Metabolites of 2-Aminophenol from Fruit Bodies of *Lepiota americana* (Agaricales)

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From the acetone extract of the North American toadstool *Lepiota americana* 2-aminophenoxazin-3-one (**1**) and a novel amino-1,4-benzoquinone derivative, lepiotaquinone (**2**), were isolated. The structure of **2** was confirmed by its preparation from 2-aminophenol and amino-1,4-benzoquinone.

Introduction

Toadstools of the genera *Lepiota* and *Macrolepiota* include species that exhibit characteristic colour changes on bruising. To the best of our knowledge the chemical background for these processes is still unknown. In this publication we describe the isolation of two metabolites of 2-aminophenol that are responsible for the dark red colour of the acetone extract from fruit bodies of *Lepiota americana*. This species is distributed in the Eastern parts of North America and occurs singly or in dense clusters in fields, sawdust piles and around stumps. The white flesh of young toadstools turns at first yellow-orange on bruising and changes later to red.

Results and Discussion

Exhaustive extraction of air-dried fruit bodies of *Lepiota americana* with acetone-water (20:1 v/v) yielded a red solution. Evaporation of the extracts and HPLC of the residue on reversed phase with H_2O/MeOH afforded a red pigment **1** and an orange compound **2** for which the name lepiotaquinone is proposed.

The HR EI-MS spectrum of the red pigment shows a molecular ion at *m/z* 212 which corresponds to the molecular formula $C_{12}H_8N_2O_2$. From the UV/Vis, IR and ^{1}H NMR data and by direct comparison with a synthetic sample the compound was identified as 2-aminophenoxazin-3-one (**1**) (Osman and Bassiouni, 1960). Phenoxazinone **1** has been isolated before from mycelial cultures of the two basidiomycetes *Calocybe gambosa* (Schlunegger *et al.*, 1976) and *Psathyrella obtusata*

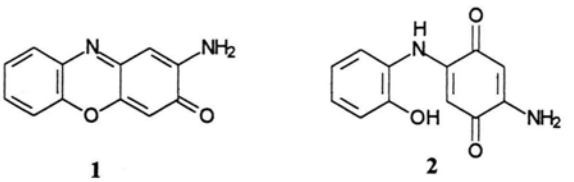

Fig. 1. 2-Aminophenoxazin-3-one (**1**) and lepiotaquinone (**2**).

(Meiss, 1987; Scherer, 1989). The compound is also formed by *Penicillium notatum* (Bär *et al.*, 1971a), the phytopathogenic ascomycete *Acrospermum viticola* (Kinjo *et al.*, 1987) and several actinomycetes (Anzai *et al.*, 1960; Gerber and Lechevalier, 1964; Gerber, 1966; Gerber, 1967). It exhibits antibiotic activity against certain Gram-positive bacteria, actinomycetes and fungi, but is inactive against Gram-negative bacteria (Gerber and Lechevalier, 1964; Meiss, 1987).

The EI-MS of lepiotaquinone (**2**) exhibits a molecular ion at *m/z* 230 in accord with the molecular formula $C_{12}H_{10}N_2O_3$. The UV/Vis spectrum of **2** shows an absorption maximum at 339 nm (MeOH) and the IR-spectrum (KBr) a strong band in the carbonyl region at 1618 cm^{-1}. A typical signal pattern for a 1,2-disubstitued benzene ring and signals for two separated olefinic protons are visible in the ^{1}H NMR spectrum. The ^{13}C NMR spectrum shows 10 signals in the aromatic region and two carbonyl signals at δ 177 and 179, which are typical for 1,4-benzoquinones carrying electron donating groups at the 2- and 5-positions (Höfle, 1976). This allowed us to identify lepiotaquinone as 2-amino-5-(2-hydroxyphenylamino)-1,4-benzoquinone (**2**).

