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SAPELENIN E AND F: NEW ACYCLIC TRITERPENOIDS FROM THE STEM BARK OF *ENTANDROPHRAGMA CYLINDRICUM*

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ABSTRACT. New acyclic triterpenoids, sapelenin E (**1**) and F (**2**) were isolated from the stem bark of *Entandrophragma cylindricum*. Their full structure including absolute configuration in sapelenin E (**1**) were determine from spectroscopic data and by chemical transformation.

KEY WORDS: *Entandrophragma cylindricum*, Sapelenin, Meliaceae, Acyclic triterpenoids

INTRODUCTION

The genus *Entandrophragma* belongs to the family Meliaceae and consists of more than 1400 species [1]. It's widespread on the African continent, south of the Sahara [2] and occupies a prominent position in traditional African medicine [3, 4]. Five species are known in Cameroon [5] and among *Entandrophragma cylindricum* (known locally as Sapele Mahogany).

In previous articles [6, 7], we reported the isolation and the structural determination of four new acyclic triterpenoids named sapelenin A-D from *Entandrophragma cylindricum*. Continuing the study of the plant, we were gratified to find the sapelenin E (**1**) and F (**2**) for which the structure of (*6R,7S*)-6,7-dihydroxy-6,7-dihydrosqualene and 6,7,10,11-tetrahydroxy-6,7,10,11-tetrahydrosqualene were proposed.

RESULTS AND DISCUSSION

The new acyclic triterpenoids (**1**) and (**2**) were obtained from the hexane extract and purification was achieved by vacuum liquid chromatography and subsequent silica gel column chromatography. Investigation of the non polar and polar fractions led to the isolation of sapelenin E (**1**) and F (**2**), respectively. Their structures were determined by interpretation of their spectral data, mainly ^{1}H and ^{13}C NMR and 2D-NMR (HMQC, HMBC) as well as by comparison with the literature values [7]. Sapelenin E was isolated as colourless oil. $[\alpha]_D^{23}$ –1.4° ($CHCl_3$, *c* 2). Its mass spectrum gives the $M^{+\cdot}$ at *m/z* 444 ($C_{30}H_{52}O_2$). The study of its IR spectrum indicated the presence of hydroxyl and carbon-carbon double bonds (3480 and 1660 cm^{-1}). The ^{13}C NMR displays 30 carbons: eight methyls, 10 methylenes, one methine bearing oxygen, five sp^2 methines and six quaternary carbons including five sp^2, and one bearing oxygen. This was confirmed by the ^{1}H NMR spectrum in which eight singlet methyls were observed, including five vinylic, and one attached to carbon bearing oxygen. The –CH(OH)-proton appears at 3.43 and the vinyl proton together at δ 5.05 (5H). Comparison of the ^{13}C NMR data of sapelenin E (**1**) with those of sapelenin B (**3**) indicated the presence of an intact geranyl-geranyl fragment **4** in **1** (Table 1). This was supported by observation of the ion at *m/z* 271 in the mass spectrum of **1**, corresponding to the geranyl-geranyl radical cation. The above evidence

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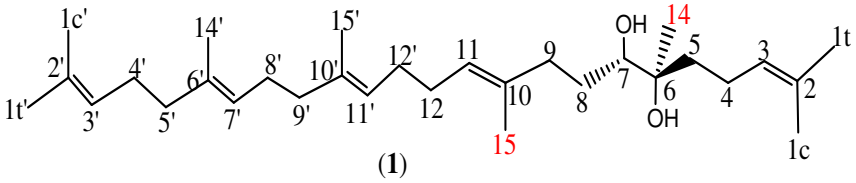
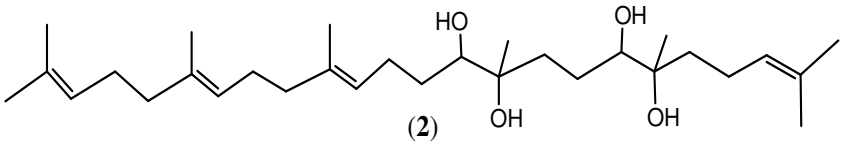
suggests that sapelenin E (**1**) is a squalene derivative in which one double bond is hydroxylated. The relative position of hydroxyl group in **1** follow from observation of correlations in an HMBC experiment between C-5, C-6, C-7 and Me-14 from the one hand, C-4, C-5 and H-3 from the another (Figure 1). At this stage the absolute configuration of C-6 and C-7 remained undetermined in compound (**1**); this was achieved as follows.

