Chemotaxonomic investigations of species of *Dermocybe* (Fr. Wünsche (Agaricales) from New Zealand, Papua New Guinea and Argentina

Gerwin Keller*) & Meinhard Moser

Institut für Mikrobiologie, Universität Innsbruck, Sternwartestraße 15, A-6020 Innsbruck, Austria

Egon Horak

Geobotanisches Institut, Eidgenössische Technische Hochschule, ETH-Zentrum, CH-8092 Zürich, Switzerland

Wolfgang Steglich

Institut für Organische Chemie und Biochemie, Universität Bonn, Gerhard-Domagk-Straße 1, D-5300 Bonn 1, Federal Republic of Germany

Abstract. – Pigmentation of 26 species of *Dermocybe* (Fr.) Wünsche from New Zealand (Horak, 1988), Australia (Horak, 1983), subantarctic and Patagonian Argentina (Moser & Horak, 1975) and Papua New Guinea (4 unnamed collections in herb. Horak, ZT) were investigated by means of thin layer chromatography. A comparison of the pigment data shows more or less specific pigment patterns (with 13 different types of pigmentation) for the species employed in this study. The importance of pigmentation and its relevance to the classification of the species examined are discussed.

For the illustrations and formal descriptions of the New Zealand species of *Dermocybe* see Horak (1988).

Introduction

Anthraquinone derivatives are found in a great variety in fruit bodies of species of the genera *Dermocybe* (Fr.) Wünsche and *Cortinarius* Fr. Their occurence and distribution have proved to be useful in differentiating infrageneric taxa (Gill & Steglich, 1987). Most of the pigments isolated from *Dermocybe* and *Cortinarius* species were identified by Steglich (1980), Steglich & Oertel (1984) and Besl & al. (1978). In recent years more and more chemical studies were done with species of *Dermocybe* and *Cortinarius* from the Southern hemisphere. Archard & al. (1985), Gill & Strauch (1985) and Gill & Smrdel (1988) have shown that an Australian

*) present address: Forstliche Bundesversuchsanstalt, Abteilung Bodenbiologie, Langgasse 64, A-6460 Imst, Austria.

species of *Dermocybe* forms anthraquinone pigments which are different from those isolated from its counterparts in the Northern Hemisphere. In a more recent study KELLER & STEGLICH (1987) report on the pigmentation of *Dermocybe canaria* which is a taxonomically isolated species from New Zealand. An unusual chromogen is reported for an Australian species of *Cortinarius* subgen. *Sericeocybe* by GILL & al. (1987).

In view of the importance of the anthraquinonic pigments and further secondary metabolites as taxonomic criteria European species of *Dermocybe* and *Cortinarius* have been chemotaxonomically studied by GABRIEL (1960a, b; 1961; 1962), GRUBER (1970), MOSER (1972), HOILAND (1980; 1983), KELLER (1982), MOSER & KELLER-DILITZ (1983), HOFBAUER (1984), STEGLICH & OERTEL (1984) and KELLER-DILITZ & al. (1985). South American species of *Dermocybe* and *Cortinarius* were investigated by GRUBER (1975). Finally KELLER & AMMIRATI (1983) report on the pigmentation of North American taxa of *Dermocybe*.

The relevance of chemical characters for the taxonomy of the Agraricales is discussed by MOSER (1985).

The objective of this study was to determine pigments and specific pigment patterns of species from New Zealand, Papua New Guinea and Australia. Since South American species of *Dermocybe* and *Cortinarius* were considered to be of importance for this study we thought it profitably to reexamine some of the Argentine species which have been investigated by GRUBER (1975) previously.

Original collections included in the present study were taxonomically described by MOSER & HORAK (1975; South America), HORAK (1983; Australia, New Zealand) and HORAK (1988; New Zealand). The specimens reported from Papua New Guinea are not named yet and are kept in the Herb. HORAK (ZT).

Material and methods

Fungal material included in this study is listed in Table 1 with collection numbers, geographic origin and location of voucher specimens. Herbaria are indicated by the appropriate abbreviations from the *Index Herbariorum*.

Extraction and chromatography of pigments: The pigments were isolated from dried carpophores by extraction with ethanol (96%). 0.1–0.3 g of dried material from each collection was finely ground and extracted with 30 ml of ethanol (96%). These extracts were submitted to thin layer chromatography (TCL) with different solvent systems (precoated silica gel TLC plates (MERCK): system I: benzene : acetic acid (glacial) 2:1, system II: ethyl acetate : methanol : H_2O 100:16.5:15.5, system III: benzene : ethyl formate : formic acid

Table 1. Chemotaxonomy of *Dermocybe*: List of analysed species
Abbrevations: ARG = Argentina; AUS = Australia; NZ = New Zealand; PNG = Papua New Guinea; IB = Herb. Innsbruck, Austria; MT = Herb. M. TAYLOR, Auckland, New Zealand; ZT = Herbarium ETHZ, Zürich, Switzerland.

