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PERIANDRIN I, A SWEET TRITERPENE-GLYCOSIDE FROM *PERIANDRA DULCIS*

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Key Word Index—*Periandra dulcis*, Leguminosae, triterpene-glycoside, periandrin I, prosapogenin, sweeteners

Abstract—Further investigation of the natural sweeteners of *Periandra dulcis* afforded a new sweet triterpene-glycoside, periandrin I, the structure of which was determined to be 3-β-*O*-[β-D-glucuronopyranosyl-(1→2)-β-D-glucuronopyranosyl]-25-al-olean-18(19)-en-30-oic acid by chemical and physicochemical evidence and also by X-ray crystallographic analysis of a derivative

INTRODUCTION

In a previous paper [1], we reported on the structures of two new sweet triterpene-glycosides, periandrin II and IV, which were obtained from the roots of *Periandra dulcis* Mart Further investigation of the roots has led us to the isolation of a new triterpene-glycoside, periandrin I (**1**), as a major sweetener of this plant

RESULTS AND DISCUSSION

Periandrin I (**1**), $C_{42}H_{62}O_{16}\ 4H_2O$, was crystallized from methanol–water as colourless plates The IR spectrum of **1** exhibited hydroxyl (3300 cm^{-1}) and carboxyl (1700 cm^{-1}) absorption bands On acid hydrolysis, **1** yielded glucuronic acid, periandric acid I (**2**, $C_{30}H_{44}O_4$, a new triterpenoid) and an unidentified triterpenoid which was formed from **2** by the acid treatment Enzymatic hydrolysis of **1** with β-glucuronidase afforded **2** and a prosapogenin (**3**, $C_{36}H_{52}O_{10}\ 0.5H_2O$), the latter being possessed of the sweet property Compound **3** is a monoglucuronide of **2** as revealed by acid hydrolysis

Methylation of **2** with diazomethane afforded a monomethyl ester (**4**) which gave a methyl ester monoacetate (**5**) with acetic anhydride in pyridine The ^{1}H NMR spectrum of **5** showed six singlet methyl signals at δ0 78, 0 83, 0 93, 0 99, 1 02 and 1 24, an acetoxymethyl signal at δ2 01, a carbomethoxymethyl signal at δ3 66, an acetoxymethine signal at δ4 51 (1H, *dd*, $J = 5$, 11 Hz), a singlet signal of an olefinic proton at δ5 19 and a formyl proton signal at δ10 27 These ^{1}H NMR data suggested that **2** was a member of the olean-18(19)-en series in which two methyl groups were replaced by a formyl group and a carboxyl group The mass spectrum of **5** showed the characteristic fragmentation of the C-ring of a Δ^{18}-amyrin derivative [2], giving rise to peaks at *m/z* 263, 262, 248 and 189 These peaks indicated the presence of a carbomethoxyl group in ring D/E and an acetoxyl group and a formyl group in ring A/B

By dissolving it in methanol, compound **2** was easily converted to an acetal (**6**) whose ^{1}H NMR spectrum showed an acetalmethyl signal at δ3 32, an acetalmethine at δ5 08 (1H, *s*) and a methine at δ3 20 (1H, *t*-like) which could be attributed to a proton on a carbon bearing an oxygen, but no formyl proton signal Compound **6** gave a monomethyl ester (**7**) with diazomethane but did not give an acetate with acetic anhydride in pyridine The ^{1}H NMR and chemical properties of **6** were the same as those of a compound having an acetal linkage between C-3 and C-25 which was synthesized from periandric acid II under the same reaction condition [1], thereby suggesting the presence of a hydroxyl group at C-3β and a formyl group at C-25 In addition, oxidation of **5** with Jones' reagent [3] followed by hydrolysis gave a dicarboxylic acid (**9**) which furnished a δ-lactone (**10**, 1720 cm^{-1}) on treatment with *p*-toluenesulphonic acid in benzene [4] The downfield shift of a proton at C-3 (δ4 05) in the ^{1}H NMR spectrum of **10** indicated that the δ-lactone was formed between C-3 and C-25, and, therefore, the hydroxyl and the formyl groups were located at C-3β and C-10, respectively

