Pergamon

PII: S0031-9422(97)00121-0

Phytochemistry, Vol. 45, No. 7, pp. 1453–1455, 1997

0031-9422/97 $17.00+0.00

RUSSULACTARORUFIN, A LACTARANE SKELETON SESQUITERPENE FROM *RUSSULA BREVIPES*

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(*Received in revised form* 11 *December* 1996)

Key Word Index—*Russula brevipes*; russulaceae; sterol; sesquiterpene lactones; lactarorufin A; russulactarorufin.

Abstract—A new sesquiterpene lactone named russulactarorufin together with lactarorufin-A and 24-ethyl-cholesta-7, 22*E*-diene-3β,5α,6β-triol have been isolated from *Russula brevipes*. The structures of the compounds were established on the basis of chemical and spectral evidence. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Russula brevipes, a short stalked white mushroom growing widely in north-western Himalayas, in woods during and after the rainy season, is reported to be an edible mushroom although it is not consumed locally [1]. It has not been investigated for its chemical constituents previously. Present phytochemical analysis has resulted in the isolation and characterization of three constituents, Rb-1, Rb-2 and Rb-3. Rb-1(**1**) and Rb-2(**2**) have been characterized as sesquiterpene lactones, while Rb-3(**3**) has been identified as 24-ethyl-cholesta-7, 22*E*-diene-3β,5α,6β-triol, reported for the first time from a fungal source [2, 3].

RESULTS AND DISCUSSION

Compound **1**, a colourless solid, mp 198°, $[\alpha]_D^{25}$ −13.9° (pyridine, 0.86), was sparingly soluble in chloroform or methanol but, readily soluble in pyridine. Molecular formula was established to be $C_{15}H_{20}O_6$ on the basis of elemental analysis and the FAB mass spectrum (*m/z* 297 $[M+H]^+$, 319 $[M+Na]^+$, 335 $[M+K]^+$, 593 $[2M+H]^+$ and 615 $[2M+Na]^+$). It was established on the basis of IR and UV spectra to be an α,β-unsaturated-γ-lactone bearing an additional free carboxyl group. Analysis of the ^{1}H NMR spectrum of **1** (see Experimental) showed the presence of two tertiary methyls (δ 1.41 and 1.54), one hydroxymethine (δ 4.70, *d*, J = 7.2 Hz), two oxymethylene protons (δ 5.09 and 5.15, *dd*, J = 17.4 Hz), two multiplets integrating for one proton each (δ 1.75 and 1.82) and a multiplet integrating for six protons (δ 2.85–3.00). The ^{13}C NMR spectrum (see Experimental) indicated the presence of four sp^2 hybridized C-atoms (δ 123.56, 163.64, 175.82 and 180.66), eleven sp^3 hybridized C-atom signals accounted for by two tertiary methyls (δ 25.45 and 25.62), four methylenes (δ 26.18, 46.94, 52.66 and 68.59), three methines (δ 41.28, 42.70 and 71.65) and two quaternary C-atoms (δ 36.82 and 72.86).

The spectral data indicated **1** to contain one lactone carbonyl (^{13}C NMR, δ 175.82; IR, 1736 cm^{-1}; UV 260 nm), one carboxyl group (^{13}C NMR, δ 180.66; IR, 1686 cm^{-1}) one oxymethylene (^{13}C NMR, δ 68.19; ^{1}H NMR, *dd* at δ 5.09 and 5.15, J = 17.4 Hz each), one hydroxymethine (^{13}C NMR, 71.65; ^{1}H NMR, δ 4.70; in the acetyl derivative the signal was shifted to δ 6.22), one oxygenated quaternary carbon atom (^{13}C NMR, δ 72.86) and two sp^2-hybridized C-atoms, α and β to a lactone carbonyl function (^{13}C NMR, δ 123.56 and 163.64). To confirm the presence of the carboxyl group, compound **1** was esterified with diazomethane; methyl ester, colourless crystals (EtOAc), mp 130°, readily soluble in $CHCl_3$, $[\alpha]_D^{25}$ +13.9° ($CHCl_3$, 0.11). Molecular composition $C_{16}H_{22}O_6$ derived from elemental analysis and FABMS. In the IR spectrum, it showed absorption at 1730 cm^{-1} indicating the presence of an ester function.

These spectral data indicated **1** to be tricyclic. On taking into account the structures of sesquiterpenoids isolated so far from mushrooms, compound **1** was thought to possess a structure in which one of the *gem*-dimethyls in lactarorufin-A [4] has been oxidized to a carboxyl group.

