Biosynthesis

DOI: 10.1002/anie.201304851

Elimination Reactions of Esters in the Biosynthesis of Polyketides and Ribosomal Peptides**

*Bartlomiej Krawczyk, Eric F. van Herwerden, Herman S. Overkleeft, and Roderich D. Süssmuth**

antibiotics · biosynthesis · elimination · lantipeptides · polyketides

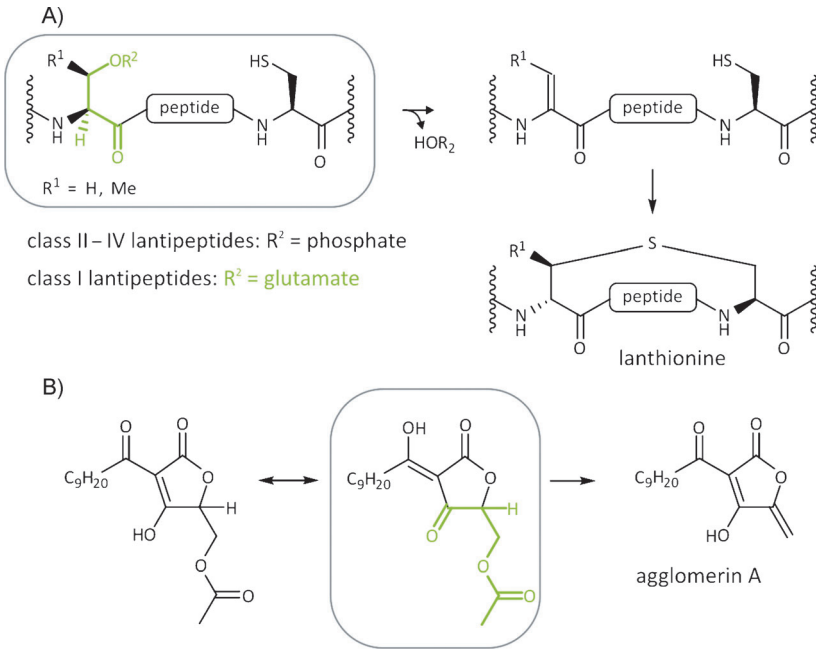
Carbon–carbon double bonds feature as characteristic structural motifs in many natural products. Nature has evolved several chemical transformations that together give rise to a wealth of functionally distinct alkenes and alkene-derived functional groups. Oxidative decarboxylation of a C-terminal cysteine residue, for example, forms the rare aminovinylcysteine (AviCys) moiety found in several lantipeptides.[1] The abstraction of dihydrogen from a saturated carbon–carbon bond, which is carried out by a number of dehydrogenases, is one of the key pathways for introducing the double bonds in unsaturated fatty acids.[2] Arguably the most widespread chemical transformation leading to the generation of a carbon–carbon double bond and the subject of this Highlight, however, is the formal elimination of water from a β-hydroxycarbonyl compound to produce an enoyl moiety. β-Elimination of water is the driving force in each of the consecutive C_2 elongation cycles that characterizes fatty acid biosynthesis. In this process, malonate as its monothioester is condensed with another thioester to give a ketone, which further undergoes reduction to the corresponding β-hydroxy thioester. Deprotonation at the α-carbon atom followed by elimination of a hydroxy group then produces the carbon–carbon double bond. This is normally reduced to the single bond, thereby setting up the system for another chain elongation event. Essentially the same process is also found in polyketide biosynthesis, a pathway present in many microorganisms for the synthesis of secondary metabolites.[3] The structural diversity of the polyketides results not only from the utilization of α-mono-substituted malonates as well as malonate, but also from the partial or complete execution of the cycle through ketoreduction of the β-carbonyl group by a ketoreductase (KR), a dehydratase (DH) mediated β-elimination, and an endoreductase (ER) mediated hydrogenation.[2]

β-Elimination to unsaturated carbonyl compounds has also emerged as a distinguishing motif in the biosynthesis of ribosomally synthesized and post-translationally modified peptides (RiPPs).[4] For example, the lantipeptides—a major class of RiPP natural products with a variety of biological activities ranging from antibacterial to antiallodynic—contain thioether bridges as distinguishing features.[4] These lanthionine residues are formed through an intramolecular 1,4-addition of a Cys side-chain thiol group to dehydroalanine (Dha) or dehydrobutyrine (Dhb). Dha and Dhb are produced by formal dehydration of either Ser (leading to Dha) or Thr (giving Dhb), in other words from two proteinogenic amino acids featuring a β-hydroxycarbonyl motive. In theory, and in analogy to fatty acid biosynthesis and polyketide biosynthesis, β-elimination of the hydroxy group would lead to the desired intermediate. In practice, however, nature employs a divergent leaving-group strategy. Since the leaving groups of choice in many biochemical reactions are phosphate derivatives (phosphate monoesters, pyrophosphates) and since Ser and Thr residues are often subject to kinase-catalyzed phosphorylation, it appears logical to invoke phosphoserine (pSer) and phosphothreonine (pThr) as initial intermediates in the formation of lanthionin residues (Scheme 1 A). This indeed appeared true for class II and IV lantipeptides, as was demonstrated by van der Donk and co-workers[5–7] and later on corroborated for class III lantipeptides.[8,9]

