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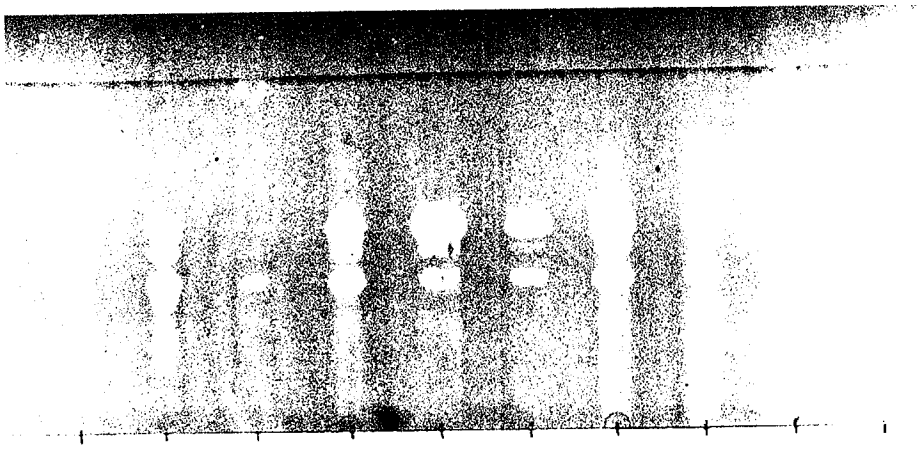
CHEMOTAXONOMIC STUDY OF ORELLANINE IN SPECIES OF *CORTINARIUS* AND *DERMOCYBE*

SYLVIE RAPIOR, CLAUDE ANDARY, AND GUY PRIVAT

Laboratoire de Botanique, Phytochimie et Mycologie, Faculté de Pharmacie, Avenue Charles Flahault, 34060 Montpellier Cedex, France

Cortinarius orellanus (Fr.) Fr. and *Cortinarius speciosissimus* Kühn. & Romagn. are mushrooms responsible for deadly poisonings in many European countries. Research on the toxins in *C. orellanus* was initiated by Grzymala (**8**) who isolated a crystalline, colorless substance which he named orellanine. Following this study, Antkowiak and Gessner (**4**) reported a bipyridine N-oxide structure for this molecule which is easily and specifically decomposed by UV light into an N,N′-deoxidized molecule, *i.e.*, orelline (**2, 5**). We carried out a systematic search for orellanine and its photodecomposition compounds in the eight *Cortinarius* species of the section *Orellani* as well as in other *Cortinarius* species from various sections of the genera *Cortinarius* Fr. and *Dermocybe* (Fr.) Wünsche that are potentially toxic for man and animals.

Our fungal material, preserved by drying, was collected in Europe, South America and North America. Voucher specimens are deposited *in herb.* WTU, TR, IB, and MPU (**10**). Methanol–water extracts of the dried specimens were prepared in the absence of UV light. An absolute ethanol extract of dried *C. fluorescens* was also prepared in daylight according to Keller-Dilitz *et al.* (**12**). The extracts were analyzed by thin-layer chromatography (TLC) on cellulose supports (Ref. 5716, Merck) in the following solvent systems (v/v): BCCE, *n*-butanol–hydrochloric acid–chloroform–water (40:20:15:3.8) (**2**), and BAW, *n*-butanol–acetic acid–water (3:1:1) (**12**). Orellanine (Rf = 0.57 in BCCE, Rf = 0.70 in BAW) and orelline (Rf = 0.42 in BCCE, Rf = 0.57 in BAW) were detected by exposure of chromatograms to UV light at 366 nm. The TLC profile of the extracts and that of an orellanine standard prepared in our laboratory (**2**) are shown in FIG. 1. Orellanine (3,3′,4,4′-tetrahydroxy-2,2′-bipyridine-N,N′-dioxide) appeared in the form of a dark spot which, after exposure for 1–3 minutes to UV light, produced a bluish-white fluorescence characteristic of orelline (3,3′,4,4′-tetrahydroxy-2,2′-bipyridine), the photodecompositon product of orellanine (**2, 5**). The results in TABLE I indicate that only seven of the eight *Cortinarius* species classified by Moser (**15**) in the section *Orellani* produce orellanine. We did not observe this product in the extract from *C. fulvaureus* Hry.



