Taylor & Francis
Taylor & Francis Group

Natural Product Research
Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: https://www.tandfonline.com/loi/gnpl20

Microporotriol, a new cadinane-type sesquiterpenoid from the cultures of the wood-decay fungus *Microporus affinis* HFG829

Zhen-Zhu Zhao, Ji-Kai Liu & He-Ping Chen

To cite this article: Zhen-Zhu Zhao, Ji-Kai Liu & He-Ping Chen (2019): Microporotriol, a new cadinane-type sesquiterpenoid from the cultures of the wood-decay fungus *Microporus affinis* HFG829, Natural Product Research

To link to this article: https://doi.org/10.1080/14786419.2019.1582038

NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2019.1582038

Taylor & Francis
Taylor & Francis Group

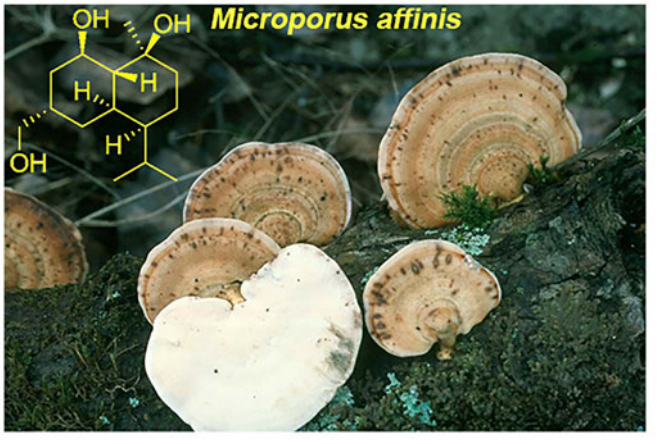
Microporotriol, a new cadinane-type sesquiterpenoid from the cultures of the wood-decay fungus *Microporus affinis* HFG829

Zhen-Zhu Zhao[a,b], Ji-Kai Liu[c,d] and He-Ping Chen[c,d]

[a]School of Pharmacy, Henan University of Chinese Medicine, Zhengzhou, People's Republic of China; [b]Collaborative Innovation Center for Respiratory Disease Diagnosis and Treatment & Chinese Medicine Development of Henan Province, Henan University of Chinese Medicine, Zhengzhou, People's Republic of China; [c]School of Pharmaceutical Sciences, South-Central University for Nationalities, Wuhan, People's Republic of China; [d]State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, People's Republic of China

ABSTRACT

A new cadinane-type sesquiterpenoid, microporotriol (**1**), together with four known compound, 5-methylresorcinol (**2**), (22*E*,24*R*)-ergosta-4,6,8(14),22-tetraen-3-one (**3**), (22*E*,24*R*)-ergosta-5,7,22-trien-3β-ol (**4**), (22*E*,24*R*)-5α,8α-epidioxy-ergosta-6,22-dien-3β-ol (**5**), were isolated from the fermentation broth of the wood decaying fungus *Microporus affinis* HFG829. The structures of the compounds were established by extensive spectroscopic methods, including 1D & 2D NMR, along with HRMS spectroscopic analysis. The relative configuration of **1** was confirmed by NMR calculation. Compound **1** was evaluated for the cytotoxicity against five human cancer cell lines.



ARTICLE HISTORY
Received 13 November 2018
Accepted 5 February 2019

KEYWORDS
Microporus affinis HFG829; polyporaceae; secondary metabolite; cadinane; sesquiterpenoid

CONTACT He-Ping Chen chenhp@mail.scuec.edu.cn

Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2019.1582038.

