Journal of Molecular Catalysis B: Enzymatic 117 (2015) 45–53

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Microbial transformations of betulinic and betulonic acids

Animesh Goswami[a,*,1], Zhiwei Guo[a,1], Thomas P. Tully[a,1], Frank A. Rinaldi[a,2], Xiaohua Stella Huang[b,3], Jacob J. Swidorski[b,4], Alicia Regueiro-Ren[b,4]

[a] Research and Development, Bristol-Myers Squibb, One Squibb Drive, New Brunswick, NJ 08903, United States
[b] Research and Development, Bristol-Myers Squibb, 5 Research Parkway, Wallingford, CT 06492, United States

ARTICLE INFO

Article history:
Received 17 March 2015
Received in revised form 16 April 2015
Accepted 20 April 2015
Available online 27 April 2015

Keywords:
Betulinic acid
Betulonic acid
Microbial transformation
Enzymatic hydroxylation
Enzymatic oxidation

ABSTRACT

Enzymatic transformation of betulinic acid by growing cells of microorganisms provided several hydroxylated and oxidized products. *Bacillus megaterium* SC16644 gave 7β,15α-dihydroxybetulinic acid, 7β,15α-dihydroxybetulonic acid, and a new compound 7β,15α,23-trihydroxybetulinic acid [3β,7β,15α,23-tetrahydroxy-lup-20(29)en-28-oic acid]. Another strain of *B. megaterium* SC6394 produced 30-oxobetulonic acid, and a mixture of 30-hydroxybetulonic acid and a new compound 7β-hydroxy-30-oxobetulonic acid [7β-hydroxy-3,30-dioxo-lup-20(29)en-28-oic acid]. Three products were obtained from the biotransformation of betulinic acid by *Streptomyces fragilis* SC16401: 7β-hydroxybetulonic acid, and two new compounds 2α,7β-dihydroxybetulinic acid [2α,3β,7β-trihydroxy-lup-20(29)en-28-oic acid] and 2-oxo-7β-hydroxy-betulinic acid [2-oxo-3β,7β-dihydroxy-lup-20(29)en-28-oic acid]. *Cunninghamella elegans* SC16025 gave 25-hydroxybetulinic acid from betulinic acid. Oxidation of betulinic acid by *Aspergillus terreus* SC16513 led to two A-ring fission products: 4-hydroxy-3,4-seco-lup-20(29)-en-3,28-dioic acid and 3,4-seco-lup-20(29), 4(23)-dien-3,28-dioic acid. *B. megaterium* SC16644 catalyzed transformation of betulonic acid provided 7β-hydroxybetulonic acid, 7β,15α-dihydroxybetulonic acid, and a new compound 7β,15α,30-trihydroxybetulonic acid [3-oxo-7β,15α,30-trihydroxy-lup-20(29)en-28-oic acid].

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Lupeol [3β-hydroxy-lup-20(29)en] **1a**, betulin [3β,28-dihydroxy-lup-20(29)en] **1b**, betulinic acid [3β-hydroxy-lup-20(29)en-28-oic acid] **1c** and betulonic acid [3-oxo-lup-20(29)en-28-oic acid] **1d** are naturally occurring pentacyclic triterpenes belonging to the lupane group isolated from many plants. Betulinic acid (**1c**) derivatives were reported to have anticancer [1–3] and anti-HIV [4–7] activities. Several semi-synthetic derivatives of betulinic acid **1c** were found to improve oral bioavailability and pharmacological activity [5–7] where the synthetic modifications were mostly centered on addition of sugars or polar groups to the C-3 hydroxyl and C-28 carboxyl groups of betulinic acid **1c** (Fig. 1; Table 1).

Several microbial transformations of betulinic acid and its derivatives were reported to prepare hydroxylated/oxygenated derivatives to improve the polarity and pharmacological properties. Microbial transformation of betulinic acid **1c** by *Cunninghamella echinulata* NRRL 5695 produced C-28 β-D-glucoside **1e** which showed no in vitro activity against melanoma [8]. Microbial transformation of betulinic acid **1c** by *Bacillus megaterium* ATCC14581 produced betulonic acid **1d**, 7β-hydroxybetulinic acid [3β,7β-dihydroxy-lup-20(29)en-28-oic acid] **1f** and 6α,7β-dihydroxybetulinic acid [3β,6α,7β-trihydroxy-lup-20(29)en-28-oic acid] **1g**; by *C. elegans* ATCC9244 gave 1β,7β-dihydroxybetulinic acid [1β,3β,7β-trihydroxy-lup-20(29)en-28-oic acid] **1h**; while *Mucor mucedo* UI4605 afforded 7β-hydroxybetulinic acid [3β,7β-dihydroxy-lup-20(29)en-28-oic acid] **1f** [9]. Another strain of *B. megaterium* ATCC13368 catalyzed the transformation of betulinic acid **1c** to four products: betulonic acid [3-oxo-lup-20(29)en-28-oic acid] **1d**, 11α-hydroxybetulonic acid [3-oxo-11α-hydroxy-lup-20(29)en-28-oic acid] **1i**, 1β-hydroxybetulonic acid [3-oxo-1β-hydroxy-lup-20(29)en-28-oic acid] **1j** and 7β,15α-dihydroxybetulinic acid

