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PERIANDRIN II AND IV, TRITERPENE GLYCOSIDES FROM *PERIANDRA DULCIS*

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Key Word Index—*Periandra dulcis*; Leguminosae; new triterpene glycosides; periandrin II; periandrin IV; sweeteners.

Abstract—Investigation of the natural sweeteners of *Periandra dulcis* afforded new sweet triterpene glycosides, periandrin II (3-β-*O*-[β-D-glucuronopyranosyl-(1→2)-β-D-glucuronopyranosyl]-25-formyl-olean-12(13)-en-30-oic acid) and periandrin IV (3-β-*O*-[β-D-glucuronopyranosyl-(1→2)-β-D-glucuronopyranosyl]-25-hydroxyolean-12(13)-en-30-oic acid). Evidence for the structures was obtained by correlation of their derivatives with known compounds.

INTRODUCTION

In search for new natural sweeteners, an aqueous extract of the root of *Periandra dulcis* Mart. (Leguminosae) was found to be sweet. *Periandra* species have been used in Brazil as a cough cure. The roots of *Periandra dulcis* were described to contain 0.38% of glycyrrhizin [1] as a sweet component. However, Takahashi [2] reported that glycyrrhizin (**3**) could not be detected in the same part of the plant. We found periandrin I, II (**1**), III and IV (**2**), as the sweet components of this plant but could not find any significant amount of glycyrrhizin (**3**). The present paper deals with the structural elucidation of the triterpene glycosides, periandrin II (**1**) and IV (**2**).

RESULTS

Periandrin II (**1**) was crystallized from MeOH–H_2O as colourless needles, $C_{42}H_{62}O_{16}\cdot 3H_2O$. The IR spectrum of **1** showed the presence of hydroxyl (3400 cm^{-1}) and carboxyl groups (1720 and 1710 cm^{-1}). Acid hydrolysis of **1** yielded glucuronic acid, periandric acid II (**4**, a new triterpenoid) and an unidentified triterpenoid, the latter being formed from **4** by acid treatment. Enzymatic hydrolysis of **1** with β-glucuronidase afforded only **4** as aglycone.

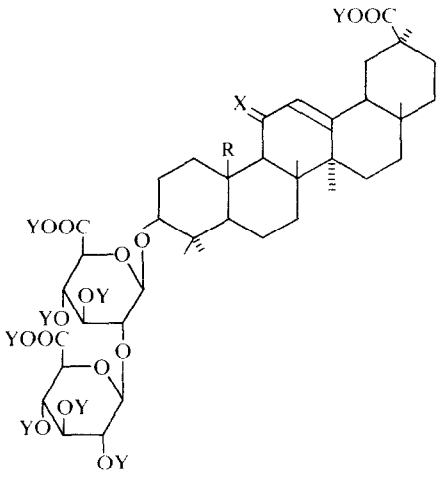
Treatment of the aglycone **4** with CH_2N_2 gave a monomethyl ester (**10**), thereby indicating the presence of a carboxyl group in **4**. Acetylation of **10** with Ac_2O in C_5H_5N gave a methyl ester monoacetate (**11**). The ^1H NMR spectrum of **11** showed six singlet methyl signals at δ 0.76, 0.82, 0.88, 0.93, 1.11 and 1.16, an acetoxymethyl signal at 2.01, a carbomethoxymethyl signal at 3.67, an acetoxymethine signal at 4.52 (1 H, *dd*, J = 5, 11 Hz), an olefinic proton signal at 5.22 and a formyl proton signal at 10.28. The MS of **4** exhibited the typical retro-Diels–Alder fragmentation of the C-ring of a Δ^{12}-amyrin derivative [3], giving rise to two peaks at *m/e* 248 and 203 (after dehydration). In the MS of **11**, these peaks were shifted to *m/e* 262 and 263, respectively. Thus, the carboxyl group of **4** must be present in rings D/E, whereas rings A/B must contain a hydroxyl group and a formyl group. These data suggested that **4** was a member of the olean-12-en-x-oic acid series whose C-25 methyl group at the A/B ring junction was replaced by a formyl group.

Compound **4** was easily converted to an acetal (**12**) by dissolving in MeOH. The ^1H NMR spectrum of **12** showed no formyl proton signal but an acetalmethyl at δ 3.37, an acetalmethine at 5.07 (1 H, *s*) and a methine at 3.22 (1 H, *t*-like) which could be attributed to a proton on a carbon bearing an oxygen. Compound **12** was converted to a monomethyl ester (**13**) by treatment with CH_2N_2, but not to an acetate with Ac_2O in C_5H_5N. From biogenetic considerations, it would be expected that the C-25 formyl group could form the acetal with the 3β-hydroxyl group but formyl groups at C-23 and C-24 could not because this would require formation of a four-membered ring.

