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Research Article

The Isolation of Important Biosynthetic Intermediate; Presqualene Alcohol and Its Acetate Derivative from *Antidesma Venosum*

Joseph J. Magadula[*1], Dulcie A. Mulholland[2, 3], Neil R. Crouch[3, 4]

[1]*Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences, P.O. Box 65001, Dar es Salaam, Tanzania*
[2]*Division of Chemical Sciences, Faculty of Health and Medical Sciences, University of Surrey, GU2 7XH, Guildford, United Kingdom.*
[3]*School of Chemistry, University of KwaZulu-Natal, Durban 4041, South Africa*
[4]*Ethnobotany Unit, South African National Biodiversity Institute, PO Box 52099, Berea Road 4007, Durban, South Africa*
**Corresponding author: magadulajanguj@yahoo.com*

ABSTRACT

Phytochemical investigations of the root bark of *Antidesma venosum* (Euphorbiaceae) afforded presqualene alcohol (**1**), presqualene acetate (**2**) together with friedelin; *epi*friedelanol, betulinic acid, toddaculin, α-tocopherol and pheophytin A. This is the first report of compound **2** to be isolated from the natural source. Furthermore, presqualene acetate (**2**) can be obtained in large amounts from this source, making it a good source of material for biosynthetic studies.

Keywords: *Antidesma venosum*, Euphorbiaceae, Isolation, presqualene alcohol, presqualene acetate, triterpenoid biosynthesis

1. INTRODUCTION

Antidesma venosum E.Mey. ex Tul. (Euphorbiaceae) is a medium-sized dioecious tree found growing along rivers and in dry lowland forests through south-Central, tropical and West Africa [1, 2]. Some authorities [3] recognize a broader circumscription for the species that includes both *A. membranaceum* Müll.Arg and *A. vogelianum* Müll.Arg. In South Africa, *A. venosum* occurs mainly in the coastal and coastal scarp forests of KwaZulu-Natal, and in the Lowveld of Mpumalanga and Limpopo Provinces [4]. Across its range, the fruits are sometimes eaten [5], whilst this species is widely employed medicinally, particularly in the treatment of various digestive tract disorders [6, 7] and abdominal pain [8]. It has also reportedly been used to treat liver complaints [3, 8], snakebite, hookworm [8] and various sexual health issues, including Sexual Transmitted Diseases [9, 10]. In Tanzania, *A. venosum* grows along the coastal forests and riverine woodlands in Mbeya, Morogoro and Tanga regions. The wood of this plant is used in Tanzania for firewood, tool handles and knife sheaths while fruits are sometimes eaten [11]

A previous analysis of the leaves of *A. venosum* collected in Dar es Salaam, Tanzania led to the isolation of the quinoline alkaloid antidesmone [12]. However, antidesmone was not obtained in these investigations despite using the special procedure for extraction of alkaloids.

2. MATERIAL AND METHODS

2.1. General

IR spectra were recorded with a Nicolet Impact 400 D spectrometer on sodium chloride plates and calibrated against an air background. The HREIMS were recorded on a Kratos high resolution MS 9/50 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity Inova 400 MHz NMR spectrometer. Chemical shifts (δ) are expressed in ppm and were referenced against the solvent resonances at 7.26 and 77.23 ppm for ^{1}H and ^{13}C NMR respectively. Optical rotations were measured at room temperature in methanol using a Perkin Elmer Polarimeter-Model 341.

2.2. Plant Material

Root bark was collected in May 2004 from a female tree growing in Kloof, South Africa and a voucher specimen (*N. Crouch 1025*, NH) retained for verification purposes at the South African National Biodiversity Institute.

2.3. Extraction and isolation

The dried, milled root bark (164 g) was successively extracted using a soxhlet apparatus for 24 hours each with hexane, dichloromethane, ethyl acetate and methanol. The hexane extract (1.93 g) was subjected to column chromatography over silica gel (Merck 9385), to yield presqualene acetate, **2** (168.0 mg), presqualene alcohol **1** (6.2

mg) [13], friedelin (14.0 mg) [14] and *epi*friedelanol (13.4 mg) [15]. Column chromatography of the dichloromethane extract (0.94 g) yielded presqualene acetate, **2** (18.0 mg), friedelin (2.8 mg) and toddaculin (14.0 mg) [16] and betulinic acid (36.8 mg) [17]. The ethyl acetate and methanol soluble extracts contained only sugars and were not analysed further. Neither alkaloids were isolated nor detected using the Dragendorf test.

