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Structure and absolute configuration of protoilludane sesquiterpenes from *Russula delica*

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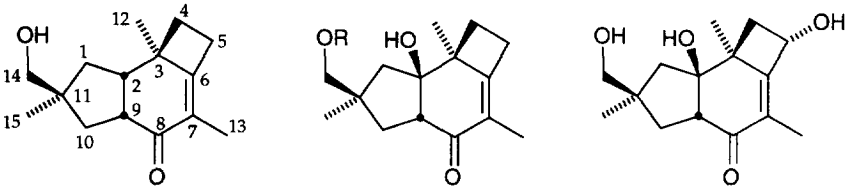
Abstract: Three new protoilludane sesquiterpenes, plorantinone A, B and C (**1**, **2a** and **3**), were isolated from injured fruit bodies of the Basidiomycete *Russula delica*, presumably formed from stearoylplorantinone B (**2b**) which was isolated from intact fruit bodies. The isolation and structure elucidation including the determination of the absolute configuration of the four compounds is reported.

A considerable number of sesquiterpenoids have been isolated from fruit bodies of species belonging to the Russulaceae family (Basidiomycotina)[1]. As a general rule, the intact fruit bodies contain fatty acid esters of a sesquiterpene[2], which, when the fruit body is injured, rapidly is hydrolysed enzymatically to the free sesquiterpene and/or further metabolised to new sesquiterpenes[3]. Several of the products formed possess bioactivities, they are, for example, pungent and antibiotic[4], and have been suggested to be part of a chemical defence system that protects the fruit bodies from parasites. Besides the possible ecological significance that such conversions may have, they can also serve as chemotaxonomic markers, and indicate the arrangement of sections inside the genera as well as distinguish closely related taxa within the same section[5]. While the *Lactarius* species belonging to the European flora have been rather thoroughly investigated[1], little has been done with the *Russula* species, in spite of the much larger number of species belonging to this genus. To date, marasmane sesquiterpenes have been isolated from *R. cuprea*[6]; a lactarane has been reported from *R. emetica*[7], while lactarane and secolactarane sesquiterpenes have been isolated from *R. sardonia*[8] and from *R. queletii*[9]. The species investigated here, *R. delica*, is especially interesting as it belongs to a group of Russulae (Compactae) which is distinguished by morphological characters that indicate primitivity[10].

The fractionation of extracts of intact fruit bodies of *R. delica* yielded the stearic acid ester **2b**, while extracts of injured specimens in addition to the free ketodiol **2a** also yielded the reduced derivative **1** and the oxidised derivative **3** (see Figure 1 for structures). As *R. delica* belongs to the section Plorantinae, we propose the names plorantinone A for compound **1**, plorantinone B for compound **2a** and plorantinone C for compound **3**. The NMR spectra of all four natural products showed large similarities, indicating a structural

relationship. The transesterification of the precursor **2b** with sodium methoxide in methanol yielded plorantinone B (**2a**) together with methyl stearate, which identity and purity was confirmed by its ^{1}H NMR spectrum and a GC-MS analysis. The structures and the relative configuration of the compounds were determined by 2D NMR techniques, COSY, NOESY, HMQC and HMBC.



1 **2** **3**

Figure 1. a: R = H; b: R = Stearoyl

The high resolution MS data of plorantinone A (**1**) indicated that it is reduced (one oxygen less) compared to plorantinone B (**2a**), and the signal for the tertiary alcohol function is missing in the ^{13}C NMR spectrum of plorantinone A (**1**). 2D NMR experiments showed that C-2 of **1** is a CH group, which explains the differences in the chemical shifts observed, and NOESY correlations between 1-Hβ and 2-H, between 10-Hβ and 9-H, between 12-H_3 and 1-Hα, 4-Hα, 5-Hα and 10-Hα, between 14-H_2 and 1-Hβ, 9-H and 10-Hβ, and between 15-H_3 and 1-Hα and 10-Hα, establish the relative stereochemistry of plorantinone A (**1**). Plorantinone C (**3**) is instead oxidised compared to plorantinone B (**2a**), and the ^{13}C NMR spectrum of **3** shows the presence of a third hydroxylated carbon. 2D NMR experiments unambiguously determined this carbon to be C-5, and NOESY correlations between 10-Hβ and 9-H, between 12-H_3 and 1-Hα, 4-Hα and 10-Hα, between 13-H_3 and 5-Hβ, between 14-H_2 and 1-Hβ, 9-H and 10-Hβ, and between 15-H_3 and 1-Hα and 10-Hα, establish the relative stereochemistry of plorantinone C (**3**). Pertinent NOESY and HMBC correlations observed with plorantinone B (**2a**) are shown in Figure 2

