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New Protoilludane Sesquiterpenes from *Lactarius violascens* +

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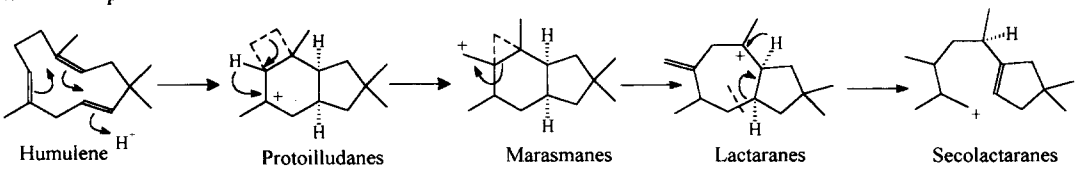
Abstract. Violascensol **1** and the corresponding 6-ketostearoyl ester **2** were isolated from the fruiting bodies of *Lactarius violascens*. Their structures were established by spectroscopic and chemical methods. Compounds **1** and **2** are the first examples of protoilludane sesquiterpenes isolated from a *Lactarius* species.

The *Lactarius* genus (order Agaricales, family Russulaceae), comprising more than 150 species world-wide distributed, is one of the largest in the subdivision Basidiomycotina of Whittaker's Kingdom of Fungi.[1,2] In the great majority of these species, sesquiterpenes of different kinds exert an important biological role, being responsible for the pungency and bitterness of the flesh and the milky juice, and the change in the air of the colour of the latex.[3] Moreover, a few sesquiterpene dialdehydes are believed to be the active principles of a chemical defence system that protects the mushrooms from parasites and predators.[4] The largest group of *Lactarius* sesquiterpenes belongs to the classes of marasmanes, lactaranes, and secolactaranes, believed to be biosynthesized from humulene through a series of skeleton rearrangements (Scheme 1).[5] Protoilludane intermediates are considered to be formed in the early steps of the biosynthetic pathway, though no representative of that class of sesquiterpenes has ever been isolated so far from a *Lactarius* species.



Scheme 1

In this paper we report the isolation of the first two *Lactarius* protoilludanes, i.e. violascensol **1** and the ester **2**, from *L. violascens* (Otto ex Fr.) Fr., an inedible species growing on Italian Apennines.[6] Specimens of the above mushroom that appeared undamaged were collected, frozen at -20°C, and extracted with EtOAc in the cold. The residue from the organic extract was redissolved in MeOH-H_2O (9:1) and extracted with hexane to remove the largest part of free hydroxy compounds. The two organic layers were then separately fractioned by multistep flash column chromatography on SiO_2. Violascensol **1** was isolated from the alcoholic fraction while the corresponding ester **2** was isolated from the hydrocarbon layer. The molecular composition of **1** [7] was established as $C_{15}H_{22}O_2$ from eims and the number of carbon signals in the NOE

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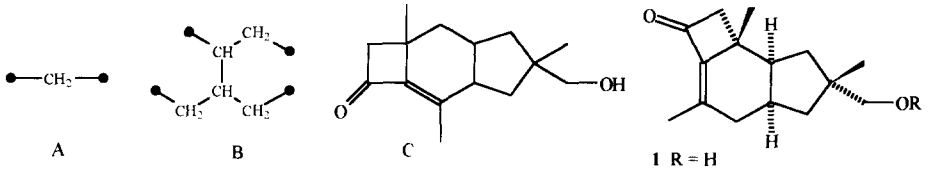
suppressed ^{13}C-NMR spectrum (Table 1), which indicated a sesquiterpenoid skeleton containing 5 degrees of unsaturation. Since **1** exhibited a primary CH_2OH group (ν_{max} 3450 cm^{-1}; ^{1}H-NMR, δ 3.35, ABq, 2H; ^{13}C-NMR δ 69.2, t), a ketone conjugated to a tetrasubstituted double bond (λ_{max} 264 nm, ν_{max} 1727 and 1667 cm^{-1}; ^{13}C-NMR δ 197.1, 150.6, and 143.2), and no additional sp^2 or sp carbons, the structure must be tricyclic. The remaining signals in the ^{13}C-NMR spectrum of **1** could be attributed , with the aid of the DEPT spectrum, to three methyls, one (δ 1.95 ppm) being vinylic and in β position to the carbonyl group, four methylene, two methine, and two nonprotonated sp^3 carbons. A ^{13}C-^{1}H COSY experiment established the positions of all of the ^{1}H-NMR signals for each of the carbons, while ^{1}H-^{1}H COSY data and selective homonuclear decoupling experiments clearly indicated two partial structures, A and B, comprising two separate spin systems. The former was an AB quartet, whose chemical shift was characteristic for a methylene (C-4) flanking the carbonyl group; the latter was the system linking C-1 to C-10 and included the H_2-8 to H-9 linkages.



