Published in final edited form as:

Nat Prod Res. 2013 September ; 27(17): 1562–1568. doi:10.1080/14786419.2012.738206.

New compounds and secondary metabolites from bioactive extracts of the fungus *Armillaria tabescens*

H.M.T. Bandara Herath^a, Melissa Jacob^a, A. Dan Wilson^b, Hamed K. Abbas^c, and N. P. Dhammika Nanayakkara^{a,*}

^aNational Center for Natural Products Research, Research Center for Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, MS 38677

^bCenter for Bottomland Hardwoods Research, USDA-Forest Service, Southern Hardwood Laboratory, Stoneville, MS 38776

^cCrop genetics and Production Research Unit, USDA-ARS, Stoneville, MS 38776

Abstract

Ethyl acetate extracts of *Armillaria tabescens* (strain JNB-OZ344) showed significant fungistatic and bacteristatic activities against several major human pathogens including *Candida albicans*, *Cryptococcus neoformans*, *Escherichia coli* and *Mycobacterium intracellulare*. Chemical analysis of these extracts led to the isolation and identification of four new compounds emestrin-F (**1**), emestrin-G (**2**), 6-O-(4-O-methyl- β -D-glucopyranosyl)-8-hydroxy-2,7-dimethyl-4*H*-benzopyran-4-one (**3**) and cephalosporolide-J (**4**) along with five other previously known compounds, emestrin (**5**), cephalosporolide-E (**6**), decarestrictine-C₂ (**7**), ergosterol and brassicasterol. Structural elucidation of all compounds was carried out by NMR and MS analysis. Antimicrobial assays revealed that compounds **1** and **5** were responsible for the observed growth-inhibitory activities of the fungal extracts against the human pathogens tested.

Keywords

Armillaria tabescens; emestrin; chromone; cephalosporolide; antimicrobial activity

1. Introduction

Armillaria tabescens is a major fungal pathogen known to cause root decay and mortality in numerous woody plant species in the southern United States, including many commercially important hardwood tree species within natural forest stands, timberlands, plantations, and urban landscapes (Wilson, Leininger, Otrrosina, Dwinell, & Schiff, 2004; Baietto, & Wilson, 2010). At least twenty species of *Armillaria* are recognized worldwide with virulence varying between species and individual strains of each species (Shaw, & Kile, 1991).

A series of biologically active protoilludane sesquiterpene aryl esters have been isolated previously from different strains of *A. tabescens* (Donnelly, Konishi, Dunne, & Cremin, 1997).

In our investigations of bioactive secondary metabolites from wood decay fungi, we found that the ethyl acetate extracts of *A. tabescens*, cultured on boiled brown rice grains, showed antimicrobial activity against the fungi *Candida albicans*, *Cryptococcus neoformans* and the

*Corresponding author. dhammika@olemiss.edu.

bacteria *Escherichia coli* and *Mycobacterium intracellulare*. Chemical analysis of these extracts led to the isolation and identification of emestrin (**5**) (Seya, Nozawa, Nakajima, Kawai, & Udagawa, 1986) and two of its new closely related analogs, emestrin-F (**1**) and emestrin-G (**2**), as the compounds responsible for this activity. In addition to these compounds, we also isolated a new chromone glycoside, [6-O-(4-O-methyl- β -D-glucopyranosyl)-8-hydroxy-2,7-dimethyl-4*H*-benzopyran-4-one] (**3**) and a new diastereomer of the cephalosporolide series, cephalosporolide-J (**4**) along with four other known compounds, cephalosporolide-E (**6**) (Ackland, Hanson, Hitchcock, & Ratcliffe, 1985), decarestrictine C₁ (**7**) (Gohrt et al., 1992), ergosterol and brassicasterol. The identification of the compounds along with physical and spectroscopic data for four new compounds and their biological activities is described.

