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SESQUITERPENES FROM *LACTARIUS BLENNIUS**

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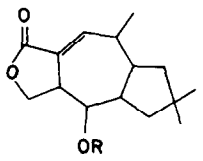
Key Word Index—*Lactarius blennius*; Russulaceae; Basidiomycetae; sesquiterpene lactones; furanosesquiterpenes; blennin A, B, C; lactarorufin A.

Abstract—Six sesquiterpene lactones were isolated from *Lactarius blennius*. The structures of two new sesquiterpenes, blennin A and blennin B were determinated by spectroscopic methods and the structure of the *seco*-compound, blennin C, is revised. The two known furan sesquiterpenes and lactarorufin A were also identified.

INTRODUCTION

In the course of our studies of Russulaceae metabolites we examined *Lactarius blennius* which is an inedible mushroom growing in late autumn in the beech woods on the Italian Apennines. Like all the toxic or inedible *Lactarius* mushrooms it has a pungent milky juice which has the property of turning from white to light grey.

We report here the structures of sesquiterpenes isolated from the ethanolic extract of this mushroom. Two sesquiterpenes named blennin A **1a** and blennin B **2** are new natural compounds and the other four **3a** [1–3], **4** [1,4], **5** [2,5] and **6** have been previously found in different species of fungi.



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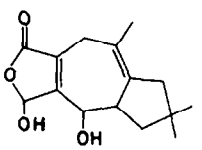

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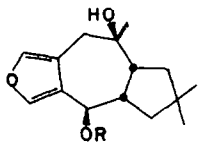

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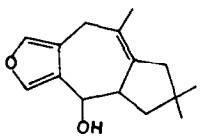

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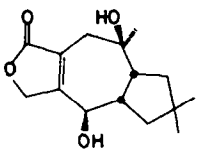

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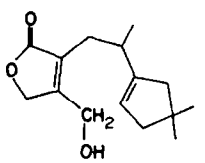

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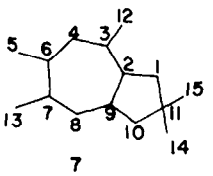
* Part 3 in the series "Fungal Metabolites". For Part 2 see De Bernardi, M., Fronza, G., Vidari, G. and Vita-Finzi, P. (1976) *Chim. Ind.* **58**, 177.



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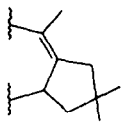

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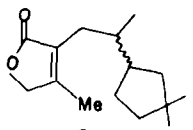

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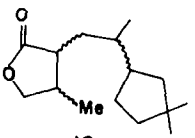

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For compound **6**, isolated from *L. scrobiculatus*, we previously proposed [3] the isomeric structure with the C=O (at C-13) close to the $-CH_2OH$; this structure corresponds to lactaronectorin [6]. The chemical and spectroscopic evidence reported here led us to revise this structure. Compound **6** isolated from *L. blennius* and from *L. scrobiculatus*, is now named blennin C.

All these compounds **1–6** have the unusual and characteristic lactarane skeleton **7** [7] which appears unique to the *Lactarius* sesquiterpenes.

RESULTS AND DISCUSSION

The work-up of the ethanolic extract of the mushrooms and isolation of the sesquiterpenes is described in the Experimental.

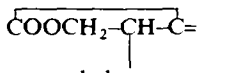
Blennin A **1a** is a yellowish oil. The MW by MS was 250 and together with the ^{13}C- and ^1H-NMR data indicated the molecular formula to be $C_{15}H_{22}O_3$. The IR spectrum (film) showed bands at 3450 cm^{-1} (OH) and at 1755, 1680 and 840 cm^{-1} for an α,β-unsaturated γ-lactone. The ^{13}C-NMR spectrum confirmed this feature by the following signals: C=O, *s*, 171.93 ppm; >C=, *s*, 126.71 ppm; –CH=, *d*, 145.71 ppm and $-CH_2O$, *t*, 69.36 ppm. The double bond was trisubstituted and in an α,β position to the C=O suggesting it to be in the seven membered ring. The ^1H-NMR spectrum showed a triplet (J = 2.5 Hz) at δ 6.67 for a vinylic proton and two

