Spectral Assignments and Reference Data

Magnetic Resonance in Chemistry

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# Tetrapterosides A and B, two new oleanane-type saponins from *Tetrapleura tetraptera*

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**From the stem bark of *Tetrapleura tetraptera*, two new oleanane-type saponins, tetrapteroside A 3-*O*-{6-*O*-[(2*E*,6*S*)-2,6-dimethyl-6-hydroxyocta-2,7-dienoyl]-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 4)-[β-D-glucopyranosyl-(1 → 2)]-β-D-glucopyranosyl}-3,27-dihydroxyoleanolic acid (1), and tetrapteroside B 3-*O*-{β-D-glucopyranosyl-(1 → 2)-6-*O*-[(*E*)-feruloyl]-β-D-glucopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 4)-[β-D-glucopyranosyl-(1 → 2)]-β-D-glucopyranosyl}-3,27-dihydroxyoleanolic acid (2), were isolated. Further extractions from the roots led to the isolation of four known oleanane-type saponins. Their structures were elucidated by the combination of mass spectrometry (MS), one and two-dimensional NMR experiments.** 

**Keywords:** NMR; $^{1}$H; $^{13}$C; 2D NMR; triterpene saponins; tetrapteroside A; tetrapteroside B; Mimosaceae; *Tetrapleura tetraptera*

## Introduction

In a continuation of the study on bioactive triterpene saponins from Cameroonian medicinal plants,[1–5] we have examined the saponin fraction of the stem bark and roots of *Tetrapleura tetraptera* Taub. (Mimosaceae), locally named Aridan. This robust tree from the forest of tropical Africa is well-known as remedies in the traditional medicine,[6] and for its strong molluscicidal activity. The previous chemical analysis of the fruit and stem bark led to the isolation of oleanane-type triterpene saponins like aridanin, a *N*-acetylglycoside triterpenoid.[7,8] In this paper, we report the isolation by successive chromatographic steps of two new triterpene saponins, tetrapterosides A (**1**) and B (**2**) (Fig. 1) from the stem bark and four known saponins from the roots,[7,8] 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)oleanolic acid (aridanin), 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)echinocystic acid, 3-*O*-[β-D-glucopyranosyl-(1 → 6)-2-acetamido-2-deoxy-β-D-glucopyranosyl]oleanolic acid, and 3-*O*-sulfoechinocystic acid. Their structures were established mainly by two-dimensional (2D) NMR (COSY, TOCSY, NOESY, HSQC, HMBC) and mass spectrometry.

## Results and Discussion

Compound **1**, a white amorphous powder, exhibited in high-resolution electrospray ionization mass spectrometry (HR-ESIMS) (positive-ion mode) a pseudo-molecular ion peak at $m/z$ = 1471,7090 $[M + Na]^+$ (calcd 1471,7085), consistent with a molecular formula of $C_{70}H_{112}O_{31}Na$. Its fast-atom bombardment mass spectrum (FABMS) (negative-ion mode) showed a quasi-molecular ion peak at $m/z$ = 1447 $[M - H]^-$, indicating a molecular weight of 1448. Other significant fragment ion peaks were observed at $m/z$ = 1281 $[(M - H) - 166]^-$, 1119 $[(M - H) - 166 - 162]^-$, 957 $[(M - H) - 166 - 162 - 162]^-$, and 795 $[(M - H) - 166 - 162 - 162 - 162]^-$, corresponding to the successive loss of one monoterpenoyl moiety, and three hexosyl moieties, respectively.

The $^{1}$H-NMR spectrum of **1** showed characteristic signals of a saponin structure composed by an aglycon part and an oligosaccharidic part. For the aglycon moiety, the $^{1}$H-NMR spectrum displayed signals for six angular methyl groups as singlets, one olefinic proton at $\delta_H$ 5.77 (br t, $J$ = 3 Hz, H-12), one oxygen bearing methine protons at $\delta_H$ 3.08 (dd, $J$ = 11.4, 3.8 Hz, H-3), and one primary alcoholic function at C-27 position, $\delta_H$ 3.72 (d, $J$ = 10.5 Hz), 3.98. In the $^{13}$C-NMR spectrum, the deshielded signal at $\delta_C$ 89.5 (C-3) in comparison with the free aglycon, suggested a glycosidic linkage at C-3. The structure of the aglycon of **1** was thus recognized to be the triterpene 3,27-dihydroxyoleanolic acid by $^{1}$H-NMR and $^{13}$C-NMR analyses