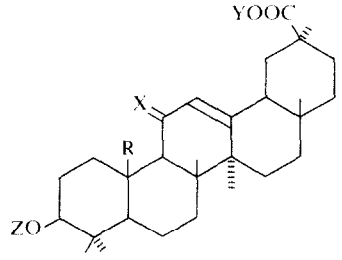
Furthermore, oxidation of **11** with Jones reagent [4] gave a lactone (**14**, 1770 cm^{-1}) which possessed no formyl group. The ^1H NMR spectrum of **14** showed an olefinic proton at δ 5.65 as a doublet (J = 4 Hz) and a methine proton at 4.79 as a triple (J = 4 Hz) which was assigned to a proton on a carbon bearing an oxygen. Double resonance experiments between the olefinic proton and the methine proton revealed that the methine proton must be assigned to the C-11 α position. It is possible that the oxygen function could be introduced to the C-11 allylic position by oxidation and then form γ-lactone with C-25. These data suggest that the formyl group must be located at C-25 in **4**.

It remained, therefore, to locate a carboxyl group on ring D or E. A methyl signal at δ 1.16 in the ^1H NMR spectrum of **11** indicated that the carboxyl group might be attached to C-20 because the chemical shift was lower than usual for methyl signals. Successive Huang-Minlon reduction [5], acetylation and methylation of **11** yielded 11-deoxoglycyrrhetic acid methyl ester acetate (**15**) which was identified with the authentic sample synthesized from glycyrrhetic acid (**6**) by means of ^1H NMR spectroscopy and TLC.

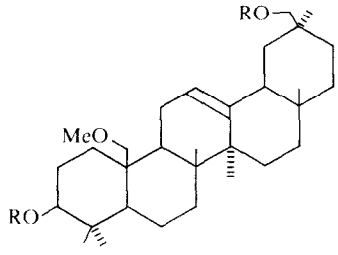
Methylation of **1** by Hakomori's method [6] gave the octa-*O*-methyl derivative (**7**) whose ^1H NMR spectrum



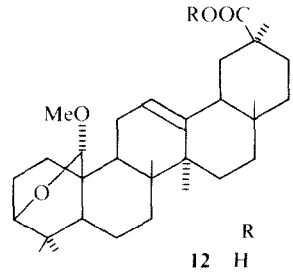
	R	X	Y
1	CHO	H_2	H
2	CH_2OH	H_2	H
3	Me	O	H
7	CHO	H_2	Me
8	CH_2OMe	H_2	Me
9	Me	O	Me



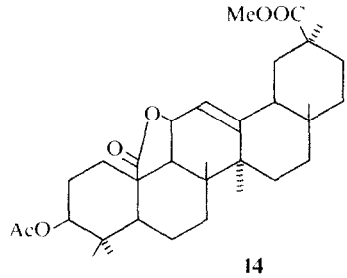
	R	X	Y	Z
4	CHO	H_2	H	H
5	CH_2OH	H_2	H	H
6	Me	O	H	H
10	CHO	H_2	Me	H
11	CHO	H_2	Me	COMe
15	Me	H_2	Me	COMe
16	CH_2OH	H_2	Me	H
17	CH_2OMe	H_2	Me	COMe



	R
18	H
19	COMe



	R
12	H
13	Me



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showed an olefinic proton at δ 5.18 (1 H, *m*), a formyl proton at 10.19 (1 H, *s*) and two anomeric protons at 4.33 (1 H, *d*, $J = 7$ Hz) and 4.64 (1 H, *d*, $J = 7$ Hz). This finding suggested that two glucuronic acid residues in **1** were linked with β-orientation. $LiAlH_4$ reduction of **7** followed by methanolysis gave methyl-3,4-di-*O*-methyl glucose and methyl-2,3,4-tri-*O*-methyl glucose. They were identified by GLC with authentic samples derived from octa-*O*-methyl glycyrrhizin (**9**) by $LiAlH_4$ reduction followed by methanolysis.

The accumulated evidence described above led us to assign the structure 3-β-*O*-[β-D-glucuronopyranosyl-(1→2)-β-D-glucuronopyranosyl]-25-formyl-olean-12(13)-en-30-oic acid to periandrin II (**1**).

