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ISOLATION OF FLAVOMANNIN-6,6′-DIMETHYL ETHER AND ONE OF ITS RACEMATES FROM HIGHER FUNGI

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Dedicated to Professor ROBERT KÜHNER on the occasion of his seventieth birthday

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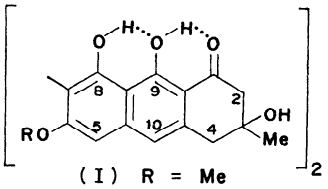
Key Word Index—*Dermocybe; Tricholoma;* Fungi; flavomannin-6,6′-dimethyl ether; dimeric tetrahydroanthracene derivatives; anthraquinone pigments.

Abstract—The main yellow pigment of *Dermocybe cinnamomeolutea, D. uliginosa* and *D. palustris* var. *sphagneti* has been shown to be flavomannin-6,6′-dimethyl ether (I). *Tricholoma flavovirens* (=*T. equestre*) contains a racemate of the same pigment. Minor pigments in *D. cinnamomeolutea* are anhydroflavomannin-quinone-6,6′-dimethyl ether (VII), questin, dermolutein, dermorubin and traces of endocrocin.

INTRODUCTION

SEVERAL species of *Dermocybe*, section *Dermocybe* Moser[1] (Cortinariaceae), contain a characteristic bright yellow intercellular pigment,[2–4] which has been considered to be an anthrone derivative.[3] This pigment is very similar to the colouring matters of *Tricholoma flavovirens* (Pers. ex Fr.) Lund. [=*T. equestre* (L. ex Fr.) Quel.] and related species (Tricholomataceae) and is responsible for the greenish-yellow appearance of some of these toadstools. We have now found that both these families of the Agaricales contain a common pigment, for which we have established structure (I).



(I) R = Me
(II) R = H

* Part VIII in the series 'Pigments of Fungi'. For Part VII see W. STEGLICH, I. PILS and A. BRESINSKY, *Z. Naturforsch.* **26B**, 376 (1971).

† Recherches chimiotaxinomiques sur les Champignons XX. For Part XIX see Ref. 10 of this article.

[1] M. MOSER, Basidiomyceten, II. Teil, "Die Röhrlinge und Blätterpilze", G. Fischer, Stuttgart (1967).
[2] R. KÜHNER, *Bull. Soc. Natur. Oyonnax* **3**, 1 (1949); *Bull. Soc. Linn. Lyon* **9**, 260 (1960).
[3] M. GABRIEL, Thèse, Université de Lyon (1965).
[4] I. GRUBER, *Z. Pilzkunde* **36**, 95 (1970); I. GRUBER, Dissertation, Universität Innsbruck (1969).

RESULTS AND DISCUSSION

Flavomannin-6,6′-dimethyl ether (I) from Dermocybe

The intercellular pigment (I) was isolated from chloroform extracts of defatted, air or freeze dried fruiting bodies of *Dermocybe cinnamomeolutea* (Orton) Mos. and *D. uliginosa* (Berk.) Mos.[5] a column of acetylated polyamide. The yields were 0·5 and 0·35% respectively. In the case of *D. palustris* var. *sphagneti* (Orton) Mos., (I) was isolated from fresh toadstools in comparable yield. In all cases it is accompanied by several anthraquinones, which are described in the Experimental.

The pigment forms bright-yellow granular microcrystals (from benzene) which show m.p. *ca.* 245–250° (decomp.) and retain a variable amount of solvent. It is strongly laevorotatory, $[\alpha]_{546}^{25}$ −1090°, and decomposes slowly on exposure of its solutions to air. The IR spectrum (KBr) shows strong bands at 3480, 3370 (OH) and 1625 cm^{-1} (broad; hydrogen bonded C=O-groups). The high resolution MS establishes the molecular formula as $C_{32}H_{30}O_{10}$. The molecular ion is of weak intensity. It readily loses two molecules of water to give the ion *m/e* 538, corresponding to the base peak and yields a prominent ion at *m/e* 507 by loss of OMe.

