Steroidal glycosides from the marine sponge *Pandaros acanthifolium*.

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Abstract

The chemical composition of the Caribbean sponge *Pandaros* sp. was investigated and led to the isolation of seven new steroidal glycosides namely pandarosides A-D (**1**, **3**, **4** and **6**) along with the three methyl esters of pandarosides A, C, and D (**2**, **5** and **7**). Their structures were characterized as 3 β -[β -glucopyranosyl-(1 $\rightarrow$ 2)- β -glucopyranosyloxyuronic acid]-16-hydroxy-5 α ,14 β -poriferast-16-ene-15,23-dione (**1**) and its methyl ester (**2**), 3 β -[β -glucopyranosyloxyuronic acid]-16-hydroxy-5 α ,14 β -poriferast-16-ene-15,23-dione (**3**), 3 β -[β -glucopyranosyl-(1 $\rightarrow$ 2)- β -glucopyranosyloxyuronic acid]-16-hydroxy-5 α ,14 β -cholest-16-ene-15,23-dione (**4**) and its methyl ester (**5**), 3 β -(β -glucopyranosyloxyuronic acid)-16-hydroxy-5 α ,14 β -cholest-16-ene-15,23-dione (**6**) and its methyl ester (**7**) on the basis of detailed spectroscopic analyses, including 2D NMR and HRESI-MS studies. Pandarosides A-D and their methyl esters (**1-7**) are all characterized by a rare 2-hydroxycyclopentenone D-ring with a 14 β configuration.

Keywords: Marine sponge; *Pandaros*; steroidal glycosides; structure elucidation, stereochemistry

1. Introduction

Saponins are an important class of natural products mainly found in terrestrial plants. They are scarcely found in the marine environment and echinoderms are the main source of this class of compounds [1]. Their presence in marine sponges was first discovered in the late 1980' [2,3]. Since then, triterpene glycosides only have been reported from the two genera *Asteropus* and *Erylus* [4,5].

As part of our ongoing work on the secondary metabolites of Caribbean marine sponges, we undertook the chemical study of a specimen of the marine sponge *Pandaros* sp. (Poecilosclerida, Microcionidae) collected at the Canyon rock off the Martinique coast. The order Poecilosclerida is known to produce a large diversity of complex guanidine alkaloids [6], and only acanthifolicin, a polyether carboxylic acid, has been isolated from *Pandaros* species [7].

Herein we report the isolation of seven steroidal glycosides named pandaroside A-D (**1**, **3**, **4** and **6**) and the methyl ester of pandarosides A, C and D (**2**, **6** and **7**) as the major constituents of a *Pandaros* sp. sponge. Their structures were elucidated by extensive spectroscopic studies including 1D- and 2D-NMR experiments (COSY, HSQC, HMBC, and NOESY), as well as HRESI-MS analyses. All new compounds share the rare 2-hydroxycyclopent-2-enone D ring with a 14 β configuration. To our knowledge, *Pandaros* sp. is the first marine organism producing steroidal glycosides with such an aglycon nucleus.

2. Experimental

2.1 General

Optical rotations were measured on a Bellingham ADP220 polarimeter. CD spectra were measured using a JASCO J-810 spectropolarimeter. IR spectra were obtained with a Perkin–Elmer Paragon 1000 FT–IR spectrometer. UV measurements were performed on a Varian Cary 300 Scan UV-Visible spectrometer. Electrospray ionisation (ESI) mass spectra were obtained with a Bruker Esquire 3000 Plus spectrometer in the positive or negative mode. High-resolution mass spectra (HRESI-MS) were obtained from a LTQ Orbitrap mass spectrometer (Thermo Finnigan). NMR experiments were performed on a Bruker Avance 500 MHz spectrometer. Chemical shifts (δ in ppm) are referenced to the carbon (δ_C 49.15) and residual proton (δ_H 3.31) signals of CD₃OD, the solvent with multiplicity (s singlet, d doublet, t triplet, m multiplet). HPLC separation and purification were carried out on a Waters 600 system equipped with a Waters 996 Photodiode Array detector coupled with a Sedex 55 ELSD (SEDERE, France), and a Waters 717 plus Autosampler. TLC was performed with

Kieselgel 60 F₂₅₄ (Merck glass support plates) and spots were detected after spraying with 10% H₂SO₄ in EtOH reagent and heating.

