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TRITERPENOID SAPONINS FROM *ERYTHRINA SIGMOIDEA**

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Key Word Index—*Erythrina sigmoidea*; Leguminosae; stem bark; saponins.

Abstract—Two new triterpenoid saponins, designated sigmoside C and D, have been isolated from the methanol extract of the stem bark of *Erythrina sigmoidea* in addition to the known soyasapogenol-B and 3-*O*-[β-D-glucopyranosyl]-sitosterol. The structures of the saponins were determined by chemical and spectroscopic means as 22-*O*-[β-D-glucopyranosyl]-soyasapogenol-B and 22-*O*-[α-L-rhamnopyranosyl]-soyasapogenol-B, respectively. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Saponins are presently being widely investigated because of the host of biological effects they exert [1–5]. Their presence in the plants of the genus *Erythrina*, widely used in Cameroon to treat syphilis, wounds, ulcers and female sterility has prompted our investigation of the saponin constituents of this group of plants, which have already been shown to be rich in flavonoids [6–10], isoflavonoids [11] and triterpenoid saponins [12]. Preliminary bioassays on the crude methanol extracts and on some pure compounds isolated from *Erythrina* species have shown antimicrobial, antimitotic and diuretic properties [13, 14].

We now report the isolation and characterization of two new triterpenoid saponins designated sigmoside-C and sigmoside-D along with the known soyasapogenol-B and 3-*O*-[β-D-glucopyranosyl]-sitosterol, isolated for the first time from *Erythrina sigmoidea*.

RESULTS AND DISCUSSION

The methanol extract of the stem bark after successive extraction with *n*-hexane and ethyl acetate, followed by repeated column chromatography on silica gel of the residue afforded compounds **1–4**.

Sigmoside C (**1**). $C_{36}H_{60}O_8$, mp, 246–248°C, $[M]^+$ at *m/z* 620 using FABMS, obtained as a powder from a solution in CH_2Cl_2 was found to be a triterpenoid from the Liebermann–Burchard test. It showed no UV-absorption band above 220 nm. A broad band in the IR spectrum at ν_{max} 3500–3350 cm^{-1} was ascribed to hydroxyl groups; bands at 1393, 1385 and 1370 cm^{-1} were attributed to gem-dimethyl and/or an isopropyl group. The presence of seven tertiary methyl singlets at δ 0.86, 0.89, 0.92, 0.95, 0.97, 1.02, 1.10, a vinylic proton centered at δ5.22 (*t*, *J* = 3.9 Hz) in the ^1H NMR spectrum and signals in the ^{13}C NMR spectrum at δ123.5 and 145.39 due to sp^2 carbons, and mass fragmentation suggested an oleanene derivative [15, 16], carrying seven methyl groups. In addition, the presence of diastereotopic protons of a hydroxymethylene group was inferred from the AB doublet (*J* = 11.79 Hz) at δ3.65 and 4.10 and was assigned to C-24 [17]. Furthermore, signals in the ^1H NMR at δ3.45 (*dd*, J_{aa} = 9.50 Hz and J_{ae} = 5.50 Hz) and at δ3.19 (*dd*, J_{aa} = 11.00 Hz and J_{ae} = 4.30 Hz) indicated the presence on the oleanene skeleton of two carbons carrying an oxygen in the equatorial position [18], one of which was placed at C-3 on biogenetic considerations.

The compound was subjected to acid hydrolysis with 7% H_2SO_4 to yield an aglycone that was identified to soyasapogenol-B (**3**) from its physical and spectral data (IR, ^1H NMR and ^{13}C NMR) [19]. The glycone component was identified by TLC and GLC of its TMSi derivative to glucose. This was confirmed by signals in ^{13}C NMR spectrum at δ102.14, 78.73, 77.70, 75.23, 71.96 and 62.99 which agree with data published for D-glucose [20]. Acetylation of compound **1** with acetic anhydride in pyridine yielded a hexaacetate (**5**) whose mass spectrum showed an $[M]^+$ ion at *m/z* 872. Mass fragments at *m/z* 525 $[M-(Glu-4Ac)]^+$ and diagnostically prominent peaks at *m/z* 308 and 564 from the retro Diels–Alder's fragmentation, suggested that the glucose was on ring D or E and the hydroxyl groups of the natural products were on C-3 and C-24. In addition, a FAB-mass

* Part 33 in the series 'Erythrina Studies'. For part 32 see ref. [11].

