with Schaap's observations. Exhaustive electrolysis of **4** at 0.93 V vs. SCE ($E^{0'}$ = +0.89 V vs. SCE)[11] in an argon- or oxygen-saturated solution produced a deep green-colored solution with passage of 1 F/mol. The deep green solution persisted for several hours in argon-saturated solution but faded in 20 min in an oxygen atmosphere to form a colorless solution. An ESR signal was observed for the green solution but disappeared as soon as the color faded. The slow reaction of these radical cations with oxygen reflects the extensive delocalization of the odd electron density in $3^{+\cdot}$ and $4^{+\cdot}$.

This reaction is an example of a continually increasing class of reactions[12] in which a chemical reaction is accelerated in the presence of an electrode. The successful isolation of the dioxetane is based upon the enhanced reactivity of an oxidized state of the substrate with molecular oxygen. This reaction provides a counter example to the $S_{RN}1$ electrode-catalyzed reaction[13] which owes its success to enhanced reactivity of a reduced state of the starting material.

We are continuing our study of the electrode-catalyzed oxidations of organic molecules to determine the generality of this reaction and we anticipate the communication of additional results in the near future.

Acknowledgment. NMR data critical for the completion of this work was graciously provided for us by Dr. Daniel Netzel of the Laramie Energy Technology Center. We also thank Professor S. F. Nelsen (University of Wisconsin—Madison) for communication of his results prior to publication.

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Direct Evidence from Multiple ^{13}C Labeling and Homonuclear Decoupling for the Labeling Pattern by Glucose of the *m*-Aminobenzoyl (C_7N) Unit of Pactamycin

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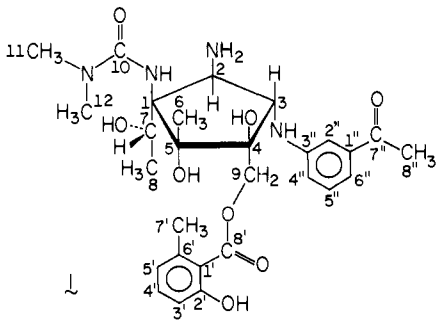
A number of structurally interesting and clinically important antibiotics contain a "C_7N" unit whose biosynthesis is believed to be related to the shikimate pathway.[1] The simplest of these C_7N units is the *m*-aminobenzoyl unit found in pactamycin (**1**).[1,2]



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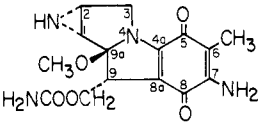
The aminobenzoquinonoid portions of mitomycin C (**2**, C-4a through C-8a, 6-methyl, N-4),[3] geldanamycin,[4] and the nearly



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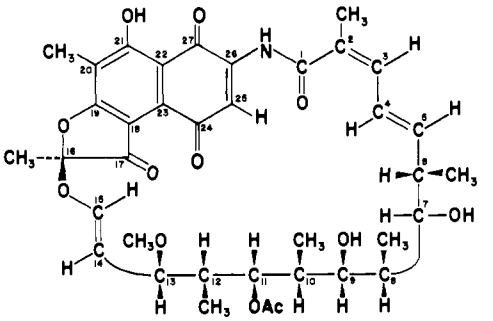
identical herbimycin[5] and asukamycin[6] are of intermediate complexity, while modified C_7N units are built into the aminonaphthoquinonoid portions of rifamycin S (**3**, C-21 through C-27)[7,8] and streptovaricin D.[9]



