Diosgenyl 2-amino-2-deoxy-β-D-galactopyranoside: synthesis, derivatives and antimicrobial activity

Henryk Myszka*1, Patrycja Sokolowska1, Agnieszka Cieślińska1, Andrzej Nowacki1, Maciej Jaśkiewicz2, Wojciech Kamysz2 and Beata Liberek1

Abstract
The synthesis of diosgenyl 2-amino-2-deoxy-β-D-galactopyranoside is presented for the first time. This synthetic saponin was transformed into its hydrochloride as well as N-acyl, 2-ureido, N-alkyl, and N,N-dialkyl derivatives. Antifungal and antibacterial studies show that some of the obtained compounds are active against Gram-positive bacteria and Candida type fungi.

Introduction
Saponins are steroid or triterpenoid glycosides found in various plants [1]. They produce a soap-like foam when shaken in aqueous solutions, which makes them useful as detergents, foaming agents and emulsifiers. Saponins are known for their structural diversity and a wide range of biological properties [2]. Among others, they display different pharmacological activities, particularly antifungal [3-6] and antitumor [7-9].

The aglycone part of a saponin is termed sapogenin. Diosgenin, yamogenin, tigogenin, smlagenin and sarsapogenin are the most abundant sapogenins in nature [10]. They are linked via a glycosidic bond to a sugar unit, mainly D-glucose. Diosgenyl glycosides constitute a very important group among spirostanol saponins. Diosgenin has a double bond between the C-5 and C-6 atoms of the spirostanol skeleton and can be found in combination with different sugars in Costus, Discorea, Paris, Solanum, Yucca, and Trillium plants [11]. The plants containing diosgenyl saponins are used in folk medicine in many countries. Such herbal medicines exhibit anti-inflammatory, antibacterial, antifungal, antiviral, diuretic and expectorant activities [2,11]. Importantly, these also help fight cancer cells [12-14].

Although saponins, in which D-galactose is directly bound with sapogenin are rarely found in plants, there are some examples of such galactosides. For example, timosaponin, isolated from an Anemarrhena asphodeloides plant, is a natural spirostanol saponin, which has D-galactose bound with sarsapogenin [15]. Tribulus plants are known to contain tigogenin linked with...
D-galactose [16]. Moreover Smilacina atropurpurea, Solanum indicum, and the genus Yucca contain smilacinicosides and funkiosides [17], indiosides [18], and elephanosides [19]. All these saponins are glycosides, where diosgenin is attached directly with D-galactose.

Besides their isolation from natural sources, the chemical syntheses of saponins have been intensively investigated to evaluate and improve their pharmacological activities [20-22]. For example, in search of new variants of steroidal glycosides, diosgenyl 2-amino-2-deoxy-β-D-glucopyranoside was synthesized [23]. Modifications of the amino group in this synthetic saponin led to analogs with promising antitumor, antifungal and antibacterial activities [24-31].

In this paper, for the first time, syntheses of diosgenyl 2-amino-2-deoxy-β-D-galactopyranoside, its hydrochloride as well as N-acyl, 2-ureido, N-alkyl, and N,N-dialkyl derivatives are presented. These new synthetic saponins were tested for their antifungal and antibacterial activities.

Results and Discussion

Chemistry

Relying on our previous experiences [30,31], we used the O-acetylated bromide 2 to synthesize diosgenyl 2-amino-2-deoxy-β-D-galactopyranoside, its hydrochloride as well as N-acyl, 2-ureido, N-alkyl, and N,N-dialkyl derivatives are presented. These new synthetic saponins were tested for their antifungal and antibacterial activities.

The synthesis of 4 started from commercially available D-galactosamine hydrochloride which was first converted to the 2-N-tetrachlorophthaloyl derivative followed by peracetylation with acetic anhydride to give fully protected galactose 1 as an anomeric mixture. The α and β anomers (3:7 molar ratio) were identified by NMR. To synthesize bromide 2, we used TiBr₄ as a bromination agent. Bromide 2 was obtained as a mixture of α and β anomers (1:4 molar ratio) and was used directly in the glycosylation. This mixture of 2 is chromatographically inseparable due to the highly reactive nature of the bromine group at the anomeric carbon, but, the anomers of 2 are readily distinguishable in the NMR spectrum (δ 6.65, d, J₁,₂ = 3.7 Hz for the α anomer and δ 6.35, d, J₁,₂ = 9.6 Hz for the β anomer).

