Bull. Chem. Soc. Ethiop. **2007**, 21(3), 373-378. ISSN 1011-3924
Printed in Ethiopia

SIGMOISIDE E: A NEW ANTIBACTERIAL TRITERPENOID SAPONIN FROM *ERYTHRINA SIGMOIDEA* (HUA)

J. Kouam[1*], X. Siewe Noundou[1], Laure B. Kouitcheu Mabeku[2], A. Meli Lannang[1], M. Iqbal Choudhary[3] and Z.T. Fomum[1]

[1]Department of Organic Chemistry, Faculty of Science, University of Yaoundé I, P. O. Box 812, Cameroon
[2]Microbiology Laboratory, Department of Biochemistry, Faculty of Science, P. O. Box 812, University of Yaoundé I, Cameroon
[3]H.E.J. Research Institute of Chemistry, International Centre of Chemical Sciences, University of Karachi-75270, Pakistan

(Received July 25, 2006; revised January 19, 2007)

ABSTRACT. Chemical analysis of the stem bark of *Erythrina sigmoidea* (Leguminoseae) yielded two known isoflavones, 6,8-diprenylgenisteine (**3**) and warangalone (**4**) as well as a new triterpenoid saponin designated sigmoiside E (**1**). Its structure was established by chemical and spectroscopic means as 16-O-β-D-galactopyranosyl maniladiol (**1**). Sigmoiside E exhibited antibacterial activity against gram-negative bacteria.

KEY WORDS: *Erythrina sigmoidea*, Stem bark, Triterpenoid, Saponin, Isoflavone, Leguminoseae

INTRODUCTION

The genus *Erythrina* (Leguminosae) is widely used in Cameroon to treat syphilis, wounds of ulcers and female sterility [1, 2]. Preliminary bioassays on the crude methanol extracts and on some pure compounds isolated from *Erythrina* species have shown antimicrobial, antimiotic and antidiueretic properties [3, 4]. Previous chemical studies in our laboratory of the neutral components of Cameroonian *Erythrina* species have resulted in the isolation and characterization of flavone, isoflavones and cinnamate esters [5-8]. Recently we reported the isolation of triterpenes and saponins from *Erythrina sigmoidea* [9-11]. Saponins form a class of compounds presently investigated because of biological effects they exert [12-14]. Some saponins have properties of hemolysis, bitterness, complex formation with cholesterol, and fish poison [15-18].

Our interest in the systematic investigation of the triterpenoid saponins of the Cameroonians medicinal plant *E. sigmoidea (Hua.*) [10, 11] has led us to the isolation of a new triterpenoid saponin designated sigmoiside E (**1**). In this paper, we report the isolation and structural elucidation of this compound as well as the preliminary antibacterial test on it.

RESULTS AND DISCUSSION

The ethyl acetate extract after successive column chromatography and preparative TLC on silica gel, afforded pure compounds (**3**) and (**4**). Isoflavones (**3**) and (**4**) were found to be 6,8-diprenyl genisteine [19] and warangalone [7] respectively by comparison of their physical (m.p. and TLC) and spectral data (IR, ^{1}H and ^{13}C NMR) with those of authentic samples.

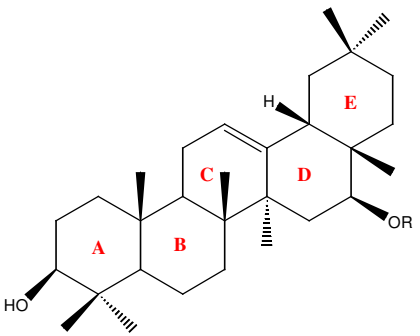
The residue of the methanol extract after successive extraction with n-hexane and ethyl acetate was chromatographed on Sephadex LH20 followed by chromatography on silica gel to yield compound (**1**).

