Panabipinoside A and panabipinoside B, two new oleanane triterpenoid saponins from the roots of Panax bipinnatifidus with nitric oxide inhibitory activity

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Abstract
Panax bipinnatifidus belongs to the ginseng genus and it is used in traditional Vietnamese and Chinese medicine. Phytochemical studies of the roots of this plant led to the isolation of two new oleanane triterpenoid saponins, panabipinoside A and panabipinoside B, and three known compounds, ginsennoside Ro, 3-O-β-D-glucopyranosyl-(1→3)-β-glucuronopyranosyl oleanolic acid, and spinasaponin A 28-O-glucoside. Their structures are established by extensive spectroscopic analysis (IR, high-resolution electrospray ionization mass spectrometry, and nuclear magnetic resonance) and by comparison of the spectral data with those reported in the literature. The anti-inflammatory activity of the isolated compounds is evaluated by their inhibition of nitric oxide production in lipopolysaccharide stimulated RAW 264.7 cells. Compounds 2–5 showed inhibitory effects on nitric oxide production with IC₅₀ values of 0.62 ± 0.09, 0.21 ± 0.04, 0.30 ± 0.03, and 0.45 ± 0.05 µg/mL, respectively, compared to value of 8.08 ± 0.09 µg/mL for the positive control compound, NG-monomethyl-L-arginine.

Keywords
Araliaceae, Panax bipinnatifidus, panabipinoside A, panabipinoside B, anti-inflammatory activity

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Introduction
Nitric oxide (NO) is a very short half-life molecule. It is known to be a ubiquitous signaling molecule involved in numerous important biological processes in the body such as neurotransmission, in the vascular system and in immune defenses. NO also plays a key role in the pathogenesis of inflammatory diseases.¹ In mammalian cells, the formation of NO is catalyzed by inducible nitric oxide synthase (iNOS) enzymes. In the presence of NADPH and oxygen, iNOSs oxidize L-arginine into L-citrulline and NO. The NO produced aids host defenses by killing invaders such as microorganisms and cancer cells. However, an excess production of NO can cause host and tissue damage which lead to acute and chronic inflammation.²,³ Thus, the level of NO produced by iNOS can be reflected in the degree of inflammation and is considered as an indicator for monitoring inflammatory processes. In addition, NO has been shown as a potential promoter of several types of cancer such as breast, cervical, lung, and gastrointestinal cancers.⁴ An understanding of NO inhibitors can be helpful in reducing the risk of cancer.