The IR spectrum of periandrin IV (**2**), a white powder, $C_{42}H_{64}O_{16}\cdot 3H_2O$, showed the presence of hydroxyl (3400 cm^{-1}) and carboxyl (1700 cm^{-1}) groups. Acid hydrolysis of **2** yielded glucuronic acid and periandric acid IV (**5**, a new triterpenoid). Enzymatic hydrolysis of **2** with β-glucuronidase also afforded **5** as the aglycone. Methylation of **5** with CH_2N_2 gave a monomethyl ester (**16**), which on acetylation with Ac_2O in C_5H_5N gave a monomethyl ester diacetate (**17**). The ^1H NMR spectra of **16** and **17** showed AB-type signals at δ 3.99, 4.08 (1 H, *d*, $J = 12$ Hz each) and 4.47, 4.50 (1 H, *d*, $J = 12$ Hz each), respectively, which could be assigned to methylene protons of a hydroxymethyl group. The similarity of periandric acid IV derivatives to periandric acid II derivatives in their ^1H NMR and MS suggested that **5** would be produced from **4** by reducing the formyl group. In fact, $NaBH_4$ reduction of **4** gave a diol. The diol and its methyl ester were identified with periandric acid IV (**5**)

and its methyl ester (**6**), respectively, by means of IR and ^{1}H NMR spectroscopy and by mmp.

Methylation of **2** by Hakomori's method [6] gave the nona-*O*-methyl derivative (**8**), whose ^{1}H NMR spectrum showed an olefinic proton at δ 5.23 (1 H, *m*) and two anomeric protons at 4.36 (1 H, *d*, J = 7 Hz) and 4.69 (1 H, *d*, J = 7 Hz). This result suggested that the two glucuronic residues in **2** were linked with the β-orientation in the same manner as in periandrin II (**1**). $LiAlH_4$ reduction followed by methanolysis of **8** resulted in liberation of methyl-3,4-di-*O*-methyl glucose and methyl-2,3,4-tri-*O*-methyl glucose, which were identical by GLC with authentic samples derived from **9**.

Methylated aglycone (**18**) produced concomitantly during methanolysis of **8** possessed a methoxyl group as shown by the ^{1}H NMR spectrum (δ 3.22). The ^{1}H NMR spectrum of the diacetate (**19**), prepared by acetylation of **18** showed a double-doublet signal at δ 4.53 due to a proton at C-3α geminal to an acetoxyl group at C-3β. Therefore, the glucuronic acid in **2** must be linked to a 3β-hydroxyl group.

Reduction of periandrin II (**1**) with $NaBH_4$ led to dihydroperiandrin II, which was methylated to nona-*O*-methyl dihydroperiandrin II. Dihydroperiandrin II and the methylated compound were identified as periandrin IV (**2**) and nona-*O*-methyl periandrin IV (**8**), respectively, by means of IR and ^{1}H NMR spectroscopy and TLC.

Consequently, periandrin IV (**2**) is assigned the structure 3-β-*O*-[β-D-glucuronopyranosyl-(1→2)-β-D-glucuronopyranosyl]-25-hydroxy-olean-12(13)-en-30-oic acid.

The sweetness of periandrin II and periandrin IV was as strong as that of glycyrrhizin.

EXPERIMENTAL

For CC, Si gel (Merck, Si gel 60, 70–230 mesh) was used. Detection of the isolated spots on TLC (Merck, pre-coated Si gel 60 F-254 or HPLC Si gel 60 F-254) was made by spraying 10% H_2SO_4 soln followed by heating.

Plant material. The roots of *Periandra dulcis* Mart. (Leguminosae) were purchased in 1976 from Moageira Botanica 'Index' Ltda. in Brazil.

Extraction and isolation. The roots (20 kg) of *P. dulcis* were extracted 3 × with H_2O at 70° for 24 hr (100 l. each). The extracts were combined and concd *in vacuo* to 6 l. and the ppt. formed was removed by filtration. EtOH (88 l.) was added to the soln and left at 5° overnight. The ppt. was separated and dissolved in H_2O (4 l.). After filtration, EtOH (46 l.) was added to the soln. Crude sweet materials (870 g) were obtained by repeated precipitation with EtOH. The crude sweet materials (100 g) were chromatographed on a Si gel column (80 × 6 cm) by use of *n*-BuOH–C_6H_6–MeOH–28% NH_4OH (4:3:3:2) as the eluent. Periandrin II was eluted earlier than periandrin IV. The periandrin II and periandrin IV fractions were chromatographed individually by using $CHCl_3$–MeOH–H_2O (25:17:3) as the eluent to get pure periandrin II and periandrin IV.

Periandrin II (**1**). Mp 216–220° (colourless plates from MeOH–H_2O). $[\alpha]_D^{28}$ + 37.0° (H_2O, *c* 0.27). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2900, 1720 (COOH or CHO), 1710 (CHO or COOH), 1400 and 1300. (Found: C, 57.10; H, 8.05. $C_{42}H_{62}O_{16}\cdot 3H_2O$ requires: C, 57.52; H, 7.82%).

