A New Norsesquiterpenoid from *Russula delica* Fr.

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Received March 19, 2003; accepted May 15, 2003

Russulanorol (1), a new norsesquiterpenoid with a novel carbon skeleton, was isolated from the fruiting bodies of *Russula delica* Fr. (Russulaceae) together with three known sesquiterpenoids. The structure of 1 was elucidated on the basis of spectral data and chemical transformation. Compound 1 is an equilibrium mixture of two stereoisomers (1a, b) on the C-11 acetal carbon.

Key words *Russula delica*; Russulaceae; norsesquiterpenoid

The Russulaceae family is one of the largest in the subdivision Basidiomycotina in Whittaker's *Kingdom of Fungi* and comprises hundreds of species.[1] While secondary metabolites occurring in the fruiting bodies of European *Lactarius* species have been well investigated,[2] the *Russula* mushrooms have received less attention, notwithstanding the larger number of existing species.[3] The constituents of *Russula delica* Fr. have been previously investigated and shown to contain protoilludane sesquiterpenoids.[4—6] It has been reported that the extract of the fruiting bodies of *R. delica* can inhibit 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced inflammatory ear edema in mice.[7] In a continuation of our investigation of the terpenoid constituents of mushrooms,[8] we describe here the isolation and structure elucidation of a new norsesquiterpenoid, russulanorol (**1**), together with three known compounds from the fruiting bodies of *R. delica*. The known compounds were identified as blennin C (**2**),[9,10] lactarolide A (**3**),[11] and furandiol (**4**),[12] respectively, by comparison of their spectroscopic data with those previously described in the literature. To the best of our knowledge, this is the first report of the latter of these compounds from *R. delica*.

