OBSERVATIONS ON PSYCHONEUROPHYSIOLOGICALLY SIGNIFICANT MUSHROOMS

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I. CLINICAL DETAILS PERTAINING TO THE INGESTION OF PANAEOLUS VENENOSUS AND PSILOCYBE CAERULESCENS MUSHROOMS

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Introduction

The exploration of the effects of psychoneurophysiologically significant mushrooms or the chemicals they contain is continuing and ostensibly expanding (1) (2) (3). In some instances the most dramatic and subjectively discernible effect was in the psychic modality of the nervous system. Probably for this reason, the term "hallucinogenic" was applied to categorize the mushroom effects with those produced by other naturally occurring plant substances (mescaline, LSD 25, Yohimbine) under experimental investigation with the advent of the psychopharmacological era which started about 1952. The term "hallucinogenic" is unfortunate since (a) it suggests the probability of having material capable of causing effects identical to those obtaining in dementia praecox which can thereby be studied at will; and (b) it misleads by placing an emphasis on the effects in the psychic modality and thereby deters observations of the effects in other areas of the nervous system or in other systems. The need for having qualified screening measurements for psychopharmacological effects is of paramount importance (4). In view of this requirement, this case study reports the clinical details as related, measured, and observed on a non-patient volunteer, Dr. GERHARD L. CLOSS, Assistant Professor of Chemistry, The University of Chicago, who was interested in personally experiencing the effects of our mushrooms whose significant chemicals he is seeking to isolate and identify in his laboratory.

Experimental Studies

Dr. CLOSS, the subject, is 30 years of age. The following was obtained in sequence.

August 17, 1958: First Experiment

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9 : 50 A.M.

Room temperature:	72° F
Room humidity:	50 %
Pulse:	80
Blood pressure:	128/60—70
Weight:	155
Pupils:	Moderately dilated; react quickly to light and accommodation

9 : 55 A.M.

Subject was requested to:	Draw A Person (DAP)
Subject was requested to:	Draw A Person of the Opposite Sex (DAPOS)

10 : 10 A.M.
Subject took 0.5 Gm. *Panaeolus venenosus* [1])

10 : 20 A.M.

Pulse:	83
Blood pressure:	112/70
Pupils:	Slightly dilated
Subject reports:	Slight dizziness

10 : 35 A.M.

Pulse:	68
Blood pressure:	120/72
Subject reports:	Slight inebriation. Turning causes dizziness. See subject's notes.

10 : 50 A.M.

Pulse:	60
Blood pressure:	118/76
Subject reports:	"The effect is decreasing. I feel normal".

11 : 20 A.M.

Pulse:	64
Blood pressure:	105/70

August 22, 1958: Second Experiment

9 : 20 A.M.
Check normal polygraphic effects. (see tracing)

9 : 30 A.M.

Pulse:	70
Blood pressure:	115/65
Weight:	155
Oral temperature:	98.3° F
Room temperature:	72° F
Room humidity:	52 %
Pupils:	Approximate size noted

9 : 36 A.M.
Subject took 1.0 Gm. of *Panaeolus venenosus* [2])

9 : 46 A.M.

Pulse:	73
Blood pressure:	110/56
Pupils:	Seemingly unchanged

[1]) Oven-dried. Identified by Drs. ROLF SINGER, University of Argentina, Tucuman, and A. H. SMITH, University of Michigan; grown by Dr. L. KNEEBONE, Pennsylvania State University.

[2]) Same as material used one August 17, 1958.

9 : 55 A.M.

Pulse:	74
Blood pressure:	110/72
Pupils:	Slightly more dilated
Subject reports:	No effects and inquires if he had been given bread crumbs.

10 : 05 A.M.

Subject reports voluntarily:	"I feel different". Slight dizziness. Tingling of fingers. Feels warm. Sweating.
Pupils:	Distincly larger
Pulse:	60
Blood pressure:	120/75

10 : 12 A.M.

Subject volunteers:	"It's a type of intoxication, hard to describe". He uses his hands to maintain equilibrium while walking. "It's more pronounced than last time. The intoxication is not unpleasant at all." Lights a cigarette.

10 : 15 A.M.

Pulse:	66
Blood pressure:	108/74
Walking:	Equilibrium disturbed
Subject reports:	"Bitter feeling at tip of tongue".

10 : 20 — 10 : 30 A.M. (polygraphic tracings)

Subject reports:	The paresthesias are still present. "The total effect is more pronounced than last time. Not in the mood to solve any complicated chemical problems. Just feel like relaxing". Attitude is one of relative indifference or diminished motivation. The effect seems to be at its height at this time.