Table 1. ^{13}C-NMR data* of compounds **1a**, **5** and **6**

	C-1, C-10	C-2, C-9	C-3	C-4	C-5	C-6	C-7	C-8	C-11	C-12	C-13	C-14, C-15
1a	47.3 *t*	51.3 *d*	34.9 *d*	145.7 *d*	171.9 *s*	126.7 *s*	45.0 *d*	75.1 *d*	36.8 *s*	20.7 *q*	69.4 *t*	30.7 *q*
	44.8 *t*	43.7 *d*										29.1 *q*
5	45.5. *t*	49.1 *d*	75.1 *s*	34.8 *t*	175.6 *s*	123.3 *s*	160.1 *s*	67.4 *d*	36.9 *s*	31.3 *q*	71.8 *t*	29.2 *q*
	45.3 *t*	46.2 *d*										26.4 *q*
6	47.7 *t*	147.0 *s*	34.1 *d*	29.9 *t*	175.5 *s*	126.6 *s*	159.7 *s*	58.0 *t*	38.3 *s*	19.1 *q*	70.6 *t*	29.9 *q*
	47.5 *t*	122.3 *d*										29.9 *q*

*25.2 MHz, $CDCl_3$, TMS. Chemical shifts in ppm. Signal multiplicity *s* = singlet, *d* = doublet, *t* = triplet, *q* = quartet obtained by "off resonance" decoupling experiments.

triplets at δ 4.54 (1H) and 4.11 (1H) (J = 9.0 Hz), which are characteristic of a methylene group in a



system [8]. The two methylene protons had identical couplings to each other and to a vicinal proton (δ 3.30 *m*). Decoupling experiments showed that this last proton was also coupled to a C<u>H</u>OH. The presence of a secondary alcohol was indicated by a doublet at 75.12 ppm (^{13}C-NMR) and by a triplet at δ 3.66 (^{1}H-NMR). The ^{1}H-NMR spectrum showed three methyl groups: two singlets (3 H each) at δ 1.08 and 1.00 (C-11 (C<u>H</u>$_3$)$_2$) and a doublet (3 H) at δ 1.11 (C-3 C<u>H</u>$_3$). The spectroscopic data of **1a** did not lead to a definitive structure. Decoupling experiments, in order to decide whether the vinylic proton was at C-4, the CHOH at C-8 and the C=O at C-5 or the vinylic proton at C-8, the CHOH at C-4, and the C=O at C-13 were not successful because of the closeness of the chemical shifts (δ 1.9–2.5) of the C-3, C-2, C-9 and OH protons.

Acetylation of blennin A gave **1b** which allowed the problem to be solved. The ^{1}H-NMR spectrum of **1b** was recorded in $CDCl_3$ and in C_6D_6. In C_6D_6 the C-3 proton was shifted to δ 1.76, far enough from the absorption of the C-9 proton (1 H, δ 2.18, *m*). By decoupling of the C-3 proton the methyl doublet became a singlet and the vinylic proton a doublet while the signal for C<u>H</u>OH remained unchanged. Furthermore when the C-9 proton was irradiated the C<u>H</u>OH signal was simplified to a doublet, while the signals of the vinylic proton and the C-3 methyl group were not affected. These experiments showed without any doubt that the vinylic proton was at C-4 and therefore the C=O was at C-5 and the C<u>H</u>OH at C-8. On these facts we assigned the structure **1a** to blennin A.

Blennin B, an oil, was found by MS and NMR spectra to have the molecular formula $C_{15}H_{20}O_4$. The IR strong bands at 1745 and 3600–3300 cm^{-1} suggested the presence of an α,β-unsaturated γ-lactone and of hydroxyl groups. In the ^{1}H-NMR spectrum (d_6-Me_2CO) the singlets of three methyls (two geminal at δ 0.91 and 1.12 and one on a double bond at δ 1.76 and the signals for the methylene groups of the cyclopentane ring (AB system centered at δ 2.2, C-1; δ 1.57, *dd* and 1.96, *dd*, C-10) were attributed to the partial structure **8** which was suggested by the great similarity to the data of **4**. Also present were one double doublet at δ 6.21 (1 H), an AB system at δ 2.76 and 3.14, two multiplets at δ 4.18 (1 H) and at *ca* δ 3 (1 H) and two doublets for 2 OH at δ 6.46 and 4.52. The signal at δ 6.21 was assigned to the proton of a –O–C<u>H</u>OH– group and that at δ 4.18 to that of a –C<u>H</u>OH–. These assignments were supported by the presence of two doublets at 97.7 and 69.48 ppm respectively in the ^{13}C-NMR spectrum. From these data we assumed that the CH linked to two oxygen atoms should be in the lactone ring which had a double bond between C-6 and C-7. Again there was the possibility of two structures, namely **2** or its isomer with the C=O at C-13 and the –O–C<u>H</u>OH– at C-5. We prefer structure **2** for blennin B because of the coupling constants of O–C<u>H</u>OH. This proton was coupled with an hydroxyl group (J = 8.0 Hz) and with another proton at δ *ca* 3 (J = 2.0 Hz). This value can be attributed to a homoallylic coupling which was possible for the protons at C-13 and C-4 in **2** but which could not be accounted for by the isomeric structure. The remaining OH group was located at C-8. These assignments are in part supported by double resonance experiments. Chemical evidence for the structure of blennin B is in progress. Compound **2** is the first compound with the lactarene skeleton containing an α,β-unsaturated γ-hydroxy lactone isolated from an extract of a mushroom.

