The Co-formation of Sesquiterpene Aldehydes and Lactones in Injured Fruit Bodies of *Lactarius necator* and *L. circellatus*. The Isolation of *epi*-Piperalol

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The sesquiterpenoid contents of intact and of injured fruit bodies of the pungent species *Lactarius necator* and *L. circellatus* (mushrooms) have been investigated. The fruit bodies originally contain fatty acid esters of the sesquiterpene velutinal (i.e. compounds **1a** and **1b**) as the only sesquiterpenoids. When the fruit bodies are injured, by grinding, the esters are enzymatically converted into sesquiterpene furans, mono- and di-aldehydes, and lactones. The pungency of the fruit bodies of both species is caused by the formation of unsaturated dialdehydes, e.g. velleral (**3**). Besides already known compounds, a new monoaldehyde, which is shown to be *epi*-piperalol (**11**), was isolated. The conversions of the fatty acid esters of velutinal in injured fruit bodies of *L. necator* and *L. circellatus* are less efficient compared with other pungent *Lactarius* species, the main part of the esters remaining unchanged.

A large number of humulene derived sesquiterpene furans, mono- and di-aldehydes, and lactones has been isolated from fruit bodies of *Lactarius* species (Basidiomycotina subdivision of Fungi).[1] While the furans always have been isolated together with aldehydes, or with lactones, there is little evidence for the co-existence of sesquiterpene aldehydes and lactones, even if both have been isolated separately from the same species. Since different investigators have used different techniques for extracting the sesquiterpenes from the fruit bodies, and some of the *Lactarius* sesquiterpenoids are chemically labile (*vide infra*), the possibility that chemical conversions occur during the extraction and the work-up of the extracts must be considered.

The dialdehydes, which, owing to the reactivity of their unsaturated 1,4-dialdehyde functionality, have a very pungent taste and are potent antimicrobial agents (among other biological activities), have been suggested to be the active principles of a chemical defense system that protects the fruit bodies from parasites.[2,3] In *L. vellereus* Fr. for example, velleral (**3**), together with isovelleral (**2**), is formed enzymatically in seconds from an apparently biologically inactive precursor, stearoylvelutinal (**1a**), when the fruit bodies are injured.[2] These conversions result in the immediate formation of pungent and toxic compounds (the dialdehydes) at the spot where the fruit body has been injured by, for instance, a bite. Subsequently, in minutes, velleral (**3**) is reduced to the less active and non-pungent monoaldehyde vellerol (**4**), and within hours vellerol (**4**) is in turn reduced to vellerdiol (**5**).[2] In *L. rufus* Scop. ex Fr., isovelleral (**2**) is the only dialdehyde formed, while piperdial (**7**) and lactardial (**14**) are formed together with velleral (**3**) in *L. piperatus* L. ex. Fr. and *L. torminosus* Schff. ex Fr.,[4] and it is intriguing that different species form different dialdehydes in response to an injury.

The furans isolated from *Lactarius* species have lately been regarded with some skepticism, as the fatty acid esters of velutinal (e.g. **1a** and **1b**) originally present in the fruit bodies have been shown to be easily degraded to a number of furans (e.g. compounds **16** and **19**) during, for instance, silica gel chromatography.[5] However, some furans, i.e. the dihydroxyfurans **16** and **18**, are formed during the enzymatic conversions of sesquiterpenes in the injured fruit bodies,[4] although only in small amounts. Furans may, in turn, be precursors to some lactones, as compound **16** has been shown to be autoxidized to lactarorufin A (**17**) when kept in an ethanol solution at room temperature of a number of days.[6] Lactones have been reported from many *Lactarius* species,[1,7] although from some only in very small amounts. One of the species that has previously yielded many lactones,[8,9] but no dialdehydes, is *L. necator* Pers. ex Fr. In order to investigate whether the pungency of the fruit bodies of *L. necator* is caused by sesquiterpene dialdehydes as in other *Lactarius* species, fruit bodies of *L. necator* were extracted by the technique developed by us for studying conversions of sesquiterpenes in the *Lactarius* species.[2] Essentially the same conversions were found to take place in another species, *L. circellatus* Fr., and the two are therefore discussed together.

