Two New Triterpenoid Saponins from Erythrina sigmoidea

J. Kouam, A. Ephrem Nkengfack, Z. Tanee Fomum, Rosa Ubillas, Michael S. Tempesta, and Michèle Meyer

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TWO NEW TRITERPENOID SAPONINS FROM *ERYTHRINA SIGMOIDEA*[1]

J. KOUAM, A. EPHREM NKENGFACK, Z. TANEE FOMUM,*

Department of Organic Chemistry, University of Yaounde, B.P 812 Yaounde, Cameroon

ROSA UBILLAS, MICHAEL S. TEMPESTA,

Department of Chemistry, University of Missouri, Columbia, Missouri 65211

and MICHÈLE MEYER

Laboratoire de Chimie du Museum National D'Histoire Naturelle, 63 Rue Buffon, 75005 Paris Cedex 05, France

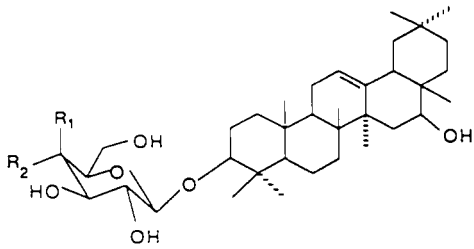
ABSTRACT.—Two new triterpenoid saponins, designated sigmoisides A [**1**] and B [**2**] have been isolated from the MeOH extract of the wood and the stem bark, respectively, of *Erythrina sigmoidea* in addition to known maniladiol. Their structures were established by chemical and spectroscopic means as 3-*O*-[β-D-galactopyranosyl]maniladiol [**1**] and 3-*O*-[β-D-glucopyranosyl]maniladiol [**2**].

Plants of the genus *Erythrina* (Leguminosae) whose decoctions of bark and roots are widely used in West African folk medicine for the treatment of female infertility, stomach pain, and gonorrhea (1–3), have recently been reported as a rich source of cinnamate esters (4,5), pterocarpan (6), and flavonoid compounds (5, 7–10). Some of these compounds, such as *n*-octacosanyl 4-hydroxy-3-methoxycinnamate, 6,8-diprenylgenistein, and auriculatin, recently reported as constituents of *Erythrina senegalensis* (4), *Erythrina excelsa* (10), and *Erythrina eriotricha* (5,11), were subjected to pharmacological screening. The results of these tests revealed that all these compounds are completely nontoxic when administrated orally and peritoneally. They possessed reflex depression, behavioral depression, and muscle relaxant properties on the central nervous system of mice and also displayed antiarrhythmic effects as cardiovascular agents in addition to their antibacterial activity against *Staphylococcus aureus* and antifungal activity against *Cladosporium herbarum* (12).

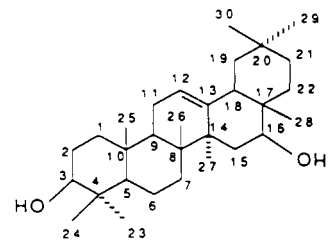
Continuing the search for new neutral components of the Cameroonian medicinal plant *Erythrina sigmoidea* Hua, we have isolated two new triterpenoid saponins designated sigmoisides A [**1**] and B [**2**] along with the known maniladiol [**3**] (13). In this paper, we report the isolation and structural elucidation of these two compounds.

RESULTS AND DISCUSSION

Repeated Si gel cc followed by preparative tlc of the MeOH extract of the wood of *E.*



1 R_1=H, R_2=OH
2 R_1=OH, R_2=H



3

[1]Part 20 in the series "Erythrina Studies."

sigmoidea afforded sigmoside A [**1**] together with the known maniladiol [**3**]. The latter was identified by physical and spectral data (ir, ^{1}H-nmr, and ^{13}C-nmr) as well as by comparison with an authentic sample of maniladiol previously isolated from *E. eriotricha* (13).

