Journal of Natural Products
Vol. 48, No. 2, pp. 279-288, Mar-Apr 1985

THE SESQUITERPENES OF *LACTARIUS VELLEREUS* AND THEIR ROLE IN A PROPOSED CHEMICAL DEFENSE SYSTEM

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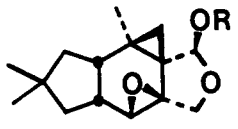
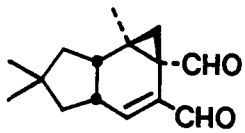
ABSTRACT.—The Basidiomycete *Lactarius vellereus* has been found to contain orginally a single sesquiterpene, velutinal (**1a**), as its stearic acid ester. When the mushroom is injured, stearoylvelutinal (**1b**) is rapidly converted to the two toxic sesquiterpene dialdehydes isovelleral (**2**) and velleral (**3**), which are then gradually reduced by the mushroom to less toxic compounds. It is suggested that these compounds constitute a chemical defense system for *L. vellereus*.

While their significance for the mushroom has generally been poorly understood, a great many sesquiterpenes with lactarane (e.g., **3**), secolactarane (e.g., **12**), and marasmane (e.g., **2**) skeletons have been isolated from the fruiting bodies of members of the genera *Lactarius* and *Russula* (1). Some of these compounds have been reported from various species of *Lactarius* as well as other genera, while others seem to be specific to one or a few species, and attempts have been made to draw chemotaxonomic conclusions on the basis of the sesquiterpenes isolated (2,3). In several cases, however, the sesquiterpenes obtained were observed to vary both qualitatively and quantitatively when different extraction procedures were used (4,5), and some compounds, for instance ethyl ethers, obtained when the extraction was performed with EtOH, were considered to be extraction artifacts (5,6).



1a R=H
1b R=Stearoyl



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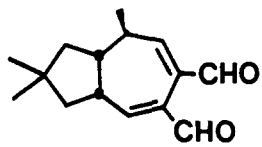

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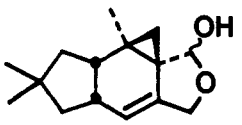

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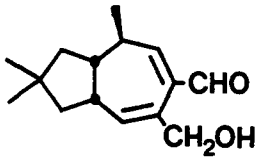

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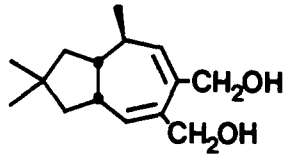

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The apparent discrepancies in the earlier results reported from different laboratories would seem to be at least partially explained by the recent discovery of a series of fatty acid esters of a very unstable sesquiterpene, velutinal (**1a**], in Basidiomycetes belonging to several genera, particularly in *Lactarius* and *Russula* (7,8). Thus, all *Lactarius* species reinvestigated so far have been found to contain velutinal (as fatty acid esters) as the only observable sesquiterpene when worked up under carefully controlled conditions. Therefore, all other sesquiterpenes, with the possible exception of trace constituents, must be secondary products formed during the collection or transport, or later during extraction and isolation. Attack by insects or snails should certainly cause similar changes. The velutinal esters undergo rapid solvolysis in pure MeOH at room temperature, and their further degradation is catalyzed even by minute traces of acids (9). The degradation of velutinal (**1a**) or its methyl acetal when adsorbed on silica gel for 2 h is summarized in Figure 1. The furans **7-12** obtained as end products have been

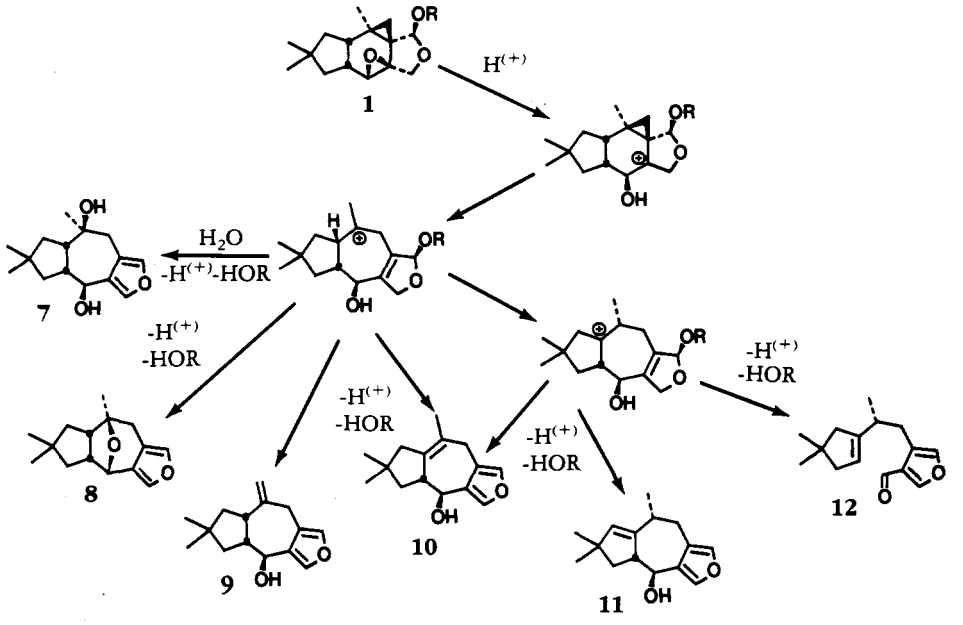


FIGURE 1. Summary of the acid catalyzed degradation of velutinal (R=H) or its methyl acetal ($R=CH_3$).

reported previously from a number of different species (1, 10) but must now be strongly suspected to be artifacts.

