Pergamon 0031-9422(95)00775-X Phytochemistry, Vol. 41, No. 4, pp. 1093–1096, 1996

0031-9422/96 $15.00 + 0.00

MONOHYDROXYLACTONES OF *LACTARIUS VELLEREUS*

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(*Received* 22 *June* 1995)

Key Word Index—*Lactarius vellereus*; Basidiomycetes; fruiting body; marasmane sesquiterpenoic lactones; structural elucidation; crystal structure.

Abstract—Reinvestigation of the monohydroxylactone fraction of an ethanolic extract of *Lactarius vellereus* resulted in the isolation of four new monohydroxylactones with the marasmane skeleton. The structures were elucidated by extensive NMR investigations and in one case confirmed by X-ray measurements.

INTRODUCTION

The chemical defence mechanism of hot tasting mushrooms involves an enzymic transformation of the natural precursor, existing in their tissues, into a series of hot tasting antifeedant sesquiterpenoids [1–4]. The fact that this process is an enzymic one is not doubted and our present paper supports this view. Investigations of several species of *Lactarius* revealed that in all of them the precursor (velutinal, **1**) which possesses the marasmane skeleton is quickly transformed into sesquiterpenes with the lactarane skeleton. However, although *L. vellereus* has such an enzymic system, in ethanol several lactones with the unchanged marasmane skeleton can be isolated [5–9]. Bearing this in mind we decided to reinvestigate the monohydroxylactone fraction of the ethanolic extract of *L. vellereus*, and in addition to the monohydroxylactones with the lactarane skeleton, four new marasmanolides were isolated.

RESULTS AND DISCUSSION

All the new compounds (**2–5**) exhibited some common features: their molecular formula, established by HR mass spectrometry, was $C_{15}H_{20}O_3$. Their IR spectra showed the same hydroxylic, carbonyl lactonic and C=C bands. Also, their UV spectra had the same absorption characteristic for a cross conjugated carbonyl-*gem*-cyclopropane double bond system. The ^{1}H NMR spectra of the compounds showed common features such as pairs of doublets of protons (H-4), lactonic methylenes (H-13, AB*q*) and methyl singlets (δ 1.5) at H-12 characteristic of the marasmane skeleton. The additional common feature was the presence of a vinyl proton absorption which showed allylic coupling with the H-13 methylene. All the data allowed assignment, of the marasmane skeleton, for compounds **2–5** and, therefore, they differed in the position of a hydroxyl group. Compounds **2** and **3** when acetylated in the cold to give monoacetyl derivatives **2a** and **3a**, respectively, whereas compounds **4** and **5** failed to yield acetates. The ^{1}H NMR spectrum of **2** exhibited, instead of the usual three signals (singlets) for the methyl groups belonging to the marasmane skeleton, only two singlets and a diprotonic signal of a hydroxymethylene (δ 3.34 *dd*). The last signal was shifted downfield by 0.5 ppm in the spectrum of its acetyl derivative **2a**. The ^{13}C NMR spectrum of **2** exhibited a triplet for a hydroxymethylene carbon at δ 71.8. Therefore **2** was assumed to be a 14-hydroxylated derivative as one of the geminal methyls, i.e., from the convex side of the molecule, was oxidized, as was found previously [10]. A NOE experiment confirmed this assumption because irradiation of the H-14 signal enhanced the signals of H-1α, H-10α and H-15. Shifts of signals of other protons were substantiated by decoupling and by ^{1}H–^{1}H and ^{1}H–^{13}C NMR correlation spectra and are all presented in Table 1.