Periandrin IV (**2**). Mp >300° (colourless powder from EtOH–H_2O. $[\alpha]_D^{22}$ + 96.0° (H_2O, *c* 2.5). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2920, 1700 (COOH), 1407 and 1045. (Found: C, 57.01; H, 7.70. $C_{42}H_{64}O_{16}\cdot 3H_2O$ requires: C, 57.39; H, 8.03%).

Acid hydrolysis of periandrin II (**1**). Periandrin II (**1**, 200 mg) was refluxed with 10% H_2SO_4 (20 ml) for 4 hr. The reaction mixture was diluted with H_2O and extracted with EtOAc. The EtOAc extractive was washed with H_2O, dried over Na_2SO_4 and evapd. Chromatography of the EtOAc extractive on Si gel eluting with *n*-hexane–Me_2CO (5:1) afforded periandric acid II (**4**, 21 mg) and an unidentified triterpenoid (63 mg). Compound **4** was crystallized from EtOH–H_2O as colourless needles, mp 245–249°. IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 2950, 1710 (COOH and CHO), 1470, 1450, 1390 and 1230. MS *m/e*: 470.3392 (M^+, calcd. for $C_{30}H_{46}O_4$, 470.3419), 452 ($M^+ - 18$), 437 ($M^+ - 18 - 15$), 408, 366, 248, 203 and 189 (base peak). The aq. layer was concd under red. pres. and subjected to TLC for identification with glucuronic acid (*n*-PrOH–nitromethane–H_2O (5:2:3), R_f = 0.17. *n*-BuOH–C_5H_5N–H_2O (6:4:3), R_f = 0.39. Naphthoresorcinol or diphenylamine–aniline as colour reagents).

Enzymatic hydrolysis of periandrin II (**1**). Periandrin II (**1**, 150 mg) was incubated with β-glucuronidase (P–L Biochemicals Inc., 100 mg) in 0.2 M NaOAc–HOAc buffer (pH 5.0, 40 ml) at 37° for 24 hr. The reaction mixture was extracted with $CHCl_3$. Chromatography of the $CHCl_3$ extract on Si gel with *n*-hexane–Me_2CO (5:1) only gave periandric acid II (**4**, 64 mg). Glucuronic acid in the aq. layer was identified with the authentic sample by TLC as described above.

Methylation of periandric acid II (**4**). A soln of **4** (50 mg) in MeOH (20 mg) was treated with ethereal CH_2N_2 at room temp. for 15 min, and treated as usual to give the monomethyl ester (**10**, 46 mg) as colourless needles from EtOH–H_2O, mp 216–218°. ^{1}H NMR (90 MHz, $CDCl_3$): δ 0.73 (3 H, *s*, Me), 0.83 (3 H, *s*, Me), 0.88 (3 H, *s*, Me), 1.02 (3 H, *s*, Me), 1.12 (3 H, *s*, Me), 1.16 (3 H, *s*, Me), 3.24 (1 H, *dd*, J = 6, 10 Hz, C-3), 3.65 (3 H, *s*, COOMe), 5.23 (1 H, *m*, C-12) and 10.20 (1 H, *s*, CHO). MS *m/e*: 484.3553 (M^+, calc. for $C_{31}H_{48}O_4$, 484.3558), 466 ($M^+ - 18$, base peak), 451 ($M^+ - 18 - 15$), 438, 407 ($M^+ - 18 - 59$), 248, 203 and 189.

Acetylation of periandric acid II *methyl ester* (**10**). Compound **10** (30 mg) was treated with Ac_2O (2 ml) in C_5H_5N (2 ml) at room temp. overnight. The reaction mixture was evapd to dryness. Monoacetate (**11**, 25 mg) was crystallized from Me_2CO as colourless needles, mp >300°. IR ν_{max}^{KBr} cm^{-1}: 2900, 1720 (OAc and COOMe), 1700 (CHO), 1440, 1370 and 1250. ^{1}H NMR (90 MHz, $CDCl_3$): δ 0.76 (3 H, *s*, Me), 0.82 (3 H, *s*, Me), 0.88 (3 H, *s*, Me), 0.93 (3 H, *s*, Me), 1.11 (3 H, *s*, Me), 1.16 (3 H, *s*, Me), 2.01 (3 H, *s*, OCOMe), 3.67 (3 H, *s*, COOMe), 4.52 (1 H, *dd*, J = 5, 11 Hz, C-3), 5.22 (1 H, *m*, C-12) and 10.28 (1 H, *s*, CHO). MS *m/e*: 526.3665 (M^+, calc. for $C_{33}H_{50}O_5$, 526.3658), 466 ($M^+ - 60$), 451 ($M^+ - 60 - 15$), 437, 407 ($M^+ - 60 - 59$), 391, 354, 263, 262 (base peak), 241 and 203.