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In each of these antibiotics, studies thus far have shown C-6 of glucose to label the C_7N unit at both carbons ortho to the carbon bearing the exocyclic carbon,[10] in agreement with a shikimate-type pathway,[11] involving condensation of phosphoenol pyruvate and erythrose 4-phosphate to 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate (DAHP) and the latter's cyclization to 5-dehydroquinic acid (DHQ). An attractive possibility for introduction of the amino group into the C_7N unit would involve transamination of the keto group of DHQ or dehydroshikimic acid (DHS), as shown in path A (Scheme I). This pathway requires that erythrose 4-phosphate provide C-2″ through C-5″ of the *m*-aminobenzoyl portion of **1** and phosphoenol pyruvate provide C-1″, C-6″, and C-7″. Path A was suggested by our earlier observation of the relatively greater labeling of C-2″ than C-6″ by [6-^{13}C]-glucose and the opposite ratio (C-6″ > C-2″) by [1-^{13}C]glucose,[1,2] a direct analogy to the relative labeling patterns in the original shikimate pathway study of Srinivasan et al.[11] Hornemann has recently suggested that the alternative path B is operative in mitomycin biosynthesis, on the basis of chemical degradation of mitomycin labeled by D-[4-^{14}C]erythrose and [3-^{14}C]pyruvate.[3] Path B, if operative in pactamycin biosynthesis, would require that erythrose 4-phosphate provide C-3″ through C-6″ of the *m*-aminobenzoyl group and phosphoenol pyruvate provide C-1″, C-2″, and C-7″.

A third possibility, combining elements of paths A and B, was suggested by White and Martinelli,[7] who argued for a DHS intermediate but for amination at C-3 of DHS, which would give

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(9) Rinehart, Jr., K. L.; Shield, L. S. *Fortschr. Chem. Org. Naturst.* **1976**, *33*, 231–307.
(10) Other natural products, including the maytansinoids and additional members of the ansamycin class[9] are presumed to contain units formed by the same pathway.
(11) Srinivasan, P. R.; Shigeura, H. T.; Sprecher, M.; Sprinson, D. B.; Davis, B. D. *J. Biol. Chem.* **1956**, *220*, 477–497.

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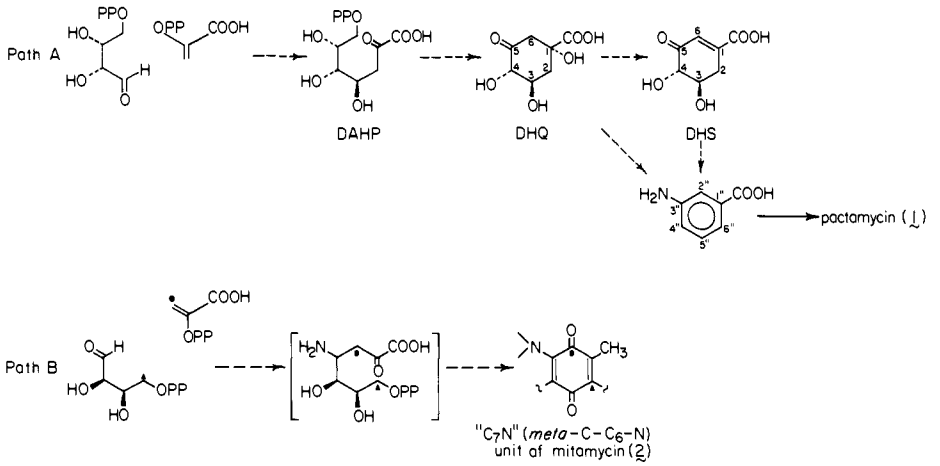
Scheme I. Alternative Pathways Proposed Previously for the C_7N Units of Pactamycin (1)[2] and Mitomycin (2)[3]



the same labeling results as path B, i.e., erythrose 4-phosphate providing C-3″ through C-6″ and phosphoenol pyruvate C-1″, C-2″, and C-7″ of the *m*-aminobenzoyl group of Scheme I.

