Phytochemistry, 1979, Vol. 18, pp. 293–298. © Pergamon Press Ltd. Printed in England. 0031-9422/79/0201-0293 $02.00/0

NEW SESQUITERPENE HYDROXYLACTONES FROM *LACTARIUS* SPECIES*

MARIA DE BERNARDI, GIOVANNI FRONZA†, GIORGIO MELLERIO, GIOVANNI VIDARI and PAOLA VITA-FINZI

Istituto di Chimica Organica dell'Università, Viale Taramelli 10, 27100 Pavia, Italy; †Centro del CNR per la Chimica delle sostanze organiche naturali Politecnico, Piazza Leonardo da Vinci 32, 20133 Milano, Italy

(*Revised received* 5 *July* 1978)

Key Word Index—*Lactarius scrobiculatus*; *Lactarius blennius*; *Lactarius pallidus*; Russulaceae; Basidiomycetes; sesquiterpene lactones; γ-hydroxybutenolides; lactarolide A and B.

Abstract—Four γ-hydroxybutenolide sesquiterpenes have been isolated from *Lactarius scrobiculatus, L. blennius* and *L. pallidus*. Their structures and absolute stereochemistry were determined by spectral data and by conversion to known compounds.

INTRODUCTION

In pursuing our research on the sesquiterpenes of *Lactarius* species we isolated four new lactaranes **1a–b** and **2a–b** from *Lactarius scrobiculatus, L. blennius* and *L. pallidus*. Some results have already been reported concerning the first two species [1–3]. Compounds **1a** and **2a** were found in all three species. The corresponding ethyl ethers **1b** and **2b** were isolated from *L. blennius* and only **2b** from *L. pallidus*.

RESULTS AND DISCUSSION

In the Experimental are reported the purification of the compounds and their physical and spectral data except for the NMR data which are tabulated in Tables 1 and 2. Since **1a** and **2a** presented very similar spectra they will be discussed together. Spectral data suggested the presence of a γ-hydroxy-α,β-unsaturated butenolide system with a double bond located at the junction of two rings. In fact, the ^{13}C-NMR spectrum showed signals of a C=O group (at 172.3 and 172.1 ppm, respectively), of two unsaturated quaternary carbon atoms (at 159.4 and 126.5 ppm and at 159.4 and 130.1 ppm) and of a carbon linked to two oxygens (at 97.3 and 98.9 ppm). Furthermore the IR spectra showed bands of an unsaturated lactone C=O (at 1745–1735, 1690–1695 cm^{-1}) and of OH groups (3340–3345 cm^{-1}). This feature recalled the structure of blennin B (**12**) [3] which was confirmed by the bathochromic shift (40–50 nm) of the UV maximum of **1a** and **2a** when KOH was added (this behaviour corresponds to the transformation of the cyclic pseudo acid to the anion of the ring opened α,β-unsaturated γ-aldehydo acid [4]) as well as by the easy loss of one acetyl from the diacetyl derivatives **1c** and **2c** respectively, on treatment with ethanolic KOH (UV shift) yielding **1d** and **2d**. The IR spectra of **1c** and **2c** showed that, besides the two acetylated hydroxyls, a tertiary OH was still present in both molecules.

The NMR data together with the oxygenated functional groups (three OH and a lactone ring) indicated the molecular formula $C_{15}H_{20}O_5$ for both compounds. On the basis of the lactarane skeleton and of the PMR data the position of the substituents could be determined. Three singlets (3H each) were attributed to the *gem.* methyls at C-11 and to the methyl at C-3 (δ1.26) which must therefore be geminal to the *tert*-OH. The chemical shift of the doublet ($J \simeq 3$ Hz, 1H) at δ 4.28 and 4.36 respectively for **1a** and **2a**, shifting down field by *ca* 1.4 ppm upon acetylation, suggested that the third OH was allylic to the butenolide double bond. Moreover on irradiation of the multiplet at δ 2.6–2.9 (cyclopentane ring methyne protons) the doublet changed to a singlet indicating the hydroxyl was at C-8. The complicated signals at δ 2.4–2.9 (4H) contained an AB system (centred at δ 2.59 for **1a** and at δ 2.86 for **2a**) which was attributed to the isolated CH_2 in position 4. Decoupling experiments showed also that the signals of the C-2, C-4 and C-9 protons were partially overlapped.

At this point it was likely that **1a** and **2a** were isomers, the isomerism arising from the possible modes of linking the O=C(↓)—O—CHOH-group to the C-6 and C-7 atoms of the lactarane skeleton, that is they had either C=O in position 5 and OH at C-13 or C=O in position 13 and OH at C-5.

Distinction between the two isomers was possible on the basis of the homoallylic coupling constant between the protons at C-4 and the protons at C-13. For instance, in the case of lactarorufin A(**8a**) [3, 5] and blennin B (**12**) [3], which have the C=O in position 5, we have observed a homoallylic coupling constant of about 2 Hz between the C-13 and C-4 protons. In the present case the C-13 proton at δ 6.11 of **1a** was a triplet (J = 1.7 Hz) collapsing into a singlet on irradiation of the two C-4 protons at δ 2.6, whereas in the case of **2a** the C-5 proton at δ 5.89 was coupled only with the *gem.* OH. It followed that the carbonyl group is placed at C-5 in compound **1a**, named lactarolide A, and at C-13 in compound **2a**, named lactarolide B.