	Dermocybe spp.	Herbarium, Nr.	Origin
1	D. alienata HORAK (1988)	PDD, 27180 (type)	NZ
	D. alienata	MT, 673	NZ
	D. alienata	MT, 839	NZ
2	D. cardinalis HORAK (1988)	PDD, 27174 (type)	NZ
	D. cardinalis	ZT, 69/327	NZ
	D. cardinalis	ZT, 76/240	NZ
	D. cardinalis	ZT, 597	NZ
	D. cardinalis	ZT, 917	NZ
3	D. castaneodisca HORAK (1988)	PDD, 27178 (type)	NZ
4	D. cramesina HORAK (1988)	PDD, 27173 (type)	NZ
5	D. egmontiana HORAK (1988)	PDD, 27177 (type)	NZ
6	D. icterinoides HORAK (1988)	PDD, 27184 (type)	NZ
	D. icterinoides	ZT, 69/250	NZ
7	D. indotata HORAK (1988)	PDD, 27182 (type)	NZ
	D. indotata	ZT, 69/216	NZ
8	D. largofulgens HORAK (1988)	PDD, 27181 (type)	NZ
9	D. leptospermarum HORAK (1988)	PDD, 27183 (type)	NZ
	D. leptospermarum	ZT, 68/354	NZ
	D. leptospermarum	ZT, 68/405	NZ
	D. leptospermarum	ZT, 69/226	NZ
10	D. olivaceonigra HORAK (1988)	PDD, 27179 (type)	NZ
11	D. splendida HORAK (1988)	PDD, 27168 (type)	NZ
	D. splendida	ZT, 71/81	NZ
12	D. purpurata HORAK & KELLER (1988)	PDD, 27171 (type)	NZ
	D. purpurata	ZT, 69/108	NZ
13	D. vinicolor HORAK (1988)	ZT, 67/188	NZ
14	D. alcalisensibilis MOSER (1975)	IB, M 5327a (type)	ARG
15	D. amoena MOSER (1975)	IB, TF 109	ARG
16	D. austronanceiensis MOSER (1975)	IB, 63/398	ARG
17	D. icterina HK. in MOS. & HK. (1975)	IB, M 100	ARG
18	D. luteostriatula MOS. & HK. (1975)	IB, 63/320	ARG
19	D. obscuroolivea var. brunnea MOSER (1975)	IB, 63/379	ARG
20	D. oliveoicterina MOSER (1975)	IB, 63/195 (type)	ARG
21	D. olivipes MOSER (1975)	IB, 63/172	ARG
22	Dermocybe sp.	ZT, 71/392	PNG
23	Dermocybe sp.	ZT, 72/94	PNG
24	Dermocybe sp.	ZT, 72/317	PNG
25	Dermocybe sp.	ZT, 72/318	PNG
26	Cort. umbonatus CLEL. & HARRIS	IB, Herb. CLEL., 11	AUS

160:40:1, system IV: chloroform:ethanol 3:1; cellulose TLC plates [self prepared, cellulose microcristalline (MERCK)]: system A: n-butanol:pyridine:H_2O 6:4:3, (v/v), thickness of layer: 0.25 mm).

Identification of pigments: (1) Pigments were identified by co-chromatography with authentic reference samples. In many cases extracts of various other *Dermocybe* and *Cortinarius* species were chromatographed simultaneously to get a better interpretation of the pigment patterns. Furthermore, all distinct pigment fractions obtained by system I were rechromatographed with systems I, II, III, IV and A. Spraying of the TLC plates with a solution of KOH (5% in methanol) resulted in specific colour reactions. Similar colour changes were obtained using magnesium acetate (5% in methanol) as reagent. – (2) Some of the pigments were identified by spectroscopic methods. 1.0–1.5 g of dried fungal material was extracted with 100 ml of ethanol (96%). The extract was filtered and the solvent was removed under reduced pressure. The extract was shaken with H_2O (30 ml) containing hydrochloric acid (1 drop) and this aqueous suspension was extracted subsequently with ethyl acetate (2–3×50 ml). The organic phase was evaporated under reduced pressure and subjected to preparative TLC on precoated TLC plates (silica gel 60 MERCK, solvent systems I, II or III). The pigment fractions were eluated with ethyl acetate, dried with Na_2SO_4 and evaporated under reduced pressure. ^{1}H-NMR, UV or mass spectra were recorded from pigments of interest.

Results

Chromatographic examination revealed that anthraquinonic pigments are present in carpophores of all species included in this study (Table 1). Furthermore, the results indicate that all species have pigment patterns which are derived from a more or less specific combination of several pigments. Thirteen distinct types of pigmentation were detected by evaluating chromatigraphic data of all 26 species investigated. In the following species with similar type of pigmentation will be treated together. The chromatographic experiments gave the following results:

1. Group A (Table 2):

New Zealand:	*D. alienata* HK. (type)
	D. alienata HK. (839, ex Herb. TAYLOR)
	D. icterinoides HK. (type)
Papua New Guinea:	*Dermocybe* sp. (ZT, 72/94)
Argentina:	*D. olivipes* MOS.
	D. obscuroolivea var. *brunnea* MOS.
	D. luteostriatula MOS. & HK.
	D. alcalisensibilis MOS. (type)

Tab. 2. Pigments of species in Group A
Symbols: +,++, +++, ++++ indicate relative intensity of pigment fraction, (+) indicates traces of pigment detectable, – indicates pigment not detected, (?): identity of pigment not certain.

Pigment pattern and properties										Relative intensity							
Nr.	Pigment	R. value X 100 in system I	II	III	IV	A	Colour in daylight	Colour in UV light (λ = 365 nm)	Colour reaction with KOH	*D. alienata* (type)	*D. alienata* (Taylor, 839)	*D. icterinoides* (type)	*D. ermocybe* sp. (ZT, 72/94)	*D. olivipes*	*D. obscurooliveа* var. *brunnea*	*D. luteostriatula*	*D. alcalisensibilis* (type)
12		65	80	32	83	98	yellow	orange	pink	–	–	++	–	++	++	(+)	(+)
		53					ochraceous	orange	pink	(+)	(+)	–	(+)	(+)	(+)	(+)	–
28	Skyrin	48	78	11	73	98	orange	brown	purple	+++	+++	++++	++++	++	+++	+++	++++
		43					brown	brown	pink	–	–	++	–	–	–	–	–
41	Endocrocin	28	22	03	08	67	yellow	ochraceous	pink	+	+	+	+	(+)	+	+	+
42	Hypericin	23	45	01	11	98	grey	bright red	grey	++	++	++	++	(+)	+	++	+
47	Dermolutein	16	15	00	09	53	yellow	orange	orange	(+)	(+)	+	+	+	++	–	–
52		08	04	00	02	56	ochraceous	brown	pink	+	+	++	++	+	+	(+)	+