In contrast to this, however, Ser/Thr dehydration in the precursor peptide during the biosynthesis of the most renowned class I lantipeptide—nisin—remained an enigma for many years. Very recently a major step forward in solving this issue was made by the van der Donk research group, who demonstrated that nature very likely employs an ester—derived from the condensation of the primary or secondary alcohol with one of the glutamate carboxylate groups—as the leaving group.[10] They demonstrated in an in vitro study that NisB, the dehydratase involved, installs Dha and Dhb in the precursor peptide in the presence of *E. coli* membrane fraction, ATP, and Mg^{2+}, but only does so when glutamate is added to the mixture. This finding is especially important since NisB homologues are also reported as the dehydration

[*] Dr. B. Krawczyk, E. F. van Herwerden, Prof. Dr. R. D. Süssmuth
Institut für Chemie, Technische Universität Berlin
Strasse des 17. Juni 124, 10623 Berlin (Germany)
E-mail: suessmuth@chem.tu-berlin.de
Prof. Dr. H. S. Overkleeft
Leiden Institute of Chemistry, Leiden University
Einsteinweg 55, 2300 RA Leiden (The Netherlands)

[**] H.S.O. acknowledges the Alexander von Humboldt Foundation for a Friedrich Wilhelm Bessel Research Award. This work was supported by the cluster of excellence UniCat (granted by the German research council-DFG).



Scheme 1. A) Glutamate ester in the biosynthesis of class I lantipeptides. B) β-Elimination of acetate as a key step in the biosynthesis of agglomerin A.

catalysts for other natural product peptides including the thiopeptides and linear azole-containing peptides (LAPs).[4] The mechanism by which the putative Ser/Thr-Glu esters are formed remains unclear and further studies are also needed to establish whether the α- or γ-Glu carboxylate is employed in the ester bond.[11,12]

As is true in many natural product synthesis campaigns, nature shows the way for the development of synthetic strategies for the construction of lantipeptides. A large variety of leaving groups has been explored in the past decades for the synthetic introduction of Dha or Dhb as both the end product and as an intermediate for a subsequent cyclization through 1,4-addition involving a cysteine thiol nucleophile. Amongst these leaving groups are mesylates, isoureas, and carbonates derived from Ser or Thr,[13] but also more unusual species such as the trimethyl ammonium salt derived from 2,3-diaminopropionic acid (Dap).[14] In related strategies, methylation of the side chain of cysteine (Cys) and selenocysteine (Sec) and subsequent oxidative elimination was shown to give Dha residues efficiently.[15] As is perhaps not surprising, since phosphate ester leaving groups are not commonly part of the synthetic organic chemistry repertoire, this strategy from nature has not been employed. Perhaps more surprisingly, the same holds true for ester leaving groups.

Leadlay and co-workers recently discovered a mechanistically closely related formal elimination of water in the biosynthesis of the multiple unsaturated furan ring in the tetratonate antibiotic agglomerin (Scheme 1 B).[16] Again, in an in vitro study with putative biosynthetic enzymes, they showed that in the first step the hydroxy group is acetylated in an acetyl-CoA-dependent process by the enzyme Agg4. In the second step catalyzed by Agg5, presumably after abstraction of the acidic α proton in the conjugated system, the acetate group is expelled to yield the final natural product. By virtue of its simplicity, nature may have provided a strategy for the laboratory synthesis of agglomerin and related metabolites. This holds true especially since, after an early synthesis of the furanone moiety by a Hoffmann elimination strategy starting from 2,3-diaminopropionic acid,[17] no new synthetic method for the construction of this moiety has appeared in the literature.

Both recent findings demonstrate that the palette of elimination reactions by which enoyl derivatives are installed either as intermediates or final moieties in natural products is much larger than was assumed. These seminal findings are of importance for several reasons. The lack of success in enzymatic in vitro reconstitution studies may be caused by the lack of appropriate components included in the experiments, rather than, for example, protein folding. Therefore, it may be wise to include, besides the enzyme and the usual cofactors, other less-obvious co-substrates or components. From the viewpoint of synthetic organic chemistry and natural product synthesis, nature continues to be a source of inspiration, not only in the diversity of the structures but also in strategies to assemble these. Finally the here-described examples comprise enoyl derivatives as both intermediates (nisin) and end products (nisin, agglomerin). Given the fact that nature is efficient in using a relatively limited number of chemical motifs in numerous biosynthesis pathways it might be worthwhile looking for the unsaturated furanone moiety not as an end motif, as in agglomerin, but rather as an intermediate that undergoes an 1,4-addition, such as in a process resembling the one leading to the lanthionins in class I/II/IV lantipeptides (thiol addition to Dha/Dhb) or

indeed as we identified in the formation of the complex triamino triacid moiety (labionin) in the class III lantipeptide, labyrinthopeptin (enolate addition to Dha/Dhb).[18]

