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# Acylated Preatroxigenin Glycosides from *Atroxima congolana*

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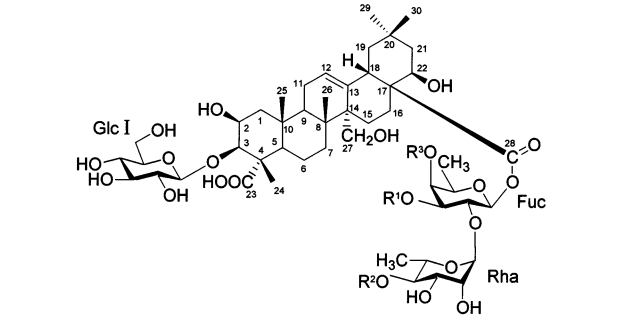
Six new acylated bisdesmosidic preatroxigenin saponins named atroximasaponins $E_1$, $E_2$ (**1**, **2**), $F_1$, $F_2$ (**3**, **4**), and $G_1$, $G_2$ (**5**, **6**) were isolated as three inseparable mixtures of the *trans*- and *cis*-*p*-methoxycinnamoyl derivatives, from the roots of *Atroxima congolana*. Their structures were established through extensive NMR spectroscopic analysis as 3-*O*-*β*-D-glucopyranosylpreatroxigenin-28-*O*-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→3)]-[4-*O*-*trans*-*p*-methoxycinnamoyl]-*β*-D-fucopyranoside (atroximasaponin $E_1$, **1**), and its *cis*-isomer, atroximasaponin $E_2$ (**2**), 3-*O*-*β*-D-glucopyranosylpreatroxigenin-28-*O*-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[6-*O*-acetyl-*β*-D-glucopyranosyl-(1→3)]-[4-*O*-*trans*-*p*-methoxycinnamoyl]-*β*-D-fucopyranoside (atroximasaponin $F_1$, **3**), and its *cis*-isomer, atroximasaponin $F_2$ (**4**), 3-*O*-*β*-D-glucopyranosylpreatroxigenin-28-*O*-*β*-D-apiofuranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-[4-*O*-*trans*-*p*-methoxycinnamoyl]-*β*-D-fucopyranoside (atroximasaponin $G_1$, **5**), and its *cis*-isomer, atroximasaponin $G_2$ (**6**), respectively.

In a previous paper,[1] we reported the isolation and characterization of eight new preatroxigenin saponins, atroximasaponins $A_1$, $A_2$, $B_1$, $B_2$, $C_1$, $C_2$, and $D_1$, $D_2$ as four inseparable mixtures from the ethanolic extract of the cortex of roots of *Atroxima congolana* E. Petit (Polygalaceae). Further investigation of the saponin fraction of this plant resulted in the isolation and structure elucidation of six new additional triterpene glycosides named atroximasaponins $E_1$, $E_2$ (**1**, **2**), $F_1$, $F_2$ (**3**, **4**), and $G_1$, $G_2$ (**5**, **6**), which were obtained as three inseparable mixtures of the respective *trans*- and *cis*-*p*-methoxycinnamoyl derivatives. This paper deals with the isolation and structure elucidation of these saponins (**1**−**6**).

## Results and Discussion

The ethanolic extract of the cortex of the roots of *A. congolana* was suspended in MeOH and purified by precipitation with $Et_2O$, yielding a crude saponin mixture.[2] This extract was subjected to Sephadex LH-20 column chromatography followed by repeated medium-pressure liquid chromatography (MPLC) on normal silica gel and semipreparative reversed-phase HPLC to afford compounds **1**−**6** as three inseparable mixtures, with each compound pair **1**/**2**, **3**/**4**, and **5**/**6** giving only one spot by HPTLC but two peaks by HPLC.

All the compounds **1**−**6** were obtained as white amorphous powders. The $^1$H and $^{13}$C NMR data of the aglycon part of **1**−**6** (Table 1) were almost superimposable with those of preatroxigenin (2*β*,3*β*,22*β*,27-tetrahydroxyolean-12-ene-23,28-dioic acid).[1−3] Acid hydrolysis of each saponin pair **1**/**2**, **3**/**4**, and **5**/**6** with 2 N TFA at 120 °C afforded artifactual aglycons of preatroxigenin (atroxigenic acid, atroxigenin, and atroxigenic acid lactone)[2,3] and rhamnose, fucose, glucose, and xylose in the case of **1**/**2** and **3**/**4** and rhamnose, fucose, glucose, and apiose in the case of **5**/**6** (TLC, GLC). The alkaline hydrolysis of each compound pair with 5% KOH at 120 °C gave the same prosapogenin previously characterized as 3-*O*-*β*-D-glucopyranosylpreatroxigenin (TLC, $^1$H and $^{13}$C NMR).[1] The mild alkaline hydrolysis of each saponin pair with 1% KOH (60 min at room temperature) yielded *trans*- and *cis*-*p*-methoxycinnamic acid (TLC, authentic sample)[1] and a compound that exhibited a lower polarity than the native one (lower $R_f$ on TLC), indicating acylation of the saponins. All these