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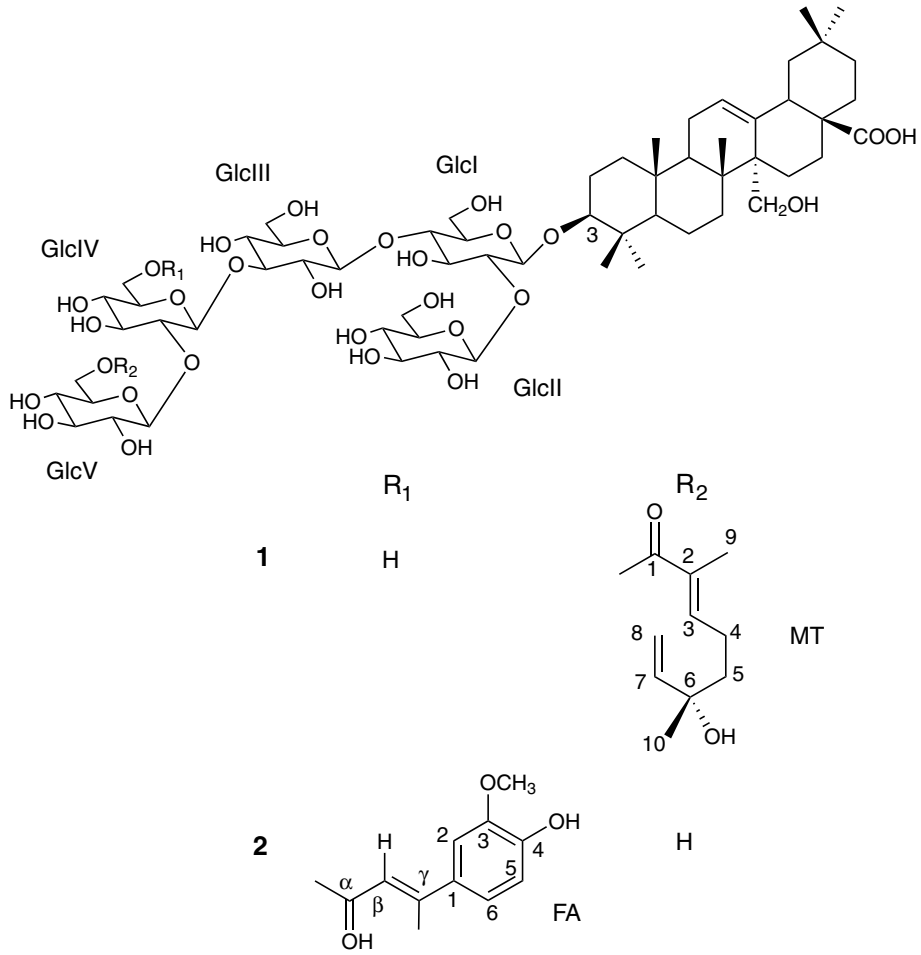


**Figure 1.** Structures of **1** and **2**.

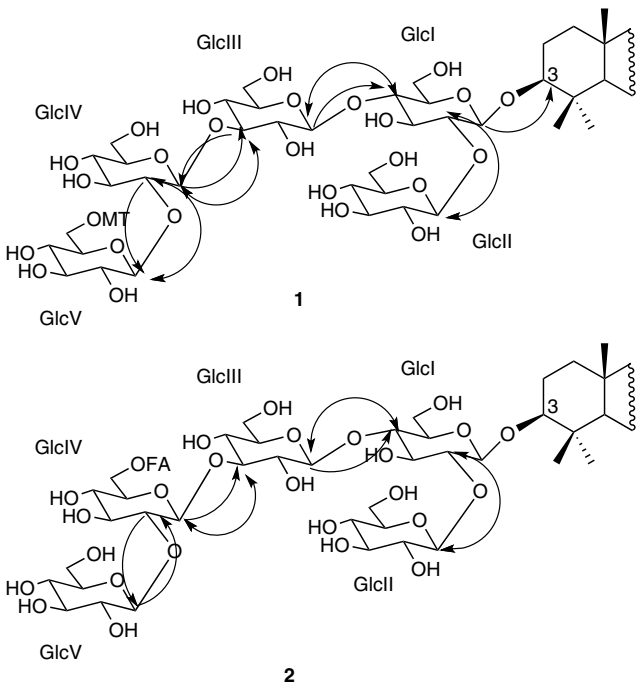


**Figure 2.** Important HMBC ( ) and NOESY ( ) correlations for **1** and **2**.

Magnetic Resonance in Chemistry

**Table 1.** $^{1}$H-NMR (600 MHz) and $^{13}$C-NMR(150 MHz) data of the aglycons of **1** and **2** in pyridine-$d_5$ ($\delta$ in ppm, $J$ in Hz)

