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Synthesis of *N*-Benzoylphenylisoserinates of Lactarorufin B (a Sesquiterpene of *Lactarius* Origin) and Its Derivatives

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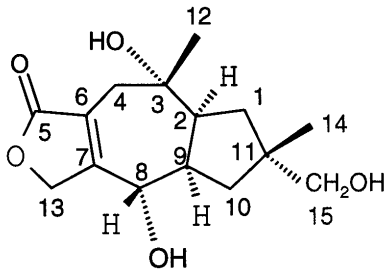
Lactarorufin B, which is a *Lactarius* sesquiterpene possessing primary, secondary and tertiary hydroxyl groups on its lactarane skeleton, was selectively transformed into (*2′R,3′S*)-*N*-benzoylphenylisoserinates, using protective group methodology described below.

Key words: lactarorufin B and its derivatives, (*2′R,3′S*)-*N*-benzoylphenylisoserinates, synthesis

Interesting biological properties [1–3] of (*2′R,3′S*)-*N*-benzoylphenylisoserinates of sesquiterpenoid alcohols of *Lactarius* origin prompted us to prepare the (*2′R,3′S*)-*N*-benzoylphenylisoserinates of lactarorufin B (**1**). So far, lactarorufin B (Fig. 1) was isolated from mushrooms of *Lactarius* genus, *i.e*. *Lactarius rufus* and *Lactarius mitissimus* [4]. Lactarorufin B is a sesquiterpenoid lactone possessing lactarane skeleton. Its molecule contains three hydroxyl groups attached to carbons 15, 8 and 3, thus, being primary, secondary and tertiary respectively. The structure of **1** was confirmed by X-ray investigation [5].

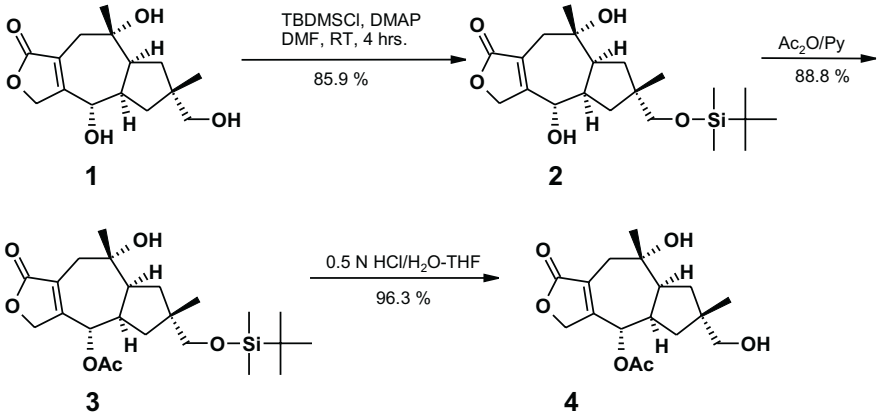


RESULTS AND DISCUSSION

In order to prepare N-benzoylphenylisoserinates of lactarorufin B (**1**) it was necessary to protect two hydroxyl groups, leaving the one for planned esterification. As it was expected, the tertiary hydroxyl group was much less reactive than the remaining two. However, introduction of (*2R,3S*)-*N*-benzoyl-3-phenyl-isoserine into position 15 required the protection of the hydroxyl group at C-8. By carrying out esterification

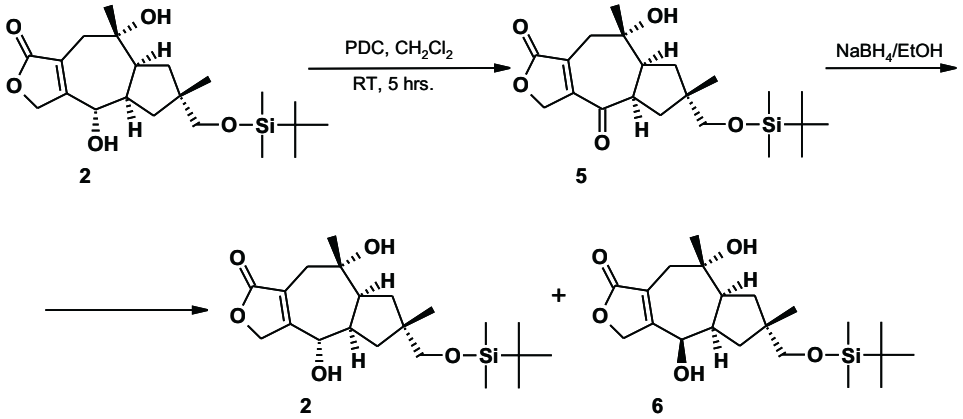
experiments it was found that the C-15 hydroxyl group is more reactive than the hydroxyl group at C-8. Therefore, a reaction sequence shown in Scheme 1 was carried out and 8-acetoxy-lactarorufin B (**4**) was prepared with a good yield:

Scheme 1

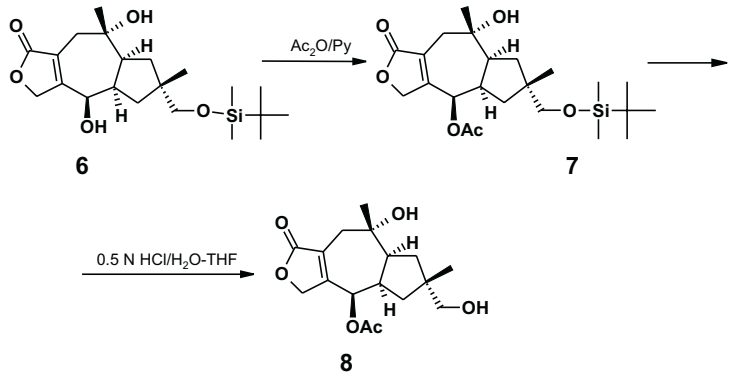


Compound **2** with protected C-15 hydroxyl group was used for the preparation of 8-*epi*-derivatives according to Scheme 2. Thus **2** oxidized with PDC gave keto derivative (**5**) in 90% yield. Compound **5**, when reduced with sodium borohydride, gave the starting **2** and its 8-*epi*-derivative (**6**) in the ratio 1:9 respectively. Similarly as in the case of **2**, compound **6** was acetylated and hydrolyzed and gave 8-*epi*-8-acetoxy-lactarorufin B (**8**) what is shown in Scheme 3.