2. Results and Discussion

Emestrin (**5**) (Seya, Nozawa, Nakajima, Kawai, & Udagawa, 1986), cephalosporolide-E (**6**) (Ackland, Hanson, Hitchcock, & Ratcliffe, 1985), decarestrictine C₁ (**7**) (Gohrt et al., 1992), brassicasterol (Lee, Lee, Cho, Baek, & Lee, 2010) and ergosterol (Kang, Wang, & Chen, 2003) were identified by comparing physical and spectroscopic data with those reported. Ergosterol is the common, predominant sterol structural component in the cell membranes of higher fungi, whereas brassicasterol is a rarer sterol found in the cell membranes of certain fungi.

Compound **1** was isolated as a white solid and its molecular formula was determined as C₂₇H₂₂N₂O₉S₂ by high-resolution electrospray ionization mass spectroscopy (HRESIMS) analysis. Comparison of the molecular formulas of **1** and emestrin (**5**) (C₂₇H₂₂N₂O₁₀S₂) indicated that the former has one oxygen atom less than the latter. The only major difference in their ¹H-NMR spectra was the replacement of an oxygenated methine signal in **5** with two geminal protons at the δ 3.95 (1H, d, J=13.2 Hz) and 3.45 (1H, d, J=13.6 Hz) in **1**. This was also evident in their ¹³C-NMR spectra with the appearance of a methylene signal (δ 36.9) in **1** in place of an oxygenated methine in **5**. The correlation of the two protons at δ 3.95 and 3.45 with the C-3 (81.4), C-4 (163.7), C-1'' (123.5), C-2'' (128.1) and C-6'' (125.7) showed in the HMBC spectra of **1**, suggested that these two protons should be at the C-7'' position. Hence, the structure of **1** was deduced as the 7''-deoxy analog of emestrin and named emestrin-F. Emestrin-F is rare in having a hydrogen in the C-7'' position instead of a hydroxy group that is typical of most emestrin analogs.

Compound **2** was also isolated as a white solid and HRESIMS analysis indicated the molecular formula C₂₇H₂₂N₂O₁₀S with one less sulfur atom compared to emestrin (C₂₇H₂₂N₂O₁₀S₂). The ¹H and ¹³C NMR spectroscopic data of both **2** and the emestrin were almost identical. Therefore, the structure of **2** was suggested to be the mono-sulfur derivative of emestrin and it was named emestrin-G.

Compound **3** was isolated as a crystalline solid and the HRMS suggested the molecular formula C₁₈H₂₂O₉. The ¹H-NMR of **3** exhibited singlets due to two aromatic protons (δ 6.12, δ 7.03) a methoxy group (δ 3.87) and two aromatic methyls (δ 2.33, δ 2.07) and signals typical of a sugar unit. The ¹³C NMR spectrum indicated resonances due to 10 low-field carbons, including one each for a carbonyl (δ 183.2) and an anomeric carbon (δ 101.7), four oxygenated methines, a oxygenated methylene, a methoxy and 2 aromatic methyls. These data indicated that **3** is a chromone glycoside. In the HMBC spectrum, the aromatic proton at δ 6.12 showed a strong correlation with carbons at δ 106.1 (C-4a), 167.8 (C-2), 20.3 (C-11) and 183.2 (C-4), while the other aromatic proton at δ 7.03 correlated with carbons at δ 110.1 (C-7), 162.0 (C-6), 106.1 (C-4a), 156.5 (C-8a) and 183.2 (C-4). Further, the correlation of the methyl protons at δ 2.07 (2-CH₃) with the carbons at δ 167.8 (C-2)

and 109.1 (C-3) and the other methyl protons at δ 2.33 (7-CH₃) with carbons at δ 159.6 (C-8), 110.1 (C-7) and 162.0 (C-6) in the HMBC of **3** suggested that the two methyl groups were on C-2 and C-7 of the chromone moiety, respectively. The methoxy group correlated a carbon in the sugar unit suggesting that the aglycone is 6,8-dihydroxy-2,7-dimethylchromone, dulcinone, which was previously isolated from a higher plant (Deachathai et al., 2006). The anomeric proton (δ 5.66) of the sugar moiety showed correlation with the C-6 carbon (δ 162.0) indicating the attachment of the sugar unit to C-6. The ¹H NMR coupling constants, COSY, HMQC and HMBC spectroscopic data confirmed that the sugar moiety is β -D-glucose and the methoxy group is attached to C-4 of the sugar. The evidence suggested the structure of **3** as 6-O-(4-O-methyl- β -D-glucopyranosyl)-8-hydroxy-2,7-dimethyl-4H-benzopyran-4-one. Even though compounds bearing 4-O-methylglucopyranosyl units have not been isolated from higher plants, several of them have previously been isolated from fungi (Hu et al., 2002; Zhang, & Xuan, 2007) and a fern (Ye, Fan, Zhang, Yin, & Zhao, 2007).