*Corresponding author. E-mail: kouamjac@yahoo.fr

Compound (**1**), sigmoiside E, (m.p. 215-217 °C), $[\alpha]_D^{22}$ -26 (c = 0.11, MeOH); was isolated as white needles from $CHCl_3$/hexane (9:1). Its molecular formula $C_{36}H_{60}O_7$ was deduced from the HRFAB-MS. Its ^1H data (see Experimental) and ^{13}C NMR data (Table 1) indicated that it was an oleanane glycoside [20, 21].

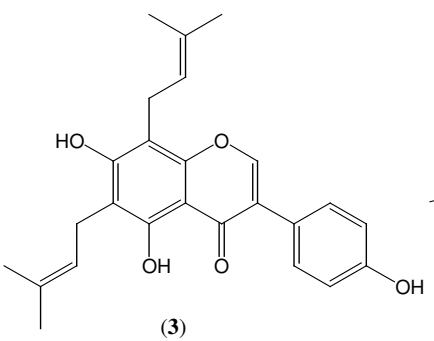
Acid hydrolysis of (**1**) yielded the aglycone, identified as maniladiol (**2**) by comparison with an authentic sample [10] and the sugar component. The latter was assumed to be D-galactose by the TLC and GLC of its trimethylsilyl derivative as well as the ^{13}C NMR spectrum in which signals at δ 100.5, 73.5, 76.8, 70.3, 76.8, 61.1 ppm agree with those published for D-galactose [10, 22, 23].

The β configuration of the D-galactopyranosyl moiety in (**1**) was deduced from the coupling constant (J = 7.2 Hz) of the anomeric proton signal at 4.87 ppm in the ^1H NMR spectrum [24]. Inspection of the mass and ^{13}C NMR spectra of (**1**) clarified the location of the sugar moiety.

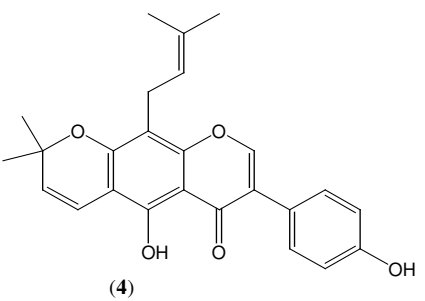


(**1**) R = gal
(**2**) R = H

(**1**): sigmoiside E and (**2**): maniladiol



(**3**)



(**4**)

(**3**): 6,8-diprenylgenisteine

(**4**): warangalone

The low resolution FAB-MS of (**1**) showed a molecular ion peak at m/z 604; mass fragments at m/z 442 $[M\text{-gal}]^+$ and diagnostically prominent peaks at m/z 208 and 396 from the retro Diels-Alder fragmentation, suggesting that the galactose was on ring D or E. On the other hand, the spectrum corresponding to the aglycone part of (**1**) showed signals essentially identical with those of (**2**) except for those due to D-ring carbons. Among these D-ring carbons signals, the

C-16 carbon signal was significantly downfield (δ 82.0, in (**2**) this signal appeared at 77.8 ppm) (Table 1). This observation implied that the galactose residue was bound through the glycosidic linkage to the C-16 hydroxyl group of the aglycone [25, 26]. This was confirmed by the 2D-HMQC (heteronuclear multiple quantum coherence) and 2D-HMBC (heteronuclear multiple bond connectivity) spectra of (**1**), particularly the 2- and 3-bonds correlation, which showed connectivity patterns between, H-16 (4.05 ppm) and C-17 (48.0 ppm), H-16 and C-15 (29.4 ppm) as well as H-1' (4.58 ppm) and C-16 (82.0 ppm).

On the basis of the above evidence, the structure of sigmoiside E was elucidated to be 16-O-β-D-galactopyranosylmaniladiol. Disc diffusion assay showed that sigmoiside E had an antimicrobial activity against *Proteus vulgaris* at the concentration of 0.2 mg/disc. The value of the inhibition zone against this microorganism was 15.5 ± 0.5 mM. The MIC value was 0.05 mg/mL.