| | R¹ | R² | R³ |
|---|---|---|---|
| Atroximasaponin $E_1$ (**1**) | Glc II | Xyl | S¹ |
| Atroximasaponin $E_2$ (**2**) | Glc II | Xyl | S² |
| Atroximasaponin $F_1$ (**3**) | Glc II Ac | Xyl | S¹ |
| Atroximasaponin $F_2$ (**4**) | Glc II Ac | Xyl | S² |
| Atroximasaponin $G_1$ (**5**) | Api | H | S¹ |
| Atroximasaponin $G_2$ (**6**) | Api | H | S² |

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**Table 1.** $^{13}C$ NMR and $^{1}H$ NMR Data of the Aglycons of **1**−**6** ($C_5D_5N$)[a−c]

| | | **1**, **2** | | **3**, **4** | | **5**, **6** | |
|---|---|---|---|---|---|---|---|
| position | DEPT | $\delta_{^{13}C}$ | $\delta_{^{1}H}$ | $\delta_{^{13}C}$ | $\delta_{^{1}H}$ | $\delta_{^{13}C}$ | $\delta_{^{1}H}$ |
| 1 | $CH_2$ | 43.4 | 1.45 (1H, m) | 43.4 | 1.36 (1H, m) | 43.9 | 1.33 (1H, m) |
| | | | 2.22 (1H, m) | | 2.22 (1H, m) | | 2.30 (1H, m) |
| 2 | CH | 69.8 | 4.62 (1H, m) | 69.6 | 4.61 (1H, m) | 69.8 | 4.70 (1H, m) |
| 3 | CH | 86.3 | 4.55 (1H, d, $J = 3$) | 85.7 | 4.47 (1H, d, $J = 3$) | 85.6 | 4.55 (1H, d, $J = 3$) |
| 4 | C | 53.2 | | 53.2 | | 52.5 | |
| 5 | CH | 51.8 | 2.06 (1H, m) | 52.1 | 2.03 (1H, m) | 52.1 | 2.13 (1H, m) |
| 6 | $CH_2$ | 20.7 | nd | 21.0 | 1.65, 1.70 | 21.0 | nd, 1.56 |
| 7 | $CH_2$ | 33.0 | 1.70, 2.10 | 33.0 | 1.68, 2.05 | 33.1 | 1.78, 2.01 |
| 8 | C | 40.3 | | 40.2 | | 40.8 | |
| 9 | CH | 48.6 | 2.10 | 48.6 | 2.10 | 49.0 | 2.31 |
| 10 | C | 36.0 | | 36.1 | | 36.7 | |
| 11 | $CH_2$ | 23.2 | 1.86, 1.90 | 23.0 | 1.90, 1.95 | 23.9 | 20.5, 2.18 |
| 12 | CH | 126.9 | 5.74 (1H, t-like) | 126.9 | 5.78 (1H, t-like) | 127.5 | 5.94 (1H, t-like) |
| 13 | C | 138.8 | | 138.8 | | 139.2 | |
| 14 | C | 47.8 | | 47.8 | | 48.2 | |
| 15 | $CH_2$ | 23.9 | nd, 1.98 | 23.9 | nd, 1.75 | 24.4 | 2.07, 2.15 |
| 16 | $CH_2$ | 23.8 | nd | 23.8 | 2.00, 1.17 | 24.3 | nd, 1.75 |
| 17 | C | 53.7 | | 53.6 | | 53.5 | |
| 18 | CH | 38.4 | 3.36 (1H, m) | 38.4 | 3.37 (1H, m) | 38.9 | 3.58 (1H, m) |
| 19 | $CH_2$ | 45.1 | 1.26, 1.65 | 45.2 | 1.30, 1.68 | 45.6 | 1.48, 1.88 |
| 20 | C | 29.7 | | 29.8 | | 30.3 | |
| 21 | $CH_2$ | 40.4 | 1.30, 1.50 | 40.3 | nd | 41.4 | 1.50, 1.67 |
| 22 | CH | 70.4 | 4.63 (1H, d, $J = 1.8$) | 70.6 | 4.63 (1H, d, $J = 1.8$) | 71.3 | 4.71 (1H, d, $J = 1.8$) |
| 23 | C | 182.5 | | 180.4 | | 180.7 | |
| 24 | $CH_3$ | 14.3 | 1.72 (3H, s) | 13.8 | 1.71 (3H, s) | 13.8 | 1.88 (3H, s) |
| 25 | $CH_3$ | 16.8 | 1.29 (3H, s) | 16.8 | 1.32 (3H, s) | 17.2 | 1.53 (3H, s) |
| 26 | $CH_3$ | 18.2 | 0.95 (3H, s) | 18.1 | 0.98 (3H, s) | 16.1 | 1.16 (3H, s) |
| 27 | $CH_2$ | 63.6 | 3.68 (1H, d, $J = 12$) | 63.7 | 3.72 (1H, d, $J = 12$) | 63.8 | 3.84 (1H, d, $J = 12$) |
| | | | 4.07 (1H, d, $J = 12$) | | 4.07 (1H, d, $J = 12$) | | 4.06 (1H, d, $J = 12$) |
| 28 | C | 174.3 | | 174.4 | | 174.3 | |
| 29 | $CH_3$ | 33.5 | 0.78 (3H, s) | 33.5 | 0.77 (3H, s) | 34.0 | 0.85 (3H, s) |
| 30 | $CH_3$ | 26.7 | 1.21 (3H, s) | 26.8 | 1.22 (3H, s) | 27.3 | 1.36 (3H, s) |