| | Compound **1** | | Compound **2** | |
|---|---|---|---|---|
| No | $^{1}$H-NMR | $^{13}$C-NMR | $^{1}$H-NMR | $^{13}$C-NMR |
| 1 | 0.83, 1.28 | 38.3 | 0.87, 1.31 | 38.2 |
| 2 | 1.75, 1.95 | 26.0 | 1.76, 1.96 | 26.0 |
| 3 | 3.08 dd, $J$ = 11.4, 3.8 | 89.5 | 3.12 dd, $J$ = 11.6, 3.8 | 89.6 |
| 4 | – | 39.3 | – | 39.5 |
| 5 | 0.81 | 55.3 | 0.83 | 55.3 |
| 6 | 1.24, 1.51 | 18.4 | 1.24, 1.50 | 18.2 |
| 7 | 1.04, 1.25 | 33.8 | 1.08, nd | 33.8 |
| 8 | – | 40.0 | – | 40.0 |
| 9 | 2.05 | 48.2 | 2.07 | 48.2 |
| 10 | – | 36.7 | – | 36.7 |
| 11 | 1.88, 2.04 | 23.6 | 1.94, 2.03 | 23.4 |
| 12 | 5.77 br t, $J$ = 3.0 | 127.6 | 5.84 br t, $J$ = 3.0 | 127.1 |
| 13 | – | 139.7 | – | 139.8 |
| 14 | – | 47.5 | – | 47.5 |
| 15 | nd | 24.1 | nd | 23.9 |
| 16 | nd | 23.5 | nd | 23.6 |
| 17 | – | 46.3 | – | 46.4 |
| 18 | 3.27 dd, $J$ = 12.5, 2.5 | 41.4 | 3.35 dd, $J$ = 12.1, 2.4 | 41.5 |
| 19 | 1.25, 1.68 t, $J$ = 12.5 | 45.3 | 1.28, 1.70 t, $J$ = 12.1 | 45.4 |
| 20 | – | 30.6 | – | 30.6 |
| 21 | 1.75, 1.89 | 33.2 | 1.76, 1.95 | 33.1 |
| 22 | 1.65, nd | 32.8 | 1.69, nd | 32.9 |
| 23 | 1.21 s | 27.6 | 1.22 s | 27.7 |
| 24 | 1.01 s | 16.4 | 1.04 s | 16.4 |
| 25 | 0.71 s | 15.4 | 0.74 s | 15.5 |
| 26 | 0.89 s | 18.5 | 0.90 s | 18.5 |
| 27 | 3.72 d, $J$ = 10.5, 3.98 | 64.1 | 3.73 d, $J$ = 10.0, 4.03 | 63.9 |
| 28 | – | 179.5 | – | 179.5 |
| 29 | 0.81 s | 32.9 | 0.83 s | 32.9 |
| 30 | 0.93 s | 23.6 | 0.96 s | 23.6 |

Overlapped proton NMR signals are reported without designated multiplicity.
Nd, not determined.

