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Retrosynthetic Analysis of Penidaleodiolide A with Relevance to Epilepsy Therapy

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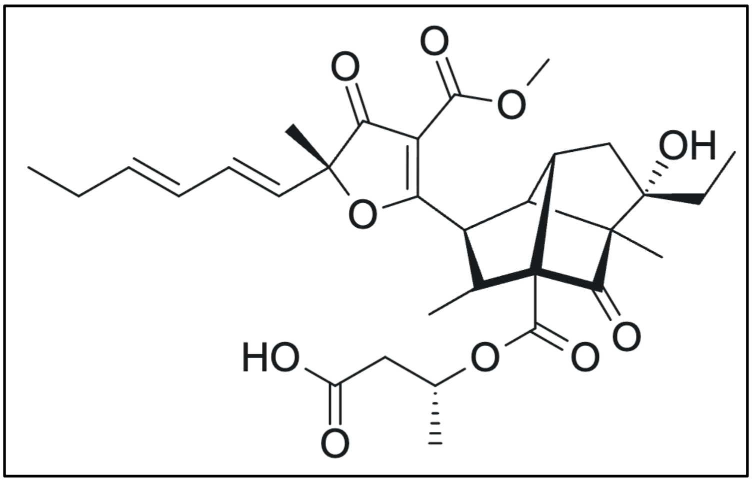
Abstract

Penidaleodiolide A, a recently discovered cage-like polyketide isolated from Penicillium daleae L3SO in association with Monotropa uniflora, has demonstrated selective and reversible inhibition of hippocampal basket neuron excitability without cytotoxicity. This unique activity highlights its promise as a novel therapeutic scaffold for drug-resistant epilepsy and other hyperexcitability-related neurological disorders. However, reliance on natural extraction from scarce fungal sources poses limitations in yield and accessibility. To address this, our study presents a retrosynthetic pathway for the laboratory synthesis of penidaleodiolide A. Through oxidation-level analysis, strategic 1,3- and 1,5-disconnections, and retro-aldol and Claisen-type fragmentations, we deconstructed the complex tricyclic scaffold into simpler and commercially available precursors, including glycolic acid, propanal, and β-hydroxybutyric acid. This systematic approach underscores the feasibility of reconstructing the molecule synthetically, offering flexibility and scalability compared to biosynthetic routes. By bridging natural product chemistry with retrosynthetic design, this work lays a foundation for future synthetic efforts toward penidaleodiolide A and supports its further pharmacological exploration as a safer, more effective treatment for neurological disorders.

1. Introduction & Literature Review



Monotropa uniflora, a holoparasitic herbaceous perennial flower that belongs to the *Ericaceae* family, is a mycoheterotroph that mainly dwells in the temperate regions of Asia, Northern America, and Northern South America. Commonly called the "ghost plant" for its pale white appearance, *M. uniflora* lacks chlorophyll and cannot photosynthesize, instead relying on a symbiotic fungal network in the rhizospheric soil for nutrients [Richard W. Lutz, 1973, S Yang, 2006]. *M. uniflora* has a longstanding ethnobotanical history of medicinal use among indigenous people, all the way to herbists in India and China. The plant is known to exhibit several pharmacological effects, including pain relief, alleviation of convulsions, epilepsy, and nervous inability. Emerging research suggests it may also ease anxiety and insomnia [Riju Aikkal, 2025].

Fungi have been pioneers in medicine discovery for the last century, yielding antibiotics, bioactive antioxidants, and other pharmaceutical agents. Filamentous fungi, in particular, have produced compounds with antimicrobial and anticancer properties. Cortinellin mycelial extract from different strains of Lentinula edodes showed anti-microbial activity against *B. cereus*, *E. coli*, *L. monocytogenes*, *S. typhimurium*, and *Staphylococcus*. Chitin has been shown to stimulate the production of anti- and pro-inflammatory cytokines [Girma Waktola Gemechu, 2024].

Among fungal metabolites, polyketides are especially significant. Polyketides, characterized by a chain or repeating ketone and methyl groups, are metabolites biosynthetically derived from a precursor molecule and are produced by polyketide synthases (PKs). These natural compounds, isolated from natural sources, PK, possess several biological and pharmacological impacts, such as cytotoxic, antibacterial, antifungal, antiparasitic, and immunosuppressive effects. Consequently, many polyketide-derived compounds are going through clinical trials [Weissman and Leadlay 2005; Hussain et al. 2017]. A study conducted by Du et al. showcased aspergillolide B, an aromatic polyketide that exhibited cytotoxicity towards leukemia. Such findings highlight the importance of further research on polyketides in drug discovery. [Du et al., 2018]

1.1 Analysis of Penidaleodiolide A

In 2024, Wang et al. isolated *Penicillium daleae* L3SO from the rhizospheric soil of *M. uniflora*. Rice fermentation of this fungus has led to the discovery of the two novel cage-like polyketides, penidaleodiolide A and B [Wang et al., 2024]. While the initial study suggests potential effects on neuronal transmission - specifically in mural hippocampal basket neurons - further study of penidaleodiolide A is still lacking.

