Bioorganic & Medicinal Chemistry 24 (2016) 6269–6275

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Tetrodecamycin: An unusual and interesting tetronate antibiotic

Tomas Gverzdys, Glenna Kramer, Justin R. Nodwell *

Department of Biochemistry, University of Toronto, 1 King's College Circle, Toronto, ON M5S 1A8, Canada

ARTICLE INFO

Article history:
Received 7 March 2016
Revised 8 May 2016
Accepted 17 May 2016
Available online 18 May 2016

Keywords:
Tetronate
Tetrodecamycin
13-Deoxytetrodecamycin
Photobacterium damselae ssp. *piscicida*
Biosynthesis
Mechanism of action
Review
Covalent

ABSTRACT

The tetrodecamycins are a group of secondary metabolites that are characterized by the presence of a tetronate ring in their structure. Originally discovered for their antibiotic activity against *Photobacterium damselae* ssp. *piscicida*, the causative agent of pseudotuberculosis in fish, this family of molecules has also been shown to have potent antibiotic activity against methicillin-resistant *Staphylococcus aureus*. Due to their small size and highly cyclized nature, they represent an unusual member of the much larger group of bioactive molecules called the tetronates. Herein, we review what is known about the mechanism of action of these molecules and also present a hypothesis for their biosynthesis. A deeper understanding of the tetrodecamycins will provide a more holistic view of the tetronate-family, provide new chemical probes of bacterial biology, and may provide therapeutic lead molecules.

1. Introduction

Since the early 1940s, more than 10^7 bacteria have been screened for their ability to produce bioactive molecules. This has resulted in the identification of more than 2000 antibiotics that are divided into a variety of classes.[1,2] While there are numerous mechanisms by which bioactive molecules can be assembled, a particularly common mechanism is the polyketide synthases (PKS). These biosynthetic machines iteratively link carbon atoms together, using acetyl-CoA, malonyl-CoA, methylmalonyl-CoA and other related precursors to form linear carbon chains. Subsequent cyclization and tailoring of the linear molecules generates compounds with a magnificent diversity of structures and wide-ranging bioactivities.[3,4] Examples that illustrate this diversity include actinorhodin (antibacterial),[5] tetracycline (antibacterial),[6] FK506 (immunosuppressant),[7] doxorubicin (chemotherapeutic),[8] amphotericin B (antifungal),[9] erythromycin (antibacterial),[10] lovastatin (anti-cholesterol drug),[11] as well as the numerous tetronate-family molecules which are the subject of this review. The bioactivities of the tetronate family range from antiviral[12] to antibacterial[13] to antitumor.[14]

The tetronates are characterized by a unique, five-membered lactone ring called a tetronate ring (Fig. 1A). This family was recently divided into three subfamilies: the linear tetronates, the spirotetronates, and the miscellaneous tetronates.[15] The linear tetronates are epitomized by molecules such as agglomerin A,[16] acaterin,[17] didehydro-acaterin,[18] RK-682,[12] pesthetoxin,[19] and tetronomycin[20] (Fig. 1B). These molecules are characterized by a tetronate ring modified with a carbon chain attached at either the C-3 or C-5 position. The attached carbon chains, synthesized as either a fatty acid or a polyketide, may be saturated or unsaturated and may include additional small rings (*e.g.*, tetronomycin). The spirotetronate molecules, themselves the topic of dedicated reviews,[21] are characterized by a spirotetronate ring system (in which a six-membered ring and the tetronate ring are linked to form a spirane) and a macrocyclic ring. This group is further divided into three groups on the basis of the size of their macrocyclic ring: the small spirotetronates have a macrocyclic ring of 11 carbons (*e.g.*, maklamicin,[22] nomimicin,[23] abyssomicin C[13]); the medium spirotetronates have a macrocyclic ring of 13 carbons (*e.g.* chlorothricin,[24] kijanimicin,[25] tetrocarcin,[26] decatromicin B[27]); and the large spirotetronates have more than 13 carbons in their macrocyclic ring (*e.g.*, quartromicin,[28] versipelostatin,[29] tetronothiodin[30]) (Fig. 1B).

Finally, the last group of tetronate molecules is the miscellaneous tetronates. As the name suggests, these are tetronate molecules which do not fit into any of the above categories. This group includes members like the artapetalins (excluded from the linear tetronate group because they are alkylated, not acylated, at the C-3 position),[31] picrodendrin B (which possess an unusual

* Corresponding author.
E-mail addresses: tomas.gverzdys@mail.utoronto.ca (T. Gverzdys), glenna.kramer@utoronto.ca (G. Kramer), justin.nodwell@utoronto.ca (J.R. Nodwell).

