Retrosynthetic Analysis of Compound 13 from Rhizopus sp. W23

Yoonje (Philip) Song[1*]

1. Chadwick International School, Yeonsu-gu, Incheon, Republic of Korea
* Corresponding author email: philipsong1022@gmail.com

Abstract

Compound 13 from Rhizopus sp. W23 is a recently discovered molecule in the deep sea, with a C25 steroid structure and possessing an unusual 7/7/6/5 tetracyclic structure bicyclo [4.4.1] scaffold. This structure significantly deviates from the conventional canonical cyclopentanoperhydrophenanthrene, and no complete retrosynthetic analysis for this molecule has been reported in the literature. A recent study suggests that this compound has the potential to treat osteoporosis by inhibiting adipogenic differentiation and osteoblast mineralization, albeit with moderate toxicity. In this work, a detailed retrosynthetic pathway of compound 13 is proposed by identifying key disconnections, strategic intermediates, and synthetic equivalents.

Keywords

Retrosynthesis; Rhizopus sp. W23; Compound 13; Osteoporosis; Natural Product

Introduction

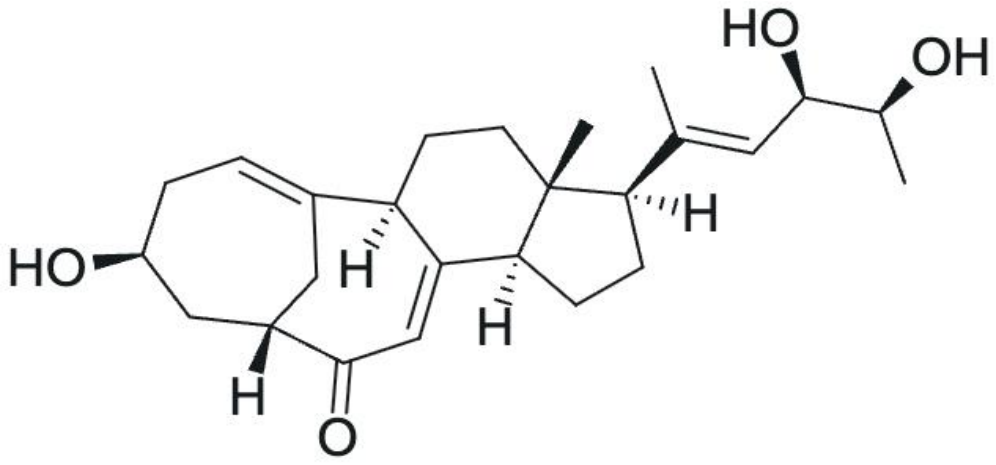


Figure 1. Compound 13 Extracted from Rhizopus sp. W23

Natural products extracted from extreme environments can often be a source for discovering diverse compounds, with their structure being not only novel but also unique. One such example is molecule 13 extracted from Rhizopus sp. W23, with the molecular formula $C_{25}H_{38}O_4$ and an Mr of 402.6 g/mol (Figure 1) (Wang et al., 2015). The fungus Rhizopus sp. W23 was found in the deep sea, which is predominantly characterized by high pressure, low temperatures, oligotrophic conditions, and minimal light exposure. Among the diverse inhabitants in the deep sea, the fungi of the genus Rhizopus have been recently recognized to have bioactive secondary metabolites. While Rhizopus species is often associated with connections to terrestrial environments, such as bread molds, Rhizopus sp. W23 is a significant deviation

from the common perception: Rhizopus sp. W23 was found in the deep sea at depths exceeding 1000 meters (Wang et al., 2015; He et al., 2022). The adaptation of the fungi to the extreme environment has allowed the development of distinctive biosynthetic gene clusters and secondary metabolites, which are often absent in terrestrial environments (Wang et al., 2020; Zhao et al., 2022). In fact, more than 180 bioactive secondary metabolites were discovered from the deep-sea fungi with diverse effects, including anticancer, antimicrobial, antifungal, antiprotozoal, and antiviral activities (Wang et al., 2015).

The recent chemical investigation on Rhizopus sp. W23 allowed the discovery of one handling artifact and six new and 12 known cyclocitrinol analogues: that is, an unusual C^{25} steroid natural product (Figure 1). Notably, these molecules present an unusual 7/7/6/5-tetracyclic scaffold bicyclo [4.4.1] ring system (He et al., 2022). In other words, the compounds are characterized by four rings that are fused, with each ring exhibiting 7, 7, 6, and 5 carbon atoms, respectively, with bicyclo [4.4.1] representing the number of carbon atoms in each of three bridges connecting the bridgehead atoms. This shows a significant deviation from the canonical cyclopentanoperhydrophenanthrene compound, a fundamental structural unit of steroids (Liu et al., 2018). To date, no complete retrosynthetic analysis for compound 13 has been proposed in the literature, making this investigation necessary. This novel structure of the compound presents a unique challenge in the synthesis but also presents a potential opportunity to be utilized in curing osteoporosis (He et al., 2022).

