Nigricanin, the First Ellagic Acid Derived Metabolite from the Basidiomycete *Russula nigricans*

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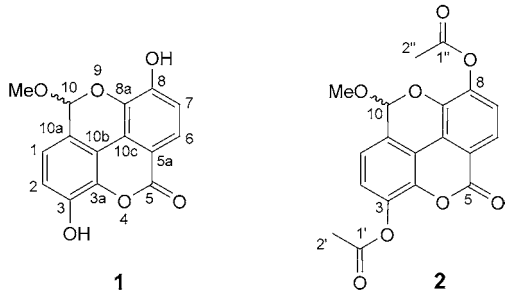
Nigricanin (**1**), the first ellagic acid related derivative from higher fungi, has been isolated from the fruiting bodies of the Basidiomycetes *Russula nigricans*. The structure of the novel compound was established by spectroscopic and chemical methods.

Introduction. – *Russula*, a fungus genus comprising hundreds of species, belongs to the Russulaceae family, which is one of the largest in the subdivision Basidiomycotina in *Whitthaker*'s kingdom of fungi [1]. While secondary metabolites occurring in the fruiting bodies of European *Lactarius* species have well been investigated, the *Russula* mushrooms have received relatively little attention [2]. Our investigations have revealed some novel sesquiterpenoids and triterpenoids, new ceramides, as well as a first N-containing aristolane derivative (lepidamine) from a few species of the genus *Russula* [3–7]. *Russula nigricans* is an inedible mushroom, whose fruiting bodies have been found to show antitumor activity [8]. However, so far, there has been no report regarding its chemical constituents.

In continuing our studies on the bioactive secondary metabolites from higher fungi, a new compound, nigricanin (**1**), was isolated from the fruiting bodies of *R. nigricans*. Here, we describe the structure elucidation of this new compound.



Results and Discussion. – Nigricanin (**1**) was isolated in the form of white crystalline needles after repeated extraction (EtOH, $CHCl_3$, $CHCl_3$/MeOH 1:1) of the fruiting bodies of *R. nigricans*, followed by repeated column chromatography (see the *Exper. Part*). HR-FAB-MS (negative mode) showed a pseudo-molecular ion at *m*/*z* 285.0382 ($[M-1]^-$, $C_{15}H_9O_6^-$; calc. 285.0399), giving rise to the molecular formula $C_{15}H_{10}O_6$. In

the IR spectrum of 1, a strong absorption band was found at 3415 cm^{-1}, indicating the presence of OH groups, as further confirmed chemically: upon treatment of **1** with Ac_2O in pyridine for 30 min at room temperature, the diacetate **2** was isolated. The EI mass spectrum of the the latter showed the M^+ peak at *m/z* 370, together with characteristic fragment ions at *m/z* 328 ($[M - CH_2CO]^+$) and 286 ($[M - 2\ CH_2CO]^+$), as well as a base peak at *m/z* 255 ($[M - 2\ CH_2CO - MeO]^+$).

The ^{13}C-NMR DEPT spectrum of nigricanin (**1**) indicated a highly conjugated lactone C=O group at δ(C) 160.1, which was supported by a strong IR band at 1705 cm^{-1}. A total of eight quaternary-C-atom signals at δ(C) 151.8, 145.6, 138.3, 136.1, 121.1, 120.5, 113.1, 112.2, as well as four methine signals at δ(C) 124.8, 123.0, 119.0, and 118.4, were assigned to the carbon skeleton. In the corresponding ^{1}H-NMR spectrum, four aromatic H-atoms were observed at δ(H) 7.79 (*d*, $J = 10.0$ Hz, H–C(6)), 7.21 (*d*, $J = 9.5$ Hz, H–C(1)), 7.18 (*d*, $J = 10.0$ Hz, H–C(7)), and 7.13 (*d*, $J = 9.5$ Hz, H–C(2)); and the signals at δ(C) 56.0 and δ(H) 3.57 (*s*, 3 H) were assigned to the 10-MeO group. ^{1}H,^{1}H-COSY and HMQC Spectra allowed the establishment of two H-atom systems, one at C(1)/C(2), the other at C(6)/C(7). From these data, in combination with the observed HMBC correlations shown in the *Figure*, the structure of nigricanin (**1**) was established as 3,8-dihydroxy-10-methoxy[1]benzopyrano[5,4,3-*cde*][1]benzopyran-5(10*H*)-one. Full assignment of the C- and H-atoms of **1** was made by 2D-NMR techniques (HMQC, HMBC and ^{1}H,^{1}H-COSY), as listed in the *Table*.