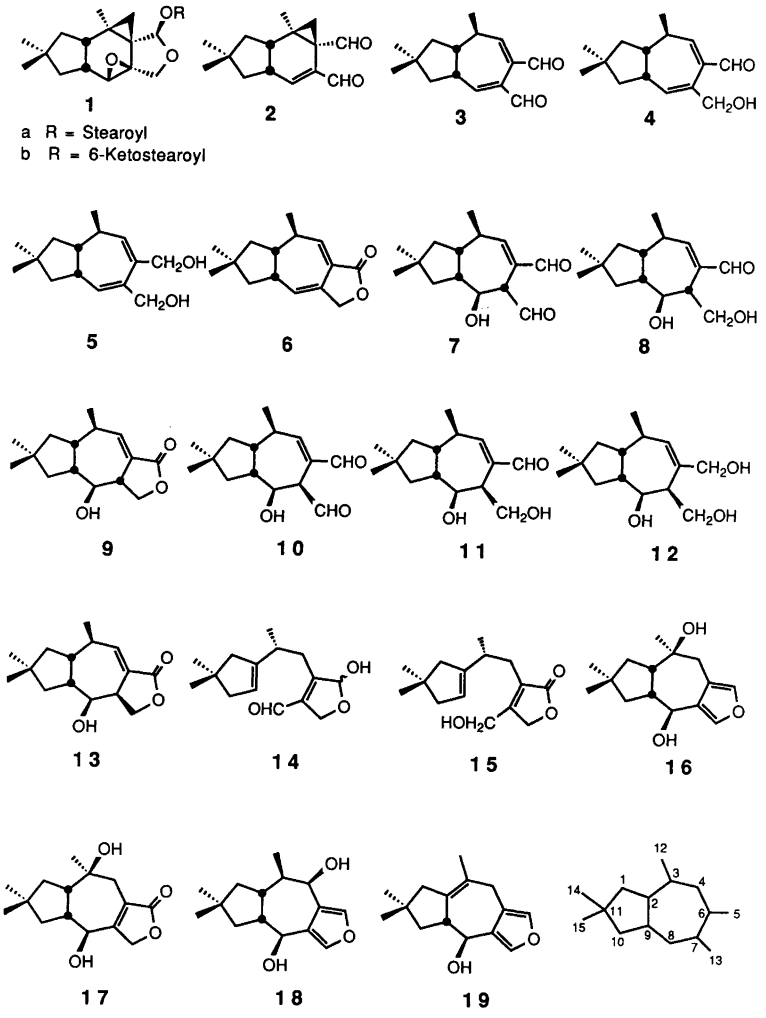
Results and discussion

It has previously been shown that the only sesquiterpenoids present in young and intact fruit bodies of *L. necator* are fatty acid esters of velutinal [approximately 10 % stearoylvelutinal (**1a**) and 90 % 6-ketostearoylvelutinal (**1b**)].[10] The same was found to be the case in fruit bodies of *L. circellatus*. When the fruit bodies of both species were injured (by being ground in a meat grinder[2]), the esters of velutinal were, to some extent, converted into free sesquiterpenes (the conversions were followed by TLC analyses of EtOAc extracts made of ground mushrooms at different times after grinding).

In contrast with the conversions of sesquiterpenes in ground fruit bodies of other *Lactarius* species, in which the esters of velutinal disappear completely after a few minutes,[2,4] the conversions in the species investigated here are less efficient. The main part of the esters of velutinal actually remains unchanged in the ground mushroom tissue, even after several hours. Consequently, only limited amounts of free sesquiterpenes were formed in ground fruit bodies of *L. necator* and *L. circellatus*, compared with other *Lactarius* species.

