Derivatization Does Not Influence Antimicrobial and Antifungal Activities of Applanoxidic Acids and Sterols from *Ganoderma* spp.

Artur Smania Jr.[a,*], Elza F. A. Smania[a], Franco Delle Monache[b], Moacir G. Pizzolatti[c], and Giuliano Delle Monache[a]

[a] Departamento de Microbiologia e Parasitologia, Universidade Federal de Santa Catarina, Florianopolis, SC 88040-900, Brazil. Fax: +55-48-331 9258. E-mail: smania@mbox1.ufsc.br, artursmania@hotmail.com

[b] Istituto di Biochimica e Biochimica Clinica, Università Cattolica Del Sacro Cuore, Roma Italy

[c] Departamento de Química, Universidade Federal de Santa Catarina, Florianopolis, Brazil

* Author for correspondence and reprint requests

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Applanoxidic acids and sterols, isolated from *Ganoderma* spp., were acetylated and/or methylated. The antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* and the antifungal activity against *Candida albicans* and *Trichophyton mentagrophytes* of the derivatives were investigated by a microdilution method, and compared with those of the natural products. Both natural and modified compounds exhibited comparable antibacterial and antifungal activities in a range of 1.0 to > 2.0 mg/ml minimal inhibitory concentration.

Key words: Applanoxidic Acids, Sterols, Derivatization, Antibacterial and Antifungal Activity

Introduction

Applanoxidic acids (**1**–**5**) are exclusive triterpene metabolites from *Ganoderma* species (Ganodermataceae Donk), while sterols (**6**–**8**) were isolated from other Basidiomycetes, as *Pycnoporus* and *Rigidoporus*, in addition to *Ganoderma* (Chen *et al.*, 1999; Chyr and Shiao, 1991; Smânia *et al.*, 2001). Their biological activities have been described previously (Gerber *et al.*, 2000; Smânia *et al.*, 1999, 2003). This paper deals with the antibacterial and antifungal activities of methyl/acetyl derivatives in comparison with those of the natural compounds.

Material and Methods

Fungi

A basidioma of *Ganoderma applanatum* was collected from decayed wood in a forest of Southern Brazil in 1995; a voucher specimen was deposited at the Herbarium FLOR, Department of Botany, Federal University of Santa Catarina, Brazil, under the number FLOR 11.470. Four basidiomata of *Ganoderma australe* were collected in Florianopolis, Santa Catarina, Brazil; voucher specimens are deposited in the same Herbarium as above, under the number FLOR 11.723, 11.727, 11.728, and 11.729.

Natural products

Applanoxidic acids A (**1**), C (**2**), F (**3**), G (**4**), and H (**5**) were isolated from *G. australe* (Gerber *et al.*, 2000). 5α-Ergost-7en-3β-ol (**6**), 5α-ergost-7,22-dien-3β-ol (**7**), and 5,8-epidioxy-5α,8α-ergost-6,22-dien-3β-ol (**8**) were isolated from both *G. applanatum* (Smânia *et al.*, 1999) and *G. australe* (Gerber *et al.*, 2000) (Fig. 1).

Acetylation of applanoxidic acids

Compounds **1**, **4**, and **5** were treated with Ac_2O in pyridine (1:1) at room temperature for 24 h, to yield the corresponding acetyl derivatives **1a**, **4a**, and **5a**, as confirmed by the appropriate signals for the OCOMe groups in ^{1}H and ^{13}C NMR spectra ($CDCl_3$, TMS as internal standard) (Fig. 1).

Methylation of applanoxidic acids

Compounds **1**–**5** were treated with a satured diazomethane solution Et_2O at room temperature for 2 h, to yield the corresponding methyl derivatives **1m**–**5m**, as confirmed by the appropriate signals for the COOMe groups in ^{1}H and ^{13}C NMR spectra (Fig. 1).

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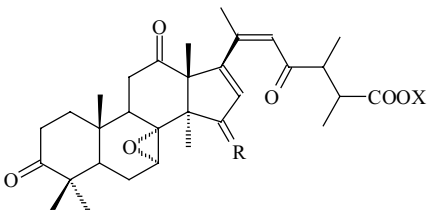
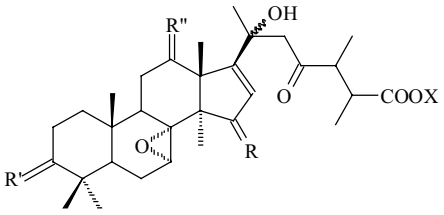
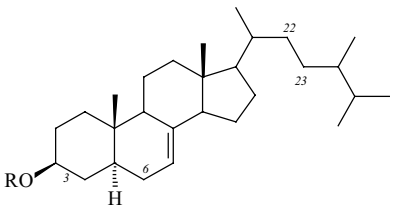


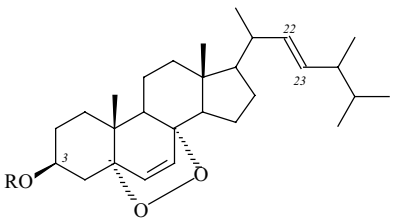
	R	X
1	H,OH	H
3	O	H
1a	H, OAc	H
1m	H,OH	Me
1d	H,OAc	Me
3m	O	Me



	R	R'	R"	X
2	O	O	O	H
4	H,OH	O	O	H
5	O	H,OH	H,OH	H
2m	O	O	O	Me
4a	H,OAc	O	O	Me
4m	H,OH	O	O	Me
4d	H,OAc	O	O	Me
5a	O	H,OAc	H,OAc	H
5m	O	H,OH	H,OH	Me
5d	O	H,OAc	H,OAc	Me



	R	
6	H	
6a	Ac	
7	H	$\Delta_{22\text{-}23}$
7a	Ac	$\Delta_{22\text{-}23}$



	R
8	H
8a	Ac

Fig. 1. Triterpenes and sterols isolated from *Ganoderma* spp. and their derivatives.

