ABSTRACT

Vietnamese ginseng, Panax vietnamensis was recently found in central Vietnam, and regarded as a new botanical species. It was used in traditional folk medicine in hill tribes for the purpose of anti-fatigue and life saving. From the rhizome and root of this plant, 37 saponins including 14 new compounds were isolated. Some of them were common to other Panax spp., but the general yields in this species were very high. Among them, an ocotillol-type saponin majonoside-R2 (MR2) was remarkable in its yield (5.3%). Structural features of new saponins were briefly introduced. The biological effects of these saponins were studied in two ways, anti-stress effect and anti-tumor promoting effect; both activities were based upon the traditional usage of this crude drug. In psychologically stressed mice, the saponin fraction of Vietnamese ginseng significantly reduced stress-related disorders (reducing sleeping time, formation of gastric lesions) and MR2 was responsible for this effect. A possible mechanism for this effect was proposed. MR2 exhibited a significant inhibitory effect on Epstein-Barr virus early antigen (EBV-EA) induced by the tumor promoter phorbol acetate. MR2 also showed potent anti-tumor-promoting activity on mouse skin and hepatic tumors.

INTRODUCTION

Vietnamese ginseng was found in the highlands of Central Vietnam in 1973, and was regarded as a new species as Panax vietnamensis Ha et Grushv. (Dung et al., 1985). This is the most southern distribution of Panax genus (Araliaceae). It is a secret medicine of the Sedang ethnic group used as a miraculous, life-saving plant drug, for the treatment of many serious diseases, for its adaptogenic activity and for enhancing body strength in long journeys in high mountains (Nham, 1989).

The discovery of this species led to the foundation of the Science Production Centre of Vietnamese Ginseng at Ho Chi Minh City. A comprehensive study on the cultivation, chemical constituents and pharmacology of Vietnamese ginseng has been carried out there with the collaboration of several Japanese Universities. In this review, the structural study of saponins and some biological activities are introduced as the result of our collaborative work.

Isolation and Structure of Saponins

The plant materials were collected at altitudes of 1500–2500 m in Central Vietnam in autumn 1978. Since no significant difference in the saponin composition of the rhizome and the root was observed in a preliminary test using TLC, both parts were combined and extracted with hot MeOH and hot 50% aqueous MeOH. The combined extract was chromatographed on a column of highly porous polymer, Diaion HP-20 (Mitsubishi Chem. Ind.), using water, MeOH, and CHCl3 as eluting solvents. The MeOH eluate (crude saponin fraction) was subjected to repeated column chromatography (CC) and high-performance liquid chromatography (HPLC) to afford daucosterin (sitosteryl glucoside), 23 known saponins and 14 new dammarane saponins, which were named vina-ginsenosides-R1–R14 (Duc et al., 1993, 1994a, 1994b).

Keywords: Adaptogen, antistress, anti-tumor promotion, cancer chemoprevention, dammarane saponin, majonoside-R2, ocotillol, Panax vietnamensis, Vietnamese ginseng, vina-ginsenoside.

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(EIMS) as a trimethylsilyl ether with those of a corresponding authentic sample or reported data. The identified compounds are eight protopanaxadiol-type (2) saponins (3–10), seven propanaxatriol-type (12) saponins (13–19), four octillol-type (21) saponins (22–25) and two oleanolic acid-type (26) saponins (27–28), and are listed in Figures 1–4 with their yields.

Most of the known saponins have been isolated from other Panax spp., though gypenoside XVII (8) (Take-moto et al., 1983) and hemsloside Ma3 (28) (Nie et al., 1984) were not previously isolated from Araliaceae, but from Cucurbitaceous plants. The yields of ginsenosides-Rb1 (3) and -Rg1 (15) were higher than those of Panax ginseng C. A. Meyer root. The high yield of an octillol saponin, majonoside-R2 (25, 5.3%), was remarkable. This saponin was previously isolated from Panax japonicus C. A. Meyer var. major (Burk.) C. Y. Wu et K. M. Feng of Chinese origin (Morita et al., 1982), but the yield was less than 0.1%.

