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PERIANDRIN III, A NOVEL SWEET TRITERPENE GLYCOSIDE FROM *PERIANDRA DULCIS*

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Key Word Index— *Periandra dulcis*; Leguminosae; triterpene glycoside; periandrin III; sweetener.

Abstract—Periandrin III, a new sweet triterpene glycoside of the roots of *Periandra dulcis*, has been shown to possess the structure 3-β-*O*-[β-D-glucuronopyranosyl-(1→2)-β-D-glucuronopyranosyl]-25-hydroxyolean-18(19)-en-30-oic acid on the basis of spectral and chemical correlation with periandrin I.

INTRODUCTION

In previous papers we reported the isolation and characterization of three new sweeteners, periandrin I, II and IV, from the roots of *Periandra dulcis* [1, 2]. Further fractionation has now afforded another new sweet triterpene glycoside, designated periandrin III.

RESULTS AND DISCUSSION

Periandrin III (**1**), $C_{42}H_{64}O_{16}\cdot 2H_2O$, was crystallized from EtOH–H_2O as colourless needles. Acidic hydrolysis of **1** yielded glucuronic acid and periandric acid III (**3**) which was a new triterpenoid. Periandric acid III (**3**) showed positive Liebermann–Burchard reaction (purple to pink) and trichloroacetic acid reaction (pink at 125°), thereby suggesting it to be a pentacyclic triterpenoid [3]. The ^{1}H NMR spectrum of periandric acid III methyl ester (**4**) indicated six singlet methyl groups (δ 0.77, 0.80, 1.00, 1.05, 1.20 and 1.24), a carbomethoxymethyl group (3.66), a hydroxymethine proton (3.27, *dd*, $J = 6$, 10 Hz), hydroxymethylene protons (AB pattern at 4.00 and 4.02, $J = 12$ Hz each) and a singlet olefinic proton (5.19). The mass spectrum of **4** exhibited significant peaks at *m/z* 262 and 223, which could be rationalized only in terms of a $\Delta^{18(19)}$-amyrin bearing a carbomethoxyl group on rings D/E and hydroxyl and hydroxymethyl groups on rings A/B.

Treatment of **4** with H_2SO_4 in $CHCl_3$ [4] gave a γ-lactone (**5**, 1765 cm^{-1}) and a δ-lactone (**6**, 1748 cm^{-1}) whose ^{1}H NMR spectra showed no signal of an olefinic proton nor of a proton on a lactone ring. This indicated that the carboxyl group should be attached at C-20.

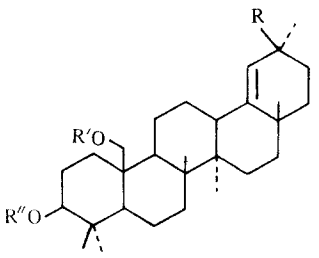
In an attempt to form an acetonide, **3** did not react with H_2SO_4 in acetone, whereas polygalacic acid methyl ester formed its acetonide under the same reaction condition [5, 6]. The mobility to form an acetonide of **3** indicates that the hydroxymethyl group in **3** could be attached at C-10.

The above structural features suggested that **3** might be the 25-dihydro compound corresponding to periandric acid I. Direct proof for the suggested structure of **3** was accomplished by converting periandric acid I with $NaBH_4$ to the corresponding dihydro compound, identical in all respects (TLC, mmp, IR and ^{1}H NMR) with **3**.

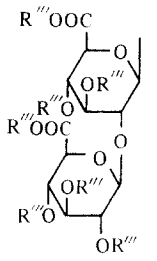
Nona-*O*-methyl periandrin III (**2**) showed in its ^{1}H NMR spectrum an olefinic proton at δ 5.15 (1H, *s*) and two anomeric protons at 4.35 (*d*, $J = 7$ Hz) and 4.70 (*d*, $J = 7$ Hz) which suggested that two glucuronic acid residues in **1** were linked with the β-orientation. $LiAlH_4$ reduction followed by methanolysis of **2** gave methyl-3,4-di-*O*-methyl glucose and methyl-2,3,4-tri-*O*-methyl glucose as methylated saccharides and a methylated aglycone (**7**). Two methylated saccharides were identified with authentic samples derived from octa-*O*-methyl periandrin I by $LiAlH_4$ reduction followed by methanolysis. Acetylation of **7** afforded a diacetate (**8**) whose ^{1}H NMR spectrum showed an acetoxyl methine proton at δ 4.50 (*dd*, $J = 6$, 9 Hz). Therefore, the glucuronic acid in **1** must be linked to a 3β-hydroxyl group.

Treatment of periandrin I with $NaBH_4$ yielded dihydro periandrin I which was identified as periandrin III (**1**) by means of IR spectroscopy and TLC.

