We are continuing our studies of these η^3-indenyl complexes in order to more completely understand the solution dynamics and the bonding in these compounds.

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Supplementary Material Available: Complete details of the X-ray diffraction experiment, listings of positional and thermal parameters, bond angles and distances, and observed and calculated structure factors for **3b** (39 pages). Ordering information is given on any current masthead page.

Biosynthesis of the Antibiotic Reductiomycin

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The antibiotic reductiomycin (**1**), a metabolite of *Streptomyces xanthochromogenus* (AM 6201),[1] consists of two unique structural units. One is a 2-amino-3-hydroxycyclopent-2-enone moiety, which is also found in the antibiotics asukamycin,[2] manumycin,[3] moenomycin,[4] senecarcin A,[5] virustomycin A,[6] bafilomycin B_1,[7] and antibiotic L-155,175.[8] The other is an unusual acetoxydihydrofuran carrying an acrylic acid side chain. In this paper we report results which establish the biosynthetic origin of these two moieties.

Labeled precursors were fed to 24-h old cultures of *S. xanthochromogenus* AM 6201 grown in 100 mL of medium (2% glucose, 2% soybean meal, 0.3% NaCl, pH 7.0) in 500-mL baffled Erlenmeyer flasks (29 °C, 300 rpm rotary shaking). Cultures were harvested 48 h later and **1** (about 150 mg/L) was extracted (ethyl acetate) from the acidified broth, purified by preparative layer chromatography (silica gel GF, $CHCl_3$–acetone 9:2), quantitated by HPLC (C-18, 5 μm, CH_3OH–H_2O 4:1), and subjected to liquid scintillation counting (Beckman LS 7500) and/or ^{13}C NMR spectroscopy (Bruker WM 300, 7.1 T, solvent $CDCl_3$). The ^{13}C NMR assignments of **1** are based on multiplicity, chemical shift theory, and characteristics of the line shapes and intensities in different solvents.

We[9] recently reported results which suggested formation of the 2-amino-3-hydroxycyclopent-2-enone moiety of asukamycin by an intramolecular cyclization of 5-aminolevulinic acid (**2**). Feeding experiments with [^{14}C]glycine point to a similar origin of this moiety in **1**. Thus, [2-^{14}C]glycine, but not [1-^{14}C]glycine, is efficiently incorporated into **1** (specific incorporation 20.9% vs. 0.8%). As in the case of asukamycin,[9] [1(4)-^{14}C]succinic acid and [5-^{14}C]-**2** were incorporated significantly (2.3% and 1.8%) but less efficiently than [2-^{14}C]glycine. The origin of the 2-amino-3-hydroxycyclopent-2-enone moiety from **2** was unequivocally proven by feeding [4,5-$^{13}C_2$]-**2** (100 mg, 90% ^{13}C per labeled carbon, Cambridge Isotopes, Woburn, MA) and observing the expected enrichments and couplings in the ^{13}C NMR spectrum of the resulting **1** (55 mg) (Table I). C-2 is twice as enriched as C-1 and C-3; in half the labeled molecules it is coupled to C-1 and in the other half to C-3. As suggested previously,[9] it seems likely that the cyclization of **2** involves an acylation by C-5 of a pyridoxal phosphate-generated α-carbanion.

Inspection of the dihydrofuran moiety suggests the possibility that the entire nine-carbon assembly, including the acetoxy group, could be derived by a ring cleavage of phenylalanine or tyrosine or a derivative thereof. In agreement with such an assumption, both ^{14}C-labeled shikimate and phenylalanine were incorporated (1.0% and 4.3%). However, [1-^{14}C]tyrosine was not incorporated at all (0.02%), and analysis of the enrichment and coupling pattern of **1** (60 mg) derived from [1,2-$^{13}C_2$]acetate (300 mg, 90% ^{13}C, British Oxygen Co., LTD, London, UK) showed that the acetoxy group is derived intact and with high efficiency from a molecule of acetate (Table I). This was clearly not in accord with the original assumption. To obtain further information on the source of the remaining seven carbon atoms of the dihydrofuran moiety, we made use of the efficient incorporation of glycerol (13–15%) and analyzed the labeling pattern of **1** (65 mg) derived from [U-$^{13}C_3$]glycerol[10,11] (300 mg, 99% ^{13}C).

