) and treated over 1 h with Br_2 (7.95 mL, 0.15 mol) in glacial HOAc (4 mL). The mixture was heated at ca. 60 °C for 24 h, cooled, and poured into water (100 mL). The resulting yellow precipitate was collected and recrystalllized from EtOH to give 11.35 g (76%) of **13**: mp 116–119 °C (lit.[15] mp 113–114 °C); ^1H NMR ($CDCl_3$, 80 MHz) δ 10.68 (1 H, brs), 7.89 (1 H, d, *J* = 2.5 Hz), 7.62 (1 H, d, *J* = 2.5 Hz).

[3,5-$^2H_5$]-2-Hydroxyaniline (11b). Sodium acetate (410 mg, 4 mmol) was dissolved in D_2O (5 mL) in a 100-mL round-bottomed flask and then lyophilized. **13** (500 mg, 1.68 mmol), 10% Pd–C (100 mg), and EtOD (70 mL) were added to the flask, and the resulting suspension was stirred under an atmosphere of D_2 at room temperature for 1 h. The suspension was filtered and the filtrate was concentrated in vacuo. The residue was extracted with EtOAc (50 mL), and the extract was washed with H_2O (2 × 3 mL) and then with saturated brine (5 mL). After drying ($MgSO_4$) and concentration in vacuo, the residue was chromatographed on silica gel (20 cm × 1.5 cm), eluting with hexane/EtOAc (1:1), and the crude product was recrystallized from benzene to give 119 mg (64%) of **11b** as yellow–brown crystals: mp 172–176 °C (lit.[55] mp 175–177 °C); ^1H NMR ($CDCl_3$, 400 MHz) δ 6.73 (1 H, s), 6.58 (1 H, s).

2-Acetamido-1,4-benzoquinone (4). A portion of **5** (34 mg, 0.2 mmol) in Et_2O (25 mL) and CH_2Cl_2 (25 mL) was treated with PbO_2 (287 mg, 1.2 mmol), and the resulting black suspension was stirred at room temperature for 12 h. The mixture was filtered through a silica gel column (10 cm × 1.5 cm), eluting with EtOAc/hexane (1:1), and the yellow fractions were combined and concentrated to give 28.1 mg (85%) of **4** as orange crystals: mp 148–149 °C dec (sealed capillary) (lit.[7] mp 142 °C); ^1H NMR (acetone-d_6, 400 MHz) δ 8.85 (1 H, brs, exch D_2O), 7.42 (1 H, d, *J* = 2.9 Hz), 6.78 (1 H, d, *J* = 10.3 Hz), 6.64 (1 H, dd, *J* = 10.2, 2.2 Hz), 2.28 (3 H, s); ^{13}C NMR (acetone-d_6, 100.6 MHz) δ 189.21, 183.37, 171.62, 140.22, 118.16, 134.70, 114.67, 24.48.

[2′,2′,2′,4,6-$^2H_5$]-2-Acetamido-1,4-benzoquinone (4a). With use of the above procedure, **5a** (193 mg, 1.13 mmol) in Et_2O (100 mL) and CH_2Cl_2 (100 mL) was oxidized with PbO_2 (1.63 g), and the product was purified (silica gel column, 15 cm × 1.5 cm) to give 164 mg (86%) of **4a** that was 89.5% deuterated at H-3 (δ 7.42), 54% deuterated at H-5 (δ 6.64), and 100% deuterated in the methyl group (δ 2.25).

Preparation of Cell-Free Extract of *S.* LL-C10037. The cells from 1.6 L of 96-h fermentations were collected by centrifugation at 4 °C (13800*g*, 20 min) and washed successively with 200-mL portions of glass-distilled H_2O, 1.0 M KCl, 0.8 M NaCl, and 0.01 M potassium phosphate buffer (pH 7.0). After each wash the cells were centrifuged as above. The cells (50-g wet weight) were then suspended in additional buffer (200 mL) and sonicated at 4 °C (power setting 9, 50% duty cycle, pulsed for 20 min). Centrifugation at 4 °C (700g, 20 min) yielded 130 mL of the cell-free extract (CFE).

