FUNGI FICTION: ANALYTICAL INVESTIGATION INTO THE CHURCH OF PSILOMETHOXIN'S ALLEGED NOVEL COMPOUND USING UPLC-HRMS

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ABSTRACT: The Church of Psilomethoxin claims to produce a novel tryptamine by adding 5-MeO-DMT to the substrate of cultivated *Psilocybe* mushrooms, which is then biosynthesized into psilomethoxin, the church's sacrament. In this study, we investigate the validity of this claim using comprehensive analytical techniques, namely ultra-performance liquid chromatography with high-resolution mass spectrometry (UPLC-HRMS). Authentic reference standards for structurally related tryptamines were used for comparison. Our findings revealed no evidence to suggest that the compound psilomethoxin is present in samples of material that the church is offering to their members online. Psilocybin, baeocystin, and psilocin, were, however, unambiguously identified in the sample, suggesting that the claims regarding the biosynthesis of psilomethoxin may be misguided. The implications of these findings should be critically considered within the context of public health and safety.

Introduction

The Church of Psilomethoxin (http://psilomethoxin.com) has recently gained attention for its claim to produce a novel compound by incorporating 5-MeO-DMT into the growth medium during the cultivation of *Psilocybe* mushrooms. This compound, referred to as psilomethoxin (**Figure 1**), is purported to have unique properties and effects compared to traditional psychedelics. According to their website, the Church states that by modifying the growth conditions and supplementing the substrate with 5-MeO-DMT, the mushrooms can biosynthesize psilomethoxin, which is alleged to offer a distinct and enhanced psychedelic experience. However, there has been no scientific evidence to support these claims and raises concerns regarding the safety and efficacy of the material being distributed.

The proposed method for producing psilomethoxin within the mushrooms challenges the established understanding of psilocybin biosynthesis, which involves a series of enzymatic reactions, including the action of the enzyme PsiH, a monooxygenase that catalyzes the hydroxylation of the indole ring of tryptamine at the four position (Figure 2).[1–6]

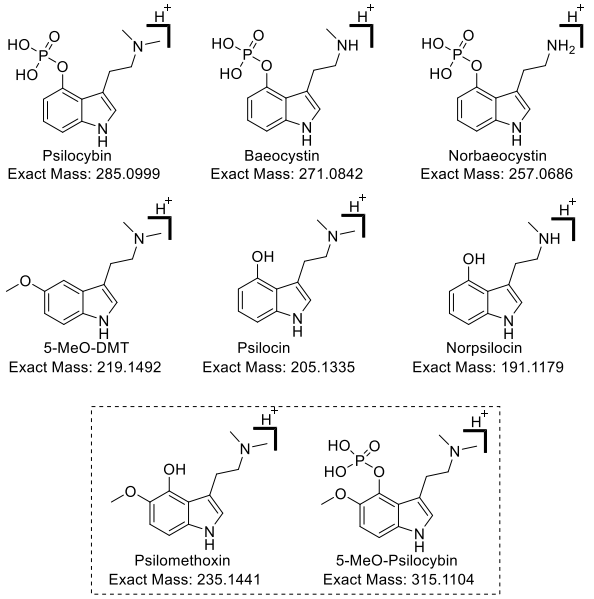

Figure 1. Structures for tryptamine references, psilomethoxin, 5-MeO-psilocybin, and corresponding monoisotopic masses for protonated adducts.

The metabolism of 5-MeO-DMT by *Psilocybe*, as claimed by the Church, would require an alternative biosynthetic pathway or modifications to the existing one. Given structural dissimilarities between 5-MeO-DMT and tryptamine, the endogenous substrate for PsiH, the substitution of 5-MeO-DMT as an enzymatic substrate to produce the purported psilomethoxin is unexpected without significant alterations to the enzymatic specificity and function. As a result, the Church's assertions regarding the production of psilomethoxin in mushrooms appear to be unsubstantiated.

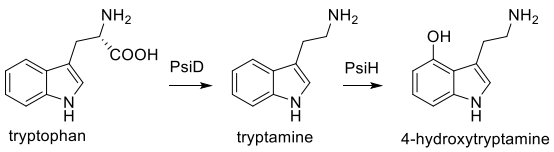

Figure 2. Enzymatic actions of PsiD/PsiH in *Psilocybe*.

