Tetrahedron Vol. 40, No. 14, pp. 2757 to 2762, 1984
Printed in the U.S.A.

0040-4020/84 $3.00 + .00
Pergamon Press Ltd.

STRUCTURAL STUDIES ON *LACTARIUS* SESQUITERPENES: STRUCTURE ELUCIDATION OF LACTARORUFINS D AND E AND CONFORMATIONAL ANALYSIS OF LACTARAN-5-OLIDES

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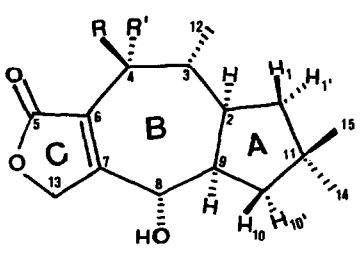
(*Received in UK* 25 *April* 1983)

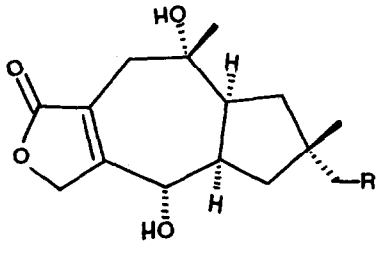
Abstract—The structures of lactarorufins D and E, two new sesquiterpenes from *Lactarius necator*, have been elucidated. Conformational analysis of lactarorufins and blennins is discussed.

HPLC analysis of monohydroxylactone content of various *Lactarius* species has shown a different chromatogram[1] for each species, which can be distinguished by this way. During our studies on *Lactarius necator* six monohydroxylactones with the lactarane skeleton have been isolated[2-4] from the ethanolic crude extract. From the same extract a fraction A, mainly containing dihydroxylactones, was also obtained. Thinking that also the dihydroxylactone pattern could be a significative chemotaxonomic marker of the genus *Lactarius*, we undertook a preliminary study of this fraction in order to devise the best conditions of HPLC separation. We found that A was indeed a complicated mixture of several compounds with close R_f values on Silica gel TLC but could be resolved by HPLC using μ Porasil columns. By means of prep HPLC, using columns packed with Lichrosorb Si 60, two new compounds, lactarorufin D **1a**, m.p. 160–162°, $[\alpha]_D^{20}$ + 93° ($CHCl_3$), and lactarorufin E **1b**, m.p. 125–130°, $[\alpha]_D^{20}$ +58° ($CHCl_3$), were separated from known lactarorufin A **2a**,[3,5] 7-OH-blennin A (sardonialactone A) **3b**,[6] and other not yet identified sesquiterpenes.

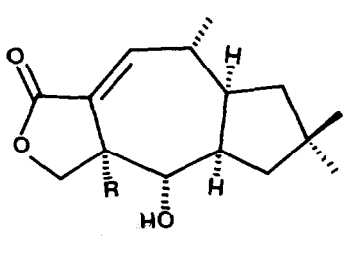
1a and **1b** showed a parent ion at m/z 266 which, together with ^{13}C-NMR and ^{1}H-NMR data, indicated the molecular formula $C_{15}H_{22}O_4$. That these two compounds were actually isomers of **2a** and **3b** and very similar to each other was also confirmed by IR bands, attributable to alcoholic OH and unsaturated γ-lactone CO stretchings, and by their ^{1}H-NMR spectra which showed signals consistent with a lactarane-like structure. In particular a doublet occurring in both cases at δ 1.10 ppm (J = 6.6 Hz for **1a** and 6.2 Hz for **1b**) was attributed to the C-12 methyl, which then must be geminal to a hydrogen, as in blennins **3**. However the appearance of the CH–OH at C-8 as a doublet (J ≅ 11.5 Hz) at δ4.60 and 4.76 respectively, and of the methylene at C-13 as a broad AB system centred at $\delta \cong 4.91$, excluded that the conjugated double bond was exocyclic to the lactone ring and demonstrated that it was between C-6 and C-7 in both compounds, as in lactarorufin A **2a**. Moreover it was anticipated from the signal at δ4.41 in **1a** and at δ 4.06 in **1b**, and from extensive decoupling experiments, that in addition to C-8 also C-4 was bearing a secondary OH group.



