

Lepidolide, a Novel *Seco*-ring-A Cucurbitane Triterpenoid from *Russula lepida* (Basidiomycetes)

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A novel compound, lepidolide **1**, was isolated from the fruiting bodies of the Basidiomycete *Russula lepida*. Its structure was established by spectroscopic and chemical means.

Introduction

The Russulaceae family is one of the largest in the subdivision Basidiomycotina in Whitthaker's kingdom of Fungi and comprises hundreds of species (Whitthaker, 1969). 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 (Vitari *et al.*, 1998). Recently, some new terpenoids and ceramides have been reported from *Russula sp.* (Tan *et al.*, 2000; Gao *et al.*, 2000, 2001). Here we report the isolation and structure elucidation of a new triterpenoid (**1**), lepidolide, from EtOH and CHCl₃-CH₃OH (1:1 v/v) extracts of the fruiting bodies of *R. lepida*.

Results and Discussion

The CHCl₃ soluble fraction of the EtOH and CHCl₃/MeOH (1:1 v/v) extract from the fruiting bodies of *R. lepida* was subjected to repeated col-

umn chromatography to afford **1** as a pale yellow oil. Its molecular formula C₃₀H₄₀O₆ was deduced by HR-FABMS (M-H, calcd: 495.2747 for C₃₀H₃₉O₆; found: 495.2762). Its IR absorption at 3245, 1700 cm⁻¹ and ¹³C NMR signals at δ 172.6 (C-26), 178.5 (C-3) ppm show the presence of two carboxylic acid groups which were further confirmed by HR-EIMS of its ethyl ester derivative **2** (M: C₃₄H₄₈O₆, calc: 552.3451; found: 552.3458) and characteristic EIMS ion peaks: *m/z* 552 [M]⁺, 506 [M-CH₃CH₂OH]⁺, 385 [M-side chain]⁺ and 262 [ring D + side chain]⁺. Four olefinic protons were revealed in the ¹H NMR spectrum of **1** at δ 5.54, 5.56, 6.86 and 6.94 ppm (Table I). Evidence for the existence of four double bonds was provided by the presence of the signals in the ¹³C NMR spectrum of **1** [109.0(CH), 122.2(C), 122.7(CH), 125.2(C), 136.9(CH), 145.4(CH), 149.6(C), 149.8(C)], and the six methyl groups were located at δ 0.80(s), 0.91(s), 1.00(s), 1.03(d, 6.4), 2.03(s), 2.04(s) in its ¹H NMR spectrum. All the above evidence and comparison of the data

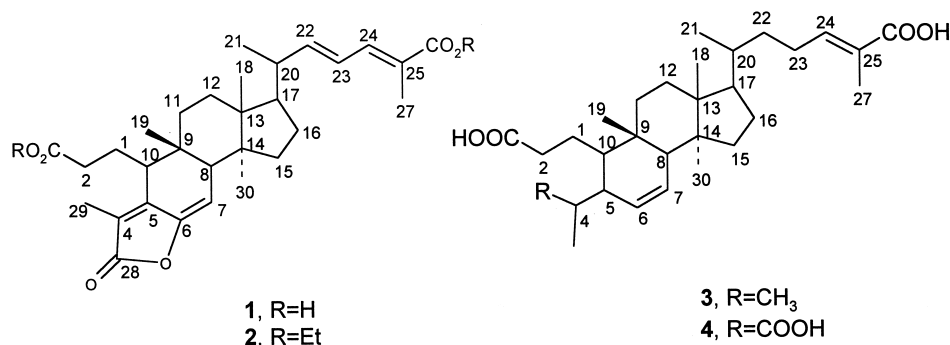


Fig. 1. Structures of **1** and **2**.

Table I. ^1H and ^{13}C -NMR data of **1** and **2** in CDCl_3 (500 MHz).

