*Phytochemistry*, Vol. 27, No. 10, pp. 3315–3317, 1988.
Printed in Great Britain.

0031-9422/88 $3.00+0.00


# 8-*EPI*-PIPERTRIOL, A LACTARANE SESQUITERPENE FROM *LACTARIUS NECATOR*

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(*Received* 22 *December* 1987)

**Key Word Index**—*Lactarius necator*; Basidiomycetes; trihydroxy lactarane sesquiterpenes; configuration determination.

**Abstract**—An ethanol extract of *Lactarius necator* gave 8-*epi*-pipertriol [(2*R**, 3*R**, 7*S**, 8*S**, 9*R**-5,8,13-trihydroxylactaran-4-ene] in addition to known sesquiterpenes. The structure of this new trihydroxy lactarane was established by $^1H$ and $^{13}C$ NMR spectroscopy and confirmed by chemical correlation with lactarorufin N.

*Lactarius necator* (Bull. em Pers. ex Fr.) Karst is a very common mushroom in European forests. It has already been investigated in our laboratories with regard to the contents of monohydroxy-[1–5] and dihydroxy sesquiterpene lactones [6]. In view of recent discoveries, we can expect the presence of trihydroxy compounds in the ethanol extract from this mushroom. In fact epipiperdial (**1**) and epipiperalol (**2**), recently isolated [7,8] from ground *L. necator*, affords trihydroxy compounds upon enzymatic reduction.

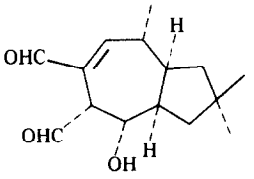
By a series of chromatographic separations (see the Experimental part) a very polar crystalline compound **3a** was isolated. The IR spectrum of **3a** showed no C=O absorption, but an intense OH band at 3300 $cm^{-1}$ and a weak but significant C=C band at 1670 $cm^{-1}$.

The elemental analysis and the NMR spectra led to the molecular formula $C_{15}H_{26}O_3$. The latter was substantiated by the EIMS spectrum of **3a**, as it exhibited $[M-H_2O]^+$ ion at *m/z* 236.

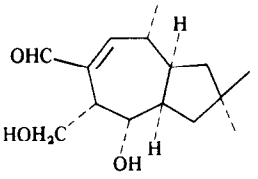
The $^{13}C$ NMR spectrum of **3a** is consistent with the presence of the lactarane skeleton possessing two $-CH_2O$ groups (triplets at 63.48 and 68.79 ppm), one $>CHO$ group (doublet at 74.82 ppm) and one disubstituted double bond (singlet at 139.4 and doublet at 135.44 ppm). The presence of the latter was confirmed by the vinylic proton signal at 5.43 ppm in the $^1H$ NMR spectrum of **3a**. One of the three methyl signals was a doublet (0.94 ppm, $J=7.0$ Hz), showing that it was attached to a secondary carbon atom. Chemical shifts and coupling constants of all protons of **3a–c** are shown in Table 1.

The presence of three OH groups in the molecule of **3a** was proved by acetylation which gave the triacetyl derivative **3b**. The IR spectrum of **3b** showed no OH bands. Furthermore the signals of five protons, highly overlapping between 3.72 and 4.05 ppm in the spectrum of **3a**, were shifted down field upon acetylation and formed five well separated sets (4.17, 4.41, 4.48, 4.62 and 5.22 ppm respectively) which were assigned to two primary and one secondary alcoholic groups. After *in situ* TAI acylation [9] of **3a**, besides the improved resolution of the CH–O signals, three NHCO proton singlets were visible in the $^1H$ NMR spectrum of **3c**, confirming the above assignments. Extensive decoupling experiments performed on both **3b** and **3c** led to the formula **3a** for this new sesquiterpene. The following stereochemical assignments could be made: H-2 and H-3 trans diaxial ($J_{2,3}=11.5$ Hz for **3b** and 11.0 Hz for **3c**), H-9 and H-8 trans diaxial ($J_{8,9}=11.5$ Hz for **3b** and 11.6 Hz for **3c**), H-8 and H-7 *cis* ($J_{7,8}=2.8$ Hz for **3b** and 2.5 Hz for **3c**).