1a R=H R'=OH
1b R=OH R'=H
1c R=R'=H



2a R=H
2b R=OH



3a R=H
3b R=OH

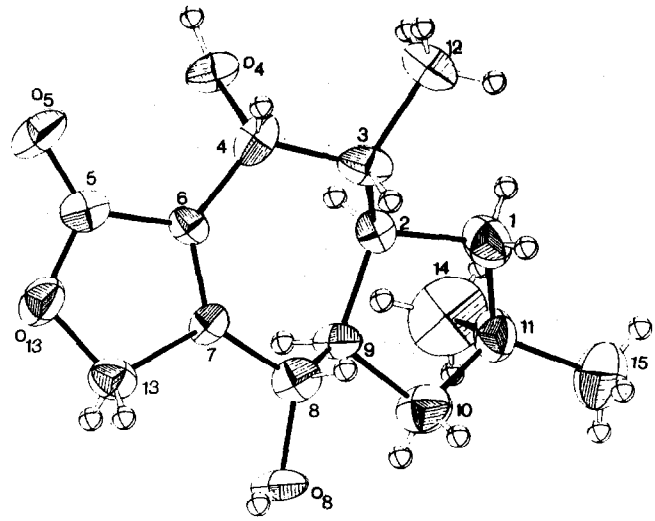


Fig. 1. ORTEP generated perspective drawing of lactarorufin D **1a** with atomic numbering. Atoms are carbons unless indicated otherwise. The H atoms are shown but not labelled. Ellipsoids are contoured to enclose 40% of the electron density. No absolute stereochemistry is implied.

In order to determine, unequivocally, the structure and relative stereochemistry, compound **1a** was subjected to single-crystal X-ray analysis. Figure 1 shows a perspective view of the structure computed from the final relative atomic coordinates. The majority of the corresponding bond lengths and angles for **1a**, listed in Table 1 and 2, agree within experimental error with each other and with those expected.†

The X-ray results demonstrate that lactarorufin D has actually a lactarane skeleton with the five membered ring A fused to the seven-membered ring in a *cis*-manner. In ring C the C=O is at C-5 and in ring B CH_3-12, OH-4 and OH-8 are *cis* to the bridge-head protons H-2 and H-9. The carbons C-3, C-4, C-6, C-7, C-8 and C-2, C-3, C-8, C-9 lie approximately in two planes (maximum deviation from the least squares plane is 0.04 and 0.02 Å, respectively) forming a dihedral angle of 123.04° between each other. Thus the cycloheptene ring B exists in the hinge conformation H(6)[7] with CH_3-12 and OH-8 in a *quasi*-equatorial and OH-4 in a *quasi*-axial orientation. The lactone ring C is essentially planar and the five membered ring A has the ^{11}E conformation‡ since

†In this paper the numbering of C_{11}-methyls is merely indicative not being supported by any biosynthetic results.

‡For conformation nomenclature of the five membered ring A we followed the rules originally given for aldofuranoses (*J. Chem. Soc.* Chem. Commun 505 (1973)).

Table 1. Intramolecular bond distances (Å) for non-hydrogen atoms of **1a** with their ESD's in parentheses

C_1 - C_2	1.535 (9)	C_6 = C_7	1.329 (9)	C_8 - O_8	1.421 (8)
C_2 - C_3	1.553 (8)	C_5 = O_5	1.204 (7)	C_9 - C_{10}	1.543 (9)
C_3 - C_4	1.519 (9)	C_5 - O_{13}	1.352 (8)	C_9 - C_2	1.541 (10)
C_3 - C_{12}	1.540 (10)	C_7 - C_{13}	1.519 (9)	C_{10}- C_{11}	1.573 (10)
C_4 - C_6	1.475 (9)	C_{13}- O_{13}	1.412 (8)	C_{11}- C_1	1.509 (9)
C_4 - O_4	1.435 (8)	C_7 - C_8	1.469 (8)	C_{11}- C_{14}	1.572 (12)
C_6 - C_5	1.461 (9)	C_8 - C_9	1.523 (8)	C_{11}- C_{15}	1.513 (11)

