Periandradulcins A, B and C: Phosphodiesterase Inhibitors from *Periandra dulcis* MART.

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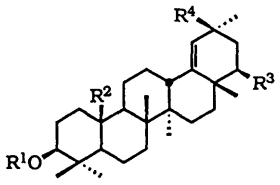
During the course of our screening of bioactive natural products, three new saponins named periandradulcins A (1), B (2) and C (3) were isolated as phosphodiesterase (PDE, EC 3.1.4.17) inhibitors from 80% MeOH extract of the roots of *Periandra dulcis* MART. (Leguminosae) by a combination of column chromatography and reversed- and normal-phase high-performance liquid chromatography (HPLC). On the basis of ^{1}H-, ^{13}C- and two-dimensional nuclear magnetic resonance (NMR) spectral data and chemical evidence, their chemical structures were characterized as 3-*O*-*β*-[*α*-L-rhamnopyranosyl(1→2)-*β*-D-xylopyranosyl(1→2)-*β*-D-glucuronopyranosyl]-30-hydroxyl-25-formylolean-18-ene-22*β*-*O*-syringate, 3-*O*-*β*-[*α*-L-rhamnopyranosyl(1→2)-*β*-D-xylopyranosyl(1→2)-*β*-D-glucuronopyranosyl]-22*β*-hydroxyl-25-formylolean-12-ene and 3-*O*-*β*-[*α*-L-rhamnopyranosyl(1→2)-*β*-D-glucopyranosyl(1→2)-*β*-D-glucuronopyranosyl]-22*β*-hydroxyl-25-formylolean-18-ene, respectively.

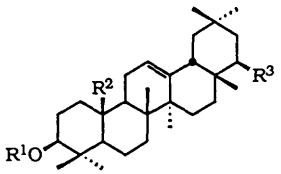
The concentrations of periandradulcins A, B and C required to give 50% inhibition (IC_{50} values) of PDE from bovine heart, were 0.033, 7.6 and 7.7 *μ*M, respectively. Compound 1 was the most potent among the known PDE inhibitors; it inhibited PDE-I (IC_{50}: 0.0022 *μ*M) twenty and forty times more effectively than PDE-II and -III, respectively.

Keywords *Periandra dulcis*; Leguminosae; triterpenoid saponin; periandradulcin A; periandradulcin B; periandradulcin C; phosphodiesterase inhibitor

Since Sutherland found cyclic adenosine monophosphate (cAMP) as a second messenger inside cells,[1] compounds that act to alter cAMP metabolism have been studied with the aim of developing of new medicinal drugs. Various synthetic compounds[2] and natural products[3] have been reported as cAMP phosphodiesterase (PDE, EC 3.1.4.17) inhibitors.

During the course of our screening of bioactive constituents from crude drugs, three new triterpene glycosides, named periandradulcins A (**1**), B (**2**) and C (**3**), were isolated as potent PDE inhibitors from the roots of *Periandra dulcis* MART. (Leguminosae). Hashimoto *et al.* had reported the