It is particularly clear from Table 2 that the pigment pattern of the species of this group are similar. The main pigment in carpophores of these species has chromatographically been identified as skyrin. The identity of this pigment was further verified by UV and ^{1}H-NMR spectroscopy of the corresponding fraction of *Dermocybe* sp. (ZT, 72/94) [Skyrin: UV λ_{max} (MeOH) nm: 222, 255, 296, 455; ^{1}H-NMR (90 MHz, D_6, –Aceton) δ: 2.37 (s, $C_{3/3}$, –Me), 6.80 (s, $C_{7/7}$, –H), 7.10 (m, $C_{2/2}$, –H), 7.31 (m, $C_{4/4}$, –H), 12.15 (s br, $C_{1/1}$, –OH), 12.88 (s br, $C_{8/8}$, –OH). As a further compound hypericin has been found on the chromatograms of all species. The identity (hypericin: UV λ_{max} (MeOH) nm: 508, 548, 588) was verified by co-chromatography with an authentic reference sample and by UV spectra from a fraction isolated from *Dermocybe* sp. (ZT, 72/94). On chromatograms of *D. obscuroolivea* var. *brunnea* and *D. icterinoides* developed with solvent system II more compounds with a hypericin-like fluorescence were detected. Two of the minor pigments found in species of this group are identical with edocrocin and dermolutein. All other pigments remain unidentified. Whereas skyrin, hypericin, endocrocin and pigment 52 were found in all species dermolutein was not detectable in carpophores of *D. alcalisensibilis* and *D. luteostriatula*. Pigment 12 was found to be present in all species except of *D. alienata* and *Dermocybe* sp. (ZT, 72/94).

2. Group B (Table 3):

New Zealand:	*D. olivaceonigra* Hk. (type)
Argentina:	*D. austronanceiensis* Mos.

It is apparent from the data in Table 3 that *D. olivaceonigra* and *D. austronanceiensis* share many important pigment characters. Their type of pigmentation is based on endocrocin, dermolutein and the unidentified pigments 12, 24, 80, 81 and 82. The pigments 80, 81 and 82 do not have colour reactions and fluorescence properties typical for anthraquinone derivatives. It is possible that these pigments are related to dihydroanthracenones like phlegmacin-8'-methylether. It is a point of interest that endocrocin and dermolutein were found on chromatograms of *D. austronanceiensis* but no fractions considered to be the glycosidic portions of these pigments were traceable. On the other hand endocrocin and endocrocinglycoside (but not dermolutein) have been found in *D. olivaceonigra*.

3. Group C (Table 4):

Argentina:	*D. icterina* Hk.
	D. oliveoicterina Mos. (type)
	D. amoena Mos. & Hk.

Table 3. Pigments of species in Group B (for symbols see Table 2)

Pigment pattern and properties										Relative intensity	
Nr.	Pigment	R. value X 100 in system I	II	III	IV	A	Colour in daylight	Colour in UV light (λ= 365 nm)	Colour reaction with KOH	*D. olivaceonigra* (type)	*D. austronanceiensis*
6	Physcion	76	78	58	80	98	yellow	orange	pink	(+)	–
82		74					brown	dark	brown	++	+
12		68	79	35	82	98	yellow	red	purple	+++	+++
24		53	79	11	62	98	yellow	yellow	purple red	–	(+)
81		42					ochraceous	dark	brown	++	(+)
41	Endocrocin	28	22	03	08	67	yellow	ochraceous	pink	++	++
47	Dermolutein	16	15	00	09	53	yellow	orange	orange red	–	++
80		24					yellow	dark	brown	+	–
63	Endocrocin-glycoside (?)	02	06	00	00	47	yellow	ochraceous	pink	+++	–

Chromatographic examination of *D. icterina*, *D. oliveoicterina* and *D. amoena* revealed that pigment patterns of these species are remarkably similar. Table 4 shows that pigment patterns differ only by some quantitative variation. Two of the pigments were identified

Table 4. Pigments of species in Group C (for symbols see Table 2)

Pigment pattern and properties										Relative intensity		
Nr.	Pigment	R. value X 100 in system I	II	III	IV	A	Colour in daylight	Colour in UV light (λ= 365 nm)	Colour reaction with KOH	*D. icterina*	*D. amoena*	*D. liveoicterina* (type)
24		53	79	11	62	98	yellow	yellow	purple red	+++	+++	+++
41	Endocrocin	28	22	03	08	67	yellow	ochraceous	pink	++	++	+++
47	Dermolutein	16	15	00	09	53	yellow	orange	orange red	+++	++	++
51		08	08	00	03	41	yellow	ochraceous	orange	+	+	+
63	Endocrocin-glycoside (?)	02	06	00	00	47	yellow	ochraceous	pink	–	–	+
79		00	05	00	00	28	yellow	orange	pink	+	(+)	+

with endocrocin and dermolutein, all others remain unknown. Endocrocin, dermolutein and pigment 24 predominated on chromatograms of each of the three species. Pigments 24 and 51 were found to be the most specific compounds of this group and do not occur in other species employed in this study. It should be considered that pigment 24 might be an oxidation product of an other chromatographic fraction.

4. Group D (Table 5):
New Zealand: *D. largofulgens* Hk. (type)

The chromatograms of *D. largofulgens* showed ten different pigment fractions but only one compound was identified with endocrocin. All others were not identical to reference samples available for comparison.

Table 5. Pigments of *D. largofulgens* Hk., Group D (for symbols see Table 2)

Nr.	Pigment	R. value X 100 in system					Colour in daylight	Colour in UV light (λ= 365 nm)	Colour reaction with KOH	Relative intensity
		I	II	III	IV	A				
3		78	79	58	81	98	yellow	pink	pink	+++
15		60	62	34	79	98	lemon yellow	orange	yellow	+++
18		59	68	26	74	79	purple red	pink	violet	+++
35		35	61	05	62	90	lemon yellow	ochraceous	yellow	+++
		30					pink	pink	violet	+
41	Endocrocin	28	22	03	08	67	yellow	ochraceous	pink	(+)
		11					yellow	pink	yellow	+
54		06	29	00	28	68	yellow	red	pink	+++
62		02	24	00	15	59	yellow	brown	violet	+
		00	23				pink	brown	violet	(+)
75		00	03	00	00	56	yellow	brown	yellow	++
		00	00	00	00	12	yellow	pink	–	(+)

5. Group E (Table 6):
New Zealand: *D. castaneodisca* Hk. (type)

Eight pigments were found on chromatograms of *D. castaneodisca* with endocrocin being the only identified pigment. All others remain unknown and rarely had homologues on the chromatograms of other species investigated.