This study aims to provide an in-depth analysis of the alleged psilomethoxin compound by comparing it to authentic reference standards of structurally related tryptamines using UPLC-HRMS. This will enable a comprehensive understanding of the composition of the material and determine whether the Church's claims are substantiated by scientific data or if the material is merely a mix of known tryptamines or other compounds.

Experimental

Sample Collection: Samples of the purported psilomethoxin material were obtained from the Church of Psilomethoxin through a donation from an anonymous church member. The samples were stored protected from light and ambient atmosphere until analysis.

Sample Preparation: The psilomethoxin samples were prepared by carefully opening the supplied capsule (**Figure 3**, 480 mg gross mass), measuring 100 mg of the tan powder, and sonicating this material in anhydrous methanol (1 mL) for 1 hour while protected from light. The extracts were then centrifuged and 100 µL of supernatant was added to 900 µL deionized water to provide dilutions for UPLC-HRMS injection.

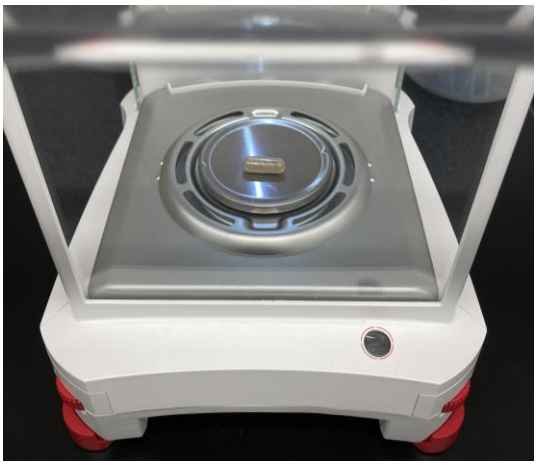

Figure 3. Material as received from the Church of Psilomethoxin

Authentic Reference Standards: Authentic reference standards for structurally related tryptamines, including psilocybin, psilocin, 5-MeO-DMT, baeocystin, norpsilocin, and norbaeocystin, were produced in-house by reported methods.[7–10] These standards were used for comparison in the UPLC-HRMS analysis to identify and quantify the presence of related compounds in the psilomethoxin samples.

UPLC-HRMS Analysis: UPLC-HRMS analysis was performed using a Waters Xevo G2-XS qToF in ESI positive mode. Analytical UPLC was performed with a Waters Acquity I-Class UPLC utilizing Waters HSS T3 (2.5 µm, 2.1 mm x 30 mm) run in gradient mode with H_2O (0.1% formic acid) and acetonitrile (0.1% formic acid) mobile phases at 0.6 mL/min.

Data Analysis: Data obtained from the UPLC-HRMS analysis were processed using Waters Unifi v1.9. Chromatograms and mass spectra were compared with those obtained from the analysis of authentic reference standards to identify the presence of related compounds in the psilomethoxin samples.

Results and Discussion

A tryptamine standard test mix was prepared, containing psilocybin, psilocin, 5-MeO-DMT, baeocystin, norpsilocin, and norbaeocystin (**Figure 1**). These compounds were added to a targeted screen library and detected based on the high-resolution mass for the protonated adducts of each.

5-MeO-psilocybin is a hypothetical compound that combines the structural features of both psilocybin and 5-MeO-DMT. It is characterized by a 4-phosphorylated indole ring. Presumably, if psilomethoxin is biosynthesized from 5-MeO-DMT through the same pathway that hydroxylates psilocin, then the subsequent step that phosphorylates psilocin to psilocybin via PsiK may also take place to produce 5-MeO-psilocybin. By also examining the potential presence of 5-MeO-psilocybin in the sample, our objective was to thoroughly assess the plausibility of the proposed metabolic pathway.

Psilomethoxin and 5-MeO-psilocybin were added to the library and targeted based on their corresponding exact monoisotopic masses. The results from the analysis of the reference standard mix can be found in **Figure 4**. The elution order for these compounds aligns predictably with reversed-phase chromatography, considering the polarity of the structures. If present in the sample, psilomethoxin would likely appear with a retention time between that of psilocin and 5-MeO-DMT at approximately 1.3-1.6 minutes.