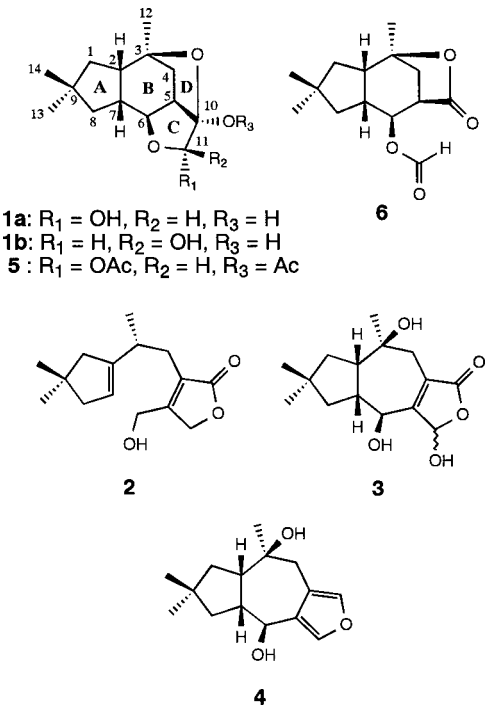

Chart 1

Compound **1** was isolated as an amorphous powder. The molecular formula was determined to be $C_{14}H_{22}O_4$ by high-resolution (HR)-electron ionization (EI)-MS. The IR spectrum showed the presence of a hydroxyl group (3370 cm^{-1}). Inspection of the ^1H- (Table 1) and ^{13}C-NMR (Table 2) spectra, obtained with the aid of ^1H–^1H shift correlation spectroscopy (^1H–^1H COSY), ^1H-detected heteronuclear multiple-quantum coherence (HMQC) and distorsionless enhancement by polarization transfer (DEPT) spectra, revealed that this compound exists in solution as a solvent-dependent mixture of two stereoisomers [**1a** (major isomer) and **1b** (minor isomer)], in a ratio of 2 : 1 and 10 : 1 in $CDCl_3$ and CD_3OD, respectively. The ^1H- and ^{13}C-NMR spectra of **1** showed signals due to two methines [**1a**; $\delta_{H,CDCl_3}$ 5.14 (1H, H-11), $\delta_{C,CDCl_3}$ 97.2 (C-11); **1b**; $\delta_{H,CDCl_3}$ 5.17 (1H, H-11), $\delta_{C,CDCl_3}$ 100.0 (C-11)], assignable to a hemiacetal group. The above spectral data suggested that **1** contains two stereoisomers (**1a**, **b**) on a hemiacetal group, which coexist at equilibrium in a certain ratio. Compound **1** was treated with acetic anhydride-pyridine to afford a sole diacetate **5**, the molecular formula of which was determined to be $C_{18}H_{26}O_6$ by HR-EI-MS. Compound **5** was used for structural analysis of **1**. The ^1H-NMR spectrum of **5** showed signals due to three tertiary methyl groups [δ 0.90 (3H, H_3-14), 1.05 (3H, H_3-13), 1.27 (3H, H_3-12)], two acetyl groups [δ 2.02 (3H, 11-$COCH_3$), 2.06 (3H, 10-$COCH_3$)], three methylenes [δ 0.99 (1H, H-1α), 1.11 (1H, H-8α), 1.53 (1H, H-8β), 1.61 (1H, H-1β), 1.75 (1H, H-4β), 2.07 (1H, H-4α)], three methines [δ 2.48 (1H, H-2), 2.58 (1H, H-7), 3.02 (1H, H-5)], an oxymethine [δ 4.34 (1H, H-6)], and an acetal [δ 6.56 (1H, H-11)]. The ^{13}C-NMR spectrum revealed 18 carbon signals that included an oxygenated quaternary carbon [δ 88.7 (C-3)] and two acetal carbons [δ 94.8 (C-11), 111.6 (C-10)]. There were six degrees of unsaturation in the molecule according to the molecular formula. Two acetyl groups accounted for two of those. Since compound **5** has no olefinic and carbonyl carbons except for the carbonyl carbon in the two acetyl groups, **5** was confirmed to be tetracyclic based on its molecular formula and degree of unsaturation. By ^1H–^1H COSY and the ^1H-detected heteronuclear multiple-bond connectivity (HMBC) spectra, the planar structure of **5** was deduced to be as shown in Fig. 1. The presence of the acetoxyl group at C-10 was indicated by the difference nuclear Overhauser effect (NOE) experiment, in which irradiation at δ 3.02 (H-5) caused NOE enhancement in the signal of the methyl protons in the acetoxyl group at C-10 (Fig. 2). The relative stereostructure was determined as follows. A molecular model reveals that, by its for-

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Table 1. ^{1}H-NMR Chemical Shifts of Compounds **1** and **5** (400 MHz)