Glycosylation of diosgenin with 2 was performed in dichloromethane by a “reverse” procedure: The glycosyl donor was added to the solution of diosgenin and the promoter (silver triflate) [31]. This procedure afforded the expected β glycoside 3 in 80% yield. The structure of 3 was determined by ¹H and ¹³C NMR supported by COSY and HSQC techniques. The β linkage in 3, as well as the ⁴C₁ conformation of the pyranose ring were confirmed by the coupling constant of the anomeric proton (J₁,₂ = 8.4 Hz). The deprotection of 3 was achieved by
using 98% hydrazine hydrate in refluxing ethanol. This procedure removes the TCP and acetyl protecting groups in a one-pot reaction and yields diosgenyl 2-amino-2-deoxy-β-D-galactopyranoside (4). Saponin 4 treated with the HCl in MeOH was converted into hydrochloride 5. To explore the influence of different modifications of the amino group in 4 on its antimicrobial activity the N-acyl (6, 7), 2-ureido (8–10), N-monoalkyl (11) and N,N-dialkyl (12, 13) derivatives of diosgenyl 2-amino-2-deoxy-β-D-galactopyranoside were also synthesized (Figure 1).

The N-acetyl derivative 6 was obtained by the treatment of 4 with acetic anhydride in methanol with Et₃N whereas N-trifluoroacetyl derivative 7 was obtained in a reaction of 4 with trifluoroacetic anhydride in pyridine. The IR, HRMS and NMR confirmed the structures of 6 and 7. For example, the IR spectra of 6 and 7 show typical amide I and amide II bands at 1715–1650 cm⁻¹. In turn, the ¹³C NMR spectrum of 7 is characterized by a quartet of the carbonyl carbon (≈157 ppm) with the Jₐₛ,F coupling constant ≈39 Hz and a quartet of the CF₃ carbon (≈117 ppm) with the Jₐₛ,F coupling constant ≈290 Hz (see Supporting Information File 1 for experimental and NMR data).

Strategies for the preparation of ureido sugars usually involve the condensation of saccharides with urea or the reaction of glycosamines, amino sugar, or aminoglycosides with isocyanates, or their equivalents such as carbamates [32,33]. Ureido saponins presented here were obtained in the reaction of ethyl isocyanate (8), chloroethyl isocyanate (9) and phenyl isocyanate (10) with the amino group of 4. Isocyanate was added to the 1:1 (v/v) chloroform–methanol solution of 4 and Et₃N each time. The yields of these reactions were very good (90% for 8) or good (60% for 9). However, phenylurea derivative 10 was isolated with only 34% yield, and the formation of various byproducts was observed in this case. The structures of saponins 8–10 were established using IR, HRMS and NMR spectra. For example, the amide I and amide II bands at 1665–1550 cm⁻¹ are visible in the IR spectra and the carbonyl carbon signals at 160–170 ppm are present in the ¹³C NMR spectra of 8–10 (see Supporting Information File 1 for experimental and NMR data).

To obtain the N-alkyl derivatives of 4, a method called “two-step reductive alkylation of amines” was chosen. This method was successfully used to prepare N-alkyl derivatives of other amino sugars, including diosgenyl aminoglycosides [34,35]. The presented reductive alkylation consists of a reaction of amine 4 with the appropriate aldehyde, which results in the respective imine subsequently reduced with sodium cyanoborohydride to the alkylated amine. Thus, the reaction of 4 with 1.5 molar excess of acetaldehyde followed by the in situ reduction with NaBH₃CN resulted in N-ethylamino (11) and N,N-diethylamino (12) saponins, which were separated by column chromatography. An analogous reaction of 4 with 3 molar excess of propionaldehyde, followed by the reduction with NaBH₃CN resulted solely in N,N-dipropylaminosaponin 13. The ¹H and ¹³C NMR spectroscopy as well as the HRMS spec-

