Analysis of Acid Catalyzed Hydrolysis Products of Cannabis (Cannabaceae) Exudates

A Senior Studies Report

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This undergraduate thesis is dedicated to my parents, Ray and Susan Peirson

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ABSTRACT

Products of reactions with phloroglucinol (1,3,5-benzenetriol) and isoprene were purified and analyzed with column and thin-layer chromatography methods. Products were compared with samples recovered from exudates of marihuana (Cannabis sativa L.) and with products of pure phloroglucinol and HCl. Results afforded the conclusion that an unknown residue present in acid hydrolysis of marihuana exudates is the product of a reaction between phloroglucinol and isoprene. Other possibilities are discussed.

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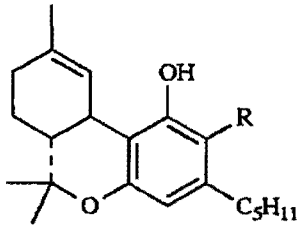
Products of reactions with phloroglucinol (1,3,5-benzenetriol) and isoprene were purified and analyzed with column and thin-layer chromatography methods. Products were compared with samples recovered from exudates of marihuana (Cannabis sativa L.) and with products of pure phloroglucinol and HCl. Results afforded the conclusion that an unknown residue present in acid hydrolysis of marihuana exudates is the product of a reaction between phloroglucinol and isoprene. Other possibilities are discussed.

INTRODUCTION

Cannabis sativa L., commonly known as marihuana (marijuana, hemp), has long inspired study, both because of its unique representation of plant life and because of its exploitation as an illicit drug. Despite the long-term human interest in the hearty plant, only as recently as the early 1940's did botanists and biochemists begin to unlock the complexity of its chemical pathways. The isolation of several terpenophenolic compounds, unique to the cannabis plant, known as cannabinols, has been an endeavor that has received much focus. The primary cannabinol of interest, Δ^9-tetrahydrocannabinol (***1***), (THC) is abundant in the plant, and is known to induce hallucinogenic and euphoric states in the human nervous system.[1]



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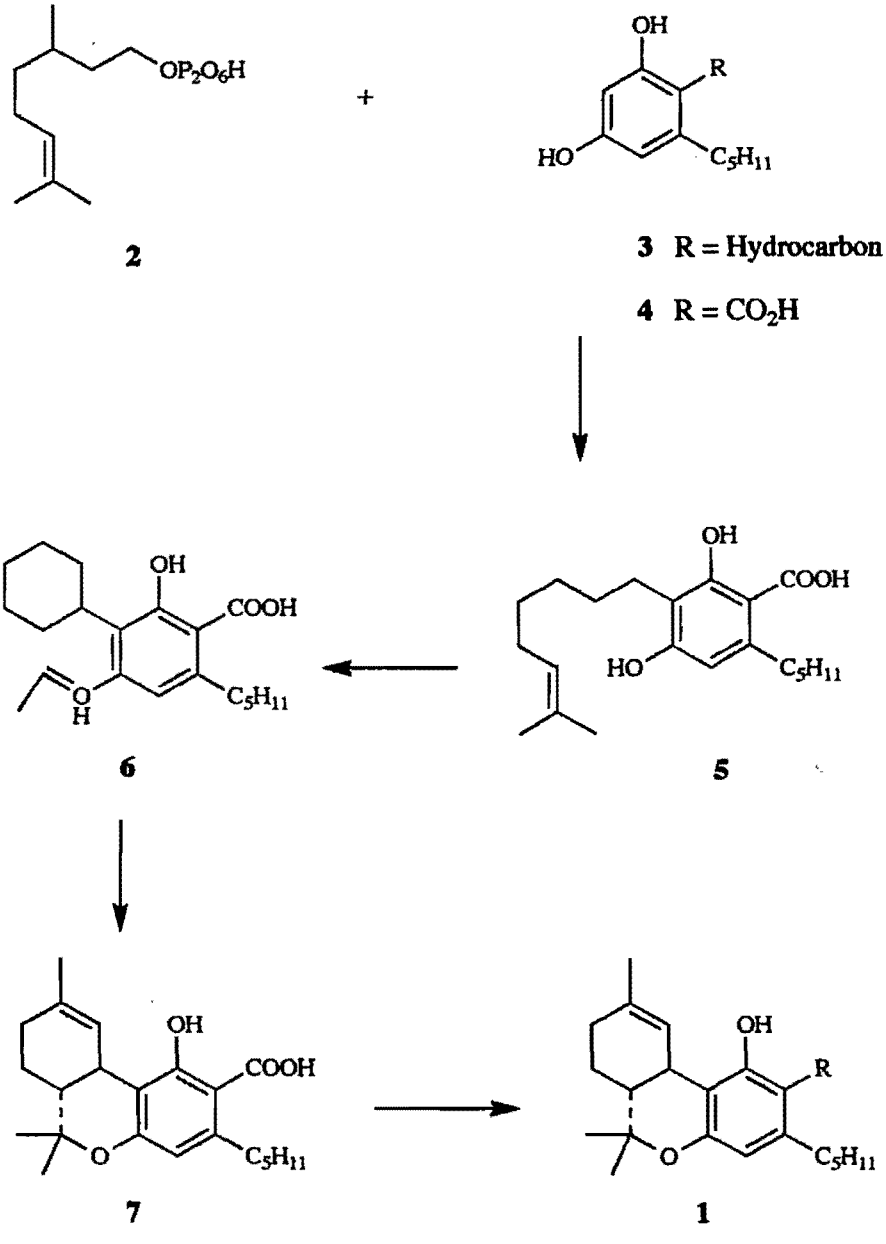
The synthesis of THC in the plant, however, is not well understood. Generally, the most accepted pathway to THC involves a reaction between geranyl pyrophosphate (***2***) and olivetol (***3***) or olivetol carboxylic acid (***4***) (olivetolic acid), to ultimately produce cannabigerolic acid (***5***) (CBGA) or cannabidiolic acid (***6***) (CBDA).[1] The latter is hypothesized to be the final precursor to Δ^9 –tetrahydrocannabinolic acid (*7*) (THCA) which leads to THC.[2] (***Scheme 1***)

