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CHEMOTAXONOMIC EVIDENCE FOR A DIVISION OF *LACTARIUS VELLEREUS* AND *L. BERTILLONII* AS DIFFERENT SPECIES

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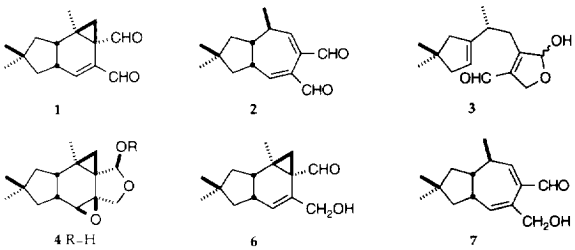
Key Word Index—*Lactarius vellereus*; *L. bertillonii*; Basidiomycotina; milk cap; chemotaxonomy; sesquiterpene; isovelleral; velleral; vellerolactone.

Abstract—The sesquiterpenes formed in the fruit bodies of *Lactarius vellereus* and *L. bertillonii* as a response to injury have been investigated. The two species are very similar, and intact fruit bodies contain the same sesquiterpenoid precursor, stearoylvelutinal. In *L. vellereus* this is converted to isovelleral (major component) and velleral, which subsequently are reduced to isovellerol and vellerol. In *L. bertillonii*, only velleral is formed as a primary product; it is subsequently reduced to vellerol which is oxidized to the end product vellerolactone. The results support the proposed removal of the taxon *bertillonii* from *L. vellereus* and its treatment as a separate species.

INTRODUCTION

A general characteristic of mushrooms belonging to the genus *Lactarius* (family Russulaceae of the Basidiomycotina subdivision of fungi), is the presence of a latex that can be observed if the fruit bodies are cut or broken. The colour and taste of the latex as well of the flesh vary between different species, a fact that has been exploited by mycologists as taxonomic markers. The chemical background to these differences has been clarified for several species. Interestingly, there seems to be a general pattern within the *Lactarius* genus, where the metabolites responsible for the characteristic differences in taste and colour are formed enzymatically from fatty acid ester precursors as a response to injury to the fruit bodies. Depending upon the precursor originally present in the fruit bodies, the *Lactarius* species and the metabolites can be divided into three major groups: The coloured latex of several species belonging to the *Dapetes* section of *Lactarius* (e.g. *L. deliciosus*, *L. deterrimus* and *L. sanguifluus*) is first carrot-coloured but slowly turns green, and this phenomenon has been shown to be due to the formation and transformation of guaiane sesquiterpenes [1]. Species belonging to the *Plinthogali* section (e.g. *L. fuliginosos* and *L. picinus*) have white and generally sweet latex and flesh. Due to the enzymatic formation of chromene derivatives as a response to injury, the latex and flesh turn reddish and bitterly acrid [2]. The third group, which is the largest, is made up by species belonging to the *Albati* and *Lactarius* sections (e.g. *L. vellereus*, *L. piperatus* and *L. scrobiculatus*). They generally have white latex, and become pungent after injury due to the rapid formation of bioactive (e.g. antimicrobial, cytotoxic, antifeedant and mutagenic activities [3, 4]) sesquiterpenes containing an unsaturated 1,4-dialdehyde functionality and with marasmane (e.g. isovelleral, **1** [5, 6]), lactarane (e.g. velleral, **2** [7]), and secolactarane (e.g. lactardial, **3** [8]) skeletons. These metabolites are all formed enzymatically from fatty acid esters of velutinal (**4**) (e.g. stearoylvelutinal, **5** [9]), which is the only sesquiterpenoid present in the intact fruit bodies. The dialdehydes are eventually (in minutes to hours) reduced enzymatically by the injured mushroom tissue, for example isovelleral (**1**) to isovellerol (**6**) and velleral (**2**) to vellerol (**7**) [10].

In addition to the aldehydes, furans (e.g. **8**) [11, 12] and lactones (e.g. **9**) [13, 14] have also been isolated from fruit bodies of the *Lactarius* species. However, several of the lactarane and secolactarane furans have later been shown to be formed *in vitro*, when the labile velutinal esters (e.g. **5**) are subjected to, for instance, silica gel chromatography or polar solvents such as acetone or methanol [15]. The furan **8**, for example, which once was believed to be a natural product may, therefore, actually



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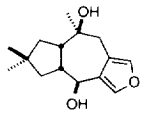

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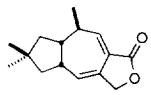

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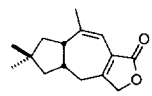