The NMR spectrum of **2a** was actually complicated by the presence of the two epimers (1:1) which equilibrate through the aldehydo acid. In the case of **1a** one of the

* Part 4 in the series "Fungal metabolites". For Part 3 see Vidari, G., De Bernardi, M., Vita-Finzi, P. and Fronza, G. (1976) *Phytochemistry* **15**, 1953.

Table 1. PMR data*

	C-4	C-4′	C-5	C-13	C-8	C-3CH$_3$
1a	2.68 *dd*	2.50 *dd*		6.11 *t*	4.28 *d*	1.26 *s*
1c ($CDCl_3$)	2.68†			6.9 *t*	5.70 *d*	1.24 *s*
1b	2.67 *dd*	2.55 *dd*		5.96 *t* 6.10 *t*	4.19 *d* 4.24 *d*	1.21 *s*
1e	2.65†			6.90 *t*	5.91 *d*	1.20 *s*
8a ($CDCl_3$)	2.70 *dt*	2.52 *dt*		4.90 *dt* 4.58 *dt* (C-13′)	4.10 *d*	1.30 *s*
8b ($CDCl_3$)	2.76 *brd*	2.48 *dt*		4.99 *dt* 4.56 *brd* (C-13′)	4.08 *dd*	1.19 *s*
3	2.74†			6.3 *t*		1.35 *s*
2a	2.94 *d*	2.78 *d*	5.89 *d*§ 5.93 *d*§		4.36 *d*§ 4.39 *d*§	1.26 *s*
2c ($CDCl_3$)	2.82 *d*	2.54 *d*	6.72 *s* 6.78 *s*		5.84 *d*	1.25 *s*
2b	2.86 *d* 2.93 *d*	2.77 *d* 2.75 *d*	5.94 *d*		4.23 *dd*§ 4.27 *dd*§	1.25 *s* 1.26 *s*
2e ($CDCl_3$)	2.60 *s*		6.71 *s* 6.74 *s*		5.74 *d*	1.25 *s* 1.27 *s*
9 ($CDCl_3$)	2.9–2.4		4.58 *s* (2H)		2.7 *m* (2H)†	1.28 *s*
10a ($CDCl_3$)	2.84 *d*	2.52 *d*	4.64 *s* (2H)		4.62 *d*	1.25 *s*
10a (C_6D_6)	2.08 *s*		3.83 *d* 4.06 *d* (C-5′)		4.70 *d*	1.09 *s*
10b ($CDCl_3$)	2.89 *d*	2.58 *d*	4.67 *s* (2H)		5.87 *br*	1.23 *s*
10c ($CDCl_3$)	2.68 *s*		4.62 *s* (2H)		4.40 *dd*	1.22 *s*
10c (C_6D_6)	1.92 *s*		3.98 *s* (2H)		4.70 *dd*	0.93 *s*
10c	2.85 *s*		4.67 *s* (2H)		4.24 *dd*	1.22 *s*
11b ($CDCl_3$)	2.94 *d*	2.75 *dd*	7.17 *m*	7.40 *d*	4.60 *br*	1.16 *s*

two epimers predominated. Examination of Dreiding models of **1a** showed that C-13 OH is probably in the more stable α-configuration and that it can form a strong hydrogen bond with the C-8 OH. However acetylation of **2a** to **2c** shifted the epimeric ratio and resulted in simpler spectra.

As regards the other two compounds, **1b** and **2b**, they were less polar (TLC R_f) but showed spectral data very similar to **1a** and **2a**. The signals relative to a γ-hydroxybutenolide ring, of a $=\overset{|}{C}-CHOH-\overset{|}{C}H-CH_2$-group and of the characteristic 3,4-disubstituted-1,1-dimethylcyclopentane system were again evident. From the MW (M^+ 310) and from the ^{13}C-NMR spectrum the molecular formula $C_{17}H_{26}O_5$ was deduced for both compounds. A comparison of the PMR data of **1a–b** and **2a–b** showed that ethylation of an OH group accounted for the further two carbon atoms.

The location of the OEt group at C-3 was deduced by

Table 2. ^{13}C-NMR data*‡

Compound	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		—OCH_2CH_3	
1a	45.8 *t*		50.3 *d*	47.1 *d*	74.9 *s*	35.3 *t*	172.3 *s*	126.5 *s*	159.4 *s*	66.5 *d*	37.3 *s*	31.2 *q*	97.3 *d*	29.4 *q*	26.9 *q*		
			50.6 *d*	47.4 *d*	74.5 *s*	35.8 *t*	172.4 *s*		158.3 *s*	67.6 *d*			100.0 *d*				
1b	45.8 *t*	45.6 *t*	49.3 *d*	47.3 *d*	81.1 *s*	29.8 *t*	172.7 *s*	125.1 *s*	160.4 *s*	66.5 *d*	37.1 *s*	24.9 *q*	97.3 *d*	29.6 *q*	27.2 *q*	57.4 *t*	15.7 *q*
			49.4 *d*	47.8 *d*						67.6 *d*		25.4 *q*	100.2 *d*				
8a	45.5 *t*	45.3 *t*	49.1 *d*	46.2 *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*	26.4 *q*		
2a	46.1 *t*		50.7 *d*	47.1 *d*	74.5 *s*	38.4 *t*	98.9 *d*	159.4 *s*	130.1 *s*	65.5 *d*	37.3 *s*	31.7 *q*	172.1 *s*	29.3 *q*	26.6 *q*		
			50.5 *d*		74.4 *s*	38.0 *t*	98.1 *d*	159.2 *s*	129.9 *s*								
2b	46.3 *t*	45.8 *t*	49.2 *d*	47.6 *d*	81.0 *s*	33.2 *t*	98.5 *d*	157.0 *s*	131.4 *s*	65.5 *d*	37.1 *s*	25.6 *q*	172.0 *s*	29.4 *q*	26.7 *q*	57.6 *t*	15.7 *q*
				47.5 *d*		32.3 *t*	97.6 *d*	156.9 *s*	131.2 *s*			25.5 *q*				57.4 *t*	
11b	45.4 *t*	45.2 *t*	49.3 *d*	46.9 *d*	80.5 *s*	28.3 *t*	142.3 *d*‡	118.5 *s*	127.2 *s*	66.7 *d*	36.8 *s*	25.0 *q*	139.8 *d*‡	29.8 *q*	27.6 *q*	56.8 *t*	15.3 *q*