Lactarius vellereus Fr. is one of the most thoroughly investigated species of mushrooms because of its sharp taste and its apparent resistance to attack by predators that normally support themselves on mushrooms, for example, some insects and snails. Among the sesquiterpenes isolated from extracts of *L. vellereus* are the two pungent dialdehydes, isovelleral (**2**) (11) and velleral (**3**) (12), which have been found to possess antimicrobial activity (13). Isovelleral was also found to be mutagenic in the Ames test (14). Isovelleral has recently been found to be an insect antifeedant (13) and a potent opossum antifeedant (15). The biological activities of **2** and **3**, and the fact that these compounds are not originally present in the mushroom but are formed during extraction in what appears to be enzymatic processes, supported our earlier proposal that they are part of a chemical defense system of *L. vellereus* (8). The enzymatic processes start when the mushroom is injured, as if the enzymes and substrate **1b** were stored apart in the intact mushroom and brought together by the injury.

To test this hypothesis, the appearance and fate of isovelleral (**2**) and velleral (**3**) in the injured mushroom was investigated by extracting deliberately injured mushrooms with hexane at different times after injury and analyzing the extracts. Two series of extractions were made, one at 22° and one at 4°, in order to investigate the temperature dependence of the processes in the injured mushroom. To compare the biological activity of the sesquiterpenes found in the extracts, their mutagenic, antibacterial, and antifungal activities were estimated by standard assays.

RESULTS AND DISCUSSION

Preliminary analysis by tlc of the extracts of *L. vellereus* made at different times after injury indicated the presence of only six sesquiterpenes. Stearoylvelutinal (**1b**) is the original compound from which the others are formed, and consequently the amount of **1b** decreases rapidly with time. Originally, fresh *L. vellereus* contain approximately

0.2% stearoylvelutinal. Five min after grinding at 22°, only small amounts of **1b** were detected by tlc. Free velutinal (**1a**) is not originally present in the mushroom, although small amounts could be observed by tlc the first minutes after grinding at 4°. In addition to isovelleral (**2**) and velleral (**3**) discussed previously, large amounts of two slightly more polar compounds were indicated by tlc. Both were isolated by silica gel chromatography and found to be compounds **4** and **5**. The reduction of **4** and **5** with KBH_4 gave the identical (nmr, optical rotation) diols that previously had been obtained by the reduction of isovelleral (11) and velleral (16). The position of the aldehyde function relative to the alcohol function was for **4** determined by the observed nOe on C(5)H when $C(12)H_3$ was irradiated, and for **5** by the observed coupling of the downfield olefinic proton (6.97 ppm) with C(3)H and the upfield olefinic proton (6.10 ppm) with C(9)H. Compounds **4** and **5** are reduced forms of isovelleral and velleral, for which we propose the names isovellerol (**4**) and vellerol (**5**). Vellerol is a new compound, while isovellerol has been isolated previously from extracts of the cultured mycelium of *Fomitopsis insularis* (17). Extracts made more than 1 h after grinding at 22° were, furthermore, shown to contain small amounts of vellerdiol (**6**), previously prepared by reduction of velleral (16). No traces of other sesquiterpenes were detected by the analytical methods used in this investigation, either in the hexane extracts or in an EtOAc extract made 2.5 min after grinding at 22°, in search for more polar sesquiterpenes.

In addition to sesquiterpenes, the hexane extracts were also found to contain fat (triolein), dimers of isovellerol (**4**) and vellerol (**5**), and stearic acid. The dimers are only observed in extracts made more than 1 h after grinding at 22° and are present only in small amounts, (<1%). They are probably not formed by enzymatic processes because isovellerol and vellerol readily dimerize when left in reagent grade solvents. It is more interesting to note the relatively small amounts of stearic acid in the extracts. When determined quantitatively, the sesquiterpenes **2-5** were typically found to constitute 80-90% of the extracts made 5 min or later after grinding at 22°. As more than 50% of the original stearoylvelutinal (**1b**) is stearic acid, and as stearic acid is easily extracted by hexane at this temperature, one would expect to find approximately equal amounts of sesquiterpenes and stearic acid. The fate of the stearic acid produced by the enzymatic conversions of stearoylvelutinal will be subject to further investigation.

From tlc is was obvious that the relative amounts of the four major sesquiterpenes **2-5** in the extracts varied considerably with the time between grinding and extraction, and a quantitative determination of compounds **2-5** was made in all extracts by hplc analysis. The results are shown in Figures 2 and 3, where the amount of each compound in percent of the total weight of the corresponding extract has been plotted against the logarithm of the time in seconds between grinding and extraction. Figures 2 and 3 show the results when extractions were made at 22° and 4°, respectively. Comparison of the variation of isovelleral (**2**) with that of isovellerol (**4**), as well as that of velleral (**3**) with that of vellerol (**5**) (see Figure 2), suggests that **4** and **5** are formed by reduction of **2** and **3** rather than directly from stearoylvelutinal (**1b**). To establish this, **2** and **3**, labeled with ^{18}O, were administered separately to two portions of freshly ground *L. vellereus* and the resulting isovellerol and vellerol were isolated. When analyzed by ms, it was found that the isovellerol, but not the vellerol, isolated from the ground mushroom that was given labeled isovelleral, had a small but significant label of ^{18}O (8%). The analogous relationship was observed for vellerol in the corresponding experiment. However, when the relative variations of compounds **2-5** are compared in Figure 3, there is a conspicuous lack of agreement between the increase of vellerol and the decrease of velleral. This may be due to poorer extractability of the more polar vellerol in hexane at this temperature, although nothing is known about how a decrease in temperature affects the enzymes involved in the reduction of isovelleral and velleral.

