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NEW SESQUITERPENES IN A PROPOSED RUSSULACEAE CHEMICAL DEFENSE SYSTEM

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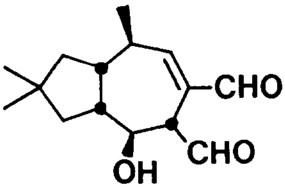
Abstract: In addition to already known sesquiterpene aldehydes, the lactaranes piperdial **1** and piperalol **2** were isolated from extracts of *Lactarius piperatus*, *L. torminosus* and *Russula queletii* (larger fungi).

The sesquiterpenes of pungent Russulaceae species (genera *Lactarius* and *Russula*) have recently been suggested to be part of a chemical defense system[1], which protects the mushrooms against parasites and mammals[2]. These species originally contain only a single sesquiterpene, the chemically very labile marasmane derivative velutinal **4a**[3,4], which occurs in the mushrooms as various fatty acid esters (e.g. **4b** and **4c**). When subjected to conditions including traces of acid (e.g. reagent grade solvents or silica gel chromatography), the velutinal derivatives **4a-c** are rapidly degraded to hydroxydihydrofuranoid and furanoid sesquiterpenes[5]. However, when fresh mushrooms are injured, the velutinal esters are enzymatically converted to unsaturated dialdehyde sesquiterpenes that are pungent and possess antimicrobial activity. When specimens of *L. vellereus* were injured by grinding, the unsaturated dialdehydes isovelleral **5** and velleral **6**, which are initially formed, are subsequently (in the course of minutes to hours) reduced by fungal enzymes to isovellerol **7** and vellerol **8**[1]. As the antimicrobial activities of **7** and **8** are less than one tenth of those of **5** and **6**[1], these mechanisms ensure that a relatively high concentration of biologically active unsaturated dialdehydes, also possessing various antifeedant activities[6], is maintained in the mushroom a short time after an injury.



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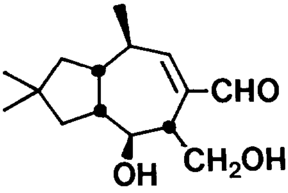

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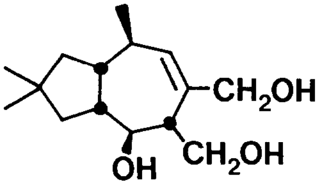

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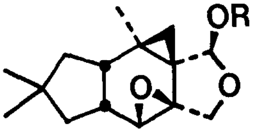
Similar analyses of other pungent Russulaceae species, made at different times after injury (grinding) by the same procedure as in the investigation with *L. vellereus*[1], showed that the velutinal esters **4b** and **4c** are converted to different sesquiterpenes in different species. In this paper we wish to report the sesquiterpenes isolated from extracts of ground Lactarius piperatus, L. torminosus and Russula queletii. In analogy with L. vellereus[1], all three species could be shown not to contain any sesquiterpene in significant amounts besides velutinal (as the stearic acid ester **4b**). When the mushrooms were ground, the new pungent unsaturated dialdehyde piperdial[7] **1** could be isolated from hexane and EtOAc extracts made within few minutes after grinding, but if the time between grinding and extraction exceeded 10 minutes only very small amounts of **1** were obtained. Piperdial **1** is a labile compound: in the presence of traces of acid (e.g. from silica gel) **1** rapidly dehydrates to velleral **6**, and upon contact with alumina, **1** is immediately degraded (as velleral **5**[1]) to as yet unidentified products. From extracts made more than 10 minutes after grinding, the C-5 reduced derivative piperalol[7] **2** could be isolated in considerable amounts. Both **1** and **2** were reduced by KBH_4 in EtOH to the same compound, pipertriol[7] **3**, and NMR experiments with piperalol **2** suggest that C(3)H and C(8)H, as well as C(7)H and C(9)H, are closely situated (NOE: 12 and 4 %, respectively). Preliminary assays indicate that the antibacterial activities of **1** and **2** are comparable with those of velleral **6** and vellerol **8**, respectively.

