Biosynthesis of Polyketide Antibiotics. Incorporation of a Pentaketide Chain Elongation Intermediate into Nargenicin

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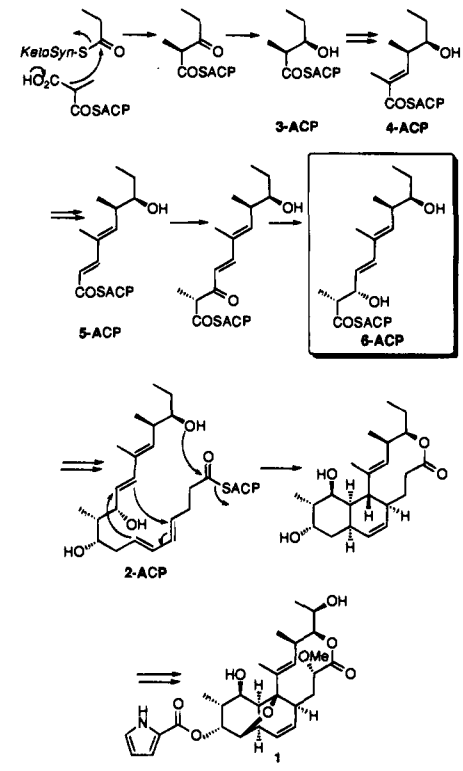
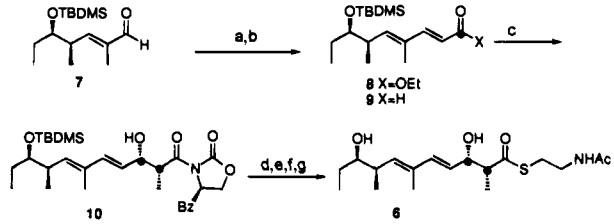
Polyketide natural products include thousands of known metabolites displaying an immense variety of structural features and possessing a wide range of physiological properties, including antibiotic, immunosuppressant, mutagenic, and toxic activities. Among the most complex of the members of this structural class are the macrolide antibiotics,[1] There is strong evidence that these partially reduced polyketides are biosynthesized by a process closely related to fatty acid biosynthesis,[2] in which a small number of biochemical reactions—β-ketoacyl thioester synthesis, β-keto reduction, dehydration, and enoyl thioester reduction—are combined in a repetitive manner to generate the characteristic polyoxygenated, branched chain polyketide backbone.[3] There is now a considerable body of biochemical and genetic evidence which supports the notion that the oxidation level and stereochemistry of the growing polyketide chain are adjusted prior to each step of chain elongation.[4–8] Subsequent late stage group transfer reactions, including hydroxylations, methylations, and glycosylations, as well as further modifications of the carbon skeleton, such as cyclizations and rearrangements, then generate the final, bioactive metabolite.

Nargenicin (**1**) is a polyketide antibiotic that has been isolated from *Nocardia argentinensis* and that contains a macrocyclic lactone fused to a *cis*-octalin ring system. We have previously carried out feeding experiments with ^{13}C-labeled precursors that have established the derivation of nargenicin from four propionate and five acetate building blocks.[9] Additional incorporation experiments with ^{13}C,^{18}O-labeled acetates and propionates, as well as with $^{18}O_2$, have also established the origin of all the oxygen atoms of nargenicin.[9] The observed labeling patterns are consistent with the now widely accepted processive model for the assembly of the parent polyketide chain, as illustrated in Scheme 1. The nonaketide product **2** generated by the polyketide synthase can undergo lactonization and intramolecular Diels–Alder cyclization, followed by oxidation at the appropriate sites and attachment of the pyrrole carboxylate to yield **1**. In further support of this proposal, we have reported the intact incorporation of a series of proposed polyketide chain elongation intermediates **3**–**5**, administered as the corresponding *N*-acetylcysteamine (NAC) thioesters.[8] We now describe the stereospecific synthesis of the NAC thioester of the proposed pentaketide intermediate **6** and the successful incorporation of **6** into nargenicin.

