BIOLOGICAL ACTIVITY OF DIOSGENYL 2-AMINO-2-DEOXY-β-D-GLUCOPYRANOSIDE HYDROCHLORIDE AND ITS *N,N*-DIALKYL DERIVATIVES

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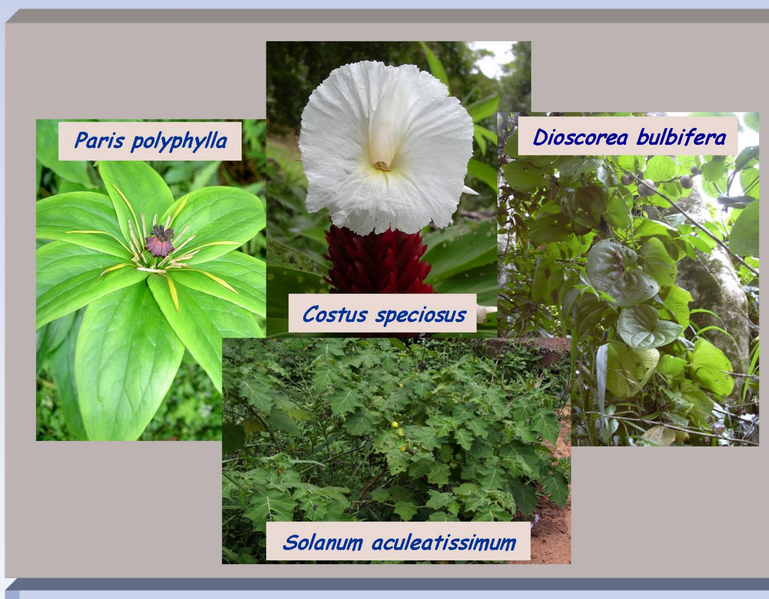


Antimicrobial activity against following reference strains: *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Rhodococcus equi* ATCC 6939, *Staphylococcus aureus* ATCC 25923, *S. epidermidis* PCM 2118, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Proteus mirabilis* PCM 543 *P. vulgaris* PCM 2668 and *Pseudomonas aeruginosa* ATCC 9027; *Candida albicans* ATCC 10231, *C. lipolytica* PCM 2680 *C. tropicalis* PCM 2681 and clinical isolates: *C. glabrata (22), C. krusei (12), C. tropicalis (13) and C. parapsilosis (19)* was evaluated. Minimum Inhibitory Concentration (MIC) was determined by the broth dilution method according to the procedures recommended by CLSI (Clinical and Laboratory Standards Institute) with Sabouraud glucose liquid medium tested fungi, while Mueller Hinton II broth was apllied for bacterial strains.

Human erythrocytes were exposed to graded concentrations of tested saponins for 2 h in order to measure their hemolytic activity.

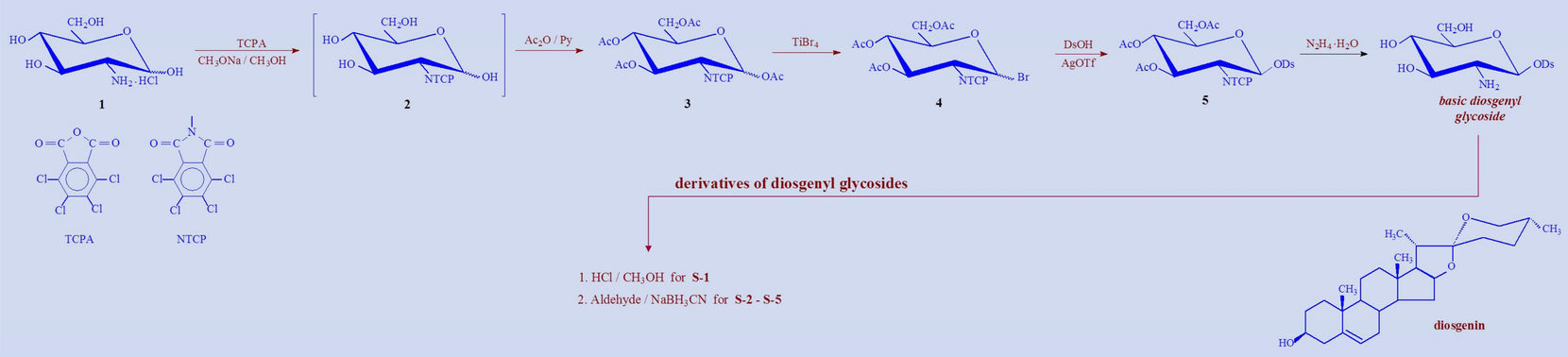
Diosgenyl glycosides are steroid saponins isolated from a variety of plants, for example *Costus*, *Dioscorea*, *Paris*, *Solanum*, *Trigonella*, *Trillium*, *Yucca*. Some of them exhibit a wide spectrum of biological activities including antifungal, antibacterial and anticancer properties. The carbohydrate residue, usually mono-, di-, tri- or tetrasaccharide, is covalently attached to the diosgenin backbone. Usually, in natural diosgenyl glycosides the first sugar connected to diosgenin is β-D-glucopyranose.