**Figure 1:** Derivatives of diosgenyl glycosides 5–13.
Evaluation of antimicrobial activity

The antimalarial in vitro activities of 5–13 were tested against two fungal strains: Candida albicans and Candida tropicalis, and against the following six Gram-positive bacteria species: Enterococcus faecalis, Enterococcus faecium, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes and Streptococcus pneumoniae. The minimum inhibitory concentrations (MICs) were determined by the broth microdilution method according to Clinical and Laboratory Standards Institute (CLSI) recommendations (for details see Supporting Information File 1). The MICs values are grouped in Table 1 and Table 2.

The presented results indicate that some of the tested saponins are characterized by selective activity. Thus, the hydrochloride of 5 inhibits the growth of the fungus genus Candida; however, this saponin is more active against C. tropicalis (MIC 4 µg/mL) than against C. albicans (MIC 64 µg/mL). In turn, hydrochloride 5 has no inhibitory activity against the tested Gram-positive bacteria. The N-trifluoroacetyl derivative of diosgenyl 2-amino-2-deoxy-β-D-galactopyranoside 7 is active solely against E. faecalis. In contrast to 7, the N-acetyl saponin 6 was found to inhibit the growth of all the tested fungi and bacteria with MICs of 8–32 µg/mL. Among the three ureido derivatives of 4, solely diosgenyl 2-(2-chloroethylureido)-2-deoxy-β-D-galactopyranoside (9) exhibits a good activity against all the tested pathogens with MICs of 8–64 µg/mL, whereas 8 was effective against C. tropicalis and E. faecium only and compound 10 does not exhibit any antimicrobial activity. Among the presented saponins, N-ethyl derivative 11 exhibits the best antimicrobial activity against all the tested microorganisms with MIC values of 2–8 µg/mL. Both N,N-diethyl (12) and N,N-dipropyl (13) derivatives are active against both fungal and almost all the bacterial strains except E. faecium PCM 1859 and S. aureus ATCC 25923. These two strains of bacteria are completely resistant to 12 and 13, whereas the growth of E. faecalis PCM 2673 as well as S. aureus ATCC 6538, and S. aureus ATCC 6538/P is inhibited by 12 and 13.

The analysis of the relationship between structure and biological activity of the presented derivatives of diosgenyl 2-amino-2-deoxy-β-D-galactopyranoside shows that N-alkylation of the amine function is the most advantageous way of modification. An introduction of one ethyl (11), two ethyl (12) or two propyl (13) groups at the amine function improves the antifungal properties of saponins 11–13 in comparison with hydrochloride 5 and the remaining compounds. In the case of antibacterial properties, N-ethyl derivative 11 is the most active among the all tested compounds. Moreover, derivative 11 acts against all tested Gram-positive bacteria. An introduction of an additional ethyl group worsens the antibacterial activity of 12 in comparison with 11, particularly against E. faecium and S. aureus ATCC 25923. Two propyl groups cause that 13 exhibits even a

### Table 1: Minimum inhibitory concentration (MIC) [µg/mL] for 5–13 against two fungi.

| Compd. | Candida albicans | Candida tropicalis |
|--------|------------------|-------------------|
| 5      | 64               | 4                 |
| 6      | 16               | 8                 |
| 7      | >1024            | >1024             |
| 8      | >1024            | 64                |
| 9      | 64               | 16                |
| 10     | >1024            | >1024             |
| 11     | 8                | 4                 |
| 12     | 4                | 2                 |
| 13     | 8                | 8                 |