Scheme 2

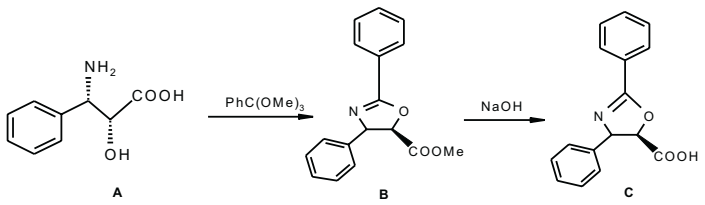


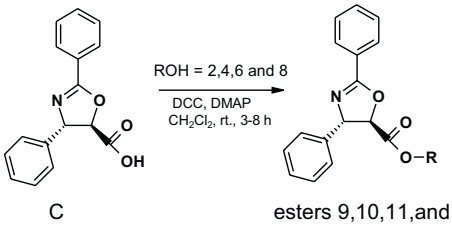
Scheme 3



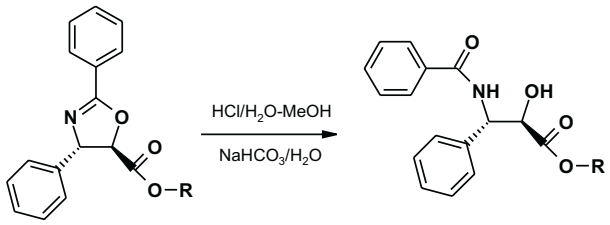
The four **2, 4, 6,** and **8** derivatives of lactarorufin B were subjected to esterification reaction with (*4S,5R)*-2,4-di-phenyl-4,5-dihydro-oxazol-5-carboxylic acid (**C)** as it was described recently [6] (Scheme 4). Compounds **9, 10, 11** and **12** were obtained respectively. The aminol fragments in esters were deprotected and gave the required (*2′R,3′S*)-*N*-benzoylphenylisoserinates **13, 14, 15** and **16** respectively (Scheme 5).

Scheme 4





Scheme 5



The structures of compounds **13, 14, 15** and **16** are shown in Fig. 1.

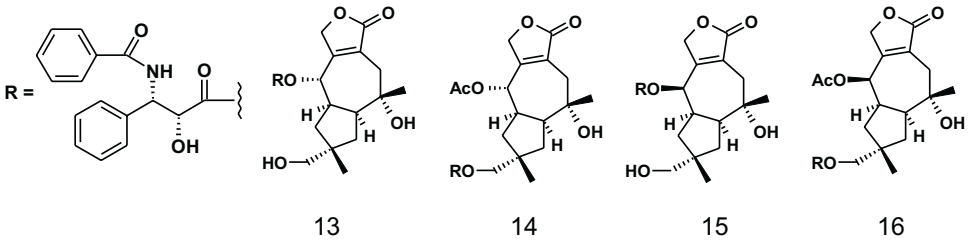


Figure 1.

The structures of all compounds were substantiated by ^{1}H NMR and ^{13}C NMR spectra, which are presented in Tables 1–4.

Table 1. ^{1}H NMR of compounds **1, 2, 3, 4, 5, 6, 7** (500 MHz, $CDCl_3$, TMS as int. standard).

Site (refr.)	**1***	**2**	**3**	**4**	**5**	**6**	**7**
1α	1.72 *dd*	1.85 *dd*	1.86 *dd*	1.90 *dd*	1.90 *dd*	1.80 *ddd*	1.77 *m*
1β	1.25 *t*	1.07 *dd*	1.41 *t*	1.44 *t*	1.16 *t*	0.99 *dd*	0.99 *dd*
2α	2.21 *q*	2.60 *m*	2.41 *m*	2.40 *m*	2.68 *m*	2.50 *m*	2.50 *m*
4a	2.43 *d*	2.68 *dt*	2.60 *m*	2.62 *d*	2.75 ABq	2.65 *dq*	2.64 *dq*
4b	2.28 *d*	2.55 *d*	2.60 *m*	*2.62 d*	–	2.43 *dt*	2.50 *m*
8α or 8β	5.56 *d*	4.13 *d*	5.88 *d*	5.87 *d*	–	5.74 br s	6.78 (brs)
9α	2.32 *m*	2.82 *m*	2.60 *m*	2.64 *m*	3.29 *m*	2.77 *m*	2.80 *m*
10α	1.50 *m*	1.79 *dd*	1.70 *dd*	1.70 *dd*	1.96 *dd*	1.08 *t*	1.08 *t*
10β	1.50 *m*	0.96 *t*	1.24 *dd*	1.30 *dd*	1.50 *dd*	1.87 *dd*	1.77 *m*
12	1.02 *s*	1.31 *s*	1.28 *s*	1.30 s	1.37 s	1.28 s	1.27 *s*
13a	4.80 *d*	4.94 *d*	4.78 *d*	4.77 *d*	5.05 *dt*	4.83 brs	4.75 *d*
13b	4.74 *d* (ABq)	4.61 *d* (ABq)	4.53 *d* (ABq)	4.54 *d*	4.78 *dq*		4.63 *d*
14	1.08 *s*	1.00 *s*	1.04 *s*	1.11 s	1.00 s	0.98 s	0.98 *s*