Panax, also known as the ginseng genus, which is a member of the Araliaceae family, consists of 18 species.³ Most species of the genus Panax are distributed in the Himalayas, Nepal, and China. Previously, the southern area of Yunnan province (China) and the northern provinces of Vietnam near the China–Vietnam border, which are located at latitude 23°N, were considered to be the distribution limit of the Asia Panax genus. Only 2 of the 18 species of this genus are native to North America. Panax is very popular in Asia and has been used as an edible food and as a
tonic and has history of use as medicine over four millennia. The chemical composition of the Panax genus can be classified into four main groups: saponins, polyacetylenes, polysaccharides, and flavonoids. Besides, there are eight other sub-groups, including phenolics, sterols, carbohydrates, proteins, amino acids, minerals, lipids, and fatty acids. Several plants of the Panax species have long been used as medicinal herbs in oriental countries, for example, P. ginseng C.A. Meyer (Korean ginseng), P. quinquefolius L. (American ginseng), and P. notoginseng F.H. Chen (Notoginseng). Panax bipinnatifidus Seem. belongs to the ginseng genus, and is distributed in China, Nepal, and Vietnam. In Vietnam, it was found in the Hoang Lien Son mountains at an altitude of 1800–2400 m and grows wild under the canopy of a tropical forest. According to traditional medicine, P. bipinnatifidus has been used to stop bleeding, to cure hemorrhages and nosebleeds, to improve memory, and to reduce cancer risk and blood sugar levels in diabetes. Recently, more than 10 saponins isolated from P. bipinnatifidus roots have been reported, all of which have the oleanane-type structure. In this study, with the aim of continuing to research on bioactive compounds in P. bipinnatifidus from Vietnam, five saponins were successfully isolated from the roots of this species including two new oleanane triterpenoid saponins, and three known compounds. The inhibition of NO production in lipopolysaccharide (LPS) stimulated RAW 264.7 cells by compounds 3–5, the glucuronopyranosyl sugar of I was attached to C-3, as confirmed by a HMBC correlation from the anomeric proton at δ 4.42 (d, J=7.5 Hz) to C-3 (δ C 91.1). The large coupling constant value of H-3 at δ 3.32 (J=12.0 Hz) confirmed the axial orientation of this proton and equatorial/β orientation of the group at C-3. In the H–H COSY spectrum, cross peaks were observed between H-1′ (δ H 4.42) and H-2′ (δ H 3.47)/H-3′ (δ H 3.62)/H-4′ (δ H 3.63)/H-5′ (δ H 3.71), which also exhibited HSQC cross peaks to the carbons at δ C 106.3, 74.8, 87.0, 71.9, and 76.7, respectively (see Supplemental Figure S8). Furthermore, a HMBC correlation of H-1′′′ (δ H 4.61) to C-3′′ (δ C 87.0) indicates that the remaining glucuronopyranosyl sugar was linked to C-3′′ of the glucuronopyranosyl moiety (see Supplemental Figure S1). The carbon signal for C-6′′ (the carboxylic carbon in the glucuronic acid moiety) was not detected because of NMR analysis in CD3OD; however, the presence of this carboxylic group was established by IR spectrometry (ν C=O: 1732 cm−1), and further confirmed by HR-ESI-MS data and by comparison of NMR spectral data (C-1′′ to C-5′′) between compound I and compounds 4 and 5 (Table 1). The glucuronopyranosyl group is typically reported in triterpene saponins from plants belonging to the Araliaceae family. All the sugar linkages in compound 1 must be in the β-form as judged from the coupling constants (J=7.5–8.0 Hz) of the anomeric protons at δ H 4.17, 4.42, and 4.61. The configurations of the sugar moieties of I were suggested by analogy with compounds 3–5, which coexist in saponins from the Panax genus, and further confirmed by the acid hydrolysis. The presence of D-glucose and D-glucuronic acid in the acid hydrolysis products of compound I were confirmed by thin-layer chromatography (TLC) analysis and by comparison of their optical rotations with those of authentic D-glucose and D-glucuronic acid. Consequently, the chemical structure of compound I was obtained as colorless amorphous powder. Its molecular formula was established as C48H67O18 by the high-resolution electrospray ionization mass spectrometry (HR-ESI-MS) quasi-molecular ion peak at m/z 941.51031 [M–H]− (calcd for [C48H67O18]−, 941.51099), indicating 10° of unsaturation (see Supplemental Figure S3). Its IR spectrum exhibited the presence of hydroxy (3401 cm−1), carbonyl (1732 cm−1), and C–O–C (1028 cm−1) groups (see Supplemental Figure S2). The 1H NMR (nuclear magnetic resonance) spectrum of I exhibited 7 methyl singlet signals at δ H 0.88, 0.91, 0.92, 1.00, 1.03, 1.09, and 1.21 (each, 3H, s), one oxygenated methylene group at 3.50 (2H, s, H-28), and a broad singlet due to one olefinic proton at δ H 5.21, corresponding to an olefin-12-ene skeleton (see Supplemental Figure S4). In addition, three sugar molecules were identified by presence of the anomeric protons at δ H 4.17 (1H, d, J=8.0 Hz), 4.42 (1H, d, J=7.5 Hz), and 