Received: June 5, 2013
Published online: August 9, 2013

[1] T. Kupke, C. Kempter, V. Gnau, G. Jung, F. Götz, *J. Biol. Chem.* **1994**, *269*, 5653 – 5659.
[2] R. H. Garrett, C. M. Grisham, *Biochemistry* Cengage Learning, **2007**.
[3] T. A. M. Gulder, M. F. Freeman, J. Piel, *Top. Curr. Chem.* **2013**, DOI: 10.1007/128 2010 113.
[4] P. G. Arnison, M. J. Bibb, G. Bierbaum, A. A. Bowers, T. S. Bugni, G. Bulaj, J. A. Camarero, D. J. Campopiano, G. L. Challis, J. Clardy, P. D. Cotter, D. J. Craik, M. Dawson, E. Dittmann, S. Donadio, P. C. Dorrestein, K.-D. Entian, M. A. Fischbach, J. S. Garavelli, U. Goeransson, C. W. Gruber, D. H. Haft, T. K. Hemscheidt, C. Hertweck, C. Hill, A. R. Horswill, M. Jaspars, W. L. Kelly, J. P. Klinman, O. P. Kuipers, A. J. Link, W. Liu, M. A. Marahiel, D. A. Mitchell, G. N. Moll, B. S. Moore, R. Müller, S. K. Nair, I. F. Nes, G. E. Norris, B. M. Olivera, H. Onaka, M. L. Patchett, J. Piel, M. J. T. Reaney, S. Rebuffat, R. P. Ross, H.-G. Sahl, E. W. Schmidt, M. E. Selsted, K. Severinov, B. Shen, K. Sivonen, L. Smith, T. Stein, R. D. Süssmuth, J. R. Tagg, G.-L. Tang, A. W. Truman, J. C. Vederas, C. T. Walsh, J. D. Walton, S. C. Wenzel, J. M. Willey, W. A. van der Donk, *Nat. Prod. Rep.* **2013**, *30*, 108 – 160.
[5] C. Chatterjee, L. M. Miller, Y. L. Leung, L. Xie, M. Yi, N. L. Kelleher, W. A. van der Donk, *J. Am. Chem. Soc.* **2005**, *127*, 15332 – 15333.
[6] Y. Goto, B. Li, J. Claesen, Y. Shi, M. J. Bibb, W. A. van der Donk, *PloS Biol.* **2010**, *8*, e1000339.
[7] Y. Goto, A. Okesli, W. A. van der Donk, *Biochemistry* **2011**, *50*, 891 – 898.
[8] W. M. Müller, T. Schmiederer, P. Ensle, R. D. Süssmuth, *Angew. Chem.* **2010**, *122*, 2486 – 2490; *Angew. Chem. Int. Ed.* **2010**, *49*, 2436 – 2440.
[9] B. Krawczyk, P. Ensle, W. M. Müller, R. D. Süssmuth, *J. Am. Chem. Soc.* **2012**, *134*, 9922 – 9925.
[10] N. Garg, L. M. A. Salazar-Ocampo, W. A. van der Donk, *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 7258 – 7263.
[11] Acyl elimination is a known feature in macrolactone biochemistry, see K. P. Bateman, K. Yang, P. Tibault, R. L. White, L. C. Vining, *J. Am. Chem. Soc.* **1996**, *118*, 5335 – 5338.
[12] Acetate elimination also features in the biosynthesis of Cys and Met from Ser/homoSer, see C. Walsh, *Enzymatic Reaction Mechanisms*, W. H. Freeman, **1979**, p. 825 – 827.
[13] a) C. Shin, Y. Yonezawa, M. Takahashi, J. Yoshimura, *Bull. Chem. Soc. Jpn.* **1981**, *54*, 1132 – 1136; b) M. J. Miller, *J. Org. Chem.* **1980**, *45*, 3131 – 3132; c) R. Ramesh, K. De, S. Chandrasekaran, *Tetrahedron* **2007**, *63*, 10534 – 10542.
[14] S. Nomoto, A. Sano, T. Shiba, *Tetrahedron* **1979**, *35*, 521 – 522.
[15] a) S. Burrage, T. Raynham, M. Bradley, *Tetrahedron* **1998**, *54*, 2831 – 2834; b) K. Hashimoto, M. Sakai, T. Okuno, H. Shirahama, *Chem. Commun.* **1996**, 1139 – 1140.
[16] C. Kanchanabanca, W. Tao, H. Hong, Y. Liu, F. Hahn, M. Samborskyy, Z. Deng, Y. Sun, P. F. Leadlay, *Angew. Chem.* **2013**, *125*, 5897 – 5900; *Angew. Chem. Int. Ed.* **2013**, *52*, 5785 – 5788.
[17] S. V. Ley, M. L. Trudell, D. J. Wadsworth, *Tetrahedron* **1991**, *47*, 8285 – 8296.
[18] K. Meindl, T. Schmiederer, K. Schneider, A. Reicke, D. Butz, S. Keller, H. Gühring, L. Vértesy, J. Wink, H. Hoffmann, M. Brönstrup, G. M. Sheldrick, R. D. Süssmuth, *Angew. Chem.* **2010**, *122*, 1169 – 1173; *Angew. Chem. Int. Ed.* **2010**, *49*, 1151 – 1154.