http://dx.doi.org/10.1016/j.bmc.2016.05.028
0968-0896/

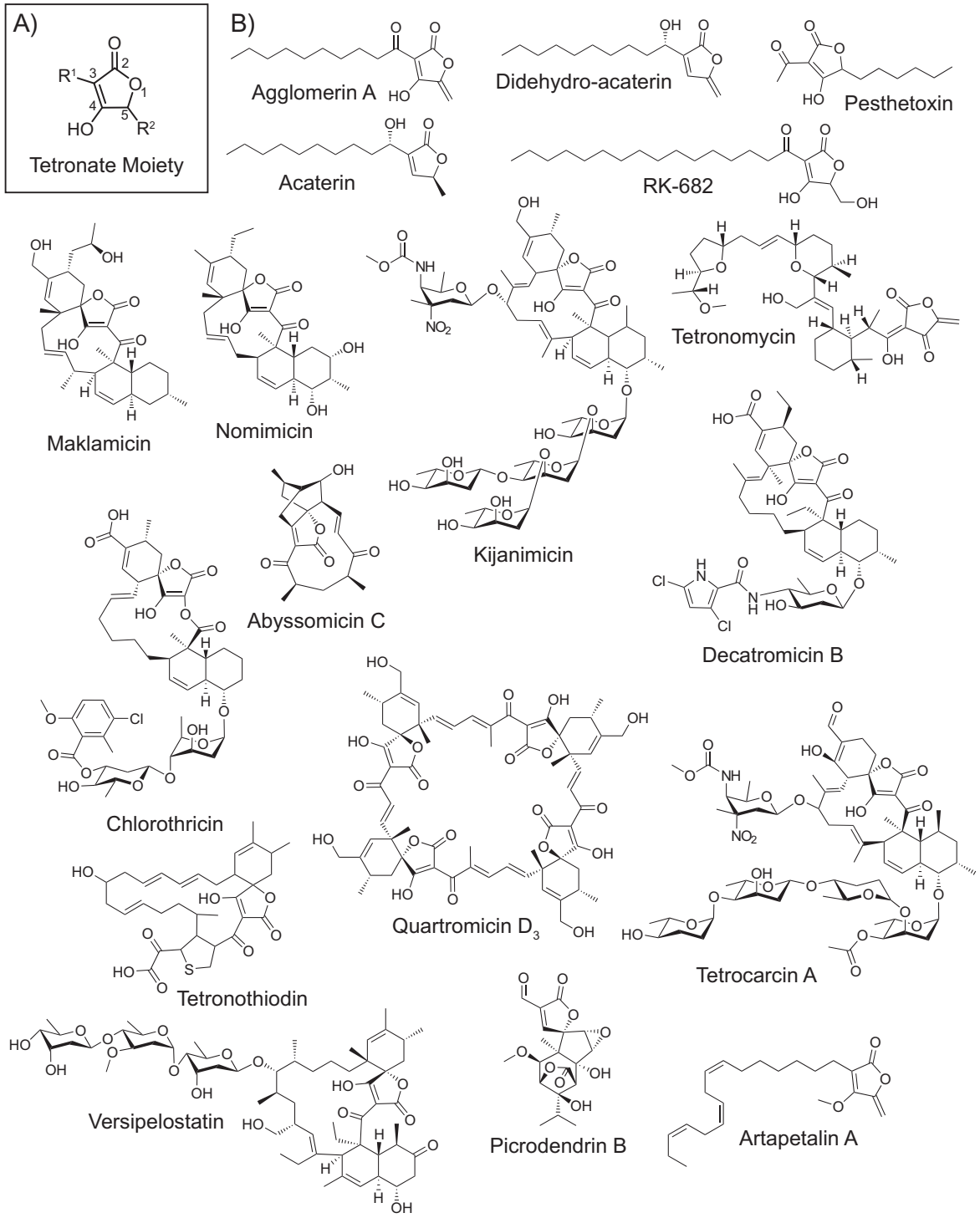


Figure 1. The tetronate family of molecules all possess a tetronate ring. (A) The tetronate ring is a 5-membered lactone ring. Modifications to the ring are commonly found at R_1 and R_2. (B) The tetronate family can be divided into three general subfamilies: the linear tetronates, the spirotetronates, and the miscellaneous tetronates. Representing the linear tetronates are agglomerin A, acaterin, tetronomycin, didehydro-acaterin, pesthetoxin, and RK-682. Representing the small spirotetronates are maklamicin, nomimicin, and abyssomicin C. Representing the medium spirotetronates are chlorothricin, tetrocarcin A, kijanimicin, and decatromicin B. Representing the large spirotetronates are quartromicin D_3, tetronothiodin, and versipelostatin. Representing the miscellaneous tetronates are picrodendrin B and artapetalin A.

spirotetronate),[32] and, our specific focus, the tetrodecamycin-group (TDM-group) molecules: tetrodecamycin (TDM), dihydro-tetrodecamycin (dhTDM), and 13-deoxytetrodecamycin (13-dTDM) (Fig. 2).[33,34]

2. History of the tetrodecamycins

TDM was first reported in 1994 in a brief letter followed later by a description of the producing strain, fermentation, isolation, and the structural elucidation of TDM and a related compound, dhTDM

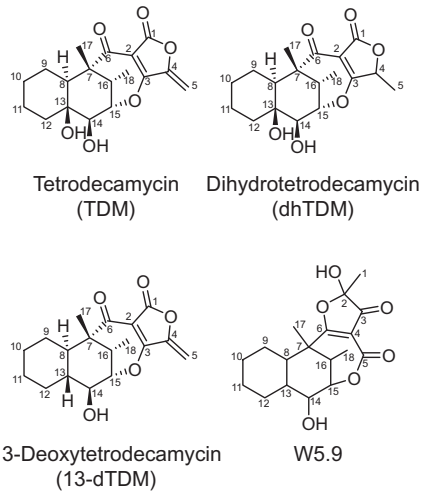


Figure 2. The tetrodecamycins are a small group of related molecules which are classified as miscellaneous tetronates.

(Fig. 2).[33,35,36] The organism that produces both of these molecules, *Streptomyces nashvillensis* MJ885-mF8, was isolated from a soil sample collected in Suginami-ku, Tokyo, Japan. Studies into this wild-isolate streptomycete were commenced with the observation that it produced anti-bacterial activity against *Photobacterium damselae* ssp. *piscicida* (formerly described as *Pasteurella piscicida*[37]). *P. damselae* ssp. *piscicida* is the causative agent of pseudotuberculosis in fish (also called pasteurellosis or photobacteriosis). Indeed, the name *piscicida* is derived from the Latin words "fish" (*piscus*) and "to kill" (*-cidus*).[38] The disease presents as a bacterial septicemia followed by the development of tubercles on internal organs that can ultimately prove fatal.[39] At the time, cultured yellowtail (*Seriola quinqueradiata*, also called Japanese amberjack) accounted for 56% of the farmed fish produced in Japan and losses due to pseudotuberculosis had significant economic impact on the industry.[40] Antibiotics were in use to treat this infection but, mirroring the same problem observed in human health, the widespread use of antibiotics was accompanied by the reciprocal development of antibiotic resistance.[41] This led to the need for new antibiotics.