[a] Multiplicities were assigned from DEPT spectra. [b] The assignments are based on the HMBC, HSQC, and DEPT experiments (150 MHz for $^{13}C$ and 600 MHz for $^{1}H$ NMR). [c] nd: not determined. Overlapped $^{1}H$ NMR signals are reported without designated multiplicity.

findings indicated that compounds **1**−**6** were bisdesmosidic saponins having the same prosapogenin (3-*O*-*β*-D-glucopyranosylpreatroxigenin). This was confirmed by the downfield chemical shifts of C-3 of the aglycon at *δ* 86.3 (**1**, **2**), 85.7 (**3**, **4**), and 85.6 (**5**, **6**) and the upfield chemical shifts at C-28 of the aglycon at *δ* 174.3 (**1**, **2**; **5**, **6**) and 85.7 (**3**, **4**), respectively.[1] Furthermore, all compounds **1**−**6** are acylated by *trans*- and *cis*-*p*-methoxycinnamoyl residues, which were identified by the $^{1}H-{}^{1}H$ COSY NMR experiment (see Tables 2 and S1).[1] These findings indicated that the compound pairs **1**/**2**, **3**/**4**, and **5**/**6** were mixtures of *trans*- and *cis*-*p*-methoxycinnamoyl preatroxigenin glycosides (2:1 for **1**/**2** and **5**/**6** and 1:1 for **3**/**4**, respectively, from the relative NMR and HPLC intensities).[1,4] Each mixture was homogeneous by HPTLC but was separated into *trans*- and *cis*-isomers by HPLC, but all attempts to separate each saponin pair by semipreparative HPLC were unsuccessful. The observed isomerization is due to the effect of light on the *p*-methoxycinnamoyl group in aqueous methanolic solution. Under these conditions, the geometrical isomeric structures of the *p*-methoxycinnamoyl groups in **1** and **2** showed tautomer-like behavior. This phenomenon has already been observed in *E* and *Z* mixtures of saponins from *Polygala senega*,[5] *Silene jenisseensis*,[4] and *Muraltia heisteria*[6] and also in the previously reported saponins of this plant.[1]

The HRESIMS (positive-ion mode) of **1**/**2** exhibited a quasimolecular ion peak at *m*/*z* 1465.6283 $[M + Na]^+$ (calcd 1465.6252), consistent with the molecular formula $C_{69}H_{102}O_{32}Na$. Their FABMS (negative-ion mode) showed a quasimolecular ion peak at *m*/*z* 1441 $[M - H]^-$, indicating a molecular weight of 1442. Other significant fragment peaks appeared at *m*/*z* 1279 $[(M - H) - 162]^-$, 695 $[(M - H) - 162 - 160 - 2 \times 146 - 132]^-$, and 533 $[(M - H) - 162 - 160 - 2 \times 146 - 132 - 162]^-$, corresponding to the loss of one hexosyl, one *p*-methoxycinnamoyl, two deoxyhexosyl, one pentosyl, and one hexosyl unit, respectively. The fragment ion peak at *m*/*z* 533 corresponded to the pseudomolecular ion of the aglycon (preatroxigenin).[1−3]

