Pigments of Fly Agaric (*Amanita muscaria*)

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The complex pigment pattern of fly agaric (*Amanita muscaria*) cap skins has been studied by LC-DAD and mass spectrometry. Among the betaxanthins the corresponding derivatives of serine, threonine, ethanolamine, alanine, Dopa, phenylalanine and tryptophan are reported for the first time to contribute to the pigment pattern of fly agarics. Betalamic acid, the chromophoric precursor of betaxanthins and betacyanins, muscaflavin and *seco*-dopas were also detected. Furthermore, the red-purple muscapurpurin and the red muscarubrin were tentatively assigned while further six betacyanin-like components could not be structurally allocated. Stability studies indicated a high susceptibility of pigment extracts to degradation which led to rapid colour loss thus rendering a complete characterization of betacyanin-like compounds impossible at present. Taking into account these difficulties the presented results may be a starting point for a comprehensive characterization of the pigment composition of fly agarics.

Key words: Amanita muscaria, Fly Agaric, Betalains

Introduction

The reddish colour of the cap skin of the toadstool fly agaric [*Amanita muscaria* (L. ex Fries) Hooker] is caused by different yellow, orange and red pigments. By repeated chromatography the pigment mixture has been fractionated into at least ten components, *i. e.* the orange musca-aurins I–VII, the yellow muscaflavin, the red-violet muscapurpurin and the red muscarubrin (Döpp *et al.*, 1971; Döpp and Musso, 1973). Structure elucidations revealed that the yellow pigments are betalamic acid-derived betaxanthins (Bx) known in higher plants, whereas the red pigments exhibited betacyanin-like properties, but were different from corresponding Caryophyllales pigments (Strack *et al.*, 2003). Musca-aurins I, II, and VII were shown to be new betaxanthins derived from ibotenic and stizolobic acids as well as histidine, respectively, musca-aurins III and IV were found to be mixtures of betaxanthins derived from acidic amino acids such as glutamic, aspartic and α-amino adipic acids, whereas musca-aurins V and VI were betaxanthin blends derived from neutral amino acids comprising glutamine, leucine, valine, proline and asparagine (Döpp *et al.*, 1982).

Recent reviews on betalains in general (Zrÿd and Christinet, 2004) and on the chemistry of natural products of *A. muscaria* in particular (Gill, 2003; Michelot and Melendez-Howell, 2003) refer still to the *A. muscaria* pigment knowledge more than 20 years ago. In the past few years a striking number of new betaxanthins, using large sets of partial synthetic standards (Kobayashi, 2002; Schliemann *et al.*, 1999; Stintzing *et al.*, 2002; Trezzini and Zryd, 1991) and sophisticated LC-MS techniques (*e. g.* Kugler *et al.*, 2004, 2007a, b; Schliemann *et al.*, 2001; Stintzing *et al.*, 2002, 2005), have been identified from higher plants. Based on this knowledge a reinvestigation of the pigment pattern from fly agaric appears to be worthwhile.

Materials and Methods

Fungus material and extraction

The cuticles of pilei from three fly agarics [*Amanita muscaria* (L. ex Fries) Hooker], found at close quarters near Halle (Saale), Germany, were manually removed 2 h after collection. After determination of their fresh weights, they were separately dropped into liquid nitrogen, crushed in a mortar and subsequently extracted with methanol/water

(8:2 v/v; 1 ml/g fresh weight). The homogenates were centrifuged (5 min, 16,000 × *g*) and the supernatants immediately taken for HPLC analyses or stored at 8 °C. Aliquots of stored samples (4 weeks old) were sent to Hohenheim University on dry ice and immediately frozen at −80 °C until LC-DAD and LC-MS analyses.

Solvents and reagents

Reagents and solvents (VWR, Darmstadt, Germany) were of analytical or HPLC grade. Amino acids and amines were from Fluka (Buchs, Switzerland) and Sigma-Aldrich (St. Louis, MO, USA).

