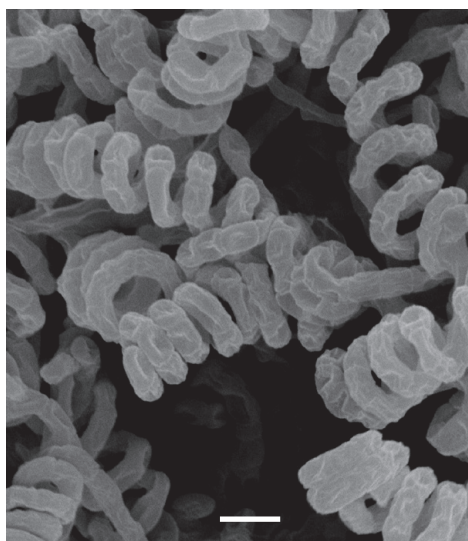


Avermectin[©]

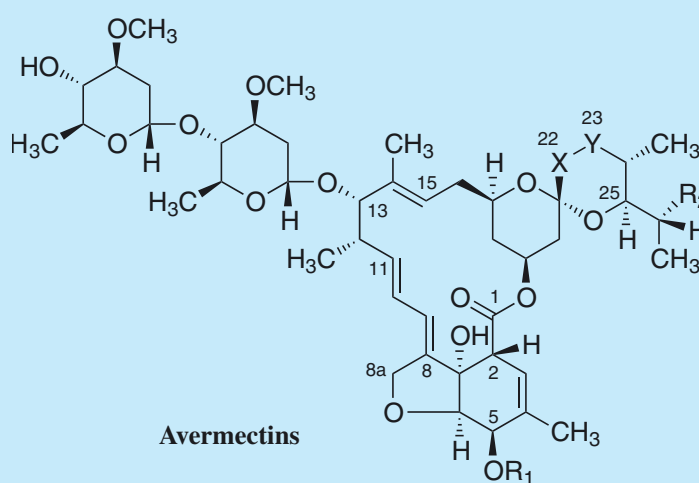
1. Discovery, producing organism and structures^{1,2)}

The discovery of avermectins was the result of a collaboration with Merck Sharp & Dohm Research Laboratories. Natural products were screened for anthelmintic activity using a direct *in vivo* test in mice infected with the nematode, *Nematospiroides dubus*.

Avermectins were isolated from the culture broth of the actinomycete strain MA-4680^T. Avermectins are a family of four closely related major components, A1a, A2a, B1a and B2a, and four minor components, A1b, A2b, B1b and B2b, which are lower homologs of the corresponding major components. The first total synthesis of avermectin A1a was achieved by Danishefsky *et al*³⁾. Since then, the total synthesis of avermectins has been reported by many groups (See Appendix-I).



Streptomyces avermectinius
(formerly *S. avermitilis*) MA-4680^T
Bar: 2 μ m



| | R ₁ | R ₂ | X-Y |
|-----------------------|-----------------|-------------------------------|------------------------------------|
| Avermectin A1a | CH ₃ | C ₂ H ₅ | CH=CH |
| A1b | CH ₃ | CH ₃ | CH=CH |
| A2a | CH ₃ | C ₂ H ₅ | CH ₂ -CH(α -OH) |
| A2b | CH ₃ | CH ₃ | CH ₂ -CH(α -OH) |
| B1a | H | C ₂ H ₅ | CH=CH |
| B1b | H | CH ₃ | CH=CH |
| B2a | H | C ₂ H ₅ | CH ₂ -CH(α -OH) |
| B2b | H | CH ₃ | CH ₂ -CH(α -OH) |

2. Physical data (Avermectin B1a)

White powder. C₄₈H₇₂O₁₄; mol wt 873.08. Sol. in CHCl₃, acetone, MeOH. Insol. in H₂O.

3. Biological activity^{1,4)}

Avermectins have potent anthelmintic and insecticidal activities. In particular, the hydrogenated product of avermectin B1, 22,23-dihydroavermectin B1 (ivermectin), is used as an important anthelmintic in veterinary medicine as well as for the control of onchocerciasis, lymphatic filariasis, strongyloidiasis, mites and scabies in humans. The hydroxyl group at the C5 position and the disaccharide moiety are essential for the potency of avermectins.