Table 1 (continuation)

15	3.12 *m*	3.34 ABq	3.29 (ABq)	3.45 *d* 3.35 *d*	3.43 s	3.33 br.s	3.27 ABq
$C\underline{H}_3CO$	–	–	2.15 *s*	2.15 s	–	–	2.05 *s*
2′	–	–			–	–	–
3′	–	–			–	–	–
t-Bu*Me*$_2$Si-	–	0.91 *s*	0.90 *s*	–	0.90 s	0.89 s	0.87 *s*
t-Bu*Me*$_2$Si-	–	0.05 *s*	0.04 *s*		0.05 s	0.04 s	0.01 *s*
N$\underline{H}$	–	–	–		–	–	–
Aro*mat*ic pro*t*ons	–	–	–		–	–	–

J (Hz) 1(*DMSO): 1α,1β = 12.7; 1α,2α = 7; 1β,2α = 12.7; 4a,4b = 16.2 (ABq); 8β,9α =7.7.
2: 1α,1β = 13.2; 1α,2α = 8.6; 1β,2α = 10.6; 4a,4b = 18.4 (ABq); 10α,10β = 12.5; 9α,10α = 7.0; 8β,9α = 3.7; 13a,13b = 17.5 (ABq); 15a,15b = 9.6.
3: 1α,1β = 12.9; 1α,2α = 6.9; 1β,2α = 13.2; 10α,10β = 13.8; 9α,10α = 6.9; 8β,9α = 10.6; 13a,13b = 17.4 (ABq); 15a,15b = 9.5.
4: 1α,1β = 14.3; 1α,2α = 8.1; 1β,2α = 13.3; 4a,4b = 10.8; 8β,9α = 10.7; 10α,10β = 13.8; 10α,9α = 7.1; 13a,13b = 17.5; 15a,15b = 10.5.
5: 1α-1β = 12.9; 1α-2α = 7.6; 4a-4b = 19.6; 10α-10β = 13.2; 10α-9α = 8.3; 10β-9α = 8.9; 13a-13b = 17.5.
6: 1α,1β = 13.1; 4a,4b = 19.0; 10α,10β = 12.7; 10β,9α = 7.3.
7: 1-1β = 13.3; 1β-2α = 10.6; 10α-10β = 12.5; 13a-13b = 17.6; 15a-15b = 9.7.

Table 2. ^{1}H NMR of compounds **8, 9, 10, 11, 12, 13, 14, 15, 16** (500 MHz, $CDCl_3$, TMS as int. standard).

Site (refr.)	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
1α	1.67 *m*	1.90 *dd*	1.86 *dd*	1.81 *dd*	1.80 *m*	1.82 *dd*	1.92 *dd*	1.71 *m*	1.77 *dd*
1β	0.90 *t*	1.46 *t*	1.51 *t*	0.99 *dd*	1.13 *t*	1.35 *t*	1.53 *t*	1.10 *t*	1.11 *t*
2α	2.43 *m*	2.44 *m*	2.40 *m*	2.58 *m*	2.57 *m*	2.38 *m*	2.57 *m*	2.52 *m*	2.56 *m*
4a	2.50 *dq*	2.61 *m*	2.55 *brs*	2.67 *d*	2.62 *dq*	2.60 *d*	2.63 *m*	2.60 *d*	2.61 *d*
4b	2.34 *(d)*	–	–	2.50 *d*	2.50 *d*	2.54 *d*	2.63 *m*	2.43 *d*	2.48 *d*
8α or 8β	6.70 *brs*	6.08 *d*	5.84 *d*	6.97 brs	6.80 *brs*	5.93 *d*	5.80 *d*	6.82 br s	6.83 br s
9α	2.71 *m*	2.69 *m*	2.64 *m*	2.97 *m*	2.89 *m*	2.64 *m*	2.64 *m*	2.86 *m*	2.96 *m*
10α	1.00 *t*	1.80 *dd*	1.70 *dd*	1.11 *t*	1.25 *t*	1.59 *dd*	1.78 *dd*	1.71 *m*	1.86 *dd*
10β	1.67 *m*	1.34 *dd*	1.34 *dd*	1.70 *dd*	1.80 *m*	1.12 *dd*	1.33 *dd*	0.99 *dd*	1.21 *t*
12	1.14 s	1.30 s	1.20 s	1.29 s	1.23 s	1.24 s	1.24 s	1.21 s	1.23 s
13a	4.67 *d*	4.71 *d*	4.74 *d*	4.69 brs	4.75 *d*	4.73 *d* 4.46 *d* *(*ABq)	4.71 *d* 4.51 *d* *(*ABq)	4.78 brs	4.69 *d*
13b	4.55 *d*	4.55 *d*	4.51 *d*	–	4.64 *d*				4.60 *(d)*
14	0.91 s	1.10 s	1.15 s	0.96 s	1.13 s	0.84 s	1.13 s	0.96 s	1.09 s
15	3.20 *brs*	3.31 s	4.04 ABq	3.28 ABq	4.13 *d* 4.01 *d*	3.14 *(*ABq)	4.0 *(*ABq)	3.27 *d* 3.23 *d*	4.06 *d* 3.94 *d*
$C\underline{H}_3CO$	1.96 s	–	2.13 s	–	2.03 s		2.16 s	–	2.02 s
2′	–	4.98 *d*	4.95 *d*	4.93 *d*	4.97 *d*	4.65 *d*	4.68 *d*	4.61 *d*	4.65 *d*
3′	–	5.48 *d*	5.45 *d*	5.42 *d*	5.46 *d*	5.65 *d*	5.75 *dd*	5.62 *dd*	5.75 *dd*
t-Bu*Me*$_2$Si-	–	0.91 s	–	0.89 s	–	–	–	–	–
t-Bu*Me*$_2$Si-	–	0.05 s	–	0.03 s	–	–	–	–	–