(Table 1) using the correlations observed in COSY, NOESY, HSQC, and HMBC spectra, and was in full agreement with literature data.[9] For the oligosaccharidic chain, the $^{1}$H-NMR spectrum showed five anomeric protons at $\delta_H$ 4.72 (d, $J$ = 7.1 Hz), 4.91 (d, $J$ = 7.6 Hz), 4.95 (d, $J$ = 7.6 Hz), 5.17 (d, $J$ = 7.8 Hz), and 5.44 (d, $J$ = 7.6 Hz), which gave correlations, in the HSQC spectrum, with anomeric carbon signals at $\delta_C$ 104.3, 103.2, 104.3, 105.7, and 102.2, respectively. The ring protons of the monosaccharide residues were assigned starting from the readily identifiable anomeric protons by means of COSY, TOCSY, NOESY, HSQC, and HMBC experiments (Table 2). Units of five ß-D-glucopyranosyl (Glc) were identified. The relatively large $^{3}J_{H-1,H-2}$ values of the anomeric protons of Glc (7.1–7.8 Hz) indicated a ß-anomeric orientation. The D configuration of Glc was determined by gas chromatography (GC) analysis.[10] Correlations observed in the HMBC spectrum between signals at $\delta_H$ 4.72 (GlcI-1) and $\delta_C$ 89.5 (C-3) confirmed the substitution at the C-3 position of the aglycone by a ß-D-glucopyranosyl moiety (GlcI). Signals at $\delta_C$ 78.8 (GlcI-2) and $\delta_C$ 80.2 (GlcI-4) in comparison with a terminal ß-D-glucopyranosyl moiety suggested a 2,4 substitution of GlcI by GlcII and GlcIII, respectively. This is confirmed by cross peaks in the NOESY spectrum between $\delta_H$ 4.27 (GlcI-2) and an anomeric signal at $\delta_H$ 5.44 (GlcII-1), and between $\delta_H$ 4.07 (GlcI-4) and another anomeric signal at $\delta_H$ 4.95 (GlcIII-1) (Fig. 2). In the HMBC spectrum, the correlation between $\delta_H$ 3.74 (GlcIII-3) and $\delta_C$ 103.2 (GlcIV-1), and the reverse correlation between $\delta_H$ (GlcIV-1) and $\delta_C$ 90.1 (GlcIII-3), and the correlation between $\delta_H$ 3.96 (GlcIV-2) and $\delta_C$ 105.7 (GlcV-1), revealed the (1 → 3) linkage between GlcIV and GlcIII and the (1 → 2) linkage between GlcV and Glc IV (Fig. 2). Moreover, the deshielded signal of GlcV-6 at $\delta_C$ 63.9 and $\delta_H$ 4.71, 5.13 showed an acylation at this position. After subtraction of the signals of the oligosaccharidic chain linked at the C-3 position, signals of a monoterpenoyl residue still remained, which acylated the GlcV-6 position (Table 3). Its NMR data are in accordance with those described in literature[11] for a (2*E*,6*S*)-2,6-dimethyl-6-hydroxyocta-2,7-dienoyl unit, already found in acylated saponins isolated from plants of the Mimosaceae family.[11,12]

The structure of **1** was thus established as 3-*O*-{6-*O*-[(2*E*,6*S*)-2,6-dimethyl-6-hydroxyocta-2,7-dienoyl]-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 4)-[β-D-glucopyranosyl-(1 → 2)]-β-D-glucopyranosyl}-3,27-dihydroxyoleanolic acid, a new oleanane-type glycoside named tetrapteroside A.

Compound **2**, a white amorphous powder, exhibited in HR-ESIMS (positive-ion mode) a pseudo-molecular ion peak at $m/z$ = 1481,6572 $[M + Na]^+$ (calcd 1481,6565), consistent with a molecular formula of $C_{70}H_{106}O_{32}Na$. Its FABMS (negative-ion mode) showed a quasi-molecular ion peak at $m/z$ = 1457 $[M - H]^-$, indicating a molecular weight of 1458. Other significant fragment ion peaks were observed at $m/z$ = 1281 $[(M - H) - 176]^-$, 1119 $[(M - H) - 176 - 162]^-$, and 957 $[(M - H) - 176 - 162 - 162]^-$, corresponding to the successive loss of one feruloyl moiety, and two hexosyl moieties, respectively.

The $^{1}$H- and $^{13}$C-NMR signals of **2** assigned from the 2D NMR spectra were almost superimposable on those of **1** except for the acyl moiety and the position of acylation (Tables 1, 2 and 3). Actually, inside the same oligosaccharidic chain, a substitution is observed at GlcIV-6 position with signals at $\delta_C$ 63.9 and $\delta_H$ 4.91, 5.18, instead of GlcV-6 position in **1**. Moreover, characteristic NMR signals of a (*E*) feruloyl unit were found corresponding to previous data from the literature (Table 3).[13]

On the basis of the above results, the structure of compound **2** was elucidated as 3-*O*-{β-D-glucopyranosyl-(1 → 2)-6-*O*-[(*E*)-feruloyl]-β-D-glucopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 4)-[β-D-glucopyranosyl-(1 → 2)]-β-D-glucopyranosyl}-3,27-dihydroxyoleanolic acid (**2**), a new saponin named tetrapteroside B.

