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Two new compounds from transgenic *Panax quinquefolium*

Jian-Hua Zhu [a,b], Rong-Min Yu [b,*], Li Yang [b], Wei-Min Li [a,*]

[a] School of Chinese Materia Medica, Guangzhou University of Chinese Medicines, Guangzhou 510006, China
[b] College of Pharmacy, Jinan University, Guangzhou 510632, China

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ABSTRACT

A new ceramide and a new poly-hydroxyl octadecenoic acid were isolated from transgenic crown galls of *Panax quinquefolium*. Their structures were elucidated as (2*S*, 3*S*, 4*R*, 20*E*)-2-[(2′*R*)-2′-hydroxyl-palmitoyl-amino]-20-hexacosene-1, 3, 4-triol (1) and 12, 13, 15-trihydroxy-9-octadecenoic acid (2) respectively on the basis of spectroscopic and chemical methods.

1. Introduction

Panax quinquefolium, commonly known as American ginseng, has been widely used for its excellent therapeutic effects. However, the field cultivation of the plant is a time-consuming and labor-intensive process. Therefore, the plant routinely faces shortage of supply and extended waiting time on the market.

The crown galls of *P. quinquefolium* is the expressing production of transgenic *P. quinquefolium*, in which the plant has been infected by *Agrabacterium tumefaciens* and the Ti plasmid in *A. tumefaciens* is introduced into the plants' cell nuclear genome [1,2]. Agrobacterium-Ti plasmids are natural gene vectors, by which a number of attempts have been made in genetic engineering of secondary metabolism in many important plants in the last few decades. Compared with callus and cell culture, the crown gall cultures grow faster, produce more active constituents, and are free of exogenous phytohormones. As part of screening bioactive constituents from transgenic crown galls of *P. quinquefolium*, the production of secondary metabolites and the potential industrial utilization with this cultures system have attracted great interest of our research group during the past decade. Eleven bioactive saponin compounds including pseudoginsenoside RT_5, ginsenoside Rh_1, Rh_2, Rg_1, Rg_3, pseudoginsenoside F_{11}, ginsenoside Re, Rd, Rb_1, Rb_3 and 20 (s)-3, 6, 12, 20, 25-tetrahydroxydammaran-6-O-α-L-rhamnopyranosyl (1←2)-β-D-glucopyranoside were isolated from the cultures [3,4], and the last one was a new natural compound. Besides, pseudoginsenoside RT_5, ginsenoside Rh_2, and Rg_3 were rare saponins in natural *P. quinquefolium*.

In this paper, we report two novel compounds, ceramide and a poly-hydroxyl octadecenoic acid, isolated from transgenic *P. quinquefolium*.

2. Results and discussion

(2*S*, 3*S*, 4*R*, 20*E*)-2-[(2′*R*)-2′-hydroxyl-palmitoyl-amino]-20-hexacosene-1, 3, 4-triol (1): white amorphous crystals, mp: 139–141 °C. The IR spectrum of compound 1 showed absorption bands of typical hydroxyl (3336 cm^{-1}), amide (1623, 1554 cm^{-1}), and $(CH_2)n$ (723 cm^{-1}) functional groups. The ^1H and ^{13}C NMR spectra of compound 1 (Table 1) indicated the presence of a secondary amide linkage (δ_H 8.56, d, 1H, $J = 8.8$ Hz; δ_C 175.1) and two long-chain aliphatic moieties of the ceramide.

* Corresponding authors. Yu is to be contacted at Tel.: +86 20 85220386; fax: +86 20 85224766. Li, Tel.: +86 20 39358290.
E-mail addresses: rongminyu99@hotmail.com (R.-M. Yu), 13925023915@139.com (W.-M. Li).

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Table 1
^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) data for compound 1 (in pyridine-d_5, δ ppm).

No.	δ_C (J=Hz)		δ_H (J=Hz)		No.	Compound 1	
	Compound 1	Compound 2	Compound 1	Compound 2		δ_C (J=Hz)	δ_H (J=Hz)
1	61.9	177.8	4.52 (*dd*, 15.0, 4.9) 4.43 (*dd*, 15.0, 4.9)		19	33.2	2.25 (*m*)
2	52.9	35.0	5.11 (*m*)	2.2 (*t*)	20	130.7	5.54 (*dt*, 15.3, 6.3)
3	76.7	26.4	4.36 (*m*)	1.58 (*m*)	21	130.6	5.47 (*dt*, 15.3, 6.3)
4	72.8	30.1	4.27 (*m*)	1.28–1.33 (*m*)	22	32.8	2.19 (*m*)
5	33.7	33.5	2.0 (*m*)	1.52 (*m*)	23	29.4	
6	26.6	26.5	1.71 (*m*)	1.28–1.33 (*m*)	24	32.0	
7	29.4–30.0	30.3	1.25–1.30 (*m*)	1.28–1.33 (*m*)	25	22.8	
8	29.4–30.0	26.1	1.25–1.30 (*m*)	1.58 (*m*)	26	14.28	0.85 (*t*, 6.49)
9	29.4–30.0	136.5	1.25–1.30 (*m*)	5.68 (*m*)	1′	175.1	
10	29.4–30.0	131.0	1.25–1.30 (*m*)	5.68 (*m*)	2′	72.4	4.69 (*m*)
11	29.4–30.0	30.5	1.25–1.30 (*m*)	1.58 (*m*)	3′	35.6	2.22 (*m*)
12	29.4–30.0	76.5	1.25–1.30 (*m*)	3.90,3.88 (*dt*)	4′	25.7	1.78 (*m*)
13	29.4–30.0	73.0	1.25–1.30 (*m*)	4.03,4.04 (*dt*)	5′–13′	29.4–30.0	1.25–1.30
14	29.4–30.0	38.3	1.25–1.30 (*m*)	1.52 (*m*)	14′	32.0	
15	29.4–30.0	75.8	1.25–1.30 (*m*)	3.40 (*m*)	15′	22.8	
16	29.4–30.0	32.7	1.25–1.30 (*m*)	1.28–1.33 (*m*)	16′	14.28	0.85 (*t*, 6.49)
17	29.4–30.0	23.6	1.25–1.30 (m)	1.28–1.33 (*m*)	NH		8.56 (*d*, 8.8)
18	29.4–30.0	14.3	1.25–1.30 (*m*)	0.89 (*t*)			

Note: NMR data were confirmed by HSQC, HMBC, and ^{1}H–^{1}H COSY.

