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Kavain Hydroxylation in Kava Metabolism: Computational Analysis

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ABSTRACT

Kava, a non-alcoholic beverage derived from the *Piper methysticum* plant, commonly also known as Kava, has a prominent role in the cultures of Oceania due to its calming and soothing effects. These psychoactive properties have sparked interest beyond Oceania and in the scientific community, who see potential for medicinal applications. However, alongside these beneficial effects, consumption of Kava has been linked to potential hepatotoxicity, raising safety concerns. Understanding the cause of this hepatotoxicity requires a detailed exploration of Kava's metabolic processes, specifically those involving kavain, its primary constituent. Recent studies have found the hydroxylation of kavain to 12-hydroxykavain as a key step in Kava's metabolic pathway. Yet, the precise mechanism of this conversion and the hydroxylation intermediates involved remain poorly understood. To address this knowledge gap, our study employed Spartan, a computational chemistry software, to model the hydroxylation process. We analyzed the stability and chemical properties of the proposed reaction intermediates, providing a detailed view of this critical phase of metabolism. Our analysis reveals that three of the five proposed intermediates appear stable, suggesting they may play significant roles in the hydroxylation process. This finding also enabled us to narrow down the possible reaction pathways of hydroxylation. Having a better understanding of the key step of Kava metabolism could guide the development of safer, more effective Kava-based products, balancing the therapeutic potential of Kava with a reduced risk profile.

Introduction

Oceania is a term used to describe the island communities of the Pacific Ocean: Polynesia, Micronesia, and Melanesia. Oceania was one of the few communities without alcoholic beverages by the time of first contact with Europeans in the 18th century (1). Instead, these Pacific Islanders consume their own staple beverage Kava, which is prepared by grinding the roots and stalks of the kava plant *Piper methysticum* (2). *Piper methysticum* grows abundantly in the islands of Polynesia and are believed to originate from Melanesia (3). Kava drinking is an integral part of Pacific Islander culture, often being the centerpiece for religious rituals, acting as a lubricant for solemn gatherings, serving as a daily social drink, or for medicinal purposes (4).

The preeminent role of Kava in Oceania is largely attributed to its outstanding pharmacological properties, and also the belief that drinking kava restores strength, soothes stomach pains, and cures ailments (5). Kava displays psychoactive properties, particularly soothing and calming effects (6), but it is not classified as a drug since its consumption leads to neither addiction nor dependency (4). Due to these properties, Kava was used in Germany before World War I to manufacture various medicines, and Europe also used Kava as a treatment for cystitis, gonorrhea, and gout around the same period (1). In recent decades, the scientific community and Western countries have also shown interest in Kava due to its potential to treat cancers (7) and symptoms associated with anxiety (8) and stress-induced insomnia (9).

However, recent research has linked heavy consumption of Kava with hepatotoxicity (10) and unexplained liver injury (11). The mechanisms leading to hepatotoxicity are still unclear but are known to be related to the metabolic processes of Kava in the human body (6). One possible suggested mechanism is related to the formation of activated metabolites that induce hepatotoxicity (10). However, before this mechanism related to hepatotoxicity can

be identified, it's important to first understand how Kava is metabolized in the human body. Kava undergoes several metabolic steps within the human body, the major one being the hydroxylation of kavain, the kavalactone found in the highest concentration in Kava (2,12). This research paper aims to investigate this hydroxylation step in the metabolic pathway of Kava, specifically aiming to understand how kavain is converted to 12-hydroxykavain, which is the main kavain metabolite found in both blood and urine (12). Through studying this hydroxylation step, it is hoped that there will be a greater understanding of Kava metabolism in a human body which may aid in finding the mechanism leading to hepatotoxicity for future research.

Composition of Kava

The pharmacological properties of Kava are thought to be primarily due to a group of compounds named kavalactones (13). The six most abundant kavalactones are kavain, dihydrokavain, methysticin, dihydromethystin, desmethoxyyangonin and yangonin (Figure 1), with kavain being the main kavalactone in Kava (2). In the following sections, all carbon numbering related to kavain will follow the scheme presented in Figure 1.

