Hue University Journal of Science: Natural Science
Vol. 133, No. 1D, 19–25, 2024

pISSN 1859-1388
eISSN 2615-9678

Non-hydrophilic components from roots of Vietnamese ginseng (*Panax vietnamensis* Ha & Grushv.)

Nguyen Thi Hoang Anh[1], Dang Thi Ngan[1], Doan Manh Dung[2], Nguyen Thi Thanh Binh[1], Nguyen Ngoc Hieu[3], Vu Van Tuan[3], Nguyen Huu Tung[3*]

[1] University of Medicine and Pharmacy, Vietnam National University – Hanoi (VNU), Hanoi, Vietnam
[2] Institute of Biotechnology and Environment, Tay Nguyen University, Buon Ma Thuot, Dak Lak, Vietnam
[3] Faculty of Pharmacy, PHENIKAA University, Hanoi 12116, Vietnam.

* Correspondence to Nguyen Huu Tung <tung.nguyenhuu@phenikaa-uni.edu.vn>

(*Received: 13 September 2023; Revised: 01 March 2024; Accepted: 02 March 2024*)

Abstract. Vietnamese ginseng is a precious medicinal herb and contains various ginsenosides as the main components. We studied the less polar constituents of the title ginseng and isolated falcarinol (**1**) and 20S-protopanaxatriol (**4**) for the first time, along with two common phytosterols: β-sitosterol (**2**) and daucosterol (**3**). Their structures were elucidated with nuclear magnetic resonance (NMR) and mass spectrometry (MS) spectra and compared with the literature data. Falcarinol was obtained from the non-polar portion of the crude residue, with a high content. The occurrence of 20S-protopanaxatriol becomes evidence for similar biosynthesis of ginsenoside in Panax spp. The obtained results contributed partly to the phytochemical database of Vietnamese ginseng and the Panax genus.

Keywords: Vietnamese ginseng, *Panax vietnamensis* Ha & Grushv., ginseng, falcarinol, 20S-protopanaxatriol

1 Introduction

Vietnamese ginseng (*Panax vietnamensis* Ha & Grushv., Araliaceae, VG in abbreviation) is considered as the most well-known in the list of Vietnamese medicinal plants and recorded in the current List of Vietnamese National Products from 2017 [1]. Vietnamese ginseng has become very attractive to research and development of healthcare products, especially in modern medicine [1, 2]. Like other ginseng, such as Korean ginseng (*P. ginseng*) and American ginseng (*P. quinquefolius*), Vietnamese ginseng has also extensively been investigated, regarding phytochemical profile and bioactive components and mostly focused on hydrophilic components with total 52 individual saponins or ginsenosides up to date [2, 3]. Among them, the typical ocotillol saponin majonoside R2 (MR2, Figure 1) represents the principal marker component with a remarkable concentration (up to 5% of the dried weight of VG and certain pharmacological effects on the central nervous system and cancer [2, 4].

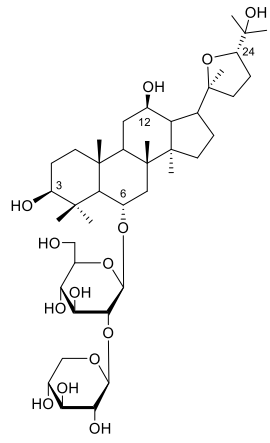

Fig. 1. Majonoside R2, main bioactive saponin of Vietnamese ginseng

2 Materials and methods

2.1 General procedures

This research employed the following methods and equipment. The NMR measurements were conducted at ambient temperature by using the JEOL ECX 400 (Jeol, Japan), and the Bruker Avance 500 NMR spectrometer (BrukerSpin, Germany) was employed with a standard pulse program. Tetramethylsilane served as the internal standard, and the chemical shift values were expressed in δ (ppm). ESI-MS experiments were performed with the Agilent 1260 TripleQuad-6420 LC-MS/MS (Agilent Technologies, USA). Column chromatography utilized silica gel 60 (particle size 230–400 mesh, Nacalai Tesque Inc., Kyoto, Japan) and YMC ODS-A gel (particle size 50 μm, YMC Co. Ltd., Kyoto, Japan). Lastly, thin layer chromatography (TLC) was conducted on Kieselgel 60 F_{254} and Silica gel 60 RP-18 F_{254S} (Aluminum plates, Merck, Darmstadt, Germany) plates, with a spraying reagent consisting of a 1% $Ce(SO_4)_2$-10% aqueous H_2SO_4 solution.

