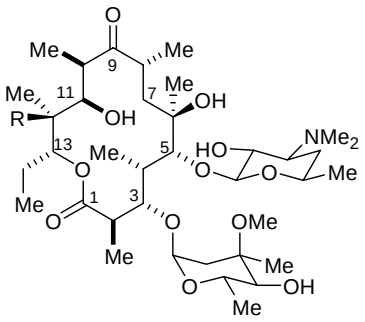
Chapter 4

Erythronolide B and the Erythromycins

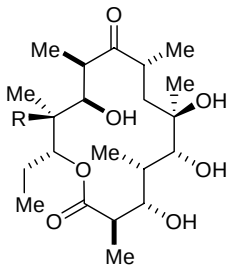
Isolation and Structure

Isolated in 1952 from the soil bacteria *actinomycetes*,[1] the erythromycins are a distinguished family of natural products by virtue of their clinically useful antibacterial properties and complex structures. Soon after their discovery, scientists elucidated the structure of erythromycin A (**1**),[2] and then erythromycin B (**2**),[3,4] through extensive degradation studies (Figure 1).[5] X-ray crystallography studies have established the three dimensional structure of these natural products.[6] The erythromycins are characterized by 14-membered macrolactones with glycosidic linkages at C(3) and C(5) to 6-deoxysugars, L-cladinose and D-desosamine, respectively. The name "erythronolide" refers to the polyketide derived-aglycone (e.g., **3** and **4**, Figure 1), while the letter codes (i.e., A, B, etc.) reflect each isomer and its order of discovery. In contrast to erythronolide A (**3**), erythronolide B (**4)** is a natural product and more importantly, the biogenic precursor of all the erythromycin isomers.



1 R = OH erythromycin A

2 R = H erythromycin B



3 R = OH erythronolide A

4 R = H erythronolide B

Figure 1. Representative members of the erythromycin macrolide family of antibiotics

Biosynthesis of Erythronolide B

Our understanding on the biosynthesis of polyketides derives mainly from extensive studies conducted on the biosynthetic mechanism of the erythromycins.[7] The first polyketide synthase genome sequenced was that of 6-deoxyerythronolide B synthase (DEBS).[8] Many *Streptomyces* polyketide synthases sequenced thereafter proved similar in structure and function to DEBS. The genes directing the synthesis of erythronolide B encode for three large multifunctional proteins: DEBS1, DEBS 2, and DEBS 3. Through a stepwise process, polyketide synthase (PKS) builds erythronolide B from simple carbon building blocks, as illustrated in Figure 2. The enzyme KS (ketosynthase) anchors the growing polyketide chain via a disulfide linkage to a cysteine residue. In one cycle of the biosynthesis, AT (acyltransferase) transfers an α-carboxylated nucleophile from the acyl-CoA to the ACP (acyl carrier protein), and acyl-KS and acyl-ACP catalyze the adol bond formation. This process can then repeat itself until the enzyme TE (thioesterase) terminates chain elongation and forms the macrocycle. Cytochrome *P*-450 uses

molecular oxygen to oxidize the C(6) position of the macrolactone (6-deoxyerythronolide B) to form erythronolide B. Subsequent steps involve attachment of the sugars to make the erythromycins. Fundamental mechanistic studies of these enzymes are ongoing, with recent efforts in this area aimed at eventually exploiting the biosynthesis of polyketides to make new macrolides because of their potential for fighting infectious diseases.[9]