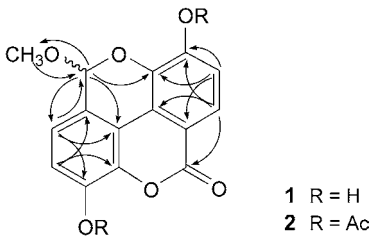


Figure. *Key HMBC interactions observed in compounds* **1** *and* **2**

Nigricanin (**1**) is a phenolic compound based on the ellagic-acid skeleton. Ellagic acid and its derivatives are widely distributed in plants, but are rare in fungi. Ellagic acid proper and its derivatives are known to display multiple biological activities such as DNA damaging [9] or acting as antioxidants [10]. In the case of actinomycete, *e.g.*, *Streptomyces chartreuses*, only the antibiotics D 329C, chartreusin, and elsamicin have been isolated; and these compounds have been reported to display antibacterial, antineoplastic, and antileukaemia activities [11–13]. Nigricanin (**1**), thus, is the first ellagic acid like compound found in higher fungi.

Experimental Part

General. Column chromatography (CC) was performed on silica gel (200–300 mesh), and thin-layer chromatography (TLC) was carried out on pre-coated silical-gel-F_{254} plates (*Qingdao Marine Chemical Ltd.*, P.R. China). Melting points (m.p.) were obtained on an *XRC-1* apparatus (*Sichuan*) and are uncorrected. Optical rotations were measured on a *HORIBA SEPA-300* polarimeter (*Horiba*, Tokyo, Japan). UV Spectra were obtained on a *UV-210* spectrophotometer; λ_{max} in nm (log ε). IR Spectra were obtained on a *Bio-Rad FTS-135* IR spectrophotometer (*Bio-Rad*, Richmond, CA) using KBr pellets; in cm^{-1}. One-dimensional ^{1}H- and

Table. *^{1}H- and ^{13}C-NMR Data of Compounds* **1** *and* **2**. At 500 and 125 MHz, resp.; in (D_6)acetone (for **1**) and $CDCl_3$ (for **2**). Chemical shifts δ in ppm, coupling constants J in Hz.

	1			**2**	
	^{1}H	^{13}C	HMBC	^{1}H	^{13}C
H–C(1)	7.21 (d, J = 9.5)	123.0	H–C(10)	7.26 (d, J = 9.5)	121.8
H–C(2)	7.13 (d, J = 9.5)	118.4		7.32 (d, J = 9.5)	124.7
C(3)		145.6	H–C(1)		137.9
C(3a)		120.5	H–C(2)		125.2
C(5)		160.1	H–C(6)		158.4
C(5a)		112.2	H–C(7)		117.4
H–C(6)	7.79 (d, J = 10)	124.8		7.98 (d, J = 10)	123.7
H–C(7)	7.18 (d, J = 10)	119.0		7.35 (d, J = 10)	124.9
C(8)		151.8	H–C(7), H–C(6)		143.2
C(8a)		136.1	H–C(2), H–C(10)		140.1
C(10)		99.9	H–C(1), MeO		98.9
C(10a)		138.3	H–C(2)		139.5
C(10b)		113.1	H–C(1), H–C(10)		112.6
C(10c)		121.9	H–C(6)		121.0
MeO	3.57 (s)	56.0	H–C(10)	3.60 (s)	56.1
C(1′)					168.3
C(1″)					168.0
Me(2′)				2.43 (s)	20.7
Me(2″)				2.45 (s)	20.4

^{13}C-NMR as well as two-dimensional NMR spectra were recorded on *Bruker AM-400* and *DRX-500* spectrometers (*Brucker*, Karlsruhe, Germany); chemical shifts δ in ppm rel. to $SiMe_4$ (=0 ppm) as internal standard, coupling constants J in Hz. Mass spectra were recorded on a *VG Autospec-3000* mass spectrometer (*VG*, UK); in m/z (rel. %).

