

Lepidamine, the First Aristolane-Type Sesquiterpene Alkaloid from the Basidiomycete *Russula lepida*

by Jianwen Tan, Zejun Dong, Lin Hu, and Jikai Liu*

Kunming Institute of Botany, the Chinese Academy of Sciences, Kunming 650204, P. R. China
(e-mail: jkliu@mail.kib.ac.cn)

A novel N-containing aristolane sesquiterpenoid compound, lepidamine (**1**), was isolated from the fruiting bodies of Basidiomycete *Russula lepida*. Its structure was established by spectroscopic means. It is the first aristolane-type sesquiterpene alkaloid isolated from nature.

Introduction. – The Russulaceae family is one of the largest in the subdivision Basidiomycotina in *Wittaker's* kingdom of fungi and comprises hundreds of species [1]. While secondary metabolites occurring in the fruiting bodies of European *Lactarius* species have well been investigated, the *Russula* mushrooms have received less attention, notwithstanding the larger number of existing species [2]. Our recent chemical constituent investigation on *Russula lepida* led to the identification of some new terpenoids [3]. As part of our studies on the active metabolites from higher fungi in Yunnan province, China [4–12], the minor constituent of *Russula lepida* were further investigated. This report deals with the isolation and structure elucidation of a novel N-containing aristolane sesquiterpenoid, lepidamine (**1**), from the EtOH and CHCl₃/MeOH 1:1 extract of the fruiting bodies of *R. lepida*.

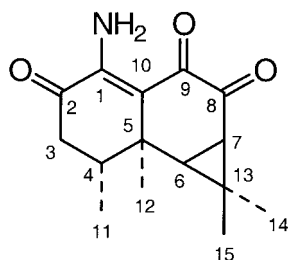


Fig. 1. Structure of lepidamine (**1**)

Results and Discussion. – The CHCl₃-soluble fraction of the EtOH and CHCl₃/MeOH 1:1 extract from the fruiting bodies of *R. lepida* was subjected to repeated column chromatography and preparative TLC to afford **1** as a pale yellow oil. HR-FAB-MS (positive mode) showed $[M + H]^+$ at m/z 262, corresponding to the formula C₁₅H₂₀NO₃ ($[M + H]^+$ 262.1453, calc. for C₁₅H₂₀NO₃ 262.1443). Fifteen signals in the ¹³C-NMR (DEPT) spectra of **1** were recognized (7 × C, 3 × CH, 1 × CH₂, 4 × CH₃), including three keto C=O C-atoms and two olefinic quaternary C-atoms, which also

suggested that there should be two H-atoms directly bound to a N-atom. This was confirmed by its IR spectrum with absorptions at 1709, 1699, 1681, 1620, and 3376, 3260 cm^{-1} . Its EI-MS showed the molecular ion peak $[M]^+$ at m/z 261 and characteristic fragment ions at m/z 233 ($[M - \text{CO}]^+$), 218 ($[M - \text{CO} - \text{CH}_3]^+$, base peak), and 149 ($[\text{C}_8\text{H}_5\text{O}_3]^+$). The signals in the $^1\text{H-NMR}$ spectra at 9.84 (br. s, 1 H) and 6.34 (br. s, 1 H) were assigned to the two N-bound H-atoms, and the signals at 1.16 (d, 3 H), 1.25 (s, 3 H), 1.28 (s, 3 H), and 1.29 (s, 3 H) were consistent with the structure of three quaternary Me groups and one tertiary Me group, respectively. $^1\text{H}, ^1\text{H-COSY}$ and HMQC spectra allowed establishment of two H-atom systems, one at C(3) through C(11), and the other at C(6) through C(7). By comparison with other aristolane sesquiterpenoids reported [3], the structure of this compound can be established as another aristolane-based sesquiterpenoid compound shown in Fig. 1. This proposed structure was further confirmed by HMBC and rational explanation of its bright-yellow color and UV spectrum, which exhibited two absorption maxima at 242 (C=C–C=O) and 409 nm (br., long conjugated system) and one weak absorption at 263.5 nm (N conjugation-related). The relative configuration was accomplished by a NOESY spectrum, in which significant NOE correlation peaks between H–C(6), and 11-Me, and 15-Me; H–C(7), and 12-Me, and 15-Me; 14-Me, and NH_b , and H–C(4) were observed (Fig. 2). All spectral data supported this structure.