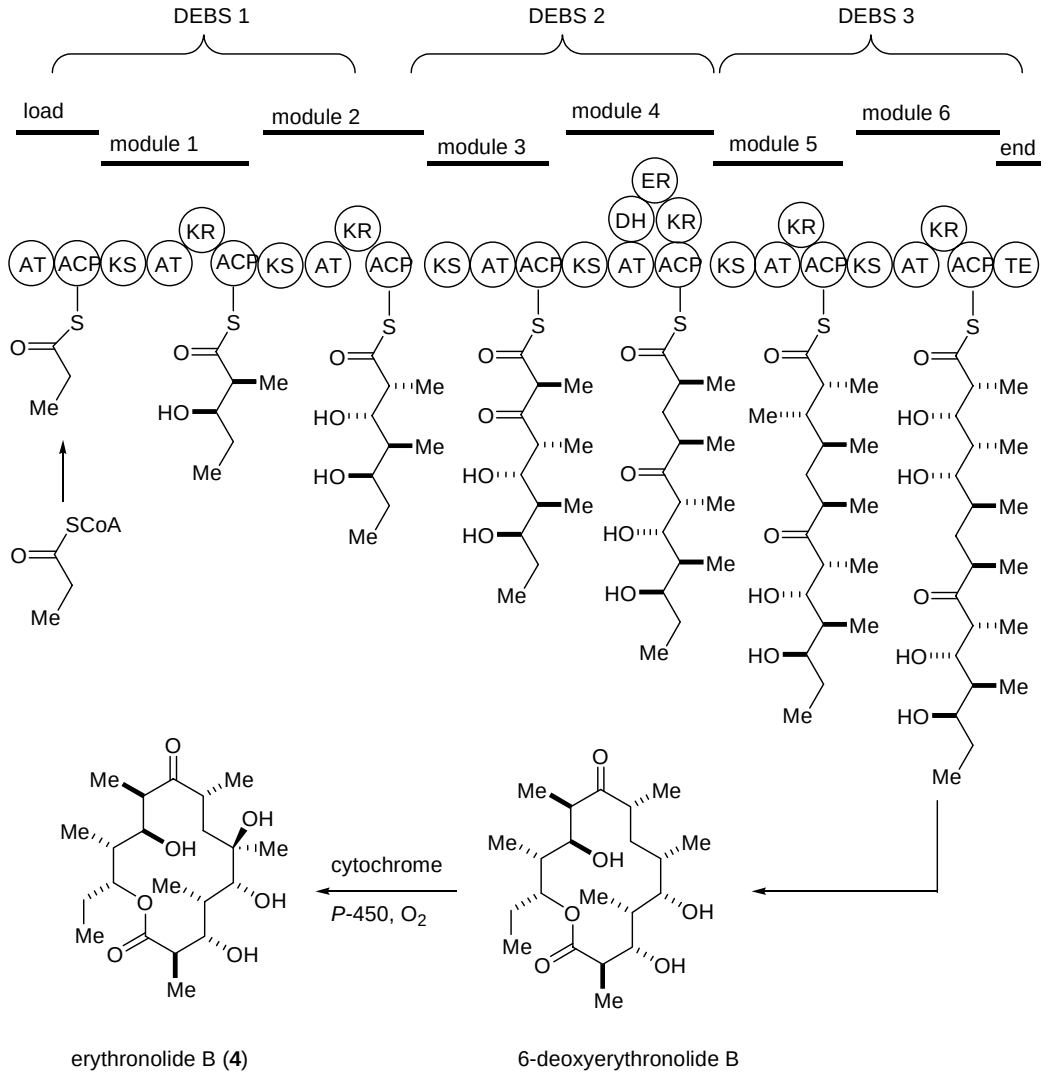


Figure 2. Predicted domain organization and biosynthetic intermediates of the erythromycin synthase. Each circle represents an enzymatic domain as follows: ACP, acyl carrier protein; AT, acyltransferase; DH, dehydratase; ER, β-ketoacyl-ACP enoyl reductase; KR, β-ketoacyl-ACP reductase; KS, β-ketoacyl-ACP synthase; TE, thioesterase

Comparison to fatty acid synthesis. Notably, the biosynthesis of polyketides bears mechanistic similarities to vertebrate fatty acid synthesis.[7] Both pathways are triggered by the Claisen condensation reaction between a starter carboxylic acid and a dicarboxylic acid (e.g., malonic or methylmalonic acid). In addition, both pathways involve the multifunctional polypetide-enzymes, KS (ketosynthases) and ACP (acyl carrier protein). Furthermore, both pathways are inhibited by a fungal product called cerulenin.[7]

Polyketide architectures, however, far exceed fatty acid structures in complexity as a result of two distinctions in their biosynthesis. First, in fatty acid synthesis three enzymatic, three steps operate in sequence to eventually form the saturated carbon chain: ketoreduction by KR (ketoacylACP reductase), dehydration by DH (dehydratase) and enoyl reduction by ER (enoyl reductase) (Figure 3). In contrast, these enzymatic steps function at various points in polyketides synthesis, resulting in greater functional group variety. Second, fatty acid synthesis uses only malonly pieces, whereas polyketide synthases incorporate more varied materials: malonly, methylmalonyl and ethylmalony extender units.

