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Stearoyldelicone, an unstable protoilludane sesquiterpenoid from intact fruit bodies of *Russula delica*

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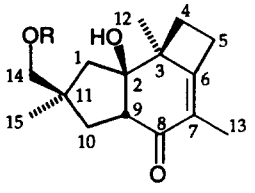
Abstract: Stearoyldelicone (**3**), containing an unusual and reactive bicyclo[4.2.0]octa-1,4-dien-3-one moiety, was isolated from the methylene chloride extracts of intact fruit bodies of the Basidiomycete *Russula delica*. **3** is unstable and easily transformed to the illudalane sesquiterpenoid **4a** during chromatography on silica gel.

Recently we reported[1] the presence of a new protoilludane sesquiterpenoid, stearoylplorantinone (**1a**), in ethyl acetate extracts of intact fruit bodies of *Russula delica* (Russulaceae family, Basidiomycotina), while extracts of injured specimens yielded the three free sesquiterpenes plorantinone A (**2a**), B (**1b**) and C (**2b**). The presence of a sesquiterpenoid fatty acid ester which as a response to injury is converted to the corresponding free sesquiterpene as well as other derivatives is in analogy with what has been found in the fruit bodies of several other Russulaceae species[2]. However, in addition to stearoylplorantinone (**1a**), we also obtained large amounts of illudalane sesquiterpenoids, e.g. compound **4a**, from the extracts of the intact specimens. As the illudalanes biosynthetically are formed from the protoilludanes, and not the reverse[3], we suspected that compound **4a** actually is a degradation product of a metabolite that is too unstable for the conditions used. In order to determine the true contents of intact fruit bodies of *R. delica*, extracts were therefore prepared with the more inert solvent methylene chloride, and the extracts were fractionated on neutral alumina gel instead of silica gel.

By this procedure it could be shown that the sesquiterpenoid contents of intact *R. delica* essentially consist of two compounds, stearoylplorantinone B (**1a**) (30 % according to the integrals in a ^{1}H NMR spectrum of the crude extract) and a new stearic acid ester for which we propose the name stearoyldelicone (**3**) (70 %). Although it was impossible to use silica gel for the purification of the latter, it is stable on neutral alumina gel and could be isolated and characterised. Its structure was determined by 2D NMR techniques, COSY, NOESY, HMQC and HMBC, and mass spectrometry. The molecular ion was clearly visible in the EI mass spectrum, and high resolution measurements confirmed the elemental composition suggested by NMR data. In the HMBC spectrum, important correlations were observed between 14-H_2 and

C-1, C-10, C-11, C-15 and C-1', between 1-H_2 as well as 10-H_2 and C-2, C-9, C-11, C-14 and C-15, between 4-H_2 and C-3, C-5, C-6 and C-12, between 5-H_2 and C-3, C-4, C-6 and C-7, between 12-H_3 and C-2, C-3, C-4 and C-6, and between 13-H_2 and C-6, C-7 and C-8. The NOESY correlations between 14-H_2 and 1-Hβ and 10-Hβ, between 15-H_3 and 1-Hα and 10-Hα, and between 12-H_3 and 1-Hα, 4-Hα and 5-Hα show that the hydroxylated methyl group (C-14) is on the opposite side of the ring system compared to C-12. The absolute configuration of plorantinone B (**1b**) is as shown in Figure 1[1], and this should consequently be the case also for stearoyldelicone (**3**).



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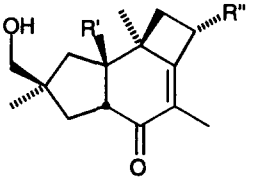

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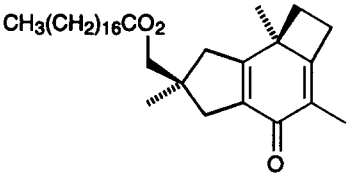

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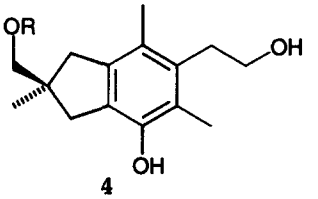