1: $R^1 = S_1$, R^2 = -CHO, R^3=-*O*-syringate, R^4 = $-CH_2OH$

2: $R^1 = S_1$, R^2 = -CHO, R^3 = -OH

3: $R^1 = S_2$, R^2 = -CHO, R^3 = -OH, R^4 = $-CH_3$

4: R^1 = H, R^2 = $-CH_3$, R^3 = -OH

5: R^1 = H, R^2 = $-CH_3$, R^3 = H, R^4 = $-CH_3$

6: R^1 = H, R^2 = $-CH_3$, R^3 = -OH, R^4 = $-CH_3$

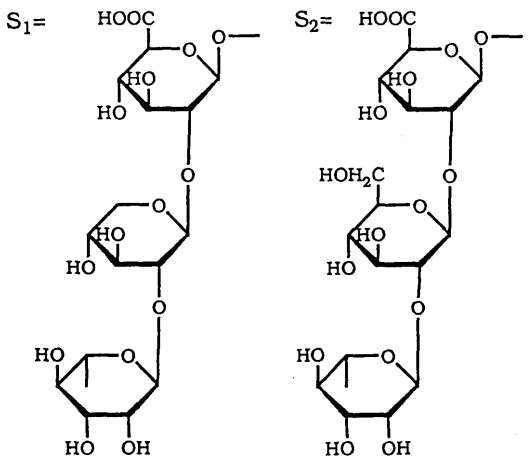


Chart 1

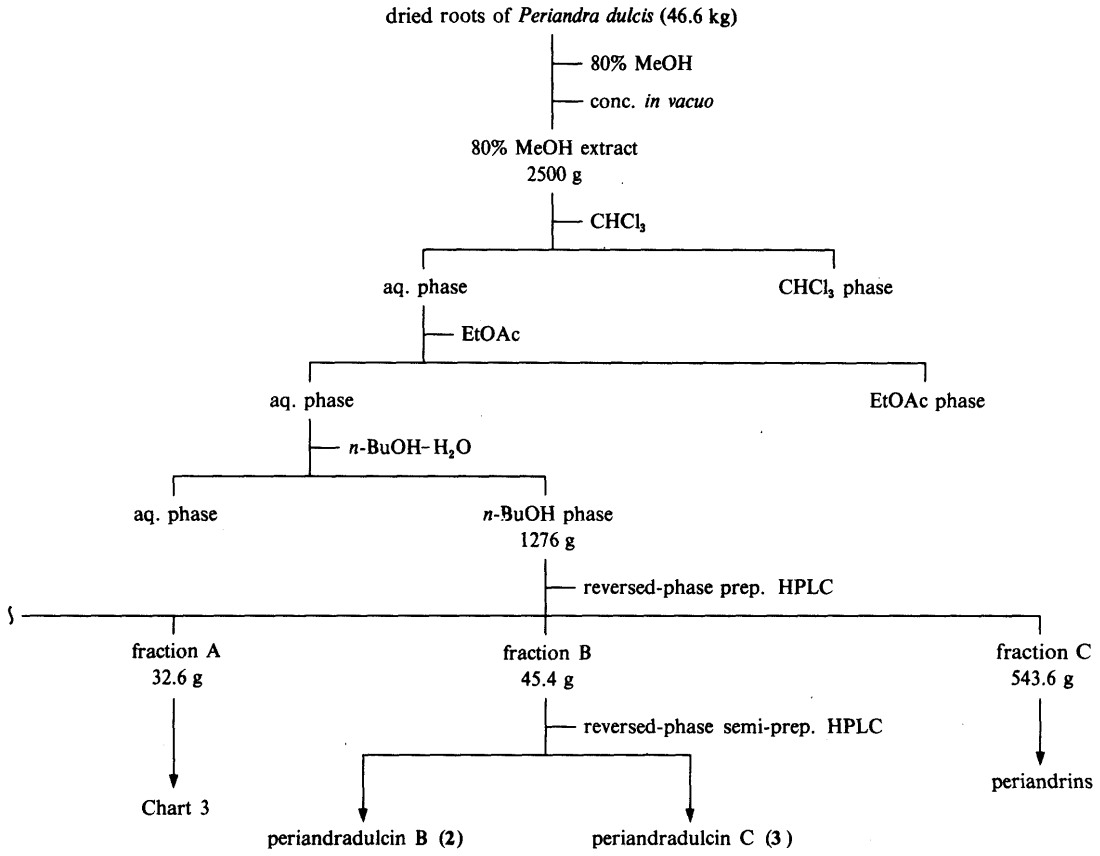


Chart 2. Isolation Procedure for Periandradulcins

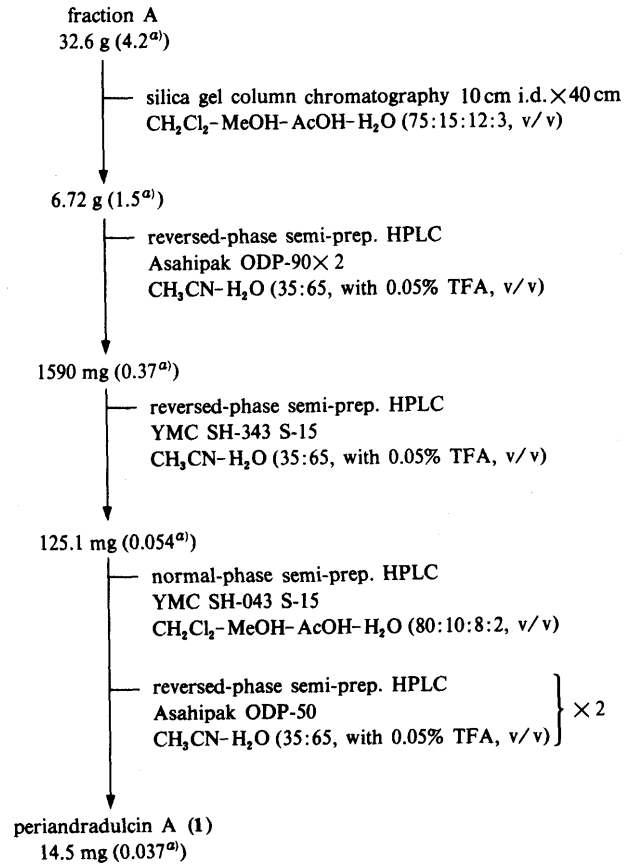


Chart 3. Isolation Procedure for Periandradulcin A (1) from Fraction A

a) IC_{50} values for PDE (μg/ml).

isolation and structural elucidation of four new saponins named periandrins I, II, III and IV as sweet constituents of this plant.[4]

In this paper, we describe the isolation, structural elucidation and PDE-inhibitory activities of periandradulcins A (**1**), B (**2**) and C (**3**).

The 80% MeOH extract from dried roots of *Periandra dulcis* was treated as shown in Chart 2 to give fractions A, B and C. Fraction A was further fractionated by column chromatography and reversed- and normal-phase high-performance liquid chromatography (HPLC) with the guidance of PDE inhibitory activity assay to obtain periandradulcin A (**1**) in 0.31 ppm yield (Chart 3). Fraction B was also fractionated by HPLC to give periandradulcins B (**2**) and C (**3**) in 25.8 and 19.2 ppm yield, respectively. Fraction C contained periandrins.[4]

The molecular weight of periandradulcin B (**2**), a white amorphous powder, was determined to be 910 by positive and negative secondary ion mass spectroscopy (SI-MS), from the peaks at *m/z* 933 for the $[M+Na]^+$ ion and at *m/z* 909 for the $[M-H]^-$ ion, respectively. The ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectrum of **2** showed signals due to seven angular methyl protons at δ 1.34, 1.27, 1.22, 1.14, 0.99, 0.98 and 0.90, one trisubstituted olefinic proton at δ 5.21 (br t), one formyl proton at δ 10.36 (br s) and three anomeric protons at δ 6.25 (d, J=1 Hz), 5.61 (d, J=7 Hz) and 5.04 (d, J=8 Hz). The ^{13}C-NMR spectrum showed two methine carbons bearing hydroxyl at δ 75.4 and a sugar moiety at δ 88.4, trisubstituted olefinic carbons at δ 144.6 (s) and 121.7 (d), a formyl carbon at δ 205.7 (d), a carbonyl carbon at δ 172.0