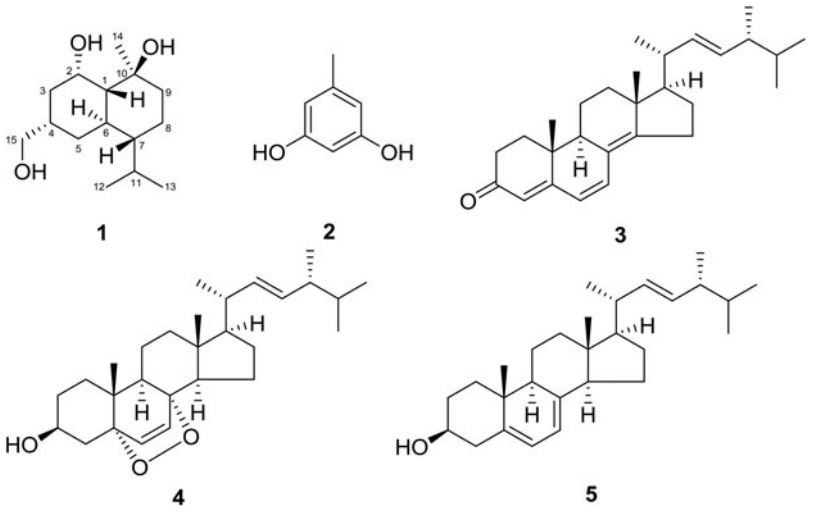
1. Introduction

Secondary metabolites from fungi are of medical, industrial and/or agricultural importance which have attracted, and continue to attract much attention from the scientific community (Schueffler and Anke 2014; Chen and Liu 2017). Fungal secondary metabolites are proved to be associate with fungal sporulation processes and development (Calvo et al. 2002), for example, zearalenone from *Fusarium graminearum* played an inducing role in sporulation and an enhancing role in perithecial formation (Wolf and Mirocha 1973), and butyrolactone I from *Aspergillus terreus* induces the sporulation and increases the production of lovastatin (Schimmel et al. 1998).

The wood-decay fungi is a class of fungi possessing the ability to produce laccases that digest moist woods and then cause them to rot. This process triggers the degradation of complex molecules to return nutrients to the soil in nature (Riley et al. 2014). However, sometimes this kind of fungi also can serve as the role of pathogenic factor for forest and mushroom-cultivation industry. The special roles of wood-decay fungi make them of great importance to be chemically investigated. The fungus *Microporus affinis* falls into the family of Polyporaceae, and always grows on the fallen woods in tropical and subtropical regions (Bi et al. 1993). However, this fungus has never been chemically studied. In an ongoing program to explore biological active substances from fungi, the culture broth of *M. affinis* HFG829 was investigated, which led to the isolation of a new cadinane-type sesquiterpenoid, together with four known compounds. Cadinane-type sesquiterpenoid is quite common as fungal metabolites, such as (+)-10α-hydroxy-4-muurolen-3-one isolated from *Favolaschia* sp. 87129 with inhibition of leukotriene biosynthesis (Zapf et al. 1996); stereumins C and D exhibiting potent nematicidal activities against *Panagrellus redivivus* (Li et al. 2008). We, herein, report the isolation, structure elucidation, and biological evaluation of the new compound.

2. Results and discussion

Compound **1** was isolated as a colourless oil. The HREIMS spectrum of **1** showed an ion peak at *m/z* 256.2038 $[M]^+$, corresponding to the molecular formula of $C_{15}H_{28}O_3$ (calcd for $C_{15}H_{28}O_3$, 256.2038). Compound **1** could not be detected by the UV detector during the separation process implying the absence of any chromophores, like the carbonyl or double bond. The ^{13}C NMR and DEPT data (Table S1) presented carbon resonances which were classified into three methyls, five methylenes (one oxygenated), six methines (one oxygenated), and one oxygenated quaternary carbons. The planar structure of **1** was established by analysis of the HSQC, HMBC, and ^1H-^1H COSY spectra. The ^1H-^1H COSY spectrum confirmed the presences of a six-membered ring (C_1−C_2−C_3−C_4−C_5−C_6) with a hydroxy group at C-2 and a moiety (C_6–C_7–C_8–C_9) which kept accordance with correlations of H-1−H-2−H_2-3−H-4−H_2-5−H-6−H-1 and H-6–H-7–H_2-8–H_2-9. The HMBC correlations from H-14 (δ_H 1.22, 3H) and 10-OH (δ_H 4.82) to C-1 (δ_C 57.8), C-10 (δ_C 73.8), and C-9 (δ_C 42.6) enabled the establishment of another six-membered ring (C_1-C_6–C_7–C_8–C_9–C_{10}), of which C-10 is substituted by a methyl and a hydroxy group. The aforementioned spectroscopic evidence revealed the existence of a decahydronaphthalene skeleton. Besides, shared HMBC correlations of



1 2 3 4 5

Figure 1. Structures of compounds **1**–**5**.