Methanol treatment of periandric acid II (**4**). Compound **4** (30 mg) was dissolved in MeOH (10 ml) and allowed to stand for 2 days. After evapn of the reaction mixture, the acetal (**12**, 23 mg) was crystallized from MeOH as colourless plates, mp 191°. IR ν_{max}^{KBr} cm^{-1}: 1695 (COOH), 1455, 1105, 1015 and 825. ^{1}H NMR (60 MHz, $CDCl_3$): δ 0.82 (3 H, *s*, Me), 1.00 (9 H, *s*, 3 × Me), 1.14 (3 H, *s*, Me), 1.19 (3 H, *s*, Me), 3.22 (1 H, *t*-like, C-3), 3.37 (3 H, *s*, OMe), 5.07 (1 H, *s*, C-25) and 5.29 (1 H, *m*, C-12).

Methylation of periandric acid II *acetal* (**12**). Compound **12** (20 mg) was treated with ethereal CH_2N_2 at room temp. for 15 min. After evapn of the reaction mixture, the acetal methyl ester (**13**, 10 mg) was crystallized from MeOH as colourless needles, mp 199°. IR ν_{max}^{KBr} cm^{-1}: 1738 (COOMe), 1462, 1180, 1108, 1020 and 932. ^{1}H NMR (60 MHz, $CDCl_3$): δ 0.80 (3 H, *s*, Me), 1.00 (9 H, *s*, 3 × Me), 1.13 (6 H, *s*, 2 × Me), 3.21 (1 H, *t*-like, C-3), 3.36 (3 H, *s*, OMe), 3.63 (3 H, *s*, COOMe), 5.07 (1 H, *s*, C-25) and 5.27 (1 H, *m*, C-12). MS *m/e*: 466.3441 ($M^+ - 32$, calc. for $C_{31}H_{46}O_3$, 466.3441, base peak), 451 ($M^+ - 32 - 15$), 407 ($M^+ - 32 - 59$), 354, 262, 204 and 189.

Oxidation of periandric acid II *methyl ester acetate* (**11**). A stirred soln of **11** (33.1 mg) in Me_2CO (70 ml) was treated with Jones reagent [4] (2.3 ml) under N_2 at room temp. for 3 hr. After adding 5% NaOAc (80 ml), the reaction mixture was extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, evapd to dryness, and chromatographed on Si gel. The elution with *n*-hexane–Me_2CO (7:1) gave a lactone (**14**, 6.2 mg) which was crystallized from EtOH as colourless needles, mp > 300°. IR ν_{max}^{KBr} cm^{-1}: 1770 (lactone) and 1715 (OAc and COOMe). ^1H NMR (90 MHz, $CDCl_3$): δ 0.81 (3 H, *s*, Me), 0.94 (3 H, *s*, Me), 1.13 (9 H, *s*, 3 × Me), 1.33 (3 H, *s*, Me), 2.07 (3 H, *s*, OCOMe), 3.71 (3 H, *s*, COOMe), 4.55 (1 H, *dd*, *J* = 5, 10 Hz, C-3), 4.79 (1 H, *t*, *J* = 4 Hz, C-11) and 5.65 (1 H, *d*, *J* = 4 Hz, C-12). MS *m/e*: 540.344 (M^+, calc. for $C_{33}H_{48}O_6$, 540.345), 525 ($M^+ - 15$), 496 (base peak), 480 ($M^+ - 60$), 278, 262, 203 and 175.

Successive Huang-Minlon reduction, acetylation and methylation of periandric acid II *methyl ester acetate* (**11**). Compound **11** (20 mg) was treated with diethylene glycol (3 ml) and 80% hydrazine hydrate in EtOH (2 ml) for 30 min at 130°. KOH (100 mg) was added and EtOH and H_2O were distilled off by raising the temp. to 200–205°, then refluxing was continued for 2.5 hr. The cooled soln was diluted with H_2O, neutralized with 1N HCl and extracted with Et_2O. The Et_2O extract, after acetylation with Ac_2O in C_5H_5N and methylation with CH_2N_2, was purified by prep. TLC (0.25 mm thickness, C_6H_6–EtOAc (19:1) as developing solvent, $CHCl_3$ as extracting solvent) to give 11-deoxoglycyrrhetic acid methyl ester acetate (**15**). The identity of **15** with an authentic sample which was prepared by PtO_2 reduction of glycyrrhetic acid methyl ester acetate was established by means of ^1H NMR spectroscopy and TLC. ^1H NMR (90 MHz, $CDCl_3$): δ 0.78 (3 H, *s*, Me), 0.87 (6 H, *s*, 2 × Me), 0.96 (6 H, *s*, 2 × Me), 1.13 (6 H, *s*, 2 × Me), 2.05 (3 H, *s*, OCOMe), 3.68 (3 H, *s*, COOMe), 4.50 (1 H, *dd*, *J* = 7, 9 Hz, C-3) and 5.27 (1 H, *m*, C-12).