6. Group F (Table 7):
New Zealand: *D. leptospermarum* Hk. (type)
D. leptospermarum Hk. (ZT, 68/354; ZT, 68/405; ZT, 69/226)

Table 6. Pigments of *D. castaneodisca* HK., Group E (for symbols see Table 2)

Nr.	Pigment	R. value X 100 in system					Colour in daylight	Colour in UV light (λ= 365 nm)	Colour reaction with KOH	Relative intensity
		I	II	III	IV	A				
7		75	79	42	82	98	yellow	pink	violet	+
20		55	77	08	79	98	yellow	dark	purple	+++
41	Endocrocin	28	22	03	08	67	yellow	ochraceous	pink	++
48		13	45	00	45	85	lemon yellow	pink	pink	++
53		07	10	00	34	48	yellow	orange	pink	++
74		00	05	00	08	39	yellow	ochraceous	pink	+
76		00	01	00	00	25	yellow	yellow	pink	+

Table 7. Pigments of *D. leptospermarum HK.*, Group F:
AFDM = Anhydroflavomannin-9.10-chinon-6.6'-dimethylether
FDM = Flavomannin-6.6'-dimethylether
(for further symbols see Table 2)

Pigment pattern and properties										Relative intensity	
Nr.	Pigment	R. value X 100 in system					Colour in daylight	Colour in UV light (λ= 365 nm)	Colour reaction with KOH	*D. leptospermarum* (type)	*D. leptospermarum* (ZT, 68/354)
		I	II	III	IV	A					
1		81	82	60	82	98	yellow	pink	purple	+++	++
23	AFDM	56	73	09	72	98	yellow	dark	purple	++	+
		41					yellow	orange	orange	++	+
37	FDM	32	63	02	63	96	lemon yellow	dark	yellow	+	+
41	Endocrocin	28	22	03	08	67	yellow	ochraceous	pink	++	++
63	Endocrocin-glycoside (?)	02	06	00	00	47	yellow	ochraceous	pink	++++	++++

The pigment patterns of *D. leptospermarum* (4 collections examined) differ only by some quantitative variation (Table 7).

One of the two major colouring compounds has been identified as flavomannin-6,6-dimethylether; anhydroflavomannin-9,10-chinon-6,6'-dimethylether and pigment 1 are considered to be oxydation products. The other dominating pigment is endocrocin which seems to be present mainly as a glycoside. No endocrocin derivatives like dermolutein or cinnalutein were found. All other pigment fractions remain unidentified.

7. Group G (Table 8):

New Zealand: *D. indotata* Hk. (type)
D. indotata Hk. (ZT, 69/216)
D. egmontiana Hk. (type)
D. vinicolor Hk. (ZT, 67/188)
D. purpurata Hk. & Keller (type)

Chromatographic examination of *D. indotata, D. egmontiana* and *D. vinicolor* reveals that these species share important pigment characters. It is apparent from Table 8 that anthraquinones of the emodin type are predominating in the carpophores of these species. With the exception of *D. purpurata* emodin itself seems to be the main colouring principle of this group. In *D. indotata* and *D. egmontiana* physcion, dermoglaucin, endocrocin and four unidentified yellow pigments have been deteted als minor pigments. In *D. egmontiana* dermocybin has been found to be an additional colouring compound.

Twelve pigments were observed on the chromatograms of *D. vinicolor* and *D. purpurata* respectively. Both taxa share important pigment characters but they differ by some qualitative and quantitative variation. On chromatograms of *D. vinicolor* emodin was found to form the main pigment fractions as aglycon and glucoside whereas emodin was absent in *D. purpurata*. Furthermore, somewhat smaller amounts of physcion, dermoglaucin and dermocybin were detectable in *D. vinicolor*, while in *D. purpurata* dermocybin and physcion were the predominating chromatographic fractions. In addition, endocrocin and dermolutein were present on chromatograms of both taxa.

8. Group H (Table 9):

New Zealand: *D. cramesina* Hk. (type)

The chromatographic features of *D. cramesina* are illustrated in Table 9. Six different pigment fractions were chromatographically separated; three of them were identified as cinnarubin, endocrocin and physcion. A further pink pigment with R_f value 0.49 in system A probably is identical with cinnarubinglycoside. Therefore, cinnarubin is considered to be the main colour compound of *D. cramesina*. Two purple pigments with Rf value 0.39 in system I (pigment 34) and 0.45 in system A (pigment 69) remain unidentified.

9. Group I (Table 10):

New Zealand: *D. splendida* Hk. (type)
D. splendida Hk. (ZT, 71/81)
Australia: *Cortinarius umbonatus* Clel. & Harris

Table 8. Pigments of species in Group G (for symbols see Table 2; -gl. = glycoside)

Pigment pattern and properties										Relative intensity				
Nr.	Pigment	R. value X 100 in system I	II	III	IV	A	Colour in daylight	Colour in UV light (λ= 365 nm)	Colour reaction with KOH	*D. indotata* (ZT, 69/216)	*D. indotata* (type)	*D. egmontiana* (type)	*D. vinicolor* (ZT, 67/188)	*D. purpurata* (type)
6	Physcion	75	78	58	80	98	yellow	orange	pink	+	++	+	+	+++
5		75	81	41	82	98	yellow	orange	pink	–	–	–	–	+
		74					yellow	orange	pink	(+)	(+)	(+)	–	–
15		62	62	34	79	98	lemon yellow	orange	yellow	(+)	(+)	(+)	–	–
		56	72	08	78	98	yellow	orange	yellow	–	–	–	(+)	++
21	Emodin	54	79	28	79	98	yellow	ochraceous	purple red	+++	++	+++	+++	–
25	Dermoglaucin	50	40	08	00	94	brown	dark	grey	+	+	+	(+)	+
32	Dermocybin	40	25	04	00	85	purple	dark purple	violet	–	–	++	(+)	++
41	Endocrocin	28	22	03	08	67	yellow	ochraceous	pink	(+)	+	–	–	–
47	Dermolutein	16	15	00	09	53	yellow	orange	orange red	–	–	–	++	++
48		13	45	00	45	85	lemon yellow	pink	pink	+	+	++	–	–
56	Emodinglucoside	07	38	00	32	85	yellow	ochraceous	red	+++	++	++	+++	–
57	Dermoglaucingl. (?)	05	14	00	00	70	yellow	dark	blue grey	++	++	+	(+)	+
58	Dermocybinglucoside	05	16	00	00	58	purple	purple	violet	–	–	+	+++	+++
63	Endocrocingl. (?)	02	06	00	00	47	yellow	ochraceous	pink	–	–	–	(+)	+
64	Dermoluteingl. (?)	02	05	00	00	37	yellow	orange red	pink	–	–	–	(+)	(+)

