is deemed thermodynamically unfeasible,[11] however. Regarding the slow enolization of IIH_T to IH_{2T}, Ogata[12] and co-workers have observed rapid equilibrium addition of a sulfur nucleophile to a fused benzoquinone followed by slow base-catalyzed enolization to the substituted hydroquinone in aqueous media.

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6-Hydroxyanthranilic Acid: A New Shikimate Pathway Product Found in the Biosynthesis of Sarubicin A

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Sarubicin A, a quinoid antibiotic isolated from various strains of *Streptomyces*,[2-4] has been characterized as **1** on the basis of physical data.[4,5] Confirmation was provided by a recent total synthesis.[6] The ^1H NMR spectrum and the nonaromatic portion of the ^{13}C NMR spectrum of **1** have been assigned.[7] We now report that the quinone portion of **1** is biosynthesized from 6-hydroxyanthranilic acid, derived by an apparently new variation of the shikimate pathway.

Previous work at The Upjohn Co. had demonstrated that glucose **2** is the direct precursor to the tetrahydropyran portion of sarubicin A and had indicated a possible shikimate origin for the quinone ring.[8] Building on this foundation, we carried out a fermentation of *Streptomyces helicus* (UC-5837) in the presence of $^{18}O_2$. A 100-mL seed broth[9] in a 500-mL flask was inoculated with spores of *S. helicus* and incubated at 32 °C for 24 h in a rotary shaker (225 rpm). A 10-mL portion was then added to each of two production broths[10] (250 mL in 1-L Erlenmeyer flasks). These were connected in series via two sterile filters to a closed system containing a burette refillable with $^{18}O_2$ (50%, obtained from Cambridge Isotopes, Inc.), a small air pump, and a CO_2 trap (aqueous KOH). Air was circulated at 2 L/min while the fermentation flasks were shaken as described above. After 72 h the fermentation was stopped; the combined broths were adjusted to pH 3 (1 N HCl), filtered, saturated with $(NH_4)_2SO_4$ (260 g), and extracted with three 500-mL portions of EtOAc. The extracts were dried (Na_2SO_4), filtered, concentrated, and chromatographed on silica gel. Elution with 10% MeOH/$CHCl_3$ gave 15.8 mg of pure **1a**.

