Phytochemistry, 1980, Vol. 19, pp. 99–101. © Pergamon Press Ltd. Printed in England. 0031-9422/80/0101-0099 $02.00/0

STEREOCHEMISTRY OF BLENNIN A AND BLENNIN D FROM *LACTARIUS BLENNIUS**

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(*Received* 30 *April* 1979)

Key Word Index—*Lactarius blennius*; Russulaceae; Basidiomycetes; structure elucidation; sesquiterpene lactones; blennin A; blennin D (2-hydroxyblennin A).

Abstract—From the ethanolic extract of *Lactarius blennius*, a new sesquiterpene blennin D (2-hydroxyblennin A) was isolated and its structure elucidated together with the stereochemistry of the already known blennin A. Treatment of blennin A mesylate with DBU afforded a compound with a new skeleton.

INTRODUCTION

In a recent paper [1] we reported, together with other sesquiterpenes isolated from *Lactarius blennius*, the structure (without stereochemistry) of blennin A (**1a**), a new sesquiterpene lactone. Blennin A seems to be a stereoisomer of lactarorufin N (**2**) which has been isolated from *Lactarius necator* [2]. The stereochemistry of the chiral centres C-2, C-3 and C-9 in lactarorufin N (**2**) could be assigned without any doubt by dehydration of **2** with $SOCl_2$ and Py to the known vellerolactone (**3**) [3, 4] whose stereochemistry has been very recently determined [5].

Further spectral studies of blennin A now show that **1a** has the stereochemistry depicted.

RESULTS AND DISCUSSION

A careful analysis of the ^{1}H NMR spectrum of **1a** showed that in the seven-membered ring H-7, H-8 and H-9 were all in a pseudoaxial configuration. In fact the signal for H-8 (δ 3.66) was a triplet with two large coupling constants ($J = 9.5$ Hz) with H-7 and H-9; H-8 must be, according to the Karplus curves [6], *anti* to both H-7 and H-9.

As far as the configuration of C-12 is concerned, it can be deduced from the coupling constant between H-3 and H-4. Literature data [7] reported the vinyl–allylic proton spin couplings of a series of olefinic substrates as a function of allylic proton conformations and a relationship between the magnitude of this coupling and the dihedral angle between the vinyl and allylic carbon–hydrogen bonds. In the case of blennin A (**1a**), the observed coupling constant ($J_{3-4} = 2.5$ Hz) corresponded to a calculated dihedral angle of about 90°. Examination of Dreiding models of **1a** with the above already fixed configuration at C-7, C-8 and C-9 showed that the estimated dihedral angle between H-3 and H-4 requires the ring junction protons H-2 and H-9 and the C-12 to be *syn* to each other, the hydroazulenic ring system having an *exo* conformation with the Me-12 group in a pseudo-equatorial position. Thus blennin A (**1a**) has the same C-2, C-3 and C-9 relative configuration as vellerolactone **3** [5].

Different attempts on **1a** to yield vellerolactone (**3**) by dehydration were unsuccessful as was treatment of blennin A mesylate (**1b**) with bases, pyrolysis of blennin A acetate, etc. These results could be foreseen as in this case dehydration could only occur through a difficult *cis*-elimination. However, it is worth reporting that treatment of blennin A mesylate (**1b**) with diazabicycloundecene (DBU) [8] afforded a product to which structure **4** could be assigned on the following data.

Compound **4** has the same TLC R_f as vellerolactone (**3**) but the spot looked blue instead of green. The MW 232 (MS) indicated **4** to be isomeric to vellerolactone (**3**) and without hydroxyls (IR confirmed the absence of OH groups).

By the ^{1}H NMR data it was possible to determine that the C-3 methyl (δ 1.68, *d*) was on the double bond coupled with an allylic coupling constant to the C-4 vinylic proton. Unlike pyrovellerolactone [3] which had two double bonds between C-3 and C-4, and C-6 and C-7, **4** still showed the coupling between the lactone methylene at C-13 (δ 4.16 and 4.28) and the C-7 proton (2.08) which absorbed at a higher field than in blennin A (3.3) [1]. The high field chemical shift and a further coupling constant of H-7 with a

* Part 7 in the series "Fungal Metabolites". For Part 6 see Andina, D., De Bernardi, M., Del Vecchio, A., Fronza, G., Mellerio, G., Vidari, G. and Vita-Finzi, P. (1980) *Phytochemistry* **19**, 93.