Three known aldehydes, velleral (**3**), vellerol (**4**), and lactardial (**14**) (identified by their spectral data[2,4,11]) were isolated, as well as one new aldehyde, *epi*-piperalol (**11**). In addition, the presence of a new dialdehyde was indicated, but, unfortunately, this compound was present only in small amounts in the ground fruit bodies, and its lability made it impossible to isolate it in a pure state completely separated from the furan **18**. However, the ^{1}H NMR spectral data suggest it to be compound **10**, the C-7 epimer of piperdial (**7**) isolated from *L. piperatus* and *L. torminosus*.[4] This is further supported by the facile conversion of the compound into piperdial (**7**), which appears to be the more stable epimer, and velleral (**3**) during chromatography on silica gel. The mixture of the metabolite believed to be compound **10** and the furan **18** was found to be pungent, while the furan **18** is tasteless by itself, indicating that compound **10** has pungency in common with the other *Lactarius* dialdehydes.

The structure of *epi*-piperalol (**11**) was elucidated by spectral methods. The NMR data (^{1}H and ^{13}C) of *epi*-piperalol (**11**) were very similar to those of piperalol (**8**),[4] indicating only a sterochemical difference between the two. The stereostructure of *epi*-piperalol (**11**) was established on



the basis of NOE NMR experiments, a large NOE (9 %) was observed on C(8)H when C(3)H was irradiated, and, in contrast with piperalol (**8**), irradiation of C(13)H_2 resulted in an NOE (12 %) on C(9)H. The recent isolation of *epi*-pipertriol (**12**) [with the same C-7 configuration as in compound **10** and *epi*-piperalol (**11**)] from fruit bodies of *L. necator,*[12] suggests that the C-5 aldehyde function of *epi*-piperalol (**11**), as is that of vellerol (**4**),[2] is slowly reduced by the mushroom tissue. The same two dihydroxyfurans (**16** and **18**) found in *L. piperatus* and *L. torminosus,*[4] were also isolated in this investigation, and, in addition, small amounts of the hydroxyfuran **19** were isolated. However, the hydroxyfuran **19** is a major furanoid product obtained when the velutinal esters **1a** and **1b** are degraded by silica gel.[5] As considerable amounts of these esters always remained in the crude extracts, and the separations were made on silica gel columns, it is plausible that the hydroxyfuran **19** isolated here is an artifact.

In addition to the aldehydes and furans, two sesquiterpene lactones that have previously been reported from *L. necator,* lactarorufin N (**13**)[8,13] and, in smaller amounts, lactaronecatorin A (**15**),[14] were isolated. Larger amounts of the two lactones could be isolated from *L. circellatus* than from *L. necator.* The isolation of vellerolactone (**6**) together with velleral (**3**) from *L. vellereus,*[11] blennin A (**9**),[15] piperdial (**7**)[4] and piperalol (**8**)[4] from *L. torminosus,* and lactarorufin N (**13**) and *epi*-piperalol (**11**) in this investigation, suggest a pattern. The lactones vellerolactone (**6**), blennin A (**9**), and lactarorufin N (**13**) could be formed by oxidation of C-5 in vellerol (**4**), piperalol (**8**), and *epi*-piperalol (**11**), or by a Cannizzaro-type reaction with the corresponding dialdehydes **3**, **7** and **10**. There were no observations made during this investigation to suggest that such reactions take place in the extracts, or during the work-up of the extracts, and the formation of lactones is therefore believed to be enzymatic. However, neither the isolation of vellerolactone (**6**) from *L. vellereus* nor blennin A(**9**) from or *L. torminosus* could be repeated in later investigations,[2,4] and there may be other factors besides injury that regulates the formation of the lactones.