Fungal Material. The fresh fruiting bodies of *Russula nigricans* were collected at Ailao Mountain, Yunnan Province, China, in July 2003, and were identified by Prof. *M. Zang*, Kunming Institute of Botany, The Chinese Academy of Sciences. A voucher specimen (HKAS 25087) was deposited at the Herbarium of the Kunming Institute of Botany, The Chinese Academy of Sciences.

Extraction and Isolation. The fresh fruiting bodies of *R. nigricans* (12 kg) were extracted first with 95% aq. EtOH (15 l). The ethanolic soln. was evaporated *in vacuo*, and the remainder was re-extracted with $CHCl_3$. The $CHCl_3$-soluble, air-dried extract (990 g) was powdered and then extracted at r.t. three times with $CHCl_3$ (2.5 l), followed by $CHCl_3$/MeOH 1:1 (2.5 l), resp. The combined extracts of all $CHCl_3$- and $CHCl_3$/MeOH-soluble fractions were concentrated *in vacuo* to afford a crude brown, oil-like residue (43 g), which was subjected to CC (SiO_2; $CHCl_3$/MeOH 100:0 → 50:50) to give several fractions. The fraction (0.14 g) eluted with $CHCl_3$/MeOH (95:5) was subjected to repeated CC (SiO_2; $CHCl_3$/MeOH 100:0 → 90:10), and further purification of the subfraction (44 mg) eluted with $CHCl_3$/MeOH 95:5 by prep. TLC (SiO_2; $CHCl_3$/MeOH 90:10) resulted in nigricanin (**1**).

3,8-Dihydroxy-10-methoxy[1]benzopyrano[5,4,3-cde*][1]benzopyran-5(10*H*)-one* (*nigricanin*; **1**). Yield: 13 mg. White cristalline needles. M.p. 224° (acetone, dec.). $[\alpha]_D^{20} = 0$ (acetone). UV (MeOH): 256.8 (4.60), 335.2 (3.93). IR (KBr): 3415, 2940, 1705, 1608, 1514, 1480, 1272, 1232, 1211, 1102, 1034. ^{1}H- and ^{13}C-NMR: see the *Table*. EI-MS: 286 (28, M^+), 285 (12, $[M-H]^+$), 256 (15, $[M-CH_2O]^+$), 255 (100, $[M-CH_3O]^+$). HR-FAB-MS: 285.0382 ($[M-H]^+$, $C_{15}H_9O_6^+$; calc. 285.0399).

Peracetylation of Compound **1**. Nigricanin (**1**; 2.5 mg) and Ac_2O (0.5 ml) were added to anh. pyridine (1.0 ml), and the mixture was allowed to react at r.t. for 30 min. Then, the mixture was diluted with H_2O (15 ml) and extracted with AcOEt (3 × 8 ml). The combined org. layers were washed again with H_2O (10 ml), dried (Na_2SO_4), and evaporated to afford 2.4 mg (74%) of *3,8-bis(acetoxy)-10-methoxy[1]benzopyrano[5,4,3*-cde*][1]benzopyran-5(10*H*)-one* (**2**). White crystalline needles. M.p. 195–197° (acetone). IR (KBr): 3440, 2933, 1765, 1744, 1616, 1427, 1370, 1271, 1212, 1171, 1090. ^{1}H- and ^{13}C-NMR: see the *Table*. EI-MS: 370 (7, M^+), 328

(18, $[M - CH_2CO]^+$), 297 (10, $[M - CH_2CO - CH_3O]^+$), 286 (33, $[M - 2\,CH_2CO]^+$), 255 (100, $[M - 2\,CH_2CO - CH_3O]^+$).

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