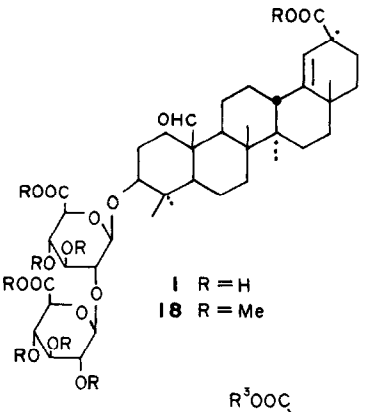
Treatment of periandric acid I monoacetate (**11**) with sulphuric acid in chloroform [5] afforded a γ-lactone (**12**, 1765 cm^{-1}) and a δ-lactone (**13**, 1720 cm^{-1}) The ^{1}H NMR spectra of **12** and **13** did not show a signal of an olefinic proton nor a proton on a lactone ring If the carboxyl group was located at C-17, the formation of only a γ-lactone and the appearance of a proton on a lactone ring in its ^{1}H NMR spectrum would be expected Thus, the carboxyl group must be attached on C-20 Comparison of the CD spectrum of **12** to that of a γ-lactone (**15**) derived from glycyrrhetic acid acetate (**14**) by the method of Barton *et al* [6] exhibited a good ap-

proximation (**12** $\Delta\epsilon_{215}+0.559$, **15** $\Delta\epsilon_{215}+0.912$) The above data suggest that the carboxyl group in periandric acid I (**2**) was located at C-20β in the same manner as in glycyrrhetic acid

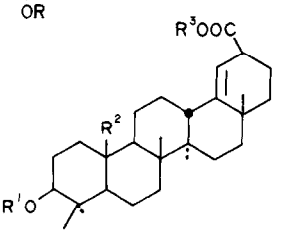
For confirmation of the structure of periandric acid I (**2**), X-ray crystallographic analysis of a *p*-bromobenzoate (**17**) was employed Compound **17** was synthesized from the alcohol **16** which was obtained by reducing an acetal methyl ester (**7**) of periandric acid I (**2**) with lithium aluminium hydride Single crystals of suitable quality for X-ray diffraction were obtained from a acetone–water solution as colourless transparent plates Crystal data triclinic, space group $P1$, $a = 7\ 520(1)$, $b = 10\ 653(1)$, $c = 11\ 192(1)$ Å, $\alpha = 99\ 13(1)$, $\beta = 75\ 88(1)$, $\gamma = 92\ 35(1)°$, $Z = 1$, $D_m = 1\ 27$ g/cm^3 (by flotation method in a potassium iodide solution), $D_x = 1\ 2611(3)$ g/cm^3 Intensities of 4150 independent reflections with 2θ less than 50° were measured by a Rigaku automatic four-cycle diffractometer, using graphite-monochromated molybdenum K_α radiation ($\lambda = 0\ 71069$ Å) Of those 2987 reflections with $|F_0| > 3\sigma(F)$ were used for the structure analysis The structure was solved by the iteration of the least-squares calculations and the Fourier syntheses with the starting Fourier map based on the bromine atom at the origin The structure was refined by the block-diagonal least-squares method The bromine atom was kept fixed at the origin The hydrogen atoms were not clearly revealed and were ignored in the refinement The final residual index R was 0 089 with anisotropic thermal parameters for the non-hydrogen atoms *

*A complete list of the refined co-ordinates is deposited at the Cambridge Crystallographic Data Centre

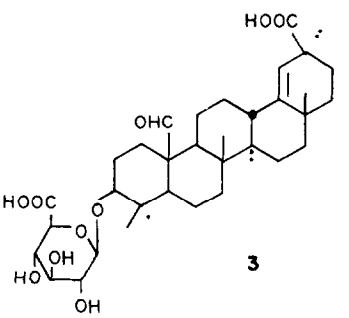
The molecular structure of **17** (Fig 1) unambiguously indicates its chemical structure as 30 - hydroxy - 3,25 - oxido - 25 - methoxy - olean - 18(19) - en - 30 - *p* - bromobenzoate, which implies that C-30



1 R = H
18 R = Me



	R¹	R²	R³
2	H	CHO	H
4	H	CHO	Me
5	Ac	CHO	Me
8	Ac	COOH	Me
9	H	COOH	Me
11	Ac	CHO	H



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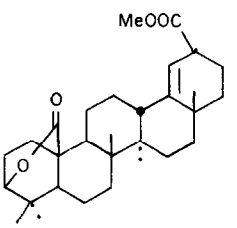
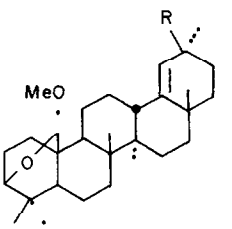
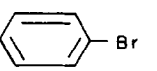


