Tetrahedron Letters,Vol.24,No.13,pp 1415-1418,1983 0040-4039/83/131415-04$03.00/0
Printed in Great Britain

VELUTINAL ESTERS OF LACTARIUS VELLEREUS AND L. NECATOR. THE PREPARATION OF FREE VELUTINAL.

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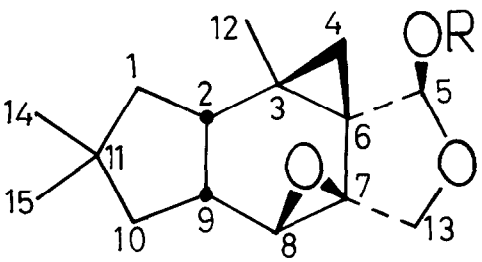
Abstract: Esters of the pentacyclic sesquiterpene velutinal have been isolated from Lactarius species, and converted to the free alcohol by base-catalyzed transesterfication in EtOH/EtO$^-$.

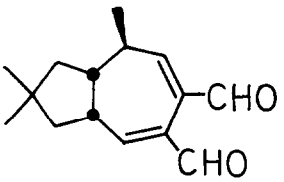
A considerable number[1] of presumably humulene derived sesquiterpenes with the lactarane (e.g. 2), marasmane (e.g. 3) and secolactarane (e.g. 4) skeletons has been obtained from Lactarius and Russula species. Recent reports indicate however that the only sesquiterpenoid originally present in L. velutinus is a very labile stearic ester, stearylvelutinal 1a[2a,b], which easily solvolyses to a number of previously isolated fungal sesquiterpenes (e.g. 4 and 7)[3].



1a R = Stearyl
1b R = 6-Ketostearyl
1c R = H
1d R = Me

Early work in this[4] and other laboratories[5] indicated that the choice of extraction method may be critical, inasmuch as the very pungent taste of L. vellereus is rapidly lost on extraction with ethanol or if the mushroom is frozen before extraction. The fact that the two pungent-tasting aldehydes 2[6] and 3[7] and the lactones 5[8] and 6[8] can be extracted from L. vellereus with hexane, but that extraction with alcohols rather yields the alcohol 7 and the ethers 8a (with MeOH) or 8b (with EtOH), led us to point out that the latter may be formed by enzymatic or other possible chemical transformations during the extraction[4]. More recently, we have found that a lipid fraction from L. vellereus, which had been freed from furanoid sesquiterpenes (NMR), afforded stearic acid and the furanoalcohols 7 and 8a as the main products upon heating in methanol. The apparent

risk of artifact formation during work-up is especially serious when chemotaxonomic conclusions are drawn from reported sesquiterpene patterns in the genus Lactarius[9]. Using the hexane extraction method, we have thus isolated velleral (2)[6] and isovelleral (3)[7] not only from L. vellereus and L. piperatus[10], but we have obtained both compounds also from L. rufus[13] and velleral (2) from L. torminosus[13], two species belonging to different sections of genus Lactarius and believed to be devoid of these aldehydes[9].



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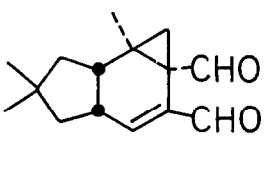

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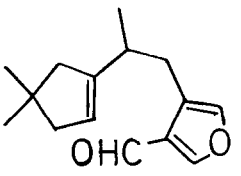

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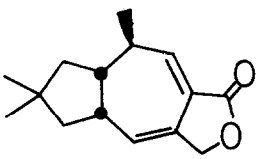

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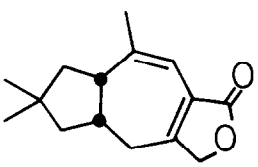

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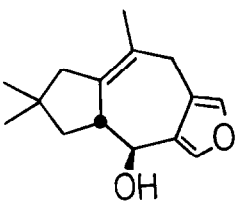

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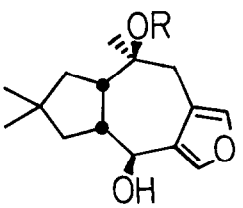

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8a R = Me
8b R = Et

The recent reports on velutinal esters[2,3] promt us to report on similar investigations which are underway in this laboratory. Stearylvelutinal (1a) was obtained from L. vellereus by extraction with hexane[6] in the cold (0°) and subsequent chromatography on SiO_2 and Al_2O_3. L. necator, on similar extraction with ethyl acetate, gave the lactarinic ester 1b as the main component, accompanied by small amounts of 1a. Catalytic transesterfication of 1a or 1b in EtOH/EtO^- afforded free velutinal 1c. In HPLC grade methanol, 1b solvolyses, in analogy with 1a[2,3] to give the methyl acetal 1d, while in reagent grade methanol or on prolonged contact with silica gel, 1a-d decompose to give different patterns of sesquiterpenes (cf. Ref.[2,3]). This work will be described in a separate paper.

When L. vellereus, L. necator or L. rufus were frozen quickly by immersion into liquid nitrogen at the collection site and extracted with hexane at below -20°, only velutinal esters and and no free sesquiterpenes were discernible chromatographically. This is in conformity with reports on other Lactarius sp.[2a,b]. When L. vellereus frozen as stated above and macerated in hexane, was allowed to warm up gradually, the aldehydes 2 and 3 appeared at -2° as the first free sesquiterpenes. It is also significant, that when a slurry of fresh L. vellereus was pre-

pared in hexane at 0°, the aldehydes 2 and 3 initially appeared in about equal concentrations, but that (2) disappeared within ca 15 min, while significant amounts of 3 remained even after 1 hr. Since both 2 and 3 are quite stable at that temperature in a wide varity of solvents and neither of them has been observed as solvolysis products from velutinal derivatives in vitro, the appearance and disappearance of 2 and 3 in the experiments above are most probably due to enzymatic reactions. Both 2 and 3 exhibit antibiotic activities[11], and 3 is a strong direct mutagen on Salmonella in Ames´test[12], while 1a is not. There is a fascinating possibility that 1a, 2 and 3 are involved in a chemical defense mechanism, in the sense that 2 and 3 are released very rapidly on attack by a parasite, but are then rendered harmless to the fungus itself by further degradation.