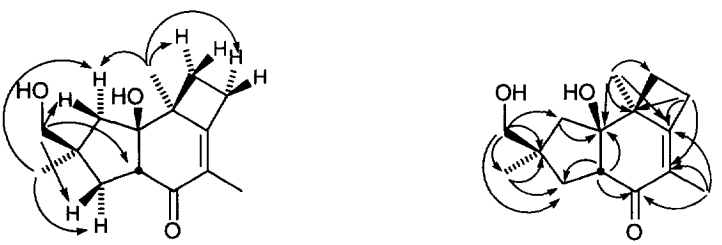


Figure 2. Pertinent NOESY (left) and HMBC (right) correlations observed with plorantinone B (**2a**).

The plorantinones contain an *s-trans* enone functionality, which is one of the most thoroughly investigated chromophores concerning chiroptical properties and can be used for the determination of absolute configuration[11]. To this purpose, it is important to establish whether the enone system is planar or not, a conformational feature that is expressed by the C=C-C=O dihedral angle, or ω. If $\omega \neq 180°$, the chromophore becomes an intrinsically dissymmetric one, and helicity rules connecting the sign of the CD bands (both n-π* and π-π*) to the absolute configuration have been formulated[12]. Conformational analysis was performed with the enantiomer of **2a** shown in Figure 1, by molecular mechanics calculations (utilising Allinger's MM3-92

force-field[13]) and by the semi-empirical MO-SCF method AM1. The conformers with the lowest energy are shown in Figure 3 (MM3 to the left and AM1 to the right). The enone system is predicted to be non-planar, MM3 calculates the angle ω to be +153° while AM1 suggests +146°. This is in agreement with the relatively low value of the molar absorption coefficient (ε = 7000 l mol^{-1} cm^{-1} at 246 nm, see Experimental), which has been calculated[14] to decrease with increasing skew angle.

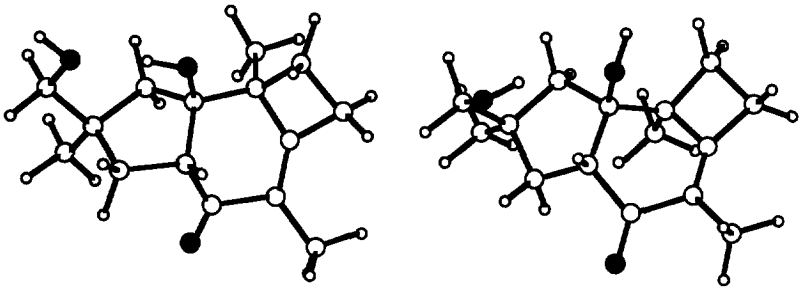


Figure 3. Minimum energy conformations of **2a** as obtained from MM3 (left) and from AM1 (right).

The major difference between the two methods is found in the conformation of the five-membered ring. MM3 suggests a small puckering (ϕ C(1)-C(11)-C(10)-C(9) = -6°), which allows the two hydroxyl groups to be at the optimal distance for hydrogen bonding ($r_{O...H}$ = 1.74 Å), while AM1 predicts a different puckering (ϕ C(1)-C(11)-C(10)-C(9) = +24°) and a larger distance between the hydroxyl groups ($r_{O...H}$ = 2.30 Å). However, the $^3J_{9\text{-}10\alpha}$ and $^3J_{9\text{-}10\beta}$ calculated[15] from the AM1 geometry are in better agreement with the coupling constants recorded in $CDCl_3$, and this is also the case for the protons of the cyclobutane ring (4-H_2 and 5-H_2), indicating that AM1 gives a better description of the lowest energy conformer of **2a**.