Methylation of periandrin II (**1**) *by Hakimori's method* [6]. Dimsyl carbanion was prepared by heating a soln of NaH (1 g) in DMSO (20 ml) under N_2 at 60° for 2 hr. The greenish soln (10 ml) was added to the soln of **1** (65 mg) in DMSO (10 ml) and stirred under N_2 for 1 hr. MeI (10 ml) was added to the total soln and the mixture left standing in the dark overnight at room temp. The reaction mixture was poured into ice-water, and extracted with Et_2O. The Et_2O extract was washed with 10% $Na_2S_2O_3$ and H_2O, and evapd to dryness. Octa-*O*-methyl periandrin II (**7**, 32 mg) was crystallized from MeOH as colourless needles, mp 243–245°. IR ν_{max}^{KBr} cm^{-1}: 1755, 1730 (COOMe) and 1700 (CHO). ^1H NMR (60 MHz, $CDCl_3$): δ 3.08 (1 H, *m*, C-3), 3.47 (6 H, 2 × OMe), 3.56 (3 H, *s*, OMe), 3.61 (6 H, *s*, 3 × OMe), 3.65 (3 H, *s*, OMe), 3.70 (3 H, *s*, OMe), 3.78 (3 H, *s*, OMe), 4.33 (1 H, *d*, *J* = 7 Hz, anomeric H), 4.64 (1 H, *d*, *J* = 7 Hz, anomeric H), 5.18 (1 H, *m*, C-12) and 10.19 (1 H, *s*, CHO). (Found: C, 63.74; H, 8.65. $C_{50}H_{80}O_{16}$ requires: C, 64.08; H, 8.60%).

$LiAlH_4$ *reduction followed by methanolysis of octa-O-methyl periandrin* II (**7**). Compound **7** (20 mg) was added to a suspension of $LiAlH_4$ (50 mg) in Et_2O (50 ml) and stirred at room temp. for 3 hr. The reaction mixture was treated with an aq. Et_2O, acidified with 20% H_2SO_4 and extracted with Et_2O. After evapn, the Et_2O extract was treated with 6% HCl–MeOH (10 ml) under reflux for 1 hr, neutralized with Ag_2CO_3, and filtered. The filtrate gave methylated monosaccharides, methyl-3,4-di-*O*-methyl glucose and methyl-2,3,4-tri-*O*-methyl glucose, which were identified with authentic samples derived from glycyrrhizin (**3**) by GLC using two systems: (1) 5% diethylene glycol succinate, 3 mm × 2 m; column temp.: 190°; carrier gas: N_2; flow rate: 60 ml/min; R_t (min): 4′ 25″ (minor), 6′ 10″ (major), 14′ 26″ (major), 17′ 12″ (minor); (2) 5% SE-30, 3 mm × 1 m; column temp.: 110°; carrier gas: N_2; flow rate: 60 ml/min; R_t (min): 8′ 44″ (minor), 10′ 39″ (major).

Acid hydrolysis of periandrin IV (**2**). Periandrin IV (**2**, 150 mg) was refluxed with 10% H_2SO_4 on a water bath for 3 hr. The reaction mixture was diluted with H_2O and extracted with EtOAc. The EtOAc extract was washed with H_2O, dried over Na_2SO_4 and evapd. Chromatography of the EtOAc extract on Si gel eluting with *n*-hexane–Me_2CO (3:2) afforded periandric acid IV (**5**, 59 mg) which was crystallized from EtOH–H_2O as colourless needles, mp 286–287°. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2900, 1700 (COOH), 1450, 1370 and 1305. ^1H NMR (90 MHz, $CDCl_3$ + CD_3OD): δ 0.82 (3 H, *s*, Me), 0.85 (3 H, *s*, Me), 1.04 (3 H, *s*, Me), 1.08 (3 H, *s*, Me), 1.15 (6 H, *s*, 2 × Me), 3.25 (1 H, *m*, C-3), 3.98 (1 H, *d*, *J* = 13 Hz, part of AB type, C-25), 4.08 (1 H, *d*, *J* = 13 Hz, part of AB type, C-25) and 5.24 (1 H, *m*, C-12). MS *m/e*: 472.3555 (M^+, calc. for $C_{30}H_{48}O_4$, 472.3553), 454 ($M^+ - 18$), 248 (base peak), 203 and 189. The aq. layer was concd under red. pres. and subjected to TLC to identify glucuronic acid (*n*-BuOH–C_5H_5N–H_2O (6:4:3), R_f = 0.39. Naphthoresolcinol as a colour reagent).