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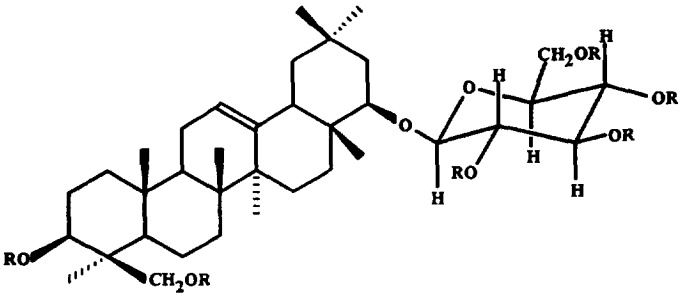
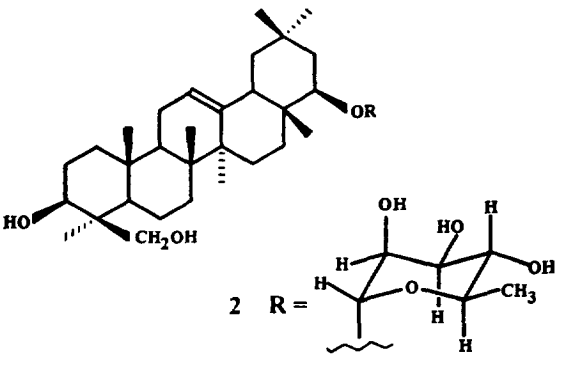
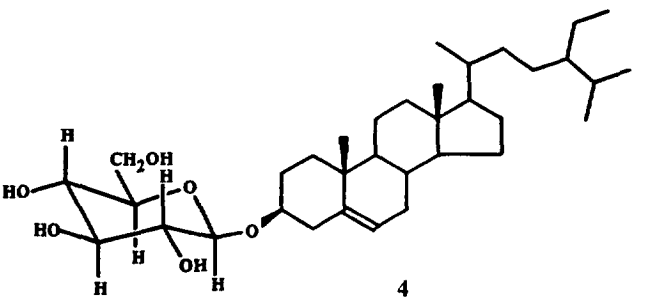


1 R = H

5 R = Ac



3 R = H



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spectrum of **5** gave a peak at *m/z* 541 corresponding to $[M-H-330]^+$. A β-D-glucopyranosyl configuration was deduced from the coupling constant ($J = 8.0$ Hz) of the anomeric proton signal at δ4.50 in the ^1H NMR [21]. 2D COSY experiments established most of the spin systems leading to the unambiguous assignment of the signals at δ4.36 and 4.14 to the diastereotopic protons of the hydroxymethylene group at C-24. From the 2D-HMQC and 2D-HMBC spectra of the acetylated derivative, particularly the 2- and 3-bond correlations, connectivity patterns were observed between the anomeric proton (H-1′) signal at δ4.50 and the signal of the C-22 carbon at δ83.50. Similar connectivities were also observed for H-2′ (δ4.99) with C-1′ (δ98.50) and C-3′ (δ74.0); H-3′ (δ5.19) with C-2′ (δ72.0) and C-4′ (δ69.0); H-4′ (δ5.07) with C-3′ (δ74.0), C-6′ (δ62.99) and C-2′ (δ72.0). This led to the assignment of the protons and carbons of the glucose and its position at C-22 (Table 1). On the basis of this evidence, the structure of compound **1** was elucidated to be 22-*O*-[β-D-glucopyranosyl]-soyasapogenol-B.

Sigmoside D (**2**). $C_{36}H_{60}O_7$, mp 220–222°C, $[M]^+$ at *m/z* 604 obtained as a white powder from a solution in methylene dichloride, was found to be a triterpenoid saponin similar to compound **1** from its positive response to the Liebermann–Burchard test and its ^1H NMR and ^{13}C NMR spectra.

Acid hydrolysis under the same conditions as mentioned above for compound **1** gave for compound **2** an aglycone identified as soyasapogenol-B and a glycone identified as L-rhamnose by TLC and GLC of its TMSi derivative. The latter was confirmed by the ^{13}C NMR spectrum in which signals at δ102.11, 75.19, 71.90, 69.80, 66.60 and 16.12 matched well with

Table 1. ^{1}H NMR and ^{13}C NMR spectra of sigmoside C (**1**), its hexaacetate (**5**) and sigmoside D (**2**)