The symmetrical structure of (I) is reflected in the simplicity of its NMR spectrum (Table 1). It shows a close similarity to that of flavomannin (II), recently isolated from *Penicillium wortmanni* Klöck.[6]

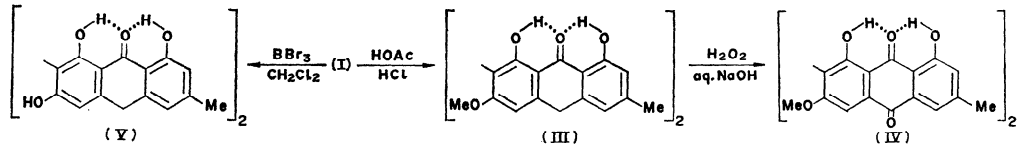
TABLE 1. NMR SPECTRA OF PIGMENT (I) AND FLAVOMANNIN (II)[6]

	Chemical shifts, δ [relative intensities]								
	2-H	4-H	5-H	10-H	C-Me	O-Me	3-OH	8-OH	9-OH
(I)*	2·77‡ [4]	2·99‡ [4]	6·63 [2]	6·88 [2]	1·38 [6]	3·76 [6]	1·88 [2]	10·00 [2]	16·13 [2]
(II)†	2·72‡	2·89	6·60	6·78	1·27	—		10·12	

* In $CDCl_3$.
† In [$^2H_6$] dimethylsulfoxide.
‡ Broad; all other signals singlets.

The 6,6′-positions of the *O*-methyl groups in (I) follow from the presence of two OH-signals at δ 10·00 and 16·13, typical of the hydrogen bonded OH groups of a 1,8-dihydroxy-2-acyl-naphthalene system.[7] This structural assignment was confirmed by dehydration of (I) with AcOH–HCl[6] to a dianhydro compound (III) and consecutive oxidation with alkaline H_2O_2 to the bianthraquinone (IV), which showed two OH signals at δ 12·07 and 12·47, characteristic of a 1,8-dihydroxyanthraquinone. The insolubility of (IV) in aqueous sodium carbonate lends further support to the proposed structure being a dehydrodimer of physcion (VI).

[5] R. ARNOLD and W. STEGLICH, unpublished. We thank Prof. M. Moser, Innsbruck, Austria, for a supply of this toadstool.
[6] J. ATHERTON, B. W. BYCROFT, J. C. ROBERTS, P. ROFFEY and M. E. WILCOX, *J. chem. Soc.* C, 2560 (1968).
[7] Compare harunganin: E. RITCHIE and W. C. TAYLOR, *Tetrahedron Letters* 1431 (1964); chromomycinone. M. MIYAMOTO, K. MORITA, Y. KAWAMATSU, S. NOGUCHI, R. MARUMOTO, M. SASAI, A. NOHARA, Y. NAKADAIRA, Y. Y. LIN and K. NAKANISHI, *Tetrahedron* **22**, 2761 (1966).



The position of the linkage between the two physcion moieties was established by comparison of the NMR spectra of peracetylated (IV) and (VI) (Table 2).

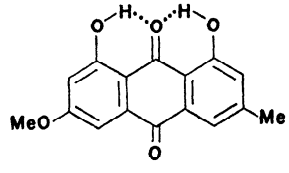
TABLE 2. NMR SPECTRA OF PERACETYLATED BIANTHRAQUINONE (IV) AND PHYSCION (VI)[8] IN $CDCl_3$

Peracetyl derivative of	Chemical shifts, δ							
	2-H	4-H	5-H	7-H	O-Me	C-Me		
(IV)	7·22*	8·04*	7·76†	—	3·93	2·12	2·40	2·50
(VI)	7·24*	8·03*	7·69‡	6·92‡	3·96		2·44 [6 H]	2·49

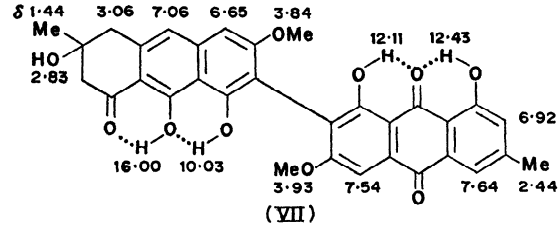
* Broad singlet.
† Singlet.
‡ Doublet, J = 2,5 Hz.