Scheme 1



Scheme 2[a]



[a] Conditions: triethyl[1-^{13}C]phosphonoacetate, *t*-BuOK, THF (95%); (b) (i) $LiAlH_4$, Et_2O (95%), (ii) MnO_2, CH_2Cl_2; (c) [2-^{13}C]propionyl-(4′*S*)-benzyloxazolidinone, *n*-Bu_2BOTf, Et_3N, CH_2Cl_2; (d) TBDMSOTf, Et_3N, CH_2Cl_2 (63% for 3 steps); (e) LiOH, H_2O_2, THF/H_2O (93%); (f) (i) $(EtO)_2P(O)N_3$, Et_3N, DMF, (ii) NAC-SH (90%); (g) HF, H_2O/CH_3CN (73%).

To prepare the desired **6**, the previously described aldehyde[8] **7** was converted to the [1-^{13}C]nonadienoic ester **8** by Emmons reaction with [1-^{13}C]triethylphosphonoacetate (Scheme 2). LAH reduction followed by oxidation of the resulting allylic alcohol gave the corresponding aldehyde **9**, which underwent enantioselective aldol condensation with the Evans reagent, [2-^{13}C]-propionyl-(4′*S*)-benzyloxazolidinone,[10] to give the (2*R*,3*S*)-2-methyl-3-hydroxy imide **10**. The latter derivative was converted to the desired NAC thioester **6**[11] by successive protection as the TBDMS ether, hydrolysis, thioesterification, and deprotection. The overall yield of the pentaketide thioester from **7** was 30%.

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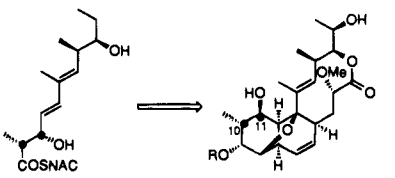
Successful incorporation of advanced polyketide chain elongation intermediates has been found to be particularly demanding. Not only must the substrate survive degradation by intracellular esterases and fatty acid oxidases, but the exogenously added precursor must be able to compete effectively with endogenously generated intermediates already covalently attached to the polyketide synthase. Experience has shown that these obstacles can be overcome by careful control of both fermentation conditions and the timing and amount of precursor administered, as well as by the use of fatty acid oxidation inhibitors and, in some cases, additives intended to enhance precursor uptake.[5-8,12-14] After extensive experimentation, we developed a protocol for feeding of the pentaketide in which 100 mg 2,6-*O*-dimethyl-β-cyclodextrin[12] was added to 200 mL of a 44-h production culture of *N. argentinensis* that had been incubated at 28 °C and 250 rpm in a 1-L DeLong flask. After another 1.5 h, a mixture of the pentaketide **6** (130 mg, 0.36 mmol) plus 4-pentynoic acid[13] (19 mg), 3-(tetradecylthio)-propanoic acid[13] (23 mg), and 60 mg of 2,6-*O*-dimethyl-β-cyclodextrin was added, and the fermentation was continued for an additional 72 h. Harvesting of the cultures and purification of the crude extract gave 7.5 mg of pure nargenicin, which was analyzed by 100.6 MHz ^{13}C NMR. The ^{13}C NMR spectrum of **1** thus obtained revealed the presence of a pair of enhanced and coupled doublets (J_{CC} = 35.4 Hz, 0.25 atom % ^{13}C enrichment) centered at δ 35.12 and 76.26, corresponding to ^{13}C label at the predicted sites, C-10 and C-11, respectively, (Scheme 3).

Scheme 3



6 **1**

The pentaketide substrate **6**, with four stereogenic centers and a conjugated *E,E*-diene, is not only the most advanced, but also by far the most complex intermediate of polyketide chain elongation which has been incorporated intact into any polyketide metabolite. The conversion of this substrate to nargenicin indicates that the polyketide synthase complex is capable of recognizing this analog of the normally enzyme-bound intermediate **6**-ACP and correctly processing it to the presumptive nonaketide product **2**. Moreover, the utilization of a substrate containing the conjugated diene moiety provides further support for the proposed intramolecular Diels-Alder cyclization mechanism which can account for the formation of the characteristic *cis*-octalin ring system of nargenicin and related metabolites.