2.2. Biological material

The marine sponge was collected off Martinique Island in Summer 2003 by SCUBA diving (Canyons de Babodie 14°45,982 N, 61°11,902 W). A voucher specimen (ORMA8362) identified by Dr Jean Vacelet, has been deposited in the Centre d'Océanologie de Marseille (Endoume, France). The sponge was kept frozen from collection until the extraction process.

2.3 Extraction and isolation

The frozen sponge (350 g) was cut into pieces of about 1 cm³ and extracted with MeOH/CH₂Cl₂ 1:1 at room temperature yielding 13 g of crude extract after solvent evaporation. The crude extract was fractionated by RP-C₁₈ flash chromatography (elution with a decreasing polarity gradient of H₂O/MeOH from 1:0 to 0:1, then MeOH/CH₂Cl₂ from 1:0 to 0:1). The H₂O/MeOH 1:3 fraction was further fractionated by RP-C₁₈ column chromatography (H₂O/MeOH from 95:5 to 0:100). The H₂O/MeOH 1:3 (195 mg) fraction was then subjected to RP-C₁₈ semi-preparative HPLC (Phenomenex, Luna C₁₈, 250 × 10 mm, 5 μm) with a gradient of H₂O/MeOH/TFA (flow 3.0 mL min⁻¹ from 28:72:0.1 to 20:80:0.1) and the subsequent mixtures were finally purified by analytical HPLC (Phenomenex, Gemini C₆-phenyl, 250 × 3 mm, 5 μm) with an isocratic mobile phase (H₂O/CH₃CN/formic acid, 60:40:0.1, flow 0.5 mL min⁻¹) to afford pure compounds **1** (3.5 mg, 10⁻³% w/w), **2** (4.8 mg, 1.4×10⁻³% w/w), **3** (5.7 mg, 1.6×10⁻³% w/w), **4** (1.7 mg, 0.5×10⁻³% w/w), **5** (1.2 mg, 0.3×10⁻³% w/w), **6** (1.8 mg, 0.5×10⁻³% w/w), and **7** (2.0 mg, 0.6×10⁻³% w/w).

Pandaroside A (**1**): 3β-[β-glucopyranosyl-(1→2)-β-glucopyranosyloxyuronic acid]-16-hydroxy-5α,14β-poriferast-16-ene-15,23-dione

White amorphous solid; $[\alpha]_D^{20} +19.1$ (*c* 0.19, MeOH); UV (MeOH): $\lambda_{\max}$ (log ϵ) 264 (4.38) nm; CD (MeOH, *c* 3.8×10⁻⁴ M) $\theta(\lambda_{\max}$ nm) -5 (220), +13 (262), -4 (289), +4 (327) mdeg; IR (thin film): $\nu_{\max}$ 3496, 1692, 1640, 1199, 1132 cm⁻¹; ¹H NMR see Table 1; ¹³C NMR see Table 2; HRESI-MS (-): *m/z* 795.4214 [M-H]⁻ (calcd for C₄₁H₆₃O₁₅, 795.4167).

Methyl ester of pandaroside A (**2**)

White amorphous solid; $[\alpha]_D^{20} +7.8$ (*c* 0.27, MeOH); ¹H NMR (500 MHz, CD₃OD) for the uronic residue: δ 4.60 (d, *J* = 7.5 Hz, H-1'), 3.43 (t, *J* = 7.5 Hz, H-2'), 3.56 (m, H-3' and H-4'), 3.85 (d, *J* = 9.0 Hz, H-5'), 3.76 (s, CH₃O-); ¹³C NMR (125 MHz, CD₃OD) for the uronic residue: 102.0 (C-1'), 82.8 (C-2'), 73.1 (C-3'), 77.1 (C-4'), 76.6 (C-5'), 171.3 (C-6'), 53.0 (CH₃O-); ESI-MS *m/z* 811.4 [M+H]⁺.