Compound **4**, a white crystalline solid, was assigned the molecular formula C₁₀H₁₆O₅ based on HRMS. The NMR spectroscopic data were similar to the previously reported spectroscopic data for cephalosporolide and HMBC correlations indicated that it has the same gross structure as cephalosporolide-C (Ackland, Hanson, Hitchcock, & Ratcliffe, 1985). The NOESY spectrum of **4** showed a good correlation between the two protons at δ 4.20 (H-3) and 3.38 (H-4), which revealed that both hydroxy groups attached to C-3 and C-4 should be on the same plane of the molecule. However, the carbon and proton signals corresponding to C-2, C-3, C-4, C-5 and C-6 positions of **4** did not match with the values obtained for cephalosporolide-C (Ackland, Hanson, Hitchcock, & Ratcliffe, 1985) which have both C-3 and C-4 hydroxy groups at the β orientation. The ¹H and ¹³C NMR values which represent the C-7, 8, 9 and 10 positions of both **4** and cephalosporolide-C were almost identical (Ackland, Hanson, Hitchcock, & Ratcliffe, 1985). This spectroscopic information suggested that both the C-3 and C-4 hydroxy groups of **4** have an α -orientation and the compound was named cephalosporolide-J. A diastereomer of **4** named cephalosporolide-G previously has been reported from a fungus (Farooq, Gordon, Hanson & Takahashi, 1995), whereas its C-4 epimer has been synthesized (Barradas, Urbano, & Carreno, 2009).

None of the compounds showed activity against *Aspergillus fumigatus*, *Candida glabrata*, and *Pseudomonas aeruginosa* at the highest test concentration. Emestrin (**5**) exhibited antifungal activity against *C. albicans*, *C. neoformans* and antibacterial activity against both *E. coli* and *S. aureus* (Table 1). Compound **1** was only active against *M. intracellulare* whereas compound **2** had no antimicrobial activity. Strong antifungal activity of emestrin (**5**) against *Penicillium expansum* and *Gibberella zeae* has been reported previously (Seya, Nozawa, Nakajima, Kawai, & Udagawa, 1986). The mode of action responsible for toxicity against certain eukaryotes (fungi) may be due to inhibition of ATP synthesis in mitochondria causing an uncoupling of oxidative phosphorylation and depression of respiration (Kawai et al., 1989).

3. Experimental

3.1. General details

Melting points were measured with a Uni-melt, Thomas Hoover capillary melting point apparatus. UV and IR spectra were obtained with a Varian-50 Bio UV visible spectrophotometer and a Bruker-Tensor-27 infrared spectrophotometer, respectively. NMR spectra were recorded using a Varian-Mercury-plus-400 or Varian Unity-Inova-600 spectrometer with CDCl₃ and methanol-*d*₄ unless otherwise stated. Mass spectra data were obtained from an Agilent Series 1100 SL equipped with an ESI source (Agilent Technologies, Palo Alto, CA, USA). Column chromatography and preparative TLC were

carried out using Merck silica gel 60 (230–400 mesh) and silica gel GF plates (20×20 cm, thickness 0.25 mm), respectively.

3.2. Fungal material

Armillaria tabescens strain accession number JNB-OZ344 was isolated from contextual hyphae of mushroom basidiomes growing on the surface roots of a live *Quercus velutina* Lam. (black oak) tree with significant root rot in southern Missouri. This strain was identified using a combination of macroscopic and microscopic morphological characters. Cultures were maintained in long-term storage under sterile water in plastic cryotubes according to methods described by Burdsall, & Dorworth (1994), prior to culture on rice grains for this study.