HPLC-DAD analyses for storage experiments (HPLC system I)

Analytical HPLC was performed on a Waters system (Milford, MA, USA), including the separation module 2690. The liquid chromatograph was equipped with a 5 μm Nucleosil C_{18} column (250 × 4 mm I. D.; Macherey-Nagel, Düren, Germany) operating at 25 °C. The following eluents and gradient system was used: A, 2 % formic acid in water; B, acetonitrile; constant gradient within 40 min from solvent A to 24% B in (A + B) at a flow rate of 1 ml/min. Using an automatic sampler 5 μl injections were performed, and the compounds were simultaneously monitored at 280, 405, 475, and 540 nm as well as maxplot between 210 and 600 nm, respectively, with a PDA detector. Data acquisition and evaluation were run with Empower 5. 0.

Identification of betalains by LC-DAD and LC-MS analyses (HPLC system II)

The aqueous methanolic extracts were carefully vacuum-dried in a SpeedVac SPD111V system (Savant, Düsseldorf, Germany) at a pressure of 10^{-6} bar and room temperature. To exclude qualitative changes in the betalain composition extracts were analyzed before and after the drying step. It is noteworthy that through removal of methanol from the extracts an improved betalain separation was achieved.

HPLC analyses of *Amanita* aqueous extracts were carried out with a Merck Hitachi LaChrom Elite HPLC system (Merck, Darmstadt, Germany) consisting of a pump L-2130, an autosampler L-2200, a JetStream column oven and a diode array detector L-2450. An analytical scale (250 × 4.6 mm I. D.) Sunfire C_{18}-reversed phase column with a particle size of 5 μm (Waters, Wexford, Ireland), fitted with a C_{18}-ODS (4 × 3.0 mm I. D.) security guard column, was used for pigment analysis, operating at a flow rate of 1 ml/min and at a temperature of 30 °C. The betaxanthin and betacyanin compositions were studied with 5% formic acid in water (v/v, eluent A) and a mixture of acetonitrile in water (80:20, v/v, eluent B). Starting isocratically with 100% A for 5 min, a linear gradient to 2% B in 2 min and isocratic elution for 3 min was performed, followed by a stepwise gradient to 6% B at 13 min, 30% B at 35 min and 100% B at 50 min before conditioning at starting conditions.

LC-MS analyses were performed on an Agilent HPLC series 1100 instrument (Agilent, Waldbronn, Germany) equipped with ChemStation software, a model G1322A degasser, a G1312A binary gradient pump, a G1329/1330A autosampler, a G1316A column oven, and a G1315A diode array detector. The HPLC system was connected in series with a Bruker Esquire 3000+ ion trap mass spectrometer (Bremen, Germany) fitted with an electrospray ionization source operating in the positive mode. Nitrogen was used as drying gas at a flow rate of 12 l/min and a pressure of 70 psi. The nebulizer temperature was set to 365 °C. Using helium as the collision gas (1.2×10^{-8} bar), collision-induced dissociation spectra were obtained with a fragmentation amplitude of 1.2 V (MS/MS).

Monitoring was performed at 280, 405, 470, and 540 nm, respectively. Identification was done by comparison with the UV-vis and mass spectrometric characteristics as well as retention times of semi-synthesized reference betaxanthins obtained according to a method described earlier (Kugler *et al.*, 2007a, b). All betaxanthin standards were checked for identity by LC-MS analyses.

Results and Discussion

Pigment stability

It was early noticed that a major challenge in the isolation of fly agaric pigments is their high susceptibility to degradation. When monitoring the stability of methanolic extracts of musca-aurins by absorption at 475 nm, a 10–15% decrease during one day at room temperature was reported. In addition, strongly acidic or alkaline conditions induced complete pigment decay within minutes (Döpp and Musso, 1973; Musso, 1979). This chemical instability was now corroborated by HPLC

