Two New Pigments from the Fruiting Bodies of the Basidiomycete *Lactarius deliciosus*

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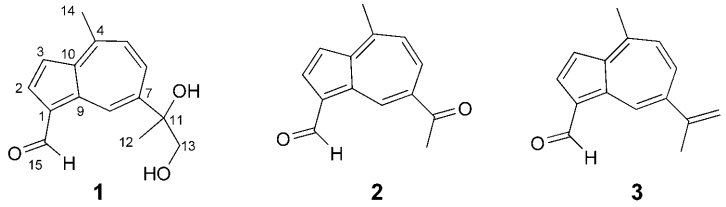
Two new, red-colored azulene pigments, 7-(1,2-dihydroxy-1-methylethyl)-4-methylazulene-1-carbaldehyde (**1**) and 7-acetyl-4-methylazulene-1-carbaldehyde (**2**), were isolated from the fruiting bodies of the basidiomycete *Lactarius deliciosus*, together with a related, known compound (**3**). Their structures were established on the basis of spectroscopic evidence including 2D-NMR experiments.

Introduction. – The mushrooms belonging to the genus *Lactarius* (family Russulaceae, Basidiomycotina) form a milky juice, when their fruiting bodies are injured. In the great majority of *Lactarius* species, different kinds of sesquiterpenes play important biological roles, being responsible for the pungency and bitterness of the milky juice, and for changes in latex color when exposed to air.

The edible fungus *Lactarius deliciosus* is widely distributed in Yunnan Province, China. The latex of the fruiting bodies of *L. deliciosus* is firstly carrot-colored, but then slowly (within minutes) darkens, and eventually turns green; these colors have previously been shown to be due to guaiane sesquiterpenes [1]. A well-known guaiane sesquiterpene is the antibiotic lactaroviolin [1–3]. Also known are the free dihydroazulene alcohol [4], its stearic acid ester [4], and lactarazulene [5], all isolated from European specimens of *L. deliciosus*. In addition, lactarofulvene [6][7] was obtained from Californian specimens of *L. deliciosus*, azulene aldehyde [2] was isolated from Indian specimens of *L. deterrimus*, and aromatic compounds [8] were obtained from liquid cultures of *L. deliciosus*.

In continuation of our research on bioactive metabolites of *Lactarius* and *Russula* species from Yunnan Province [9–13], we herein report the isolation and structure elucidation of two new pigments, compounds **1** and **2**, which were obtained together with the known compound 4-methyl-7-(1-methylethenyl)azulene-1-carbaldehyde (**3**) [1][2] from the fruiting bodies of *L. deliciosus*.

Results and Discussion. – The fresh fruiting bodies of *L. deliciosus* (10 kg) were first extracted with acetone and then with $CHCl_3$/MeOH 1:1 at ambient temperature. The combined organic phase was evaporated to afford a deep-brown gum (200 g), which was partitioned between H_2O and AcOEt. The organic layer was concentrated *in vacuo* to afford a residue (80 g), which was subjected to repeated column chromatography to afford **1**–**3**.



Compound **1** was obtained as a purple-red powder. HR-ESI-MS Experiments indicated the molecular formula $C_{15}H_{16}O_3$ (calc. for $[M+Na]^+$: 267.0997; found: 267.0997), with eight degrees of unsaturation. The ^{1}H- and ^{13}C-NMR data of **1** were similar to those of **3** [1][2], which suggested that the two compounds shared the same azulene substitution pattern. The following difference between **1** and **3** was evident: the two OH groups at C(11) (δ(C) 76.4 (*s*)) and C(13) (δ(C) 71.1 (*t*)) of **1** were absent in **3** (δ(C) 129.9 (*s*, C(11)), 116.7 (*t*, C(13))).

The HMBC spectrum of **1** (*Figure*) demonstrated key ^3J correlations between H–C(15) and C(2) and C(9); between H–C(14) and C(5) and C(10); between H–C(12) and C(7) and C(13); and between H–C(13) and C(7). These data were in accord with an azulene skeleton. Further, there were cross-peaks between H–C(2) and H–C(3), and between H–C(5) and H–C(6) in the ^{1}H,^{1}H-COSY spectrum of **1**.

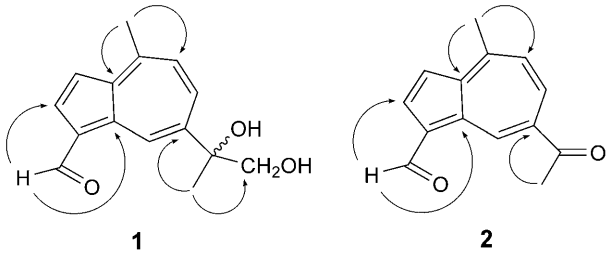


Figure. *Key HMBC correlations for* **1** *and* **2**

From the above data, the structure of compound **1** was established as 7-(1,2-dihydroxy-1-methylethyl)-4-methylazulene-1-carbaldehyde.

Compound **2** was obtained as a brown-red powder. Its HR-ESI-MS data indicated the molecular formula $C_{14}H_{12}O_2$ (calc. for $[M+H]^+$: 213.0915; found: 213.0914). By comparison of the ^{1}H- and ^{13}C-NMR data of **2** with those of **3**, it was shown that there was an additional oxo group at C(11) in **2** [δ(C) 199.0 (*s*)] compared to **3** [δ(C) 129.9 (*s*)]. Again, there were cross-peaks between H–C(2) and H–C(3), and between H–C(5) and H–C(6) in the ^{1}H,^{1}H-COSY spectrum.

