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THE ISOLATION OF A GUAIANE SESQUITERPENE FROM FRUIT BODIES OF *LACTARIUS SANGUIFLUUS*

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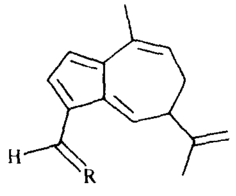
Key Word Index—*Lactarius sanguifluus*; Agaricaceae; mushroom; guaiane sesquiterpene; sangol.

Abstract—The isolation and structure elucidation of a novel guaiane alcohol, formed together with related aldehydes and alcohols from fatty acid ester precursors in injured fruit bodies of the Basidiomycete *Lactarius sanguifluus*, is reported.

The fruit bodies of the *Lactarius* species belonging to the Dapetes Fr. section (Basidiomycotina subdivision of Fungi) have yielded a series of sesquiterpenoids with a guaiane skeleton, which appear to be enzymatically converted into each other in injured fruit bodies [1, 2]. The fruit bodies of *Lactarius deliciosus* Fr. and *L. deterrimus* Gröger for instance, originally contain the stearic acid ester **1a** (together with small amounts of the corresponding linoleic acid ester [1]) as the only sesquiterpenoid. If the fruit bodies of these species are injured, the ester **1a** is converted to the aldehyde **1b**, to the free alcohol **1c**, as well as to lactaroviolin **2b** and deterrol **2c** [1]. The stearic ester of the latter, i.e. the ester **2a**, has never been detected in the fruit bodies of *L. deliciosus* and *L. deterrimus*, but was instead isolated from the fruit bodies of *L. indigo* (Schw.) Fr. [3] together with lactaroviolin **2b**. Recently, the aldehyde **3b** was isolated from the fruit bodies of *L. sanguifluus* Paulet ex Fr. [4], a species that previously has yielded the ester **1a**, the alcohol **1c**, lactaroviolin **2b**, as well as an unidentified azulene called 'lipophiles lactaroviolin' [2]. It was not clear whether the aldehyde **3b** is present as such in the fruit bodies or if it is formed enzymatically from a precursor, and in order to establish this we performed an investigation of both the initial sesquiterpenoid contents of the fruit bodies of *L. sanguifluus*, as well as of the nature of any new compounds formed in injured specimens.

In accordance with the situation observed in the fruit bodies of *L. deliciosus* and *L. deterrimus*, no traces of the free sesquiterpenes **1b**, **1c**, **2b**, **2c** or **3b** could be detected by TLC analysis of hexane extracts of young and undamaged fruit bodies of *L. sanguifluus* (extracted directly after collection). Instead, the presence of the ester **1a** could be demonstrated by comparison of ^{1}H NMR and TLC data with an original sample isolated from *L. deliciosus*. Besides the yellow ester **1a**, the presence of an equally nonpolar but red compound was indicated, but due to the instability of the compounds, the limited amounts available, and their similar chromatographic properties, it was not possible to separate the two completely. However, inspection of the ^{1}H NMR spectrum of a purified fraction of the unidentified red compound, and comparison of this with those of the other sesquiterpenoids discussed here, strongly suggest that it is the ester **3a**. The only difference (except for the signals of the fatty acid protons) compared to the ^{1}H NMR spectrum of the new alcohol **3c** (for which we propose the name sangol, structure discussed below), is that the signal for H$_2$-15 was shifted downfield from δ4.58 in the spectrum of sangol **3c**, to δ5.04 in the spectrum of the compound believed to be ester **3a**. We believe that this compound is identitical to 'lipophiles lactaroviolin' [2]. Sangol **3c** was isolated by rapid silica gel chromatography from an ethyl acetate extract of fruit bodies of *L. sanguifluus* that had



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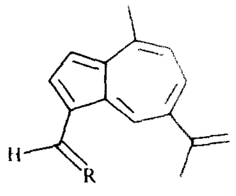