Pandaroside B (3): 3 β -[β -glucopyranosyloxyuronic acid]-16-hydroxy-5 α ,14 β -poriferast-16-ene-15,23-dione

White amorphous solid; $[\alpha]_D^{24} +12.3$ (c 0.16, MeOH); UV (MeOH): $\lambda_{\max}$ (log ϵ) 264 (3.50) nm; CD (MeOH, c 3.9 $\times 10^{-4}$ M) θ nm ($\lambda_{\max}$) -3 (220), +10 (262), -4 (285), +4 (327) mdeg; IR (thin film): $\nu_{\max}$ 3338, 1731, 1677, 1199 cm^{-1} ; ^1H NMR see Table 1; ^{13}C NMR see Table 2; HRESI-MS (-): m/z 633.3625 [M-H] $^-$ (calcd for $\text{C}_{35}\text{H}_{53}\text{O}_{10}$, 633.3639).

Pandaroside C (4): 3 β -[β -glucopyranosyl-(1 $\rightarrow$ 2)- β -glucopyranosyloxyuronic acid]-16-hydroxy-5 α ,14 β -cholest-16-ene-15,23-dione

White amorphous solid; $[\alpha]_D^{20} +35.2$ (c 0.10, MeOH); UV (MeOH): $\lambda_{\max}$ (log ϵ) 264 (3.42) nm; CD (MeOH, c 3.9 $\times 10^{-4}$ M) θ ($\lambda_{\max}$ nm) -8 (220), +7 (262), -3 (285), +4 (330) mdeg; IR (thin film): $\nu_{\max}$ 3385, 1679, 1201, 1176 cm^{-1} ; ^1H NMR see Table 1; ^{13}C NMR see Table 2; HRESI-MS (-): m/z 767.3834 [M-H] $^-$ (calcd for $\text{C}_{39}\text{H}_{59}\text{O}_{15}$, 767.3854).

Methyl ester of pandaroside C (5)

White amorphous solid; $[\alpha]_D^{20} +24.8$ (c 0.10, MeOH); ^1H NMR (500 MHz, CD_3OD) for the uronic residue: δ 4.60 (d, $J = 7.5$ Hz, H-1'), 3.44 (t, $J = 7.5$ Hz, H-2'), 3.56 (m, H-3' and H-4'), 3.85 (d, $J = 9.0$ Hz, H-5'), 3.76 (s, $\text{CH}_3\text{O-}$); ^{13}C NMR (125 MHz, CD_3OD) for the uronic acid residue: 102.0 (C-1'), 82.8 (C-2'), 73.1 (C-3'), 77.1 (C-4'), 76.6 (C-5'), 171.3 (C-6'), 53.0 ($\text{CH}_3\text{O-}$); ESI-MS m/z 783.5 [M+H] $^+$.

Pandaroside D (6): 3 β -(β -glucopyranosyloxyuronic acid)-16-hydroxy-5 α ,14 β -cholest-16-ene-15,23-dione

White amorphous solid; $[\alpha]_D^{20} +45.1$ (c 0.10, MeOH); UV (MeOH): $\lambda_{\max}$ (log ϵ) 264 (3.85) nm; CD (MeOH, c 5.0 $\times 10^{-4}$ M) θ ($\lambda_{\max}$ nm) -12 (218), +10 (262), -6 (287), +5 (330) mdeg; IR (thin film): $\nu_{\max}$ 3412, 1697, 1677, 1196 cm^{-1} ; ^1H NMR see Table 1; ^{13}C NMR see Table 2; HRESI-MS (-): m/z 605.3313 [M-H] $^-$ (calcd for $\text{C}_{33}\text{H}_{49}\text{O}_{10}$, 605.3326).

Methyl ester of pandaroside D (7)

White amorphous solid; $[\alpha]_D^{20} +35.0$ (c 0.10, MeOH); ^1H NMR (500 MHz, CD_3OD) for the uronic residue: δ 4.44 (d, $J = 8.0$ Hz, H-1'), 3.16 (dd, $J = 9.0, 8.0$ Hz, H-2'), 3.36 (t, $J = 9.0$ Hz, H-3'), 3.50 (t, $J = 9.0$ Hz, H-4'), 3.83 (d, $J = 9.0$ Hz, H-5'), 3.76 (s, $\text{CH}_3\text{O-}$); ^{13}C NMR (125 MHz, CD_3OD) for the uronic residue: 103.0 (C-1'), 74.9 (C-2'), 77.4 (C-3'), 73.3 (C-4'), 76.8 (C-5'), 171.5 (C-6'), 52.9 ($\text{CH}_3\text{O-}$); ESI-MS m/z 621.4 [M+H] $^+$.