Table 2 (continuation)

NH	–	–	–	–	–	7.92 *d*	7.04 *d*	7.15 *d*	7.00 *d*
Aromatic protons	–	10H 7.31–8.10 *m*	10H 7.32–8.11 *m*	10H 7.33–8.11 *m*	10H 7.33–8.13 *m*	10H 7.26–7.90 *m*	10H 7.28–7.77 *m*	10H 7.2–7.77 *m*	10H 7.29–7.76 *m*

8: (J/Hz): 1α,1β = 12.2; 4a,4b = 19.0; 10α,10β = 12.7; 13a,13b = 17.7.

9 (J/Hz): 1α,1β = 12.8; 1α,2α = 6.8; 8α,9α = 11.1; 10α,9α = 6.9; 10α,10β = 13.9; 13a-13b = 17.4; 2′-3′ = 6.2.

10 (J/Hz): 1α,1β = 13.3; 1α,2α = 6.8; 8α,9α = 10.7; 10α,10β = 14.0; 10α,9α = 7.0; 13a,13b = 17.5; 15a,15b = 10.7; 2′,3′ = 6.1.

11 (J/Hz): 1β,2α = 13.1; 4a,4b = 19.1; 10α,10β = 12.4; 15a,15b = 9.7; 2′,3′ = 6.2.

Table 4. ^{13}C NMR of compounds **9, 10, 11, 12, 13, 14, 15, 16** (125.7 MHz, $CDCl_3$).

C (site)	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
1	37.5 *t*	37.9 *t*	39.7 *t*	40.1 *t*	37.5 *t*	38.4 *t*	40.0 *t*	40.6 *t*
2	53.5 *d*	52.8 *d*	49.4 *d*	48.9 *d*	41.6 *d*	52.8 *d*	49.1 *d*	49.0 *d*
3	70.8 *s*	70.8 *s*	73.8 *s*	73.6 *s*	70.7 *s*	71.4 *s*	73.9 *s*	73.7 *s*
4	33.4 *t*	34.1 *t*	34.5 *t*	34.5 *t*	34.0 *t*	35.0 *t*	34.5 *t*	34.5 *t*
5	174.0 *s*	174.1 *s*	174.3 *s*	174.5 *s*	174.8 *s*	174.1 *s*	174.8 *s*	174.5 *s*
6	125.1 *s*	124.9 *s*	122.7 *s*	122.3 *s*	125.1 *s*	125.4 *s*	122.3 *s*	122.3 *s*
7	158.8 *s*	159.0 *s*	158.0 *s*	158.8 *s*	158.5 *s*	158.5 *s*	158.5 *s*	158.9 *s*
8	72.4 *d*	70.2 *d*	74.2 *d*	71.8 *d*	72.0 *d*	70.2 *d*	74.7 *d*	71.8 *d*
9	41.8 *d*	41.6 *d*	44.0 *d*	44.0	52.2 *d*	42.3 *d*	43.8 *d*	44.0 *d*
10	38.7 *t*	39.3 *t*	37.8 *t*	38.4 *t*	38.7 *t*	39.3 *t*	37.5 *t*	38.3 *t*
11	40.9 *s*	39.3 *s*	43.3 *s*	41.8 *s*	40.5 *s*	39.3 *s*	43.1 *s*	41.6 *s*
12	30.4 *q*	29.4 *q*	32.2 *q*	32.1 *s*	28.6 *q*	28.6 *q*	32.2 *q*	32.2 *q*
13	69.0 *t*	69.3 *t*	70.4 *t*	70.5 *t*	69.5 *t*	69.6 *t*	70.8 *t*	70.5 *t*
14	26.8 *q*	26.4 *q*	24.4 *q*	24.5 *q*	25.3 *q*	26.2 *q*	24.1 *q*	24.5 *q*
15	71.4 *t*	72.6 *t*	68.6 *t*	70.5 *t*	69.6 *t*	73.8 *t*	68.3 *t*	71.9 *t*
1′	169.7	170.3 *s*	167.0 *s*	170.3 *s*	171.7 *s*	173.1 *s*	171.9 *s*	173.1 *s*
2′	82.8 *d*	83.2 *d*	83.0 *d*	83.1 *d*	73.3 *d*	73.2 *d*	73.5 *d*	73.2 *d*
3′	74.9 *d*	74.8 *d*	74.9 *d*	74.8 *d*	55.3 *d*	54.8 *d*	55.1 *d*	54.7 *d*
$\underline{C}H_3CO$-	–	20.4 *q*	–	20.6 *q*	–	20.5 *q*	–	20.8 *q*
$CH_3\underline{C}O$-	–	170.1 *s*	–	169.7 *s*	–	170.1 *s*	–	169.8 *s*
-O-$\underline{C}$(Ph)=N-	164.0 *s*	159.0 *s*	163.9 *s*	164.0 *s*	167.7 *s*	166.9 *s*	167.4 *s*	166.6 *s*
$(\underline{Me})_3CMe_2Si$-	25.9 *q*	–	25.8 *q*	–	–	–	–	–
$(Me)_3\underline{C}Me_2Si$-	18.3 *s*	–	18.1 *s*	–	–	–	–	–
$(Me)_3C\underline{Me}_2Si$-	–5.4 *q*	–	–5.6 *q*	–	–	–	–	–
Ar-	140.6 *s*	140.9 *s*	140.5 *s*	140.9 *s*	136.3 *s*	138.6 *s*	138.5 *s*	138.8 *s*
	126.6 *s*	126.6 *s*	126.4 *s*	126.7 *s*	133.3 *s*	133.9 *s*	133.6 *s*	133.9 *s*
	132.2 *d*	132.0 *d*	132.1 *d*	132.1 *d*	131.9 *d*	131.8 *d*	132.0 *d*	131.9 *d*
	129.1 *d*	128.9 *d*	129.0 *d*	128.9 *d*	128.4 *d*	128.8 *d*	128.9 *d*	128.8 *d*
	128.7 *d*	128.6 *d*	128.6 *d*	128.7 *d*	128.3 *d*	128.0 *d*	128.7 *d*	128.6 *d*
	128.6 *d*	128.5 *d*	128.5 *d*	128.5 *d*	127.7 *d*	128.6 *d*	128.2 *d*	128.0 *d*
	128.4 *d*	128.1 *d*	128.3 *d*	128.2 *d*	127.0 *d*	127.1 *d*	127.2 *d*	127.0 *d*
	126.4 *d*	126.4 *d*	126.3 *d*	126.4 *d*	126.6 *d*	126.9 *d*	127.0 *d*	126.9 *d*