Enzyme hydrolysis of periandrin IV (**2**). Periandrin IV (**2**, 100 mg) was treated with β-glucuronidase (P-L Biochemicals Inc., 100 mg) in 0.2 M NaOAc–HOAc buffer (pH 5.0, 50 ml) at 37° for 24 hr. The reaction mixture was extracted with EtOAc. Chromatography of the EtOAc extract on Si gel eluting with *n*-hexane–Me_2CO (3:2) gave periandric acid IV (**5**, 31 mg). Glucuronic acid in the aq. layer was identified with the authentic sample by TLC as described above.

Methylation of periandric acid IV (**5**). Compound **5** (60 mg) was treated with ethereal CH_2N_2 in MeOH (20 ml) for 15 min at room temp. After evapn, monomethyl ester (**16**, 48 mg) was crystallized from EtOH–H_2O, mp 222–223°. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 2900, 1700 (COOMe) 1440, 1360, 1200 and 1150. ^1H NMR (90 MHz, $CDCl_3$): δ 0.80 (3 H, *s*, Me), 0.85 (3 H, *s*, Me) 1.04 (3 H, *s*, Me), 1.06 (3 H, *s*, Me), 1.13 (6 H, *s*, 2 × Me), 3.25 (1 H, *dd*, *J* = 8, 9 Hz, C-3), 3.66 (3 H, *s*, COOMe), 3.99 (1 H, *d*, *J* = 12 Hz, part of AB type, C-25), 4.08 (1 H, *d*, *J* = 12 Hz, part of AB type, C-25) and 5.25 (1 H, *m*, C-12). MS *m/e*: 486.3651 (M^+, calc. for $C_{31}H_{50}O_4$, 486.3703), 468 ($M^+ - 18$), 411, 262 (base peak), 237, 203 and 189.

Acetylation of periandric acid IV *methyl ester* (**16**). Compound **16** (40 mg) was treated with Ac_2O (2 ml) in C_5H_5N (2 ml) at room temp. overnight. After evapn of reaction mixture, methyl ester diacetate (**17**, 41 mg) was obtained as a white powder, mp 98–103°. IR ν_{max}^{KBr} cm^{-1}: 2900, 1700 (OAc and COOMe), 1440, 1360, 1230 and 1150. ^1H NMR (90 MHz, $CDCl_3$): δ 0.78 (3 H, *s*, Me), 0.92 (6 H, *s*, 2 × Me), 1.02 (3 H, *s*, Me), 1.13 (6 H, *s*, 2 × Me), 2.05 (6 H, *s*, 2 × OCOMe), 3.68 (3 H, *s*, COOMe), 4.47 (1 H, *d*, *J* = 12 Hz, part of AB type, C-25), 4.48 (1 H, *m*, C-3), 4.50 (1 H, *d*, *J* = 12 Hz, part of AB type, C-25) and 5.28 (1 H, *m*, C-12). MS *m/e*: 570.3893 (M^+, calc. for $C_{35}H_{54}O_6$, 570.3896), 510 ($M^+ - 60$), 450 ($M^+ - 60 - 60$), 437, 315, 302, 262 (base peak), 247, 241 and 202.

$NaBH_4$ *reduction of periandric acid* II (**4**). A stirred soln of **4** (19 mg) was treated with a suspension of $NaBH_4$ (100 mg) in EtOH (2 ml) at room temp. for 48 hr and added with H_2O (30 ml). The reaction mixture was treated with SK-1B (OH⁻, 2 g, Mitsubishi Chemical Industries Ltd.) and filtered to remove the resin. The filtrate was then extracted with $CHCl_3$, which was washed with H_2O and dried over Na_2SO_4. The dried $CHCl_3$ extract was evapd to dryness. The diol was crystallized from MeOH–H_2O as colourless needles and identified with periandric acid IV (**5**) by means of IR spectroscopy and mmp.

Methylation of diol. A soln of diol (4.6 mg) in $CHCl_3$ (10 ml) was treated with ethereal CH_2N_2 at room temp. for 10 min. The reaction mixture was evapd under red. pres. to give a monomethyl ester (5 mg). Identity with periandric acid IV methyl ester (**16**) was established by means of IR and ^1H NMR spectroscopy.

