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Biotransformation of Quinovic Acid Glycosides by Microbes: Direct Conversion of the Ursane to the Oleanane Triterpene Skeleton by *Nocardia* sp. NRRL 5646

Zhi-Hong Cheng,[†] Bo-Yang Yu,*[,†] Geoffrey A. Cordell,[‡] and Sheng-Xiang Qiu*[,§]

Department of Complex Prescription of TCM, China Pharmaceutical University, Nanjing 210038, People 's Republic of China, Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, 833 South Wood Street, Chicago, Illinois 60612, and Department of Chemistry, Box 1134, Washington University, One Brookings Drive, St. Louis, Missouri 63130

boyangyu@hotmail.com; sqiu@wustl.edu

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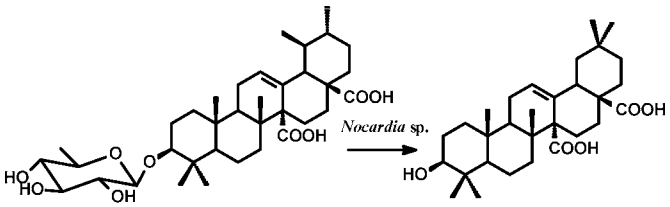
ABSTRACT



Quinovic acid 3-*O*-quinovoside **(1)** Cincholic acid **(3)**

Quinovic acid glycosides were microbially deglycosylated by a *Nocardia* sp. to their aglycon quinovic acid and its biogenetic counterpart, cincholic acid (3), via an unprecedented carbon skeleton rearrangement involving a methyl group migration. The structures of the metabolites were established by ESI-LC/MS and 2D-NMR techniques.

Natural products continue to deliver candidates to drug development pipelines over a wide range of therapeutic areas, and their structural diversity is critical to the success of natural product drug discovery.[1] It is widely believed that microbial biotransformation is a very useful approach to expand the chemical diversity of natural products.[2] There are several reports on natural product biotransformation using *Nocardia* sp. NRRL 5646, but none has been conducted on triterpenes and/or their glycosides.

Recently, we reported on the isolation and structure elucidation of two 27-nor-triterpenoid glycosides along with several quinovic acid glycosides (Figure 1) from the bark of *Mitragyna inermis* (Willd.) O. Kuntze (Rubiaceae), a famous folk medicine in West Africa traditionally used for treating stomach, intestinal disorders, and hepatic diseases.[3,4] In this study, the main constituent, quinovic acid 3-*O*-*β*-D-quinovopyranoside (quinovic acid 3-*O*-*β*-6-deoxy-D-glucopyranoside, **1**), was subjected to microbial transformation with the aim to generate new and more potent derivatives. The most commonly used microbe, *Nocardia* sp., was chosen on the basis of their documented abilities to effect carboxylic acid and aldehyde reduction reactions, to effect phenol methylation,[5−7] and to hydroxylate the methyl group of the

[†] China Pharmaceutical University.
[‡] University of Illinois at Chicago.
[§] Washington University.

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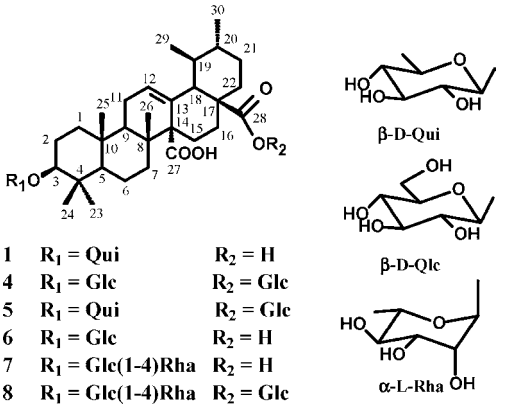


Figure 1. Structures of quinovic acid glycosides from *M. inermis*.

substrates,[2,8] respectively. Several analogues of quinovic acid glycosides were also employed to examine the specificity of this enzyme system. Herein, we describe the biotransformation results of the saponins from *M. inermis* by *Nocardia* sp. The structures of the metabolites were characterized by mass and NMR spectroscopic methods. This is the first report of the methyl group migration and deglycosylation of triterpene glycosides by *Nocardia* sp.