### Table 2: Minimum inhibitory concentration (MIC) [µg/mL] for 5–13 against Gram-positive bacteria.

| Compd. | E. faecalis S. aureus ATCC 25923 | S. aureus ATCC 6538 | S. aureus ATCC 6538/P | S. epidermidis | S. pyogenes | S. pneumoniae |
|--------|---------------------------------|---------------------|-----------------------|----------------|------------|--------------|
| 5      | >1024                           | >1024               | >1024                 | >1024          | >1024      | >1024        |
| 6      | 16                              | 8                   | 16                    | 32             | 8          | 8            |
| 7      | 32                              | >1024               | >1024                 | >1024          | >1024      | >1024        |
| 8      | 512                             | 16                  | >1024                 | >1024          | >1024      | >1024        |
| 9      | 16                              | 8                   | 16                    | 16             | 32         | 8            |
| 10     | >1024                           | >1024               | >1024                 | >1024          | >1024      | >1024        |
| 11     | 8                               | 2                   | 4                     | 4              | 8          | 2            |
| 12     | 16                              | >1024               | >1024                 | 16             | 64         | 8            |
| 13     | 64                              | >1024               | >1024                 | 64             | 128        | 32           |
weaker inhibitory effectiveness than 12. These findings are in full agreement with our previous findings concerning N-alkyl and N,N-dialkyl derivatives of diosgenyl 2-amino-2-deoxy-β-D-glucopyranoside [31]. Monoethyl derivatives of both glucosamine and galactosamine are the most active compounds against Gram-positive bacteria in the tested groups, regardless of the fact that hydrochloride of aminogalactoside 5 is completely inactive with respect to the tested bacteria whereas analogous hydrochloride of aminoglucoside is relatively active.

Transformation of the 2-amino group into a 2-ureido group rather negatively influences both antifungal and antibacterial activities of the presented saponins. Among the ureido compounds (8–10) only chloroethylyureido derivative 9 exhibits weak inhibitory activity against tested Gram-positive bacteria. Its ethylureido analog 8 is inactive in respect of both fungi and bacteria, likewise phenylethylyureido derivative 10. It would seem that an electronegative Cl atom improves the inhibitory properties of ureido saponins against Gram-positive bacteria. However, the influence of the more electronegative three F atoms on an inhibitory effectivity of trifluoroacetyl derivative 7 is opposite. If N-acetyl derivative 6 is relatively active against tested fungi and bacteria, its N-trifluoroacetyl analog 7 does not act at all. The presented results show how complicated the relationships between structure and biological activity are.

Conclusion

Diosgenyl 2-amino-2-deoxy-β-D-galactopyranoside, as well as its two N-acyl, three 2-ureido and three N-alkyl derivatives are reported. N-Alkylation of the amine function seems to be the most advantageous way of modification. Among the tested compounds, the N-alkyl derivatives are the most active against tested fungi and the N-ethyl derivative exhibits the best inhibitory activity against Gram-positive bacteria. The latter finding is in full agreement with our previous findings concerning the N-ethyl derivative of D-glucosamine. Apart from N-ethyl also N-acetyl and 2-chloroethylyureido derivatives exhibit activity against Gram-positive bacteria. The change of D-glucosamine into D-galactosamine in diosgenyl 2-amino-2-deoxy-β-D-glycopyranoside impairs the antimicrobial properties of its hydrochloride.

Acknowledgements

This research was financed by the European Union within the European Regional Development Fund – Grant UDA-P01.01.02.-14-102/09 and DS/530-8457-D603-17.

References

1. Chindo, B. A.; Adzu, B.; Gamananiel, K. S. Saponins: structural diversity, properties and applications. In Saponins: properties, applications and health benefits; Koh, R.; Tay, I., Eds.; Nova Science Publishers Inc.: New York, 2012; pp 1–50.