Methylation of periandrin IV (**2**) *by Hakomori's method* [6]. A soln of **2** (55 mg) in DMSO (10 ml) was treated with dimsyl carbanion soln (10 ml) under N_2 with stirring. MeI (5 ml) added

and the mixture stirred in the dark overnight. Work-up gave nona-*O*-methyl periandrin IV (**8**, 43 mg) as a white powder (crystallization failed), mp 190–192°. ^{1}H NMR (60 MHz, $CDCl_3$): δ 0.80 (3 H, *s*, Me), 0.85 (3 H, *s*, Me), 1.03 (6 H, *s*, 2 × Me), 1.12 (6 H, *s*, 2 × Me), 3.20 (3 H, *s*, OMe), 3.48 (6 H, *s*, 2 × OMe), 3.57 (3 H, *s*, OMe), 3.63 (6 H, *s*, 2 × OMe), 3.67 (3 H, *s*, OMe), 3.73 (3 H, *s*, OMe), 3.78 (3 H, *s*, OMe), 4.36 (1 H, *d*, J = 7 Hz, anomeric proton), 4.69 (1 H, *d*, J = 7 Hz, anomeric proton) and 5.23 (1 H, *m*, C-12).

$LiAlH_4$ *reduction followed by methanolysis of nona-O-methyl periandrin* IV (**8**). A soln of **8** (20 mg) in Et_2O was treated with $LiAlH_4$ (50 mg), stirred for 4 hr, and treated as above. The reaction mixture was refluxed for 1 hr with 6% HCl–MeOH (10 ml) and neutralized with Ag_2CO_3. Methylated aglycone (**18**, 10 mg) was precipitated during concn of the filtrate, and was crystallized from Me_2CO as colourless plates, mp 145–148°. ^{1}H NMR (60 MHz, $CDCl_3$): δ 0.84 (6 H, *s*, 2 × Me), 0.88 (3 H, *s*, Me), 1.02 (6 H, *s*, 2 × Me), 1.26 (3 H, *s*, Me), 3.22 (3 H, *s*, OMe), 3.50 (2 H, C-30), 3.60 (2 H, C-25) and 5.15 (1 H, *m*, C-12). The mother layer gave methylated monosaccharides which were identified with methyl-3,4-di-*O*-methyl glucose and methyl-2,3,4-tri-*O*-methyl glucose by GLC using two systems: (1) 5% diethylene glycol succinate, 3 mm × 2 m; column temp.: 190° carrier gas: N_2; flow rate: 60 ml/min; R_t (min): 4′25″ (minor), 6′10″ (major), 14′12″ (major) and 17′12″ (minor). (2) 5% SE-30, 3 mm × 1 m; column temp. 110°; carrier gas: N_2; flow rate: 60 ml/min; R_t (min): 8′44″ (minor) and 10′39″ (major).

Acetylation of 3,30-*dihydroxy*-25-*methoxy*-*olean*-12(13)-*ene* (**18**). Compound **18** (9 mg) was treated with Ac_2O (1 ml) in C_5H_5N (1 ml) at room temp. overnight. The reaction mixture was evapd to dryness to yield the diacetate (**19**, 9.5 mg) as a colourless powder (crystallization failed), mp 190–192°. ^{1}H NMR (90 MHz, $CDCl_3$): δ 0.82 (3 H, *s*, Me), 0.89 (6 H, *s*, 2 × Me), 1.08 (3 H, *s*, Me), 1.11 (3 H, *s*, Me), 1.13 (3 H, *s*, Me), 2.02 (3 H, *s*, OCOMe), 2.04 (3 H, *s*, OCOMe), 3.21 (3 H, *s*, OMe), 3.59 (1 H, *d*, J = 12 Hz, part of AB type, C-25), 3.61 (1 H, *d*, J = 12 Hz, part of AB type, C-25), 3.98 (2 H, *s*, C-30), 4.53 (1 H, *dd*, J = 6, 8 Hz, C-3) and 5.15 (1 H, *m*, C-12). MS *m/e*: 556.4109 (M^+, calc. for $C_{35}H_{56}O_5$, 556.4111), 496 ($M^+ - 60$), 451 ($M^+ - 60 - 46$), 276 (base peak), 216, 203 and 189.

$NaBH_4$ *reduction of periandrin* II (**1**). A soln of **1** (56 mg) in H_2O (20 ml) was treated with $NaBH_4$ (560 mg) with stirring at room temp. for 48 hr. The reaction mixture was treated with SK-1B (OH^-, 7 g, Mitsubishi Chemical Industries Ltd.) and filtered. The filtrate was evapd to give a colourless powder of dihydroperiandrin II (43 mg). Identity with periandrin IV (**2**) was established by means of IR spectroscopy and TLC.

Methylation of dihydroperiandrin II *by Hakomori's method* [6]. Dihydroperiandrin II (40 mg) was methylated with NaH (500 mg) and MeI (10 ml) in DMSO (10 ml) as described previously. Nona-*O*-methyl dihydroperiandrin II was obtained as a powder which was identified with nona-*O*-methyl periandrin IV (**8**) by means of ^{1}H NMR and IR spectroscopy and TLC.

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