Lilacinone, a Red Aminobenzoquinone Pigment from *Lactarius lilacinus*

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A red pigment, lilacinone (**1**), was isolated from fruit bodies of the toadstool *Lactarius lilacinus*. Its structure was established by 2D NMR and APCIMS methods. Compound **1** is a novel type of fungal aminobenzoquinone pigment and may be biosynthetically derived from three molecules of anthranilic acid.

In the course of our studies on *Lactarius* pigments[1,2] we became interested in *Lactarius lilacinus* Fr. (Russulaceae) (German: Lila Milchling), a rare toadstool found in wet alder woods in Europe. In this paper, we describe the isolation and structural elucidation of lilacinone (**1**), the pigment responsible for the violet color of the fruit bodies.

Lilacinone (4 mg) was extracted from frozen fruit bodies (500 g) of *L. lilacinus* with acidified MeOH at 25 °C and purified by repeated chromatography on Sephadex LH-20. The UV/vis spectrum of **1** exhibited absorption maxima at 210, 250, 354, and 519 nm. No EIMS (70 eV) could be recorded due to the highly polar nature of the compound. In contrast, **1** exhibited a $[M + H]^+$ ion at *m/z* 498 in the LC/APCIMS. The presence of 22 carbons in the ^{13}C NMR and 15 hydrogens in the ^1H NMR spectrum permitted derivation of the molecular formula $C_{22}H_{15}N_3O_{11}$. The ^{13}C NMR spectrum of **1** contained a methoxy signal at δ_C 56.5 and 21 carbon signals between δ_C 81.2 and 188.5; these were assigned to four methine (δ_C 98.4, 106.3, 122.7, and 125.7) and 17 quaternary carbon atoms.

The ^1H NMR spectrum exhibited three singlets at δ_H 3.76 (OCH_3), 5.95 (CH), and 5.98 (CH), two doublets at 7.17 (CH) and 7.53 (CH), and eight additional signals between δ_H 9.71 and 13.93 for deuterium-exchangeable NH, NH_2, CO_2H, or OH protons. According to the HMQC spectrum, there were two aromatic *ortho*-protons at δ_H 7.17 and 7.53 ($^3J_{HH} = 8.5$ Hz) correlated with the carbons at δ_C 122.7 and 125.7, respectively. The HMBC spectrum revealed that the exchangeable proton at δ_H 10.71 belonged to a phenolic OH group coupled to carbons at δ_C 118.9, 122.7, 134.5, and 149.9. The latter signal was attributed to the phenolic carbon, and the strong coupling of the OH proton to the carbon at δ_C 122.7 suggested the direct neighborhood of the OH group to the aromatic proton at δ_H 7.17.

The exchangeable proton at δ_H 9.95 exhibited strong HMBC correlations to carbons at δ_C 118.9 and 125.7 as well as a weaker one to a carbon at δ_C 127.1. The fact that additional couplings to substructure B were visible identified this proton as an NH group, which, on account of the strong ^3J coupling, must be *ortho* to the aromatic proton at δ_H 7.53 (δ_C 118.9). The latter displayed a weak $^4J_{CH}$ coupling to an ester group at δ_C 168.3, which allowed us to put it adjacent to the aromatic carbon at δ_C 118.9. Likewise, the proton at δ_H 7.17 exhibited a weak $^4J_{CH}$ coupling to a quaternary carbon at δ_C 81.2, which connected it to the ring carbon at δ_C 134.5. These results were in agreement with substructure A (Figure 1).

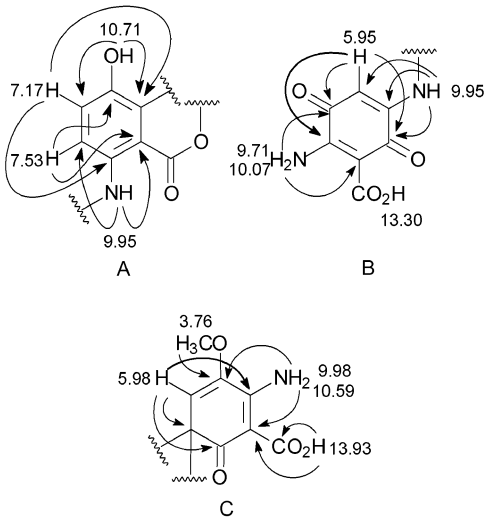

Figure 1. Substructures A, B, and C of lilacinone (**1**) with selected HMBC correlations.