EXPERIMENTAL

Lactarorufin B (**1**) was isolated from ethanolic extract of *Lactarius rufus* collected in Łomianki forest near Warsaw. The mushrooms were authenticated by a mycologist Prof. A. Skirgiełło (Warsaw University). The ethanolic extract and the isolation of **1** was carried out according to the procedures reported in our previous papers [7,8].

Lactarorufin B (1). TLC: R_f = 0.2 (hexane/ethyl acetate/methanol 47.5:47.5:5); $[\alpha]_D^{20}$ +24 (*c* 1.0, EtOH); UV (EtOH) λ_{max} 218 nm (ε 10100); IR ($CHCl_3$) ν_{max} 3420, 3356, 3265, 1732, cm^{-1}; ^1H NMR (Table 1).

15-*O*-*t*-Butyldimethylsilyl-lactarorufin B (2). Lactarorufin B (**8**, 200 mg, 0.71 mmole), dissolved in DMF (2 ml), was treated with DMAP (173 mg, 1.42 mmol) and *t*-butyldimethylsilyl chloride (118 mg, 0.78 mmole) in methylene chloride (2 ml). Reaction was carried out at RT, and was monitored with TLC (hexane/ethyl acetate 1:1). After 5 h the reaction mixture was evaporated and the residue was purified by column chromatography on silica gel (hexane/ethyl acetate 65:35), and gave pure **2** (238.6 mg, 86%

yield); TLC: R_f = 0.32 (hexane/ethyl acetate – 1:1); m.p. 41–47°C; $[\alpha]_D^{20}$ = +13.12 (*c* 1.0, $CHCl_3$); UV λ_{max}^{EtOH} nm: 204 (ε 11197); IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 3362, 2955, 2931, 2858, 1749, 1671; ^1H NMR (Table 1); ^{13}C NMR (Table 3); MS (EI, 70 eV), *m/z* (rel. int., %): 396 (M^+, 0.3), 322 (5), 321 (18), 303 (2), 291 (8), 247 (6), 246 (4), 245 (4), 231(7), 230 (17), 229 (100), 228 (5), 217 (2), 213 (4), 211 (5), 205 (3), 203 (10), 201 (13), 199 (5), 189 (4), 187 (28), 186 (9), 185 (52), 183 (15), 175 (10), 173 (16), 171 (7), 170 (15), 161 (15), 159 (12), 157 (16), 143 (19), 121 (22), 119 (11), 109 (23), 105 (18), 93 (15), 91 (10), 75 (54), 73 (32), 43 (37); MS (LSIMS, HR) *m/z*: $(M+Na)^+$ 419.22297 calc. for $C_{21}H_{36}O_5SiNa$: 419.22411.

8-*O*-Acetyl-15-*O*-*t*-butyldimethylsilyl-lactarorufin B (3). Compound **2** was acetylated according to standard procedure. The reaction product was purified by column chromatography on silica gel (hexane/ethyl acetate 7:3) and gave **3**. Yield 89%. TLC: R_f = 0.35 (hexane/ethyl acetate – 7:3); oil; $[\alpha]_D^{20}$ = +3.14 (*c* 1.0, $CHCl_3$); UV λ_{max}^{EtOH} nm: 220 (ε 10417); IR $\lambda_{max}^{CHCl_3}$ cm^{-1} : 3590, 2956, 2859, 1753, 1687; ^1H NMR (Table 1); ^{13}C NMR (Table 3); MS (LSIMS, HR) *m/z*: $(M+Na)^+$ 461.23267 calc. for $C_{23}H_{38}O_6SiNa$: 461.23354.