We have synthesized a diosgenyl glycosides containing D-glucosamine derivatives as a carbohydrate residue. These glycosides have not been found in natural sources so far. Nine of them were tested for their antifungal, antibacterial and hemolytic activity.

Material and Methods

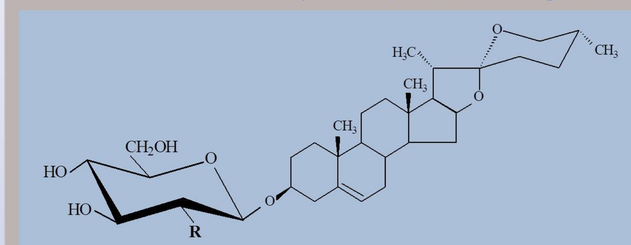
D-Glucosamine hydrochloride (**1**) was converted into **3** as follows: (*i*) neutralization of hydrochloride by a stoichiometric amount of 0.5 M methanolic solution of sodium methoxide, with immediate acylation of the 2-amino group with tetrachlorophthalic anhydride; (*ii*) *O*-acetylation, after evaporation of methanol, of the crude product with acetic anhydride in pyridine furnished the TCP-protected per-*O*-acetyl derivative **3**, as an anomeric mixture. Treatment of the *N*-protected per-*O*-acetylated derivative **3** with excess of $TiBr_4$ in 10:1 dichloromethane/ethyl acetate resulted in the glucosyl bromide **4**. Glycosylation of diosgenin with 3,4,6-tri-*O*-acetyl-2-deoxy-2-tetrachlorophthalimido-α,β-D-glucopyranosyl bromide (**4**) in dichloromethane under nitrogen at room temperature in the presence of silver triflate gave the glycoside **5** in good yield. The reaction is stereospecific and prefer formation of β-glucoside.

Treatment of **5** with excess of hydrazine hydrate results in only basic glycoside without protecting groups. For biological purposes this glycoside was converted into hydrochloride **S-1**. The saponins **S-2** ÷ **S-5** were synthesized from basic glycoside in reaction with appropriate aldehyde and $NaBH_3CN$. The structure of all new compounds was established on basis of the ^1H and ^{13}C NMR spectroscopy.



		Fungi			Gram positive bacteria					Gram negative bacteria					Hemolytic activity
	R	*C. albicans*	*C. lipolytica*	*C. tropicalis*	*B. subtilis*	*E. faecalis*	*R. equi*	*S. aureus*	*S. epidermidis*	*E. coli*	*K. pneumoniae*	*P. mirabilis*	*P. vulgaris*	*P. aeruginosa*	
S-1	$NH_2 \cdot HCl$	2	2	0,5	8	16	16	16	16	1024	512	> 1024	> 1024	1024	256
S-2	$N(CH_3)_2$	2	2	1	32	32	16	32	32	1024	512	> 1024	> 1024	1024	128
S-3	$N(CH_2CH_3)_2$	2	2	2	8	32	64	32	64	1024	1024	> 1024	> 1024	> 1024	256
S-4	$N(CH_2CH_2CH_3)_2$	4	8	4	16	8	16	8	32	1024	1024	> 1024	> 1024	> 1024	128
S-5	$N(CH_2CH_2CH_2CH_3)_2$	8	32	16	16	8	16	8	8	1024	512	> 1024	> 1024	1024	128

Minimum inhibitory concentration [mg/L]



	C. glabrata			*C. krusei*			*C. parapsilosis*			*C. tropicalis*		
	range	50%	90%	range	50%	90%	range	50%	90%	range	50%	90%
S-1	0.25-8	2	4	0.5-1024	16	1024	1-512	2	4	0.5-1024	4	1024
S-2	0.5-8	4	4	2-1024	16	1024	0.5-128	1	2	2-1024	4	1024
S-3	0.5-8	4	4	2-1024	64	1024	1-256	2	4	2-1024	4	512
S-4	0.5-256	4	8	4-1024	1024	1024	2-256	4	4	4-1024	8	1024
S-5	1-512	8	16	8-1024	1024	1024	4-256	8	16	4-1024	128	1024

Results

Tested saponins demonstrated rather poor antibacterial activity towards gram-negative strains. The growth of gram positive bacteria and fungi was inhibited at concentrations 0.5 – 128 mg/L. Strains of *Candida sp.* were the most susceptible to tested compounds. Therefore MIC assay was performed for clinical isolates of *C. glabrata, C. krusei, C. tropicalis, and C. parapsilosis*. The test was carried out also for conventional antifungal agents: amphotericin B, clotrimazole, fluconazole, itraconazole, natamycin, nystatin (data not shown).

Among clinical strains of *C. krusei* and *C. tropicalis* we have identified numerous isolates resistant to conventional antifungals at applied concentrations (0,025-1024 mg/L). Those strains were inhibited by saponins used at the highest concentrations (1024 mg/L). Obtained compounds presented very strong activity towards clinical isolates of *C. glabrata* and *C. parapsilosis* comparable or stronger than conventional antimicrobials.

Tested glycosides did not exhibit hemolytic activity towards human erythrocytes while applied at their microbiogically active concentrations. Results of presented study suggest potential application of saponins as future antifungal agents and encourage to continue the study on steroidal saponins and their potential application for the treatment of candidemia.

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