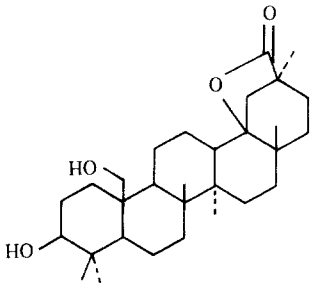
The accumulated evidence described above led us to assign the structure 3-β-*O*-[β-D-glucuronopyranosyl-(1→2)-β-D-glucuronopyranosyl]-25-hydroxyolean-18(19)-en-30-oic acid to periandrin III (**1**). Periandrin III was as sweet as periandrin I, II, IV and glycyrrhizin.



	R	R′	R″
1	COOH	H	A
2	COOMe	Me	B
3	COOH	H	H
4	COOMe	H	H
7	CH_2OH	Me	H
8	CH_2OAc	Me	Ac



A R‴ = H
B R‴ = Me



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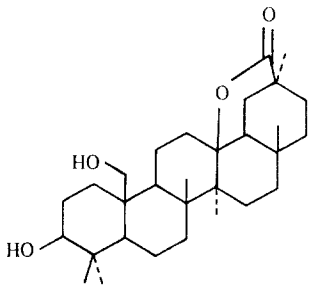

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EXPERIMENTAL

TLC was carried out over HPLC Si gel 60F-254. Detection of the isolated spots on TLC was achieved by spraying with 30% H_2SO_4 soln followed by heating.

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

Extraction and isolation. The powdered roots (18 kg) of *P. dulcis* were extracted with 80% MeOH (40 l.) at 80° for 4 days. After filtration, the extract was concd *in vacuo* to 5 l. and filtered. The filtrate was ajusted to pH 2.0 and the ppt. was collected. The crude sweet material (600 g) was extracted from the ppt. with *n*-BuOH–Et_2O (3:1). The crude sweet material (75 g) was chromatographed on a Si gel colum using *n*-BuOH–C_6H_6–MeOH–28% NH_4OH (4:3:3:2) and $CHCl_3$–MeOH–H_2O (25:17:3) as the eluents to yield pure periandrin III (200 mg).

Periandrin III (**1**). Mp > 300°; $[\alpha]_D^{18}$ −24.5° (H_2O, *c* 1.1); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1700 (COOH), 1600, 1175 and 1040. (Found: C, 58.07; H, 8.17. $C_{42}H_{64}O_{16}\cdot 2H_2O$ required: C, 58.59; H, 7.96%.)

Acidic hydrolysis of periandrin III (**1**). Periandrin III (**1**, 50 mg) was refluxed with 10% H_2SO_4 (10 ml) for 4 hr. The reaction mixture was diluted with H_2O and extracted with EtOAc. Chromatography of the EtOAc extract on Si gel eluting with *n*-hexane–Me_2CO (3:2) afforded periandric acid III (**3**, 15 mg, colourless needles from Me_2CO–H_2O). Mp 265–267°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3350 (OH), 1700 (COOH) and 1305. ^1H NMR (90 MHz, $CDCl_3 + CD_3OD$): δ 0.79 (6H, *s*, 2 × Me), 1.01 (3H, *s*, Me), 1.07 (3H, *s*, Me), 1.23 (3H, *s*, Me), 1.26 (3H, *s*, Me), 3.25 (1H, *m*, H-3), 3.99 (2H, *s*, H-25) and 5.19 (1H, *s*, H-19). The material in the aq. layer was subjected to TLC in order to identify glucuronic acid. [*n*-PrOH–nitromethane–H_2O (5:2:3). R_f = 0.39. *n*-BuOH–C_5H_5N–H_2O (6:4:3), F_f = 0.17. Naphthoresorcinol or diphenylamine–aniline as colour reagents.]

Methylation of periandric acid III (**3**). Compd **3** (10 mg) was esterified with ethereal CH_2N_2. Working-up in the usual way and crystn from MeOH yielded the methyl ester (**4**, 8 mg) as colourless needles, mp 239–240°. ^1H NMR (90 MHz, $CDCl_3$): δ 0.77 (3H, *s*, Me), 0.80 (3H, *s*, Me), 1.00 (3H, *s*, Me), 1.05 (3H, *s*, Me), 1.20 (3H, *s*, Me), 1.24 (3H, *s*, Me), 3.27 (1H, *dd*, *J* = 6, 10 Hz, H-3), 3.66 (3H, *s*, COOMe), 4.00 (1H, *d*, *J* = 12 Hz, part of AB type, H-25), 4.02 (1H, *d*, *J* = 12 Hz, part of AB type, H-25) and 5.19 (1H, *s*, H-19). MS *m/z*: 486.368 (M^+, calc. for $C_{31}H_{50}O_4$, 486.371), 468 $[M-18]^+$, 455 $[M-37]^+$, 427 $[M-59]^+$, 262, 248, 223, 203 and 189 (base peak).