Structure Elucidation of New Saponins, Vina-ginsenosides R1–R14
The chemical structures of new saponins, vina-ginsenosides R1–R14, were elucidated using chemical and spectroscopic means, especially MS, and several modes of NMR measurement were very useful. Only the characteristic features and an outline of the structure determination of each compound are described as follows.

Vina-ginsenosides R1 (29) and R2 (30) were octillol saponins with an acetyl group on the sugar chain at C-6 (Duc et al., 1993). The acetylated position was determined by EI-MS of TMS derivatives and acylation shift regularities of 13C NMR (Yamasaki et al., 1977).

Vina-ginsenoside R3 (31) was the only compound which lacked OH at the C-12 position among the all saponins isolated from this species. Enzymatic hydrolysis was useful to identify its aglycone, dammarenediol II.

Vina-ginsenoside R4 (32) was the first example of a propanaxatriol saponin that has a glycosyl linkage at C-3. The glycosylation shift rule (Kasai et al., 1977) was used to determine the location of the glycosyl bond.

Vina-ginsenosides R5 (33) and R6 (34) were rare examples of saponins with an α-glucosyl moiety. Coupling constants (J values) of the anomeric protons were critical to judge the anomeric bond.

Vina-ginsenoside R7 (35) was xylosyl ginsenoside Rd. The position of the xylosyl moiety was determined by MS and by comparison of the 13C NMR with a known compound that has the same side chain.
Fig. 2. Protopanaxatriol-type saponins isolated from *Panax vietnamensis*, previously known from other plant sources.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Yield (%)</th>
<th>R&lt;sub&gt;1&lt;/sub&gt;</th>
<th>R&lt;sub&gt;2&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>ginsenoside-Re</td>
<td>0.17</td>
<td>- Glc&lt;sup&gt;2&lt;/sup&gt; - Rha</td>
<td>- Glc</td>
</tr>
<tr>
<td>20-gluco-ginsenoside-Rf</td>
<td>0.01</td>
<td>- Glc&lt;sup&gt;2&lt;/sup&gt; - Glc</td>
<td>- Glc</td>
</tr>
<tr>
<td>ginsenoside-Rg&lt;sub&gt;1&lt;/sub&gt;</td>
<td>1.37</td>
<td>- Glc</td>
<td>- H</td>
</tr>
<tr>
<td>ginsenoside-Rh&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0.008</td>
<td>- Glc</td>
<td>- H</td>
</tr>
<tr>
<td>[20(S) + 20 (R)]</td>
<td>0.013</td>
<td>- Glc&lt;sup&gt;2&lt;/sup&gt; - Rha</td>
<td>- Glc</td>
</tr>
<tr>
<td>Pseudo-ginsenoside-RS&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0.36</td>
<td>- Glc&lt;sup&gt;2&lt;/sup&gt; - Xyl</td>
<td>- Glc</td>
</tr>
<tr>
<td>notoginsenoside-R&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0.01</td>
<td>- Glc</td>
<td>- Glc&lt;sup&gt;6&lt;/sup&gt; - α-Glc</td>
</tr>
<tr>
<td>notoginsenoside-R&lt;sub&gt;6&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 3. Ocotillol-type saponins isolated from *Panax vietnamensis*, previously known from other plant sources.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Yield (%)</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>pseudo-ginsenoside-RT&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.065</td>
<td>- Glc</td>
</tr>
<tr>
<td>24(S)-pseudo-ginsenoside-F&lt;sub&gt;11&lt;/sub&gt;</td>
<td>0.005</td>
<td>- Glc&lt;sup&gt;2&lt;/sup&gt; - Rha</td>
</tr>
<tr>
<td>majonoside-R1</td>
<td>0.14</td>
<td>- Glc&lt;sup&gt;2&lt;/sup&gt; - Glc</td>
</tr>
<tr>
<td>majonoside-R2</td>
<td>5.29</td>
<td>- Glc&lt;sup&gt;2&lt;/sup&gt; - Xyl</td>
</tr>
</tbody>
</table>
Vina-ginsenoside R8 (36) had the side chain with a migrated double bond on introduction of OH at C-25. The structure was confirmed through comparison of the $^{13}$C NMR of a similar compound, majonoside F4 (37, structure not illustrated), which is a 3-O- and 20-O-di $\beta$-d-glucoside of the same aglycone (Feng et al., 1987). Vina-ginsenoside R9 (38) also had the double bond migrated side chain as in 36, and the structure was determined through comparison with the $^{13}$C NMR of majonoside-F1, 24-epimer (Feng et al., 1987). The difference in the $\delta$ value arose from the opposite chirality at the C-24 position. Photosensitized oxidation of ginsenoside Rd (7) gave three products, 36, 38 and its 24-epimer. This reaction not only confirmed the structures of vina-ginsenosides R8 and R9, but also implied the biogenesis of these saponins (Duc et al., 1994a).