## Experimental

### General

Optical rotations values were recorded on a AA-OR automatic polarimeter. HR-ESIMS (positive-ion mode) was carried out on a Q-TOF 1-micromass spectrometer. FABMS were conducted in the negative-ion mode on a Jeol SX-102 instrument. Medium-pressure liquid chromatography (MPLC) was performed on a Gilson pump M 305, with Büchi glass column (460 mm × 25 mm and 460 mm × 15 mm), a Büchi precolumn (110 mm × 15 mm), using silicagel 60 (Merck, 15 −40 µm). Vacuum liquid chromatography (VLC) was carried out using reversed-phase RP-18 (25 −40 µm) and silica gel 60 (63 −200 µm) (Merck). TLC and HPTLC employed



  

**Table 2.** $^{1}$H-NMR (600 MHz) and $^{13}$C-NMR (150 MHz) data of the sugar moieties of **1** and **2** in pyridine-$d_5$ ($\delta$ in ppm, $J$ in Hz)

| | Compound **1** | | Compound **2** | |
|---|---|---|---|---|
| No | $^{1}$H-NMR | $^{13}$C-NMR | $^{1}$H-NMR | $^{13}$C-NMR |
| GlcI-1 | 4.72 d, $J = 7.1$ | 104.3 | 4.71 d, $J = 7.6$ | 104.1 |
| 2 | 4.27 t, $J = 8.3$ | 78.8 | 4.31 | 79.0 |
| 3 | 4.23 t, $J = 9.3$ | 77.1 | 4.20 | 77.2 |
| 4 | 4.07 d, $J = 9.3$ | 80.2 | 4.11 | 79.9 |
| 5 | 3.80 | 75.6 | 3.68 | 75.5 |
| 6 | 4.04, 4.33 | 62.4 | 4.06, 4.38 | 62.3 |
| GlcII-1 | 5.44 d, $J = 7.6$ | 102.2 | 5.50 d, $J = 7.4$ | 102.3 |
| 2 | 3.93 | 74.5 | 4.04 | 74.0 |
| 3 | 4.15 t, $J = 9.0$ | 77.4 | 4.20 | 77.2 |
| 4 | 4.03 | 71.0 | 3.85 t, $J = 8.1$ | 69.8 |
| 5 | 3.74 | 77.2 | 3.81 | 77.0 |
| 6 | 4.32, 4.40 dd, $J = 11.3, 2.0$ | 61.5 | 4.33, 4.43 | 61.2 |
| GlcIII-1 | 4.95 d, $J = 7.6$ | 104.3 | 5.00 d, $J = 7.8$ | 104.1 |
| 2 | 3.94 | 74.5 | 4.00 | 74.3 |
| 3 | 3.74 | 90.1 | 3.84 t, $J = 7.4$ | 89.9 |
| 4 | 3.79 | 70.0 | 4.08 | 70.5 |
| 5 | 3.90 | 78.0 | 3.95 | 78.0 |
| 6 | 4.13, 4.33 | 61.9 | 4.20, 4.40 | 61.8 |
| GlcIV-1 | 4.91 d, $J = 7.6$ | 103.2 | 5.02 d, $J = 7.1$ | 103.0 |
| 2 | 3.96 | 84.5 | 4.03 | 84.6 |
| 3 | 4.22 d, $J = 9.3$ | 76.9 | 4.27 | 76.8 |
| 4 | 3.98 | 70.8 | 3.98 | 70.5 |
| 5 | 3.90 | 78.0 | 4.10 | 75.2 |
| 6 | 4.11, 4.44 br d, $J = 10.2$ | 62.0 | 4.91 dd, $J = 11.4, 5.9$<br>5.18 dd, $J = 11.2, 6.4$ | 63.9 |
| GlcV-1 | 5.17 d, $J = 7.8$ | 105.7 | 5.26 d, $J = 7.8$ | 105.8 |
| 2 | 4.03 | 75.9 | 4.10 | 75.2 |
| 3 | 4.17 | 77.3 | 4.25 | 77.0 |
| 4 | 3.79 | 70.2 | 4.04 | 70.5 |
| 5 | 3.99 | 75.9 | nd | nd |
| 6 | 4.71, 5.13 br d, $J = 11.9$ | 63.9 | 4.15, 4.43 | 61.8 |

