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GUAIANE SESQUITERPENE FROM *LACTARIUS SANGUIFLUUS*

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Key Word Index—*Lactarius sanguifluus*; Basidiomicetes; guaiane sesquiterpene.

Abstract—The isolation of pigments from the fungus *Lactarius sanguifluus* and the determination of their structures is reported. The structure of a new compound with a guaiane skeleton has been elucidated by 2D NMR spectroscopy.

INTRODUCTION

The metabolites isolated from *Lactarius* species (Russulaceae, Basidiomicetes) are mainly sesquiterpenoids with the lactarane type of skeleton [1] which formally may be derived from humulene. However, only a few guaiane or related aromatic azulene sesquiterpenoids have been found in these fungi [1, 2]. The last group of sesquiterpenoids have been found in the species that contain a true, highly coloured latex. The only such species studied chemically so far, are the orange *L. deliciosus* [1] the deep blue *L. indigo* [3] and the apricot *L. deterrimus* [2]. In this paper, we wish to report the isolation and characterization of pigments **1** and **2** from *L. sanguifluus*. *L. sanguifluus* is edible and occurs in autumn in pine forests, together with the more widespread *L. deliciosus*.

RESULTS AND DISCUSSION

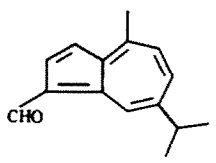
Fresh fungus was extracted with cold acetone, the extracts concentrated *in vacuo* and the residue was then extracted with diethyl ether. The etheral extract was chromatographed on silica gel, yielded two coloured zones which were blood-red and red-violet, in order of elution. The blood-red pigment **2** was obtained as a single spot on TLC, while the red-violet compound **1** was obtained as a mixture with fatty acids. After methylation, the pigment was purified by repeated chromatography.

The spectral data of **1** were in excellent agreement with published values for this compound [2].

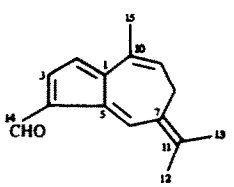
Compound **2** was sensitive to benzene and very sensitive to methanol and bases. It had the molecular formula $C_{15}H_{16}O$, from the high resolution mass measurement of the parent ion. One down-field singlet at δ9.88 in the ^{1}H NMR spectrum and the presence of bands at 2918, 2853 and 1635 cm^{-1} in the IR spectrum indicated the presence of an α,β-unsaturated aldehyde group. The UV spectrum with λ_{max} at 287, 390 and 480 nm indicated a more extended conjugation. The ^{1}H NMR spectrum (Table 1), recorded in C_6D_6 solution, showed three methyl signals, two singlets at δ1.50 and 1.58 and one broad singlet at 1.85, all assigned to vinyl methyl groups. Also present were a doublet at δ2.81 (2H; J = 6.8 Hz) and four olefinic protons at 5.55 (*br t*, J = 6.8 Hz), 6.22 (*br d*, J = 2.93 Hz), 6.82 (*d*, J = 2.93 Hz) and 9.02 (*br s*).

The proton–proton correlation (COSY) NMR spectrum [4–7] which identifies the pairs of resonances that are coupled together allowed us to readily establish the partial structure **A**. Moreover, the COSY spectrum showed that the two olefinic protons at δ6.82 and 6.22 were coupled only between themselves, while the aldehydic proton was not coupled. The presence in the ^{13}C NMR spectrum (Table 1) of four doublets and six singlets in the sp^2 region indicated the presence of five double bonds in the molecule. Hence, compound **2** having eight degrees of unsaturations, must possess a carbobicyclic skeleton. The spectral data quoted so far are in accordance with 1,3,5,7,(11),9-pentaenyl-14-guaianal (**2**) which fits very well with biogenetic considerations. Moreover, the two dimensional exchange spectroscopy [8–10] (NOESY) spectrum exhibited the presence of NOEs between (a) the vinyl proton at δ6.22 (H-2) and the methyl at 1.85 (H-15); (b) the methylene at δ2.81 and the methyl at 1.50 (H-13); (c) the proton at δ9.02 (H-6) and the methyl at 1.58 (H-12); (d) the aldehydic proton at δ9.88 and the vinyl proton at 6.82 (H-3). These data afforded an additional proof for the proposed structure. Finally, compound **2** was transformed into **1** with Pd/Al_2O_3 in refluxing decalin.