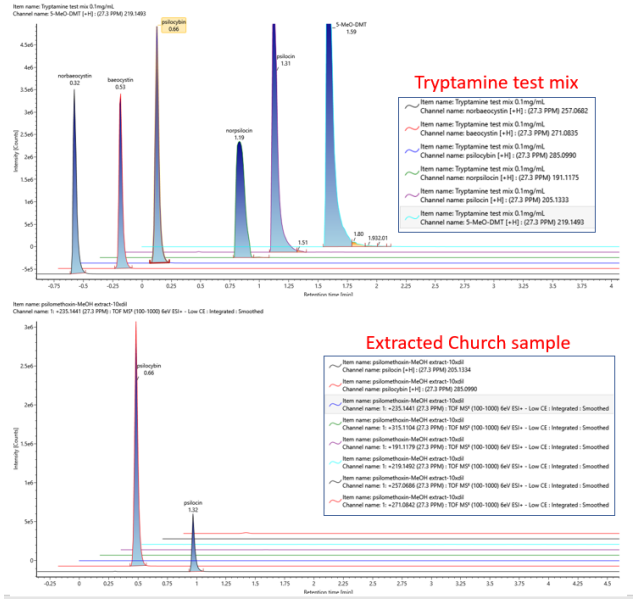

Figure 4. (Top) Selected ion chromatograms for tryptamine test mix. (Bottom) Selected ion chromatograms for Church of Psilomethoxin sample indicating that psilocybin (m/z 285.0999) and psilocin (205.1335) were readily extracted and within detection thresholds, whereas blue trace (m/z 235.1441) corresponding to psilomethoxin monoisotopic protonated mass was indistinguishable from the baseline noise.

The same screen was applied to the extracted sample from the Church. Upon analysis, only psilocybin and psilocin were