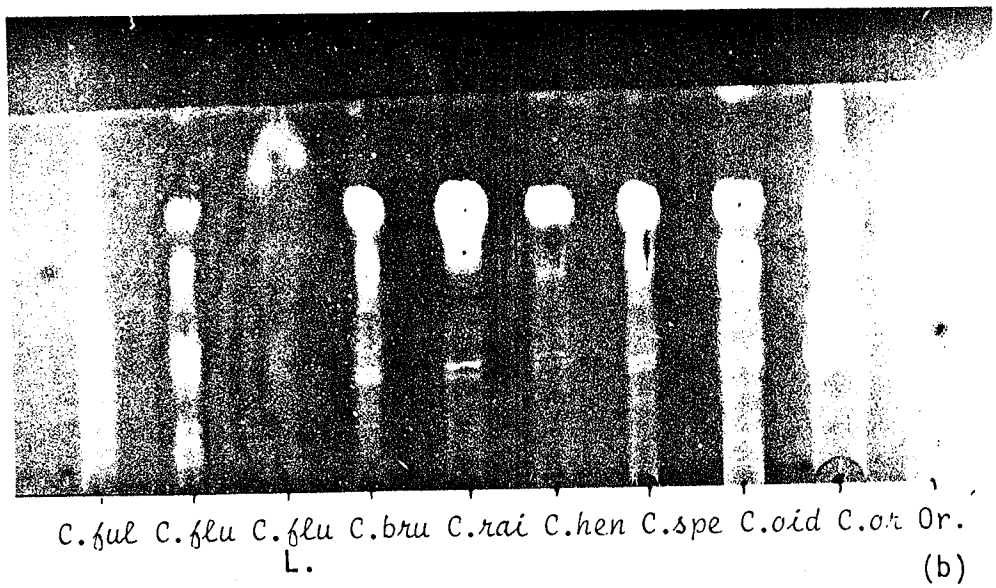


FIG. 1. Thin-layer chromatography on cellulose of various extracts of *Cortinarius* species, section *Orellani*. a. Solvent BCCE: *n*-butanol–hydrochloric acid–chloroform–water (40:20:15:3.8); b. solvent BAW: *n*-butanol–acetic acid–water (3:1:1). Or.: Orellanine; *C. or.*: *C. orellanus*; *C. oid.*: *C. orellanoides*; *C. spe.*: *C. speciosissimus*; *C. hen.*: *C. henrici*; *C. rai.*: *C. rainierensis*; *C. bru.*: *C. brunneofulvus*; *C. flu.*: *C. fluorescens*; *C. ful.*: *C. fulvaureus*. All the extracts were prepared in the absence of UV light except for the *C. flu.* extract (L) which was prepared in daylight.

TABLE I

DETECTION OF ORELLANINE IN *CORTINARIUS* SPECIES, SUBGENUS *LEPROCYBE* SECTION *ORELLANI*

Species[a]	Collection number	Location[b]	Collection site	Orellanine[c]	Collection date
C. brunneofulvus Fr. *ss.* Bres.	B/13	TR	Piné, Italy	+	August, 1927
C. fluorescens Hk.	PN 46	IB	Puerto Natales, Chile	+	March, 1963
C. fulvaureus Hry.	Hry 49/108	MPU	Montereau, France	−	October, 1936
C. henrici Reum.	Reu 243/548	MPU	Forêt de Boult, France	+	July, 1978
C. orellanoides Hry.	Hry 80767/107	MPU	Frasne, France	+	October, 1980
	Hry 3258t/106	MPU	Fontainebleau, France	+	September, 1955
	Bon 90940/542	MPU	Bellême, France	+	September, 1983
	Tre 209831/541	MPU	Le Mans, France	+	September, 1983
C. orellanus (Fr.) Fr.	Bon 14	MPU	Bédarieux, France	+	October, 1977
	Rio 29	MPU	Mende, France	+	September, 1979
	And 33	MPU	Bédarieux, France	+	October, 1984
	Che 40	MPU	Lons-le-Saunier, France	+	September, 1983
	And 75	MPU	Saint-Laurent-de-Cerdans, France	+	October, 1986
C. rainierensis Smith & Stuntz	Stz 3998	WTU	Washington, U.S.A.	+	August, 1948
C. speciosissimus Kühn. & Romag.	And 90	MPU	Le Mans, France	+	September, 1983
	Che 91	MPU	Thonon-les-Bains, France	+	October, 1984
	Che 92	MPU	Lons-le-Saunier, France	+	October, 1984
	Bon 3929/544	MPU	Bellême, France	+	September, 1963

[a] According to the classification by Moser (**1983**).
[b] Voucher specimens are deposited in the Herbarium at the University of Innsbruck (IB), Montpellier (MPU), Trento (TR), Washington (WTU).
[c] Presence of orellanine, +; absence of orellanine, −.