All three species were also found to produce velleral **6** and vellerol **8**, in analogy with L. vellereus, although no traces of isovelleral **5** or isovellerol **7** could be detected. In addition, the secolactarane lactardial **9**[7] could be isolated from all three species in amounts comparable to piperdial **1** and velleral **6**. The corresponding reduced lactarol **10** could also be isolated, although only in small amounts and in extracts made more than 30 minutes after grinding. Lactardial **9**, which is pungent but considerably less antibacterial than for instance velleral **6**[6], was originally obtained together with the corresponding furan lactaral **11**[5] as a degradation product of the velutinal derivatives **4a–c**[4]. Lactarol **10** is the KBH_4 reduction product of both **9** and **11**[5,8], but small amounts of **10** are also obtained when the velutinal esters **4b** and **4c** are degraded by adsorption on alumina[1]. The possibility that the lactardial **9** and lactarol **10** isolated from extracts of injured Russulaceae species are artifacts formed by degradation of the velutinal esters must therefore be considered. However, several observations suggest that this is not the case for the lactardial **9** and lactarol **10** isolated in this investigation. For example, pungency such as that of lactardial **9**, has been suggested to be significant for the deterrent activity of these species[2]. Lactardial **9** and lactarol **10** have not been identified in extracts of all Russulaceae species containing velutinal esters (for example L. vellereus), which suggests that they are formed by specific enzymatic processes in the three species investigated here. Furthermore, of the numerous other products always formed in addition to lactardial **9** and lacarol **10** by the degradation of velutinal derivatives[1,5], only small amounts of the furandiol **12** could be identified in the extracts of L. piperatus, L. torminosus and R. queletii. However, in extracts made more than 30 minutes after grinding, small or trace amounts of several as yet unidentified furanoid sesquiterpenes were indicated. The identity and origin of these furans, which have not been observed in degradation experiments in vitro[1,5], will be the subject of further investigations. None of the sesquiterpene lactones previously reported from L. torminosus[9] could be detected.



a: R = H

b: R = $CO(CH_2)_{16}CH_3$

c: R = $CO(CH_2)_4CO(CH_2)_{11}CH_3$

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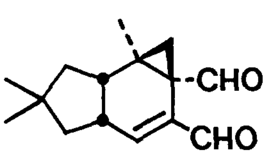

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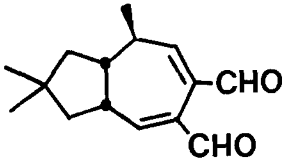

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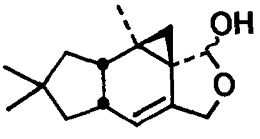

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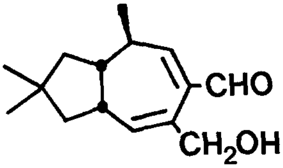

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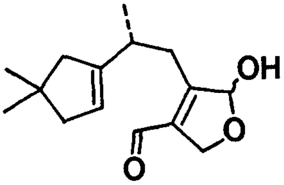

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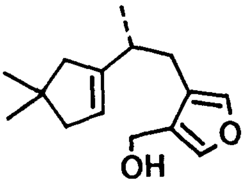

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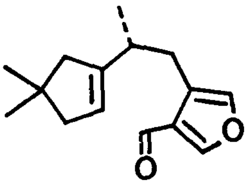

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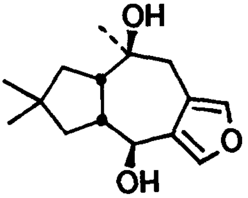

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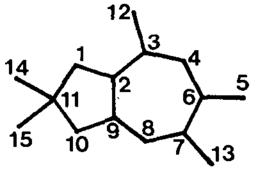

The lactarane skeleton

The combination of pungent sesquiterpenes piperdial 1, velleral 6 and lactardial 9, has also been indicated for a number of other Russulaceae species by tlc and ^1H NMR analysis of crude extracts. Yet other Russulaceae species instead form only isovelleral 5 and velleral 6, while for instance L. quietus appears to produce only isovelleral 5 as a pungent principle. Interestingly, preliminary results indicate that ground L. necator produces the C-7 epimers of piperdial 1 and piperalol 2. So far, the pattern of pungent sesquiterpenes produced by one species does not appear to vary from year to year or between different places of growth, although this has not been investigated in detail. It may therefore be possible to use the pattern of pungent sesquiterpenes produced as a response to injury as a character in chemotaxonomic studies.