Acknowledgment. This work was supported by a grant from the National Institutes of Health, GM22172.

Supplementary Material Available: Spectroscopic data for unlabeled and ^{13}C-labeled **8-10** and other intermediates in the synthesis of **6** (7 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(11) (2*R*,3*S*,8*R*,9*R*,4*E*,6*E*)-2,6,8-Trimethyl-3,9-dihydroxy-4,6-undecadienoic acid, *N*-acetylcysteamine thioester (**6**): R_f = 0.31 (10% MeOH/$CHCl_3$); IR(neat) ν 3379.2 (br), 1667.6, 1098.7, 966.4, 748.1, 668.4 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.27 (d, 1 H, J = 15.56 Hz, C_5-H), 5.83 (br, 1 H, NH), 5.55 (dd, 1H, J = 6.73, 15.60 Hz, C_4-H), 5.36 (d, 1 H, J = 9.96 Hz, C_7-H), 4.46 (m, 1 H, C_3-H), 3.42 (m, 2 H, N-CH_2), 3.33 (m, 1 H, C_9-H), 3.02 (m, 2 H, S-CH_2), 2.82 (m, 1 H, C_2-H), 2.58 (m, 1 H, C_8-H), 1.98 (s, 3 H, $COCH_3$), 1.75 (s, 3 H, C_6-CH_3), 1.60-1.52 (m, 1 H, one of C_{10}-H_2), 1.50-1.42 (m, 1 H, one of C_{10}-H_2), 1.21 (d, 3 H, J = 7.06 Hz, C_2-CH_3), 1.00 (d, 3 H, J = 6.71 Hz, C_8-CH_3), 0.95 (t, 3 H, J = 7.40 Hz, C_{11}-H_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.21, 170.35, 137.01, 136.44, 132.73, 125.91, 75.35, 73.70, 54.04, 39.41, 38.63, 28.61, 27.46, 23.17, 16.00, 12.84, 12.04, 10.31; $[\alpha]_D$ = -4.72° (*c* 1.95, $CHCl_3$); HRMS (FAB) [M + Na]$^+$ calcd 380.1871, found 380.1866. [2,3-$^{13}C_2$]-**6**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.27 (dd, 1 H, J_{HH} = 15.60 Hz, J_{CH} = 6.41 Hz, C_5-H), 4.45 (d of m, 1 H, J_{CH} = 145.57 Hz, C_3-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 73.68 (enriched, d, J = 36.00 Hz), 54.06 (enriched, d, J = 36.00 Hz); MS (FAB) [M + Na]$^+$ 382.

(12) Robinson has recently reported that addition of 2,6-*O*-dimethyl-β-cyclodextrin to cultures of *Streptomyces cinnamonensis* both increases the yield of the polyether monensin and allows intact incorporation of a triketide NAC thioester precursor: Patzelt, H.; Gaudet, V.; Robinson, J. A. *J. Antibiot.* **1992**, *45*, 1806-1808. Patzelt, H.; Robinson, J. A. *J. Chem. Soc., Chem. Commun.* **1993**, 1258-1260. In the case of nargenicin, we found that addition of 2,6-*O*-dimethyl-β-cyclodextrin to cultures of *N. argentinensis* doubled the production of nargenicin from 3-4 to 7.5 mg/200 mL.

(13) Vederas has described the use of a number of inhibitors of fatty acid β-oxidation, including 4-pentynoic acid and 3-(tetradecylthio)propanoic acid: Li, Z.; Martin, M.; Vederas, J. C. *J. Am. Chem. Soc.* **1992**, *114*, 1531-1533. Yoshizawa, Y.; Li, Z.; Reese, P. B.; Vederas, J. C. *J. Am. Chem. Soc.* **1990**, *112*, 3212-3213.

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