Overlapped proton NMR signals are reported without designated multiplicity.
Nd, not determined.

precoated silica gel 60$F_{254}$ plates (Merck). The following TLC solvent system $CHCl_3$–MeOH–AcOH –$H_2O$ (60 : 32 : 0.5 : 6.5) was used. The spray reagent was Komarowsky reagent, a mixture (5 : 1) of *p*-hydroxybenzaldehyde (2% in MeOH) and ethanolic $H_2SO_4$ (50%).

### Plant material

The stem bark of *T. tetraptera* was collected at Eloundem, near Yaoundé, Cameroon, in November 2006, and identified by Dr P. Nana, botanist of the National Herbarium of Cameroon (NHC), Yaoundé, where a voucher specimen (No. 5567) was deposited.

### NMR spectroscopy

The NMR spectra were recorded on a Varian UNITY Inova 600 spectrometer equipped with 5-mm probes. Samples were dissolved in 160 ml of $C_5D_5N$ and 20 ml of $D_2O$ and transferred into 5-mm NMR tubes (Shigemi). The $^{1}$H and $^{13}$C-NMR spectra (at 600 and 150 MHz respectively) were measured at 303 K. Chemical shifts are given on the $\delta$ scale and referenced to the residual solvent signals ($\delta_H = 7.19$, $\delta_C = 123.5$). Coupling constants ($J$) are in Hz. For 2D experiments, Varian software using pulse field gradient were applied. The pulse conditions in $C_5D_5N$ were as follows: for the $^{1}$H-NMR spectrum, observation frequency (OF) = 599.88 MHz, acquisition time (AQ) = 4.202 s, relaxation delay (RD) = 5.0 s, 90 pulse width = 10.0 µs, spectral width (SW) = 7798.8 Hz, Fourier transform (FT) size = 65 536; for the $^{13}$C-NMR spectrum, OF = 150.854 MHz, AQ = 0.453 s, RD = 1.547 s, 90 pulse width = 15.8 µs, SW = 36 182.7 Hz, line broadening (LB) = 1.0 Hz, FT size = 65 536; for the COSY spectrum, AQ = 0.131, $F_2$ = 2048, $F_1$ = 256, RD = 0.369, SW = 7798.8 Hz; for the NOESY spectrum, AQ = 0.131, $F_2$ = 2048, $F_1$ = 256, RD = 0.369, SW = 7798.8 Hz, mixing time = 500 ms; for the TOCSY spectrum, AQ = 0.131, $F_2$ = 2048, $F_1$ = 256, RD = 0.369, SW = 7798.8 Hz, mixing time = 60 ms; for the HSQC spectrum, AQ = 0.131, RD =0.369, $F_1$ = 36 182.7 Hz, $F_1$ = 7798.8 Hz; for the HMBC spectrum, AQ = 0.131, spectra frequency (SF) = 599.880 MHz, RD = 0.369, delay time (DE) = 50 ms, $F_1$ = 36 182.7 Hz, $F_1$ = 7798.8 Hz.

**Table 3.** $^{1}$H-NMR (600 MHz) and $^{13}$C-NMR (150 MHz) data of the monoterpenoyl moiety (MT) of **1** and the feruloyl moiety (FA) of **2** in pyridine-$d_5$ ($\delta$ in ppm, $J$ in Hz)