Horeau's method [8-10] was applied to **1** in order to determine the configuration at the C-6 and C-7. A mixture of **1** with an excess of 2-phenylbutyric anhydride and DMAP in methylene chloride showed an immediate evolution of the optical rotation in the (-) sense, thus including the preferential esterification by the (+) antipode of the acid. Silica column chromatography coupled with an optical rotation detector [11] allowed the isolation of levorotatory 2-phenylbutyric acid. According to Horeau's method, when (-)-*R*-2-phenylbutyric acid accumulates in the mixture (*i.e.* when the (-)-(*S*)-acid is the preferential esterifying acid), the C-7 secondary hydroxyl has the (*S*) configuration. The (*R*) configuration in C-6 is consistent with formation of the glycol system by epoxidation followed by trans opening of the epoxide[6]. The above evidence established the structure of sapelenin E as (**1**), having a (6*R*,7*S*) absolute configuration.

Sapelenin F (**2**), a fine powder, exhibited IR bands at 3480 (OH) and 1660 (C=C) cm^{-1}. The molecular formula of **2** was deduced as $C_{30}H_{54}O_4$ by mass spectrometry (EIMS: M^{+} at *m/z* 478), ^{1}H and ^{13}C NMR data. The formation of a *bis*-acetonide (**5**), upon treatment of **2** with 2,2-dimethoxypropane and *p*-toluenesulfonic acid in acetone, indicated the presence of two vicinal diol: the ^{13}C chemical shift of the acetal carbons (δ 106.5 and 106.7 ppm) revealed that the acetonide had a five membered ring [12]. The ^{1}H NMR of **5** showed four additional methyl signals and a small downfield shift of the CH(OR) proton (δ 3.69 ppm). The carbons involved in acetonide formation were readily identified by downfield shifts of 6 ppm (see Experimental). Comparison of the ^{13}C data of sapelenin F (**2**) with those of sapelenin A (**6**) indicated the presence of an intact farnesyl fragment (**7**) in **2** (Table 1). This was supported by observation of the ion at *m/z* 205 in the mass spectrum of **2**, corresponding to the farnesyl radical cation. The above information suggests that sapelenin F (**2**) is a squalene derivative in which two internal and consecutive double bonds are hydroxylated. The relative position of hydroxyl group in (**2**) follow from observation of correlations in an HMBC experiment between C-5, C-6, C-7 and Me-14 from the one hand, C-9, C-10, C-11 and Me-15 from the another (Figure 2).



(**1**)



(**2**)

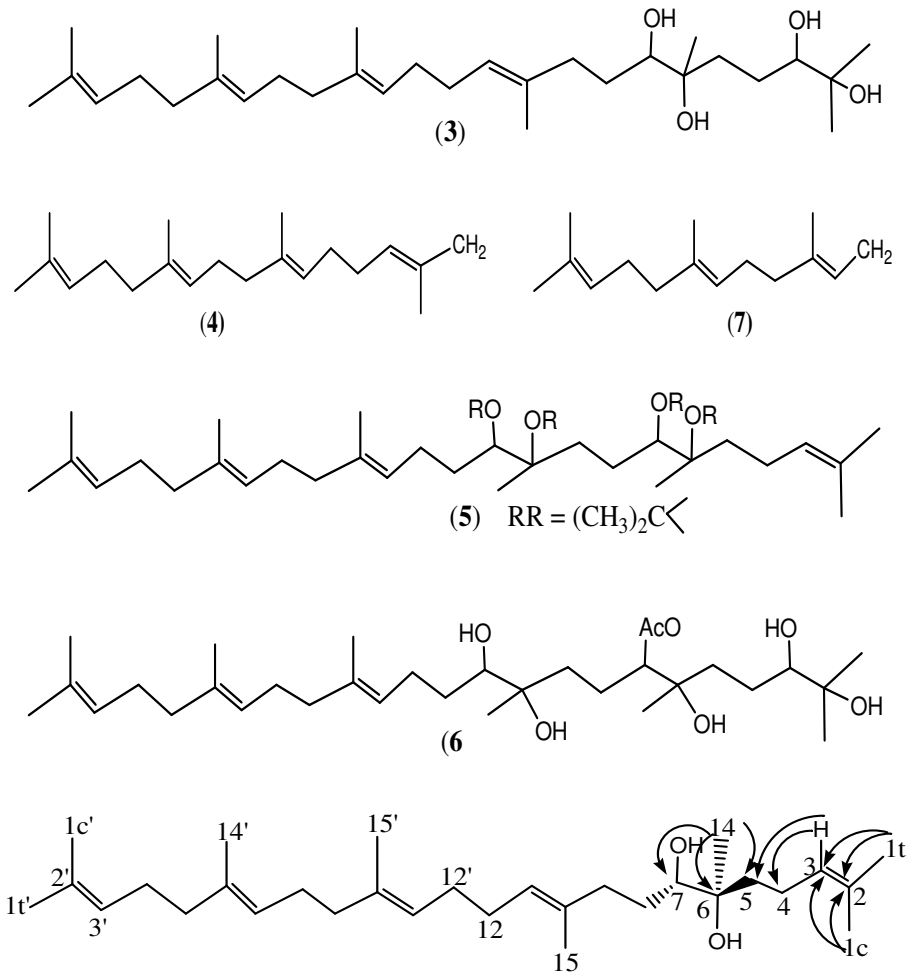


Figure 1. HMBC correlations observed for compound (**1**).