* Corresponding author at: Chemical Development, New Brunswick, NJ, United States. Tel.: +1 732 227 6225; fax: +1 732 227 3994.
E-mail address: animesh.goswami@bms.com (A. Goswami).
[1] Chemical Development, New Brunswick, NJ, United States.
[2] Analytical & Bioanalytical Development, New Brunswick, NJ, United States.
[3] Discovery Analytical Sciences, Wallingford, CT, United States.
[4] Discovery Chemistry, 5 Research Parkway, Wallingford, CT, United States.

http://dx.doi.org/10.1016/j.molcatb.2015.04.012
1381-1177/© 2015 Elsevier B.V. All rights reserved.

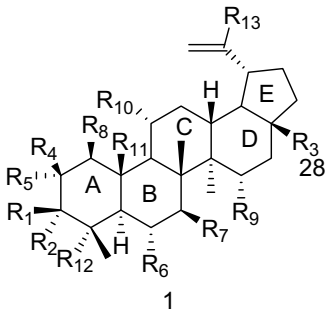
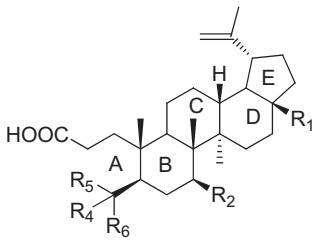


Fig. 1. Structures of starting materials and biotransformation products. See Table 1 for R groups.



2a, R_1 = CH_2OH, R_2 = H, R_4=OH, R_5=R_6=CH_3
2b, R_1 = COOH, R_2 = H, R_4=OH, R_5=R_6=CH_3
2c, R_1 = OH, R_2 = OH, R_4=OH, R_5=R_6=CH_3
2d, R_1 = COOH, R_2 = OH, R_4+R_5=CH_2, R_6=CH_3

Fig. 2. Structures of biotransformation products obtained via cleavage of A-ring.

[3β,7β,15α-trihydroxy-lup-20(29)en-28-oic acid] **1k** [10]. Microbial transformations by *Chaetomium longirostre* provided 4,28-dihydroxy-3,4-seco-lup20(29)en-3-oic acid **2a** and 4-hydroxy-3,4-seco-lup20(29)en-3,28-dioic acid **2b** from betulin **1b**, and 4,7β,17-trihydroxy-3,4-seco-28-norlup-20(29)en-3-oic acid **2c** and 7β,15α-dihydroxybetulonic acid [7β,15α-dihydroxy-3-oxo-lup-20(29)en-28-oic acid] **1l** from betulonic acid **1d** [11] where the compounds **2a**–**c** were formed by the cleavage of ring A (Fig. 2). Biotransformations of both betulinic **1c** and betulonic acids **1d** by several fungi were studied [12] – 7β,15α-dihydroxybetulonic acid [7β,15α-dihydroxy-3-oxo-lup-20(29)en-28-oic acid] **1l**, 7β-hydroxybetulonic acid [3-oxo-7β-hydroxy-lup-20(29)en-28-oic acid] **1m** and 7β,30-dihydroxybetulonic acid [7β,30-dihydroxy-3-oxo-lup-20(29)en-28-oic acid] **1n** were produced by betulonic acid **1d** and *Arthrobotrys* sp., 15α-hydroxybetulonic acid [3-oxo-15α-hydroxy-lup-20(29)en-28-oic acid] **1o** was produced by betulinic acid **1c** and *Colletotrichum* sp., 7β,15α-dihydroxybetulonic acid [7β,15α-dihydroxy-3-oxo-lup-20(29)en-28-oic acid] **1l** and 15α-hydroxybetulonic acid [3-oxo-15α-hydroxy-lup-20(29)en-28-oic acid] **1o** were obtained from betulonic acid **1d** and *Colletotrichum* sp., 25-hydroxybetulonic acid [3-oxo-25-hydroxy-lup-20(29)en-28-oic acid] **1p** from *Chaetophoma* sp. and betulonic acid **1d**, and both *Chaetophoma* sp. and *Dematium* sp. transformed betulinic acid **1c** to betulonic acid **1d**. Biotransformation of betulonic acid **1d** by *Nocardia* sp. NRRL 5646 gave methyl ester of betulonic acid [methyl 3-oxo-lup-20(29)en-28-oate] **1q** and methyl ester of 2α-acetoxybetulonic acid [methyl 2α-acetoxy-3-oxo-lup-20(29)en-28-oate] **1r** [13]. Both betulinic acid **1c** and betulonic acid **1d** were converted to their respective methyl esters **1s** and **1t**, respectively, by *Nocardia* sp. NRRL 5646 [13,14]