We also isolated two furanosesquiterpenes **3a** and **4** which have been already found in other fungi [1–4]. The lactarorufin A **5** [2,5] was also identified. The structures of **3a** and its acetyl derivative, **3b**, were determined by comparison of their spectroscopic data with those of authentic samples obtained from *L. scrobiculatus* [3] and *L. necator* [2]. The spectroscopic data of **4** were identical to the published data [4]. Lactarorufin **5** was identified by the spectroscopic data [5]. The ^{13}C-NMR spectrum and the decoupling experiments in the ^{1}H-NMR analysis confirmed the revised structure **5** [2]. Considering blennin C the following definitive evidence allowed us to assign to it the structure **6**. Catalytic hydrogenation (PtO_2) of **6** afforded a mixture of compounds of MW 236 **9** and 238 **10**. They were separated by PLC. Compounds **9** and **10** were actually mixtures of stereoisomers, however, this fact did not affect the interpretation of the spectroscopic data. Hydrogenation not only reduced the double bonds of **9** and **10** but also reduced the CH_2OH group to Me. In the IR spectrum **9** still showed the bands characteristic of an α,β-unsaturated γ-lactone (1758 and 1680 cm^{-1}). In the ^{1}H-NMR spectrum four CH_3 signals were present at δ 0.97 *s* and 1.02 *s* C-11 $(CH_3)_2$, 0.78 *d*, $J_{3\text{-}12}$ = 7.0 Hz (C-3 Me) and 2.02 *s* (C-7 Me). This last chemical shift is characteristic of a β-methyl group on a cyclic –C=CH–C=O system. It is known [9,10] that while an α-Me absorbs at δ *ca* 1.6–1.9, a β-Me absorbs at δ *ca*. 2.0–2.15. In the case of **9** the Me is in a β position only when the C=O is at C-5. Final evidence of the structure of **6** was given by the ^{1}H-NMR spectrum (C_6D_6) of **10**, the dihydroderivative of **9**. The signal for the C-7 proton was located at δ 1.7 (1 H). The irradiation of this

proton caused the decoupling of the C-7 Me doublet and at the same time of the lactone ring CH_2 signals. Irradiation at δ 1.02 (C-4 proton) allowed the identification of the C-6 proton at δ 2.16 as a doublet (J = 6.5 Hz) due to the coupling to the C-7 proton. The lack of further coupling of the C-6 proton and the coupling of the C-7 proton to both Me and CH_2O groups indicated that the CH_2O was at C-13 and therefore the C=O was at C-5. It is interesting that the revision of the structures of lactarorufin A **5** [2] and Blennin C **6** lead to the conclusion that the lactarolactones have mostly the lactone C=O at C-5. Few examples of the isomeric lactone structure are known apart from the epoxylactone and the diene lactone isolated from *L. scrobiculatus* [3,8]. The occurrence of the two kinds of lactone (at C-5 and at C-13) is not surprising if the precursor is really a β,β'-disubstituted furan ring which could be oxidized at either of its two free positions (α or α'). It may be worthwhile to investigate if the γ-hydroxy-α,β-unsaturated lactone **2** is the natural intermediate in the formation of the lactone skeleton from a furan compound.