TABLE I. ^{13}C-NMR Chemical Shift Values (δ) of Aglycone Moieties of **1**, **2**, **3** and Related Compounds[a]

No.	2	4	3	6	5	1
1	32.5	39.2	33.5	39.0	38.5	33.6
2	27.3	28.2	27.6	27.0	27.4	27.7
3	88.4	78.1	88.7	79.0	79.0	88.5
4	39.6	39.5	40.2	39.0	39.0	39.5
5	54.1	55.9	54.4	55.5	55.7	54.4
6	17.4	18.9	17.7	18.5	18.3	17.7
7	33.1	33.3	34.0	34.5	34.7	34.7
8	39.9	40.1	40.5	39.5	40.8	40.5
9	49.9	48.1	53.6	51.2	51.3	53.5
10	52.6	37.3	52.8	37.0	37.3	52.8
11	23.9	23.9	21.9	21.1	21.2	21.7
12	121.7	122.5	26.8	26.6	26.2	26.5
13	144.6	144.9	38.5	38.4	39.0	38.5
14	42.2	42.5	42.8	42.5	43.4	42.7
15	26.7	26.5	28.1	27.4	27.6	27.8
16	28.6	28.7	35.1	34.4	37.7	33.9
17	37.8	38.0	40.1	40.6	34.4	34.7
18	45.3	45.4	142.5	141.5	142.8	143.6
19	46.6	46.9	129.4	129.3	129.8	127.0
20	30.8	30.9	33.7	33.5	32.3	40.1
21	42.2	42.3	42.3	41.1	33.4	33.6
22	75.4	75.6	75.4	76.5	37.4	78.4
23	26.5	28.8	26.8	28.0	28.0	26.8
24	15.4	15.9	15.8	15.4	15.4	15.8
25	205.7	16.6	205.6	16.1	16.1	205.6
26	19.3	17.3	18.4	16.7	16.7	18.3
27	25.6	25.8	14.9	14.7	14.6	15.0
28	28.6	28.8	21.4	21.0	25.3	19.7
29	33.1	33.3	32.1	31.8	31.3	25.1
30	21.0	21.2	30.1	29.9	29.2	72.1

a) Spectra were measured in pyridine-d_5 and assignment of the signals was performed by comparison with reference data.[6]

TABLE II. ^{13}C-NMR Chemical Shift Values (δ) of Sugar Moieties of **1**, **2** and **3**[a]

No.	1	2	3
Glucuronic acid-1	105.4	105.2	105.4
Glucuronic acid-2	79.2	78.5	78.8[b]
Glucuronic acid-3	76.3	76.4	76.6
Glucuronic acid-4	73.7	73.3	73.6
Glucuronic acid-5	78.4	78.9	78.8[b]
Glucuronic acid-6	172.8	172.0	173.4
Xylose-1	102.9	102.7	
Xylose-2	79.3	79.0	
Xylose-3	78.8	78.6	
Xylose-4	71.6	71.4	
Xylose-5	66.6	66.6	
Glucose-1			102.2
Glucose-2			79.5
Glucose-3			77.4
Glucose-4			72.4
Glucose-5			78.8
Glucose-6			63.4
Rhamnose-1	102.3	101.9	102.1
Rhamnose-2	72.4	72.1	72.4
Rhamnose-3	72.8	72.4	72.8
Rhamnose-4	74.4	74.1	74.3
Rhamnose-5	69.7	69.3	69.5
Rhamnose-6	19.0	18.8	19.0

a) Spectra were measured in pyridine-d_5. b) Overlapped.

and three anomeric carbons at δ 105.2, 102.7 and 101.9 (Tables I and II). These results suggested that **2** is a Δ^{12}-oleanene triterpene triglycoside with hydroxyl and formyl groups.

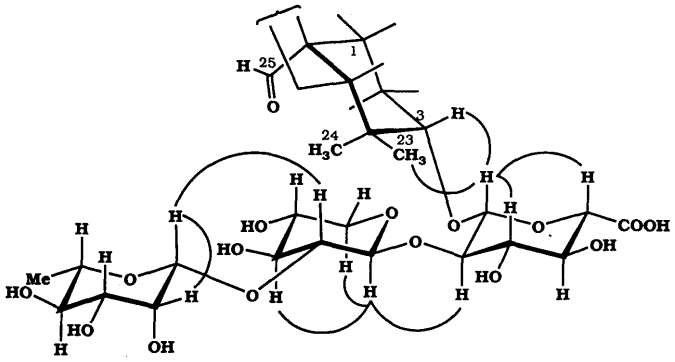


Fig. 1. NOE Correlations in the Sugar Moiety of **2** (Pyridine-d_5)

The product obtained by Huang-Minlon reduction of **2** was identical with sophoradiol (**4**) on ^{13}C-NMR comparison.[5]

A W-type long-range coupling between the formyl proton at δ 10.36 (br s) and 1α-H proton at δ 0.76 (br t), which was comfirmed by the ^{1}H–^{1}H correlation spectrum (^{1}H–^{1}H COSY), indicated that the position of the formyl group was at 25-C.