Experimental

The fruit bodies of *L. necator* and *L. circellatus* were collected near Lund in the autumn of the years between 1984 and 1988, and ethyl acetate extracts were prepared and worked up as described previously.[2,4] The approximate amounts of sesquiterpenes isolated from extracts of fruit bodies that had been ground for 15 min (in mg per kg fresh fruit body) were 2 for velleral (**3**), 20 for vellerol (**4**), 20 for lactardial (**14**), 100 for *epi*-piperalol (**11**), 10 for the dihydroxyfurans **16** and **18**, 2 for the monohydroxyfuran **19**, 20–80 for lactarorufin N (**13**), and 2–5 for lactaronecatorin A(**15**). ^{1}H NMR spectra were recorded with a Varian XL-300 spectrometer, the coupling constants (J) being given in Hz. ^{13}C NMR spectra, proton noise-decoupled and coupled, were recorded with the same spectrometer. The chemical shifts are reported in ppm with tetramethylsilane as an internal standard. The IR spectrum was recorded with a Perkin–Elmer 257 spectrophotometer, the UV spectrum with a Cary 219 spectrophotometer, and the high resolution mass spectrum was recorded with a Jeol DX-303 mass spectrometer.

1,2,3,3a,4,5,8,8a-Octahydro-4-hydroxy-5-hydroxymethyl-2,2,8-trimethylazulene-6-carbaldehyde (**10**, *epi*-piperalol) was obtained as a colourless oil by SiO_2 chromatography of an EtOAc extract of *L. necator* and *L. circellatus.* $[\alpha]_D^{24}$ = −62° (*c* 2.0 in chloroform). Anal. $C_{15}H_{24}O_3$: C, H. MS [EI 70 eV *m/z* (% rel. int.)]: 234 (36, $M-H_2O$), 204 (68), 189 (18), 187 (11), 175 (16). Found ($M-H_2O$), 234.1612. Calc. for $C_{15}H_{22}O_2$: 234.1620. ^{1}H NMR (300 MHz, $CDCl_3$): 9.26 [s, C(5)H], 6.46 [d, C(4)H, $J_{3,4}$ = 2.2], 3.88 [dm, C(8)H, $J_{8,9}$ = 11], 3.82 [m, C(13)H_2], 3.30 [dddd, C(7)H, $J_{3,7}$ = 2, $J_{7,8}$ = 2, $J_{7,13a}$ = 5.9, $J_{7,13b}$ = 7.9], 2.67 [m, C(9)H], 2.50 [m, C(3)H], 2.07 [m, C(2)H], 1.86 [ddd, C(10)H_a, $J_{1a,10a}$ = 1.9, $J_{9,10a}$ = 6.5, $J_{10a,10b}$ = 12], 1.80 [ddd, C(1)H_a, $J_{1a,1b}$ = 13.1, $J_{1a,2}$ = 8.0, $J_{1a,10a}$ = 1.9], 1.39 [dd, C(10)H_b, $J_{9,10b}$ = 12, $J_{10a,10b}$ = 12], 1.32, [dd, C(1)H_b, $J_{1a,1b}$ = 13.1, $J_{1b,2}$ = 6.9], 1.14 [d, C(12)H_3, $J_{3,12}$ = 7.2], 1.10 and 1.01 [s, C(14)H_3 and C(15)H_3]. ^{13}C NMR (76 MHz, $CDCl_3$): 195.6 [C(5)], 163.1 [C(4)], 140.2 [C(6)], 74.8 [C(8)], 63.3 [C(13)], 48.4 and 46.0 [C(1) and C(10)], 44.4, 44.3 and 42.9 [C(2), C(7), and C(9)], 37.0 [C(11)], 36.4 [C(3)], 30.1 and 27.9 [C(14) and C(15)], 21.5 [C(12)]. Ir (KBr): 3400, 2960, 1750, and 1690 cm^{-1}. UV [abs. ethanol (log ε)]: 231 nm (3.67).

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