10

be an artefact. Unlike the furans, the lactones have never been observed to be formed *in vitro*, neither from the velutinal esters nor from the aldehydes. Vellerolactone (**9**) and pyrovellerolactone (**10**) have been isolated from *L. vellereus* and *L. pergamenus* [13, 14], although their existence has since then become something of an enigma. In later investigations they have only appeared occasionally and in small amounts in extracts of *L. vellereus*, even when the fruit bodies were collected at the same habitat and identical work-up procedures were used as previously [13, 14]. In contrast, the formation of isovellerol (**1**) and velleral (**2**) and their reduced congeners **6** and **7** has always been found to be consistent. The fact that vellerolactone (**9**) is present in only some extracts indicates that it is not formed during the work-up of crude extracts. The possibility that it is formed by autooxidation of the C-5 aldehyde group of vellerol (**7**) followed by intramolecular lactone formation seems less likely, as analysis of a crude extracts of *L. vellereus* stored at $-25°$ for two years and consisting mainly of vellerol did not contain any vellerolactone (**9**).

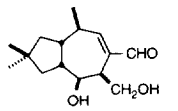
The botanical classification of *L. vellereus* has been discussed recently [16]. The *L. vellereus* group is now considered to consist of several species and varieties [16] although in Europe the name *L. vellereus* is frequently used for the whole complex or for the commonest taxon of this polytypic aggregate in each author's study area. In Fennoscandia two varieties/species have been discussed; *L. vellereus* (Fr.: Fr.) Fr. and *L. bertillonii* (Neuh. ex Z. Schaefer) M. Bon, and a recent study substantiated their taxonomic status as different species [16]. Some differences between the two species in Fennoscandia are their distribution, their time for fructification and the taste of their latex. Their appearances to the naked eye are rather similar but there are a few distinguishing macroscopic as well as microscopic characteristics [16].

In Fennoscandia *L. bertillonii* is more northerly distributed and fructifies between the middle of July until early October, while *L. vellereus* is more southerly distributed and produces fruit bodies with about one month delay compared to *L. bertillonii*. However, their distribution is such that in many areas they can be found together. The latex of *L. bertillonii* has an immediate acrid taste even when separate from flesh, while the latex (but not the flesh) of *L. vellereus* has a mild taste [16]. It thus occurred to us that the occasional appearance of vellerolactone (**9**) could be explained if it is only produced by for instance *L. bertillonii* but not by *L. vellereus*, and if the two species grow together.

RESULTS AND DISCUSSION

Fruit bodies of *L. vellereus* and *L. bertillonii* were collected at different habitats and carefully identified according to their taxonomic keys [16]. In order to avoid mix-ups, the sesquiterpenes produced in response to injury (simulated by grinding the fruit bodies in a meat grinder) were studied from only one fruit body at a time (see Experimental for details). Intact specimens of both species contain the precursor stearoylvelutinal (**5**) as the only sesquiterpenoid, identified by comparison of ^{1}H NMR data and TLC analysis. When injured, the fruit bodies of *L. vellereus* produce isovellerol (**1**) and velleral (**2**) which subsequently are reduced to isovellerol (**6**) and vellerol (**7**). There were no traces of vellerolactone (**9**) in the extracts of *L. vellereus*. However, isovellerol (**1**), which is the major product of *L. vellereus*, was not produced at all by *L. bertillonii* as a response to injury, instead velleral (**2**), vellerol (**6**) and vellerolactone (**9**) were identified in the extract of this species. In extracts made 5 min after injury the ratio between **2**:**6**:**9** is approximately 1:2:2, while velleral (**2**) has disappeared and vellerolactone (**9**) is the major extractable sesquiterpenoid component (*ca* 75%) 30 min after injury. Velleral (**2**) is formed in seconds after injury and is completely converted to vellerol (**6**) in minutes, identical to the situation in *L. vellereus* [10]. It is reasonable to assume that vellerolactone (**9**), which appears to be the end-product in *L. bertillonii*, is formed by oxidation and lactonization of vellerol (**6**). It is also reasonable that its formation is enzymatic, as several other *Lactarius* species which produce velleral (**2**) and vellerol (**6**), for instance *L. piperatus* and *L. necator*, do not form vellerolactone (**9**). Similar transformation of lactarane monoaldehydes into lactones has also been observed in *L. necator* where lactarorufin N (**12**) is produced from epi-piperalol (**11**) [17], and in *L. scrobiculatus* where the lactone **14** is produced from scrobicalol (**13**) [18]. Pyrovellerolactone (**10**) was not detected in the extracts of *L. bertillonii*. However, it has previously been shown that it is formed from vellerolactone (**9**) by heating [13, 14] and may, therefore, be an artefact produced during work-up of the extracts.