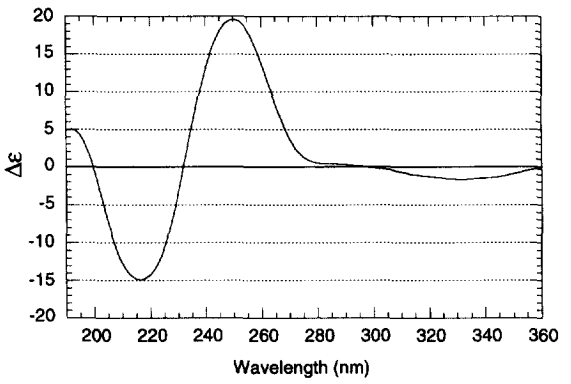


Figure 4. CD spectrum of plorantinone B (**2a**) in CH_3CN.

Table 1. ^{1}H NMR data (δ; mult.; *J*) for plorantinone A (**1**), B (**2a**) and C (**3**) in $CDCl_3$.

H	1	2a	3
1α	1.50; dd; 14.5, 4.7	2.01; d; 15.0	1.89; d; 15.0
1β	1.69; dd; 14.5, 9.4	1.65; d; 15.0,	1.52; dd; 15.0, 1.3
2	2.69; ddd; 10.0, 9.4, 4.7	-	-
4α	1.89; m	1.63; m	1.54; dd; 12.4, 2.8
4β	1.88; m	2.71; m	2.81; dd; 12.4, 7.4
5α	3.01; dddq; 16.3, 7.5, 7.5, 1.7	2.96; m	-
5β	2.74; dddq; 16.3, 6.7, 4.8, 0.8	2.74; m	4.83; dd; 7.4, 2.8
9	2.87; m; 13.2, 10.0, 7.9	3.02; dd; 8.5, 12.8	2.89; dd; 12.8, 8.6
10α	1.43; dd; 13.2, 13.2	1.49; dd; 13.5, 12.8	1.37; dd; 13.5, 12.8
10β	2.09; dd; 13.2, 7.9	2.22; dd; 13.5, 8.5	2.09; ddd; 13.5, 8.6, 1.3
12	1.28; s	1.30; s	1.29; s
13	1.63; dd; 1.7, 0.8	1.60; m	1.63; d; 0.6
14	3.38/3.30; d; 10.6	3.49; s	3.37; s
15	1.11; s	0.99; s	0.90; s

Table 2. ^{13}C NMR data (δ; mult.) for plorantinone A (**1**), B (**2a**) and C (**3**) in $CDCl_3$.

C	1	2a	3
1	35.5; t	45.9; t	45.0; t
2	46.8; d	83.0; s	82.6; s
3	46.1; s	49.9; s	47.3; s
4	35.0; t	26.4; t	37.0; t
5	28.0; t	27.0; t	68.3; d
6	168.6; s	168.0; s	166.2; s
7	125.8; s	125.2; s	127.8; s
8	201.4; s	200.8; s	203.1; s
9	51.5; d	62.6; d	63.1; d
10	38.8; t	40.2; t	39.9; t
11	44.4; s	42.9; s	43.0; s
12	24.0; q	26.4; q	27.3; q
13	10.1; q	9.8; q	9.5; q
14	70.7; t	72.0; t	71.0; t
15	24.4; q	24.8; q	24.5; q