8-*O*-Acetyl-lactarorufin B (4). Compound **3** (55 mg, 0.125 mmole) dissolved in THF (4 ml) was hydrolyzed with 0.5 *N* HCl aq. solution (2 ml). The reaction was carried out at RT until **3** disappeared (TLC, methylene chloride/acetone 6:4). Subsequently the reaction mixture was diluted with water (20 ml) and extracted with chloroform (3 × 30 ml). The extract was dried over $MgSO_4$, and the solvent evaporated, leaving a residue. This was chromatographed on silica gel using methylene chloride/ acetone (7:3) solvent system. Thus **4** (39.2 mg, 96% yield) was obtained. TLC: R_f = 0.35 (CH_2Cl_2/Me_2CO – 6:4); m.p. 131–136°C; $[\alpha]_D^{20}$ = +10.80 (*c* 0.5, $CHCl_3$); UV: λ_{max}^{EtOH} nm: 191 (ε 4118), 217 (ε 11673); IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 3629, 3442, 2932, 2873, 1753, 1688; ^1H NMR (Table 1); ^{13}C NMR (Table 3); MS (ESI, HR) *m/z*: $(M+Na)^+$ 347.1478 calc. for $C_{17}H_{24}O_6Na$: 347.1465.

8-Dihydro-15-*O*-*t*-butyldimethylsilyl-lactarorufin B (5). Compound **2** (72 mg, 0.182 mmole), dissolved in methylene chloride (8 ml), was treated with PDC (205 mg, 0.55 mmole), powdered molecular sieves 4A (50 mg) and sodium acetate (anhydrous) (15 mg, 0.19 mmole). Reaction was carried out at RT, with continuous stirring, and was monitored with TLC (hexane/ethyl acetate 1:1). When the reaction is finished (*ca*. 2.5 hrs), the reaction mixture was filtered through Celite, the precipitate washed with methylene chloride, and the combined filtrates evaporated leaving a residue. This was purified on silica-gel chromatographic column using hexane/ethyl acetate 7:3 solvent system. Proper fractions were collected, evaporated and gave **5** (64 mg, 89%). TLC: R_f = 0.75 (hexane/ethyl acetate – 1:1); oil; $[\alpha]_D^{20}$ = –23.74 (*c* 1.0, $CHCl_3$); UV λ_{max}^{EtOH} nm: 234 (ε 8055); IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 3598, 2932, 2858, 1760, 1670; ^1H NMR (Table 1); ^{13}C NMR (Table 3); MS (EI, 70 eV), *m/z* (rel. int., %): 394 (M^+, 5), 338 (16), 337 (53), 320 (10), 319 (33), 301 (5), 291 (8), 275 (6), 249 (5), 247 (6), 246 (17), 245 (100), 244 (10), 227 (20), 217 (13), 215 (8), 203 (24), 201 (16), 199 (15), 189 (6), 187 (7), 185 (11), 183 (12), 177 (6), 175 (8), 173 (11), 171 (9), 161 (9), 159 (8), 157 (10), 153 (6), 145 (7), 143 (8), 131 (6), 121 (14), 119 (34), 115 (6), 105 (11), 93 (11), 89 (15), 81 (6), 75 (79), 73 (42), 59 (11), 57 (9), 43 (46); MS (EI, HR) *m/z*: M^+ 394.21908 calc. for $C_{21}H_{34}O_5Si$: 394.21755.

8-*epi*-15-*O*-*t*-Butyldimethylsilyl-lactarorufin B (6). Compound **5** (40 mg, 0.1 mmole) dissolved in ethanol (4 ml) was reduced with $NaBH_4$, the reaction was monitored by TLC (hexane/ethyl acetate 1:1). Subsequently the reaction mixture was treated with water (20 ml), and the reaction products were extracted with chloroform (3 x 30 ml). Upon the removal of the solvent the crude residue was subjected to chromatography on Si-gel using methylene chloride/acetone (7:3) solvent system. Two compounds were obtained: 1. 15-*O*-*t*-butyldimethylsilyl-lactarorufin B (**2**, 4 mg, 7%) and 2. a new compound – 8-*epi*-15-*O*-*t*-butyldimethylsilyl-lactarorufin B (**6**, 37 mg, 92%). The ratio of both isomers **2** and **6** in the reaction mixture was established by HPLC with the use of RI detector and it was 1:9. Compound **6**: TLC: R_f = 0.30 (hexane/ethyl acetate – 1:1); m.p. 67–69°C; $[\alpha]_D^{20}$ = –4.28 (*c* 1.0, $CHCl_3$); UV λ_{max}^{EtOH} nm: 218 (ε 21795); IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 3604, 2956, 2932, 2859, 1746, 1687; ^1H NMR (Table 1); ^{13}C NMR (Table 3); MS (ESI, HR) *m/z*: $(M+Na)^+$ 419.2224 calc. for $C_{21}H_{36}O_5SiNa$: 419.2241.

8-*epi*-*O*-Acetyl-15-*O*-*t*-butyldimethylsilyl-lactarorufin B (7). Compound **6** was acetylated by standard procedure. The reaction product was purified by column chromatography on Si-gel using hexane/ethyl acetate 7:3 to 6:4 gradient solvent system. Yield 97%. TLC: R_f = 0.66 (hexane/Me_2CO – 6:4); m.p. 162–165°C; $[\alpha]_D^{20}$ = –26.12 (*c* 1.0, $CHCl_3$); UV λ_{max}^{EtOH} nm: 217 (ε 12795); IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 3597, 2955, 2933, 2859, 1748, 1689; ^1H NMR (Table 1); ^{13}C NMR (Table 3); MS (ESI, HR) *m/z*: $(M+Na)^+$ 461.2335 calc. for $C_{23}H_{38}O_6SiNa$: 461.2330.