Compound **1** ($C_{36}H_{56}O_9$, M_r = 632.84) was fed to a panel of microbes according to the standard two-stage fermentation protocol.[9] Two microorganisms, *Nocardia* sp. and *Streptomyces griseus*, were found to exert the highest capacity for catalyzing the transformation, as revealed from the analysis of the biotransformation products by high-performance thin-layer chromatography (HPTLC), developed with $CHCl_3$−CH_3OH (10:1 and 5:1, v/v), respectively, and visualized by spraying with 10% sulfanilic acid in ethanol and heated for 2 min. This report focuses on the biotransformation with *Nocardia* sp.NRRL 5646.

A 200 mg sample of **1** was used for the preparative-scale incubation for 120 h. The incubation cultures were filtered, and the broth was extracted with EtOAc. The pooled EtOAc solution was dried over anhydrous Na_2SO_4, and the solvent was removed in a vacuum. The resulting residue was subjected to silica gel chromatographic separation which afforded two metabolites, M-1 (**2**, 40% yield based on the starting material **1**) and M-2 (**3**, 20%).

M-1(**2**) was obtained as white powder. Extensive mass and NMR spectroscopic studies revealed it to be quinovic acid (**2**) on the basis of its almost superimposable NMR data (Table 1) with literature values.[10] Similarly, M-2 (**3**) was

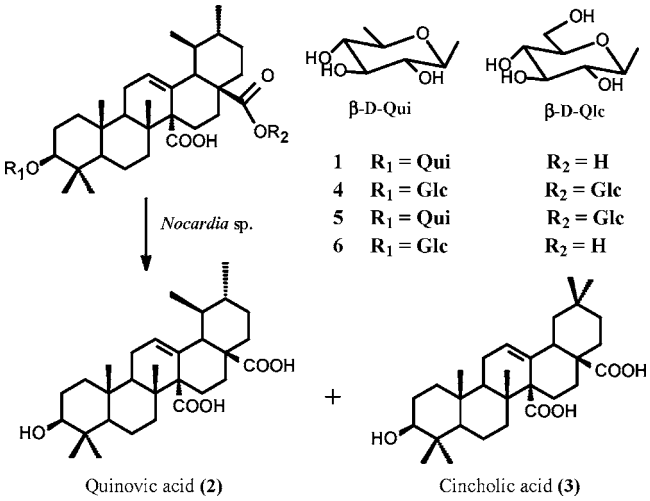
Table 1. NMR Spectral Data of Compounds **1**−**3**[a]

C	1	2	3	C	1	2	3
1	39.28	39.92	39.84	19	39.42	39.92	44.81
2	26.31	25.60	25.69	20	37.67	38.13	31.57
3	88.38	78.57	78.64	21	30.51	31.25	34.79
4	39.00	40.02	39.92	22	36.93	38.01	31.57
5	55.72	56.53	56.44	23	27.92	29.25	28.82
6	18.77	19.55	19.55	24	17.00	17.13	17.13
7	34.47	38.37	38.20	25	16.50	17.20	17.20
8	39.95	40.73	40.59	26	18.52	18.86	19.55
9	47.13	47.97	48.39	27	178.01	178.57	179.07
10	37.04	37.75	38.03	28	180.07	180.62	180.79
11	23.27	24.03	24.18	29	18.84	19.43	33.83
12	128.90	129.68	126.68	30	21.30	21.93	24.03
13	134.04	134.78	138.84	1′	106.65		
14	56.70	57.51	57.35	2′	75.83		
15	26.78	27.02	26.14	3′	78.31		
16	25.46	26.14	25.60	4′	76.83		
17	48.66	49.37	48.31	5′	72.60		
18	54.86	55.63	44.99	6′	18.19		

[a] Recorded in pyridine-d_5.

readily identified as cincholic acid by NMR data (Table 1) on comparison with those reported.[11] It is particularly noteworthy that the triterpene derivatives **2** and **3** (Scheme 1) have a different skeleton. Biogenetically, **2** belongs to the

Scheme 1. Biotransformation of Quinovic Acid Glycosides by *Nocardia* sp.



ursane while **3** belongs to the oleanane group, the two most predominant types of natural triterpenes found in plants.[12,13] This implies that there must be an isomerization enzyme system present in *Nocardia* sp. responsible for this conversion involving methyl migration. This is the first successful direct conversion of the ursane to oleane skeleton, a reaction

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which should be very difficult, if not impossible, to conduct through organic synthesis.