In our earlier work with the production of pactamycin by *Streptomyces pactum* var. *pactum*, we demonstrated good incorporation of C-6 of glucose into the *m*-aminobenzoyl unit,[2] and thus, it seemed possible that we could use fully labeled glucose[12] to decide between paths A and B. If we assume that C-4 through C-6 (and, to a lesser extent, C-3 through C-1) of glucose provides C-1 through C-3 of phosphoenol pyruvate and that C-3 through C-6 of glucose provides C-1 through C-4 of erythrose 4-phosphate, then we would expect the carbons of one unit (pyruvate, erythrose) to be coupled to other adjacent carbons in the *same* unit but not to those in the other unit should a relatively small amount of [U-^{13}C]glucose be added to the fermentation medium in the presence of a large amount of unlabeled glucose. Indeed, that experiment has been carried out to provide direct evidence in agreement with path A[2] but eliminating path B[3] for the biosynthesis of pactamycin.[13]

Streptomyces pactum was incubated under the conditions and in the production medium (8 flasks, 100 mL each) described previously.[2] After 48 h mycelia were harvested, centrifuged, washed, and resuspended in fresh production medium (4 flasks, 100 mL each) containing the same concentration of inorganic salts plus 0.8 g of unlabeled sodium acetate, 0.5 g of glucose labeled with ^{13}C to the extent of 84% at each carbon, and 3.5 g of unlabeled glucose. After 72 h of shaking beyond the addition of precursor, the medium was adjusted to pH 8.3 and shaking was continued for 36 h. The combined broths were worked up as before[2] to give 12 mg of [^{13}C]pactamycate (**4**). Homonuclear ^{13}C–^{13}C coupling observed is given in Table I.

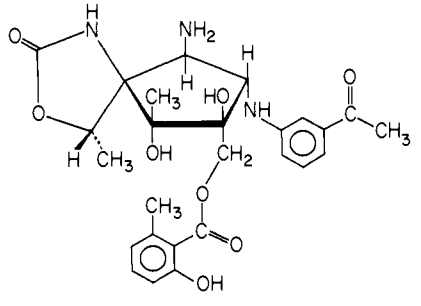


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Table I. Homonuclear Coupling of Carbons of the *m*-Aminoacetophenone Unit of Pactamycate (4) Derived from [U-^{13}C] Glucose

carbon	chemical shift, ppm[a]	J_{CC}, Hz
C-1″	139.0	58, 62
C-2″	112.2	64
C-3″	149.2	64, 58
C-4″	118.1	60, 58
C-5″	129.8	60
C-6″	117.8	58
C-7″	*b*	
C-8″	26.6	singlet

[a] Spectrum obtained in CD_3COCD_3 solvent, on a Varian XL-100 spectrometer. [b] Not observed due to solvent.

The critical carbons in distinguishing between paths A and B are C-1″, which should couple to either C-6″ (path A) or C-2″ (path B), C-2″, which should couple to either C-3″ (path A) or C-1″ (path B), and C-6″, which should couple to either C-1″ (path A) or C-5″ (path B). In addition, path A requires C-3″ to be coupled to *both* C-2″ and C-4″ but C-5″ to *only* C-4″, while path B requires C-3″ to be coupled to *only* C-4″ but C-5″ to be coupled to *both* C-4″ and C-6″.

All couplings observed are in accord with the predictions of path A. Carbon-5″ (like C-6″ and unlike C-4″) is a doublet (J_{CC} = 60 Hz) coupled to C-4″, which is coupled to C-3″ as well as C-5″ (Table I). Similarly, C-2″ (a doublet, J_{CC} = 64 Hz) can be seen from the coupling constants in Table I to be coupled to C-3″ (a multiplet) rather than to C-1″, while C-6″ (a doublet) is coupled to C-1″ (a multiplet) rather than to C-5″. Thus, path A is clearly followed by *Streptomyces pactum* in the biosynthesis of pactamycin.[13]