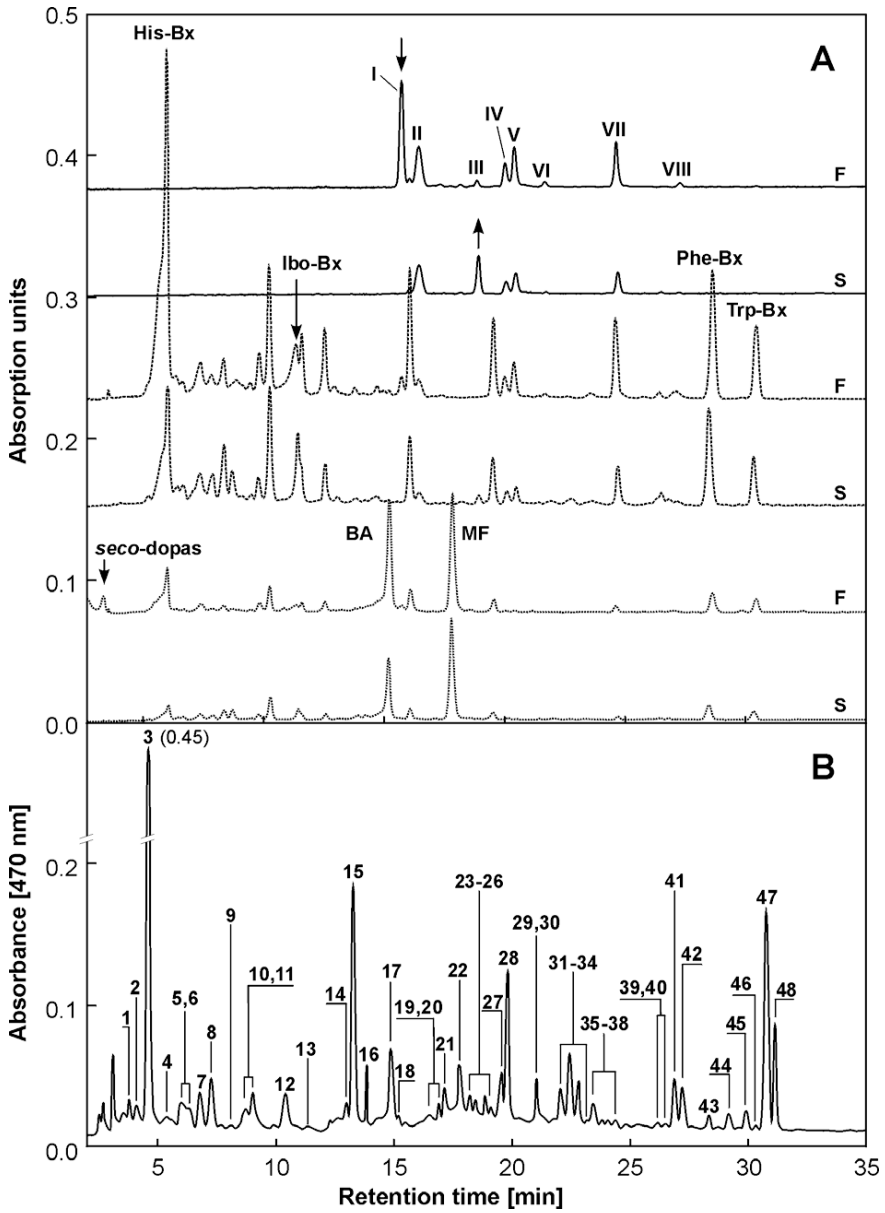

Fig. 1. (A) HPLC patterns of fresh (F) and stored (28 d, 8 °C; S) extracts from cap skin of *Amanita muscaria* (HPLC system I). Detection of betacyanin-like components (I–VIII) was performed at 540 nm (solid line). Detection of betaxanthins was done at 475 nm (dashed line) and precursors were monitored at 405 nm (dotted line). Downward directed arrows refer to complete disappearance of pigments. Upward directed arrows indicate an increase of the respective betacyanin-like pigment. The concentration changes of selected pigments are summarized in Table I. BA, betalamic acid; MF, muscaflavin. (B) HPLC pigment pattern of extracts from cap skin of *Amanita muscaria* at 470 nm (HPLC system II). Peak assignment is given in Table II.

analyses of sequential injections of aliquots of the same extracts at 20 °C and after storage at 8 °C (Fig. 1A). Betalamic acid readily identifiable by its typical fronting and muscaflavin as the predominant compound at 405 nm, the *seco*-dopas (410 nm) representing their biosynthetic precursors, the betaxanthins (475 nm) and the betacyanin-like compounds (540 nm) showed decreasing

Table I. Peak area changes (%) of *A. muscaria* pigments during storage (analyzed by HPLC system I).