Table 2. Bond angles (°) for non-hydrogen atoms of **1a** with their ESD's in parentheses

C_{11}-C_1-C_2	107.4 (7)	C_4 -C_6 -C_7	128.8 (7)	C_7 -C_8 -O_8	112.6 (7)
C_1 -C_2-C_3	111.8 (7)	C_5 -C_6 -C_7	109.7 (7)	C_9 -C_8 -O_8	109.9 (6)
C_1 -C_2-C_9	103.6 (6)	C_6-C_5-O_{13}	108.3 (6)	C_8 -C_9 -C_2	117.2 (6)
C_3 -C_2-C_9	118.8 (6)	C_6-C_5-O_5	130.3 (9)	C_8 -C_9 -C_{10}	110.7 (7)
C_2 -C_3-C_4	114.6 (7)	O_5 -C_5-O_{13}	121.4 (8)	C_2 -C_9 -C_{10}	107.5 (7)
C_2 -C_3-C_{12}	110.5 (7)	C_5-O_{13}-C_{13}	109.7 (6)	C_9 -C_{10}-C_{11}	104.4 (7)
C_4 -C_3-C_{12}	110.5 (8)	O_{13}-C_{13}-C_7	105.5 (6)	C_{10}-C_{11}-C_1	100.4 (7)
C_3 -C_4-C_6	116.8 (7)	C_{13}-C_7 -C_6	106.7 (6)	C_{10}-C_{11}-C_{14}	109.2 (10)
C_3 -C_4-O_4	110.7 (6)	C_{13}-C_7 -C_8	121.3 (7)	C_{10}-C_{11}-C_{15}	111.8 (10)
C_6 -C_4-O_4	110.3 (7)	C_6 -C_7 -C_8	131.8 (8)	C_1 -C_{11}-C_{14}	110.6 (10)
C_4 -C_6-C_5	121.4 (7)	C_7 -C_8 -C_9	113.0 (6)	C_1 -C_{11}-C_{15}	113.2 (9)

the four atoms C-1, C-2, C-9 and C-10 are almost coplanar (torsion angle +4.6°) while C-11 is located out of this plane, with CH_3-14 in a pseudo-axial and CH_3-15 in a pseudo-equatorial configuration. Two molecules are contained in the unit cell and are related by a two fold screw-axis; a hydrogen bond (2.74 Å) links the oxygen of the carbonyl group to OH-4.

Although lactarorufin E did not give crystals suitable for X-ray analysis, we could assign the stereostructure **1b** to this sesquiterpene by a careful examination of the complete set of ^{1}H-NMR parameters (Table 3). They will be discussed along with those of **1a**, allowing also the preferred conformation of both compounds in solution to be inferred (Fig. 2a). We deduced that lactarorufin E **1b** has the same relative configuration of lactarorufin D **1a** at C-2, C-3, C-8 and C-9 from the large values of $^3J(2, 3)$ and $^3J(8, 9)$ in both compounds (indicating a trans quasi-axial orientation of these protons). It was then clear that **1a** and **1b** are epimers at C-4. The value of $^3J(3, 4) = 10.2$ Hz for **1b** and 1.8 Hz for **1a** suggested that OH-4 is quasi-axial in lactarorufin D **1a** and quasi-equatorial, anti to H-2, in lactarorufin E **1b**. The 1,3-syn-diaxial relationship thus existing in **1a** between OH-4 and H-2 was also confirmed[8] by the marked downfield shift of H-2 in **1a** with respect to **1b** ($\Delta\delta \cong 0.57$ ppm in $CDCl_3$ and *ca* 1.03 ppm in C_6D_6). These data, together with the NOE (*ca* 3%) observed for H-3 by irradiation of H-8 (indicating the closeness of these protons), showed that the ring B of lactarorufin D and E adopts in solution the hinge conformation H(6), similar to that of **1a** in the solid state. Similarly, the cyclopentane ring A preferred conformation for **1a-b** could be established by considering the characteristic values of the coupling constants of the C-1 and C-10 protons. The corresponding signals are split in two different sets (2H each) occurring at two different regions of the ^{1}H-NMR spectra.