*25.2 MHz. $CDCl_3$ for **8a** and **11b**, d_6-Me_2CO for **1a–b** and **2a–b**. Chemical shifts in ppm from TMS. Signal multiplicity obtained by 'off resonance' decoupling experiments.

† In the case of **1a–b** and **2a–b** the presence of two epimeric hemiacetals causes a doubling of many signals.

‡ The assignments of these signals may be reversed.

C-11 CH_3	CH_3CO	OCH_2CH_3‡		$J_{4\text{-}4'}$	$J_{8\text{-}9}$	$J_{13\text{-}4}$	$J_{13\text{-}4'}$	Others
1.04 *s*, 1.02 *s*				19.0	3.7	1.7	1.7	5.20 (1H *br*, OH); 5.94 (1H *br*, OH); 6.68 (1H *br*, OH).
1.09 *s*, 1.00 *s*	2.05 *s* 2.09 *s*				7.0	~1	~1	
1.06 *s*, 1.03 *s*		3.59 3.24	1.10	20.0	3.0	1.3	1.3	
1.13 *s*, 1.05 *s*	2.05 *s* 2.10 *s*	3.36 3.27	1.10		9.0	~1	~1	
1.04 *s*, 1.02 *s*				20.0	3.0	2.6	2.6	$J_{13'\text{-}4} = J_{13'\text{-}4'} = 2.6$; $J_{13'\text{-}13} = 17.0$
1.04 *s*, 1.01 *s*		3.23 3.56	1.14	19.0	3.0	2.5	2.5	$J_{13'\text{-}4} = 1.0\, J_{13'\text{-}4'} = 2.5$; $J_{13\text{-}13'} = 17.0\, J_{8\text{-}OH} = 11.5$; 5.66 (*d*, C_8-O<u>H</u>)
1.09 *s*, 1.04 *s*						1.5	1.5	6.70 (1H, *br*, OH)
1.00*s*, 1.00 *s*				20.0	3.0			5.7 (2H, *br*, 2OH); 6.66 (1H, *br*, OH)
1.06 *s*, 1.00 *s*	2.11 *s* 2.18 *s*			19.0	4.0			
1.02 *s*, 1.02 *s*		3.54†	1.12	20.0	3.0			5.32 *d* (C_8-O<u>H</u>) 6.63 *d* and 6.77 *d* (C_5-O<u>H</u>) $J_{5\text{-}OH} = 7.0$ $J_{8\text{-}OH} = 11.5$
1.03 *s*, 0.99 *s*	2.12 *s* 2.18 *s*	3.52†	1.13		7.0			
1.07 *s*, 1.00 *s* 1.02 *s*, 1.00 *s*				19.0	4.0			
0.86 *s*, 0.79 *s*					3.0			$J_{5\text{-}5'} = 17.0$
1.03 *s*, 0.98 *s*	2.09 *s*			19.0				
1.02 *s*, 1.00 *s*		3.35 3.63	1.19		3.0			5.35 *d* (C_8-O<u>H</u>) $J_{8\text{-}OH} = 11.3$
0.82*s*, 0.78 *s*		3.0†	1.02		3.0			5.30 *d* (C_8-O<u>H</u>) $J_{8\text{-}OH} = 11.3$
1.00 *s*, 1.00 *s*		3.53†	1.10		2.5			5.17 *d* (C_8-O<u>H</u>) $J_{8\text{-}OH} = 11.3$
0.99 *s*, 0.99 *s*		3.34 3.53	1.03	17.0				$J_{5\text{-}13} = 2.0\, J_{5\text{-}4'} = 1.5$

* 100 MHz—Chemical shifts quoted in δ units relative to TMS ($\delta = 0$). J in Hz. d_6-Me_2CO if not otherwise indicated. Signals of C-1 and C-10 methylenes and C-2 and C-9 methynes are complicated multiplets within δ 1.30–1.80 and δ 2.50–3.00 respectively for all compounds. Some signals are doubled for the simultaneous presence of two epimeric hemiacetals.

† Centre of the signal.

‡ Analysed as ABX_3 system.

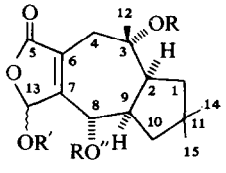
§ Data determined by decoupling experiments.