From the acidic hydrolysate of **2**, three sugars were identified as rhamnose, xylose and glucuronic acid by HPLC. Fragment ions at *m/z* 763 and 631 in the negative SI-MS of **2** were assumed to be due to $[M-\text{rhamnose}-H]^-$ and $[M-\text{rhamnose}-\text{xylose}-H]^-$, respectively, suggesting that **2** possesses a terminal rhamnosyl-xylosyl moiety in the carbohydrate residue. All proton and carbon signals of the sugar moiety were assigned on the basis of ^{1}H–^{1}H COSY and ^{1}H–^{13}C correlation (^{1}H–^{13}C COSY) spectroscopies (Table II). Glycosylation shifts which were observed at C-1—3 of glucuronic acid and xylose suggested that the linkage patterns of the sugars should be 1—2. These conclusions were supported by the nuclear Overhauser effect (NOE) correlations (Fig. 1) observed in the phase-sensitive nuclear Overhauser effect 2D spectroscopy (NOESY) spectra.

Therefore, the structure of **2** was concluded to be 3-*O*-β-[α-L-rhamnopyranosyl(1→2)-β-D-xylopyranosyl(1→2)-β-D-glucuronopyranosyl]-22β-hydroxyl-25-formyloleanan-12-ene.

Periandradulcin C (**3**) was a pale brown amorphous powder, of which the molecular weight was 940 as determined by positive and negative SI-MS, which showed peaks at *m/z* 963 for the $[M+Na]^+$ ion and at *m/z* 939 for the $[M-H]^-$ ion, respectively. Its ^{1}H- and ^{13}C-NMR spectra were similar to those of **2** except for the olefinic proton at δ 4.90 (br s) and the olefinic carbons at δ 142.5 (s) and 129.4 (d) which were similar to those of Δ^{18}-oleanene triterpenes such as geramanicol (**5**)[6] (Table I). Thus, **3** is a triglycoside of a Δ^{18}-oleanene triterpene having hydroxyl and formyl groups. The product (**6**) obtained by Huang-Minlon reduction showed an M^+ ion at *m/z* 442 in the electron impact mass spectrum (EI-MS). The ^{1}H-NMR spectrum of **6** showed signals of an olefinic proton as a broad singlet (δ 4.83), eight angular methyl protons as singlets (δ 1.07, 1.01, 1.00, 0.97, 0.96, 0.88, 0.77 and 0.73) and two methine protons on carbon equatorially bearing a hydroxyl group as double doublets [δ 3.63 (J=7, 10 Hz)

and 3.20 ($J=10$, 6 Hz)]. These assignments as well as the EI-MS fragmentations (*m/z* 220, 206, 205 and 175) suggested **6** to be 3β-hydroxylolean-18-ene with a hydroxyl group at 15α, 16β, 21α or 22β.[7] By ^{13}C-NMR comparison of **6** and **5**, signals due to 17-, 21- and 22-C were seen to be shifted downfield by 6.2, 7.7 and 39.1 ppm, respectively, and those due to 16-, 18- and 28-C were shifted upfield by 3.3, 1.3 and 4.3 ppm, while other carbon signals were not much changed (Table I). From these data, **6** was characterized as 3β,22β-dihydroxyolean-18-ene.

The position of the formyl group was suggested to be 25-C on the basis of ^{1}H–^{1}H COSY data similar to those for **2**. On acidic hydrolysis of **3**, the sugars were identified as rhamnose, glucose and glucuronic acid by HPLC. Fragment ions at *m/z* 793 and 631 in negative SI-MS, assumed to be due to $[M-\text{rhamnose}-H]^-$ and $[M-\text{rhamnose}-\text{glucose}-H]^-$, respectively, indicated that the sugar moiety of **3** was rhamnosyl, glucosyl and glucuronyl. From the NMR data (^{1}H-NMR, ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY and phase-sensitive NOESY), all carbon signals of the sugar moiety of **3** were assigned as shown in Table II.

Therefore, the chemical structure of **3** was concluded to be 3-*O*-β-[α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl]-22β-hydroxyl-25-formyl-olean-18-ene.

Periandradulcin A (**1**) was a pale brown amorphous powder, of which the molecular weight was 1106 as determined by positive and negative SI-MS, which showed peaks at *m/z* 1129 for the $[M+Na]^+$ ion and at *m/z* 1105 for the $[M-H]^-$ ion, respectively. The ^{1}H-NMR spectrum of **1** showed signals of six angular methyl protons at δ 1.42, 1.41×2, 1.08, 0.93 and 0.90, one trisubstituted olefinic proton as broad singlet at δ 5.21, one formyl proton at δ 10.38 (br s) and three anomeric protons at δ 6.42 (d, $J=1$ Hz), 5.72 (d, $J=7$ Hz) and 5.04 (d, $J=8$ Hz). The ^{13}C-NMR spectrum (Table I) showed signals of olefinic carbons at δ 143.6 (s) and 127.0 (d), a formyl carbon at δ 205.6 (d), two methine carbons bearing ester and sugar linkage at δ 78.4 and 88.5, a carbonyl carbon at δ 172.8 and three anomeric carbons at δ 105.4, 102.9 and 102.3. Besides these NMR signals, eight protons (except that of the hydroxyl group) and nine carbons were assignable as those of syringic acid.