EXPERIMENTAL

Extraction and isolation of sesquiterpenes from L. blennius. 10.5 kg of fresh mushrooms collected in October 1974 on the Apennines of Oltrepò (Pavia) were extracted with EtOH and left at −30°. After one month the extract was filtered and evaporated to remove the EtOH. The aq. liquor was extracted with CH_2Cl_2 and with EtOAc. The collected organic layers were evap. to dryness *in vacuo* to give 31 g of a crude residue that was dissolved in MeOH. At −30° insol. white materials, free of sesquiterpenes, precipitated and were filtered off. The MeOH soln was diluted with H_2O and repeatedly partitioned with pentane to remove the lipid components. The residue (12 g) of the hydroalcoholic phase was chromatographed on a Kieselgel 60 (Merck, 70–230 mesh) column with mixtures of C_6H_6–EtOAc and EtOAc–MeOH. Sesquiterpenes in the fractions were visualized as differently coloured spots by spraying the Si gel TLC plates with a vanillin–H_2SO_4 soln. Sesquiterpenes were eluted from the column in the order: **4**; **1a** and **6**; **2**; **3a**; **5**. Fractions containing mixtures of sesquiterpenes were rechromatographed on column or on PLC. Other sesquiterpenes were detected by TLC but not isolated.

Blennin A **1a**. **1a** was separated from **6** by a Kieselgel 60 column eluted with *iso*-Pr_2O: 38 mg. Yellowish oil, $[\alpha]_D^{20}$ + 49.9° ($CHCl_3$), λ_{max}^{EtOH} nm (lg ϵ): 222 (3.85), ν_{max} (thin film) cm^{-1}: 3450, 1755, 1680, 840. ^{1}H-NMR (100 MHz, $CDCl_3$, TMS): δ 1.00 (3 H, *s*, C-11 Me); 1.08 (3 H, *s*, C-11 Me); 1.11 (3 H, *d*, J_{12-3} = 7.0 Hz, C-12); 1,2–1.9 (4 H, *m*, C-1 and C-10); 1.9–2.5 (4 H, *m*, C-2, C-9, C-3, OH); 3.3 (1 H, *m*, C-7); 3.66 (1 H, *t*, $J_{7-8} = J_{8-9}$ = 9.5 Hz, C-8); 4.11 (1 H, *t*, $J_{13-13'} = J_{13-7}$ = 9.0 Hz, C-13); 4.54 (1 H, *t*, $J_{13-13'} = J_{13'-7}$ = 9.0 Hz, C-13'); 6.67 (1 H, *t*, $J_{3-4} = J_{4-7}$ = 2.5 Hz, C-4). ^{13}C-NMR. see Table 1. MS (probe) 70 eV *m/e* (rel. int.): 250 M^+ (36), 235 (M-Me; 17); 232 (M-H_2O, 4), 221 (32), 217 (12), 165 (16), 154 (27), 135 (24), 126 (83), 123 (44), 109 (69), 95 (71), 81 (71), 69 (44), 55 (71), 43 (67), 41 (100).

Acetylation of **1a** *to give* **1b**. Acetylation of 11 mg of **1a** in 0.5 ml C_5H_5N with 0.5 ml Ac_2O at room temp. overnight followed by the usual work-up afforded 11 mg of **1b**. $[\alpha]_D^{20}$ + 63.8° ($CHCl_3$) ν_{max} (thin film) cm^{-1}: 1765 (γ-lactone C=O), 1740 (COMe), 1680 (C=C). ^{1}H-NMR (100 MHz, C_6D_6, TMS): δ 0.70 (3 H, *d*, J_{3-12} = 7.0 Hz, C-12); 0.82 (3 H, *s*, C-11 Me); 0.92 (3 H, *s*, C-11 $\underline{CH_3}$); 0.9–1.6 (4 H, *m*, C-1 and C-10); 1.62 (3 H, *s*, MeCO); 1.76 (2 H, *m*, C-2 and C-3); 2.18 (1 H, *m*, C-9); 2.80 (1 H, *m*, C-7); 3.55 (1 H, *t*, $J_{13-13'} = J_{13-7}$ = 9.0 Hz, C-13); 3.94 (1 H, *t*, $J_{13-13'} = J_{13'-7}$ = 9.0 Hz, C-13'); 5.02 (1 H, *t*, $J_{8-9} = J_{7-8}$ = 10.25 Hz, C-8); 6.54 (1 H, *dd*, J_{3-4} = 1.3 Hz, J_{4-7} = 3.0 Hz, C-4).