The proposed structure was confirmed by a straightforward synthesis. Addition of one equivalent of 2-aminophenol (**6**) to two equivalents of amino-1,4-benzoquinone (**5**) yielded an orange product that was in every respect identical with

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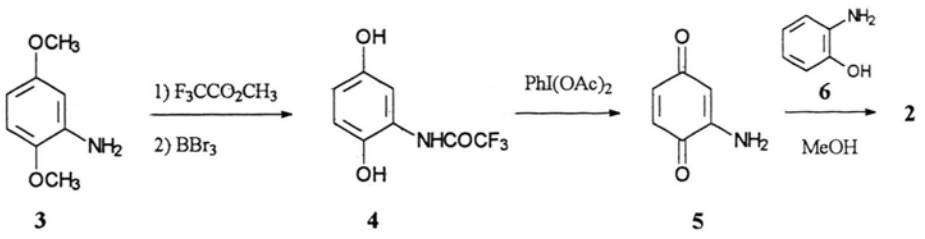

Scheme 1. Synthesis of lepiotaquinone (**2**).

natural lepiotaquinone (**2**). Amino-1,4-benzoquinone (**5**) has recently been obtained by the addition of sodium azide to 1,4-benzoquinone (Pachatouridis *et al.*, 1998). We found that quinone **5** can be smoothly prepared in pure form by oxidation of *N*-(2′,5′-dihydroxyphenyl)-2,2,2-trifluoroacetamide (**4**) with iodobenzene diacetate in methanol (Pelter and Elgendy, 1988). Under these conditions quinone formation and cleavage of the trifluoroacetyl protecting group take place and the aminobenzoquinone **5** is obtained in 40% yield. The compound must be handled with care since it is rapidly transformed into brown products on standing at room temperature. The trifluoroacetyl derivative **4** can be easily obtained from commercially available 2,5-dimethoxyaniline (**3**) by *N*-trifluoroacetylation (Steglich and Hinze, 1976) and subsequent demethylation with boron tribromide (Gould *et al.*, 1989).

Both pigments **1** and **2** are closely related and appear to be derived biogenetically from 2-aminophenol (**6**) (Anzai *et al.*, 1960; Bär *et al.*, 1971b). Like other quinonoid compounds from macromycetes the pigments of *Lepiota americana* are originally present as colourless leuco derivatives (Gill and Steglich, 1987) which undergo rapid oxidation when the fruit bodies are bruised or dried.

Experimental

General

M.ps.: Kofler hotstage apparatus, uncorr. CC: TLC: silica gel 60 F_{254} (Merck), petrol ether-EtOAc (1:1 v/v). Analytical HPLC: Waters 600 E pump and system controller coupled with a diode array detector 990+. Preparative HPLC: Waters 590 EF pump and system controller 680 coupled with a Knauer Variable Wavelength Monitor. UV: Perkin-Elmer Lambda spectrophotometer. IR: Bruker FTIR spectrophotometer IFS 45. NMR: Bruker AMX-300 and AMX-600 spectrometers, chemical shifts in δ [ppm] rel. to [D_6]DMSO (δ_H 2.49, δ_C 39.5), CD_3OD (δ_H 3.35, δ_C 49.3) and $CDCl_3$ (δ_H 7.26, δ_C 77.0) as internal standard. EI-MS: Finnigan MAT 90 instrument.

Plant material

Lepiota americana was collected in September 1996 near Harvard Square in Boston, Mass. (leg. et det. N. Arnold). Voucher specimens were deposited in the Herbarium of the Department of Chemistry, University of München.

Extraction and isolation

The air-dried toadstools (121 g) were exhaustively extracted with acetone-water (20:1 v/v) at room temperature. The combined extracts were evaporated under reduced pressure at 30 °C and kept at −20 °C under an argon atmosphere. The crude extract was separated by HPLC using a RP 18 column [Macherey-Nagel, 5 μm, 250×4.5 mm or 7 μm, 25×20 mm; gradient: H_2O (100%) in 40 min to MeOH (100%), R_t (**1**) = 34.9 min, R_t (**2**) = 29.5 min]. The yields were 7 mg of 2-amino-phenoxazin-3-one (**1**) and 6 mg of lepiotaquinone (**2**).