Osteoporosis is a non-communicable disease affecting the bones, caused by a decrease in the mineral density and mass of the bones (Pierce et al., 2019). At the cellular level, it arises from the molecular imbalance between osteoclasts, or the bone-resorbing cells, and osteoblasts, or the bone-forming cells (He et al., 2022). However, the newly discovered cyclocitrinol analogues, particularly compounds 13 and 19, have been shown to enhance the mineralization of the osteoblasts with moderate cytotoxicity while inhibiting adipogenic differentiation of bone marrow mesenchymal stem cells, or BMSCs, in vitro (He et al., 2022). That is, while the stem cells of osteoporotic patients transform into adipocytes, or fat cells, instead of bone cells, the compounds 13 and 19 can prevent this (He et al., 2022; Zhu et al., 2023). These activities indicate that compound 13 may have the potential to restore the formation of the bones in osteoporotic patients, specifically by shifting the differentiation balance toward bone formation rather than fat accumulation within the marrow. Importantly, these effects were observed at relatively low concentrations (5-20 μM) (He et al., 2022). As a result, it suggests the possible development of an anti-osteoporosis cure from the newly identified molecule. However, it is important to note that the findings on the potential for anti-osteoporosis effects are based on in-vitro studies, suggesting that the therapeutic effects need to be further validated through clinical application. Therefore, its promise should be considered long-term rather than immediate. In fact, proposing a potential retrosynthetic pathway can possibly foster the production of sufficient quantities of the compound in order to further evaluate its biological potential and synthesize compounds with greater pharmacological properties.

Considering their marine origins, rare molecular structure, and positive bioactivity, the newly discovered compounds are a compelling case for retrosynthetic analysis, which could offer a method for synthesizing them in the lab, thereby facilitating future drug development. Thus, this paper seeks to analyze the structural features of compound 13, specifically, and propose a possible retrosynthetic pathway. Yet for clarity and consistency throughout this study, compound 13, which was extracted from Rhizopus sp. W23, is referred to as Rhizopus sp. W23-13.

Results and Discussion

Retrosynthetic Analysis

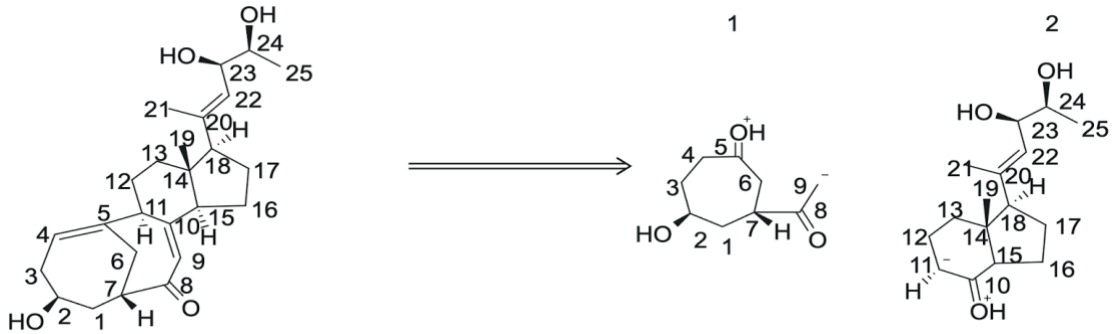


Figure 2. Key Retrosynthesis of Rhizopus sp. W23-13

In the retrosynthetic analysis of this molecule, a key disconnection was made between C4 = C5 and C9 = C10 (Figure 2). In the forward synthesis of compound 13, the transformation of the molecule can be observed through oxidation analysis: carbons C5 and C10 represent alcohol functionalities that may originate from nucleophilic addition to carbonyl precursors, while C4 and C9 correspond to reduced carbon centers. Therefore, the structure of compound 13 may potentially be constructed through two intramolecular carbon-carbon bond formation reactions through the nucleophilic attack at electrophilic carbonyl carbons based on the analysis.

Specifically, this reaction may include nucleophilic carbon species at C11 and C9 attacking electrophilic carbonyl carbons at C5 and C10, respectively. Although it is ideal for both reactions to occur simultaneously, allowing the bond formation to co-occur, this may not occur in reality due to steric and kinetic limitations. Therefore, a sequential step may be necessary.

If an attack by a carbon nucleophile at C9 on the carbonyl carbon C10 occurs first, the carbonyl undergoes nucleophilic addition to form an alkoxide intermediate, which yields a secondary alcohol as a result of protonation. This transformation eliminates the electrophilic characteristic of carbonyl carbon, decreasing the probability of further nucleophilic attack by influencing the reactivity of adjacent centers. Consequently, the bond formation between C11 and C5 becomes less favorable.

In contrast, if the bond formation between C11 and C5 occurs first, this reaction does not significantly impact the favorability of the reaction between C9 and C10, as there are minimal conjugative or inductive effects due to the distance between C5 and C9. Therefore, if a sequential step is required, the preferred sequence would be the initial formation of the bond between C11 and C, followed by the reaction between C9 and C10.

This disconnection was further supported as it produced two stable intermediates. Both compounds 1 and 2 have reactive centers positioned alpha to carbonyl groups, allowing stabilization through resonance.