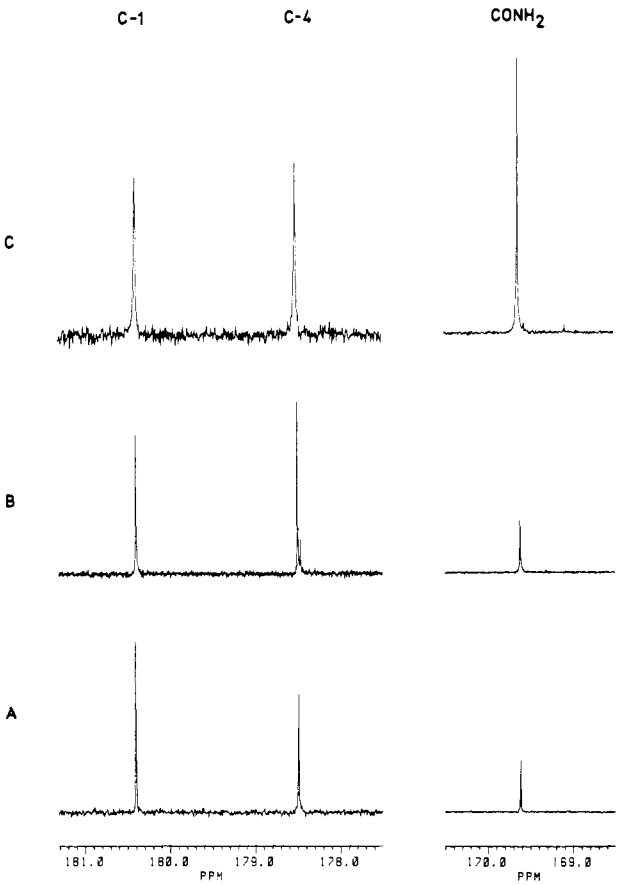

Figure 1. Waltz decoupled ^{13}C NMR spectra, 100.6 MHz, of sarubicin A taken on a Bruker AM 400 spectrometer. (A) Natural abundance **1** (SW = 22 727 Hz, SI = TD = 64 K, AQ = 1.44 s, NS = 24000, PW = 36°). (B) ^{18}O-labeled **1a** (SW = 25 000 Hz, SI = TD = 128 K, AQ = 2.62 s, NS = 25927, PW = 36°). (C) ^{13}C-labeled **1b** (NS = 28 000, other parameters same as for (A)). The amplitude for the 177.5–181.4 ppm region is 5 times that of the 168.5–170.5 ppm region in all three spectra.

Examination of the EI mass spectrum of **1a** indicated that one ^{18}O label had been incorporated, and fragments[7] at m/z 252, 235, and 207 led us to believe that the label was in one of the quinone carbonyls. In order to identify the precise location of the label, we intended to use the expected isotope shift of the ^{13}C NMR resonance.[11] However, it was first necessary to overcome the problem of carbonyl resonances 7–15 Hz wide that were encountered repeatedly with various samples of **1**. Fortunately, when a warm, dilute aqueous solution of **1** was filtered through a small portion of Chelex, interfering paramagnetic ions were apparently removed. After lyophilization, the 100-MHz ^{13}C NMR spectrum of a portion of the sample in Me_2SO-d_6 gave excellent narrow lines (Figure 1A). This was repeated for **1a** and the 178.50 ppm resonance was found to be accompanied by a smaller peak 0.03 ppm (3.45 Hz) upfield (Figure 1B).

To assign the ^{13}C NMR resonances of the quinone ring, a second portion of the deionized natural abundance sample was exchanged 3 times with ethanol-d_1, dried thoroughly, and combined with an unexchanged, deionized sample in Me_2SO-d_6. From the deuterium-induced isotope shifts of the ^{13}C NMR resonances[12] thus obtained, the resonances at 180.0 and 178.5 ppm could be unequivocally assigned to C-1 and C-4, respectively. The C-4 resonance showed five additional lines (upfield shifts of 0.01, 0.03,

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(10) The medium for the production culture consisted of glucose, 5.0 g, $(NH_4)_2SO_4$, 1.0 g, $CaCO_3$, 5.0 g, trace salts, 1.0 mL, and water to 1.0 L. The trace salts stock was made up of $MgSO_4 \cdot 7H_2O$, 200 g, $MnSO_4 \cdot H_2O$, 5.0 g, $ZnSO_4 \cdot H_2O$, 10.0 g, $FeSO_4 \cdot 7H_2O$, 6.0 g, $CoCl_2 \cdot 6H_2O$, 2.0 g, and deionized water to 1 L. Slechta, L., private communication.

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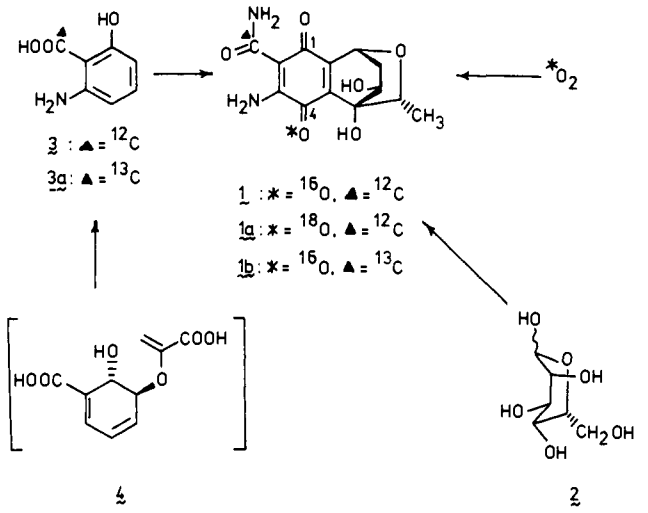
Scheme I