Condition	405 nm			475 nm			540 nm		
	Seco-dopas	Betalamic acid (**30**)	Muscaflavin (**38**)	Ibo-Bx	Phe-Bx (**47**)	Trp-Bx (**48**)	BC-like (**I**) R_t = 15.8 min	BC-like (**II**) R_t = 16.5 min	BC-like (**III**) R_t = 18.9 min
1 h, 20 °C[a]	−29.2 ±1.6	−7.9 ±2.6	−0.7 ±0.5	−1.1 ±0.5	−3.6 ±0.8	−6.3 ±1.9	−4.0 ±1.1	−3.0 ±1.9	+17.3 ±0.9
28 d, 8 °C[b]	−100	−65.7	−42.8	−100	−46.0	−52.9	−100	−49.0	+521
40 d, 8 °C[b]	−100	−72.1	−50.4	−100	−54.0	−66.7	−100	−55.9	+441

[a] Data obtained from separate fly agaric extracts (n = 3).
[b] Data obtained from one representative fly agaric extract; variation of duplicate determinations < 5%.

peak areas after storage with some peculiarities. The *seco*-dopas, known to be chemically very labile (Terradas and Wyler, 1991), depleted fastest, probably involving a partial transformation to betalamic acid and muscaflavin, thus causing an overall slower drop of the latter. As earlier reported (Döpp and Musso, 1973), betaxanthins are unstable compounds decreasing upon storage. A component assigned as ibotenic acid-betaxanthin (Ibo-Bx) by co-injection analysis completely disappeared after 4 weeks at 8 °C and could not be detected by LC-MS any more. Instead its decarboxylation product muscimol-betaxanthin was detected, a compound earlier observed as an artefact during partial synthesis and purification of Ibo-Bx (Döpp *et al.*, 1982). The instability of the betacyanin-like pigments from fly agarics were in the same range as the betaxanthins (Table I). The prevailing compound with the highest polarity (R_t 15.8 min, **I**; HPLC system I) showed an absorption maximum at λ_{max} 558 nm, hitherto not described in fresh extracts. Its level decreased rapidly in favour of a less polar compound (R_t 18.9 min, **III**; HPLC system I) with spectral properties (λ_{max} 545 nm) previously allocated to the purple muscapurpurin (Musso, 1979; Wagner, 1986). After 4 weeks at 8 °C compound **I** was completely undetectable, but led to a five-fold increase of compound **III** (Fig. 1A), the level of which dropped afterwards.

Whereas almost full proof for the identity of betaxanthins can be obtained by comparison with the UV-vis, retention time as well as ESI-MS fragmentation data of semi-synthetic reference compounds, the assignment of the red betacyanin-like components from the complex pigment mixture requires time-consuming extensive chromatographic isolation and purification at slightly acidic conditions, which are known to lead to inevitable and considerable losses, even if performed in a cold lab (Stintzing *et al.*, 2004). Therefore, the present study includes both red and yellow pigments, but focuses on the betaxanthin pattern of fly agaric pigments.