The $^{1}H$ NMR spectrum of **1**/**2** displayed signals for five anomeric protons at *δ* 6.22 (br s), 6.01 (d, $J = 8.4$ Hz), 4.97 (d, $J = 7.3$ Hz), 4.92 (d, $J = 7.0$ Hz), and 4.76 (d, $J = 7.0$ Hz), which correlated in the HSQC spectrum with $^{13}C$ NMR signals at *δ* 100.4, 93.7, 104.5, 103.5, and 106.2, respectively. The ring protons of the monosaccharide residues were assigned starting from the readily identifiable anomeric protons by means of the COSY, TOCSY, HSQC, and HMBC NMR experiments (Table S1), and the sequence of the oligosaccharide chains was obtained from the HMBC and NOESY experiments. Evaluation of spin−spin couplings and chemical shifts allowed the identification of one α-rhamnopyranosyl (Rha), one *β*-fucopyranosyl (Fuc), two *β*-glucopyranosyl (Glc), and one *β*-xylopyranosyl (Xyl) unit, respectively. The absolute configurations of these sugar residues were determined to be D for Glc, Xyl, and Fuc and L for Rha by GC analysis of chiral derivatives of sugars in an acidic hydrolysate.[7]

The $^{1}H$ and $^{13}C$ NMR signals in the molecules **1**/**2** corresponding to the 3-*O*-*β*-D-glucopyranosyl moiety and the 28-*O*-α-L-rhamnopyranosyl (1→2)-[4-*O*-*trans*-*p*-methoxycinnamoyl]-*β*-D-fucopyranosyl moiety and the *cis*-isomer form were almost superimposable to those of atroximasaponins $A_1$, $A_2$, $B_1$, $B_2$, $C_1$, $C_2$, and $D_1$, $D_2$, consistent with the proposed sequence.[1] In addition, HMBC correlations between $\delta_H$ 4.76 (d, $J = 7.0$ Hz) (Xyl-1) and $\delta_C$ 83.3 (Rha-4) and between $\delta_H$ 4.04 (Rha-4) and $\delta_C$ 106.2 (Xyl-1) showed that the Xyl unit was linked to the Rha unit at C-4. This was also supported by a NOESY cross-peak between $\delta_H$

**Table 2.** $^{13}C$ NMR Data of the Sugar and Acid Moieties of Compounds **1**−**6** ($C_5D_5N$)[a,b]

| | | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| 3-*O*-Glc I | 1 | 103.5 | 103.5 | 103.8 | 103.8 | 104.8 | 104.8 |
| | 2 | 74.1 | 74.1 | 74.0 | 74.0 | 74.7 | 74.7 |
| | 3 | 76.2 | 76.2 | 76.7 | 76.7 | 77.6 | 77.6 |
| | 4 | 69.3 | 69.3 | 69.6 | 69.6 | 71.0 | 71.0 |
| | 5 | 77.1 | 77.1 | 77.1 | 77.1 | 77.8 | 77.8 |
| | 6 | 61.3 | 61.3 | 61.2 | 61.2 | 62.0 | 62.0 |
| 28-*O*-sugars | | | | | | | |
| Fuc | 1 | 93.7 | 93.7 | 93.8 | 93.8 | 94.4 | 94.4 |
| | 2 | 71.3 | 71.1 | 71.0 | 71.0 | 72.5 | 72.5 |
| | 3 | 82.7 | 82.7 | 82.7 | 82.7 | 81.1 | 81.1 |
| | 4 | 74.1 | 74.0 | 73.1 | 72.9 | 73.7 | 73.4 |
| | 5 | 70.5 | 70.4 | 70.7 | 70.6 | 71.0 | 70.8 |
| | 6 | 15.7 | 15.6 | 16.1 | 15.9 | 16.1 | 16.0 |
| Rha | 1 | 100.4 | 100.3 | 100.4 | 100.3 | 101.9 | 101.8 |
| | 2 | 70.5 | 70.4 | 70.7 | 70.6 | 72.2 | 72.2 |
| | 3 | 71.3 | 71.3 | 71.2 | 71.2 | 71.9 | 71.9 |
| | 4 | 83.3 | 83.4 | 83.7 | 83.7 | 73.0 | 73.0 |
| | 5 | 67.5 | 67.6 | 67.7 | 67.7 | 70.0 | 70.0 |
| | 6 | 17.7 | 17.8 | 17.8 | 17.8 | 18.5 | 18.4 |
| Xyl | 1 | 106.2 | 106.2 | 106.1 | 106.1 | | |
| | 2 | 75.0 | 75.0 | 75.0 | 75.0 | | |
| | 3 | 76.7 | 76.7 | 76.4 | 76.4 | | |
| | 4 | 70.3 | 70.3 | 70.2 | 70.2 | | |
| | 5 | 66.1 | 66.1 | 66.1 | 66.1 | | |
| Glc II | 1 | 104.5 | 104.5 | 103.9 | 103.9 | | |
| | 2 | 74.0 | 74.0 | 74.0 | 74.0 | | |
| | 3 | 76.6 | 76.6 | 76.7 | 76.7 | | |
| | 4 | 70.2 | 70.2 | 69.7 | 69.7 | | |
| | 5 | 76.6 | 76.6 | 76.7 | 76.7 | | |
| | 6 | 61.8 | 61.8 | 63.3 | 63.3 | | |
| Ac at Glc II C-6 | | | | 20.3, 172.0 | 20.2, 171.0 | | |
| Api | 1 | | | | | 112.5 | 112.5 |
| | 2 | | | | | 78.1 | 78.1 |
| | 3 | | | | | 80.2 | 80.2 |
| | 4 | | | | | 75.3 | 75.3 |
| | 5 | | | | | 64.4 | 64.4 |
| acid | (1′,1″) | 167.8 | 166.5 | 166.8 | 166.1 | 167.1 | 166.1 |
| | (2′,2″) | 114.5 | 115.7 | 114.7 | 116.2 | 115.4 | 116.2 |
| | (3′,3″) | 145.9 | 144.9 | 145.1 | 144.0 | 145.6 | 145.0 |
| | (4′,4″) | 126.4 | 126.6 | 126.5 | 126.9 | 126.9 | 127.0 |
| | (5′, 9′;5″,9″) | 130.0 | 132.4 | 130.0 | 132.5 | 130.2 | 133.0 |
| | (6′,8′;6″,8″) | 114.3 | 113.5 | 114.3 | 113.7 | 114.5 | 113.8 |
| | (7′,7″) | 161.5 | 160.4 | 161.4 | 160.4 | 161.8 | 161.1 |
| OMe | (7′,7″) | 55.2 | 55.0 | 55.2 | 55.0 | 55.3 | 55.1 |