2.2 Plant materials

The root samples, aged between 4 and 6 years, were obtained from the Ngoc Linh mountain site located in Quang Nam Province in October, 2019. These samples were subjected to taxonomic identification by Dr. Do Ngoc Dai from the Department of Forestry at Nghe An University of Economics. The specimens (Code: SVN-2019-01) were stored at two academic institutions in Hanoi, Vietnam: the University of Medicine and Pharmacy (UMP), Vietnam National University, and the Faculty of Pharmacy, PHENIKAA University.

2.3 Extraction and isolation

A sample of VG root (100 g) was subjected to sonication in a solution of 80% aqueous EtOH (400 mL × 4 h × 3 times). The resulting extracts were mixed and concentrated under decreased pressure. The crude extract (35.6 g, water content 10.6%) was suspended in 200 mL of water and subsequently partitioned with ethyl acetate (EtOAc) and water-saturated n-butanol (n-BuOH) in three sequential steps, each using 200 mL of solvent. After removing the solvent under vacuum, a residue of 6.8 g from the EtOAc extract and 16.9 g from the n-BuOH extract was obtained.

Subsequently, a portion consisting of ethyl acetate residue (6.0 g) was exposed to a column packed with silica gel (diameter: 50 mm, length: 150 mm). The column was eluted with a stepwise gradient of hexane-ethyl acetate (v/v, 20:1 to 2:1) with a total volume of 2000 mL. This process resulted in the separation of the mixture into seven distinct fractions (Fr.1 to Fr.7), which were subjected to TLC analysis. Compound 1 (colorless syrup, 155 mg) was obtained by subjecting Fr.1 (350 mg) to re-chromatography using a silica gel column (Φ20 mm × 300 mm) with a hexane-EtOAc mobile phase (20:1, v/v, 500 mL). Compound 2 (white powder, 25 mg) was obtained by subjecting Fr.2 (300 mg) to additional chromatography on a silica gel column (Φ20 mm × 300 mm) using a hexane-CH_2Cl_2 solvent mixture (10:1, v/v, 600 mL). Afterwards, a sample of Fr.5 (210 mg) was introduced into a silica gel column (diameter 20 mm × length 300 mm) with a mixture of hexane and ethyl acetate (4:1, v/v, 400 mL) as the eluent. Subsequently, the eluate was further subjected to a reversed-phase C18 column (diameter 20 mm × length 400 mm) with a mixture of methanol and water (7:2, v/v, 450 mL) as the eluent. This process resulted in the isolation of compound 4 (white amorphous powder, 9 mg). Subsequently, a sample of Fr.6 (360 mg) was submitted to a silica gel column chromatography (20 mm diameter × 300 mm length) with a solvent mixture of $CHCl_3$-MeOH (12:1, v/v, 450 mL). The resulting fractions were further purified via

pISSN 1859-1388
eISSN 2615-9678

crystallization in methanol, resulting in the isolation of compound 3 as a white powder with a yield of 18 mg.

Falcarinol [(9*Z*)-heptadeca-1,9-dien-4,6-diyn-3-ol] (**1**): colorless syrup; ESI-MS: *m*/*z* 245.3 $[M + H]^+$ (MF: $C_{17}H_{24}O$; MW: 244.4); ^{1}H-NMR ($CDCl_3$, 500 MHz): δ 5.93 (1H, ddd, *J* = 17.0, 10.0, 5.5 Hz; H-2), 5.50 (1H, m, H-10), 5.45 (1H, dt, *J* = 17.0, 1.1 Hz, H-1a), 5.38 (1H, m, H-9), 5.24 (1H, br d, *J* = 10.0 Hz, H-1b), 4.90 (1H, br d, *J* = 5.5 Hz, H-3), 3.02 (2H, dd, *J* = 6.5, 0.5 Hz, H-8), 2.02 (2H, q, *J* = 7.0 Hz, H-11), 1.35–1.25 (10H, overlapped, 5×CH_2, H-12→H-16), 0.88 (3H, t, *J* = 6.5 Hz, H-17). ^{13}C-NMR ($CDCl_3$, 125 MHz): δ 117.1 (C-1), 136.2 (C-2), 63.6 (C-3), 74.3 (C-4), 71.3 (C-5), 64.0 (C-6), 80.4 (C-7), 17.8 (C-8), 121.9 (C-9), 133.1 (C-10), 27.2 (C-11), 29.1–29.3 (3C, C-12, 13, 14), 31.9 (C-15), 22.7 (C-16), 14.1 (C-17).