TDM and dhTDM were isolated from ethyl acetate extracts of *S. nashvillensis* in liquid culture. The structures of TDM, dhTDM, and 14-*O*-acyltetrodecamycin (a semi-synthetic derivative of TDM) were determined by NMR (Fig. 2). The structure revealed a tetracyclic molecule with a 6,6,7,5-membered ring system: the pair of six-membered rings are arranged in a decalin system, the seven-membered ring is a heterocycle containing an oxygen, and the five-membered ring is a tetronate ring.[33,36]

One of the characteristic features of TDM is the presence of an *exo*-methylene attached to the tetronate ring at position C-5 (Fig. 2). In contrast, dhTDM possesses a methyl group at this same position. The presence of this *exo*-methylene is interesting due to its infrequent occurrence in the other tetronate ring-containing compounds (Fig. 1) and because it also forms a potential Michael acceptor that could influence the molecule's bioactivity (discussed further below). Bioassays performed with TDM confirmed that it had strong antibiotic activity against *P. damselae* ssp. *piscicida* (1.56–6.25 μg mL^{-1}, 4.67–18.7 μM) as well as antibiotic activity against a number of strains of *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), *Micrococcus luteus*, and *Bacillus subtilis* (6.25–12.5 μg mL^{-1}, 18.7–37.4 μM). Interestingly, despite the potent activity of TDM against *P. damselae* ssp. *piscicida* (a Gram-negative organism), it showed no antibiotic activity against other Gram-negative organisms tested. dhTDM showed almost no antibiotic activity against any of the organisms tested, including *P. damselae* ssp. *piscicida*. TDM has been reported to have no toxicity in mice up to 100 mg kg^{-1} when introduced by intraperitoneal injection, though due to a lack of materials and methods pertaining to this result, this information should be considered with care. In vitro cytotoxicity studies were never conducted.[33,36]

A follow-up study by the same group attempted to improve the bioactivity of TDM by making modifications to the 14-OH position and the *exo*-methylene.[42] A patent was filed for the molecule and its derivatives (patent number JPH0892256), though there is no evidence that the molecule was ever used in the clinic or for aquaculture. Following these studies, no further work on the molecule was reported until 2000, at which time a number of groups attempted to synthesize the molecule. An initial retrosynthetic analysis of tetrodecamycin suggested that this structure could be synthesized from a decalin derivative and a tetronic acid derivative, inspiring the first efforts toward the synthesis of tetrodecamycin.[43] This strategy made two features of the molecule important targets in these early synthetic studies: the asymmetric synthesis of the novel seven-membered ring core,[43–45] and the synthesis of the decalin portion of the molecule.[46–48] These efforts culminated with the successful total synthesis of tetrodecamycin 2006 through a pinacol cyclization mechanism.[49]

In 2015, we published the first report of a new, naturally-occurring tetrodecamycin since the initial 1995 report of TDM and dhTDM. The producer strain, *Streptomyces* species strain WAC04657, was identified from a panel of wild-isolate *Streptomyces* because of its ability to inhibit the growth of MRSA. The molecule responsible for the activity, 13-dTDM, differs from TDM by the lack of a hydroxyl group at C-13 on the decalin ring (Fig. 2). The change in the decalin ring results in marginally greater activity against Gram-positive organisms (from 1 to 8 μg mL^{-1}, 3.14 to 25.2 μM) when compared to TDM.[34]

Given the anti-MRSA activity, the presence of a Michael acceptor, and the lack of knowledge about the TDM-group molecules, we went on to identify the biosynthetic gene cluster (the *ted* genes) that encodes 13-dTDM.[50] We identified the same cluster in three other strains of *Streptomyces*. Surprisingly, none of these organisms produced 13-dTDM, though two of them, *Streptomyces atroolivaceus* ATCC 19725 and *Streptomyces globisporus* ssp. *globisporus* NRRL B-2293, produced TDM and dhTDM. For reasons unknown, the third organism, *Streptomyces* sp. LaPpAH-202, did not produce any TDM-group molecules. A surprising discovery that came out of this work was the identification of a novel molecule, W5.9, also produced by the *ted* genes in *S.* str. WAC04657 but lacking the canonical tetronate ring expected from TDM-group molecules. At the current time, we believe this molecule may be a shunt product produced by the cluster.

3. Mechanism of action

At present, the mechanism of action and molecular target of the TDM-group molecules are unknown, although the potentially reactive *exo*-methylene stands out as a clue. Both TDM and 13-dTDM possess a Michael acceptor composed of the conjugated system that extends through C-6/C-1, C-2, C-3, C-4 and C-5 (Fig. 2). Due to the presence of the electronegative carbonyls at C-6 and C-1, the *exo*-methylene at C-5 is expected to act as an electrophile in a nucleophilic addition reaction (Fig. 3). The only difference between dhTDM, which has no antibiotic activity, and TDM is the lack of this *exo*-methylene. Notably, dhTDM still possesses a Michael acceptor formed by C-6/C-1, C-2, and C-3. This strongly suggests that the *exo*-methylene is important for bioactivity. Indeed, this idea has been proposed on several occasions based on similar evidence.[42,44] Further, since thiol compounds can be easily added to the *exo*-methylene,[42] one group has hypothesized