4.61 (1H, d, J=7.5 Hz), as well as by the typical signals of the sugars appearing in the range of δ H 2.95–3.63. Analyses of the 13C NMR, heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation (HMBC) spectra of I indicated that this compound had 48 carbons, including 30 from the oleane skeleton and 18 of the three sugar molecules, which was consistent with the HR-ESI-MS results (see Supplemental Figures S5–S7). Oxygenated methylene, methine carbonyl, and double bond groups were identified at δ C 77.8, 91.1, and 123.6 (CH)/145.8 (C), respectively, while three anomeric carbons were identified at δ C 105.3, 105.0, and 106.3. In the HSQC spectrum, protons H-1′ (δ H 4.17), H-2′ (δ H 3.20), H-3′ (δ H 3.35), H-4′ (δ H 3.32), H-5′ (δ H 3.32), and H-6′ (δ H 3.68/3.78) had cross peaks with the carbons at δ C 105.3, 75.1, 77.8, 71.5, 78.2, and 62.8, respectively. In addition, COSY cross peaks of H-1′/H-2′/H-3′/H-4′/H-5′/H-6′ were observed. The above evidence together with HMBC correlations from H-1′ (δ H 4.17) to C-28 (δ C 77.8), as well as from H-28 (δ C 3.50) to C-1′ (105.3) indicated one glucopyranosyl sugar attached to C-28 by an ether linkage. Furthermore, the HMBC correlations from H-28 (δ C 3.50) to C-16 (δ C 22.7)/C-17 (δ C 37.8)/C-18 (δ C 44.2)/C-22 (δ C 33.0) exactly confirmed the location of the 28-CH2O-group. As with compounds 3–5, the glucuronopyranosyl sugar of I was attached to C-3, as confirmed by a HMBC correlation from the anomeric proton at δ H 4.42 (d, J=7.5 Hz) to C-3 (δ C 91.1). The large coupling constant value of H-3 at δ C 3.32 (J=12.0 Hz) confirmed the axial orientation of this proton and equatorial/β orientation of the group at C-3. In the H–H COSY spectrum, cross peaks were observed between H-1′ (δ H 4.42) and H-2′ (δ H 3.47)/H-3′ (δ H 3.62)/H-4′ (δ H 3.63)/H-5′ (δ H 3.71), which also exhibited HSQC cross peaks to the carbons at δ C 106.3, 74.8, 87.0, 71.9, and 76.7, respectively (see Supplemental Figure S8). Furthermore, a HMBC correlation of H-1′′′ (δ H 4.61) to C-3′′ (δ C 87.0) indicates that the remaining glucuronopyranosyl sugar was linked to C-3′′ of the glucuronopyranosyl moiety (see Supplemental Figure S1). The carbon signal for C-6′′ (the carboxylic carbon in the glucuronic acid moiety) was not detected because of NMR analysis in CD3OD; however, the presence of this carboxylic group was established by IR spectrometry (ν C=O: 1732 cm−1), and further confirmed by HR-ESI-MS data and by comparison of NMR spectral data (C-1′′ to C-5′′) between compound I and compounds 4 and 5 (Table 1). The glucuronopyranosyl group is typically reported in triterpene saponins from plants belonging to the Araliaceae family. All the sugar linkages in compound 1 must be in the β-form as judged from the coupling constants (J=7.5–8.0 Hz) of the anomeric protons at δ H 4.17, 4.42, and 4.61. The configurations of the sugar moieties of I were suggested by analogy with compounds 3–5, which coexist in saponins from the Panax genus, and further confirmed by the acid hydrolysis. The presence of D-glucose and D-glucuronic acid in the acid hydrolysis products of compound I were confirmed by thin-layer chromatography (TLC) analysis and by comparison of their optical rotations with those of authentic D-glucose and D-glucuronic acid. Consequently, the chemical structure of compound I was established as 3β,28-dihydroxyolean-12-ene-3-O-[β-D-glucuronopyranosyl-(1→3)-β-D-glucuronopyranosyl]-28-O-β-D-glucopyranoside, a new compound and named panapipinoside A (Figure 1). Compound 2 was obtained as colorless amorphous powder. Its molecular formula was established as C48H67O18 by the HR-ESI-MS quasi-molecular ion peaks at m/z 1073.54980 [M-H]− (calcd for [C48H67O18]−, 1073.55325), indicating 11° of unsaturation (Supplemental Figure S10). The IR spectrum of 2 exhibited the presence of hydroxy (3406 cm−1), carbonyl (1732 cm−1), and C–O–C (1029 cm−1) groups (see Supplemental Figure S9). The NMR spectra of 2 (measured in CD3OD) were similar to that of I, suggesting that 2 is a derivative of I. The 28-CH2O- and C-12/C-13 double bond groups were identified...
Two new oleanane triterpenoid saponins, named panabipinoside A (1) and panabipinoside B (2), and three known compounds, ginsenoside Ro (3), 3-O-β-D-glucopyranosyl-(1→3)-β-D-glucuronopyranosyl oleanolic acid (4), and spinasaponin A 28-O-glucoside (5), were isolated from the roots of *Panax bipinnatifidus* by various chromatographic methods. Their structures were established by extensive spectroscopic analysis (UV, IR, HR-ESI-MS, and NMR) and by comparison of their spectral data with those reported in the literature. The anti-inflammatory activities of compounds 1–5 were evaluated by their inhibition on NO production in LPS stimulated RAW 264.7 cells. Compounds 2–5 showed inhibitory effects on NO production with IC_{50} values of 0.62 ± 0.09, 0.21 ± 0.04, 0.30 ± 0.03, and 0.45 ± 0.05 µg/mL, respectively, compared to the value of 8.08 ± 0.09 µg/mL for the positive control compound, L-NMMA (see Tables 2 and 3).