Table 9. Pigments of *D. cramesina* HK., Group H (for symbols see Table 2)

Nr.	Pigment	R. value X 100 in system					Colour in daylight	Colour in UV light (λ = 365 nm)	Colour reaction with KOH	Relative intensity
		I	II	III	IV	A				
6	Physcion	75	78	58	80	98	yellow	orange	pink	+
33	Cinnarubin	42	21	04	08	62	pink	pink	blue purple	+++
34		39	19	02	07	58	violet	red	blue purple	+++
41	Endocrocin	28	22	03	08	67	yellow	ochraceous	pink	(+)
67	Cinnarubinglycoside (?)	02	04	00	00	49	pink	purple	violet	+++
69		02	03	00	00	45	pink	red	violet	+

Table 10. Pigments of species in Group I (for symbols see Table 2)

Pigment pattern and properties										Relative intensity		
Nr.	Pigment	R. value X 100 in system					Colour in daylight	Colour in UV light (λ = 365 nm)	Colour reaction with KOH	*D. splendida* (type)	*D. splendida* (ZT, 71/81)	*Cort. umbonatus*
		I	II	III	IV	A						
3	6-methylxantho-purpurin-3-methylether	75	79	58	81	98	lemon yellow	yellow	orange	+++	+++	+++
11	Austrocortinin	68	76	50	79	86	pink	orange	violet			
22	Desoxyaustrocortilutein	54	73	15	79	96	orange	ochraceous	pink	(+)	(+)	(+)
29	Desoxyaustrocortirubin	48	73	09	71	87	purple	purple red	violet	(+)	+	+
30	Austrocortilutein	45	69	06	71	95	yellow	yellow	orange red	+	+	+++
30a		43	67	04	68	93	yellow	yellow	orange red	−	−	++
38	Austrocortirubin	32	55	02	66	79	purple	red	violet	+	++++	++
			19				yellow	orange	orange	(+)	+	−
			17				yellow	orange	pink	+	+	+
72		00	11	00	03	58	yellow	lemon yellow	yellow	(+)	(+)	−

The chromatographic features of this group are shown in Table 10. The main pigment fractions of *D. splendida* HORAK (1983) are identical with 6-methylxanthopurpurin-3-methylether and austrocortinin. The identity of these compounds was proved by UV and mass spectroscopy [6-methylxanthopurpurin-3-methylether: UV λ_{max} (EtOH) nm: 224, 252, 263, 280, 338, 421; Ms (200°C) m/z (rel. int.): 284 (21), 268 (100), 255 (2), 254 (2), 239 (12), 238 (8), 225 (9), 210 (10), 197 (8), 182 (6); $C_{16}H_{12}O_5$, found 284.0674, calc. 284.0681, MS; austrocortinin: UV λ_{max} (EtOH) nm: 229 sh, 260, 299, 455 sh, 479, 512; MS (220°C) m/z (rel. int.): 284 (100), 268 (4), 266 (15), 255 (11),

254 (12), 241 (15), 238 (13), 227 (2), 226 (3), 213 (8), 210 (14), 185 (2), 182 (3); $C_{16}H_{12}O_5$, found 284.0686, calc. 284.0681, MS]. Furthermore, austrocortilutein, austrocortirubin, desoxyaustrocortilutein and desoxyaustrocortirubin were identified as minor constituents in *D. splendida*.

Examination of *Cortinarius umbonatus* showed that this South Australian species has many pigment fractions in common with *D. splendida*. 6-methylxanthopurpurin-3-methylether, austrocortirubin and austrocortilutein are found to be the major compounds in carpophores of *C. umbonatus*. Austrocortirubin must be regarded as the most predominating colour compound in fruitbodies of *D. splendida* which also occurs in Western Australia (Horak, 1983).

10. Group J (Table 11):

New Zealand: *D. cardinalis* Hk. (type)
cf. also ZT, 69/327; ZT, 76/240; ZT, 597; ZT, 917.

Ten different pigment fractions were distinguished on chromatograms of *D. cardinalis*. The chromatographic data are summarized in Table 11. The pigments range in colour from yellow to purple and most of them remain unidentified and are very specific to this species. Pigments 16 and 17 must be considered to be the major colouring substandes of *D. cardinalis*. However, endocrocin and dermolutein are the only identified pigments. Two further chromatographic fractions are probably identical with the glycosides of endocrocin and dermolutein.

Table 11. Pigments of *D. cardinalis* Hk., Group J (5 collections examined) (for symbols see Table 2)

Nr.	Pigment	R. value X 100 in system					Colour in daylight	Colour in UV light (λ= 365 nm)	Colour reaction with KOH	Relative intensity
		I	II	III	IV	A				
5		75	81	41	82	98	yellow	brown	pink	++
8		72	72	26	80	98	violet	dark	violet	++
13		65	78	11	80	98	violet	pink	violet	+
16		60	76	08	76	96	yellow	dark	pink	+++
17		59	69	06	78	93	purple	pink	blue grey	+++
41	Endocrocin	28	22	03	08	67	yellow	ochraceous	pink	++
47	Dermolutein	16	15	00	09	53	yellow	orange	orange red	++
52		08	04	00	02	56	yellow	brown	brown	+++
63	Endocrocinglycoside (?)	02	06	00	00	47	yellow	ochraceous	pink	+
64	Dermoluteinglycoside (?)	02	05	00	00	37	yellow	orange red	pink	++

11. Group K (Table 12):
Papua New Guinea: *Dermocybe* sp. (TZ, 72/317)

It is apparent from the data in Table 12 that *Dermocybe* sp. (ZT, 72/317) is a very complex coloured species. None of the chromatographic fractions were identical to any of the reference samples employed in this study. Pigment 34 of purple colour was the most prominent chromatographic fraction. Pigments 19, 22, 27, 36 and 55 were detected with somewhat smaller amounts whereas the remaining fractions were present with low intensities. It is noteworthy that pigments of this *Dermocybe* have a wide colour spectrum which ranges from lemon yellow to violet.