The ^{1}H NMR spectrum of **3** exhibited signals of three methyl protons characteristic of the marasmane skeleton, and one proton doublet at δ 3.61, which was shifted downfield in the ^{1}H NMR spectrum of **3a**. This was the signal of the proton attached to the carbon bearing a secondary hydroxyl group. A signal for a similar carbon also existed in ^{13}C NMR spectrum of **3**, which was additionally confirmed by a ^{1}H–^{13}C NMR correlation spectrum. Irradiation of the signal of H-8 allowed identification of the signal of H-9α and irradiation simplified the signal at δ 3.61, thus allowing location of the secondary hydroxyl group at C-9 and not at C-1. Its stereochemistry followed from the small coupling constant ($J_{H9\alpha,10\alpha}$ = 2 Hz) and the NOE experiment where irradiation of H-10α enhanced the signals of H-9α, H-2α

Table 1. ^{1}H NMR spectra of **2**, **2a**, **3**, **3a**, **3u**, **4** and **5** (500 MHz, $CDCl_3$, TMS as int. standard)

Site	2	2a	3	3a	3u	4	5
1a	1.82 (*ddd*)	1.80 (*ddd*)	1.68 (*ddd*)	1.70 (*ddd*)	1.76 (*ddd*)	1.75 (*dd*)	1.71 (*dd*)
1b	1.07 (*t*)	1.13 (*t*)	1.25 (*t*)	1.28 (*t*)	1.33 (*t*)	1.03 (*t*)	1.58 (*d*)
2a	2.45 (*dt*)	2.47 (*dt*)	2.83 (*dt*)	2.78 (*dt*)	2.85 (*dt*)	2.45 (*dd*)	—
4a	1.61 (*d*)	1.60 (*d*)	1.48 (*d*)	1.49 (*d*)	1.56 (*d*)	2.00 (*d*)	1.74 (*d*)
4b	1.42 (*d*)	1.43 (*d*)	1.44 (*d*)	1.45 (*d*)	1.47 (*d*)	1.59 (*d*)	1.46 (*d*)
8	5.06 (*q*)	5.05 (*q*)	5.11 (*q*)	5.19 (*q*)	5.19 (*q*)	5.29 (*t*)	5.18 (*t*)
9a	2.54 (*m*)	2.54 (*m*)	2.43 (*m*)	2.43 (*m*)	2.59 (*m*)	—	2.39 (*m*)
10a	1.93 (*dd*)	1.92 (*dd*)	3.61 (*d*)	4.67 (*d*)	4.77 (*d*)	1.83 (ABq)	2.21 (*dd*)
10b	1.35 (*dd*)	1.40 (*dd*)	—	—	—	1.88 (ABq)	1.47 (*d*)
12	1.48 (*s*)	1.48 (*s*)	1.46 (*s*)	1.49 (*s*)	1.49 (*s*)	1.49 (*s*)	1.56 (*s*)
13a	4.89	4.88	4.87	4.89	4.90	4.92 (ABq)	4.95 (ABq)
13b	4.94	4.94	4.94	4.95	4.96	4.99 (ABq)	4.85 (ABq)
14	3.34 (*dd*)	3.82 (*dd*)	1.05 (*s*)	1.06 (*s*)	1.10 (*s*)	1.11 (*s*)	1.18 (*s*)
15	1.00 (*s*)	1.02 (*s*)	1.00 (*s*)	1.02 (*s*)	1.12 (*s*)	0.92 (*s*)	1.01 (*s*)
Ac	—	2.09 (*s*)	—	2.06 (*s*)	NH 8.30 (*s*)	—	—

J (Hz) – **2**: 1α,1β = 13.0; 1α,2α = 7.2; 1β,2α = 12.7; 4α,4β = 3.8; 8,13a = 1.8; 8,13b = 2.5; 8,9α = 2.1; 9α,10α = 7.6; 9α,10β = 13.4; 10α,10β = 13.4; 13a,13b = 17.6 (ABq).

2a: 1α,1β = 13.1; 1α,2α = 7.2; 1β,2α = 12.8; 4α,4β = 3.8; 8,13a = 1.8; 8,13b = 2.5; 8,9α = 2.0; 9α,10α = 7.6; 9α,10β = 13.5; 10α,10β = 13.5; 13a,13b = 16.6 (ABq). 9α,13a = 3.1; 9α,13b = 4.4. NOE: irradiation of H-14 enhanced 1α,10α and 15.

3: 1α,1β = 12.6; 1α,2α = 6.9; 1β,2α = 12.7; 4α,4β = 3.9; 8,13a = 1.8; 8,13b = 2.5; 9α,10α = 1.2; 9α,13a = 3.2; 9α,13b = 4.5; 13a,13b = 13.3(ABq).