In Vitro Enzymatic Formation of 16. Substrate (**5**, 20 mg) and NADH (50 mg) in 100 mL of H_2O/acetone (96:4) was incubated at 28 °C and 250 rpm with a 24-h-old CFE (50 mL, 1 M potassium phosphate, pH 6.5). A 12-h extractive workup and preparative TLC purification yielded **4** (6 mg) and **16** (6 mg), whose structures were confirmed by ^1H NMR spectroscopy.

(53) Baessler, A. *Chem. Ber.* **1884**, *17*, 2118.
(54) *Beilstein* Vol. 13, 371.
(55) *Dictionary of Organic Compounds*, 5th ed.; Chapman and Hall: London, 1982; vol. 1, p 304.

Acknowledgment. Dr. Donald Borders, Lederle Laboratories, Pearl River, NY, is thanked for a strain of *S.* LL-C10037 and for a sample of LL-C10037α. Rodger Kohnert is thanked for obtaining some of the ^2H NMR spectra. This work was supported by Public Health Service Research Grant GM 31715 and National Science Foundation Grant 8711102 to S.J.G. The multinuclear Bruker AM 400 NMR spectrometer was purchased in part through grants from the National Science Foundation (Grant No. CHE-8216190) and from the M. J. Murdock Charitable Trust to Oregon State University.

Registry No. **1**, 93752-54-6; **1a**, 121055-91-2; **1b**, 121055-92-3; **1d**, 121055-94-5; **2**, 548-93-6; **4**, 4053-51-4; **4a**, 121055-95-6; **4b**, 121055-96-7; **5**, 93525-28-1; **5a**, 121055-97-8; **5b**, 121055-98-9; **6**, 102-56-7; **6a**, 121055-99-0; **7**, 3467-59-2; **7a**, 121056-00-6; **8**, 20734-68-3; **8a**, 121056-01-7; **9**, 614-80-2; **9a**, 121056-02-8; **9a** (methyl ether), 121056-05-1; **9b**, 121056-03-9; **10**, 90-04-0; **10a**, 121056-04-0; **11**, 95-55-6; **11b**, 121056-06-2; **12**, 88-75-5; **13**, 15969-09-2; **14**, 10539-14-7; **15**, 121055-90-1; **15a**, 121056-07-3; **16**, 112458-00-1.

Cobalt Carbonyl Catalyzed Reactions of Cyclic Ethers with a Hydrosilane and Carbon Monoxide. A New Synthetic Reaction Equivalent to Nucleophilic Oxymethylation[1]

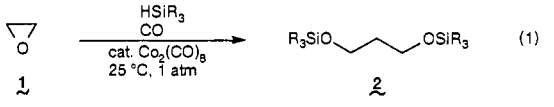
Toshiaki Murai,[†] Eiji Yasui,[†] Shinzi Kato,[†] Yoshio Hatayama,[‡] Satoshi Suzuki,[‡] Yasuhiro Yamasaki,[‡] Noboru Sonoda,[‡] Hideo Kurosawa,[‡] Yoshikane Kawasaki,[‡] and Shinji Murai*[,‡]

Contribution from the Department of Chemistry, Faculty of Engineering, Gifu University, Yanagido, Gifu 501-11, Japan, and Department of Applied Chemistry, Faculty of Engineering, Osaka University, Suita, Osaka 565, Japan. *Received February 16, 1989*

Abstract: Siloxymethylative ring opening of cyclic ethers has been attained by a new catalytic system of $HSiR_3/CO/Co_2(CO)_8$. The reaction generally proceeded at room temperatures under 1 atm of CO. The carbon monoxide was incorporated into the product as a part of siloxymethyl group. The reactivity of cyclic ethers decreased in the order of 4 > 3 > 5 >> 6 and 7 membered ring. Among the hydrosilanes ($HSiMe_3$, $HSiEt_2Me$, and $HSiEt_3$), the highly reactive $HSiMe_3$ allowed the use of lower reaction temperature leading to high product selectivities. The regiochemical course of the reaction depended on the substituents of the oxiranes. The reaction of monosubstituted oxiranes having electron-withdrawing groups, such as hydroxy, acetoxy, and benzoyloxy, resulted in a highly regioselective ring opening at the primary carbon center. While *tert*-butylethylene oxide reacted at the primary carbon, styrene oxide reacted at the secondary center. The stereochemical course of the reaction was demonstrated to be trans in the cases of cycloalkene oxides and *cis*- and *trans*-2-butene oxides. The regio- and stereoselective ring opening of allylic alcohol epoxide derivatives has been attained when their hydroxy group was converted into monochloroacetoxy group. Rare examples of incorporation of carbon monoxide into tertiary carbon centers were observed for the ring opening of geminal dialkyl-substituted oxiranes. The importance of $R_3SiCo(CO)_4$ (**3**) as a key catalyst species and the reaction mechanism have been discussed. An acylcobalt carbonyl intermediate generated by the stoichiometric reaction of $R_3SiCo(CO)_4$ (**3**) with *tert*-butylethylene oxide was intercepted by a 1,3-diene.