The broad-band ^1H-decoupled ^{13}C NMR spectrum indicated extensive enrichment and coupling throughout the molecule (Table I). The absolute ^{13}C enrichment of the methyl carbon of the 2′-acetoxy group was determined by integration of the methyl proton signal and its ^{13}C satellites in the ^1H NMR spectrum; the integral of the corresponding ^{13}C signal was then used as the reference against which other enrichments were calculated. As expected for the known metabolism of glycerol, pairs of carbon atoms were derived intact from glycerol; these were the same ones which originated from intact acetate units, i.e., the acetoxy group, C-1/C-5 and C-3/C-4. One additional pair of coupled carbon atoms was observed at C-2′/C-3′. A coupled assembly of three carbon atoms, indicative of intact incorporation of a glycerol unit, was detected at C-3″/C-4′/C-5′, as evidenced by a 11-Hz coupling between C-3″ and C-5′. The 4′ signal showed a pattern of at least eight lines instead of the expected four for a doubly coupled carbon. This implied the presence of another coupled two-carbon or three-carbon system involving C-3′, exhibiting the same $J_{2'3'}$. A 2-D INADEQUATE experiment[12] clearly showed that C-3′ was indeed coupled to both C-2′ and C-4′ with a coincident coupling constant of 41 Hz. The projection from the INADEQUATE spectrum at δ 114.68 (C-4′) showed an eight-line pattern, expected for a superimposition of two arrays of three coupled carbon atoms. The two possible arrangements of an intact three-carbon unit involving C-3′ and C-4′, i.e., C-3′/C-4′/C-5′ and C-3′/C-4′/C-3″, were distinguished by spectral simulation (Bruker PANIC routine) using the known δ and J values and proper weighting with respect to intensity. There was close correspondence of the observed pattern to that calculated for the superimposition of a C-3″/C-4′/C-5′ and a C-3′/C-4′/C-5′ coupling pattern and a poor match with the other alternative, C-3″/C-4′/C-5′ plus C-3″/C-4′/C-3′. Another coupled three-carbon assembly was detected at C-1″/C-2″/C-3″. Thus, [U-$^{13}C_3$]glycerol gives rise to two species of **1**, one showing the labeling pattern **a** and the other pattern **b** (Scheme I).

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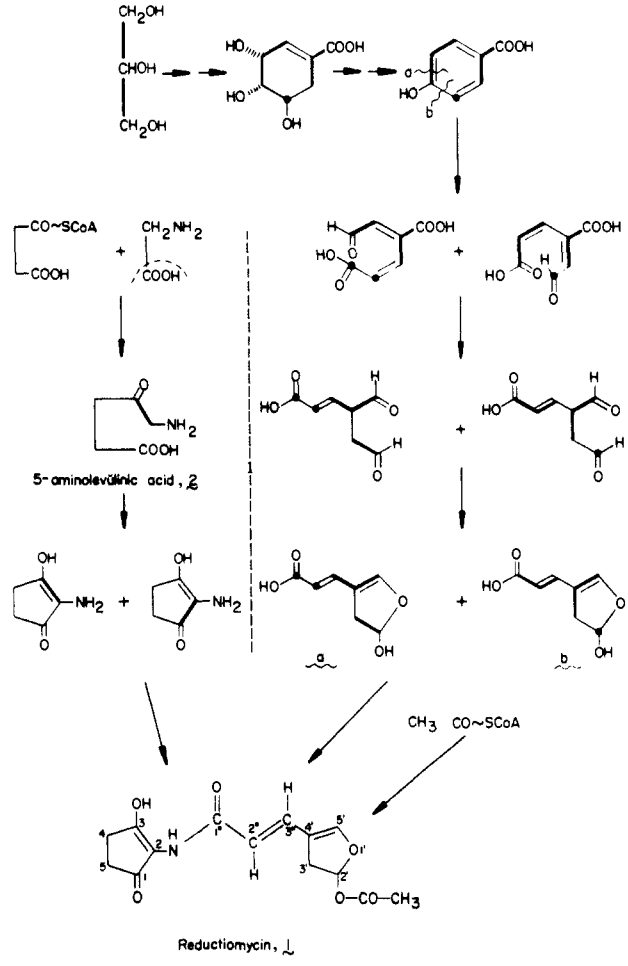
Table I