Vina-ginsenosides R10 (39) and R11 (40) were similar saponins which had a pyran ring in their side chains just like panaxadiol (acid hydrolysis product of protopanaxadiol saponin). The stereochemistry of the side chain was disclosed by NOE and HMBC in the NMR, as well as by Jones' oxidation reaction.

Vina-ginsenosides R12 (43) and R13 (44) had vicinal diol systems in their side chains. EI-MS and HMBC were useful to determine the structure of the side chain. For the decision of the sugar linkage of 43, $^1$H-$^1$H COSY and NOESY techniques were effectively used.

Vina-ginsenoside R14 (45) was an ocotillol saponin and the aglycone was closely related to (20S)-protopanaxatriol oxide II. By detailed observation of $^{13}$C NMR, introduction of the OH at 26-C was deduced. NOE experiments disclosed the stereochemistry (Duc et al., 1994b). Structures of these new saponins isolated from *Panax vietnamensis* are summarized in Figure 5.

**Bioactivity**

Anti-tumor Promoting Activity

To search for possible anti-tumor promoters (cancer chemopreventive agents), several plant extracts were screened using the inhibitory effects on Epstein-Barr virus early antigen (EBV-EA) activation induced by 12-O-tetradecanoylphorbol-13-acetate (TPA) (Konoshima et al., 1998). The methanol extract of *Panax vietnamensis* showed significant inhibitory activity (37.9% at 100 $\mu$g/ml). Since the activity was concentrated in the saponin fraction, the major saponins of this plant were tested, i.e., protopanaxadiol-type saponins: ginsenosides-Rb1 (3, 2.0%), -Rd (7, 0.87%); protopanaxatriol-type saponins: ginsenosides-Re (13, 0.17%) and ginsenoside-Rg1 (15, 1.37%); and ocotillol-type saponins: majonoside R1 (24, 0.14%) and majonoside-R2 (MR2, 25, 5.3%). Among them, the major saponin, MR2, exhibited the strongest inhibitory effects on EBV-
Fig. 5. New saponins isolated from *Panax vietnamensis* (Duc et al., 1993, 1994a, 1994b).
EA activation (50% inhibition at 100 molar ratio/TPA) as is shown in Figure 6. This activity was much higher than that of glycyrrhetic acid (Mizutani, 1994) which is known to be a potent anti-tumor promoter.

The effects of MR2 on the cell cycle of Raji cells treated with TPA were examined by flow cytometry. By the treatment with MR2, the S phase ratio of Raji cells was increased, but the ratio of G2/M phases was decreased in a dose-dependent manner. The inhibition mechanism of MR2 against cancer promotion of TPA was through influencing the cell cycle.

