CONSTITUENTS OF *ERYTHRINA SIGMOIDEA*

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ABSTRACT. The study of the secondary metabolites of the chloroform extract of the stem wood of Erythrina sigmoidea afforded a new compound α-sophoradiol (**1**), and the known compounds: acetate of β-amyrin (**2**), acetate of oleanolic acid (**3**), n-isocosanyl-isocosanoate (**4**), and n-hexatetracontanol (**5**). Their structures were characterised by spectroscopic methods.

KEY WORDS: Erythrina sigmoidea, Triterpenoids, Flavonoids, Isoflavonoids

INTRODUCTION

Natural compounds are presently being widely investigated because of the host of biological effects they exert [1-3]. Their presence in the genus *Erythrina* is widely used in Cameroon to treat syphilis, wounds of ulcers and female sterility [4-5]. *Erythrina sigmoidea* has already been shown to be rich in flavonoids [6-7], isoflavonoids [8], and triterpenoids saponins [9-10]. We report herein the isolation and characterisation of a new compound α-sophoradiol (**1**), in addition to the known compounds identified as acetate of β-amyrin (**2**), acetate of oleanolic acid (**3**), *n*-isocosanyl-isocosanoate (**4**), and *n*-hexatetracontanol (**5**) isolated for the first time from *Erythrina sigmoidea*. The use of two dimensional (2D) techniques enabled the completed assignment of the structures.

RESULTS AND DISCUSSIONS

Secondary metabolites were isolated from the chloroform extract of the stem wood. Compounds (**2**), (**3**), (**4**), and (**5**) were identified by comparison of their spectral data with those reported in the literature for acetate of β-amyrin (**2**) [11], acetate of oleanolic acid (**3**) [12], *n*-isocosanyl-isocosanoate (**4**) [13], and *n*-hexatetracontanol (**5**) [14].

Compound (**1**), α-sophoradiol, was obtained as light powder. Its molecular formula $C_{30}H_{50}O_2$ was deduced from the CI and the EI mass spectra which indicated the respective ions of *m/z* 443 [M + H]$^+$, *m/z* 460 [M + NH$_4$]$^+$ and 442 [M]$^+$. IR spectrum of compound (**1**) showed absorptions at 3500 cm^{-1} (free OH), 1650 cm^{-1} (C=C), 1350-1380 cm^{-1} (C(CH$_3$)$_2$), 1105 cm^{-1} (ether linkage). The mass spectrum also showed two important fragments at *m/z* = 424 and 406 indicating the loss of two water molecules suggesting that its skeleton contained free hydroxyl group.

The ^{1}H NMR spectrum in CDCl$_3$ showed signals of eight methyls at δ 0.77, 0.85, 0.89, 0.92, 0.95, 0.97, 1.02, 1.09; a vinylic proton centered at δ 5.20 (t, J = 3.52 Hz); an allylic proton at δ

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2.17, two protons geminal to secondary group at δ 3.42 (dd, J = 13.68, 4.28 Hz) and δ 3.22 (dd, J = 11.00, 6.50 Hz).

$CH_3-(CH_2)_{44}-CH_2OH$ $CH_3-(CH_2)_{17}-CH_2COO-CH_2-(CH_2)_{18}-CH_3$
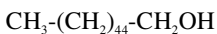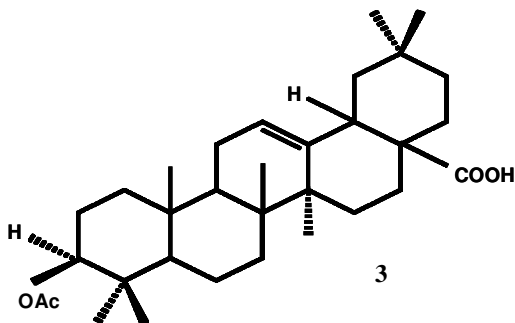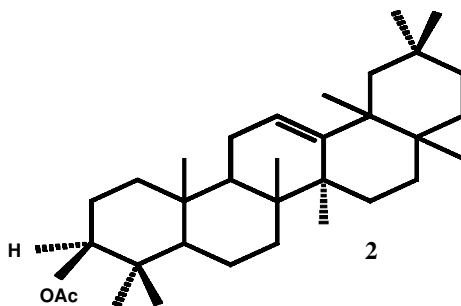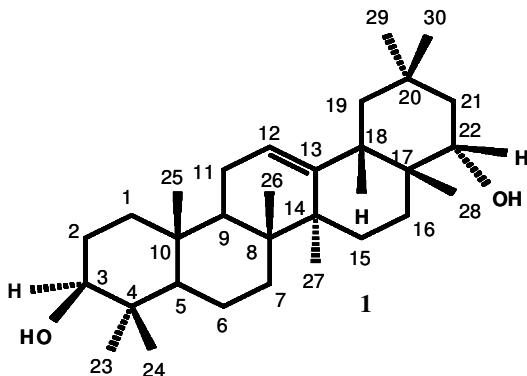
4   **5**

