# Sigmoiside F and Propyloxyamyrin, Two New Triterpenoid Derivatives from *Erythrina sigmoidea* (Fabaceae)

Jacques Kouam[a], Alain L. Meli[a], Muhammad I. Choudhary[b], and Zacharias T. Fomum[a]

[a] Department of Organic Chemistry, Faculty of Science, University of Yaoundé I, P. O. Box 812, Cameroon
[b] H. E. J. Research Institute of Chemistry, International Centre of Chemical and Biological Sciences, University of Karachi, Pakistan

Reprint requests to Dr. Jacques Kouam. E-mail: kouamjac@yahoo.fr

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A new triterpenoid saponin, olean-12-ene-3$\beta$,19$\beta$,24-triol-3-*O*-$\beta$-D-glucopyranoside, named sigmoiside F and a new triterpenoid, 3$\beta$-*n*-propyloxy-$\beta$-amyrin, were isolated from the methanolic extract of the stem bark of *Erythrina sigmoidea*. Their structures were characterized by chemical and spectral analysis.

*Key words: Erythrina sigmoidea*, Fabaceae, Spectrum, Triterpenoids, Saponins

## Introduction

Recently, we reported that the stem bark and wood extracts of *Erythrina sigmoidea* contained maniladiol-3-*O*-$\beta$-D-glucopyranoside, maniladiol-3-*O*-$\beta$-D-galactopyranoside, maniladiol-16-*O*-$\beta$-D-glucopyranoside, soyasapogenol-B-22-*O*-$\beta$-D-glucopyranoside, and soyasapogenol-B-22-*O*-$\alpha$-L-rhamnopyranoside [1 – 3]. In continuation of our studies on the West African medicinal plants and our interest in the systematic investigation of the triterpenoid derivatives of Erythrina, we now report the isolation and characterization of a new triterpenoid saponin and a new triterpenoid designated sigmoiside F (**1**) and propyloxyamyrin (**3**), respectively, from the stem bark of *Erythrina sigmoidea* (Fabaceae). Hydrolysis of **1** yielded the new aglycon **2**.

## Results and Discussion

The methanolic extract of the stem bark of *Erythrina sigmoidea* was subjected to column chromatography to yield the new compounds **1** and **3**. We now wish to describe the structures of the two new compounds.

Compound **1** (sigmoiside F), m. p. 245 – 247 °C, isolated as white powder from $CH_2Cl_2$/MeOH 9.5/0.5 (v : v), gave a positive response to the Lieberman-Burchard test. Its FAB mass spectrum showed a quasi-molecular ion at *m/z* = 643.4184 $[M+Na]^+$, which suggested *m/z* ($[M]^+$) to be 620.4288, corresponding to the molecular formula $C_{36}H_{60}O_8$. The IR spectrum of **1** exhibited strong bands at 3600 – 3300 $cm^{-1}$ ascribable to hydroxyl groups. Acetylation of **1** with acetic anhydride in pyridine yielded the penta-acetyl derivative **1a** consistent with the presence of five hydroxyl groups. Signals at $\delta$ = 122.6 and 144.7 in the $^{13}C$ NMR spectrum ascribable to $sp^2$ carbons together with an anomeric carbon signal at $\delta$ = 101.5 indicated **1** to be an olean-12-ene glycoside with a single monosaccharide moiety [4] (Table 1).

Acid hydrolysis of sigmoiside F (**1**) yielded the aglycone **2** and a sugar component. The latter was assumed to be D-glucose by the TLC and GLC analysis of its trimethylsilylated derivative. Compound **2**, m. p. 212 – 213 °C, displayed a peak for $[M]^+$ at *m/z* = 458.3759 consistent with the molecular formula $C_{30}H_{50}O_3$. Its $^{13}C$ NMR data (Table 1) indicated the presence of an olean-12-ene derivative.

The IR spectrum of **2** showed the hydroxyl group band at $\nu$ = 3250 $cm^{-1}$. Acetylation of **2** in the same manner as described above for **1** afforded a triacetyl derivative which indicated **2** to have three hydroxyl groups. The low resolution mass spectrum of **2** exhibited a pair of diagnostically important mass peaks at *m/z* = 234 (base peak) and 224 typical of the retro-Diels Alder fragmentation in ring C of an olean-12-ene derivative [5]. The fragmentation ions showed that two hydroxyl groups were confined to ring A or B and one hydroxyl group to ring D or E.