SCHEME 1



2

3 R = Hydrocarbon

4 R = CO_2H

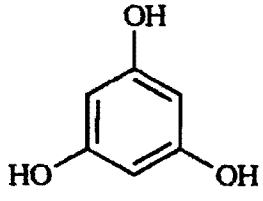
5

6

7

1

Olivetol, however, is not detected in any stage in the life of the marihuana plant. Also, olivetol is not found within the cannabis petioles where the production of THC is suspected. Such petiole exudates exhibit a reddish color suggesting the presence of oxidized phenols. A study for phenols by Hammond and Mahlberg indicates the abundant presence of phloroglucinol (***8***) as the only phenol in the cannabis glands. Since olivetol is not in the crucial glandular tissue involved with THC synthesis, it is suspected that phloroglucinol is the phenol precursor to CBGA and CBDA in the reaction with geranyl pyrophosphate, rather than olivetol.[3]



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Hammond and Mahlberg report that the Harborne and Williams[4] method for analyzing and purifying THC produces an unknown by-product. After the acid hydrolysis and work-up they observed an unknown, more dense, and reddish compound resting at the bottom of the test tube.[5]

Recently, Ahluwalia et al., investigated the reaction of isoprene (***9***) with phloroglucinol catalyzed by orthophosphoric acid.[6] Isoprene is a terpene known to naturally exist in laticifers, including the laticifers within the marihuana plant and it may be the starting point for the synthesis of monoterpenes. As discussed above, it is

believed that a monoterpene, such as geranol or geranyl pyrophosphate reacts with olivetol or phloroglucinol to lead to produce compounds that eventually lead to THC.[7] Dichromans (***10***), (***11***), and (***12***) are reported as products in Ahluwalia's condensation reaction. (***Scheme 2***)

This study compares the products of the Ahluwalia reaction with the compounds isolated from the acid hydrolysis of phloroglucinol and hydrochloric acid in an attempt to identify the unknown by-product observed by Hammond and Mahlberg.

SCHEME 2

RESULTS AND DISCUSSION

Ahluwalia et al. report that compounds (***10***), (***11***), and (***12***) were obtained by adding a solution of isoprene (1.5ml) in light petroleum (5.0 ml) to a stirred mixture of phloroglucinol (2.0 g), orthophosphoric acid (85%, 2.0 ml), and light petroleum (5.0 ml) at 30-35 °C over two hours. The extract was washed with water, dried (Na_2SO_4), and distilled. The mixture was found by thin-layer chromatography (TLC) to be three separate compounds. The products were then separated by column chromatography.[6]

Initially, we observed some difficulties with the administration of phloroglucinol because orthophosphoric acid and phloroglucinol make a thick slurry that is difficult to stir. The phloroglucinol does not go completely into solution and remains a whitish color. After isoprene was added to the phosphoric acid and phloroglucinol solution, the slurry became yellow. Before the end of the reaction it was a deep purple color.