Table 12. Pigments of *Dermocybe* sp. (ZT, 72/317), Group K (for symbols see Table 2)

Nr.	Pigment	R. value X 100 in system					Colour in daylight	Colour in UV light (λ = 365 nm)	Colour reaction with KOH	Relative intensity
		I	II	III	IV	A				
12		65	80	33	80	98	yellow	pink	purple	(+)
19		58	44	08	29	64	lemon yellow	orange	purple	++
22		54	74	15	80	72	yellow	orange	violet	++
27		51	25	05	10	70	violet	orange	blue grey	++
		45					violet	pink	violet	(+)
34		39	19	01	10	58	violet	red	blue grey	+++
36		32	27	01	06	66	orange	purple	purple	++
40		29	28	00	07	48	violet	pink	blue grey	+
		27					violet	pink	blue grey	(+)
		11					yellow	orange red	pink	+
55		06	13	00	00	51	yellow	pink	pink	++
60		04	06	00	00	53	pink	red orange	violet	+
68		02	04	00	00	48	orange	orange	violet	+

Table 13. Pigments of *Dermocybe* sp. (ZT, 72/318), Group L (for symbols see Table 2)

Nr.	Pigment	R. value X 100 in system					Colour in daylight	Colour in UV light (λ = 365 nm)	Colour reaction with KOH	Relative intensity
		I	II	III	IV	A				
3		78	79	58	81	98	yellow	pink	pink	+++
5		75	78	45	82	98	yellow	pink	pink	+
19		56	77	08	78	98	yellow	dark	purple red	+++
		32	68	06	71	98	yellow	dark	grey	++
		25					pink	pink	violet	(+)
		23	20	01	08	98	yellow	dark	yellow	(+)
44		21	22	03	06	70	purple red	pink	violet	+++
68		02	06	00	01	49	purple red	pink	violet	++++
		02	05	00	00	46	yellow	dark	yellow	(+)
78		00	00	00	00	26	pink	pink	blue grey	+

12. Group L (Table 13):
Papua New Guinea: *Dermocybe* sp. (ZT, 72/318)

Examination of this *Dermocybe* showed that none of the chromatographic fractions were identical with any of the reference samples used in this study (see Table 13). Pigments 3, 19, 44 and 68 (yellow or red colour) were predominating on the chromatograms. All further pigments were detected with smaller chromatographic intensities.

13. Group M (Table 14):
Papua New Guinea: *Dermocybe* sp. (ZT, 71/392)

The chromatographic features for the third Papuan *Dermocybe* examined are summarized in Table 14. The major fraction of this species is identical to physcion. Further pigments which have been identified with lower intensities are emodin, dermoglaucin and endocrocin. A yellow pigment of unknown identity was found to form a strong fraction with R_f value 0.67 in system A and is probably a glycoside. In addition, two yellow pigments were detectable with R_f values 0.54 and 0.58 in system A only with low intensities in daylight but with a bright yellow fluorescence under UV light.

Table 14. Pigments of *Dermocybe* sp. (ZT, 71/392), Group M (for symbols see Table 2)

Nr.	Pigment	R. value X 100 in system					Colour in daylight	Colour in UV light (λ= 365 nm)	Colour reaction with KOH	Relative intensity
		I	II	III	IV	A				
6	Physcion	75	78	58	80	98	yellow	orange	pink	++++
15		62	62	34	79	98	lemon yellow	orange	yellow	(+)
21	Emodin	54	79	28	79	98	yellow	ochraceous	purple red	+
25	Dermoglaucin	50	40	08	00	94	brown	dark	grey	++
41	Endocrocin	28	22	03	08	67	yellow	ochraceous	pink	++
		15					violet	dark	blue grey	(+)
70		00	21	00	06	67	lemon yellow	orange	orange	+++
72		00	11	00	03	58	yellow	turquoise	yellow	+ (UV ++++)
73		00	06	00	03	54	yellow	turquoise	yellow	+ (UV ++++)

Discussion

A comparison of the chemical features and morphological characters described for the Australasian species investigated reveals that all species belonging to a chemical group (A–M) are found to have close morphological and thus taxonomic affinities. There is a

similar congruence between pigmentation and morphology as it is reported for species of *Dermocybe* and *Cortinarius* from the Northern Hemisphere.

Diversity of the *Dermocybe* species from the Southern Hemisphere is indicated by a multitude of types of pigmentation which is reported here for a comparatively small number of species investigated. Chemotaxonomic studies on further species will certainly expose more types of pigmentation. Diversity is also found in the morphology of the species

The present taxonomic structure of *Dermocybe* (and various sections of *Cortinarius*) is strongly influenced by chemotaxonomic data (MOSER, 1985). In this respect it seems to be reasonable to trace for further chemical and morphological relationships between the groups (A–M) of this study and the established sections and subsections of *Dermocybe* (and *Cortinarius*).

Chemical group A with *alienata*-type of pigmentation shows to have chemical and morphological affinities to subsect. *Atrovirentes* of sect. *Scauri* FR. which is chemically characterized by atrovirin, flavomannin, skyrin and probably by hypericin (HOFBAUER, 1983; STEGLICH & OERTEL, 1984). Only skyrin and hypericin were identified for group A since chromatographic proof of atrovirin and flavomannin was not possible. Further studies of species of group A with fresh fungal material and authentic reference samples will probably show evidence of these pigments. A close morphological relationship of group A and subsect. *Atrovirentes* is indicated by several characters. Most species of both groups correspond both in colour and shape of their fruit bodies and in spore form and spore ornamentation. Furthermore, most of the species have gelatinized and/or rather thin cuticular hyphae on the pileus and recruit their ectomycorrhiza partners in the Fagales.