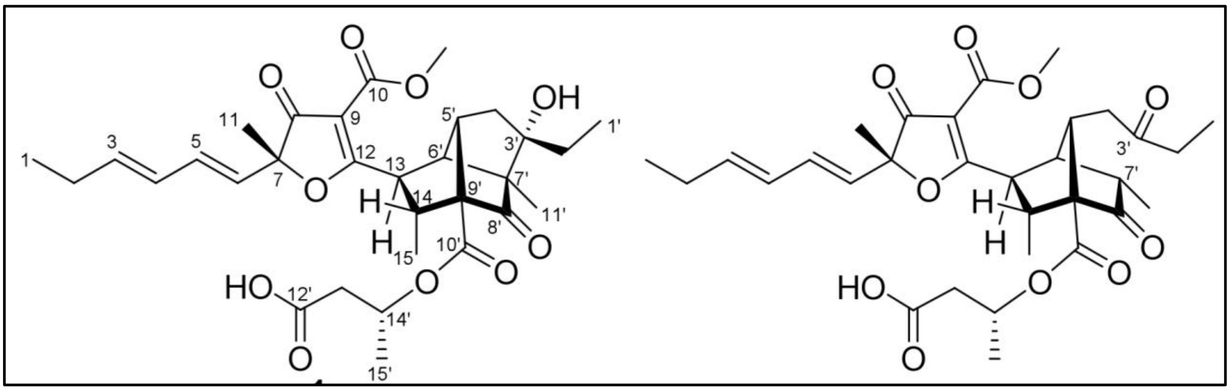


Fig. 1 . Chemical structures of penidaleodiolide A (left) and B (right), as identified by Wang et al. (2024). The shown carbon numbering is from the original source and is not used in this study.

Epilepsy is a chronic neurological disorder characterized by recurrent, unprovoked seizures, caused by abnormal electrical activity in the brain. Affecting approximately 50 million individuals globally, it is the second most prevalent neurological condition. Seizures not only cause direct damage to some regions of the brain but also, in the long term, increase risks of cognitive decline, memory loss, depression, and anxiety. Despite substantial research in anticonvulsant treatments, many patients remain resistant to available medications, underscoring the need for novel and targeted therapies. Recent research has highlighted the central role of hippocampal inhibitory circuits, especially basket neurons, in the pathophysiology of epilepsy [Sloviter, 1987]. Basket neurons are crucial in maintaining the balance of excitation and inhibition within hippocampal networks; abnormal activity of these interneurons can contribute to the generation and propagation of seizures.

Penidaleodiolide A has demonstrated a significant inhibitory effect on the action potentials of murine hippocampal basket neurons, resulting in a dose-dependent decrease in the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) [Wang et al., 2024]. Although the precise role of hippocampal alterations in epilepsy remains to be fully elucidated, the hippocampus - often described as the brain region with the “least seizure threshold” [Chauhan et al., 2022] - is clearly a critical area for further investigation. Targeted modulation of hippocampal basket neurons by penidaleodiolide A may modulate pathological network synchrony underlying epileptic seizures, thereby representing a promising therapeutic strategy for epilepsy management, especially in drug-resistant cases [American Epilepsy Society, 2006]. Moreover, the compound’s lack of cytotoxicity against nerve cells suggests potential as a safer alternative for existing antiepileptic drugs, which often cause significant side effects.

However, natural extraction of penidaleodiolide A from *M. uniflora* is not ideal due to its onerous methodology and the scarcity of the plant in the wild. This research builds on Wang et al.’s study by proposing a retrosynthetic pathway for laboratory synthesis of penidaleodiolide A, with attention to its relevance in epilepsy treatment.

Through rice fermentation of *P. daleae* L3SO, Penidaleodiolide A (1) was isolated as a yellow oil with the molecular formula $C_{31}H_{40}O_{10}$ and molecular mass of 572.65, determined by high-resolution mass spectrometry, corresponding to 12 degrees of unsaturation. ^{1}H and ^{13}C NMR spectroscopy revealed seven methyl groups, four methylenes, nine methines, and eleven quaternary carbons, including multiple olefinic and carbonyl resonances. Key ^{1}H–^{1}H COSY and HMBC correlations from H_3-1 → H_2-2 → H-3 → H-4 → H-5 → H-6 established an ethyl-substituted 1,3-butadiene motif. In contrast, correlations from H_3-11 and methoxy protons to C-10, along with the chemical shifts of C-9 and C-12, indicated a gregatin A-like alkylated furanone core.

The proposed biosynthesis for the compound relies on the fungus’s polyketide synthases, surrounding enzymes, and the cellular environment; control over the reaction sequence and yields is heavily limited. Thus, retrosynthesis is imperative, as it provides control, flexibility, and scalability, while still being able to mimic the key transformations.

2. Results

2.1 Oxidation Level Analysis

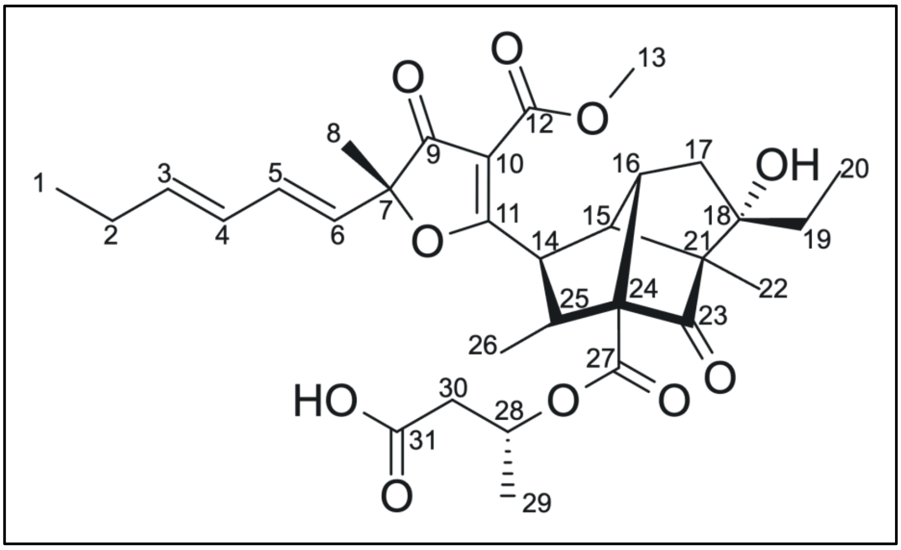


Fig. 2 . Chemical structure of penidaleodiolide A with carbon atoms renumbered.

For clarity, the carbon atoms were renumbered for this study (Fig. 2).