10



6 R = H
7 R = COOMe
16 R = CH_2OH
17 R = CH_2OCO



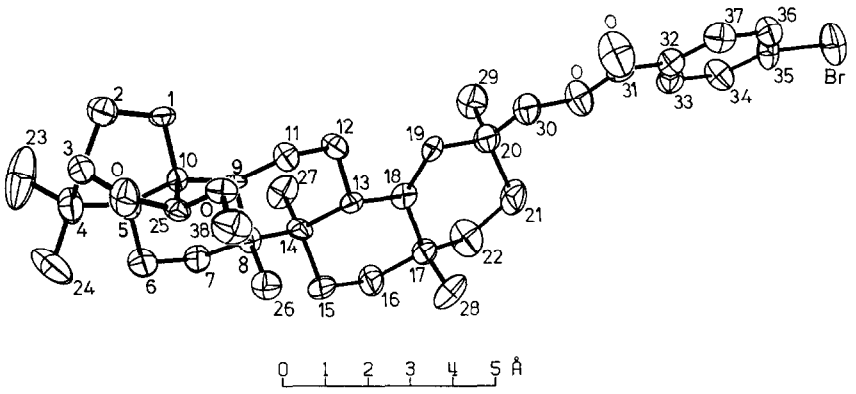
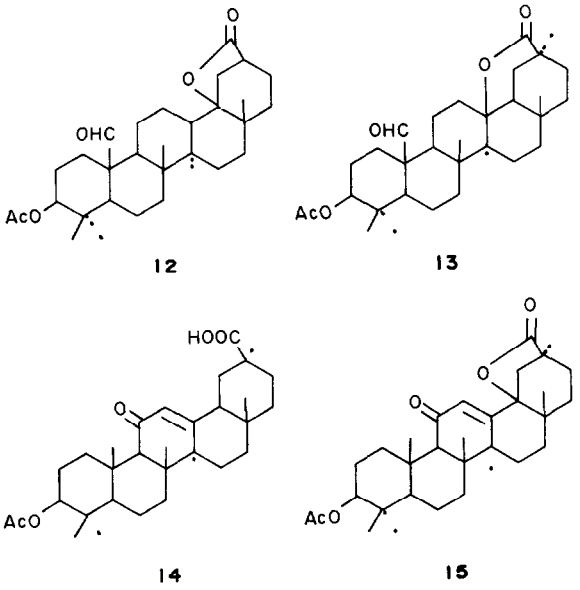


Fig 1 Molecular structure and atom numbering scheme for *p*-bromobenzoate (17)



was the carbon atom of the carboxyl group in 2 The bond lengths and angles are in an expected range in view of their estimated standard deviations The C-18–C-19 length of 1 316 Å shows a localized double bond of C-18=C-19 The B, C and D rings assume a chair form, whereas ring A assumes a boat form Ring E is distorted from the chair form due to C-18=C-19 The ring junctions of A/B, B/C and C/D are *trans* Consequently, the structure of the original aglycone (2) is designated as a 3-β-hydroxy-25-al-olean-18(19)-en-30-oic acid

Methylation of 1 by Hakomori's method [7] yielded an octa-*O*-methyl derivative (18) whose ^{1}H NMR spectrum showed an olefinic proton at δ5 12 (1H, *m*) and two anomeric protons at δ4 30 (1H, *d*, $J = 7$ Hz) and δ4 64 (1H, *d*, $J = 7$ Hz) This finding suggested that two glucuronic acid residues in 1 were linked with a β-orientation Lithium aluminium hydride reduction of 18 followed by methanolysis liberated methyl-3,4-di-*O*-methyl glucose and methyl-2,3,4-tri-*O*-methyl glucose They were identified by GC with authentic samples derived from octa-*O*-methyl glycyrrhizin by lithium aluminium hydride 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 - al - olean - 18(19) - en - 30 - oic acid to periandrin I (1) The sweetness of periandrin I (1) was as strong as those of periandrin II, IV and glycyrrhizin

EXPERIMENTAL

^{1}H NMR 60, 90 or 200 MHz with TMS as int standard, MS direct inlet system, 70 eV Mps are uncorr Si gel was used for CC and TLC Detection of the isolated spots on TLC was by spraying with 10 or 30% H_2SO_4 followed by heating

Plant material The roots of *P dulcis* Mart were purchased in 1976 from Moageira Botanica 'Index' in Brazil

Extraction and isolation The roots (20 kg) were extracted with H_2O (3 × 100 l) at 70° for 24 hr The extracts were concd to 6 l and the ppt removed by filtration The filtrate was treated with EtOH (88 l) and left at 5° overnight After removal, the ppt was dissolved in H_2O (4 l) and EtOH (46 l) added Crude sweet materials (870 g) were obtained by repeated pptation with EtOH The crude sweet materials (100 g) were subjected to CC on Si gel (80 × 6 cm) with *n*-BuOH–C_6H_6–MeOH–28% NH_4OH (4 3 3 2) as the eluent Periandrin I (1) was purified by repetition of the CC step

Periandrin I (1) Mp > 300° (colourless plates from MeOH–H_2O), $[\alpha]_D^{20}$ −23 0° (H_2O, *c* 1 0) IR ν_{max}^{KBr} cm^{-1} 3300 (OH), 2900, 1700 (COOH), 1360 and 1030 (Found C, 56 52, H, 7 61 $C_{42}H_{62}O_{16}$ $4H_2O$ requires C, 56 36, H, 7 88%)