Group B with *austronanceiensis*-type of pigmentation relates to species of subsect. *Percomes* of sect. *Scauri* FR. which – to some extent – are similar in pigmentation patterns and morphology of fruit bodies. The identity of the main pigment of *D. austronanceiensis* and *D. olivaceonigra* remains unclear. Moreover, pigment 24 is suspected to be an oxidation product of another chromatographic fraction. It might be identical with anhydrophlegmacin-9.10-chinon-8'-methylether since pigment 80 showed chromatographic features which are typical to phlegmacin-8'methylether. Chromatographical data of pigment 24 itself correspond with those reported for anhydrophlegmacin-9.10-chinon-8'-methylether. In addition to phlegmacin-8'-methylether endocrocin and dermolutein are typical to species of subsect. *Percomes* (HOFBAUER, 1983; STEGLICH & OERTEL, 1984). These compounds are also found in species of group B. Morphological characters give final support of a close taxonomic

relationship. *D. austronanceiensis, D. olivaceonigra* and most species of subsect. *Percomes* have similar characters regarding colour and shape of the fruit body and form and ornamentation of the spores respectively.

For the time being the identity of the main pigments of group C with *icterina*-type of pigmentation remains unclear. It is assumed that this pigment might be an oxidized form of an other pigment which probably has a dihydro-anthracenone structure. Since the identification of the relevant pigments is still insufficient and clear relationships to other taxonomic groups are missing this group should be maintained in *Dermocybe* subgen. *Icterinula* Mos. & Hk. (1975).

The evaluation of pigments in *D. castaneodisca* and *D. largofulgens* raised similar difficulties as pointed out for the foregoing group. It is clear from the chromatographic data that both species have distinctive pigment patterns not corresponding to any other pigmentation group. Evidence in pigmentation only suggests that these species might be placed in subgen. *Icterinula* Mos. & Hk which is also strongly supported by morphological characters. We are convinced that the systematic structure of the subgen. *Icterinula* Mos. & Hk. should be rearranged because pigmentation and morphological characters of several taxa support a close relationship to species of sect. *Scauri* Fr. As already emphasized sect. *Pauperae* Mos. & Hk. of subgen. *Icterinula* Mos. & Hk. should be grouped with subsect. *Atrovirentes*. Furthermore, subsect. *Percomes* should be placed near *D. austronanceiensis* and related species. In addition the species auf group C, D, and E should be kept in *Icterinula* Mos. & Hk. until the identity of most of the pigments is elucidated by chemical investigation. These species show bivalent affinities not only to taxa of sect. *Pauperae* Mos. & Hk. but also to those of sect. *Scauri* Fr. As soon as the molecular structure of the pigments in *D. icterina, D. chrysophthalma* Mos. in Mos. & Hk. (1975; from Argentina), *D. castaneodisca* and *D. largofulgens* (both from New Zealand) is known then their taxonomic position can be established accordingly.

There is some chemotaxonomic evidence that New Zealand *D. leptospermarum* Hk. has close affinities to the European *D. schaefferi* Bres. (Moser, 1986: = *D. carpineti* Mos., ined.) and related species. The relationships between group F and species of sect. *Holoxanthae* Mos. are apparent due to their content of flavomannin-6,6'-dimethylether and endocrocin. The indication regarding species affinity obtained by chromatographic experiments corroborates the morphological evidence. Similar characters in colour and form of fruit body and in shape and ornamentation of the spores confirm a

close relationship. Therefore, *D. leptospermarum* is considered to represent a member of sect. *Holoxanthae* Mos.

The group G consists of four species which have pigment characters in common with species of section *Sanguineae* Kühn. & Romagn. ex Mos. Derivatives of emodin and endocrocin are responsible for the colours in these species. The occurence of this compounds appear to be an useful chemotaxonomic criterion for grouping. Since all species share important pigment characters, they are considered to possess taxonomic connections. Since the morphological data observed on the fruit bodies also support taxonomic links it is suggested that all species listed in group G belong to sect. *Sanquineae* Kühn. & Romagn. ex Mos.

The most common pigment of *D. cramesina* is cinnarubin which is also reported from *C. cinnabarina* (Fr.) Wünsche and related species (Steglich & Reininger, 1972; Keller & Ammirati, 1983). This evidence might be regarded as a chemotaxonomic indication for a possible relationship. Since morphological differences between *D. cramesina* and *D. cinnabarina* are rather inconspicuous the two taxa are obviously close taxonomic allies.

D. splendida and *Cortinarius umbonatus* were placed in group I. The relationships of these species are established by their content of 6-methylxantopupurin-3-methylether, austrocortinin, austrocortilutein, austrocortirubin and related pigments which are herewith reported for the first time from a red Australian *Dermocybe* (Archard & al., 1985; Gill & Strauch, 1985; Gill & Smrdel, in press). The pigment similarities support a close relationship between these species, while affinities to other groups were not found. In group I no inconsistancy between morphological evidence and chromatographical data was found.

Chromatographic examination of *D. cardinalis, Dermocybe* sp. (ZT, 72/317) and *Dermocybe* sp. (ZT, 72/318) revealed that each one of these species (group J, K and L) is characterized by complex and unique pigments. Successful identification of these pigments was achieved in few cases only. To our opinion all pigments are anthrachinons which appear to be useful chemotaxonomic criteria for their identification and separation from taxa in related groups.

The spectrum of pigment patterns recorded for *Dermocybe* species included in this report is quite large and shows a greater diversity than it is known from European and North American taxa of this genus. Three groups of pigment patterns have close or direct relationships to established infrageneric categories of *Dermocybe*. *D. indota, D. egmontiana, D. purpurata,* and *D. vinicolor* can be regarded as members of sect. *Holoxanthae* Mos. or *Sanguineae* Kühn. & Romagn. ex Mos. respectively. The pigments observed in *D.*

cramesina indicate a taxonomic affinity to *D. cinnabarina* (Fr.) Wünsche and its related taxa.

The pigments observed in *D. splendida, D. cardinalis, Dermocybe* sp. (ZT, 72/317) and *Dermocybe* sp. (ZT, 72/318, both from Papua New Guinea) are classified as unique concerning their chemical composition and thus their taxonomic value(s).