The $^1H$ and $^{13}C$ NMR spectra of **2** displayed signals for one hydroxymethyl group [$\delta_H$ = 3.41(d)

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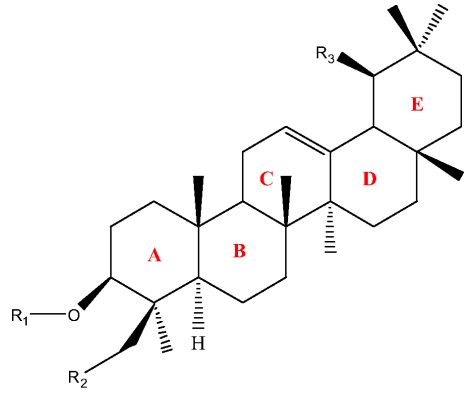
Table 1. $^{13}$C NMR data of sigmoiside F (**1**), olean-12-ene-1$\beta$-24-diol (**2**), propyloxyamyrin (**3**), and $\beta$-amyrin (**4**).

| Carbon No. | **1** ([D$_6$]-DMSO, 75 MHz) | **2** ([D$_6$]-DMSO, 75 MHz) | **3** ($CDCl_3$, 100 MHz) | **4** ($CDCl_3$, 100 MHz) |
|---|---|---|---|---|
| C-1 | 39.1 $CH_2$ | 39.7 $CH_2$ | 38.6 $CH_2$ | 38.7 $CH_2$ |
| C-2 | 27.6 $CH_2$ | 27.7 $CH_2$ | 27.3 $CH_2$ | 27.3 $CH_2$ |
| C-3 | 81.8 CH | 79.8.CH | 78.5 CH | 79.0 CH |
| C-4 | 42.5 C | 42.1 C | 38.8 C | 38.8 C |
| C-5 | 55.6 CH | 55.9 CH | 55.2 CH | 55.3 CH |
| C-6 | 18.9 $CH_2$ | 18.4 $CH_2$ | 18.4 $CH_2$ | 18.5 $CH_2$ |
| C-7 | 33.1 $CH_2$ | 33.1 $CH_2$ | 32.8 $CH_2$ | 32.8 $CH_2$ |
| C-8 | 39.6 C | 39.7 C | 38.1 C | 38.8 C |
| C-9 | 47.9 CH | 47.7 CH | 47.7 CH | 47.7 CH |
| C-10 | 37.2 C | 37.4 C | 36.1 C | 37.6 C |
| C-11 | 23.9 $CH_2$ | 23.8 $CH_2$ | 23.5 $CH_2$ | 23.6 $CH_2$ |
| C-12 | 122.6 CH | 122.3 CH | 121.7 CH | 121.8 CH |
| C-13 | 144.7 C | 143.9 C | 145.2 C | 145.1 C |
| C-14 | 42.4 C | 42.8 C | 41.7 C | 41.8 C |
| C-15 | 26.2 $CH_2$ | 26.9 $CH_2$ | 26.2 $CH_2$ | 26.2 $CH_2$ |
| C-16 | 26.2 $CH_2$ | 25.9 $CH_2$ | 27.2 $CH_2$ | 27.0 $CH_2$ |
| C-17 | 31.6 C | 32.7 C | 32.5 C | 32.5 C |
| C-18 | 47.5 CH | 47.7 CH | 47.2 CH | 47.4 CH |
| C-19 | 77.8 CH | 77.3 CH | 46.8 $CH_3$ | 46.9 $CH_2$ |
| C-20 | 30.6 C | 30.5 C | 31.1 C | 31.1 $CH_2$ |
| C-21 | 33.3 $CH_2$ | 33.1 $CH_2$ | 34.8 $CH_2$ | 34.8 $CH_2$ |
| C-22 | 37.3 $CH_2$ | 37.4 $CH_2$ | 37.0 $CH_2$ | 37.2 CH |
| C-23 | 21.3 $CH_3$ | 20.0 $CH_3$ | 28.1 $CH_3$ | 28.2 $CH_3$ |
| C-24 | 63.5 $CH_2$ | 64.5 $CH_2$ | 15.5 $CH_3$ | 15.5 $CH_3$ |
| C-25 | 16.2 $CH_3$ | 16.1 $CH_3$ | 15.6 $CH_3$ | 15.6 $CH_3$ |
| C-26 | 16.6 $CH_3$ | 16.8 $CH_3$ | 16.8 $CH_3$ | 16.9 $CH_3$ |
| C-27 | 25.9 $CH_3$ | 25.4 $CH_3$ | 26.0 $CH_3$ | 26.0 $CH_3$ |
| C-28 | 27.7 $CH_3$ | 27.7 $CH_3$ | 28.4 $CH_3$ | 28.4 $CH_3$ |
| C-29 | 23.5 $CH_3$ | 23.7 $CH_3$ | 33.3 $CH_3$ | 33.3 $CH_3$ |
| C-30 | 23.7 $CH_3$ | 22.4 $CH_3$ | 23.7 $CH_3$ | 23.7 $CH_3$ |
| C-1′ | Glu 101.5 CH | | 63.1 $CH_2$ | |
| C-2′ | 74.4 CH | | 25.1 $CH_2$ | |
| C-3′ | 77.5 CH | | 14.3 $CH_3$ | |
| C-4′ | 71.2 CH | | | |
| C-5′ | 77.7 CH | | | |
| C-6′ | 62.1 $CH_2$ | | | |