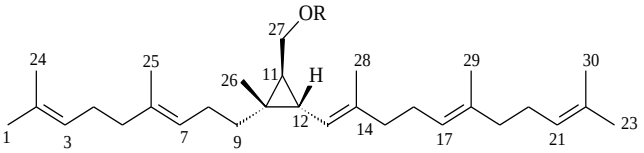
2.4. Hydrolysis of presqualene acetate (2)

Compound **2** (50.0 mg) was dissolved in methanol (5 ml) and 5 % KOH (10 ml) was added. The mixture was heated under reflux for 3 hrs, cooled and acidified by adding 2M H_2SO_4 to attain a pH of 3. Na_2CO_3 solution was added until no further effervescence was observed. The basic solution was extracted with diethyl ether to yield presqualene alcohol (**1**) (16 mg, 32 % yield).

Presqualene acetate (**2**); Colourless oil; $[\alpha]^{25}_D$: +10.6° ($CHCl_3$, c 0.232); IR (ν_{max}^{NaCl} cm^{-1}): 2965, 2922, 1742, 1451, 1366, 1238, 1024; HREIMS: *m/z* 468.39977 (calc. for $C_{32}H_{52}O_2$ 468.39977); GCMS *m/z* (rel. int.): 468 (2), 408 (2), 215 (5), 203 (17), 137 (12), 69 (100); ^{1}H and ^{13}C NMR data: Table 1

3. RESULTS AND DISCUSSION

The HREIMS of compound **1** showed a molecular formula of $C_{30}H_{50}O$ indicating six degrees of unsaturation. The ^{13}C NMR spectrum showed the presence of five alkene double bonds, seven vinyl and one additional methyl groups, an oxymethylene carbon resonance and three unusual upfield resonances that could be ascribed to carbons of a cyclopropane ring [δ_C 26.3 (C); δ_C 35.0 (CH); δ_C 29.0 (CH)]. The NOESY spectrum showed correlations between the H-12, 3H-26 and 2H-27, indicating they were *cis* to each other.



1 R=H
2 R=Ac
3 R=**PP**

Fig 1: Structures of compound 1-3

A literature search indicated compound **1** to be a presqualene alcohol [13]. This compound has been synthesized from farnesyl pyrophosphate in incubations with yeast microsomes from which inhibitors of phosphatase have been omitted [18]. It has also been detected in rat liver cells using pulse labelling experiments [19] while a stereoselective total synthesis of (+)-presqualene alcohol has been reported [1]. The only previous isolations of compound **1** from a plant source were two reports from *Salvia divinorum* [19, 20].

The NMR spectra of compound **2** were very similar to those of compound **1**. However, the molecular formula determined by HR-EIMS was found to be $C_{32}H_{52}O_2$. The ^{1}H NMR spectrum showed an additional singlet resonance at δ_H 2.03, additional resonances at δ_C 171.3 and 21.1 in the ^{13}C NMR spectrum and an absorption at 1742 cm^{-1} in the IR spectrum. This indicated that it was an acetylated derivative of compound **1**. Further evidence was the presence of a fragment ion at $[M-60]^+$ due to the loss of an acetic acid unit seen in the mass spectrum (Fig. 2)