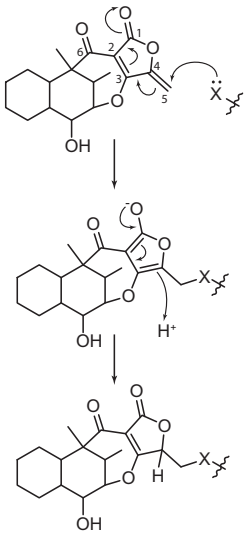


Figure 3. The proposed mechanism by which the tetrodecamycins could bind covalently to their target. The conjugated system composed of C-1/C-6, C-2, C-3, C-4, and C-5 forms a possible Michael acceptor. Due to the presence of the electronegative carbonyls, C-5 is able to act as an electrophile in a nucleophilic addition reaction, potentially with a proteinaceous target. This results in the formation of a covalent bond with the target molecule. "X" denotes the nucleophile attached to the target biomolecule.

that the mechanism of action involves a Michael-type addition with a functional cysteine residue of a target protein.[45]

There are several synthetic studies that provide further insight into how TDM might work. In these studies, derivatives or semi-structures of TDM were tested for their bioactivity (Fig. 4). In one of these papers, the authors reported making modifications to two different places in the structure of TDM: the 14-OH position and the *exo*-methylene.[42] To the 14-OH position, various benzene groups were added as either ethers or esters. These derivatives exhibited only minor changes to their activity, ranging from a 2-fold decrease to 4-fold increase. The compound that showed the greatest increase in activity against *S. aureus*, compound **1**, was modified with a naphthalene moiety (0.78 μg mL^{-1}, 1.61 μM). Given the hydrophobic nature of naphthalene, the modification could cause better targeting of the molecule to the bacterial membrane, thus suggesting that the target of TDM may be at this location.

The addition of bromines or thiols to the *exo*-methylene (molecules **2**–**4**) reduced the compound's antibiotic activity against *S. aureus* though the results are somewhat contradictory. While compound **4** lost all activity, compounds **2** and **3** retained some antimicrobial activity, an observation that is difficult to rectify with the view that the *exo*-methylene's reactivity is essential. Possible explanations include the idea that the *exo*-methylene can be restored via a retro-Michael reaction, thus regenerating the unmodified TDM which subsequently restores the bioactivity.[45] Alternatively, it is possible that some TDM-group molecules may be able to function through another mechanism independent of the Michael acceptor. Neither of these ideas have been tested at this time.

Additional evidence about TDM's function came from work involving partial structures of TDM.[45] It was shown that molecule **5** had no bioactivity against *S. aureus*, while one of the synthetic precursors, molecule **8**, did. Therefore, while the *exo*-methylene is necessary for antibiotic activity, it is not sufficient. This suggests that the molecule exerts its activity through a specific binding

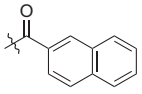
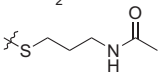
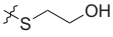
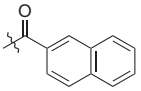
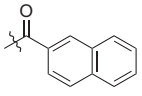
Molecule	MIC (μg mL^{-1}) *S.a.*	*P.d.*	Side Groups
TDM:	6.25	1.56	R= H
1:	0.78	>100	R= 
dhTDM:	>100	50	R_1= H R_2= H
2:	12.5	6.25	R_1=  R_2= H
3:	25	6.25	R_1=  R_2= H
4:	>100	12.5	R_1= Br R_2= Br
5:	>128	nt	R_1= H R_2= H
6:	8	nt	R_1=  R_2= H
7:	128	nt	R_1= H R_2= 
8:	4	nt	

Figure 4. Synthetic derivatives and partially synthesized variants of tetrodecamycin. Minimum inhibitory concentrations (MICs) are measured against either *S. aureus* (*S.a.*) or *P. damselae* ssp. *piscicida* (*P.d.*). "nt" denotes that the molecule was not tested against that organism. The MICs of molecules TDM, dhTDM, and **1**–**4** were measured against *S. aureus* FDA209P while compounds **5**–**8** were measured against *S. aureus* ATCC 25923.[42,45]

reaction. This would be in contrast to a promiscuous action in which the compound simply reacts covalently with multiple targets on the cell surface.[51,52] Further derivatives of **5**, including molecule **6** (which was active) and molecule **7** (which was not active), support the idea that the molecule's overall structure and chemical properties are important, consistent with a specific molecular target rather than promiscuous action.

An important question is why TDM showed bioactivity against the Gram-negative organism *P. damselae* ssp. *piscicida*, but not against *Escherichia coli*, *Klebsiella pneumoniae*, or *Pseudomonas aeruginosa*.[33,35] Interestingly, some TDM-derivatives that showed increased activity against *S. aureus* showed no bioactivity against *P. damselae* ssp. *piscicida* (*e.g.*, molecule **1**). This and other examples where there is a lack of concordance between activity against *S. aureus* and *P. damselae* (e.g. molecules **2**, **3**, and **4**) could be consistent the TDMs having more than one mechanism of action. While this is rare and, again, untested, there is precedent for this idea in the case of streptomycin.[53]

4. Biosynthesis

Careful examination of the *ted* cluster and comparison to well-studied tetronate molecules[54] has allowed us to propose a biosynthetic pathway for the TDM-group molecules (Fig. 5). The carbon backbone of the TDM-group molecules is produced by a type I PKS. Bioinformatic analysis of the PKS genes suggests that the backbone is synthesized by 7 modules (1 loading module and 6 extension modules) and that most of the integrated monomers are derived from malonyl CoA with the exception of a methylmalonyl CoA used by module 5. Release of the backbone from the final module is expected to be concurrent with formation of the tetronate ring.[50]