10 : 32 A.M.

Subject is asked to:	Draw A Person which he attempts but comments: "I have difficulty concentrating on one spot with my eyes. It seems as if I am not able to draw at all today". Stops drawing. His hands feel warm and completely wet from sweat. He lolls back. Claims to sense anxiety and states: "I can't figure out (name) the problems going on in the lab now".

10 : 37 A.M.

Pulse:	58—60
Blood pressure:	108/70
Oral temperature:	97.9—98° F
Pupils:	Still slightly dilated
Walking:	Unsteady: "It is not like from weakness, but disturbed equilibrium".

10 : 48 A.M.

Subject was requested to:	DAPOS. His movements are active now, and he is showing his more usual interest in responding.

10 : 55 A.M.

Subject eats some fresh seedless grapes and a plum.

Subject reports:	No gastro-intestinal effects but states that paresthesias are still present in his fingers.

11 : 00 A.M.

	Subject is smoking. Now is motivated and capable of discussing the work of his laboratory.
Pulse:	54
Blood pressure:	116/76
Hands:	Warm, but sweating less.
Pupils:	Still slightly dilated but react briskly to light now.
Subject reports:	"The intrinsic sensation is much less and equilibrium is better".

11 : 30 A.P.

Pulse:	54
Blood pressure:	110/74

12 : 00 Noon

Pulse:	56
Blood pressure:	110/74

12 : 45 P.M. (lunch)

Pupils:	Dilated. Give distress in outdoor light.

1 : 30 P.M.

Pulse:	62
Blood pressure:	110/64
Pupils:	Nearly original size.
Subject drinks lemonade	

2 : 00 P.M.

Pulse:	72
Blood pressure:	114/70
Pupils:	Nearly normal. Out-door light gives no distress.

2 : 45 P.M.

Pulse:	66
Blood pressure:	118/64
Oral temperature:	98.1° F
Pupils:	Normal

August 29, 1958: Third Experiment

Room temperature:	71° F
Room humidity:	58 %

9 : 35 A.M.

Pulse:	70—71
Blood pressure:	112/60
Weight:	154 plus
Oral temperature:	98.3° F
Pupils:	Observed for size and tested for light and accommodation reflex.

9 : 45 A.M.

Subject ingested 1.0 Gm. oven-dried, granulated *Psilocybe caerulescens* (i.e. prepared and identified in the same way as the *Panaeolus venenosus* used in the previous experiments).

9 : 50 A. M.(yawning)

Pulse:	77
Blood pressure:	110/56

10 : 05 A.M.

Pulse:	72
Blood pressure:	108/64
Subjective effects:	None reported

10 : 15 A.M.
Pulse: 64
Blood pressure: 104/60
Pupils: Slightly dilated
Skin effects: None
Equilibrium: Questionably affected
Subjective effects: "Unpleasant sensation in the tube from mouth to stomach". Subject was given some water.

10 : 20—10 : 25 A.M.
Subject smoking a cigarette.

10 : 27 A.M.
Pulse: 64
Blood pressure: 102/74
Subjective effects: Subject is yawning and complains of some dizziness but mainly fatigue.

10 : 35 A.M.
Subject comments: „The equilibrium system is not as affected. There is some sense of inebriation but more like the 0.5 Gm. portion of *Panaeolus venenosus*. The main feeling-tone at this time is tiredness". There is yawning and a drooping of eyelids. This yawning and somnolence with *Psilocybe* was observed by the author upon himself (5) and another subject (3).

10 : 39 A.M.
Pulse: 65
Blood pressure: 108/70
Subjective effects: "The sensations are different from *Panaeolus*. This makes me feel terribly tired. This is like the normal state of being tired. With *Panaeolus*, it was a relaxed feeling".

10 : 50 A.M.
Pulse: 64
Blood pressure: 105/70
Subjective effects: "I have had some unpleasant effects in the stomach, not serious, but noticeable. The main sensation is that I wish to take a nap. That's very pronounced". At this moment a stranger entered the office-suite, and Dr. C. stood up to observe a demonstration. A manner of intoxication seemed to prevail.

11 : 05 A.M.
Pulse: 65
Blood pressure: 108/68
Subjective effects: "I have an intoxicated feeling. The equilibrium isn't much affected. I feel strange and very drunk now". Facial expression appears drawn or anxious. Dr. C. does not report any visual hallucinations or any haloeffects as he looks at the light.

11 : 10 A.M.
Subject was attached to the polygraphic apparatus, since the effects appeared to have reached a significant stage or one of strong intoxication. The polygraph had run only a few minutes when:
Subject reported: "Something strange is happening. I feel real drunk, and I have strong anxieties".