Our results are in good agreement with Henry who stated that *C. fulvaureus* was a "*hinnuleus* with no ring" (**13**) and we suggest that this *Cortinarius* species should not be classified in the section *Orellani*. Furthermore, we did not find any trace of orellanine in 28 species of *Cortinarius* belonging to the subgenera *Cortinarius* Fr., *Leprocybe* Mos. (excluding section *Orellani*) and *Phlegmacium* (Fr.) Fr., or in 13 *Dermocybe* species (TABLES II, III). It is interesting to note that among the species that do not produce orellanine, several have been found to be toxic to some animal species (**16**, **18**).

In our study, the presence of orellanine was detected in *C. fluorescens* Hk.; whereas Keller-Dilitz *et al.* (**12**) were not able to find this substance in their *C. fluorescens* extract. Their failure to detect the toxin can probably be explained by examining the extraction and chromatographic conditions they used. First of all, the extraction of orellanine must be carried out in the dark due to its photodecomposition into orelline (**2**, **5**). Secondly, the quantity of orellanine isolated varies with the nature of the extraction solvent. We noted that water and the water-alcohol solutions (methanol or ethanol) proved to be better extraction solvents than alcohol alone. It should be noted that fluorescence of orellanine under UV light is brighter in the presence of inorganic acids such as HCl contained in the BCCE solvent (**2**, **6**). The detection limit of orellanine on cellulose chromatograms is then approximately 10 ng (**2**). Thus, when extracts with weak concentrations of orellanine prepared in daylight are analyzed, it is difficult to detect the fluorescence on chromatograms developed in a solvent such as BAW (**12**) (FIG. 1).

When the *Cortinarius* species with a high level of orellanine, *i.e.*, *C. orellanus*, *C. orellanoides* or *C. speciosissimus* were compared with *C. fluorescens*, the latter seemed to contain much less orellanine than the others. It would appear that *C. fluorescens* had been badly stored since we have detected higher orelline contents in this

TABLE II

SEARCH FOR ORELLANINE IN *CORTINARIUS* SPECIES, SUBGENERA *CORTINARIUS*, *LEPROCYBE* (EXCLUDING SECT. *ORELLANI*) AND *PHLEGMACIUM*[a]

Subgenus	Sections	Species[b]	Collection number[c]	Collection site	Collection date
Cortinarius Fr.		*C. violaceus* (L.: Fr.) Fr.	And 148	Bédarieux, France	October, 1979
		C. hercynicus (Pers.) Mos.	Mel 77497/438	Trossingen, Germany	September, 1977
Leprocybe Mos.	*Leprocybe*	*C. ignipes* Mos.	Che 133	La Salvetat-sur-Agout, France	November, 1978
		C. cotoneus Fr.	Rap 214	Montarnaud, France	November, 1982
		C. melanotus Kalchbr.	Hry 230	Ganges, France	October, 1982
		C. psittacinus Mos.	And 240	Bédarieux, France	October, 1976
		C. venetus (Fr.: Fr.) Fr.	Che 242	Saint-Laurent-le-Minier, France	October, 1976
	Zinziberati	*C. zinziberatus* (Fr.) Fr.	Che 1879/219	Montpellier	November, 1976
	Raphanoidei	*C. valgus* Fr.	Mel 859/442	Oslo, Norway	September, 1985
		C. raphanoides (Fr.) Fr.	Che 252	Oyonnax, France	October, 1983
	Bolares	*C. rubicundulus* (Rea) Pearson	Bon 433	Desvres, France	August, 1972
		C. bolaris (Pers.: Fr.) Fr.	Rap 257	Bédarieux, France	October, 1982
	Limonei	*C. saniosus* (Fr.) Fr.	Bon 70351/434	Forêt de Reno, France	September, 1970
		C. gentilis (Fr.) Fr.	Bon 12091303/426	Regensburg, Germany	September, 1972
		C. humicola (Quél.) R. Mre.	Bon 429	Bellême, France	September, 1970
		C. limonius (Fr.: Fr.) Fr.	Bon 50905/427	Saint-Dié, France	September, 1965
		C. callisteus (Fr.) Fr.	Bon C3930/421	Bellême, France	September, 1963
		C. tophaceus (Fr.) Fr.	Bon 71091401/437	Brenne, France	September, 1971
Phlegmacium (Fr.) Fr.	*Scauri* Fr. subsection *Orichalcei*	*C. odorifer* Britz	Bla 22A/564	Thonon-les-Bains, France	October, 1984
		C. xanthophyllus Cke.	Che 1387/552	Clapiers, France	December, 1970
		C. rufoolivaceus Fr.	Che 59/549	Montarnaud, France	November, 1967
		C. aureofulvus Mos.	Che 2747/550	Espinouse, France	October, 1967
		C. olearioides Hry.	Che 3122/551	Espinouse, France	October, 1980
		C. auroturbinatus (Secr.) Lge.	Bla 27A/565	Saint-Gervais-la-Forêt, France	October, 1984
		C. odoratus (Joguet *ex* Mos.) Mos.	Bla 28A/566	Thonon-les-Bains, France	October, 1984
		C. atrovirens Kalchbr.	Bla 29A/567	Thonon-les-Bains, France	October, 1984
		C. vitellinus Mos.	Che 279	Oyonnax, France	October, 1983
		C. splendens Hry.	Che 280	Oyonnax, France	October, 1983

[a] We were not able to detect orellanine in any of these species.

