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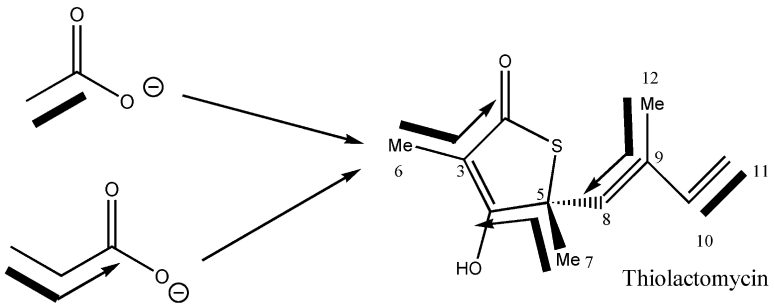

Thiolactomycin

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Biosynthetic Origins of the Natural Product, Thiolactomycin: A Unique and Selective Inhibitor of Type II Dissociated Fatty Acid Synthases

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Thiolactomycin (TLM, **1**) (TLM numbering uses IUPAC nomenclature of giving the lowest locant to heteroatoms in heterocycles, and is different from the initial numbering used on TLM.[1a]) was initially isolated from a *Nocardia* sp. (ATCC 31319) found in a Japanese soil sample[1−4] This unusual thiolactone antibiotic has low toxicity and is active against Gram-positive, and -negative bacteria, including both aerobes and anaerobes. TLM has also been shown to be effective in murine infectious disease models,[2−6] and to have encouraging anti-malarial activity.[7] In all of these cases, evidence indicates that TLM acts as a selective and reversible inhibitor of the dissociated type II fatty acid synthase (FAS).[7−10] As this FAS plays an essential role in biosynthesis of membrane lipids, the individual components have attracted significant interest as valid antibacterial targets.[9,11] In *Escherichia coli*, the β-ketoacyl synthase (KAS I, FabB) of the FAS has been unequivocally demonstrated to be the target for TLM; missense mutations in FabB or overexpression of FabB both lead to TLM resistance.[12,13] Important interactions between TLM and the FabB active site have been obtained from a recent enzyme−inhibitor cocrystal structure.[14] TLM has thus emerged as an attractive lead for developing new antibacterial agents.[15]

Several synthetic strategies have been developed for generation of TLM and TLM analogues. The initial synthesis reported provided racemic TLM,[16] while a subsequent asymmetric approach provided the (5*S*)-isomer.[17] Recently, an asymmetric synthesis of the natural (5*R*) isomer of TLM from (2*R*)-alanine has been developed.[18] This method is flexible and will allow substituents on the thiolactone to be varied in a combinatorial mode.[18] Some synthetic TLM analogues have already been shown to be effective FAS inhibitors. For instance, TLM analogues with extended hydrocarbon chains at C-5 have been shown to have enhanced anti-mycobacterial activity.[6,19] These compounds also inhibit the pea (*Pisum sativum*) FAS.[20] Furthermore, C-3 acetyl TLM analogues also bearing lipophilic C-5 substituents are active against *Staphyloccocus aureus* and *Pasteurella multocida*.[15] Despite the significance of these results, the biosynthetic pathway for TLM has not been elucidated. In this study, we show that the backbone of this unique thiolactone is synthesized from acetate- and propionate-derived building blocks in a manner consistent with a polyketide biosynthetic process, suggesting that combinatorial biosynthesis may provide an alternative approach for generation of novel and useful TLM analogues.

Nocardia sp. ATCC 31319 was grown as a two-stage fermentation process in shake flask cultures (29 °C and 210 rpm). A 10% inoculum for the 3-day seed culture[3] (substituting 2 g/L of meat extract for 5 g of "Boullion") was used to inoculate the production medium.[21] After 3 days, TLM was extracted from the acidified fermentation broth (pH 3) with ethyl acetate and purified by reverse-phase HPLC. Gradient HMQC and HMBC spectra were collected on unlabeled TLM (10 mg) and used to provide the unequivocal ^{13}C and ^{1}H NMR assignments. The ^{13}C assignments (Table 1) are consistent with those initially reported for TLM,[1] with the exception of a switch in assignment of resonances associated with C-3 and C-9. [2,3-$^{13}C_2$]Propionate was added as a sterile solution at 24 and 48 h to the second-stage fermentation, at a final concentration of 5 mM. A total of 3.2 mg of TLM was isolated from 1 L of broth and analyzed by ^{13}C NMR. No change was observed in the relative intensities for each of the ^{13}C natural abundance signals. However, doublets surrounding C-3, C-6 ($^{1}J_{^{13}C-^{13}C}$ 46.3 Hz), C-5, C-7 ($^{1}J_{^{13}C-^{13}C}$ 35.1 Hz), and C-9, C-12 ($^{1}J_{^{13}C-^{13}C}$ 41.6 Hz) unambiguously demonstrated intact incorporation of C-2 and C-3 of the labeled propionate into these three pairs of adjacent carbons at a level of approximately 1.5% (Figure 1). An analogous incorporation experiment conducted using [1,2-$^{13}C_2$]acetate provided 12.1 mg of pure, labeled TLM from 2 L of broth. In this case, the ^{13}C NMR analysis revealed doublets surrounding the natural abundance signals for just C-10 and C-11 ($^{1}J_{^{13}C-^{13}C}$ 69.4 Hz), demonstrating approximately 1% intact incorporation of the labeled acetate into these specific adjacent carbons of TLM.