Insert Tables 1 and 2

3. Results and discussion

3.1. Structural elucidation

The CH₂Cl₂/MeOH (1:1) extract of the Caribbean marine sponge *Pandaros* sp. was fractionated by RP-C₁₈ flash chromatography and one fraction was further subjected to RP-C₁₈ column chromatography and purified by RP-C₁₈ semi-preparative HPLC to yield seven new compounds **1–7**. The NMR spectroscopic features of metabolites **1–7** (Tables 1 and 2) were similar and suggested sugar residues linked to a steroidal moiety.

Insert Figure 1.

Pandaroside A (1): Compound **1** (Figure 1) was isolated as a white amorphous solid with a molecular formula of C₄₁H₆₄O₁₅ which was established by the HRESIMS spectrum (m/z 795.4214 [M-H]⁻, $\Delta = 5.9$ ppm). The bands at 3496, 1692, and 1640 cm⁻¹ in the IR spectrum suggested the presence of hydroxyl and carbonyl moieties. The UV spectrum of **1** showing a maximum at 264 nm (log ϵ 4.38) suggested the presence of a conjugated carbonyl function. The ¹³C NMR spectrum of **1** displayed 41 carbon signals, 29 corresponding to the aglycon part and 12 to the diglycoside. The ¹H NMR spectrum exhibited characteristic steroid signals: two tertiary methyl groups at δ_H 0.81 (s, H-19) and 1.17 (s, H-18), three secondary methyl groups at δ_H 0.89 (d, $J = 6.5$ Hz, H-26), 0.90 (d, $J = 6.5$ Hz, H-27), and 1.15 (d, $J = 7.0$ Hz, H-21), and one primary methyl group at δ_H 0.76 (t, $J = 7.5$ Hz, H-24²). All these data were consistent with a poriferastane/stigmastane skeleton. The ¹³C NMR spectrum confirmed the presence of two anomeric carbons at δ_C 101.9 (C-1') and 105.3 (C-1'') and also revealed the presence of a tetra-substituted double bond at δ_C 151.5 (C-16) and 154.9 (C-17), a carboxylic acid at δ_C 172.4 (C-6'), and two carbonyls at δ_C 206.1 (C-15) and 215.8 (C-23). The usual A,B and C rings of the steroidal aglycon part was established by COSY and HMBC correlations, and by comparison with literature data. The key H-14/H-20/H-22/H-18/C-17, and /H-20/H-14/H-8/C-16 HMBC correlations allowed us to build the unusual cyclopentenone D ring of the aglycon part (Figure 2). A hydroxyl at C-16 was the only possibility to fit the molecular formula. The 2-hydroxycyclopentenone D ring was further confirmed by comparison with the ¹³C NMR data of stemmosides E and F [δ_C 43.3 (C-13), 54.5 (C-14), 206.7 (C-15), 152.2 (C-16), 152.9 (C-17), 25.5 (C-18) ppm], the other unique examples of natural compounds exhibiting such a D ring [8]. The structure of the side chain was deduced from the key C-23/H-20/H-22/H-24/H-24¹ HMBC correlations. The relative