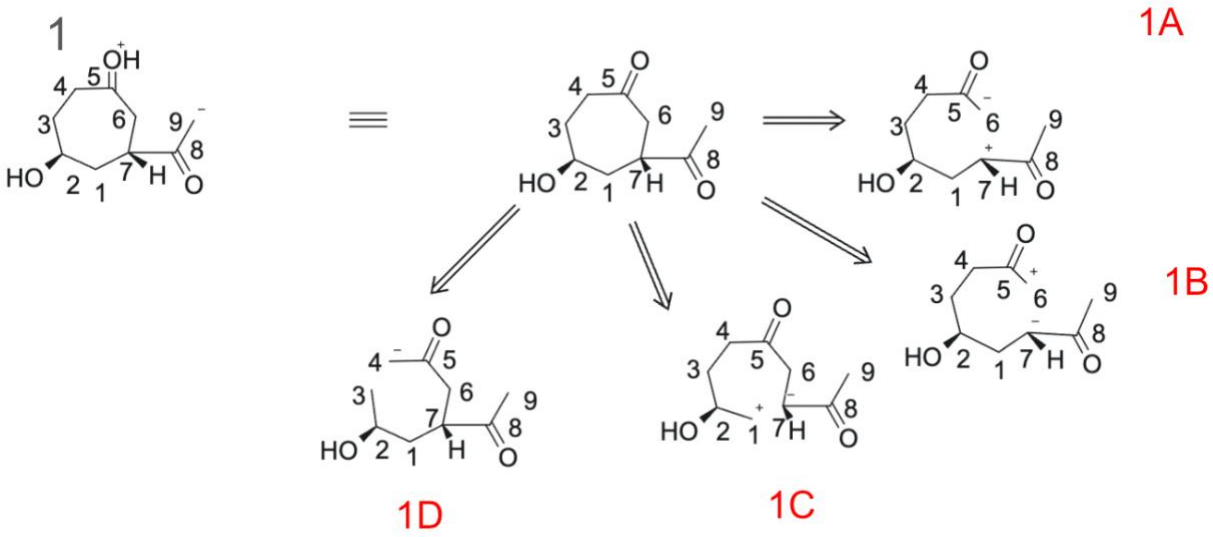


Figure 3. Possible Disconnections of Compound 1

Further retrosynthetic analysis focused on compound 1 as a next step. Four possible disconnection strategies of compound 1 were considered, with each approach including the cleavage of the bond in the 2,3 relationship relative to one of the two ketones (Fig. 3). Compounds 1A and 1B were derived by cleavage between two alpha carbons adjacent to the carbonyl groups. Although 1A and 1B have the same disconnection site, they present opposite charge assignments, which can be rationalized through enolate resonance structure. In contrast, compound 1C was created by the cleavage of the bond between the 2,3-relationship between the alpha and beta carbons relative to the carbonyl group attached to C8, while compound 1D involved the same pattern applied to the carbonyl group attached to C5.