Sigmoside A [**1**], mp 239–241°, $[\alpha]^{22}D$ −16°, obtained as colorless needles from MeOH, responded positively to the Liebermann-Burchard test. Its ir spectrum exhibited strong hydroxyl absorption bands at 3540–3200, 1065, and 1030 cm^- and *gem*-dimethyl groups at 1378 and 1363 cm^{-1}. The negative ion fabms of [**1**] showed a quasi molecular ion peak at *m/z* 603 $[M-H]^-$ while its broad-band-decoupled ^{13}C-nmr spectrum and ^{13}C-DEPT spectrum exhibited the presence of 36 carbon signals (-CH_3 × 8, -CH_2 × 9, -CH × 4, -C- × 7, -CH_2O × 1, -CHO × 7) (Table 1). These data indicated **1** to have the molecular formula $C_{36}H_{60}O_7$. This substance was subjected to acid

TABLE 1. ^{13}C-nmr (75.4 MHz, DMSO-d_6) Assignments for Sigmoside A [**1**], Sigmoside B [**2**], and Maniladiol [**3**].

Carbon	Compound		
	1	**2**	**3**
C-1	38.3 t	39.1 t	38.6 t
C-2	27.1 t	27.3 t	27.7 t
C-3	80.9 d	81.8 d	78.9 d
C-4	38.4 s	38.5 s	38.8 s
C-5	54.8 d	55.6 d	55.4 d
C-6	18.0 t	18.8 t	18.5 t
C-7	32.5 t	33.2 t	32.6 t
C-8	41.6 s	40.9 s	39.7 s
C-9	47.1 d	47.9 d	47.7 d
C-10	37.3 s	37.3 s	36.9 s
C-11	23.1 t	23.4 t	23.7 t
C-12	121.0 d	122.6 d	122.3 d
C-13	143.9 s	144.7 s	144.2 s
C-14	42.0 s	42.4 s	41.7 s
C-15	27.6 t	27.7 t	28.1 t
C-16	77.1 d	77.8 d	77.8 d
C-17	46.1 s	46.7 s	46.8 s
C-18	44.9 d	42.4 d	41.3 d
C-19	45.9 t	45.7 t	46.4 t
C-20	30.6 s	30.8 s	31.1 s
C-21	36.4 t	37.2 t	35.8 t
C-22	32.3 t	33.1 t	32.1 t
C-23	28.3 q	29.0 q	28.4 q
C-24	16.1 q	16.8 q	15.7 q
C-25	15.4 q	16.1 q	16.0 q
C-26	16.8 q	17.6 q	16.9 q
C-27	25.1 q	25.8 q	26.1 q
C-28	28.2 q	28.4 q	28.5 q
C-29	32.3 q	33.2 q	33.1 q
C-30	20.6 q	21.3 q	22.3 q
C-1′	Gal 100.7 d	Glu 101.5 d	
C-2′	73.6 d	74.4 d	
C-3′	76.8 d	77.8 d	
C-4′	70.3 d	71.2 d	
C-5′	76.8 d	77.5 d	
C-6′	61.3 t	63.5 t	

hydrolysis with 2 N H_2SO_4 to yield aglycone **3** along with a carbohydrate component. The latter was identified as galactose by tlc and glc of its TMSi derivative. This was confirmed by the signal in fabms of **1** at *m/z* 441 $[M-H-162]^-$ corresponding to the loss of hexosyl moiety from the molecular ion, as well as by ^{13}C-nmr spectrum of sigmoiside A in which signals at δ 100.7, 73.6, 76.8, 70.3, 76.8, and 61.3 ppm agree well with data published for D-galactose (14–16).

Aglycone **3** was characterized as maniladiol by comparison of its physical and spectroscopic data with those of an authentic sample of maniladiol also isolated from the same source. The ^{13}C-nmr assignments of **1** (Table 1) were made on the basis of the known related compounds (17,18) as well as the observed multiplicities in the DEPT spectrum of this compound. The α-D-pyranosyl configuration of the galactose moiety in **1** was deduced from the coupling constant ($J=7.1$ Hz) of the anomeric proton signal at δ 4.89 in the ^1H-nmr spectrum; the ^{13}C-nmr spectrum showed an anomeric carbon signal at δ 100.7 ppm.

For clarification of the location of the galactose moiety, the ^{13}C-nmr spectrum was inspected. The spectrum corresponding to the aglycone part of **1** showed signals essentially identical with those of **3** except for those due to the A-ring carbons. Among these A-ring carbon signals, the C-3 carbon signal was significantly downfield (δ 80.9; in **3**, this signal appeared at δ 78.9 ppm), and the C-2 and C-4 carbon signals were slightly shifted upfield (Table 1). The observations implied that the galactose molecule was bound through the glycoside linkage to the C-3 hydroxyl group of the aglycone **3** (18–20). On the basis of the above evidence, the structure of sigmoiside A [**1**] was elucidated to be 3-*O*-[β-D-galactopyranosyl]maniladiol.