Fragment ions at *m/z* 924 and 778, assumed to be due to $[M-\text{syringic acid}-H]^-$ and $[M-\text{syringic acid}-\text{rhamnose}-H]^-$, respectively, were observed in the negative SI-MS along with fragment ions at *m/z* 959 and 827, assumed to be due to $[M-\text{rhamnose}-H]^-$ and $[M-\text{rhamnose}-\text{xylose}-H]^-$, respectively. Methyl syringate was obtained from **1** by treatment with sodium methoxide, and was confirmed to be identical with an authentic specimen by HPLC, thin layer chromatography (TLC) and EI-MS. Therefore, **1** was suggested to be Δ^{18}-oleanene triterpene triglycoside syringate.

In the ^{1}H-NMR spectrum, a double doublet signal at δ 4.90 (22α-H) in **3** was observed at δ 5.63 in **1**. This downfield shift suggested that the ester linkage with syringic acid was at 22-C. The signals at δ 3.70 and 3.64 (hydroxymethyl, AB-quartet, $J=12$ Hz) showed NOE correlation with δ 5.21 (19-H), 2.33 (21α-H), 1.97 (21β-H) and 1.41 (29-H), and the signal at δ 1.41 (29-H) showed NOE correlation with δ 5.63 (22α-H) and 0.90 (27-H), as shown in Fig. 2. On ^{13}C-NMR

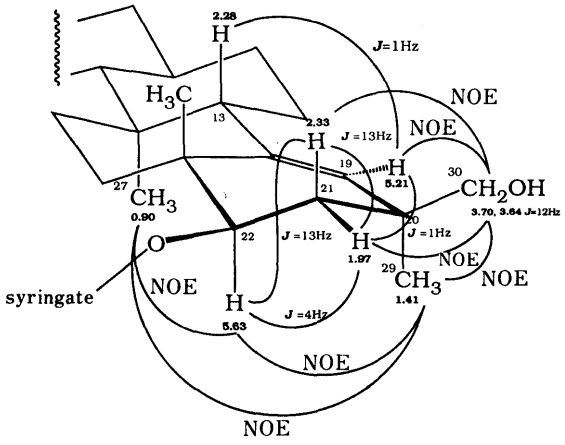


Fig. 2. ^{1}H-NMR Chemical Shift Values (δ), Spin Coupling Constants and NOE Correlation for the E-Ring of **1** (Pyridine-d_5)

comparison of **3** and **1** (Table I), signals due to 20-C, 22-C and 30-C were shifted downfield by 6.4, 3.0 and 42.0 ppm, respectively and those due to 17-C, 19-C, 21-C and 29-C shifted upfield by 5.4, 2.4, 8.7 and 7.0 ppm, respectively, while other carbon signals were almost unchanged. Thus, the hydroxymethyl carbon and the ester linkage position with syringic acid were concluded to be at 30-C and 22-C, respectively.

The position of the formyl group was suggested to be 25-C on the basis of ^{1}H–^{1}H COSY data, as in the case of **2**. On acidic hydrolysis of **1**, released sugars were identified as rhamnose, xylose and glucuronic acid by HPLC. The chemical shift values of all proton and carbon signals of the sugar moiety, which were assigned by ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY and phase-sensitive NOESY, were in good agreement with those of periandradulcin B (**2**) (Table II). Therefore, the chemical structure of **1** was characterized as 3-*O*-β-[α-L-rhamnopyranosyl(1→2)-β-D-xylopyranosyl-(1→2)-β-D-glucuronopyranosyl]-30-hydroxyl-25-formyl-olean-18-ene-22β-*O*-syringate.

Periandradulcin A (**1**) is the first example of a syringate of a saponin to be isolated from a natural source. This is also the first time that periandradulcins B (**2**) and C (**3**) have been found in nature.

The concentrations of **1**, **2** and **3** required to give 50% inhibition (IC_{50} values) of PDE from bovine heart were determined to be 0.033, 7.6 and 7.7 μM, respectively. Periandradulcin A (**1**) was thus two hundred times more potent than the others, and is the most potent among the known PDE inhibitors.

Three kinetically distinct PDEs PDE-I, PDE-II and PDE-III, were separated from bovine heart by diethylaminoethyl (DEAE)-cellulose chromatography.[8] Papaverine, which is a well known PDE inhibitor, was not specific for any of these PDEs. The novel vasodilating agent vinpocetine[9] specifically inhibited PDE-I, which was activated by Ca^{2+} and calmodulin. The novel positive inotropic agent milurinone was reported to inhibit most effectively PDE-III, which is a cAMP-specific PDE, among the three PDEs.[10] The IC_{50} value of **1** for PDE-I was 0.0022 μM, which is twenty and forty times less than those for the other PDEs (Table III). Thus, **1** is a specific inhibitor of PDE-I like vinpocetine, but the activity of the former was 500 times that of the latter. One of the important

TABLE III. IC_{50} Values (μM) of **1** and Typical Known Inhibitors for the PDEs Derived from Bovine or Guinea Pig Hearts[a]

