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TWO NEW BIOACTIVE DITERPENES FROM *LEPISTA SORDIDA*

XENIA MAZUR, UTA BECKER, TIMM ANKE* and OLOV STERNER*†

Lehrbereich Biotechnologie der Universität Kaiserslautern, Paul Ehrlich-Straße 23, D-67663 Kaiserslautern, Germany; †Division of Organic Chemistry 2, University of Lund, P.O.B. 124, S-221 00 Lund, Sweden

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Key Word Index—*Lepista sordida*; basidiomycete; fermentation; diterpene; lepistal; lepistol; biological activities.

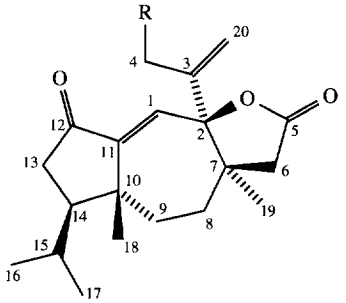
Abstract—In a screening for inducers of the differentiation of human leukaemic cells, two new active diterpenoids were isolated from fermentations of the basidiomycete *Lepista sordida*. The structural elucidation by spectroscopic methods and the biological activities of both metabolites are described. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

The arrest of uncontrolled growth leading to differentiated stages and eventually apoptosis offers an attractive concept for the treatment of leukaemias. During a screening of fungal cultures for new metabolites capable of inducing morphological and physiological differentiation of HL 60 and U 937 cells, the presence of two differentiation-inducers was detected in the ethyl acetate extracts of the culture fluids of the basidiomycete *Lepista sordida* (Fr.) Sing. 92029. In this paper we describe the production, isolation, structural elucidation and biological activity of these metabolites which we have named lepistal and lepistol.

RESULTS AND DISCUSSION

The fermentation of the fungus and isolation of the two active compounds is described in the experimental section. The structures of lepistal (**1**) and lepistol (**2**) were determined by spectroscopy. HR-mass spectra as well as ^1H and ^{13}C NMR spectra (NMR data given in Tables 1 and 2) established the elemental composition to be $C_{20}H_{26}O_4$ for lepistal (**1**) and $C_{20}H_{28}O_4$ for lepistol (**2**). 2D heteronuclear correlation NMR spectroscopy was used to elucidate the basic structures and NOESY correlations determined the relative stereochemistry. Pertinent HMBC and NOESY correlations observed with lepistal (**1**) are summarized in Fig. 1, the corresponding correlations were observed also with lepistol (**2**). Especially important are the correlations from H_2-6 and H_3-19 which show that both C-6 and C-19 are attached to the same quaternary carbon (C-7), and from H_3-18 which placed the five- and seven-membered rings together. The diterpene skeleton of



1, R = =O
2, R = —OH

Table 1. ^1H (500 MHz) NMR data (δ, mult., J) for lepistal (**1**) and lepistol (**2**)

H	1	2
1	6.58 *s*	6.55 *s*
4a	9.66 *s*	4.17 *d*; 13.2
4b	—	4.14 *d*; 13.2
6a	2.48 *d*; 17.7	2.68 *d*; 17.5
6b	2.34 *d*; 17.7	2.29 *d*; 17.5
8a	1.74 *m*	1.56 *m*
8b	1.60 *dd*; 6.7, 15.0	1.60 *m*
9a	2.08 *dd*; 8.0, 15.0	2.02 *dd*; 6.3, 13.4
9b	1.65 *m*	1.64 *m*
13a	2.46 *dd*; 7.9, 18.8	2.42 *dd*; 7.8, 18.8
13b	2.14 *dd*; 12.6, 18.8	2.13 *dd*; 12.8,18.8
14	1.68 *m*	1.60 *m*
15	1.82 *dqq*; 6.6, 6.6, 6.6	1.79 *dqq*; 6.6, 6.6, 6.6
16	1.04 *d*; 6.6	1.02 *d*; 6.6
17	0.95 *d*; 6.6	0.92 *d*; 6.6
18	1.17 *s*	1.16 *s*
19	1.00 *s*	1.05 *s*
20*Z*	6.33 *s*	5.57 *s*
20*E*	6.55 *s*	5.23 *s*

*Authors to whom correspondence should be addressed.

