by arclightshroom

EXTRACTION OF PSILOCIN FROM PSYCHOACTIVE MUSHROOMS AND SYNTHESIS OF PSILACETIN

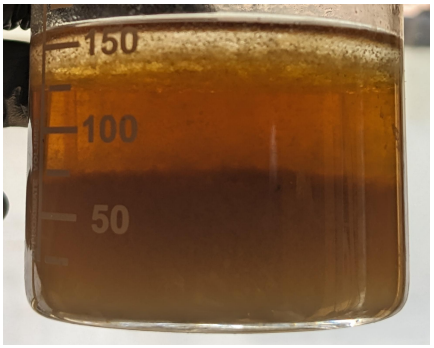
Psychedelic mushrooms are possibly the most popular psychedelic there is due to their history, extensive clinical research and easiness to source. Psilacetin, or 4-Aco-DMT is a convenient form of storage for the mushroom's main alkaloid, which is otherwise too sensitive to be kept as a pure extract. This work serves to explore the conversion of mushrooms to shelf-stable psilacetin with various acetylation methods, from the traditional acetic anhydride acetylation to GAA with metal catalysts.

Extracting and isolating psilocin

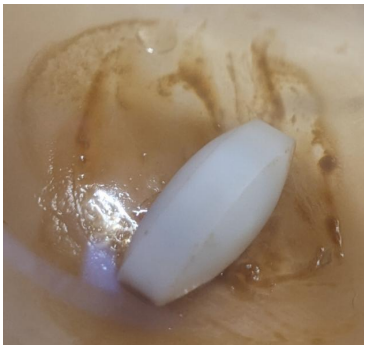
Acetic acid extraction:

10g of dried Psilocybe cubensis are crushed into a fine powder and stirred in 10% acetic acid for an hour. The sludge is then heated to 70°C and kept at temperature for 10 minutes, after which the fungal matter is removed with vacuum filtration through cotton. After reaching room temperature concentrated ammonia is slowly added with stirring until pH 8, then extracted as quickly as possible with 2x20mL ethyl acetate. The organic layer was washed with brine and dried with sodium sulfate. 180mg of crude psilocin is recovered as a brown crust after solvent removal in a warm water bath under vacuum.
NOTE: Psilocin can be recrystallized from boiling hexane or DCM:hexane (1:3) to yield white crystals, but I prefer to purify the more stable product later.