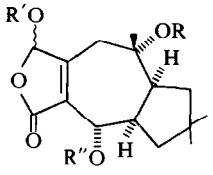
the presence of two acetylable hydroxyls (at C-8 and on the butenolide ring) and of a tertiary C-3 CH_3 (*s* at δ 1.21 and 1.25) which must then be *geminal* to the last oxygenated substituent. According to this attribution, the ^{13}C-NMR signal of this CH_3 is shifted to higher field by $\simeq$6 ppm in comparison with **1a** and **2a** because of the γ-effect of the alkyl substituent.

Again, as previously described for **1a**, we could establish that **1b** was 3-*O*-ethyllactarolide A, on the basis of the homoallylic coupling constant (J = 1.3 Hz) shown by the C-13 proton at $\delta \simeq 6$ with the isolated CH_2 group centred at δ 2.61.

The NMR data of **2b** were complicated by the almost equal amount of the two epimers which caused the doubling of some signals. Nevertheless the absence of homoallylic coupling constants of the C-5 proton at δ 5.94 led to us to conclude that **2b** was 3-*O*-ethyllactarolide B.

The oxidation with CrO_3 or $Na_2Cr_2O_7$ of lactarolide A and B, with the aim of obtaining the same anhydride was unsuccessful. Compound **1a** yielded the 8-dehydro-



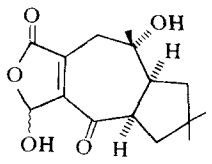


1a R = R′ = R″ = H
1b R = Et; R′ = R″ = H
1c R = H; R′ = R″ = COMe
1d R = R′ = H; R″ = COMe
1e R = Et; R′ = R″ = COMe

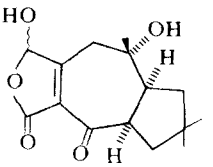
2a R = R′ = R″ = H
2b R = Et; R′ = R″ = H
2c R = H; R′ = R″ = COMe
2d R = R′ = H; R″ = COMe
2e R = Et; R′ = R″ = COMe

derivative (3) in good yields while from **2a** very complicated mixtures were obtained; in this case structure **4** was tentatively assigned to a compound isolated in a very poor yield.

From the reduction of the butenolide ring of **1a** and **2a** with three different reagents either the same compounds or compounds with known stereochemistry were obtained. Reaction of **1a** with $LiBH_4$ in refluxing THF led



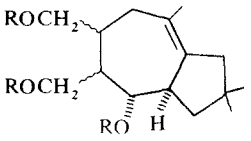
3



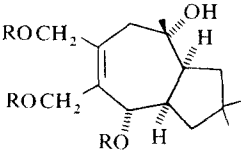
4

mainly to **5a** in very low yields by reduction of the lactone ring (**5b**; PMR: *m*, 4H, δ 3.9–4.3, $2CH_2O$) and dehydration of the *tert*-OH (**5b**; PMR: *s*, 3H, δ 1.6, CH_3—C=).

Reduction of **1a** with $LiBH_4$ at room temp. gave a mixture of the tetraols **6a** and **7a**. In this case reduction



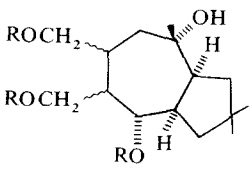
5a R = H
5b R = COMe



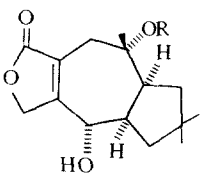
6a R = H
6b R = COMe

of the hydroxybutenolide system occurred in part along with saturation of the conjugated double bond (see in the Experimental the PMR of the mixture of the acetyl derivatives). These results confirmed the instability of these molecules towards the strong reducing hydrides, as already observed in the case of lactarorufin A (**8a**) with $LiAlH_4$ [5].

A milder reduction of **1a** with $NaBH_4$ afforded only lactarorufin A (**8a**) [5–7] which was identified by comparison with an authentic sample [3]. The similarity of



7a R = H
7b R = COMe



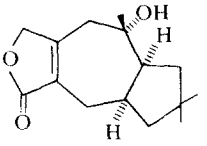
8a R = H
8b R = Et

the α_D value also indicated the absolute stereochemistry of **1a** as shown in the formula. By treatment of **2a** with the same reagent, two compounds **9** and **10a** were obtained. To the former the structure **9** (5,8-desoxylactarolide B) was assigned on the basis of the following data: (a) MW 250 (MS) indicating that two CHOH groups had been reduced to CH_2; (b) the absence in the PMR spectrum of the signals of CHOH; (c) the presence in the IR of a *tertiary* OH and of an α,β-unsaturated-γ-lactone (1740 and 1678 cm^{-1}); (d) two sharp singlets for the lactone CH_2O group (2H, δ 4.58) and for the CH_3 at C-3 (3H, δ 1.28).

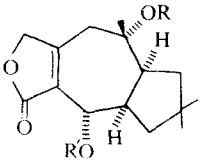
In the second product (**10a**) reduction occurred only on the hemiacetalic CHOH. In fact, by acetylation with Ac_2O/Py, a monoacetyl derivative (**10b**) was obtained as was clearly demonstrated by the OH band in the IR and the PMR chemical shift of the C-8 proton. It is interesting to note that the PMR spectrum of **10a**, recorded in $CDCl_3$, showed two isolated CH_2 groups. An AB system centred at δ 2.68 was attributed to the CH_2 at C-4 and a singlet at δ 4.64 to the lactone methylene. By contrast in C_6D_6, the C-4 protons became a singlet (δ 2.08), while the other methylene gave an AB system (δ 3.95). In any case, as expected, the CH_2O signals were not further coupled.