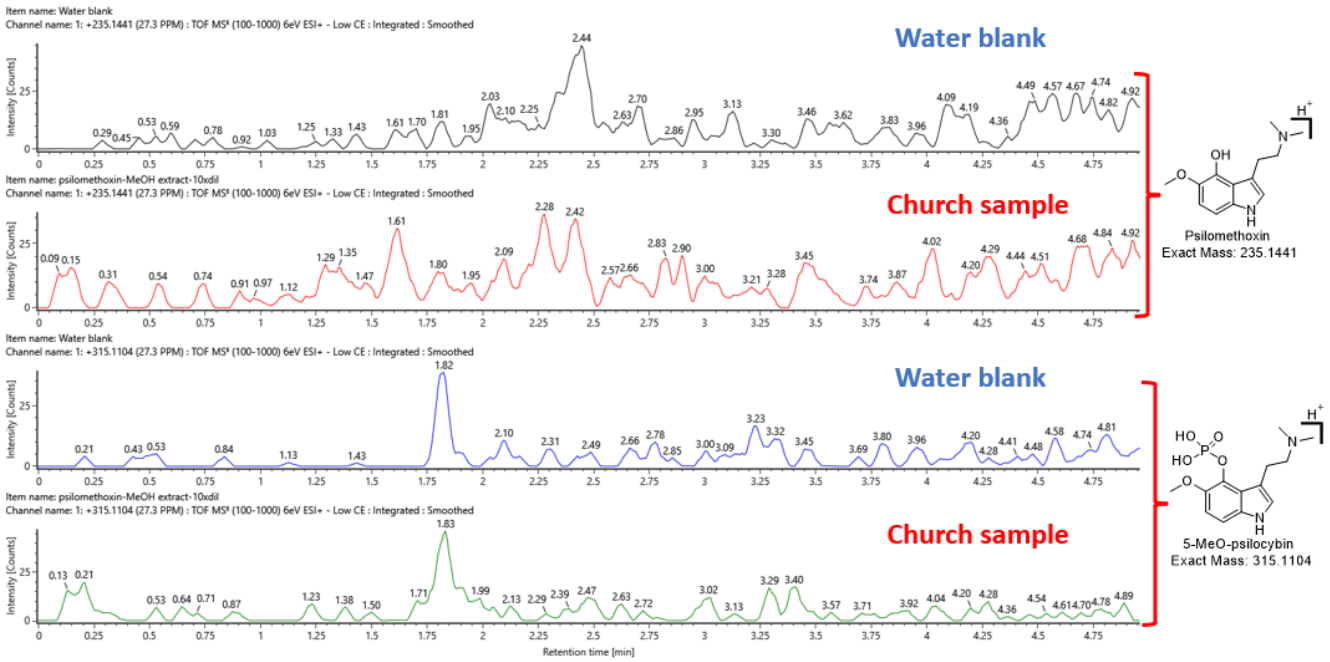

Figure 5. Expanded view of selected ion traces for psilomethoxin (m/z: 235.1441) and 5-MeO-psilocybin (m/z: 315.1104) with church sample compared to water blank runs indicating target compound detection was indistinguishable from baseline noise

detected with about the same sensitivity as the test mix (**Figure 4**). A trace amount of baeocystin was also detectable in the sample. However, close inspection of the selected ion chromatograms for psilomethoxin and 5-MeO-psilocybin were indistinguishable from blank runs (**Figure 5**), indicating that these compounds were not detected in the sample. Given the robust recovery and detection response from both psilocybin and psilocin in this extracted sample, if psilomethoxin or 5-MeO-psilocybin were present at levels even several orders of magnitude lower than the known tryptamines, a signal would have been readily detected.

These results strongly indicate that the material distributed by the Church of Psilomethoxin does not contain the novel compound psilomethoxin (or 5-MeO-psilocybin) as claimed. Instead, the sample mainly consists of known tryptamines and other natural products expected to be present in dried *Psilocybe* mushrooms, specifically psilocybin and psilocin, with a trace amount of baeocystin.

Given that psilomethoxin was not detected in the sample, we conducted additional targeted analysis to investigate whether the Church might be adding other psychoactive substances to the material, which might account for the atypical effects self-reported by users in a growing number of reports online. This analysis aimed to determine if the sample had been potentially adulterated with other psychoactive research chemicals. HRMS was employed to compare additional ions identified in the sample against comprehensive libraries of common synthetic psychoactive tryptamines and phenethylamines. The results provided no indication of the presence of adulterating substances within the sample, other than the indicated materials corresponding to small molecule substances expected to be present in a methanolic extract of dried fungi. Taken together, these data lead us to consider the likely possibility that the reported unique effects could be attributed to the placebo effect. As the users' expectations and beliefs surrounding the compound might have influenced their perception of its effects, the placebo effect may adequately explain the observed experiences, which were likely elicited by a low dose of "Magic Mushrooms."

Conclusion

Considering the experimental results obtained through the UPLC-HRMS analysis of the Church of Psilomethoxin's sample, it is evident that their claims of producing a novel compound, psilomethoxin, by incorporating 5-MeO-DMT into the substrate of cultivated *Psilocybe* mushrooms are more akin to "fungi fiction" than reality. The absence of any detectible psilomethoxin or the hypothetical compound 5-MeO-psilocybin in the analyzed sample demonstrates that the Church's assertions are not yet supported by scientific evidence.

Instead, our analysis revealed that the material distributed by the Church primarily contains known tryptamines, specifically psilocybin and psilocin, with a trace amount of baeocystin. The lack of evidence of novel compounds in the sample coupled with the implausibility of the proposed biosynthetic pathway suggests that the Church of Psilomethoxin is engaging in misleading marketing practices and may be misrepresenting the material that they are distributing.

It is crucial for the scientific community to continue scrutinizing such claims and provide accurate information to the public, ensuring that the distribution and use of psychedelic substances are based on factual data and not on unsubstantiated assertions made by organizations like the Church of Psilomethoxin.

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Notes

The authors are employed by a non-profit medical research organization that is currently conducting clinical trials with psilocybin. The organization had no role in the design, analysis, or interpretation of the present study. This research was conducted in the interest of advancing scientific understanding and does not directly benefit any ongoing trials conducted by the organization.

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