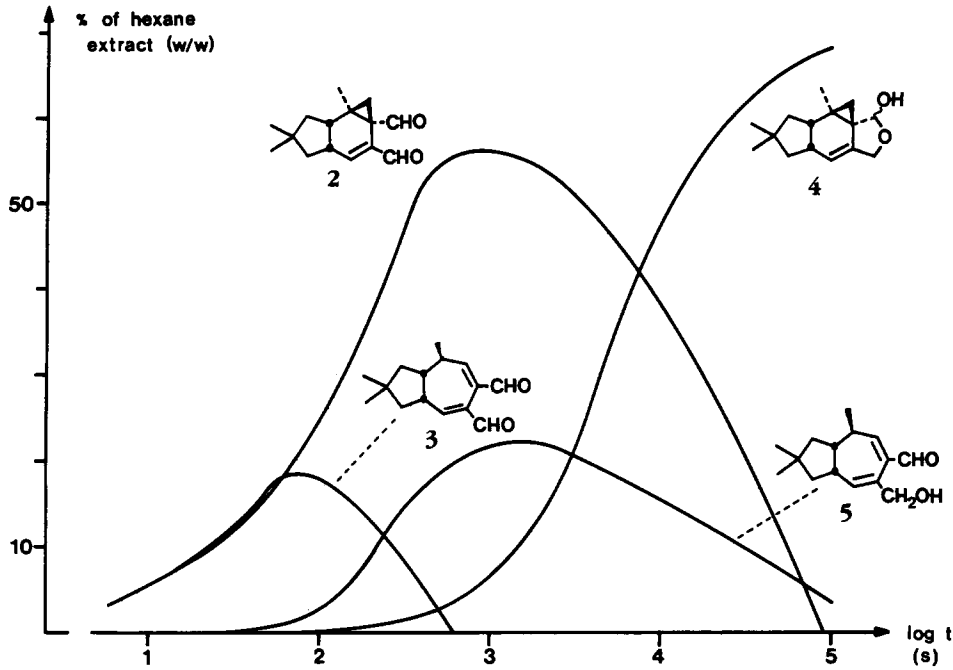


FIGURE 2. The variations of isovelleral (**2**), velleral (**3**), isovellerol (**4**), and vellerol (**5**) in hexane extracts of ground *Lactarius vellereus*, at different times after grinding at 22°.

Time after grinding:	10sec	1min	2.5min	5min	11min	30min	1h	2h	24h
% of compound **2**:	6.2	17	28	51	49	56	51	21	1.5
3:	5.6	17	12	14	—	—	—	—	—
4:	—	—	1.3	2.2	2.8	11	24	42	68
5:	—	1.4	3.6	14	20	22	20	16	6.4

The mutagenicity of isovelleral (**2**) in the *Salmonella*/microsome assay, as a potent direct-acting mutagen, has already been demonstrated (14). Isovellerol (**4**) was also found to be mutagenic, although more than ten times less than isovelleral, and requiring metabolic activation by rat liver microsomal enzymes. As this in vitro metabolism easily performs oxidations, it seems reasonable to assume that the mutagenicity of isovellerol may be due to its oxidation back to isovelleral. This is strongly supported by the almost identical appearance of the mutagenicity profile (14) of the two compounds. Stearoylvelutinal (**1b**, velleral (**3**) (14), vellerol (**5**) and vellerdiol (**6**) were not found to be mutagenic at all. The antibacterial and antifungal activities of compounds **1b, 2, 3, 4, 5,** and **6** are presented in Table 1. The toxicity of isovelleral and that of velleral are in the same range, and in every case more than 10 times greater than that observed for **1b, 4, 5** and **6**. The considerable reduction of the toxicity in the assays used here, of isovelleral and velleral by the chemical reduction of the C-13 aldehyde function indicates the importance of the latter for the biological activity of **2** and **3**.

As previously shown (8), and also clearly indicated by Figures 2 and 3, velutinal, as its stearic acid ester **1b**, is the only sesquiterpene originally present in significant amounts in *L. vellereus*. The biological activity of **1b** appears very low, although its low solubility in H_2O may affect the results obtained in the assays used here. The very rapid enzymatic conversion of stearoylvelutinal (**1b**) to the antimicrobial compounds isovelleral (**2**) and velleral (**3**) by the mushroom as a response to injury should certainly act as a