Betacyanin-like compounds

Whereas higher plants of the order Caryophyllales accumulate betacyanins mainly derived from betanidin (Strack *et al.*, 2003), pigments with very similar absorption properties were detected in fly agaric extracts, but could not be related to plant betacyanins. HPLC analyses of fresh extracts prepared from three individual fly agarics growing at close quarters revealed that two of them contained five major (**I**, **II**, **IV**, **V**, **VII**) and three minor (**III**, **VI**, **VIII**) red pigments, whereas in the third extract only the major betacyanin-like pigments were found (data not shown). Two of them could be tentatively assigned as muscapurpurin (**29**) and muscarubrin (**42**), respectively, on the basis of the UV-vis spectra reported earlier (Döpp *et al.*, 1971; Table II). The protonated molecular ion of **29** (*m/z* 419) corresponds to the involvement of a *cyclo*-stizolobic structure proposed by Zahn (1978) and Musso (1979), whereas the *m/z* 407 of **27** (betacyanin-like, **II**) is compatible with the pyrroline 2-carboxylic acid derivative proposed by Gill and Steglich (1987) on the basis of the results by Wagner (1986). Since these betacyanin-like compounds turned out to be unstable and reference compounds are not available, no isolation and purification was performed for a closer structural assignment in the present work.

Betaxanthin profile characterization

After optimization of the gradient system, about 50 pigments could be separated, 15 of which could

Table II. Retention time and LC-DAD-MS^n data of betalamic acid and muscaflavin, yellow betaxanthins (Bx) as well as red-violet betacyanin-like pigments from fly agaric cap skin extracts.

Peak no.	Retention time [min]	LC-DAD λ_{max} [nm]	$[M+H]^+$ (m/z)	MS^2 (m/z)[a]	MS^3 (m/z)[a]	Pigment assignment (trivial name)
1[b]	3.8	250, 467	425	381	115	Betaxanthin-like
2[b]	4.2	266, 465	421	173	116	Betaxanthin-like
3	4.7	259, 471	349	305	217	Histidine-Bx (Musca-aurin VII)
4[b]	5.6	249, 280, 473	268	136	136	Betaxanthin-like
5[b]	6.2	260, 469	268	136	–[c]	Betaxanthin-like
6[b]	6.5	250, 466	541	379	–[c]	Betaxanthin-like
7	7.0	210, 253, 468	299	194	194	Serine-Bx
8[b]	7.5	257, 341, 466	542	524	437	Betaxanthin-like
9[b]	8.4	255, 339, 480	284	152	–[c]	Betaxanthin-like
10[b]	9.0	251, 470	347	199	199	Betaxanthin-like
11	9.4	252, 469	340	323	277	Glutamine-Bx (Vulgaxanthin I)
12[b]	10.2	249, 470	–[c]	–[c]	–[c]	Betaxanthin-like
13	10.6	258, 460	255	168	168	Ethanolamine-Bx
14	13.1	259, 465	313	269	135	Threonine-Bx
15[d]	13.4	260, 466	310	277	231	4,5-Dihydromuscimol-Bx
16[b,e]	13.7	245, 449, 471	376	148	130	Betaxanthin-like
17	15.0	214, 264, 469	308	264	264	Muscimol-Bx
18[b]	15.1	245, 336, 468	600	556	494	Betaxanthin-like
19[a]	16.6	247, 472	617	599	580	Betaxanthin-like
20[b]	16.9	249, 469	389	345	299	Betaxanthin-like
21	17.2	253, 467	283	150	–[c]	Alanine-Bx
22[d]	17.8	196, 260, 473	421	375	301	Stizolobic acid-Bx (Musca-aurin II)
23[b]	18.2	258, 334, 474	554	510	215	Betaxanthin-like
24[b]	18.4	238, 267, 474	565	547	547	Betaxanthin-like
25[b]	18.9	237, 278, 472	421	375	194	Betaxanthin-like
26[b]	19.1	236, 262, 482	389	343	281	Betaxanthin-like
27[b]	19.6	262, 300, 530	407	363	316	Betacyanin-like (**II**)
28	19.8	237, 261, 471	391	347	299	Dopa-Bx (Dopaxanthin)
29[d]	21.1	259, 324, 542	419	375	331	Betacyanin-like (**III**) (Muscapurpurin)
30	21.3	236, 258, 405	212	166	121	Betalamic acid
31[e]	22.0	–[c]	343	299	255	Methionine-Bx
32[b]	22.0	267, 336, 516	389	345	299	Betacyanin-like (**IV**)
33[b]	22.4	263, 341, 471	490	307	261	Betaxanthin-like
34[b]	22.6	246, 337, 515	582	538	226	Betacyanin-like (**V**)
35[b]	30.0	264, 339, 481	582	494	378	Betaxanthin-like
36[b]	23.2	263, 329, 473	602	558	200	Betaxanthin-like
37[b]	23.4	263, 476	403	359	315	Betaxanthin-like
38	23.6	237, 252, 404	212	166	110	Muscaflavin
39[b,e]	24.4	264, 470	212	166	110	Betaxanthin-like
40[e]	26.8	–[c]	311	193	152	Valine-Bx
41[b]	27.0	273, 338, 516	582	492	404	Betacyanin-like (**VI**)
42[d]	27.4	266, 368, 499	403	359	313	Betacyanin-like (**VII**) (Muscarubrin)
43[b]	28.4	265, 339, 476	375	357	225	Betaxanthin-like
44[b]	29.5	252, 342, 515	582	538	345	Betacyanin-like (**VIII**)
45[b]	30.0	265, 351, 477	414	185	158	Betaxanthin-like
46	30.5	265, 337, 471	325	194	121	Leucine-Bx
47	31.0	234, 262, 471	359	315	106	Phenylalanine-Bx
48	31.3	266, 472	398	354	266	Tryptophan-Bx