Table 1. Revised ^{13}C Assignments for Thiolactomycin

carbon	^{13}C chemical shift (ppm) ($CHCl_3$)	attached hydrogens (no.)
C-2	197.3	0
C-3	110.9	0
C-4	180.2	0
C-5	55.8	0
C-6	8.1	3
C-7	30.2	3
C-8	129.5	1
C-9	140.8	0
C-10	141.0	1
C-11	114.5	2
C-12	12.5	3

These results are consistent with a pathway in which an acetate-derived starter unit is elongated by three propionate/methylmalonate-extender units in a manner established for polyketide synthases (PKS).[22−24] On the basis of a PKS model, a linear tetraketide product, (4*E*,6*E*)-2,4,6-trimethyl-3-oxo-octanoic acid (**2**), potentially activated as an ACP (acyl carrier protein-R′ in Figure 1) thioester, can be envisioned. This biosynthetic intermediate could then be transferred to an enzyme (R″ in Figure 1) that contains a cysteine persulfide in its active site, releasing the ACP. The resulting disulfide-linked polyketide product (**3**) could then undergo a unique $\Delta^{4,6}\Delta^{5,7}$ isomerization that would simultaneously cyclize and transfer this sulfur to the polyketide chain, generating TLM (Figure 1). An alternative cyclization pathway, proceeding from **2** through a

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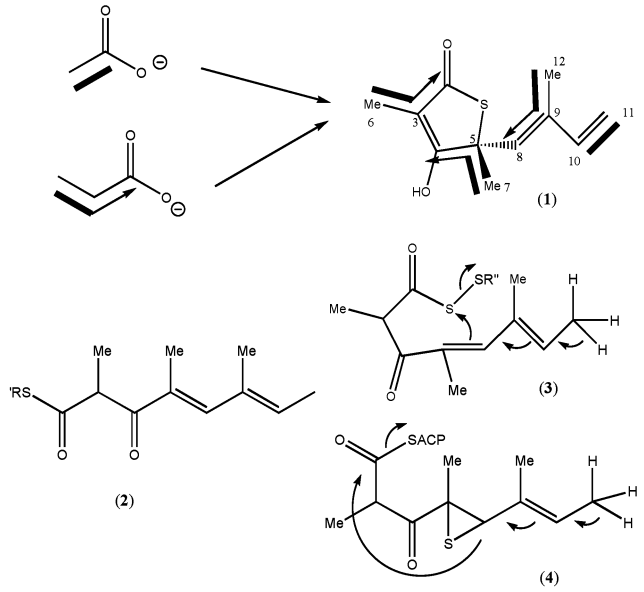

Figure 1. Biosynthetic origins of thiolactomycin (1). Bold bonds indicate intact incorporation of adjacent ^{13}C atoms from dual labeled acetate and propionate. Putative pathway intermediates (**2**−**4**) are also shown.

thiirane intermediate (**4**), can also be envisioned. In either case the sulfur atom of TLM is likely to be obtained by desulfuration of L-cysteine, (potentially analogous to the process catalyzed by IscS in *E. coli*).[25] Consistent with this hypothesis we observed radiolabeled TLM when our fermentations of *Nocardia* sp. ATCC 31319 were carried out in the presence of ^{35}S-labeled cysteine (see Supporting Information).

Streptomyces sp. Y-0834H[26] also produces TLM and may do so in an analogous fashion. In this organism, analogues containing ethyl substituents at C-5 (834-B1) and both C-3 and C-5 (thiotetromycin) have been reported. This substituent variation in PKS-derived products is well established and is the result of the use of either ethylmalonate- or methylmalonate-derived extender units during biosynthesis.[27,28]

Exciting progress with the generation of hybrid PKSs in recent years suggests that the TLM biosynthetic process may be engineered for production of novel analogues with improved activity or pharmacological properties. We are in the process of cloning the TLM biosynthetic gene cluster to both address this possibility and investigate the unique final steps of TLM biosynthesis. We have cloned numerous separate PKS biosynthetic gene clusters from the producing strain, *Nocardia* sp. ATCC 31319, and are currently determining which is responsible for TLM production. The results of these analyses will be reported in due course.