3a: 1α,1β = 13.7; 1α,2α = 7.1; 1β,2α = 12.7; 4α,4β = 3.7; 8,13a = 1.7; 8,13b = 1.5; 9α,10α = 2.0; 9α,13a = 3.1; 9α,13b = 4.6; 1313a,13b = 16.6 (ABq). 9α,13a = 3.1; 9α,13b = 4.4. NOE: irradiation of H-10α enhanced 9α,2α and 1α.

3u: 1α,1β = 12.7; 1α,2α = 6.9; 1β,2α = 12.7; 4α,4β = 4.0; 8,13a = 1.8; 8, 13b = 2.5; 9α,10α = 1.2; 9α,13a = 3.2; 9α,13b = 4.5; 13a,13b = 13.3 (ABq).

4: 1α, 1β = 13.1; 1α,2α = 7.5; 1β,2α = 13.0; 4α,4β = 3.2; 8,13a = 1.8; 8,13b = 2.4; 10α,10β = 13.3 (ABq); 13a,13b = 13.6 (ABq). NOE: irradiation of 2α enhanced 10α and 1α.

5: 1α,1β = 14.0; 1α,9α = 1.7; 4α,4β = 4.0; 8,13a = 1.8; 8,13b = 2.4; 10α,10β = 13.0; 13a,13b = 13.3 (ABq). NOESSY confirmed the stereochemistry.

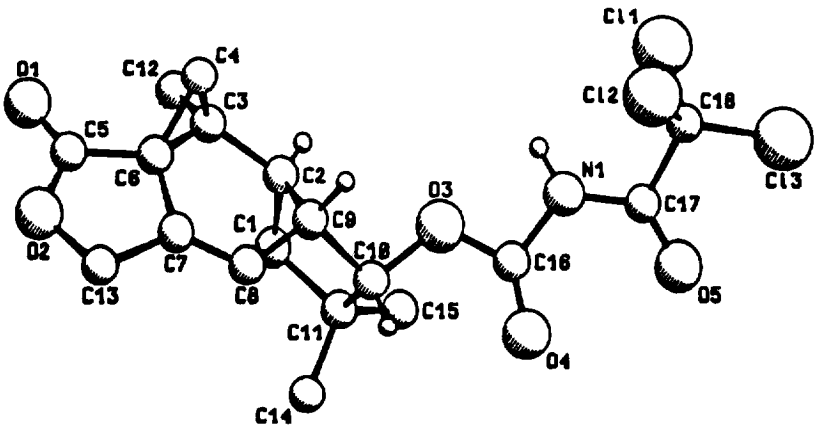


Fig. 1. Three-dimensional structure of **3u**. Crystal data: $C_{18}H_{20}O_5NCl_3$: $M_r = 436.72$, orthorhombic, space group $P2_12_12_1$, cell constants $a = 10.3101(7)$, $b = 11.465(2)$, $c = 16.580(1)$ Å; $V = 1959.8(4)$ Å^3; $Z = 4$; $d_x = 1.48$ g cm^{-3}; $F(OOO) = 904$; The final $R = 0.066$, $R_W = 0.086$ [$W = 1/(\sigma^2 + 0.0005\ F^2)$]. X-ray data are deposited at the Cambridge Crystallographic Data Centre.

and H-1α. It has been assumed that in all the compounds the six- and five-membered ring junction was *cis*. Since **3** reacted with trichloroacetyl isocyanate to give a crystalline derivative, X-ray analysis was undertaken and confirmed our structural assignments (Fig. 1).