2. Sparag, S. G.; Light, M. E.; van Staden, J. J. Ethnopharmacol. 2004, 94, 219–243. doi:10.1016/j.eph.2004.05.016

3. Ionizzi, M.; Lanzotti, V.; Ranalli, G.; De Marino, S.; Zollo, F. J. Agric. Food Chem. 2002, 50, 4310–4316. doi:10.1021/jf0116911

4. Zhang, J.-D.; Cao, Y.-B.; Xu, Z.; Sun, H.-H.; An, M.-M.; Yan, L.; Chen, H.-S.; Gao, P.-H.; Wang, Y.; Jia, X.-M.; Jiang, Y.-Y. Biol. Pharm. Bull. 2005, 28, 2211–2215. doi:10.1248/bpb.28.2211

5. Yang, C.-R.; Zhang, Y.; Jacob, M. R.; Khan, S. I.; Zhang, Y.-J.; Li, X.-C. Antimicrob. Agents Chemother. 2006, 50, 1710–1714. doi:10.1128/AAC.50.5.1710-1714.2006

6. Chapagain, B. P.; Wiesman, Z.; Taror (Lahkim), L. Ind. Crops Prod. 2007, 26, 109–115. doi:10.1016/j.indcrop.2007.02.005

7. Man, S.; Gao, W.; Zhang, Y.; Huang, L.; Liu, C. Filoterpapia 2010, 81, 703–714. doi:10.1016/j.filote.2010.06.004

8. Pérez-Labrada, K.; Brouard, I.; Estévez, I.; Marrero, M. T.; Estévez, F.; Bermejo, J.; Rivera, D. G. Biolog. Med. Chem. 2012, 20, 2690–2700. doi:10.1016/j.bmc.2012.02.026

9. Liu, Z.; Gao, W.; Jing, S.; Zhang, Y.; Man, S.; Wang, Y.; Zhang, J.; Liu, C. J. Ethnopharmacol. 2013, 149, 422–430. doi:10.1016/j.eph.2013.06.033

10. Thakur, M.; Melzig, M. F.; Fuchs, H.; Weng, A. Bot. Targets Ther. 2011, 1, 19–29.

11. Yang, C.-R.; Tanaka, O. Advances in Plant Glycosides, Chemistry and Biology; Elsevier: Amsterdam, 1999.

12. Hou, S. J.; Zou, C. C.; Zhou, L.; Xu, P.; Yu, D. Q.; Lei, P. S. Chin. Chem. Lett. 2007, 18, 769–772. doi:10.1016/j.ccl.2007.05.007

13. Wang, B.; Chun, J.; Liu, Y.; Han, L.; Wang, Y.-s.; Joo, E.-J.; Kim, Y.-S.; Cheng, M.-s. Org. Biomol. Chem. 2012, 10, 8822–8834. doi:10.1039/c2ob26579f

14. Bansal, R.; Acharya, P. C. Chem. Rev. 2014, 114, 6986–7005. doi:10.1021/cr4002935

15. Fang, M.; Gu, L.; Gu, G.; Fang, J. J. Carbohydr. Chem. 2012, 31, 197–202. doi:10.1080/07328303.2011.639966

16. Gu, G.; An, L.; Fang, M.; Guo, Z. Carbohydr. Res. 2014, 383, 21–26. doi:10.1016/j.carres.2013.10.015

17. Yang, S.-L.; Liu, X.-K.; Wu, H.; Wang, H.-B.; Qing, C. Steroids 2009, 74, 7–12. doi:10.1016/j.steroids.2008.08.008

18. Gao, J.; Li, X.; Gu, G.; Sun, B.; Cui, M.; Ji, M.; Lou, H.-X. Bioorg. Med. Chem. Lett. 2011, 21, 622–627. doi:10.1016/j.bmcl.2010.12.046

19. Zhang, Y.; Zhang, Y.-J.; Jacob, M. R.; Li, X.-C.; Yang, C.-R. Phytochemistry 2008, 69, 264–270. doi:10.1016/j.phytochem.2007.06.015

20. Yu, B.; Zhang, Y.; Tang, P. Eur. J. Org. Chem. 2007, 5145–5161. doi:10.1002/ejoc.200700542

21. Zhao, Y.; An, L.; Gu, G.; Guo, Z. J. Carbohydr. Chem. 2014, 33, 152–168. doi:10.1080/07328303.2014.900564

22. Yang, Y.; Laval, S.; Yu, B. Adv. Carbohydr. Chem. Biochem. 2014, 71, 137–226. doi:10.1016/B978-0-12-800128-8.00002-9

Supporting Information

Supporting Information File 1

Experimental procedures for the preparation of compounds 1–13, spectroscopic data and information on the method of determination of the minimum inhibitory concentration.