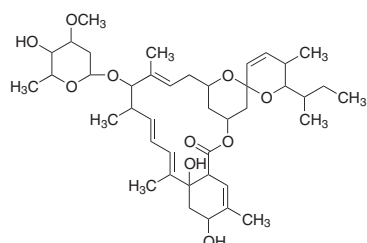
Numbers of nematodes and anthelmintic efficacy in lambs treated with dihydroavermectin B1a*

| Parasite | Group 1 (control) | | Group 2 (25 μ g/ml) | | | Group 3 (50 μ g/ml) | | |
|----------------------------|-------------------|-------|-------------------------|------|--------------|-------------------------|------|--------------|
| | Range (Min-max) | Mean | Range (Min-max) | Mean | Efficacy (%) | Range (Min-max) | Mean | Efficacy (%) |
| <i>H. contortus</i> | 1,550–11,605 | 7,216 | 0–45 | 10 | > 99 | 0 | 0 | > 99 |
| <i>T. axei</i> | 0–110 | 41 | 0–5 | 2 | > 99 | 0 | 0 | > 99 |
| <i>O. ciecumcincta</i> | 135–1,340 | 699 | 0–30 | 7 | > 99 | 0 | 0 | > 99 |
| <i>T. colubriformis</i> | 3,555–6,130 | 4,843 | 0–5 | 1 | > 99 | 0 | 0 | > 99 |
| <i>Cooperia spp</i> | 250–1,685 | 714 | 0–10 | 2 | > 99 | 0 | 0 | > 99 |
| Immature (abomasum) | 420–7,590 | 2,463 | 0 | 0 | > 99 | 0 | 0 | > 99 |
| Immature (small intestine) | 160–945 | 470 | 0 | 0 | > 99 | 0 | 0 | > 99 |

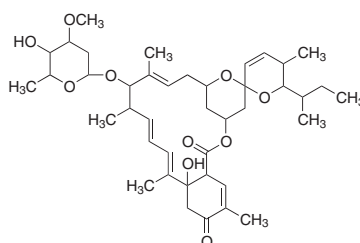
*Modified from Ref. 4

4. New metabolites related to avermectin⁵⁻⁷⁾

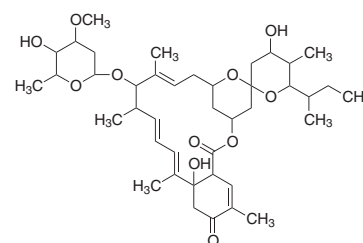
The following novel avermectin metabolites were obtained from blocked mutants of the producer.



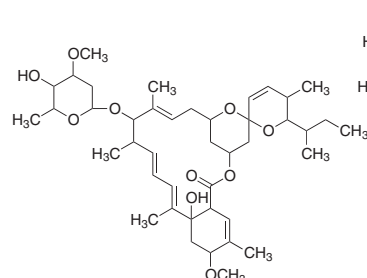
4'-Deoleandrosyl-6,8a-seco-6,8a-deoxy AVM B1a



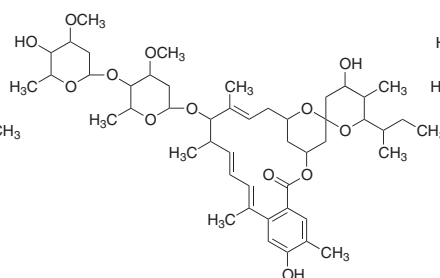
4'-Deoleandrosyl-6,8a-seco-6,8a-deoxy-5-oxo AVM B1a



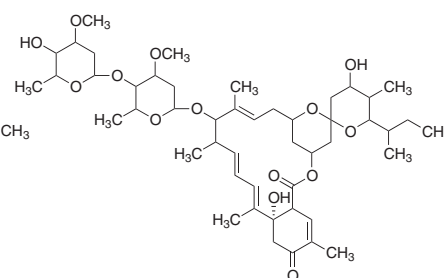
4'-Deoleandrosyl-6,8a-seco-6,8a-deoxy-5-oxo AVM B2a