We have identified several compounds synthesized from betulinic acid **1c** with HIV maturation inhibitory activity [15–21]. In order to improve the water solubility, oral bioavailability and pharmacological properties, we sought to improve the polarity of betulinic acid **1c** core by hydroxylation/oxygenation via microbial transformations. This manuscript contains details of our work on the transformations of betulinic acid (**1c**) by two bacteria *B. megaterium*, *Streptomyces fragilis* and two fungi *C. echinulata* and *Aspergillus terreus*, and of betulonic acid (**1d**) by *B. megaterium*. A

Table 1
R groups in Fig. 1.

Compd	R_1	R_2	R_1+R_2	R_3	R_4	R_5	R_4+R_5	R_6	R_7	R_8	R_9	R_{10}	R_{11}	R_{12}	R_{13}
1a	OH	H	NA	CH_3	H	H	NA	H	H	H	H	H	CH_3	CH_3	CH_3
1b	OH	H	NA	CH_2OH	H	H	NA	H	H	H	H	H	CH_3	CH_3	CH_3
1c	OH	H	NA	COOH	H	H	NA	H	H	H	H	H	CH_3	CH_3	CH_3
1d	NA	NA	O	COOH	H	H	NA	H	H	H	H	H	CH_3	CH_3	CH_3
1e	OH	H	NA	COOX	H	H	NA	H	H	H	H	H	CH_3	CH_3	CH_3
1f	OH	H	NA	COOH	H	H	NA	H	OH	H	H	H	CH_3	CH_3	CH_3
1g	OH	H	NA	COOH	H	H	NA	OH	OH	H	H	H	CH_3	CH_3	CH_3
1h	OH	H	NA	COOH	H	H	NA	H	OH	OH	H	H	CH_3	CH_3	CH_3
1i	NA	NA	O	COOH	H	H	NA	H	H	H	H	OH	CH_3	CH_3	CH_3
1j	NA	NA	O	COOH	H	H	NA	H	H	OH	H	H	CH_3	CH_3	CH_3
1k	OH	H	NA	COOH	H	H	NA	H	OH	H	OH	H	CH_3	CH_3	CH_3
1l	NA	NA	O	COOH	H	H	NA	H	OH	H	OH	H	CH_3	CH_3	CH_3
1m	NA	NA	O	COOH	H	H	NA	H	OH	H	H	H	CH_3	CH_3	CH_3
1n	NA	NA	O	COOH	H	H	NA	H	OH	H	H	H	CH_3	CH_3	CH_2OH
1o	NA	NA	O	COOH	H	H	NA	H	H	H	OH	H	CH_3	CH_3	CH_3
1p	NA	NA	O	COOH	H	H	NA	H	H	H	H	H	CH_2OH	CH_3	CH_3
1q	NA	NA	O	$COOCH_3$	H	H	NA	H	H	H	H	H	CH_3	CH_3	CH_3
1r	NA	NA	O	$COOCH_3$	H	OAc	NA	H	H	H	H	H	CH_3	CH_3	CH_3
1s	OH	H	NA	$COOCH_3$	H	H	NA	H	H	H	H	H	CH_3	CH_3	CH_3
1t	NA	NA	O	$COOCH_3$	H	H	NA	H	H	H	H	H	CH_3	CH_3	CH_3
1u	NA	NA	O	CH_2OH	H	H	NA	H	H	H	H	H	CH_3	CH_3	CH_3
1v	OH	H	NA	COOH	H	H	NA	H	OH	H	OH	H	CH_3	CH_2OH	CH_3
1w	NA	NA	O	COOH	H	H	NA	H	OH	H	OH	H	CH_3	CH_3	CH_2OH
1x	NA	NA	O	COOH	H	H	NA	H	H	H	H	H	CH_3	CH_3	CHO
1y	NA	NA	O	COOH	H	H	NA	H	OH	H	H	H	CH_3	CH_3	CHO
1z	NA	NA	O	COOH	H	H	NA	H	H	H	H	H	CH_3	CH_3	CH_2OH
1aa	OH	H	NA	COOH	H	OH	NA	H	OH	H	H	H	CH_3	CH_3	CH_3
1bb	OH	H	NA	COOH	NA	NA	O	H	H	H	H	H	CH_3	CH_3	CH_3
1cc	OH	H	NA	COOH	H	H	NA	H	H	H	H	H	CH_2OH	CH_3	CH_3

NA, not applicable; X, β-D-glucose.

Download English Version:

https://daneshyari.com/en/article/69708

Download Persian Version:

https://daneshyari.com/article/69708

Daneshyari.com