	IC_{50} values (μM)			
	1[b]	Papaverine[b]	Vinpocetine[b]	Milurinone[c,d]
PDE-I	0.0022	11.7	22.8	310
PDE-II	0.042	2.26	71.3	220
PDE-III	0.083	1.75	>285	2.50

a) cAMP (1 μM) was used as a substrate. *b*) PDEs from bovine heart were used. *c*) PDEs from guinea pig heart were used. *d*) Reported data.[10]

vasodilating mechanisms of vinpocetine is thought to be specific inhibition of PDE-I.[9] So, **1** may be expected to show a vasodilating activity.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Ultraviolet (UV) spectra were recorded on a Shimadzu UV-240 spectrometer and optical rotations were measured on a JASCO DIP-181 digital polarimeter. NMR spectra were recorded on Bruker AM-500 (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR) and Bruker AC-200 (200 MHz for ^{1}H-NMR and 50 MHz for ^{13}C-NMR) instruments, using tetramethylsilane (TMS) as an internal standard. Chemical shifts are shown in δ (ppm) and multiplicities are indicated as follows: singlet = s, doublet = d, triplet = t, multiplet = m and broad = br. Coupling constants (J) are shown in Hz. All 1D and 2D plus sequences were run using Bruker standard software. EI-MS and SI-MS were taken on a Shimadzu QP-1000 mass spectrometer and a Hitachi M-2000 mass spectrometer, respectively. TLC analysis were performed on pre-coated Kieselgel 60 F_{254} plates (Merck) and spots were detected under UV irradiation (254 nm) and by spraying 10% H_2SO_4 solution followed by heating. Column chromatographies were carried out on Silica gel 60 (230—400 mesh, Nacalai Tesque Inc.) and YMC gel ODS-AQ S-50 (50 μm, YMC Co.). Preparative HPLC were carried out on two columns (47 mm i.d. × 300 mm) packed with YMC gel ODS-AQ S-50 (50 μm, YMC Co.) with a Waters System 500 Prep. LC System. Semi-preparative HPLC separations were carried out on a YMC SH-343 S-15 column (20 mm i.d. × 250 mm, ODS, 15 μm, YMC Co.), YMC SH-043 S-15 column (20 mm i.d. × 250 mm, silica gel, 15 μm, YMC Co.), YMC A023 column (10 mm i.d. × 250 mm, silica gel, YMC Co.) or Asahipack ODP-90 column (21.5 mm i.d. × 300 mm, octadecylpolymer, 9 μm, Asahi Chemical Industry Co., Ltd.) with a Waters M600 pump, Waters U6K injector and Shimadzu SPD-6AV ultraviolet-visual detector.

Isolation The dried roots of *Periandra dulcis* (46.6 kg) were extracted with 80% MeOH. The concentrated extract (2500 g) was dissolved in H_2O and extracted successively with $CHCl_3$, EtOAc and water-saturated *n*-BuOH. The *n*-BuOH layer was evaporated *in vacuo* to give the crude saponin (1276 g). The crude saponin fraction was subjected to preparative HPLC [conditions: column, as described above; mobile phase, CH_3CN–H_2O (35 : 65, v/v) containing 0.05% trifluoroacetic acid (TFA); flow rate, 150 ml/min; detector, refractive index] to give fractions A (32.6 g), B (45.4 g) and C (543.6 g) as shown in Chart 2. Fraction A was further subjected to silica gel column chromatography and normal- and reversed-phase semi-preparative HPLC as shown in Chart 3 to give periandradulcin A (**1**, 14.5 mg). A part of fraction B (4.54 g) was subjected to reversed-phase semi-preparative HPLC [conditions: column, YMC SH-343 S-15 column; mobile phase, CH_3CN–H_2O (35 : 65, v/v) containing 0.05% TFA; flow rate, 10 ml/min; detector, 207 nm UV absorbance] to give periandradulcin B (**2**, 120.0 mg) and periandradulcin C (**3**, 89.0 mg). Fraction C (543.6 g) mainly contained periandrin II, which was identified by HPLC and TLC comparisons with an authentic sample.

Periandradulcin A (1) A pale brown amorphous powder, $[\alpha]_D^{25}$ −55.0° (c = 0.2, MeOH), mp 220—225 °C (dec.). UV λ_{max}^{MeOH} nm (log ε): 277 (3.88), 206 (4.21). ^{1}H-NMR (pyridine-d_5) δ: 10.38 (1H, br s, –CHO), 7.82 (2H, s, syringate-2), 6.42 (1H, d, J = 1 Hz, Rha-1-H), 5.72 (1H, d, J = 7 Hz, Xyl-1-H), 5.63 (1H, dd, J = 13, 4 Hz, 22α-H), 5.21 (1H, br s, 19-H), 5.04 (1H, d, J = 8 Hz, Glc.A-1-H), 5.02 (1H, dt, J = 6, 9 Hz, Rha-5-H), 4.78 (1H, dd, J = 1, 4 Hz, Rha-2-H), 4.70 (1H, dd, J = 4, 9 Hz, Rha-H-3), 4.62 (2H, m, Glc.A-3-H overlapped with Glc.A-5-H), 4.49 (1H, dd, J = 9, 10 Hz, Glc.A-4-H), 4.42 (1H, dd, J = 9, 8 Hz, Glc.A-2-H), 4.36 (1H, t, J = 9 Hz, Rha-4-H), 4.35 (1H, dd, J = 4, 11 Hz, Xyl-5-H), 4.31 (1H, dd, J = 7, 8 Hz, Xyl-2-H), 4.21 (2H, m, Xyl-3-H overlapped with Xyl-4-H), 3.91 (6H, s, syringate-OCH$_3$), 3.70 (1H, d, J = 12 Hz, H-30), 3.64 (1H, d, J = 12 Hz, H-30), 3.59 (1H, dd, J = 11, 12 Hz, Xyl-5-H), 3.38 (1H, dd, J = 4, 12 Hz, 3-H), 2.58 (1H, br d, J = 14 Hz, 1β-H), 2.33 (1H, t, J = 13 Hz, 21α-H), 2.28 (2H, 13-H overlapped with 2β-H), 1.97 (1H, ddd, J = 13, 4, 1 Hz, 21β-H), 1.84 (3H, d, J = 6 Hz, Rha-6-H), 1.42 (3H, s, 28-H), 1.41 (6H, s, 23-H overlapped with 29-H), 1.08 (3H, s, 24-H), 0.93 (3H, s, 26-H), 0.90 (3H, s, 27-H), 0.75 (1H, br t, J = 12 Hz, 1α-H). ^{13}C-NMR (pyridine-d_5): Tables I and II.

