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ISOLATION OF 12-HYDROXYCARYOPHYLLENE-4,5-OXIDE, A SESQUITERPENE FROM *LACTARIUS CAMPHORATUS**

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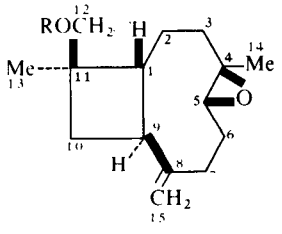
Key Word Index—*Lactarius camphoratus*; Basidiomycetes; 12-hydroxycaryophyllene-4,5-oxide; caryophyllene derivatives; sesquiterpenes.

Abstract—A new sesquiterpene, 12-hydroxycaryophyllene-4,5-oxide, has been isolated from the ethanolic extract of *Lactarius camphoratus*. The structure, stereochemistry and absolute configuration were determined by a combination of spectral data and single-crystal X-ray analysis of the *p*-bromobenzoate derivative.

INTRODUCTION

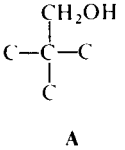
During the last decade the structures of many sesquiterpenes isolated from *Lactarius* mushrooms have been reported. The sesquiterpenes commonly found in *Lactarius* species include the guaianes [1], the lactaranes and related compounds [2–5], and drimanes [6]. In our continuing search for new sesquiterpenes from mushrooms, we examined the contents of *Lactarius camphoratus* and have isolated 12-hydroxycaryophyllene-4,5-oxide (**1**), the first naturally occurring C-12 oxygenated caryophyllene. In addition, the isolation of **1** constitutes the first finding of the caryophyllane skeleton in *Lactarius* species.



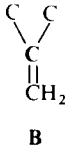
1 R = H
2 R = Ac
3 R = *p*-BrC$_6$H$_4$CO

RESULTS AND DISCUSSION

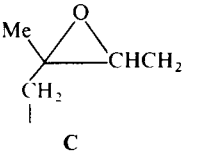
Chromatography on silica gel of an ethanolic extract of *L. camphoratus* employing chloroform–acetone (9:1) resulted in isolation (0.06%) of a new sesquiterpene. 12-Hydroxycaryophyllene-4,5-oxide (**1**), $C_{15}H_{24}O_2$, M^+ at *m/z* 236, was obtained as an amorphous solid, mp 48–53°, $[\alpha]_D^{23}$ −69.9° (*c* 1.0, $CHCl_3$). The 220 MHz ^1H NMR spectrum ($CDCl_3$) of **1** exhibited two one-proton singlets at δ 4.90 and 5.02 which, together with IR ($CHCl_3$) bands at 1625 and 890 cm^{-1}, indicated the presence of an exocyclic methylene. The presence of a two-proton singlet at δ 3.36 coupled with absorptions at 3620 and 3450 cm^{-1} in the IR spectrum suggested the presence of a hydroxymethyl group attached to a quaternary carbon. Further confirmation that a single OH function was present was obtained by transformation of **1** into the corresponding acetate **2**, $C_{17}H_{26}O_3$, M^+ at *m/z* 278, mp 80–83°, $[\alpha]_D^{23}$ −46.4° (*c* 1.0, $CHCl_3$). Examination of the ^1H NMR spectrum of **2** revealed a single acetate and a downfield shift of the C-12 methylene protons [^1H NMR ($CDCl_3$) δ 3.84 (*s*, 2H), 2.06 (*s*, 3H)]. The presence of the 4,5-epoxide was strongly suggested by a three-proton singlet in the ^1H NMR spectrum at δ 1.20, whose carbon atom appeared at 15.10 (*q*) in the ^{13}C NMR spectrum, and the appearance of a one-proton doublet of doublets (J = 10, 4 Hz) centred at 2.93. In addition, the ^1H NMR revealed a three-proton singlet at δ 1.04 (C-15 Me), which shifted downfield to 1.17 in the corresponding *p*-bromobenzoate **3** (see below). The above data coupled with the ^{13}C NMR data (see Experimental) led to three obvious partial structures **A**–**C**.