Acid hydrolysis of periandrin I (1) 1 (210 mg) was refluxed with 10% H_2SO_4 (150 ml) for 3 hr The cooled reaction mixture was extracted with $CHCl_3$ (3 × 100 ml) The $CHCl_3$ extracts were washed with H_2O and evaporated CC of the residue on Si gel eluting with *n*-hexane–Me_2CO (5 1) afforded periandric acid I (2, 25 mg) and an unidentified triterpenoid (37 mg) Compound 2 was crystallized from EtOH–H_2O as colourless needles, mp 267–268° IR ν_{max}^{KBr} cm^{-1} 3400 (OH), 2930, 1700 (COOH and CHO), 1450 and 1380, ^{1}H NMR (90 MHz, $CDCl_3$) δ0 75 (6H, *s*, 2 × Me), 0 98 (3H, *s*, Me), 1 03 (6H, *s*, 2 × Me), 1 28 (3H, *s*, Me), 3 25 (1H, *dd*, $J = 5$, 11 Hz, $W_{1/2} = 20$ Hz, C-3), 5 20 (1H, *s*, C-19) and 10 25 (1H, *s*, CHO), MS *m/z* 452 326275 [M − 18]$^+$ (calc for $C_{30}H_{44}O_3$, 452 328998), 407 [M − 18 − 45]$^+$, 248, 236, 234, 221, 218, 203 and 189 (base peak) The aq layer was concd and

the presence of glucuronic acid shown by TLC (*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 I (**1**) **1** (320 mg) was incubated with β-glucuronidase (400 mg, P-L Biochemicals Inc) in 0 2 M NaOAc–HOAc buffer (pH 5 0, 100 ml) at 37° for 24 hr A $CHCl_3$ extract of the reaction mixture was chromotographed on Si gel using *n*-hexane–Me_2CO (5 1) as the eluent Periandric acid I (**2**, 82 4 mg) was isolated and crystallized from Me_2CO–H_2O as colourless needles The presence of glucuronic acid in the aq layer was shown by TLC as described above The supernatant of the aq layer after centrifugation (4000 rpm) was concd and chromatographed on HP-20 resin (20 ml, Mitsubishi Chemical Industries Ltd) After washing with H_2O, the absorbed compounds were eluted with 80% EtOH (100 ml) Chromatography of the recovered material from the eluate on Si gel eluting with CH_2Cl_2–EtOH–MeOH–40% HOAc (8 3 2 1) afforded prosapogenin (**3**) By treating with HP-20 resin for desalting, compound **3** was purified as a white powder, mp 218–221° IR ν_{max}^{KBr} cm^{-1} 3300 (OH), 2900, 1700 (COOH and CHO), 1440, 1360 and 1020 (Found C, 65 81, H, 8 56 $C_{36}H_{54}O_{10}$ 0 5H_2O requires C, 65 93, H, 8 54%)

Acid hydrolysis of prosapogenin (**3**) **3** (4 mg) was treated with 10% H_2SO_4 (15 ml) at 100° for 1 hr The reaction mixture was extracted with $CHCl_3$ (2 × 20 ml) The $CHCl_3$ extract was washed with H_2O, dried over Na_2SO_4 and evaporated The aglycone in the $CHCl_3$ extract was identified with periandric acid I (**2**) and an unidentified triterpenoid by TLC (*n*-hexane–Me_2CO, 2 1) The aq layer was treated with $Ba(OH)_2$ and $BaSO_4$ and the ppt was removed Glucuronic acid in the supernatant was identified by TLC as described above

Methylation of periandric acid I (**2**) A soln of **2** (106 3 mg) in $CHCl_3$ (50 ml) was treated with CH_2N_2 at room temp for 10 min After evaporation of the reaction mixture, the monomethyl ester (**4**, 33 mg) was crystallized from EtOH–H_2O as colourless needles, mp 196–197° IR ν_{max}^{KBr} cm^{-1} 3500 (OH), 2950, 2860, 1710 (COOMe), 1450, 1385, 1305, 1265, ^1H NMR (90 MHz, $CDCl_3$) δ0 74 (3H, *s*, Me), 0 76 (3H, *s*, Me), 0 97 (3H, *s*, Me), 1 02 (3H, *s*, Me), 1 03 (3H, *s*, Me), 1 23 (3H, *s*, Me), 3 20 (1H, *dd*, *J* = 5, 11 Hz, C-3), 3 65 (3H, *s*, COOMe), 5 16 (1H, *s*, C-19), 10 24 (1H, *s*, CHO)