On this evidence we affirmed that **10a** (and therefore also **2a**) must have the C=O at C-13. The structure of **10a** is then isomeric to that of lactarorufin A [6] and is exactly the same as previously assigned to this sesquiterpene [5].

Similar treatment of **1b** and **2b** with $NaBH_4$ afforded the 3-*O*-ethyl lactarorufin A (**8b**) and 3-*O*-ethyl-5-desoxylactarolide B (**10c**), as demonstrated by analogous arguments.



9



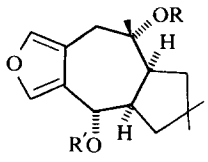
10a R = R′ = H
10b R = H; R′ = COMe
10c R = Et; R′ = H

The DIBAL-H reduction [8] of the lactone ring of **1a** and **2a** yielded in both cases the already known furandiol (**11a**) [9] that we also isolated from *Lactarius* species [2, 3]. The same reaction on the ethyl derivatives **8b** and **10c** afforded the 3-*O*-ethylfuran alcohol (**11b**) [10], identical to the compound we isolated from *L. pallidus* [11]. Finally we demonstrated that **11a** and **11b** had the same stereochemistry by the synthesis of the 3,8-di-*O*-ethyl derivative **11c** from both compounds [11]. In this way by correlation with the known absolute configuration of lactarorufin A [6, 7], the stereochemistry of all our sesquiterpenes was completely established as shown in the formulae. In previous papers [2, 3] only the relative configuration of **11a** has been indicated.

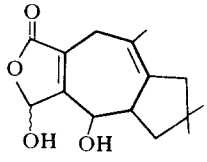
Compounds **1a–b** and **2a–b**, along with blennin B (**12**) [3], are the only representatives of the class of γ-hydroxybutenolides isolated until now from *Lactarius* mushrooms.

γ-Hydroxybutenolides such as **1a** and **2a** can well be precursors of the two kinds of lactones found in the *Lactarius* species: those with the carbonyl at C-5 and the few until now isolated only from *L. scrobiculatus* [1, 2] with the carbonyl at C-13. By analogy with the behaviour of other furan compounds [12], an alcoholic solution of **11a** was exposed to air in the light and the reaction was followed by TLC. A few days later the TLC showed, besides the spots of the starting material and of other polar products, those corresponding to lactarorufin A (**8a**) and to lactarolide A (**1a**) and B (**2a**). It is interesting to note that even traces of 5-desoxylactarolide B (**10a**) were never detected. These experiments confirmed the possibility of transforming lactarane furans into the corresponding lactones and it will be worth while to investigate how these interconversions occur in nature.

In conclusion we believe that **1a** and **2a** are genuine natural products, even if air or light could not be excluded during the work-up of the mushrooms and the isolation procedures. In fact their quantitative distribution varied very irregularly in different lots of materials and not always could they be found together with **11a** in other *Lactarius* species [11]. On the other hand the 3-*O*-ethyl derivatives **1b** and **2b** are probably artefacts due to the solvent of extraction, as was already demonstrated in the case of **11b** [10].



11a R = R′ = H
11b R = Et; R′ = H
11c R = R′ = Et



12

EXPERIMENTAL

All mps were determined with a Fisher–Johns hot plate and are uncorr. Chromatography was carried out on Si gel (Kieselgel HR 60 Merck) and monitored by TLC or GLC. The compounds were visualized as coloured spots by spraying the plates with a vanillin/H_2SO_4 soln. The yields reported correspond to chromatographically pure compounds.

Isolation of sesquiterpenes **1a** *and* **2a** *from* L. scrobiculatus. The last fractions of an ethanolic extract, separated as previously described by column chromatography [1, 2], contained two compounds (**1a** and **2a**) which were visualized as red spots on TLC with a very close R_f. The mixture of **1a** and **2a** was chromatographed many times on columns or on PLC in order to obtain pure compounds. Separation was afforded using as eluent C_6H_6–EtOAc (1:1) or *iso*-Pr_2O–EtOAc (from 2:1 to 1:1) or $CHCl_3$–EtOAc (2:3 and 1:1). Similarly **1a** and **2a** were obtained from an EtOH extract of *L. blennius* and *L. pallidus*.

Lactarolide A (**1a**): 170 mg, white solid, mp 153–155° (CH_2Cl_2–pentane), red spot. $[\alpha]_D^{20}$ +59.84° (Me_2CO), λ_{max}^{EtOH} nm (log ε): 214 (3.87), $\lambda_{max}^{EtOH/KOH}$ nm (log ε): 254 (3.75), ν_{max}^{nujol} cm^{-1}: 3340 (OH), 1745 (butenolide C=O), 1695 (C=C). MS (probe) 70 eV, *m/e* (rel. int.): 282 (M^+, <1), 264 (16), 246 (16), 231 (10), 228 (23), 215 (20), 213 (16), 203 (19), 168 (22), 155 (100), 134 (23), 122 (28), 107 (19), 98 (39), 84 (28), 74(29), 69 (20), 55 (32.7), 43 (46), 41 (40).