The relative configuration of the molecule was confirmed by the observed NOEs for **3b**. Thus irradiation of H-




**1**

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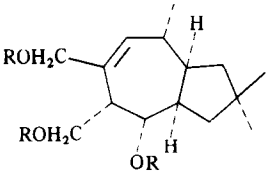



**3a** R = H
**3b** R = Ac
**3c** R = $CCl_3NHCO$

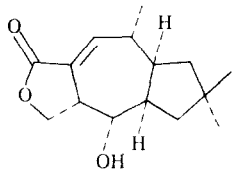
Table 1. $^1$H NMR spectral data of compounds **3a**–**3c** (300 MHz, $CDCl_3$, $\delta$, TMS as int. standard)

| H | 3a* | 3b† | 3c§ |
|---|---|---|---|
| 1α | 1.68 *ddd* | 1.71 *ddd* | 1.79 *ddd* |
| 1β | 1.27 *dd* | 1.22 *dd* | 1.30 *dd* |
| 2 | 1.95 *m* | ~2.05 *m*‡ | 2.05 *m* |
| 3 | 2.24 *m* | 2.35 *m* | 2.39 *m* |
| 4 | 5.43 *brs* | 5.59 *brd* | 5.78 *brd* |
| 5 | 3.91 *dt* | 4.62 *dt* | 4.61 *dt* |
| 5′ | 3.99 *dt* | 4.41 *brd* | 4.95 *dt* |
| 7 | 2.75 *m* | 2.78 *tt* | 3.02 *brt* |
| 8 | 3.86 *dd* | 5.22 *dd* | 5.31 *dd* |
| 9 | 2.55 *m* | 2.60 *tdd* | 2.67 *m* |
| 10α | 1.78 *ddd* | 1.53 *ddd* | 1.64 *ddd* |
| 10β | 1.34 *t* | 1.20 *t* | 1.37 *t* |
| 12 | 0.94 *d* | 0.99 *d* | 1.04 *d* |
| 13 | 3.79 *dd* | 4.17 *dd* | 4.49 *dd* |
| 13′ | 3.95 *dd* | 4.48 *dd* | 4.64 *dd* |
| 14 | 1.06 *s* | 1.04 *s* | 1.07 *s* |
| 15 | 0.97 *s* | 0.94 *s* | 0.99 *s* |

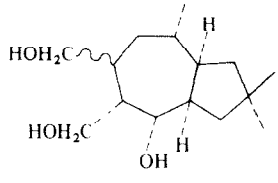
*In $Me_2CO$-$d_6$ †OAc: 2.05*s*, 2.054*s*, 2.065*s*. ‡obscured by OAc signals. §200 MHz. NH:8.54*s*, 8.61*s*, 8.78*s*.

*J*(Hz) **3a**: 1α, 1β = 13; 1α, 2 = 7.8; 1α, 10α = 2; 1β, 2 = 6.8; 5, 5′ = 12.2; 5, 4 ≃ 5, 3 = 1.2; 5′, 4 ≃ 5′, 3 = 1.4; 7, 8 = 2.5; 8, 9 = 10.5; 3, 12 = 7; 10α, 10β ≃ 10β, 9 = 12; 10α, 9 = 6.4; 13, 13′ = 10.2; 13, 7 = 6; 13′, 7 = 7; **3b**: 1α, 1β = 13; 1α, 2 = 8; 1α, 10α = 2; 1β, 2 = 7.2; 2, 3 = 11.5; 2, 9 = 9.7; 3, 12 = 7; 3, 4 = 2; 5, 5′ = 12.2; 5, 4 ≃ 5, 3 < 1; 5′, 4 ≃ 5′, 3 = 1.3; 7, 8 = 2.8; 7, 3 = 2.3; 7, 13 = 5.5; 7, 13′ = 5; 8, 9 ≃ 9, 10β = 11.5;9, 10α = 6.3; 10α, 10β = 12; 13, 13′ = 11.3. **3c**: 1α, 1β = 13.3; 1α, 2 = 8.0; 1α, 10α = 1.8; 1β, 2 = 6.9; 2, 3 = 11; 2, 9 = 9.6; 3, 12 = 7.1; 3, 5 = 0.8; 3, 5′ = 2.2; 4, 5 = 0.7; 4, 5′ = 2; 5, 5′ = 12.1; 7, 8 = 2.5; 7, 13 = 5.9; 7, 13′ = 6.8; 8, 9 = 11.6; 9, 10α = 6.5; 9, 10β = 12; 10α, 10β = 12.3; 13, 13′ = 11.3.

8 showed marked effect on H-7 (7.8%), and H-3 (8.8%), whereas the signals of the H-2 and H-9 protons were not affected. The final proof of the entire stereostructure of compound **3a** was obtained by chemical correlation with lactarorufin N (**4**) [2,5]. Lithium aluminium hydride reduction of **4** gave a triol which was identical in all respects with the natural compound **3a**. The major product of the reaction was accompanied by minor amounts of the two C-6 epimeric triols **5** resulting from the C-4, C-6 double bond reduction.