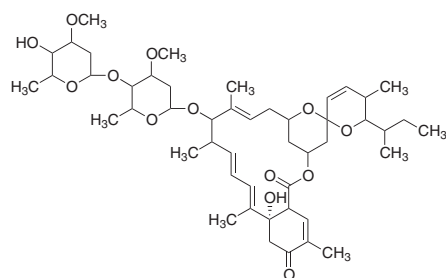
4'-Deoleandrosyl-6,8a-seco-6,8a-deoxy AVM A1a



6,8a-seco-6,8a-deoxy-2,5-anhydro AVM B2a



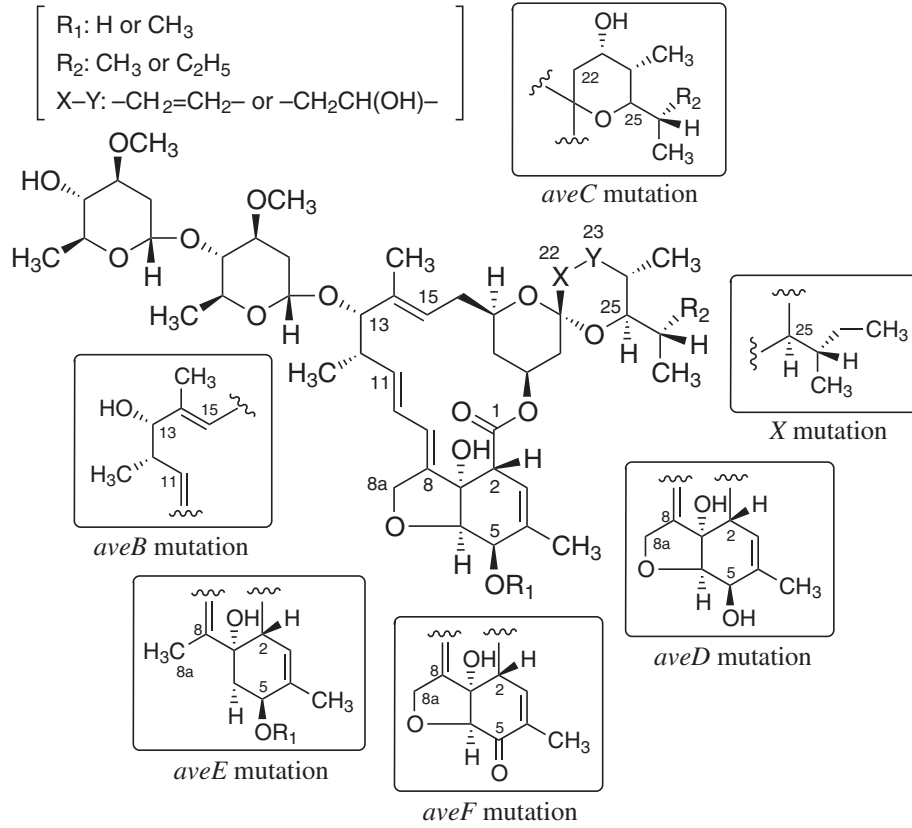
5-Oxo AVM B2a



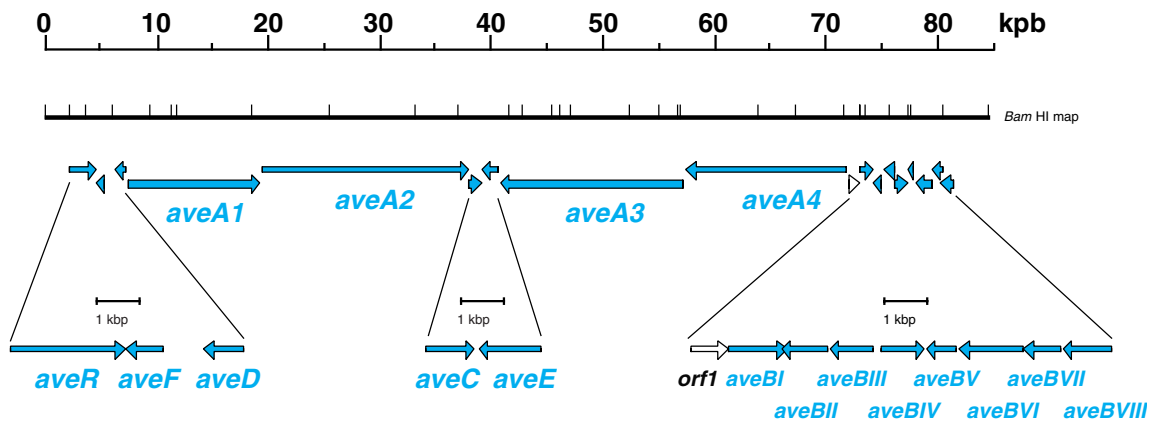
5-Oxo AVM B1a

5. Biosynthesis^{8-21,26-31)}

Eight kinds of genes involving avermectin biosynthesis, *aveA1*, *aveA2*, *aveA3*, *aveA4* (polyketide synthase), *aveB* (glycosylation), *aveC* (C-22,23 dehydration?), *aveD* (C-5 O-methylation), *aveE* (C-6, 8a furan ring formation), *aveF* (C-5 keto reduction), and *aveR*(regulation), were characterized and mapped on the chromosome as shown below.