Compounds **4** and **5** failed to produce acetyl derivatives. Their ^{13}C NMR spectra showed the presence of quaternary carbons substituted with hydroxyls in their molecules (Table 2). The ^{1}H NMR spectra of both **4** and **5** exhibited signals of protons at C-4 which, together with

Table 2. ^{13}C (125.7 MHz) NMR spectra of **2**, **2a**, **3**, **4** and **5**

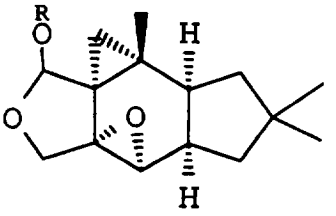
Site	2	2a	3	4	5
1	38.8 *t*	39.4 *t*	43.7 *t*	44.6 *t*	45.5 *t*
2	43.0 *s*	43.0 *s*	39.5 *s*	47.4 *d*	86.1 *s*
3	33.5 *s*	33.3 *s*	32.2 *s*	32.9 *s*	31.5 *s*
4	30.7 *t*	30.7 *t*	31.7 *t*	36.2 *t*	36.2 *t*
5	177.7 *s*	177.5 *s*	177.6 *s*	177.1 *s*	176.9 *s*
6	27.5 *s*	27.6 *s*	27.8 *s*	28.4 *s*	37.7 *s*
7	133.6 *s*	134.2 *s*	134.1 *s*	140.6 *s*	141.3 *s*
8	117.7 *d*	117.5 *d*	120.3 *d*	117.6 *d*	119.9 *d*
9	38.2 *d*	38.2 *d*	47.3 *d*	79.9 *s*	47.2 *s*
10	42.5 *t*	42.9 *t*	87.1 *d*	58.1 *t*	50.9 *t*
11	40.6 *s*	40.7 *s*	41.7 *s*	35.6 *s*	37.3 *s*
12	16.8 *q*	16.8 *q*	16.6 *q*	17.3 *q*	13.3 *q*
13	69.1 *t*	69.1 *t*	69.1 *t*	68.3 *t*	72.9 *t*
14	71.8 *t*	72.6 *t*	23.6 *q*	32.5 *q*	33.2 *q*
15	26.6 *q*	27.1 *q*	30.0 *q*	31.3 *q*	33.5 *q*
Ac	—	20.9 *q*, 171.4 *s*	—	—	—

the UV spectral data, confirmed their marasmane skeleton. Therefore, the quaternary hydroxyl groups had to be attached to carbons C-2 or C-9. The distinction was accomplished by decoupling experiments and ^1H–^1H NMR correlation spectra. When the signal at δ 5.29 (H-8 vinyl proton) was irradiated only the signals of the AB*q* of H-13 (allylic coupling) were simplified. Therefore, in the molecule of **4** the hydroxyl group is located at C-9. Conversely, irradiation of the signal at δ 5.18 in the spectrum of **5** simplified the signals of H-13 (AB*q*) and the signal of the angular proton at C-9, thus allowing location of the hydroxyl group in the molecule of **5** at C-2. A pattern of allylic H-13, H-8 and homoallylic H-13, H-9α couplings was observed in the ^1H NMR spectra of **2**, **2a**, **3**, **3a** and **5**, whereas in the spectrum of **4** only allylic coupling was exhibited. All the ^1H and ^{13}C NMR data unequivocally supported the structures **2**–**5** and are presented in Tables 1 and 2.

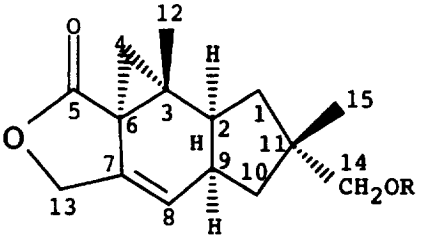
EXPERIMENTAL

Lactarius vellereus was collected in October 1993 in Zalesie mixed forest near Warsaw and was authenticated by the mycologist Prof. A Skirgiełło (Warsaw University). A specimen (Voucher no. 32660) is deposited at the Department of Systematics and Geography of Plants, University of Warsaw.