The spectral data compiled so far agreed fairly well with the proposed structure (**1**). The ^{1}H NMR spectrum of **1**-methyl ester revealed some interesting features. In **1**, quasi-axial protons, on C-1 and C-10 (one each), were observed as multiplets at δ 1.82 and

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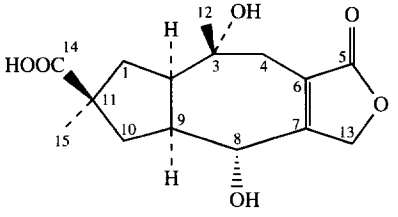
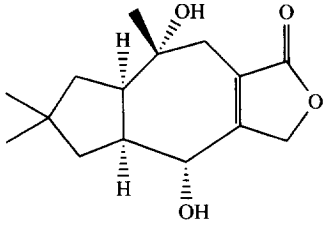
1.75. However, in the case of the methyl ester these absorptions were found to be at higher field than methyls i.e. δ 1.08 (t, J = 12.6 Hz) and 1.15 (q, J = 7.2 Hz). The assignments were amply supported by ^{1}H–^{1}H COSY spectra of the ester. The high field absorptions in the methyl ester could only be accounted for, if the protons fall in the shielding cone of the ester carbonyl. In the parent acid, hydrogen bonding between the carboxyl group and a hydroxyl group at C-3/C-8, oriented the carbonyl group in such a rigid conformation that the protons remained out of the shielding cone of the carbonyl group. Since the stereochemistry of hydroxyl groups at C-3/C-8 in lactarorufin-A [4] has been well established, the proposition of extra shielding of the quasi-axial protons at C-1 and C-10 by ester carbonyl is amply supported by a study of Dreiding models if the carboxyl group is oriented in the equatorial position anti to the C-2 and C-9 protons.

We propose russulactarorufin as the trivial nomenclature name for this novel isolate.



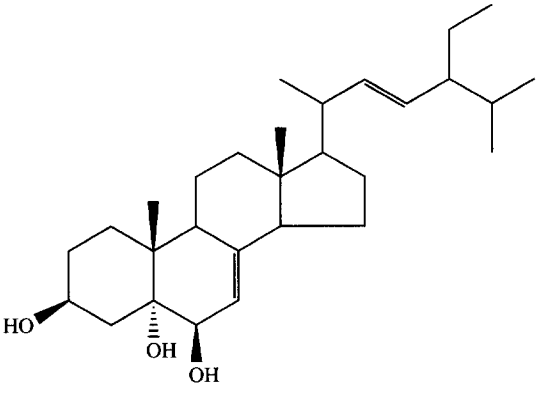
1

Compound **2**, a colourless crystalline solid, mp 163°, $[\alpha]_D^{25}$ +18.2° (MeOH, 0.1) was readily soluble in chloroform. The molecular formula was found to be $C_{15}H_{22}O_4$ from the FAB mass spectrum and elemental analytical data. Absorption at 1740 and 1692 cm^{-1} in the IR spectrum indicated the presence of an α,β-unsaturated-γ-lactone moiety in the molecule. It yielded a monoacetate on acetylation, which on crystallization from methanol was obtained as a colourless crystalline solid, mp 115°, $[\alpha]_D^{25}$ +18.6° ($CHCl_3$, 0.23). Physical constants and spectral data (UV, IR, ^{1}H and ^{13}C NMR) for **2** were in complete agreement with those reported for lactarorufin A. Compound **2**, was thus identified to be lactarorufin-A, previously reported from *Russula sardonia* [4] and *Lactarius rufus* [5].



2

Compound **3**, a colourless amorphous solid, mp 180°, $[\alpha]_D^{25}$ −4.4° (MeOH, 0.1) was soluble in methanol and pyridine, IRU$_{max}^{KBr}$ cm^{-1} 3450 (—OH), 1605(=) and 1380 (*gem*-dimethyl) and tested positive for steroidal skeleton. The molecular formula was determined to be $C_{29}H_{48}O_3$ from the M^+ ion in the EI-mass spectrum and from the elemental analytical data. Compound **3** yielded a diacetate, mp 153–54°, $[\alpha]_D^{25}$ −126.6° ($CHCl_3$, 0.03), the molecular composition was determined to be $C_{33}H_{52}O_5$ on the basis of the FAB-mass spectrum and elemental analytical data. A critical comparison of physical constants and spectral data (IR, UV, ^{1}H and ^{13}C NMR) revealed that **3** was 24-ethylcholesta-7, 22*E*-diene-3β,5α,6β-triol, reported earlier from marine scallops *Myriapora truncata* [2] and *Patinopecten yessoensis* [3]. However, its isolation from a fungal source has not been reported previously.