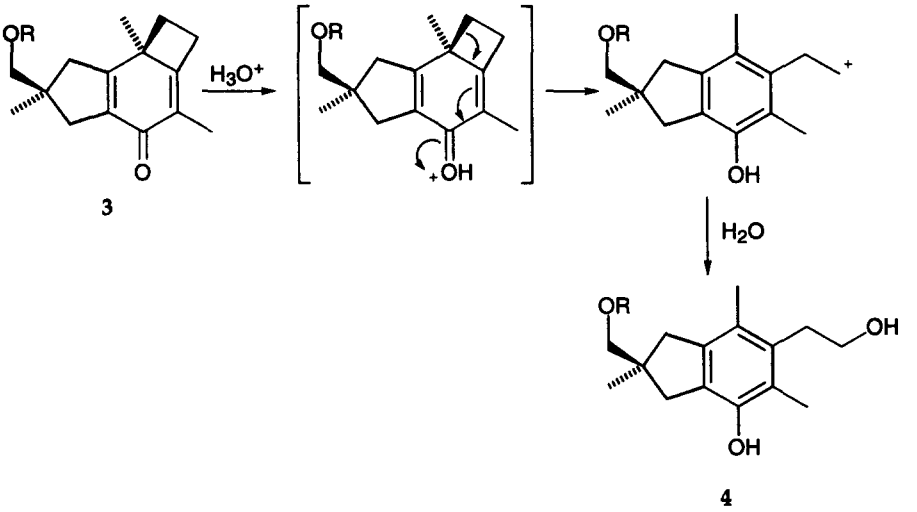
4

Figure 1. a: R = stearoyl, R' = R" = H; b: R = H, R' = R" = OH

Stearoyldelicone (**3**) contains a bicyclo[4.2.0]octa-1,4-dien-3-one moiety, which to our knowledge never has been reported previously and that is not present in any reported natural product[4]. The doubly activated keto function of **3** is responsible for the chemical lability of **3**. Upon adsorption on silica gel, and even during a normal TLC experiment, **3** is cleanly transformed to the illudalane **4a**, a new compound which structure was determined by spectroscopy and by its transesterfication to the free illudalane **4b** in K^+MeO^-/MeOH. Presumably, traces of acid present in the silica gel protonate the keto oxygen, and the aromatisation of the 6-membered ring cause the formation of a primary carbocation (as suggested in Figure 2) which rapidly adds water to form **4**. Whether stearoyldelicone (**3**) also reacts with nucleophiles during neutral conditions, and thereby for example may possess mutagenic activity, remains to be investigated.

Protoilludane sesquiterpenes with a keto function at C-8 are rare, the only example listed in reference 4 are coprinolone and its 6,7-didehydro derivative isolated from *Coprinus psychromorbidus*[5]. On the other hand, aromatic illudalane sesquiterpenes that are hydroxylated at C-4 and C-8 are common[4], and the possibility that they are formed chemically from protoilludanes similar to stearoyldelicone (**3**) should be considered. Stearoyldelicone (**3**) is another example of the presence of an acid labile sesquiterpenoid in the fruit bodies of Russulaceae species. The first reported was stearoylvelutinal, present in large amounts in for example *Lactarius vellereus*, which is a precursor in an advanced chemical defence system that protects the fruit bodies from parasites[6]. In contact with silica gel, stearoylvelutinal is degraded to a series of

sesquiterpene furans[7], which no longer are considered to be true natural products, *via* a carbocation formed after the protonation of an epoxide oxygen.



3 4

Figure 2. R = stearoyl.

EXPERIMENTAL

Extraction and isolation: Fruit bodies of *Russula delica* Fr. were collected in the vicinity of Nanjing in the summer of 1997. They were immediately brought to the laboratory, where a methylene chloride extract was prepared as reported previously[1]. TLC analyses were made on Merck Kieselgel 60 F_{254} SiO_2 plates developed with MTBE:hexane mixtures and visualised by spraying with anisaldehyde:sulfuric acid and warming to 120 °C. The pure compounds were isolated by chromatography on neutral alumina gel (Beckman activity grade II-III) eluted with mixtures of MTBE:hexane. From 1 kg of fruit bodies approximately 40 mg stearoylplorantinone B (**1a**) and 90 mg stearoyldelicone (**3**) were 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.

Stearoyldelicone (**3**) was obtained as a colourless oil, $[\alpha]_D^{22}$ = -9° (c 1.6, $CHCl_3$). MS, *m/z* (% rel. int.): 498.4065 (M^+, 58, $C_{33}H_{54}O_3$ requires 498.4073), 214 (92), 201 (100), 186 (66), 171 (24), 157 (20), 84 (14), 43 (18). UV (cyclohexane) λ_{max} (ε): 273 nm (2300, sh), 247 nm (9700). NMR ($CDCl_3$) ^1H: 3.91 (1H,

