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

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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 O2 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, 2.23 Thus, although it bears clear similarities to the polyketide-derived isoperoxacin, 3,45 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).

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 known6—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′,4,6-2H5]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% deuteriation at C-4 and at C-6, with quantitative mass recovery. This was then acetylated with [3H]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 ceric ammonium nitrate10 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

Scheme I

Scheme II

is prone to oxidation, but could be purified by careful recrystallization.

References

(3) We have established the correct absolute stereochemistry as shown (unpublished results).
(7) These compounds had previously been reported: Kehrmann, F.; Bhattacharian, G. Chem. Ber. 1998, 31, 2399. However, the melting point of the material purported to be 5 was 80 °C lower than that of ours.
Biosynthesis of Antibiotic LL-C10037α

Scheme III

\[
\begin{align*}
6 & \xrightarrow{\text{i}} 6a \xrightarrow{\text{ii}} 7a \\
\end{align*}
\]

\*(i) \text{D}_2\text{O}/\text{TFA-d}/\text{MeOD}/80^\circ\text{C}; (ii) \text{CD}_3\text{COCl/}3\text{N/CH}_2\text{C}_2; (iii) \text{BBr}_3/\text{CH}_2\text{Cl}_2\rightarrow -78^\circ\text{C}\text{ to room temperature}; (iv) \text{PbO}_2/\text{CH}_2\text{Cl}_2\rightarrow\text{Et}_2\text{O}.

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

<table>
<thead>
<tr>
<th>Substrate</th>
<th>\text{CH}_2\text{OD}</th>
<th>\text{CH}_3\text{OD}</th>
<th>4 \text{ M DCI}, 110^\circ\text{C}</th>
<th>3 \text{ M DCI}, 80^\circ\text{C}</th>
</tr>
</thead>
<tbody>
<tr>
<td>\text{OCH}_3\text{NO}_2</td>
<td>NR</td>
<td>NR</td>
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<tr>
<td>\text{OCH}_3\text{NH}_2</td>
<td>NR</td>
<td>NR</td>
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<td>-</td>
</tr>
<tr>
<td>\text{OCH}_3\text{OAc}</td>
<td>NR</td>
<td>NR</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>\text{OH} \text{NH}_2 \text{Ac}</td>
<td>NR</td>
<td>NR</td>
<td>H-4, H-6</td>
<td>-</td>
</tr>
<tr>
<td>\text{OH} \text{OAc}</td>
<td>NR</td>
<td>NR</td>
<td>H-4, H-6</td>
<td>-</td>
</tr>
<tr>
<td>\text{OH} \text{NH}_2 \text{Ac}</td>
<td>NR</td>
<td>NR</td>
<td>H-4, H-6</td>
<td>-</td>
</tr>
<tr>
<td>\text{OCH}_3\text{COOH} \text{Bu}</td>
<td>NR</td>
<td>NR</td>
<td>H-4, H-6</td>
<td>-</td>
</tr>
</tbody>
</table>

\*
Room temperature for 24 h. \*60^\circ\text{C} for 24 h. \*80^\circ\text{C} for 12 h. \*60^\circ\text{C} for 48 h.

A portion of 5a (100 mg) was fed to a 200-mL fermentation of \text{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 \text{^1H NMR spectroscopy} (Figure 1B).\(11\)

\[
\begin{align*}
6a & \xrightarrow{\text{i}} 10 \xrightarrow{\text{ii}} 10a \\
\end{align*}
\]

\*(i) 3 \text{ M DCI/Δ}; (ii) \text{CD}_3\text{COCl/}3\text{N/CH}_2\text{C}_2; (iii) \text{BBr}_3/\text{CH}_2\text{Cl}_2\rightarrow -78^\circ\text{C}\text{ 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 DCI/D_2O at reflux and yielded the hydrochloride salt [4,6-\text{^2H}_2]8a. Whereas the free base is very unstable,\(12\) the

(11) All \text{^1H 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.

Scheme VI

\[
\begin{array}{c}
\text{9a} \\
\text{9b}
\end{array}
\]

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 \(^1\)H NMR (Scheme IV, Figure 1C). The incorporation of \(\text{8a} \) into 1b had been 0.5%.