Table 2. ^{13}C (125 MHz) NMR data (δ, mult.) for lepistal (**1**) and lepistol (**2**)

C	1	2	C	1	2
1	127.3 *d*	129.3 *d*	11	151.0 *s*	151.8 *s*
2	89.3 *s*	90.9 *s*	12	205.9 *s*	206.2 *s*
3	149.2 *s*	148.4 *s*	13	39.4 *t*	39.6 *t*
4	192.0 *d*	63.0 *t*	14	48.7 *d*	48.8 *d*
5	175.7 *s*	176.6 *s*	15	28.4 *d*	28.3 *d*
6	40.9 *t*	41.2 *t*	16	24.1 *q*	24.0 *q*
7	45.9 *s*	45.6 *s*	17	21.8 *q*	21.8 *q*
8	36.8 *t*	37.4 *t*	18	21.8 *q*	21.6 *q*
9	30.9 *t*	31.0 *t*	19	25.8 *q*	24.9 *q*
10	46.4 *s*	46.4 *s*	20	137.6 *t*	116.6 *t*

lepistal (**1**) and lepistol (**2**), called trichoaurantiane [1], was unknown until recently when similar diterpenes were reported from *Tricholoma aurantium* [1–3] and *T. fracticum* [3]. These all contain a hydroxy or acetoxy group in position 8, and lepistal (**1**) is in fact trichoaurantianolide A [1] lacking the 8-acetoxy group while lepistol (**2**) is deoxytrichoaurantianolide B [2] (or deoxytrichaurantin [3]). The NMR data of lepistal (**1**) and lepistol (**2**) are in agreement with those reported for the related compounds [1–3], except of course for C-8/8-H. It should be noted that both *Tricholoma* and *Lepista* belong to the family Tricholomataceae (Agaricales).

At a concentration of 0.2 μg ml^{-1} lepistal induces the differentiation of 20% of the HL-60 cells into granulocyte-monocyte-like cells and a differentiation of 18% of the U 937 cells into the monocyte-like cells. Lepistol induces differentiation of 30% of the HL-60 cells and 14% of the U 937 cells at concentrations of 20 μg ml^{-1} and 10 μg ml^{-1}, respectively. Cytotoxic activity (lysis of cells) of **1** and **2** were observed at 1 μg ml^{-1} and 50 μg ml^{-1}, respectively. Lepistal (**1**) exhibits antibacterial and antifungal activities (minimal inhibitory concentrations in μg ml^{-1}: *Streptomyces* sp. ATCC 23836, 5; *Bacillus subtilis* 50; *Rhodotorula glutinis*, 5; *Saccharomyces cerevisiae* S288c, *Penicillium notatum*, *Fusarium oxysporum*, 19; *Mucor miehei*, *Nematospora coryli*, 50). No antibacterial and only weak antifungal activities (*Nematospora coryli* and *Rhodotorula glutinis*) were observed for lepistol (**2**) at concentrations of 100 μg ml^{-1}. Therefore the aldehyde function of **1** is considered to contribute substantially to the biological activities of lepistal.

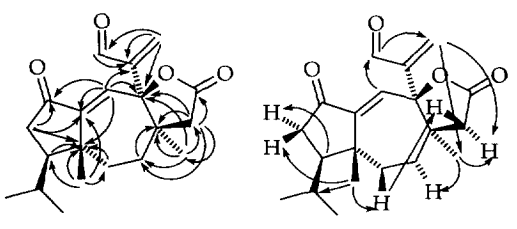


Fig. 1. HMBC (left) and NOESY (right) correlations observed with lepistal (**1**). The corresponding correlations were observed with lepistol (**2**).