Acetylation of applanoxidic acid methyl esters

Compounds **1m**, **2m** and **5m** were acetylated, as described above, to yield **1d**, **2d**, and **5d**. The reactions were confirmed by the appropriate signals for the OCOMe groups in ^{1}H and ^{13}C NMR spectra (Fig. 1).

Acetylation of sterols

5α-Ergost-7-en-3β-ol (**6**), 5α-ergost-7,22-dien-3β-ol (**7**), and 5,8-epidioxy-5α,8α-ergost-6,22-dien-3β-ol (**8**) yielded by acetylation the derivatives **6a**–**8a**, respectively, as confirmed by ^{1}H and ^{13}C NMR spectra (Fig. 1).

Antibacterial test

The eight natural products and the fourteen derivatives were tested against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 using a microdilution method and tetracycline as a reference agent. All the compounds were dissolved in dimethylsulfoxide (DMSO) and diluted (2.0–0.0156 mg/ml) in a Mueller-Hinton broth. 100 μl from each dilution, as well as 100 μl of the vehicle (Mueller-Hinton broth plus DMSO), were poured in one of the 96 wells of a sterilized microplate. Each well was inoculated with 5 μl of bacterial inoculum (10^6 CFU/ml). The procedure was performed in duplicate and the microdilution trays were incubated at 36 °C for 18 h. The optical density was read in an ELISA apparatus while the microbial growth was confirmed with INT (*p*-iodonitrotetrazolium violet). The minimal inhibitory concentration (MIC), defined as the lowest concentration for each substance that produced inhibition of bacterial growth, is reported in Table I.

Table I. Antibacterial and antifungal activities (MIC*) of *Ganoderma* spp. metabolites and their derivatives.

Compound	Bacteria		Fungi	
	E. coli	*S. aureus*	*C. albicans*	*T. mentagrophytes*
1	2.0	2.0	> 2.0	0.5
1a	2.0	2.0	2.0	2.0
1m	2.0	2.0	2.0	2.0
1d	2.0	1.0	1.0	1.5
2	2.0	1.0	2.0	2.0
2m	2.0	2.0	2.0	2.0
3	> 2.0	> 2.0	1.0	1.0
3m	2.0	2.0	1.0	1.25
4	2.0	2.0	1.0	> 2.0
4a	2.0	2.0	2.0	2.0
4m	2.0	1.0	2.0	2.0
4d	2.0	> 2.0	> 2.0	1.5
5	> 2.0	1.0	2.0	1.0
5a	2.0	2.0	1.0	1.0
5m	2.0	2.0	> 2.0	1.25
5d	2.0	2.0	> 2.0	1.0
6	2.0	2.0	> 2.0	> 2.0
6a	2.0	2.0	2.0	1.0
7	2.0	2.0	> 2.0	> 2.0
7a	> 2.0	> 2.0	> 2.0	> 2.0
8	1.0	1.0	2.0	2.0
8a	> 2.0	> 2.0	> 2.0	> 2.0
Reference[a]	0.002	0.001	0.1	0.0006

* Minimal inhibitory concentration (mg/ml).
[a] Tetracycline (for bacteria) and fluconazole (for fungi).

Antifungal test

The same substrates were tested against *Candida albicans* and *Trichophyton mentagrophytes* using the microdilution method and fluconazole as a reference agent. The same procedure as above was used for a 5 μl fungal inoculum (10^5 CFU/ml). Incubation was at 30 °C for 72 h. MIC values, as the lowest concentration for each substrate resulting in the absence of fungal growth, are also reported in Table I.

Results and Discussion

As shown in Table I, the Gram-positive strain *S. aureus* was slightly more sensitive than the Gram-negative strain *E. coli*, while the dermatophyte *T. mentagrophytes* was also slightly more sensitive than the yeast *C. albicans*. The antimicrobial activities of natural and modified compounds were comparable. Derivatization of triterpenes influenced the MIC value only in a few cases and the change did not concern more than one dilution, whereas acetylation of sterols was essentially negative. For the antifungal activities, which varied in the same range (1.0 to > 2.0 mg/ml), similar considerations are valid.

In conclusion, the structural modifications did not promote a better interaction of the substrates with the target. Actually, the co-administration of natural products with alkaloids (efflux pump inhibition) remains a good perspective for the use of these secondary metabolites as prototypes for new antibiotics (Bambeke *et al.*, 2003; Tegos *et al.*, 2002).

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