and 4.21(d), $\delta_C$ = 64.5] and a secondary carbinolic methine group [$\delta_H$ = 3.46 (dd), $\delta_C$ = 79.8] in agreement with the presence of diastereotropic protons of a hydroxymethylene group at C-24 and a 3 $\beta$(eq) hydroxy group, respectively [2]. Signals of a second carbinolic methine group were observed [$\delta_H$ = 4.18 (d), $\delta_C$ = 77.3 (C-19)]. The comparison of the C-19 carbon chemical shift with the $\delta$ values of the corresponding carbon atom in the related compounds with 19 $\alpha$(ax) hydroxy groups [6] showed an upfield shift of about 4 ppm for C-19 in **2** due to the $\gamma$-*gauche* interaction. From this observation the orientation of 19-OH was assumed to be $\beta$(eq). Therefore, the structure of **2** was proved to be olean-12-ene-3$\beta$,19$\beta$,24-triol. To our knowledge, this compound has not been described previously in the literature (Fig. 1).




(**1**) $R_1$ = Glu $R_2$ = $R_3$ = OH

(**2**) $R_1$ = H $R_2$ = $R_3$ = OH

(**3**) $R_1$ = $-CH_2-CH_2-CH_3$ $R_2$ = $R_3$ = H

Fig. 1. Structures of sigmoiside F (**1**), olean-12-ene-1$\beta$-24-diol (**2**) and propyloxyamyrin (**3**).

The configurations of the D-glucopyranosyl residue were regarded to be $\beta$ by the $J$ value (7.8 Hz) of its anomeric proton signal at $\delta_H$ = 4.62 [7]. The downfield shift of the C-3 signal in **1** as compared with the chemical shift of the corresponding carbon atom in olean-12-ene-3$\beta$,19$\beta$,24-triol (see Table 1) indicated that the glucose residue was bound through the glycosidic linkage to the C-3 hydroxyl group of the aglycone [8]. On the basis of the above findings the structure of sigmoiside F (**1**) was assigned to be olean-12-ene-3$\beta$,19$\beta$,24-triol-3-*O*-$\beta$-D-glucopyranoside (Fig. 1).

Compound **3**, named propyloxyamyrin (m. p. 102 – 104 °C,) has the molecular formula $C_{33}H_{56}O$ (*m/z* = 468.3330, $[M]^+$). It responded positively to the Lieberman-Burchard test. The IR absorption at $\nu$ (KBr) = 1031 $cm^{-1}$ indicated the presence of an ether function in **3**. The $^1$H NMR spectrum showed eight tertiary methyl signals ($\delta$ = 0.78, 0.82, 0.85, 0.92, 0.95, 0.98, 1.12, and 1.24), and an olefinic proton triplet ($\delta$ = 5.17) while the $^{13}$C NMR spectrum exhibited $sp^2$ carbon signals at 121.7 and 145.2 ppm. These signals were compatible with an olean-12-ene skeleton [4].

The $^1$H and$^{13}$C NMR spectra of **3** displayed signals for one *O*-methylene group [$\delta_H$ = 3.62 (q), $\delta_C$ = 63.1], a methylene group [$\delta_H$ = 2.35 (m), $\delta_C$ = 25.1] and a methyl group [$\delta_H$ = 1.53 (t), $\delta_C$ = 14.3] in agreement with the presence of an *n*-propyloxy group. In the mass spectrum, the peak at *m/z* = 426 was ascribed

to the loss of a propyl group from the molecular ion at *m/z* = 468. The presence of one oxygen atom in **3** together with biogenetic considerations suggested the propyloxy group to be located at C-3. A signal at $\delta$ = 3.20 (dd, $J_{ae}$ = 4.4 Hz, $J_{aa}$ = 10.6 Hz) in the $^1$H NMR spectrum was assigned to the 3 $\alpha$(axial) methine proton [2]. Comparison of the $^{13}$C NMR signals of compound **3** with those of $\beta$-amyrin **4** [6] (see Table 1) showed that **3** is 3$\beta$-*n*-propyloxy-$\beta$-amyrin (Fig. 1).