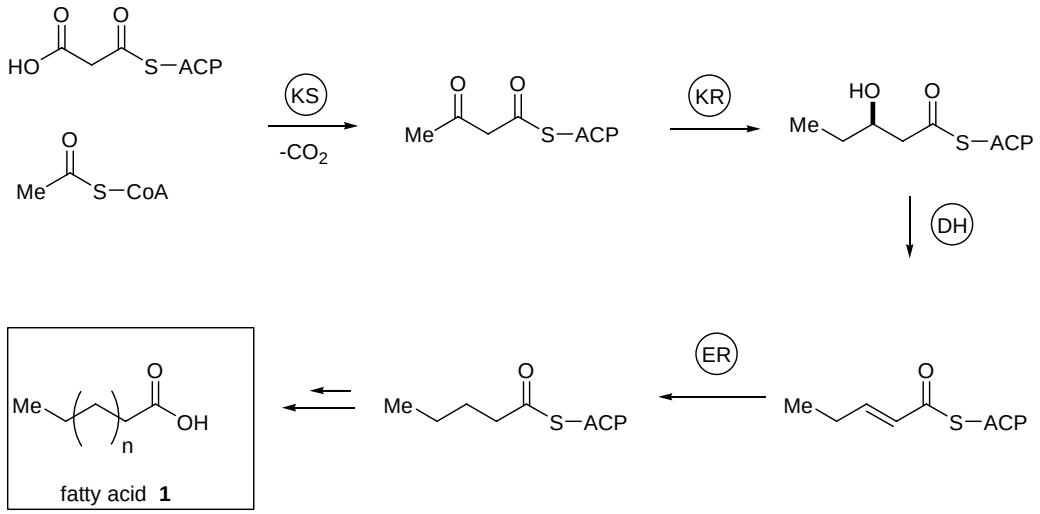


Figure 3. Biosynthesis of fatty acids involves the three enzymatic steps by KR (ketoacylACP reductase), DH (dehydratase) and ER (enoyl reductase)

Clinical Usage

Over the past forty years, erythromycin A, commonly referred to as erythromycin, has been used to fight a variety of infections including pneumonia, diphtheria, pertussis, chlamydia, trachomatis, and conjunctivitis.[10] As one of the oldest and safest antibiotics, erythromycin continues to be a useful alternative to penicillin. This antibiotic acts by reversibly binding the 50S ribosomal subunit of a susceptible microorganism.[11] Because ribosomes are responsible for protein synthesis in a cell, binding to the 50S ribosome inhibits the microorganism's growth. Recently, the structural basis for this binding event was reported; an X-ray crystal structure of eythromycin bound to the ribosome reveals that the antibiotic participates in hydrogen bonding to six adjacent nitrogenous bases of the nucleotides in the 23S RNA ribozyme (a subunit of the 50S ribosome).[12]

Although erythromycin has been widely used over the past four decades, improvement in its biological and chemical properties is still needed and pursued. The

current drawbacks of erythromycin include acid sensitivity, poor intestinal absorption, low tissue and cellular penetration, poor digestive tolerance, and undesirable interactions with other drugs. Under acidic conditions (e.g. in the stomach), erythromycin decomposes to two weaker antibiotics. As such, absorption of the drug is variable and difficult to predict. Because of its acid sensitivity and complex structure, modification of erythromycin has been difficult.

Concluding Remarks

Antibiotic-resistant strains of bacteria are still a major concern to public health. Unfortunately, the erythromycin structures are complex and difficult to modify. As such, chemical tools that facilitate the production of erythromycin-like antibiotics will increase our ability to treat resistant bacterial strains.

References

(1) McGuire, J. M.; Bunch, R. L.; Anderson, R. C.; Boaz, H. E.; Flynn, E. H.; Powell, H. M.; Smith, J. W. *Antibiotics and Chemotherapy* **1952**, *2*, 281–283.

(2) (a) Wiley, P. F.; Gerzon, K.; Flynn, E. H.; Sigal, M. V.; Weaver, O.; Quarck, U. C.; Chauvette, R. R.; Monahan, R. *J. Am. Chem. Soc.* **1957**, *79*, 6062–6070. (b) Wiley, P. F.; Weaver, O. *J. Am. Chem. Soc.* **1956**, *78*, 808–810. (c) Flynn, E. H.; Sigal, M. V.; Wiley, P. F.; Gerzon, K. *J. Am. Chem. Soc.* **1954**, *76*, 3121–3131. (d) Sigal, M. V.; Wiley, P. F.; Gerzon, K.; Flynn, E. H.; Quarck, U. C.; Weaver, O. *J. Am. Chem. Soc.* **1956**, *78*, 388–395. (e) Gerzon, K.; Flynn, E. H.; Sigal, M. V.; Wiley, P. F.; Monahan, R.; Quarck, U. C. *J. Am. Chem. Soc.* **1956**, *78*, 6396–6408.