By NOEDS experiments (irradiation of H-8 caused a positive NOE of *ca* 3% on the cis proton H-10) we attributed the upper field resonances (at *ca* 1.3 ppm in $CDCl_3$) to H-1 and H-10 and the lower field (*ca* 1.75 ppm in $CDCl_3$) signals to H-1′ and H-10′. The latter two are mutually interacting by a long-range coupling of 2.3 Hz and thus must adopt a pseudo-equatorial orientation in order to allow the four bonds $H_{1'}$–C_1-C_{11}-C_{10}-$H_{10'}$ to form the required planar W pathway.[9] Furthermore a long range coupling constant of *ca* 0.8 Hz was observed between H-1 and H-10 and one of the methyl group at C-11, thus indicating[10] a trans-diaxial relationship between these interacting groups. These observations allowed both to infer that H-1 and H-10 have a pseudo-axial orientation on the cyclopentane ring and to assign the proper chemical shifts to the two geminal methyls. Finally, employing the DAERM method,[11] the following torsion angles of each CH–CH_2 fragment of the cyclopentane ring were calculated from the vicinal coupling constants: $\Phi_{1,2} = 164°$, $\Phi_{1',2} = 39°$, $\Phi_{9,10} = 152°$ and $\Phi_{9,10'} = 27°$ for **1a** and $\Phi_{1,2} = 164°$,

Table 3. ^{1}H-NMR chemical shifts and coupling constants[a] of **1a** and **1b**

	1a		1b			1a	1b
	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D_6	J	C_6D_6	$CDCl_3$
H-1	1.25	0.91	1.32	b	1,1′	12.0	~12
H-1′	~1.70	1.45	1.78	~1.43	1,2	12	11.0
H-2	~2.58	2.53	2.01	~1.50	1′,2	6.8	6.5
H-3	~1.70	1.22	1.20	~1.60	2,3	11.3	11.4
H-4	4.41	4.22	4.06	3.81	3,4	1.8	10.2
H-8	4.60	3.79	4.76	3.90	4,8	~1.3	2.4
H-9	~2.58	2.19	2.48	1.81	4,13	0.8	2.4
H-10	1.40	~0.98	1.35	0.82	4,13′	2.0	1.5
H-10′	~1.70	1.25	1.78	1.31	8,13	1.6	1.5
H-13	4.97	4.55	4.96	4.29	8,13′	1.2	1.5
H-13′	4.84	4.36	4.87	4.48	13,13′	18.1	18.6
CH_3-12	1.10	0.99	1.10	1.09	8,9	11.5	11.2
CH_3-14	0.98	0.82	0.97	0.77	2,9	10.2	10.0
CH_3-15	1.09	0.97	1.11	0.94	9,10	8.6	8.8
					9,10′	7.8	7.5
					10,10′	12.5	12.5
					1′,10′	2.3	2.3[c]
					3,12	6.6	6.2
					1,14	~0.8	~0.8
					10,14	~0.8	~0.8

[a] Coupling constants (Hz) are reported only for the solvent in which less overlapping occurs.

[b] Overlapped to the methyl signal.

[c] Taken from the C_6D_6 solution.