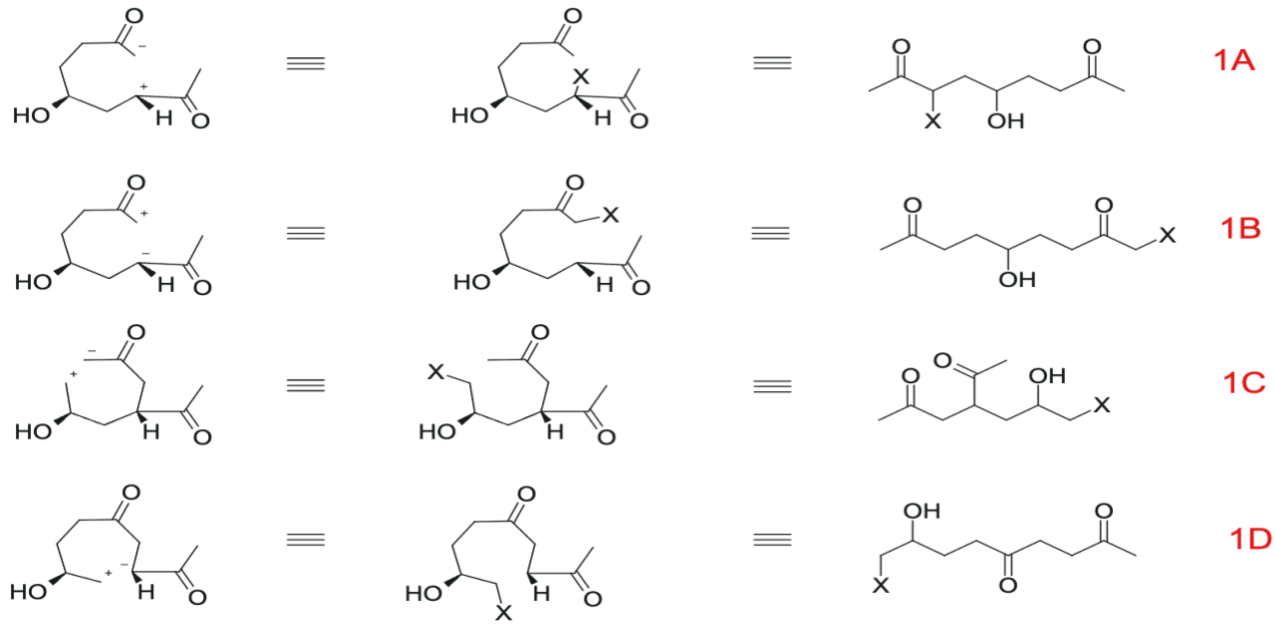
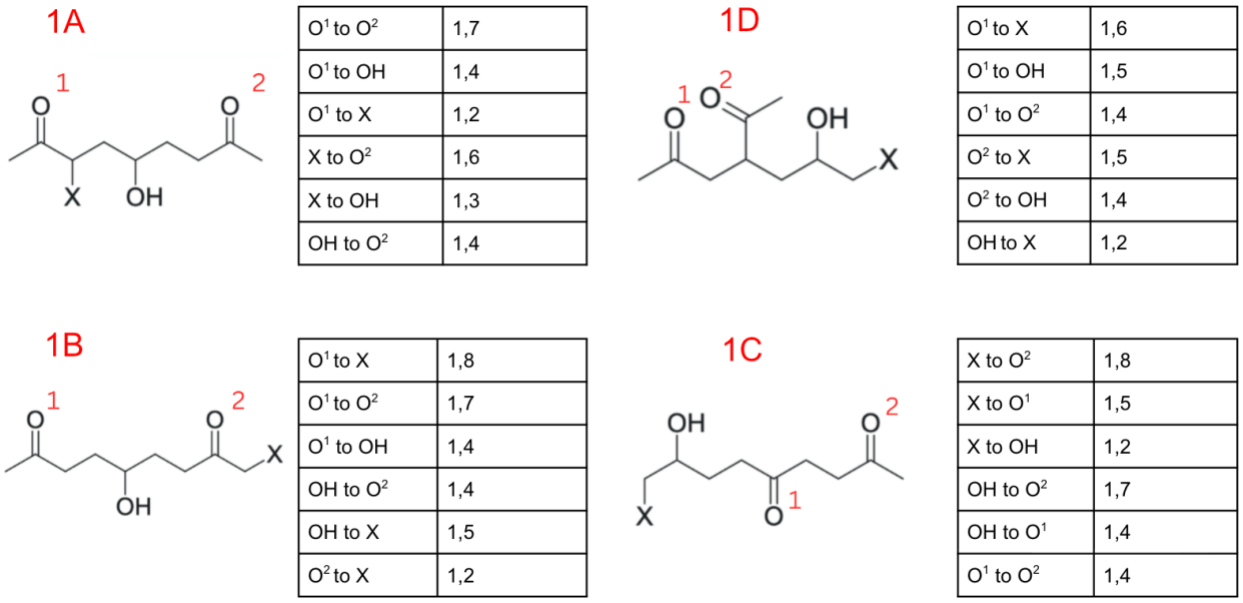


Figure 4. Linear Forms of Synthetic Equivalents

To evaluate each of these pathways, each compound was converted into its corresponding synthetic equivalents. In these representations, the electrophilic synthons were transformed into alkyl halides, as expressed in “X”, while nucleophilic carbons in synthons were represented as neutral carbonyl-containing compounds, including enolate precursors (Fig. 4). As all disconnections involve the cleavage of the seven-membered ring system, the intermediates can be represented as linear chains, facilitating the comparison of synthetic availability through clear visualization.



O^1 to O^2	1,7
O^1 to OH	1,4
O^1 to X	1,2
X to O^2	1,6
X to OH	1,3
OH to O^2	1,4

O^1 to X	1,6
O^1 to OH	1,5
O^1 to O^2	1,4
O^2 to X	1,5
O^2 to OH	1,4
OH to X	1,2

O^1 to X	1,8
O^1 to O^2	1,7
O^1 to OH	1,4
OH to O^2	1,4
OH to X	1,5
O^2 to X	1,2

X to O^2	1,8
X to O^1	1,5
X to OH	1,2
OH to O^2	1,7
OH to O^1	1,4
O^1 to O^2	1,4

Figure 5. Dioxygenation Pattern Analysis

To further evaluate the feasibility of each method of disconnection, dioxygenation pattern analysis was conducted (Fig. 5). This analysis allows to identify 1,3 and 1,5 positions between oxygen-containing functional groups in the synthetic equivalents. These positions are crucial in identifying further disconnection sites due to the compatibility with established bond-forming reactions. In particular, 1,5-dicarbonyl systems are essential in intermediates, as they can form an intramolecular carbon-carbon bond through enolate-mediated reactions, including aldol condensations or cyclizations.

The two oxygens in each synthetic equivalent were assigned a number, either 1 or 2, for clarity and consistency. This analysis allowed an evaluation of the relative effectiveness of each possible disconnection strategy.

Through this analysis, it was discovered that pathways A, B, and C present only one favorable relationship of either of the two positions, while pathway D demonstrates two distinct 1,5 dioxygenation patterns. As the existence of two reactive sites increases the probability of successful cyclization, pathway D was selected as the most favorable disconnection strategy.