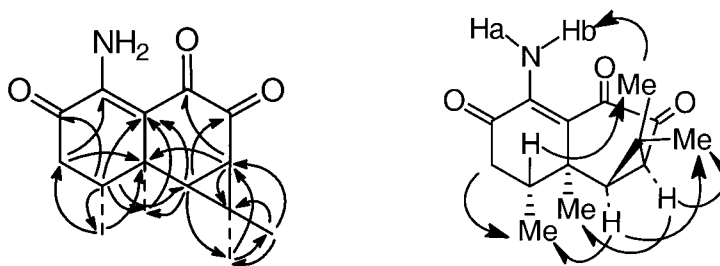


Fig. 2. Significant HMBC and NOESY correlations of **1**

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Experimental Part

General. IR Spectra: *Bio-Rad FTS-135* spectrometer, KBr pellets. 1D- and 2D-NMR spectra: in CDCl_3 with *Bruker AM-400* and *DRX-500* with TMS as internal standard, respectively. EI- and FAB-MS were carried out with a *VG-Auto Spec-3000* spectrometer.

Mushroom Material. The fresh fruiting bodies of *Russula lepida* Fr. were harvested at the Ailao Mountain in Yunnan Province, China, in July 1998. The botanical identification was made by Prof. *Liu Pei-Gui*, Kunming Institute of Botany, the Chinese Academy of Sciences, where a voucher specimen was deposited.

Extraction and Isolation. The fresh fruiting bodies of *Russula lepida* (dry weight after extraction 475 g) were extracted twice with 95% EtOH, then $\text{CHCl}_3/\text{MeOH}$ 1:1, CHCl_3 (4 \times), and BuOH (4 \times). The CHCl_3 extract (17 g) was fractionated by CC (silica gel (207 g), 200–300 mesh; $\text{CHCl}_3/\text{MeOH}$ 99:1, 99:5, and 90:10) to afford several fractions. The fraction (2.5 g) from $\text{CHCl}_3/\text{MeOH}$ 99:1 was further purified by repeated CC and prep. TLC (petroleum ether/acetone 55:45) to give the pure compound **1** (11 mg).

Lepidamine (1). Pale yellow oil. $[\alpha]_D^{25} = +125$ ($c = 0.36$, CHCl_3). UV (Et_2O): 242.0 (strong), 409.5 (middle and broad), 263.5 (weak). IR (KBr): 3376 and 3260 (NH_2), 2958, 2928, 2874, 1709, 1699, 1681, 1620, 1481, 1457,

1377, 1274, 1201, 1117, 910, 619. ¹H-NMR (CDCl₃): 9.84 (br. s, NH_a), 6.34 (br. s, NH_b); 2.53–2.58 (m, CH₂(3)); 2.42 (m, H–C(4)); 2.20 (d, J = 8.0, H–C(7)); 1.71 (d, J = 8.0, H–C(6)), 1.29 (s, Me(14)), 1.28 (s, Me(12)), 1.25 (s, Me(15)), 1.16 (d, Me(11)). ¹³C-NMR: 195.1 (C(2)); 193.1 (C(8)); 185.8 (C(9)); 147.1 (C(1)); 118.1 (C(10)); 41.1 (C(4)); 40.1 (C(6)); 39.3 (C(7)); 37.1 (C(5)); 36.6 (C(3)); 30.4 (C(15)); 30.1 (C(13)); 25.4 (C(12)); 16.2 (C(14)); 16.0 (C(11)). HR-EI-MS: 262.1453 ([M + H]⁺, C₁₅H₂₀NO₃; calc. 262.1443), 218.1167 ([M – Me – CO]⁺, C₁₃H₁₆NO₂; calc. 218.1181), 149.0224 (C₈H₈O₃; calc. 149.0239). EI-MS: 261 (40, M⁺), 246 (28, [M – Me]⁺), 233 (27, [M – CO]⁺), 218 (100, [M – CO – Me]⁺), 204 (12), 190 (24), 176 (27), 162 (15), 149 (52), 137 (17), 122 (18), 107 (17), 94 (26), 77 (23), 67 (35).

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