by grants from the National Institutes of Health (AI-11490) and U.S. Army Armament Research and Development Command. We thank George Maynard for carrying out the calculations.

Communications

Structure and Biosynthesis of Novel Antibiotics, Aurantinins A and B Produced by *Bacillus aurantinus*

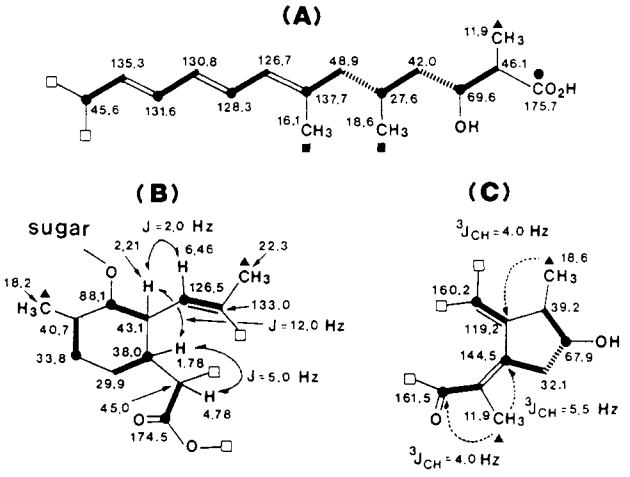
Summary: Structures of aurantinins A (1) and B (2), novel antibacterial antibiotics isolated from bacterium, have been determined in part by biosynthetic means using ^{13}C-labeled precursors.

Sir: The aurantinins A (1) and B (2)[1] comprise a novel polyketide antibiotic complex produced by *Bacillus aurantinus* Masuma and Ōmura sp. nov. which show antimicrobial activity against Gram-positive bacteria, especially anaerobes. In the present report, we describe structure elucidation of the antibiotics based on NMR analysis and biosynthetic means using ^{13}C-labeled precursors.

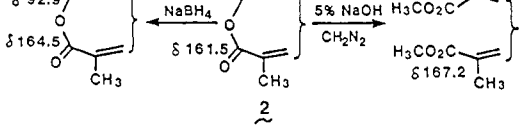
Antibiotics 1 and 2 were isolated from the ethyl acetate extract of the cultured broth by Sephadex LH-20 followed by centrifugal liquid chromatography, preparative silica gel thin-layer chromatography, and then Sephadex LH-20. Antibiotics 1 and 2 possess the following physicochemical properties. 1: mp 139 °C; $[\alpha]^{25}_D$ +126° (*c* 1.0, MeOH); FD-MS, *m/z* 636 (M^+); $C_{38}H_{52}O_8$.[2] 2: mp 98 °C; $[\alpha]^{25}_D$ +124° (*c* 0.33, MeOH); FD-MS, *m/z* 780 (M^+); $C_{44}H_{60}O_{12}$. The UV absorption (λ_{max}^{EtOH} 268, 278, 287, and 320 nm) of the aurantinins suggested the presence of a triene chromophore.

Methanolysis of 2 afforded the same monomethyl ester 3 [mp 94–96 °C; $[\alpha]^{25}_D$ +148° (*c* 0.35, MeOH); FD-MS, *m/z* 650 (M^+)] as a product obtained by methylation of 1 with CH_2N_2, indicating that the aglycon part of 2 is identical with that of 1. Comparative ^{13}C NMR spectral data for 1 and 2 suggested the existence of a novel ulose (a ketone carbonyl at δ 206.5, an anomeric carbon at δ 105.1, three oxygenated carbons at δ 78.0, 77.8, and 72.8, and a methyl at δ 18.4) in 2. $^1H/^1H$ and $^1H/^{13}C$ COSY spectral analyses assigned 6-deoxy-β-*ribo*-hexopyranos-3-ulose as the sugar. The ^{13}C spectrum of 2 indicated the presence of eight methyls, five methylenes, eight methines, six oxygenated methines, six double bonds, and four carbonyls.