stereochemistry of the aglycon part was established on the basis of detailed NOESY spectrum analysis. Observation of H-8/H-18/H-19 NOEs placed these protons on the same side. Whereas the characteristic chemical shift of C-19 at δ_C 11.2 was in agreement with the *trans-trans* relative configuration for the A/B and B/C ring junctions, the chemical shift of C-18 at δ_C 25.9 did not allow us to assign C-D ring junction unambiguously. A small H-14/H-18 NOE suggested a *cis* C/D junction. This unusual β configuration at C-14 was further confirmed by examination of previous studies which showed that a downfield signal for H-7 α at δ_H 2.17 (1H, qd, $J = 12.5, 4.5$ Hz) was consistent with a *cis* C-D junction [9]. This downfield resonance of H-7 α could be explained because it lies in the deshielding cone of the carbonyl group. The configuration at H-3 was assigned as α on the basis of its vicinal coupling constants of $J = 11.5$ and 4.5 Hz (δ_H 3.64 ppm, tt). For the configurational assignment of the steroidal aglycon part we decided to use quantum-mechanical calculation of the CD spectrum which was then compared with the experimental one [10,11]. The cyclopentenone and the ketone gave rise to three chromophores with alternative signs [327 ($\Delta\epsilon = +2.5$), 290 (-7.5), 262 ($+10.2$) nm] on the experimental CD spectrum. The similar ^{13}C NMR data of **1** with those of phytosterone, the unique example of 23-oxocholestane suggested a C-24 *R* configuration for **1** [12], and the usual absolute configurations for the tetracycle of steroids suggested an α orientation for H-14. A conformational analysis was performed on this stereoisomer with the DFT method at B3LYP/6-31+G* level which gave 43 conformers of relative energies below 2 kcal mol^{-1} . Because of the high number of conformers the calculation was run on the lowest energy DFT structure (Figure 3) [13]. A very good agreement was observed between the experimental CD spectrum of **1** in the gas phase and the DFT calculated structure of **1** with this stereochemistry which allowed us to propose this absolute configuration for the aglycon part of **1**.

Insert Figures 2 and 3

Compound **1** contained two sugar residues at C-3 evidenced by the two anomeric protons at δ_H 4.60 (1H, d, $J = 7.5$ Hz, H-1') and 4.56 (1H, d, $J = 8.0$ Hz, H-1''), one being an uronic derivative due to the presence of a carboxylic acid signal at δ_C 172.4. The COSY spectrum and the coupling constants indicated that the two sugar residues were a glucose and a glucuronic acid [8,14]. A C-3/H-1' HMBC correlation placed the glucuronic acid at the C-3 position of the aglycon, whereas a H-1''/C-2' HMBC correlation indicated that the glucose was linked to the glucuronic acid through the oxygen at C-2'. The large J values of the signals

H-1' and H-1'' (d, 7.5 and d, 8.0 Hz) showed that both sugars and the aglycon were connected through β -glycosidic linkages. The limiting quantity of **1** did not allow us to assign the absolute configuration of the sugar residues.

A minor compound **2** was isolated during the purification process of **1**. The m/z at 811.4 $[M+H]^+$ indicated the presence of an additional methylene unit in comparison with **1**. The 1H and ^{13}C NMR data were almost identical to those of **1** except for the new signals at δ_H 3.76 (s, H_3 -CO) and δ_C 53.0 (O- $\underline{C}H_3$) which suggested the presence of a methoxy group. Compound **2** was shown to be the methyl ester of the glucuronic acid of pandaroside A (**1**) by the observation of a strong C-6'/ H_3 CO HMBC correlation. Firstly **2** was thought to be an artefact of the purification process of **1**. Its presence was evidenced by LC-MS after extraction by $CH_2Cl_2/MeOH$ but we assumed that esterification could not occur during this step. We then concluded that the methyl ester **2** was produced by the organism or its associated microorganisms and is not an artefact of the purification process.

Pandaroside B (3): Compound **3** was isolated as a white amorphous solid with a molecular formula of $C_{35}H_{54}O_{10}$ which was deduced from the HRESI-MS spectrum (m/z 633.3625 $[M-H]^-$, $\Delta=-1.2$ ppm). A detailed comparison of NMR data of the aglycon part of compound **3** with those of compounds **1** and **2** revealed that all compounds possessed the same skeleton. The presence of only one anomeric proton at δ_H 4.43 (d, $J = 8.0$ Hz, H-1') indicated that only one sugar residue was connected at C-3. COSY correlations and the signal at δ_C 171.9 allowed us to prove that the sugar residue was the same glucuronic acid as in **1**.