3.3. Culture, extraction and purification

Armillaria tabescens strain JNB-OZ344 was cultured and incubated for 21 days at 26 C followed by 7 days at 10 C on 750 g of previously boiled brown rice grains. The rice substrate was inoculated with 4.5% potato dextrose agar (PDA) plugs containing mycelium of the fungus. Accumulated mycelial growth on the rice substrate was extracted three times with 500 ml aliquots of ethyl acetate. The combined organic extract was evaporated to dryness (18.5 g) and thoroughly washed with hexane. The hexane-insoluble portion (1.35 g), later showing antimicrobial activity, was subjected to chromatography on a silica gel column using hexane/ethyl acetate/methanol gradient to yield nine major fractions. The activity was concentrated in fourth and fifth fractions which eluted with hexane/ethyl acetate (80:20). They were combined and separated by preparative TLC on silica gel using hexane/ethyl acetate 7:3 as the solvent (3 developments) to yield compounds **1** (17 mg), **2** (15 mg), and **5** (27 mg). Fraction 6 which eluted with ethyl acetate: hexane (3:7) was separated by preparative TLC on silica gel using hexane: ethyl acetate (1:1) to yield compounds **4** (11 mg) and **7** (15 mg). Compound **3** (13 mg) which eluted with ethyl acetate was purified by preparative TLC on silica gel using CH₂Cl₂: MeOH (98:2) as the solvent. Compounds **6** (19 mg), ergosterol (24 mg) and brassicasterol (41 mg) were isolated from fraction 3 by preparative TLC using hexane/ethyl acetate (7:3).

Emestrin-F (1)—¹H NMR (CDCl₃) δ: 7.89 (1H, d, J = 1.6 Hz, 6''-H), 7.76 (1H, dd, J = 8.8, 2.0 Hz, 7'-H), 7.77 (1H, d, J = 1.2 Hz, 3'-H), 7.06 (1H, dd, J = 8.0, 2.0 Hz, 2''-H), 7.02 (1H, d, J = 8.8 Hz, 6'-H), 6.91 (1H, d, J = 8.4 Hz, 3''-H), 6.89 (1H, brs, 10-H), 6.32 (1H, dd, J = 8.4, 2.4 Hz, 8-H), 5.62 (1H, dd, J = 8.4, 2.4 Hz, 5a-H), 5.55 (1H, brs, OH), 5.29 (1H, s, 11-H), 4.92 (2H, m, 6-H, 7-H), 4.01 (3H, s, 8'-CH₃), 3.95 (1H, d, J = 13.2 Hz, 7''-Ha), 3.45 (1H, d, J = 13.6 Hz, 7''-Hb), 3.22 (3H, s, N-CH₃); ¹³C NMR (Pyridine-D₅) δ: 166.42 (C-1), 81.4 (C-3), 163.7 (C-4), 61.9 (C-5a), 76.3 (C-6), 108.4 (C-7), 138.2 (C-8), 143.1 (C-10), 114.2 (C-10a), 77.2 (C-11), 81.4 (C-11a), 28.1 (C-12), 165.7 (C-1'), 123.5 (C-2'), 122.9 (C-3'), 146.4 (C-4'), 154.9 (C-5'), 113.1 (C-6'), 125.6 (C-7'), 56.5 (C-8'), 123.5 (C-1''), 128.1 (C-2''), 118.3 (C-3''), 150.3 (C-4''), 145.8 (C-5''), 125.7 (C-6''), 36.9 (C-7''); HRESIMS [M-1]⁻ m/z 581.0662 (calcd for C₂₇H₂₁N₂O₉S₂, 581.0688)