In order to elucidate the C–C connectivity of the aglycon part of 2, biosynthetic studies were performed with ^{13}C labeled precursors. The feeding experiments[3] using ^{13}C-labeled acetate and L-methionine clearly indicated an alternating labeling pattern typical of a polyketide. The ^{13}C spectrum of aurantinin B labeled with [1-^{13}C]acetate showed strong enrichment for 16 carbon signals (δ 175.7, 174.5, 161.5, 160.2, 144.5, 137.7, 131.6, 128.3, 126.5, 88.1, 69.6, 67.9, 45.6, 38.0, 33.8, and 27.6). In the feeding experiment with [2-^{13}C]acetate, 17 carbon signals (δ 135.3, 133.0, 130.8, 126.7, 119.2, 118.5, 48.9, 46.1, 45.0, 43.1, 42.0, 40.7, 39.2, 32.1, 29.9, 18.6, and 16.1) including two methyl carbons were enriched. The ^{13}C spectrum of [1,2-^{13}C]-acetate labeled aurantinin B exhibited additional satellite peaks for all carbon signals except for the carbons arising from methionine as the biosynthetic precursor and the sugar moiety. The observation of intra- and intermolecular ^{13}C–^{13}C coupling patterns of acetate units in the 2D INADEQUATE spectrum[4] and LSPD (^1H and ^{13}C long-range selective decoupling) experiment permitted derivation of partial structures A, B, and C. Regarding the connectivity



between structures B and C, the existence of an acid anhydride moiety in 2 was derived from the ^{13}C NMR data[5] of dihydroaurantinin B obtained by $NaBH_4$ reduction of



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(2) The molecular formula, $C_{35}H_{54}O_9$ for aurantinin A reported in ref 1 should be revised to $C_{38}H_{52}O_8$ from FD mass and ^{13}C NMR spectral data of its triacetae and monomethyl ester.

(3) The ^{13}C precursors (0.2%, w/v), 98% enriched [1-^{13}C]acetate, [2-^{13}C]acetate, [1,2-^{13}C]acetate, [1-^{13}C]propionate, and [*methyl*-^{13}C]L-methionine were added to a 6-h fermentation broth (media: glycerol, 0.8%; starch, 0.9%; soybean meal, 2.0%; dry yeast, 0.3%; NaCl, 0.5%; $(NH_4)_2SO_4$, 0.2%; K_2HPO_4, 0.1%; $CaCO_3$ 0.3%; pH 7.0) and the cultivation continued at 27 °C for 42 h. ^{13}C labeled aurantinins (2–4 mg) were isolated from the cultured broth (1-2 L) by the isolation procedure described in this paper. ^1H and ^{13}C NMR spectra were measured with a Varian XL-400 in deuterioacetone.

(4) 2D-INADEQUATE spectrum of [1,2-$^{13}C_2$]acetate labeled aurantinin B was taken under the following conditions: number of accumulations, 2050; J_{CC} = 60 Hz; number of increments, 64; delay time, D_1 = 3 s; total acquisition time, 122 h.

(5) The existence of the anhydride moiety in 2 was confirmed by the following ^{13}C NMR data:

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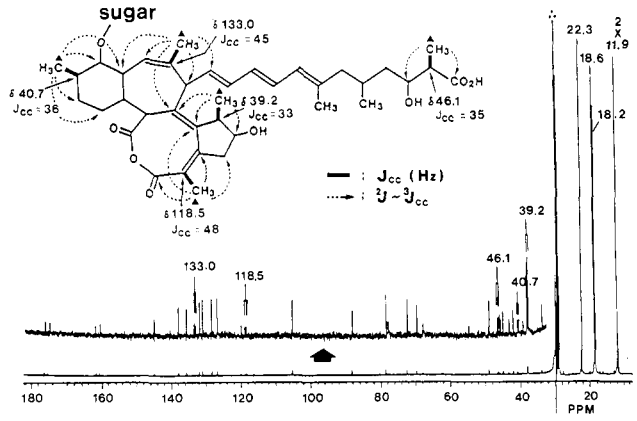

Figure 1. ^{13}C NMR spectrum and ^{13}C–^{13}C coupling pattern of [*methyl*-^{13}C]-L-methionine labeled aurantinin B.