Lactarolide B (**2a**): 120 mg, white solid, mp 212–216° (Me_2CO–Et_2O) red violet spot, $[\alpha]_D^{20}$ −3.53° (Me_2CO), λ_{max}^{EtOH} nm (log ε): 213 (3.84), $\lambda_{max}^{EtOH/KOH}$ nm (log ε): 264 (3.74), ν_{max}^{nujol} cm^{-1}: 3345 and 3300(OH) 1735 (butenolide C=O), 1692 (C=C). MS (probe) 70 eV, *m/e* (rel. int.): 282 (M^+, <1), 264 (5), 246 (13.5), 231 (6), 228 (4.5), 221 (4), 218 (12), 217 (11), 204 (9.5), 203 (12.5), 190 (12.5), 185 (9), 175 (11), 167 (9), 147 (9), 140 (9), 125 (9), 121 (9), 107 (9), 105 (7), 95 (19), 91 (20), 81 (19), 79 (19), 77 (14), 69 (20), 67 (14), 65 (12), 55 (29), 53 (19), 51 (9), 43 (100), 41 (56).

Acetylation of **1a**, **1c** *and* **1d**. 8,13-Diacetyllactarolide A (**1c**) was obtained in quantitative yields by acetylation of **1a** with Ac_2O and was not purified. $[\alpha]_D^{20}$ −7.9° ($CHCl_3$), $\nu_{max}^{thin\ film}$ cm^{-1}: 3520 (OH), 1780–1750 (butenolide C=O and MeCO), 1700 (C=C), λ_{max}^{EtOH} nm (log ε): 220 (3.84). The UV spectrum recorded soon after the addition of a few drops of ethanolic KOH to **1c** in EtOH showed a maximum at 231 nm, but after *ca* 0.5 hr the band was shifted to 254 nm. Usual work-up of the soln afforded **1d** (8-acetyllactarolide A): mp 143–147° (*iso*Pr_2O–pentane), λ_{max}^{EtOH} nm (log ε): 210 (3.90), $\lambda_{max}^{EtOH/KOH}$ nm (log ε): 254 (3.78), ν_{max}^{KBr} cm^{-1}: 3350 and 3200 (OH), 1750 and 1735 (C=O), 1690 (C=C).

Acetylation of **2a**, **2c** *and* **2d**. Acetylation of **2a** (20 mg) by the usual procedure gave quantitative yields of 5,8-diacetyllactarolide B (**2c**) which was not further purified. $[\alpha]_D^{20}$ −24.84° (Me_2CO). λ_{max}^{EtOH} nm (log ε): 215 (3.84). Deacetylation of **2c** with base to **2d** (8-acetyllactarolide B) proceeded rapidly. The reaction was easily followed by observing the increasing intensity of a new band in the UV spectrum at 262 nm.

Oxidation of **1a** *to 8-dehydrolactarolide A*(**3**) *with* $Na_2Cr_2O_7$–H_2SO_4. A soln of 25 mg of **1a** in 1 ml of Me_2CO was cooled in an ice bath and treated, under stirring, with a few drops of cooled Jones' soln until the orange colour was persistent. The mixture was kept at room temp. for 1 hr. Then Me_2CO was evapd and the soln extracted exhaustively with CH_2Cl_2. After evapn of the solvent the residue was separated by CC (CH_2Cl_2–EtOAc 2:1) to yield 21 mg of **3**, white solid, mp 176–178° (Et_2O–pentane), yellow-red spot, $[\alpha]_D^{20}$ +27.86 (Me_2CO), λ_{max}^{EtOH} nm (log ε): 230 (3.89), 216 sh (3.82), $\lambda_{max}^{EtOH/KOH}$ nm (log ε): 313 (4.05), ν_{max}^{KBr} cm^{-1}: 3410 and 3240 (OH), 1760, 1690 and 1675 (—OCO—C=C—CO system). MS (probe) 70 eV, *m/e* (rel. int.): 280 (M^+, <1), 265 (4.5), 262 (20), 247 (7.5), 244 (19), 237 (10.5), 229 (18.5), 220 (16.5), 219 (26), 218 (16), 206 (9), 201 (17.5), 189 (5.5), 175 (8), 173 (8), 166 (20), 161 (11.2), 145 (15), 141 (9.5), 128 (10), 123 (21), 122 (13.5), 107 (19), 105 (17), 95 (23), 91 (38), 79 (18), 77 (16), 69 (14), 55 (22), **43** (100), 41 (45).

The same compound **3** was obtained by oxidation of **1a** with $PyHCrO_3Cl$ or with CrO_3–AcOH.

Reduction of **1a** *with* $LiBH_4$ *in refluxing THF*. **1a** treated with $LiBH_4$ in THF was refluxed for 0.5 hr under N_2. After acidification with diluted HCl, the reaction mixture was extracted with EtOAc. The MS of the residue showed besides the peak at *m/e* 236 ($M^+ - H_2O$) for **5a**, a higher peak at *m/e* 252 (M^+) indicating the presence of a small amount of the 6,7-dehydro derivative of **5a**. Acetylation of **5a** yielded **5b**. PMR($CDCl_3$, δ): 1.6 (*s*, 3H, CH_3—C=): 2.15 (*s*, 6H) and 2.4 (*s*, 3H, $3CH_3CO$); 3.9–4.3 (*m*, 4H, CH_2O).