To investigate the capability and specificity of the enzyme system in *Nocardia* sp. for cleaving sugar linkages, another five, previously isolated, triterpenoid glycosides, including mono-, di-, and bisdesmosidic glycosides (with both an *O*-glycosidic linkage at C-3 position and an ester glycosidic linkage at C-28 position), were employed for parallel biotransformation under the same incubation conditions. It was found that compound **6** can be metabolized to quinovic acid (**2**) and cincholic acid (**3**) at a rate similar to that of **1**, indicating there is no selectivity between D-glucose and 6-deoxy-D-glucose (D-quinovose). Compounds **4** and **5** were also converted to **2** and **3** when the incubation time was extended to 240 h, implying that an ester glucose linkage at the C-28 position can be also cleaved by *Nocardia* sp., but at a slower rate compared to the C-3 sugar linkage. It is interesting to note that compounds **7** and **8**, with inner L-rhamnoses in the C-3 sugar linkage chain, could not be metabolized to **2** or **3**. Nevertheless, the positive electrospray LC/MS analysis of the incubation media detected one unrecognized metabolism product, M-3, from **7**, and two metabolism products, M-4 and M-5, from **8**. M-3 exhibited sodiated and pseudomolecular ions at *m*/*z* 655 $[M + Na - 162]^+$ and 633 $[M + H - 162]^+$, implying it to be a deglucosyl derivative of the parent **7**. M-4 and M-5 exhibited sodiated molecular ions at *m*/*z* 817 $[M + Na - 162]^+$ and 655 $[M + Na - 162 - 162]^+$, respectively, indicating they were formed by loss of one and two glucoses from the parent **8**. This suggested the high specificity for D-glucose over L-rhamnose for biotransformation with *Nocardia* sp., which provides an alternative way to identify the sugar chain composition of glycosides. Furthermore, the cleavage rate difference between the C-3 D-glucose and the C-28 D-glucose, as observed in compounds **5** and **6**, also affords a strategy to facilitate the structure elucidation of unknown glycosides.

It seems that at least two activities were involved in the quinovic acid glycosides when incubated with microbe *Nocardia* sp., namely, a glycosidase to cleave the sugar chain and an isomerization enzyme to induce the methyl migration. Interestingly, when compound **2** was incubated with the microbe *Nocardia* sp. under the same conditions, for 240 h, no cincholic acid (**3**) was detected by TLC and HPLC by comparison with reference samples. The explanation for this observation could be either the blockade of the access of the substrate to the active domain of the isomerization enzyme simply due to the lower hydrophilicity of triterpene acid with regard to the glycosides; or more likely, deglycosidation and methyl migration are two consecutive processes with the assumption that the two catalytic active domains are proximate, which is consistent with the observation that no methyl migration was detected for compounds **7** and **8**. Namely, the quinovic acid glycosides with D-glucose and 6-deoxy-D-glucose, as in compounds **1** and **4**−**6**, bind to the highly hydrophilic domain of glycosidase where the selective deglycosidation takes place, and the deglucosyl products successively bind to the less hydrophilic active domain of the isomerization enzyme where the methyl migration was catalyzed. As for compounds **7** and **8**, the partial deglycosyl products (M-3, M-4, and M-5) are still too hydrophilic to bind to the active domain of the isomerization enzyme. The unique catalytic capability of *Nocardia* sp. to cause methyl group rearrangement in triterpene glycosides deserves further exploration to enhance the chemical diversity of triterpene glycisides or possibly even other natural products.

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Supporting Information Available: Experimental details for the incubation and separation of metabolites from incubation mixture. This material is available free of charge via the Internet at http://pubs.acs.org.

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