The NH group formed a bridge to a second ring in the molecule as indicated by HMBC correlations to carbons at δ_C 98.4, 146.7, 175.9, and 179.3. The strong correlation with the methine carbon at δ_C 98.4 (δ_H 5.95) placed this group adjacent to the carbon carrying the NH group. The proton at δ_H 5.95 showed correlations to carbons at δ_C 94.7, 146.7, 156.7, 175.9, and 179.3, the latter two belonging to carbonyl groups. If one considers the chemical shifts of these carbons, a 1,4-benzoquinone system could be proposed for substructure B (Figure 1). Both the NH and the methine protons exhibited strong $^3J_{CH}$ couplings to the C=O signal at δ_C 179.3, which indicated the presence of a −CH=C(NH−R)−CO− moiety and, from the HMBC correlations mentioned above, the second C=O at δ_C 175.9 had to be attached to the CH group. Two exchangeable protons at δ_H 9.71 and 10.07 showed identical $^3J_{CH}$ couplings to the ^{13}C signals at δ_C 94.7 and 175.9 and belonged therefore to a NH_2 group. According to these strong $^3J_{CH}$ couplings, the carbon atom connected to the NH_2 group (δ_C 156.7) was localized between the C=O group at δ_C 175.9 and a carbon at δ_C 94.7, which carries the carboxy group at δ_C 167.9.

Substructure C (Figure 1) was revealed by considering the remaining methine proton at δ_H 5.98 (δ_C 106.3), which showed HMBC correlations to carbons at δ_C 81.2, 93.5, 134.5, 146.8, 161.0, and 188.5. Since the carbon at δ_C 134.5

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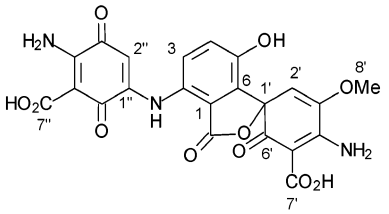
belongs to substructure A, a connection was achieved between that part of the molecule. Furthermore, the quaternary carbon at δ_C 81.2 had to be placed next to the methine group. Since the protons of the methoxy group at δ_H 3.76 exhibited a NOESY correlation to the CH proton at δ_H 5.98, it was possible to identify a −CH=C(OMe)− unit. These results together with the HMBC correlations given in Figure 1 were in accordance with substructure C. It explains the strong downfield shift of the signal at δ_C 161.0, which is caused by the vinylogous amide carbon.

A combination of the substructures A, B, and C leads to structure **1** for lilacinone. Despite the presence of the stereogenic spiro center, **1** is racemic and exhibits neither an optical rotation nor CD effects.



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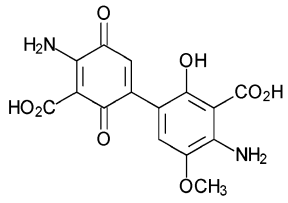

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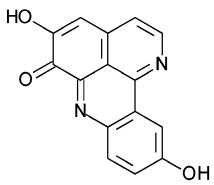

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Lilacinone (**1**) is structurally related to blennione (**2**), the green aminobenzoquinone pigment of *Lactarius blennius*.[2] The biosynthesis of **1** probably involves the addition of 5-hydroxyanthranilic acid to the quinone derived from 3,6-dihydroxyanthranilic acid, followed by phenolic coupling with 3-methoxy-6-hydroxyanthranilic acid and oxidative closure of the spirolactone ring. Interestingly, the polar pigments from *Lactarius* species lilacinone (**1**), blennione (**2**),[2] and necatorone (**3**)[1] are all derived from anthranilic acid.