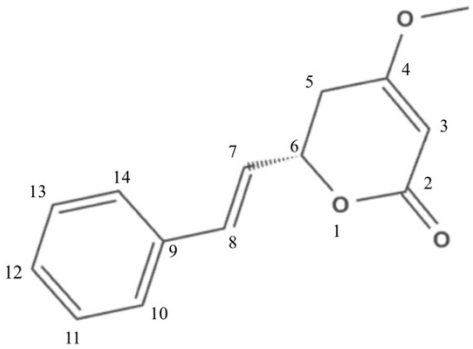

Figure 1. Skeletal representation of kavain molecule

Metabolism of Kava

Köppel et al. (14) identified the following metabolites related to urinary excreted kavain in rats following oral administration: hippuric acid, *p*-hydroxybenzoic acid, 4-hydroxy-6-hydroxyphenyl- 5-hexen-2-one, 4-hydroxy-6- phenyl-5-hexen-2-one, dihydrokavain, and 12-Hydroxykavain (12,14,15), which also supported the findings by Rasmussen et al. (16) who conducted a similar study. To see if there was any correlation between Kava metabolism in rats and humans, Tarbah et al. (12) performed a study on kavain metabolites in human urine after oral administration. Upon analysis, a different set of metabolites was identified in human urine compared to rat urine, but 12-hydroxykavain was a metabolite that was prevalent in human urine as well as rat urine. 12-hydroxykavain and its glucuronide and sulfate derivatives were also found in the highest concentrations in the urine samples. These findings suggested that hydroxylation at the C-12 position of the phenyl ring in kavain to form 12-hydroxykavain was a major step in metabolism of kavain (Figure 2). Wang et al. (17) found that CYP2C19, a liver enzyme protein, was the primary enzyme responsible for kavain bioactivation and hydroxylation.

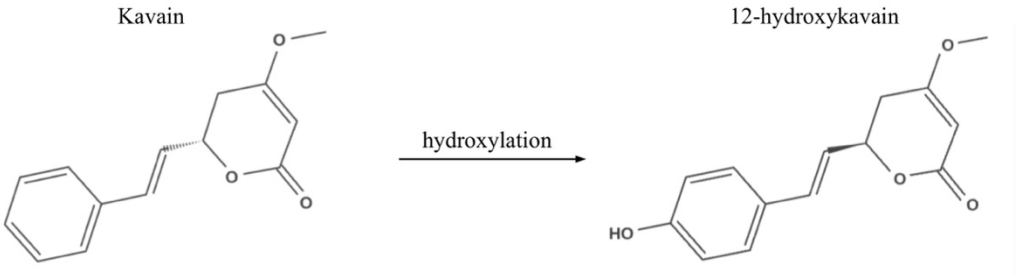

Figure 2. Hydroxylation of kavain to form 12-hydroxykavain

Aromatic Hydroxylation

The possible mechanism pathways (Figure 3) for hydroxylation were found by Trager et al. (18). The oxygen atom of the iron porphyrin attaches to the para position due to better resonance stabilization and less steric hindrance compared to the ortho position (19). The basis of this study will center around exploring the chemical properties of the five kavain hydroxylation intermediates depicted below (Figure 3).

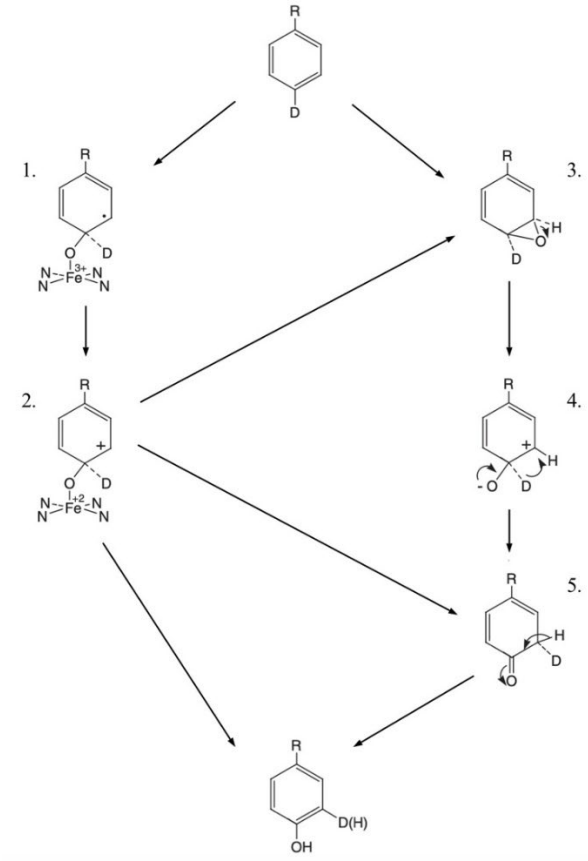

Figure 3. Possible mechanism pathways of aromatic hydroxylation of kavain adapted from Trager et al. (18)

Trager et al. (18) suggest that intermediates 1 and 2 are bonded to an iron porphyrin each. For this study, we used an iron porphyrin model derived from the National Center for Biotechnology Information (20) (Figure 4).

