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 *Witthaker*'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 $CHCl_3$/MeOH 1:1 extract of the fruiting bodies of *R. lepida.*

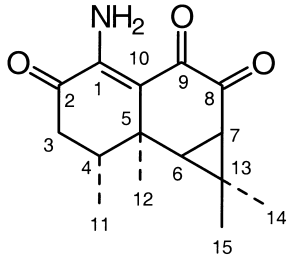

Fig. 1. *Structure of lepidamine* (**1**)

Results and Discussion. – The $CHCl_3$-soluble fraction of the EtOH and $CHCl_3$/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 $C_{15}H_{20}NO_3$ ($[M+H]^+$ 262.1453, calc. for $C_{15}H_{20}NO_3$ 262.1443). Fifteen signals in the ^{13}C-NMR (DEPT) spectra of **1** were recognized ($7\times C$, $3\times CH$, $1\times CH_2$, $4\times CH_3$), 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 ($[C_8H_5O_3]^+$). 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 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 explaination 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.

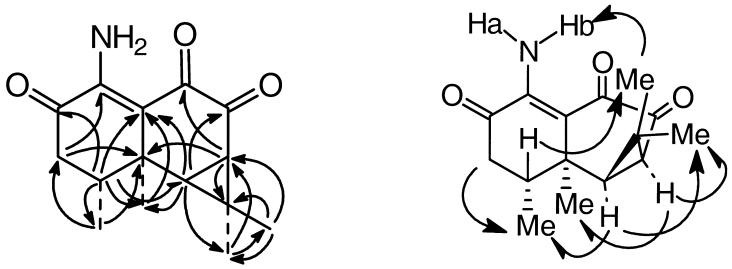

Fig. 2. *Significant HMBC and NOESY correlations of* **1**

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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 $CHCl_3$/MeOH 1:1, $CHCl_3$ (4 ×), and BuOH (4 ×). 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. $[\alpha]_D = +125$ ($c = 0.36$, $CHCl_3$). 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. ^{1}H-NMR ($CDCl_3$): 9.84 (br. *s*, NH_a), 6.34 (br. *s*, NH_b); 2.53–2.58 (*m*, CH_2(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)). ^{13}C-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 ($[M+H]^+$, $C_{15}H_{20}NO_3$; calc. 262.1443), 218.1167 ($[M-Me-CO]^+$, $C_{13}H_{16}NO_2$; calc. 218.1181), 149.0224 ($C_8H_5O_3$; 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).

REFERENCES

[1] R. H. Witthaker, *Science* **1969**, *163*, 160.
[2] G. Vitari, Z. Che, L. Garlaschelli, *Tetrahedron Lett.* **1998**, *39*, 6073.
[3] Tan Jianwen, Dong Zejun, Liu Jikai, *Helv. Chim. Acta* **2000**, *83*, 3191.
[4] Liu Jikai, *Hetereocycles* **2002**, *57*, 157.
[5] Ding Zhihui, Li Jingping, Liu Jikai, Lu Yang, Cheng Wang, Zheng Qitai, *Helv. Chim. Acta* **2002**, *85*, 882.
[6] Liu Jikai, Tan Jianwen, Dong Zejun, Wang Xianhua, Liu Peigui, *Helv. Chim. Acta* **2002**, *85*, 1439.
[7] Hu Lin, Liu Jikai, *Z. Naturforsch.*, **2001**, *56c*, 983.
[8] Hu Lin, Gao Jinming, Liu Jikai, *Helv. Chim. Acta* **2001**, *84*, 3342.
[9] Ding Zhihui, Dong Zejun, Liu Jikai, *Helv. Chim. Acta* **2001**, *84*, 259.
[10] Gao Jinming, Hu Lin, Liu Jikai, *Steroids* **2001**, *66*, 771.
[11] Gao Jinming, Dong Zejun, Liu Jikai, *Lipids* **2001**, *36*, 175.
[12] Gao Jinming, Lin Hu, Dong Zejun, Liu Jikai, *Lipids* **2001**, *36*, 521.

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