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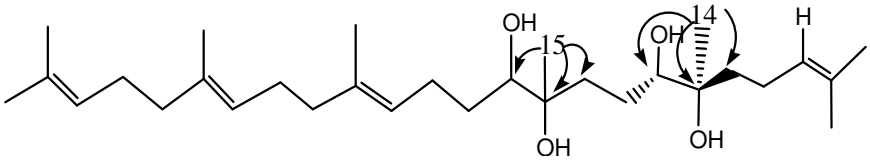

Figure 2. HMBC correlations observed for compound (**2**).

Table. ^{13}C NMR data of compounds (**1**), (**2**), (**3**) and (**6**) in $CDCl_3$.

C N°	**1**	**2**	**3**	**6**	C N°	**1**	**2**	**3**	**6**
1t'	25.6	25.6	26.6	25.8	1t	25.6	25.6	26.4	26.2
1c'	17.6	17.6	17.8	17.8	1c	15.9	16.0	23.3	23.5
2'	131.1	131.4	131.1	131.4	2	131.1	131.1	74.5	73.0
3'	124.2	124.3	124.3	124.5	3	124.2	124.3	78.7	78.7
4'	26.7	26.6	26.6	26.9	4	22.0	22.0	25.2	23.0
5'	39.7	39.5	38.6	39.9	5	38.7	38.8	32.8	35.3
6'	135.2	135.1	135.1	135.1	6	74.8	74.4	73.2	73.7
7'	124.0	124.0	124.0	123.9	7	76.8	77.4	78.6	80.5
8'	26.6	26.7	26.5	26.8	8	29.5	24.8	28.4	24.8
9'	39.7	39.5	38.6	39.8	9	36.8	35.7	36.8	39.6
10'	135.1	136.2	135.1	136.2	10	134.6	75.0	134.8	74.6
11'	124.0	123.8	124.1	124.3	11	124.8	77.7	124.8	78.6
12'	28.2	25.1	28.1	25.4	12	28.1	31.5	28.2	31.9
14'	15.9	16.0	15.8	16.1	14	21.0	20.09	23.2	24.8
15'	15.9	16.0	15.8	16.3	15	15.9	20.09	15.8	21.6
					CH_3CO				171.1; 21.1

EXPERIMENTAL

General. M.p.: uncorr. Infrared spectra were taken on a Bomem, Hart-Mann and Braun, MB-series spectrophotometer. ^1H and ^{13}C NMR were obtained using a Bruker AC-300 (^1H 300.1 MHz and ^{13}C 75.4 MHz) spectrometer. Chemical shifts are given in δ value (ppm) with TMS as internal standard. Mass spectra were recorded on a Jeol JMS D300 spectrometer at 70 eV. TLC was carried out on silica gel. The triterpenoid compounds were detected by spraying with 50% solution of H_2SO_4 in H_2O following by heating.

Plant material. The bark of *Entandrophragma cylindricum* was collected from Awae (Cameroon) in December 1991 and identified by Mr Mesili Paul. A voucher specimen (N° 54965/SFR/CAM) was deposited at the National Herbarium of Cameroon, Yaounde, Cameroon.

Extraction and isolation. The air-dried and finely material (10 kg) was extracted with n-hexane (15 L) at room temperature. The extract was evaporated to dryness (103 g) of which 70 g was fractionated on a silica gel column using a hexane-AcOEt gradient and collecting 200 mL fractions. Frs 19-30 were collected (1 g) and rechromatographed repeatedly on silica gel using the same solvent system to yield sapelenin E (**1**) (35 mg). Frs 88-101 (700 mg) were rechromatographed on a silica gel column using a CH_2Cl_2-MeOH gradient to yield sapelenin F (**2**) (41 mg).

Sapelenin E (**1**). Colourless oil. $[\alpha]_D^{23}$ –1.4° ($CHCl_3$, *c* 2). IR ν_{max} (NaCl) cm^{-1}: 3480 (OH), 1660 (C=C). EIMS *m/z* (% int. rel.): 139 (100), 271 (8), 444 (0.5). ^1H NMR ($CDCl_3$): δ 5.05 (m, (5H), H-3', H-7', H-11', H-11 and H-3), 3.43 (dd, J = 10.4 and 3.6 Hz, H-7), 1.68 (s, Me-1t', Me-1t), 1.62 (s, Me-1c', Me-1c), 1.60 (s, Me-14', Me-15' Me-15), 1.15 (Me-14).