	1		**5**		**2**	
C	^{1}H(400 MHz, *J* (Hz))	^{13}C(75 MHz)	^{1}H(500 MHz, J (Hz))	^{13}C(75 MHz)	^{1}H(400 MHz, J (Hz))	^{13}C(75 MHz)
01		38.13		38.13		38.13
02		28.85		29.39		28.85
03	3.45 *dd*, *J* = 5.5, 9.5	81.73	4.58 *dd*, *J* = 5.0, 10.5	80.60	3.2 *dd*, *J* = 5.5, 9.0	81.73
04		37.77		40.83		37.77
05		55.26		55.26		55.26
06		19.80		19.80		19.80
07		34.50		34.50		34.50
08		39.72		39.72		39.72
09		48.00		48.00		49.10
10		36.86		36.86		36.86
11		24.80		24.80		24.80
12	5.22 *t*, *J* = 3.9	123.50	5.22 *t*, *J* = 3.5	123.00	5.22 *t*, *J* = 3.9	123.50
13		145.39		145.32		145.39
14		42.09		48.00		42.09
15		26.85		26.85		24.58
16		29.39		28.85		29.34
17		37.92		37.92		38.12
18		46.00		46.50		46.90
19		47.49		47.49		47.47
20		31.26		31.26		31.27
21		38.00		38.00		37.87
22	3.19 *dd*, *J* = 4.3, 11.0	83.50	3.38 *dd*, *J* = 4.3, 12.0	83.50	3.15 *dd*, *J* = 4.3, 11.0	83.29
23		23.20		21.08		23.20
24a	3.65 *d*, *J* = 11.79		4.36 *d*, *J* = 11.5		4.10 *d*, *J* = 11.79	
24b	4.10 *d*, *J* = 11.79	65.31	4.14 *d*, *J* = 11.5	65.40	3.65 *d*, *J* = 11.79	62.95
25		16.60		16.60		16.17
26		17.52		17.52		17.64
27		25.58		25.38		25.64
28		21.08		23.20		21.08
29		33.00		33.00		33.62
30		28.00		28.70		28.70
1′	4.2 *d*, *J* = 7.9	102.14	4.5 *d*, *J* = 8.0	98.50	4.25 *d*, *J* = 1.4	102.11
2′		75.23	4.99 *t*, *J* = 8.0	72.00	3.85 *t*, *J* = 3.7	71.90
3′		78.37	5.19 *t*, *J* = 9.5	74.00	3.65 *t*, *J* = 10	69.80
4′	3.15–3.65	71.96	5.07 *t*, *J* = 10.0	69.00		
5′	Protons of glucose	77.70	3.65 *m*	71.7	3.3 *t*, *J* = 9.7	75.19
6′a			4.14 *dd*, *J* = 12; *J* = 2.5		3.5 *m*	66.60
6′b		62.99	4.22 *dd*, *J* = 11.6, *J* = 5.0	63.00	0.95 *d*, *J* = 6	16.12

those published for L-rhamnopyranosides [22]. Mass fragment peaks at *m/z* 458 (M^+ − rhamnosyl), at *m/z* 334 by retro Diels–Alder's fission indicated that the rhamnose moiety is attached on ring D or E. A doublet in the ^{1}H NMR spectrum at δ4.25 (*J* = 1.4 Hz) assigned to the anomeric proton, confirmed the α-L-rhamnose configuration [23]. The ^{13}C-DEPT spectrum exhibited the presence of seven tertiary methyl groups at δ33.62, 28.70, 25.64, 23.20, 21.08, 17.64, and 16.17. An additional secondary methyl group at δ16.12 replaced the signal for the carbon of the hydroxymethylene group of the glucose in compound **1** (Table 1), further indicating that the glycone in compound **2** is an α-L-rhamnose. The rhamnose moiety was placed at C-22 from a close similarity observed between the carbon signals in compounds **1** and **2** and the 2D COSY experiment. The structure of compound **2** was therefore elucidated as 22-*O*-[α-L-rhamnopyranosyl]-soyasapogenol-B.

Compound **3**. $C_{30}H_{50}O_3$, mp 258°C, $[M]^+$ at *m/z* 458 which also gave a positive Liebermann–Burchard test was identified as soyasapogenol-B from its IR, ^{1}H NMR and ^{13}C NMR spectra [19].

Compound **4**. $C_{35}H_{60}O_6$, mp 259°C, $[M]^+$ at *m/z* 576 was identified as 3-*O*-[β-D-glucopyranosyl]-sitosterol [24].

EXPERIMENTAL

UV spectra were obtained on a Unicam SP 1700 spectrophotometer. IR spectra were recorded on a Nicolet 7199 FT instrument. ^{1}H NMR spectra were measured on a Bruker WH-360 spectrometer or a Bruker WH-400 spectrometer with residual $CHCl_3$ in

$CDCl_3$ or MeOH in CD_3OH employed as the internal standard. ^{13}C NMR spectra were measured on a Bruker WH-300 spectrometer of a Bruker WH-400 spectrometer with $CDCl_3$ or CD_3OD employed as int. standard (assigned as 77.00 ppm or 49.00 ppm downfield from TMS). Mps were measured on a Kofler hot-stage apparatus and were uncorr.