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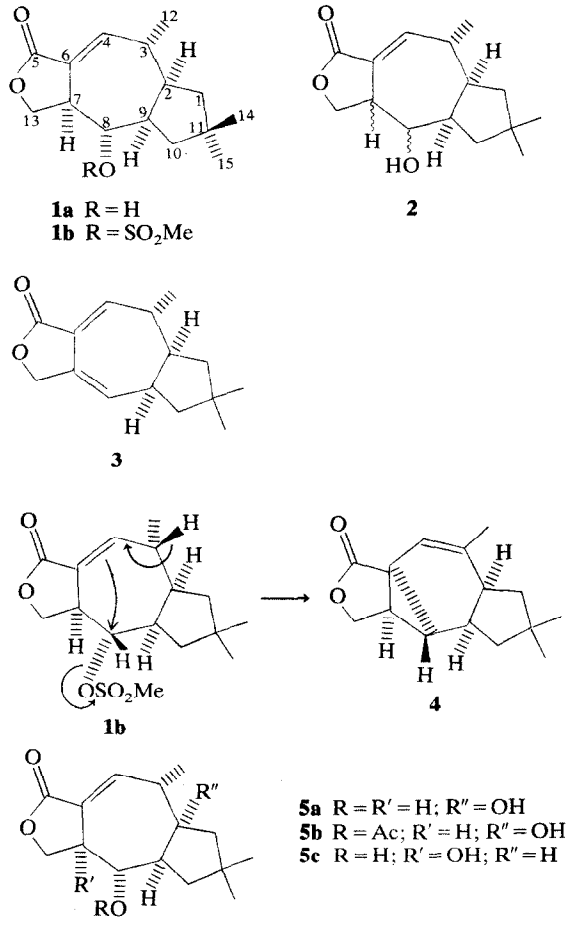

Scheme 1.

proton whose signal was covered by the *gem*-dimethyl group (δ 1.00 and 1.02), as was indicated by decoupling experiments, led to the inference that a new bond between C-6 and C-8 was formed giving rise to a cyclopropane ring. Decoupling experiments determined the coupling constants and confirmed the attribution of the signals as reported in the Experimental.

The probable mechanism of the reaction is indicated in Scheme 1 by a formal elimination of a molecule of methansulphonic acid from C-3 and C-8 and rearrangement of the bonds of **1b** to yield **4**.

Together with the elucidation of the stereochemistry of blennin A, we determined the structure of blennin D (**5a**), a new sesquiterpene lactone isolated from the EtOH extract of *Lactarius blennius* [1].

The MW 266 (MS) together with ^{13}C NMR and ^{1}H NMR spectra indicated the molecular formula to be $C_{15}H_{22}O_4$. The IR spectrum showed OH bands (at 3400 cm^{-1}) and the characteristic absorptions of an α,β-unsaturated lactone (at 1748, 1680 and 825 cm^{-1}). The ^{13}C NMR spectrum was quite similar to that of blennin A (Table 1) and showed a trisubstituted double bond (*s*, 171.4 and *d*, 141.9 ppm) and three carbon atoms linked to oxygens indicating the presence of a lactone CH_2—O (*t*, 69.2 ppm), a CHOH (*d*, 75.4 ppm) and a >C—OH (*s*, 83.5 ppm). As expected acetylation of **5a** yielded the monoacetate **5b** which still exhibited a band for a free tertiary hydroxyl group (3440 cm^{-1}) in the IR spectrum. We assigned to **5a** the structure of 2-hydroxyblennin A on the following data.

The ^{1}H NMR spectrum located the double bond in position C-4 C-6 (α,β to the C=O at C-5) because the vinylic proton (*t*, δ 6.51) was coupled to the same methine at C-3 which gave rise to a doublet for C-12 (1.24). The methylene protons of the lactone ring exhibited two triplets (4.56 and 4.11, $J = 8.5$ Hz) because of the identical coupling constant to each other and to the vicinal proton at C-7 (3.3–3.7, *m*) [9].

Furthermore a triplet for the CHOH group (δ 3.62, $J = 9.5$ Hz) recalled the previously discussed pattern of the *anti* configuration of H-8, both with H-7 and H-9. Therefore the only quaternary carbon atom free to accomodate the tertiary hydroxyl was C-2. In fact it is possible to observe in the ^{13}C NMR spectrum of **5a** the down-field shift of the C-1, C-3 and C-9 signals and the upfield shift of the C-4, C-11 and C-12 signals, with respect to the corresponding chemical shifts in **1a**, because of the known β- and γ-effect exerted by hydroxyls on the neighbouring carbon atoms [10]. Moreover, in the ^{1}H NMR spectrum an isolated AB system (centred at δ 1.77, $J_{AB} = 14.0$ Hz) was evident for the C-1 protons. These assignments were supported by extensive decoupling experiments both on **5a** and **5b**.