β-sitosterol (**2**): white powder; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 5.32 (1H, br d, *J* = 5.2 Hz, H-6), 3.50 (1H, m, H-3), 0.98 (3H, s, H-19), 0.90 (3H, d, *J* = 6.4 Hz, H-21), 0.84 (3H, d, *J* = 7.2 Hz, H-29), 0.80 (3H, d, *J* = 6.8 Hz, H-27), 0.78 (3H, d, *J* = 6.4 Hz, H-26), 0.65 (3H, s, H-18); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 33.1 (C-1), 33.5 (C-2), 71.4 (C-3), 39.4 (C-4), 140.4 (C-5), 121.3 (C-6), 31.5 (C-7), 31.5 (C-8), 49.7 (C-9), 36.1 (C-10), 20.7 (C-11), 41.9 (C-12), 41.9 (C-13), 56.4 (C-14), 23.9 (C-15), 27.9 (C-16), 55.6 (C-17), 11.6 (C-18), 19.4 (C-19), 35.8 (C-20), 18.6 (C-21), 39.4 (C-22), 25.6 (C-23), 45.4 (C-24), 29.1 (C-25), 19.0 (C-26), 18.4 (C-27), 22.9 (C-28), 11.5 (C-29).

Daucosterol (**3**): white powder; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 5.31 (1H, br d, *J* = 5.2 Hz, H-6), 4.36 (1H, d, *J* = 8.0 Hz, H-1-Glc), 3.50 (1H, m, H-3), 0.98 (3H, s, H-19), 0.91 (3H, d, *J* = 6.4 Hz, H-21), 0.84 (3H, d, *J* = 7.2 Hz, H-29), 0.80 (3H, d, *J* = 6.8 Hz, H-27), 0.79 (3H, d, *J* = 6.4 Hz, H-26), 0.66 (3H, s, H-18); ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 33.7 (C-1), 35.9 (C-2), 75.5 (C-3), 39.5 (C-4), 140.0 (C-5), 121.9 (C-6), 31.6 (C-7), 32.1 (C-8), 49.9 (C-9), 36.4 (C-10), 20.8 (C-11), 42.1 (C-12), 41.9 (C-13), 56.5 (C-14), 24.0 (C-15), 27.9 (C-16), 55.8 (C-17), 11.6 (C-18), 19.4 (C-19), 35.9 (C-20), 18.6 (C-21), 39.4 (C-22), 25.8 (C-23), 45.6 (C-24), 29.3 (C-25), 19.4 (C-26), 18.9 (C-27), 22.8 (C-28), 11.5 (C-29), 100.9 (Glc-1), 73.3 (Glc-2), 76.2 (Glc-3), 69.9 (Glc-4), 78.9 (Glc-5), 61.6 (Glc-6).

(20*S*)-Protopanaxatriol [(20*S*)-Damara-24-en-3β,6β,12β,20β-tetraol] (**4**): white amorphous powder; ESI-MS: *m*/*z* 477.3 $[M + H]^+$ (MF: $C_{30}H_{52}O_4$; MW: 476.7); ^{1}H-NMR (Pyridine-d_5, 500 MHz): δ 5.04 (1H, br t, J = 6.5 Hz, H-24), 4.42 (1H, m, H-6), 3.96 (1H, m, H-12), 3.54 (1H, m, H-3); 0.85, 0.98, 0,98, 1.03, 1.13, 1.43, 1.48, 1.64 (8 × CH_3, all s, H_3-30, 19, 29, 18, 21, 26, 27, 28). ^{13}C-NMR (Pyridine-d_5, 125 MHz): δ 39.7 (C-1), 27.2 (C-2), 78.8 (C-3), 40.8 (C-4), 62.2 (C-5), 68.1 (C-6), 47.9 (C-7), 41.6 (C-8), 50.5 (C-9), 40.8 (C-10), 31.8 (C-11), 71.4 (C-12), 48.7 (C-13), 52.0 (C-14), 30.4 (C-15), 27.5 (C-16), 55.2 (C-17), 17.8 (C-18), 17.5 (C-19), 73.4 (C-20), 26.2 (C-21), 36.3 (C-22), 23.4 (C-23), 126.7 (C-24), 131.2 (C-25), 26.2 (C-26), 18.0 (C-27), 32.4 (C-28), 16.9 (C-29), 18.1 (C-30).