On the basis of the above in vitro assay, an in vivo assay was carried out. The inhibitory effect of MR2 on the two-stage carcinogenesis test of mouse skin tumor was evaluated using 7,12-dimethylbenz[a]anthracene (DMBA) as an initiator, and TPA as a promoter. The papilloma production promoted by TPA was significantly decreased and delayed by the pre-treatment of 85 nmol of MR2 (Fig. 7). The activity of MR2 was higher than that of glycyrrhetic acid (Tokuda, et al. 1986). The inhibitory activity of MR2 was also observed when a non-TPA type tumor promoter, fumonsin B1, was used as a promoter (Konoshima et al., 1998).

MR2 also exhibited potent anti-tumor-promoting activity in a two-stage carcinogenesis test of mouse hepatic tumor using N-nitrosodiethylamine (DEN) as an initiator and phenobarbital (PB) as a promoter (Table 1). Further, MR2 exhibited a remarkable inhibitory effect on two-stage carcinogenesis test of mouse skin induced by nitric oxide (NO) donor/TPA or peroxynitrite/TPA (Konoshima et al., 1999).

Anti-stress Activity

Vietnamese ginseng has been known to have an anti-fatigue effect and some adaptogenic activity (Nham et al., 1995). The notion of an adaptogen is an ambiguous concept, but it is recognized as prophylaxis which re-establishes homeostasis by increasing non-specific resistance against mental, physical and chemical stress.

We have investigated the effect of Vietnamese ginseng (VG) and its major saponin, majonoside-R2 (MR2, 25), on behavioral and pathophysiological responses induced by psychological stress in mice and elucidated possible neuronal mechanisms underlying the action of the Vietnamese ginseng (Huong et al., 1995). For exposing mice to psychological stress, the communication box method (Ogawa et al., 1990) was employed. The important feature of this method is that an animal exposed to physical stress such as electronic footshock can induce socio-psychological stress in other animals by using an intraspecies emotional communication. Nociceptive threshold was estimated by the tail-pinch method. Gastric lesions were evaluated after exposing mice to the stress for 16 h (Huong et al., 1996c). After the administration of pentobarbital, the duration of loss of righting reflex was measured as the sleeping time (50 mg/kg, i.p.).

Pretreatment with VG extract (100 mg/kg, p.o.) or VG saponin (6.25–25 mg/kg, p.o.) suppressed the
antinociception caused by the stress. MR2 (3.1–12.5 mg/kg) also attenuated the stress-induced antinociception. Both flumazenil and picrotoxin completely blocked the antagonistic effects of MR2 on opioid antinociception. It is likely the MR2 suppresses the stress-induced antinociception due to the modulation of the activity of opioid systems (Huong et al., 1996a, 1996b, 1997a). Pretreatment with VG extract, MR2 (6.2 and 12.5 mg/kg, p.o.), diazepam or naloxone exhibited protective actions against the stress-induced gastric lesions, while Panax ginseng extract (50 mg/kg, p.o.) failed to suppress the gastric lesions (Table 2). Psychological stress shortened the duration of sleep induced by pentobarbital. VG extract (50 mg/kg, p.o.), VG saponin (25 mg/kg, p.o.) and MR2 (3.1–12.5 mg/kg, p.o.) each restored the hypnotic activity of pentobarbital to the level of unstressed control mice (Table 3). Panax ginseng extract (50–100 mg/kg, p.o.) showed no effect on pentobarbital sleep in both the stressed and control mice. The effect of MR2 on the stress-induced decrease in pentobarbital sleep was antagonized by numazenil. These results suggest that the GABA<sub>A</sub>-benzodiazepine receptor complex participates in the effect of MR2 on the stress-induced decrease in pentobarbital sleep (Huong et al., 1996c).

Apart from the communication box mediated stress, social isolation stress caused by being housed individually also induced a decrease in pentobarbital sleep,
and MR2 significantly attenuated this effect. The response was mediated by the neurosteroid site on the GABAA receptor complex in mice (Huong et al., 1997b).

The cumulative experiments strongly suggested that MR2 might be responsible for the effects that are characteristic of the crude drug. It is noteworthy that MR2 showed no effect on the pentobarbital sleep duration in unstressed control, while diazepam (a minor tranquilizer) prolonged pentobarbital sleep duration in both unstressed and stressed mice.