3

EXPERIMENTAL

Analytical methods. Mps uncorr; NMR; 300 MHz (^{1}H) and 75 MHz (^{13}C) Bruker AM 300; EIMS on JEOL JMS D-300 and FABMS on JEOLS 102 equipment; optical rotations on Perkin Elmer Model 241 digital polarimeter; IR spectra in Hitachi 270-30 IR spectrometer; UV spectra on Hitachi 320 UV spectrometer; Elemental analysis on Carlo Erba Stroneutazione elemental analysis model-1106. A voucher specimen of the plant material has been deposited in the Herbarium of Regional Research Laboratory, Jammu, India.

Extraction and isolation of **1**, **2** *and* **3**. Air and oven dried (40°), finely powdered fruit bodies of *Russula brevipes* (600 g) were extracted with petrol (60–80°) in a Soxhlet for 24 hr. The defatted material was extracted with MeOH for 40 hr. The MeOH extract after concn *in vacuo* yielded a thick brownish-red residue, which on standing gave a dark orange coloured solid, which yielded white coloured solid, mannitol, on crystallization from Me_2CO. The mannitol free MeOH extract, after removal of the MeOH, was dissolved in water. The aq. soln was partitioned with $CHCl_3$, EtOAc and *n*-BuOH, successively. The solvent extracts were dried over anhydrous Na_2So_4 and concd

under red. pres. yielding RBC ($CHCl_3$ fr.) 2 g, RBE (EtOAc fr.) 5 g and RBB (*n*-BuOH fr.) 2 g.

RBE on repeated CC over silica gel (mesh size 100–120, 100–200, 200–400) using hexane–$CHCl_3$–MeOH mixts of steadily increasing polarity yielded two TLC homogeneous frs. Frs eluted with $CHCl_3$–MeOH (99:1) on concn and crystallization from EtOAc yielded fine crystals of **1** (80 mg). The residue from frs eluted with $CHCl_3$–MeOH (4:1), displayed a distinct yellow spot on TLC. The residue on crystallization from EtOAc yielded a colourless solid of **2** (50 mg). RBC was chromatographed over silica gel (60–120 mesh) using $CHCl_3$–MeOH mixts in increasing polarity as eluant. Frs eluted with $CHCl_3$–MeOH (19:1) were pooled and rechromatographed, yielding a white amorphous solid of **3** (20 mg).

Compound **1**. Colourless crystalline solid (EtOAc), mp 198°, $[\alpha]_D^{25}$ −13.9° (pyridine, *c* 0.86); molecular composition from FABMS and elemental analysis $C_{15}H_{20}O_6$; FABMS *m/z* (rel. int.): 593 $[2M+H]^+$ (59), 335 $[M+K]^+$ (10), 319 $[M+Na]^+$ (41), 297 $[M-H]^+$ (83), 279 $[M+H-H_2O]^+$ (83), 261 $[M+H-2H_2O]^+$ (100). IR ν_{max}^{KBr} cm^{-1}: 3450–3100, 1736, 1686; UV λ_{max}^{MeOH} nm: 210, 260; ^1H NMR (pyridine-d_5): δ 1.41 (3H, *s*, CH_3), 1.54 (3H, *s*, CH_3), 1.75 (1H, *m*, H-10), 1.82 (1H, *m*, H-1), 2.85–3.00 (6H, *m*, H-10; H-1; H_2-4; H-2 and H-9), 4.70 (1H, *d*, *J* = 7.2 Hz, H-8), 5.09 and 5.15 (2H, *dd*, *J* = 17.4 Hz each H-13); ^{13}C NMR, (pyridine-d_5): δ 52.66 (C-1), 41.28 (C-2), 72.86 (C-3), 26.18 (C-4), 175.82 (C-5), 123.56 (C-6), 163.64 (C-7), 71.65 (C-8), 42.70 (C-9), 46.94 (C-10), 36.83 (C-11), 25.45 (C-12), 68.59 (C-13), 180.66 (C-14), 25.62 (C-15).