Acetylation of periandric acid I monomethyl ester (**4**) **4** (108 mg) was treated with Ac_2O (10 ml) in C_5H_5N (10 ml) at room temp overnight The reaction mixture was evaporated to dryness The monoacetate (**5**, 33 5 mg) was crystallized from Me_2CO as colourless needles, mp > 300° IR ν_{max}^{KBr} cm^{-1} 2830, 1720 (COOMe), 1700 (OCOMe), 1440, 1375 and 1250, ^1H NMR (90 MHz, $CDCl_3$) δ0 78 (3H, *s*, Me), 0 83 (3H, *s*, Me), 0 93 (3H, *s*, Me), 0 99 (3H, *s*, Me), 1 02 (3H, *s*, Me), 1 24 (3H, *s*, Me), 2 01 (3H, *s*, OCOMe), 3 66 (3H, *s*, COOMe), 4 51 (1H, *dd*, *J* = 5, 11 Hz, C-3), 5 19 (1H, *s*, C-19), 10 27 (1H, *s*, CHO), MS *m/z* 526 366528 $[M]^+$ (calc for $C_{33}H_{50}O_5$, 526 365770), 466 $[M-60]^+$, 437 $[M-59-30]^+$, 407 $[M-59-60]^+$, 263, 262, 248, 203, 189 (base peak)

Acetylation of periandric acid I (**2**) A soln of **2** (15 mg) in C_5H_5N (3 ml) was treated with Ac_2O (3 ml) at room temp overnight The reaction mixture was evaporated to dryness The monoacetate (**11**, 14 mg) was crystallized from EtOH–H_2O as colourless needles, mp 290° (decomp) IR ν_{max}^{KBr} cm^{-1} 3250 (OH), 2900, 1720 (OCOMe), 1690 (COOH), 1440, 1360, 1250, ^1H NMR (90 MHz, $CDCl_3$) δ0 76 (3H, *s*, Me), 0 81 (3H, *s*, Me), 0 91 (3H, *s*, Me), 0 98 (3H, *s*, Me), 1 01 (3H, *s*, Me), 1 24 (3H, *s*, Me), 1 99 (3H, *s*, OCOMe), 4 45 (1H, *dd*, *J* = 6, 12 Hz, C-3), 5 12 (1H, *s*, C-19), 10 17 (1H, *s*, CHO), MS *m/z* 512 349276 $[M]^+$ (calc for $C_{32}H_{48}O_5$, 512 350122), 467 $[M-45]^+$, 452 $[M-60]^+$, 423 $[M-60-29]^+$, 408 $[M-59-45]^+$, 263, 234, 203, 189 (base peak)

MeOH *treatment of periandric acid I* (**2**) **2** (64 mg) was dissolved in MeOH (10 ml) and allowed to stand for 2 days at room temp The reaction mixture was evaporated to dryness The acetal (**6**, 43 mg) was crystallized from MeOH as colourless needles, mp 204–206° ^1H NMR (90 MHz, $CDCl_3$) δ0 72 (3H, *s*, Me), 0 97 (6H, *s*, 2 × Me), 1 03 (6H, *s*, 2 × Me), 1 25 (3H, *s*, Me), 3 20 (1H, *t*, C-3), 3 32 (3H, *s*, OMe), 5 08 (1H, *s*, C-25), 5 11 (1H, *s*, C-19) (Found C, 73 88, H, 10 13, $C_{31}H_{48}O_4$ H_2O requires C, 74 06, H, 10 03%)

Methylation of periandric acid I acetal (**6**) A soln of **6** (65 mg) in $CHCl_3$ (30 ml) was treated with CH_2N_2 and allowed to stand for 10 min The reaction mixture was evaporated to dryness The methyl ester (**7**, 33 mg) was crystallized from Me_2CO–H_2O as colourless needles, mp 163° IR ν_{max}^{KBr} cm^{-1} 2900, 1740 (COOMe), 1445, 1380, 1240, 1115, 859, ^1H NMR (90 MHz, $CDCl_3$) δ0 73 (3H, *s*, Me), 0 96 (3H, *s*, Me), 0 99 (3H, *s*, Me), 1 04 (3H, *s*, Me), 1 08 (3H, *s*, Me), 1 23 (3H, *s*, Me), 3 21 (1H, *t*, *J* = 2, 2 Hz, C-3), 3 35 (3H, *s*, OMe), 3 61 (3H, *s*, COOMe), 5 10 (1H, *s*, C-25), 5 12 (1H, *br s*, C-19), MS *m/z* 466 3462 $[M-32]^+$ (calc for $C_{31}H_{46}O_3$, 466 3447), 451 $[M-32-15]^+$, 438 $[M-32-28]^+$, 423, 406, 395, 379 $[M-32-28-59]^+$, 373, 249, 248, 247, 221, 218, 215, 201, 190, 189 (base peak), 175