[a] The assignments are based on the COSY, TOCSY, NOESY, HSQC, and HMBC experiments (150 MHz for $^{13}C$ and 600 MHz for $^{1}H$ NMR). Multiplicities were assigned from DEPT spectra.

4.76 (d, $J$ = 7.0 Hz) (Xyl-1) and $\delta_H$ 4.04 (Rha-4). This terminal Xyl was confirmed by its $^{1}H$ and $^{13}C$ NMR data (Tables 2 and S1). Another HMBC correlation observed between $\delta_H$ 4.97 (d, $J$ = 7.3 Hz) (Glc II-1) and $\delta_C$ 82.7 (Fuc-3) indicated that Glc II was attached to the Fuc unit at C-3. This was confirmed by a NOESY cross-peak between $\delta_H$ 4.43 (Fuc-3) and 4.97 (d, $J$ = 7.3 Hz) (Glc II-1). Thus, the structures of **1**/**2** were established as 3-*O*-*β*-D-glucopyranosylpreatroxigenin-28-*O*-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→3)]-[4-*O*-*trans*-*p*-methoxycinnamoyl]-*β*-D-fucopyranoside (atroximasaponin $E_1$, **1**) and its *cis*-isomer, atroximasaponin $E_2$ (**2**).

The HRESIMS (positive-ion mode) of compounds **3**/**4** exhibited a quasimolecular ion peak at *m*/*z* 1507.6367 [M + Na]$^+$ (calcd 1507.6358), consistent with the molecular formula of $C_{71}H_{104}O_{33}Na$. Their FABMS (negative-ion mode) showed a quasimolecular ion peak at *m*/*z* 1483 [M − H]$^-$, indicating a molecular weight of 1484. Other significant ion peaks in the FABMS appeared at *m*/*z* 1321-[(M − H) − 162]$^-$, 695 [(M − H) − 162 − 42 − 160 − 2 × 146 − 132]$^-$, and 533 [(M − H) − 162 − 42 − 160 − 2 × 146 − 132 − 162]$^-$, corresponding to the loss of one hexosyl, one acetyl, one *p*-methoxycinnamoyl, two deoxyhexosyl, one pentosyl, and one hexosyl unit, respectively. The fragment ion peak at *m*/*z* 533 corresponded to the pseudomolecular ion of the preatroxigenin.[1−3]

The $^{1}H$ and $^{13}C$ NMR data of **3**/**4** (Tables 1, 2, and S1) assigned from TOCSY, HSQC, and HMBC experiments were similar to those of **1**/**2**, except for the appearance of one additional acetyl group. The location of the acetyl group at Glc II-C-6 was determined by TOCSY and COSY experiments, starting from the anomeric $^{1}H$ NMR signal of Glc II at $\delta$ 4.94 (d, $J$ = 7.7 Hz). The downfield shifts observed in the HSQC spectrum for the Glc II−H-6/Glc II−C-6 resonances at $\delta_H$ 4.74, 4.54/$\delta_C$ 63.3 proved the primary alcoholic function of Glc II−6-OH to be acetylated. On the basis of the above observations, the structures of **3**/**4** were determined as 3-*O*-*β*-D-glucopyranosylpreatroxigenin-28-*O*-*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[6-*O*-acetyl-*β*-D-glucopyranosyl-(1→3)]-[4-*O*-*trans*-*p*-methoxycinnamoyl]-*β*-D-fucopyranoside (atroximasaponin $F_1$, **3**) and its *cis*-isomer, atroximasaponin $F_2$ (**4**).

