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SHORT COMMUNICATION

SQUALENE AND AMENTOFLAVONE FROM *ANTIDESMA LACINIATUM*

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ABSTRACT. Squalene, (2*E*, 7ξ,11ξ)-phyt-2-en-1-ol and amentoflavone have been isolated from the extract of the leaves of *Antidesma laciniatum*. Their structures were elucidated using spectroscopic methods. This is the first report of these compounds from *Antidesma* species.

KEY WORDS: *Antidesma laciniatum*, Euphorbiaceae, Squalene, Amentoflavone, (2*E*, 7ξ,11ξ)-Phyt-2-en-1-ol

INTRODUCTION

Antidesma is a relatively homogeneous genus of dioecious shrubs and trees in the Old World tropics. Out of 170 *Antidesma* species in the world, less than ten species are found in Africa. These include *A. venosum*, *A. laciniatum*, *A. chevalieri*, *A. membranaceum*, *A. madagascariensis* and *A. vogelianum* [1]. The interest on the investigation of *Antidesma* species is growing considerably due to its various applications in traditional medicine [2]. Antidesmone, a quinoline-type alkaloid isolated from *A. membranaceum* [3] and its derivatives are highly potent against *Leishmania donovani* and *Trypanosoma cruzi* [4]. In addition, strong fungitoxic activity of antidesmone has been reported [3]. The compounds isolated from *Antidesma* species include triterpenoids, cyclopeptide alkaloids, steroids, phenolic acids, megastigmanes, lignans, flavonoids and quinolide-type alkaloids [3]. The above features prompted us to investigate the leaves of *A. laciniatum*. Except for the study of the essential oil of this plant [5], which showed activity against strain of W-2 of *Plasmodium falciparum* [6], there is no prior report on the chemistry of this species.

RESULTS AND DISCUSSION

The leaves of *A. laciniatum* Muell. Arg. (Euphorbiaceae) were extracted with 80% aqueous methanol, and the solution was concentrated *in vacuo* to the aqueous phase and then partitioned using $CHCl_3$-MeOH-H_2O (4:4:1); the $CHCl_3$ layer, after concentration, was subjected to extensive chromatography to afford squalene (**1**) [7], (2*E*, 7ξ,11ξ)-phyt-2-en-1-ol (**2**) [8], sitosterol (**3**) [9] and the biflavonoid amentoflavone (**4**) [10, 11].

Squalene (**1**) was obtained as colorless oil. Its structure was elucidated by analysis of its NMR (^{1}H, ^{13}C, DEPT, HMBC, HSQC and COSY), IR, mass spectra and comparison with

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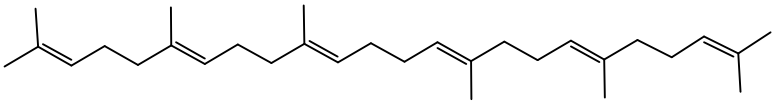
literature reports [7]. Squalene is the main constituent of shark liver oil, yeast and many vegetable oils [12] and it has also been reported from higher plants [13]. Squalene has shown hypocholesterolaemic activity and preventive and therapeutic efficacy on tumor proliferation [12]. This is the first report of squalene from the genus *Antidesma*.



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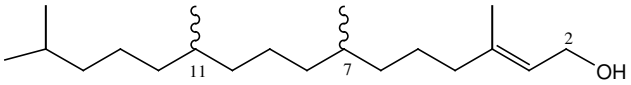

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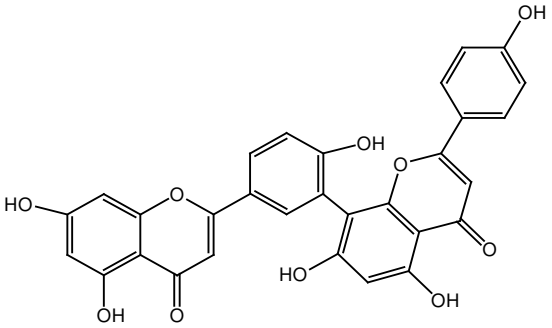

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Compound **2** was obtained as colorless oil. The structure was determined as (2*E*, 7ξ,11ξ)-phyt-2-en-1-ol using ^{1}H, ^{13}C, DEPT, HMBC, HSQC and COSY spectra. Its NMR data agree with those reported in the literature [8, 14].

Compound **3** was obtained as white crystalline needles. The interpretation of its 1D and 2D NMR data led to its identification as β-sitosterol. The ^{13}C NMR data and melting point are in good agreement with those reported in the literature [9]. β-Sitosterol has also been reported to occur in *A. diandrum* [3].

Compound **4** was obtained as a yellow amorphous solid. Its structure was obtained by the interpretation of the NMR data (^{1}H, ^{13}C, DEPT, HMBC, HSQC and COSY) as well as mass spectra. The ^{1}H and ^{13}C NMR data were very similar with those hitherto reported for amentoflavone [10, 11]. This is the first report of this compound from the genus *Antidesma*.

It is worth pointing out that antidesmone and its derivatives have not been detected in the extract of *A. laciniatum* during this work. This correlates well with the observations of Buske *et al.* [3] on the occurrence of antidesmone and its derivatives in different species of *Antidesma* collected from Cameroon.