1,2,3,3a,4,5,8,8a-Octahydro-4-hydroxy-2,2,8-trimethyl-azulene-5,6-dicarbaldehyde 1 (piperdial) was obtained as a colourless oil from EtOAC extracts of L. piperatus and L. torminosus by SiO_2 flash chromatography. Insufficient amounts were obtained for elemental analysis and ^{13}C NMR. $[\alpha]_D^{23}$ = +77° (c 0.8 in diethylether); MS, m/e (rel. int.) 250 (M^+ 1%), 232 (4%), 217 (3%), 204 (23%), 91 (45%), 55 (67%), 41 (100%); UV (ethanol): 232 nm (9300); IR (KBr): 3450, 2960, 1730 and 1690 cm^{-1}. ^1H NMR[10] 9.70 and 9.41, s, C(5)H and C(13)H; 6.68, d, C(4)H, J_{3-4}=5.0; 4.35, ddd, C(8)H, J_{7-8}=9.4, J_{8-9}=9, J_{8-OH}=3; 3.77, d, C(7)H, J_{7-8}=9.4; 2.52, m, C(3)H; 2.45, d, OH, J_{8-OH}=3; 2.23, m, C(9)H and C(2)H; 1.77, dd, C(1)Ha (or C(10)Ha), J=13, J=7; 1.60, m, C(10)Ha (or C(1)Ha; 1.48, dd, C(1)Hb (or C(10)Hb), J=7.9, J=13; 1.21, m, C(10)Hb (or C(1)Hb; 1.12, d, C(12)H_3, J_{3-12}=6.5; 1.09 and 0.93, s, C(14)H_3 and C(15)H_3.

1,2,3,3a,4,5,8,8a-Octahydro-4-hydroxy-5α-hydroxy-methyl-2,2,8-trimethyl-azulene-6-carbaldehyde **2** (piperalol) was obtained as a colourless oil from EtOAc extracts of *L. piperatus* and *L. torminosus* by SiO_2 and Al_2O_3 chromatography. $[\alpha]_D^{23}$ = +57° (c 4.3 in diethylether); Elemental analysis: Calculated for $C_{15}H_{24}O_3$: C 71.4, H 9.59; Found C 71.1, H 9.41; MS, m/e (rel. int.) 234 (M^+-18 7%), 219 (3%), 204 (15%), 109 (35%), 81 (48%), 55 (65%), 41 (100%); UV (ethanol): 238 nm (9400); IR (KBr): 3380, 2960 and 1680 cm^{-1}. ^1H NMR[10] 9.30, s, C(5)H; 6.52, d, C(4)H, J_{3-4}=5.4; 4.12, dd, C(8)H, J_{7-8}=8.6, J_{8-9}=10.1; 3.85, dd, C(13)Ha, J_{7-13a}=3.6, $J_{13a-13b}$=10.4; 3.59, dd, C(13)Hb, J_{7-13b}=7.2, $J_{13a-13b}$=10.4; 2.88, ddd, C(7)H, J_{7-8}=8.6, J_{7-13a}=3.6, J_{7-13b}=7.2; 2.61, m, C(3)H; 2.17, m, C(2)H and C(9)H; 1.73, dd, C(1)Ha or C(10)Ha; 1.55, m, C(1)Hb and C(10)Ha, or C(10)Hb and C(1)Ha; 1.21, m, C(1)Hb or C(10)Hb; 1.10, d, C(12)H_3, J_{3-12}=9.7; 1.08 and 0.92, s, C(14)H_3 and C(15)H_3. 91 MHz ^{13}C NMR ($CDCl_3$) ppm (TMS): 194.3 C(5); 162.5 C(4); 140.3 C(6); 74.3 C(8); 65.0 C(13); 48.4, 47.4 and 43.4 C(2), C(7) and C(9); 46.4 and 45.4 C(1) and C(10); 37.4 C(11); 33.8 C(3); 29.6 and 26.9 C(14) and C(15); 19.6 C(12).

Pipertriol **3** was obtained as a white crystalline solid, mp 158-160°C by KBH_4 reduction of **1** and **2** in EtOH. $[\alpha]_D^{24}$ = +62° (c 0.2 in methanol). MS, m/e (rel. int.): 236 (M^+-18 12%), 206 (45%), 175 (57%), 153 (100%), 123 (66%), 109 (47%), 95 (100%), 81 (56%), 69 (40%), 55 (44%) and 43 (40%); UV (ethanol): no maximum above 220 nm; IR (KBr): 3200, 2940, 1060, 1030 and 1000 cm^{-1}. ^1H NMR[10] 5.46, d, C(4)H, J_{3-4}=5.4; 3.6-4.1, m, C(5)H_2, C(7)H, C(8)H and C(13)H_2; 3.37, m, C(3)H; 2.22 and 2.10, m, C(2)H and C(9)H; 1.67, d, C(1)H_2 (or C(10)H_2), J_{1-2}=7.9; 1.53, dd, C(10)Ha (or C(1)Ha), J_{9-10a}=6.5, $J_{10a-10b}$=11.9; 1.20, dd, C(10)Hb (or C(1)Hb), J_{9-10b}=11.9, $J_{10a-10b}$=11.9; 1.10 and 0.98, s, C(14)H_3 and C(15)H_3; 0.95, d, C(12)H_3, J_{3-12}=6.8.

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10. 360 MHz PMR ($CDCl_3$): ppm (TMS), multiplicity, J (Hz).

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