### Material and methods

#### General

Optical rotations were measured on a Jasco P2000 polarimeter. IR spectra were recorded on a Spectrum Two Fourier transform infrared (FTIR) spectrometer. HR-ESI-MS were measured on an Agilent 6530 Accurate Mass Q-TOF LC/MS or a Thermo Scientific Q Exactive tm: Focus Hybrid Quadrupole-Orbitrap. NMR spectra were recorded on a Bruker 500 MHz spectrometer. Preparative high-performance liquid chromatography (HPLC) were run on an Agilent 1100 system including a quaternary pump, an autosampler, a DAD detector, and a preparative HPLC column YMC J'sphere ODS-H80 (4 µm, 20×250 mm). Anisocratic mobile phase with a flow rate of 3 mL/min was used for pre-HPLC. Compounds were monitored at wavelengths of 205, 230, 254, and 280 nm. Flash column chromatography was performed using silica gel, reversed phase C-18, and Dianion HP-20 resins as the stationary phase. TLC was carried out on pre-coated silica gel 60 F_{254} and RP-18 F_{254} plates. The spots were detected by spraying with an aqueous solution of 5% H_{2}SO_{4} followed by heating with a heat gun.

#### Plant material

The roots of *Panax bipinnatifidus* Seem. were collected at Sapa, Lao Cai, Vietnam, in May 2019, and identified by Dr Do Ngoc Dai, Department of Forestry, Nghe An University of Economics. A voucher specimen (coded: SVD-BK2019) was deposited at the School of Chemical Engineering, Hanoi University of Science and Technology.
Table 1. The NMR data of compounds 1–5.