The HRESIMS (positive-ion mode) of compounds **5**/**6** exhibited a quasimolecular ion peak at *m*/*z* 1303.5695 [M + Na]$^+$ (calcd 1303.5724) consistent with the molecular formula of $C_{63}H_{92}O_{27}Na$. Their FABMS (negative-ion mode) showed a quasimolecular ion peak at *m*/*z* 1279 [M − H]$^-$, indicating a molecular mass of 1280, or 162 mass units less than compounds **1** and **2**. Two other significant ion peaks

appeared at *m/z* 1119 [(M − H) − 160]$^-$ and 533 [(M − H) − 160 − 2 × 146 − 132 − 162]$^-$, corresponding to the loss of one *p*-methoxycinnamoyl, two deoxyhexosyl, one pentosyl, and one hexosyl unit, respectively.

The $^1$H NMR spectrum of **5**/**6** showed four proton anomeric signals at $\delta$ 6.14 (d, $J$ = 8.4 Hz), 6.08 (br s), 5.81 (br s), and 5.00 (d, $J$ = 7.7 Hz), which gave correlations with $^{13}$C NMR signals in the HSQC spectrum at $\delta$ 94.4, 101.9, 112.5, and 104.8, respectively. Evaluation of spin−spin couplings and chemical shifts from the 2D NMR data for the sugar moieties of **5**/**6** and GC analysis of chiral derivatives of the sugars in the acidic hydrolysate of **5**/**6** allowed the identification of one α-L-rhamnopyranosyl (Rha), one β-D-fucopyranosyl (Fuc), one β-D-apiofuranosyl (Api), and one β-D-glucopyranosyl (Glc) unit, respectively. In the HMBC spectrum, a correlation between the $^1$H NMR signal at $\delta_H$ 6.14 (d, $J$ = 8.4 Hz) (Fuc-1) and the $^{13}$C NMR signal at $\delta_C$ 174.3 (aglycon-28) demonstrated a glycosidic ester linkage of the Fuc unit to the C-28 of the aglycon. The location of the *p*-methoxycinnamoyl group at Fuc-4 was determined by TOCSY and COSY experiments, starting from the anomeric $^1$H NMR signal of Fuc at $\delta$ 6.14 (d, $J$ = 8.4 Hz). The downfield shifts observed in the HSQC spectrum for the Fuc (H)4/Fuc(C)4 resonances at $\delta_H$ 5.87/$\delta_C$ 73.7 showed the secondary alcoholic function of OH−C(4) of Fuc to be acylated. In the HMBC spectrum a correlation between signals at $\delta_H$ 6.08 (br s) (Rha-1) and $\delta_C$ (72.5) (Fuc-2) and a reverse correlation between $\delta_H$ (4.72) (Fuc-2) and $\delta_C$ (101.9) (Rha-1) revealed a (1→2) linkage between these two sugars. The $^1$H and $^{13}$C NMR signals of a terminal Rha were assigned.

The HMBC correlation observed between $\delta_H$ 4.36 (Fuc-3) and $\delta_C$ 112.5 (Api-1) indicated that the Api unit was linked to a Fuc residue at C-3. This was confirmed by a NOESY cross-peak between $\delta_H$ 4.36 (Fuc-3) and 5.81 (br s) (Api-1). On the basis of these results the structures of **5**/**6** were established as 3-*O*-β-D-glucopyranosylpreatroxigenin-28-*O*-β-D-apiofuranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-[4-*O*-*trans*-*p*-methoxycinnamoyl]-β-D-fucopyranoside (atroximasaponin $G_1$, **5**) and its *cis*-isomer, atroximasaponin $G_2$ (**6**).