Mushrooms used for the experiment



Gelatinous mass precipitating at high pH

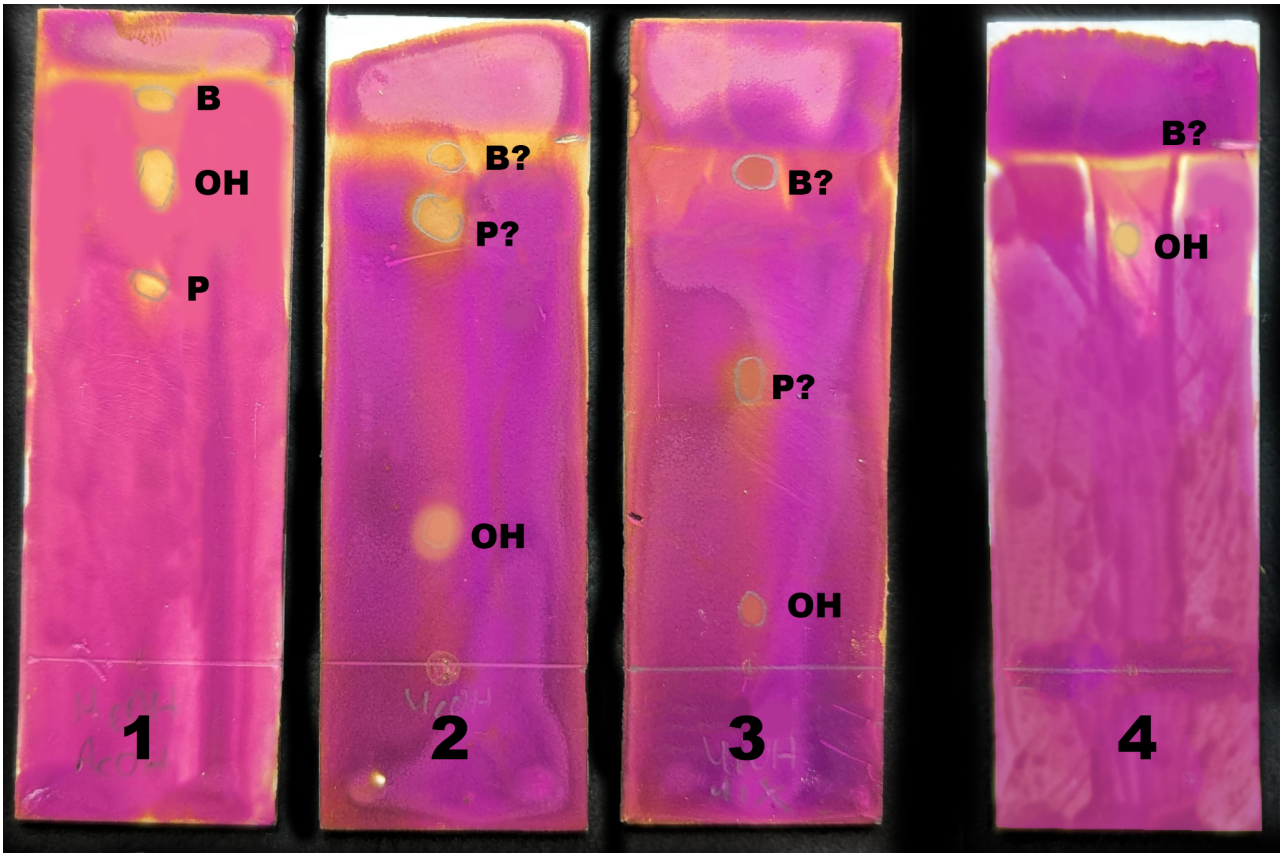


Crude psilocin residue

Methanol extraction:

10g of dried Psilocybe cubensis are crushed into a fine powder and stirred overnight in 150mL 75% methanol. 5mL of concentrated HCl are added and the mixture is refluxed for 15 minutes with a balloon of nitrogen, after which the mixture is quickly vacuum filtered over a fritted glass funnel and the filtrate distilled dry under vacuum. To the brown residue a mixture of 10mL DCM and 10mL 3% HCl is added, the organic phase is discarded and the aqueous basified to pH 8 with concentrated ammonia. The cloudy solution is extracted with DCM, which is dried over sodium sulfate and removed under vacuum to yield 215mg of psilocin as a tan residue.

TLC analysis:
Visualized with $KMnO_4$ stain



1. Crude methanol extract, eluent EtOH:GAA:water (12:3:5): Identification plate 1, Rf doesn't match the one from the paper but they used n-butanol instead of ethanol. Distance and order of the points is identical though, they are just closer to the top
    - Psilocybin (P) Rf: 0.65
    - Psilocin (OH) Rf: 0.81
    - Baeocystin (B) Rf: 0.96
2. Crude methanol extract, eluent MeOH:water (98:2): Identification plate 2, Rf of psilocin matches perfectly (Rf = 0.2); paper doesn't give Rfs for psilocybin and baeocystin, but i assume that they are the top points. B? Rf = 0.96, P? Rf = 0.86.
3. Crude methanol extract, eluent MeOH:hexane:NH_3 (20:10:1): I made up this solvent system. The order of the points is the same as the previous but they are better separated. OH Rf = 0.1, B? Rf = 0.44, P? Rf = 0.92.
4. DCM extract after HCl treatment, eluent EtOH:GAA:water (12:3:5): Only psilocin present, psilocybin spot undetected. Possible baeocystin contamination. OH Rf = 0.81.

DISCUSSION & NOTES: While the methanol extraction is slower, it's my go-to method due to the ease of its workup. The acetic acid extract is very thick due to the presence of cell lysate and proteins; this makes filtering slower, emulsion-prone when solvent-extracted and if the pH becomes too basic a large amount of jelly like substance precipitates out making things even more difficult. The methanol extract also yields a cleaner product, probably because of less air exposure due to the time saved during filtration and extraction.
Psilocin is very prone to oxidative degradation once dephosphorylated, so it has to be handled quickly and used immediately after. Inert atmosphere is not necessary but makes things

easier. In any case the entire procedure after the acidic dephosphorylation of psilocybin must be carried out quickly and diligently.
In case an emulsion forms during solvent extraction, the quickest way to resolve it is to separate as best as possible the emulsified layer, take as much water out as possible by adding brine (small amounts multiple time works best), then add a large amount of sodium sulfate and stir for a bit.
100% methanol is not a very good solvent for extraction, according to literature (cited below) the optimal extraction solvent is dilute aqueous acetic acid or 75% short alcohols (methanol, ethanol).
Albino mushrooms are better for isolation purposes, as they don't contain pigments that get carried over with the alkaloids.