|   | δ\_C (m\_c) | δ\_H (m\_c in Hz) | δ\_C (m\_b) | δ\_H (m\_b in Hz) | δ\_C (m\_d) | δ\_C (m\_c) |
|---|-------------|--------------------|-------------|--------------------|-------------|-------------|
| 1 | 40.0        | 0.99 (m)/1.62 (m)  | 40.0        | 0.96 (m)/1.61 (m)  | 38.3        | 39.7        |
| 2 | 26.9        | 1.72 (m)/1.91 (m)  | 26.9        | 1.70 (m)/1.87 (m)  | 25.3        | 26.9        |
| 3 | 91.1        | 3.21 (dd, 12.0, 4.5)| 91.0        | 3.21 (dd, 12.0, 4.5)| 88.1        | 91.0        |
| 4 | 40.2        | –                  | 40.2        | –                  | 38.6        | 40.3        |
| 5 | 57.0        | 0.80 (d, 12.5)     | 57.0        | 0.81 (d, 12.5)     | 55.1        | 57.0        |
| 6 | 19.3        | 1.47 (m)/1.59 (m)  | 19.2        | 1.45 (m)/1.58 (m)  | 17.8        | 19.3        |
| 7 | 33.8        | 1.40 (m)/1.57 (m)  | 33.8        | 1.40 (m)/1.59 (m)  | 32.3        | 34.0        |
| 8 | 41.2        | –                  | 41.2        | –                  | 41.3        | 40.6        |
| 9 | 49.0        | 1.60 (m)           | 49.0        | 1.60 (m)           | 47.1        | 49.0        |
|10 | 37.8        | –                  | 37.8        | –                  | 36.2        | 37.9        |
|11 | 24.7        | 1.90 (m)/1.92 (m)  | 24.7        | 1.90 (m)/1.92 (m)  | 22.9        | 24.5        |
|12 | 123.6       | 5.21 (br s)        | 123.6       | 5.20 (br s)        | 121.7       | 123.7       |
|13 | 145.8       | –                  | 145.8       | –                  | 143.4       | 145.2       |
|14 | 42.9        | –                  | 42.9        | –                  | 40.9        | 43.0        |
|15 | 26.9        | 1.00 (m)/1.70 (m)  | 27.0        | 1.03 (m)/1.70 (m)  | 27.2        | 28.8        |
|16 | 22.7        | 1.41 (m)/1.89 (m)  | 22.7        | 1.42 (m)/1.90 (m)  | 22.5        | 24.1        |
|17 | 37.8        | –                  | 37.8        | –                  | 45.9        | 47.6        |
|18 | 44.2        | 2.03 (dd, 13.0, 3.5)| 44.2        | 2.01 (dd, 13.0, 3.5)| 40.8        | 42.8        |
|19 | 47.7        | 1.07 (m)/1.81 (m)  | 47.7        | 1.07 (m)/1.83 (m)  | 45.5        | 47.3        |
|20 | 31.8        | –                  | 31.8        | –                  | 30.3        | 31.6        |
|21 | 35.3        | 1.16 (m)/1.33 (m)  | 35.3        | 1.16 (m)/1.33 (m)  | 33.2        | 34.9        |
|22 | 33.0        | 1.52 (m)/1.60 (m)  | 33.0        | 1.51 (m)/1.60 (m)  | 31.6        | 33.8        |
|23 | 28.5        | 1.09 (s)           | 28.5        | 1.08 (s)           | 27.6        | 28.5        |
|24 | 16.7        | 0.88 (s)           | 17.1        | 0.87 (s)           | 16.1        | 17.0        |
|25 | 16.2        | 1.00 (s)           | 16.2        | 0.99 (s)           | 15.2        | 15.9        |
|26 | 17.6        | 1.03 (s)           | 17.6        | 1.02 (s)           | 16.7        | 17.4        |
|27 | 26.6        | 1.21 (s)           | 26.6        | 1.20 (s)           | 25.5        | 26.4        |
|28 | 77.8        | 3.50 (s)           | 77.9        | 3.50 (s)           | 175.2       | 181.8       |
|29 | 33.8        | 0.91 (s)           | 33.8        | 0.91 (s)           | 32.7        | 33.6        |
|30 | 24.2        | 0.92 (s)           | 24.2        | 0.92 (s)           | 23.3        | 24.0        |
|28-O-Glc 1 | 105.3       | 4.17 (d, 8.0)      | 105.3       | 4.16 (d, 7.5)      | 94.1        | 95.7        |
|   | 75.1        | 3.20 (dd, 9.0, 7.5)| 75.1        | 3.20 (dd, 9.0, 7.5)| 72.4        | 73.9        |
|   | 77.8        | 3.35 (m)\*         | 78.2        | 3.35 (m)\*         | 76.7        | 78.2        |
|   | 71.5        | 3.32 (m)\*         | 71.7        | 3.31 (m)\*         | 69.8        | 71.1        |
|   | 78.2        | 3.32 (m)\*         | 78.0        | 3.23 (m)\*         | 77.7        | 78.6        |
|   | 62.8        | 3.68 (m)\*\*/3.87 (m)\* | 62.8        | 3.69 (m)\*\*/3.87 (m)\* | 60.8        | 62.5        |
|3-O-GluA 1 | 106.3       | 4.42 (d, 7.5)      | 106.4       | 4.39 (d, 8.0)      | 103.7       | 106.5       |
|   | 74.8        | 3.47 (dd, 9.0, 7.5)| 76.5        | 3.56 (dd, 9.0, 8.0)| 81.4        | 74.9        |
|   | 87.0        | 3.62 (m)\*         | 82.2        | 3.78 (m)\*         | 76.7        | 86.7        |
|   | 71.9        | 3.63 (m)\*         | 74.8        | 3.88 (m)\*         | 72.1        | 72.0        |
|   | 76.7        | 3.71 (d, 9.0)      | 77.9        | 3.25\*             | 73.0        | 76.5        |
|   | 62.8        | 3.68 (m)\*\*/3.87 (m)\* | 62.3        | 3.70 (m)\*\*/3.83 (m)\* | 60.7        | 62.6        |
|4-O-Ara(f) 1 | 107.9       | 5.24 (br s)        | 107.9       | 5.24 (br s)        | 107.9       | 104.9       |
|   | 82.0        | 4.03 (br s)        | 79.5        | 3.82\*            | 87.3        | 4.40 (m)    |
|   | 63.3        | 3.66 (m), 3.71 (m)  | 63.3        | 3.66 (m), 3.71 (m)  | 63.3        | 3.66 (m), 3.71 (m) |