[b] According to the classification by Moser (1983).

[c] We would like to thank Professors J. F. Ammirati and M. Moser and Drs. F. Bellù, H. Blanchecotte, M. Bon, G. Chevassut, J. Melot, F. Trescol, and P. Reumaux for exsiccata.

TABLE III

SEARCH FOR ORELLANINE IN *DERMOCYBE* SPECIES[a]

Sections	Species[b]	Collection number	Collection site	Collection date
Dermocybe,	*D. carpineti* Mos. *ined.*	Bon 740822/423	Desvres, France	April, 1974
	D. palustris (Mos.) Mos.	Bla 2A/568	Thonon-les-Bains, France	October, 1984
	D. uliginosa (Berk.) Mos.	Che 207	Bédarieux, France	October, 1986
	D. crocea (Schff.) Mos. (=*C. cinnamomeolutescens* Hry.)	Hry 177	Bédarieux, France	October, 1977
Holoxantha,	*D. malicoria* (Fr.) Ricken	Bon 740914/430	Le Puy, France	September, 1974
	D. luteomarginata (*ined.*) (=*D. croceifolia ss.* Mos., 1978)	Bon 183	Paris, France	November, 1977
Malicoria	*D. cinnamomea* (L.: Fr.) Wünsche	And 481	Ganges, France	October, 1976
	D. cinnamomeobadia (Hry.) Mos.	And 153	Bédarieux, France	November, 1978
	D. cinnamofulva (Hry.)	Hry 160	Bédarieux, France	October, 1977
Sanguinei	*D. semisanguinea* (Fr.) Mos.	Che 397	Truscas, France	October, 1984
	D. phoenicea (Bull.: Mre.) Mos.	Che 2856/343	Saint-Laurent-le-Minier, France	October, 1976
	D. sanguinea (Wulf.: Fr.) Wünsche	Rap 371	Bédarieux, France	October, 1982
	D. cinnabarina (Fr.) Wünsche	Bon 424	Bellême, France	September, 1979

[a] We were not able to detect orellanine in any of these species.
[b] According to the classification by Moser (1983).

mushroom. We have noted that the orelline content is always higher in old or badly preserved mushrooms by comparison with mushrooms that have been recently picked and correctly dried and stored. On the basis of our results, we believe that *C. fluorescens* can be definitively classified in the section *Orellani*. Moreover, the fact that we found orellanine in voucher specimens of *C. brunneofulvus* Fr. *ss.* Bres., *C. henrici* Reum., *C. orellanoides* Hry. and *C. rainierensis* Smith & Stuntz confirms once again that this molecule is stable in exsiccates that are over 60 years old. In contrast, orellanine in solution undergoes photodegradation more rapidly than *in situ* in the mushroom (**2**).

On the basis of statistical studies on the size and form of spores from only two exsiccatae, Hoiland (**10**) established that *C. speciosissimus* is synonymous with *C. orellanoides,* the latter name having nomenclatural priority. Pöder and Pipitz (**17**) showed in a critical review of the literature based exclusively on macroscopic, microscopic, macrochemical and ecological data that *C. speciosissimus* is in fact synonymous with *C. speciosus* Favre, *C. orellanoides, C. brunneofulvus* and *C. henrici.* Moreover, Moser (pers. comm., 1982) found *C. henrici* to be synonymous with *C. speciosissimus.* This notion is confirmed by Melot (**14**) who, after bibliographic research, also showed *C. rubellus* Cke. to be synonymous with *C. orellanoides.*

It is well established that the use of statistical methods requires analysis of a large number of samples. Microscopic and macrochemical data must be considered with caution given the lack of standardization of the operating conditions. Consequently, these investigative methods can only rarely result in the synonymization of species names. As has already been shown by several authors (**1, 3, 7, 11**), we also insist on the importance of chemical markers for accuracy of systematic classification. On the basis of our results showing very similar TLC profiles of mushroom extracts from all species of the section *Orellani* in the genus *Cortinarius* and *Dermocybe* a better circumscription of this section is now possible.

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Key Words: orellanine, orelline, *Cortinarius, Orellani,* chemotaxonomy.

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