Table 4. ^{1}H-NMR chemical shifts and coupling constants (Hz) of **3a** and **3b**

	3a		3b			3a	3b
	$CDCl_3$	C_6D_6	$CDCl_3$	C_6D_6	J	C_6D_6	C_6D_6
H-1	1.41	1.01	1.36	0.95	1,1'	13.5	13.2
H-1'	1.75	1.35	1.80	1.39	1,2	6.5	6.5
H-2	2.18	1.63	2.17	1.72	1',2	7.5	7.7
H-3	2.38	~1.76	2.34	1.63	2,3	10.5	10.5
H-4	6.70	6.58	6.83	6.61	3,4	2.5	2.2
H-7	3.30	2.54	=	=	8,9	10.0	10.0
H-8	3.68	2.93	3.68	3.03	13,13'	9.0	10.0
H-9	2.38	~1.76	2.68	2.30	2,9	8.5	8.7
H-10	1.37	0.92	1.43	1.06	9,10	11.2	12.2
H-10'	1.79	1.39	1.80	1.50	9,10'	6.5	6.5
H-13	4.55	4.15	4.35	4.07	10,10'	12.2	12.2
H-13'	4.11	3.70	4.26	3.89	1',10'	1.5	1.7
CH_3-12	1.13	0.72	1.16	0.72	3,12	7.0	7.0
CH_3-14	1.02	0.83	1.02	0.84	7,13	9.0	=
CH_3-15	1.11	0.93	1.11	0.94	7,13'	9.0	=
					7,4	3.2	=
					7,3	4.5	=
					7,8	10.3	=

Table 5. ^{13}C-NMR data of blennin A **3a** and sardonialactone A **3b**[+]

	3a	multiplicity	3b	multiplicity
C-1	47.3[a]	t	47.8[a]	t
C-10	44.8[a]	t	44.7[a]	t
C-2	47.3[b]	d	45.0[b]	d
C-9	51.3[b]	d	43.1[b]	d
C-3	34.9	d	36.1	d
C-4	145.7	d	150.1	d
C-5	171.9	s	171.4	s
C-6	126.7	s	129.1	s
C-7	45.0	d	77.1	s
C-8	75.1	d	76.8	d
C-11	36.8	s	36.8	s
C-12	20.7	q	20.8	q
C-13	69.4	t	77.6	t
C-14	30.7[c]	q	30.5[c]	q
C-15	29.1[c]	q	28.6[c]	q

[+]Chemical shifts in ppm. 25.2 MHz, $CDCl_3$, TMS=0. Signal multiplicity, s=singlet, d=doublet, t=triplet, q=quartet obtained by "off-resonance" decoupling experiments.

a, b, c = assignments can be reversed.

$\Phi_{1',2} = 39°$, $\Phi_{9,10} = 154°$ and $\Phi_{9,10'} = 29°$ for **1b**. From the examination of the Dreiding models these angles correspond, approximately, to the envelope conformation ^{11}E, slightly distorted toward the $^{11}T_1$ conformation, in good agreement with the X-ray results.

During these conformational studies on lactarane lactones we have also examined, in more detail, the already known blennin A **3a**[12] and 7-OH-blennin A (sardonialactone A) **3b**.[6] The complete set of high resolution ^{1}H-NMR parameters, obtained in $CDCl_3$ and/or C_6D_6, is reported in Table 4. They confirm the previously assigned[6,12] relative configurations at C-2,. C-3, C-7, C-8 and C-9. In particular the *syn* relationship existing between H-3 and H-8, the CH_3-12 being in a pseudo-equatorial orientation, received further evidence from the positive NOE effect (*ca* 4%) observed for H-3 by irradiating H-8 and, in the case of blennin A **3a**, from the magnitude of homoallylic coupling constant $^4J(3, 7) = 4.5$ Hz. In fact this value

implies that both H-3 and H-7 form a dihedral angle near 90° with the plane of the double bond.[13] Moreover the stereochemistry assigned to the quaternary OH-7 in sardonialactone A **3b**, already proved by chemical correlation[6] of **3b** with vellerolactone,[14] was in complete agreement with the H-9 and C-9 chemical shifts. In fact the down field shift experienced by H-9 in **3b** with respect to **3a** ($\Delta\delta$ 0.3 ppm in $CDCl_3$ and 0.54 ppm in C_6D_6) can be very well explained by invoking the 1-3-syn-diaxial interaction between OH-7 and H-9. Furthermore, in the ^{13}C-NMR spectrum (Table 5), both C-2 and C-9 are shifted upfield going from **3a** to **3b**. The chemical shifts were assigned to C-2 and C-9 on the assumption that C-9 should be affected by the C-7 substituent more markedly than C-2. On this basis the upfield shift experienced by C-9 (8.2 ppm) is in good agreement with the effect exerted by an axial OH group on a γ-gauche carbon.[15]

The preferred conformations of the two blennins **3a-b** in solution (Fig. 2b) are not significantly affected by the different substitution at C-7, as shown by the great similarities of the corresponding coupling constants (Table 4).