Sigmoiside B [**2**], mp 135–138°, $[\alpha]^{22}D$ −27°, was obtained as white powder from the MeOH extract of *E. sigmoidea* stem bark and responded positively to Liebermann-Burchard reaction. Its ir and ^{13}C-nmr spectral data were very similar to those of **1**, suggesting **2** to be a congener of **1**. On acid hydrolysis under the same conditions as mentioned above for compound **1**, compound **2** gave **3** and D-glucose, which were identical by tlc, glc, and ir with authentic samples. The latter was confirmed by the ^{13}C-nmr spectrum, in which signals at δ 101.5, 74.4, 77.8, 71.2, 77.5, and 63.5 matched well with those published for D-glucopyranoside (19). The configuration at the anomeric position of the glucose moiety was easily assigned to β from the coupling constant of the anomeric proton signal at δ 4.85 ppm (1H, d, $J=6.8$ Hz), while the location of the glucose molecule in **2** was established by ^{13}C-nmr analysis. It was concluded from the anomeric carbon signal at δ 101.4 ppm and the C-3 carbon signal at δ 81.8 ppm and glycosylation shift of the C-2 and C-4 carbon signal (17,18) that the C-1′ of the glucose moiety was bound to the C-3 hydroxyl group of the aglycone through glycosidic linkage. Thus, the structure of sigmoiside B [**2**], which is an isomer of sigmoiside A [**1**], has been determined to be 3-*O*-[β-glucopyranosyl]maniladiol.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURE.—All mp's were determined on a Kofler hot stage apparatus and were uncorrected. Fabms spectra were obtained with Kratos MS-25 with DS-55 data system and on JEOL JMS-DX 303 mass spectrometer, collision gas Xe (ion gun conditions: 6 kV and 10 mA) and matrix glycerol or thioglycerol. Optical rotations were measured on a Perkin-Elmer 241 polarimeter at room temperature. Ir spectra were run on a Nicolet 20DBX. Si gel GF_{254} (Merck) and Si gel 60 (70–230 mesh ASTM) (Merck) were used for tlc and cc, respectively. All nmr experiments were performed on a Nicolet NT-300wB or 300 Bruker spectrometer equipped with 5 mm ^1H and ^{13}C probe operation at 300.06 and 75.45 MHz, respectively. Samples were run in DMSO-d_6, and chemical shifts, expressed in ppm, were referenced to internal TMS (0.0 ppm) for ^1H-nmr and to deuterated solvent (DMSO-d_6) for ^{13}C-nmr.

PLANT MATERIAL.—*E. sigmoidea* wood and stem bark were collected in July 1986 at Foumban, Cameroon. An herbarium specimen documenting the collection was identified at the National Herbarium, Yaounde Cameroon, and is deposited there.

EXTRACTION AND ISOLATION OF SIGMOISIDE A [**1**].—The dried ground wood of *E. sigmoidea* (5 kg) was successively extracted in a Soxhlet extractor with MeOH. The resulting extract (900 g) was concentrated and partitioned with EtOAc. Cc of the EtOAc extract over Si gel gave, on elution with $CHCl_3$, maniladiol [**3**] (0.62 g). With $CHCl_3$ containing 15% MeOH, cc of the EtOAc extract yielded impure sigmoiside A (0.53 g), which was subjected to a repeat of column procedure followed by preparative tlc over Si gel, using $CHCl_3$-Me_2CO (3:2) as eluent to give sigmoiside A [**1**] (0.10 g).

Maniladiol [**3**] was identified by comparison of its mp 224° [lit. (13) 221°] and spectral data (ir, ^1H nmr, and ^{13}C nmr) with those of an authentic sample.

Sigmoiside A [**1**].—Colorless needles (EtOAc/MeOH), mp 239–241°, $[\alpha]^{22}D$ −16° (c = 0.04, MeOH); ir ν max (KBr) 3540–3200, 1378, 1363, 1065, 1030 cm^{-1}; ^1H nmr (DMSO-d_6) 0.68 (3H, s, Me), 0.81 (3H, s, Me), 0.86 (3H, s, Me), 0.88 (3H, s, Me), 0.89 (3H, s, Me), 0.90 (3H, s, Me), 0.98 (3H, s, Me), 1.10 (3H, s, Me), 3.63 (1H, dd, J_{ae} = 5.45 Hz, J_{aa} = 10.5 Hz, H-3), 3.81 (1H, m, H-16), 4.08–4.68 (5H sugar protons), 4.89 (1H, d, J = 7.1 Hz, H-1′ of galactose), 5.19 (1H, t, J = 6.4 Hz, H-12); ^{13}C nmr see Table 1; negative fabms *m/z* (rel. int.) $[M-H]^-$ 603 (20), $[M-H-162]^-$ 441 (16), 233 (100), 215 (39), 207 (25), 121 (30).