H-12 (δ_H 0.89, 3H) and H-13 (δ_H 0.71, 3H) to C-11 (δ_C 27.2)/C-7 (δ_C 48.7) suggested that an isopropanyl group attached to C-7. The ^{1}H-^{1}H COSY correlations between H-4 (δ_H 1.97)/H-15 (δ_H 3.52, 2H)/15-OH (δ_H 3.60) indicated that a hydromethyl group is connected to C-4. Thus, the planar structure of **1** was established as shown in Figure 1.

The relative configuration of **1** was determined by the ROESY spectrum. The close chemical shifts of H-1 (δ_H 1.16) and H-6 (δ_H 1.19) made it impossible to directly determine the fused pattern of the two six-membered rings. The two rings were determined to be *trans*-fused by the ROESY correlations of H-1/H-3β/H-5β, and H-5α/H-6 (Figure S2), which was further defined by coupling constant $J_{1,6}$ = 11.4 Hz indicative of $J_{ax\text{-}ax}$ in cyclohexane. Furthermore, the ROESY correlations of H-15/H-2/H-6, H-2/H$_3$-14, and H-11/H-6/H$_3$-12 suggested that both 2-OH, 10-OH and H-7 are β oriented, while the 15-CH$_2$OH are α oriented. Therefore, the relative configuration of **1** was established as 1*R**,2*R**,4*S**,6*R**,7*R**,10*S**.

Computational methods have accelerated the structural determination of natural products (Lodewyk et al. 2012). NMR calculation showed superiority over traditional methods in discriminating stereoisomers of natural products (Micco et al. 2010; Grimblat and Sarotti 2016). Due to the ^{1}H NMR of most of the terpenoids always presented overlapping signals which posed challenges in determining the relative configuration only by the ROESY spectrum. Therefore, the relative configuration of **1** was confirmed by NMR calculation. A conformation search of **1** by MMFF94s force field gave 20 conformers with population higher than 1% (Goto and Osawa 1989, 1993). All these conformers were optimized at B3LYP/6-31G(d) level of theory and were further subjected to NMR calculation on PCM-B972/pcSseg-1 level of theory in acetone on Gaussian 09 program (Frisch et al. 2010). As shown in Figure 2, the results showed that the correlation coefficient (R^2) between the calculated and experimental data from linear regression analysis was 0.9945. There was only one outlier higher than 3 ppm between the calculated and experimental data ($\Delta\delta = 3.1$ (C-6)), the mean absolute deviation (MAD) and the root-mean-square deviation (RMSD) were 0.47 and

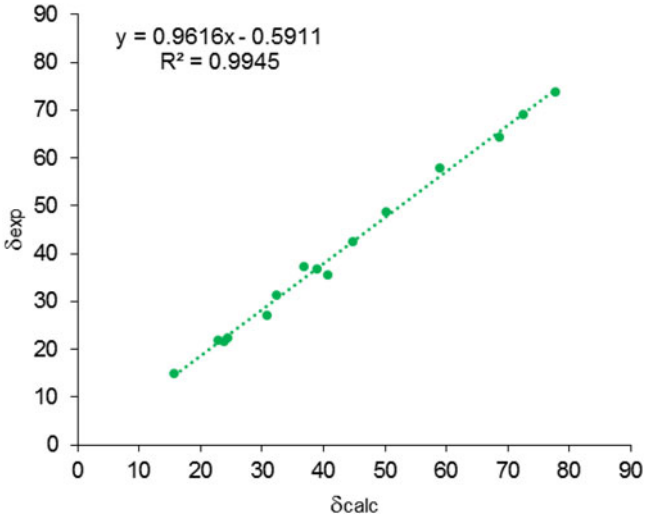


Figure 2. Regression analysis of experimental vs. calculated ^{13}C NMR chemical shifts of **1** at B972/pcSseg-1 level; linear fitting was shown as line.

0.93 ppm, respectively (Supplementary material). Thus, the NMR calculation results supported the conclusion of the ROESY spectrum in assigning the relative configuration of **1**.