(Continued)
Extraction and isolation

The air-dried roots of *P. bipinnatifidus* (1.0 kg) were ultrasonically extracted in C₂H₅OH (3 L × 3, 80 °C). After filtration, the extracts were concentrated in vacuo to dryness. The obtained C₂H₅OH residue (350 g) was suspended in H₂O (1 L) and then partitioned with n-hexane and ethyl acetate (each 2.5 L × 3), successively, to give n-hexane and ethyl acetate soluble fractions in the weights of 82.5 and 35.72 g, and the water layer. The water layer was subjected to a Dianion HP-20 column and eluted stepwise using a mixture of H₂O and C₂H₅OH (100:0 → 4:96; v/v) to give four fractions (fr. 1.1–fr. 1.5). After checking these fractions by TLC, the sub-fraction 1.3 (3 g) was first separated on a reverse phase C-18 resin column chromatography, eluting with acetone/water (1/1.2, v/v) to give seven smaller fractions, 1.3A–G. Fraction 1.3B was further purified by HPLC using a J’sphere ODS H-80, 250 mm × 20 mm column, MeCN in H₂O (20%, v/v), and a flow rate of 3 mL/min to yield compound 3 (29.5 mg). Next, fraction 1.3D was chromatographed on HPLC using the same conditions to obtain compounds 2 (12.0 mg) and 5 (20.0 mg). Fraction 1.3E was purified by HPLC using a J’sphere ODS H-80, 250 mm × 20 mm column, MeCN in H₂O (23%, v/v), and a flow rate of 3 mL/min to yield compound 1 (14.0 mg). Finally, fraction 1.3G was purified by HPLC using the same conditions to give compound 4 (11.0 mg).