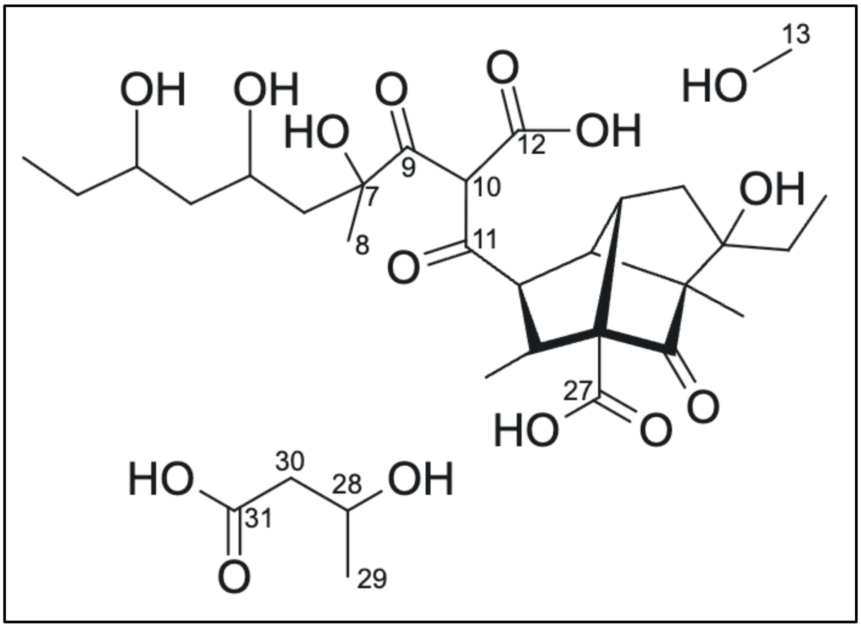


Fig. 3 . Illustration of the separation of CH_3OH and $C_4H_8O_3$ from penidaleodiolide A

Oxidation level analysis was performed on Penidaleodiolide A. As shown in Fig. 3, two small organic molecules were isolated: CH_3OH (methanol) with C13, and $C_4H_8O_3$ (β-hydroxybutyric acid) with C28, C29, C30, and C31, each as a consequence of the oxidation of C12 and C27, respectively, into acid. Both methanol and β-hydroxybutyric acid were found to be commercially available. Although represented as one possible outcome in Fig. 3, the C=C double bond between C10 and C11 (as in before the oxidation level analysis) presents two possible scenarios for the oxidation level analysis, illustrated below (Fig. 4).

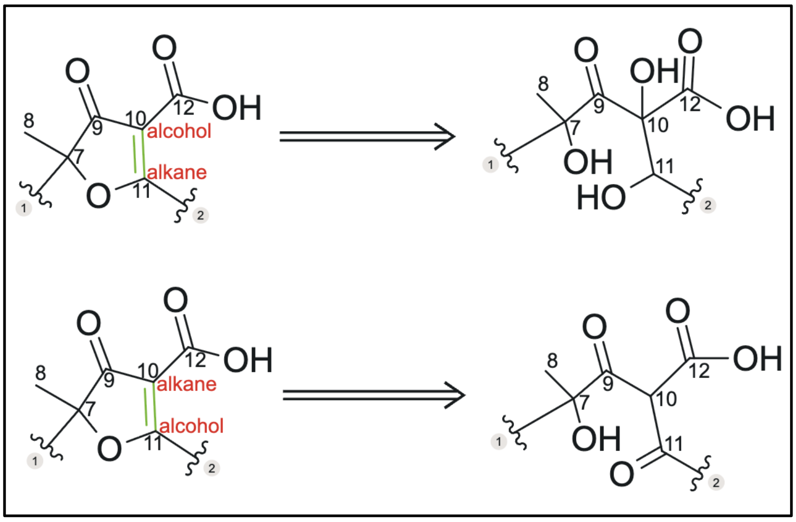


Fig. 4 . Two possible oxidation outcomes of the C10=C11 double bond. The two outcomes lead to two possible methods: the first pathway utilizes method 1, and the second utilizes method 2.

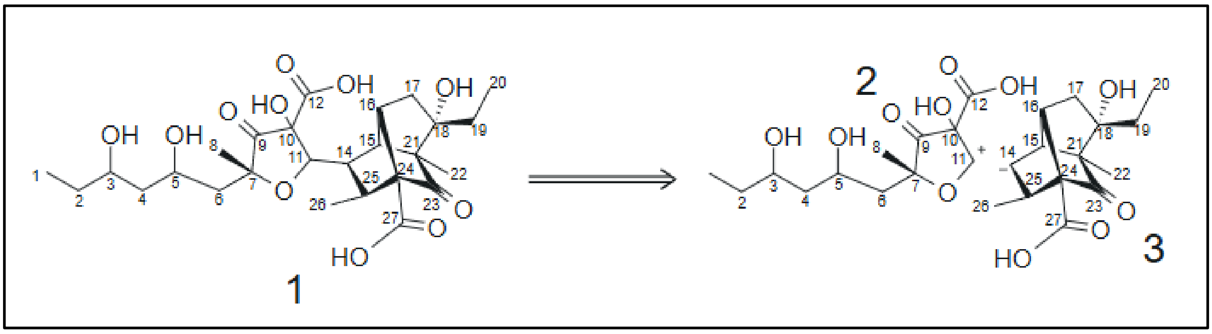


Fig. 5 . Unstable disconnection of the CC bond between C11 and C14

For Figure 5, breaking the C11–C14 bond isolates an anion on carbon 14; not being attached to any electronegative atom makes the molecule unstable.