The ^{13}C and DEPT NMR spectra of (**1**) displayed six quaternary carbons, nine methylene, three methine, eight methyl groups in the region 15-35 ppm, two secondary oxygenated carbons at 79.00 and 76.58 ppm, of which one carbon carrying an oxygen was placed at the C-3 on biogenetic considerations [15] and a hydroxyl group was placed in β-position [16]. A trisubstituted double bond at 122.46 and 143.87 ppm due to sp^2 carbons which were completely

assigned on the basis of the 2D-HMBC and COSY spectra. Mass fragment peaks at m/z = 234 and 208, by retro Diels Alder's fission, indicated that the second hydroxyl group is attached on ring D or E.

Thus the skeleton of compound (**1**) proved to be that of a typical Δ^{12}-oleanene. Furthermore, the position of the second alcoholic function was determined by HMBC NMR and ^{1}H NMR, ^{1}H-^{1}H DQF COSY as follows: the HMBC spectrum of (**1**) clearly indicated correlations of the proton H-22 (δ 3.42) and C-20 (δ 30.51); H-22 (δ 3.42) and C-18 (δ 44.72); H-18 (δ 2.17) and C-22 (δ 76.58); H-22 (δ 3.42) and C-16 (δ 29.70); H-18 (δ 2.17) and C-16 (δ 29.70); H-18 (δ 2.17) and C-28 (δ 28.17); H-18 (δ 2.17) and C-20 (δ 30.51); H-22 (δ 3.42) and C-28 (δ 28.17); CH$_3$-30 (δ 0.92) and C-18 (δ 44.72). The COSY spectrum indicated correlations between H-22 (δ 3.42) and H-21 α/β (δ 2.20); H-22 (δ 3.42) and H-18 (δ 2.17).

Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) data (in ppm) of α-sophoradiol (**1**) in (CDCl$_3$) and β-sophoradiol in (pyridine-d$_5$).

N° carbons	α-Sophoradiol		β-Sophoradiol
	^{13}C NMR	^{1}H NMR	^{13}C NMR
1	36.63		39.20
2	27.23	2.08 (m)	28.20
3	79.00	3.22 (J = 11.00, 6.50 Hz)	78.10
4	37.50		39.40
5	55.20		55.90
6	18.37		18.90
7	32.85		33.30
8	39.90		40.10
9	47.62		48.10
10	36.90		37.30
11	23.49		23.90
12	122.46	5.20 (t, J = 3.52 Hz)	122.50
13	143.87		144.90
14	42.08		42.50
15	25.88		26.50
16	29.70		28.70
17	37.38		38.00
18	44.72	2.17 (J = 10.20 , 4.60 Hz)	45.40
19	46.12		46.90
20	30.51		30.90
21	41.46	2.20 (J = 12.20, 3.80 Hz)	42.30
22	76.58	3.42 (J = 13.68 , 4.28 Hz)	75.60
23	28.09	0.89	28.80
24	15.60	0.77	15.90
25	15.60	0.97	16.60
26	16.94	1.02	17.30
27	20.00	1.09	25.80
28	28.17	0.85	28.80
29	32.17	0.95	33.30
30	25.43	0.92	21.20

Physical properties of compound (**1**) were compared to β-sophoradial [17]. The ^{1}H and ^{13}C NMR spectra of compound (**1**) in CDCl$_3$ were compared to the ^{1}H [17] and ^{13}C NMR spectral data of β-sophoradiol in pyridine-d$_5$ (Table 1) [18]. The ^{13}C signal of C-22 (δ 76.58) of compound (**1**) revealed that the hydroxyl bearing C-22 is less shielded by the adjacent axial hydroxyl [19-20]. The carbinyl carbons containing an axial hydroxyl group are less shielded than their equatorial counterparts because of the strong 1,3-diaxial interactions Me…OH. The proton H-18 indicated the β-configuration because of the lack of γ-gauche interaction with C-27 methyl group.