Emestrin-G (2)—¹H NMR (CDCl₃) δ: 8.71 (1H, brs, 6''-H), 8.35 (1H, brs, 3'-H), 7.86 (1H, d, J = 8.4 Hz, 7'-H), 7.02 (1H, d, J = 8.4 Hz, 6'-H), 6.93 (1H, d, J = 8.0 Hz, 2''-H), 6.83 (1H, s, 10-H), 6.80 (1H, d, J = 8.0 Hz, 3''-H), 6.36 (1H, d, J = 8.4 Hz, 8-H), 5.39 (1H, d, J = 8.4 Hz, 5a-H), 5.32 (1H, brd, J = 8.0 Hz, 6-H), 5.22 (1H, brd, J = 12.0 Hz, 7''-H), 5.00 (1H, dd, J = 8.0, 2.0 Hz, 7-H), 4.81 (1H, s, 11-H), 4.43 (1H, brd, J = 12.4 Hz, 7''-OH), 4.06 (3H, s, 8'-CH₃), 3.53 (3H, s, N-CH₃); ¹³C NMR (CDCl₃) δ: 168.1 (C-1), 77.6 (C-3), 165.5 (C-4), 59.0 (C-5a), 74.8 (C-6), 109.8 (C-7), 139.0 (C-8), 143.0 (C-10), 108.3 (C-10a), 79.8 (C-11), 83.1 (C-11a), 28.8 (C-12), 165.2 (C-1'), 122.6 (C-2'), 123.2 (C-3'), 147.2 (C-4'), 154.5 (C-5'), 112.0 (C-6'), 127.5 (C-7'), 55.5 (C-8'), 127.0 (C-1''), 130.3 (C-2''), 114.4

(C-3''), 149.6 (C-4''), 146.4 (C-5''), 126.8 (C-6''), 76.6 (C-7''); HRESIMS [M-1]⁻ m/z 565.0902 (calcd for C₂₇H₂₁N₂O₁₀S, 565.0917)

6-O-(4-O-methyl-β-D-glucopyranosyl)-8-hydroxy-2,7-dimethyl-4H-

benzopyran-4-one (3)—Mp 132–134 °C, ¹H NMR (pyridine-D₆) δ: 13.57 (1H, s, OH), 7.03 (1H, s, 5-H), 6.12 (1H, s, 3-H), 5.66 (1H, dd, J = 7.6, 1.6 Hz, 1'-H), 4.34 (3H, m, 2', 3', 6'-H), 4.20 (1H, dd, J = 12.0, 4.4 Hz, 6'-H), 4.00 (1H, m, 5'-H), 3.89 (1H, overlapped, 4'-H), 3.87 (3H, s, OCH₃), 2.33 (3H, s), 2.07 (3H, s). ¹³C NMR (pyridine-D₆) δ: 183.2 (C-4), 167.8 (C-2), 162.0 (C-6), 159.6 (C-8), 156.6 (C-8a), 110.2 (C-7), 109.1 (C-3), 106.1 (C-4a), 101.7 (C-1'), 93.9 (C-5), 80.5 (C-4'), 78.4 (C-3'), 78.1 (C-5'), 75.1 (C-2'), 62.0 (C-6'), 60.9 (OCH₃), 20.3 (2-CH₃), 8.2 (7-CH₃). HRESIMS [M+1]⁺ m/z 383.0983 (calcd for C₁₈H₂₃O₉, requires 383.1342).

Cephalosporolide-J (4)—¹H NMR (CDCl₃) δ: 5.09 (1H, m, 9-H), 4.20 (2H, m, 3-H, OH), 3.38 (1H, m, 4-H), 2.96 (1H, brs, OH), 2.90 (1H, dd, J = 17.4, 3.4 Hz, 2-H_a), 2.76 (1H, dd, J = 18.4, 4.8 Hz, 5-H_a), 2.69 (1H, dd, J = 18.4, 2.8 Hz, 5-H_b), 2.34 (3H, m, 2-H_b, 7-H_a, 7-H_b), 2.05 (2H, m, 8-H_a, 8-H_b), 1.37 (3H, s, 10-CH₃); ¹³C NMR (CDCl₃) δ: 214.4 (C-6), 169.5 (C-1), 74.9 (C-4), 72.1 (C-9), 68.7 (C-3), 43.3 (C-5), 40.6 (C-7), 38.8 (C-2), 33.6 (C-8), 19.4 (C-10). HRESIMS [M+1]⁺ m/z 217.1047 (calcd for C₁₀H₁₇O₅, 217.1076).

