The Occurrence of Tryptamine Derivatives in *Psilocybe semilanceata*

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The content of tryptamine derivatives in *Psilocybe semilanceata,* a popular hallucinogenic mushroom, was measured by high-performance liquid chromatography. Most of the 52 samples have been collected at several localities in Switzerland during a 1–5 year period. The content of psilocybin and baeocystin varied in the range of 0.21–2.02% and 0.05–0.77%, respectively, whereas only traces of psilocin were present. The variability of the alkaloid level depending on origin, year of collection, size and part of mushrooms is discussed.

Psilocybe semilanceata (Fr.) Quél. (Strophariaceae) is growing wild in Middle and North Europe, North America etc. and is known as a hallucinogenic mushroom for about 10 years. A marked increase can be observed in the abuse – by ingestion of dried fruit-bodies – of this potent narcotic and therefore in many countries illicit drug. The main indole alkaloids are psilocybin and baeocystin, both phosphorylated tryptamine derivatives. Psilocybin is responsible for the psychotropic potency of *P. semilanceata,* whereas the pharmacological effects of baeocystin are still unknown. The presence of psilocybin in european *P. semilanceata* was first reported by Hofmann, Heim and Tscherter in 1963 [1], whereas baeocystin was isolated in 1979 [2] and recently characterized by spectroscopic methods [3]. Further analytical work with samples from several european countries revealed considerable quantities of these tryptamine derivatives [4–7].

It was the aim of the presented study to screen and compare the content of tryptamine derivatives in a large number of *P. semilanceata* samples of different origin following a standardized procedure. Our interest was especially focussed on the variability of the alkaloid level depending on origin (location), year of collection, size and part of mushrooms.

Materials and Methods

Mushroom samples

Most of the analyzed samples of *Psilocybe semilanceata* (Fr.) Quél. (Strophariaceae) were collected between 1983 and 1987 in Switzerland at localities A–L: (A) Tramelan (Canton of Berne), (B) Les Verrières (Canton of Neuchâtel), (C) Ponts de Martel (Canton of Neuchâtel), (D) Napf (Canton of Luzern), (E) Habkern (Canton of Berne), (F) Kiental (Canton of Berne), (G) Gurnigel (Canton of Berne), (H) Les Avants (Canton of Vaud), (I) Gstaad (Canton of Berne), (K) Blonay (Canton of Vaud), (L) Schwarzsee (Canton of Freiburg). The main habitats of *P. semilanceata* were wet grassy fields and uncultivated pastures in the vicinity of firs and pines at an altitude of about 1000–1500 m. They usually grew on manured soil but not directly on dung. Immediately after harvesting the fruit-bodies (carpophores) were deep-frozen, lyophilized and stored at −20 °C.

Some samples were confiscated at illegal drug markets by drug enforcement authorities or obtained through Nestec Ltd. (Vevey, Switzerland).

Quantitation of tryptamine derivatives

The qualitative and quantitative determination of psilocybin (4-phosphoryloxy-N,N-dimethyltryptamine), baeocystin (4-phosphoryloxy-N-methyltryptamine) and psilocin (4-hydroxy-N,N-dimethyltryptamine) in mushroom samples was done by high-performance liquid chromatography with photodiode array detection (HPLC-PAD) as published earlier [8].

Results and Discussion

Psilocybin, the main psychotropic tryptamine derivative of *Psilocybe semilanceata,* baeocystin, demethyl analog and possibly a biogenetic precursor of psilocybin [3], and psilocin could be identified in all 52 mushroom samples by high-performance liquid

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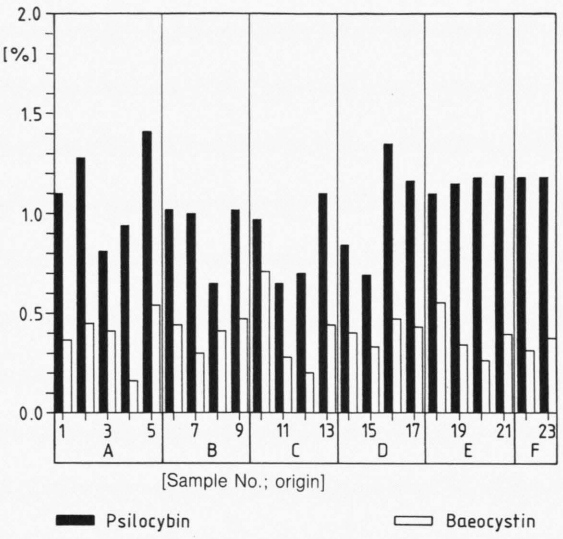