## Experimental Section

**General Experimental Procedures.** The instruments and techniques used for IR spectra, 1D and 2D NMR spectra ($^1$H−$^1$H COSY, TOCSY, NOESY, HSQC, and HMBC) (600 MHz for $^1$H and 150 MHz for $^{13}$C NMR spectra), and fast-atom bombardment (FABMS) (negative-ion mode, thioglycerol matrix) were previously described.[1] HRESIMS was carried out on a Q-TOF 1 micromass spectrometer. GC analysis was carried out on a Termoquest gas chromatograph using a DB-1701 capillary column (30 m × 0.25 mm, i.d.) (J & W Scientific); detection, FID; detector temperature, 250 °C; injection temperature, 230 °C; initial temperature was maintained at 80 °C for 5 min and then raised to 270 °C at the rate of 15 °C/min; carrier gas, He. TLC, HPTLC, MPLC, and HPLC were carried out by using previously reported conditions.[1]

**Plant Material.** The cortex of the roots of *Atroxima congolana* was collected from the Democratic Republic of Congo, in the Eala Forest in March 1990. A voucher specimen under the reference H. Breyne No. 1865 is deposited in the Herbarium of the National Botanical Garden of Brussels, Belgium.

**Extraction and Isolation.** The dried powdered root cortex (2 kg) was macerated with 80% EtOH and further submitted to boiling for 3 h. The EtOH extract was filtered and evaporated to dryness. The residue was dissolved in MeOH (1500 mL). After filtration, the MeOH solution was concentrated and purified by precipitation with $Et_2O$ (3 × 1500 mL). The resulting residue was washed with $Et_2O$, dried, solubilized in water (1200 mL), and submitted to dialysis for 4 days and then lyophilized. After decolorization with charcoal and filtration, the residue was dissolved in MeOH and purified again by precipitation with $Et_2O$, yielding a crude saponin mixture (124.6 g). Of this mixture, 4.0 g was submitted to column chromatography (Sephadex LH-20 ) and then to successive MPLC (silica gel 60 (15−40 μm, $CHCl_3$−MeOH−$H_2O$ (32:17:3 and 65:35:10, lower phase)), followed by semipreparative HPLC (isocratic, 28% MeCN−$H_2O$ with 0.06% $CF_3COOH$ for 30 min; flow rate 4.5 mL/min), yielding **1**/**2** ($t_R$ 12.70, 13.76 min) (14 mg), **3**/**4** ($t_R$ 15.30, 16.24 min) (11 mg), and **5**/**6** ($t_R$ 11.58, 12.03 min) (35 mg). Each compound pair was isolated as an amorphous powder, which gave fluorescence quenching zones under UV light at 254 nm and violet-blue fluorescence under UV light at 365 nm by TLC without any chemical treatment.

**3-*O*-β-D-Glucopyranosylpreatroxigenin-28-*O*-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-[4-O-*trans*-*p*-methoxycinnamoyl]-β-D-fucopyranoside (atroximasaponin $E_1$, 1) and its *cis*-isomer, atroximasaponin $E_2$ (2):** IR (KBr) $\nu_{max}$ 3405 (OH), 2926 (CH), 1750 and 1740 (CO ester groups), 1710 (CO carboxylic acid), 1634 (C=C), 1610, 1600, 1560, 1500 cm$^{-1}$; $^1$H NMR (pyridine-$d_5$, 600 MHz) and $^{13}$C NMR (pyridine-$d_5$, 150 MHz), see Tables 2 and S1; HRESIMS positive-ion mode *m/z* 1465.6283 [M + Na]$^+$ (calcd for $C_{69}H_{102}O_{32}Na$, 1465.6252); negative FABMS (thioglycerol matrix) *m/z* 1441 [M − H]$^-$, 1279 [(M − H) − 162]$^-$, 695 [(M − H) − 162 − 160 − 2 × 146 − 132]$^-$, 533 [(M − H) − 162 − 162 − 160 − 2 × 146 − 132 − 162]$^-$; TLC $R_f$ 0.45 (system a); blue spot by spraying with Komarowsky reagent.

**3-*O*-β-D-Glucopyranosylpreatroxigenin-28-*O*-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-[6-*O*-acetyl-β-D-glucopyranosyl-(1→3)]-[4-*O*-*trans*-*p*-methoxycinnamoyl]-β-D-fucopyranoside (atroximasaponin $F_1$, 3) and its *cis*-isomer, atroximasaponin $F_2$ (4):** IR (KBr) $\nu_{max}$ 3406 (OH), 2927 (CH), 1723 and 1740 (CO ester groups), 1710 (CO carboxylic acid), 1636 (C=C), 1580, 1500, 1420 cm$^{-1}$; $^1$H NMR (pyridine-$d_5$, 600 MHz) and $^{13}$C NMR (pyridine-$d_5$, 150 MHz), see Tables 2 and S1; HRESIMS positive-ion mode *m/z* 1507.6367 [M + Na]$^+$ (calcd for $C_{71}H_{104}O_{33}Na$, 1507.6358); negative FABMS (thioglycerol matrix) *m/z* 1483 [M − H]$^-$, 1321 [(M − H) − 162]$^-$, 695 [(M − H) − 162 − 42 − 160 − 2 × 146 − 132]$^-$ and 533 [(M − H) − 42 − 162 − 162 − 160 − 2 × 146 − 132 − 162]$^-$; TLC $R_f$ 0.51 (system a); blue spot by spraying with Komarowsky reagent.