The signals of the aromatic protons 2-H and 4-H are almost identical with regard to position and shape in both spectra, but in the spectrum of the dimer a singlet at δ 7·76, from the 5-H, replaces a doublet and there is no signal from a proton at C-7. This defines the structure of the bianthraquinone as 7,7'-bi-physcion (IV). The diamagnetic shielding of one of the acetyl signals (δ 2·12) is in accord with position α to the biphenyl linkage.[6]

(I) is therefore flavomannin-6,6'-dimethyl ether, and this was proven by direct comparison. Treatment of (I) with BBr_3 yielded dianhydroflavomannin (V),[6] identical with an authentic sample, kindly provided by Dr. J. C. Roberts, Nottingham.



(VI)



(VII)

(I) and flavomannin show close correspondence in their ORD curves (Fig. 1). The absolute configurations of the binaphthyl system and the asymmetric centers at C-3 and C-3' remain to be determined. One of the minor pigments in *D. cinnamomeolutea* was shown to be anhydroflavomanninquinone-6,6-'dimethyl ether (VII). Its structure follows from the spectroscopic data, especially the high resolution MS and the NMR values indicated in the formula. The possibility that (VII) is an artefact, formed from (I) during the isolation procedure can not be completely ruled out at present.

[8] W. STEGLICH and W. LÖSEL, *Tetrahedron* **25**, 4391 (1969).

(atrop±)-Flavomannin-6,6′-dimethyl ether from Tricholoma[9]

Chromatography of the crude pigments of *Tricholoma flavovirens* on silica yielded a dimeric anthraquinone which was shown to be (±)-7,7′-biphyscion.[10] This compound is an artefact, formed during the isolation procedure from a flavomannin type precursor. The genuine pigment may easily be isolated following the technique described for *Dermocybe*. It

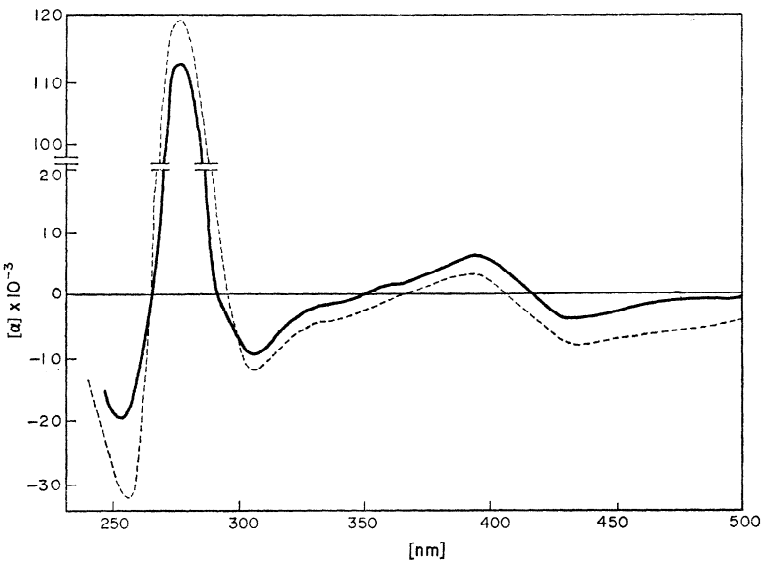


FIG. 1. ORD curves of (−)-flavomannin-6,6′-dimethyl ether and (– – –) flavomannin.

forms bright-yellow crystals and shows nearly complete agreement in its UV, IR, NMR and MS data with flavomannin-6,6′-dimethyl ether. It differs, however, in its higher m.p. of 290–330° (decomp.) and in being less soluble in chloroform and other solvents.