Methyl ester of **1**. An ethereal soln of CH_2N_2 (5 ml) was added to **1** (25 mg) and the mixt. allowed to stand overnight at 0°. Removal of solvent gave a residue, crystallization (EtOAc) yielded colourless solid, mp 130°, $[\alpha]_D^{25}$ +13.6° ($CHCl_3$ *e*, 0.11), molecular composition $C_{16}H_{22}O_6$, derived from FABMS and elemental analytical data. IR ν_{max}^{KBr} cm^{-1}: 3400, 1730, 1692. ^1H NMR ($CDCl_3$): δ 1.08 (1H, *t*, *J* = 12.6 Hz, H-1), 1.15 (1H, *q*, *J* = 7.2 Hz, H-10), 1.19 (6H, *s*, 2 × CH_3), 2.27 (1H, *m*, H-1), 2.50 (5H, *m*, H-10; H_2, H-2; H-9), 3.7 (3H, *s*, $-OCH_3$), 4.13 (1H, *bs*, H-8), 4.60 (1H, *d*, *J* = 17.4 Hz, H-13) and 4.95 (1H, *d*, *J* = 17.4 Hz, H-13); ^{13}C NMR ($CDCl_3$): δ 34.50 (C-1), 41.30 (C-2), 74.60 (C-3), 49.10 (C-4), 175.50 (C-5), 123.40 (C-6), 159.90 (C-7), 66.80 (C-8), 42.00 (C-9), 46.20 (C-10), 48.40 (C-11), 30.60 (C-12), 71.60 (C-13), 177.40 (C-14), 24.50 (C-15) and 52.20 ($-OCH_3$). FABMS: *m/z* (rel. int.): 643 $[2M+Na]^+$ (3), 62 $[2M+H]^+$ (55), 349 $[M+K]^+$ (7), 333 $[M+Na]^+$ (21), 311 $[M+H]^+$ (62), 293 $[M+H-H_2O]^+$ (83), 275 $[M+H]-2H_2O]^+$ (100), 260 $[M+H-2H_2O-Me]^+$ (7).

Acetylation of **1**. Compound **1**, (10 mg), pyridine (0.5 ml) and Ac_2O (1 ml) were left overnight at room temp. Usual processing of the reaction mixt. and crystallization of the residue from EtOAc yielded colourless crystals, insoluble in $CHCl_3$, MeOH but soluble in pyridine, mp 195°, $[\alpha]_D^{25}$ +0.23° (pyridine, *c* 0.12). Molecular composition $C_{17}H_{22}O_7$, derived from FABMS and elemental analysis. IR ν_{max}^{KBr} cm^{-1}: 3400, 2950, 1760, 1715. ^1H NMR (pyridine-d_5); δ 1.41 (3H, *s*, $-CH_3$), 1.59 (3H, *s*, $-CH_3$), 1.68 (1H, *m*, H-1), 1.86 (1H, *t*, *J* = 12.00 Hz, H-10), 2.13 (3H, *s*, $-OCOCH_3$), 2.81 (6H, *m*, H-4; H-3; H-9; H-10), 4.77 (2H, AB*q*, *J* = 17.4 Hz, H_2-13), 6.22 (1H, *d*, *J* = 10.2 Hz, H-8).

Compound **2**. Colourless crystalline solid, mp 163°, $[\alpha]_D^{25}$ +18.2 (MeOH, 0.1), readily soluble in $CHCl_3$. Molecular composition $C_{15}H_{22}O_4$ derived from FABMS ($[M+Li]^+$ at *m/z* 273) and elemental analysis. Absorption maxima at 260 nm (MeOH) in UV spectrum and strong absorptions at 1740 and 1692 cm^{-1} in IR spectrum indicated the presence of an α,β-unsaturated-γ-lactone moiety in the molecule. Monoacetate mp 115°, $[\alpha]_D^{25}$ −18.6° ($CHCl_3$, 0.23) (^1H and ^{13}C NMR of **2** were in complete agreement with those reported for lactarorufin-A).

Compound **3**. White amorphous solid, mp 180° $[\alpha]_D^{25}$ −4.4° (MeOH, *c* 0.1), soluble in MeOH and pyridine. IR ν_{max}^{KBr} cm^{-1}: 3450, 1605 and 1380. Molecular ion peak at *m/z* 444 in EIMS and elemental analysis data were in good agreement with the molecular formula $C_{29}H_{48}O_3$.

Acetylation of **3**. On usual acetylation procedure, 3 yielded a diacetate, white solid, crystallized from MeOH, mp 153–54°, $[\alpha]_D^{25}$ −126.6° ($CHCl_3$, *c* 0.03), IR ν_{max}^{KBr} cm^{-1}: 3450, 1720, 1605 and 1380. ^1H and ^{13}C NMR were in total agreement with those reported for 24-ethylcholesta-7,22*E*-diene-3β,5α,6β-triol, isolated earlier from marine scallops *Myriapora truncata* and *Patinopecten yessoensis*.

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