(3) (a) Gerzon, K.; Monahan, R.; Weaver, O.; Sigal, M. V.; Wiley, P. F. *J. Am. Chem. Soc.* **1956**, *78*, 6412–6413. (b) Wiley, P. F.; Sigal, M. V.; Weaver, O.; Monahan, R.; Gerzon, K. *J. Am. Chem. Soc.* **1957**, *79*, 6070–6074. (c) Wiley, P. F.; Gale, R.; Pettinga, C. W.; Gerzon, K. *J. Am. Chem. Soc.* **1957**, *79*, 6074–6077.

(4) In 1969, Perun assigned the complete ^{1}H NMR spectrum for erythronolide B, and in 1982, Neszmelyi confirmed the ^{13}C NMR spectrum.see,: (a) Perun, T. J.; Egan, R. S. *Tetrahedron Lett.* **1969**, 387. (b)

(5) (a) Wiley, P. F.; Gerzon, K.; Flynn, E. H.; Sigal, M. V.; Weaver, O.; Quarck, U. C.; Chauvette, R. R.; Monahan, R. *J. Am. Chem. Soc.* **1957**, *79*, 6062–6070. (b) Wiley, P. F.; Weaver, O. *J. Am. Chem. Soc.* **1956**, *78*, 808–810. (c) Flynn, E. H.; Sigal, M. V.; Wiley, P. F.; Gerzon, K. *J. Am. Chem. Soc.* **1954**, *76*, 3121–3131. (d) Sigal, M. V.; Wiley, P. F.; Gerzon, K.; Flynn, E. H.; Quarck, U. C.; Weaver,

O. *J. Am. Chem. Soc.* **1956**, *78*, 388–395. (e) Gerzon, K.; Flynn, E. H.; Sigal, M. V.; Wiley, P. F.; Monahan, R.; Quarck, U. C. *J. Am. Chem. Soc.* **1956**, *78*, 6396–6408.

(6) Absolute configuration and confirmation of orginal structural determination of the erythromycin family was based on X-ray structure of a erythromycin A derivative, see: Harris, D. R.; McGeachi.Sg; Mills, H. H. *Tetrahedron Lett.* **1965**, 679.

(7) For recent comprehensive reviews of modular PKS genetics and biochemistry, see (a) Cane, D.E. (Guest editor) *Chem. Rev.* **1997**, *97*, (7). (b) Khosla, C.; Gokhale, R. S.; Jacobsen, J. R.; Cane, D. E. *Annu. Rev. Biochem.* **1999**, *68*, 219–253. (c) Stauton, J.; Wilkinson, B. In *Comprehensive Natural Products Chemistry, Polyketides and Other Secondary Metabolites Including Fatty Acids and Their Derivatives;* Sankawa, U., Ed.; Elsevier: Oxford, 1999; Vol.1, pp 95–532. (d) Ikeda, H.; Omura, S. In *Macrolide Antibiotics. Chemistry, Biology and Practice*; Omura, S., Ed.; Academic Press: San Diego CA 2002; pp 286–326.

(8) (a) Cortes, J.; Haydock, S. F.; Roberts, G. A.; Bevitt, D. J.; Leadlay, P. F. *Nature* **1990**, *348*, 176–178. (b) Donadio, S.; Staver, M. J.; McAlpine, J. B.; Swanson, S. J.; Katz, L. *Science* **1991**, *252*, 675–679. (c) Pieper, R.; Luo, G. L.; Cane, D. E.; Khosla, C. *Nature* **1995**, *378*, 263–266.

(9) For leading references, see: Yin, Y. F.; Lu, H. X.; Khosla, C.; Cane, D. E. *J. Am. Chem. Soc.* **2003**, *125*, 5671–5676.

(10) Ikeda, H.; Omura, S. In *Macrolide Antibiotics. Chemistry, Biology and Practice*; Omura, S., Ed.; Academic Press: San Diego CA 2002; pp 364.

(11) (a) Ikeda, H.; Omura, S. In *Macrolide Antibiotics. Chemistry, Biology and Practice*; Omura, S., Ed.; Academic Press: San Diego CA 2002; p. 455.

(12) Schlunzen, F.; Zarivach, R.; Harms, R.; Bashan, A.; Tocilj, A.; Albrecht, R.; Yonath, A.; Franceschi, F. *Nature* **2001**, *413*, 814–821.