Reduction of **1a** *with* $LiBH_4$. In a similar way the reaction at room temp. afforded a mixture of **6a** and **7a** which were acetylated to **6b** and **7b**. PMR of **6b** ($CDCl_3$, δ): 1.26 (*s*, 3H, CH_3—C—O), 4.58 and 4.84 (AB system, 2H, J = 12.0 Hz, CH_2O); 4.58 and 4.88 (AB system, 2H, J = 12.0 Hz, CH_2O); 5.56 (*d*, 1H, J = 11.0 Hz, CHO-) PMR of **7b**: 1.29 (*s*, 3H, CH_3—C—O); 3.9–4.2 (*m*, 4H, $2CH_2O$); 3.24 (*m*, 1H, CHO—).

Reduction of **1a** *with* $NaBH_4$ *to yield lactarorufin A* (**8a**) [5, 7]. 21 mg of **1a** were treated with 25 mg of $NaBH_4$ in EtOH for 15 min. The reaction mixture was then poured into ice and acidified with 5% HCl. After evapn of EtOH *in vacuo*, the H_2O soln was neutralized with $NaHCO_3$ and extracted with $CHCl_3$. Evapn of the solvent gave 17 mg of crude residue. By purification on CC 12 mg of **8a** were obtained, mp 166–168° (Et_2O–hexane), $[\alpha]_D^{20}$ +6.28 ($CHCl_3$). The product was identified as lactarorufin A by comparison with an authentic sample [3].

$NaBH_4$ *reduction of* **2a**. 18 mg of **2a** were treated with 20 mg of $NaBH_4$ in EtOH for 20 min. The usual work-up, as described for **1a**, gave 15 mg of a mixture of products; **9** (4 mg) and **10a** (5 mg) were obtained by separation on CC (eluent: $CHCl_3$–EtOAc 1:1) **9** (5,8-*desoxylactarolide B*): pale yellow oil, violet spot, $[\alpha]_D^{20}$ + 31° (Me_2CO), λ_{max}^{EtOH} nm (log ε): 219 (3.84), ν_{max}^{film} cm^{-1}: 3350 (OH), 1740 (butenolide C = O), 1678 (C=C). MS (probe) 70 eV: 250 (M^+), 235, 232, 217, 139 (100), 122, 121, 112, 111. **10a** (5-*desoxylactarolide B*): mp 68–72° (crude), green-blue spot, $[\alpha]_D^{20}$ +9.53° (Me_2CO), λ_{max}^{EtOH} nm (log ε): 217 (3.92), ν_{max}^{nujol} cm^{-1}: 3330 (OH), 1745 (butenolide C=O), 1675 (C=C). MS (probe) 70 eV, *m/e* (rel. int.): 248 ($M^+ - H_2O$, 19), 233 (13), 217 (9), 215 (10), 206 (9), 205(9), 191 (17), 174 (15), 169 (12), 151 (15), 145 (9), 141 (10), 123 (10), 119 (9), 107 (10), 105 (14), 95 (28), 91 (16), 83 (15), 81 (13), 77 (15), 69 (20), 67 (10), 59 (18), 57 (13), 55 (27), 53 (15), 43 (100), 41 (48). Acetylation of **10a** (2 mg) with Ac_2O/Py gave quantitative yields of 8-*acetyl-5-desoxylactarolide B* (**10b**): $[\alpha]_D^{20}$ +22° ($CHCl_3$), ν_{max}^{film} cm^{-1}: 3450 (OH), 1760 (C=O), 1685 (C=C), 1240 (acetate C—O).

Diisobutylaluminium hydride (*DIBAL-H*) *reduction of* **1a** *and* **2a**. 20 mg of **2a**, dissolved in dry THF, and cooled to −10°, were treated with stirring and under N_2 with an excess of DIBAL-H in THF and left for 1 hr at −10°–0°C. Then the mixture was acidified with 10% H_2SO_4, diluted with H_2O and extracted with Et_2O. Usual work-up of the organic phase gave a residue which was purified by PLC (GF_{254} Merck; C_6H_6–EtOAc 1:1). Besides unreacted **2a**, 4.5 mg of furandiol **11a** [9] was obtained, $[\alpha]_D^{20}$ +28.5° ($CHCl_3$) (lit. [2] $[\alpha]_D^{20}$ +30.8° ($CHCl_3$)). The same procedure was followed in the case of **1a**, giving **11a**, $[\alpha]_D^{20}$ +23° ($CHCl_3$).

Isolation and purification of **1b** *and* **2b**. CC of an ethanolic extract of *L. blennius* [3], eluted with C_6H_6–EtOAc mixtures separated **1b** from the more polar **2b**. Similarly **2b** was obtained from an ethanolic extract of *L. pallidus* [11] which had been worked up in the same way as that of *L. blennius* [3]. 3-O-*ethyllactarolide A* (**1b**), white solid, mp 171–173° (Et_2O–pentane), red spot, $[\alpha]_D^{20}$ +18.47 (Me_2CO), λ_{max}^{EtOH} nm (log ε): 206 (4.08), $\lambda_{max}^{EtOH/KOH}$ nm (log ε): 252 (3.94), ν_{max}^{nujol} cm^{-1}: 3350 and 3310 (OH),