A



B



C

Evidence that these subunits were part of a caryophyllene skeleton was strongly suggested by the presence of one-proton triplet at δ 1.84 (J = 10 Hz) and a one-proton quartet centred at 2.68 (J = 10 Hz) which were assigned to the protons at C-1 and C-9, respectively [7].

Having established the presence of the caryophyllene ring system, we resorted to single-crystal X-ray analysis in order to determine unambiguously the detailed structure and stereochemistry of **1**. X-ray analysis of the *p*-bromobenzoate derivative **3** of 12-hydroxycaryophyllene-4,5-oxide established unequivocally the stereochemistry depicted in Fig. 1.

* Part XIII in the series "Constituents of Higher Fungi". For Part XII, see Daniewski, W. M. and Król, J., *Pol. J. Chem.* (in press).

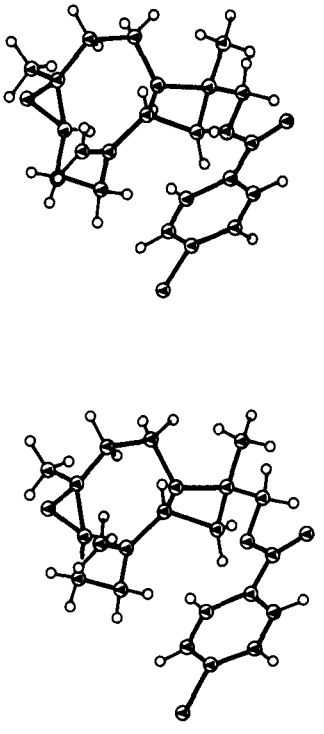


Fig. 1.

Compound **3** crystallized in space group $P2_12_12_1$ with $a = 7.354$ (2), $b = 23.739$ (6) and $c = 11.250$ (3) Å at $-162°$; $D_{calc} = 1.418$ g/cm^3 for $Z = 4$. A continuous θ–2θ scan at a rate of 4°/min over a range of 2° + dispersion and 2-sec background counts was used to collect the 2609 unique amplitudes on a Picker goniostat. The structure was solved by Patterson and Fourier techniques and refined by full-matrix least squares. At the present stage of refinement the residuals are $R(F) = 0.067$ and $Rw(F) = 0.050$. The figure as shown corresponds to the proper enantiomorph as determined by the anomalous dispersion terms of the bromine atom scattering factors.

EXPERIMENTAL

Isolation of 12-*hydroxycaryophyllene*-4,5-*oxide*. Fresh *Lactarius camphoratus* (Bull ex Fr.)Fr. (450 g) were crushed and immersed in EtOH (2 l.) and left for 2 weeks. Filtration followed by evaporation of the aq. EtOH *in vacuo* provided 45 g of a crude extract. The residue was dissolved in an Et_2O–H_2O (1:1) mixture (0.5 l.) and the H_2O layer was extrd (60 hr) continuously with Et_2O. The combined Et_2O extracts were evapd under red. pres. leaving 10 g of a residue which was purified on 50 g of activity III alumina using 0.5 l. C_6H_6–EtOH (7:3) as the solvent system. After removal of the solvent the residue (4.5 g) was chromatographed (medium pressure) on 160 g Si gel using a $CHCl_3$–Me_2CO gradient eluting system. The chromatography was monitored by TLC ($CHCl_3$–Me_2CO, 9:1). Fractions possessing R_f values of 0.35 were combined and gave 285 mg of pure 12-hydroxycaryophyllene-4,5-oxide ($C_{15}H_{24}O_2$) as an amorphous solid, mp 48–53°; $[\alpha]_D^{23}$ −69.9° (*c* 1.0, $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3620, 3450, 1625, 890; ^{1}H NMR (220 MHz) ($CDCl_3$): δ 1.04 (3 H, *s*, H-13), 1.20 (3 H, *s*, H-14), 1.84 (1 H, *t*, J = 10 Hz, H-1), 2.68 (1 H, *q*, J = 10 Hz, H-9), 2.93 (1 H, *dd*, J = 10 Hz, 4 Hz, H-5), 3.36 (2 H, *s*, H-12), 4.90 (1 H, *s*, H-15), 5.02 (1 H, *s*, H-15); ^{13}C NMR ($CDCl_3$): 15.10 (*q*, C-14), 25.81 (*q*, C-13), 28.06 (*t*, C-6), 28.13 (*t*, C-3)*, 32.20 (*t*, C-2) and C-10), 36.69 (*s*, C-11), 36.98 (*t*, C-7, 42.98 (*d*, C-1), 46.26 (*d*, C-9), 57.93 (*s*, C-4), 61.78 (*d*, C-5), 68.58 (*t*, C-12), 111.16 (*t*, C-15), 149.78 (*s*, C-8)*; MS *m/z*: 236 $[M]^+$.

