DIOSGENYL 2-AMINO-2-DEOXY-β-D-GLUCO-PYRANOSIDE HYDROCHLORIDE AND ITS *N*-ACYL ANALOGUES

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INTRODUCTION

Dioscorea villos

Saponins are structurally complicated compounds which are typically glycosides of steroids, triterpenes or steroid alkaloids. They are believed to be the main constituents of many plant drugs and are therefore used in folk medicine. Naturally occurring diosgenyl glycosides belong to the group of saponins. They are used in traditional medicine as an antidiabetes and antihyperglycemia agents and 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.

Trigonella foenum-graecum L

SYNTHESIS

Synthesized by us 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.

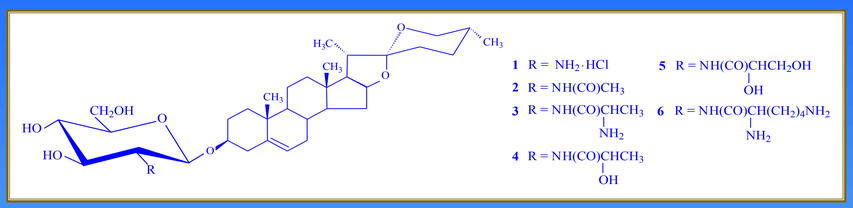
N-Acyl derivatives were synthesized from diosgenyl glycoside hydrochloride **1** in reaction with: acetic anhydride in methanol (**2**), Fmoc-L-alanine (**3**), lactic acid (**4**), glyceric acid (**5**) and Fmoc-L-lysine (**6**). DIC (diizopropylocarbodiimide) was used as a coupling reagent and HOBt to avoid racemization. Fmoc was removed by a treatment with freshly prepared 20% piperidine/DCM.

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

MICROBIOLOGY

In biological set of experiments we have investigated the antibacterial and antifungal effect of presented saponins. Minimum inhibitory concentration (MIC) was determined for reference strains of the following bacteria: (*B. subtilis* ATCC 6633, *E. faecalis* ATCC 29212, *R. equi* ATCC 6939, *S. aureus* ATCC 25923, *S. epidermidis* PCM 2118, *Escherichia coli* ATCC 25922, *K. pneumoniae* ATCC 700603, *P. mirabilis* PCM 543, *P. vulgaris* PCM 2668, *P. aeruginosa* ATCC 9027) and the following fungi: (*C. albicans* ATCC 10231, *C. tropicalis* PCM 2681, *C. lipolytica* PCM 2680).

Minimum inhibitory concentration was determined by the broth microdilution method according to the procedures recommended by CLSI. MIC was taken as the lowest drug concentration at which a noticeable growth was inhibited. The experiments were performed in triplicate.



Compound	R	*Fungi*			*Bacteria G+*					*Bacteria G-*				
		Candida albicans	*Candida lipolytica*	*Candida tropicalis*	*Bacillus subtilis*	*Enterococcus faecalis*	*Rhodococcus equi*	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Escherichia coli*	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Proteus vulgaris*	*Pseudomonas aeruginosa*
1	$NH_2 \cdot HCl$	2	2	0,5	8	16	16	16	16	1024	512	> 1024	> 1024	1024
2	$NH(CO)CH_3$	256	-	256	> 1024	512	512	> 1024	> 1024	1024	1024	> 1024	> 1024	1024
3	$NH(CO)CH(NH_2)CH_3$	4	2	1	8	4	8	4	4	1024	> 1024	> 1024	> 1024	1024
4	$NH(CO)CH(OH)CH_3$	512	256	256	1024	1024	512	1024	1024	1024	> 1024	> 1024	> 1024	1024
5	$NH(CO)CH(OH)CH_2OH$	8	2	1	1024	256	64	256	512	1024	> 1024	> 1024	> 1024	1024
6	$NH(CO)CH(NH_2)(CH_2)_4NH_2$	64	32	16	32	64	64	64	32	1024	> 1024	1024	> 1024	1024

Results

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

- saponins **1**, **3** and **5** are active against *Candida* species,
- glycoside **3** is the most effective compound against Gram positive bacteria and fungi,
- compound **6** presents medium antimicrobial activity against both G+ bacteria and fungi, while **5** is strong antifungal and weak antibacterial agent,
- the *N*-acetyl derivative (compounds **2**) and saponin **4** are inactive,
- Gram negative bacteria turned out to be resistant to presented saponins.

INNOVATIVE ECONOMY
NATIONAL COHESION STRATEGY

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