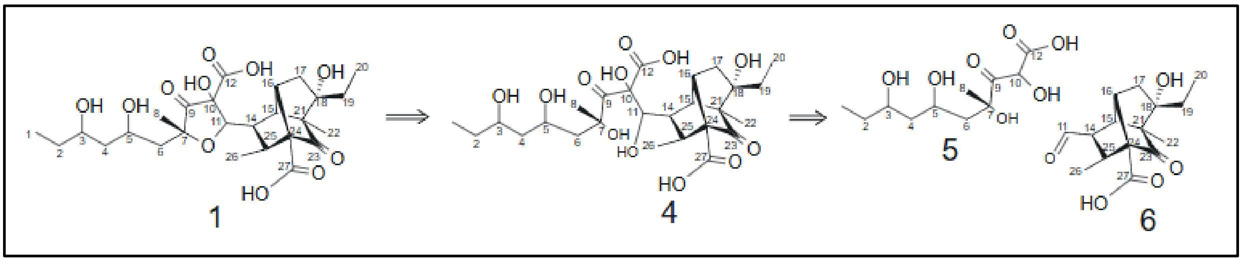


Fig. 6 . Division of the molecule into two for easier retrosynthesis

Alternatively, assigning C10 the alcohol oxidation level also leaves C11 as an alcohol~~,~~separating the oxygen into two alcohol groups. The lone pair of the oxygen from C11–OH attacks C10–C11, isolating the **tricyclic** ring (C14~C31) from the remaining chain(C1~C13). Therefore, breaking this bond is preferable because it allows for the generation of two stabilised alcohol groups and effectively disconnects the complex tricyclic core of the molecule, resulting in the electronic stabilisation and simplification of the overall molecular structure for further retrosynthetic analysis.

For the lower pathway, by proposing a C=O bond at C11, the number of 1,3-relationships, which was previously one, increases to three. Each oxygen atom at positions C9, C11, and C12 is in a 1,3-relationship with the other two, creating three distinct 1,3-relationships. This implies that there are three potential 1,3-disconnections for separating the tricyclononane part from the rest of the molecule.

2.2 Retrosynthesis of Chain

2.2.1 Method 1

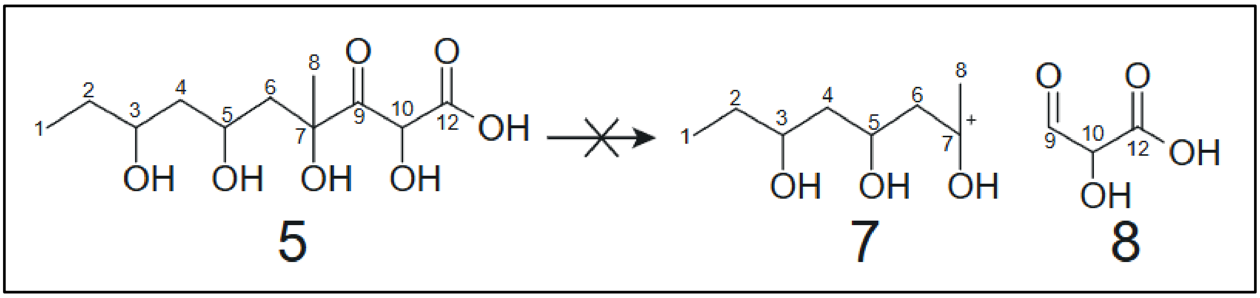


Fig. 7 . Unfavorable option of chain disconnection

By being attached to an alcohol group, both C7 and C9 have partial positive charges. Therefore, disconnecting the bond between C7 and C9, as in Fig. 7, would be unfavorable because the two carbons have a similar electronegativity.

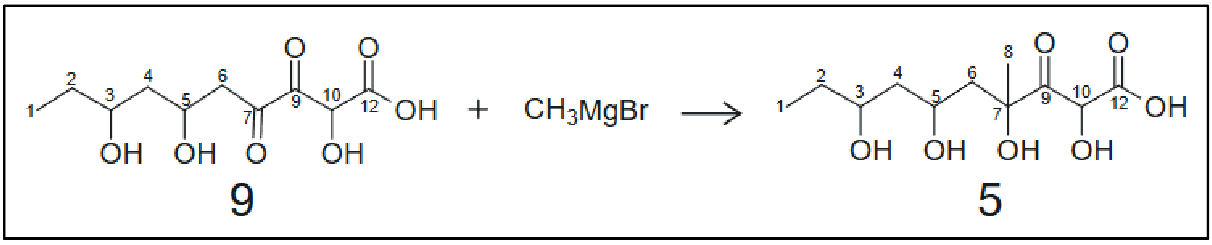


Fig. 8 . Methyl Grignard reaction pathway for the first chain method

Instead, when going from molecule 9 to 5 in Fig. 8, the methyl Grignard reaction can be used to remove a methyl group and turn the alcohol group attached to C7 into a ketone. The methyl group C8 detaches. Figures 9 and 10 present numerous opportunities for 1,3 disconnections, indicating a more reactive behavior, and will therefore be used for the remainder of the reaction.

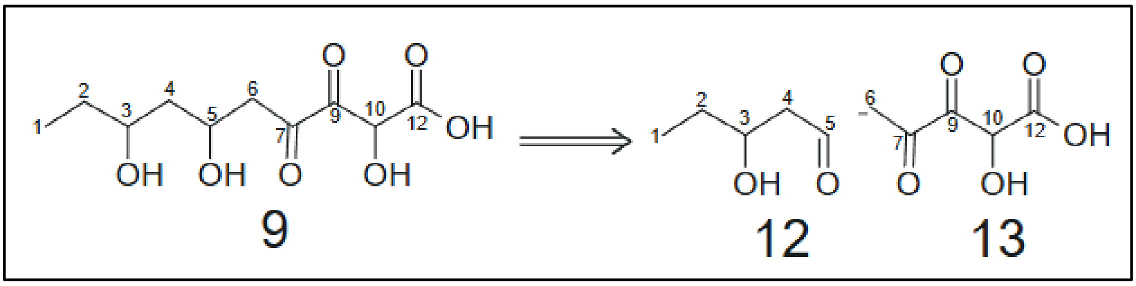


Fig. 9 . Alternative option for the disconnection of the first chain method

Using an electron from the alcohol group attached to C5, I broke the bond between C5 and C6, separating the molecule into two. By disconnecting the bond, C6 gets a negative charge, and because it is alpha to the carbonyl, it is more stable.