The pigment pattern described for *Dermocybe* sp. (ZT, 71/392) indicate that physcion, emodin, dermoglaucin and endocrocin are the compounds responsible for the colour of its fruit bodies (group M). On account of the properties of these pigments it seems that this Papua New Guinean species is classified best in sect. *Sanguineae* Kühn. & Romagn. ex Mos. This supposed taxonomic concept, however, is not supported by the remainder of its pigments and/or morphologically relevant features of the carpophores. Two yellow fractions with a xanthon-like fluorescence in UV light, the structure of the pileal cuticle and the morphology of the spores rather suggest that *Dermocybe* sp. (ZT, 71/391) should be placed in *Cortinarius* subgen. *Leprocybe* Mos.

Acknowledgements

This research project was supported by the „Fond zur Förderung der wissenschaftlichen Forschung" (Projekt Nr. 1909, Vienna, Austria) and by a fellowship to the first author from the „Alexander von Humboldt-Stiftung" (Bonn, FRG). The authors thank Dr. M. Gill, University of Melbourne (Parkville, Australia) for the generous supply of austrocortirubin and related compounds used as reference samples in this study.

References

Archard, M., Gill, M. & Strauch., R. J. (1985). New anthraquinones from the genus *Cortinarius*. – Phytochemistry 24: 2755–2758.

Besl, H., Halbauer, R. & Steglich, W. (1987). Neue Anthrachinonfarbstoffe aus *Cortinarius armillatus* und *C. miniatopus* (Agaricales). – Z. Naturf. 33c: 294–295.

Gabriel, M. (1960a). Pigments des Cortinaires des Cinnamomei et Sanguinei. – Bull. Soc. Myc. Fr. 76: 208–215.

— (1960b). Deuxième contribution à la connaissance de la pigmentation des Cortinaires des groupes Sanguinei et Cinnamomei. – 5 Ann. Univ. Lyon 11–12: 67–76.

— (1961). Troisième contribution à l'étude des pigments des groupes Sanguinei et Cinnamomei. – Bull. Soc. Myc. Fr. 77: 262–272.

— (1962). Pigments des Cortinaires du groupe Olivascentes. – Bull. Soc. Myc. Fr. 78: 359–366.

Gill, M., Gimenez, A. & Strauch, R. J. (1987). 6-Nitro-iso-vanillic acid, an unusual chromogen from the genus *Cortinarius*. – Phytochemistry 26: 2815–2817.

— & SMRDEL, A. F. (1988). Deoxyaustrocortirubin and deoxyaustrocortilutein, tetrahydroanthraquinones from the genus *Cortinarius*. – Phytochemistry, in press.
— & STEGLICH, W. (1987). Pigments of Fungi (Macromycetes). – Progr. Chem. Org. Nat. Products 51: 1–317.
— & STRAUCH, R. J. (1985). New tetrahydroanthraquinones from the genus *Cortinarius*. – Tetrahedron Lett. 26: 2593–2596.
GRUBER, I. (1969). Fluoreszierende Stoffe der *Cortinarius*-Untergattung *Leprocybe*. – Zeitschr. f. Pilzk. 35: 249–262.
— (1970). Anthrachinonfarbstoffe in der Gattung *Dermocybe* und Versuch ihrer Auswertung für die Systematik. – Zeitschr. f. Pilzk. 36: 95-112.
— (1975). Papierchromatographische Pigmentanalyse von südamerikanischen Dermocyben und Cortinarien. – In: MOSER, M. & HORAK, E. (1975). *Cortinarius* FR. und nahe verwandte Gattungen in Südamerika. – Beihefte zur Nova Hedwigia 52: 524–540.
HOFBAUER, C. (1984). Chemotaxonomische Untersuchungen in der Untergattung *Phlegmacium*. – Thesis, University of Innsbruck.
HOILAND, K. (1980). *Cortinarius* subgen. *Leprocybe* in Norway. – Norw. J. Bot. 27: 101–126.
— (1983). *Cortinarius* subgen. *Dermocybe*. – Opera Botanica 71: 1–112.
HORAK, E. (1983). Mycogeography in the South Pacific region: Agaricales, Boletales. – Aust. J. Bot. Suppl. Ser. 10: 1–41.
— (1988). New species of *Dermocybe* (Agaricales) from New Zealand. – Sydowia 40: 81.
KELLER, G. (1982). Pigmentationsuntersuchungen bei europäischen Arten aus der Gattung *Dermocybe* (FR.) WÜNSCHE. – Sydowia 35: 110-126.
— & AMMIRATI, J. F. (1983). Chemotaxonomic significance of anthraquinone derivatives in North American species of *Dermocybe*, sect. Sanguineae. – Mycotaxon 18: 357–377.
— & STEGLICH, W. (1987). 4-Aminophyscion, an anthraquinone derivative from *Dermocybe* (Agaricales). – Phytochemistry 26: 2119–2121.
KELLER-DILITZ, H., MOSER, M. & AMMIRATI, J. F. (1985). Orellanine and other fluorescent compounds in the genus *Cortinarius* sect. Orellani. – Mycologia 77: 667–673.
MOSER, M. (1972). Die Gattung *Dermocybe* (FR.) WÜNSCHE. – Schweiz. Z. Pilzkd. 50: 153–167.
— (1985). The relevance of chemical characters for the taxonomy of Agaricales. – Proc. Indian Acad. Sci. (Plant Sci.) 94: 381–386.
— (1986). *Cortinarius schaefferi* BRES. – Boll. Gr. mycol. Bresadola 29: 100–104.
— & HORAK, E. (1975). *Cortinarius* FR. und nahe verwandte Gattungen in Südamerika. – Beihefte zur Nova Hedwigia 52: 1–628.
— & KELLER-DILITZ, H. (1983). *Cortinarius raphanoides* PERS.: FR. and related species. – Cryptogamie, Mycologie 4: 41–50.
STEGLICH, W. (1980). Pigments of Higher Fungi (Macromycetes). – In: CZYGAN, F. C. (ed.) (1980). Pigments in plants. – 2nd ed. – Stuttgart: Gustav Fischer. – pp. 393–412.
— & OERTEL, B. (1984). Untersuchungen zur Konstitution und Verbreitung der Farbstoffe von *Cortinarius*, Untergattung *Phlegmacium* (Agaricales). – Sydowia 37: 284–295.
— & REINIGER, W. (1972). Anthrachinon-Pigmente aus *Dermocybe cinnabarina* (FR.) WÜNSCHE. – Chem. Ber. 105: 2922–2927.