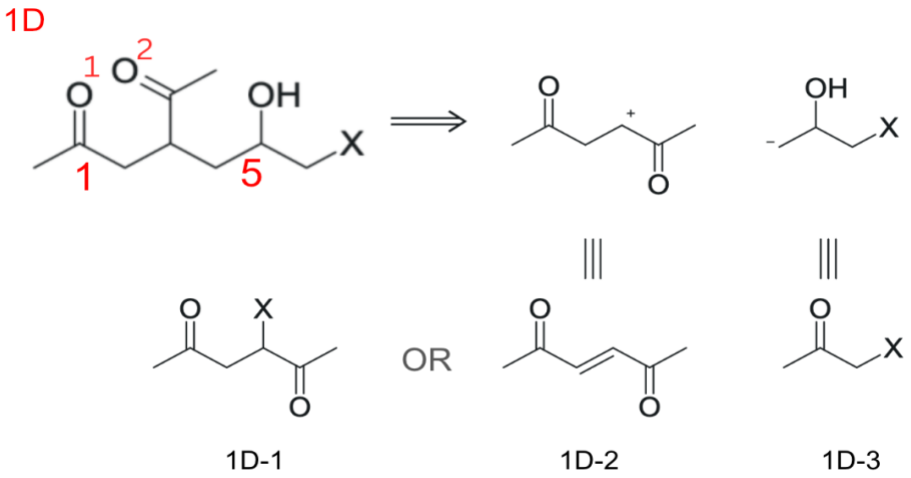


Figure 6. Synthetic Equivalents of Compound 1D

Within the synthetic equivalent 1D, two potential 1,5-disconnection sites were identified (Fig. 6). However, the disconnection between ketone 1 and alcohol functionalities was utilized instead of the 1,5 relationship between ketone 2 and the halide (Fig. 6). This allowed the disconnection between ketone 1 and the alcohol functionalities, specifically between the 3,4 positions relative to the carbonyl group. This disconnection leads to the formation of two separate compounds with complementary reactivity: one electrophilic and one nucleophilic.

In the subsequent representation of the corresponding synthetic equivalents of the two compounds, the electrophilic fragment can be represented as an alkyl halide or as an α,β-unsaturated carbonyl compound, while the nucleophilic fragment can be transformed into an enolate precursor derived from a ketone. As a result, this analysis leads to three potential compounds (1D-1, 1D-2, and 1D-3), which can form a carbon–carbon bond in appropriate conditions

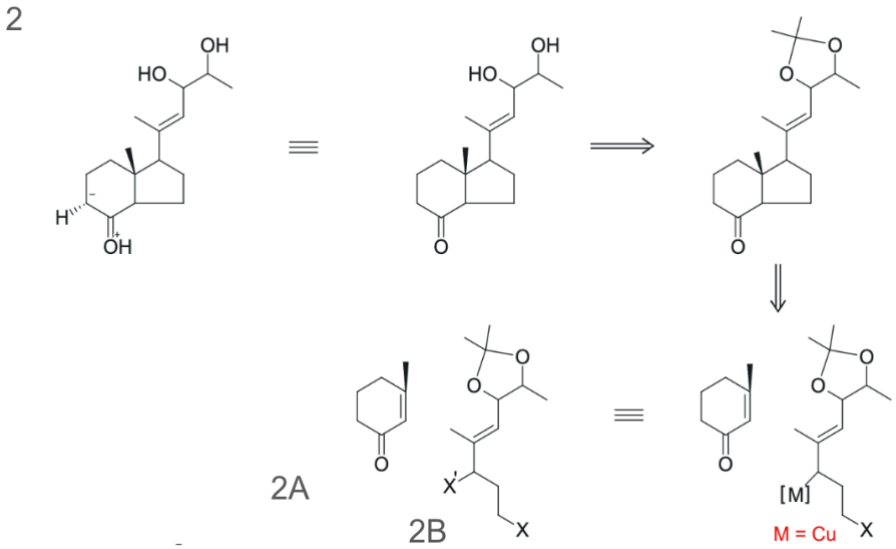


Figure 7. Retrosynthesis of Compound 2

The retrosynthesis of compound 2 (Fig. 7) was then theorized as compound 1 was fully disconnected. Firstly, the charged intermediate compound 2 was converted into its neutral synthetic equivalents by replacing protonated oxygen species with ketones and by removing the formal charge.

To prevent undesired side reactions during subsequent steps, the vicinal diol located at the side chain was converted to an acetonide as a protection through acid-catalyzed ketone formation with acetone. This transformation leads to the formation of a five-membered cyclic ketal, replacing the hydroxyl groups and enhancing the chemical stability of further reaction conditions.

Subsequently, the six-membered ring was disconnected from the rest of the compound, generating two separate compounds. This cleavage represents the use of an organocuprate reagent (Gilman reagent, R2CuLi), which can undergo a selective 1,4-addition in the forward synthesis through nucleophilic addition to the β-carbon of α,β-unsaturated carbonyl compounds. Consequently, this disconnection yields the two synthetic equivalents: 2A and 2B.