3 Results and discussion

The VG material was subjected to extraction by using 80% ethanol. Subsequently, a series of experiments involving partitioning and chromatographic techniques were conducted. These experiments led to the isolation of four compounds from the ethyl acetate (EtOAc) residue. The isolated compounds were falcarinol (**1**), which was classified as a polyyne/polyacetylene, and two sterols: β-sitosterol (**2**) and daucosterol (**3**). Additionally, a dammarane triterpene, specifically (20S)-protopanaxatriol (**4**), was also isolated. The structures of the compounds, depicted in Figure 2, were determined by using NMR and MS spectra. These findings were further validated by comparing with existing data from the literature. The two phytosterols, β-sitosterol (**2**) and

daucosterol (**3**), were confirmed by using co-TLC with the respective reference compounds, and their ^{1}H and ^{13}C NMR data were consistent with the reported data [5]. β-Sitosterol (**2**) and daucosterol (**3**) commonly exist in the plant kingdom and are previously reported in the *Panax* spp. [3].

Compound **1** was obtained as a yellowish syrup and concentrated in the less polar portion of the crude extract by TLC monitoring. The ^{1}H NMR spectrum of **1** showed the downfield-shifted signals of olefinic and oxygenated methine protons at δ 5.93 (1H, ddd, *J* = 17.0, 10.0, 5.5 Hz), 5.50 (1H, m), 5.45 (1H, dt, *J* = 17.0, 1.1 Hz), 5.38 (1H, m), 5.24 (1H, br d, *J* = 10.0 Hz), and 4.90 (1H, br d, *J* = 5.5 Hz), the signals of methylene groups in an overlapped cluster at δ 1.25–1.35 (5 × CH_2, 10H), and the signal of one typical terminal methyl group at δ 0.88 (3H, t, *J* = 6.5 Hz). The ^{13}C NMR spectrum of **1** showed seventeen carbon resonances including one upfield-shifted signal (δ 14.1, CH_3), four midfield-shifted signals (δ 60–80) for acetylene and oxygenated carbons as well as four olefinic carbons at δ 115–140. Furthermore, the ESI-MS spectrum of **1** reveals a quasimolecular ion peak at *m*/*z* 245.3 $[M+H]^+$, consistent with the molecular formula of $C_{17}H_{24}O$ (MW 244). According to the above analysis and detailed comparison of the spectroscopic data with those published [6], compound **1** was identified as falcarinol (Figure 2), the main component in the less polar fraction of VG.

Compound **4** was isolated as a white amorphous powder and appeared as purple spots via TLC visualization with a 10% aqueous H_2SO_4 solution. The overall NMR spectra of **4** proposed a structure of a dammarane-type triterpenoid. The ^{1}H NMR spectrum of **4** showed signals of one olefinic proton (δ 5.04, br t, *J* = 6.5 Hz), three oxymethine groups [δ 3.54 (1H, m), 3.96 (1H, m), and 4.42 (1H, m)], and eight quaternary methyl groups [δ 0.85, 0.98, 0.98, 1.03, 1.13, 1.43, 1.48, 1.64 (8 × CH_3, all s)] as the features for a dammarane triterpene as protopanaxatriol [3, 7]. The ^{13}C-NMR spectrum of **4** revealed 30 carbon resonances of the triterpene molecule including two olefinic carbons (δ 126.7 and 131.2) and four oxygenated carbons (δ 68.1, 71.4, 73.4, and 78.8). The ^{1}H and ^{13}C NMR data of **4** from the above analysis were compatible to those of (20*S*)-damara-24-en-3β,6β,12β,20β-tetraol. In addition, the ESI MS spectrum of **4** showed a molecular ion peak at *m*/*z* 477.3 $[M+H]^+$, consistent with the molecular formula of $C_{30}H_{52}O_4$ (MW 476). According to these findings and comparison of spectroscopic data with those in the literature [7], the structure of **4** including stereochemistry was unambiguously deduced as (20*S*)-damara-24-en-3β,6β,12β,20β-tetraol or (20*S*)-protopanaxatriol (Figure 2).