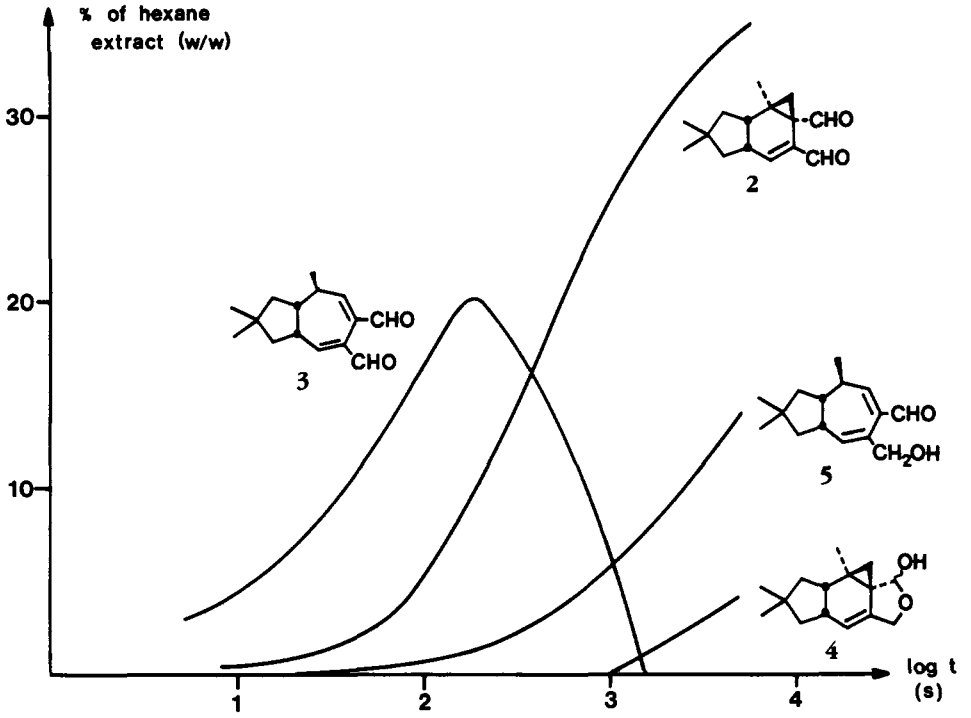


FIGURE 3. The variations of isovelleral (**2**), velleral (**3**), isovellerol (**4**), and vellerol (**5**) in hexane extracts of ground *Lactarius vellereus*, at different times after grinding at 4°.

Time after grinding:	10sec	1min	3min	6min	15min	30min	60min
% of compound **2**:	0.4	2.4	11	20	29	26	34
3:	4.2	12	20	15	10	—	—
4:	—	—	—	—	—	2.0	5.9
5:	—	0.4	1.0	2.2	4.7	8.6	12

deterrant to many predators, as demonstrated for the opossum rat (15). However, it is reasonable that isovelleral and velleral are also toxic to the mushroom itself, as indicated by their antifungal activity. Indeed, both the fact that they are not formed until the mushroom is injured and their subsequent enzymatic reduction to the considerably less toxic compounds isovellerol (**4**) and vellerol (**5**), and eventually to the diol **6**, would have the effect of saving the mushroom from unnecessarily prolonged contact with its own defense chemicals.

The compounds **2-5** are in no way unique to *L. vellereus* but are found in many, though not all, of the *Lactarius* and *Russula* species investigated so far (8). Each species

TABLE 1. The Relative Antimicrobial Activity of Compounds **1b-6**, Determined with the Kirby-Bauer Growth Inhibition Assay.

Test organisms	Compounds					
	1b	**2**	**3**	**4**	**5**	**6**
Escherichia coli	>1000[a]	15	50	>1000	>1000	>1000
Micrococcus luteus	>1000	2	15	300	300	>1000
Candida utilis	>1000	2	2	50	100	>1000

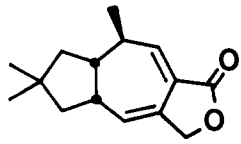
[a]μg.

seems to exhibit its own characteristic pattern, with the following variable parameters: a) the fatty acids in the velutinal esters; b) the amount of velutinal (**1a**) originally present in the mushroom; c) the relative amount of isovelleral (**2**) and velleral (**3**) formed; d) the rate of the enzymatic conversions; e) the formation of other sesquiterpenes, presumably derivatives of **2** and **3**.

Russula queletii, for instance, which contains less velutinal ester than *L. vellereus*, produces velleral (**3**) and vellerol (**5**) but no isovelleral (**2**) and isovellerol (**4**), and, in addition, it also produces other sesquiterpenes not discussed in this paper. Since **2** and **3** have a very sharp taste, and since mycologists frequently classify mushrooms according to pungency, one would expect that pungent Russulaceae species could produce isovelleral and/or velleral, while nonpungent ones could not. This is, however, not generally true, as demonstrated for *Lactarius quietus*. This species is frequently described as having a mild or slightly bitter taste. This is true for a quick test, but if the sample is kept in the mouth for at least a minute, a sharp taste is clearly felt. Grinding specimens of *L. quietus* at room temperature and extracting the ground mushroom at different intervals confirmed this: appreciable amounts of isovelleral, accompanied by isovellerol, were first detected by tlc 5 min after grinding (no velleral or vellerol was produced by *L. quietus*).