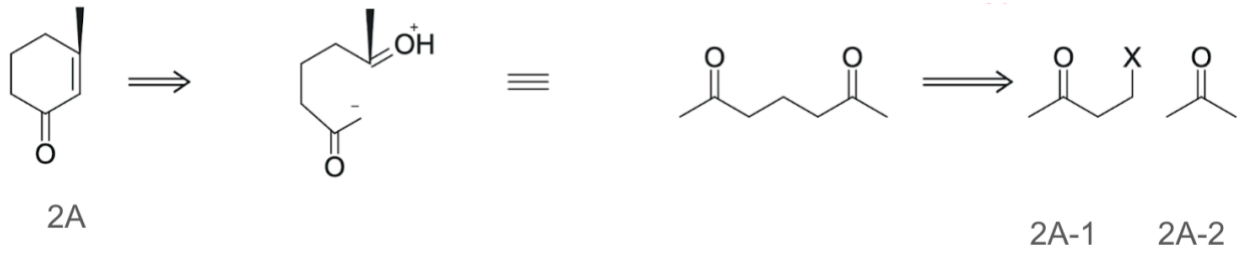


Figure 8. Retrosynthesis of 2A-1

As two separate compounds were generated, the retrosynthetic pathway of compound 2A was first considered (Fig. 8). The retrosynthetic analysis begins with the cleavage of the six-membered ring, which represents the reversal of an intramolecular carbon-carbon bond-forming reaction. The disconnection of the carbon double bond leads to the formation of a protonated oxygen atom with a double bond while assigning a negative charge on the opposite side of the ring. As this disconnection leads to the formation of the linear chain, the synthetic equivalent can be considered in the linear form, specifically forming a linear diketone system presenting a 1,5-dicarbonyl arrangement. The diketone arrangement is synthetically valuable, as it readily undergoes enolate-mediated cyclization reactions. Exploiting this feature, the chain can be further decoupled into simpler precursors: 2A-1 and 2A-2. These two compounds can be reconnected by establishing a carbon-carbon bond-formation reaction in the forward synthesis.

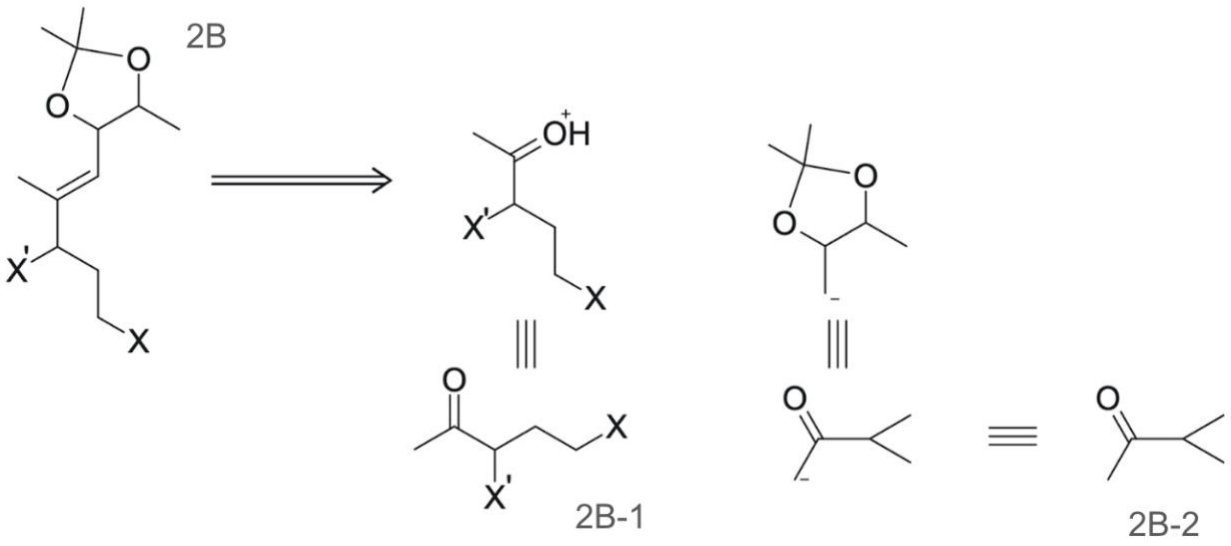


Figure 9. Retrosynthesis of Compound 2B

Subsequently, the retrosynthetic analysis of compound 2B was conducted (Fig. 9). As compound 2B involved a double bond between two carbon atoms, oxidation analysis can be utilized by considering the alkene functionality. Conceptual oxidative cleavage of the alkene would generate two carbonyl-containing fragments (ketones or aldehydes, depending on substitution). Disconnection of the alkene therefore reveals a carbonyl fragment on the alcohol-bearing side, with its synthetic equivalent representing a ketone, leading to the formation of the compound 2B-1. On the opposite side, the removal of a formal charge and the deprotection of the acetonide yield a fragment that contains a ketone, representing a tertiary carbon center. As a result, the compound 2B-2 could be further theorized.