Periandradulcin B (2) A white amorphous powder, $[\alpha]_D^{25}$ +12.0° (c = 1.0, MeOH), mp 225—227 °C (dec.). UV λ_{max}^{MeOH} nm (log ε): 209 (3.56). *Anal.* Calcd for $C_{47}H_{74}O_{17}\cdot H_2O$: C, 60.76; H, 8.25. Found: C, 60.70; H, 8.45. ^{1}H-NMR (pyridine-d_5) δ: 10.36 (1H, br s, –CHO), 6.25 (1H, d, J = 1 Hz, Rha-1-H), 5.61 (1H, d, J = 7 Hz, Xyl-1-H), 5.21 (1H, br t, J = 4 Hz, 11-H), 5.04 (1H, d, J = 8 Hz, Glc.A-1-H), 5.02 (1H, dt, J = 6, 9 Hz, Rha-5-H), 4.77 (1H, dd, J = 1, 4 Hz, Rha-2-H), 4.69 (1H, dd, J = 4, 9 Hz, Rha-H-3), 4.60 (2H, m, Glc.A-3-H overlapped with Glc.A-5-H), 4.49 (1H, dd, J = 9, 10 Hz, Glc.A-4-H), 4.40 (1H, dd, J = 9, 8 Hz, Glc.A-2-H), 4.35 (1H, t, J = 9 Hz, Rha-4-H), 4.34 (1H, dd, J = 4, 11 Hz, Xyl-5-H), 4.30 (1H, dd, J = 7, 8 Hz, Xyl-2-H), 4.20 (2H, m, Xyl-3-H overlapped with Xyl-4-H), 3.72 (1H, dd, J = 3, 7 Hz, 22α-H), 3.57 (1H, dd, J = 11, 12 Hz, Xyl-5-H), 3.33 (1H, dd, J = 4, 10 Hz, 3-H), 1.80 (3H, d, J = 6 Hz, Rha-6-H), 1.34 (3H, s, 23-H), 1.27 (3H, s, 27-H), 1.22 (3H, s, 29-H), 1.14 (3H, s, 30-H), 0.99 (3H, s, 24-H), 0.98 (3H, s, 28-H), 0.90 (3H, s, 26-H), 0.76 (1H, br t, J = 12 Hz, 1α-H). ^{13}C-NMR (pyridine-d_5): Tables I and II.

Periandradulcin C (3) A pale brown powder, $[\alpha]_D^{25}$ −17.4° (c = 0.5, pyridine), mp 205—210 °C (dec.). UV λ_{max}^{MeOH} nm (log ε): 208 (3.70). *Anal.* Calcd for $C_{48}H_{76}O_{18}\cdot H_2O$: C, 59.17; H, 8.20. Found: C, 58.95; H, 8.45. ^{1}H-NMR (pyridine-d_5) δ: 10.30 (1H, br s, CHO), 6.29 (1H, d, J = 1 Hz, Rha-1-H), 5.73 (1H, d, J = 7 Hz, Glc-1-H), 4.90 (1H, br s, 18-H), 3.25 (1H, dd, J = 4, 11, 3-H), 1.73 (3H, d, J = 6 Hz, Rha-6-H), 1.34 (3H, s, 23-H), 1.24 (3H, s, 26-H), 1.10 (3H, s, 30-H), 1.03 (3H, s, 24-H), 1.01 (3H, s, 29-H), 0.93 (3H, s, 28-H), 0.86 (3H, s, 27-H), 0.71 (1H, br t, J = 12 Hz, 1α-H). ^{13}C-NMR (pyridine-d_5): Tables I and II.

General Procedure of Acidic Hydrolysis The solution of pure saponin (1 mg) in 10% H_2SO_4 was refluxed for 3 h. After removal of the $CHCl_3$-soluble portion, the aqueous phase was neutralized with 1 N NaOH solution and analyzed by HPLC [column, TSKgel Sugar-AXI (4.6 mm i.d. × 150 mm, Tosoh Co.); mobile phase, FUNABEC-B solution (borate–ethanolamine complex, Funakoshi Pharmaceutical Co., Ltd.); detector, Shimadzu RF535 fluorescence detector (Ex = 342 nm, Em = 432 nm); flow rate, 0.4 ml/min; column temperature, 60 °C. Retention times: rhamnose, 14.92 min; xylose, 40.28 min; glucose, 49.82 min]. Glucuronic acid was identified by comparison with an authentic sample on another HPLC system [column, Carbopac PAI (4 mm i.d. × 250 mm, Dionex Co.); mobile phase, 100 mM NaOH–200 mM NaOH, 1.0 M NaOAc–200 mM NaOH (35 : 15 : 50, v/v); detector, Dionex PAD-2 plused amperometric detector; flow rate, 0.8 ml/min; column temperature, room temperature. Retention time: glucuronic acid, 12.34 min].