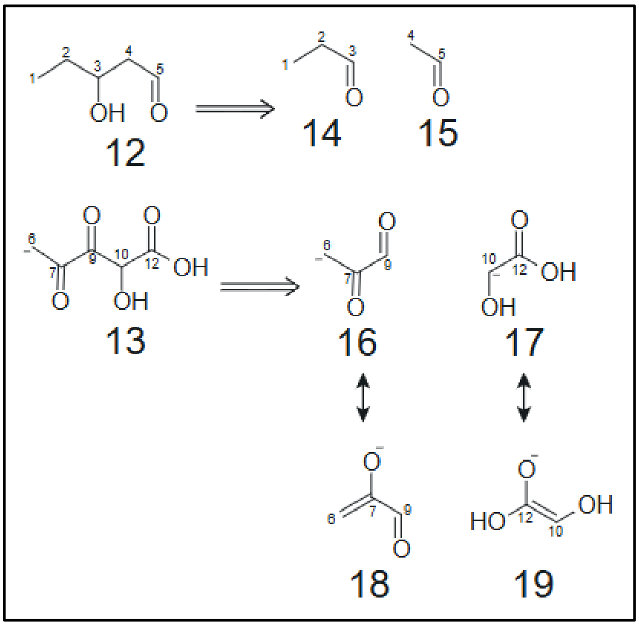


Fig. 10 . Further retrosynthesis of the chain using method 1

In the same way, we disconnected the C3-C4 bond. Similarly, the bond between C9 and C10 disconnects. The resulting smaller molecules 16 and 17 would be better represented by their resonance structures (18 and 19, respectively), as enolates tend to be more stable than structures with a lone anion on a carbon, such as molecules 16 and 17.

2.2.2 Method 2

A 1,3-disconnection between carbons C10 and C11 was employed to achieve the retrosynthetic simplification of the target molecule, following the strategy outlined in Figure 4. This strategic bond cleavage generates two primary fragments: a linear chain and a tetracyclic core, which are depicted in Figure 11.

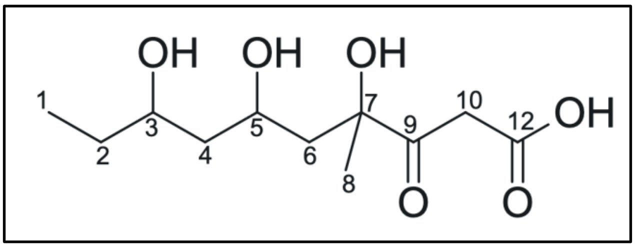


Fig. 11 . Resulting chain structure from pathway two (Fig. 4)

Disconnection strategies

1. C6–C7 disconnection

This bond disconnection is a retro-aldol reaction. The cleavage between C6 and C7 is rationalised by recognising that the resultant fragments can be united via an aldol condensation in the forward (synthetic) pathway. Mechanistically, the electron-rich enolate (arising from deprotonation α to a carbonyl group) functions as the nucleophile, attacking an electrophilic carbonyl carbon to forge the C–C bond. Electrons from the C–C σ bond move towards the oxygen, forming a carbonyl where there was a hydroxyl group. Hence, this step would result in an enolate synthon and a carbonyl synthon.

2. C9–C10 disconnection (1,3-disconnection)

The disconnection splits the molecule into an ester or acid derivative and a carbonyl-containing fragment. The enolate generated from one fragment can react with the electrophilic carbonyl carbon of an ester or a thioester. In the forward direction would be a Claisen condensation. Disconnection at this site is favorable due to the 1,3 relationship between C9 and C12, putting C10 at the alpha position, and it simplifies the molecule by yielding two smaller carbonyl fragments.

2.3 Retrosynthesis of Ring

Although we had two different methods for separating the chain from the ring (Fig. 4), both methods resulted in the same ring structure, so we decided to use the same retrosynthetic analysis for the ring structure.

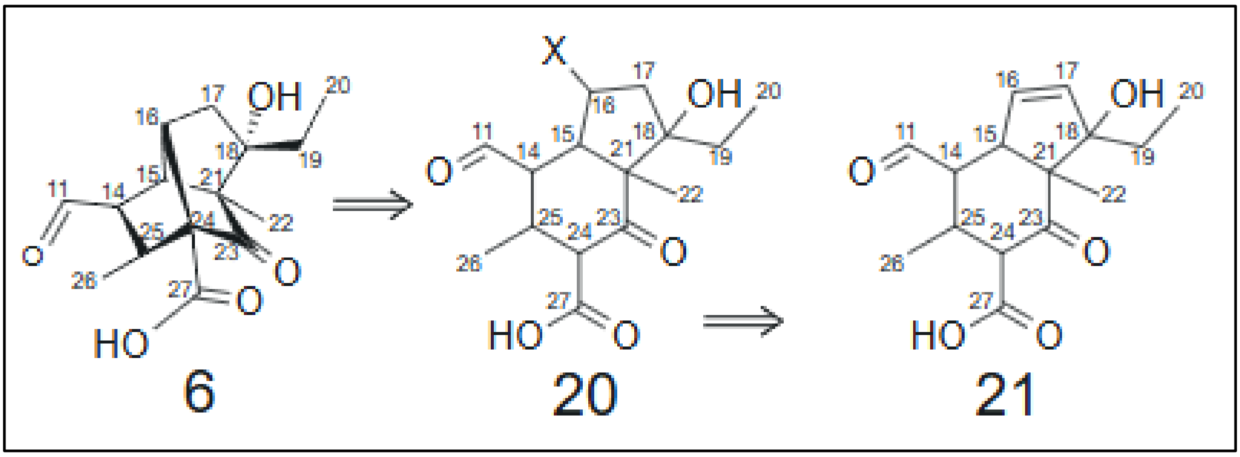


Fig. 12 . Disconnection of C16 and C24, in tricyclononane ring

Breaking the bond between common carbons C16 and C24 separates the molecule into one pentane and one hexane ring. There, because the positive charge is assigned to C16, a halide, which is the synthetic equivalence of a carbon with a plus charge, replaces the positive charge, which is then replaced by an olefin, as halides tend to be reactive.