2-Aminophenoxazin-3-one (**1**): dark red needles, m.p. 235 °C; 235 °C (Osman and Bassouini, 1960). – TLC: R_f 0.29. – UV/Vis (MeOH): λ_{max} (log ε) = 430 (4.188), 236 (4.322), 203 nm (4.467). – IR (KBr): ν = 3334 (m), 1602 (s), 1497 (m), 1458 (m), 1204 (m), 850 (w), 737 (w), 698 (w), 584 cm^{-1} (w). – ^1H NMR (300 MHz, $CDCl_3$): δ = 6.02 (s, 2H, NH_2), 6.32 (s, 1H, 1-H), 6.38 (s, 1H, 4-H), 7.27–7.34 (m, 3H, 5-H, 6-H, 7-H), 7.66 (d, J = 8.0 Hz, 1H, 8-H). – ^{13}C NMR (150 MHz, CD_3OD): δ = 99.5 (C-1), 104.8 (C-4), 117.2 (C-6), 126.6 (C-8), 128.8 (C-9), 130.4 (C-7), 134.9 (C-9a), 144.0 (C-5a), 149.0 (C-

4a), 149.9 (C-10a), 151.5 (C-2), 182.3 (C-3). - EI-MS: *m/z* (%) = 212 (100) [M^+], 186 (5), 185 (39), 184 (20), 157 (5), 144 (5), 92 (7).

Lepiotaquinone (**2**): orange crystals, m.p. 224 °C. – TLC: R_f 0.40. – UV/Vis (MeOH): λ_{max} (log ε) = 339 nm (2.739). – IR (KBr): ν = 3435 (s), 2924 (w), 2854 (w), 1618 (m), 1567 (m), 1518 (m), 1459 (m), 1356 (m), 1276 (w), 1203 (w), 1104 (w), 1046 (w), 749 (w), 444 cm^{-1} (m). – ^1H NMR (600 MHz, [D_6]DMSO): δ = 5.45 (s, 1H, 3-H), 5.54 (s, 1H, 6-H), 6.83 (t, *J* = 7.8 Hz, 1H, 5′-H), 6.94 (d, *J* = 7.8 Hz, 1H, 3′-H), 7.03 (t, *J* = 7.2 Hz, 1H, 2′-H), 7.15 (s, br, 1H, NH), 7.25 (d, *J* = 7.2 Hz, 1H, 6′-H), 7.90 (s, br, 1H, NH), 8.68 (s, br, 1H, NH), 10.17 (s, br, 1H, OH). – ^{13}C NMR (150 MHz, [D_6]DMSO): δ = 94.6 (C-3), 95.1 (C-6), 115.8 (C-3′), 119.3 (C-5′), 122.1 (C-2′), 124.6 (C-4′), 126.1 (C-1′), 146.1 (C-5), 149.4 (C-6′), 152.5 (C-2), 177.4 (C-4), 179.2 (C-1). – HR EI-MS: *m/z* (%) = 230.0686 (100, [M^+], $C_{12}H_{10}N_2O_3$ requires 230.0681), 214 (30), 213 (16), 201 (39), 185 (26), 160 (65), 159 (15), 133 (38), 109 (28), 80 (14), 70 (14), 68 (15), 65 (15), 44 (13).

N-*(2′,5′-Dihydroxyphenyl)-2,2,2-trifluoroacetamide* (**4**): A mixture of 2,5-dimethoxyaniline (**3**) (7.66 g, 0.05 mol), *N,N,N′,N′*-tetramethylguanidine (9.26 ml, 0.08 mol), and ethyl trifluoroacetate (27.3 ml, 0.27 mol) was kept for 1 h in an ultrasound bath. The solvent was removed *in vacuo* and the residue dissolved in 2 N HCl. Extraction of the aqueous solution with EtOAc (3×), drying of the combined organic phases ($MgSO_4$) and evaporation of the solvent afforded *N*-(2′,5′-dimethoxyphenyl)-2,2,2-trifluoroacetamide as colourless crystals (10.3 g, 83%), m.p. 66 °C; m.p. 66–66.2 °C (Russell *et al.*, 1991).