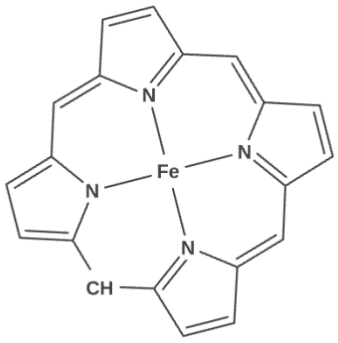

Figure 4. Skeletal representation of iron porphyrin adapted from National Center for Biotechnology Information (20)

Figure 3 illustrates atom D as a hydrogen atom and R as the remaining components of the kavain molecule. Based on the same figure (Figure 3), we also inferred the hydroxylation kavain intermediates as follows:

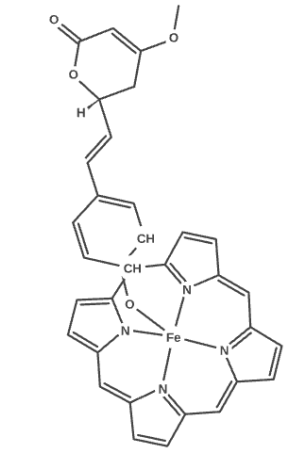

Figure 5. Skeletal representation of Intermediate 1

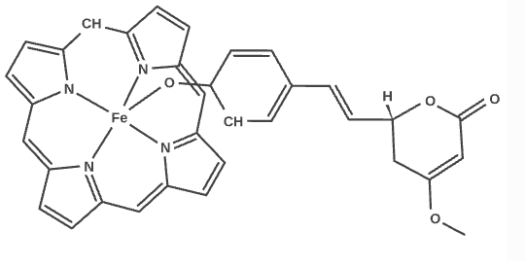

Figure 6. Skeletal representation of Intermediate 2

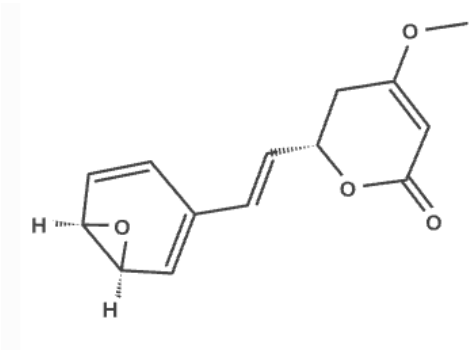

Figure 7. Skeletal representation of Intermediate 3

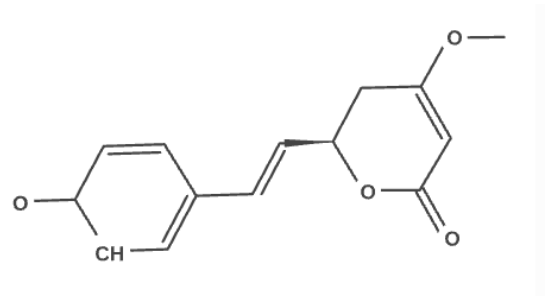

Figure 8. Skeletal representation of Intermediate 4

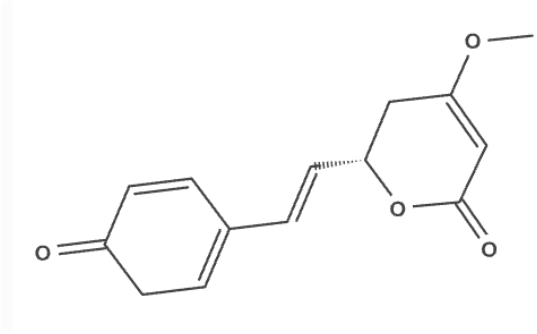

Figure 9. Skeletal representation of Intermediate 5

Methods

We used the Spartan program, a molecular modeling software developed by Wavefunction Inc., to model the hydroxylation intermediates in this study. The software offers various setups including Energy, Equilibrium State Geometry, Transition State Geometry, Equilibrium Conformer, Conformer Distribution, and Energy Profile. Each of these setups serves a different function and we used them in conjunction with semi-empirical quantum chemical models such as PM3, Hartree-Fock, density functional, and MP2 to calculate the lowest energy arrangement for a kavain-related molecule.

In this study, we chose the Equilibrium Geometry setup along with a PM3 calculation. We used the Equilibrium Geometry setup to calculate the lowest potential energy state in all dimensions of a specific hydroxylation intermediate. Equilibrium Geometry calculates the forces acting on each atom based on their potential energy surface and determines how to adjust the positions of those atoms to minimize the total potential energy. It then repeatedly adjusts the positions of the atoms until a local minimum on the potential energy surface is found, where the forces on all atoms reach approximately zero.