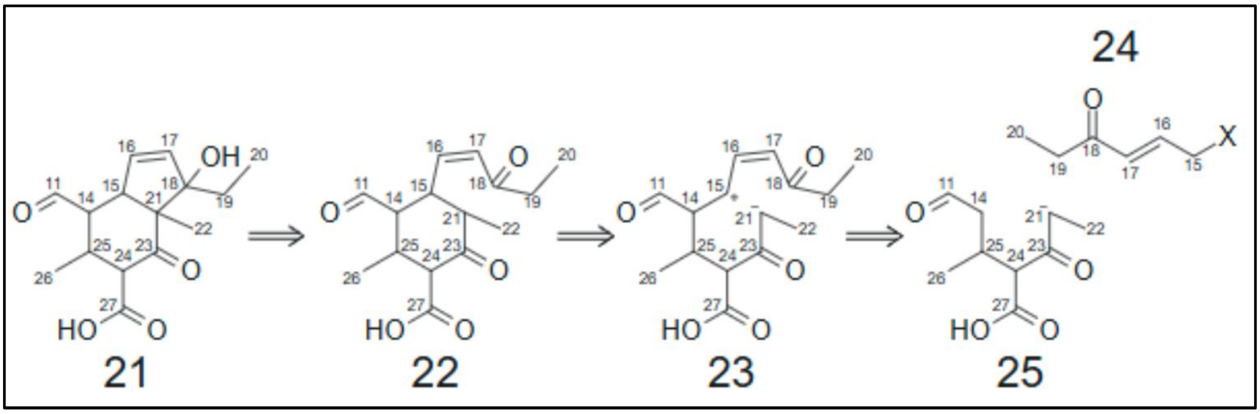


Fig. 13 . Disconnection of multiple bonds from the bicyclic structure to make two separate chains

Using the electrons from the alcohol group attached to C18, the bond between C21 and C18 breaks, the alcohol group on C18 turning into a ketone. Upon 1,5-disconnection, the bond between C15 and C21 breaks, leaving a positive charge on C15 and a negative charge on C21, which is easily replaced by a hydrogen due to its alpha position on the carbonyl. The positive charge is replaced with a halide, and upon doing so, we are left with two molecules, molecules 24 and 25.

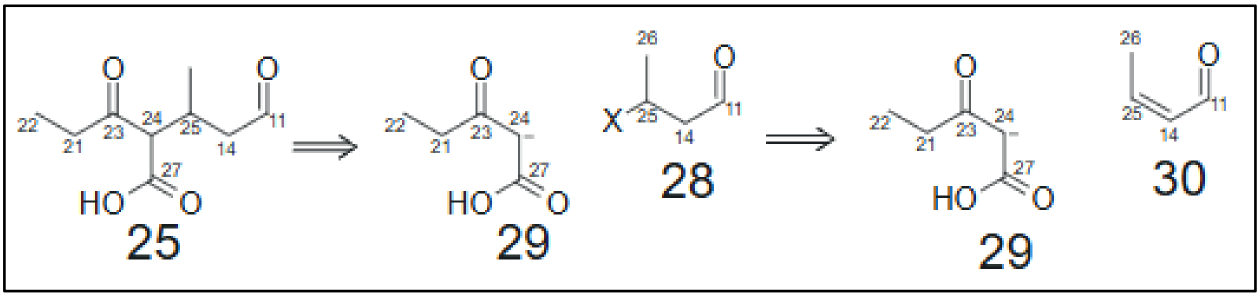


Fig. 14 . Further retrosynthesis of the ring part of Penidaleodiolide A

As shown in Fig. 14, through 1,3-disconnection on molecule 25, the bond connecting C24 and C25 is broken, leaving an isolated positive charge on C25, which turns into an olefin, and a negative charge on C24, which is paired with a hydrogen.

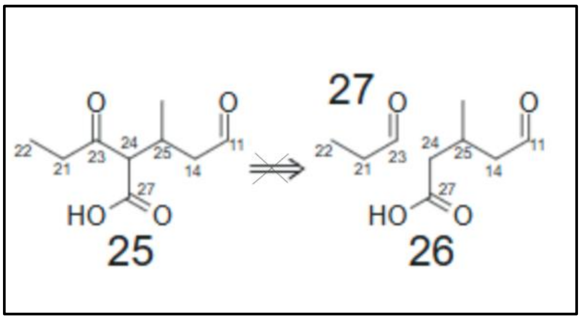


Fig. 15 . Less favorable retrosthetic pathway, resulting in two molecules with a significant difference in size

Breaking bonds along other C–C connections, such as the bond between C23 and C24 (Fig. 15), proves to be less favorable because it results in two molecules that differ significantly in size.