Hands:	Cool but sweating
Subject was removed from the polygraph.	
Pulse:	72
Blood pressure:	105/64
Oral temperature:	98° F

Because of subjective sensations described above, he was given fresh fruit (grapes and plums).

11 : 40 A.M.	Reading taken immediately after Dr. C. ate the fresh fruit.
Pulse:	48
Blood pressure:	98/55
Pupils:	Dilated
11 : 50 A.M.	
Subjective effects:	"I feel cold, even my hands and nose". There is evidence of some shivering.
Room temperature:	Still 72° F
Room humidity:	52 %
11 : 55 A.M.	
Pulse:	56
Blood pressure:	105/68
Oral temperature:	98.3° F
Subjective effects:	Intoxication symptoms subsided rapidly after this.
12 : 25 P.M.	
Pulse:	58
Blood pressure:	104/62

Subject ate lunch immediately after reading.

1 : 40 P.M.	
Pulse:	74
Blood pressure:	112/64
Pupils:	Still slightly dilated but reacted quickly to light stimulus.

Summary

There were noticeable similarities and differences in the effects of *Panaeolus venenosus* and *Psilocybe caerulescens* which appeared to have no correlation with the quantity of raw mushroom ingested.

In the three experiments reported here, the subjective and objective effects developed within about the same time interval after ingestion; namely, 25 to 30 minutes, with one exception. Yawning, which was observed here and elsewhere as occurring only with the *Psilocybe* material, usually within 5 to 10 minutes, proceeds until other symptoms and signs present themselves.

Reported symptoms or subjective effects which occurred only or mainly with *Panaeolus* were (a) a mild or moderate relaxing (tranquil) type of inebriation, (b) disturbed equilibrium, (c) either diminished motivation or blocking in the psychic (thought) process, and (d) paresthesias.

Features which obtained only or mainly with *Psilocybe* were (a) the aforementioned yawning in the induction period (b) "burning" sensation in the esophageal and stomach areas, (c) a feeling

of extreme tiredness, and (d) a feeling-tone of anxiety bordering on collapse associated with a sense of strong intoxication.

The factors occurring in common were (a) the extreme reduction in the pulse rate, slightly more with *Psilocybe*, (b) the steadiness of the systolic and diastolic components of the blood pressure except for a very period of drop in both diastole and systole with *Psilocybe* during the period of strong intoxication, (c) dilation of the pupils, (d) a drop in body temperature, seemingly more marked with *Panaeolus*, but producing a much more subjective effect (feeling of cold) during the period of *Psilocybe* intoxication, and (e) sweating.

With *Panaeolus* there was a general drop in the total personality integration, but the internal sensation was a pleasant and relaxed feeling; whereas, with *Psilocybe* there was actually disorganization associated with the feeling of panic and disagreeable intoxication.

A most significant feature is that Dr. CLOSS at no time perceived or reported effects that could be categorized as hallucinatory.

Formulation

Obviously, it is unwise and perhaps unnecessary to try to correlate clinical observations where unknown chemicals or chemical complexes are ingested.

But the author has had considerable experience with psychoneuropharmacological (psychoneurotropic) material and offers the following tentative opinion. The relaxing subjective effects, the hypothermia, and diminished pulse found with *Panaeolus* resemble the effects attributed to serotonin. But the inebriation and the pupillary mydriasis cannot be explained as effects of serotonin. Some of the effects observed here (and elsewhere) (1) (3) with *Psilocybe* are probably due to the serotonin-related-psilocybin already isolated (6) from *Psilocybe mexicana* (HEIM) which probably also obtains in *Psilocybe caerulescens*. (Refer to Part II by Dr. CLOSS) However, it seems unlikely that psilocybin will be found to have caused the signs of mydriasis and cerebral or cortical intoxication

Probably several chemical compounds will be found to be psychoneurophysiologically effective in each significant mushroom. In addition, undoubtedly, the relative differences in the biochemistry which obtain from individual to individual may account further for differences elicited, alleged or reported, including extrasensory perception and hallucinosis, in situations where so-called "sacred" (2) (7) mushroom material has been ingested.

II. STUDIES TOWARD THE ISOLATION OF THE ACTIVE CONSTITUENTS OF PANAEOLUS VENENOSUS

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The observed clinical effects described in Part I of this communication seemed to justify an attempt at the isolation and

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characterization of the compounds which are responsible for the remarkable action of *Panaeolus venenosus* upon the nervous system. The guiding principle in this work was the consideration that it would be highly desirable to obtain any of the compounds in their original form, unchanged from the state in which they occur in the mushroom. Since it seemed reasonable to expect that the active compounds might possess easily hydrolizable groups which could be of importance to their physiological activity, isolation and purification procedures had to be chosen to avoid any acid or base treatment.