As far as the stereochemistry of blennin D (**5a**) is concerned, the magnitudes of the coupling constants J_{7-8}, J_{8-9} and J_{3-4}, were very similar to those found for blennin A (**1a**) [1] and clearly suggested, for the same reasons, that **5a** had the same relative configuration as blennin A. Furthermore the very high-field chemical shift of C-12 (16.2 ppm) in the ^{13}C NMR spectrum indicated that the pseudo-equatorial methyl and the C-2 OH must by *syn* and therefore, notwithstanding the substitution at C-2, the stereochemistry of the ring function for **1a** and **5a** is identical. It is noteworthy that very recently we isolated from *Russula sardonia* [4] another hydroxyl derivative of blennin A, sardonialactone A (**5c**) (7-hydroxyblennin A) with identical stereochemistry to **1a** and **5a**.

EXPERIMENTAL

The isolation procedure of sesquiterpenes from *Lactarius blennius* has already been described in a previous paper [1].

Table 1. ^{13}C NMR data of blennin A (**1a**) and blennin D (**5a**)*

	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*[a]	44.8*t*[a]	47.3*d*[b]	51.3*d*[b]	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*[c]	29.1*q*[c]
5a	56.2*t*	45.3*t*	83.5*s*	60.3*d*	39.8*d*	141.9*d*	171.4*s*	127.7*s*	44.1*d*	75.4*d*	35.1*s*	16.2*q*	69.2*t*	30.6*q*[c]	29.1*q*[c]

* 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.

a, *b* and *c* = assignments can be reversed.

Dehydration of blennin A through **1b** *to* **4**. To a Py soln of **1a** (22 mg), $MeSO_2Cl$ (32 mg) was added and the mixture left at room temp. for 4 hr. Ice was then added, the product extracted with CH_2Cl_2, and washed with dil. HCl, aq. $NaHCO_3$ and H_2O. The dried organic layer, filtered on a Florisil column, afforded pure blennin A mesylate **1b** in quantitative yield. IR ν_{max} cm^{-1}: 1760 (CO), 1680 (C=C), 1360 and 1172 (O—SO_2). MS (probe 70 eV *m/e* (rel. int.): 328 (M^+, <1), 313 (M−15, <1), 249 (M−$MeSO_2$, 75), 233(25), 232 (M−$MeSO_3H$, 37.5), 217(26), 203(11), 189(10), 187(18), 185(9), 177(9), 176(13), 175(11), 173(14), 167(10), 161(11), 159(17), 149(24), 147(11), 145(15), 137(15), 136(12), 135(25), 134(10), 133(13), 132(10), 131(19), 129(13), 125(18), 124(10), 123(27), 122(26), 121(21), 119(20), 117(18), 115(13), 113(13), 112(12), 111(22), 110(10), 109(33), 108(33), 107(29), 105(29), 99(14), 98(11), 97(35), 96(20), 95(55), 94(17), 93(45), 85(35), 83(45), 82(21), 81(68), 79(33), 77(31), 73(14), 71(58), 70(29), 69(100), 68(18), 65(14), 60(14), 58(11), 57(90), 56(23), 53(21), 49(11), 43(70), 41(83).

To **1b** (8 mg) dissolved in 1 ml dry C_6H_6 was added DBU (few drops) and the soln was refluxed under a N_2 stream for 40 min. Evapn of C_6H_6 left a residue which was poured into ice and HCl and extracted with CH_2Cl_2. The dried organic layer was percolated through a Florisil column yielding 4 mg of **4** which showed an identical R_f on TLC plates (eluent CH_2Cl_2) with vellerolactone, but by spraying with vanillin–H_2SO_4 soln gave a blue spot instead of the green spot of **3**. Compound **4** was a thick oil, $[\alpha]_D^{20}$ −4.2° (CH_2Cl_2); IR ν_{max}^{film} cm^{-1}: 1770 (CO), 1670, 1650 (C=C), 1378, 1326 (gem.-dimethyl group). MS (probe) 70 eV *m/e* (rel. int.): 232 (M^+, 74), 217 (M−15, 49), 204 (M−28, 16), 189(19), 187(16), 176(61), 173(54), 159(27), 149(28), 145(36), 131(47), 119(34), 117(49), 115(23), 105(40), 97(21), 95(25), 91(59), 83(26), 81(33), 79(25), 77(36), 71(37), 69(68), 67(22), 58(29), 57(91), 55(69), 43(100), 41(97). ^1H NMR (100 MHz, $CDCl_3$, TMS): δ 1.00 (3H, *s*, C-11 Me), 1.02 (3H, *s*, C-11 Me), 1.1–1.65 (4H, *m*, C-1 and C-10), 1.68 (3H, *d*, $J_{4-12} \simeq 1$ Hz, C-12), 2.08 (1H, *d* × *t*, $J_{7-13} = 4.5$ Hz, $J_{7-13'} = 1.2$ Hz, $J_{7-8} = 4.0$ Hz, C-7), 2.28 (1H, *m*, C-2), 2.64 (1H, *m*, C-9), 4.16 (1H, *dd*, $J_{13-13'} = 9.0$ Hz, $J_{13'-7} = 1.2$ Hz, C-13'), 4.28 (1H, *dd*, $J_{13-13'} = 9.0$ Hz, $J_{13-7} = 4.5$ Hz, C-13), 5.74 (1H, *br s*, C-4). Decoupling experiments indicated the presence of the C-8 proton at *ca.* δ 1, covered by the singlets of the methyl groups.