0.04, 0.05, and 0.06 ppm), while the C-1 resonance showed one additional line shifted 0.03 ppm upfield. The multitude of lines for C-4 was due to the presence of NH_2, NHD, and ND_2 at C-3, as well as two definable orientations for the NHD due to hydrogen bonding. The unexpected shift for the C-1 resonance was due to a deuterium isotope effect from the amide hydrogen through hydrogen bonding to the carbonyl oxygen. Confirmation for an isotope shift through a hydrogen bond was obtained from an analogous experiment with partially exchanged methyl anthranilate, in which case the normal ester carbonyl was accompanied by two additional lines (upfield shifts of 0.01 and 0.02 ppm).[13] Therefore, we have concluded that the ^{18}O label from the biosynthetic experiment was located at C-4.

Although not previously a known natural product, we next tested 6-hydroxyanthranilic acid **3** as a logical precursor to the quinone ring. By use of an efficient four-step procedure,[14] [^{13}COOH]-6-hydroxyanthranilic acid (**3a**) was prepared, and 13-mg portions were added to each of three 200-mL production broths at 23, 35, and 47 h after they had been inoculated with a seed culture. Workup at 70 h afforded 33.8 mg of pure **1b**. The 100-MHz ^{13}C NMR spectrum of **1b**, obtained under identical conditions as for a natural abundance sample, showed a clean 7% enrichment in ^{13}C for the amide carbonyl (Figure 1C), demonstrating the intact incorporation of **3a**.[15]

The results presented here reveal a new aromatic amino acid which would most reasonably be derived from the shikimate pathway via isochroismic acid (**4**),[16] as shown in Scheme I. This will be tested as part of our continuing studies of sarubicin biosynthesis.

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Absolute Rate Constants for Reaction of Phenyl, 2,2-Dimethylvinyl, Cyclopropyl, and Neopentyl Radicals with Tri-*n*-butylstannane. Comparison of the Radical Trapping Abilities of Tri-*n*-butylstannane and -germane[1]

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Intramolecular radical cyclizations have become important in organic synthesis because the generally high regio- and stereoselectivities are complemented by the fact that there is no need to "protect" many types of functional groups.[4-7] For cyclizations involving carbon-centered radicals the usual procedure involves a free-radical chain reaction between a suitable precursor (e.g., a bromide) and tri-*n*-butylstannane:

$$n\text{-Bu}_3\text{Sn}\cdot + \text{R}_1\text{Br} \rightarrow n\text{-Bu}_3\text{SnBr} + \text{R}_1\cdot$$

$$\text{R}_1\cdot \xrightarrow{k_c} \text{c-R}_2\cdot$$

$$\text{R}_1\cdot + n\text{-Bu}_3\text{SnH} \xrightarrow{k_H^{Sn}} \text{R}_1\text{H} + n\text{-Bu}_3\text{Sn}\cdot$$

$$\text{c-R}_2\cdot + n\text{-Bu}_3\text{SnH} \rightarrow \text{c-R}_2\text{H} + n\text{-Bu}_3\text{Sn}\cdot$$

The yield of cyclized product, c-R_2H, is dependent on the concentration of tin hydride and on the ratio of the rate constants for hydrogen abstraction by $R_1\cdot$ and for its cyclization, i.e., k_H^{Sn}/k_c. Detailed synthetic strategies can be improved by kinetic information regarding the reactions involved and, in particular, by a knowledge of the magnitude of k_H^{Sn}. Absolute rate constants for this reaction have been measured for $R_1\cdot$ = methyl, *n*-alkyl, *sec*-alkyl, and *tert*-alkyl radicals by the rotating sector method at room temperature[8] and, more reliably and over a range of temperature, by the laser flash photolytic technique.[9] The growing importance of cyclizations involving carbon-centered radicals that are considerably more reactive than the alkyls, e.g., phenyl-,[10,11] vinyl-,[12-14] and cyclopropyl-centered[15] radicals, has generated a need for absolute values of k_H for these classes of radicals.

Results and Discussion. Kinetic data for phenyl, 2,2-dimethylvinyl, and cyclopropyl radicals were obtained by monitoring the growth of the *n*-$Bu_3Sn\cdot$ radical at 400 nm[9,16] following the

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