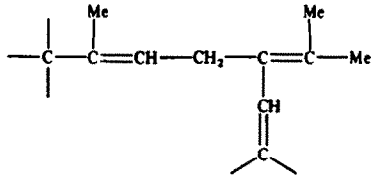
Using heteronuclear shift-correlations [11, 12] we obtained the carbon–carbon connectivities, as a further proof of the proposed structure. From the heteronuclear shift-correlation experiment, using a delay time of 3.58



1



2



A

Table 1. Carbon-13 and proton NMR chemical shifts for compound 2*

Carbon	$^{13}C(CDCl_3)$	$^{1}H(C_6D_6)$	$^{1}H(CDCl_3)$
1	144.20 *s*	—	—
2	126.70 *d*	6.22 *dd* (2.93, 0.98)	6.48
3	147.18 *d*	6.82 *d* (2.93)	7.27
4	134.50 *s*	—	—
5	136.01 *s*	—	—
6	144.52 *d*	9.02 *br s*	8.67
7	147.65 *s*	—	—
8	29.81 *t*	2.81 *d* (6.8)	3.07
9	124.99 *d*	5.55 *tq* (6.8, 1.7)	5.83
10	133.21 *s*	—	—
11	127.34 *s*	—	—
12	21.93 *q*†	1.58 *s*	2.00‡
13	22.87 *q*	1.50 *s*	2.05‡
14	186.59 *d*	9.88 *s*	9.78
15	21.79 *q*†	1.85 *br d* (1.7)	2.00‡

*Coupling constants (Hertz) are in parentheses.
†Assignments may be reversed.
‡The assignments were obtained by NOE difference.

msec in Bruker's microprogram, the direct ^{1}H–^{13}C correlations were obtained. By choosing delay times appropriate for transferring magnetization, via geminal (^{1}H–C–^{13}C) or vicinal (^{1}H–C–C–^{13}C) couplings the long-range ^{1}H–^{13}C correlations were obtained. Moreover, using a delay time of 41.6 msec, only vicinal correlations were observed, while with a delay of 84.0 msec both geminal and vicinal correlations were observed. The recognition of geminal from vicinal couplings was possible by considering the NMR data so far reported. Unfortunately, no correlation appeared in both long-range correlation experiments for the proton at δ 5.83, but this was not an hindrance for the solution of problem. The carbon–carbon connectivity was deduced from the heteronuclear correlations (Table 2) and the ^{13}C chemical shifts were assigned as reported in Table 1.

The mild extraction and purification conditions and the difficult transformation of 2 into 1 support the occurrence of the azulene compound (1) in the fungus in accordance with previous reports [2, 3].

