DOI 10.51231/2667-9507-2024-007-03-70-75

Dynamics of β-glucosidase enzymatic activity in Yucca gloriosa L. leaves

Tsisana Giorgadze

European University, Tbilisi, Georgia

Abstract

The steroidal sapogenin tigogenin from Yucca gloriosa L. leaves is a raw material for the synthesis of steroidal hormonal preparations. Tigogenin is spirostan sapogenin. In the plant intact leaves are synthesized furostanol glycosides, which are transformed into spirostanol glycosides by the action of the enzyme **β-**glucosidase. The maximum of **β**-glucosidase enzymatic activity is observed in July and August. The results have a great significance for optimization of tigogenin production technology.

KEYWORDS: β-glucosidase; Yucca gloriosa L.; tigogenin; furostanol glycosides; spirostanol glycosides

Introduction

As early as in the 70s of the last century, I. Kutateladze Institute of Pharmacochemistry of the Georgian Academy of Sciences found that from all the species of the genus Yucca, introduced in our area for the decorative purposes, the leaves of Yucca gloriosa L. deserve particular attention. Leaves generally biosynthesize single aglycone-produced steroidal glycosides; this has simplified tigogenin isolation from the raw [1-4]. In cooperation with the All-Union Scientific Research Chemical-Pharmaceutical Institute, tigogenin was transformed into the initial products of the synthesis of steroidal hormonal drugs: 5α-pregnen-16-en-3β-20-oneacetate and 5α-androstan-3β-ol-17-ol acetate. The synthesis of a number of hormonal preparations was carried out from the latter: dihydrotestosterone and the ether derivatives thereof: Althesin, Dexamethasone, Mestranol, Methylandrostenol, anti-tubercular isonicotinic hydrazones and other 5α-steroids. Tigogenin from Yucca gloriosa was recognized as the most cost-effective industrial raw material for the synthesis of 5α-steroids [5-13].

The Institute of Pharmacochemistry developed a highly effective method of vegetative propagation of Yucca gloriosa; 200ha plantations were set up in Eastern Georgia in accordance with the elaborated agrarian recommendations [14].

As is known, steroidal glycosides are accumulated in plant in two forms: in furo – and spirostanol. For the isolation of tigogenin as a spirostanol aglycone from the plant, it is important that furostanols be converted into the spiro-form. This process in plant is carried out under the influence of β-glucosidase endogen enzyme. Our researches revealed that furostanolic glycosides in the leaves of Yucca gloriosa are localized in the epidermis of the leaf, while enzyme is active in the leaf mesophyll [15].

Two forms of β-glucosidase enzyme were found in the leaves of Yucca gloriosa: the first one catalyzes the hydrolysis of both, oligofurostanosides and 4-nitrophenyl-β-D-glucopyranoside synthetic substrate, while the second form shows the activity only towards the synthetic substrate. The first form of β-glucosidase exhibits a greater affinity for the natural substrate than for the synthetic substrate [16,17].

The purpose of the present paper was to study the dynamics of β-glucosidase enzyme activity in the leaves of Yucca gloriosa.

Materials and methods

The upper, middle and lower layers of the young Yucca gloriosa plant growing in Georgia were used as a research object. The dynamics was studied for 3 years during the vegetation period of the plant, in June, July and August. β-glucosidase activity was determined by the commonly known method [18]. The sum of furostanol glycosides of Yucca gloriosa isolated from Yucca gloriosa leaves through a commonly known method was used as a natural substrate [19]. 4-nitrophenyl-β-D-glucopyranoside by the Czech "La-Chema" company was used as a synthetic substrate.The amount of enzyme catalyzing 1nmol substrate hydrolysis per 1 minute was taken as a unit of glucosidase activity, while the specific activity was calculated per 1mg of protein. Enzyme activity was measured threefold. The results are presented in the table.