Previously, besides stearoylvelutinal (**1b**), isovelleral (**2**) and velleral (**3**), the furans **7** (5), **9** (10), **10** (5), and **12** (18), and the lactones vellerolactone (**13**) (19) and pyrovellerolactone (**14**) (19) were isolated in considerable amounts from extracts of *L. vellereus*. The furans must now, as discussed in the introduction, be considered to be mainly artifacts formed from the degradation of velutinal derivatives during extraction and workup (9). The previously proposed (20) biosynthetic link to the furan via isovelleral may therefore be removed from consideration. The two lactones **13** and **14** could, however, never be detected in the extracts of *L. vellereus* prepared in this investigation, neither as natural products nor as artifacts. This is quite puzzling inasmuch as both were previously routinely isolated from hexane extracts of *L. vellereus*. It must, however, be emphasized that the specimens of *L. vellereus* investigated here were collected from a single habitat, different from those that afforded *L. vellereus* producing the lactones **13** and **14**. From the notable intraspecific variations in mutagenic activity observed (21), it is evident that chemical differences exist in specimens of the same species, even when they grow close to each other. Work is now underway to clarify the reasons for the apparent irregular appearance of vellerolactone (**13**) and pyrovellerolactone (**14**), and their eventual role in the mechanisms discussed above.

During the course of this work, a number of chemical conversions of compounds **1-5** were observed under conditions normally encountered during workup. As such conversions are potential sources of artifacts, one must be aware of their existence and exert strict control on any operation where they may occur. The degradation of velutinal derivatives on SiO_2 or in reagent grade MeOH (9), for example, is primarily responsible for the furans **7-12**. If stearoylvelutinal (**1b**) is degraded by adsorption on Al_2O_3, significant amounts of isovellerol (**4**) and reduced lactaral (lactarol) (**15**) (22) were formed in addition to the furans. Isovellerol may therefore under certain circumstances be an artifact itself. When preparative chromatography of velleral (**3**) on Al_2O_3 was attempted, **3** was found to be degraded rapidly to more than ten as yet unidentified compounds. On being stored at $-30°$ for months as a noncrystalline mixture together with isovellerol (**4**), vellerol (**5**), and stearic acid, both isovelleral (**2**) and velleral (**3**) are oxidized at a significant rate, to 9-hydroxyisovelleral (**16**) and 9-hydroxyvelleral (**17**). Compounds **16** and **17** are new, and their structures could be elucidated by spectral comparison with isovelleral and velleral. The insertion of hydroxyl groups in **16** and **17**



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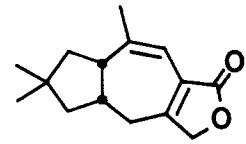

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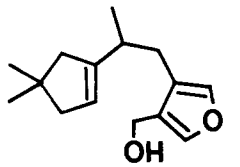

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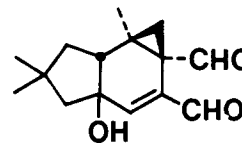

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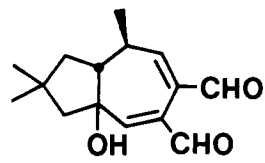

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was clearly indicated, and their position could be established to C-9 by decoupling and nOe experiments. The enzymatic processes that are triggered by an injury may also be affected by a number of conditions. For example, freezing and thawing the mushrooms prior to extraction partially inactivates the enzymes of *L. vellereus*, so that the amount of isovelleral and velleral formed is reduced. The solvent used for extraction is critical because alcoholic solvents affect the enzymes, probably due to rapid denaturation, more than water-immiscible solvents like hexane or EtOAc.

Naturally, it is impossible to prove conclusively that the appearance and disappearance of isovelleral (**2**) and velleral (**3**) in ground specimens of *L. vellereus* constitute a chemical defense system. The componds discussed in this paper may have other as yet unidentified functions, although in some instances they act as defense chemicals and protect the mushroom against parasites. It is very interesting to note that, while some species produce both isovelleral and velleral, others produce only one or the other. If part of a chemical defense system, such variations may be explained by differences between isovelleral and velleral in biological activity and a subsequent adaption of different species to different environments.

EXPERIMENTAL

L. vellereus was collected in the south of Sweden in September 1982 and 1983. Only young specimens that appeared unaffected by parasites were chosen. To simulate injury, approximately 1 kg of such mushrooms was ground in a meat grinder, without the addition of solvent, whereupon 50-g portions of the ground mushroom were extracted with 100 ml of hexane, and the time elapsed between grinding and extraction was recorded for each portion. Two series of extractions were made, one at 22° and one at 4°. A portion was also extracted with EtOAc 2.5 min after grinding at 22° to reveal the presence of polar sesquiterpenes that are poorly extracted by hexane. All solvents used were freshly distilled, and all extracts were immediately evaporated to dryness below room temperature and stored at −70°.

Tlc experiments were performed on Merck Kieselgel 60 F_{254} SiO_2 plates (prewashed with Et_2O), developed with EtOAc-hexane mixtures, and visualized by spraying with anisaldehyde-H_2SO_4 and warming to 120°. The hplc analytical system was composed of a 20 cm column packed with 5 μ LiChrosorb Si 100, and an Optilab Multiref 901 RI-detector in connection with a Hewlett Packard HP 3390 integrator. The eluent in the hplc experiments was 15% EtOAc in hexane and the flow rate 1 ml/min. To avoid contamination of the hplc column by stearic acid, CH_2N_2 in Et_2O was added to all extracts prior to analysis. Test runs showed that the sesquiterpenes **2-5** were not affected by the CH_2N_2.

The labeling of isovelleral (**2**) and velleral (**3**) with ^{18}O was performed by adding 100 mg of $^{18}O\,H_2O$ and a trace of trifluoroacetic acid to a solution of 100 mg of the corresponding sesquiterpene in 1 ml of Et_2O. The exchange was monitored by ms to completion, whereupon the Et_2O solution was separated and evaporated. The labeled compounds were dissolved in a small amount of MeOH and administered, at room temperature, separately to ca. 100-g batches of freshly ground mushroom, each of which was extracted with hexane 60 min later.