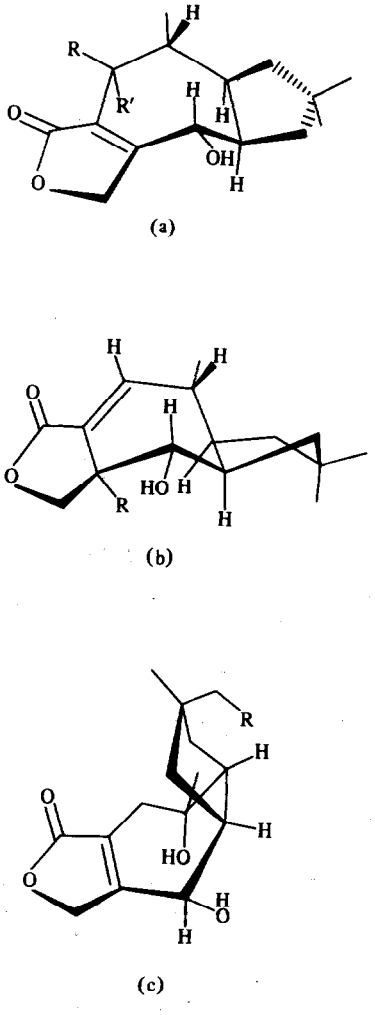


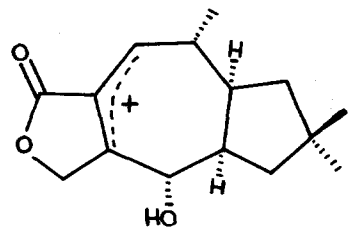
Fig. 2. Preferred conformations in solution: (a) for lactarorufin D (R=H, R'=OH) and lactarorufin E (R=OH, R'=H); (b) for blennin A (R=H) and sardonialactone A (R=OH), (c) for lactarorufin A (R=H) and lactarorufin B (R=OH).

In particular the long range coupling constants $^4J(1', 10')$, $^4J(1, 14)$ and $^4J(10, 14)$, although slightly smaller than those of **1a-b** (CH_3-14 is significantly broader than CH_3-15, but no resolved splittings have been obtained), indicate a pseudo-equatorial orientation of H-1', H-10' and CH_3-15, and a pseudo-axial orientation of H-1, H-10 and CH_3-14. Moreover the calculated torsional angles for the CH-CH_2 moieties of the cyclopentane ring are as follows: $\Phi_{1,2} = 146°$, $\Phi_{1',2} = 21°$, $\Phi_{9,10} = 164°$, $\Phi_{9,10'} = 39°$ for **3a** and $\Phi_{1,2} = 145°$, $\Phi_{1',2} = 20°$, $\Phi_{9,10} = 166°$, $\Phi_{9,10'} = 41°$ for **3b**. Such values for the torsional angles are qualitatively in agreement with the twisted conformation $^{11}T_{10}$ for the ring A, where C-10 is pushed over and C-11 under the plane C_1-C_2-C_9 (considering the formulae **3a-b** as drawn). In this conformation the dihedral angle between H-2 and H-9 is *ca* 20–30°, whereas in the conformation ^{11}E of lactarorufins D and E is close to 0° (Fig. 2a). Accordingly the value of the vicinal coupling constant $^3J(2, 9)$ decreases from *ca* 10 Hz in **1a-b** to *ca* 8.5 Hz in **3a-b**.