Experimental Section

General Experimental Procedures. Evaporation of the solvents was performed under reduced pressure using a rotary evaporator. Column chromatography: Sephadex LH-20 (Pharmacia). UV: Perkin-Elmer Lambda spectrophotometer. NMR: Bruker AMX-600 spectrometer (^{1}H at 600.1, ^{13}C at 150.9 MHz), chemical shifts in δ relative to DMSO-d_6 as internal standard. The presence of traces of AcOH was beneficial for the quality of the NMR spectra. LC/APCIMS: Gynkotek-HPLC equipped with a Nucleosil RP-18 column (Macherey-Nagel, 250 × 2 mm, 5 μm, operation temperature 40 °C, flow 250 μL/min [solvent A: 99.9% H_2O/0.1% AcOH; solvent B: MeOH; gradient 100% A → 100% B in 20 min]) coupled with a Finnigan TSQ 7000, Finnigan API ion source interface, positive APCI mode, ionization 4.5 kV, capillary temperature 200 °C, mass range 50−800 mu, multiplier 1000 V (scan modus). MS/MS: argon collision gas 2.0 mbar, sheath gas (N_2) 2.9 bar, multiplier 1400 V, collision energy automatically rotated at −20, −30, −40 eV.

Toadstool. Fruit bodies of *L. lilacinus* (leg. et det. N. Arnold) were collected in October 1998 in a marshy alder grove 15 km north of Geisenfeld (Bavaria). The toadstools were frozen after collecting. A voucher specimen is kept in the herbarium of the Ludwig-Maximilians-Universität München, Department Chemie.

Isolation Procedure. A 500 g sample of the frozen fruit bodies was crushed and extracted with MeOH (2 × 500 mL) at room temperature for 30 min. The brownish red extract was then concentrated in a vacuum at 40 °C, yielding a red residue, which was dissolved in 5 mL of MeOH and a few drops of 2 N HCl under sonification. During this procedure most of the sugars remained undissolved. Repeated chromatography on Sephadex LH-20 with MeOH and a few drops of 2 N HCl yielded 2−3 mg of lilacinone (**1**).

Lilacinone (1): red solid: mp >300 °C (dec); $[\alpha]^{25}_D$ ±0° (*c* 0.10, MeOH); UV/vis (MeOH) λ_{max} (log ϵ) 210 (4.06), 250 (4.11), 309 (sh, 3.70), 354 (3.82), 519 (3.24) nm; ^{1}H NMR (DMSO-d_6) δ 13.93 (1H, s, 7′-CO_2H), 13.30 (1H, br s, 7″-CO_2H), 10.71 (1H, s, 5-OH), 10.59 (1H, s, 4′-NH_2), 10.07 (1H, s, 4″-NH_2), 9.98 (1H, s, 4′-NH_2), 9.95 (1H, s, 2-NH), 9.71 (1H, s, 4″-NH_2), 7.53 (1H, d, J = 8.5 Hz, H-3), 7.17 (1H, d, J = 8.5 Hz, H-4), 5.98 (1H, s, 2′-H), 5.95 (1H, s, 2″-H), 3.76 (3H, s, OCH_3); ^{13}C NMR (DMSO-d_6) δ 188.5 (C, C-6′), 179.3 (C, C-6″), 175.9 (C, C-3″), 168.8 (C, C-7′), 168.3 (C, C-7), 167.9 (C, C-7″), 161.0 (C, C-4′), 156.7 (C, C-4″), 149.9 (C, C-5), 146.8 (C, C-3′), 146.7 (C, C-1″), 134.5 (C, C-6), 127.1 (C, C-2), 125.7 (CH, C-3), 122.7 (CH, C-4), 118.9 (C, C-1), 106.3 (CH, C-2′), 98.4 (CH, C-2″), 94.7 (C, C-5″), 93.5 (C, C-5′), 81.2 (C, C-1′), 56.5 (CH_3, C-8′); LC/APCIMS t_R = 16.4 min (detection, UV at λ = 250 nm and APCIMS), *m*/*z* 498 [M + H]$^+$; LC/APCIMS/MS (parent ion *m*/*z* 498, 20 eV) *m*/*z* (%) 498 (4), 480 (100), 462 (15), 418 (2).

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References and Notes

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