**4**



**5**

## EXPERIMENTAL

*Lactarius necator* was collected in September 1985 and 1986 in Zalesie mixed forests near Warsaw and was authenticated by Prof. A. Skirgiello, Warsaw University.

Fresh mushrooms (28.5 kg) were soaked in EtOH (40 l) and left for 2 weeks. Subsequently the mushrooms were filtered, ground and soaked in a new portion of EtOH (20 l) for 5 days. After filtration the EtOH extracts were combined and evapd *in vacuo* leaving a tarry residue. This was dissolved in $H_2O$–$Et_2O$, 1:1. The 2 layers were sepd and the aq. layer extracted with $Et_2O$ in a continuous extraction apparatus for 60 hr. The $Et_2O$ extracts were combined, evapd and partitioned between hexane and 60% aq. EtOH. The aq. EtOH layer was evapd and gave a residue (21 g) which was chromatographed on silica gel (Kieselgel Merck, 0.040–0.063 mm, 800 g) with a $C_6H_6$–$Me_2CO$ gradient system. After elution of dihydroxy compounds (lactarorufin A [2] and isolactarorufin [10]) the column was washed with $Me_2CO$ (2 l). The $Me_2CO$ fraction (4.2 g) was separated with a silica gel CC (210 g) by using $CHCl_3$–$Me_2CO$, 3:2, as eluent. Fractions possessing $R_f$ 0.35–0.45 (TLC:silica gel GF 254, Merck), visualized as red spots with EtOH anisaldehyde–0.5% $H_2SO_4$, were collected. After evaporation, separation of the residue (2.7 g) by HPLC (Lichroprep RP-18 25–40 μ; 300 × 16 mm stainless steel columns; MeOH–$H_2O$, 3:2; Varian RI detector; repeated separations) gave 650 mg pure **3a**.

8-epi-*Pipertriol* (**3a**). Colourless crystals, mp 108–114°, $[\alpha]_D^{20}$–36.6° ($CHCl_3$; *c* 1), $[\alpha]_{578}^{20}$–39.0° ($CHCl_3$; *c* 1). IR $\nu_{max}^{nujol}$ cm$^{-1}$: 3300, 1670; $^1$H NMR given in Table 1; $^{13}$C NMR (75.47 MHz, $Me_2CO$-$d_6$): $\delta$ 139.4 *s* (C-6); 135.4 *d* (C-4); 74.8 *d* (C-8); 68.8*t* (C-5); 63.5 *t* (C-13); 50.2 *d*, 45.7 *d* and 44.7 *d* (C-7), (C-9) and (C-2); 48.8 *t* and 46.9 *t* (C-1) and (C-10); 37.3 *s* (C-11); 35.7 *d* (C-3); 30.7 *q* and 28.6 *q* (C-14) and (C-15); 22.7 *q* (C-12); EIMS (probe) 70 eV *m/z* (rel. int.): 254 $[M]^+$ (not observed), 236 $[M-H_2O]^+$ (3.9), 218 $[M-2H_2O]^+$ (6.0), 206 $[M-H_2O-CH_2O]^+$ (25.1), 189 (14.1), 175 (55.8), 161 (16.7), 153 (100), 139 (17.9), 123 (55.7), 109 (46.6), 105 (39.8), 95 (83.8), 81 (49.5), 69 (35.5), 55 (58.9), 41 (66.5). (Found: C,70.61; H, 10.4. $C_{15}H_{26}O_3$ requires: C.70.82; H, 10.32%).

*In situ* acylation of **3a** was performed by the addition of a small excess of trichloroacetyl isocyanate (TAI) directly into the NMR sample tube [9] and the product **3c** was characterized by $^1$H NMR spectrum (Table 1) without isolation.

8-epi-*Pipertriol triacetate*. Acetylation of **3a** with $Ac_2O$/Pyridine as usual gave the triacetate **3c** as a colourless oil in quantitative yield, $[\alpha]_D^{20}$–81.2° ($CHCl_3$; *c* 1). IR $\nu_{max}^{nujol}$ cm$^{-1}$ no OH band, 1735; $^1$H NMR given in Table 1; EIMS (probe) 15 eV *m/z* (rel. int.): 380 $[M]^+$ (not observed), 320 $[M-AcOH]^+$ (0.4), 260 $[M-2AcOH]^+$ (12.5), 218 $[M-2AcOH-CH_2CO]^+$ (30.9), 200 $[M-2AcOH-CH_2CO-H_2O]^+$ (100), 187 (21.4), 185 (21.5), 157 (4.3), 144 (11.9), 95 (2.9), 81 (0.7), 43 (7.9); EIMS (probe) 70 eV *m/z* (rel. int.): 380 $[M]^+$ (not observed), 260 $[M-2AcOH]^+$ (5.5), 218 $[M-2AcOH-CH_2CO]^+$ (12.5), 200 $[M-2AcOH-CH_2O-H_2O]^+$ (44.8), 187 (13.9), 185 (12.0), 157 (4.6), 144 (7.6), 95 (7.1), 81 (7.4), 55 (9.3), 43 (100).