Plant material. Erythrina sigmoidea (Hua) stem bark was collected at Foumban, Cameroon, in May 1988. An herbarium specimen documenting the collection was identified at the National Herbarium, Yaounde, Cameroon and is on deposit there.

Extraction and isolation. The ground stem bark of *E. sigmoidea* (7.5 kg) was extracted with MeOH. The MeOH extract (750 g) after concn under red. pres. was successively extracted with *n*-hexane (4 × 500 ml) and EtOAc (4 × 500 ml) to give 51 g and 386 g of extracts, respectively. The residue (300 g) was subjected to dry column flash CC (6 × 50 g) using hexane–EtOAc and EtOAc–MeOH in increasing polarity as eluent. A total of 130 frs of 200 ml each were collected for every 50 g portion of extract and combined on the basis of TLC. Frs 100–120 (36 g) were purified using CC on silica gel with $CHCl_3$ and $CHCl_3$–MeOH in increasing polarity as eluent. From a total of 70 frs of 100 ml each, fractions 14–26 were combined on the basis of TLC and further purified using prep. TLC. MeOH–$CHCl_3$ (1:4) to give compound **1** (45 mg) and compound **2** (43 mg). Frs 8–18 (39 g) were chromatographed on silica gel using $CHCl_3$–MeOH (95:5) to give compound **3** (20 mg) and compound **4** (45 mg).

Sigmoside C (**1**). Powder (45 mg); mp 246–248°; $[\alpha]_D^{25}$ −13.0° ($CHCl_3$; *c* 0.05); IR ν_{max} KBr cm^{-1}: 3500–3350 (OH); 1640 (C=C); 1393, 1385, 1370 (gem dimethyl). ^1H NMR; ^{13}C NMR. Table 1.

Acid hydrolysis of sigmoside C. The sample (30 mg) was dissolved in 7% H_2SO_4 and refluxed on a water bath for 4 hr. The reaction mixture was diluted with 30 ml of H_2O and extracted with CH_2Cl_2. Evapn of solvent followed by purification of the residue by prep. TLC over silica gel with toluene–Me_2CO (10:3) as eluent gave a white compound identified as soyasapogenol-B by comparison with an authentic sample (TLC, IR mp, NMR).

Identification of sugar. The aq. phase after extraction with CH_2Cl_2 was neutralized with 1N NaOH and evapd in vacuum. H_2O was added to the residue and the mixt. was again evapd in vacuum in order to remove all the impurities. The residue obtained was compared with standard sugars by TLC using *n*-BuOH–toluene–pyridine–H_2O (5:1:3:3) (BTPW). The sugar was detected with aniline hydrogen phalate, and shown to consist of D-glucose. For GLC analysis the residue was dissolved in TRISIL (0.05 ml; *N*-(trimethylsilyl)-imidazole in pyridine), left at room temp. for 15 min, and analysed by GLC on a SHIMADZU GC-GA gas chromatograph, glass column 2.6 mm × 2 m packed with 1.5% SE-30 on chromasorb w, detector FID injection temp. 150°, carrier gas N_2 (40 ml min^{-1}). The GLC peaks of the silylated derivative of the residue and of glucose had the same retention time (R_t 4.9 min).

Acetylation of sigmoside C. The sample (10 mg) was dissolved in 5 ml pyridine and 5 ml Ac_2O added to the soln which was kept overnight. Work-up gave 9.5 mg of acetate (Table 1).

Sigmoside D (**2**). Powder (43 mg); mp 220–222°; $[\alpha]_D^{25}$ −19.0° ($CHCl_3$; *c* 0.02); IR ν_{max} KBr cm^{-1}: 3500–3355 (OH); 1642 (C=C). ^{1}NMR, ^{13}C NMR (Table 1).

Identification of sugar. The sample (30 mg) was hydrolysed as described above, and the aglycone was identified as soyasapogenol-B by comparison with an authentic sample.

The sugar was identified as L-rhamnose by comparison of its trimethylsilylated derivative to that of standard sugars, using GLC as described above.

Soyasapogenol B (**3**). Powder; mp 246–288°; IR, ^1H NMR, ^{13}C NMR were in agreement with lit. [19].

3-O-[α-D-*glycopyranosyl*]-*sitosterol* (**5**). Powder; mp 259°; IR, ^1H NMR, ^{13}C NMR were in agreement with lit. [24].

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