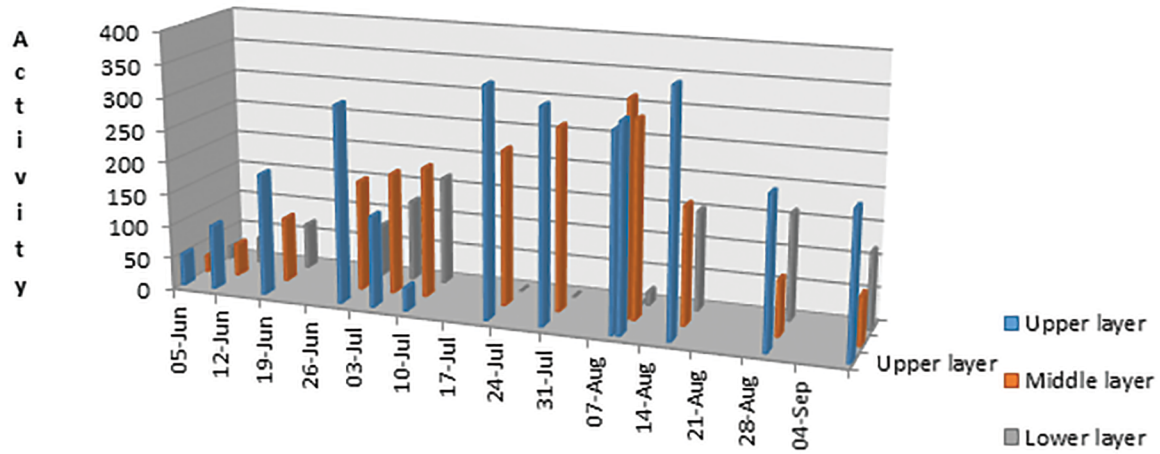
TABLE. The dynamics of β-glucosidase form 1 activity in Yucca gloriosa leaves during the plant vegetation period (the average of three parallel testing)

Date	Activity /Upper layer/	Activity /Middle layer/	Activity /Lower layer/
June 5	50	26	20
June 10	100	50	40
June 18	188	100	70
June 30	304	170	80
July 5	140	186	124
July 10	36	200	166
July 22	350	238	0
July 30	326	280	0
August 9	300	330	0
August 10	314	300	20
August 17	370	180	152
August 30	230	84	162
September 10	220	74	116

The table shows the three year data average.

Results and discussion

Experimental studies have shown that β-glucosidase form 1 specific activity in the leaves of Yucca gloriosa varies considerably during the plant vegetation period.



	05-Jun	10-Jun	18-Jun	30-Jun	05-Jul	10-Jul	22-Jul	30-Jul	09-Aug	10-Aug	17-Aug	30-Aug	10-Sep	
Upper layer	50	100	188	304	140	36	350	326	300	314	370	230	220	
Middle layer	26	50	100	170	186	200	238	280	330	300	180	84	74	
Lower layer	20	40	70	80	124	166	0	0	0	20	152	162	116	

Fig.1.The dynamics of β-glucosidase form 1 activity in Yucca gloriosa leaves during the plant vegetation period.

In the upper and lower layer leaves, the β-glucosidase form 1 activity increased several times in the period from May till August, while in the middle-layer leaves – it has abruptly increases during the mentioned period. Starting from September, the enzyme activity dropped and was practically not observed during the winter period. The maximum enzyme activity was reported in July-August.

The obtained results are of great importance for determining the period for collecting raw material, as well as for the fermentation conditions in order to achieve maximum tigogenin extraction.