Supporting Information Available: ^{13}C NMR spectra of acetate and propionate labeled thiolactomycin. HPLC-analyses of radiolabeled TLM produced from incorporation experiments using [^{35}S]-L-cysteine and [1-^{14}C]propionate (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Sasaki, H.; Oishi, H.; Hayashi, T.; Matsuura, I.; Ando, K.; Sawada, M. *J. Antibiot.* **1982**, *35*, 396−400.
(2) Miyakawa, S.; Suzuki, K.; Noto, T.; Harada, Y.; Okazaki, H. *J. Antibiot.* **1982**, *35*, 411−9.
(3) Oishi, H.; Noto, T.; Sasaki, H.; Suzuki, K.; Hayashi, T.; Okazaki, H.; Ando, K.; Sawada, M. *J. Antibiot.* **1982**, *35*, 391−395.
(4) Noto, T.; Miyakawa, S.; Oishi, H.; Endo, H.; Okazaki, H. *J. Antibiot.* **1982**, *35*, 401−410.
(5) Hayashi, T.; Yamamoto, O.; Sasaki, H.; Okazaki, H. *J. Antibiot.* **1984**, *37*, 1456−1461.
(6) Kremer, L.; Douglas, J. D.; Baulard, A. R.; Morehouse, C.; Guy, M. R.; Alland, D.; Dover, L. G.; Lakey, J. H.; Jacobs, W. R., Jr.; Brennan, P. J.; Minnikin, D. E.; Besra, G. S. *J. Biol. Chem.* **2000**, *275*, 16857−16864.
(7) Waller, R. F.; Keeling, P. J.; Donald, R. G.; Striepen, B.; Handman, E.; Lang-Unnasch, N.; Cowman, A. F.; Besra, G. S.; Roos, D. S.; McFadden, G. I. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 12352−12357.
(8) Nishida, I.; Kawaguchi, A.; Yamada, M. *J. Biochem.* **1986**, *99*, 1447−1454.
(9) Heath, R. J.; White, S. W.; Rock, C. O. *Prog. Lipid Res.* **2001**, *40*, 467−497.
(10) Jackowski, S.; Murphy, C. M.; Cronan, J. E.; Rock, C. O. *J. Biol. Chem.* **1989**, *264*, 7624−7629.
(11) Payne, D. J.; Warren, P. V.; Holmes, D. J.; Ji, Y.; Lonsdale, J. T. *Drug Discovery Today* **2001**, *6*, 537−544.
(12) Tsay, J. T.; Rock, C. O.; Jackowski, S. *J. Bacteriol.* **1992**, *174*, 508−513.
(13) Jackowski, S.; Zhang, Y. M.; Price, A. C.; White, S. W.; Rock, C. O. *Antimicrob. Agents Chemother.* **2002**, *46*, 1246−1252.
(14) Price, A. C.; Choi, K. H.; Heath, R. J.; Li, Z.; White, S. W.; Rock, C. O. *J. Biol. Chem.* **2000**, *276*, 6551−6559.
(15) Sakya, S. M.; Suarez-Contreras, M.; Dirlam, J. P.; O'Connell, T. N.; Hayashi, S. F.; Santoro, S. L.; Kamicker, B. J.; George, D. M.; Ziegler, C. B. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2751−2754.
(16) Wang, C. J.; Salvino, J. M. *Tetrahedron Lett.* **1984**, *25*, 5243−5246.
(17) Chambers, M. S.; Thomas, E. J. *J. Chem. Soc., Perkin Trans. 1* **1997**, 417−431.
(18) McFadden, J. M.; Frehywot, G. L.; Townsend, C. A. *Org. Lett.* **2002**, *4*, 3859−3862.
(19) Douglas, J. D.; Senior, S. J.; Morehouse, C.; Phetsukiri, B.; Campbell, I. B.; Besra, G. S.; Minnikin, D. E. *Microbiology* **2002**, *148*, 3101−3109.
(20) Jones, A. L.; Herbert, D.; Rutter, A. J.; Dancer, J. E.; Harwood, J. L. *Biochem. J.* **2000**, *347*, 205−209.
(21) Maiese, W. M.; Lechevalier, M. P.; Lechevalier, H. A.; Korshalla, J.; Goodman, J.; Wildey, M. J.; Kuck, N.; Greenstein, M. *J. Antibiot.* **1989**, *42*, 846−851.
(22) Katz, L. *Chem. Rev.* **1997**, *97*, 2557−2575.
(23) Staunton, J.; Weissman, K. J. *Nat. Prod. Rep.* **2001**, *18*, 380−416.
(24) Rawlings, B. J. *Nat. Prod. Rep.* **2001**, *18*, 231−81.
(25) Lauhon, C. T.; Kambampati, R. *J. Biol. Chem.* **2000**, *275*, 20096−20103.
(26) Sato, T.; Suzuki, K.; Kadota, S.; Abe, K.; Takamura, S.; Iwanami, M. *J. Antibiot.* **1989**, *42*, 890−896.
(27) Liu, H.; Reynolds, K. A. *J. Bacteriol.* **1999**, *181*, 6806−6813.
(28) Liu, H.; Reynolds, K. A. *Metab. Eng.* **2001**, *3*, 40−48.

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