	1	2	1	2
1	22.5	22.6	2.10, 2.04	2.06, 2.04
2	35.6	36.0	2.65, 2.51	2.60, 2.44
3	178.5	172.6		
4	122.2	122.7		
5	149.6	149.6		
6	149.8	149.9		
7	109.0	108.9	5.54(d, 6.4)	5.53(d, 6.4)
8	48.7	50.0	2.53(d, 6.4)	2.53(d, 6.4)
9	38.5	38.4		
10	40.7	40.9	2.99(bs)	2.87(bs)
11	30.6	30.6	1.95, 1.85	1.92, 1.86
12	30.2	30.2	1.75, 1.59	1.74, 1.60
13	46.1	46.2		
14	50.4	50.3		
15	34.0	34.0	1.30, 1.28	1.27, 1.24
16	27.9	27.8	1.75, 1.29	1.73, 1.28
17	49.9	50.2	1.57	1.58
18	15.6	15.8	1.00(s)	1.01(s)
19	26.5	26.6	0.91(s)	0.92(s)
20	34.6	34.3	2.70(m)	2.76(m)
21	20.5	20.4	1.03(d, 6.4)	1.02(d, 6.4)
22	145.4	143.6	5.56(t, 11.2)	5.49(t, 11.2)
23	122.7	122.5	6.94(t, 11.2)	6.86(t, 11.2)
24	136.9	134.0	6.86(d, 11.2)	6.73(d, 11.2)
25	125.2	126.7		
26	172.9	167.8		
27	20.9	21.1	2.03(s)	2.02(s)
28	171.7	171.6		
29	10.0	10.0	2.04(s)	2.04(s)
30	18.5	18.5	0.80(s)	0.80(s)
31		60.2		4.16(q, 6.8)
32		14.2		1.29(t, 6.8)
33		60.6		4.22(q, 5.6)
34		14.3		1.31(t, 5.6)

with the previous triterpenoid compounds isolated from the same fungus (Tan *et al.*, 2000) together with the aid of 2D NMR (HMQC, COSY and HMBC) of **1** and **2** (Fig. 2) led to structure **1** for compound **1**, which represents a novel *seco*-ring-A cucurbitane triterpenoid. In previous paper the structures of compounds **3** and **4** were reported (Tan *et al.*, 2000). The configuration at C-20 for

compounds **3** and **4** was suggested as 20*R*-stereochemistry since the location of the chemical shift for H-21 in ^1H NMR (Nes *et al.*, 1977; Nes *et al.*, 1991). When we compared the structure of compound **1** with the structures of compounds **3** and **4**, it is quite clear that they belong to the same structure cluster. The big difference is only that a heterocycle was formed between C-28 and C-6 in compound **1**.

Experimental

General

Optical rotations were taken from a JASCO DIP-300 with 1 cm cell. UV spectral: *UV-210* spectrometer, λ_{max} (log ϵ). EI and FAB-MS were carried out with a VG-Auto Spec-3000 spectrometer. IR spectra were recorded with a *Bio-Rad FTS-135* spectrometer with KBr pellets. 1D and 2D-NMR spectra were measured in CDCl_3 with a *Bruker DRX-500* using TMS as internal standard, δ in ppm, *J* in Hz.

Mushroom material

The fruiting bodies of *Russula lepida* Fr. were purchased in the market of Kunming, Yunnan Province in 1999. The botanical identification was made by Prof. Liu Pei-Gui at Kunming Institute of Botany, the Chinese Academy of Sciences, where a voucher specimen was deposited.

Extraction and isolation

The fruiting bodies of *R. lepida* (dry weight 7.0 kg) were extracted three times with CHCl_3 -MeOH (1:1 v/v) and CHCl_3 at room temperature. The residue obtained by removal of the solvent *in vacuo* was partitioned between water and CHCl_3 . The CHCl_3 portion (151 g) was fractionated by silica gel (200–300 mesh) chromatography eluted with CHCl_3 -MeOH (from 100:0 to 60:40) to

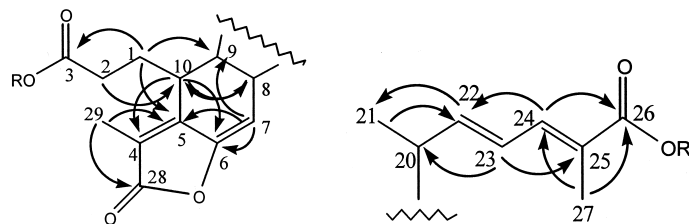


Fig. 2. Selected HMBC Correlations for **1** (R = H) and **2** (R = C_2H_5).

afford several fractions. The fraction (4.5 g) from CHCl₃-CH₃OH (95:5 v/v) was purified by repeated silica gel chromatography and reverse phase chromatography (RP-8) to give 23 mg of compound **1**.