The new catalytic reaction system of $HSiR_3/CO/Co_2(CO)_8$ can bring about incorporation of carbon monoxide into olefins and various oxygen-containing compounds.[2] It has been shown that carbon monoxide is incorporated into the products in the form of formyl, siloxymethylidene (=$CHOSiR_3$), or other carbafunctional groups. For example, conversions of alkenes to siloxymethylidene alkanes,[2,3] aldehydes to higher α-siloxy aldehydes,[2,4] alkyl acetates to siloxymethylidene alkanes,[5] and cyclobutanones to disiloxycyclopentenes[6] have been attained by the reaction of these substrates with $HSiR_3$ and CO in the presence of $Co_2(CO)_8$. These reactions proceed generally at 140 °C and 50 atm of carbon monoxide, and in some cases[1,5] at 200 °C and 50 atm.

In contrast to these results, we have found that a catalytic reaction of oxiranes with $HSiR_3/CO/Co_2(CO)_8$ takes place under surprisingly mild conditions, i.e., at 25 °C under 1 atm.[7] More interesting is the form of the carbafunctional group introduced. The incorporated carbon monoxide is converted to a siloxymethyl group in the product (eq 1) instead of previously observed formyl or siloxymethylidene group.



(1)

1 2

From the synthetic point of view, the present reaction can be regarded formally as a nucleophilic oxymethylation ($ROCH_2^-$). The reaction should provide a simple method for the construction of 1,3-diol units, an important building block in natural product

[†] Gifu University.
[‡] Osaka University.

(1) For the previous paper of this series, see: Chatani, N.; Ikeda, T.; Sano, T.; Sonoda, N.; Kurosawa, H.; Kawasaki, Y.; Murai, S. *J. Org. Chem.* **1988**, *53*, 3387.
(2) For reviews, see: (a) Murai, S.; Sonoda, N. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 837. (b) Murai, S.; Seki, Y. *J. Mol. Catal.* **1987**, *41*, 197.
(3) (a) Seki, Y.; Hidaka, A.; Murai, S.; Sonoda, N. *Angew. Chem., Int. Ed. Engl.* **1977**, *16*, 174. (b) Seki, Y.; Murai, S.; Hidaka, A.; Sonoda, N. *Ibid.* **1977**, *16*, 881. (c) Seki, Y.; Hidaka, A.; Makino, S.; Murai, S.; Sonoda, N. *J. Organomet. Chem.* **1977**, *140*, 361.
(4) Murai, S.; Kato, T.; Sonoda, N.; Seki, Y.; Kawamoto, K. *J. Organomet. Chem.* **1979**, *18*, 393.
(5) (a) Chatani, N.; Murai, S.; Sonoda, N. *J. Am. Chem. Soc.* **1983**, *105*, 1370. (b) Chatani, N.; Fujii, S.; Yamasaki, Y.; Murai, S.; Sonoda, N. *J. Am. Chem. Soc.* **1986**, *108*, 7361.
(6) Chatani, N.; Furukawa, H.; Kato, T. Murai, S.; Sonoda, N. *J. Am. Chem. Soc.* **1984**, *106*, 430.
(7) Preliminary results have been reported: Murai, T.; Kato, S.; Murai, S.; Toki, T.; Suzuki, S.; Sonoda, N. *J. Am. Chem. Soc.* **1984**, *106*, 6093.

0002-7863/89/1511-7938$01.50/0