These studies provide evidence for the anti-stress effects of Vietnamese ginseng, especially in psychosomatic disorders caused by psychological stress as well as the important role of MR2 in the effects of this plant drug.

Majonoside-R2 (25) is not only abundant in this plant, but it also plays important roles in both bioactivities. Chemically, octillol can be obtained by peracid epoxidation of the corresponding dammarenene derivative with a linear side chain (Fig. 8). In this reaction, the unstable intermediate epoxide cannot be isolated, and it affords a 24R and 24S mixture. In Vietnamese ginseng, only the 24S saponin was isolated, and the probable precursor, notoginsenoside-R2 (46), was not isolated, but a fair amount of corresponding 20 β-glucoside was found. The cumulative experiments strongly suggested that MR2 might be responsible for the effects that are characteristic of the crude drug. It is noteworthy that MR2 showed no effect on the pentobarbital sleep duration in unstressed control, while diazepam (a minor tranquilizer) prolonged pentobarbital sleep duration in both unstressed and stressed mice.

Table 2. Effects of Vietnamese ginseng (VG) extract, VG-saponin and majonoside-R2 (MR2) on the psychological stress-induced decrease in pentobarbital-induced sleep in mice (Huong et al., 1996c).

<table>
<thead>
<tr>
<th>Dose mg/kg</th>
<th>Sleeping time (min)</th>
<th>unstressed</th>
<th>stressed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>73.0 ± 1.9</td>
<td>56.2 ± 2.3</td>
<td></td>
</tr>
<tr>
<td>VG-extract</td>
<td>50</td>
<td>70.3 ± 4.5</td>
<td>79.5 ± 5.3*</td>
</tr>
<tr>
<td>VG-saponin</td>
<td>25</td>
<td>75.5 ± 4.0</td>
<td>71.3 ± 4.7*</td>
</tr>
<tr>
<td>MR2</td>
<td>6.2</td>
<td>73.4 ± 2.0</td>
<td>80.0 ± 6.3*</td>
</tr>
</tbody>
</table>

*P < 0.01 compared with vehicle treatment.

Mice were divided into two groups and the stressed group was exposed to psychological stress for 30 min. Test drugs were administered p.o. 1 h before stress exposure. Each datum represents the mean ± S.E.M. of 12–15 mice.

Table 3. The protective effect of Vietnamese ginseng (VG) extract and majonoside-R2 against the psychological stress-induced gastric lesions (Huong et al., 1996c).

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dose (mg/kg)</th>
<th>Lesion incidence</th>
<th>Lesion severity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>unstressed</td>
<td>stressed</td>
</tr>
<tr>
<td>Vehicle</td>
<td>7/40</td>
<td>30/39</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>VG-ext</td>
<td>25</td>
<td>1/12</td>
<td>1/12**</td>
</tr>
<tr>
<td>Majonoside-R2 (25)</td>
<td>6.2</td>
<td>3/14</td>
<td>1/13**</td>
</tr>
<tr>
<td>Naloxone</td>
<td>5</td>
<td>3/14</td>
<td>2/14**</td>
</tr>
<tr>
<td>Diazepam</td>
<td>10</td>
<td>3/14</td>
<td>4/12**</td>
</tr>
</tbody>
</table>

Mice were divided into 2 groups, and the stressed group was exposed to psychological stress for 16 hr. Gastric lesion incidence was expressed as the ratio of the number of animals with lesion score of >2 to the number of animals used. Lesion severity was expressed as the mean score ± SEM of 12-14 mice. Test drugs except naloxone were administered p.o. 1 hr before stress exposure. Naloxone was injected i.p. 10 min before stress exposure. *P < 0.05, **P < 0.01 compared with the vehicle treatment.

Fig. 8. Formation of octillol saponin.
coside, notoginsenoside-R1 (18) was isolated. Biochemical study and metabolism of MR2 are in progress in our laboratory.

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