EXPERIMENTAL

General. Mp's: electro thermal digital melting point apparatus. IR: Perkin-Elmer BX spectrometer; UV: Shimadzu UV/VIS-160. NMR: Bruker 400 MHz Avance spectrometer; solvents: $CDCl_3$ and DMSO-d_6 (400 MHz for ^1H and 125 MHz for ^{13}C). The high resolution ESI mass spectra were obtained from a Bruker Apex III Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (Bruker Daltonics, Billerica, USA) equipped with an Infinity™ cell, a 7.0 Tesla superconducting magnet (Bruker, Karlsruhe, Germany), an RF-only hexapole ion guide and an external electrospray ion source (Agilent, off axis spray). The sample solutions were introduced continuously via a syringe pump with a flow rate of 120 μL h^{-1}. The 70 eV EI mass spectra were obtained from an AMD 402 (AMD Intectra) mass spectrometer.

The adsorbent used for flash column chromatography (FCC) and medium pressure liquid chromatography (using SEPARO AB type apparatus) was silica gel 60 (230-400 mesh, Merck). Prep. TLC was carried out on 0.75 mm thick layer silica gel PF_{254} (Merck) coated (20 cm x 20 cm) glass plates.

Plant material. The leaves of *Antidesma laciniatum* (Euphorbiaceae) were collected in August 2004 in Cameroon at Mount Kala (altitude 1800 m), about 15 km from Yaoundé. The plant was authentified by Mr. Nana, Botanist at the National Herbarium, Yaoundé, Cameroon where a voucher specimen has been deposited.

Extraction and isolation. The leaves of *A. laciniatum* were air-dried and ground to a powder (2.6 kg) which was macerated with 80% aqueous methanol (7.5 L) for 48 h. After concentration in vacuo to the aqueous phase (1.8 L), it was treated with $CHCl_3$/MeOH/H_2O (4:4:1) (2.5 L). The lower organic phase was separated and dried with $MgSO_4$, concentrated in vacuo to yield 36 g of extract. 6 g of this extract was subjected to FCC (silica gel, 140 g) and eluted successively with the mixtures of petrol-$CHCl_3$ and $CHCl_3$-EtOAc, each of increasing polarity.

Squalene (**1**) (37 mg) was obtained directly from the fraction eluted with petrol-$CHCl_3$ (80:20) as a colorless oil. IR (KBr) ν_{max} 2925, 1670, 1449, 1379 cm^{-1}. UV ($CHCl_3$) λ_{max} blank. ^1H and ^{13}C NMR ($CDCl_3$) data were identical to those previously reported [7]. EIMS m/z (rel. int.) 410 (4), 341 (7), 273 (3), 149 (32), 136 (48), 121 (43), 109 (34), 95 (49), 81 (79), 69 (100), 67 (39), 41 (54).

The fraction obtained from the FCC with $CHCl_3$ was passed through Sephadex LH-20 using $CHCl_3$-MeOH (50:50) as eluent and further purified through prep. TLC (eluent petrol-EtOAc 80:20) to afford (2*E*, 7ξ,11ξ)-phyt-2-en-1-ol (**2**) (25 mg) as a colorless oil. UV ($CHCl_3$) λ_{max} blank. IR (KBr) ν_{max} 3435, 2928, 1666, 1463, 1379, 1215 cm^{-1}. ^1H and ^{13}C NMR ($CDCl_3$) identical to literature [8, 14].

The fraction eluted with $CHCl_3$-EtOAc (85:15) was chromatographed over SEPARO AB-type apparatus using petrol-EtOAc of increasing polarity as eluent. Sub-fractions collected from the column with light petrol-EtOAc (85:15) gave a solid which was further recrystallysed with petrol-$CHCl_3$ to afford β-sitosterol (56 mg) as white crystals, m.p. 137-139 °C. UV ($CHCl_3$) λ_{max} blank. IR (KBr) ν_{max} 3434, 2960, 1638, 1469, 1382, 1062 cm^{-1}. ^1H and ^{13}C NMR ($CDCl_3$) identical to literature [9].

Fractions obtained from the FCC with $CHCl_3$/EtOAc (40:60) to pure EtOAc were combined and passed trough Sephadex LH-20 eluted with $CHCl_3$-MeOH (50:50) to afford amentoflavone as yellow amorphous solid (18 mg). UV (MeOH) λ_{max} (log ε) 335.0 (2.26), 212.0 (3.15) nm. IR (KBr) ν_{max} 3435-3100 (broad), 1730, 1654, 1579, 1492, 1286, 1246, 1167 cm^{-1}. ^1H and ^{13}C NMR (DMSO-d_6) identical to those found in the literature [10, 11]. HR-ESIMS (positive mode) m/z 561.07800 $[M + Na]^+$ (calcd. 561.15668 for $C_{30}H_{18}O_{10}Na$); HR-ESIMS (negative mode) m/z

537. 08163 $[M-H]^+$; EIMS m/z (rel. int.) 537.4 (100) $[M-H]^+$, 339.3 (15), 325.3 (20), 311.4 (12), 255.3 (14), 179.2 (8), 151.3 (72), 137 (34).

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