We chose PM3 because it was the most accurate calculation method applicable to systems with transition metals like the iron in the iron porphyrin used in this study. PM3 finds the most stable set of molecular orbital coefficients and then adjusts atom positions until it finds the lowest possible energy configuration. PM3 also involves approximations to the time independent Schrödinger equation $H\Psi = E\Psi$, which is the fundamental wave equation in quantum mechanics that calculates and provides an understanding of the energy levels and wavefunctions of a quantum system.

To create a hydroxylation intermediate, we either combined a pre-existing kavain molecule with an iron porphyrin or formed new bonds with oxygen/oxide atoms and broke existing double bonds using the various bond manipulation functions within the program. We drafted the intermediate and then minimized its energy using the "E minimize" function before running it through the Equilibrium Geometry and PM3 calculation to ensure accuracy of the result. After completing the calculations, we analyzed the hydroxylation intermediates for any outstanding chemical traits. We used the "measure distance" and "measure angle" functions to analyze the geometry of the intermediates, and the Higher Occupied Molecular Orbitals (HOMOs) function and electrostatic potential map to assess any signs of atomic orbital overlap and electron density (21).

Results

Analysis of the reactant kavain and the product 12-hydroxykavain

Kavain

Kavain contains cyclic ester, ether, and phenyl functional groups. Although analysis of its electrostatic potential map showed that the highest electron density was found at the cyclic ester group (Figure 10), the reactivity of kavain was found to mainly occur at the phenyl ring (17). This could have potentially been explained by analysis of HOMOs, which showed that there was strong atomic orbital overlapping and hence high electron density around the phenyl ring particularly at the para position (Figure 11).

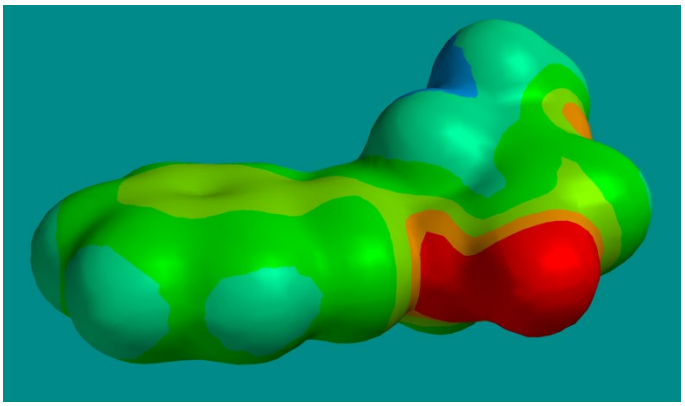

Figure 10. Electrostatic potential map showing high electron density at cyclic ester group

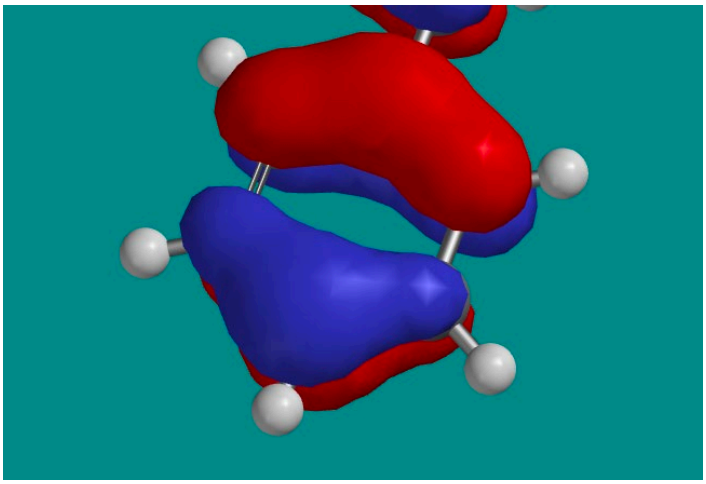

Figure 11. HOMOs showing overlapping atomic orbitals at phenyl ring

12-hydroxykavain

Addition of a hydroxy group O-H at C-12 position added to the presence of orbitals around the ring (Figure 12). Analysis of HOMOs also showed that there was strong electron density on one side of the oxygen atom despite significantly less density on the other side due to bonding with a hydrogen atom (Figure 13). This illustrated that the oxygen atom in O-H was still prone to attack by electrophiles and reinforced the finding by Tarbah et al. (12) that described 12-hydroxykavain existing in its glucuronide and sulfate forms.