To establish the absolute configuration of **1**, the specific rotations calculations on possible structure of (1*R*,2*R*,4*S*,6*R*,7*R*,10*S*)-**1** was calculated at b3lyp/6-311g(d,p) level of theory with PCM model in methanol based on previous B3LYP/6-31G(d)-optimized geometries on Gaussian 09 program. As a result, the calculated specific rotation was + 67.7°, while the experimental value is + 17.5°. Although the calculated specific rotation and the experimental one showed same sign, however, it was insufficient to determine the absolute configuration not only by the large magnitude discrepancy, but also by the possible racemic nature of terpenoids (Finefield et al. 2012). In order to verify if compound **1** was optically pure or not, we should plan to carry out chiral HPLC analysis of **1**. However, the shortage of sample as well as the absence of any chromophores hampered the plan.

In conclusion, compound **1** was determined as a cadinane-type sesquiterpenoid substituted by three hydroxy groups while devoid of any chromophores. Finally, compound **1** was trivially named as microporotriol.

A benzene derivative 5-methylresorcinol (**2**) (Monde et al. 1998), three ergostane derivatives, (22*E*,24*R*)-ergosta-4,6,8(14),22-tetraen-3-one (**3**), (22*E*,24*R*)-ergosta-5,7,22-trien-3β-ol (**4**) (Yang et al. 2010), (22*E*,24*R*)-5α,8α-epidioxy-ergosta-6,22-dien-3β-ol (**5**) (Kobori et al. 2006), were encountered in this research. Their structures were determined by comparison of the NMR data with those of reported ones in the literature.

Compound **1** was evaluated for its cytotoxicity against the five human cancer cell lines, human myeloid leukaemia cell line (HL-60), human hepatocellular carcinoma cell line (SMMC-7721), lung cancer cell line (A549), breast cancer cell line (MCF-7), and human colon cancer (SW-480). However, compound **1** displayed no significant cytotoxicity against the five human cancer cell lines.

3. Experimental

3.1. General experimental procedures

Optical rotations were obtained on a JASCO P-1020 digital polarimeter (Horiba, Kyoto, Japan). IR spectra were obtained using a Bruker Tensor 27 FT-IR spectrometer (Bruker Optics, Inc., Billerica, MA) with KBr pellets. 1D and 2D NMR spectra were obtained on a Bruker Avance III 600 MHz spectrometer (Bruker Corporation, Karlsruhe, Germany). HREIMS was measured on Waters Xevo TQ-S and Waters Autospec Premier P776 mass spectrometers (Waters, Milford, MA, USA). Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden) and silica gel (Qingdao Haiyang Chemical Co., Ltd) were used for column chromatography (CC).

3.2. Fungal material

The fungus *M. affinis* HFG829 was collected from Mang Mountain National Forest Park, Chenzhou City, Hunan Province in 2006. The strain of *M. affinis* HFG829 in this study was isolated from the fresh fruiting bodies and kept on potato-dextrose-agar (PDA) culture medium. The strain was identified by Prof. Yu-cheng Dai, who is a mushroom specialist of Beijing Forestry University. A voucher specimen (No. Dai2006_2) was deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences. The culture medium to ferment this fungus consisted of glucose (5%), peptone from porcine meat (0.15%), yeast powder (0.5%), KH_2PO_4 (0.05%), and $MgSO_4$ (0.05%). Sixty Erlenmeyer flasks (500 ml) each containing 350 mL of above-mentioned culture medium were inoculated with *M. affinis* HFG829 strains, respectively. Fermentation were carried out on rotatory shakers at 25 °C and 150 rpm for 25 days in dark environment.

3.3. Extraction and isolation

The culture broth (20 L) of *M. affinis* HFG829 was filtered and concentrated to 3 liters followed by partitioned between EtOAc and water four times to give an EtOAc layer. Meanwhile, the mycelia were extracted with EtOH (95%) for three times. The EtOAc layer together with the mycelium extract were concentrated under reduced pressure to afford a crude extract (8.0 g). This residue was separated by silica gel CC eluting with $CHCl_3$/MeOH (0:100→100:0) to give ten main fractions (A–J).

Subfraction C was separated by repeated silica gel CC, and further purified by Sephadex LH-20 CC (acetone), and then eluted with petroleum ether/ethyl acetate (80:20→0:100) to yield compounds **3** (2.0 mg), **4** (2.8 mg), and **5** (1.0 mg).