Huang-Minlon Reduction of Periandradulcin B (2) A solution of **2** (20 mg) in diethyleneglycol (20 ml) and 80% hydrazine hydrate (10 ml) with KOH (700 mg) was refluxed on an oil bath (160 °C) for 24 h. After the removal of excess hydrazine hydrate, the reaction mixture was refluxed at 210 °C for 24 h following by extraction with $CHCl_3$. The $CHCl_3$ extract was purified by semi-preparative HPLC [column, YMC-A023; mobile phase, hexane–iso-PrOH (95 : 5, v/v); flow rate, 5 ml/min; detection, 207 nm UV absorption] to furnish **4** (5.20 mg), of which the ^{13}C-NMR chemical shift values were identical with those of sophoradiol.[5]

Huang-Minlon Reduction of Periandradulcin C (3) A solution of **3** (20 mg) in diethyleneglycol (20 ml) and 80% hydrazine hydrate (20 ml) with KOH (700 mg) was refluxed on oil bath (160 °C) for 54 h. After removal of excess hydrazine hydrate, the reaction mixture was refluxed at 210 °C for 12 h following by extraction with $CHCl_3$. The $CHCl_3$ extract was purified by semi-preparative HPLC [column, YMC-A023; mobile phase, hexane–iso-PrOH (99 : 1, v/v); flow rate, 5 ml/min; detection, 207 nm UV absorption] to furnish **6** (5.30 mg).

3β,22β-Dihydroxyolean-18-ene (6) Colorless plates ($CHCl_3$–MeOH), $[\alpha]_D^{25}$ +8.0° (c = 0.1, $CHCl_3$), mp 200—203 °C. EI-MS m/z: 442 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 4.83 (1H, br s, 18-H), 3.63 (1H, dd, J = 10, 7 Hz, 22α-H), 3.20 (1H, dd, J = 10, 6, 3-H), 1.07, 1.01, 1.00, 0.97, 0.96, 0.88, 0.77, 0.73 (each 3H, s). ^{13}C-NMR ($CDCl_3$) Table I.

Cleavage of the Ester Linkage of Periandradulcin A (1) A solution of **1** (1 mg) in 10% sodium methoxide (1 ml) was kept at 70 °C for 12 h, then poured into ice-water, neutralized with AcOH and extracted with $CHCl_3$

(10 ml × 3). The product from the $CHCl_3$ extract was purified by preparative TLC [silica gel, hexane–EtOAc (2:1, v/v)] and confirmed to be identical with authentic methyl syringate in HPLC, TLC and EI-MS.

Preparation of Methyl Syringate Syringic acid (400 mg, Tokyo Kasei Co., Ltd.) was refluxed with consentrated H_2SO_4 (0.1 ml) and anhydrous MeOH (4 ml) for 5.5 h. The reaction mixture was poured into ice-water and extracted with Et_2O. The product from the Et_2O extract was purified by silica gel column chromatography [hexane–EtOAc (1:4, v/v)], to furnish methyl syringate (372.3 mg, 86.9% yield). Colorless needles ($CHCl_3$–MeOH), mp 104—105 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.61 (2H, s, 2, 6-H), 3.88 (3H, s, $-COOCH_3$), 3.81 (6H, s, $-OCH_3$). ^{13}C-NMR ($CDCl_3$) δ: 167.0 (s, $-COOCH_3$), 148.5 (s, 3, 5-C), 142.6 (s, 4-C), 120.0 (s, 1-C), 107.8 (d, 2, 6-C), 56.2 (q, $-OCH_3$), 51.7 (q, $-COOCH_3$).

Assay of PDE-Inhibitory Activity Activity of PDE was determined by a modification of the method described by Thompson and Appleman[11] and Brooker *et al.*[12] The reaction mixture (400 μl), containing 50 mM Tris–HCl (pH 8.0), [^{3}H]cAMP or [^{3}H]cGMP and a sample dissolved in 4% dimethyl sulfoxide, was incubated with PDE derived from bovine heart (Boehringer Mannheim GmbH) for 10 min at 30 °C and then boiled for 5 min. In the assay for PDE-I, 0.1 mM Ca^{2+} and calmodulin (5 units, Sigma) were added to the reaction mixture. Snake vemon (5′-nucleotidase, Sigma) (20 μM, 0.2 U) was added to the boiled reaction mixture and the whole was incubated for another 10 min at 37 °C, then 1 ml of Dowex AG1 × 2 resin (200—400 mesh) was added. After the resin had absorbed unchanged [^{3}H]cAMP or [^{3}H]cGMP, it was precipitated by centrifugation, and the radioactivity of the supernatant containing [^{3}H]adenosine or [^{3}H]guanosine formed during the incubation was measured with a liquid scintillation counter. The IC_{50} value is concentration of the compound required to give 50% inhibition.

Fractionation of PDEs PDE derived from bovine heart (Boehringer Mannheim GmbH) was fractionated on a DEAE-cellulose (Seikagaku Kogyo Co., Ltd.) column (0—0.4 M sodium acetate linear gradient) to furnish PDE-II and PDE-III. PDE-I was obtained by purification of activator-deficient PDE derived from bovine heart (P-I fraction, Sigma) on a DEAE-cellulose column.

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