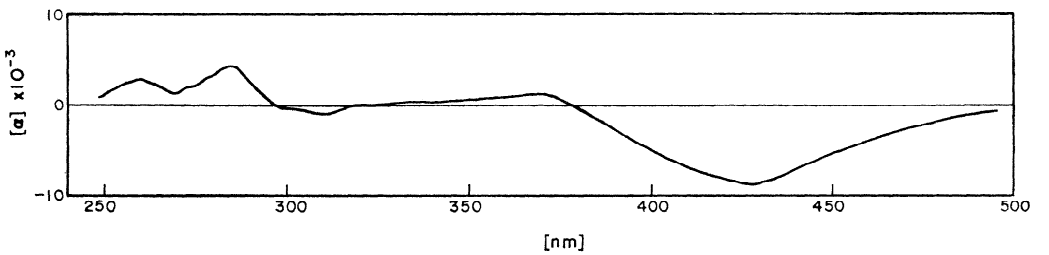


FIG. 2. ORD curve of (atrop±)-flavomannin-6,6′-dimethyl ether.

Lack of optical activity at 546 nm indicates that the *Tricholoma* pigment is (I), racemic at least at the binaphthyl linkage. This is supported by the optical properties of the bianthraquinones (IV) derived from (I) and the *Tricholoma* pigment. The former is strongly laevorotatory, $[\alpha]_{546}^{25}$ −640°, the latter optically inactive. The fact that optical activity of the

[9] The term (atrop±) is proposed to indicate a modification, racemic at the chiral biaryl system.
[10] K. GLUCHOFF, N. ARPIN, M.-P. DANGY-CAYE, P. LEBRETON, W. STEGLICH, E. TÖPFER, H. POURRAT, F. REGERAT et D. DERUAZ, *Compt. Rend.* **274**, 1739 (1972).

Tricholoma pigment may be detected at shorter wavelengths by an ORD measurement (Fig. 2) indicates a definite absolute configuration for the chiral centers at C-3 and C-3′. The pigment therefore may be designated as (atrop±)-flavomannin-6,6′-dimethyl ether.

EXPERIMENTAL

IR spectra were measured in KBr pellets, and NMR spectra in $CDCl_3$ with TMS as the internal standard The chemical shifts are given in δ. The UV spectra were measured in EtOH, and the MS on a A.E.I. MS9 spectrometer with direct inlet at 70 eV. The acetylated polyamide used was purchased from Macherey and Nagel, Duren (Germany).

Dermocybe cinnamomeolutea was collected in September 1970 near Gauting, Bavaria and *D. palustris* var. *sphagneti* near Geltendorf, Bavaria. The help of Profs. M. Moser, Innsbruck, and A. Bresinsky, Munich, in identifying the toadstools is gratefully acknowledged.

Isolation procedure. Finely ground freeze dried sporophores of *D. cinnamomeolutea* (135 g, from 1·4 kg fr. material) were shaken with light petrol. (1·5 l.) for 3 days at 20°. The defatted material was then extracted by shaking with CH_2Cl_2 (1·5 l.) for 2 days at room temp. The extraction was repeated and the combined extracts were evaporated under reduced pressure (fraction I, 3·0 g). A new extraction with EtOH (1·5 l., 3 days, 20°) yielded fraction II.

Fraction I was chromatographed on acetylated polyamide. C_6H_6 eluted 3 overlapping yellow zones (fraction 1a), C_6H_6–$CHCl_3$ (1:1) flavomannin-6,6′-dimethyl ether (I) and $CHCl_3$ questin (20 mg), identical with an authentic sample.[11]