Fig. 1. Variation of the content of psilocybin and baeocystin in *P. semilanceata* in dependence on origin and year of collection.

chromatography and photodiode array detection (Tables I–IV, Fig. 1). The quantitation showed psilocybin and baeocystin contents in the range of 0.21–2.02% (mean value, $\bar{x}$: 1.01%) and 0.05–0.77% ($\bar{x}$: 0.37%), respectively. The baeocystin level was always below the psilocybin level. Similar psilocybin (0.55–1.96%) and baeocystin levels (0.05–0.34%) have been found in *P. semilanceata* collected in Norway [9]. In no sample the psilocin content was higher than 0.01%. Psilocin is thought to be a biogenetic or thermal dephosphorylation product of psilocybin, formed enzymatically or more often by inappropriate drying and storage.

To study the influence of the geographical origin and the vegetation cycles on the alkaloid content of *P. semilanceata,* 23 mushroom samples of 6 different locations (see Table I: Nos. 1–23) were collected over a 2–5 year period and analyzed under standardized conditions. As demonstrated with Table I and Fig. 1 there is no correlation between location A–F and the percentage of the main tryptamine derivatives psilocybin and baeocystin. The content of psilocybin and baeocystin varied from 0.65 to 1.18% ($\bar{x}$: 0.95%) and 0.28 to 0.41% ($\bar{x}$: 0.33%), respectively. Samples originating from location A–D (Nos. 1–17) showed also a considerable variation in the alkaloid levels from one vegetation period to the other. For example in samples Nos. 1–5 of location A a psilocybin content of 0.81–1.41% ($\bar{x}$: 1.11%; coefficient of variation, c.v.: 22%) and a baeocystin content of 0.16–0.54% ($\bar{x}$: 0.38%; c.v.: 37%) was measured within a 5-year period. An interesting fact is with 1.10–1.19% ($\bar{x}$: 1.16%; c.v.: 2.9%) the much smaller range of variation in the psilocybin content of samples 18–23, collected at location E and F. Only at these locations the samples have always been collected within the same group of mushrooms (same mycelium?), growing on a very small and limited area, whereas at location A–D the mushrooms were scattered over a large area. 5 other localities in Switzerland (G–L) are represented by samples Nos. 24–28, each collected only in one single vegetation period.

Samples Nos. 29–34, which have been confiscated or collected in other countries, were not used for the comparative study, as their history is not known.

Location A was chosen to determine the variation of tryptamine derivatives in 10 mushrooms of about the same size and collected at the same time (Table II: Nos. 35–44). The psilocybin content varied between 0.53 and 1.19% ($\bar{x}$: 0.91%; c.v.: 22.3%), the baeocystin content between 0.21 and 0.77 ($\bar{x}$: 0.44%; c.v.: 45.5%). The results illustrate that for comparative studies at least 10 samples per location should be analyzed.

As shown in Table III more psilocybin is accumulated relative to the dry weight in stipes than in caps of *P. semilanceata,* collected at three different locations. For example ten stipes of sample No. 45 with a total dry weight of 73 mg contained 1.58% psilocybin, whereas in the caps (No. 46) with a total dry weight of 158 mg only 1.17% were measured. The relative baeocystin content of stipes was at two different locations about 60% lower than that of the corresponding caps (Nos. 45–48). At another location no significant difference was observed (Nos. 49 and 50). One earlier study [9] has found similar psilocybin levels in stipes and caps and also lower baeocystin content in stipes, whereas according to another study [10] caps contained more psilocybin than stipes. Since not much is known about enzyme activities, transport systems, storage sites etc. within the biosynthesis of *Psilocybe* tryptamine derivatives an interpretation of these data is not yet possible.

To check the influence of the size of *P. semilanceata* on the content of tryptamine derivatives, a group of fruit-bodies with about a 5 mm-diameter (No. 51:

Table I. The content of tryptamine derivatives in *P. semilanceata* of different origin.