## Experimental Section

*General experimental procedures*

Melting points are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter at r. t. NMR experiments were performed on a Bruker WH-400 or 300 spectrometer. Samples were dissolved in $CDCl_3$ or [$D_6$]-DMSO and chemical shifts, expressed in ppm, were referenced to internal TMS (0.0 ppm). The FABMS spectrum was obtained with a Kratos MS 25 instrument with a DS-55 data system, and collision gas Xe (ion gun conditions 6 KV and 10 mA). EI mass spectra (at 70 eV) were recorded on a JEOL MSRoute mass spectrometer. IR spectra were run from KBr pellets on a Nicolet 20 DBX instrument. A glass column with dimensions 2.6 mm × 2 m packed with 1.5 % SE-30 on chromosorb W (carrier gas $N_2$) was used for GLC analysis on a Shimadzu GC-GA gas chromatograph. Si gel $GF_{254}$ (Merck) and Si gel 60 (70 – 230 mesh ASTM) (Merck) were used for the CC and TLC experiments, respectively.

*Plant material*

*Erythrina sigmoidea* (Fabaceae) stem bark was collected in Abagana-Nigeria, in January 2000. The plant was identified at the National Herbarium, Yaounde, where a reference sample is deposited (26645SRF.Cam).

*Extraction and isolation of compounds **1** and **3***

Air-dried and crushed stem bark of *Erythrina sigmoidea* (Fabaceae) (3.5 kg) was exhaustively extracted with MeOH and the extract concentrated to dryness under reduced pressure to yield a brown semi-solid residue (275 g). The residue was fractionated between hexane, EtOAc and MeOH. A 12 g portion of the MeOH solubles (98 g) [a 17 g portion of the EtOAc solubles (150 g)] was subjected to CC on silica gel (200 g) using an eluent of *n*-hexane-ethyl/acetate-methanol of increasing polarity. Fractions eluted with hex/AcOEt/MeOH 30/69/1 [with EtOAc/hex 1/4] afforded a residue which was recrystallized from $CH_2Cl_2$/MeOH 9.5/0.5 [EtOAc/hex 4/1] to yield compound **1** (66 mg) [compound **3** (200 mg)] as a colorless powder [as colorless needles].

*Sigmoiside F (**1**)*

M. p. 245 – 247 °C. – $[\alpha]_D^{22}$ = –30 (*c* = 0.11, MeOH). – IR (KBr) $\nu$ = 3600 – 3300, 1395, 1360, 1050, 1000 cm$^{-1}$. – $^1$H NMR (300 MHz, [$D_6$]-DMSO): $\delta$ = 0.78 (s, 3H, -$CH_3$), 0.86 (s, 3H, -$CH_3$), 0.89 (s, 3H, -$CH_3$), 0.95 (s, 3H, -$CH_3$), 0.98 (s, 3H, -$CH_3$), 0.99 (s, 3H, -$CH_3$), 1.22 (s, 3H, -$CH_3$), 3.40 (d, $^2J$ = 11.5 Hz, 1H, 24a-H), 3.61 (dd, $^3J$ = 5.2, 8.1 Hz, 1H, 3-H), 4.10 (d, $^3J$ = 3.3 Hz, 1H, 19-H), 4.22 (d, $^2J$ = 11.5 Hz, 1H, 24b-H), 4.62 (d, $^3J$ = 7.8 Hz, 1H, 1′-H of glucose), 5.21 (br s, 1H, 12-H). – $^{13}$C NMR: see Table 1. – HRMS (FAB, 6 kV): *m/z* = 643.4184 (calcd. 643.4185 for $C_{36}H_{60}O_8Na$, $[M+Na]^+$).

*Acetylation of compound **1***

Compound **1** (12 mg) was dissolved in a 1/1 mixture of pyridine/$Ac_2O$ and warmed up slightly. On standing overnight and on usual work-up it yielded the penta-acetyl derivative **1a** (14.5 mg). – M. p. 178 – 180 °C. – IR (KBr): $\nu$ = 1725, 1395, 1400, 1100, 1000 cm$^{-1}$. – HRMS (EI, 70 eV): *m/z* = 830.4815 (calcd. 830.4817 for $C_{46}H_{70}O_{13}$, $[M]^+$).