(I) was rechromatographed (1·09 g) and recrystallized from benzene or toluene (yield 0·68 g). Bright-yellow granular microcrystals, m.p. 245–250° (decomp.), contains variable amounts of solvent. $[\alpha]_{546}^{25}$ −1090° (*c*, 0·27 in $CHCl_3$); λ_{max} 412 (ε 22 400); 274 (65 000); 236 nm (30 000); ν 3480; 3370 (*m*); 2915 (*w*); 1625 (*ss*, broad); 1575 (*sh*); 1495 (*m*); 1460 (*s*); 1400 (*s*, broad); 1340 (*s*); 1265 (*m*); 1210 (*m*); 1200 (*m*) 1170 (*m*); 1095 (*s*, broad); 1060 (*w*); 995 (*w*); 952 (*w*); 920 (*w*); 890 (*w*); 860 (*w*); 828 (*w*); 810 (*w*); MS: *m/e* 574 (14·6%) [M^+, $C_{32}H_{30}O_{10}$];[12] 556 (60·8%); 538 (100%) [$C_{32}H_{26}O_8$]; 521 (8·8%); 507 (97%); 498 (8·8%); 283 (13·1%); 269 (22%); *m/2e* 253·5 (17·5%); *m/e* 239 (7·4%). (Found: C, 67·51; H, 5·56. Required for $C_{32}H_{30}O_{10}$: C, 66·88; H, 5·26.)

Rechromatography of fraction Ia gave as first cut, traces of (−)-7,7-biphyscion (IV), followed by a few mg of an orange pigment, which could be identified by high resolution MS as $C_{32}H_{24}O_9$ [dianhydroflavomanninquinone-6,6′-dimethyl ether (?)]. The main fraction was recrystallised from benzene to yield anhydroflavomanninquinone-6,6′-dimethyl ether (VII) (120 mg), m.p. 256–258° (decomp.); λ_{max} 455 (shoulder); 410; 276; 220 nm; +NaOH: 525; 400; 273; 5 nm; ν 3360 (*w*); 2905 (*w*); 1668 (*w*); 1647 (*sh*); 1621 (*ss*); 1595 cm^{-1}; MS: *m/e* 570 (37%) [M^+, $C_{32}H_{26}O_{10}$]; 552 (100%) [$C_{32}H_{24}O_9$]; 535 (14%); 521 (95%); 503 (8·7%); 297 (10%); 283 (7·4%); *m/2e* 276 (17·5%); *m/e* 270 (10%); *m/2e* 260·5 (9·6%).

The pigments of fraction Ia may at least partially be artefacts. This is indicated by the fact, that a forerun very similar to fraction Ia may be obtained by rechromatography of (I) exposed to air for several months or standing in $CHCl_3$ for a few days.

Fraction II was dissolved in EtOAc and extracted with phosphate buffer (2·45 g $Na_2HPO_4 \times 2H_2O$, 2 g $(NH_4)_2SO_4$ and 100 g H_2O). The extracts were acidified and extracted with EtOAc. Evaporation of the solvent gave 1·02 g crude acids, which were separated on acetylated polyamide. Acetone eluted dermolutein (240 mg), MeOH dermorubin (50 mg), identical with authentic samples.[13] The crude pigments contained traces of endocrocin, identified by TLC comparison.

The pigments were isolated from fresh sporophores of *D. palustris* var. *sphagneti* (140 g) by extraction of the ground toadstools with EtOH, evaporation of the solvent and defatting the residue with light petrol. Working up as described above yielded 54 mg (I). The acids were identified as dermolutein and dermorubin.

Dianhydroflavomannin-6,6′-dimethyl ether (III): Flavomannin-6,6′-dimethyl ether (I) (120 mg) was refluxed for 3 hr with 20 ml HOAc and 3 ml conc. HCl. The precipitate was isolated by centrifugation, washed with H_2O and dried over P_2O_5. Crystallization from CH_2Cl_2 yielded 100 mg, m.p. 300–325° (decomp.); λ_{max} 346 (ε 35 400); 320 (shoulder, 22 050); 271·5 (29 000); 260 (30 200); 241·5 nm (35 400); ν 3390; 1620 (shoulder); 1600; 1563 cm^{-1}. (Found: C, 69·43; H, 5·14. Required for $C_{32}H_{26}O_8.1H_2O$: C, 71·36; H, 4·86%.)