EXPERIMENTAL

General procedures. TLC was carried out using pre-coated silica gel F_{254} plates (Merck). Kieselgel 60 (Merck) was used for chromatography. IR spectra were recorded on a Perkin–Elmer Model 257 Infracord. UV spectra were recorded on a Shimatzu and Bausch-Lomb Spectronic 210. Low-resolution and high-resolution MS were recorded on an AEI MS-30 and on an AEI MS-50 spectrometers, respectively. ^{1}H and ^{13}C NMR spectra were recorded at 270 and 125 MHz on a Bruker WH-270 and a Bruker WM-500, respectively, both under ASPECT 2000 control, with TMS as internal standard. The 2D NMR spectra were obtained using Bruker micro-programs. A 0.28 M soln of 2 in C_6D_6 was used to record the COSY-90 and NOESY spectra, while $CDCl_3$ was used to record the 2D heteronuclear correlations. The COSY-90 spectrum was obtained by co-addition of 16 scans at each of 256 t_1 values. A 512 × 1024 data matrix had been Fourier transformed with sine–bell filters in both domains, using DISN 85 software. The digital resolution was 5.06 Hz/point in both domains. The ^{1}H NOESY spectrum was obtained by co-addition of 16 scans at each of 512 t_1 values, with the mixing time τ_m = 1 sec ± 180 msec (randomly modulated). A 1024 × 2048 data matrix was Fourier transformed with sine–bell filters in both domains. The digital resolution was 2.90 Hz/point in both domains. The 2D heteronuclear correlations were obtained by co-addition of 160 scans at each of 256 t_1 values, with $J_{C\text{-}H}$ = 140 Hz; 12 Hz or 6 Hz for polarization transfer, to obtain direct or long-range C–H correlation, respectively. A 512 × 2048 data matrix was Fourier transformed with Lorentz–Gauss (LB1 = −4.0, GB1 = 0.15; LB2 = −10.0, GB2 = 0.30) filters in both domains. The digital resolution was 9.76 and 21.23 Hz/point, in F1 and F2 domains, respectively.

Extraction and isolation of compounds. Fresh fungus *Lactarius sanguifluus* (80 g, dry wt, after extraction) collected in November 1985 in the pine forest of Pollino (Potenza), was extracted with 2 l. cold Me_2CO (× 3). The extracts were concd *in vacuo* and the residue was extracted with Et_2O (3 × 300 ml). After evapn of

Table 2. ^{1}H–^{13}C correlations for compound 2 in $CDCl_3$ solution

	δ_C*			
δ_H	^{1}H–^{13}C	^{1}H–C–C–^{13}C†	^{1}H–C–^{13}C†	^{1}H–C–C–^{13}C‡
9.78	186.59	136.01	—	—
8.67	144.52	134.50; 29.81	—	—
7.27	147.18	186.59; 136.01	126.70	144.20
6.48	126.70	136.01; 134.50	147.18	—
5.83	124.99	—	—	—
3.07	29.81	144.52; 133.21; 127.34	147.65; 124.99	144.52
2.05	22.87	147.65	127.34	147.65
	21.79	147.65; 144.20;	133.21; 127.34	147.65;
2.00	21.93	124.99		144.20

*Chemical shifts of carbons coupled with proton.
†Coupling observed using J = 6 Hz.
‡Coupling observed using J = 12 Hz.

solvent a dark red gum (4.5 g) was obtained, that was applied to a column (5 × 100 cm) of silica gel. The column was eluted with a solvent gradient system from petrol 40–70° to Et_2O. Fractions of 40 ml were collected. Fractions 35–40 contained **2** (80 mg, 0.1% dry wt), single spot on TLC. Fractions 47–55 were combined to give a solid that was methylated with CH_2N_2 and rechromatographed on silica gel, using C_6H_6–EtOAc (9:1) as eluent, to obtain **1** (15 mg, 0.02% dry wt). All spectral data of **1** were in excellent agreement with published values [2].

1,3,5,7(11),9-*Pentaenyl*-14-*guaianal* (**2**). UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 287 (3.98), 382 sh (4.02), 390 (4.08) and 480 (3.70); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2918, 2853, 1635, 1462 and 1408; MS *m/z* (rel. int.): 212.1224 (100) (M^+ calc. for $C_{15}H_{16}O$, 212.1201), 197 $[M-Me]^+$ (18), 183 $[M-CHO]^+$ (14), 169 (60); ^1H NMR and ^{13}C NMR, see Table 1.

Transformation of compound **2**. Compound **2** (30 mg) was dissolved in decalin (2 ml) in presence of Pd-Al_2O_3 (5 mg) and the mixture was kept at reflux for 5 min. After filtration of the catalyst and elimination of the solvent *in vacuo*, the residue was chromatographed on a silica gel column, using petrol (40–70°)–Et_2O (8:2) as eluent, to obtain a compound (4 mg) identical in all respects with the natural product **1**.

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