It should be pointed out that the experiments to be described are preliminary in nature and were designed to establish the best conditions for the isolation and purification of the compounds occurring in the mushroom. As a second stage, it is planned to work up larger quantities of the mushroom in order to obtain sizeable amounts of the compounds and test their activities in similar experiments as described in Part I. Unforeseen difficulties, however, prevented us from securing a large enough supply of *P. venenosus* to continue our investigations on a larger scale and made further characterizations of the isolated products impossible at the present time. The total amount of dried mushrooms which were available for our studies was less than 12 grams.

Paper Chromatography Studies on Extracts from Several Panaeolus and Psilocybe Species

At the outset it was necessary to establish whether the extractable compounds from *P. venenosus* were identical with those occurring in other *Panaeolus* species and particularly whether psilocybin (6) would be among them. Paper chromatography proved to be a valuable and reliable tool in these investigations.

The methanol extracts of the well-dried fruiting bodies were partially evaporated in vacuum, and fatty material was removed by extraction with hexane [1]). The methanolic solutions were then spotted on Whatman No 1 paper in suitable concentrations. As eluting solvent, isopropanol-water 9:1 was found most satisfactory. Spraying with a solution of p-dimethylamino-benzaldehyde (Ehrlich's reagent) followed by exposure to hydrogen chloride vapor, was used as detecting reagent. In several chromatograms Dragendorff's alkaloid reagent was used for detection.

Figure 1 shows the chromatograms of extracts from *P. venenosus*, *P. sphrinctrinus*, *Psil. mexicana*, *Psil. cubensis*, and *Psil. caerulescens*. All three *Psilocybe* species investigated show a strong spot at Rf 0.63 which produces a bluish-grey color with Ehrlich's reagent indicating the presence of psilocybin (6). A few very faint spots at

[1]) Subsequent paper chromatograms of these extracts did not show any spots with the developing reagents used.

lower Rf show the presence of minor constituents in much smaller concentrations.

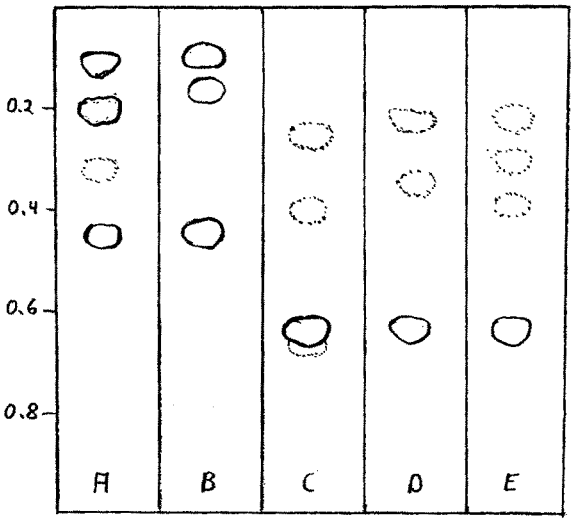

Fig. 1

Paper Chromatograms of A. *P. venenosus*, B. *P. sphrinctrinus*, C. *Psil. mexicana*, D. *Psil. cubensis*, E. *Psil. caerulescens*. Solvent: Isopropanol — water 9 : 1. Detecting reagent: Ehrlich's reagent.

The chromatograms of the *Panaeolus* species show clearly that psilocybin is absent. The chromatogram of *Panaeolus venenosus* has three strong spots at Rf 0.44, 0.20, and 0.11. The last two show up in blue color with Ehrlich's reagent, whereas the first appears as a bright yellow spot. The presence of at least one minor constituent is indicated by a faint spot at Rf 0.32. *Panaeolus sphrinctrinus* gives rise to a chromatogram with three intense spots at Rf 0.43 (yellow), 0.17 (red), and 0.09 (bluishgrey). It appears that the spots at Rf 0.4 in the chromatograms of both Panaeoli are produced by the same compound as judged by their identical Rf and color reactions with Ehrlich's reagent. However, the other major constituents in both *Panaeolus* species seem to differ from each other since they appear at different Rf values and produce different colors with the developing reagent. From these results it can be concluded that the extractable constituents of *Panaeolus venenosus* are totally different from the constituents of the *Psilocybe* species and partially different from the extracts obtained from *P. sphrinctrinus*.