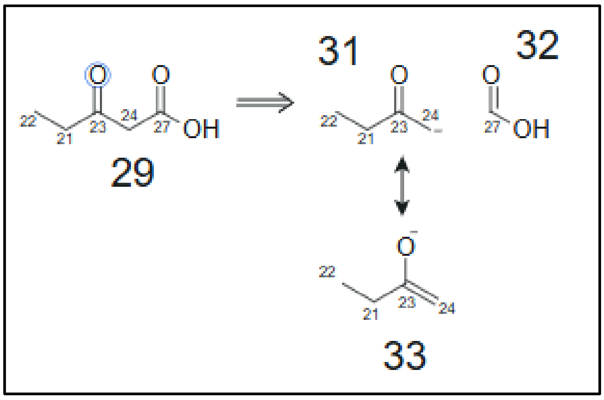


Fig. 16 . Disconnection of C24–C27 bond to isolate enolate

With the help of the electrons from the alcohol group attached to C27, upon disconnecting the carbon-carbon bond between C24 and C27 of molecule 29 (Fig. 16), the molecule separates into two, in which molecule 31 has a resonance structure that proves to be more stable due to the formation of an enolate. If we use 1,3-disconnection and assign charges to C23 and C24, the resulting molecule with the positive charge would be reactive and thus unstable due to the halide group. Therefore, disconnection using the lone pair is a more efficient method of disconnecting.

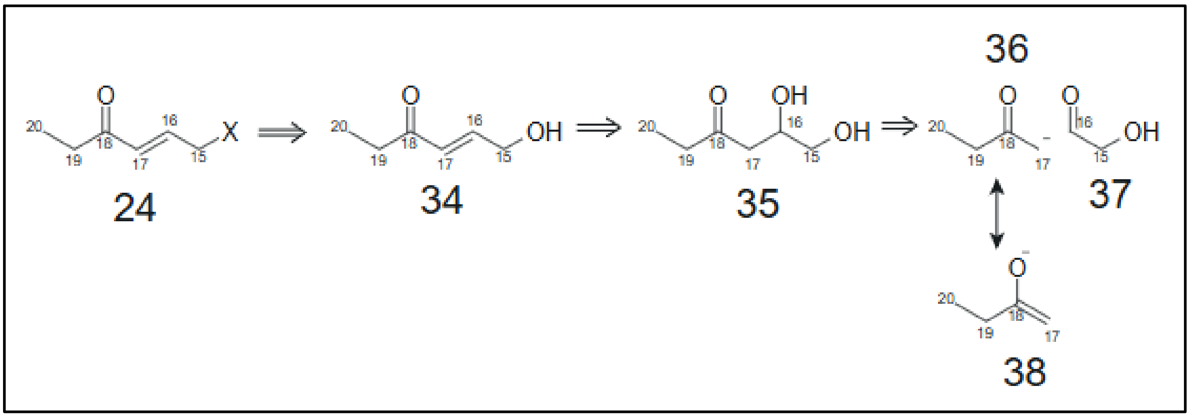


Fig. 17 . Final step of the retrosynthetic pathway of the ring

Molecule 24, detached from the chain earlier, can be disconnected further. An alcohol group substitutes for the halide, and the double bond extends into an alcohol group. Through 1,3-disconnection using the lone pair from the alcohol group, the molecule disconnects into two.

The following molecules are the results of our retrosynthesis of penidaleodiolide A.