6-Ketostearylvelutinal[14], 1b, was obtained as a colourless oil from an EtOAc extract of L. necator by SiO_2 and Al_2O_3 chromatography. α_D^{26} = +54.8° (c 2.4 in diethylether); UV (hexane): no maximum above 210 nm; IR (neat): 1720 and 1740 (C=O). 360 MHz ^{1}H NMR ($CDCl_3$) ppm (TMS), multiplicity, J (Hz): 6.24, s, C(5)H; 4.25, d, C(13)H_a, $J_{13a-13b}$=10.1; 4.15, d, C(13)H_b, $J_{13a-13b}$=10.1; 2.81, d, C(8)H, J_{8-9}=0.9; 2.33-2.45, m, C(2´)H_2, C(5´)H_2 and C(7´)H_2; 2.22, ddd, C(2)H, J_{1a-2}= 6.2, J_{1b-2}=13, J_{2-9}=6.2; 1.90, m, C(9)H; 1.81, dd, C(10)H_a, J_{9-10a}=8.0, $J_{10a-10b}$= 13.8; 1.50-1.69, m, C(3´)H_2, C(4´)H_2, C(8´)H_2, C(1)H_a and C(10)H_b; 1.24-1.28, m, C(9´)H_2-C(17´)H_2; 1.22, s, C(12)H_3; 1.06, dd, C(1)H_b, J_{1a-1b}=13, J_{1b-2}=13; 1.06 and 1.04, s, C(14)H_3 and C(15)H_3; 0.88, t, C(18´)H_3, $J_{17´-18´}$=6.8; 0.86, d, C(4)H_a, J_{4a-4b}=5.2; 0.48, d, C(4)H_b, J_{4a-4b}=5.2. 91 MHz ^{13}C NMR ($CDCl_3$) ppm (TMS): 208.3 C(6´); 172.9 C(1´); 99.6 C(5); 69.8 C(13); 65.5 C(7); 58.4 C(8); 46.4 and 45.8 C(1) and C(10); 43.3 and 38.6 C(2) and C(9); 42.9 and 42.2 C(5´) and C(7´); 36.8 C(11); 34.2 C(2´); 31.9 C(16´); 31.8 and 31.6 C(14) and C(15); 31.2 C(6); 29.6-29.2 C(9´)-C(15´); 24.9 C(3); 24.3, 23.9, 23.1 and 22.7 C(3´), C(4´), C(8´) and C(17´); 20.4 C(12); 17.5 C(4); 14.1 C(18´). Primed numbers refers to 6-ketostearyl carbons.

Velutinal[14], 1c, was obtained as a colourless oil by ethanolysis at 25° of 1a or 1b in 1 mM NaOEt/EtOH and rapid chromatography on prewashed SiO_2 and Al_2O_3. α_D^{26}= +39.6° (c 1.9 in diethylether); MS 70 eV, m/e (rel. int.): 250 (M^+ 32%), 232 (64%), 217 (50%), 203 (48%), 135 (50%), 123 (100%); UV (hexane): no maximum above 210 nm; IR (neat): 3420 (OH). 360 MHz ^{1}H NMR ($CDCl_3$) ppm (TMS), multplicity, J (Hz): 5.28, d, C(5)H, J_{5-OH}=6.7; 4.25, d, C(13)H_a, $J_{13a-13b}$=10.1; 4.10, d, C(13)H_b, $J_{13a-13b}$=10.1; 2.90, d, OH, J_{5-OH}=6.7; 2.78, s, C(8)H; 2.23, ddd, C(2)H, J_{1a-2}= 6.5, J_{1b-2}=12, J_{2-9}=6.5; 1.92, m, C(9)H; 1.80, dd, C(10)H_a, J_{9-10a}=8.3, $J_{10a-10b}$= 13.7; 1.64, dd, C(1)H_a, J_{1a-1b}=12.0, J_{1a-2}=6.5; 1.58, dd, C(10)H_b, J_{9-10b}=1.3, $J_{10a-10b}$=13.7; 1.16, s, C(12)H_3; 1.05, dd, C(1)H_b, J_{1a-1b}=12, J_{1b-2}=12; 1.04 and 1.03, s, C(14)H_3 and C(15)H_3; 0.83, d, C(4)H_a, J_{4a-4b}=5.0; 0.56, d, C(4)H_b, J_{4a-4b}=5.0. 91 MHz ^{13}C NMR ($CDCl_3$) ppm (TMS): 99.3 C(5); 68.2 C(13); 66.4 C(7);

58.2 C(8); 46.4 and 45.8 C(1) and C(10); 43.4 and 38.6 C(2) and C(9); 36.7 C(11); 32.0 C(6); 31.7 and 31.6 C(14) and C(15); 23.8 C(3); 20.3 C(12); 17.5 C(4).

Acknowledgement

This work was supported by the Swedish Natural Research Council, grant No. K-KU 2070-104 and by the International Seminar for Chemistry, Uppsala.

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13. Unpublished results.
14. Both compounds gave satisfactory elemental analysis.

(Received in UK 20 January 1983)