Proton	**1a** (major)[a]	**1b** (minor)[a]	**1a** (major)[b]	**1b** (minor)[b]	**5**[a]
1	α 1.02 (1H, dd, 12.2, 9.3)[c]	α 1.02 (1H, dd, 12.2, 9.3)	α 1.09 (1H, dd, 12.7, 9.3)	α 1.09 (1H, dd, 12.7, 9.3)	α 0.99 (1H, dd, 12.9, 9.3)
	β 1.63 (1H, m)	β 1.63 (1H, m)	β 1.58 (1H, ddd, 12.7, 8.3, 2.2)	β 1.58 (1H, ddd, 12.7, 8.3, 2.2)	β 1.61 (1H, ddd, 12.9, 8.3, 2.2)
2	2.45 (1H, m)	2.45 (1H, m)	2.33 (1H, dddd, 10.5, 9.3, 8.3, 2.0)	2.33 (1H, dddd, 10.5, 9.3, 8.3, 2.0)	2.48 (1H, dddd, 10.5, 9.3, 8.3, 2.0)
4	α 2.01 (1H, d, 12.4)	α 2.04 (1H, d, 12.7)	α 2.07 (1H, d, 12.7)	α 2.03 (1H, d, 12.8)	α 2.07 (1H, d, 12.4)
	β 1.77 (1H, ddd, 12.4, 4.4, 1.7)	β 1.81 (1H, ddd, 12.7, 4.4, 2.0)	β 1.75 (1H, ddd, 12.7, 3.7, 2.0)	β 1.78 (1H, ddd, (12.8, 3.7, 2.0)	β 1.75 (1H, ddd, 12.4, 3.9, 2.0)
5	2.79 (1H, dd, 8.5, 4.4)	2.90 (1H, dd, 8.3, 4.4)	2.76 (1H, dd, 8.5, 3.7)	2.76 (1H, dd, 8.5, 3.7)	3.02 (1H, dd, 8.5, 3.9)
6	4.34 (1H, d, 8.5)	4.13 (1H, d, 8.3)	4.28 (1H, d, 8.5)	4.07 (1H, d, 8.5)	4.34 (1H, d, 8.5)
7	2.56 (1H, m)	2.56 (1H, m)	2.43 (1H, ddd, 13.4, 10.5, 7.6)	2.51 (1H, ddd, 13.4, 10.5, 7.6)	2.58 (1H, ddd, 13.4, 10.5, 7.8)
8	α 1.11 (1H, dd, 12.7, 12.7)	α 1.06 (1H, dd, 13.2, 13.2)	α 1.19 (1H, dd, 13.4, 12.2)	α 1.19 (1H, dd, 13.4, 12.2)	α 1.11 (1H, dd, 13.4, 12.2)
	β 1.55 (1H, m)	β 1.55 (1H, m)	β 1.50 (1H, ddd, 12.2, 7.6, 2.2)	β 1.50 (1H, ddd, 12.2, 7.6, 2.2)	β 1.53 (1H, ddd, 12.2, 7.8, 2.2)
11	5.14 (1H, d, 4.1)	5.17 (1H, d, 12.0)	4.95 (1H, s)	5.06 (1H, s)	6.56 (1H, s)
12	1.29 (3H, s)	1.32 (3H, s)	1.25 (3H, s)	1.30 (3H, s)	1.27 (3H, s)
13	1.05 (3H, s)	1.04 (3H, s)	1.06 (3H, s)	1.05 (3H, s)	1.05 (3H, s)
14	0.90 (3H, s)	0.90 (3H, s)	0.92 (3H, s)	0.92 (3H, s)	0.90 (3H, s)
11-OH	3.07 (1H, d, 4.1)	3.74 (1H, d, 12.0)			
10-$COCH_3$					2.06 (3H, s)
11-$COCH_3$					2.02 (3H, s)

a) Measurement in $CDCl_3$. *b*) Measurement in CD_3OD. *c*) Coupling constants (J in Hz) are given in parentheses.

Table 2. ^{13}C-NMR Chemical Shifts of Compounds **1** and **5** (100 MHz)

Carbon	**1a** (major)[a]	**1b** (minor)[a]	**1a** (major)[b]	**1b** (minor)[b]	**5**[a]
1	45.5	45.7	46.4	46.6	45.4
2	46.4	46.5	47.9	47.9[c]	46.0
3	86.4	87.1	86.8	87.2	88.7
4	29.5	30.9	30.4	31.1	29.5
5	46.8	48.5	47.0	50.6	46.52
6	78.0	78.9	79.5	79.5	77.8
7	42.7	42.6	44.3	44.0	42.2
8	46.9	47.8	47.6	47.9[c]	46.45
9	38.3	38.2	39.2	39.1	38.3
10	111.3	109.5	112.3	110.5	111.6
11	97.2	100.0	98.5	101.3	94.8
12	24.0	24.3	24.4	24.4	23.9
13	29.1	28.9	29.4	29.2	29.1
14	26.2	25.9	26.7	26.4	26.1
10-CO$\underline{C}H_3$					21.1[d]
10-$\underline{C}OCH_3$					168.9
11-CO$\underline{C}H_3$					21.2[d]
11-$\underline{C}OCH_3$					169.4

a) Measurement in $CDCl_3$. *b*) Measurement in CD_3OD. *c*) Signals overlapped. *d*) Assignments are interchangeable.