As far as the conformation of the ring B in **3a-b** is concerned, it should be essentially similar to that of **1a-b**. In fact the values of $^3J(2, 3)$ and $^3J(8, 9)$, although slightly smaller than in **1a-b**, indicate that these protons are in a trans pseudo-axial relationship. The small decrease of the $^3J(2, 3)$ and $^3J(8, 9)$ values in **3a-b** with respect to **1a-b** is related to the change of the conformation of ring A from ^{11}E to $^{11}T_{10}$.

It is interesting to compare the conformations of lactarorufins D and E **1a-b** (Fig. 2a) and blennins **3a-b** (Fig. 2b) with those of lactarorufins A and B **2a-b** (Fig. 2c).[16] In **2a-b** CH_3-12 is geminal to an OH group and has the (relative) configuration opposite to that of **1a–b** and **3a-b**, i.e. it is trans to the bridgehead hydrogens H-2 and H-9 and to OH-8. These structural changes have a marked effect on the molecular shape of **2a-b** (compare Fig. 2c with Figs. 2a and b). The cycloheptene ring B adopts again a hinge conformation H(6)[7] but is folded in the opposite way to that of **1a-b** and **3a-b** (considering the formulae **1-3** as drawn). By this way OH-3 and OH-8, both pseudoaxially oriented, approach very closely to each other, so they can form a strong intramolecular H-bond. Thus the molecules **2a-b** take the form of a shell, with the cyclopentane ring A bent inside the concave face of the ring B., CH_3-15 being lying almost over the γ-lactone ring. On the contrary, in both lactarorufins **1a-b** and in blennins **3a-b** the cyclopentane ring A is exo to ring B and the two five membered rings A and C are stretched in opposite directions with respect to the central ring B.

Lactarorufins D **1a** and E **1b** are the first examples of lactarane lactones bearing an oxygenated function at C-4. They have the same stereochemistry at C-3 of blennins **3a-b** and of the recently[17] isolated 3-epi-3-deoxylactarorufin A **1c**, that is CH_3-12 is syn to the hydrogens H-2 and H-9. This feature suggests that both **1a-c** and **3a-b** can arise from the same advanced presursor **4**, where the stereochemistry at C-2, C-3, C-8 and C-9 has been already fixed in some previous step of the biosynthetic pathway. A formal attack by a hydride ion either to C-4 or to C-7 can then lead to **1c** or to **3a**, while **1a-b** and **3b** can be formed by an analogous reaction of an OH^- ion either on C-4 or on C-7.



EXPERIMENTAL

All m.ps are uncorrected and were determined with a Fisher–Johns hot plate. IR spectra were recorded on a Perkin–Elmer 257 grating spectrophotometer and PMR spectra with a Bruker 300 MHz spectrometer and are calibrated in ppm (δ) downfield from TMS as an internal standard.[13]C-NMR spectra were obtained with a Varian XL-100 spectrometer. Mass spectra were run on a DuPont 21-492B instrument. Specific rotations were taken with a Perkin–Elmer automatic polarimeter. Preparative HPLC was carried out on a modified Waters instrument equipped with a RI detector, using 3/8 in. semipreparative columns packed with Lichrosorb Si 60 (15–25μ) and as eluant the mixture $CHCl_3$–hexane–isoprOH, 12:12:1.

The isolation procedure of sesquiterpenes from *Lactarius necator* has already been described in previous papers[2-4] dealing with the structure elucidation of monohydroxylactones. When a fraction, more polar of those examined previously and containing several compounds with close R_f values on TLC (Merck 60 GF_{254} plates; eluant: C_6H_6–Me_2CO, 4:1), was submitted to prep. HPLC, lactarorufin D **1a**, sardonialactone A **3b** and lactarorufin E **1b** were eluted in this order after lactarorufin A **2a** and could be obtained in a pure form. PMR data of **1a-b** and **3a-b** are reported in Tables 3 and 4. [13]C-NMR spectrum of **3b** is reported in Table 5.