*Reduction of lactarorufin N* (**4**). **4** (51 mg), dissolved in $Et_2O$ (50 ml), was added slowly to $LiAlH_4$ (120 mg) in $Et_2O$ (50 ml) at 0°. The reaction mixture was stirred for 0.5 hr at 0°, then treated with satd $Na_2SO_4$ aq. soln, filtered, and the Al salts were rinsed with a large amount of $Et_2O$. The $Et_2O$ phases were bulked, dried ($MgSO_4$) and filtered. Removal of $Et_2O$ gave a mixture which was separated by prep. HPLC using a pack of 5 stainless steel columns (300 × 8 mm each, filled with Lichrosorb Si 60, 10μ; $CHCl_3$–hexane–*iso*-PrOH, 9:9:2, as eluent; Varian RI detector). Pure triol **3a** (15 mg) was obtained, identical with the natural

compound ($[\alpha]_D^{20}$, co-TLC, IR, NMR). An inseparable mixture of the two C-6 epimers **5** was also isolated.

*Acknowledgements*—This work has been done according to a scientific co-operation program between CNR (Italy) and the Polish Academy of Sciences (Poland), which provided financial support and fellowships to two of us (W. D. and G.V.). Partial financial aid was also given by the MPI (40%). We are grateful to Mr W. Ignacak for expert technical assistance.

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*Phytochemistry*, Vol. 27, No. 10, pp. 3317–3319, 1988.
Printed in Great Britain.

0031 9422/88 $3.00+0.00


# BICYCLOGERMACRENE TYPE SESQUITERPENOID FROM THE LIVERWORT *CONOCEPHALUM CONICUM*

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(*Received in revised form* 11 *March* 1988)

**Key Word Index**—*Conocephalum conicum*; Conocephalaceae; Hepaticae; bicyclogermacren-13-al; bicyclogermacrene; sesquiterpene aldehyde; bicyclo[8.1.0]undecane.

**Abstract**—From the thalloid liverwort *Conocephalum conicum*, a new sesquiterpene aldehyde has been isolated and its structure has been elucidated as bicyclogermacren-13-al by extensive 2D NMR spectroscopy.

## INTRODUCTION

Two chemical studies on the thalloid liverwort *Conocephalum conicum* have been documented in the past decade [1]. The liverworts, however, can be regarded as one of the plant sources to search for biologically interested substances, as they produce a number of terpenoids [2, 3] and aromatic compounds [3], and also large amounts of material are readily available. As part of our program [4], we have re-examined *Conocephalum conicum* from Japan and have isolated a new sesquiterpene aldehyde (**1**) named bicyclogermacren-13-al. This paper describes the structure of the new sesquiterpene.

## RESULTS AND DISCUSSION

A combination of column chromatography on silica gel, Sephadex LH-20, and HPLC of an ether extract of *C. conicum* led to the isolation of a new labile sesquiterpene (**1**) as a colourless oil.

Compound **1** had the molecular formula $C_{15}H_{22}O$ ($M^+$ at $m/z$ 218.1650) indicating five degrees of unsaturation. The IR, $^1H$ NMR and $^{13}C$ NMR spectra (Table 1) indicated the presence of a formyl group (1690 $cm^{-1}$; $\delta$9.60 *s*). One tertiary methyl group ($\delta$1.27 *s*), two olefinic methyl groups ($\delta$1.50 and 1.69, each *s*) which were long range coupled with the olefinic protons at $\delta$4.96 (2 H, *m*) on two trisubstituted double bonds ($\delta$122.2 and 125.8, each *d*, 131.3 and 139.8, each *s*). These spectral data showed that **1** was a bicyclosesquiterpene. The 2D $^1H$–$^1H$ and $^{13}C$–$^1H$ COSYs were extensively examined to clarify the connectivity of each proton in **1**. One of the two olefinic proton signals ($\delta$122.2 and 125.8) eventually appeared at the same field ($\delta$4.96) and displayed a coupling to the signals at $\delta$2.13 (*dd*, $J$ = 11.7, 8.8 Hz), which were further coupled with the high field signals at $\delta$1.43 (*ddd*, $J$ = 12.5, 8.8, 2.9 Hz). Moreover, this methine signals had cross peaks with the two geminal proton signals ($\delta$1.94 and 2.05; $\delta$25.8), each of which showed additional cross peaks with the methylene signals ($\delta$1.75 and 2.52;