Table 1. ^{13}C NMR (75.4 MHz, DMSO-d_6) spectra of sigmoiside E (**1**) and maniladiol (**2**).

Carbon	Compounds	
	(**1**)	(**2**)
C-1	38.3 CH_2	38.6 CH_2
C-2	28.3 CH_2	27.7 CH_2
C-3	77.7 CH	78.9 CH
C-4	39.0 C	38.8 C
C-5	54.8 CH	55.4 CH
C-6	18.0 CH_2	18.5 CH_2
C-7	32.5 CH_2	32.6 CH_2
C-8	40.0 C	39.7 C
C-9	47.8 CH	47.7 CH
C-10	37.5 C	36.9 C
C-11	23.0 CH_2	23.7 CH_2
C-12	121.2 CH	122.3 CH
C-13	144.0 C	144.2 C
C-14	41.6 C	41.7 C
C-15	29.4 CH_2	28.1 CH_2
C-16	82.0 CH	77.8 CH
C-17	48.0 C	46.8 C
C-18	42.0 CH	41.3 CH
C-19	47.1 CH_2	46.4 CH_2
C-20	31.9 C	31.1 C
C-21	35.7 CH_2	35.8 CH_2
C-22	32.2 CH_2	32.1 CH_2
C-23	28.3 CH_3	28.4 CH_3
C-24	15.4 CH_3	15.7 CH_3
C-25	16.1 CH_3	16.0 CH_3
C-26	16.3 CH_3	16.9 CH3
C-27	27.0 CH_3	26.1 CH_3
C-28	28.4 CH_3	28.5 CH_3
C-29	33.3 CH_3	33.1 CH_3
C-30	22.5 CH_3	22.3 CH_3
C-1'	gal 100.5 CH	
C-2'	73.5 CH	
C-3'	76.8 CH	
C-4'	70.3 CH	
C-5'	76.8 CH	
C-6'	61.1 CH_2	

EXPERIMENTAL

General experimental procedure. All mps were determined on a Kofler hot stage apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter at room temperature. FABMS spectra were obtained with Kratos MS 25 with DS-55 data system and on JEOLJMS-DX 303 mass spectrometer, collision gas Xe (ion gun conditions 6 kV and 10 mA) and matrix glycerol or thioglycerol. IR spectra were run on a Nicolet 20 DBX, Sephadex LH20, Si gel GF_{254} (Merck) and Si gel 60(70-230 mesh ASTM) (Merck) were used for the CC and TLC, respectively. NMR experiments were performed on a Nicolet NT 300wB or 300 Bruker spectrometer equipped with 5 mm ^{1}H and ^{13}C probe at 300.06 and 75.45 MHz, respectively. Samples were run in DMSO-d_6 or $CDCl_3$ and chemical shifts, expressed in ppm, were referenced to internal TMS (0.0 ppm) for ^{1}H NMR and to deuterated solvent DMSO-d_6 (40.9 ppm) for ^{13}C NMR.

Plant material. *Erythrina sigmoidea* (Hua) stem bark was collected at Foumban, Cameroon, in May 2002. The plant was identified at the National Herbarium, Yaounde where a reference (26645SRF.Cam) sample was deposited.