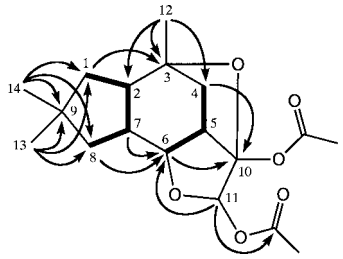

Fig. 1. ^{1}H–^{1}H COSY (Bold Lines) and HMBC (Full-Line Arrows) Correlations for **5**

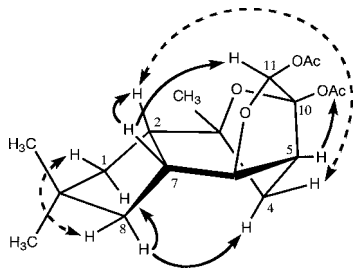

Fig. 2. NOEs (Full-Line Arrows) and W-Type Coupling (Dotted-Line Arrows) in **5**

mation, the tricyclic system (rings B, C, and D) itself sets up the relative configurations at the chiral centers C-3, C-5, C-6, and C-10 (Fig. 2). In the difference NOE experiment, irradiation at δ 2.58 (H-7) caused NOE enhancement in the signals of H-2 and H-11. These NOEs implied a *cis*-junction for the A/B rings and an α-orientation of the acetoxyl group at C-11 (Fig. 2). Furthermore, irradiation at δ 1.11 (H-8α) caused NOE enhancement in the signals of H-1α and H-4α. These NOEs exhibited the conformation of **5** depicted in Fig. 2. This conformation was supported by W-type long-range couplings between H-1β and H-8β (J=2.2 Hz), and between H-2 and H-4β (J=2.0 Hz, Fig. 2). The relative stereostructure of **5** is therefore represented as shown in the formula. Consequently, compound **1** is an equilibrium mixture of two stereoisomers (**1a**, **b**) on the C-11 acetal carbon.[13]

To determine the absolute stereochemistry of **1**, the following derivatization was performed. Treatment of **1** with pyridinium chlorochromate (PCC)–Al_2O_3 in benzene gave a γ-lactone **6**. The circular dichroism (CD) spectrum of **6** showed a positive Cotton effect at 217.5 nm ($\Delta\varepsilon$ +3.28). The application of the lactone sector rule[14] to **6** suggests that the expected sign of the Cotton effect should be positive (Fig. 3). Thus the absolute stereostructure of **1** is represented as shown in the formula.

Compound **1** has a previously unknown carbon skeleton: we propose the name "russulane" for this new skeleton. A

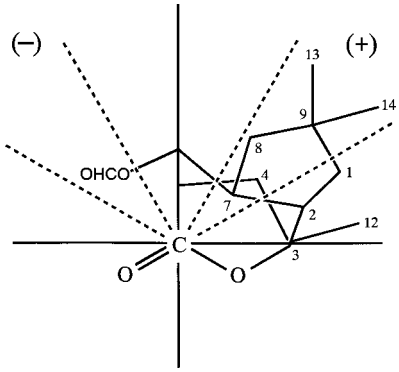

Fig. 3. Lactone Sector Projection of **6**

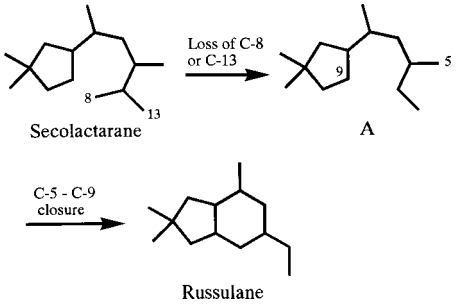

Fig. 4. Possible Biogenetic Pathway for the Carbon Framework of **1**

possible biosynthetic pathway for the carbon framework of **1** is shown in Fig. 4. The loss of a carbon atom (C-8 or C-13) from secolactarane gives rise to a monocyclic carbon skeleton **A**, with a six-membered ring construction between C-5 and C-9, resulting in the formation of a russulane-type carbon skeleton. The cooccurrence of **1** with **2** in the same mushroom reinforces this hypothesis.