1740 (butenolide C=O), 1692 (C=C). MS (probe) 70 eV, *m/e* of the main peaks: 310 (M^+), 292 ($M^+ - H_2O$), 277 ($M^+ - H_2O - CH_3$), 265 ($M^+ - OEt$), 264 ($M^+ - H_2O - C_2H_4$), 168 (base peak, $M^+ - H_2O - C_2H_4 -$ ), 122, 95, 43. 3-O-*ethyllactarolide B* (**2b**), white solid, mp 180–183° (Me_2CO–pentane), red spot, $[\alpha]_D^{20}$ +2.10 (Me_2CO), λ_{max}^{EtOH} nm (log ε): 206 (4.04), $\lambda_{max}^{EtOH/KOH}$ nm (log ε): 265 (3.75), ν_{max}^{nujol} cm^{-1}: 3390 (OH), 1760 (C=O), 1690 (C=C). MS (probe) 70 eV, *m/e* (rel. int.): 310 (M^+, <1), 264 (15), 246 (23), 231 (12), 228 (15), 218 (28), 213 (88), 203 (30), 195 (34), 190 (38), 175 (13), 167 (22), 162 (14), 139 (14), 123 (18), 107 (18), 95 (26), 91 (20), 81 (25), 69 (20), 55 (28), 43 (100), 41 (41).

$NaBH_4$ *reduction of* **1b** *to* 3-O-*ethyllactarorufin A* (**8b**). 43 mg of **1b** were treated with $NaBH_4$ as previously described for **1a**. From the reaction residue **5b** (21 mg) was obtained as a viscous oil by CC (eluent C_6H_6–EtOAc 4:1). Blue-green spot, $[\alpha]_D^{20}$ −17° ($CHCl_3$), λ_{max}^{EtOH} nm (log ε): 218 (3.85), ν_{max}^{film} cm^{-1}: 3400 (OH), 1758 (butenolide C=O), 1680 (C=C). MS (probe) 70 eV, *m/e* (rel. int.): 294 (M^+, 7), 279 (3), 261 (3), 248 (41), 233 (10), 206 (15), 169 (14), 151 (15), 122 (23), 109 (22) 95 (20), 81 (16), 69 (14), 55 (22), 43 (100), 41 (36).

$NaBH_4$ *reduction of* **2b** *to* 3-O-*ethyl*-5-*deoxylactarolide B* (**10c**). From 60 mg of **2b**, treated with $NaBH_4$ and worked up in the usual way **10c** (15 mg) was obtained; violet spot, mp 149–152° (Et_2O–pentane), $[\alpha]_D^{20}$ +9.1° ($CHCl_3$), λ_{max}^{EtOH} nm (log ε): 216 (3.89), ν_{max}^{KBr} cm^{-1}: 3380 (OH), 1750 (C=O), 1685 (C=C). MS (probe) 20 eV, *m/e* (rel. int.): 294 (M^+, 1), 276 (12), 249 (13), 248 (41), 235 (17), 233 (18), 231 (25), 230 (22), 217 (33), 215 (13), 206 (11), 205 (15), 198 (11), 197 (100), 192 (12), 191 (35), 190 (16), 153 (10), 151 (26), 123 (17), 95 (21), 43 (21).

DIBAL-H reduction of **8b** *and* **10c**. The above described procedure was followed for reducing **8b** and **10c** with DIBAL-H. From 15 mg of **8b**, after purification by PLC (GF_{254} Merck, C_6H_6-EtOAc 2:1), 5.2 mg of furanol **11b** were obtained, $[\alpha]_D^{20}$ +20.6° ($CHCl_3$), ν_{max}^{film} cm^{-1}: 3420 (OH), 1540 and 885 (furan), identical to an authentic sample of **11b** [10] from *L. pallidus* [11]. From 12 mg of **10c**, 3.3 mg of the same compound were obtained: $[\alpha]_D^{20}$ +17.3° ($CHCl_3$).

Acknowledgements—We thank Dr. Gianernesto Leoni of the Mycological Control Service (Milano) for the identification of the mushrooms.

REFERENCES

1. Vidari, G., Garlaschelli, L., De Bernardi, M., Fronza, G. and Vita-Finzi, P. (1975) *Tetrahedron Letters* 1773.
2. De Bernardi, M., Fronza, G., Vidari, G. and Vita-Finzi, P. (1976) *Chim. Ind.* **58**, 177.
3. Vidari, G., De Bernardi, M., Vita-Finzi, P. and Fronza, G. (1976) *Phytochemistry* **15**, 1953.
4. Appel, H. H., Bond, R. P. M. and Overton, K. H. (1963) *Tetrahedron* **19**, 635.
5. Daniewski, W. M. and Kocór, M. (1971) *Bull. Acad. Polon. Sci., Ser. Sci. Chim.* **19**, 553.
6. Daniewski, W. M., Kocór, M. and Król, J. (1976) *Rocz. Chem.* **50**, 2095.
7. Bogucka-Ledóchowska, M., Hempel, A., Dauter, Z., Konitz, A., Borowski, E., Daniewski, W. M. and Kocór, M. (1976) *Tetrahedron Letters* 2267.
8. Minato, H. and Nagasaki, T. (1966) *J. Chem. Soc.* (C) 377.
9. Nozoe, S., Matsumoto, H. and Urano, S. (1971) *Tetrahedron Letters* 3125.
10. Magnusson, G., Thorén, S., Dahmén, J. and Leander, K. (1974) *Acta Chem. Scand. Ser. B* **28**, 841.
11. De Bernardi, M., Fronza, G., Mellerio, G., Vidari, G. and Vita-Finzi, P., unpublished data.
12. Burke, B. A., Chan, W. R., Magnus, K. E. and Taylor, D. R. (1969) *Tetrahedron* **25**, 5007.