Pandaroside C (4): Compound **4** was isolated as a white amorphous solid with a molecular formula of $C_{39}H_{60}O_{15}$ which was deduced from the HRESI-MS spectrum (m/z 767.3834 $[M-H]^-$, $\Delta=-1.9$ ppm). The 1H and ^{13}C NMR data were also quite similar to those of pandaroside A (**1**), which indicated the occurrence of a similar steroidal saponin skeleton. Compared to **1**, **4** had a 28 amu difference which suggested the loss of two methylene units. Differences were easily located on the aglycon side chain where the lack of the characteristic signal at δ_H 0.76 (t, $J = 7.5$, H_3 -24²) suggested that the ethyl was absent from C-24. The AB signals at δ_H 2.96 (dd, $J = 17.5$, 4.5 Hz, H-22a) and 2.79 (dd, $J = 17.5$, 9.0 Hz, H-22b) were also replaced by a signal at δ_H 2.85 (m, H-22a and b).

A minor compound **5** was isolated during the purification process of **4**, which was assigned as the methyl ester of **4** in a manner similar to that used to assign the structure of compound **2**.

Pandaroside D (6): Compound **6** was isolated as a white amorphous solid with a molecular formula of $C_{33}H_{50}O_{10}$ which was deduced from the HRESI-MS spectrum (m/z 605.3313 $[M-H]^-$, $\Delta=-1.19$ ppm). Comparison of NMR data of the **4** and **6** aglycon parts clearly indicated

that **6** also possessed a cholestane skeleton. The sugar moiety of **6** was identical to that of **3** with only one glucuronic acid at C-3.

Here also, a minor compound **7** was isolated during the purification process of **6**, which was assigned as the methyl ester of **6** in a manner similar to that used to assign the structure of compound **2**.

All absolute and relative configurations of compounds **2-7** were assumed to be the same as for **1** because of very similar NOESY and CD spectra.

For all the isolated compounds, no antitumor activity against three human tumor cell lines (A549 lung cancer cells, HT29 colonic cancer cells, and MDA-MB-231 breast cancer cells) was detected below 10 $\mu\text{g mL}^{-1}$.

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Supplementary information: picture of the sponge specimen, ^1H , ^{13}C , COSY, HSQC, HMBC spectra of **1**, ^1H , ^{13}C , HMBC spectra of **2**, ^1H , ^{13}C spectra of **3** and **4**, ^1H spectrum of **5**, ^1H , ^{13}C spectra of **6** and **7**.

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$$\Delta\epsilon(E) = \frac{1}{2.297 \times 10^{-39}} \frac{1}{\sqrt{2\pi\sigma}} \sum_i \Delta E_i R_i e^{-\left(\frac{E - \Delta E_i}{2\sigma}\right)^2}$$

where σ is the width of the band at $\frac{1}{e}$ height and ΔE_i and R_i are the excitation energies and the rotatory strengths for transition i , respectively, $\sigma = 0.20$ eV and R_{vel} were used.

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Table 1 - ^1H NMR data (500 MHz, CD_3OD) for compounds **1**, **3**, **4**, **6**: δ in ppm (*multiplicity*, *J* in Hz)