References

1. Jiménez GG, Durán AG, Macías FA, Simonet AM. Structure, Bioactivity and Analytical Methods for the Determination of Yucca Saponins Molecules 2021;26(17): 5251. doi: https://doi.org/10.3390/molecules26175251.
2. Favel A, Kemertelidze E, Benidze M, Fallague K, Regli P. Antifungal activity of steroidal glycosides from Yucca gloriosa L. Phytotherapy Research. 2005;19(2):158-161. doi: https://doi.org/10.1002/ptr.1644.
3. Kemertelidze ÉP, Benidze MM, Skhirtladze AV. Steroid compounds from Yucca gloriosa L. introduced into Georgia and their applications. Pharmaceutical Chemistry Journal. Published: 2009;43:45-47. DOI:10.1007/s11094-009-0230-2.
4. Kemertelidze E, Pkheidze T, Grinenko G, Menshova N. Tigogenin, a vegetable raw-material for synthesis of steroidal structures preparation. Planta Medica. 1979;36(3):365-366.
5. Nadaraia N, Barbakadze N, Mulkijanyan K, Mshvildadze V, et al. Legault Jean. Synthesis of some novel nitrogen-containing 5α-steroids based on tigogenin. Bulletin of the Georgian National Academy of Sciences. 2021;15(3):65-71.
6. Nadaraia N, Barbakadze N, Kakhabrishvili M, Mulkijanyan K, Mshvildadze V, et al. Legault Jean. (2022). Synthesis of New 5α-Steroidal Hydrazones from Tigogenin. Bulletin of the Georgian National Academy of Sciences. 2022;16(2):129-134.
7. Merlani MI, Amiranashvili LSh, Men'shova NI, Kemertelidze EP. Synthesis of 5α – androstan-3β,17β-diol from tigogenin. Chem. Nat. Comp. 2007;43(1):97-99. doi: https://doi.org/10.1007/s10600-007-0041-1.
8. Merlani MI, Amiranashvili LS, Mulkidzhanyan KG et al. Synthesis and antituberculosis activity of certain steroidal derivatives of the 5α-series. Chem Nat Compd. 2008;44: 618-620. doi: https://doi.org/10.1007/s10600-008-9126-8.
9. Merlani MI, Amiranashvili LS, Kemertelidze, EP. et al. Synthesis and antimycobacterial activity of some steroidal derivatives of tigogenin. Chem Nat Compd. 2009;45:389-392. doi: https://doi.org/10.1007/s10600-009-9335-9.
10. Barbakadze NN, Nadaraia NS, Kakhabrishvili ML, et al. Synthesis from Tigogenin of 17β-Amino-5α-Androstan-3β-Ol Peptide Derivatives. Chem Nat Compd. 2016;52:445-447. doi: https://doi.org/10.1007/s10600-016-1669-5.
11. Nadaraia NS, Kakhabrishvili ML, Onashvili EO, et al. Synthesis of Several 5α-Androstano[17,16-d]Pyrazolines from Tigogenin.

Chem Nat Compd. 2014;50:1024-1028. doi: https://doi.org/10.1007/s10600-014-1151-1.

12. Merlani MI, Amiranashvili LS, Kemertelidze EP. Synthesis of Several 5α-D-Homosteroid Derivatives Based on Tigogenin. Chem Nat Compd. 2014;50:480-482. doi: https://doi.org/10.1007/s10600-014-0992-y.
13. Kemertelidze E. Biologically Active Compounds and Original Remedies from Plants Growing in Georgia. Bulletin of the Georgian National Academy of Sciences. 2007;175(1).
14. Gurielidze KG, Giorgadze TA, Kemertelidze EP, Pruidze GN. Location of olligofurostanosides and their hydrolase in the leaves of Yucca gloriosa. Fiziol. Rast. 1992:39:300-304.
15. Giorgadze Ts, Gurielidze K, Kemertelidze E, Pkheidze T. Some Properties of Yucca Gloriosa L. Leaves Glucohydrolases Catalyzing Degradation of Oligofurostanosides. Bulletin of the Academy of Sciences of Georgia. 1991;144(3):425-428.
16. Gurielidze KG, Giorgadze TsA, Kemertelidze EP, Pruidze GN. Location of Oligofurostanosides and their Hydrolase in the Leaves of Yucca Gloriosa L. Fiziol. Rast. 1992;39:300-304.
17. Gurielidze KG, Paseshnichenko VA, Vasileva IS. Glucohydrolases of Leaves and Rhizomes of Dioscorea Deltoidea. Biochemistry.1987;52(4):562.
18. Gurielidze KG, Vasil'eva IS, Paseshnichenko VA, Vorob'ev AS, Nosov AM.
19. Method for obtaining oligofurostanozides. certificate of authorship. 1995. SU 1453667 A1. eLIBRARY ID: 41073525. https://elibrary.ru/bgjqeu.