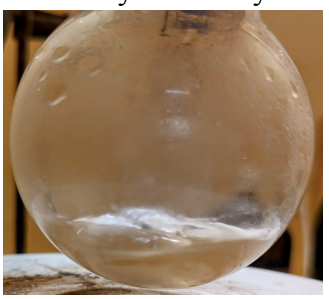
Synthesis of 4-Aco-DMT

Acetic anhydride acetylation:
To the 215mg psilocin residue is added 121mg of acetic anhydride (1.1 molar eq.) and 9mg sodium acetate (10% molar eq.). The solution is then stirred at room temperature over a nitrogen balloon overnight. 15mL of water were then added with strong stirring until all of the acetic anhydride was destroyed, followed by concentrated NaOH solution to pH 10 and extraction with 3x20mL DCM. The organic layer is dried with sodium sulfate and desolventized under vacuum to yield 4-Aco-DMT freebase as an off-white solid. The residue is redissolved in minimal hot methanol (approx. 15mL, add until dissolution), 86mg fumaric acid in 5mL methanol is added and everything is stirred until all the solids dissolved. After approx. half of the methanol has evaporated 265mg of the product precipitates as white fluffy crystals, final yield 73%.


Clean fumarate precipitating from methanol

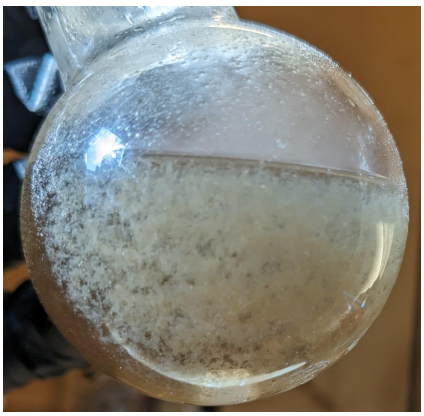

Anhyd. ethanol washing of impure fumarate

NOTE: Impure yellow fumarate can be purified by triturating in anhydrous ethanol, which will dissolve the colored impurity. Nitrogen significantly improves the yield and quality of the final product, which barely needs any further purification.



8h reaction time with (1) and without (2) nitrogen atmosphere.

Cobalt chloride acetylation:
To 200mg psilocin is added 10mL GAA and 12mg $CoCl_2 \cdot 6H_2O$ (5% molar eq.), the solution is then stirred at 75°C for 24h over a nitrogen balloon. After cooling to room temperature a concentrated ice cold solution of sodium hydroxide is added until pH 10, then 10mL of DCM are added with stirring and the biphasic mixture filtered over cotton. The aqueous layer is extracted with 10mL more DCM, the pooled organic extracts dried over sodium sulfate and desolventized. The residue is redissolved in hot methanol and treated with 80mg fumaric acid. Solvent removal and absolute ethanol washings yielded 135mg 4-Aco-DMT fumarate with a 38% yield.
NOTE: Blowing air to evaporate the methanol introduced a significant amount of water that kept the fumarate salt dissolved. To fix this the solution was evaporated to dryness with heat and the brown residue stirred in 2mL absolute ethanol, then filtered and the powder washed with 1mL more ethanol. This process effectively gets rid of the impurities leaving white crystals behind.
After addition of NaOH, insoluble cobalt hydroxide precipitates as a brownish powder. The majority of it gets removed with the first filtration, but a small amount gets dissolved in the organic solvent. After the DCM was removed and the residue redissolved in methanol all the cobalt salts stay behind as a brown precipitate. Filtering the cloudy methanol gets rid of metal residue, leaving behind a clear slightly yellow solution. A simple way to test if metal contamination is present is to simply drop a sample of 4-Aco-DMT fumarate in conc. HCl, if there's residual cobalt the solution will turn bright green.
The theoretical GAA volume needed is 2mL for 200mg psilocin, but I wanted to work with larger volumes. For scaling up one should consider 1mL per 100mg.