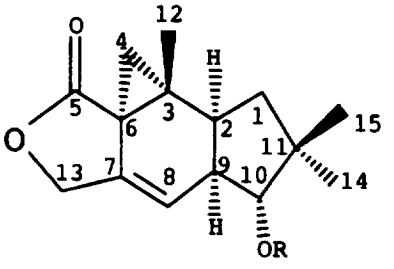
Isolation of compounds **2**, **3**, **4** *and* **5**. Prepn of an EtOH extract of *L. vellereus*, its purification and coarse chromatography are as reported previously [10]. The fr. containing monohydroxylactones [TLC: R_f 0.3–0.5, C_6H_6–Me_2CO (4:1)] was collected and the residue (2.2 g) obtained after evapn of the solvent was rechromatographed on silica gel using a hexane–EtOAc gradient (20–30%) system. The chromatography was monitored by TLC (hexane–EtOAc, 7:3) and 3 frs were obtained: 1 (R_f 0.41–0.87, 38 mg), 2 (R_f 0.16–0.45, 1477 mg), 3 (R_f 0.05–0.15, 597 mg). Fr. 3 was by sepd HPLC using an efficient column (20 × 300 mm) filled



1 R=stearate (Velutinal)

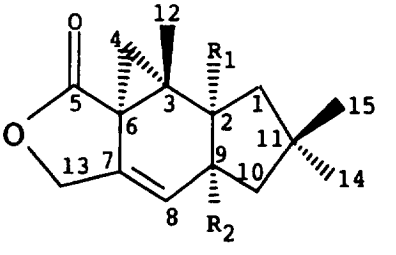


2 R=H, **2a** R=Ac



3 R=H, **3a** R=Ac

3u R=OCNHCOCCl$_3$



4 R_1=H, R_2=OH

5 R_1=OH, R_2=H

with LiChrosorb (10 μm), which was eluted with hexane–EtOAc (4:1). The K' values reported below were obtained first using an analyt. column eluted with hexane–EtOAc (41:9). The following compounds were obtained.

13,14-*Dihydroxy-marasm*-7(8)-*en*-5-*oic acid* γ-*lactone* (**1**). Compound **1** (120 mg, $K' = 15.5$) obtained as oil; $[\alpha]_D^{20} = +83.2°$ ($CHCl_3$, *c* 1.17); UV λ_{max} nm: 229.7 (ε 3240), 286.6 (ε 396); IR ν_{max}^{film} cm^{-1}: 3440, 1766, 1689. HRMS: $[M]^+$ 248.14059, calc. for $C_{15}H_{20}O_3$ $[M]^+$ 248.14124. MS 70 eV *m/z* (rel.int): 248 $[M]^+$ (4), 230 (47), 215 (31), 201 (85), 185 (20), 171 (26), 157 (100), 143 (35), 129 (32), 120 (23), 105 (24), 91 (28), 77 (12), 65 (4), 55 (7), 43 (10). 171 (26), 157 (100), 143 (35), 129 (32), 120 (23), 105 (24), 91 (28), 77 (12), 65 (4), 55 (7), 43 (10).

14-*Acetoxy*-13-*hydroxy-marasm*-7(8)-*en*-5-*oic acid* γ-*lactone* (**2a**). Compound **2a** was prepd by standard acetylation of **2**. Oil; $[\alpha]_D^{20} = +64.4°$ ($CHCl_3$, *c* 1.3); IR ν_{max}^{film} cm^{-1}: 1768, 1737, HRMS: $[M]^+$ 290.15145, calc. for $C_{17}H_{22}O_4$ 290.15181. MS 70 eV, *m/z* (rel. int.): 290 $[M]^+$ (60), 248 (16), 230 (94), 215 (64), 201 (100), 185 (43), 172 (42), 157 (97), 143 (58), 129 (49), 120 (77), 115 (27), 111 (23), 105 (43), 91 (49), 77 (29), 69 (25), 55 (27), 43 (98).

10β,13-*Dihydroxy-marasm*-7(8)-*en*-5-*oic acid* γ-*lactone* (**3**). Compound **3** (158 mg, $K' = 17.8$) obtained as oil; $[\alpha]_D^{20} = +61.8°$ ($CHCl_3$, *c* 1.57) ; UV λ_{max} nm: 229.7 (ε 2195) , 282.4 (ε 151) ; IR ν_{max}^{film} cm^{-1}: 3452, 1767, 1692. HRMS: $[M]^+$ 248.14171, calc. for $C_{15}H_{20}O_3$ $[M]^+$ 248.14124. MS 70 eV *m/z* (rel.int.): 248 $[M]^+$ (100), 233 (40), 230 (14), 215 (16), 192 (30), 187 (25), 177 (82), 163 (45), 147 (26), 138 (32), 131 (25), 119 (31), 91 (400), 77 (23), 72 (16), 65 (10), 53 (10), 43 (25).