3.4 Antimicrobial assay

Test organisms include the fungi *Candida albicans* ATCC 90028, *Candida glabrata* ATCC 90030, *Cryptococcus neoformans* ATCC 90113, and *Aspergillus fumigatus* ATCC 204305 and the bacteria *Staphylococcus aureus* ATCC 29213, methicillin-resistant *Staphylococcus aureus* ATCC 33591 (MRSA), *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, and *Mycobacterium intracellulare* ATCC 23068 and were obtained from the American Type Culture Collection (ATCC, Manassas, VA). Susceptibility testing was performed using a modified version of CLSI methods [National Committee on Clinical Laboratory Standards (NCCLS, 2002a, 2002b, 2003, 2006)] as described by Samoylenko et al. (2009). Two drug controls included in each assay were ciprofloxacin (ICN Biomedicals, Ohio) for bacteria and amphotericin B (ICN Biomedicals, Ohio) for fungi. IC₅₀s (concentrations that afford 50% inhibition relative to controls) are calculated using XLfit 4.2 software (IDBS, Alameda, CA) using fit model 201. The MIC (minimum inhibitory concentration) is defined as the lowest test concentration that allows no detectable growth.

Acknowledgments

The authors wish to thank Ms. Marsha Wright for the biological testing. This work was supported by the NIH, NIAID, Division of AIDS, Grant No. AI 27094 and the USDA Agricultural Research Service Specific Cooperative Agreement No. 58-6408-1-603.

References

- Ackland MJ, Hanson JR, Hitchcock PB, Ratcliffe AH. Journal of the Chemical Society, Perkin Transactions. 1985; 1:843–847.
- Baietto M, Wilson AD. Relative in vitro wood decay resistance of sapwood from landscape trees of southern temperate regions. Horticulture Science. 2010; 45:401–408.
- Barradas S, Urbano A, Carreno MC. Concise enantioselective synthesis of the ten-membered lactone cephalosporolide G and its C-3 epimer. Chemistry A European Journal. 2009; 15:9286–9289.
- Burdsall HH, Dorworth EB. Preserving cultures of wood-decaying Basidiomycotina using sterile distilled water. Mycologia. 1994; 86:275–280.