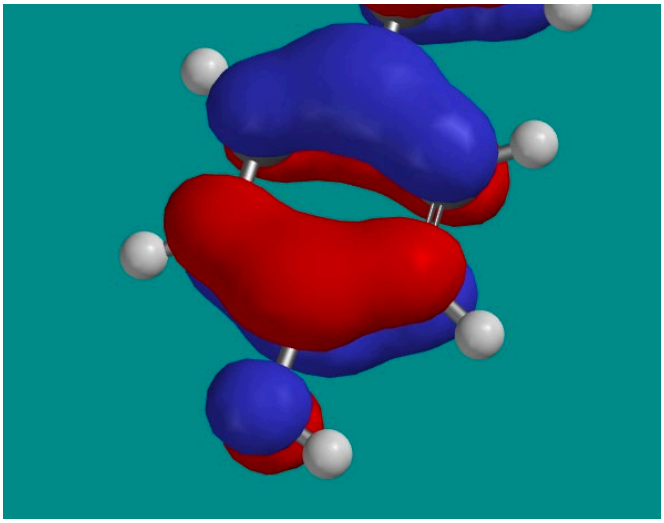

Figure 12. HOMOs showing atomic orbitals at oxygen of O-H group

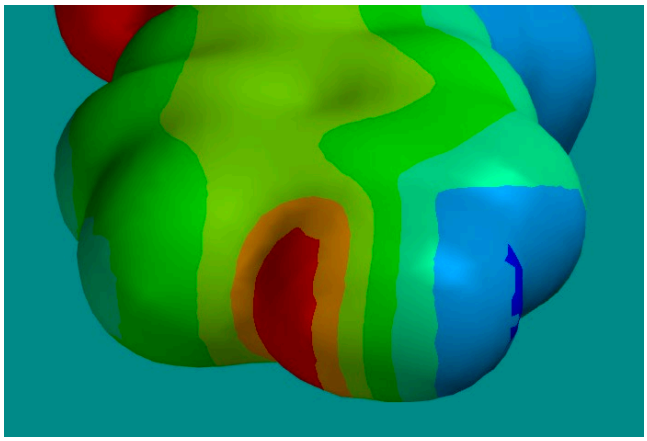

Figure 13. Electrostatic potential map showing strong electron density at the oxygen atom from the O-H group

Chemical Analysis of the Five Hydroxylation Intermediates

Intermediate 1

A strong feature of Intermediate 1 was the relatively close proximity between the iron atom of the iron porphyrin and C-11 of the phenyl ring, with a distance of 2.113Å. This short distance suggested that there was potentially some form of bonding between the two atoms. This hypothesis was further reinforced upon analysis of the interactions between the HOMOs of atoms where there was an evidently large atomic orbital overlap (Figure 14). This bonding was significant in that it also dictated the orientation of the kavain molecule as it bonded with the iron porphyrin molecule. It also seemed to affect the way in which the oxygen atom of the iron porphyrin attached to the kavain molecule, which can be seen through the bond angle of 97.47° around the oxygen atom of the iron porphyrin (Figure 15).