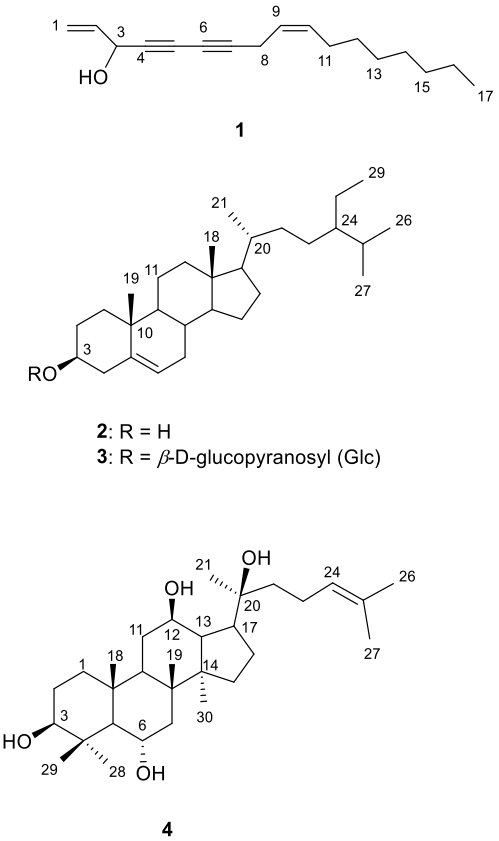

Fig. 2. Chemical structures of the isolated compounds (**1**–**4**) from Vietnamese ginseng

pISSN 1859-1388
eISSN 2615-9678

To our best knowledge, it is noteworthy that this isolation of falcarinol (**1**) and (20*S*)-protopanaxatriol (**4**) from VG was conducted for the first time. The TLC evidence and mass isolation of falcarinol further supported a high content of falcarinol in VG, and falcarinol could be isolated from the nonpolar portion of the crude residue. The result of falcarinol in VG further supported a high content of falcarinol and polyacetylens in the *Panax* species, such as *P. ginseng*, *P. stipuleanatus*, *P. notoginseng*, and *P. quinquefolius* [8, 9].