We extracted the products of the first experiment with benzene, and after removal of solvent, a very small amount of yellowish oil remained. The water layer, however, was very concentrated and eventually turned dark purple. This was consistent throughout the study. One low-polarity compound (Product **A**) with an R_f of 0.05 was identified with TLC. The purple water layer revealed a compound with a high polarity R_f of 0.77 (Product **B**).

To avoid the problems experienced in the first experiment, subsequent reactions were performed in the absence of light and such a radical color difference was not noted. The water layer was allowed to present in light and it eventually turned the dark purple color. It seems likely, then, that the purple color observed in the water layer is the result of the reaction mixture's exposure to light.

Ethanol was added to the second experiment to bring phloroglucinol into solution. The flask was covered with aluminum foil to protect the reaction from light and after the work-up both the product from the benzene layer (Product **A**) and the product from the water layer (product **B**) were also kept in darkness. Product **A** had an R_f of 0.11. It strongly adhered to the wall of the test tube and was an oil with a red-yellow color. The water layer was re-extracted in chloroform and TLC revealed a compound (Product **B**) with a high polarity R_f of 0.7. It was observed to be an oil with a red color after purification using column chromatography.

In order to eliminate the possibility that our products were not the result of a reaction between isoprene and phloroglucinol, we performed a control experiment to test our methods. Pentane was added to phloroglucinol and phosphoric acid under the same conditions as before. No color change was observed and the water layer remained yellowish and did not change color. Also, no compound was isolated in TLC, suggesting that any compounds found after the reaction were, indeed, the products of a reaction between phloroglucinol and isoprene.

In order to determine phloroglucinol's stability under the acid conditions used to purify marihuana exudates, we ran an experiment with only phloroglucinol and hydrochloric acid. Pure phloroglucinol was heated with hydrochloric acid for twenty minutes and the only result was a yellowish solution as before. No compound was observed with TLC and after a work-up in chloroform, no color change was noted. It is clear then, that the unknown, red compound observed after treating marihuana exudates with HCL is not produced by hydrolysis of phloroglucinol.

The unknown, more dense, red compound observed at the bottom of the test tube after treating marihuana exudates with acid was provided by Dr. C. Thomas

Hammond of Monmouth College in Illinois and was compared with TLC to the combined products of our reaction. Two compounds were identified and had R_f values of 0.06 and 0.8, respectively. Compared to the R_f values of products **A** and **B** (0.05 0.77, respectively), the TLC data strongly suggests that the unknown residue from the marihuana exudates is the product of isoprene and phloroglucinol. Also, the deep red color of the supplied exudate is not dissimilar to the deep purple color of the products synthesized in our lab.

This study leaves many other possibilities open that would explain the unknown residue. Because many chemicals are found in the exudate, it is still possible that a reaction occurs between two constituents in the exudate with an acid as a catalyst. However, because of the tested similarity between the polarity of the two identified compounds it seems that any other explanation other than a reaction involving isoprene and phloroglucinol is unlikely. Furthermore, if reason exists to suspect phloroglucinol alone is the primary precursor to the residue, then an analysis of protonation of phloroglucinol would be advantageous. It is important to note, however, that we did not detect any products indicative of a reaction between pure phloroglucinol and hydrochloric acid in our own control. Also, it is important to discuss the significance of light in the reactions. Our reactions performed in the presence of light turned a dark purple. Still, reactions in darkness do not turn a dark color, and the water layers remain an amber color until they are exposed to light. Certain explanations for this phenomenon may lie with the properties of isoprene or phloroglucinol since both chemicals are sensitive to light. Our reaction occurred in the absence of light, but the yield was not as high as reactions exposed to light. This suggests that light is a catalyst to this reaction.

Furthermore, it is important to recognize that our experiments did not reveal the third compound described by Ahluwalia et al. It is possible that only two compounds are identified because one R_f may represent two compounds. Compounds (***10***) and (***11***) have similar structures and they may have similar R_f values.