Detailed studies on tetronate ring formation have been carried out for tetronomycin,[55] RK-682,[56] agglomerin,[57] and quartromicin.[58,59] Given that homologues of all the tetronate biosynthesis proteins have been found in the *ted* cluster, it is likely that tetronate ring formation follows the same pathway in the TDM-group molecules. The first step involves taking 1,3-bisphosphoglycerate from primary metabolism and loading it onto a conserved cysteine residue of TedF1, an FkbH-family protein. During this step, both phosphates are removed to yield a glyceryl moiety linked to TedF1. Next, this glyceryl moiety is transferred to TedF2, a dedicated acyl carrier protein. TedF5, a FabH-like enzyme, then uses the glyceryl-*S*-TedF2 to catalyze the release of the carbon backbone from the PKS and subsequently generate the immature tetronate ring (molecule **9**). The final step in tetronate ring formation involves elimination of 5-OH from **9** to form the *exo*-methylene. This step involves an acetylation-elimination step in which TedF3, an acetyl

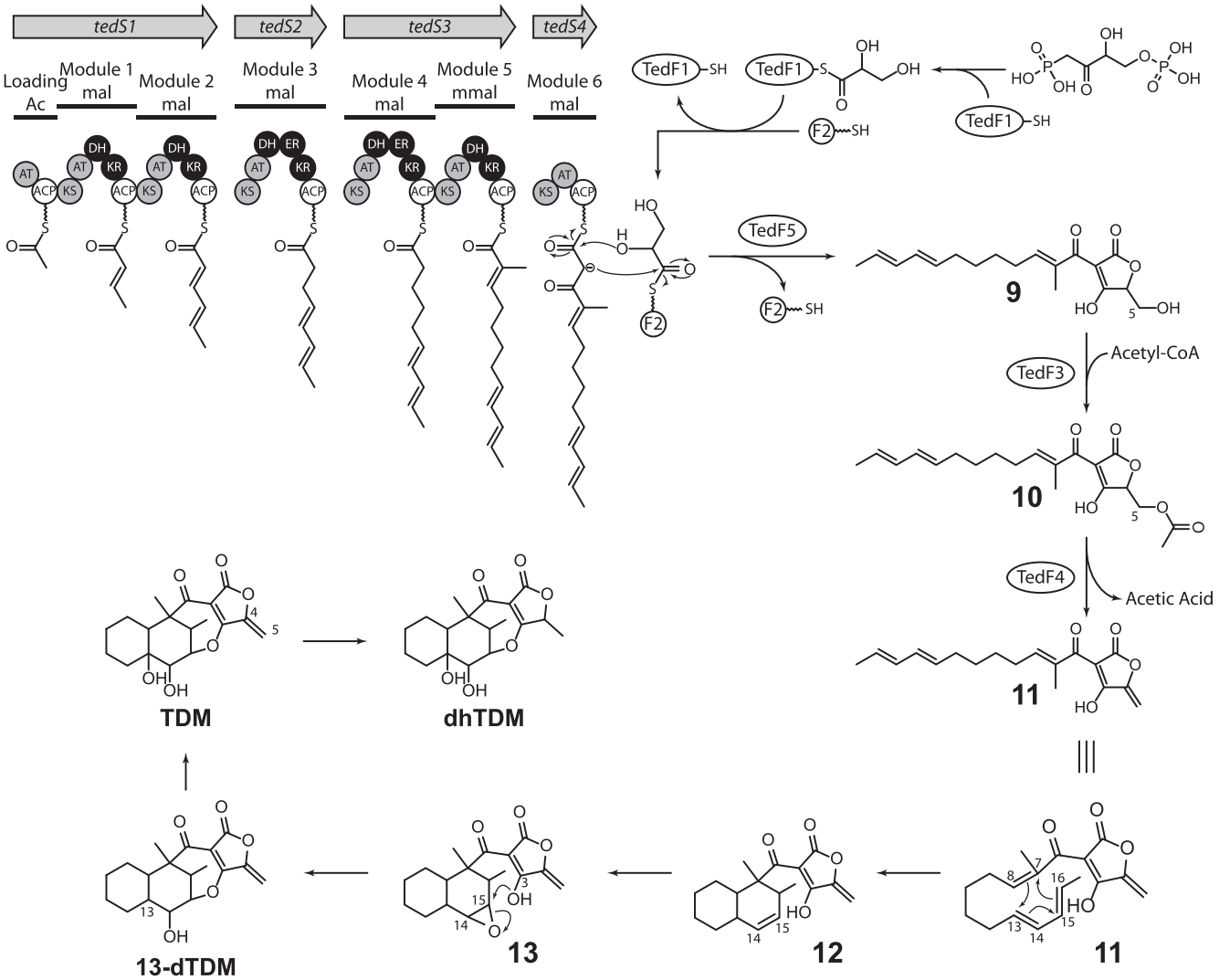


Figure 5. Proposed biosynthesis for 13-deoxytetrodecamycin, tetrodecamycin, and dihydrotetrodecamycin. Details of the biosynthesis are described in Section 4.

transferase, catalyzes the addition of an acetyl group to the hydroxyl (molecule **10**) followed by elimination of acetic acid from this position by TedF4, an α/β hydrolase, thus generating the *exo*-methylene (molecule **11**).