*Extraction and isolation of compounds **1**, **3** and **4***. The ground stem bark of *Erythrina sigmoidea* (Hua) (5 kg) was immersed in MeOH (25 L) and kept for 72 hours. The MeOH extract (400 g) after concentration under reduced pressure was successively extracted with n-hexane (5 x 500 mL) and ethyl acetate (5 x 500 mL) to give 40 g and 251 g of extract, respectively. 100 g portion of the ethyl acetate extract was subjected to column chromatography on silica gel (400 g) using an eluent of hexane-ethyl acetate-methanol of increasing polarity. A total of 210 fractions of 250 mL each were collected and combined on the basis of TLC. Fractions 95-122 (9 g) were again chromatographed on silica gel (100 g) using an eluent of hexane-ethyl acetate-MeOH in increasing polarity. From a total of 105 fractions of 50 mL each, fractions 70-105 were combined on the TLC basis and further purified by preparative TLC. MeOH/CH_2Cl_2/cyclohexane (3:5:2) to give compound (**3**) 15 mg and (**4**) 22 mg. 60 g portion of the residue (105 g) of the methanol extract after successive extraction with n-hexane and ethyl acetate was subjected to CC on Sephadex LH20 (250 g) using methanol as eluent. 50 fractions of 250 mL each were collected and combined on the basis of TLC. Fractions 15-21 (11 g) were again chromatographed in the same manner as described for the residue. From a total of 60 fractions of 50 mL each, fractions 20-22 (500 mg) were further purified using preparative TLC. MeOH/$CHCl_3$/toluene (5:4:1) to give compound (**1**) (20 mg). The analysis of the second portion (43 g) of the residue of the methanol extract (105 g) in the same manner as described above for the first portion (60 g) yielded 14 mg of (**1**).

*Sigmoiside E (**1**).* White needles ($CHCl_3$/hexane 9:1); m.p. (215-217 °C); $[\alpha]_D^{22}$ -26 (c = 0.11, MeOH); IR ν_{max} (KBr) 3540-3200, 1390, 1365, 1330 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.68 (Me, s), 0.81 (Me, s), 0.87 (Me, s), 0.98 (Me, s), 1.07 (Me, s), 1.28 (Me, s), 1.44 (Me, s), 1.47 (Me, s), 3.58 (1H, dd, Jae = 5.4 Hz, Jaa = 11.0 Hz, H-3) 4.05 (1H, dd, Jae = 4.3 Hz, Jaa = 11.7 Hz, H-16), 4.02-4.58 sugar protons), 4.87 (1H, d, J = 7.2 Hz, H-1' of galactose), 5.20 (1H, br t, H-12); ^{13}C NMR (Table 1); HRFAB-MS m/z: 604.4338 (calcd for $C_{36}H_{60}O_7$: 604.4339); LRFAB-MS m/z (rel. int.) $[M]^+$ 604 (22), $[M-H-162]^+$ (14), 442 (18), 441(15), 396 (60), 360 (34), 244 (26), 208 (28), 233 (100).

Acid hydrolysis of sigmoiside E. The sample (15 mg) was dissolved in 7 % H_2SO_4 and refluxed on a water bath for 4 hours. The reaction mixture was diluted with 15 mL H_2O and extracted with CH_2Cl_2. Evaporation of the solvent followed by the purification of the residue by prep. TLC over silica gel with toluene/Me_2CO (10:3) as eluent gave a white compound identified as

maniladiol (**2**) (6.5 mg) by comparison of its physical and spectral data (IR, ^{1}H, and ^{13}C) with those of an authentic sample isolated from the same plant [10].

Identification of the carbohydrate unit. The aq. phase after extraction with CH_2Cl_2 was neutralised with 1 M NaOH and evaporated *in vacuo*. Distilled H_2O was added to the residue and the mixture was again evaporated in order to remove all the impurities. The residue obtained was compared with standard sugars (galactose, glucose, rhamnose) by TLC using n-BuOH/toluene/pyridine/H_2O (5:1:3:3) (BTPW). The sugar was detected with aniline hydrogen phthalate, and shown to consist of D-galactose. For GLC analysis, the above residue was dissolved in TRISIL Z [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 x 2 m packed with 1.5 % SE-30 on Chromosorb W, detector FID, injection temp. 180 °C, column temp. 150 °C, carrier gas N_2 (40 mL min^{-1}). The GLC peaks of the silylated derivative of the residue and of galactose had the same retention time (4.8 min).