The stereochemistry of C-22 center of compound (**1**) was established through NOESY spectral analysis (Figure 1) which indicated the spatial connectivities between H-22 (δ 3.42) and H-18 (δ 2.17); H-22 (δ 3.42) and CH$_3$-30 (δ 0.92), H-18 (δ 2.17) and CH$_3$-30 (δ 0.92). H-22 (δ 3.42) and CH$_3$-28 (δ 0.85); H-18 (δ 2.17) and CH$_3$-28 (δ 0.85); CH$_3$-30 (δ 0.92) and CH$_3$-28 (δ 0.85). These observations revealed that H-22 and H-18 have β-configuration. From the above spectroscopic studies, compound (**1**) was established as olean-12-en-3β,22α-diol.
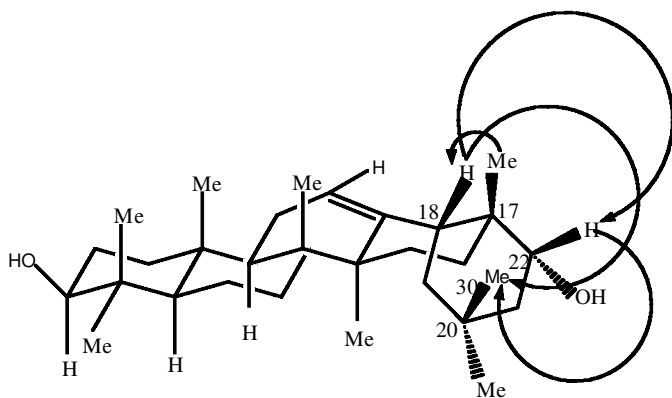

Figure 1. NOESY correlations in compound **1**.

EXPERIMENTAL

General. IR spectra were recorded on a Nicolet 7199FT instrument. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 300 spectrometer at 400 MHz and 100 MHz, respectively, with residual CHCl$_3$ in CDCl$_3$ employed as the internal standard. The ^{1}H and [13]C (broad band and DEPT) chemical shifts are expressed in ppm relative to TMS but measured against the central solvent peak at δ 7.26 and δ 77.00. Melting points were measured on a Kofler apparatus and are uncorrected.

The homonuclear [1]H-^{1}H shift correlated two-dimensional diagrams were obtained using the COSY-45 pulse sequence. NOESY experiments were performed on a Bruker AC 300 using steady-state difference experiments. Long range 2D heteronuclear shift correlation spectra were recorded using inverse mode correlation via heteronuclear zero and double quantum with a low-pass J-tilter to suppress one-band correlation in the operating Bruker software. The CI-mass spectra were recorded with (Finningan PSQ 700) direct flow injection of sample dissolved in

MeOH for positive ionisation (4.5 kV). EI-mass spectra were carried out with an LKB machine (70 eV) with direct inlet.

Plant material. *Erythrina sigmoide*a (hua) stem wood was collected at Foumban in Cameroon, in May 1998. A voucher specimen is deposited in the National Herbarium in Yaounde, Cameroon.

Extraction and isolation of constituents. The ground stem wood of *E. sigmoidea* (4 kg) was extracted continuously with chloroform. The CHCl$_3$ (22 g) extract was obtained on removal of the solvent under reduced pressure. A total of 75 fractions of 50 mL were collected and combined on the basis of TLC. The pure compounds were obtained from the combined fractions either by direct recrystallisation or after purification by CC or preparative TLC in MeOH/CHCl$_3$ and gave α-sophoradiol (**1**) (30 mg), acetate of β-amyrin (**2**) (100 mg), acetate of oleanolic acid (**3**) (400 mg), *n*-isocosanyl isocosanoate (**4**) (300 mg), and *n*-hexatetracontanol (**5**) (200 mg).

Physical and spectroscopic properties of the isolated compounds of chloroform extracts

α-Sophoradiol (1). Light powder MeOH-CHCl$_3$ (30 mg); m.p. 268-269 °C; IR v_{max} cm^{-1}: 3500 (OH), 1650 (C=C), 1350-1380 (C(CH$_3$)$_2$), 1105 (ether linkage); ^{1}H NMR (CDCl$_3$) (Table 1); ^{13}C NMR (CDCl$_3$) (Table 1); CI-MS: *m/z* 443 [M + H]$^+$, EI-MS: *m/z* (%) 442 (M$^+$, 10); 234 (100); 216 (8); 208 (16).