Starting Materials of Rhizopus sp. W23-13

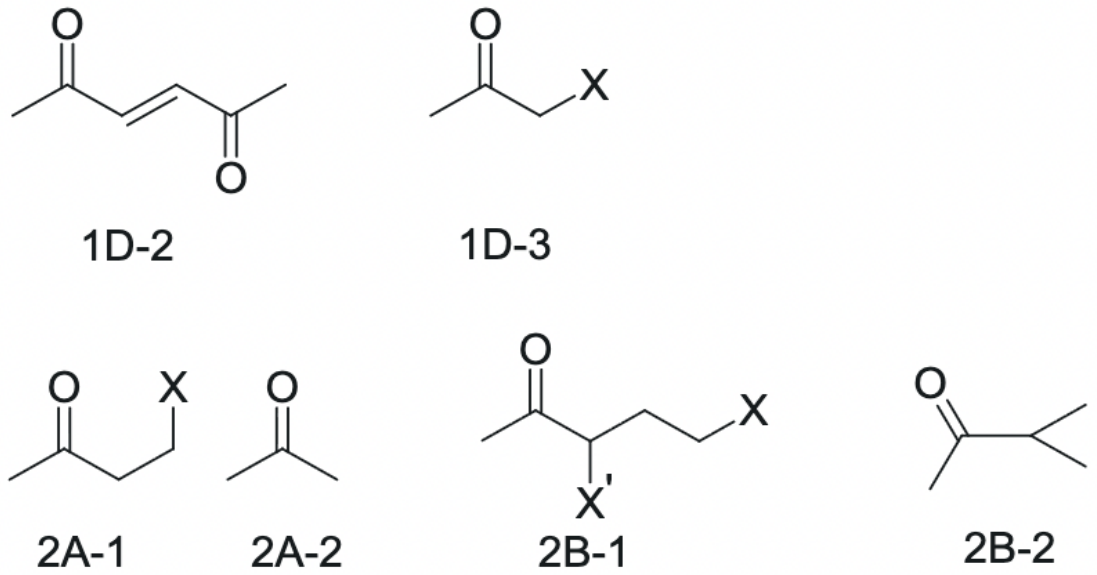


Figure 10. Theorized Synthesis of Rhizopus sp. W23-13

In summary, during the synthesis of Rhizopus sp. W23-13, a total of 6 key precursor molecules are required: 1D-2, 1D-3, 2A-1, 2A-2, 2B-1, and 2B-2. The synthetic pathway begins with the carbon-carbon bond formation reaction between 1D-2 and 1D-3 (or even 1D-1 and 1D-3), which undergo a reaction to generate the intermediate 1D. Although 1D-1 can also be utilized to react with 1D-3, 1D-2 may be preferred due to its symmetrical structure. In parallel, 2A-1 and 2A-2 undergo a carbon-carbon formation reaction to yield the intermediate 2A, while 2B-1 and 2B-2 act as precursors to form molecule 2B.

Once these intermediates are in place, the synthesis should proceed through sequential combination. Specifically, intermediates 2A and 2B should undergo a reaction to produce compound 2, which is a significant fragment of the target molecule.

Finally, the last step involves the convergent coupling of compounds 1 and 2, resulting in the production of the natural product, Rhizopus sp. W23-13. This step is especially essential as the two main compounds created from independent synthesis lead to the final product. This not only improves efficiency but also ensures that the structure of the compound is generated in a controlled pathway.

Discussion

Alternative Retrosynthetic Pathway

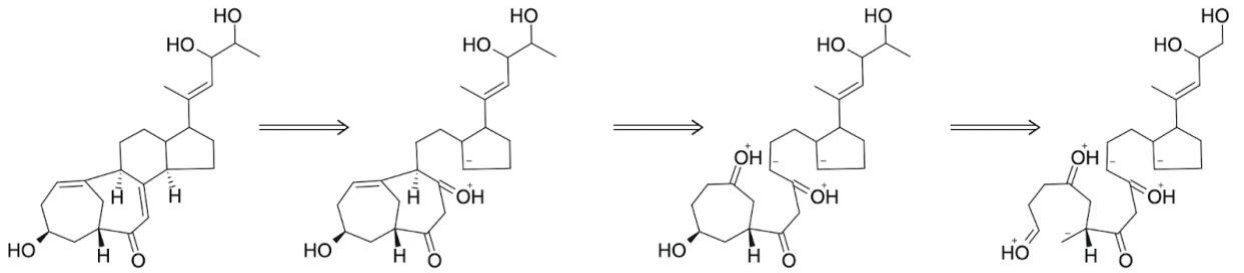


Fig 11. Alternative Retrosynthetic Pathway (Rejected)

An alternative retrosynthetic pathway was also considered during the retrosynthetic analysis (Fig. 11). The theorized basic mechanism of this retrosynthesis involves the disconnection across alkene and alcohol functionalities. However, this retrosynthesis pathway leads to intermediates with unstabilized carbanionic synthons. These species lack an adjacent electron-withdrawing group or resonance stabilization. As a result, this chemical structure is very likely to be highly unstable and synthetically unfavorable. In fact, no resonance nucleophilic precursors can be identified for these intermediates when converted into their synthetic equivalents. Therefore, it was concluded that the presence of isolated anions makes this retrosynthetic pathway infeasible and thus rejected.