Disc diffusion assay. The sample was dissolved in DMSO to a final concentration of 20 mg/mL. Sterile paper discs (6 mm of diameter) prepared from Whatman No. 1 filter paper were impregnated with 10 µL of that solution as described by Edward [27]. Each disc contained 200 µg of compound (**1**). These paper discs were kept in an incubator at 37 °C for 24 hours to evaporate the solvent. Similarly, paper disc containing standard concentration of antibiotic (gentamicin) as described by Matsen [28] was prepared and used for the susceptibility test. Negative control was also prepared using the same solvent employed to dissolve the sample. Antimicrobial tests were then carried out by disc diffusion method.

100 µL of the suspension of the tested microorganism (*Proteus vulgaris*) containing 10^6 CFU/mL, prepared from an overnight Mueller Hinton agar culture was used to seed each prepared and dried Mueller Hinton agar plate. The discs were arranged and firmly pressed on the agar surface of each seeded plate. These plates, after staying at 4 °C for 2 hours were incubated aerobically at 37 °C for 24 hours. Antimicrobial activity was evaluated by measure the zone of inhibition against the tested microorganism.

Determination of MIC. A broth macrodilution susceptible assay was used for the determination of MIC [29]. All the tests were performed in peptone water with red phenol supplemented with glucose 1 % (w/v) (PPG 1 %). The tested microorganism (*Proteus vulgaris*) was cultured overnight at 37 °C in Mueller Hinton. Then, it was suspended in normal saline (NaCl 9 ‰), adjusted to 0.5 Mc Farland standard turbidity and suspended in PPG 1 % to give a final density of 5 x 10^5 CFU/mL.

For the susceptibility test, in the first step, 2 mL of PPG 1 % was distributed from the second to the 7^{th} 5-mL sterile test tubes. Sigmoiside E was initially dissolved in 0.5 % Tween 80 and then in the PPG 1 % to reach the highest concentration 0.6 mg/mL to be tested. 4 mL of this suspension was added in the 1^{st} test tube of the 5-mL test tube and then 2 mL of scalar dilution was transferred from the 2^{nd} to the 6^{th} test tubes. The 7^{th} test tube was considered as growth control since no sigmoiside E solution was added. Then, 1 mL of microbial suspension was added to each test tube. The final volume of each 5-mL test tube was 3 mL. The final concentration of the sample adopted to evaluate the antimicrobial activities was included from 0.4 mg/mL (1^{st} test tube) to 0.0125 mg/mL (6^{th} test tube). Gentamicin at the concentration range of 0.32 to 0.000019 mg/mL was prepared in (PPG 1 %) and used as standard drug for the positive control. The content of each test tube was mixed and then incubated under normal atmospheric condition at 37 °C for 24 hours. The bacterial growth was indicated by the colour change of the test tube content from the red to yellow. The MIC was defined as the lowest concentration of the extract to inhibit the growth of microorganims (1^{st} red test tube content) and

confirmed by platting 5 µL sample from that red test tube on Mueller Hinton agar. The sample testing in this study was screened two times.

AKNOWLEDGEMENTS

We are indebted to the International Centre of Chemical Sciences (University of Karachi-75270 Pakistan) for technical assistance.