Oxidation of periandric acid I methyl ester acetate (**5**) **5** (95 5 mg) was dissolved in Me_2CO (70 ml, distilled on $KMnO_4$) and treated with Jones' reagent [5] (2 3 ml) at room temp for 3 hr with stirring The reaction mixture was mixed with 5% NaOAc (80 ml) and extracted with $CHCl_3$ The $CHCl_3$ extract was washed with H_2O, dried over Na_2SO_4 and evaporated Chromatography of the $CHCl_3$ extract on Si gel eluting with *n*-hexane–Me_2CO (7 1) gave **8** (32 mg) which was crystallized from EtOH as colourless needles, mp 270–272° IR ν_{max}^{KBr} cm^{-1} 3200, 2900, 1720 (OCOMe), 1700 (COOH), 1420, 1365, 1240, ^1H NMR (90 MHz, $CDCl_3$) δ0 72 (3H, *s*, Me), 0 90 (3H, *s*, Me), 0 92 (3H, *s*, Me), 1 02 (3H, *s*, Me), 1 07 (3H, *s*, Me), 1 26 (3H, *s*, Me), 2 04 (3H, *s*, OCOMe), 3 68 (3H, *s*, COOMe), 4 46 (1H, *dd*, *J* = 5, 11 Hz, C-3), 5 22 (1H, *s*, C-19), MS *m/z* 542 357920 $[M]^+$ (calc for $C_{33}H_{50}O_6$, 542 360684), 483 $[M-59]^+$, 438 $[M-45-59]^+$, 423 $[M-59-60]^+$, 249, 235, 234, 203, 189 (base peak)

Hydrolysis of compound **8** A soln of **8** (25 3 mg) in EtOH (5 ml) was mixed with alcoholic 1 M KOH (5 ml) and refluxed for 2 hr The reaction mixture was cooled and treated with SK-1B resin (H^+, Mitsubishi Chemical Industries Ltd) After removal of resin by filtration, the filtrate was evaporated to dryness and chromatographed on Si gel Elution with *n*-hexane–Me_2CO (3 1) gave a dicarboxylic acid (**9**, 5 4 mg) which crystallized from EtOH–H_2O as colourless needles, mp > 300° IR ν_{max}^{KBr} cm^{-1} 3300 (OH), 2900, 1700 (COOH), 1445 and 1380, ^1H NMR (90 MHz, $CDCl_3$) δ0 75 (3H, *s*, Me), 0 81 (3H, *s*, Me), 1 02 (3H, *s*, Me), 1 04 (3H, *s*, Me), 1 09 (3H, *s*, Me), 1 25 (3H, *s*, Me), 3 23 (1H, *m*, C-3), 5 22 (1H, *s*, C-19), MS *m/z* 468 322134 $[M-18]^+$, (calc for $C_{30}H_{44}O_4$, 468 323912), 423 $[M-45]^+$, 248, 234, 219, 189 (base peak)

Lactonization of dicarboxylic acid (**9**) A soln of **9** (10 8 mg) in dry C_6H_6 (20 ml) was mixed with *p*-TsOH (20 mg) The mixture was slowly distilled within 15 min on a water bath The reaction mixture was diluted with C_6H_6 (50 ml) and washed with 5% Na_2CO_3 (30 ml) and H_2O (3 × 30 ml) The C_6H_6 layer was dried on Na_2SO_4 and evaporated under red pres to give a δ-lactone (**10**, 5 5 mg) which was

crystallized from EtOH–H_2O as colourless needles, mp > 300° IR ν_{max}^{KBr} cm^{-1} 3200, 2900, 1720 (δ-lactone), 1700 (COOH), 1440, 1380, ^1H NMR (90 MHz, $CDCl_3$) δ0 73 (3H, *s*, Me), 1 01 (3H, *s*, Me), 1 08 (6H, *s*, 2 × Me), 1 21 (3H, *s*, Me), 1 30 (3H, *s*, Me), 4 05 (1H, *m*, C-3), 5 21 (1H, *s*, C-19), MS *m/z* 468 322134 $[M]^+$ (calc for $C_{30}H_{44}O_4$, 468 323912), 423 $[M-45]^+$, 248, 234, 219, 203, 189 (base peak)