[a] Most intensive ion is presented.
[b] No assignment possible.
[c] No or no unambiguous signal.
[d] Preliminary assignment.
[e] Traces.

be assigned to betaxanthin structures (Fig. 1B; Table II). Some of them were long known (glutamine-Bx, **11**; Piattelli *et al*., 1965) or were reported previously to occur in higher plants such as histidine-Bx (**3**), serine-Bx (**7**) (Kugler *et al.*, 2004; Stintzing *et al.*, 2002, 2005), ethanolamine-Bx (**13**) and threonine-Bx (**14**) (Kugler *et al*., 2007b), alanine-Bx (**21**) (Kugler *et al.*, 2004), Dopa-Bx (**28**) and leucine-Bx (**46**) (Kugler *et al.*, 2004, 2007a, b; Stintzing *et al.*, 2002, 2005), phenylalanine-Bx (**47**) and tryptophan-Bx (**48**) (Kugler *et al.*, 2004, 2007a, b; Schliemann *et al.*, 2001; Stintzing *et al.*, 2002). Methionine-Bx (**31**), only recently identified in cactus pears (Stintzing *et al.*, 2005), in red and yellow beetroots and in yellow Swiss chard (Kugler *et al.*, 2007b), as well as valine-Bx (**40**) could only be detected in traces. Serine-Bx (**7**), threonine-Bx (**14**), ethanolamine-Bx (**13**), alanine-Bx (**21**), Dopa-Bx (**28**), phenylalanine-Bx (**47**) and tryptophan-Bx (**48**) are reported for the first time to contribute to the pigment pattern of fly agaric, while histidine-Bx (musca-aurin VII, **3**) and the betaxanthins derived from neutral amino acids such as glutamine (**11**), valine (**40**) and leucine (**46**) were in correspondence with the *Amanita* betaxanthin patterns of pooled material reported by Döpp *et al.* (1982). In contrast to Döpp *et al.* (1982) the betaxanthins derived from glutamic, aspartic, and α-amino-adipic acids as well as proline and asparagine could not be corroborated for our extracts by co-injection experiments with reference compounds (data not shown). Unlike the UV-vis data of the fresh extracts, no *seco*-dopas with expected *m/z* values of 238 could be detected in the stored extract. Among the pigments, musacaflavin (**38**), the structural isomer of betalamic acid (**30**), was detected representing the major pigment at 405 nm (Fig. 1A). The presence of stizolobic acid-Bx (musca-aurin II, **22**) was tentatively assigned by comparison with UV-vis data from the literature (Döpp *et al.*, 1982) and its typical mass spectrum (Table II). Unfortunately, no co-injection experiment was possible due to lack of commercially available stizolobic acid for preparation of the corresponding Bx standard. Noteworthy, ibotenic acid-Bx (musca-aurin I; Döpp *et al.*, 1982) could only be found in fresh extracts, while its decarboxylated conjugate muscimol-Bx (**17**) was obviously more stable and thus unambiguously identified in all extracts. According to Döpp and co-workers (1982), muscimol-Bx was detected as an artefact in partial synthesis of musca-aurin I, although its genuine presence would be plausible due to the presence of free muscimol in fly agaric extracts (Krogsgaard-Larsen *et al.*, 1981; Tsujikawa *et al*., 2007) and its spontaneous conjugation with betalamic acid to form the respective betaxanthin (Schliemann *et al.*, 1999). Moreover, a muscimol-Bx derivative, 4,5-dihydromuscimol-Bx (**15**), could be tentatively assigned for the first time as a genuine compound in the present study (Table II). Remarkably, the betalamic acid conjugates of stizolobic acid (**22**), muscimol (**17**), 4,5-dihydromuscimol (**15**), as well as ibotenic acid are restricted to *Amanita* and have not yet been reported to occur in higher plants. Other yellow compounds with absorption maxima typical for betaxanthins listed in Table II could not be more closely assigned by comparison with literature values and co-injection experiments with betalamic acid conjugates of amino acids and amines (Kugler *et al.*, 2004, 2007a, b; Schliemann *et al.*, 1999; Stintzing *et al.*, 2002, 2005). It remains unclear, whether these compounds represent hygroaurins (Bresinsky and Kronawitter, 1986; Fugmann, 1985), the muscaflavin analogues of betaxanthins. In our first approach 15 betaxanthins were assigned, but the major part of the betaxanthin-like pigments remained to be structurally elucidated. The large number of betaxanthin-like pigments suggests that an obvious restriction of the conjugate formation between betalamic acid and amino compounds does not exist indicative for a spontaneous reaction (Schliemann *et al.*, 1999). Considering the chemical susceptibility of betalains and the noted different pigment composition of individual fly agarics, in all probability also depending of the developmental state, a good deal of work must be done for complete understanding of fly agaric's pigment biochemistry.