A B C

1 R = H
2 R = $OC(CH_2)_4CO(CH_2)_{11}CH_3$

When the partial structures were compared with the two and three bond C-H connectivity data from COLOC experiments (Fig.1) the structure of violascensol could unambiguously be assembled as **1**. The important linkages which established the structure of the ring system were: (i) those about the angular methyl at C-3 connecting C-5 and C-6 to C-12 via C-4; (ii) that from C-4 to H-2, which ruled out the alternative sterpurane like structure C, also excluded by the coupling (J=1.5 Hz) between H_3 -13 and βH-8; (iii) those linking C-10 to C-14 via C-11, and (iv) that from C-14 to H_2 -15 which enabled the two terminal methylenes of the partial structure B to be connected to a gem substituted quaternary carbon of a cyclopentane ring. The relative stereochemistry of **1** was obtained from NOE and NOESY spectra. The important positive NOE's are shown in Fig. 2 and placed H_2-4, H-2, and H-9 on the same face as H_2-15, that is on the opposite side of H_3-14 and H_3-12.

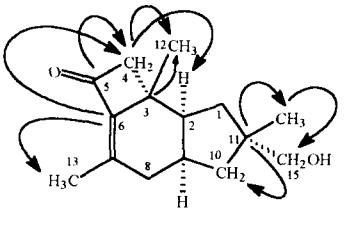


Fig. 1 COLOC correlations (J = 5 Hz)

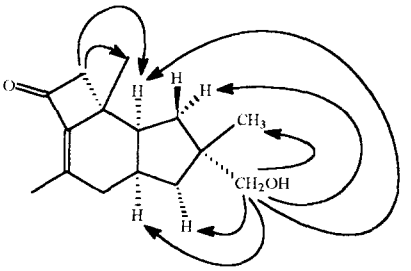


Fig 2 Selected NOE and NOESY correlatio

Chiroptical properties of skewed α,β unsaturated ketones are often used for determining the absolute configuration of organic compounds on the basis of simple empirical helicity rules.[8,9] The CD spectrum of violascensol showed a negative CD band at 332 nm which was attributed to the n $\rightarrow$ π^* transition of the dissymmetric enone chromophore; however, we promptly realized that the sign of this band could not be straightly connected to the absolute configuration of compound **1**. In fact, a Dreiding model of **1** clearly

showed that the above CD band was strongly affected not only by the helicity of the *cisoid* enone chromophore, but also by the chirality sense of the rigid twisted cyclobutanone framework. Unfortunately, these two effects make opposite contributions to the CD band and leave, therefore, room for considerable ambiguity in determining the absolute configuration of violascensol. Therefore, the latter was assumed to correspond, for biosynthetic reasons, to that of related marasmane and lactarane *Lactarius* sesquiterpenes. [5] The same absolute configuration was also proposed for naturally occurring protoilludanes. [10-14]

Table 1- $^{1}H^{a}$ and ^{13}C-NMR[b, c, d] Data ($CDCl_3$) for Compounds **1** and **2**

	1		**2**	
assignment	^{13}C	$^{1}H^{e}$	$^{13}C^{f}$	$^{1}H^{g,h}$
1	36.7 (2)	α 1.38 dd β 1.68 ddd	36.6 (2)	α 1.47 dd β 1.74 ddd
2	47.3 (1)	2.17 ddd	47.1 (1)	2.23 m[i]
3	36.7 (0)	--	36.6 (0)	---
4	60.9 (2)	2.62 (ABq)	60.8 (2)	2.68 (ABq)
5	197.1 (0)	---	196.9 (0)	---
6	150.6 (0)	---	150.5 (0)	---
7	143.2 (0)	---	143.1 (0)	---
8	35.7 (2)	α 2.20 dd β 1.77 dddq	35.6 (2)	α 2.25 dd β 1.82 ddq
9	41.7 (1)	2.35 ddddd	41.5 (1)	2.4 m[i]
10	43.5 (2)	α 0.99 dd β 1.83 ddd	43.9 (2)	α 1.08 dd β 1.88 ddd
11	45.3 (0)	---	43.8 (0)	---
12	20.3 (3)	1.15 s	20.3 (3)	1.17 s
13	20.3 (3)	1.95 d	20.3 (3)	2.02 d
14	24.3 (3)	1.05 s	24.8 (3)	1.13 s
15	69.2 (2)	3.35 (ABq)	69.7 (2)	3.90 (ABq)