- Deachathai S, Mahabusarakam W, Phongpaichit S, Taylor WC, Zhang YJ, Yang CR. Phenolic compounds from the flowers of *Garcinia dulcis*. *Phytochemistry*. 2006; 67:464–469. [PubMed: 16325214]
- Donnelly DMX, Konishi T, Dunne O, Cremin P. Sesquiterpene aryl esters from *Armillaria tabescens*. *Phytochemistry*. 1997; 44:1473–1478.
- Farooq A, Gordon J, Hanson JR, Takahashi JA. Two C10 lactones from *Cephalosporium aphidicola*. *Phytochemistry*. 1995; 38:557–558.
- Gohrt A, Zeeck A, Hutter K, Kirsch R, Kluge H, Thiericke R. Secondary metabolites by chemical screening. 9. Decarestrictines, a new family of inhibitors of cholesterol biosynthesis from *Penicillium*. II. Structure elucidation of the decarestrictines A to D. *The Journal of Antibiotics*. 1992; 45:66–73. [PubMed: 1548191]
- Hu F, Schmidt K, Stoyanova S, Zengzhi L, Gräfe U, Hamburger M. Radical scavengers from the entomogenous Deuteromycete *Beauveria amorpha*. *Planta Medica*. 2002; 68:64–65. [PubMed: 11842330]
- Kang J, Wang HQ, Chen RY. Studies on the constituents of the mycelium produced by fermenting culture of *Flammulina velutipes* (W. Curt.:Fr.) Singer (Agaricomycetideae). *International Journal of Medicinal Mushrooms*. 2003; 5:391–396.
- Kawai K, Ishizaki K, Nakamaru T, Hisada K, Nozawa Y, Kawai KI. Toxicity of emestrin, a new macrocyclic dithiodioxopiperazine mycotoxin, to mitochondrial function. *Mycotoxin Research*. 1989; 5:2–8. [PubMed: 23605226]
- Lee JW, Lee DY, Cho JG, Baek NI, Lee YH. Isolation and identification of sterol compounds from the Red Kohlrabi (*Brassica oleracea* var. *gongylodes*) Sprouts. *Journal of Applied Biological Chemistry*. 2010; 53:207–211.
- NCCLS. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard M27-A2. National Committee on Clinical Laboratory Standards. 2002a; 22(15)
- NCCLS. Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; approved standard, M38-A. National Committee on Clinical Laboratory Standards. 2002b; 22(16)
- NCCLS. Susceptibility testing of mycobacteria, nocardia, and other aerobic actinomycetes; approved standard, M24-A. National Committee on Clinical Laboratory Standards. 2003; 23(18)
- NCCLS. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard, Seventh Edition M7-A7. National Committee on Clinical Laboratory Standards. 2006; 26(2)
- Seya H, Nozawa K, Nakajima S, Kawai K, Udagawa AS. Studies on fungal products. Part 8. Isolation and structure of emestrin, a novel antifungal macrocyclic epidithiodioxopiperazine from *Emericella striata*. X-Ray molecular structure of emestrin. *Journal of the Chemical Society, Perkin Transactions*. 1986; 1:109–116.
- Shaw, CG.; Kile, GA. *Armillaria Root Disease*. Washington DC: Agricultural Handbook No. 691, USDA Forest Service; 1991.
- Samoylenko V, Jacob MR, Khan SI, Zhao J, Tekwani BL, Midiwo JO, Walker LA, Muhammad I. Antimicrobial, antiparasitic and cytotoxic spermine alkaloids from *Albizia schimperiana*. *Natural Product Communications*. 2009; 4:791–796. [PubMed: 19634324]
- Wilson, AD.; Leininger, TD.; Orosina, WJ.; Dwinell, LD.; Schiff, NM. The impact and control of major southern forest diseases. In: Rauscher, HM.; Johnsen, K., editors. *Southern forest science: Past, present, and future*. Asheville, NC: Gen. Tech. Rep. SRS-75. USDA Forest Service, Southern Research Station; 2004. p. 161-178.
- Ye W, Fan C, Zhang L, Yin Z, Zhao S. A new phenolic glycoside from the roots of *Lygodium japonicum*. *Fitoterapia*. 2007; 78:600–601. [PubMed: 17570612]
- Zhang SW, Xuan LJ. Five aromatics bearing a 4-*O*-methylglucose unit from *Cordyceps cicadae*. *Helvetica Chimica Acta*. 2007; 90:404–410.

- (1) $n=2$, $R=H$, Emestrin-F
 (2) $n=1$, $R=OH$, Emestrin-G
 (5) $n=2$, $R=OH$, Emestrin

3

4

6

7

Figure 1.

Table-1

Antimicrobial activity^a of compounds **5** and **1** from *Armillaria tabescens*

Compound	<i>C. albicans</i>		<i>C. neoformans</i>		<i>M. intracellulare</i>		<i>E. coli</i>		<i>S. aureus</i>		MRSA	
	IC ₅₀	MIC	IC ₅₀	MIC	IC ₅₀	MIC	IC ₅₀	MIC	IC ₅₀	MIC	IC ₅₀	MIC
5	3.94	^b	0.6	2.5	-	-	2.21	-	4.55	20	2.21	10
1	-	-	3.24	-	1.18	1.25	-	-	-	-	-	-
Amphotericin B ^d	0.42	1.25	0.43	1.25	NT ^c	NT	NT	NT	NT	NT	NT	NT
Ciprofloxacin ^d	NT	NT	NT	NT	0.41	0.5	0.007	0.03	0.12	0.25	0.14	0.50

^aIC₅₀ and MIC (minimum inhibitory concentration) values are in µg/mL.^b-not active at the highest test concentration of 20 µg/mL.^cNT: not tested^dPositive control