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

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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 Wolpert’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*.

**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 the¹³C-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 quartery 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 – CO]$^+$), 218 ([M – CO – CH$_3$]$^+$, base peak), and 149 ([C$_9$H$_6$O$_3$]$^+$). The signals in the $^1$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. $^1$H,$^1$H-COSY and HMOC 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.

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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 CHCl$_3$/MeOH 1:1, CHCl$_3$ (4 x), and BuOH (4 x). The CHCl$_3$ extract (17 g) was fractionated by CC (silica gel (207 g), 200±300 mesh; CHCl$_3$/MeOH 99:1, 99:5, and 90:10) to afford several fractions. The fraction (2.5 g) from CHCl$_3$/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$_2$O): 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,
H-NMR (CDCl₃): 9.84 (br. s, N Hα), 6.34 (br. s, N Hβ); 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)); 1.28 (s, Me(14)); 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/C₁₃₅H/C₁₃₅C 15H₂₀NO₃; calc. 262.1443), 218.1167 ([M/C₀Me/C₀CO/C₁₃₅C 13H₁₆NO₂; calc. 218.1181), 218.1181 (C₈H₅O₃; calc. 218.1181). EI-MS: 261 (40, [M/C₁₃₅]), 246 (28, [M/C₀Me]), 233 (27, [M/C₀CO/C₁₃₅]), 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).

REFERENCES


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