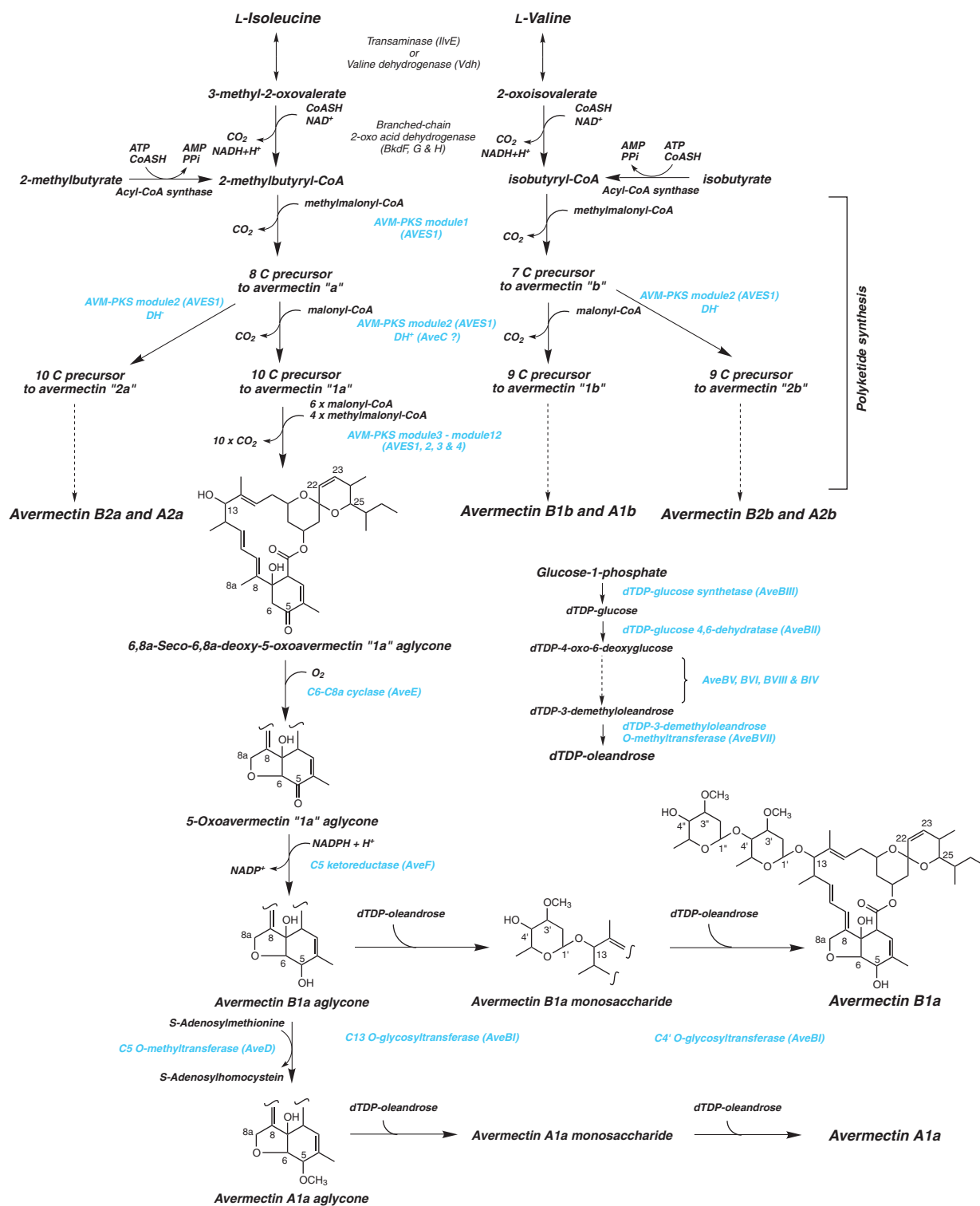


Characteristics of Mutants



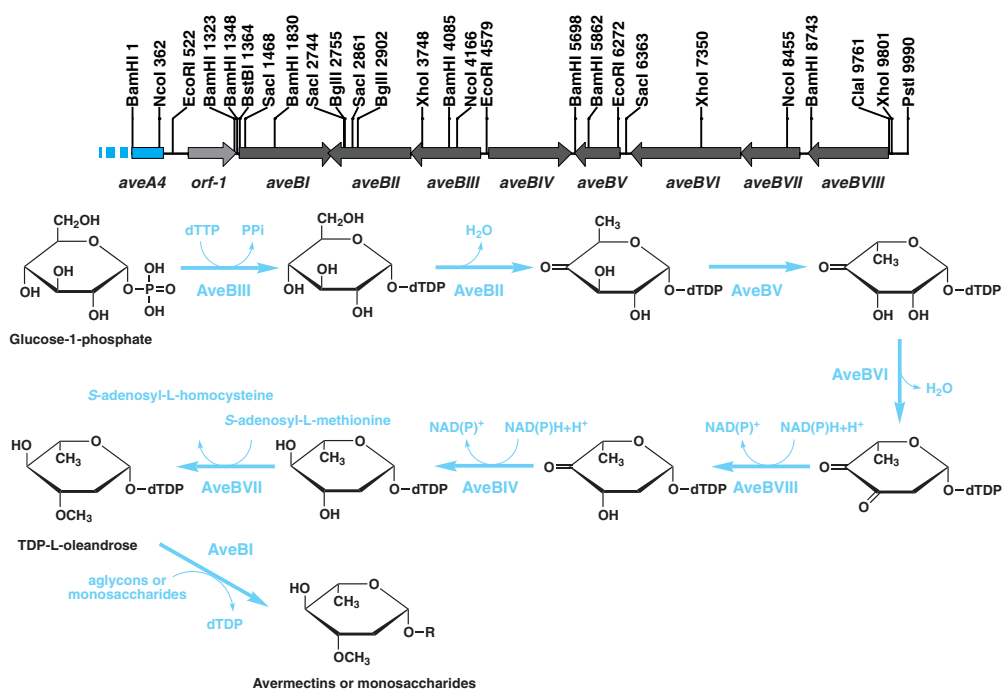
Organization of the gene cluster for avermectin biosynthesis.

The direction of transcription and relative sizes of the ORFs deduced from analysis of the nucleotide sequence are indicated.