8-*epi*-O-Acetyl-lactarorufin B (8). Compound **8** was obtained from **7** according to the procedure described for **4**. Compound **8** was purified by column chromatography using hexane/acetone 6:4 solvent system. Yield 66%. TLC: R_f = 0.29 (hexane/Me_2CO – 6:4); m.p. 202–203.5°C (~190°C sub.); $[\alpha]_D^{20}$ = –43.04 (*c* 0.5, $CHCl_3$); UV λ_{max}^{EtOH} nm: 217 (ε 11441); IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 3629, 2936, 1749, 1689; ^{1}H NMR (Table 2); ^{13}C NMR (Table 3); MS (ESI, HR) *m/z*: $(M+Na)^+$ 347.1455 calc. for $C_{17}H_{24}O_6Na$: 347.1465.

Preparation of oxazoline methyl ester (B): Phenylisoserine(R,S) (2.870 g, 0.013 mole) was suspended in dry toluene (50 ml) in round bottom flask (200 ml) equipped with magnetic stirrer and a still head. The reaction was heated to boiling and 15 ml of toluene was distilled off. Subsequently trimethylorthobenzoate (5 ml, 0.029 mole) was added and the volatile components were removed slowly by distillation during 8 hours. Residual solids were filtered off and the filtrate evaporated in rotavap. The resulting oil was purified by chromatography over silica-gel in hexane/ethyl acetate gradient solvent system. Proper fractions were collected and gave pure ester **B** (1.5 g, 41% theoretical yield). $[\alpha]_D^{20}$ +14.2 (c 1.0, EtOH); ^{1}H NMR ($CDCl_3$, 200 MHz) δ_H 3.90 (s, 3H), 4.96 (d, J = 6.5 Hz, 1H), 5.50 (d, J = 6.5 Hz, 1H), 7.30–7.62 (m, 8H), 8.10–8.20 (m, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ_C 52.68, 74.59, 83.07, 126.39, 126.72, 127.97, 128.15, 128.22, 128.39, 128.64, 128.79, 131.86, 141.04, 163.89, 170.55.

The unidentified residual solids by treatment with another portion of trimethyl ortho benzoate in presence of p-toluenesulphonic acid in toluene can be converted to the desired ester **B.** In this way overall yield can be improved up to 66%.

(4S,5R) 2,4-Diphenyl-4,5-dihydro-oxazol-5-carboxylic acid (C): Compound **B** (1.520 g, 0.0054 mole) dissolved in methanol (8 ml) was treated with sodium hydroxide (0.33 g, 0.00835 mole) dissolved in methanol (20 ml) and water (0.5 ml) at room temperature. The reaction was followed by TLC and when the starting material was gone (5 hrs), hydrochloric acid (6 ml, 5%) was added to the reaction mixture to obtain the pH value equal 6. Precipitated solid was filtered, washed with water and dried to give 1.23 g of the desired product with 86% yield. m.p. = 207–211 °C, $[\alpha]_D^{20}$ = –9.97 (c 1.01, 0.05 N NaOH) ,^{1}H NMR (DMSO-d_6, 200 MHz) δ_H 5.00 (d, J = 6.4 Hz, 1H), 5.41 (d, J = 6.4 Hz, 1H), 7.20–7.70 (m, 8H), 7.90–8.10 (m, 2H); ^{13}C NMR (DMSO-d_6, 50 MHz) δ_C 73.78, 82.42, 126 53, 126.64, 127.79, 128.18, 128.71, 128.80, 132.09, 141.54, 162.86, 171.45.

General method for the synthesis of esters of (4S,5R)-2,4-diphenyl-4,5-dihydro-oxazol-5-carboxylic acid and sesquiterpenoid alcohols of *Lactarius* origin. A sesquiterpenoid alcohol (1 mmole) the acid (**C**) (1.1 mmole) DMAP (18.3 mg, 0.15 mmole) and DCC (247.2 mg, 1.2 mmole) were dissolved in methylene chloride (25 ml) in a round bottom flask. The reaction was carried out at room temperature and was monitored by TLC. When the reaction was completed (3–12 hrs) the contents of the flask were fitered, the solution evaporated *in vacuo* and the residue purified by chromatography to give the desired esters.

(4S,5R)-2,4-Diphenyl-4,5-dihydro-oxazol-5-carboxylic acid ester with 8-*O*-15-t-butyldimethylsilyl-lactarorufin B (9). Compound **9** was obtained from **2** according to the general method described above. Yield 95%. TLC: R_f = 0.38 (hexane/ethyl acetate – 7:3); m.p. 83–87°C; $[\alpha]_D^{20}$ = –22.32 (*c* 1.0, $CHCl_3$); UV λ_{max}^{EtOH} nm: 193 (ε 74373); IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 2956, 2932, 2858, 1756, 1655; ^{1}H NMR (Table 2); ^{13}C NMR (Table 4); MS (LSIMS, HR) *m/z*: $(M+Na)^+$ 668.2990 calc. for $C_{37}H_{47}O_7NNaSi$: 668.3014.

(4S,5R)-2,4-Diphenyl-4,5-dihydro-oxazol-5-carboxylic acid ester with 15-*O*-8-*O*-acetyl-lactarorufin B (10). Compound **9** was obtained from **4** according to the general method described above. TLC: R_f = 0.22 (hexane/Me_2CO – 7:3); m.p. 81–86°C; $[\alpha]_D^{20}$ = + 2.58 (*c* 1.0, $CHCl_3$); UV λ_{max}^{EtOH} nm: 193 (ε 65328); IR λ_{max}^{KBr} cm^{-1} : 3462, 2954, 1752, 1654; ^{1}H NMR (Table 2); ^{13}C NMR (Table 4); MS (ESI, HR) *m/z*: $(M+H)^+$ 574.2480 calc. for $C_{33}H_{36}O_8Ni$: 574.2435.

