Synthesis of *N*-acyl and Ureido Derivatives of Diosgenyl Glycoside with Potent Antifungal and Antibacterial Activity

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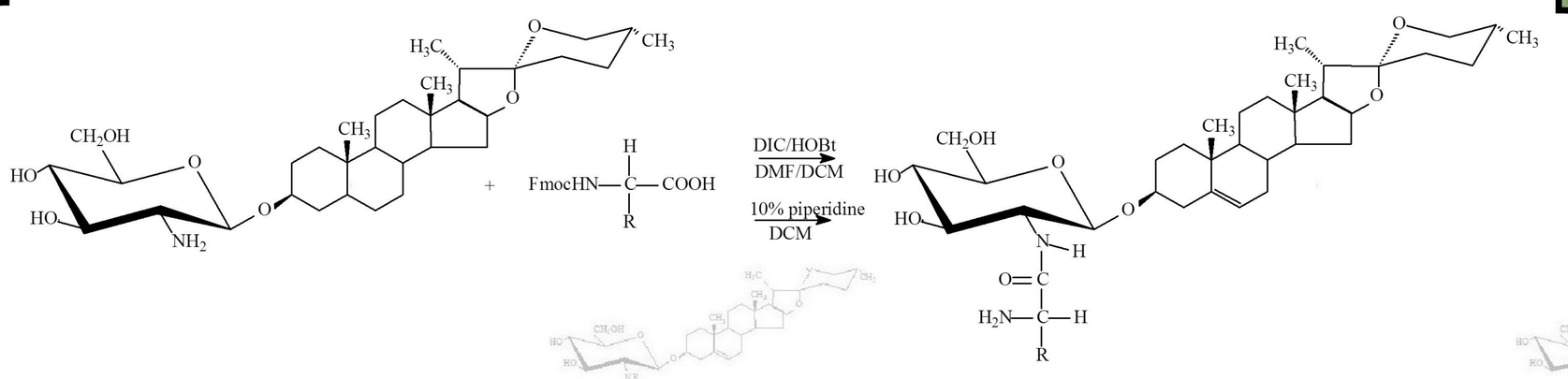
Introduction

Saponins are natural glycosides which possess a wide range of pharmacological properties. Diosgenyl glycosides are steroid saponins isolated from a variety of plants, for example *Costus, Dioscorea, Paris, Solanum, Trillium, Yucca*.

They are used in traditional medicine as an antidiabetes and antihyperglycemia agents, medical material to treat malaria, helminthes infections and snake bites. Some of diosgenyl glycosides exhibit a wide spectrum of biological activities including antifungal, antibacterial and anticancer properties.



Material and Methods

Synthesized glycosides consists of diosgenin and D-glucosamine residue. Such saponins have not been found in natural sources. Our synthetic strategy is based on the preparation of glycosyl donors, coupling of the respective donors with diosgenin, deprotection of the NH_2 and OH groups and finally obtaining of *N*-acyl derivatives.

N-Acyl derivatives were synthesized from diosgenyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride (Ref.) in reaction with: Ac-glycine (**1**), Fmoc-sarcosine (**2**), Fmoc-glycine (**3**), Fmoc-L-alanine (**4**), Fmoc-L-serine (**5**), Fmoc-L-threonine (**6**), ethyl isocyanate (**7**), 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-isocyanato-β-D-glucopyranose (**8**). Fmoc was removed by a treatment with freshly prepared 10 % piperidine/DCM, while acetyl groups by a treatment with sodium methoxide in methanol.

The structure of our products were confirmed by IR, ^{1}H and ^{13}C NMR spectroscopy and mass spectrometry.

Microbiology

Minimum Inhibitory Concentration (MIC) was determined for eight steroidal saponins. MIC was determined for reference strains of the following bacteria: *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* PCM 2118 and the following fungi: *Candida albicans* ATCC 10231, *Candida lipolytica* PCM 2680, *Candida tropicalis* PCM 2681.

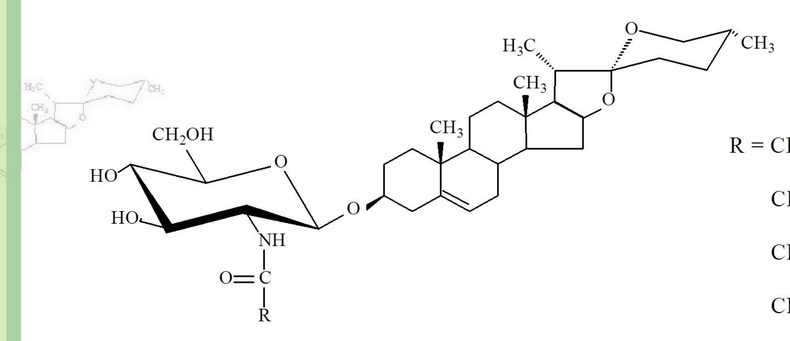
MIC was determined by the broth dilution method according to the procedures recommended by CLSI (Clinical and Laboratory Standards Institute). Bacteria were exposed to the saponins at adequate concentrations diluted in Mueller Hinton II broth (Becton Dickinson) while for fungi Sabouraud glucose liquid medium (Sigma-Aldrich) was applied. Polystyrene 96-well plates (Greiner Bio-One) were incubated for 18 h at 37˚C (bacteria) or 48 h at 25˚C (fungi). MIC was taken as the lowest drug concentration at which a noticeable growth was inhibited.

The experiments were performed in triplicate.

Results

Synthesized saponins exhibit various degrees of activity against fungi and bacteria:

- saponins **Ref.**, **2** and **4** are active against *Candida* species,
- glycoside **4** is the most effective compound against G+ bacteria and fungi,
- compounds **3**, **5**, **6** presents medium antimicrobial activity against both G+ bacteria and fungi,
- the *N*-acetyl derivative (compounds **2**) and saponin **8** are inactive,
- Gram negative bacteria turned out to be resistant to presented saponins.



R = CH_2NHAc	**1**	$CH(NH_2)CH_2OH$	**5**
CH_2NHCH_3	**2**	$CH(NH_2)CH(OH)CH_3$	**6**
CH_2NH_2	**3**	$NHCH_2CH_3$	**7**
$CH(NH_2)CH_3$	**4**	NH-D-Glc	**8**

Table 1. Antifungal and antibacterial activity of steroidal saponins

		Ref.	1	2	3	4	5	6	7	8
Fungi	*C. albicans*	2	1024	4	64	4	4	4	8	1024
	C. lipolytica	2	256	4	64	2	4	4	4	128
Bacteria Gram +	*B. subtilis*	8	1024	4	64	8	8	32	16	1024
	E. faecalis	16	1024	4	64	4	16	16	4	1024
	S. aureus	16	1024	4	64	4	8	16	8	1024
	S. epidermidis	16	1024	4	64	4	4	16	1024	1024

INNOVATIVE ECONOMY
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