No.	1	3	4	6
1 β	1.68 (<i>m</i>)	1.64 (<i>m</i>)	1.68 (<i>m</i>)	1.64 (<i>m</i>)
1 α	0.86 (<i>m</i>)	0.86 (<i>m</i>)	0.86 (<i>m</i>)	0.86 (<i>m</i>)
2 α	1.86 (<i>m</i>)	1.83 (<i>m</i>)	1.86 (<i>m</i>)	1.84 (<i>m</i>)
2 β	1.49 (<i>m</i>)	1.50 (<i>m</i>)	1.49 (<i>m</i>)	1.50 (<i>m</i>)
3	3.64 (<i>tt</i> , 11.5 4.5)	3.63 (<i>m</i>)	3.64 (<i>m</i>)	3.63 (<i>m</i>)
4 α	1.72 (<i>m</i>)	1.72 (<i>m</i>)	1.72 (<i>m</i>)	1.72 (<i>m</i>)
4 β	1.28 (<i>m</i>)	1.28 (<i>m</i>)	1.28 (<i>m</i>)	1.28 (<i>m</i>)
5	1.04 (<i>m</i>)	1.04 (<i>m</i>)	1.04 (<i>m</i>)	1.04 (<i>m</i>)
6 α	1.35 (<i>m</i>)	1.35 (<i>m</i>)	1.35 (<i>m</i>)	1.35 (<i>m</i>)
6 β	1.30 (<i>m</i>)	1.30 (<i>m</i>)	1.30 (<i>m</i>)	1.30 (<i>m</i>)
7 α	2.17 (<i>qd</i> , 12.5 4.5)	2.17 (<i>m</i>)	2.17 (<i>m</i>)	2.17 (<i>m</i>)
7 β	1.66 (<i>m</i>)	1.65 (<i>m</i>)	1.66 (<i>m</i>)	1.65 (<i>m</i>)
8	1.94 (<i>m</i>)	1.93 (<i>m</i>)	1.94 (<i>m</i>)	1.93 (<i>m</i>)
9	0.88 (<i>m</i>)	0.87 (<i>m</i>)	0.88 (<i>m</i>)	0.87 (<i>m</i>)
11 β	1.39 (<i>m</i>)	1.37 (<i>m</i>)	1.39 (<i>m</i>)	1.37 (<i>m</i>)
11 α	1.17 (<i>m</i>)	1.15 (<i>m</i>)	1.17 (<i>m</i>)	1.15 (<i>m</i>)
12 β	1.55 (<i>m</i>)	1.55 (<i>m</i>)	1.55 (<i>m</i>)	1.55 (<i>m</i>)
12 α	1.47 (<i>m</i>)	1.47 (<i>m</i>)	1.47 (<i>m</i>)	1.47 (<i>m</i>)
14	1.83 (<i>d</i> , 4.5)	1.83 (<i>d</i> , 4.5)	1.83 (<i>d</i> , 4.5)	1.83 (<i>d</i> , 4.5)
18	1.17 (<i>s</i>)	1.15 (<i>s</i>)	1.17 (<i>s</i>)	1.15 (<i>s</i>)
19	0.81 (<i>s</i>)	0.81 (<i>s</i>)	0.81 (<i>s</i>)	0.81 (<i>s</i>)
20	2.88 (<i>m</i>)	2.83 (<i>m</i>)	2.83 (<i>m</i>)	2.83 (<i>m</i>)
21	1.15 (<i>d</i> , 4.5)	1.14 (<i>d</i> , 7 Hz)	1.15 (<i>d</i> , 4.5)	1.14 (<i>d</i> , 4.5)
22a	2.96 (<i>dd</i> , 17.5 4.5)	2.87 (<i>dd</i> , 17.5, 4.5)	2.85 (<i>m</i>)	2.85 (<i>m</i>)
22b	2.79 (<i>dd</i> , 17.5 9.0)	2.79 (<i>dd</i> , 17.5, 8.5)		
24	2.23 (<i>ddd</i> , 10.0 7.5 4.0)	2.23 (<i>m</i>)	2.31 (<i>d</i> , 7)	2.31 (<i>d</i> , 7)
24 ¹ a	1.57 (<i>m</i>)	1.57 (<i>m</i>)	2.10 (<i>m</i>)	2.08 (<i>m</i>)
24 ¹ b	1.51 (<i>m</i>)	1.51 (<i>m</i>)		
24 ²	0.76 (<i>t</i> , 7.5)	0.76 (<i>t</i> , 7.5)		
25	1.88 (<i>m</i>)	1.88 (<i>m</i>)		
26/27	0.89 (<i>d</i> , 6.5)	0.89 (<i>d</i> , 6.5)	0.89 (<i>d</i> , 6.5)	0.89 (<i>d</i> , 6.5)
	0.90 (<i>d</i> , 6.5)	0.90 (<i>d</i> , 6.5)	0.90 (<i>d</i> , 6.5)	0.90 (<i>d</i> , 6.5)
1'	4.60 (<i>d</i> , 7.5)	4.43 (<i>d</i> , 8.0)	4.60 (<i>d</i> , 7.5)	4.43 (<i>d</i> , 8.0)
2'	3.45 (<i>dd</i> , 9.0 8.0)	3.18 (<i>dd</i> , 9.0 8.0)	3.45 (<i>dd</i> , 9.0 8.0)	3.18 (<i>dd</i> , 9.0 8.0)
3'	3.57 (<i>t</i> , 9.5)	3.36 (<i>t</i> , 9.0)	3.57 (<i>t</i> , 9.5)	3.36 (<i>t</i> , 9.0)
4'	3.56 (<i>d</i> , 10.0)	3.51 (<i>t</i> , 9.0)	3.56 (<i>d</i> , 10.0)	3.51 (<i>t</i> , 9.0)
5'	3.79 (<i>t</i> , 9.5)	3.78 (<i>d</i> , 9.5)	3.79 (<i>t</i> , 9.5)	3.78 (<i>d</i> , 9.5)
1''	4.56 (<i>d</i> , 8.0)	3.23 (<i>dd</i> , 9.0 8.0)	4.56 (<i>d</i> , 8.0)	3.37 (<i>t</i> , 9.0)
2''	3.23 (<i>dd</i> , 9.0 8.0)		3.23 (<i>dd</i> , 9.0 8.0)	
3''	3.37 (<i>t</i> , 9.0)		3.37 (<i>t</i> , 9.0)	
4''	3.33 (<i>m</i>)		3.33 (<i>m</i>)	
5''	3.27 (<i>ddd</i> , 12.0 5.0 2.5)	3.70 (<i>dd</i> , 11.5 5.0)	3.27 (<i>ddd</i> , 12.0 5.0 2.5)	3.84 (<i>dd</i> , 11.5 2.5)
6''a	3.84 (<i>dd</i> , 11.5 2.5)		3.84 (<i>dd</i> , 11.5 2.5)	
6''b	3.70 (<i>dd</i> , 11.5 5.0)		3.70 (<i>dd</i> , 11.5 5.0)	

