Arch. Pharm. (Weinheim) *320*, 767–768 (1987)

Selective Enzymatic Transformation of Lanatoside A

Selektive enzymatische Umsetzung von Lanatosid A

Mihajlo Stanković* and Siniša Djordjević

* Faculty of Technology, University of Niš, and Pharmaceutical and Chemical Works „Zdravlje" 16 000 Leskovac, Yugoslavia

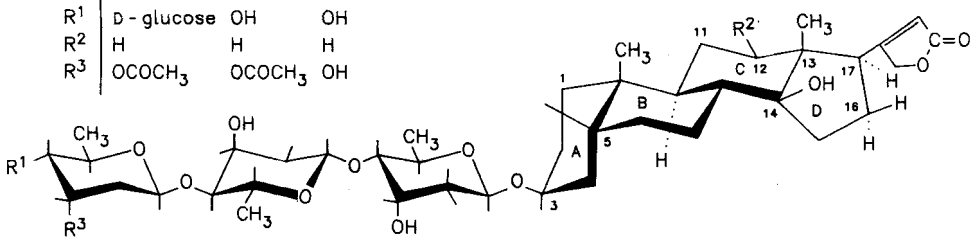
Received March, 27th, 1987

The transformations of the cardiac glycosides lanatoside A, B and C can be performed enzymatically with digilanidase (β-glucosidase) and acetyl-esterase from plant, animal or microbial sources[1–4]. In this paper the transformation with enzymes from plant sources (barleycorns, hemp- and flax-seed) as well as with a comercial preparation of biocellulase (Biocon GmbH Kolbemoor) was investigated, wich has not been used for this purpose yet. The enzyme preparations used were prepared by our procedure[5]. From the investigation of the degree and products of enzymatic hydrolysis of **1a** in aqueous suspension (37 °; enzyme preparation: substrate = 8:1; pH 6.8–7.0; 48 h) it was established that the selective biotransformation of **1a** to acetyl-digitoxine **(1b)** and digitoxine **(1c)** occured with the enzyme preparation from flax-seed and the comercial biocellulase, respectivelly. The other two enzyme preparations investigated had significantly less activity (three to four times) and were not selective, i. e. a mixture of **1b** and **1c** was obtained (table 1).

The kinetic investigation of the enzymatic hydrolysis and the change of the enzyme activity during the hydrolysis under optimum conditions showed that the maximum degrees of hydrolysis of **1a** with the enzyme preparation from flax seed and with biocellulase (65 % and 68 %, respectivelly) were obtained after 48 h.

	1a	1b	1c
R^1	D - glucose	OH	OH
R^2	H	H	H
R^3	$OCOCH_3$	$OCOCH_3$	OH

Selective enzymic transformation of Lanatoside A



Tab. 1: Products and grades of biotransformation of 1a

Enzyme preparations	Products	Grade biotransformation (%)	1c:1b
Biocellulase	**1c**	68	0:100
From flax seed	**1b**	65	100: 0
From barley seed	**1b + 1c**	15	80: 20
From hamp seed	**1b + 1c**	15	51: 49

0365-6233/87/0808-767 $ 02.50/0

1b and **1c** were separated from **1a** using column chromatography (alumina; chloroform and chloroform/MeOH 1:1). According to their physico-chemical properties (m.p.; $[\alpha]^{28}$; UV- and IR-spectra; colour reactions) the produced glycosides **1b** and **1c** correspond to the standards of these substances.

Experimental

Preparation of enzyme: By our procedure[5]

Choice of selective enzyme preparation and investigation of conditions and kinetics of enzymatic transformation

The enzyme preparation and **1a** (50 mg) were suspended in water and incubated by varying the parameters of enzymatic transformation (pH, temp., quantity of enzyme preparation, reaction time). The reaction products **1a** were extracted with chloroform/MeOH (4:1; 10 ml) and the extracts were evaporated to dryness. The contents of **1b**, **1c** and **1a** were determined by TLC followed by a spectrophotometric method[6, 7].

Preparative production of **1b** *and* **1c**

Enzymatic hydrolysis of **1a** (5 g) was performed under optimum conditions using the enzyme preparation from flax seed and biocellulase. The glycosides were extracted from the reaction medium with chloroform/MeOH (4:1), and the solvents were evaporated in vacuo. The glycosides were separated by column chromatography with alumina and chloroform to eluate **1b** and **1c** or using chloroform/MeOH (1:1) to eluate **1a**. The fractions were analyzed qualitatively by TLC[6, 7]. The separated glycosides were recrystallized from MeOH.

References

1 Hagers Handbuch der Pharmazeutischen Praxis, vollständige (4.) Neuausgabe, IV Bd., S. 581, Springer Verlag, Berlin–Heidelberg–New York 1973.
2 G. Baumgarten, Herzwirksame Glycoside, S. 48, VEB Georg Thieme, Leipzig 1963.
3 A. Stoll and W. Kreis, Helv. Chim. Acta *17*, 592 (1934).
4 M. Wichtl, Pharmazie in unserer Zeit *2*, 33 (1978).
5 M. Stanković, S. Djordjević, S. Stanković and O. Stojanović, Hem. Ind. *37*, 241 (1983); C. A. *100*, 33210e (1984).
6 B. Pekić and S. Djordjević, Arh. Farm. *20*, 1 (1970); C. A. *73*, 91269r (1970).
7 B. Pekić, Acta Pharm. Jugoslav. *18*, 141 (1968); C. A. *73*, 48568a (1970). [KPh 439]

Verantwortlich für die Redaktion: Prof. Dr. W. Wiegrebe, Pharmazeutisches Institut der Universität Regensburg, Universitätsstraße 31, Postfach 397, D-8400 Regensburg. – Anzeigenleitung: R. J. Roth, D-6940 Weinheim – VCH Verlagsgesellschaft mbH (Geschäftsführer: Prof. Dr. Helmut Grünewald und Hans Dirk Köhler), Postfach 1260/1280, D-6940 Weinheim – Alle Rechte, insbesondere die der Übersetzung in fremde Sprachen, vorbehalten. Kein Teil dieser Zeitschrift darf ohne schriftliche Genehmigung des Verlages in irgendeiner Form – durch Photokopie, Mikrofilm oder irgendein anderes Verfahren – reproduziert oder in eine von Maschinen, insbesondere von Datenverarbeitungsmaschinen verwandbare Sprache übertragen oder übersetzt werden. – All rights reserved (including those of translation into foreign languages). No part of this issue may be reproduced in any form – photoprint, microfilm, or any other means – nor transmitted or translated into a machine language without the permission in writing of the publishers. – Von einzelnen Beiträgen oder Teilen von ihnen dürfen nur einzelne Vervielfältigungsstücke für den persönlichen und sonstigen eigenen Gebrauch hergestellt werden. Die Weitergabe von Vervielfältigungen, gleichgültig zu welchem Zweck sie hergestellt werden, ist eine Urheberrechtsverletzung. – Die Wiedergabe von Gebrauchsnamen, Handelsnamen, Warenbezeichnungen u. dgl. in dieser Zeitschrift berechtigt nicht zu der Annahme, daß solche Namen ohne weiteres von jedermann benutzt werden dürfen. Es handelt sich häufig um gesetzlich eingetragene Warenzeichen, auch wenn sie in dieser Zeitschrift nicht als solche gekennzeichnet sind. – Satz: Rheinhessische Druckwerkstätte, Alzey. – Unverlangt zur Rezension eingehende Bücher werden nicht zurückgesandt.