*Acid hydrolysis of sigmoiside F (**1**)*

Sigmoiside F (42 mg) was dissolved in 7 % $H_2SO_4$ and refluxed on a water bath for 4 h. Water (30 mL) was added to the reaction mixture which was then extracted with $CHCl_3$. Evaporation of the solvent followed by the purification of the residue by prep. TLC over silica gel with EtOAc/hex 8/2 as eluent gave compound **2** (21 mg). M. p. 212 – 213 °C. – $[\alpha]_D^{22}$ = +66° (*c* = 0.25, $CHCl_3$). – IR (KBr) $\nu$ = 3250, 1462, 1381, 1357 cm$^{-1}$. – $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 0.78 (s, 3H, -$CH_3$), 0.86 (s, 3H, -$CH_3$), 0.94 (s, 3H, -$CH_3$), 1.06 (s, 3H, -$CH_3$), 1.10 (s, 3H, -$CH_3$), 1.11 (s, 3H, -$CH_3$), 1.22 (s, 3H, -$CH_3$), 3.41 (d, $^2J$ = 11.2 Hz, 1H, 24a-H), 3.46 (dd, $^3J$ = 5.1, 8.4 Hz, 1H, 3-H), 4.18 (d, $^3J$ = 3.3 Hz, 1H, 19-H), 4.21 (d, $^2J$ = 11.2 Hz, 1H, 24b-H), 5.29 (t, $^3J$ = 3.2 Hz, 1H, 12-H). – $^{13}$C NMR: see Table 1. – HRMS (EI, 70 eV): *m/z* = 458.3759 (calcd. 458.3760 for $C_{30}H_{50}O_3$, $[M]^+$). – MS (EI, 70 eV): *m/z* (%) = 458 (6) $[M]^+$, 224 (18), 219 (88), 176 (60), 234 (100), 440 (6).

*Acetylation of compound **2***

Compound **2** (10 mg) was acetylated in the same manner as described for compound **1** to give the triacetyl derivative **2a** (10.9 mg). – M. p. 117 – 118 °C. – IR (KBr): $\nu$ = 1715, 1502, 1200, 1100 cm$^{-1}$. – HRMS (EI, 70 eV): *m/z* = 584.4077 (calcd. 584.4077 for $C_{36}H_{56}O_6$, $[M]^+$).

*Identification of the carbohydrate unit*

The aqueous solutions were neutralized with 1N NaOH and evaporated *in vacuo*. The residues obtained were ex-

amined by TLC using standard sugars, *n*-BuOH/toluene/pyridine/$H_2O$ 5/1/3/3 (BTPW) as eluent, and aniline hydrogen phthalate as the detection agent. GLC analysis followed the procedure [2].

*Propyloxyamyrin* ***3***

M. p. 102 – 104 °C. – $[\alpha]_D^{22} = +56°$ ($c$ = 0.25, $CHCl_3$). – IR (KBr): $\nu$ = 2916, 2848, 1462, 1381, 1357, 1032 $cm^{-1}$. – $^1H$ NMR (400 MHz, $CDCl_3$): $\delta$ = 0.78 (s, 3H, -$CH_3$), 0.82 (s, 3H, -$CH_3$), 0.85 (s, 3H, -$CH_3$), 0.92 (s, 3H, -$CH_3$), 0.95 (s, 3H, -$CH_3$), 0.98 (s, 3H, -$CH_3$), 1.12 (s, 3H, -$CH_3$), 1.24 (s, 3H, -$CH_3$), 1.53 (s, 3H, -$OCH_2CH_2CH_3$), 2.35 (m, 2H, -$OCH_2CH_2CH_3$), 3.20 (dd, $^3J$ = 4.4, 10.6 Hz, 1H, 3-H), 3.62 (q, $^3J$= 4.4 Hz, 2H, -$OCH_2CH_2CH_3$), 5.17 (t, $^3J$ = 3.4 Hz, 1H, 12-H). – $^{13}C$ NMR: see Table 1. – HRMS (EI, 70 eV): *m/z* = 468.3330 (calcd. 468.3331 for $C_{33}H_{56}O$, $[M]^+$). – MS (EI, 70 eV): *m/z* (%) = 468 (5) $[M]^+$, 426 (10), 218 (100).

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