REFERENCES

1. Zipcy, E. *J. Agric. Trop. Bot. Appli.* **1976**, 23, 1.
2. Dalziel, J.M. *The Useful Plants of West Tropical Office, Crown Agents for the Colonies*. Vol. 2, Burkill, H.M. (Ed.); Kew Botanical Gardens: London; **1993**; pp 140-141.
3. Biyiti, L.; Pesando, D.; Puiseux-Dao, S.; Girard, J.-P. and Payan, P. *Toxicon*. **1990**, 28, 373.
4. Biyiti, L.; Pesando, D.; Puisieux-Dao, S. *Planta Med.* **1998**, 2, 126.
5. Fomum, Z.T.; Ayafor, J.F.; Mbafor, J.T. *Tetrahedron lett.* **1983**, 24, 4127.
6. Fomum, Z.T.; Ayafor, J.F.; Mbafor, J.T.; Mbi, C.M. *J. Chem. Soc., Perkin Trans*. I, **1986**, 33.
7. Fomum, Z.T.; Ayafor, J.F.; Wandji, J. *Phytochemistry* **1985**, 24, 3075.
8. Nkengfack, A.E.; Sanson, D.R.; Tempesta, M.S.; Fomum, Z.T. *J. Nat. Prod.* **1989**, 52, 320.
9. Ndom, J.C.; Kouam; Vardamides, J.C.; Wansi, J.D.; Kamdem, W.A.; Mbafor, T.J.; Zacharias, T.F. *Bull. Chem. Soc. Ethiop*. **2001**, 15, 2001.
10. Kouam, J.; Nkengfack, A.E.; Fomum, Z.T.; Ubillas, R.; Tempesta, M.S. *J. Nat. Prod.* **1991**, 54, 1288.
11. Mbafor, J.T.; Ndom, J.C.; Fomum, Z.T. *Phytochemistry* **1997**, 44, 1151.
12. Bloor, S.J.; Qi, L. *J. Nat. Prod.* **1994**, 57, 1354.
13. Pengsuparg, T.; Cai, L.; Fong, H.H.S.; Kinghorn, A.D.; Pezzuto, J.M.; Wani, M.C.; Wall, M.E. *J. Nat. Prod.* **1994**, **57**, 415.
14. Recio, M.D.C.; Just, M.J.; Giner, R.M.; Manez, S.; Rios, J.L.; Hostettmann, K. *J. Nat. Prod.* **1995**, 58, 140.
15. Lee, M.-R.; Chen, C.M.; Hwang, B.-H.; Hsu, L.-M. *J. Mass Spectrom.* **1999**, 34, 804.
16. Cui, M.; Sun, W.; Song, F.; Liu, Z.; Liu, S. *Rapid Commun. Mass Spectrom.* **1999**, 13, 873.
17. Van Setten, D.C.; ten Hove, G.J.; Wiertz, E.J.H.J.; Kamerling, J.P.; van de Werken, G. *Anal. Chem*. **1988**, 70, 4401.
18. Chludil, H.D.; Seldes, A.M.; Maier, M.S. *J. Nat. Prod*. **2002**, **65**, 153.
19. Singhal, A.K.; Sharma, R.P.; Thyagarajan, G.; Hertz, W.; Govindan, S.V. *Phytochemistry* **1980**, 19, 929.
20. Budzikiewicz, H.; Wilson, J.M.; Djerassi, C. *J. Am. Chem. Soc*. **1963**, 85, 3688.
21. Ogunkoya, L. *Phytochemistry* **1981**, 20, 121.
22. Carmely, S.; Roll, M.; Loya, Y.; Kashman, Y. *J. Nat. Prod*. **1989**, 52, 167.
23. Lin, C.; Chung, M.-I.; Lin, S.-Y. *Phytochemistry* **1987**, 26, 305.
24. Arriva, F.; Wollenweber, E.; Shober, I.; Sostal, P.; Braun, S. *Phytochemistry* **1986**, 25, 719.
25. Miama, G.A.; Al Hazimi, M.G. in *Essays on Science*, Seshari, R.T. (Ed.), Jamdard Foundation Press: Pakistan; **1986**, p 171.
26. Stothers, J.B. *Carbon 13-NMR Spectroscopy*, Academic Press: New York; **1972**, p 461.
27. Edward, D.I. in *Antimicrobial Action*, 1st ed., Macmillan Press: London; **1980**; pp 8-30.
28. Matsen, I.M. *Bacterial susceptibility testing and assay* in *Clinical Diagnosis and Laboratory Methods*. Henry, J.B. (Ed.), WB Saunders: Philadelphia; **1979**; pp 1900-1903.
29. Vanden, B.D.A.; Vlietinck, A.J. *Methods in Plant Biochemistry: Screening Methods for Antibacterial and Antiviral Agents from Higher Plants*, Dey, P.Mp.; Harbone, J.B. (Eds.), Academic Press: London; **1991**; pp 47-69.