From 2,2',2,4,6-\(^2\)H\(_{5}\)-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 1e (45 mg). Examination of the \(^1\)H 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 more efficiently incorporated into 1b. The latter was apparently then more efficiently incorporated into 1. The lower enrichment at H-3 of 1 was believed to reveal an NH\(^{13}\) shift in the hydroxylation of the aromatic ring. On the basis of unrecovered 9a, a 0.54% incorporation of the acetyl residue was obtained.

In order to confirm the NH shift and simultaneously test 2-aminophenol, 11, as an intermediate, [3,5-\(^2\)H\(_2\)]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 or 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 with the sample derived from 5a, H-3, H-5, and the methyl group were labeled with deuterium in proportion to the material fed,

\[\text{llb} \rightarrow \text{ld}\]

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-CL10037a. 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

\[\text{4a} \rightarrow \text{le}\]

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 [4,6-2H2]5b and [2,2',2'-2H3]4b to the same fermentation. In this case If, 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 antibiotic LL-C10037a was 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 KCI 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, 1635 was detected by TLC of the ethyl acetate extracts. For clear identification of the product, a 24-h-old CFE was added to a 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 1H 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, the enzyme is most likely a flavin monoxygenase.

The cofactor requirements of this enzyme, acetamidobenzoquinone (ABQ) monoxygenase, were investigated with 4 as the substrate, and the results are presented in Table III. From entries 1 and 2 it is clear that O2 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 and have also found that either coenzyme will reduce 4 chemically although this is a slower reaction than the

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

<table>
<thead>
<tr>
<th>compd fed</th>
<th>amt* fed, mg</th>
<th>positions labeled, % enrichmentb</th>
<th>amt obtained, mg</th>
<th>positions labeled, % enrichmentb</th>
<th>% incorp</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td>100.0</td>
<td>H-4, 60</td>
<td>53.4</td>
<td>H-5, 15.1</td>
<td>9.10</td>
</tr>
<tr>
<td>5a</td>
<td>100.0</td>
<td>H-6, 89</td>
<td>47.6</td>
<td>H-5, 11.3</td>
<td>0.47</td>
</tr>
<tr>
<td>5a</td>
<td>100.0</td>
<td>H-2', 100</td>
<td>45.2 (1c)</td>
<td>H-5, 0.67</td>
<td>0.54</td>
</tr>
<tr>
<td>5a</td>
<td>100.0</td>
<td>H-4, 92</td>
<td>89.0 (9a)</td>
<td>H-5, 0.19 (based on H-5)</td>
<td></td>
</tr>
<tr>
<td>5a</td>
<td>100.0</td>
<td>H-6, 92</td>
<td>26.6 (1d)</td>
<td>H-6, 0.22 (based on H-6)</td>
<td>0.13</td>
</tr>
<tr>
<td>5a</td>
<td>100.0</td>
<td>H-5, 100</td>
<td>98.0 (9b)</td>
<td>H-2', 0.09</td>
<td></td>
</tr>
<tr>
<td>5a + 4b</td>
<td>32.0 each</td>
<td>H-4, 57</td>
<td>21.0</td>
<td>H-5, 6.0</td>
<td>1.60</td>
</tr>
<tr>
<td>5a + 4b</td>
<td>32.0 each</td>
<td>H-6, 93</td>
<td>53.0</td>
<td>H-5, 3.27</td>
<td>10.20 (5b)</td>
</tr>
<tr>
<td>5a + 4b</td>
<td>32.0 each</td>
<td>H-2', 88</td>
<td></td>
<td>H-2', 4.10</td>
<td>7.90 (4b)</td>
</tr>
</tbody>
</table>

*Fed as an aqueous or aqueous ethanol solution to 200 mL of production broth 96 h after inoculation. *Determined from the 1H NMR spectrum. *Worked up 24 h after feeding. *Determined by 1H NMR analysis; see ref 11. *Compound (no. of scans): 1a (6976), 1b (16309), 1c (15358), 1d (39414), 1e (22706), If (14983). *Quantiﬁed by comparison of integrals with that from 25 μL of t-BuOH added to each sample.
Table III. Epoxidation of Acetamidobenzoquinone (ABQ, 4) by Cell-Free Preparation of S. LL-C10037