EXTRACTION AND ISOLATION OF SIGMOISIDE B [**2**].—The dried ground stem bark of *E. sigmoidea* (6 kg) was successively extracted with petroleum ether, $CHCl_3$, and MeOH in a Soxhlet extractor. Concentration of the MeOH extract under reduced pressure gave a dark brown gum (150 g). Part of this residue (100 g) was chromatographed on Si gel (900 g) eluted with *n*-hexane and increasing amounts of EtOAc in *n*-hexane. A total of 60 fractions of 150 ml per fraction were collected and combined on the basis of tlc. Combined fractions 40–54, obtained by elution with hexane-EtOAc (2:3), were subjected to repeated cc over Si gel followed by reversed-phase tlc, eluted with MeCN-H_2O (10:3), to yield sigmoiside B [**2**] (0.37 g) and maniladiol [**3**] (0.70 g).

Sigmoiside B [**2**].—White powder; mp 135–138°, $[\alpha]^{22}D$ −27° (c = 0.11, MeOH); ir ν max (KBr) 3650–3200, 1385, 1360, 1070, 1040 cm^{-1}; ^1H nmr (DMSO-d_6) δ 0.68 (3H, s, Me), 0.80 (3H, s, Me), 0.83 (3H, s, Me), 0.85 (3H, s, Me), 0.88 (3H, s, Me), 0.92 (3H, s, Me), 0.98 (3H, s, Me), 1.14 (3H, s, Me), 3.58 (1H, m, H-3), 3.71 (1H, m, H-16), 4.10–4.62 (5H sugar protons), 4.85 (1H, d, J = 6.8 Hz, H-1′ of glucose), 5.21 (1H, m, H-12); ^{13}C nmr see Table 1; negative fabms *m/z* (rel. int.) $[M-H]^-$ 603 (24), $[M-H-162]^-$ 441 (14), 233 (100), 215 (31), 207 (33), 121 (65).

ACID HYDROLYSIS OF SIGMOISIDE A.—The sample (13 mg) was dissolved in 7% H_2SO_4 and refluxed on an H_2O bath at 100° for 4 h. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was evaporated to dryness and purified by preparative tlc over Si gel with toluene-Me_2CO (10:3) as eluent. The aglycone was isolated and identified as maniladiol [**3**] through direct comparison with an authentic sample (tlc, mp, ir).

IDENTIFICATION OF SUGAR.—The H_2O layer was neutralized with 1 N NaOH and concentrated in vacuo, distilled H_2O was added to the residue, and the mixture was evaporated again in vacuo. The residue obtained was compared with standard sugars by tlc using *n*-BuOH–toluene–pyridine–H_2O (5:1:3:3) (BTPW) and shown to consist of D-galactose, $[\alpha]D$ +83° (c = 0.10, H_2O). The sugar was detected with aniline hydrogen phthalate. For gc analysis, the above residue was dissolved in TRISIL Z [0.05 ml; *N*-(trimethylsilyl)imidazole in pyridine], left at room temperature for 15 min, and analyzed by glc [on a Shimadzu GC-GA gas chromatograph, glass column, 2.6 mm × 2 m packed with 1.5% SE-30 on chromosorb W, detector FID, injection temperature 180°, column temperature 150°, carrier gas N_2 (40 ml/min)]. Glc peak of silylated derivative (Rt 4.8 min) co-eluted with that of the silylated standard.

HYDROLYSIS OF SIGMOISIDE B [**2**].—Sigmoiside B [**2**] (10 mg) was hydrolyzed with 7% H_2SO_4 (6 ml) under reflux for 4 h and worked up in the same way as **1** to furnish **3** and D-glucose. The sugar residue was identified by comparison with standard sugars by tlc using BTPW and by glc analysis of its TMSi derivative (Rt 5.8 min) as described above for galactose.

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