Caryophyllene-4,5-*oxide*-12-*acetate* (**2**). Compound **2** was obtained in near-quantitative yield by acetylation of **1** employing standard procedures. Thus 50 mg (0.21 mmol) of 12-hydroxycaryophyllene-4,5-oxide gave 57 mg (97%) of pure **2** as a crystalline substance, mp 80–83°; $[\alpha]_D^{23}$ −46.4° (*c* 1.0, $CHCl_3$); IR ν_{max}^{Nujol} cm^{-1}: 1725, 1630; ^{1}H NMR (220 MHz) $CDCl_3$: δ 1.60 (3 H, *s*, H-13), 1.18 (3 H, *s*, H-14), 1.81 (1 H, *t*, J = 10 Hz, H-1), 2.06 (3 H, *s*, OCOMe), 2.68 (1 H, *q*, J = 10 Hz, H-9), 2.92 (1 H, *dd*, J = 10 Hz, 4 Hz, H-5), 3.77 (2 H, *s*, H-12), 4.84 (1 H, *s*, H-15), 4.94 (1 H, *s*, H-15), MS *m/z*: 278 $[M]^+$. (Found: C, 73.21; H, 9.42. $C_{17}H_{26}O_3$ requires: C, 73.34; H, 9.34%.)

Caryophyllene-4,5-*oxide*-12-p-*bromobenzoate* (**3**). 12-Hydroxycaryophyllene-4,5-oxide (60 mg, 0.25 mmol) dissolved in 5.0 ml pyridine was treated with 77 mg (0.35 mmol) *p*-bromobenzoyl chloride dissolved in 3.0 ml pyridine. The reaction was heated at reflux for 15 min. The reaction mixture was evapd to dryness under high vacuum and the residue was chromatographed on Si gel. Elution with hexane–EtOAc provided 80 mg (75%) pure **3** as a crystalline compound, mp 88–90°; $[\alpha]_D^{23}$ −16.3° (*c* 1.0, $CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1710, 1630; ^{1}H NMR (220 MHz) $CDCl_3$: δ 1.18 (3 H, *s*, H-13), 1.21 (3 H, *s*, H-14), 1.90 (1 H, *t*, J = 10 Hz, H-1), 2.75 (1 H, *q*, J = 10 Hz, H-9), 2.84 (1 H, *dd*, J = 10 Hz, 4 Hz, H-5), 4.09 (2 H, *s*, H-12), 4.89 (1 H, *s*, H-15), 5.01 (1 H, *s*, H-15), 7.70 (4 H, AB*q*, J = 8 Hz, $\Delta\nu_{XB}$ = 70 Hz); MS *m/z*: 419 $[M]^+$. (Found: C, 63.13; H, 6.58. $C_{22}H_{27}BrO_3$ requires: C, 63.01; H, 6.48%.)

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* Assignments may be reversed.