To test the conclusion reached regarding path A from splitting patterns and coupling constants alone, the sample of pactamycate labeled by [U-^{13}C]glucose was subjected to homonuclear decoupling (JEOL FX-400, CD_3COCD_3 solvent). As expected from path A, irradiation at 139.7 ppm (C-1″) collapsed the doublet spanning C-6″ (118.1 ppm) without affecting the doublet spanning C-2″ (112.9 ppm), while irradiation at 149.9 ppm (C-3″) collapsed the doublet spanning C-2″ (112.9 ppm) to a singlet.[14]

Scheme I shows path A leading to *m*-aminobenzoic acid. Although we had assumed the latter compound to be incorporated into pactamycin, since the methyl group of the *m*-aminoacetophenone unit is derived from the methyl carbon of acetate,[2] it had not been demonstrated previously. We have now shown this to be the case by adding *m*-amino[*ring*-U-^{14}C]benzoic acid to growing *S. pactum* and isolating the pactamycate, which contains 11.5%

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(13) While these results apply strictly only to pactamycin, we presume they are valid for other C_7N-containing antibiotics.

(14) In addition, irradiation at 112.9 ppm (C-2″) appeared to alter the pattern for C-3″ (149.9 ppm) without affecting that for C-1″ (139.7 ppm), though poor signal-to-noise ratio for these peaks prevented more definitive descriptions of the patterns.

of the added label. Very recently, 3-amino-5-hydroxybenzoic acid has been shown to be incorporated into mitomycin[15] and the ansamycin antibiotics actamycin[16] and rifamycin S[17] and, most pertinently, to be a part of the starter unit for rifamycin S.[18] A decision as to whether 3-amino-5-hydroxybenzoic acid is also a precursor of *m*-aminobenzoic acid must await the outcome of additional experiments.

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(17) Ghisalba, O.; Nüesch, J. *J. Antibiot.* **1981**, *34*, 64–71.
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Electron Spin Resonance–High-Performance Liquid Chromatography Study of Organometallic Free Radical Reactions: Separation and Characterization of the Rhenium Carbonyl–*o*-Quinone Radical Complex

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The successful use of high-performance liquid chromatography (HPLC) to separate nitroxide and other organic oxygen- and nitrogen-centered radicals has recently been reported.[1,2] Because of the wide potential applications of HPLC to chemical, biochemical, and organometallic studies and the established technique of ESR in characterizing free radicals and paramagnetic intermediates involved in chemical reactions, we have currently initiated a series of investigations of organometallic free radical reactions in solution by using an integral general-purpose HPLC–ESR apparatus recently assembled in our laboratory. We present here our first successful application of this HPLC–ESR technique to the separation and characterization of an interesting organometallic radical complex, the rhenium carbonyl–3,5-di-*tert*-butyl-*o*-quinone complex which is readily formed during the photolysis of a benzene solution containing both the parent quinone and $Re_2(CO)_{10}$. The conclusive results shed some light on the primary photochemical process between the rhenium carbonyl and the quinone, but the demonstrated usefulness of the HPLC–ESR technique in organometallic free radical studies should be of wider general interest.

The principal components of the integral HPLC–ESR apparatus consist of a positive-displacement pump coupled to a homemade pulse dampener and a Valco sample-injection six-port valve. All columns were packed in our laboratory; the general-purpose column was packed with Whatman Partisil-10 and measured 3 mm × 60 mm. The components were mounted on a platform next to the ESR bridge of a Varian V4500 X-band ESR spectrometer with 100-kHz field modulation. The connection between the HPLC column and the ESR cavity is made by Teflon tubing which is coiled around a quartz tube inserted into the cavity.

(1) T. Reich, K. S. Chen, and J. K. S. Wan, *J. Chromatogr.*, **206**, 139 (1981).
(2) K. Makino, *J. Phys. Chem.*, **84**, 1012, 1016 (1980); K. Makino, N. Suzuki, F. Moriya, S. Rokushika, and H. Hatano, *Anal. Lett., Part A*, **13**, 301 (1980).