<table>
<thead>
<tr>
<th>entry</th>
<th>assay components</th>
<th>products (TLC)</th>
<th>% 16b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NADPH, CFE</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>NADPH, CFE</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>NADH</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>NADPH</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>CFE</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>NADH, CFE</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>NADH, CFE</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>NADPH, CFE</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

aConditions: substrate, 0.1 mM; cofactors, 1.0 mM; buffer, 0.1 M; total volume, 10 mL; time, 20 h; temperature, 30 °C. Determined by chromatography of extracts, and UV quantitation.

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 isooxydopine,2 3 (see Scheme I), and phyllostine,2 9, 22, from blocked mutants of P. urticae and demonstrated2 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 epoxidease has never been isolated.51

No advanced studies on the biosynthesis of the polyketide-derived epoxylacton terretum and the related epoxquinone teeriacid52 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 isooxydopine 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; 13C NMR spectra were taken at 20 and 100.6 MHz on Varian FT 80 and Bruker AM 400 spectrometers. 1H NMR spectra were obtained at 61.4 MHz on a Bruker AM 400 spectrometer. All 13C NMR spectra were broad-band decoupled and 1H NMR spectra were proton decoupled and run unlocked. Five-millimeter NMR tubes were used for all NMR measurements. 1H and 13C NMR samples were referenced with TMS, CH3CN, or t-BuOH. 1H NMR samples were prepared in D2O-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 1H in the methyl groups).

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

Melting points were taken on a Buchi 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 F254 (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 5 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% CaCO3 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 flask) consisting of 1.0% glucose, 0.4% yeast extract, 2.0% molasses (Granda's Famous light unsulfured), and 0.1% CaCO3 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 H2O/ethanol mixture and added in a sterile manner through micropore filters after ca. 96 h.


(42) Forrester, P. I.; Gaucher, G. M. Biochemistry 1972, 11, 1108.
(51) Neither [14C]gentisyl alcohol nor [14C]gentisaldehyde quinone 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 isooxydopine and phyllostine. Gaucher, G. M. Personal communication.
Isolation. The cultures were filtered through cheesecloth and Celite, and the filtrate was adjusted to pH 4.7 with solid KH$_2$PO$_4$. This was then saturated with (NH$_4$)$_2$SO$_4$ 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 I, which was crystallized from MeOH.

\[ \text{[4,6-H$_2$]-2,5-Methoxyaniline} \]

6a. 2,5-Dimethoxyaniline (600 mg, 3.92 mmol), MeOH (20 mL), D$_2$O (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.

\[ \text{[4,6-H$_2$]-2,5-Methoxyaniline} \]

With use of the above procedure, a mixture of 10a (360 mg, 2.3 mmol), acetyl chloride (217 mg, 2.8 mmol), and Et$_3$N (542 mg, 5.6 mmol) in CH$_2$Cl$_2$ (30 mL) gave deuterated 2-methoxyaniline (280 mg, 74%).

\[ \text{[2,2',2'-6'-H$_3$]-2,5-Methoxyaniline} \]

With use of the above procedure, a mixture of 10b (360 mg, 2.3 mmol), acetyl chloride (217 mg, 2.8 mmol), and Et$_3$N (542 mg, 5.6 mmol) in CH$_2$Cl$_2$ (30 mL) gave deuterated 2-methoxyaniline (280 mg, 74%).

\[ \text{2-Hydroxyacetanilide} \]

2-Hydroxyacetanilide (41 mg, 0.25 mmol) in dry CH$_2$Cl$_2$ (15 mL) under an Ar atmosphere was treated at -78 °C with solid BBr$_3$/CH$_2$Cl$_2$ (300 mL, 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. mp 209 °C); $^1$H NMR (aceto-d$_2$, 400 MHz) δ 8.89 (1 H, s, exch D$_2$O), 9.41 (1 H, br, exch D$_2$O), 7.39 (1 H, dd, J = 8.1, 1.3 Hz), 7.03 (1 H, d, J = 6.8 Hz), 8.19 (1 H, d, J = 8.1 Hz), 6.80 (1 H, m), 2.21 (3 H, s); $^{13}$C NMR (aceto-d$_2$, 100.6 MHz) δ 171.22, 149.42, 127.72, 126.61, 122.84, (20.51, 118.96, 23.49.