Progressing from the linear PKS backbone of intermediate **11** to the recognizable tetrodecamycin scaffold would require two distinct tasks: formation of the decalin ring, and the closure of the seven-membered heterocycle (Fig. 5). In the literature, an intramolecular Diels–Alder has been proposed for the formation of the decalin ring of maklamicin[60] and could likewise be responsible for the formation of the decalin rings in other tetronates including nomimicin, chlorothricin,[61] kijanimicin,[62] tetrocarcin A,[63] and decatromicin B (Fig. 1B). Following suit, we postulate that the decalin ring in the TDM natural products could also be formed by an intramolecular Diels–Alder reaction. In this process, the C-7/C-8 alkene in intermediate **11** would act as a dienophile in the cycloaddition with the diene at C-13 to C-16, yielding the decalin ring in intermediate **12**. Enzymes known to catalyze intramolecular Diels–Alder reactions have been identified.[64–69] Interestingly, the enzyme TedJ shows homology to previously identified Diels–Alderases,[70] suggesting that this protein may be responsible for formation of the decalin ring.

Next, the heterocyclic ring central to the tetrodecamycin scaffold would be formed. Based on a similar reaction which has been suggested for abyssomicin (Fig. 1B),[71] we propose that the cyclization resulting in this heterocyclic ring would proceed through two steps. First, the alkene present at C-14/C-15 in intermediate **12** would undergo an epoxidation giving intermediate **13**. A nucleophilic attack on the epoxide C-15 by the oxygen on C-3 would result in a seven-membered heterocyclic ring and simultaneously open the epoxide, which upon protonation, would give a hydroxyl on C-14. The resulting product, 13-dTDM, could then proceed through other tailoring steps to give a spectrum of analogues in the TDM-group including TDM and dhTDM.

In *S.* str. WAC04657, 13-dTDM appears to be the end-product of this biosynthetic pathway,[34] yet it is a logical next step to add a hydroxyl to C-13 to generate TDM. Next, reduction of the *exo*-methylene to a methyl would generate dhTDM. While our proposed biosynthesis suggests that dhTDM is the end product, it brings about an interesting question: why would a biosynthetic gene cluster capable of making potent antibiotics then convert the molecules into an inactive form? Attempts to find enzymes which catalyze the conversion of didehydro-acaterin into acaterin (Fig. 1B) were unable to find a specific protein responsible for this activity.[72,73] Thus, it is possible that the reduction of TDM's *exo*-methylene into a methyl may be the result of a non-enzymatic process that generates dhTDM as an unintended byproduct.

5. Conclusions

Our intent in this paper has been to review what is known in the relatively scant literature of the tetrodecamycin family and to propose some cogent hypotheses for their biosynthesis and mechanism of action. While there is a great deal left to learn about this family, their potent action against resistant pathogens such as MRSA suggests that they merit continued investigation. Identifying their mechanism of action could lead to new molecular targets for antimicrobial development.

Acknowledgements

We thank Stefanie Mak and Dr. Sheila Elardo for advice during the writing process. Work on 13-dTDM was supported by grants from the Canadian Institutes for Health Research (MOP-57684) and from Cystic Fibrosis Canada.