Lepidolide (**1**), pale yellow oil, $[\alpha]_D^{23}$: 166.5° (CHCl₃ 0.1); UV(Et₂O) $\lambda_{\max}(\log \epsilon)$: 278.5 nm (3.86); HR-FABMS m/z : 495.2762 [M-H]⁺, for C₃₀H₄₀O₆-H, (calcd: 495.2747); EI-MS m/z (rel. int): 496 M⁺ (25), 478 [M-H₂O]⁺ (37), 452 [M-CO₂]⁺ (10), 357 [M-C₈H₁₀O₂ (side chain)]⁺ (52), 339 (12), 302 (16), 247 (30), 234 (47), 215 (34), 201 (16) 189 (37), 175 (55), 161 (70), 147 (48), 121 (66), 95 (100); IR ν_{\max} (KBr) cm⁻¹: 3425, 2954, 1761, 1700, 1627, 1457, 1417, 1380; ¹H and ¹³C-NMR see Table I.

15 mg of compound **1** were added into 0.25 ml ice cold SOCl₂ and stirred at room temperature for 30 min. Then, 0.5 ml of absolute EtOH was added to the reaction mixture and stirred at room temperature for 10 min subsequently, 10 ml of ice-cold H₂O was added and then the aqueous solution was extracted with 6 ml AcOEt for three times. The combined AcOEt extracts were then

washed with 8 ml water for two times and evaporated *in vacuo* to dryness. The residue was re-dissolved in 0.5 ml CHCl₃, and the extract subjected to normal silica-gel column chromatography. Elution was performed by CHCl₃/MeOH (99:1 v/v) and eventually 14.6 mg of compound **2** were obtained.

Lepidolide ethyl ester (**2**), pale yellow oil, $[\alpha]_D^{23}$: 115.6 (CHCl₃ 0.1); UV(Et₂O) $\lambda_{\max}(\log \epsilon)$: 276.5 nm (3.88); EI-MS m/z (rel. int): 552 M⁺ (15), 506 [M-EtOH]⁺ (13), 385 [M-C₁₀H₁₄O₂ (side chain)]⁺ (17), 331 (5), 303 (6), 291 (7), 273 (9), 262 (154), 215 (16) 189 (14), 175 (27), 161 (25), 147 (22), 139 (46), 121(35), 95 (100); IR ν_{\max} (KBr) cm⁻¹: 2951, 1764, 1708, 1628, 1460, 1376; ¹H and ¹³C-NMR see Table I.

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- Gao J.-M., Dong Z.-J. and Liu J.-K. (2001), A new ceramide from the Basidiomycete *Russula cyanoxantha*. *Lipids* **36**, 175–180.
- Gao J.-M., Hu L., D. Z.-J. and Liu J.-K. (2001), New glycosphingolipid containing an unusual sphingoid base from the Basidiomycete *Polyporus ellisii*. *Lipids* **36**, 521–527.
- Gao J.-M., Dong Z.-J. and Liu J.-K. (2000), The constituents of the Basidiomycete *Russula cyanoxantha*. *Acta Botanica Yunnanica* **22**, 85–89.
- Nes, W. D., Wong, R. Y., Benson, M. and Akihisa, T. (1991), Conformational Analysis of 10 α -Cucurbitadienol. *J. Chem. Soc. Chem. Commun.* 1272–1274.
- Nes, W. D., Varkey, T. E. and Krevitz, K. (1977), The Stereochemistry of Sterols at C-20 and Its Biosynthetic Implications. *J. Amer. Chem. Soc.* **99**, 260–262.
- Tan J.-W., Dong Z.-J. and Liu J.-K. (2000), New terpenoids from Basidiomycetes *Russula lepida*. *Helv. Chim. Acta* **83**, 3191–3197.
- Vitari, G., Che Z. and Garlaschelli L. (1998), New nardosinane and aristolane sesquiterpenes from the fruiting bodies of *Russula lepida*. *Tetrahedron Lett.* **39**, 6073–6076.
- Whitthaker R. H. (1969), New concepts of kingdoms of organisms. *Science* **163**, 150–160.