Evaluation of Synthetic Feasibility

While most of the proposed retrosynthetic pathways are mostly feasible, the initial step of the retrosynthetic pathway (Fig. 2) creates a significant synthetic challenge, as it requires the cleavage of two bonds in the retrosynthesis, implying the formation of two carbon-carbon bonds in the forward synthesis. Therefore, a synthetic pathway with successive formation of bonds is likely to be more favorable.

During the synthesis of compound 13, it is more favorable for the reaction between C11 and C5 to occur prior to the bond formation between C9 and C10 as the C11-C5 reaction maintains the electrophilicity of the carbonyl functionality at C10 and prevents the loss of reactivity. However, if the bond formation between C9 and C10 occurs first, this reaction would lead to a reduction in the electrophilicity of this site and prevent subsequent reaction between C11 and C5.

Although challenges such as conformational rigidity, geometric strain, and competing side reactions may be potentially possible, this pathway remains plausible based on mechanistic and electronic considerations.

Impacts & Future Directions

The retrosynthesis proposed herein is the first published retrosynthetic analysis. As the global burden of osteoporosis constantly increases, reaching 41.5 million cases in 2019, and causes fracture-related disabilities, it is essential to develop a novel treatment for osteoporosis. (Zhu et al., 2023) While existing treatments for osteoporosis predominantly focus on inhibiting the differentiation of osteoclasts, the newly found compound can enhance the formation of the bond by fostering osteoblast activity with moderate side effects. (He et al., 2022) This highlights that it could potentially become the leading material for anti-osteoporosis.

Further research should focus on the experimental validation of the proposed pathway of the retrosynthesis through laboratory synthesis. At the same time, researching alternative synthetic strategies of Rhizopus sp. W23-13 is also important in order to identify more efficient and sustainable methods for the (retro)synthetic pathway. Upon successful synthesis, experiments on preclinical in vitro cell models and in vivo models should also be conducted in order to foster its therapeutic potential.

Conclusion

The restroynthesis of Rhizopus sp. W23-13 provides a possible pathway to break down the complex tetracyclic structure into simpler synthetic equivalents. The analysis provides key disconnections and a protective strategy that could allow laboratory synthesis of the compound. Given its ability to enhance osteoblast activity and prevent adipogenic differentiation of BMSCs, Rhizopus sp. W23-13 presents its potential as a novel candidate for anti-osteoporosis.

Acknowledgements

The author gratefully acknowledges Professor Brian Stoltz from Caltech University for his guidance and feedback throughout the project. This research was supported by the CRI program, which provided the necessary resources and facilities.

References

He, Z.-H., Xie, C.-L., Wu, T., Yue, Y.-T., Wang, C.-F., Xu, L., Xie, M.-M., Zhang, Y., Hao, Y.-J., Xu, R., & Yang, X.-W. (2022). Tetracyclic Steroids Bearing a Bicyclo[4.4.1] Ring System as Potent Antiosteoporosis Agents from the Deep-Sea-Derived Fungus Rhizopus sp. W23. Journal of Natural Products, 86(1), 157–165. https://doi.org/10.1021/acs.jnatprod.2c00866

Liu, J., Wu, J., Fan, J.-H., Yan, X., Mei, G., & Li, C.-C. (2018). Asymmetric Total Synthesis of Cyclocitrinol. Journal of the American Chemical Society, 140(16), 5365–5369. https://doi.org/10.1021/jacs.8b02629

Pierce, J. R., Begun, D. L., Westendorf, J. J., & McGee-Lawrence, M. E. (2019). Defining osteoblast and adipocyte lineages in the bone marrow. BONE, 118, 2–7. https://doi.org/10.1016/j.bone.2018.05.019

Wang, Y.-N., Meng, L.-H., & Wang, B.-G. (2020). Progress in Research on Bioactive Secondary Metabolites from Deep-Sea Derived Microorganisms. Marine Drugs, 18(12), 614. https://doi.org/10.3390/md18120614

Wang, Y.-T., Xue, Y.-R., & Liu, C.-H. (2015). A Brief Review of Bioactive Metabolites Derived from Deep-Sea Fungi. Marine Drugs, 13(8), 4594–4616. https://doi.org/10.3390/md13084594

Zhao, G., Tang, W., Zhang, J., Shi, P., Li, Y., Wang, J., Shen, Q., Si, H., Jiang, L., Yu, X., Zhu, H., Chen, G., Zhang, X., & Jia, H. (2022). Deep-Sea-Derived Fungi as Valuable Producers of Cytotoxic Secondary Metabolites and Their Leads Potential. Frontiers in Marine Science, 9. https://doi.org/10.3389/fmars.2022.929561

Zhu, Z., Yu, P., Wu, Y., Wu, Y., Tan, Z., Ling, J., Ma, J., Zhang, J., Zhu, W., & Liu, X. (2023). Sex Specific Global Burden of Osteoporosis in 204 Countries and Territories, from 1990 to 2030: An Age-Period-Cohort Modeling Study. The Journal of Nutrition, Health and Aging, 27(9), 767–774. https://doi.org/10.1007/s12603-023-1971-4

Authors

Yoonje (Philip) Song is a junior at Chadwick International School with research interests in medicine, chemistry, and public health. He aims to explore the intersection of science and healthcare through academic research and future studies