(4S,5R)-2,4-Diphenyl-4,5-dihydro-oxazol-5-carboxylic acid ester with 8-epi-*O*-15-*O*-t-butyldimethylsilyl-lactarorufin B (11). Compound **11** was prepared from **6** according to the general method described above. TLC: R_f = 0.26 (hexane/Me_2CO – 8:2); m.p. 80–84°C; $[\alpha]_D^{20}$ –46.56 (*c* 1.0, $CHCl_3$); UV λ_{max}^{EtOH} nm: 193 (ε 67405), 243 (16968); IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 2957, 2932, 1752, 1655; ^{1}H NMR (Table 2); ^{13}C NMR (Table 4); MS (ESI, HR) *m/z*: $(M+H)^+$ 646.3208 calc. for $C_{37}H_{48}O_7NSi$: 646.3195.

(4S,5R)-2,4-Diphenyl-4,5-dihydro-oxazol-5-carboxylic acid ester with 15-*O*-8-*epi*-*O*-acetyl-lactarorufin B (12). Compound **12** was obtained from **8** according to the general procedure described above. TLC: R_f = 0.37 (hexane/Me_2CO – 6:4); m.p. 82–87°C; $[\alpha]_D^{20}$ = –13.72 (*c* 1.0, $CHCl_3$); IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 2962, 1750, 1655; ^{1}H NMR (Table 2); ^{13}C NMR (Table 4); MS (ESI, HR) *m/z*: $(M+H)^+$ 574.2454 calc. for $C_{33}H_{36}O_8Ni$: 574.2435.

General method for the hydrolysis of esters of (4S,5R)-2,4-diphenyl-4,5-dihydro-oxazol-5-carboxylic acid and sesquiterpenoid alcohols of *Lactarius* origin into N-benzoyl-(2S,3R)-phenyl-isoserinates: A sesquiterpenoid alcohol (4S,5R)-2,4-diphenyl-oxazol-5-carboxylate (0.2 mmole) dissolved in methanol (25 ml) was chilled to 0°C and treated with 0.5 N aq. HCl solution (2.5 ml). The reaction must be carried out at 0°C. After completion of the reaction the solution was neutralised with the aid of saturated aq. sodium hydrogen carbonate solution (pH = 7) and left for 15 min. Subsequently the solution was diluted with water (75 ml) and extracted with chloroform (3 × 40 ml). The chloroform extract was dried over magnesium sulphate, filtered and evaporated to dryness leaving a residue, which was purified by chromatography in proper solvent system and gave desired N-benzoyl-(*2R,3S)*-phenylisoserinate.

Lactarorufin B-8-*O*-N-benzoyl-(2′R,3′S)-phenylisoserinate (13). TLC: R_f = 0.16 (hexane/Me_2CO – 1:1); m.p. 141–147°C; $[\alpha]_D^{20}$ = –8.0 (*c* 0.5, $CHCl_3$); UV λ_{max}^{EtOH} nm: 194 (ε 58040), 220 (ε 21310); IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 3440, 2932, 2544, 2401, 1757, 1657; ^1H NMR (Table 2); ^{13}C NMR (Table 4); MS (ESI, HR) *m/z*: $(M+Na)^+$ 572.2277 calc. for $C_{31}H_{35}O_8NNa$: 572.2255.

Lactarorufin B-8-*O*-acetyl-15-*O*-N-benzoyl-(2′R,3′S)-phenylisoserinate (14). TLC: R_f = 0.22 (hexane/Me_2CO – 6:4); m.p. 78–81°C; $[\alpha]_D^{20}$ = +10.52 (*c* 1.0, $CHCl_3$); UV λ_{max}^{EtOH} nm: 203, 219; IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 3441, 2937, 1754, 1664; ^1H NMR (Table 2); ^{13}C NMR (Table 4); MS (ESI, HR) *m/z*: $(M+Na)^+$ 614.2380 calc. for $C_{33}H_{37}O_9NNa$: 614.2361.

Lactarorufin B-epi-8-*O*-N-benzoyl-(2′R,3′S)-phenylisoserinate (15). TLC: R_f = 0.14 (hexane/Me_2CO – 1:1); m.p. 98–104°C; $[\alpha]_D^{20}$ = –10.32 (*c* 1.0, $CHCl_3$); UV λ_{max}^{EtOH} nm: 195 (ε 46945), 218 (ε 21651); IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 3596, 3440, 2937, 2873, 1745, 1664; ^1H NMR (Table 2); ^{13}C NMR (Table 4); MS (ESI, HR) *m/z*: $(M+Na)^+$ 572.2227 calc. for $C_{31}H_{35}O_8NNa$: 572.2255.

Lactarorufin B-epi-8-*O*-acetyl-15-*O*-N-benzoyl-(2′R,3′S)-phenylisoserinate (16). TLC: R_f = 0.19 (hexane/Me_2CO – 6:4); m.p. 116–120°C; $[\alpha]_D^{20}$ = –9.16 (*c* 1.0, $CHCl_3$); UV λ_{max}^{EtOH} nm: 193 (ε 66502); IR $\lambda_{max}^{CHCl_3}$ **cm**$^{-1}$: 3598, 3518, 3443, 2967, 1747, 1668; ^1H NMR (Table 2); ^{13}C NMR (Table 4); MS (ESI, HR) *m/z*: $(M+Na)^+$ 614.2351 calc. for $C_{33}H_{37}O_9NNa$: 614.2361.

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