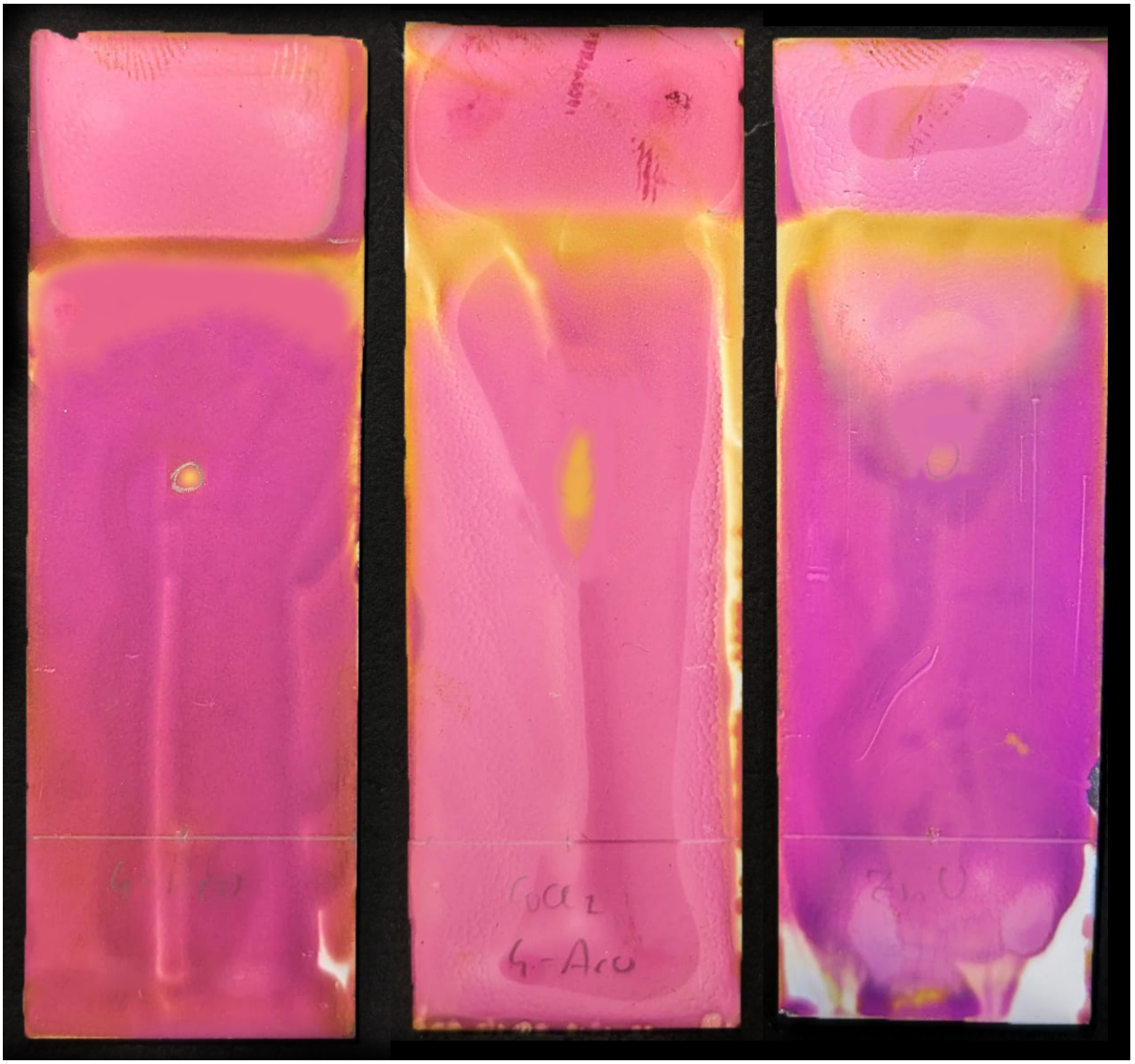


4-Aco-DMT freebase

Zinc oxide nanoparticles acetylation:
To 121mg psilocin is added 5mL GAA and 3mg ZnO (5% molar eq.), the solution is then refluxed for 3 hours over a nitrogen balloon. Workup is done identically to the cobalt chloride run, to yield a few mgs of 4-Aco-DMT, yield was around 5%, too depressing to be calculated exactly.
NOTE: One of the possible reasons for my failure was the use of standard ZnO instead of ZnO nanoparticles, but I wanted to see the result with standard zinc. ZnO nanoparticles can be prepared as followed:
110mg of zinc acetate are dissolved in 60mL IPA, and a cold solution of 40mg NaOH in 15mL IPA is added with stirring. The solution is heated in the microwave for 5 minutes, then

centrifuged to yield a white powder, which is heated at 600°C for 1 hour to (allegedly) yield ZnO nanoparticles in a 70% yield.
This is the procedure reported in the paper, which I didn't try myself, as it was too much work compared to the other routes. The paper reports 2x yield with nanoparticles compared to bulk ZnO, but 2x of 5 is still a 10% yield.

TLC analysis:
Eluent, MeOH:GAA:H_2O (75:10:15), visualized with $KMnO_4$ stain



1. Acetic anhydride run, DCM extract, prior to purification. Single spot, Rf = 0.53, matches with literature.
2. Cobalt chloride run, DCM extract, prior to purification.
3. Zinc oxide run, DCM extract, prior to purification. Faint spot. I noticed that all the samples had a large band that traveled with the solvent system, consistent with the B? band on the extraction TLC, but bigger. Considering that it's bigger here, especially in the runs with lower yields, I suspect this is some decomposition byproduct of psilocin. Fortunately it disappears after the EtOH washings.

Sources:

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