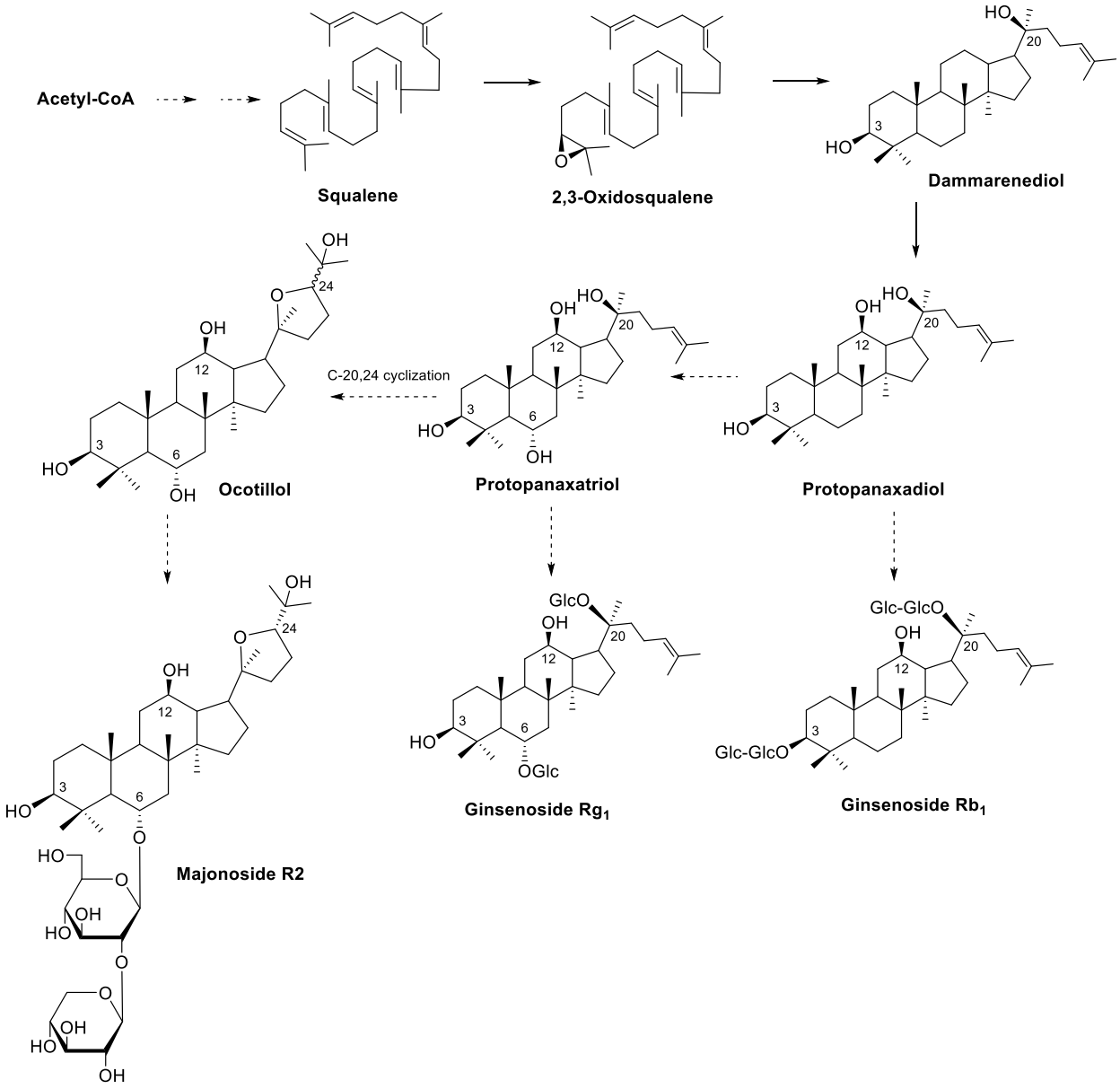

Fig. 3. Proposed biosynthetic pathway of propanaxatriol and principal ginsenosides of Vietnamsese ginseng (Ginsenoside Rb_1, Ginsenoside Rg_1 and Majonoside R2)

Regarding (20*S*)-protopanaxatriol (**4**) and in concern of chemistry, (20*S*)-protopanaxatriol (**2**) is the aglycone part of major ginsenosides (e.g. ginsenoside Rg1 and ginsenoside Re) and as an intermediate precursor for occotillol-type saponins in VG (e.g. majonoside R1 and majonoside R2) as the experimental organic synthesis [10, 11]. In relation to the process of

biosynthesis, it has been observed that the production of ginsenoside in *Panax spp*. takes place within the terpenoid pathway, utilizing the farnesyl group derived from squalene. Han et al. thoroughly reported that the conversion of dammarenediol-II [12] to protopanaxadiol and the conversion of protopanaxadiol to protopanaxatriol are facilitated under the enzymatic activity of cytochrome P450 [13]. A correlation between biosynthetic enzyme activity, gene expression, ginsenoside concentration, ginsenoside profiles, related gene expression, and the expression of genes associated with ginsenoside production was investigated [14, 15]. The biochemical pathways responsible for the production of dammarane-type and ocotillol-type triterpenes exhibit a close association. The proposed biosynthesis pathways of ginsenosides are depicted in Figure 3. However, the conversion of protopanaxadiol to ocotillol, particularly in VG, lacks clarity and requires further investigation at the molecular level.

4 Conclusions

Our present investigation on the organic portion of crude residue of Vietnamese ginseng resulted in the isolation of falcarinol (**1**) and (20*S*)-protopanaxatriol (**4**) for the first time, together with two common phytosterols: β-sitosterol (**2**) and daucosterol (**3**). Their structures were identified by using extensive spectroscopic data and compared with those reported in the literature. Falcarinol (**1**) represents a high concentration in Vietnamese ginseng and exists in the non-polar fraction. These results contributed partly to the phytochemical profile of *P. vietnamensis* and ginseng.