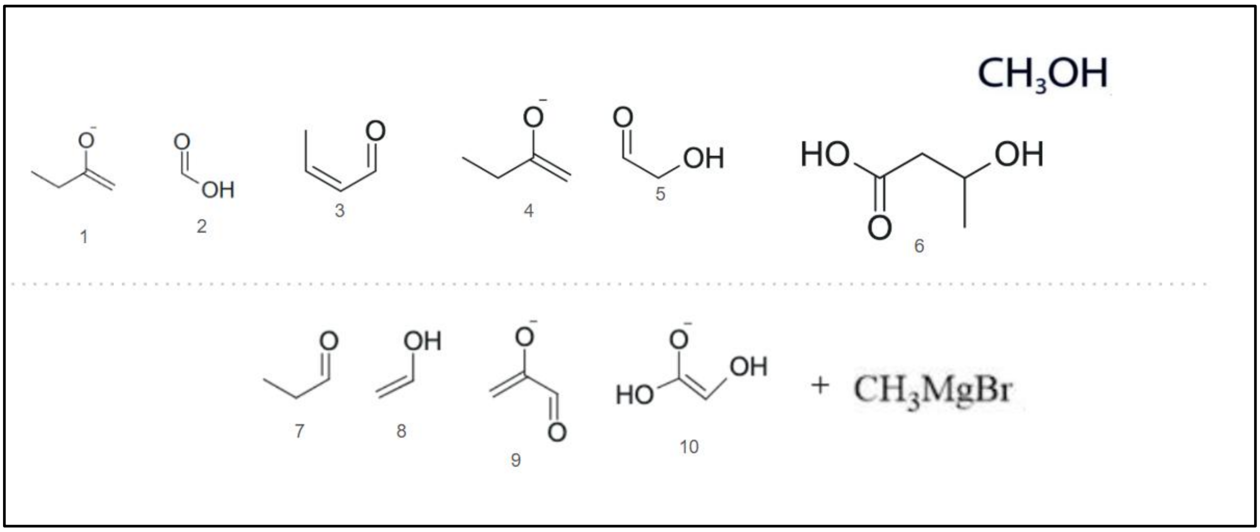


Fig. 18 . Final products of Penidaleodiolide A’s retrosynthesis

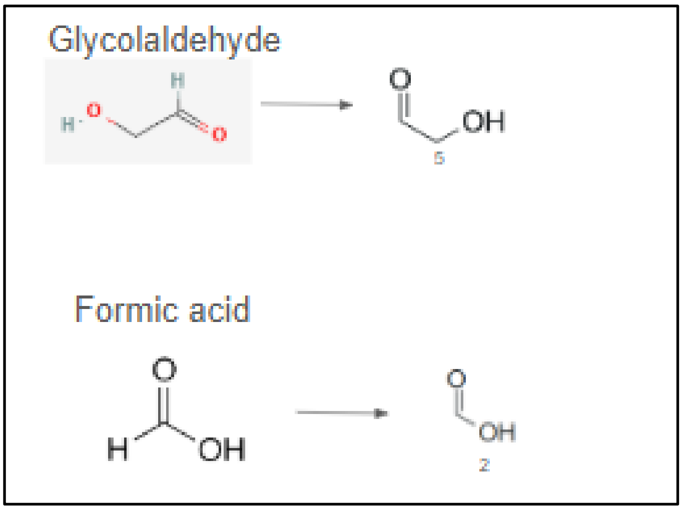


Fig. 19 . Synthetic equivalent glycoaldehyde and formic acid

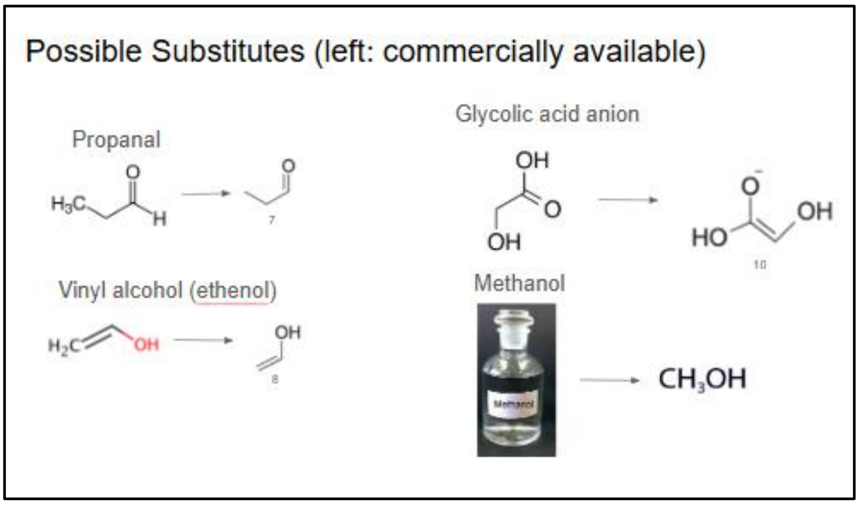


Fig. 20 . Commercially available synthetic equivalents

To aid with the synthesis process, commercially available compounds with the same or similar molecular formula and properties as the starting materials of the retrosynthesis of penidaleodiolide A have been identified. Propanal can substitute molecule 7, which is available from lab suppliers (Sigma Aldrich, Thermo Fischer, etc.)—sold as a high-purity reagent, flammable, and volatile. Glycolic acid anion can substitute for molecule 10, vinyl alcohol (ethanol) for molecule 8, beta-hydroxybutyric acid for molecule 6, glycolaldehyde for molecule 5, and formic acid for molecule 2. Molecules 1, 4, 9, and 10 have relatively stable and simple structures, and thus can be synthesized under simple procedures.

3.1 Result

Wang et al. subjected penidaleodiolide A to a variety of bioassays, including NF-κB signaling pathway regulation, cytotoxicity against human cancer cell lines, acetylcholinesterase inhibition, and effect on electrophysiological recordings of mouse brain slices, as the results can be seen in Figure 4. The tests revealed that compound 1 selectively and reversibly reduced hippocampal excitability without harming neurons, making it a promising scaffold and lead compound for developing treatments for epilepsy, Alzheimer's, and other hyperexcitability-related neurological disorders [Jin, Y. Cai, 2019; Bang, 2021; Jung, J, 2021]. The exact mechanism underlying the neuronal transmission regulation activity remains to be investigated.

3.2 Discussion

3.2.1 Challenges in Synthetic Implementation

Despite the retrosynthetic feasibility of penidaleodiolide A, several challenges may arise in the forward synthetic implementation. To make the desired ring structure, in Fig. 11, it is crucial to connect the bond between C16 and C24. However, connecting this bond in a synthetic setting may be challenging due to the difficulty in creating an environment that allows C24 to target and attack C16. Based on Fig. 21, C16 and C17 are structurally equal, and thus, the selective targeting of C24 may be challenging to replicate in a lab setting. Another difficulty that arises is stereochemical control, as the compound's multiple chiral centers—especially within the tricyclic cage—require highly selective conditions to achieve correct spatial configuration and avoid racemization. Additionally, ring-closure reactions, specifically the formation of the bridged tricyclononane core, may suffer from unfavorable strain or low yields due to entropic and steric constraints. The functional group compatibility throughout synthesis also presents difficulties, as reactive intermediates such as enolates or halides may cause side reactions or rearrangements. Addressing these challenges will likely require iterative optimization and potentially biomimetic or catalytically guided strategies to improve overall yield and stereochemical fidelity.

3.2.2 Conclusion

In conclusion, this study extends the research done by Wang and his team, providing a retrosynthetic analysis of the cage-like polyketide isolated from the rice fermentation of the fungus Penicillium daleae, which was extracted from the soil of the glowing plant *M. uniflora*. As Wang and his team explored the therapeutic implications of the compound on epilepsy and other neurological disorders, the retro-synthetic approach proves to be a step toward synthesizing the molecule in a lab environment, in contrast to the proposed biosynthetic pathways.

Existing epilepsy treatments are limited in the sense that 30-40% of patients remain drug-resistant, unable to control their seizures. More so, many treatments can cause neurotoxicity. Long-term exposure to Valproate during pregnancy is associated with autism spectrum disorder [Cynthia L Harden, 2013], both levetiracetam and perampanel have been reported to induce irritability and aggression [Wrede et. al, 2021], and extended use of certain Anti-Seizure Medications has been linked to lower IQ in children exposed in utero [Madley-Dowd et. al, 2024]. Thus, unlike existing treatments, penidaleodiolide A's lack of cytotoxicity against nerve cells makes it a promising compound for epilepsy treatment. As the current isolation process is not able to result in high yields of the compound due to M. uniflora's scarcity and the duration of the process, being able to synthesize the compound in a lab setting is imperative. This retrosynthetic analysis serves as a stepping stone for the subsequent synthesis process, laying the foundation for further research in epilepsy treatment.

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