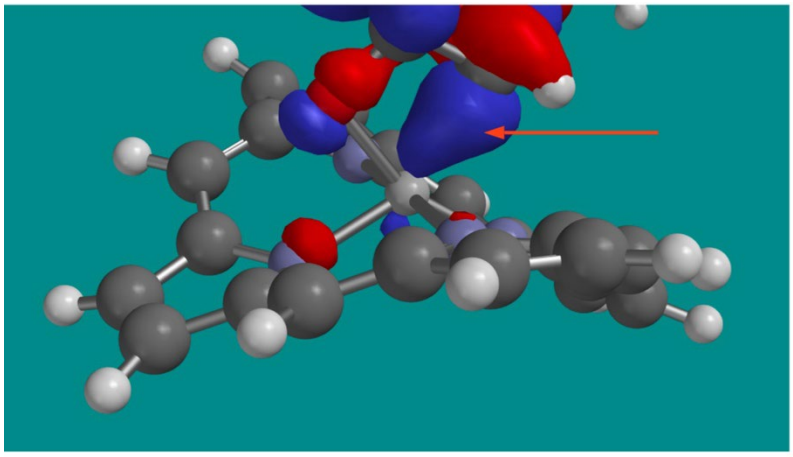

Figure 14. HOMOs showing atomic orbital overlap between C-11 and iron atom

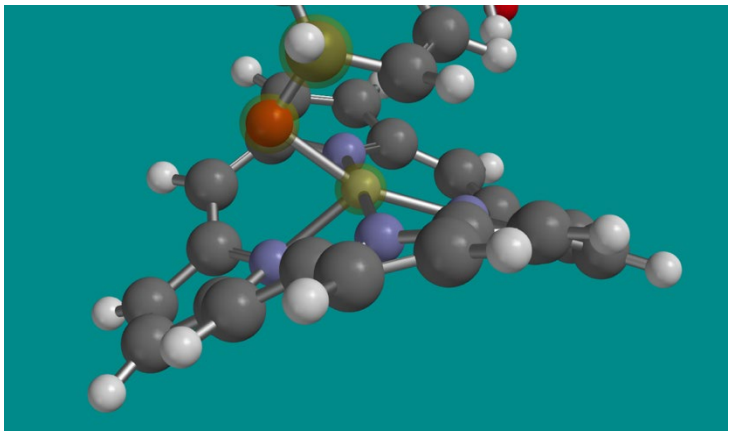

Figure 15. Bond angle around oxygen

Intermediate 2

Intermediate 2 also shared similar features to Intermediate 1. The distance between the C-11 atom of the phenyl ring and the iron atom was 2.029Å, which was a shorter distance than that found in Intermediate 1. This suggested an even stronger form of bonding between the two atoms. However, the bond angle around the oxygen atom measured at a larger angle of 99.08° than Intermediate 1. These two findings were slightly contradictory in that it was hypothesized that a shorter distance and thus stronger bonding would also lead to a smaller angle. The bond lengths measured around the oxygen atom of the iron porphyrin were almost identical to those in Intermediate 1, so the nuance in bond angles must have been related to some other factor. Upon analysis of HOMOs, there was a significant atomic orbital overlap spanning across from C-11 atom of the phenyl ring to the iron and oxygen atom of the iron porphyrin that was not found in Intermediate 1 (Figure 16). This orbital overlap could be a possible explanation for the different bond angles; however, more future research into this area is still needed.

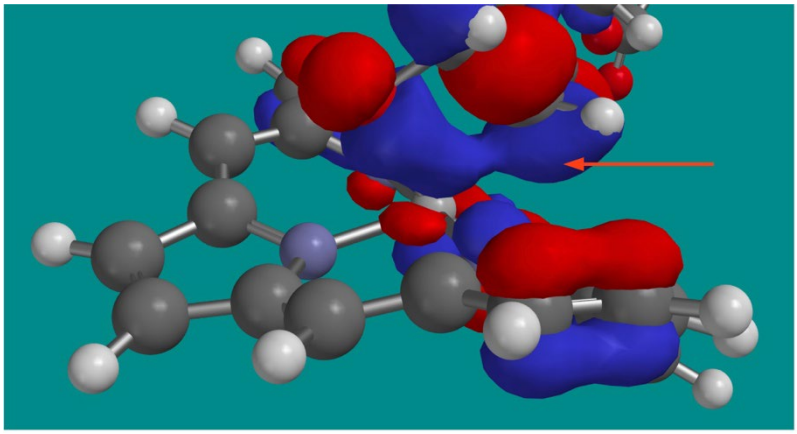

Figure 16. HOMOs showing significant atomic orbital overlap between C-11, iron and oxygen atoms

Intermediate 3

The main feature of Intermediate 3 was the triangular arrangement of the oxygen atom and the two adjacent C-11 and C-12 atoms of the phenyl ring. There was a cis arrangement where both hydrogens on the C-11 and C-12 carbons

were on the same side. The bond angles were all relatively close at 58.40, 58.46, and 63.14° while the distance of C-11 to oxygen and C-12 to oxygen were both approximately 1.440Å, showing that it could have almost been considered as an isosceles triangle (Figure 17). Considering how close the atoms were to each other and the isosceles triangle formation the atoms were in, this arrangement seemed rather unstable and unlikely to exist in human conditions.