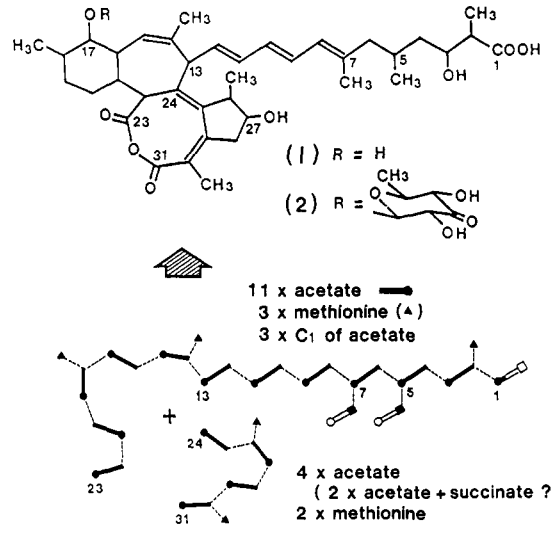

Figure 2. Structures and biosynthetic building units of aurantinins A (1) and B (2).

2 and the product of 2 by alkaline hydrolysis followed by methylation with CH_2N_2. Furthermore, the connectivity among structures A, B, and C was provided by ^{13}C spectral analysis of [methyl-^{13}C]-L-methionine labeled aurantinin B. The ^{13}C spectrum exhibited a high incorporation (90% or more) for five methyl carbons (δ 22.3, 18.6, 18.2, and 11.9 × 2). The location of these methyls was assigned to the tail position of acetate units in the polyketide chain because each carbon (δ 133.0, 118.5, 46.1, 40.7 and 39.2) attached directly to the five ^{13}C enriched methyl carbons appeared as a doublet. The observation of the C–C couplings from the enriched methyls to adjacent carbons from one to three bonds revealed the validity of a polycyclic structure for the aglycon of 2, as shown in Figure 1. This appears to be the first example in which high level incorporation by means of feeding experiments with [*methyl*-^{13}C]methionine was used effectively for structure determination of a microbial metabolite. It is noteworthy that 2 involves an acid anhydride moiety and a novel sugar, in addition to a polyketide skeleton containing four rings with five, six, seven, and eight members and a triene.

Antibiotics 1 and 2 seem to be built up biosynthetically from two polyketide chains. A long chain originates from 11 acetate units, three C_1 units arising from methionine, two C_1 units at C-5 and C-7 from acetate via decarboxylation, and one C_1 unit at C-1 from acetate being a starter unit. A short chain consists of four acetate units in which a succinate formed by "tail to tail condensation" of two acetate units might be the starter unit, and two C_1 units from methionine, as shown in Figure 2. The presence of a methyl group formed by decarboxylation of acetate seems to be common to true bacterial metabolites, as also reported in biosynthetic studies of myxopyronin.[6] The only exception to this is the virginiamycin family of antibiotics from actinomycetes.[7] Recently, Zimmerman et al. reported isolation and structure determination of the unusual macrolide antibiotics difficidin and oxydifficidin which were produced by a true bacterium, *Bacillus subtilis*.[8] Taking into consideration the findings detailed here for aurantinin biosynthesis, we can speculate that the tentative building units for difficidin consist of thirteen acetate units, three methionines, and two C_1 units arising from acetates.[8]

Acknowledgment. We are indebted to Professor T. J. Simpson, University of Edinburgh, for useful discussions.

Supplementary Material Available: Complete assignments of ^1H and ^{13}C chemical shifts in 400-MHz NMR are provided for compounds 1 and 2 (1 page). Ordering information is given on any current masthead page.

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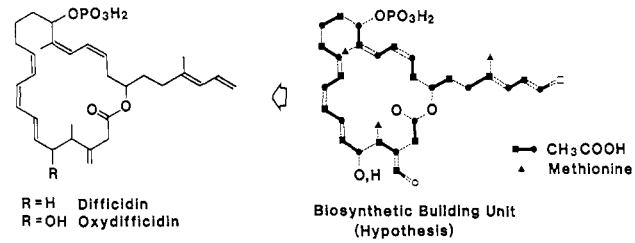

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Received January 29, 1988

Synthesis of 2-Imidoglycolic Acids and a New Heterobifunctional Cross-Linking Agent, *N*-Succinimidyl 2-Maleimidoglycolate

Summary: The reaction of five-membered cyclic imides with glyoxylic acid produces the corresponding 2-imidoglycolic acids. The *N*-hydroxysuccinimide ester of 2-maleimidoglycolic acid is introduced as a new heterobifunctional cross-linking agent for protein modification.

Sir: The reaction of amides and carbamates with glyoxylic acid monohydrate in either acetone or diethyl ether is known to produce the corresponding 2-amido and 2-car-

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