H_2SO_4 treatment of periandric acid I monoacetate (**11**) A cooled soln of H_2SO_4 (1 ml) in $CHCl_3$ (2 5 ml) was added to a previously cooled soln of **11** (11 mg) in $CHCl_3$ (2 ml) and allowed to stand at −13° for 15 min The reaction mixture was poured into 10% NaOAc (50 ml) and extracted with $CHCl_3$ The $CHCl_3$ extract was washed with H_2O, dried on Na_2SO_4 and evaporated to dryness Chromatography of the $CHCl_3$ extract on Si gel eluting with *n*-hexane–Me_2CO (15 1) resulted in separation of the γ- and δ-lactones The γ-lactone (**12**, 5 mg) was crystallized from EtOH–H_2O as colourless needles, mp > 300° IR ν_{max}^{KBr} cm^{-1} 2900, 1765 (γ-lactone), 1720 (OCOMe), 1440, 1365, 1250, ^1H NMR (90 MHz, $CDCl_3$) δ0 80 (3H, *s*, Me), 0 92 (3H, *s*, Me) 0 98 (9H, *s*, 3 × Me), 1 14 (3H, *s*, Me), 2 02 (3H, *s*, OCOMe), 4 52 (1H, *dd*, *J* = 5, 11 Hz, C-3), 10 17 (1H, *s*, CHO), MS *m/z* 512 349276 $[M]^+$ (calc for $C_{32}H_{48}O_5$, 512 350122), 452 $[M-60]^+$, 423 $[M-60-29]^+$, 279, 248, 243, 203, 189 (base peak), CD $\Delta\epsilon_{215}$ + 0 559 (MeOH, *c* 4 56 × 10^{-4}) The δ-lactone (**13**, 3 mg) was crystallized from EtOH–H_2O as colourless needles, mp > 300° IR ν_{max}^{KBr} cm^{-1} 2870, 1720 (δ-lactone and OCOMe), 1440, 1360, 1250, 1155, ^1H NMR (90 MHz, $CDCl_3$) δ0 84 (3H, *s*, Me), 0 93 (6H, *s*, 2 × Me), 1 13 (3H, *s*, Me), 1 28 (3H, *s*, Me), 1 34 (3H, *s*, Me), 2 05 (3H, *s*, OCOMe), 4 55 (1H, *m*, C-3), 10 19 (1H, *s*, CHO), MS *m/z* 512 3453 $[M]^+$ (calc for $C_{32}H_{48}O_5$, 512 3501), 452 $[M-60]^+$, 423 $[M-60-29]^+$, 235, 221, 203, 189, 175, 98 (base peak)

Lactonization of glycyrrhetic acid acetate (**14**) **14** (1 g) was treated with excess of oxalyl chloride (3 ml) at room temp for 30 min After evaporation, the residue in C_6H_6 (10 ml) was shaken vigorously with 2 8% NH_4OH for 5 min Cyclohexane (10 ml) was added and the shaking was continued for a further 5 min The ppt of the amide was filtered and dried The amide, I_2 (3 g) and $Pb(OAc)_4$ (5 g) in C_6H_6 (100 ml)–$CHCl_3$ (30 ml) soln were irradiated in a Pyrex flask at 15° with a 200 W high-pressure mercury lamp for 5 hr After filtration and washing with $CHCl_3$, the filtrate was evaporated The residue in 80% EtOH (50 ml) containing KOH (2 5 g) was refluxed for 2 hr After evaporation of EtOH, H_2O (100 ml) was added and the resulting soln was extracted with EtOAc (2 × 50 ml) The aq soln was acidified with 1 M H_2SO_4 and heated for 2 hr After neutralization, the reaction mixture was extracted with EtOAc The EtOAc extract was acetylated with Ac_2O in C_5H_5N at room temp overnight Chromatography of the reaction mixture on Si gel eluting with C_6H_6–EtOAc (1 1) afforded the γ-lactone (**15**, 29 mg) which was crystallized from EtOAc, mp > 300° IR ν_{max}^{KBr} cm^{-1} 2950, 1740 (γ-lactone), 1730 (OCOMe), 1680 (–C=C–CO–), 1640 (–C=C–CO–), 1460, 1385, 1365, 1250, 1030, 990, ^1H NMR (200 MHz, $CDCl_3$) δ0 83 (3H, *s*, Me), 0 88 (6H, *s*, 2 × Me), 1 13 (3H, *s*, Me), 1 16 (3H, *s*, Me), 1 18 (3H, *s*, Me), 2 06 (3H, *s*, OCOMe), 4 52 (1H, *dd*, *J* = 5, 10 Hz, C-3), 5 68 (1H, *s*, C-12), UV λ_{max}^{EtOH} nm (ε) 250 (9731 5), CD $\Delta\epsilon_{215}$ + 0 912 (MeOH, *c* 2 06 × 10^{-3})

$LiAlH_4$ reduction of periandric acid I acetal monomethyl ester (**7**) A suspension of $LiAlH_4$ (44 mg) in Et_2O (10 ml) was added to a soln of **7** (51 mg) in Et_2O (20 ml) and stirred overnight at room temp The reaction mixture was added to aq Et_2O, acidified with 1 M HCl and the Et_2O removed by evaporation The residue was extracted with $CHCl_3$ The $CHCl_3$ extract was washed with H_2O and evaporated to dryness The alcohol dihydroperiandric acid I acetal **16** (28 mg) was crystallized from Me_2CO–H_2O as colourless needles, mp 190–192° ^1H NMR (60 MHz, $CDCl_3$) δ0 75 (3H, *s*, Me), 0 93 (3H, *s*, Me), 0 97 (3H, *s*, Me), 1 02 (3H, *s*, Me), 1 06 (3H, *s*, Me), 1 09 (3H, *s*, Me), 3 23 (1H, *br s*, C-3), 3 27 (2H, CH_2O), 3 37 (3H, *s*, OMe), 4 89 (1H, *s*, C-25), 5 11 (1H, *s*, C-19)