The CD spectrum of plorantinone B (**2a**) is shown in Figure 4. It shows a negative band at long wavelengths, with $\Delta\varepsilon_{max}$ = -1.6 l mol^{-1} cm^{-1} at 331 nm (n-π* transition or R-band), and a positive band $\Delta\varepsilon_{max}$ = + 19.5 at 250 nm (I π-π* transition or K-band). According to the helicity rule of skewed 2-cyclohexenones[12], the enantiomer showing a negative Cotton Effect of the R-band and a positive CE of the K-band is assigned to the one having a positive ω dihedral angle. For plorantinone B (**2a**), the stereochemistry shown in Figure 1 therefore corresponds to the absolute configuration, i.e. 2-(*R*), 3-(*S*), 9-(*R*), 11-(*R*). Note also the occurrence of a negative CD band centered at 216 nm (II π-π* band), which is almost as intense as the 250 nm band. Gawronski reported[11a,b] the sign of this CD band in polycyclic cyclohexenones as to be strongly sensitive to the configuration at the allylic carbon atom(s); the author proposed that a positive CE of the II π-π* band is correlated to a positive dihedral angle C=C-C-R. This is

again in agreement with the absolute configuration shown in Figure 1, where a negative C(7)-C(6)-C(3)-Me(12) angle is found (-105° by MM3 and -83° by AM1), if we suppose that this feature gives a dominant contribution to the observed negative CE. The same absolute configuration was proposed by Arnone et al.[16] for the protoilludane 3-epi-illudol, also on the basis of chiroptical methods, and it is in agreement with absolute configuration of naturally occuring marasmane sesquiterpenes[17], which biogenetically are derived from protoilludanes by ring-contraction.

Among the species belonging to Russulaceae, the only protoilludanes so far reported were isolated from *Lactarius violascens*[1], belonging to the section Uvidi. In addition, protoilludane sesquiterpenes have been reported from several other families of Basidiomycetes, for instance Tricholomataceae (*Armillaria*[18,19] *Clitocybe*[16]) and Coprinaceae (*Coprinus*[20]).

EXPERIMENTAL

Extraction and isolation: Fruit bodies of *Russula delica* Fr. were collected in the vicinity of Nanjing in the autumn of 1996. They were immediately brought to the laboratory, where ethyl acetate extracts of intact as well as injured (ground in a meat grinder and extracted 30 minutes later) specimens were prepared according to reference 3. TLC analyses were made on Merck Kieselgel 60 F_{254} SiO_2 plates developed with ethyl acetate:heptane mixtures and visualised by spraying with anisaldehyde:sulfuric acid and warming to 120 °C. The pure compounds were isolated by chromatography in silica gel columns eluted with mixtrues of ethyl acetate:heptane. From 1 kg of injured fruit bodies 45 mg plorantinone A (**1**), 55 mg plorantinone B (**2a**), and 30 mg plorantinone C (**3**) was obtained.

Spectroscopy: ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) were recorded at room temperature with a Bruker ARX500 spectrometer with an inverse multinuclear 5 mm probehead equipped with shielded gradient coil. The spectra were recorded in $CDCl_3$, and the solvent signals (7.26 and 77.0 ppm, respectively) were used as reference. The chemical shifts (δ) are given in ppm, and the coupling constants (J) in Hz. COSY, HMQC and HMBC experiments were recorded with gradient enhancements using sine shaped gradient pulses. For the 2D heteronuclear correlation spectroscopy the refocusing delays were optimised for $^1J_{CH}$=145 Hz and $^nJ_{CH}$=10 Hz. The raw data were transformed and the spectra were evaluated with the standard Bruker UXNMR software (rev. 941001). Mass spectra were recorded with a Jeol SX102 spectrometer, while the UV and the IR spectra were recorded with a Varian Cary 2290 and a Perkin Elmer 298 spectrometer. The melting point (uncorrected) were determined with a Reichert microscope, and the optical rotations were measured with a Perkin-Elmer 141 polarimeter at 22 °C. CD spectra were recorded on a Jasco J-720 spectropolarimeter.

Plorantinone A (**1**) was obtained as white crystals, m.p. 104-106 °C ($CHCl_3$). $[\alpha]_D^{22}$ = -8° (c 0.5, $CHCl_3$). UV (CH_3CN) λ_{max} (ε): 246 nm (9700). IR (KBr): 3410, 2945, 2920, 2860, 2695, 1675, 1630, 1465, 1415, 1370, 1350, 1325, 1120, 1055, 1045 cm^{-1}. MS, *m/z* (% rel. int.): 234.1629 (M^+, 59, $C_{15}H_{22}O_2$ requires 234.1620), 219 (14), 205 (71), 203 (52), 201 (38), 187 (100), 173 (25), 159 (27), 79 (25). NMR data are given in Tables 1 and 2.