Acetate of β-amyrin (2). Crystallised as colourless needles from CHCl$_3$ (100 mg); m.p. 240-241 °C (lit. [11] m.p. 241-242 °C, [α]$_D$ 81.0); C$_{32}$H$_{52}$O$_2$. IR v_{max} cm^{-1}: 3450 (OH), 1735 (C=O, ester), 1650 (C=C), 1360-1365 (C(CH$_3$)$_3$). ^{1}H NMR (CDCl$_3$) δ: 0.83 (3H, s, CH$_3$); 0.86 (3H, s, CH$_3$); 0.87 (3H, s, CH$_3$); 0.88 (3H, s, CH$_3$); 0.96 (3H, s, CH$_3$); 0.97 (3H, s, CH$_3$); 1.07 (3H, s, CH$_3$); 1.13 (3H, s, CH$_3$), 2.40 (3H, s, CH$_3$); 4.52 (1H, dd, J = 10.50, 5.80 Hz, H-3); 5.19 (t, J = 3.50 Hz, H-12). ^{13}C NMR (CDCl$_3$) δ: 38.28 (C-1); 26.96 (C-2); 80.99 (C-3); 37.71 (C-4); 55.31 (C-5); 18.31 (C-6); 32.64 (C-7); 38.80 (C-8); 47.61 (C-9); 36.85 (C-10); 23.58 (C-11); 121.69 (C-12); 145.21 (C-13); 41.72 (C-14); 23.62 (C-15); 26.14 (C-16); 46.86(C-17); 47.40 (C-18); 46.80 (C-19); 31.07 (C-20); 37.15 (C-21); 32.74 (C-22); 28.08 (C-23); 16.85 (C-24); 15.60 (C-25); 16.73 (C-26); 25.73 (C-27); 28.43 (C-28); 33.73 (C-29); 25.99 (C-30); 21.34 (COOCH$_3$); 170.99 (COOCH$_3$). MS: *m/z* (%) 468 (M$^+$, 9); 250 (6); 218 (16); 207 (6); 43 (100).

Acetate of oleanolic acid (3). Crystallised as colourless powder from CHCl$_3$ (400 mg); m.p. 260-262 °C; (lit. [12] m.p. 259-260 °C); C$_{32}$H$_{50}$O$_4$. IR v_{max} cm^{-1}: 3450 (OH), 3000 (OH, broad band), 1740 (C=O, ester), 1350-1380 (C(CH$_3$)$_2$). ^{1}H NMR (CDCl$_3$) δ: 0.80 (3H, s, CH$_3$); 0.85 (3H, s, CH$_3$); 0.89 (3H, s, CH$_3$); 0.92 (3H, s, CH$_3$); 1.02 (3H, s, CH$_3$); 1.07 (3H, s, CH$_3$); 1.13 (3H, s, CH$_3$); 2.15 (3H, s, CH$_3$); 4.15 (1H, dd, J = 11.50, 6.00 Hz, H-3); 5.13 (1H, t, J = 3.50 Hz, H-12). ^{13}C NMR (CDCl$_3$) δ: 39.07 (C-1); 25.54 (C-2); 79.00 (C-3); 37.50 (C-4); 55.27 (C-5); 17.78 (C-6); 32.50 (C-7); 39.98 (C-8); 47.43 (C-9); 37.17 (C-10); 22.90 (C-11); 122.90 (C-12); 143.64 (C-13); 41.70 (C-14); 27.66 (C-15); 23.54 (C-16); 46.66 (C-17); 41.07 (C-18); 45.79 (C-19); 30.67 (C-20); 33.75 (C-21); 32.11 (C-22); 27.96 (C-23); 16.20 (C-24); 15.33 (C-25); 16.90 (C-26); 25.82 (C-27); 183.10 (COOH); 33.05 (C-29); 23.43 (C-30); 21.42 (COOCH$_3$); 172.10 (COOCH$_3$). MS: *m/z* (%) 498 (M$^+$, 6); 248 (5); 207 (6); 43 (100) 204 (16).

*n-Isocosanyl isocosanoate (**4**)*. Crystallised as colourless powder from $CHCl_3$ (300 mg); m.p. 69-70 °C; (lit. [13] m.p. 70-72 °C); $C_{40}H_{80}O_2$. IR ν_{max} cm^{-1}: 2900, 1730, 720. ^{1}H NMR ($CDCl_3$) δ: 0.85 (6H, t, 2CH$_3$); 1.25 (70H, s, 35CH$_2$); 2.15 (2H, t, CH$_2$CO); 4.05 (2H, t, CH$_2$OCO). MS: *m/z* (%) 592 (M$^+$, 6); 325 (25); 295 (100); 265 (50); 281 (8).

*n-Hexatetracontanol (**5**)*. Crystallised as colourless needles from MeOH-$CHCl_3$ (200 mg); m.p. 271-272 °C; (lit. [14] m.p. 270 °C); $C_{46}H_{94}O$. IR ν_{max} cm^{-1}: 2900, 1045, 720. ^{1}H NMR ($CDCl_3$) δ: 0.86 (3H, t, CH$_3$); 1.22 (88H, s, (CH$_2$)$_{44}$); 3.27 (2H, t, CH$_2$OH).

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