Sapelenin F (**2**). Fine powder, m.p. 72-73°C, $[\alpha]_D^{23}$ +4.8° ($CHCl_3$, *c* 2). IR ν_{max} (NaCl) cm^{-1}: 3480 (OH), 1660 (C=C). EIMS *m/z* (% int. rel.): 201 (100), 205 (3), 478 (0.5). ^1H NMR ($CDCl_3$): δ 5.08 (m, (4H), H-3', H-7', H-11' and H-3), 3.40 (d, J = 13.2 Hz, H-7 and H-11),

1.67 (s, Me-1t', Me-1t), 1.65 (s, Me), 1.61 (s, Me), 1.60 (s, Me-1c', Me-1c), 1.12 (Me-14), 1.10 (Me-15).

Preparation of bis-acetonide (**5**). This compound was prepared by dissolving sapelenin F (13 mg) in acetone (10 mL), adding one equivalent of 2,2-dimethoxypropane and a catalytic amount of *p*-toluenesulfonic acid. The reaction was complete at room temperature in 15 minutes. Hexane was added and the organic phase washed with saturated $NaHCO_3$ and dried over Na_2SO_4. Removal of the solvent gave the *bis*-acetonide as clear oil.

Compound (**5**). Oil. ^{1}H NMR ($CDCl_3$): 5.14-5.05 (m, (4H), H-3', H-7', H-11' and H-3), 3.69 (d, J = 13.2 Hz, H-7 and H-11), 1.68 (s, Me-1t', Me-1t), 1.65 (s, Me), 1.62 (s, Me), 1.60 (s, Me-1c', Me-1c), 1.07 (s, Me-14), 1.10 (s, Me-15), 1.41, 1.37 (s, -O-C(Me)2-O-). ^{13}C NMR ($CDCl_3$): 25.4 (Me-1t, Me-1t'), 17.4 (Me-1c), 131.1 (C-2', C-2), 124.1 (C-3'), 26.4 (C-4'), 39.3 (C-5'), 135.0 (C-6'), 123.9 (C-7'), 26.5 (C-8'), 39.3 (C-9'), 136.0 (C-10'), 123.6 (C-11'), 25.0 (C-12'), 15.9 (C-14'), 15.9 (C-15'), 15.9 (C-1c), 21.0 (C-4), 37.8 (C-5), 81.4 (C-6), 82.4 (C-7), 23.8 (C-8), 35.7 (C-9), 81.1 (C-10), 82.6 (C-11), 30.9 (C-12), 21.1 (C-15), 21.1 (C-14), 28.1, 28.2 (-OC(Me)$_2$O-).

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REFERENCES

1. Kamga, C. *Thèse de 3ème cycle*, *Chimie Organique*, Université de Yaoundé: Cameroon; **1980**.
2. Tchouankeu, J.C. *Thèse de 3ème cycle*, *Chimie Organique*, Université de Yaoundé : Yaoundé; **1989**.
3. Aubreville, A. *Flore Forestière Soudano-guinéenne*, Societé d'Editions Géographiques, Maritimes et Coloniales: Paris; **1950**; p 377.
4. Arigoni, D.; Barton, D.H.R.; Caglioti, C.; Corey, E.J.; Ferrini, P.G.; Glatier, E.R.; Jeger, D.; Melera, A.; Pradam, S.K.; Shaffner, F.; Sternhell, S.; Templeton, J.F.; Tobinaga, S. *Experiencia* **1960**, 16, 41.
5. Letouzey, R. in: *Notice de la carte géographique du Cameroun*, Int. de la carte géog., Int. de veg.: Toulouse; **1985**, 4, 98.
6. Ngnokam, D.; Massiot, G.; Nuzillard, J.M.; Connolly, J.D.; Tsamo, E.; Morin, C. *Phytochemistry* **1993**, 34, 1603.
7. Ngnokam, D.; Massiot, G.; Bliard, C.; Tsamo, E. *Nat. Prod. Lett.* **1995**, 5, 189.
8. Horeau, A. *Tetrahedron Lett.* **1962**, 965.
9. Horeau, A. *Tetrahedron Lett.* **1975**, 31, 1307.
10. Barbier, M.; Devys, M.; Bousquet, J.F.; Kollmann, A. *Phytochemistry* **1994**, 35, 955.
11. Chiral detector: Knauer France, reference: 1000
12. Buchanan, J.C.; Chacon-Fuertes, M.E.; Edgar, A.R.; Moorhouse, S.J.; Rawson, I.D.; Wightman, R.H. *Tetrahedron Lett.* **1980**, 21, 1793.