Lactarorufin D **1a**. White needles from $CHCl_3$, m.p. 160–162°, $[\alpha]_D^{20}$ +93° ($CHCl_3$, c = 1). $\tilde{\nu}_{max}$($CHCl_3$) 3700, 3620, 3490, 1755, 1695 cm^{-1}; EI-mass spectrum(70 eV) *m/z*(rel. intensity) 266(2)M, 251(9), 248(25), 233(21), 230(27), 220(18), 219(45), 215(18), 202(15), 187(13), 175(20), 174(12), 160(12), 153(20), 152(27), 151(12), 149(12), 143(31), 142(51), 141(18), 140(16), 139(16), 136(13), 135(13), 126(13), 125(47), 124(100), 123(35), 122(20), 121(14), 119(13), 114(12), 113(16), 109(43), 108(12), 107(23), 105(17), 97(45), 96(41), 95(57), 93(17), 91(25), 83(20), 82(13), 81(37), 79(27), 77(27), 69(57), 68(36), 67(42), 65(14), 57(30), 56(18), 55(78), 53(33), 43(53), 41(78).

Lactarorufin E **1b**. White needles from $CHCl_3$, m.p. 125–130°, $[\alpha]_D^{20}$ +58° ($CHCl_3$, c = 1). $\tilde{\nu}_{max}$($CHCl_3$) 3690, 3630, 1740, 1690 cm^{-1}; EI-mass spectrum(70 eV) *m/z*(rel. intensity) 266(2)M, 251(12), 248(27), 237(12), 233(25), 230(25), 221(12), 220(15), 219(47), 215(16), 205(13), 202(14), 175(14), 153(19), 152(28), 151(15), 149(15), 142(47), 141(25), 140(20), 139(17), 135(15), 125(40), 124(100), 123(35), 122(18), 121(16), 119(15), 113(27), 111(11), 109(40), 107(22), 105(16), 97(42), 96(36), 95(54), 93(17), 91(22), 85(13), 83(20), 82(11), 81(46), 79(23), 77(29), 69(60), 68(32), 67(45), 65(13), 57(29), 56(14), 55(75), 53(30), 43(49), 41(81).

Sardonialactone A (7-OH-blennin A) **3b**. White needles from $CHCl_3$, m.p. 163.5-164.5°, $[\alpha]_D^{25}$ −47.8° (Me_2CO, c = 0.5), has IR and PMR spectra and TLC mobilities identical with an authentic sample.[6]

X-ray analysis of **1a**. Crystals suitable for X-ray analysis were obtained by recrystallization of **1a** from $CHCl_3$–hexane. A least squares fitting of twenty-five 2θ values, measured on a Philips PW 1100 diffractometer, revealed that the unit cell has monoclinic symmetry with the lattice constants: $a = 17.825(5)$, $b = 6.091(2)$, $c = 6.765(2)$ Å; $\beta = 93.81(1)°$; $D_{calc} = 1.20$ g/cm^3. The systematic extinctions were uniquely accommodated by space group $P2_1$ with two formula units. All unique diffraction maxima were recorded in the range $2 \leqslant 2\theta \leqslant 21°$ using graphite monochromated Mo Kα radiation ($\lambda = 0.71073$Å). Lorentz and polarization corrections were applied and correction for spheres was made for absorption. Out of 873 independent reflections measured, 695 were considered observed ($|F_0| \geqslant 3\sigma|F_0|$). The structure was solved by the MULTAN 78[18] direct phase determination procedure using the 145 normalized structure factors having the largest E-values. The phase set having the highest reliability index revealed the positions of seventeen of the nineteen non-hydrogen atoms. The remaining two non hydrogen atoms were found using a F-map. Some of the hydrogen atoms were located by a difference F-map and the others were calculated. Scattering factors were those listed in the International Tables for X-ray crystallography.[19] A full matrix least squares refinement, in which the non-hydrogen atoms were included with anisotropic thermal parameters and the hydrogen atoms with fixed isotropic thermal parameters converged to a final R value of 0.0543.

Acknowledgements—We warmly thank Dr. G. Mellerio and Dr. D. Vercesi, University of Pavia, respectively for the mass spectra and specific rotation measurements. This work was supported by the CNR, Rome (grant Relazioni internazionali) and the Polish Academy of Sciences.

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