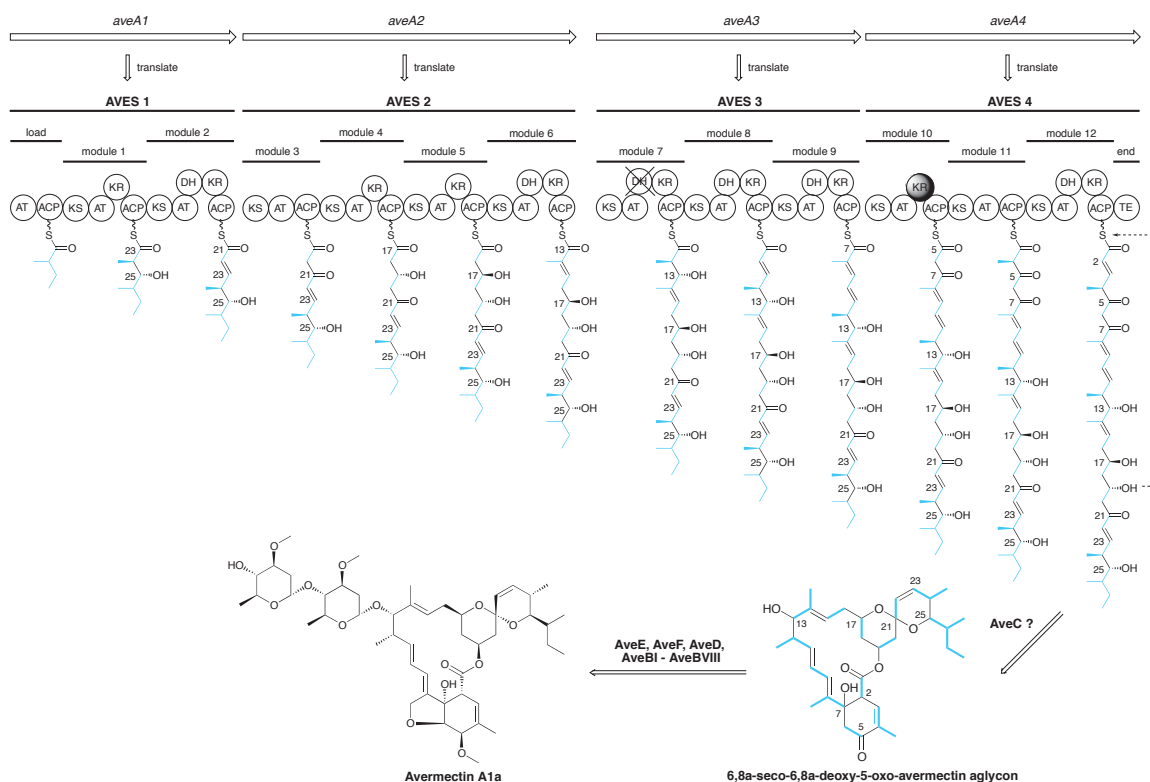
Proposed biosynthetic pathway of avermectins in *S. avermitilis*.

The 12 modules of avermectin PKS (AVES1-4) act sequentially to add 12 acyl units (seven acetate and five propionate units). The initial stage of avermectin biosynthesis, designated as "initial aglycon", is considered to be up until the formation of 6,8a-seco-6,8a-deoxy-5-oxoavermectin aglycon. The middle stage of biosynthesis is a post-polyketide modification from AveE to AveD and the last stage is glycosylation.



Scheme for the biosynthesis of L-oleandrose.

The putative gene products believed to be associated with various steps are indicated at respective points in the pathway.



Model for 6,8a-seco-6,8a-deoxy-5-oxoavermectin aglycon formation and predicted domain structure of the avermectin PKS.

Each circle represents an enzymatic domain in the PKS multifunctional polypeptide. Abbreviations: AT, acyltransferase; DH, dehydratase; KR, β -ketoacyl-ACP reductase; KS, β -ketoacyl-ACP synthase; TE, thioesterase. The reaction order from module 7 to 9, and from 10 to 12 in *aveA3* and *aveA4*, respectively, is drawn in the direction opposite to the gene order on the genome. The crossed-out domain in module 7 is nonfunctional. The shaded domain in module 10 does not function in polyketide-chain elongation.

6. Mode of action^{22-24,32-38)}

The molecular target of avermectins is a glutamate-gated chloride channel²²⁻²⁴⁾. Cully *et al.* reported in nematodes and in insects to be a target of the antiparasitic agent avermectins and revealed the molecular basis underlying it³⁶⁾.

Recently, Althoff *et al.*, Ghosh *et al.*, and Hibbs and Gouaux reported an understanding mechanism of avermectins-binding site and atomic interaction of the target molecule by X-ray crystallography analysis³²⁻³⁴⁾.

7. Avermectin and Ivermectin are commercially available as anthelmintic agents for both human and animals as well as being widely used for agriculture purposes. Ivermectin has become an invaluable tool for two global elimination initiatives, against human onchocerciasis and lymphatic filariasis^{25,39-44)}.

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