**3-*O*-β-D-Glucopyranosylpreatroxigenin-28-*O*-β-D-apiofuranosyl-(1→3)-[α-L-rhamnopyranosyl-(1→2)]-[4-*O*-*trans*-*p*-methoxycinnamoyl]-β-D-fucopyranoside (atroximasaponin $G_1$, 5) and its *cis*-isomer, atroximasaponin $G_2$ (6):** IR (KBr) $\nu_{max}$ 3404 (OH), 2927 (CH), 1723 and 1740 (CO ester groups), 1710 (CO carboxylic acid), 1636 (C=C), 1580, 1500, 1420, 1260, 1090 cm$^{-1}$; $^1$H NMR (pyridine-$d_5$, 600 MHz) and $^{13}$C NMR (pyridine-$d_5$, 150 MHz), see Tables 2 and S1; HRESIMS positive-ion mode *m/z* 1303.5695 [M + Na]$^+$ (calcd for $C_{63}H_{92}O_{27}Na$, 1303.5724); negative FABMS (thioglycerol matrix) *m/z* 1279 [M − H]$^-$, 1119 [(M − H) − 160]$^-$, 533 [(M − H) − 160−2 × 146−132−162]$^-$; TLC $R_f$ 0.62 (system a); blue spot by spraying with Komarowsky reagent.

**Acid Hydrolysis.** A solution of each compound pair (5 mg of each) in $H_2O$ (2 mL) and 2 N aqueous $CF_3COOH$ (5 mL) was refluxed on a water bath for 3 h. After this period, the reaction mixture was diluted with $H_2O$ (15 mL) and extracted with $CH_2Cl_2$ (3 × 5 mL). The combined $CH_2Cl_2$ extracts were washed with $H_2O$ and then evaporated to dryness in vacuo. Evaporation of the solvent gave the artifactual aglycons of preatroxigenin.[2,4] After repeated evaporations to dryness of the aqueous layer with MeOH until neutral, the residue of sugars was dissolved in anhydrous pyridine (100 μL) and L-cysteine methyl ester hydrochloride (0.06 mol/L) was added. The mixture was stirred at 60 °C for 1 h, then 150 μL of HMDS−TMCS (hexamethyldisilazane−trimethylchlorosilane, 3:1) was added, and the mixture was stirred at 60 °C for another 30 min. The precipitate was centrifuged off, and the

supernatant was concentrated under a $N_2$ stream. The residue was partitioned between *n*-hexane and $H_2O$ (0.1 mL each), and the hexane layer (1 $\mu$L) was analyzed by GC. D-Glucose, D-xylose, D-fucose, and L-rhamnose for **1**/**2** and **3**/**4** were detected in each case by co-injection of the hydrolysate with standard silylated samples, giving single peaks at 18.75, 13.57, 12.18, and 13.21, respectively. By the same manner, identification of D-glucose, D-fucose, D-apiose, and L-rhamnose was carried out for **5**/**6**, giving single peaks at 18.77, 12.20, 14.50, and 13.23, respectively.

**Alkaline Hydrolysis.** Each saponin pair (5 mg) was refluxed with 5% aqueous KOH (10 mL) for 2 h. The fraction mixture was adjusted to pH 6 with dilute HCl and then extracted with $H_2O$-saturated BuOH (3 × 10 mL). The combined BuOH extracts were washed with ($H_2O$) and evaporated to yield the prosapogenin, which was identified as 3-*O*-$\beta$-D-glucopyranosylpreatroxigenin (TLC, $^{13}$C NMR) in comparison with an authentic sample.[1]

**Mild Alkaline Hydrolysis.** Each saponin pair was hydrolyzed with 1% aqueous KOH at room temperature. After 1 h, the mixture was neutralized with dilute HCl and extracted with $Et_2O$. The $Et_2O$ layer gave *trans*- and *cis*-*p*-methoxycinnamic acids, which were identified by TLC (authentic sample).[1,6] The aqueous layer was extracted with $H_2O$-saturated BuOH, yielding the deacylated saponin.

**Supporting Information Available:** Figure S1 of HMBC correlations of the aglycon of compound pairs **1**/**2**, **3**/**4**, and **5**/**6** and Table S1 of $^1$H NMR data of compounds **1**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

## References and Notes

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