Acknowledgement

This research was funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 108.05-2019.01.

References

1. Dung HT, Grushvitski I V. A new species of the genus Panax (Araliaceae) from Vietnam Ha et Grushv. Bot J Vietnam. 1985;518-22.
2. Tung NH, Thuong PT. Vietnamese ginseng (Panax vietnamensis Ha & Grushv.): Phylogenetic, phytochemical and pharmacological profiles. Pharmacogn Rev. 2019;13(26):59-62.
3. Yang W, Hu Y, Wu W, Ye M, Guo D. Saponins in the genus Panax L. (Araliaceae): A systematic review of their chemical diversity. Phytochemistry. 2014;106:7-24.
4. Konoshima T, Takasaki M, Ichiishi E, Murakami T, Tokuda H, Nishino H, et al. Cancer chemopreventive activity of majonoside-R2 from Vietnamese ginseng, Panax vietnamensis. Cancer Lett. 1999;147(1–2):11-6.
5. Chang IM, Yun HS, Yamasaki K. Revision of 13C NMR assignments of β-sitosterol and β-sitosteryl-3-O-β-D-glucopyranoside isolated from Plantago asiatica seed. Kor J Pharmacog. 1981;12:12-24.
6. Corell M, Sheehy E, Evans P, Brunton N, Valverde J. Absolute Configuration of Falcarinol (9 Z -heptadeca-1,9-diene-4,6-diyn-3-ol) from Pastinaca Sativa. Nat Prod Commun. 2013; 8(8):1934578X1300800.
7. He C, Zhou D, Li J, Han H, Ji G, Yang L, et al. Identification of 20(S)-protopanaxatriol metabolites in rats by ultra-performance liquid chromatography coupled with electrospray ionization quadrupole time-of-flight tandem mass spectrometry and nuclear magnetic resonance spectroscopy. J Pharm Biomed Anal. 2014;88:497-508.
8. Xia P, Li J, Wang R, Zhang Y, Guo H, Yan X, et al. Comparative study on volatile oils of four Panax genus species in Southeast Asia by gas chromatography–mass spectrometry. Ind Crops Prod. 2015;74:478-84.

pISSN 1859-1388
eISSN 2615-9678

9. Ru W, Wang D, Xu Y, He X, Sun Y-E, Qian L, et al. Chemical constituents and bioactivities of Panax ginseng (C. A. Mey.). Drug Discov Ther. 2015;9(1):23-32.

10. Cao Y, Wang K, Xu S, Kong L, Bi Y, Li X. Recent Advances in the Semisynthesis, Modifications and Biological Activities of Ocotillol-Type Triterpenoids. Molecules. 2020; 25(23):5562.

11. Liu J, Xu Y, Yang J, Wang W, Zhang J, Zhang R, et al. Discovery, semisynthesis, biological activities, and metabolism of ocotillol-type saponins. J Ginseng Res. 2017;41(3):373-8.

12. Han J-Y, Kim H-J, Kwon Y-S, Choi Y-E. The Cyt P450 Enzyme CYP716A47 Catalyzes the Formation of Protopanaxadiol from Dammarenediol-II During Ginsenoside Biosynthesis in Panax ginseng. Plant Cell Physiol. 2011;52(12):2062-73.

13. Han J-Y, Hwang H-S, Choi S-W, Kim H-J, Choi Y-E. Cytochrome P450 CYP716A53v2 Catalyzes the Formation of Protopanaxatriol from Protopanaxadiol During Ginsenoside Biosynthesis in Panax Ginseng. Plant Cell Physiol [Internet]. 2012;53(9):1535-45.

14. Yin J, Zhang D, Zhuang J, Huang Y, Mu Y, Lv S. Study on the Correlation between Gene Expression and Enzyme Activity of Seven Key Enzymes and Ginsenoside Content in Ginseng in Over Time in Ji'an, China. Int J Mol Sci [Internet]. 2017; 18(12):2682.

15. Wang K-Y, Liu W-C, Zhang M-P, Zhao M-Z, Wang Y-F, Li L, et al. Expression of saponin biosynthesis related genes in different tissues of Panax quinquefolius. China J Chinese Mater medica. 2018 Jan; 43(1):65-71.