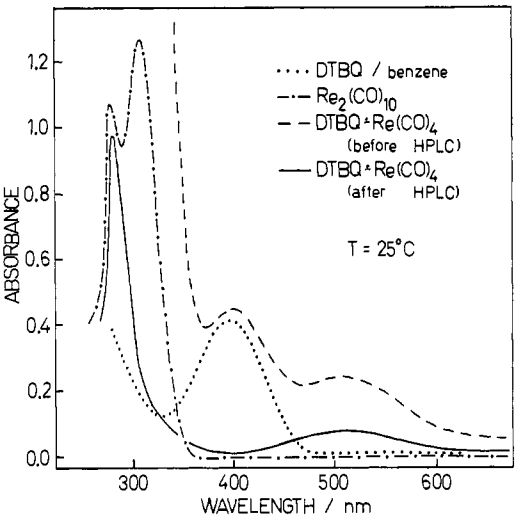
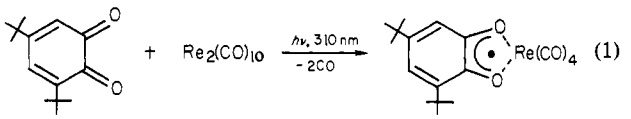


Figure 1. Electronic absorption spectra of DTBQ, $Re_2(CO)_{10}$, and the DTBQ–$Re(CO)_4$ radical complex in benzene, before and after HPLC separation. The band at 278 nm is probably due to Re → CO charge transfer transition,[3] which should remain before and after the complexation with DTBQ.

A standard radical sample placed in a capillary tube is inserted into the quartz tube in the cavity without disturbing the Teflon sample coil. The magnetic field is manually set by the standard sample and locked in by a Varian F-8A fluxmeter. The standard is then removed before the HPLC operation begins. A Narda GaAs microwave preamplifier was installed at the signal front end to enhance the detection sensitivity. Normally the HPLC–ESR apparatus is run in a dual-detector configuration with the sample solution routed from the ESR cavity directly into either a Varian SF330 double-beam spectrofluorometer or a Hilgawatt infrared spectrophotometer.

When a green benzene solution containing $Re_2(CO)_{10}$ and 3,5-di-*tert*-butyl-*o*-benzoquinone (DTBQ) was irradiated by a 200-W mercury superpressure lamp equipped with a monochromator set at 310 nm and the solution was continuously degassed by nitrogen, the color of the solution was changed to red due to the formation of the DTBQ–$Re(CO)_4$ radical complex.



HPLC was then used to separate the radical complex from the reactants and any other secondary products/radicals. The eluent of the column was monitored by ESR as well as by either IR or UV–vis absorption/emission spectrophotometry. Benzene was used as the eluting solvent. Typically and at a flow rate of 0.6 mL/min, the retention time for the DTBQ–$Re(CO)_4$ radical complex is 130 s while that of the parent quinone is 410 s, as monitored by ESR and visible absorption, respectively. Evaporation of the eluent containing the radicals only leaves a paramagnetic solid, which is confirmed by ESR as the DTBQ–$Re(CO)_4$ complex.

The electronic absorption spectra shown in Figure 1 illustrate the potential of HPLC to separate the organometallic radical complex from the parent compounds. The visible band of the quinone (λ_{max} 395 nm) is shifted upon complexation with the rhenium carbonyl to lower energy (λ_{max} 505 nm). This is expected as the quinone π electrons are delocalized onto the rhenium carbonyl moiety. The $\sigma_b \rightarrow \sigma^*$ band[3] of the $Re_2(CO)_{10}$ (λ_{max} ~308 nm) is not present in the complex, as expected.

The emission spectrum of the DTBQ–$Re(CO)_4$ radical complex was recorded in benzene at 25 °C. It shows a mirror image relationship to the absorption spectrum, having band centers at

(3) M. S. Wrighton and D. S. Ginley, *J. Am. Chem. Soc.*, **97**, 2065 (1975).

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