Standard assays were employed to evaluate the antibacterial and antifungal activity, and the mutagenicity of the compounds **1b, 2, 3, 4, 5,** and **6**. Antibacterial and fungicidal activity were investigated by the Kirby-Bauer growth inhibition method (23) with the gram negative strain *Escherichia coli*, the gram-positive strain *Micrococcus luteus*, and the fungus *Candida utilis*. A comparative value in μg, of the antimicrobial activity of each compound was obtained by extrapolating the linear relationship between the diameter of the inhibition zone and the logarithm of the amount of each compound, to zero inhibition zone. The mutagenicity was investigated with the *Salmonella*/microsome assay (24) (Ames test) on strains TA 98, TA 2637, and TA 100, both in the presence and absence of metabolic activation (5% rat liver S-9 fraction).

^1H-nmr spectra were recorded with a Nicolet NM-360 spectrometer (360 MHz). The coupling constants J are given in Hertz. ^{13}C-nmr spectra, proton noise-decoupled and coupled, were recorded with the same spectrometer (91 MHz). Chemical shifts are reported in ppm with TMS as internal standard and $CDCl_3$ (filtered through Na_2CO_3 to remove any acidic impurities) was used as solvent throughout. Melting points were obtained with a Kofler hot-stage apparatus and are uncorrected. Ir spectra were determined with a Perkin-Elmer 257 spectrophotometer. Optical rotations were measured with a Perkin-Elmer 1541 automatic polarimeter (1 dm cell). Uv spectra were recorded with a Cary 219 spectrophotometer in EtOH. Mass spectra (ei) were recorded with a Varian MAT 112 at 70 eV.

The numbering system of the lactarane, secolactarane, and marasmane skeletons are depicted in Figure 4. All previously known compounds were isolated by the described procedures, and their chemical and physical properties were in accord with data reported in the literature.

The physical data reported previously for isovellerol (**4**) (17) are insufficient, and thus a full set of data is given here. Isovellerol (200 mg) was obtained as a colorless oil by SiO_2 chromatography of a hexane extract of 1 kg *L. vellereus* that had been ground 1 h before extraction. In common organic solvents, isovellerol exists as a mixture of approximately equal amounts of the three forms shown in Figure 5. $[\alpha]^{24}D = +7{,}4°$ ($c = 1.0$ in Et_2O); Elemental analysis: Calculated for $C_{15}H_{22}O_2$: C 76.9, H 9.46, Found: C 76.6, H 9.41; ms *m/z* (rel. int.) 234 (42%), 219 (82%), 173 (71%), 119 (60%), 105 (77%), 91 (100%), 77 (55%), 55 (53%), 41 (88%); uv 216 nm (8300); ir (KBr) 3400, 2940, 2880, 1700, 1450, 1120, 920, 740 cm^{-1}. ^1H nmr 9.38, 5.30 and 5.26, s, C(5)H; 5.26, s, 4.92, d, $J_{8\text{-}9} = 1.8$ and 4.85, s, C(8)H; 4.68, 4.66 and 4.32, m, C(13)Ha; 4.59, 4.35 and 4.21, m, C(13)Hb; 2.54-2.36, m, C(2)H and C(9)H; 1.85-0.96, C(1)H_2, C(4)Ha and C(10)H_2; 1.33, 1.27 and 1.20, s, C(12)H_3; 1.02, 1.02, 1.00 and 0.98, s, C(14)H_3 and C(15)H_3; 0.85-0.81, m, C(4)Hb. ^{13}C nmr 202.3, 102.3 and 98.9 C(5); 139.3, 138.4 and 135.3 C(7); 128.4, 116.0 and 115.9 C(8); 69.0, 68.5 and 65.7 C(13); 48.1, 48.1, 47.7, 44.5, 44.4 and 44.1 C(1) and C(10); 42.9, 42.2, 42.2, 39.1, 38.5 and 38.2 C(2) and C(9); 37.4 C(11); 36.0, 35.2 and 33.9 C(6); 32.0, 32.0, 32.0, 31.9, 31.8 and 31.8 C(14) and C(15); 31.7, 26.2 and 22.0 C(4); 25.0, 24.4 and 22.0 C(3); 22.0, 20.2 and 19.2 C(12).