From these data, the structure of **2** was established as 7-acetyl-4-methylazulene-1-carbaldehyde, as further confirmed by HMBC experiments (*Figure*).

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Experimental Part

General. UV Spectra: *UV-210* spectrometer; λ_{max} (log ε) in nm. IR: *Perkin-Elmer-577* spectrometer, with KBr pellets; in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: *Bruker AM-400* and *DRX-500* spectrometers; δ in ppm, *J* in Hz. MS: *VG Autospec-3000* mass spectrometer; in *m*/*z* (rel. %).

Fungal Material. The fresh fruiting bodies of *L. deliciosu*s were collected at Ciba country, Yunnan province, P. R. China, in November 2005. A voucher specimen (No. HMAS 52851) was deposited at the Herbarium, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, P. R. China

Extraction and Isolation. The fresh fruiting bodies of *L. deliciosus* (10 kg) were first extracted with acetone (3×) and then $CHCl_3$/MeOH 1:1 (2×) at r.t. The combined extract was evaporated to afford a deep-brown gum (200 g), which was partitioned between H_2O and AcOEt. The org. layer was concentrated *in vacuo*, and the resulting residue (80 g) was subjected to repeated column chromatography (CC) (SiO_2; petroleum ether/acetone 100:0, 98:2, 95:5, 90:10, 80:20, 50:50, 0:100). The fraction eluted with petroleum ether/acetone 98:2 (21.6 g) was further purified by repeated CC (1. SiO_2, petroleum ether/acetone 3:1; 2. *Sephadex LH-20*, $CHCl_3$/MeOH 1:1) to afford compounds **1** (3 mg), **2** (7 mg), and **3** (10 mg).

7-(1,2-Dihydroxy-1-methylethyl)-4-methylazulene-1-carbaldehyde (**1**). Light-red powder. UV/VIS (MeOH): 228 (4.22), 312 (4.32), 381 (3.89), 699 (0.61). IR (KBr): 3411 (OH), 2924, 1726 (CHO), 1630 (C=C), 1385. ^1H-NMR (400 MHz, $CDCl_3$): 10.24 (*s*, CHO), 9.93 (*d*, *J* = 1.8, H–C(8)); 8.19 (*dd*, *J* = 10.8, 1.8, H–C(6)); 8.16 (*d*, *J* = 4.2, H–C(2)); 7.57 (*d*, *J* = 10.8, H–C(5)); 7.30 (*d*, *J* = 4.2, H–C(3)); 4.06 (*d*, *J* = 11.2, H_a–C(13)); 3.88 (*d*, *J* = 11.2, H_b–C(13)); 2.95 (*s*, Me(14)); 1.70 (*s*, Me(12)). ^{13}C-NMR (125 MHz, $CDCl_3$): 187.1 (*d*, C(15)); 149.2 (*s*, C(4)); 145.5 (*s*, C(7)); 143.7 (*s*, C(9)); 142.2 (*d*, C(2)); 138.5 (*s*, C(1)); 136.1 (*d*, C(8)); 135.7 (*d*, C(6)); 130.9 (*d*, C(5)); 126.4 (*s*, C(10)); 116.2 (*d*, C(3)); 76.4 (*s*, C(11)); 71.1 (*t*, C(13)); 27.0 (*q*, C(14)); 24.9 (*q*, C(12)). HR-ESI-MS: 267.0997 ($[M+Na]^+$, $C_{15}H_{16}NaO_3^+$; calc. 267.0997).

7-Acetyl-4-methylazulene-1-carbaldehyde (**2**). Red-brown powder. UV/VIS (MeOH): 243 (4.38), 313 (4.21), 406 (3.88), 463 (2.87), 509 (2.89), 526 (2.89), 778 (2.56). IR (KBr): 3000–3100, 2924, 2720, 1674 (CO), 1654, 1240. ^1H-NMR (400 MHz, $CDCl_3$): 10.43 (*s*, CHO)); 10.29 (*d*, *J* = 1.4, H–C(8)); 8.52 (*dd*, *J* = 10.9, 1.4, H–C(6)); 8.24 (*d*, *J* = 4.2, H–C(2)); 7.58 (*d*, *J* = 10.9, H–C(5)); 7.51 (*d*, *J* = 4.2, H–C(3)); 3.00 (*s*, Me(14)); 2.86 (*s*, Me(12)). ^{13}C-NMR ($CDCl_3$, 125 MHz): 199.0 (*s*, C(11)); 187.5 (*d*, C(15)) 153.8 (*s*, C(4)); 143.1 (*s*, C(9)); 141.9 (*d*, C(2)); 138.1 (*d*, C(6)); 137.2 (*d*, C(8)); 137.1 (*s*, C(1)); 134.6 (*s*, C(7)); 130.2 (*d*, C(5)); 129.2 (*s*, C(10)); 119.5 (*d*, C(3)); 27.3 (*q*, C(12)); 25.3 (*q*, C(14)). HR-ESI-MS: 213.0914 ($[M+H]^+$, $C_{14}H_{13}O_2^+$; calc. 213.0915).

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