[a] 300MHz, δ_H values in ppm from TMS. [b] 75.5MHz; δ_C values in ppm relative to $CDCl_3$ at 77.0. [c] The number in parentheses indicates the number of hydrogens attached to the corresponding carbon and was determined from DEPT experiments. [d] Carbon assignments were confirmed by ^{1}H-^{13}C COSY spectra. [e] *J*'s (Hz) for **1**: 1α,1β=13.5; 1α,2=9.3, 1β,2=8.5; 1β,10β=1.8; 2,9=11.0; 4,4'=16.0; 8α,8β=15.0; 8α,9=6.8; 8β,9=9.8; 8β,13=1.5: 8β,4=1.0; 9,10α=11.3; 9,10β=7.3; 10α,10β=12.8; 15,15'=11.0. [f] ^{13}C-NMR signals for the 6-oxostearoyl moiety: 210.8 (C=O), 173.5 (COO), 42.8, 42.1, 41.5, 34.0, 31.8, 29.5, 29.5, 29.4, 29.3, 29.2, 29.1, 24.4, 23.8, 23.1, 22,6 (one CH_2 each), 14.0 (CH_3). [g] ^{1}H-NMR signals for the 6-oxostearoyl moiety: 0.88 (t,3H, *J*=6.5, H_3 -18'), 1.25 (brs, 18H, $(CH_2)_{9'-17'}$), 1.5-1.68 (m, 6H, H_2 -3', -4', -8'), 2.3-2.5 (m, 6H, H_2 -2', -5', -7'). [h] *J*'s (Hz) for **2**: 1α,1β=13.5; 1α,2=9.5; 1β,2=8.5; 1β,10β=1.7; 2,9=11.0; 4,4'=16.0; 8α,8β=15.0; 8α,9=6.5; 8β,9=10.0; 8β,13=1.4, 9,10α=11.5; 9,10β=7.3: 10α,10β=13.0: 15,15'=11.0. [i] Hidden by other signals and unearthed by COSY experiments.

The structure of compound **2**[15] was readily established by direct comparison of the NMR data with those of **1** (see Table 1). The downfield shift of the ABq for the H_2-15 group and the characteristic signals of a long chain fatty acid ester in the ^{1}H-NMR spectrum of **2** were indicative of the 6-oxostearic ester moiety at C-15. On exposure to K_2CO_3 in MeOH, **2** gave violascensol **1** and 6-oxostearic acid (lactarinic acid), identical with an authentic sample, [16] while acylation of **1** with 6-oxostearoyl chloride[16] afforded **2**, confirming the structure. Violascensol **1** showed a weak activity against bacterial strains and *Artemia salina*.

In conclusion, the finding of violascensol **1** and the ester **2** in *L. violascens* demonstrates that protoilludane sesquiterpenes may be biosynthesized in *Lactarius* cells and gives credit to the biosynthesis proposed for *Lactarius* sesquiterpenes (Scheme 1). It is likely that protoilludane intermediates are rapidly

metabolized in the cells of most *Lactarius* species and thus escape detection, while *L. violascens* is probably devoid of the enzymes needed for transforming the protoilludane skeleton into more elaborate structures.

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6. The structures of compounds **1** and **2** were first disclosed at the XXI Italian Meeting on Organic Chemistry (1993); Abstract Book, P 57, p. 125.
7. Violascensol **1** : sticky colourless oil, $[\alpha]^{D}_{20}$ -106.4 (*c*=0.9, $CHCl_3$); ^{1}H- and ^{13}C-NMR data (Table 1); UV (MeOH) λ_{max} (log ε) 264 nm (4.09); CD (MeOH) λ_{max} (Δε) 244 (-6.0), 260 (+4.6), 332 (-1.7) nm; IR (neat) ν_{max} 3449, 2933, 1727, 1667, 1461, 1369, 1183, 1042 cm^{-1}; EIMS *m/z* (rel.intensity) $[M^+]$ 234.1618 (100, calc. for $C_{15}H_{22}O_2$ 234.1619), 219 (75), 201 (54), 173 (40), 161 (37), 159 (38), 145 (39), 133 (21), 119 (58), 105 (52), 95 (48), 93 (39), 91 (45), 79 (46), 67 (38), 55 (29), 41 (40).
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15. Ester **2** : sticky colourless oil, $[\alpha]^{D}_{20}$ -35.6 (*c*=0.4, CH_2Cl_2); ^{1}H- and ^{13}C-NMR data (Table 1); IR (neat) ν_{max} 2927, 2855, 1733, 1672, 1459, 1370, 1242, 1174, 1100, 1051, 720 cm^{-1}; EIMS *m/z* (rel. intensity) $[M^+]$ 514 (5), 486 (5), 281 (10), 263 (8), 233 (12), 216 (52), 201 (83), 188 (100), 173 (80), 159 (24), 119 (26), 95 (21), 57 (38), 55 (33), 43 (38).
16. Authentic samples of 6-oxostearic acid and the corresponding chloride were prepared as shown below. Experimental details will be reported elsewhere.