Blennin B **2**. Obtained as oil (18 mg), $[\alpha]_D^{20}$ + 70.78° (Me_2CO), ν_{max} (thin film) cm^{-1}: 3600–3300 (OH), 1745 (α,β-unsaturated-γ-lactone). ^{1}H-NMR (100 MHz, d_6-Me_2CO, TMS): δ 0.91 (3 H, *s*, C-11 Me); 1.12 (3 H, *s*, C-11 Me); 1.57 (1 H, *dd*, $J_{10-10'}$ = 13.0 Hz, J_{9-10} = 4.0 Hz, C-10); 1.76 (3 H, *s*, C-12); 1.96 (1 H, *dd*, $J_{10-10'}$ = 13.0 Hz, $J_{9-10'}$ = 7.0 Hz, C-10'); 2.12 (1 H, *d*, $J_{1-1'}$ = 14.0 Hz, C-1); 2.28 (1 H, *d*, $J_{1-1'}$ = 14.0 Hz, C-1'); 2.76 (1 H, *d*, $J_{4-4'}$ = 20.0 Hz, C-4); *ca* 3 (1 H, *m*, C-9); 3.14 (1 H, *d*, $J_{4-4'}$ = 20.0 Hz, C-4'); 4.18 (1 H, *m*, C-8); 4.52 (1 H, *d*, J_{8-OH} = 8.0 Hz, OH-8); 6.21 (1 H, *dd*, J_{13-OH} = 8.0 Hz, J_{13-4} = 2.0 Hz, C-13); 6.46 (1 H, *d*, J_{13-OH} = 8.0 Hz, OH-13). MS (probe) 70 eV *m/e* (rel. int.): 264 M^+ (1.7), 246 (8.3), 244 (9.1), 229 (30), 201 (62.5), 185 (32), 173 (32), 157 (12), 142 (37), 128 (26.6), 115 (35), 105 (21.6), 91 (35), 57 (30), 43 (100).

Lactarorufin A **5**. mp 168–169°, $[\alpha]_D^{20}$ + 12.05° ($CHCl_3$) (lit. [5] mp 156–158°, $[\alpha]_D^{20}$ + 7°). ^{13}C-NMR: see Table 1.

Blennin C **6**. For the spectroscopic data see [3]; for the ^{13}C-NMR signals see Table 1.

Hydrogenation of **6** *to yield* **9** *and* **10**. Hydrogenation of 41 mg **6** with PtO_2–AcOH (5 hr) gave a mixture of **9** and **10**. These compounds were separated by PLC on Kieselgel plates by elution with C_6H_{12}–EtOAc = 4:1.5.

Compound **9**: 20 mg; ν_{max} (thin film): 1758 (C=O), 1680 (C=C) cm^{-1}. ^{1}H-NMR (100 MHz, $CDCl_3$, TMS): δ 0.78 (3 H, *d*, J_{3-12} = 7.0 Hz, C-12); 0.97 (3 H, *s*, C-11 Me); 1.02 (3 H, *s*, C-11 Me); 2.02 (3 H, *s*, C-8); 4.63 (2 H, *s*, C-13). MS (probe) 70 eV, *m/e* (rel. int.): 236 M^+ (<1), 112 (100).

Compound **10**: 3 mg; ν_{max} (thin film): 1780 (C=O) cm^{-1}. ^{1}H-NMR (100 MHz, C_6D_6, TMS): δ 0.53 (3 H, *d*, J_{7-8} = 7.0 Hz, C-8); 0.81 (3 H, *d*, J_{3-12} = 7.0 Hz, C-12); 0.98 (3 H, *s*, C-11 Me); 1.04 (3 H, *s*, C-11 Me); 1.7 (1 H, *m*, C-7); 2.16 (1 H, *q*, $J_{6-7} = J_{6-4} = J_{6-4'}$ = 6.5 Hz, C-6); 3.32 (1 H, *dd*, $J_{13-13'}$ = 8.5 Hz, J_{13-7} = 2.0 Hz, C-13); 3.55 (1 H, *dd*, $J_{13-13'}$ = 8.5 Hz, $J_{13'-7}$ = 5.5 Hz, C-13'). MS (probe) (70 eV, *m/e* (rel. int.): 238 M^+ (<1), 113 (100), 100 (97.5), 97 (11.5), 95 (17.5), 85 (62), 83 (21), 81 (15), 69 (37), 67 (12), 57 (16), 55 (57), 43 (64), 41 (46).

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