Plorantinone B (**2a**) was obtained as white crystals, m.p. 38-40 °C (CH_3CN). $[\alpha]_D^{22}$ = -14° (c 1.0, $CHCl_3$). UV (CH_3CN) λ_{max} (ε): 246 nm (7000). IR (film): 3400, 2950, 2920, 2860, 1680, 1640, 1465, 1370, 1335, 1250, 1145, 1040, 1020, 1010, 990 cm^{-1}. MS, *m/z* (% rel. int.): 250.1577 (M^+, 36, $C_{15}H_{22}O_3$ requires 250.1569), 233 (23), 219 (27), 201 (30), 123 (27), 122 (100), 107 (28), 95 (27), 94 (46), 79 (49), 43 (32), 28 (41). NMR data are given in Tables 1 and 2.

Stearoylplorantinone B (**2b**) was obtained as a colourless oil, $[\alpha]_D^{22}$ = -5.5° (c 0.9, $CHCl_3$). MS, *m/z* (% rel. int.): 516.4199 (M^+, 34, $C_{15}H_{22}O_2$ requires 516.4178), 498 (7), 460 (20), 250 (17), 232 (14), 214 (18), 201 (17), 176 (14), 122 (100), 95 (11), 43 (10). NMR ($CDCl_3$) ^1H: 4.09 (1H, d, H-15a, $J_{15a\text{-}15b}$ = 10.9 Hz), 4.00

(1H, d, H-15b), 3.01 (1H, m, H-5α), 2.99 (1H, dd, H-9, $J_{9\text{-}10\alpha}$ = 13.3 Hz, $J_{9\text{-}10\beta}$ = 8.3 Hz), 2.77 (1H, m, H-5β), 2.71 (1H, m, H-4β), 2.35 (2H, t, H-2'), 2.34 (1H, m, H-10β, $J_{10\alpha\text{-}10\beta}$ = 13.5 Hz), 2.04 (1H, d, H-1α, $J_{1\alpha\text{-}1\beta}$ = 15.2 Hz), 1.67 (3H, s, H-13), 1.65 (4H, br, H-4α, H-1β and H-3'), 1.51 (1H, dd, H-10α), 1.34 (3H, s, H-12), 1.27 (14H, br, H-4'-17'), 1.09 (3H, s, H-14), 0.89 (3H, t, H-18'). ^{13}C: 199.5 (C-8), 174.4 (C-1'), 166.9 (C-6), 125.9 (C-7), 83.1 (C-2), 71.2 (C-15), 62.0 (C-9), 50.3 (C-3), 45.4 (C-1), 41.7 (C-11), 40.0 (C-10), 34.6 (C-2'), 32.1 (C-3'), 29.9-29.4 (C-4'-15'), 27.0 (C-5), 26.7 (C-4), 26.5 (C-12), 25.3 (C-14), 25.1 (C-16'), 22.9 (C-17'), 14.3 (C-18'), 10.0 (C-13).

Plorantinone C (3) was obtained as white crystals, m.p. 89-91 °C (CH_3CN). $[\alpha]_D^{22}$ = -11° (c 0.5, $CHCl_3$). UV (CH_3CN) λ_{max} (ε): 243 nm (7800). IR (KBr): 3540, 3360, 2950, 2920, 2860, 1635, 1370, 1325, 1310, 1245, 1215, 1140, 1060, 1040, 1025, 1000, 985 cm^{-1}. MS, *m/z* (% rel. int.): 266 (M^+, 2), 248.1416 (M^+ - H_2O, 100, $C_{15}H_{20}O_3$ requires 248.1412), 219 (100), 201 (69), 187 (80), 173 (41), 159 (24), 148 (25), 138 (23), 128 (21), 95 (23), 91 (18), 81 (18), 43 (18). NMR data are given in Tables 1 and 2.

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