Experimental

General Procedures Optical rotations were determined using a JASCO DIP-360 digital polarimeter. CD spectra were measured on a JASCO J-720 spectropolarimeter. IR spectra were recorded with a Perkin-Elmer FT-IR 1725X IR spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL JNM-LA 400 (400 and 100 MHz, respectively) spectrometer. Chemical shifts are given on a δ (ppm) scale, with tetramethylsilane as the internal standard. HR-EI-MS were recorded on a JEOL JMS-DX 303 mass spectrometer. Column chromatography was carried out on Kieselgel 60 (230—400 mesh, Merck). HPLC was carried out on a Tosoh HPLC system (pump, CCPD; detector, RI-8010) using a TSK gel ODS-120T (7.8 mm i.d.×30 cm) column (Tosoh).

Fungal Material The fruiting bodies of *R. delica* (from Morioka, Iwate Prefecture, Japan) were purchased in a food market.

Extraction and Isolation The fresh fruit bodies of *R. delica* (1.7 kg) were extracted four times with Et_2O at room temperature for 2 weeks. The Et_2O extract (11.2 g) was chromatographed on a silica gel column using hexane–EtOAc (7 : 3—1 : 7), EtOAc, and MeOH to afford 41 fractions. Fraction 10 was purified by preparative HPLC [column temperature, 40 °C; mobile phase, MeOH–H_2O (4 : 1); flow rate, 1.0 ml/min] to give **2** (0.6 mg) and **4** (4.1 mg). Fraction 19 was purified by preparative HPLC [column temperature, 40 °C; mobile phase, MeOH–H_2O (1 : 1); flow rate, 1.5 ml/min] to give **1** (15.0 mg) and **3** (2.0 mg).

Russulanorol (**1**): Amorphous powder. IR ν_{max} $CHCl_3$ cm^{-1}: 3370. HR-EI-MS *m/z*: 254.1540 (M^+, Calcd for $C_{14}H_{22}O_4$: 254.1518). ^{1}H-NMR (400 MHz, $CDCl_3$ and CD_3OD): see Table 1. ^{13}C-NMR (100 MHz, $CDCl_3$ and CD_3OD): see Table 2.

Acetylation of 1 Compound **1** (6.7 mg) was treated overnight with 0.5 ml of acetic anhydride and 0.5 ml of pyridine. The product was purified by HPLC [column temperature, 40 °C; mobile phase, MeOH–H_2O (7 : 2); flow rate, 1.0 ml/min], giving **5** (7.8 mg).

Compound **5**: Colorless oil. $[\alpha]_D^{21}$ −38.5° (c=0.8, $CHCl_3$). IR ν_{max} $CHCl_3$ cm^{-1}: 1751. HR-EI-MS *m/z*: 338.1718 (M^+, Calcd for $C_{18}H_{26}O_6$: 338.1729). ^{1}H-NMR (400 MHz, $CDCl_3$): see Table 1. ^{13}C-NMR (100 MHz, $CDCl_3$): see Table 2.

Oxidation of 1 To a solution of compound **1** (6.9 mg) in benzene (10 ml), PCC–Al_2O_3 (67.5 mg) was added and the mixture was stirred for 2 h at room temperature. The reaction mixture was filtered and the filtrates were evaporated. The product was purified by HPLC [column temperature, 40 °C; mobile phase, MeOH–H_2O (1 : 1); flow rate, 1.5 ml/min], giving **6** (3.4 mg).