| | Compound **1** | | Compound **2** | |
|---|---|---|---|---|
| NO | $^{1}$H-NMR | $^{13}$C-NMR | $^{1}$H-NMR | $^{13}$C-NMR |
| MT-1 | – | 168.0 | – | – |
| 2 | – | 127.6 | – | – |
| 3 | 7.12 t, $J = 7.3$ | 143.6 | – | – |
| 4 | 2.33, 2.44 | 23.8 | – | – |
| 5 | 1.70, 1.79 | 41.0 | – | – |
| 6 | – | 72.1 | – | – |
| 7 | 6.08 dd, $J = 17.4, 10.7$ | 145.7 | – | – |
| 8 | 5.11 d, $J = 10.9$, 5.43 d, $J = 17.8$ | 111.6 | – | – |
| 9 | 1.93 s | 12.5 | – | – |
| 10 | 1.42 s | 27.8 | – | – |
| FA-$\alpha$ | – | – | – | 167.5 |
| $\beta$ | – | – | 6.69 d, $J = 15.7$ | 114.8 |
| $\gamma$ | – | – | 8.01 d, $J = 15.7$ | 145.4 |
| 1 | – | – | – | 127.0 |
| 2 | – | – | 7.28 s | 111.0 |
| 3 | – | – | – | 148.8 |
| 4 | – | – | – | 150.0 |
| 5 | – | – | 7.23 d, $J = 8.1$ | 115.5 |
| 6 | – | – | 7.25 d, $J = 8.1$ | 123.1 |
| 3-OMe | – | – | 3.91 s | 55.8 |

Overlapped proton NMR signals are reported without designated multiplicity.

### Extraction and isolation

The dried powdered stem barks of *T. tetraptera* (300 g) were extracted with MeOH (400 ml × 3, 3 h) in a soxhlet. This MeOH extract was concentrated to dryness and gave a dark residue (13 g) which was dissolved in water (250 ml) and partitioned with *n*-BuOH saturated with water (250 ml × 3). The *n*-BuOH extract (6.1 g) was subjected to VLC on RP-18 (25 −40 µm) with $H_2O$ containing increasing amounts of MeOH. The fractions eluted with $H_2O$–MeOH (5 : 5) (240 mg) and pure MeOH were combined and submitted to VLC on silica gel ($CHCl_3$–MeOH–$H_2O$, 60 : 32 : 6.5). The resulting residue (1.6 g) was then subjected to successive MPLC on silica gel column eluted with $CHCl_3$–MeOH–$H_2O$ (60 : 32 : 6.5 and 70 : 30 : 5) yielding compounds **1** (7.7 mg) and **2** (9.5 mg).

The dried powdered roots of *T. tetraptera* (300 g) were extacted according to the same protocol. The *n*-BuOH extract (3.1 g) was submitted to successive MPLC on silica gel column eluted with $CHCl_3$–MeOH–$H_2O$ (40 : 10 : 1) to afford aridanin (14.7 mg), 3-*O*-(2-acetamido-2-deoxy-$\beta$-D-glucopyranosyl)echinocystic acid (15.5 mg), 3-*O*-[$\beta$-D-glucopyranosyl-(1 → 6)-2-acetamido-2-deoxy-$\beta$-D-glucopyranosyl]oleanolic acid (11.6 mg), and 3-*O*-sulfoechinocystic acid (8.7 mg).

**Tetrapteroside A (1)**: Amorphous powder. $[\alpha]_D^{25}$ −30° ($c$ = 0.50, MeOH). For $^{1}$H-NMR and $^{13}$C-NMR data, see Tables 1–3. HR-ESIMS $m/z$ = 1471,7090 $[M + Na]^+$ (calcd 1471,7085). FABMS $m/z$ = 1447 $[M - H]^-$, 1281 $[(M - H) - 166]^-$, 1119 $[(M - H) - 166 - 162]^-$, 957 $[(M - H) - 166 - 162 - 162]^-$, 795 $[(M - H) - 166 - 162 - 162 - 162]^-$.

**Tetrapteroside B (2)**: Amorphous powder. $[\alpha]_D^{25}$ −45° ($c$ = 0.64, MeOH). For $^{1}$H-NMR and $^{13}$C-NMR data, see Tables 1–3. HR-ESIMS $m/z$ = 1481,6572 $[M + Na]^+$ (calcd 1481,6565). FABMS $m/z$ = 1457 $[M - H]^-$, 1281 $[(M - H) - 176]^-$, 1119 $[(M - H) - 176 - 162]^-$, 957 $[(M - H) - 176 - 162 - 162]^-$.

### Acid hydrolysis

Two milligrams of each saponin was refluxed with 2-N aqueous $CF_3COOH$ (5 ml) for 2 h. After extraction with $CH_2Cl_2$ (3× 5 ml), the aqueous layer was repeatedly evaporated to dryness with MeOH until neutral. One glucose was identified by comparison with an authentic sample on TLC in $CHCl_3$–MeOH–$H_2O$ (8 : 5 : 1). The D configuration of glucose was determined by GC analysis using the method previously described.[10]

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