p-Bromobenzoylation of dihydroperiandric acid I acetal (**16**) To a soln of **16** (38 mg) in C_6H_6 (20 ml) containing five drops of C_5H_5N, *p*-bromobenzoylchloride (43 mg) was added The reaction mixture was refluxed for 7 min, cooled and evaporated under red pres The *p*-bromobenzoate (**17**) was purified by TLC (0 25 mm thickness, *n*-hexane–Me_2CO (20 1) as developing solvent, Me_2CO as extracting solvent), mp 178–179°, as colourless plates (from Me_2CO–H_2O) ^1H NMR (60 MHz, $CDCl_3$) δ0 73 (3H, *s*, Me), 0 95 (3H, *s*, Me), 0 98 (3H, *s*, Me), 1 03 (3H, *s*, Me), 1 06 (6H, *s*, 2 × Me), 3 23 (1H, *br s*, C-3), 3 37 (3H, *s*, OMe), 3 85–4 07 (2H, C-30), 4 90 (1H, *s*, C-25), 5 10 (1H, *br s*, C-19), 7 57 (2H, *d*, *J* = 8 Hz, Ph C-2′ and C-6′), 7 85 (2H, *d*, *J* = 8 Hz, Ph C-3′ and C-5′)

Methylation of periandrin I (**1**) *by Hakomori's method* Dimsyl carbanion was prepared by heating a soln of NaH (2 5 g) in DMSO (50 ml) under N_2 at 60° for 2 hr The greenish soln was added to a soln of **1** (110 mg) in DMSO (5 ml) and stirred under N_2 for 1 hr MeI (5 ml) was added and the soln left standing in the dark overnight at room temp The reaction mixture was poured into ice–H_2O and extracted with Et_2O The Et_2O extract was washed with 10% $Na_2S_2O_3$ and H_2O and evaporated to dryness Octa-*O*-methyl periandrin I (**18**, 91 mg) was crystallized from MeOH as colourless needles, mp 227–229° IR ν_{max}^{KBr} cm^{-1} 1758 (COOMe), 1445, 1380, 1103, 1092, 1040, ^1H NMR (90 MHz, $CDCl_3$) δ3 00 (1H, *m*, C-3), 3 45 (6H, *s*, 2 × OMe), 3 54 (3H, *s*, OMe), 3 59 (6H, *s*, 2 × OMe), 3 62 (3H, *s*, OMe), 3 68 (3H, *s*, OMe), 3 76 (3H, *s*, OMe), 4 30 (1H, *d*, *J* = 7 Hz, anomeric H), 4 64 (1H, *d*, *J* = 7 Hz, anomeric H), 5 12 (1H, *br s*, C-19), 10 14 (1H, *br s*, CHO) (Found C, 63 62, H, 8 73 $C_{50}H_{80}O_{16}$ requires C, 64 08, H, 8 60%)

$LiAlH_4$ reduction followed by methanolysis of octa-O-methyl periandrin I (**18**) **18** (38 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 aq Et_2O, acidified with 20%H_2SO_4 and extracted with Et_2O The Et_2O extract was evaporated to dryness The reaction product was obtained as white powder ^1H NMR (60 MHz, $CDCl_3$) δ0 76 (3H, *s*, Me), 0 83 (3H, *s*, Me), 0 90 (3H, *s*, Me), 0 94 (3H, *s*, Me), 1 06 (3H, *s*, Me), 1 22 (3H, *s*, Me), 3 00–3 32 (8H, 4 × CH_2O), 3 51 (3H, *s*, OMe), 3 57 (6H, *s*, 2 × OMe), 3 62 (6H, *s*, 2 × OMe), 4 22 (1H, *d*, *J* = 7 Hz, anomeric H), 4 68 (1H, *d*, *J* = 7 Hz, anomeric H), 4 80 (1H, *br s*, C-19) The reaction product was treated with 6% HCl–MeOH (10 ml) under reflux for 1 hr, neutralized with Ag_2CO_3, and filtered The filtrate gave the 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 by GC using two systems (1) 5% diethyleneglycol succinate, 3 mm × 2 m, temp 190°, carrier gas N_2, flow rate 60 ml/min, RR_t (min), 4 min 25 sec (minor), 6 min 10 sec (major), 14 min 26 sec (major), 17 min 12 sec (minor), (2) 5% SE-30, 3 mm × 1 m, column temp 110°, carrier gas N_2, flow rate 60 ml/min, RR_t (min) 8 min 44 sec (minor), 10 min 39 sec (major)

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