(−)-7,7′-Biphyscion (IV). A mixture of dianhydroflavomannin-6,6′-dimethyl ether (III) (90 mg), 1 N NaOH (30 ml) and 30% H_2O_2 (0·5 ml) was warmed 3–5 hr to 60° until TLC showed that (III) had completely disappeared. Exhaustive extraction of the acidified solution with EtOAc (1 l.) and evaporation of the H_2O-washed and dried extracts yielded crude (IV), which was purified by chromatography on a short column of

[11] A. MAHMOODIAN and C. E. STICKINGS, *Biochem. J.* **92,** 369 (1964).
[12] All molecular formulae given in brackets were determined by high resolution measurements.
[13] W. STEGLICH, W. LÖSEL and V. AUSTEL, *Chem. Ber.* **102,** 4104 (1969).

acetylated polyamide (eluent benzene). Recrystallization from EtOAc gave 39 mg (IV); m.p. > 350° (decomp.): $[\alpha]_{546}^{20}$ −640°; $[\alpha]_{578}^{20}$ −1235° (*c*, 0·06 in EtOAc). λ_{max} 461 (shoulder); 444; 310 (shoulder); 284; 270; 242 nm (in $CHCl_3$); +NaOH: 550; 310; 248 nm; ν 3470; 2935; 2857; 1675; 1626; 1600; 1560 cm^{-1}; MS: *m/e* 566 (71·5%) [M^+, $C_{32}H_{22}O_{10}$] 549 (7·5%); 535 (100%) [$C_{31}H_{19}O_9$]; 521 (4·6%); 520 (6·3%); 517 (6·3%); 506 (4·4%); 505 (6·3%); 491 (6·6%); 297 (39%) [$C_{17}H_{13}O_5$]; 283 (12·5%) [$C_{16}H_{11}O_5$]; 268 (3·9%); *m/2e* 267·5 (7%); *m/e* 267 (6·3%); *m/2e* 261·5 (7%).

(−)-7,7′-*Biphyscion tetraacetate*. (−)-7,7′-Biphyscion (10 mg) and anhydrous NaOAc (15 mg) were refluxed with Ac_2O (1·5 ml) for 2 hr. After removal of the solvent *in vacuo*, the product was distributed between H_2O and $CHCl_3$. It was isolated from the organic layer, crystallized from HOAc–H_2O and used for the NMR spectrum; NMR, IR and MS are identical with the (±)-derivative (*vide infra*). The (±)-derivative was analogously prepared from (±)-7,7′-biphysicion (75 mg).[10] Yellow crystals from HOAc–H_2O (57 mg); m.p. 275° (decomp.); $[\alpha]_{546}^{20}$: ±0° (*c*, 0·2 in EtOAc); ν 1788; 1771; 1682; 1668; 1610; 1590 cm^{-1}; MS: *m/e* 734 (0·15%); 692 (7·0%); 650 (38·0%); 608 (41·0%); 590 (14·3%); 566 (100%); 549 (43·0%); 548 (81·0%); 535 (98·0%); 521 (7·2%); 520 (12·0%); 519 (9·5%); 517 (8·1%); 505 (11·0%); 491 (11·6%); 297 (32·0%). (Found: C, 64·55; H, 4·29. Required for $C_{40}H_{30}O_{14}$: C, 65, 39; H, 4·11.)

Isolation of (*atrop*±)-*flavomannin*-6,6′-*dimethyl ether*. Frozen sporophores of *Tricholoma flavovirens*, which had been stored at −20°, were finely ground and exhaustively extracted with EtOH. After acidification with 2 N HCl, the pigment was completely transferred into $CHCl_3$. The organic phase was washed twice with H_2O (H_2O eliminated by freezing), concentrated and chromatographed on a column of acetylated polyamide. C_6H_6 eluted mainly (±)-7,7′-biphyscion besides other oxidation products, C_6H_6–$CHCl_3$ (1:1) eluted (atrop±)-flavomannin-6,6′-dimethyl ether, which was crystallized from $CHCl_3$. It constitutes *ca.* 1% of the dry weight of the toadstool (determined spectrophotometrically at 416 nm (ϵ 22 600) on $CHCl_3$ extracts of 3 different samples).

Acknowledgements—This investigation was supported by the Deutsche Forschungsgemeinschaft. We should like to thank Professor J. C. Roberts for a generous gift of flavomannin and dianhydroflavomannin, Mrs. I. Meloch for running the MS and Miss I. Pils for technical assistance.