EXPERIMENTAL

Fermentation and isolation. Lepista sordida strain 92029 was isolated from the spore print of a fruiting body collected in southern France. The specimen showed the characteristics of the genus [4] and, except for the smaller size, the species [5]. A Herbarium specimen and mycelial cultures are deposited in the culture collection of the LB Biotechnologie, Kaiserslautern. For maintenance on agar slants the fungus was grown on YMG medium (yeast extract 0.4%, malt extract 1.0%, glucose 0.4%, pH 5.5). Fermentations were carried out in 20 l of YMG medium in a Biostat U fermentor with stirring (120 rmp) and aeration (2 l air min^{-1}) 22°. After 200 hr of fermentation when the antifungal activity in the culture fluid had reached its maximum, the Mycelia were separated from the culture fluid (17 l) by filtration and discarded. Lepistal and lepistol were adsorbed from the culture fluid on to HP 21 resin (Mitsubishi) and eluted with Me_2CO. The crude extract (2.0 g) was applied to a column (25 × 2.5 cm) containing silica gel (0.063–0.2 mesh, Merck 60). **1** was obtained by elution with cyclohexane–EtOAc (7:3) and **2** was obtained by elution with cyclohexane–EtOAc (1:1). Yields: 7.5 mg of lepistal (**1**) and 6.8 mg of lepistol (**2**).

Spectroscopy. ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz): room temp., $CDCl_3$, Bruker ARX 500 spectrometer with an inverse 5 mm probe equipped with a shielded gradient coil. COSY, HMQC and HMBC experiments were performed with gradient enhancements using sine shaped gradient pulses, and for the 2D heteronuclear correlation spectroscopy the refocusing delays were optimised for $^1J_{CH} = 145$ Hz and $^2J_{CH} = 10$ Hz. The raw data were transformed and the spectra were evaluated with the standard Bruker UXNMR software (ref. 941001). The chemical shifts are given in ppm (with the solvent peaks at 7.26 and 77.0 ppm serving as reference) and the coupling constants J in Hz. EI-MS: 70 eV.

Lepistal (**1**). Oil, $[\alpha]_D$ +37° (*c* 0.4 in $CHCL_3$). UV λ_{max}^{MeOH} nm (e): 246 (9600); IR ν_{max}^{KBr} cm^{-1}: 3348, 2985, 2964, 2944, 2919, 2858, 1793, 1783, 1715, 1695, 1643, 1617, 1457, 1437, 1413, 1377, 1368, 1294, 1289, 1275, 1246, 1205, 1182, 1130, 1104, 1046, 1030, 1010, 997, 987, 966, 927, 896, 832; NMR: Tables 1 and 2; MS: *m/z* (rel. int.): 330.1844 $[M]^+$ (85) ($C_{20}H_{26}O_4$ requires 330.1831), 301 (35), 287 (28), 271 (33), 269 (32), 232 (30), 191 (32), 164 (100).

Lepistol (**2**). Oil, $[\alpha]_D$ +73° (*c* 0.3 in $CHCl_3$). UV λ_{max}^{MeOH} nm (e): 246 nm (7900); IR ν_{max}^{KBr} cm^{-1}: 3477, 2965, 1780, 1714, 1635, 1457, 1410, 1378, 1287, 1248, 1206, 1182, 1170, 1128, 1017, 996, 964, 928, 895; NMR: Tables 1 and 2; MS: *m/z* (rel. int.) 332.1973 $[M]^+$ (49) ($C_{20}H_{28}O_4$ requires 332.1987), 317 (17), 314 (12), 271 (20), 216 (45), 167 (23), 165 (18), 139 (100).

Biological assays. The antimicrobial, cytotoxic, haemolytic activities were assayed according to ref. [6]. The induction of morphological and physiological differentiation of HL-60 cells (ATCC CCL 240, human promyelocytic leukaemia) and U 937 cells (ATCC CRL

151, human histiocytic lymphoma) was assayed by NBT reduction and by counting of the blue stained differentiated cells as described previously [7].

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