Compound **6**: Colorless oil. $[\alpha]_D^{20}$ −44.5° (c=0.3, $CHCl_3$). CD (c=1.91×10^{-4}, MeOH) $\Delta\varepsilon$ (nm): +3.28 (217.5). IR ν_{max} $CHCl_3$ cm^{-1}: 1766, 1724. HR-EI-MS *m/z*: 252.1386 (M^+, Calcd for $C_{14}H_{20}O_4$: 252.1362). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.02 (3H, s, H_3-14), 1.15 (3H, s, H_3-13), 1.32 (1H, dd, J=13.2, 12.7 Hz, H-1α), 1.41 (1H, dd, J=14.1, 4.1 Hz, H-8α), 1.45 (3H, s, H_3-12), 1.51 (1H, dd, J=12.7, 6.8 Hz, H-1β), 1.88 (1H, ddd, J=14.1, 8.8, 1.0 Hz, H-8β), 2.08 (1H, ddd, J=12.4, 5.6, 1.7 Hz, H-4β), 2.14 (1H, d, J=12.4 Hz, H-4α), 2.48 (1H, dddd, J=10.5, 9.5, 8.8, 4.1 Hz, H-7), 2.66 (1H, dddd, J=13.2, 10.5, 6.8, 1.7 Hz, H-2), 2.90 (1H, dd, J=5.4, 3.2 Hz, H-5), 4.93 (1H, dd, J=9.5, 3.2 Hz, H-6), 8.10 (1H, s, H-11). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 23.8 (C-12), 30.0 (C-14), 30.9 (C-13), 36.1 (C-4), 39.7 (C-9), 40.9 (C-7), 43.3 (C-1), 45.6 (C-5), 45.9 (C-8), 48.7 (C-2), 76.1 (C-6), 87.2 (C-3), 160.7 (C-11), 174.9 (C-10).

Acknowledgments We are grateful to Mr. S. Sato and Mr. T. Matsuki of this university for providing the mass and NMR spectra.

References and Notes

1) Whittaker R. H., *Science*, **163**, 150—160 (1969).
2) Daniewski W. M., Vidari G., "Progress in the Chemistry of Organic Natural Products," Vol. 77, ed. by Herz W., Falk H., Kirby G. W., Moore R. E., Tamm Ch., Springer-Verlag, Wien, 1999, pp. 70—171.
3) Tan J., Dong Z., Liu J., *Helv. Chim. Acta*, **83**, 3191—3197 (2000).
4) Clericuzio M., Fu J., Pan F., Pang Z., Sterner O., *Tetrahedron*, **53**, 9735—9740 (1997).
5) Clericuzio M., Pan F., Han F., Pang Z., Sterner O., *Tetrahedron Lett.*, **38**, 8237—8240 (1997).
6) Clericuzio M., Han F., Pan F., Pang Z., Sterner O., *Acta Chem. Scand.*, **52**, 1333—1337 (1998).
7) Yasukawa K., Kanno H., Kaminaga T., Takido M., Kasahara Y., Kumaki K., *Phytother. Res.*, **10**, 367—369 (1996).
8) Yaoita Y., Machida K., Kikuchi M., *Chem. Pharm. Bull.*, **47**, 894—896 (1999).
9) De Bernardi M., Fronza G., Vidari G., Vita-Finzi P., *La Chimica e L' Industria*, **58**, 177—178 (1976).
10) De Bernardi M., Fronza G., Scilingo A., Vidari G., Vita-Finzi P., *Tetrahedron*, **42**, 4277—4283 (1986).
11) De Bernardi M., Fronza G., Mellerio G., Vidari G., Vita-Finzi P., *Phytochemistry*, **18**, 293—298 (1979).
12) Kobata K., Kano S., Shibata H., *Biosci. Biotech. Biochem.*, **59**, 316—318 (1995).
13) The configuration of the hydroxyl group at C-11 of **1a** (major isomer) was determined to be α by the difference NOE experiment in CD_3OD, in which irradiation at δ 2.43 (H-7) caused NOE enhancement in the signal of H-11.
14) Jennings J. P., Klyne W., Scopes P. M., *J. Chem. Soc.*, **1965**, 7229—7237 (1965).