Subfraction F was separated by Sephadex LH-20 CC (acetone) to obtain three subfraction (F1–F3). Subfraction F3 was purified by silica gel CC eluting with petroleum ether/acetone (50:50→0:100) to give compound **2** (5.0 mg).

Subfraction G was purified by silica gel CC eluting with petroleum ether/acetone (40:60→0:100) to afford compound **1** (4.0 mg).

3.4. Microporotriol (1)

Colourless oil; $C_{15}H_{28}O_3$; [α]14D + 17.5° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3422, 2956, 2927, 2856, 1631, 1460, 1384, 1172, 1130, 1047 cm^{-1}; HREIMS **1**: *m/z* 256.2038 $[M]^+$ (calcd for $C_{15}H_{28}O_3$, 256.2038). ^1H NMR (600 MHz, acetone-d_6) data: δ_H 1.16 (dd, *J* = 11.4, 11.4 Hz, H-1), 3.81 (br. ddd, *J* = 11.4, 11.4, 5.7 Hz, H-2), 1.43 (ddd, *J* = 12.3, 12.3, 5.7 Hz, H-3β), 1.99 (overlapped, H-3α), 1.97 (overlapped, H-4), 0.95 (ddd, *J* = 13.5, 11.5, 5.2 Hz, H-5β), 2.04 (overlapped, H-5α), 1.19 (overlapped, H-6), 1.01 (dddd, *J* = 13.0, 13.0, 3.4, 3.4 Hz, H-7), 1.52 (dddd, *J* = 13.0, 3.0, 3.0, 3.0 Hz, H-8β), 1.05 (dddd, *J* = 13.0, 13.0, 13.0, 3.0 Hz, H-8α), 1.47 (ddd, *J* = 12.7, 12.7, 3.6 Hz, H-9β), 1.66 (ddd, *J* = 12.7, 3.0, 3.0 Hz, 9α), 1.99 (m, H-11), 0.89 (d, *J* = 7.2 Hz, H-12), 0.71 (d, *J* = 7.2 Hz, H-13), 1.22 (s, H-14), 3.52 (m, H-15), 4.88 (br. s, 2-OH), 4.82 (br. s, 10-OH), 3.60 (t, *J* = 4.8 Hz, 15-OH) (Table S1); ^{13}C NMR (150 MHz, acetone-d_6) data: δ_C 57.8 (CH, C-1), 69.2 (CH, C-2), 37.4 (CH_2, C-3), 36.9 (CH, C-4), 31.4 (CH_2, C-5), 35.5 (CH, C-6), 48.7 (CH, C-7), 21.7 (CH_2, C-8), 42.6 (CH_2, C-9), 73.8 (C, C-10), 27.2 (CH, C-11), 21.9 (CH_3, C-12), 15.0 (CH_3, C-13), 22.4 (CH_3, C-14), 64.4 (CH_2, C-15) (Table S2).

3.5. Cytotoxicity assay

Compound **1** was evaluated for cytotoxicity against five human cancer cell lines by MTS (an analogue of MTT) method. The five human cancer cell lines included human myeloid leukaemia cell line (HL-60), human hepatocellular carcinoma cell line (SMMC-7721), lung cancer cell line (A549), breast cancer cell line (MCF-7), and human colon cancer (SW-480). The cell line SMMC-7721 was bought from China Infrastructure of Cell Line Resources (Beijing, China), and the remaining others from American Type Culture Collection (ATCC, Manassas, VA, USA). In these tests, DDP and taxol were used as the positive controls. Each tumour cell line was exposed to the test compound at a concentration of 40 μM in triplicates for 48 h.

4. Conclusions

In conclusion, the secondary metabolites of the culture broth of the wood decaying fungus *Microporus affinis* have been investigated for the first time. A new cadinane-type sesquiterpenoid, along with four known compounds are reported in this research. Compound **1** was devoid of cytotoxicity against the five human cancer cell lines.

Acknowledgements

We thank Analytical & Measuring Center, School of Pharmaceutical Sciences, South-Central University for Nationalities for MS and NMR spectra tests.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was financially supported by the State Key Laboratory of Phytochemistry and Plant Resources in West China (No. P2017-KF01), National Natural Science Foundation of China (No. 81773590), and National Key Technologies R&D Program of China (2017YFC1704007).

References

Bi Z, Zheng G, Li T. 1993. The macrofungus flora of China's Guangdong Province. Hong Kong: The Chinese University Press.