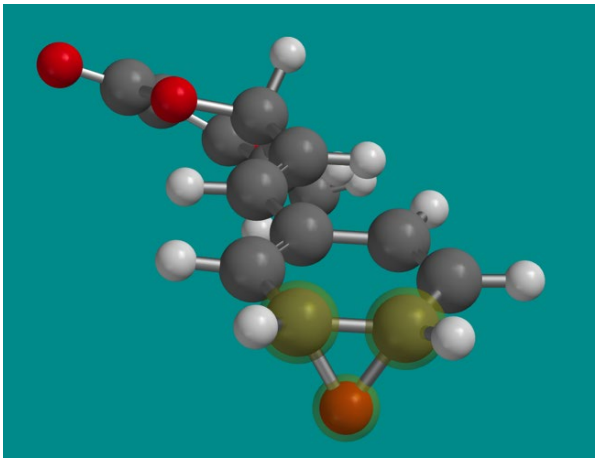

Figure 17. Isosceles triangular arrangement between C-11, C-12 and oxygen atoms

Intermediate 4

The most prominent feature of Intermediate 4 was the negatively charged oxide bonded to the C-12 atom. The bond angle between oxide, C-12 and C-11 (Figure 18) measured 58.45° which appeared rather irregular. A possible explanation for this arrangement was through analysis of HOMOs, where the electron repulsion created by the two electron dense pi bonds in the phenyl ring (Figure 19) forced the oxide into an awkward position. Additionally, it was interesting to note that this bond angle of 58.45° around this C-12 position practically did not change from the corresponding angle of 58.46° in Intermediate 3. The position of this oxide also prevented both the hydrogen atoms on C-11 and C-12 from remaining in planar positions (Figure 20). Such an arrangement would likely be too unstable to exist under human conditions.

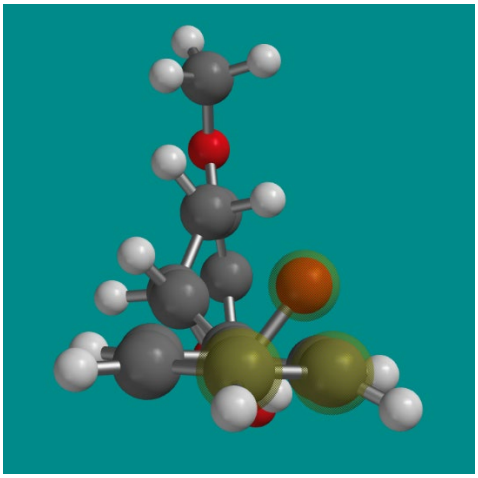

Figure 18. Bond angle around C-12 atom

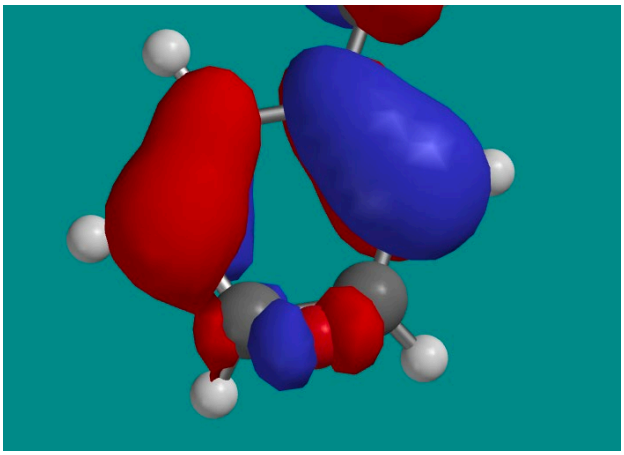

Figure 19. HOMOs showing pi bond orbitals at phenyl ring

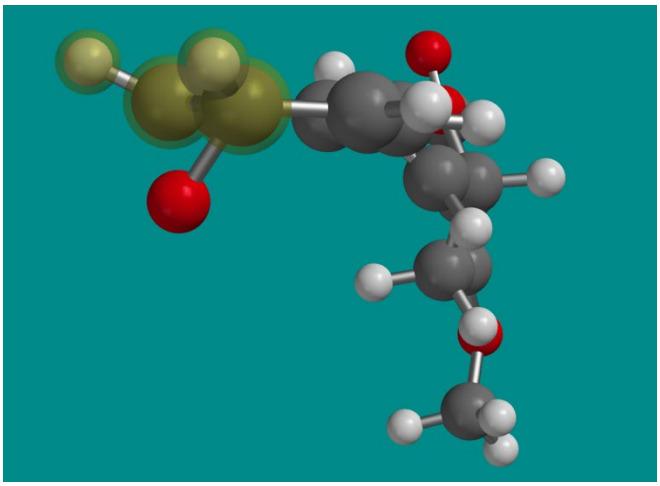

Figure 20. C-11 and C-12 atoms with non-planar hydrogen atoms

Intermediate 5

Intermediate 5 contained a ketone group and appeared to be relatively stable.

Energy

A comparison of the energy values (heats of formation) of the five intermediates was also recorded below (Table 1). Both Intermediates 1 and 2 had the most negative energy values while Intermediates 3 and 4 had the least negative. This further reinforced the instability of Intermediates 3 and 4.