Sulphuric acid treatment of periandric acid III *methyl ester* (**4**). Cooled H_2SO_4 (1 ml) in $CHCl_3$ (2.5 ml) was added to a previously cooled soln of **4** (10 mg) in $CHCl_3$ (2 ml) and the mixture allowed to stand at −13° for 15 min. The reaction mixture was added to 10% NaOAc (50 ml) and the mixture extracted with $CHCl_3$. After evapn of the $CHCl_3$ extract, chromatography on a Si gel column eluting with *n*-hexane–Me_2CO (15:1) resulted in sepn of γ-lactone (**5**, 5 mg) and δ-lactone (**6**, 3 mg). The γ-lactone was crystallized from $CHCl_3$–MeOH, mp > 300°. IR ν_{max}^{KBr} cm^{-1}: 3480 (OH),

1765 (γ-lactone), 1455, 1390, 1160 and 910. ^{1}H NMR (90 MHz, $CDCl_3$): δ 0.78 (3H, *s*, Me), 0.97 (3H, *s*, Me), 1.00 (3H, *s*, Me), 1.04 (3H, *s*, Me), 1.13 (3H, *s*, Me), 1.17 (3H, *s*, Me), 3.26 (1H, *dd*, $J = 8, 9$ Hz, H-3) and 3.95 (2H, *s*, H-25). MS *m/z*: 472.358 $[M]^+$ (calc. for $C_{30}H_{48}O_4$, 472.355), 454 $[M-18]^+$, 423$[M-18-31]^+$, 410$[M-44]^+$, 248 (base peak), 237, 221 and 189. The δ-lactone was crystallized from $CHCl_3$–MeOH, mp >300°. IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1748 (δ-lactone), 1110, 1030 and 950. MS *m/z*: 472.357 $[M]^+$ (calc. for $C_{30}H_{48}O_4$, 472.355), 454$[M-18]^+$, 436, 424$[M-18-30]^+$, 248, 237 and 221 (base peak).

Reduction of periandric acid I. Periandric acid I (20 mg) was treated with $NaBH_4$ (100 mg) in EtOH (20 ml) at room temp. for 48 hr. After usual work-up, the diol was crystallized from MeOH as colourless needles and identified with periandric acid III (**3**) by means of IR spectroscopy and mmp.

Permethylation of periandrin III (**1**). Compound **1** (15 mg) was methylated by Hakomori's method [7]. Work-up gave nona-*O*-methyl periandrin III (**2**, 9 mg) as a white powder. ^{1}H NMR (90 MHz, $CDCl_3$): δ3.17 (3H, *s*, OMe), 3.46 (6H, *s*, 2 × OMe), 3.53 (3H, *s*, OMe), 3.60 (6H, *s*, 2 × OMe), 3.62 (3H, *s*, OMe), 3.70 (3H, *s*, OMe), 3.78 (3H, *s*, OMe), 4.35 (1H, *d*, $J = 7$ Hz, anomeric H), 4.70 (1H, *d*, $J = 7$ Hz, anomeric H) and 5.15 (1H, *s*, H-19).

Reduction followed by methanolysis of nona-O-methyl periandrin III (**2**). A soln of **2** (8 mg) in Et_2O was treated with $LiAlH_4$ (50 mg). The crude product which was worked-up in the usual way was refluxed for 1 hr with 6% HCl–MeOH (10 ml) and the mixture neutralized with Ag_2CO_3. Methylated aglycone (**7**) was precipitated during concentration of the filtrate. The mother layer gave methylated monosaccharides which were identified with methyl-3,4-di-*O*-methyl glucose and methyl-2,3,4-tri-*O*-methylglucose derived from octa-*O*-methylperiandrin I by GLC using two systems. (1) Column: 5% diethyleneglycol succinate, 3 mm × 2 m; column temp.: 190°; carrier gas: N_2; flow rate: 60 ml/min; R_t(min): 4 min 25 sec(minor), 6 min 10 sec(major), 14 min 26 sec(major) and 17 min 12 sec(minor). (2) Column: 5% SE-30, 3 mm × 1 m; column temp.: 110°; carrier gas: N_2; flow rate: 60 ml/min; R_t(min): 8 min 44 sec(minor) and 10 min 39 sec(major).

Acetylation of 3,30-*dihydroxy*-25-*methoxyolean*-18(19)-*ene* (**7**). Compd **7** was treated with Ac_2O in C_5H_5N at room temp. overnight. The reaction mixture was evapd to yield the diacetate (**8**). ^{1}H NMR (90 MHz, $CDCl_3$): δ 0.71 (3H, *s*, Me), 0.84 (6H, *s*, 2 × Me), 0.98 (3H, *s*, Me), 1.00 (3H, *s*, Me), 1.09 (3H, *s*, Me), 2.02 (6H, *s*, 2 × OCOMe), 3.22 (3H, *s*, OMe), 3.52 (2H, *s*, H-25), 3.72 (1H, *d*, $J = 12$ Hz, part of AB type, H-30), 3.85 (1H, *d*, $J = 12$ Hz, part of AB type, H-30), 4.50 (1H, *dd*, $J = 6, 9$ Hz, H-3) and 4.84 (1H, *s*, H-19).

Reduction of periandrin I. A soln of periandrin I (11 mg) in H_2O (5 ml) was treated with $NaBH_4$ (110 mg) at room temp. for 48 hr. Working-up in the usual way yielded a colourless powder of dihydroperiandrin I (7 mg). Indentity with periandrin III (**1**) was established by means of IR spectroscopy and TLC.

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