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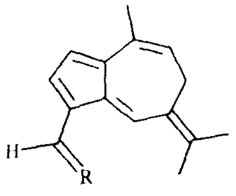
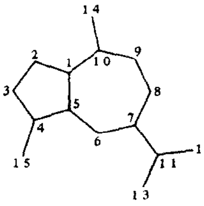


3

a R = $H,OCO(CH_2)_{16}Me$
b R = O
c R = H,OH



been ground 30 min prior to extraction. It showed the same chromatographic properties as the alcohols **1c** and **2c**, and is just as unstable as compound **1c**. Its structure could be established by analysis of the spectroscopic data, and especially by comparing its ^{1}H NMR data with those of compounds **1b**, **1c**, **2b**, **2c** and **3b**. Compared with the corresponding aldehyde **3b** [4], all signals in the spectrum of sangol **3c** appeared with the expected multiplicity and coupling constants, except for the δ9.78 singlet integrating for one proton which had been replaced by a δ4.58 singlet integrating for two protons. In addition, the olefinic protons were slightly upshifted in the spectrum of the alcohol **3c**, which is expected if C-15 is a hydroxymethyl group instead of a formyl group. Actually, the observed differences between the ^{1}H NMR spectra of the two compounds **3b** and **3c** are almost identical to the reported differences between the spectra of the aldehyde **1b** [1] and the alcohol **1c** [5]. In conclusion, it appears as if the fruit bodies of the *Lactarius* species belonging to the Dapetes section originally contain fatty acid esters of at least one guaiane sesquiterpene (i.e. compounds **1a**, **2a** and/or **3a**), and that these esters are converted enzymatically into the aldehydes **1b**, **2b** and/or **3b** and the alcohols **1c**, **2c** and/or **3c** if the fruit bodies are injured. The enzymatic conversions appear to be relatively simple, like ester hydrolysis and oxidation of a primary alcohol to an aldehyde, but the fact that lactaroviolin **2b** and deterrol **2c** are formed also in mushrooms which do not contain the ester **2a** originally, show that other oxidations also take place.

EXPERIMENTAL

Fruit bodies of *L. sanguifluus* were collected near Karlstadt (F.R.G.) in September 1986, and near Caussols (Alpes Maritimes) (France) in October 1987. The extraction of the fresh mushrooms and the work-up of the extracts were made in the same way as described previously [1]. ^{1}H NMR (300 MHz) spectra were run in $CDCl_3$, J is given in Hz and the chemical shifts in ppm relative to tetramethylsilane. The UV spectrum was recorded with a Cary 219 spectrophotometer in EtOH, and the mass spectrum (EI) was obtained at 70 eV.

8-*Hydro*-1-*hydroxymethyl*-4-*methyl*-7-isopropylideneazulene (**3c**) (*sangol*). 2 mg, was obtained as a dark red oil after repeated chromatography on silica gel. UV λ_{max}^{EtOH} nm (log ε): 239 (4.06), 280 (3.83), 343 (3.86), 445 (2.67); ^{1}H NMR: δ7.56 (1H, *s*, H-6), 6.42 (1H, *d*, $J_{2,3} = 2.4$, H-2 or H-3), 6.34 (1H, *m*, H-2 or H-3), 5.59 (1H, *dt*, $J_{8,9} = 7.2$, $J_{9,14} = 1.2$, H-9), 4.58 (2H, *s*, H-15), 3.07 (2H, *d*, $J_{8,9} = 7.2$, H-8), 1.98 and 1.93 (3H + 3H, *s*, H-12 and H-13), 1.94 (3H, *d*, $J_{9,14} = 1.2$, H-14); EIMS (probe) 70 eV *m/z* (rel. int.): 214 (M^+) (25), 155 (13), 143 (15), 129 (46), 115 (19), 73 (100), 60 (99); insufficient amounts were obtained for ^{13}C NMR and elemental analysis.

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