Table 2 - ^{13}C NMR data (125 MHz, CD_3OD) for compounds **1**, **3**, **4**, **6**: δ in ppm.

No.	1	3	4	6
1	37.5	37.5	37.5	37.5
2	30.1	30.1	30.1	30.1
3	80.9	79.8	80.9	80.0
4	35.5	35.4	35.5	35.4
5	45.7	45.7	45.7	45.7
6	30.3	30.3	30.3	30.3
7	31.4	31.4	31.4	31.4
8	35.2	35.2	35.2	35.2
9	45.9	45.9	45.9	45.9
10	38.3	38.3	38.3	38.3
11	20.0	20.0	20.0	20.0
12	32.7	32.7	32.7	32.7
13	43.8	43.8	43.7	43.7
14	56.2	56.2	56.2	56.2
15	206.1	206.1	206.1	206.1
16	151.4	151.5	151.4	151.4
17	154.9	154.9	154.9	154.6
18	25.9	25.9	25.9	25.9
19	11.2	11.2	11.2	11.2
20	27.4	27.3	28.1	28.1
21	18.0	18.0	18.0	18.0
22	49.0	49.0	48.1	48.1
23	215.8	215.8	212.4	212.4
24	61.9	61.9	53.4	53.4
24 ¹	22.3	22.3		
24 ²	12.4	12.4		
25	30.8	30.9	25.8	25.8
26/27	20.0	20.0	22.9	22.9
	21.5	21.5	22.9	22.9
1'	101.9	102.9	101.9	102.9
2'	82.8	74.8	82.8	74.8
3'	72.9	77.5	72.9	77.5
4'	77.2	73.2	77.2	73.2
5'	76.4	76.6	76.4	76.6
6'	172.4	171.9	172.4	171.9
1''	105.3		105.3	
2''	76.2		76.2	
3''	77.7		77.7	
4''	71.4		71.4	
5''	78.3		78.3	
6''	62.7		62.7	

Figure 1 – Structure of pandarosides A-D and their methyl esters (**1-7**).

Figure 2 – Key HMBC correlations for a portion of **1**.

Figure 3 – Measured (gas phase) and calculated CD spectra for the aglycon part of pandaroside A (**1**)

Figure 1.

Figure 2.

Figure 3.