References and notes

1. Baltz, R. H. *J. Ind. Microbiol. Biotechnol.* **2006**, *33*, 507.
2. Berdy, J. *J. Antibiot. (Tokyo)* **2005**, *58*, 1.
3. Fischbach, M. A.; Walsh, C. T. *Chem. Rev.* **2006**, *106*, 3468.
4. Watve, M. G.; Tickoo, R.; Jog, M. M.; Bhole, B. D. *Arch. Microbiol.* **2001**, *176*, 386.
5. Wright, L. F.; Hopwood, D. A. *J. Gen. Microbiol.* **1976**, *96*, 289.
6. Chopra, I.; Roberts, M. *Microbiol. Mol. Biol. Rev. MMBR* **2001**, *65*, 232.
7. Kino, T.; Hatanaka, H.; Miyata, S.; Inamura, N.; Nishiyama, M.; Yajima, T.; Goto, T.; Okuhara, M.; Kohsaka, M.; Aoki, H. *J. Antibiot. (Tokyo)* **1987**, *40*, 1256.
8. Arcamone, F.; Cassinelli, G.; Fantini, G.; Grein, A.; Orezzi, P.; Pol, C.; Spalla, C. *Biotechnol. Bioeng.* **1969**, *11*, 1101.
9. Donovick, R.; Gold, W.; Pagano, J. F.; Stout, H. A. *Antibiot. Annu.* **1955**, *3*, 579.
10. McGuire, J. M.; Bunch, R. L.; Anderson, R. C.; Boaz, H. E.; Flynn, E. H.; Powell, H. M.; Smith, J. W. *Antibiot. Chemother. Northfield Ill* **1952**, *2*, 281.
11. Endo, A. *J. Antibiot. (Tokyo)* **1980**, *33*, 334.
12. Roggo, B. E.; Petersen, F.; Delmendo, R.; Jenny, H. B.; Peter, H. H.; Roesel, J. *J. Antibiot. (Tokyo)* **1994**, *47*, 136.
13. Bister, B.; Bischoff, D.; Ströbele, M.; Riedlinger, J.; Reicke, A.; Wolter, F.; Bull, A. T.; Zähner, H.; Fiedler, H.-P.; Süssmuth, R. D. *Angew. Chem., Int. Ed.* **2004**, *43*, 2574.
14. Morimoto, M.; Fukui, M.; Ohkubo, S.; Tamaoki, T.; Tomita, F. *J. Antibiot. (Tokyo)* **1982**, *35*, 1033.
15. Vieweg, L.; Reichau, S.; Schobert, R.; Leadlay, P. F.; Süssmuth, R. D. *Nat. Prod. Rep.* **2014**, *31*, 1554.
16. Shoji, J.; Sakazaki, R.; Hattori, T.; Matsumoto, K.; Uotani, N.; Yoshida, T. *J. Antibiot. (Tokyo)* **1989**, *42*, 1729.
17. Naganuma, S.; Sakai, K.; Hasumi, K.; Endo, A. *J. Antibiot. (Tokyo)* **1992**, *45*, 1216.
18. Hatano, E.; Hashidoko, Y.; Deora, A.; Fukushi, Y.; Tahara, S. *Biosci. Biotechnol. Biochem.* **2007**, *71*, 1601.
19. Kimura, Y.; Kouge, A.; Nakamura, K.; Koshino, H.; Uzawa, J.; Fujioka, S.; Kawano, T. *Biosci. Biotechnol. Biochem.* **1998**, *62*, 1624.
20. Keller-Juslén, C.; King, H. D.; Kuhn, M.; Loosli, H. R.; Pache, W.; Petcher, T. J.; Weber, H. P.; von Wartburg, A. *J. Antibiot. (Tokyo)* **1982**, *35*, 142.
21. Lacoske, M. H.; Theodorakis, E. A. *J. Nat. Prod.* **2015**, *78*, 562.
22. Igarashi, Y.; Ogura, H.; Furihata, K.; Oku, N.; Indananda, C.; Thamchaipenet, A. *J. Nat. Prod.* **2011**, *74*, 670.
23. Igarashi, Y.; Iida, T.; Oku, N.; Watanabe, H.; Furihata, K.; Miyanouchi, K. *J. Antibiot. (Tokyo)* **2012**, *65*, 355.
24. Keller-Schierlein, W.; Muntwyler, R.; Pache, W.; Zähner, H. *Helv. Chim. Acta* **1969**, *52*, 127.
25. Waitz, J. A.; Horan, A. C.; Kalyanpur, M.; Lee, B. K.; Loebenberg, D.; Marquez, J. A.; Miller, G.; Patel, M. G. *J. Antibiot. (Tokyo)* **1981**, *34*, 1101.
26. Tomita, F.; Tamaoki, T.; Shirahata, K.; Kasai, M.; Morimoto, M.; Ohkubo, S.; Mineura, K.; Ishii, S. *J. Antibiot. (Tokyo)* **1980**, *33*, 668.
27. Momose, I.; Iinuma, H.; Kinoshita, N.; Momose, Y.; Kunimoto, S.; Hamada, M.; Takeuchi, T. *J. Antibiot. (Tokyo)* **1999**, *52*, 781.
28. Tsunakawa, M.; Tenmyo, O.; Tomita, K.; Naruse, N.; Kotake, C.; Miyaki, T.; Konishi, M.; Oki, T. *J. Antibiot. (Tokyo)* **1992**, *45*, 180.
29. Park, H.-R.; Furihata, K.; Hayakawa, Y.; Shin-ya, K. *Tetrahedron Lett.* **2002**, *43*, 3941.
30. Ohtsuka, T.; Kudoh, T.; Shimma, N.; Kotaki, H.; Nakayama, N.; Itezono, Y.; Fujisaki, N.; Watanabe, J.; Yokose, K.; Seto, H. *J. Antibiot. (Tokyo)* **1992**, *45*, 140.
31. Wong, H. F.; Brown, G. D. *Phytochemistry* **2002**, *59*, 99.
32. Ozoe, Y.; Akamatsu, M.; Higata, T.; Ikeda, I.; Mochida, K.; Koike, K.; Ohmoto, T.; Nikaido, T. *Bioorg. Med. Chem.* **1998**, *6*, 481.
33. Tsuchida, T.; Iinuma, H.; Nishida, C.; Kinoshita, N.; Sawa, T.; Hamada, M.; Takeuchi, T. *J. Antibiot. (Tokyo)* **1995**, *48*, 1104.
34. Gverzdys, T.; Hart, M. K.; Pimentel-Elardo, S.; Tranmer, G.; Nodwell, J. R. *J. Antibiot. (Tokyo)* **2015**, *68*, 698.
35. Tsuchida, T.; Sawa, R.; Iinuma, H.; Nishida, C.; Kinoshita, N.; Takahashi, Y.; Naganawa, H.; Sawa, T.; Hamada, M.; Takeuchi, T. *J. Antibiot. (Tokyo)* **1994**, *47*, 386.
36. Tsuchida, T.; Iinuma, H.; Sawa, R.; Takahashi, Y.; Nakamura, H.; Nakamura, K. T.; Sawa, T.; Naganawa, H.; Takeuchi, T. *J. Antibiot. (Tokyo)* **1995**, *48*, 1110.
37. Thyssen, A.; Grisez, L.; van Houdt, R.; Ollevier, F. *Int. J. Syst. Bacteriol.* **1998**, *48*, 1145.
38. Janssen, W. A.; Surgalla, M. J. *J. Bacteriol.* **1968**, *96*, 1606.
39. Romalde, J. L. *Int. Microbiol.* **2002**, *5*, 3.
40. Kusuda, R.; Kawai, K. *Fish Pathol.* **1998**, *33*, 221.
41. Kim, E. H.; Aoki, T. *Microbiol. Immunol.* **1993**, *37*, 103.
42. Tsuchida, T.; Iinuma, H.; Nakamura, K. T.; Nakamura, H.; Sawa, T.; Hamada, M.; Takeuchi, T. *J. Antibiot. (Tokyo)* **1995**, *48*, 1330.
43. Paintner, F. F.; Bauschke, G.; Kestel, M. *Tetrahedron Lett.* **2000**, *41*, 9977.
44. Paintner, F. F.; Allmendinger, L.; Bauschke, G.; Polborn, K. *Synlett* **2002**, 1308.
45. Paintner, F. F.; Allmendinger, L.; Bauschke, G.; Berns, C.; Heisig, P. *Bioorg. Med. Chem.* **2003**, *11*, 2823.
46. Paintner, F. F.; Bauschke, G.; Polborn, K. *Tetrahedron Lett.* **2003**, *44*, 2549.
47. Warrington, J. M.; Barriault, L. *Org. Lett.* **2005**, *7*, 4589.
48. He, J.; Tchabanenko, K.; Adlington, R. M.; Cowley, A. R.; Baldwin, J. E. *Eur. J. Org. Chem.* **2006**, *2006*, 4003.
49. Tatsuta, K.; Suzuki, Y.; Furuyama, A.; Ikegami, H. *Tetrahedron Lett.* **2006**, *47*, 3595.
50. Gverzdys, T.; Nodwell, J. R. *J. Bacteriol.* **2016**. JB.00140.
51. Singh, J.; Petter, R. C.; Baillie, T. A.; Whitty, A. *Nat. Rev. Drug Disc.* **2011**, *10*, 307.