\[ \text{2-[3,4,6-H$_3$]-2-Hydroxyacetanilide} \]

As described above, 2-[3',4',6'-H$_3$]-2-hydroxyacetanilide (280 mg, 1.65 mmol) was deprotected with 1 M BBr$_3$/CH$_2$Cl$_2$ (2.0 mL, 0.00 mmol) to yield 247 mg (96%) of 9a (mp 206-208 °C).

\[ \text{2,5-Dimethoxyacetanilide} \]

With use of the above procedure, 6a (560 mg, 3.6 mmol), acetyl chloride (381 mg, 4.7 mmol), and Et$_3$N (473 mg, 4.7 mmol) in CH$_2$Cl$_2$ (30 mL) yielded 80% of 2,5-dimethoxyacetanilide (288 mg, 62%).

\[ \text{2,5-Dihydroxyacetanilide} \]

6a. 2,5-Dihydroxyaniline (90 mg, 0.6 mmol) was dissolved in CH$_2$Cl$_2$ (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$_2$Cl$_2$ (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 H$_2$O (100 mL), extracted with EtOAc (5 X 20 mL), and washed with saturated brine. The combined organic fractions were dried (Na$_2$SO$_4$ and concentrated in vacuo to give 83 mg (100%) of nearly pure 2,5-dimethoxyacetanilide.

\[ \text{[2,2',2',4',6-2H$_5$]-2,5-Dihydroxyacetanilide} \]

With use of the above procedure, 6a (560 mg, 3.6 mmol), acetyl chloride (381 mg, 4.7 mmol), and Et$_3$N (473 mg, 4.7 mmol) in CH$_2$Cl$_2$ (30 mL) yielded 2,5-dimethoxyacetanilide (288 mg, 62%).

\[ \text{2-Acetamido-1,4-benzoquinone} \]

2-Acetamido-1,4-benzoquinone (4a) as a white solid. Recrystallization from EtOH yielded 660 mg (95%) of nearly pure 2-acetamido-1,4-benzoquinone (4a).

\[ \text{2,5-Dimethoxyacetanilide} \]

With use of the above procedure, 6a (193 mg, 1.13 mmol) in Et$_2$O (25 mL) and CH$_2$Cl$_2$ (25 mL) was treated with PdO$_2$ (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 x 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. mp 175-177 °C).
Cobalt Carbonyl Catalyzed Reactions of Cyclic Ethers with a Hydrosilane and Carbon Monoxide. A New Synthetic Reaction Equivalent to Nucleophilic Oxymethylation

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Abstract: Siloxymethylative ring opening of cyclic ethers has been attained by a new catalytic system of HSiR3/CO/CO2(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 7-membered ring > 6-membered ring > 5-membered ring. Among the hydrosilanes (HSiMe3, HSiEt2Me, and HSiEt3), the highly reactive HSiMe3 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.

The new catalytic reaction system of HSiR3/CO/CO2(CO)8 can bring about incorporation of carbon monoxide into olefins and various oxygen-containing compounds. It has been shown that carbon monoxide is incorporated into the products in the form of formylsiloxymethylidene (=CHOSiR3), or other carbafunctional groups. For example, conversions of alkenes to siloxymethylidene alkanes,2,3 aldehydes to higher siloxymethylidene aldehydes,4 alkyl acetates to siloxymethylidene alkanes,5 and cyclobutanones to disiloxycyclopentenones6 have been attained by the reaction of these substrates with HSiR3 and CO in the presence of CO2(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 HSiR3/CO/CO2(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.

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\text{HSiR}_3 + \text{CO} \rightarrow \text{R}_3\text{Si(CHOSiR)}(\text{CO})_8
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From the synthetic point of view, the present reaction can be regarded formally as a nucleophile oxymethylation (ROCH2-).

The reaction should provide a simple method for the construction of 1,3-diol units, an important building block in natural product synthesis.

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