Velleral (**5**) (150 mg) was obtained as a colorless oil by SiO_2 chromatography of a hexane extract of *L. vellereus* that had been ground 1 h before extraction. $[\alpha]^{24}D = +149°$ ($c = 1.2$ in Et_2O); Elemental analysis: Calculated for $C_{15}H_{22}O_2$: C 76.9, H 9.46, Found: C 77.0, H 9.62; ms *m/z* (rel. int.) 234 (56%), 201 (35%), 173 (36%), 117 (42%), 105 (55%), 91 (100%), 77 (60%), 55 (56%), 41 (92%); uv 217 nm (19700) and 253 nm (6100); ir (KBr) 3400, 2960, 2880, 1680, 1470, 1220, 1120, 920, 740 cm^{-1}. ^1H nmr 9.36, s, C(5)H; 6.97, d, C(4)H, $H_{3\text{-}4} = 6.1$; 6.10, d, C(8)H, $J_{8\text{-}9} = 2.2$; 4.21, d, C(13)Ha, $J_{13a\text{-}13b} = 11.9$; 4.06, d, C(13)Hb, $J_{13a\text{-}13b} = 11.9$; 2.73-2.59, m, C(2)H and C(9)H; 2.30, ddd, C(3)H, $J_{2\text{-}3} = 10.8$, $J_{3\text{-}4} = 6.1$, $J_{3\text{-}12} = 6.5$; 1.84, dd, C(1)Ha (or C(10)Ha), $J_{1a\text{-}2} = 7.9$, $J_{1a\text{-}1b} = 11.9$; 1.58, dd, C(10)Ha (or C(1)Ha), $J_{9\text{-}10a} = 5.0$, $J_{10a\text{-}10b} = 11.9$; 1.44, dd, C(10)Hb (or C(1)Hb), $J_{9\text{-}10b} = 7.2$, $J_{10a\text{-}10b} = 11.9$; 1.34, dd, C(1)Hb (or C(10)Hb), $J_{1a\text{-}1b} = 11.9$, $J_{1b\text{-}2} = 11.9$; 1.15, d, C(12)H_3, $J_{3\text{-}12} = 6.5$; 1.09 and 0.94, s, C(14)H_3 and C(15)H_3. ^{13}C nmr 194.7 C(5); 166.1 C(4); 143.6 C(8); 141.9 and 134.3

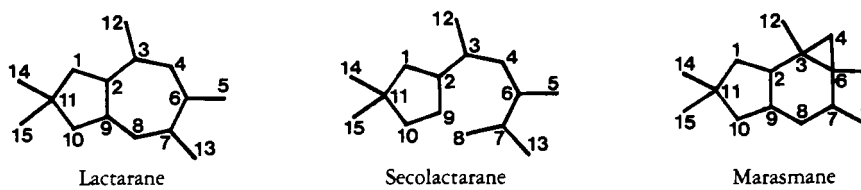
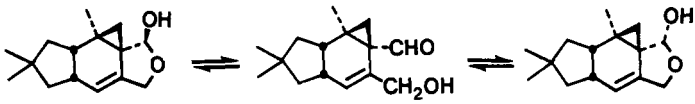


FIGURE 4. Numbering System of Ring Skeletons of Sesquiterpenes of *Lactarius vellereus*.



~1:1:1

FIGURE 5

C(6) and C(7); 65.3 C(13); 60.6 C(3); 49.1 and 47.4 C(1) and C(10); 39.6 and 38.4 C(2) and C(9); 37.9 C(11); 29.7 and 27.7 C(14) and C(15); 18.4 C(12).

9-Hydroxyisovelleral (**16**) (15 mg) was obtained as a colorless oil by SiO_2 chromatography of a partly worked up hexane extract (0.85 g) of *L. vellereus* kept in a freezer (−30°) for 4 months. Compound **16** was also formed in a 50% yield, when isovelleral (**2**) was adsorbed on alumina for 5 h in daylight and, in small amounts, in a hexane solution of **2** kept at room temperature for 2 weeks. $[\alpha]^{24}D = +92°$ (c = 1.6 in Et_2O); Elemental analysis: Calculated for $C_{15}H_{20}O_3$: C 72.6, H 8.12, Found C 72.3, H 8.22; ms *m/z* (rel. int.) 248 (100%), 230 (52%), 164 (47%), 149 (63%), 146 (58%), 105 (49%), 91 (67%), 77 (55%), 55 (60%); uv 243 nm (5000); ir (KBr) 3420, 2960, 2880, 1680, 1200, 1180, 920 and 740 cm^{-1}. ^1H nmr 9.67 and 9.56, s, C(5)H and C(13)H; 6.51, d, C(8)H, $J_{2\text{-}8}$ = 0.4; 2.69, dd, C(2)H, $J_{1a\text{-}2}$ = 7.6, $J_{1b\text{-}2}$ = 13.0; 2.02, d, C(10)Ha, $J_{10a\text{-}10b}$ = 13.7; 1.95, d, C(10)Hb, $J_{10a\text{-}10b}$ = 13.7; 1.94, d, C(4)Ha, $J_{4a\text{-}4b}$ = 4.3; 1.90, dd, C(1)Ha, $J_{1a\text{-}1b}$ = 13.0, $J_{1a\text{-}2}$ = 7.6; 1.37, d, C(4)Hb, $J_{4a\text{-}4b}$ = 4.3; 1.16, 1.14, and 1.00, s, C(12)H_3, C(14)H_3 and C(15)H_3; 1.13, dd, C(1)Hb, $J_{1a\text{-}1b}$ = 13.0, $J_{1b\text{-}2}$ = 13.0. ^{13}C nmr 197.3 and 192.9 C(5) and C(13); 148.7 C(8); 142.0 C(7); 76.9 C(9) (recorded in acetone-d_6); 57.3 C(10); 47.6 C(2); 46.3 C(1); 36.0, 35.5, and 34.1 C(3), C(6) and C(11); 31.6 and 31.3 C(14) and C(15); 30.8 C(4); 19.1 C(12).