Blennin D (**5a**) was visualized as a light-blue spot by spraying the GF_{254} TLC plates with a vanillin–H_2SO_4 soln. **5a** was only slightly more polar than the isomeric lactarorufin A [1] on TLC, and the two compounds were separated by CC on Kieselgel 60HR Merck (eluent $CHCl_3$–Me_2CO, 11:3). 10.5 kg of fresh mushrooms gave *ca* 25 mg of **5a**. $[\alpha]_D^{20}$ + 51°(Me_2CO); IR ν_{max} cm^{-1}: 3400(OH), 1748(CO), 1680 and 835(C=C). MS (probe, 70°) 70 eV, *m/e* (rel. int.): 266 (M^+, <1), 251 (M−Me, <1), 248 (M−H_2O, 20), 233 (M−Me−H_2O, 4), 230 (M−$2H_2O$, 6), 215 (M−$2H_2O$−Me, 10), 203(18), 189(6), 173(8), 155(14), 141(14), 125(64), 124(20), 123(16), 121(14), 109(20), 107(16), 105(14), 97(24), 95(26), 93(18), 91(27), 83(24), 81(46), 79(40), 77(24), 69(32), 67(26), 65(16), 57(22), 55(55), 53(38), 43(85), 41(100), ^{13}C NMR see Table 1. ^1H NMR (100 MHz, $CDCl_3$, TMS): δ 1.05 (3H, *s*, C-11 Me), 1.16 (3H, *s*, C-11 Me), 1.24 (3H, *d*, $J_{3-12} = 7.5$ Hz, C-12), 0.9–1.4 (2H, *m*, C-10), 1.67 (1H, *dd*, $J_{AB} = 14.0$ Hz and $J_{long\ range} = 1.0$ Hz, C-1), 1.87 (1H, *d*, $J_{AB} = 14.0$ Hz, C-1'), 2.20–2.75 (4H, *m*, C-3, C-9, 2OH), 3.3–3.7 (1H, *m*, C-7), 3.62 (1H, *t*, $J_{7-8} = J_{8-9} = 9.5$ Hz, C-8), 4.11 (1H, *t*, $J_{13-13'} = J_{13-7} = 8.5$ Hz, C-13), 4.56 (1H, *t*, $J_{13-13'} = J_{13'-7} = 8.5$ Hz, C-13'), 6.51 (1H, *t*, $J_{3-4} = 2.1 \simeq J_{4-7} = 2.5$ Hz, C-4).

Acetylation of **5a** *to* **5b**. Acetylation of 11 mg **5a** in 0.5 ml Py with 0.2 ml Ac_2O at room temp. overnight, followed by the usual work-up, afforded 11 mg **5b**, which was not further purified. IR ν_{max} cm^{-1}: 3440 (OH), 1765 (γ-lactone CO), 1730 (O—COMe), 1690 (C=C). MS (probe, 60°) 70 eV *m/e*: 308 (M^+), 266, 248, (M−HOAc), 203, 125, 43 (base peak). ^1H NMR (100 MHz, C_6D_6, TMS): δ 0.83 (3H, *d*, $J_{12-3} = 7.5$ Hz, C-12), 0.83 (3H, *s*, C-11 Me), 1.04 (3H, *s*, C-11 Me), 0.8–1.5 (4H, *m*, C-1 and C-10), 1.59 (3H, *s*, MeCO), 1.85 (1H, *m*, C-3), 2.2–2.6 (1H, *m*, C-9); 3.0–3.4 (1H, *m*, C-7), 3.56 (1H, *t*, $J_{13-13'} = J_{13-7} = 8.5$ Hz, C-13), 3.96 (1H, *t*, $J_{13-13'} = J_{13'-7} = 8.5$ Hz, C-13'), 4.99 (1H, *t*, $J_{7-8} = J_{8-9} = 10.0$ Hz, C-8), 6.36 (1H, *t*, $J_{3-4} = 2.1 \simeq J_{4-7} = 2.5$ Hz, C-4).

Acknowledgements—We thank Consiglio Nazionale delle Ricerche and Ministero della Pubblica Istruzione (Italy) for partial financial support and Mr. M. Angarano of the 'Bresadola' Mycological Group (Trento) for collecting the mushrooms.

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