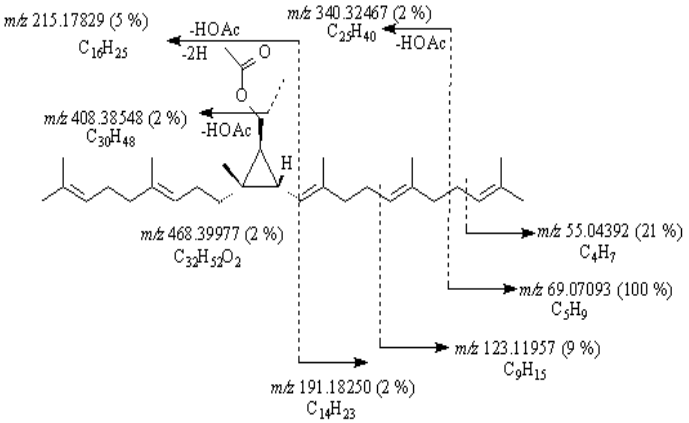


Fig. 2: Fragmentation patterns of compound 2

The ^{13}C NMR spectrum showed the presence of five trisubstituted double bonds while the ^{1}H NMR spectrum showed seven vinyl methyl group singlets (δ_H 1.56-1.66) and seven coupled methylene groups (between δ_H 1.2 and 2.1) indicating the presence of an isoprenoid structure as for compound **1**. As with compound **1** and from the molecular formula, one ring was necessary in the molecule, hence 7 degrees of unsaturation. Using a combination of COSY, HMBC and NOESY spectra, the chain from C-1 to C-9 and associated protons could be assigned. The H-9 resonance only showed coupling to H-8. The HMBC spectrum showed that the C-9 resonance showed correlations with a methyl group proton resonance (δ_H 1.09, 3H-26), and two methine proton resonances (δ_H 0.88 H-11; δ_H 1.16 H-12). Coupling was seen in the COSY spectrum between the 2H-27 (δ_H 4.28 and 3.92) and the H-11 resonance and then between the H-11 and the H-12 resonances. All resonances for the remainder of the chain could then be assigned using COSY, NOESY and HMBC spectra. The NOESY spectrum showed that C-26, C-27 and H-12 were *cis*, and H-11 was on the same plane of the molecules as C-9 and C-13. Other NMR data is given in Table 1. Compound **2** was the unacetylated form of compound **1** and the two compounds were readily interconverted by hydrolysis or acetylation.

Table 1: NMR data (400MHz) of 2 ($CDCl_3$, J in Hz)

Position	δ_C	δ_H	Position	δ_C	δ_H
1	17.7 (CH_3)	1.57 s	17	124.3 (CH)	5.11 m**
2	131.2 (C)	-	18	134.8 (C)	-
3	124.2 (CH)	5.09 m**	19	39.6 (CH_2)	1.95 m
4	26.6 (CH_2)	2.04 m*	20	26.7 (CH_2)	2.05 m*
5	39.7 (CH_2)	1.96 m	21	124.5 (CH)	5.08 m**
6	135.0 (C)	-	22	131.3 (C)	-
7	124.4 (CH)	5.10 m**	23	17.7 (CH_3)	1.57 s
8	25.1 (CH_2)	2.05 m*	24	25.7 (CH_3)	1.65 s
9	36.5 (CH_2)	1.22 m	25	15.9 (CH_3)	1.57 s
10	26.4 (C)	-	26	18.7 (CH_3)	1.09 s
11	30.7 (CH)	0.88 ddd (5.4, 6.7, 8.9)	27	65.5 (CH)	3.92 dd (8.9, 11.7)
					4.28 dd (6.7, 11.7)
12	29.4 (CH)	1.16 dd (5.4, 7.8)	28	16.6 (CH_3)	1.66 s
13	122.7 (CH)	4.89 dd (1.1, 7.8)	29	16.0 (CH_3)	1.57 s
14	137.3 (C)	-	30	25.7 (CH_3)	1.65 s
15	39.7 (CH_2)	2.02 m*	$COCH_3$	171.3 (C)	-
		1.97 m			
16	26.6 (CH_2)	2.05 m*	$COCH_3$	21.1 (CH_3)	2.03 s

**, ** overlapping signals*

The elucidated chemical profile of material from Southern Africa (current report) and that from East Africa [21] reflects substantial constituent diversity across the geographic range of *A. venosum*. To the authors' knowledge, this is the third plant source of presqualene alcohol (**1**), and the first of its acetate (compound **2**) from a plant source. The biosynthesis of squalene from the tail to tail addition of two farnesyl pyrophosphate goes *via* the intermediate presqualene pyrophosphate, **3**. This compound, a necessary precursor of all triterpenoids, occurs in the root bark of *A. venosum* in both the free alcohol and acetylated form. Fair amounts of the acetate (5 % of dried plant material) can be isolated from the South African chemotype, which can readily be converted into the free alcohol for further biosynthetic studies.

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