d, H-15a, $J_{15a\text{-}15b}$ = 10.7 Hz), 3.86 (1H, d, H-15b), 3.22 (1H, dddd, H-5α, $J_{5\alpha\text{-}5\beta}$ = 14.2 Hz, $J_{5\alpha\text{-}4\alpha}$ = 9 Hz, $J_{5\alpha\text{-}4\beta}$ = 9 Hz, $J_{5\alpha\text{-}13}$ = 1.6 Hz), 2.74 (1H, ddd, H-5β, $J_{5\beta\text{-}4\alpha}$ = 2.6 Hz, $J_{5\beta\text{-}4\beta}$ = 7.7 Hz), 2.55 (1H, ddd, H-10β, $J_{10\alpha\text{-}10\beta}$ = 16.5 Hz, $J_{10\beta\text{-}1\alpha}$ = 2 Hz, $J_{10\beta\text{-}1\beta}$ =2 Hz), 2.48 (1H, ddd, H-1β, $J_{1\alpha\text{-}1\beta}$ = 17.6 H, $J_{1\beta\text{-}10\alpha}$ = 2 Hz), 2.42 (1H, ddd, H-10α, $J_{10\alpha\text{-}1\alpha}$ = 2 Hz), 2.32 (1H, ddd, H-1α), 2.30 (2H, t, H-2', $J_{2'\text{-}3'}$ = 7.6 Hz) 1.97 (1H, ddd, H-4α, $J_{4\beta\text{-}4\beta}$ = 9 Hz), 1.75 (3H, d, H-13), 1.70 (1H, ddd, H-4β), 1.59 (2H, m, H-3'), 1.42 (3H, s, H-12), 1.25 (28H, br, H-4'-17'), 1.18 (3H, s, H-14), 0.88 (3H, t, H-18', $J_{17'\text{-}18'}$ = 7.2 Hz). ^{13}C: 186.7 (C-8), 174.2 (C-1'), 164.1 (C-6), 162.3 (C-2), 137.6 (C-9), 126.3 (C-7), 71.2 (C-15), 49.9 (C-3), 43.0 (C-1), 41.9 (C-11), 40.3 (C-10), 34.6 (C-2'), 32.1 (C-3'), 31.2 (C-5), 29.9-29.4 (C-4'-15'), 27.3 (C-4), 25.3 (C-12), 25.2 (C-16'), 24.6 (C-14), 22.9 (C-17'), 14.3 (C-18'), 10.4 (C-13).

Compound **4a** was obtained as a white amorphous solid. $[\alpha]_D^{22}$ = -0.5° (c 0.4, $CHCl_3$). UV (cyclohexane) λ_{max} (ε): 284 nm (1000), 275 (910), 222 (6500, sh), 204 (30,000). EI-MS, m/z (rel. int.): 516 (M^+, 39), 233 (58), 232 (100), 215 (27), 202 (54), 201 (77), 187 (42), 173 (14), 57 (13), 43 (19), 28 (36). NMR ($CDCl_3$) ^1H: 4.01 (2H, s, H-15), 3.73 (2H, t, $J_{4\text{-}5}$ = 7.5 Hz, H-4), 2.96 (2H, t, H-5), 2.87 (1H, d, $J_{1a\text{-}1b}$ = 16.0 Hz, H-1a), 2.85 (1H, d, $J_{10a\text{-}10b}$ = 15.5 Hz, H-10a), 2.64 (1H, d, H-1b), 2.60 (1H, d, H-10b), 2.31 (2H, t, $J_{2'\text{-}3'}$ = 7.5 Hz, H-2'), 2.22 (3H, s, H-13), 2.16 (3H, s, H-12), 1.61 (2H, br, H-3'), 1.25 (28H, br, H-4'-17'), 1.20 (3H, s, H-14), 0.88 (3H, t, H-18'). ^{13}C: 174.5 (C-1'), 148.7 (C-8), 140.6 (C-2), 134.6 (C-6), 125.4 (C-9), 125.0 (C-3), 121.2 (C-7), 71.6 (C-15), 62.5 (C-4), 43.5 (C-1), 43.2 (C-11), 39.9 (C-10), 34.8 (C-2'), 33.5 (C-5), 32.3 (C-3'), 30.1-29.8 (C-4'-15'), 25.4 (C-16' and C-14), 23.1 (C-17'), 16.3 (C-12), 14.5 (C-18'), 12.2 (C-13).

Compound **4b** was obtained as white crystals, mp = 157-158°. $[\alpha]_D^{22}$ = +2.5° (c 0.3, MeOH). EI-MS, m/z (rel. int.): 250 (M^+, 61), 219 (100), 201 (49), 187 (16), 173 (9). IR (KBr), cm^{-1}: 3350, 2940, 2910, 2860, 1620, 1580, 1460, 1445, 1315, 1290, 1260, 1135, 1100, 1090, 1035. NMR (70% $CDCl_3$, 30% CD_3OD) ^1H: 3.56 (2H, t, $J_{4\text{-}5}$ = 8.0 Hz, H-4), 3.41 (2H, s, H-15), 2.87 (2H, t, H-5), 2.78 (1H, d, $J_{1a\text{-}1b}$ = 15.8 Hz, H-1a), 2.73 (1H, d, $J_{10a\text{-}10b}$ = 16.0 Hz, H-10a), 2.52 (1H, d, H-10b), 2.50 (1H, d, H-1b), 2.17 (3H, s, H-13), 2.10 (3H, s, H-12), 1.12 (3H, s, H-14). ^{13}C: 149.1 (C-8), 140.6 (C-2), 134.1 (C-6), 126.1 (C-9), 124.6 (C-3), 121.9 (C-7), 70.6 (C-15), 61.6 (C-4), 44.4 (C-11), 43.1 (C-1), 39.9 (C-10), 33.4 (C-5), 24.8 (C-14), 15.8 (C-12), 11.9 (C-13).

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