It can thus be concluded that vellerolactone (**9**) is not produced by *L. vellereus* as first reported [13, 14] but by the closely related species *L. bertillonii*. The random occurrence of fruit bodies of *L. bertillonii* among those of *L. vellereus* and the mix-up of the very similar species explains the occasional presence of vellerolactone (**9**) as well as variations in the ratio between isovellerol (**1**) and velleral (**2**). In addition, the proposed taxonomic treatment of the taxon *bertillonii* as separate species [16] is



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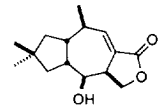

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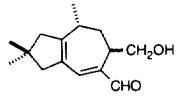

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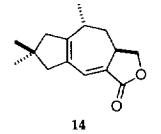

14

chemotaxonomically substantiated by the results presented in this investigation.

EXPERIMENTAL

Fruit bodies of *Lactarius vellereus* (Fr.: Fr.) Fr. were collected in beech woods in the vicinity of Lund in south Sweden, while fruit bodies of *Lactarius bertillonii* (Neuh. ex Z. Schaefer) M. Bon were collected in the vicinity of Stockholm in central Sweden. The fruit bodies were identified by comparison of the taste of the latex, appearance of the spores and the tips of pileal hairs [16]. The fruit bodies were cut in half, one half was used for the identification and (in order to simulate injury to the fruit bodies) the other half was ground in a meat grinder without the addition of any solvent. The mush was extracted in different experiments after 5, 15, 30, 45 and 90 min with EtOAc. The EtOAc was dried with Na_2SO_4 and concd, whereafter the crude extracts were dissolved in $CDCl_3$ and analysed by ^1H NMR. Identification of **1**, **2**, **6**, **7** and **9** was made by comparison with ^1H NMR spectra of the pure compounds, and by TLC analysis, and their relative concentrations in the extracts were estimated by integration of the NMR signals in the aldehyde and olefin area.

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REFERENCES

1. Bergendorff, O. and Sterner, O. (1988) *Phytochemistry* **27**, 97.
2. De Bernardi, M., Vidari, G. and Vita Finzi, P. (1992) *Tetrahedron* **48**, 7331.
3. Sterner, O., Carter, R. E. and Nilsson, L. M. (1987) *Mutation Res.* **188**, 169.
4. Camiazine, S., Resch, J., Eisner, T. and Meinwald, J. (1983) *J. Chem. Ecol.* **10**, 1439.
5. List, P. and Hackenberg, H. (1969) *Arch. Pharmaz.* **302**, 125.
6. Magnusson, G., Thorén, S. and Wickberg, B. (1972) *Tetrahedron Letters* 1105.
7. Magnusson, G., Thorén, S. and Drakenberg, T. (1973) *Tetrahedron* **29**, 1621.
8. Sterner, O., Bergman, R., Franzén, C. and Wickberg, B. (1985) *Tetrahedron Letters* **26**, 3163.
9. Favre-Bonvin, J., Gluchoff-Fiasson, K. and Bernillon, J. (1982) *Tetrahedron Letters* **23**, 1907.
10. Sterner, O., Bergman, R., Kihlberg, J. and Wickberg, B. (1985) *J. Nat. Prod.* **48**, 279.
11. Nozoe, S., Matsumoto, H. and Urano, S. (1971) *Tetrahedron Letters* 3125.
12. Magnusson, G., Thorén, S., Dahmen, J. and Leander, K. (1974) *Acta Chem. Scand. B* **28**, 841.
13. Magnusson, G. and Thorén, S. (1973) *Acta Chem. Scand. B* **27**, 1573.
14. Magnusson, G. and Thorén, S. (1973) *Acta Chem. Scand. B* **27**, 2396.
15. Sterner, O., Bergman, R., Kihlberg, J., Oluwadiya, J., Wickberg, B., Vidari, G., De Bernardi, M., De Marchi, F., Fronza, G. and Vita-Finzi, P. (1985) *J. Org. Chem.* **50**, 950.
16. Kytövuori, I. and Korhonen, M. (1990) *Karstenia* **30**, 33.
17. Sterner, O. (1989) *Acta Chem. Scand. B* **43**, 694.
18. De Bernardi, M., Garlashelli, L., Toma, L., Vidari, G. and Vita Finzi, P. (1993) *Tetrahedron* **49**, 1489.