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Bresinsky A. and Kronawitter I. (1986), Zur Kenntnis der Hygrocybenpigmente. Z. Mykol. **52**, 321–334.

Döpp H. and Musso H. (1973), Isolierung und Chromophore der Farbstoffe aus *Amanita muscaria.* Chem. Ber. **106**, 3473–3482.

Döpp H., Grob W., and Musso H. (1971), Über die Farbstoffe des Fliegenpilzes (*Amanita muscaria*). Naturwissenschaften **58**, 566.

Döpp H., Maurer S., Sasaki A. N., and Musso, H. (1982), Die Konstitution der Musca-aurine. Liebigs Ann. Chem. **2**, 254–264.

Fugmann B. (1985), Neue niedermolekulare Naturstoffe aus Höheren Pilzen (Basidiomyceten). Isolierung, Strukturaufklärung und Synthese. PhD thesis, Bonn.

Gill M. (2003), Pigments of fungi (Macromycetes). Nat. Prod. Rep. **20**, 615–639.

Gill M. and Steglich W. (1987), Pigments in fungi (Macromycetes). In: Fortschritte der Chemie organischer Naturstoffe, Vol. 51 (Herz W., Grisebach H., Kirby G. W., and Tamm C., eds.). Springer-Verlag, Wien, New York, pp. 74–286.

Kobayashi N. (2002), Contributions to betalain biochemistry. New structures, condensation reactions, and vacuolar transport. PhD thesis, Halle. http://sundoc.bibliothek.uni-halle.de/diss-online/02/03H029/prom.pdf

Krogsgaard-Larsen P., Brehm L., and Schaumburg, K. (1981), Muscimol, a psychoactive constituent of *Amanita muscaria*, as a medicinal chemical model structure. Acta Chim. Scand. B **35**, 311–324.

Kugler F., Stintzing F. C., and Carle R. (2004), Identification of betalains from differently coloured Swiss chard (*Beta vulgaris* ssp. *cicla* [L.] Alef. cv. "Bright Lights") by high-performance liquid chromatography-electrospray ionization mass spectrometry. J. Agric. Food Chem. **52**, 2975–2981.