10β-*Acetoxy*-13-*hydroxy-marasm*-7(8)-*en*-5-*oic acid* γ-*lactone* (**2a**). Compound **2a** was obtained by acetylation of **3** under standard conditions. Oil; $[\alpha]_D^{20} = +29.5$ ($CHCl_3$, *c* 1.1); IR ν_{max}^{film} cm^{-1}: 1769, 1734, HRMS: $[M]^+$ 290.15146, calc. for $C_{17}H_{22}O_4$ 290.15181. MS 70 eV *m/z* (rel. int.): 290 $[M]^+$ (1), 248 (21), 230 (94), 215 (87), 201 (46), 187 (30), 171 (47), 157 (47), 143 (32), 120 (48), 105 (28), 91 (42), 77 (26), 43 (100).

10β-[N-*Trichloroacetyl-carbamate*]-13- *hydroxy-marasm*-7-*en*-5-*oic acid* γ-*lactone* (**3**). Compound **3** was prepd from **3** and trichloroacetyl isocyanate under standard conditions. Mp 203–208°; $[\alpha]_D^{20} = +10.5°$ ($CHCl_3$; *c* 0.8); IR ν_{max}^{KBr} cm^{-1}: 3160, 2966, 1799, 1753, 1731, 1684, HRMS: $[M - CCl_3CONCO]^+$ 248.14742, calc. for $C_{15}H_{20}O_3$ 248.14124. MS 70 eV *m/z* (rel. int) : 436 $[M]^+$ (0.1), 322 (0.1), 248 (9), 230 (100), 215 (73), 201 (33), 188 (21), 171 (20), 157 (20), 143 (11), 120 (20), 105 (8), 91 (11).

9α,13-*Dihydroxy-marasm*-7(8)-*en*-5-*oic acid* γ-*lactone* (**4**). Compound **4** (33 mg, $K' = 14.6$) obtained as oil; $[\alpha]_D^{20} = +2.91°$ ($CHCl_3$, *c* 1.63); UV λ_{max} nm: 231.1 (ε 3191) , 275.5 (ε 562); IR ν_{max}^{film} 3400, 1766, 1688. HRMS: $[M]^+$ 248.1420, calc. for $C_{15}H_{20}O_3$ $[M]^+$ 248.14124. MS 70 eV *m/z* (rel. int.): 248 $[M]^+$ (48), 233 (36), 230 (32), 215 (100), 203 (32), 192 (35), 187 (32), 174 (44), 164 (33), 149 (32), 133 (24), 119 (37), 105 (50), 91 (50), 77 (44), 69 (19), 55 (28), 43 (42).

2α,13-*Dihydroxy-marasm*-7(8)-*en*-5-*oic acid* γ-*lactone* (**5**). Compound **5** (33 mg, $K' = 6.4$) obtained as oil; $[\alpha]_D^{20} = +33.4°$ ($CHCl_3$, *c* 1.7); UV λ_{max} nm: 196 (ε 5760), 227 (ε 2555), 285 (ε 696); IR ν_{max}^{film} cm^{-1}: 3452, 1760, 1616; HRMS: $[M]^+$ 248.1430, calc. for $C_{15}H_{20}O_3$ $[M]^+$ 248.14124; MS 70 eV *m/z*: 248 $[M]^+$ (84), 233 (71), 215 (28), 205 (62), 190 (37), 175 (31), 159 (41), 156 (41), 145 (35), 131 (24), 119 (55), 105 (81), 91 (61), 77 (37), 55 (28), 43 (100).

Acknowledgements—The authors wish to express their thanks to the Institute of Organic Chemistry, Polish Academy of Sciences, and the Swedish Natural Science Research Council for coordination and financial support.

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