To *N*-(2′,5′-dimethoxyphenyl)-2,2,2-trifluoroacetamide (5 g, 0.02 mol) in dry CH_2Cl_2 (250 ml) was added dropwise at –78 °C BBr_3 (4.62 ml, 0.05 mol). After 1 h stirring at –78 °C, the mixture was allowed to warm to room temp., and the stirring was continued for further 6 h. Then, water (100 ml) was carefully added at 0 °C and the solvent removed *in vacuo*. The aqueous residue was extracted with EtOAc, and the extracts were washed with saturated NaCl solution. Evaporation of the dried ($MgSO_4$) organic phases afforded the diol **4** as a colourless powder (4.30 g, 97%), m.p. 179 °C. – TLC: R_f 0.69. – ^1H NMR (300 MHz, [D_6]DMSO): δ = 6.55 (dd, *J* = 9, 3 Hz, 1H), 6.75 (d, *J* = 9 Hz, 1H), 6.88 (d, *J* = 3 Hz, 1H), 8.92 (1H, OH), 9.17 (1H, NH), 10.26 (1H, OH). – ^{13}C NMR (75.5 MHz, [D_6]DMSO): δ = 112.0, 114.2, 115.9 (q, *J* = 288 Hz, CF_3), 116.4, 122.5, 142.9, 149.5, 154.6 (q, *J* = 36.2 Hz, CO). – EI-MS: *m/z* (%) = 219 (28) [M^+], 204 (11), 137 (19), 124 (10), 123 (100), 95 (28), 82 (31), 69 (16), 68 (21), 67 (16), 54 (31), 53 (12), 44 (11).

2-Amino-1,4-benzoquinone (**5**): To a stirred solution of **4** (770 mg, 3.48 mmol) in CH_2Cl_2 (10 ml) was added at room temperature $PhI(OAc)_2$ (1.29 g, 4.00 mmol) in a single portion. After 1 min, the reaction mixture was partitioned between EtOAc (40 ml) and H_2O (40 ml). The organic layer was separated, dried ($NaSO_4$) and concentrated *in vacuo*. The resulting orange solid was purified by chromatography (silica gel, EtOAc-petrol ether, 2:1 v/v) to yield **5** (170 mg, 40%) as an orange solid, m.p. 145 °C; 148 °C (Pachatouridis *et al.*, 1998). – TLC: R_f 0.33. – UV (MeOH): λ_{max} (log ε) = 302 (3.481), 202 nm (3.999). – IR (KBr): ν = 3436 (m), 3247 (m), 3091 (w), 3066 (w), 3005 (w), 1695 (m), 1671 (s), 1596 (m), 1509 (s), 1426 (w), 1370 (m), 1325 (s), 1237 (s), 1204 (m), 1172 (w), 1097 (m), 1041 (w), 1009 (w), 904 (m), 895 (m), 840 (w), 772 (w), 735 (w), 636 (w), 592 (w), 490 (w), 439 (w), 427 cm^{-1} (w). – ^1H NMR (300 MHz, $CDCl_3$): δ = 5.04 (br. s, 2H, NH_2), 5.75 (s, 1H, 3-H), 6.61–6.62 (m, 2H, 5-H, 6-H). – ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 102.4, 132.4. 139.4 (C_{ar}), 146.5 (C_q), 171.2 (CO), 173.9 (CO). – EI-MS: *m/z* (%) = 123 (100) [M^+], 109 (11), 96(9), 95 (41), 82 (35), 69 (13), 68 (20), 67 (19), 55 (15), 54 (37), 53 (14), 44 (15), 43 (19).

Analysis for $C_6H_5NO_2$ (123.11):

Calcd.	C 58.54	H 4.09	N 11.38%,
Found	C 58.60	H 4.25	N 11.39%.

2-Amino-5-(2-hydroxyphenylamino)-1,4-benzoquinone, lepiotaquinone (**2**): To a stirred solution of **5** (66 mg, 0.50 mol) in MeOH (15 ml) was added 2-aminophenol (**6**) (29 mg, 0.27 mmol) at room temperature. After 1 h, the reaction mixture was concentrated *in vacuo*. The red solid was purified by chromatography (silica gel, EtOAc-petrol ether, 2:1 v/v) to yield **2** (13 mg, 21%) as an orange solid. The spectral data (^1H NMR, ^{13}C NMR, MS, IR, UV/Vis) were identical with those of lepiotaquinone from *L. americana*. The identity of both samples was confirmed by direct chromatographic comparison (TLC, HPLC).

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