^{1}H NMR spectrum of compound 1 (Table 1) showed the presence of two terminal methyl groups at δ 0.85 (*t*, 6H, J=6.49 Hz), methylenes at δ 1.25 (br s), an amide proton signal at δ 8.56 (d, 1H, J=8.8 Hz, NH), and signals of a *trans*-olefinic bond at δ 5.47 (br dt, 1H, J=15.3, 6.3 Hz) and δ 5.54 (br dt, 1H, J=15.3, 6.3 Hz). Meanwhile, six characteristic signals of geminal protons to hydroxyl groups were also observed at δ 4.69 (*m*, 1H), 4.52 and 4.43 (*dd*, each 1H, J=15.0, 4.9 Hz), 4.36 (*m*, 1H), 4.27 (*m*, 1H).

Another signal at low field was observed at δ 5.11 (*m*, 1H) for a methine proton vicinal to the nitrogen atom of the amide group. The data indicated a phytosphingolipid structure. Furthermore, ^{13}C NMR spectra of compound 1 (Table 1) showed one quaternary carbon at δ 175.1 (CONH), two olefinic methine carbons at δ 130.6 and 130.7 (C=C), five methines at δ 52.9 (CHNH), 76.7 (CHOH), 72.8 (CHOH), 72.4 (CHOH), 72.4 (CHOH), and one methylene at δ 61.9 (CH_2OH).

Methanolysis of compound 1 yielded a fatty acid methyl ester (FAME), and a long-chain base (LCB). FAME was identified as methyl 2-hydroxypalmitate by means of GC/MS analysis, and the absolute configuration at C-2′ was determined to be *R* from the specific rotation.

The presence of a 1, 3, 4-trihydroxy unsaturated C_{26} long-chain base was deduced from the ^{1}H–^{1}H COSY and MS data. The signal at δ 8.56 gave a cross-peak with the signal at δ 5.11 (H-2) in the ^{1}H–^{1}H COSY spectrum of compound 1, which, in turn, showed cross-peaks with methylene protons (H-1) at δ 4.52 and 4.43 and δ 4.36 (H-3). The latter correlated with the signal at δ 4.27 (H-4). The negative ESI-MS of compound 1 displayed a $[M-H]^-$ peak at *m/z* 680, in accordance with the molecular formula $C_{42}H_{83}NO_5$. The negative ESI-MS of the LCB obtained from the methanolysis of compound 1 gave a $[M-H]^-$ ion at *m/z* 426. These findings indicated that the phytosphingosine moiety was 2-amino-hexacosene-1, 3, 4-triol.

In order to determine the position of the double bond in the phytosphingosine moiety, $KMnO_4$ oxidation was performed on the LCB. Oxidation afforded n-caproic acid which was determined by GC-MS analysis. This allowed the localization of the double bond at C-20. This result was further confirmed by the intense fragment ion at *m/z* 71 $[C_5H_{11}]^+$, 97 $[C_7H_{13}]^+$, 111 $[C_8H_{15}]^+$ in EI-MS. The trans (*E*) configuration of the double bond was shown by the coupling constant (J=15.3 Hz) between H-20 and H-21 in the ^{1}H NMR spectrum, and the chemical shifts of the carbons next to the double bond at δ 33.4 and 33.1 (C-7 and C-10) in the ^{13}C NMR spectrum, which have to be observed at δ≈27 in (*Z*) isomers and at δ≈32 in (*E*) isomers [5].

The chemical shift of the H-2 signal and the ^{13}C chemical shifts of C-1–C-4, C-1′ and C-2′ of ceramides were especially suitable for determination of the absolute stereochemistry of the phytosphingosine moiety [6,7]. The chemical shift of H-2 (δ 5.11) and the carbon chemical shifts at δ 61.9 (C-1), 52.9 (C-2), 76.7 (C-3), 72.8 (C-4), 175.1 (C-1′) and 72.4 (C-2′) in compound 1 were virtually identical with those of the reported data of other (2*S*, 3*S*, 4*R*)-phytosphingosine moieties [8,9]. These results clearly indicated that the 1, 3, 4-trihydroxyphytosphingosine moiety in compound 1 possesses the 2*S*, 3*S*, 4*R* configuration. Therefore, compound 1 was determined to be (2*S*, 3*S*, 4*R*, 20*E*)-2-[(2′*R*)-2′-hydroxyl-palmitoyl-amino]-20-hexacosene-1, 3, 4-triol (Fig. 1).

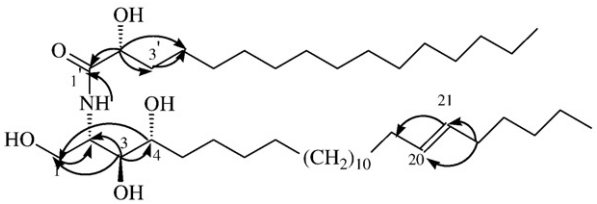

Fig. 1. The structure and key HMBC correlations of compound 1.