Table 1. Energy Values of the five hydroxylation intermediates

Hydroxylation Intermediate:	Energy Values (kJ/mol):
Intermediate 1	-1023.42
Intermediate 2	-1013.04
Intermediate 3	-256.09
Intermediate 4	-254.15
Intermediate 5	-403.90

Discussion

After analyzing the Spartan calculations, Intermediates 1, 2 and 5 all seemed to be stable intermediates while Intermediates 3 and 4 were both unstable in their own ways and thus were unlikely to be viable intermediates that could exist in the hydroxylation mechanism. This was further reinforced by the fact Intermediates 3 and 4 had the highest set of energy values at –256.09 and –254.15 kJ/mol making them the most unstable (Table 1). Referring to Figure 3 with these findings in mind, the pathway involving Intermediates 3 and 4 can be ruled out, meaning that the remaining pathway of kavain to Intermediate 1 to Intermediate 2 to Intermediate 5 to 12-hydroxykavain would be more plausible. Alternatively, another pathway would be kavain to Intermediate 1 to Intermediate 2 to 12-hydroxykavain which would not even require the ketone (Intermediate 5) to be formed.

Having a stronger understanding of the metabolic pathway of Kava and in particular its major hydroxylation step could provide hints to the cause of hepatotoxicity of Kava ingestion. It is hoped that future studies on hepatotoxicity might use these findings on the suggested hydroxylation intermediates and mechanism pathway as a potential starting point for their research.

As for future research, experimental validation of these proposed hydroxylation pathways is needed. In vitro studies using human liver microsomes or in vivo studies using animal models could be performed to corroborate the findings from our computational analysis. Furthermore, the potential hepatotoxic effects of the metabolites produced during these hydroxylation processes should be investigated.

It would also be beneficial to investigate the specific role of the CYP2C19 enzyme in the hydroxylation process, as this could provide further insights into the metabolism of Kava and possibly pave the way for the development of targeted interventions to modify this process.

Lastly, while this study focused on the hydroxylation of kavain, future research could also explore the metabolic pathways of the other kavalactones in Kava to provide a more comprehensive understanding of its pharmacokinetics.

Conclusion

In this study, we utilized computational chemistry software, Spartan, to model the hydroxylation process of kavain, a key constituent of Kava, a beverage integral to Pacific Island cultures. The aim was to gain insight into the potential hepatotoxicity linked with Kava consumption, believed to be associated with its metabolic processes. Our investigation revealed that out of the five proposed intermediates involved in the hydroxylation process, three displayed stability, suggesting their significant roles in the process. This finding has allowed us to propose plausible reaction pathways for kavain hydroxylation.

While this study offers substantial insights into the metabolism of Kava, it is important to note that the results are based on computational models which may not fully emulate the conditions in the human body. The intermediates

modeled in our study might differ from those produced in laboratory synthesis, highlighting the need for further research involving both computational simulations and laboratory analyses. Despite these limitations, this research provides a more detailed understanding of the kavain hydroxylation step, a crucial phase in Kava metabolism.

Although Tarbah et al. (12) suggested a reaction pathway for the kavain intermediates found in the whole Kava metabolism process, there is still yet to be any published studies related specifically to the hydroxylation mechanism and this paper is likely to be the first. We believe our findings will guide future studies aiming to identify the mechanism leading to hepatotoxicity, thereby contributing to the development of safer and more effective Kava-based products. As research continues to unfold, it is crucial to bear in mind the cultural significance of Kava in Oceania and the potential for its use in treating various health conditions. Therefore, a balanced approach is needed, one that respects cultural traditions while also considering potential health risks and benefits. This research signifies a step forward in our understanding of Kava metabolism and provides a foundation for future studies in this important area.

Limitations

We used the Spartan program to simulate possible arrangements for the hydroxylation intermediates. However, these simulated arrangements may not match the intermediates produced by laboratory synthesis in future studies. Furthermore, the calculations we performed may not have fully replicated conditions in the human body. While we chose the Equilibrium Geometry setup as the most suitable for our study, the geometry optimization process might have constrained certain bond lengths and angles due to the theoretical assumptions inherent in the program.

The PM3 calculation was an approximation and might not have captured all aspects of the molecular behavior of the hydroxylation intermediates. More accurate representations of the intermediates might have been provided by Density Functional and Hartree-Fock calculations. However, due to the large size of the intermediates, the laptop we used could not process calculations more accurately than PM3. Future studies also using computational programs like Spartan should consider other forms of calculation as well and potentially compare the differences in the intermediates produced.

The Spartan manual (21) also did not provide any advice or suggestions for selection of different combinations of setups and calculations. Although we believe that Equilibrium Geometry and PM3 was the best combination for this study, we do not rule out the possibility that a different setup might have provided more accurate models of the hydroxylation intermediates.

Acknowledgments

I'd like to thank Professor Lectka for his help and constant dedication over the past few months. I'd also like to thank my high school chemistry teacher Mr. Haq for providing me with valuable tips for writing this paper.

Conflict of Interest

The author of this paper states that there were no conflicts of interest

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