Panabipinoside A (1):  
Colorless amorphous powder, [α]₂⁰°: +12.2 (c 0.1, MeOH); IR (KBr): v_max 3401 (broad), 2944, 1732, 1612, 1076, 1028 cm⁻¹; HR-ESI-MS: m/z 941.51031 [M–H]⁻ (calcd for [C₄₈H₇₁O₁₈]⁻, 941.51099).

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**Table 1.** (Continued)

| NMR: nuclear magnetic resonance; nd: not detected.
| *Measured at 125 MHz.
| †Measured at 500 MHz.
| ‡Measured in CD₃OD.
| §Measured in dimethyl sulfoxide d₆ (DMSO-d₆).
| *Indicates overlapped signals.
| ††Signals were detected from heteronuclear multiple bond correlation (HMBC) spectra.

**Figure 1.** Chemical structures of compounds 1–5.

**Table 2.** Effects of compounds 1–5 (20 µg/mL) on NO productions in LPS stimulated RAW 264.7 cells.

| Comp. | Inhibition (%) | Cell viability (%) |
|-------|---------------|-------------------|
| 1     | 74.45 ± 3.56  | 35.86 ± 3.09      |
| 2     | 93.75 ± 2.55  | 87.27 ± 3.16      |
| 3     | 74.40 ± 4.22  | 93.77 ± 4.07      |
| 4     | 96.89 ± 3.88  | 94.94 ± 1.45      |
| 5     | 80.70 ± 4.35  | 103.41 ± 2.03     |
| L-NMMA* | 70.08 ± 2.18  | 92.46 ± 1.08      |

| NO: nitric oxide; LPS: lipopolysaccharide; L-NMMA: N⁶-monomethyl-L-arginine.  
| Positive control compound. |

**Table 3.** Effects of compounds 2–5 on NO productions in LPS stimulated RAW 264.7 cells.

| Comp. | IC₅₀ (µg/mL) |
|-------|-------------|
| 2     | 0.62 ± 0.09 |
| 3     | 0.21 ± 0.04 |
| 4     | 0.30 ± 0.03 |
| 5     | 0.45 ± 0.05 |
| L-NMMA* | 8.08 ± 0.90  |

| NO: nitric oxide; LPS: lipopolysaccharide; L-NMMA: N⁶-monomethyl-L-arginine.  
| Positive control compound. |

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J’sphere ODS H-80, 250 mm × 20 mm column, MeCN in H₂O (20%, v/v), and a flow rate of 3 mL/min to yield compound 3 (29.5 mg). Next, fraction 1.3D was chromatographed on HPLC using the same conditions to obtain compounds 2 (12.0 mg) and 5 (20.0 mg). Fraction 1.3E was purified by HPLC using a J’sphere ODS H-80, 250 mm × 20 mm column, MeCN in H₂O (23%, v/v), and a flow rate of 3 mL/min to yield compound 1 (14.0 mg). Finally, fraction 1.3G was purified by HPLC using the same conditions to give compound 4 (11.0 mg).
Panabipinoside B (2):

Colorless amorphous powder, $[\alpha]_{D}^{25} +16.7$ (c 0.1, MeOH); IR (KBr): $\nu_{\text{max}}$ 3406 (broad), 2945, 1732, 1613, 1077, 1029 cm$^{-1}$; HR-ESI-MS: $m/z$ 1073.54980 [M–H]$^-$ (calcd for [C$_{53}$H$_{85}$O$_{22}$], 1073.55325).

$^{1}$H NMR (CD$_3$OD, 500 MHz) and $^{13}$C NMR (CD$_3$OD, 125 MHz) data: see Table 1.

Nitric oxide assay

Refer to Supplemental Material

Acid hydrolysis and confirmation of the monosaccharides

Refer to Supplemental Material

Declaration of conflicting interests

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Ethical approval

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Informed consent

There are no human subjects in this article and informed consent is not applicable.

Human and animal rights

This article does not contain any studies with human or animal subjects.

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Supplemental material

Supplemental material for this article is available online.

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