52. Jost, C.; Nitsche, C.; Scholz, T.; Roux, L.; Klein, C. D. *J. Med. Chem.* **2014**, *57*, 7590.
53. Davis, B. D. *Microbiol. Rev.* **1987**, *51*, 341.
54. Tao, W.; Zhu, M.; Deng, Z.; Sun, Y. *Sci. China Chem.* **2013**, *56*, 1364.
55. Sun, Y.; Hong, H.; Gillies, F.; Spencer, J. B.; Leadlay, P. F. *Chembiochem. Eur. J. Chem. Biol.* **2008**, *9*, 150.
56. Sun, Y.; Hahn, F.; Demydchuk, Y.; Chettle, J.; Tosin, M.; Osada, H.; Leadlay, P. F. *Nat. Chem. Biol.* **2010**, *6*, 99.
57. Kanchanabanca, C.; Tao, W.; Hong, H.; Liu, Y.; Hahn, F.; Samborskyy, M.; Deng, Z.; Sun, Y.; Leadlay, P. F. *Angew. Chem., Int. Ed.* **2013**, *52*, 5785.
58. He, H.-Y.; Pan, H.-X.; Wu, L.-F.; Zhang, B.-B.; Chai, H.-B.; Liu, W.; Tang, G.-L. *Chem. Biol.* **2012**, *19*, 1313.
59. Wu, L.-F.; He, H.-Y.; Pan, H.-X.; Han, L.; Wang, R.; Tang, G.-L. *Org. Lett.* **2014**, *16*, 1578.
60. Daduang, R.; Kitani, S.; Hashimoto, J.; Thamchaipenet, A.; Igarashi, Y.; Shin-ya, K.; Ikeda, H.; Nihira, T. *Microbiol. Res.* **2015**, *180*, 30.
61. Jia, X.-Y.; Tian, Z.-H.; Shao, L.; Qu, X.-D.; Zhao, Q.-F.; Tang, J.; Tang, G.-L.; Liu, W. *Chem. Biol.* **2006**, *13*, 575.
62. Zhang, H.; White-Phillip, J. A.; Melançon, C. E.; Kwon, H.; Yu, W.; Liu, H. *J. Am. Chem. Soc.* **2007**, *129*, 14670.
63. Fang, J.; Zhang, Y.; Huang, L.; Jia, X.; Zhang, Q.; Zhang, X.; Tang, G.; Liu, W. *J. Bacteriol.* **2008**, *190*, 6014.
64. Zheng, Q.; Tian, Z.; Liu, W. *Curr. Opin. Chem. Biol.* **2016**, *31*, 95.
65. Hashimoto, T.; Hashimoto, J.; Teruya, K.; Hirano, T.; Shin-ya, K.; Ikeda, H.; Liu, H.; Nishiyama, M.; Kuzuyama, T. *J. Am. Chem. Soc.* **2015**, *137*, 572.
66. Klas, K.; Tsukamoto, S.; Sherman, D. H.; Williams, R. M. *J. Org. Chem.* **2015**, *80*, 11672.
67. Sato, M.; Yagishita, F.; Mino, T.; Uchiyama, N.; Patel, A.; Chooi, Y.-H.; Goda, Y.; Xu, W.; Noguchi, H.; Yamamoto, T.; Hotta, K.; Houk, K. N.; Tang, Y.; Watanabe, K. *Chembiochem. Eur. J. Chem. Biol.* **2015**, *16*, 2294.
68. Kato, N.; Nogawa, T.; Hirota, H.; Jang, J.-H.; Takahashi, S.; Ahn, J. S.; Osada, H. *Biochem. Biophys. Res. Commun.* **2015**, *460*, 210.
69. Zheng, Q.; Guo, Y.; Yang, L.; Zhao, Z.; Wu, Z.; Zhang, H.; Liu, J.; Cheng, X.; Wu, J.; Yang, H.; Jiang, H.; Pan, L.; Liu, W. *Chem. Biol.* **2016**, *23*, 352.
70. Tian, Z.; Sun, P.; Yan, Y.; Wu, Z.; Zheng, Q.; Zhou, S.; Zhang, H.; Yu, F.; Jia, X.; Chen, D.; Mándi, A.; Kurtán, T.; Liu, W. *Nat. Chem. Biol.* **2015**, *11*, 259.
71. Gottardi, E. M.; Krawczyk, J. M.; von Suchodoletz, H.; Schadt, S.; Mühlenweg, A.; Uguru, G. C.; Pelzer, S.; Fiedler, H.-P.; Bibb, M. J.; Stach, J. E. M.; Süssmuth, R. D. *Chembiochem. Eur. J. Chem. Biol.* **2011**, *12*, 1401.
72. Nakano, S.; Sakane, W.; Oinaka, H.; Fujimoto, Y. *Bioorg. Med. Chem.* **2006**, *14*, 6404.
73. Sekiyama, Y.; Araya, H.; Hasumi, K.; Endo, A.; Fujimoto, Y. *Nat. Prod. Lett.* **1997**, *11*, 61.