Column Chromatogram of the Extract Obtained from Panaeolus Venenosus

Encouraged by the good resolution obtained on paper, it was decided to try the separation on a larger scale by chromatography on a cellulose column. The methanol extract from 11 grams of dried and finelypowdered mushrooms was evaporated under vacuum. Some of the high molecular weight material was removed from an aqueous solution of the residue by precipitation with ethanol. The resulting solution was evaporated under reduced pressure and the residue chromatographed on cellulose powder. As eluting solvent, isopropanol-water 9:1 was followed by 95 % ethanol. In good agreement with the paper chromatography studies, three major fractions were obtained. Paper chromatography of these fractions demonstrated their correspondence to the three major spots of the paper chromatogram of the total extract. A fourth fraction, corresponding to the minor spot on paper at Rf 0.32, could be recognized but was not further investigated because of the very small quantity of this material. Each of the major fractions was rechromatographed several times, and two of the three compounds were finally obtained crystalline.

"Compound I", first compound to be eluted from the column, could be shown to be identical with the substance producing the yellow spot on paper at Rf 0.44. It was obtained from ethanolethyl acetate in tiny needles and could be further purified by sublimation in vacuum. The compound melts at 136° C, shows no ultra violet absorption, and has the typical characteristics of a hydrohalide (insoluble in nonpolar solvents, infra red spectrum with strong absorption at 3300—3000 and 1600 cm^{-1} and ionic chlorine).

The next larger fraction to be eluted from the column could be shown to correspond to the blue spot on paper with Rf 0.20. This *"Compound II"* was obtained crystalline, after considerable losses, on purification by rechromatographing it several times on cellulose. The compound decomposes above 250° C, is soluble in water, insoluble in nonpolar solvents, and could be recrystallized from ethanol. The compound is not extractable either from acidic or basic solutions, suggesting acidic and basic functions in the molecule. Not enough material was available to obtain a quantitative elementary analysis. The ultra violet absorption spectrum (Figure 2) shows maxima at 220, 265, and 288 mu. The band positions and relative intensities agree well with the spectrum to be expected for a benzene hydroxylated indole chromophore. The blue color reaction with Ehrlich's reagent supports this conclusion. It should be noted that the spectrum is almost identical with the spectrum of psilocybin (6). This correlation indicates that Compound II possesses the same chromophore as psilocybin, an esterified 4-hydroxy indole system. This near relation to psilocybin seems to suggest that Compound II is the active principle of *P.*

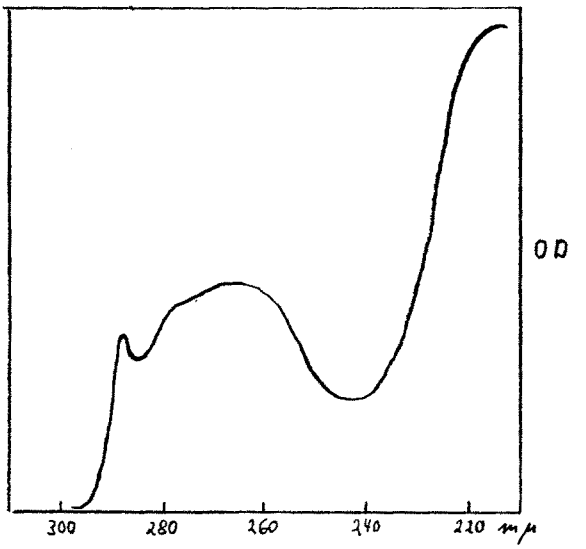

Fig. 2
Ultra Violet Absorption Spectrum of Compound II in Methanol.

venenosus although actual tests of its activity could not be carried out because of the small amount isolated. From its greater solubility in water, higher melting point, and smaller Rf value, it can be concluded that the compound is more polar than psilocybin. From the ultra violet spectrum of the total extract which is very similar to the spectrum of Compound II and shows the characteristic band at 288 mu very clearly, the total amount of Compound II present in the mushroom can be estimated to 0.1—0.3 %.

The last fraction to be eluted from the column did not yield a crystalline compound. Paper chromatography demonstrated that it corresponds to the blue spot at Rf 0.11. The ultra violet absorption spectrum of this material showed a maximum at 260 mu. The material was not further investigated.

Summary

The extracts of *P. venenosus*, *P. sphrinctrinus*, *Psil. mexicana*, *Psil. cubensis*, and *Psil. caerulescens* have been compared by paper chromatography. It was found that neither Panaeoli contain psilocybin, the main constituent of the three *Psilocybe* species. The extract from *P. venenosus* has been chromatographed, and two of the three major constituents were purified and obtained crystalline. One of these compounds could be shown to possess the same chromophore as psilocybin, a 4-oxygenated indole system, and seems most likely to be the active compound of the mushroom.

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