Finally, it would be worthwhile to examine our compounds with analytical instrumentation, primarily nuclear magnetic resonance spectrometry, gas chromatography and mass spectrometry, because these procedures would further elucidate the structures of our compounds. Future analysis may more clearly identify the unknown residue, but our study strongly suggests that it is the direct result of a reaction between phloroglucinol and isoprene.

EXPERIMENTAL

Methods and Materials

Samples were weighed on a Mettler analytical balance. Melting points were obtained using a Mel-Temp capillary melting point apparatus. Thin-layer chromatography was performed using Riedler-de Hähn chromatography plates with silica on aluminum. All plates were developed with sublimated iodine. Column chromatography was performed using silica gel (60-120 mesh) obtained from Aldrich chemical as the stationary phase.

All solvents, including benzene, ethyl-acetate, pentane, and chloroform were purchased from Aldrich Chemical Company and were used with no further purification. All reagents including our phloroglucinol and isoprene were also obtained from the Aldrich Chemical Company. Marihuana exudate samples were supplied by Dr. Charles T. Hammond of Monmouth College in Illinois. The exudates were acid treated in his lab before we received them.

Experiment One

Phloroglucinol (2.6 g, 0.017 mol) and orthophosphoric acid (2.0 ml) was added to pentane (5.0 ml) at room temperature, and a slight yellow color was noted. The mixture was heated in a water bath to 27 °C while being stirred. A mixture of isoprene (1.5 ml, 0.015 mol) and pentane (5.0 ml) was slowly added over two hours. One hour into the reaction a stable temperature of 30 °C was attained. The solution was allowed to cool to room temperature and was stirred over night. It was neutralized with a 5%

$NaHCO_3$ solution. The mixture was extracted with benzene (3x20ml). The combined slightly yellow benzene layers were washed with water (2x15ml) and dried for at least ten minutes over anhydrous $MgSO_4$.

Both layers were analyzed with TLC. The compound in the combined benzene layers, product **A,** were observed to have an R_f of 0.05 in benzene-ethyl acetate (9:1). Product **A** was not isolated. The compound in the water layer, product **B** had an R_f 0.11 in benzene-ethyl acetate (9:1). Product **B** was purified using column chromatography, and isolated as a yellow oil.

Experiment Two

Ethanol (8.0 ml) was added to phloroglucinol (2.0 g, 0.016 mol) and orthophosphoric acid (2.0 ml) and a slight yellow color was noted. The flask was covered with aluminum foil to prevent exposure to light. The mixture was stirred while being brought to 35 °C. The mixture of isoprene (1.7 ml, 0.017 mol) and pentane (5.0 ml) was added slowly over two hours and the complete slurry was allowed to cool to room temperature and then was stirred over night. The mixture was neutralized with 5% $NaHCO_3$ and extracted in benzene (3x20 ml) which afforded an amber water layer. The benzene layers were washed with water (2x15 ml) and dried over $MgSO_4$.

The combined benzene layers were analyzed with TLC and then combined with the benzene layer from reaction one. A compound with an R_f of 0.11 (product **A**) was detected. The water layer was re-extracted in chloroform (2x15 ml) and washed with water (2x15 ml). The chloroform layer was dried over $MgSO_4$ and concentrated. It was analyzed with TLC and a compound with an R_f of 0.7 (compound **B**) was observed

and then purified using column chromatography (benzene-ethyl acetate, 5:1). Product **B** was combined with that from before.

Experiment Three

Pentane (10.0 ml) was slowly added to phloroglucinol (2.0 g, 0.016 mol) and orthophosphoric acid (2.0 ml) over two hours at 35 °C. No color change was observed, and the solution was allowed to cool to room temperature. The apparatus was covered with aluminum foil and left to stir over night. After being neutralized in 5% $NaHCO_3$ the solution was extracted in chloroform (3x20 ml) and the benzene layer was washed in water (2x15 ml). After being dried in $MgSO_4$ for 10 minutes, TLC was performed and no compounds were detected.

Comparison

The red exudates provided by Hammond were analyzed by TLC (benzene-ethyl acetate, 9:1) and compared to products from experiments one and two. One low polarity compound with an R_f of 0.06 (Compound **A**) was observed, and we also observed one high polarity compound with an R_f of 0.8 (Compound **B**).

ENDNOTES

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