9-Hydroxyvelleral (**17**) (4 mg) was obtained as a white crystalline solid, mp 131-133°, together with 9-hydroxyisovelleral (**16**) from the frozen hexane extract of *L. vellereus*. Compound **17** was also formed in small amounts when a hexane solution of velleral (**3**) was left at room temperature for 2 weeks. Too small amounts of the pure compound were obtained to obtain elemental analysis and a ^{13}C-nmr spectrum. $[\alpha]^{24}D = -31°$ (c = 1.5 in Et_2O); ms *m/z* (rel. int.) 248 (14%), 230 (51%), 201 (42%), 146 (39%), 91 (49%), 77 (52%), 69 (53%), 55 (100%); uv 240 nm (9700); ir (KBr) 3450, 2960, 1700, 1670, 1460, 1220, 1070, and 870 cm^{-1}. ^1H nmr 9.51 and 9.50, s, C(5)H and C(13)H; 7.15, s, C(8)H; 7.01, d, C(4)H, $J_{3\text{-}4}$ = 6.1; 2.72, ddd, C(2)H, $J_{1a\text{-}2}$ = 11.9, $J_{1b\text{-}2}$ = 11.9, $J_{2\text{-}3}$ = 6.1; 2.26, ddd, C(3)H, $J_{2\text{-}3}$ = 6.1, $J_{3\text{-}4}$ = 6.1, $J_{3\text{-}12}$ = 6.8; 2.01, d, C(10)Ha, $J_{10a\text{-}10b}$ = 14.0; 1.86, d, C(10)Hb, $J_{10a\text{-}10b}$ = 14.4; 1.84, dd, C(1)Ha, $J_{1a\text{-}1b}$ = 11.9, $J_{1a\text{-}2}$ = 11.9; 1.36, dd, C(1)Hb, $J_{1a\text{-}1b}$ = 11.9, $J_{1b\text{-}2}$ = 11.9; 1.17, d, C(12)H_3, $J_{3\text{-}12}$ = 6.8; 1.15 and 1.08, s, C(14)H_3 and C(15)H_3.

ACKNOWLEDGMENTS

This work was supported by the Swedish Research Council, grant no. K-KU 2070-104. The authors thank Dr. Robert E. Carter for helpful linguistic criticisms.

LITERATURE CITED

1. W.A. Ayer and L.M. Browne, *Tetrahedron*, **37**, 2199 (1981).
2. E. Seppa and K. Widen, *Ann. Bot. Fennici*, **17**, 56 (1980).
3. W. Daniewski, M. Kocor, T. Januszewski, and A. Rymkiewicz, *Pol. J. Chem.*, **55**, 807 (1981).
4. P.G. List and H. Hackenberg, *Arch. Pharmaz.*, **302**, 125 (1969).
5. G. Magnusson, S. Thoren, J. Dahmen, and K. Leander, *Acta Chem. Scand. B*, **28**, 841 (1974).
6. M. De Bernardi, G. Fronza, G. Mellerio, G. Vidari, and P. Vita Finzi, *Phytochemistry*, **18**, 293 (1979).
7. J. Favre-Bonvin, K. Gluchoff-Fiasson, and J. Bernillon, *Tetrahedron Lett.*, **23**, 1907 (1982).
8. O. Sterner, R. Bergman, E. Kesler, L. Nilsson, J. Oluwadiya, and B. Wickberg, *Tetrahedron Lett.*, **24**, 1415 (1983).
9. O. Sterner, R. Bergman, J. Kihlberg, J. Oluwadiya, B. Wickberg, G. Vidari, M. Bernardi, F. Marchi, G. Fronza, and P. Vita Finzi, *J. Org. Chem.*, (accepted for publication).
10. J. Kihlberg, R. Bergman, L. Nilsson, O. Sterner, and B. Wickberg, *Tetrahedron Lett.*, **24**, 4631 (1983).
11. G. Magnusson, S. Thoren, and B. Wickberg, *Tetrahedron Lett.*, **12**, 1105 (1972).
12. G. Magnusson and S. Thoren, *Tetrahedron*, **29**, 1621 (1973).
13. G. Magnusson, University of Lund, personal communications, 1984.
14. O. Sterner, R. Bergman, E. Kesler, G. Magnusson, L. Nilsson, B. Wickberg, E. Zimersson, and G. Zetterberg, *Mutation Res.*, **101**, 269 (1982).

15. S. Camazine, J. Resch, T. Eisner, and J. Meinwald, *J. Chem. Ecology*, **10**, 1439 (1983).
16. G. Magnusson and S. Thoren, *Acta Chem. Scand.*, **27**, 2396 (1973).
17. S. Nozoe, H. Kobayashi, S. Urano, and J. Furukawa, *Tetrahedron Lett.*, **16**, 181 (1977).
18. G. Magnusson and S. Thoren, *Tetrahedron*, **30**, 1431 (1974).
19. G. Magnusson and S. Thoren, *Acta Chem. Scand.*, **27**, 1573 (1973).
20. J. Froborg and G. Magnusson, *Tetrahedron*, **34**, 2027 (1978).
21. C. Franzen, R. Bergman, L. Nilsson, O. Sterner, and B. Wickberg, *Mutation Res.* (submitted for publication).
22. J. Froborg, G. Magnusson, and S. Thoren, *Acta Chem. Scand. B*, **28**, 255 (1974).
23. A. Delaat, "Microbiology for the Allied Health Professions," 2nd ed., Philadelphia: Lea and Febiger, 1979, p. 360.
24. B. Ames, J. McCann, and E. Yamasaki, *Mutation Res.*, **31**, 347 (1975).

Received 10 August 1984