No.	Origin[a]	Time of collection	% (calc. per dry weight)[b] Psilocybin	Baeocystin
1	A	Oct. 1983	1.10	0.36
2		Sept. 1984	1.28	0.45
3		Sept. 1985	0.81	0.41
4		Sept. 1986	0.94	0.16
5		Oct. 1987	1.41	0.54
6	B	Oct. 1984	1.02	0.44
7		Oct. 1985	1.00	0.30
8		Sept. 1986	0.65	0.41
9		Oct. 1987	1.02	0.47
10	C	Sept. 1984	0.97	0.71
11		Oct. 1985	0.65	0.28
12		Sept. 1986	0.70	0.20
13		Oct. 1987	1.10	0.44
14	D	Oct. 1984	0.84	0.40
15		Oct. 1985	0.69	0.33
16		Sept. 1986	1.35	0.47
17		Oct. 1987	1.16	0.43
18	E	Oct. 1984	1.10	0.55
19		Oct. 1985	1.15	0.34
20		Sept. 1986	1.18	0.26
21		Oct. 1987	1.19	0.39
22	F	Oct. 1985	1.18	0.31
23		Sept. 1986	1.18	0.37
24	G	Oct. 1985	1.17	0.35
25	H	1982	1.18	0.60
26	I	1983	0.42	0.33
27	K	1984	0.62	0.32
28	L	1985	0.93	0.38
29	Switzerland[c]	1983	0.41	0.10
30		1986	0.84	0.29
31	Pacific Coast/U.S.A.	1980	0.83	0.23
32	Narden/Holland	1982	0.53	0.41
33	Lübeck/W.-Germany	1985	1.13	0.40
34	France[c]	1985	0.29	0.05

[a] For localities A–L (sample No. 1–28) see "Material and Methods".
[b] Average of 10 mushrooms.
Psilocin content: $\leqq 0.01\%$ (detection limit: 0.002%, corresponding to about 10 ng).
[c] Sample confiscated by police on the illegal drug market.

Table II. Variation of tryptamine derivatives in *P. semilanceata* originating from the same location and collected at the same time[a].

No.	% (calc. per dry weight)[b] Psilocybin	Baeocystin
35	0.81	0.51
36	1.07	0.60
37	1.10	0.47
38	1.04	0.77
39	0.92	0.34
40	0.90	0.26
41	1.19	0.40
42	0.53	0.21
43	0.89	0.53
44	0.67	0.32

[a] Locality A (see "Material and Methods"), collected Sept. 1985.
[b] Psilocin content: $\leqq 0.01\%$.

"small") and another group of fruit-bodies with about a 15 mm-diameter (No. 52: "big"), both collected at the same time and at the same location, were analyzed. No difference was found between the psilocybin content of small and big fruit-bodies, whereas the baeocystin content was about 20% higher in small carpophores (Table IV). Earlier studies [4, 6, 11] have shown higher psilocybin contents for small fruit-bodies.

Table III. Comparison of the content of tryptamine derivatives in stipes and caps of *P. semilanceata*.

No.	Origin[a]	Part of mushroom	Time of collection	% (calc. per dry weight)[b] Psilocybin	Baeocystin
45	A	Stipe	Sept. 1985	1.58	0.17
46		Cap		1.17	0.41
47	E	Stipe	Oct. 1985	1.61	0.13
48		Cap		1.15	0.30
49	G	Stipe	Oct. 1985	2.02	0.30
50		Cap		1.32	0.29

[a] For localities see "Material and Methods".
[b] Average of 10 mushrooms.
Psilocin content: $\leqq 0.01\%$.

Table IV. Comparison of the content of tryptamine derivatives in small and big fruit-bodies of *P. semilanceata*.

No.	Origin[a]	Size of fruit-body	Time of collection	% (calc. per dry weight)[b] Psilocybin	Baeocystin
51	A	Small[c]	Sept. 1985	1.17	0.31
52		Big[d]		1.15	0.40

[a] For locality see "Material and Methods".
[b] Average of 10 mushrooms.
Psilocin content: $\leqq 0.01\%$.
[c] About 5 mm, 8–14 mg (diameter, dry weight).
[d] About 15 mm, 30–45 mg (diameter, dry weight).

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