[http://www.beilstein-journals.org/bjoc/content/supplementary/1860-5397-13-227-S1.pdf]
23. Bednarczyk, D.; Kaca, W.; Myszka, H.; Serwecińska, L.; Smiatacz, Z.; Zaborowski, A. Carbohydr. Res. 2000, 328, 249–252. doi:10.1016/S0008-6215(00)00199-3
24. Myszka, H.; Bednarczyk, D.; Najder, M.; Kaca, W. Carbohydr. Res. 2003, 338, 133–141. doi:10.1016/S0008-6215(02)00407-X
25. Kaskiw, M. J.; Tassotto, M. L.; Th'ng, J.; Jiang, Z.-H. Bioorg. Med. Chem. 2008, 16, 3209–3217. doi:10.1016/j.bmc.2007.12.022
26. Kaskiw, M. J.; Tassotto, M. L.; Mok, M.; Tokar, S. L.; Pycko, R.; Th'ng, J.; Jiang, Z.-H. Bioorg. Med. Chem. 2009, 17, 7670–7679. doi:10.1016/j.bmc.2009.09.046
27. Cirioni, O.; Myszka, H.; Dawgul, M.; Ghiselli, R.; Orlando, F.; Silvestri, C.; Brescini, L.; Kamysz, W.; Guerrieri, M.; Giacometti, A. J. Med. Microbiol. 2011, 60, 1337–1343. doi:10.1099/jmm.0.031708-0
28. Wang, B.; Liu, Y.; Wang, Y.; Liu, X.; Cheng, M.-S. Bioorg. Med. Chem. Lett. 2012, 22, 7110–7113. doi:10.1016/j.bmcl.2012.09.075
29. Fernández-Herrera, M. A.; López-Muñoz, H.; Hernández-Vázquez, J. M. V.; Sánchez-Sánchez, L.; Escobar-Sánchez, M. L.; Pinto, B. M.; Sandoval-Ramírez, J. Eur. J. Med. Chem. 2012, 54, 721–727. doi:10.1016/j.ejmech.2012.06.027
30. Bednarczyk, D.; Walczewska, A.; Grzywacz, D.; Sikorski, A.; Liberek, B.; Myszka, H. Carbohydr. Res. 2013, 367, 10–17. doi:10.1016/j.carres.2012.11.020
31. Walczewska, A.; Grzywacz, D.; Bednarczyk, D.; Dawgul, M.; Nowacki, A.; Kamysz, W.; Liberek, B.; Myszka, H. Beilstein J. Org. Chem. 2015, 11, 869–874. doi:10.3762/bjoc.11.97
32. Jiménez Blanco, J. L.; Ortega-Caballero, F.; Ortiz Mellet, C.; García Fernández, J. M. Beilstein J. Org. Chem. 2010, 6, No. 20. doi:10.3762/bjoc.6.20
33. McKay, M. J.; Nguyen, H. M. Carbohydr. Res. 2014, 385, 18–44. doi:10.1016/j.carres.2013.08.007
34. Cai, J.; Davison, B. E.; Gianellin, C. R.; Thaisrivongs, S.; Wibley, K. S. Carbohydr. Res. 1997, 300, 109–117. doi:10.1016/S0008-6215(97)00039-6
35. Liberek, B.; Melcer, A.; Osuch, A.; Wakieć, R.; Milewski, S.; Wiśniewski, A. Carbohydr. Res. 2005, 340, 1876–1884. doi:10.1016/j.carres.2005.05.013

License and Terms

This is an Open Access article under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The license is subject to the Beilstein Journal of Organic Chemistry terms and conditions: (http://www.beilstein-journals.org/bjoc)

The definitive version of this article is the electronic one which can be found at: doi:10.3762/bjoc.13.227