Kugler F., Stintzing F. C., and Carle R. (2007a), Characterisation of betalain patterns of differently coloured inflorescences from *Gomphrena globosa* L. and *Bougainvillea* sp. by HPLC–DAD–ESI–MS^n. Anal. Bioanal. Chem. **387**, 637–648.

Kugler F., Stintzing F. C., and Carle R. (2007b), Studies on betaxanthin profiles of vegetables and fruits from the Chenopodiaceae and Cactaceae. Z. Naturforsch. **62c**, 311–318.

Michelot D. and Melendez-Howell L. M. (2003), *Amanita muscaria*: chemistry, biology, toxicology, and ethnomycology. Mycol. Res. **107**, 131–146.

Musso H. (1979), The pigments of fly agaric, *Amanita muscaria*. Tetrahedron **35**, 2843–2853.

Piattelli M., Minale L., and Prota G. (1965), Pigments of Centrospermae. III. Betaxanthins from *Beta vulgaris* L. Phytochemistry **4**, 121–125.

Schliemann W., Kobayashi N., and Strack D. (1999), The decisive step in betaxanthin biosynthesis is a spontaneous reaction. Plant Physiol. **119**, 1217–1232.

Schliemann W., Cai Y., Degenkolb T., Schmidt J., and Corke H. (2001), Betalains of *Celosia argentea*. Phytochemistry **58**, 159–165.

Stintzing F. C., Schieber A., and Carle R. (2002), Identification of betalains from yellow beet (*Beta vulgaris* L.) and cactus pear (*Opuntia ficus-indica* (L.) Mill.) by high-performance liquid chromatography-electrospray ionization mass spectrometry. J. Agric. Food Chem. **50**, 2302–2307.

Stintzing F. C., Conrad J., Klaiber I., Beifuss U., and Carle R. (2004), Structural investigations on betacyanin pigments by LC NMR and 2D NMR spectroscopy. Phytochemistry **65**, 415–422.

Stintzing F. C., Herbach K. M., Moßhammer M. R., Carle R.,Yi W., Sellappan S., Akoh C. C., Bunch R., and Felker, P. (2005), Color, betalain pattern and antioxidant properties of cactus pear (*Opuntia* sp.) clones. J. Agric. Food Chem. **53**, 442–451.

Strack D., Vogt T., and Schliemann W. (2003), Recent advances in betalain research. Phytochemistry **62**, 247–269.

Terradas F. and Wyler H. (1991), 2,3- and 4,5-secodopa, the biosynthetic intermediates generated from *L*-Dopa by an enzyme system extracted from the fly agaric, *Amanita muscaria* L., and their spontaneous conversion to muscaflavin and betalamic acid, respectively, and betalains. Helv. Chim. Acta **74**, 124–140.

Trezzini G. F. and Zryd J.-P. (1991), Characterization of some natural and semi-synthetic betaxanthins. Phytochemistry **30**, 1901–1903.

Tsujikawa K., Kuwayama K., Miyaguchi H., Kanamori T., Iwata Y., Inoue H., Yoshida T., and Kishi T. (2007), Determination of muscimol and ibotenic acid in *Amanita* mushrooms by high-performance liquid chromatography and liquid chromatography-tandem mass spectrometry. J. Chromatogr. B **852**, 430–435.

Wagner I. (1986), Beitrag zur Strukturaufklärung des rotvioletten Fliegenpilzfarbstoffes Muscapurpurin. PhD thesis, Karlsruhe.

Zahn M. (1978), Zur Konstitution, Synthese und Biosynthese von Muscapurpurin, dem rotvioletten Farbstoff aus der Huthaut des Fliegenpilzes (*Amanita muscaria*). PhD thesis, Karlsruhe.

Zrÿd J.-P. and Christinet L. (2004), Betalains. In: Plant Pigments and their Manipulation. Annual Plant Reviews, Vol. 14 (Davies K. M., ed.). Blackwell, Oxford, pp. 185–213.