^{13}C chem shift, ppm	assignment	^{13}C enrichments, %; J_{cc}, Hz, in **1** derived from [U^{13}-C_3]glycerol	[1,2-$^{13}C_2$]acetate	[4,5-$^{13}C_2$]-**2**[a]	4-hydroxy[7-^{13}C]-benzoic acid
20.92	CH_3CO	4.35; 60	11.5; 60		
25.52	4	4.35; 45	15.6; 45		
32.14	5	4.35; 40	15.6; 40		
34.19	3′	6.7; 41	3.1		
98.45	2′	4.7; 41	3.1		
114.68	4′	6.3; 78, 64, 41	2.2		
115.09	2	3.7	3.2	3.7; 62.6, 80	
115.35	2″	3.5; 68, 73.5	1.8		
135.48	3″	5.9; 64, 73.5, 11	2.1		
150.52	5′	6.7; 78, 11	2.1		64
165.71	1″	4.2; 68	2.4		
169.53	CH_3CO	3.8; 60	11.5; 60		
173.90	3	4.7; 45	12.5; 45	1.8; 80	
197.56	1	3.95; 40	11.5; 40	1.8; 62.6	

[a] Signals for which no figures are given showed no significant enrichment.

Scheme I



These results point to the shikimate pathway as the source of the dihydrofuran moiety of **1**. [U-$^{13}C_3$]Glycerol will label shikimate as shown in Scheme I. Coupling pattern **b** implies ring cleavage between C-4 and C-5 of shikimate or a metabolite thereof. The additional presence of coupling pattern **a** reflects cleavage between C-3 and C-4, indicating that the substrate must be a symmetrical product containing all seven carbon atoms of shikimate. 4-Hydroxybenzoate was considered a plausible candidate for this ring cleavage. This notion was tested by synthesizing 4-hydroxy[7-^{13}C]benzoate[13] and feeding it (400 mg, 99% ^{13}C) to *S. xanthochromogenus*. A 64% enrichment solely in C-5′ of the resulting **1** (85 mg) confirmed that indeed 4-hydroxybenzoate or a closely related product, e.g., the corresponding aldehyde, must be the substrate for the ring cleavage reaction leading to the formation of the dihydrofuran moiety of **1**. The conclusions are summarized in Scheme I in terms of a likely pathway to reductiomycin. These results add another example to the list of natural products that arise by cleavage of an aromatic ring, like the betacyanins[14] or the anthramycin family of antibiotics.[15]

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Registry No. 1, 68748-55-0; **2**, 106-60-5; $HOCH_2CH(OH)CH_2OH$, 56-81-5; HOAc, 64-19-7; *p*-$HOC_6H_4CO_2H$, 99-96-7.

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Degradation and Detoxification of Organophosphonates: Cleavage of the Carbon to Phosphorus Bond

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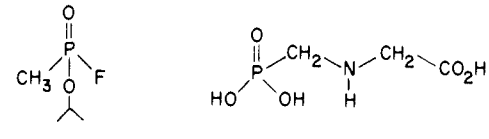
Ranging from the acetylcholinesterase inactivator sarin (**1**), to the herbicide glyphosate (**2**), organophosphonates rank among



1 2

the most biocidal of all organic molecules. Organophosphonates are characterized by a carbon atom covalently bonded to phosphorus. This bond is inert to vigorous acid and base hydrolytic conditions as well as to the action of phosphatase. Nonetheless, *Escherichia coli*[1,2] can cleave carbon to phosphorus bonds. This

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