Calvo AM, Wilson RA, Bok JW, Keller NP. 2002. Relationship between secondary metabolism and fungal development. Microbiol Mol Biol Rev. 66(3):447–459.

Chen HP, Liu JK. 2017. Secondary metabolites from higher fungi. Prog Chem Org Nat Prod. 106: 1–201.

Finefield JM, Sherman DH, Kreitman M, Williams RM. 2012. Enantiomeric natural products: Occurrence and Biogenesis. Angew Chem Int Ed Engl. 51(20):4802–4836.

Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Scalmani G, Barone V, Mennucci B, Petersson GA, et al. 2010. Gaussian 09, revision C.01. Wallingford, CT: Gaussian, Inc.

Goto H, Osawa E. 1989. Corner flapping: a simple and fast algorithm for exhaustive generation of ring conformations. J Am Chem Soc. 111(24):8950–8951.

Goto H, Osawa E. 1993. An efficient algorithm for searching low-energy conformers of cyclic and acyclic molecules. J Chem Soc Perkin Trans. 2:187–198.

Grimblat N, Sarotti AM. 2016. Computational chemistry to the rescue: Modern toolboxes for the assignment of complex molecules by GIAO NMR calculations. Chem Eur J. 22(35): 12246–12261.

Kobori M, Yoshida M, Ohnishi-Kameyama M, Takei T, Shinmoto H. 2006. 5alpha,8alpha-Epidioxy-22E-ergosta-6,9(11),22-trien-3beta-ol from an edible mushroom suppresses growth of HL60 leukemia and HT29 colon adenocarcinoma cells. Biol Pharm Bull. 29(4):755–759.

Li GH, Duan M, Yu ZF, Li L, Dong JY, Wang XB, Guo JW, Huang R, Wang M, Zhang KQ. 2008. Stereumin A-E, sesquiterpenoids from the fungus *Stereum* sp. CCTCC AF 207024. Phytochemistry. 69(6):1439–1445.

Lodewyk MW, Siebert MR, Tantillo DJ. 2012. Computational prediction of ^{1}H and ^{13}C chemical shifts: A useful tool for natural product, mechanistic, and synthetic organic chemistry. Chem Rev. 112(3):1839–1862.

Micco SD, Chini MG, Riccio R, Bifulco G. 2010. Quantum mechanical calculation of NMR parameters in the stereostructural determination of natural products. Eur J Org Chem. 2010: 1411–1434.

Monde K, Satoh H, Nakamura M, Tamura M, Takasugi M. 1998. Organochlorine compounds from a terrestrial higher plant: Structures and origin of chlorinated orcinol derivatives from diseased bulbs of *Lilium maximowiczii*. J Nat Prod. 61(7):913–921.

Riley R, Salamov AA, Brown DW, Nagy LG, Floudas D, Held BW, Levasseur A, Lombard V, Morin E, Otillar R. 2014. Extensive sampling of basidiomycete genomes demonstrates inadequacy of the white-rot/brown-rot paradigm for wood decay fungi. Proc Natl Acad Sci USA. 111: 14959–14959.

Schimmel TG, Coffman AD, Parsons SJ. 1998. Effect of butyrolactone I on the producing fungus, *Aspergillus terreus*. Appl Environ Microbiol. 64(10):3707–3712.

Schueffler A, Anke T. 2014. Fungal natural products in research and development. Nat Prod Rep. 31(10):1425–1448.

Wolf JC, Mirocha CJ. 1973. Regulation of sexual reproduction in *Gibberella zeae* (*Fusarium roxeum* "*graminearum*") by F-2 (Zearalenone). Can J Microbiol. 19(6):725–734.

Yang XL, Zhu YC, Fang ST, Jiang MY. 2010. Chemical constitutes of *Termitomyces schimperi* collected from Africa. Nat Prod Res Dev. 22:972–975.

Zapf S, Wunder A, Anke T, Klostermeyer D, Steglich W, Shan R, Sterner O, Scheuer W. 1996. (+)-10 alpha-Hydroxy-4-muurolen-3-one, a new inhibitor of leukotriene biosynthesis from a Favolaschia species. Comparison with other sesquiterpenes. Z Naturforsch C, J Biosci. 51(7–8): 487–492.