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A new triterpene and stigmasterol from *Anthostema madagascariense* (Euphorbiaceae)

Volasoa Herilalaina Victorine Rambeloson [1*], Léa Herilala Rasoanaivo [2], Anne Wadouachi [3], Amelie Raharisololalao [4]

1. UG 1 Department of Chemistry, Faculty of Science, University of Fianarantsoa, Madagascar, P.O. Box 1264, Fianarantsoa 301, Madagascar, [Email: vlrambeloson@gmail.com; +261324327885]
2. Department of Organic Chemistry, Faculty of Science, University of Antananarivo, Madagascar, P.O. Box 906, Antananarivo 101, Madagascar, [Email: learasoa3@yahoo.fr; +261324022036]
3. Laboratory of Antimicrobial Glycochemistry and Agroressources FRE CNRS 3517 University of Picardie Amiens France, [Email: anne.wadouachi@u-picardie.fr]
4. Department of Organic Chemistry, Faculty of Science, University of Antananarivo, Madagascar, P.O. Box 906, Antananarivo 101, Madagascar, [Email: amelie_raharisololalao@yahoo.fr; +261320257754]

Purification of the ethyl acetate fraction from the stem bark of *Anthostema madagascariense* (Euphorbiaceae) resulted in the isolation of triterpene namely 3-acetoxy-olean-9(11)-en-28- oic acid and a sterol namely Stigmasterol. The structures of the two products isolated compounds were characterized on the basis of extensive spectral data NMR (1D and 2D) and in the comparison with the literature data. The compounds are reported for the first time from this plant.

Keyword: *Anthostema madagascariense,* Triterpene, Sterol, Euphorbiaceae.

1. Introduction

Anthostema madagascariense is a flowering plant species of the Family Euphorbiaceae. *Anthostema* is a small genus with 3 species of which 2 in continental Africa and 1 in Madagascar: *Anthostema madagascariense* is endemic of Madagascar. Evergreen, monoecious shrub to medium-sized tree up to 30 m tall, with abundant white latex in all parts; bole branchless for up to 12 m or more, generally straight and regular, up to 70 cm in diameter; bark surface densely fissured, reddish to blackish [4, 5, 9, 10, 11, 12].

A decoction of the stem bark of Anthostema *madagascariense* is used to treat fever, cough and liver.

According to available literature, no phytochemical research work has been carried out on this plant. We now report the isolation of triterpene and sterol on the ethyl acetate extract from the stem bark of the *Anthostema madagascariense*.

2. Materials and Methods

2.1 General

1D(1H, 13C, DEPT) and 2D (1H-1H COSY, 1H-13C HSQC, 1H-13C HMBC) NMR spectra were recorded on a Bruker Varian 300 NMR and 600 NMR operating respectively at 300.15/100.6 MHz and 600.15/100.6 MHz using CDCl3 and CD3OD as solvent and TMS as an internal standard. Column chromatography (CC) was carried out on silica gel 60F254 (Merck) in glass blades. Thin layer chromatography (TLC) was performed on precoated TLC plates (Merck, silica 60F254) and detection wavelength (254 and 365 nm) was used. Mass spectra were measured with waters 2995/2975-Micromass Q-Tof micro

spectrometer (ES+-MS) and Agilent 5975 spectrometer (EI-MS).

2.2. Plant material

Anthostema madagascariense Baill. (Euphorbiaceae) was collected in July 2012 in Farafangana Manombo, Vatovavy Fitovinany's Region Madagascar and was identified by botanists at the Parc National Botanic and Zoologique Tsimbazaza, Antananarivo, Madagascar where a voucher specimen has been deposited in the Herbarium.

2.3 Extraction and isolation:

The stem barks of *Anthostema madagascariense* Baill. were dried, ground, reduced on powder. The powder was macerated successively with hexane, ethyl acetate and methanol. The solvents were evaporated under reduced pressure to obtain crude residue: green, green and red solid gum (2.05 g; 7.02 g; 7.27 g).

The ethyl acetate extract was subjected to column chromatography (silicagel, 3 x 80 cm) and was eluted with a gradient profil of hexane and acetone to give 280 main fractions. The fractions 31- 32 were obtained after 660 mL elution with hexane and acetone (90:10), and the fractions 119- 138 were obtained after 2700 mL and after elution with hexane and acetone (40:60).

The purification of fractions 31-32 (compound1) and the 119-138 (compound 2) were carried out by column chromatography on Sephadex LH-20 with CDCl3-MeOH (80:20).

3. Result and discussion: reported in tables 1 and 2

The dried and ground stem bark of *Anthostema madagascariense* was extracted successively with hexane, ethyl acetate and methanol. The ethyl acetate extract was repeatedly chromatography over Si gel and purified over Sephadex LH-20 to give two compounds a triterpene (**1**) and a sterol (**2**).

Compound (**1**) was obtained as a white cottony needle; it gave a positive Liebermann-Burchard test. Its HREIMS spectrum showed a molecular peak at *m/z 4*98.37 and 13C NMR exhibited thirty two signals in accordance with the molecular formula C32H50O4.

The 1H-NMR (Table 1) showed eight methyls, as singlets respectively at δ 0.76, 0.80, 0.83, 0.85, 0.87, 0.88, 1.18 and 2.00 ppm. A signal at δ 3.96 ppm attributable to a proton which is carried by a carbon bonded to a heteroatom. In the spectrum, there were also present an olefinic proton at δ 5.44 ppm. The presence of the singulet (3H) at δ 2.00 ppm indicated that the molecule contained a acetyle group. So that it consisted to a double bond. It was confirmed by the presence of the signal at 171.00 ppm [3] (DEPT). Further, in compound (**1**) there was a signal at 183.86 ppm consisting to acid group COOH [2] (DEPT).

The DEPT spectrum showed thirty two carbon atoms which belong to different groups, carbonyl carbon of carbon acetate at δ 171.00 ppm (C-1') , an acid at δ 183.86 ppm (C-28) and two olefinic carbons at δ 160.56 ppm (C-9) and δ 116.62 ppm (C-11). The signal δ 80.72 ppm a carbonyl signal is attributed to the carbon at position C-3. Thirty carbon atoms correspond to the basic skeleton triterpene where acetate is fixed: 10 methylene carbons, 8 methyl carbons, 5 methynique carbons and 9 quaternary carbons of which an acid.

As is observed the existence of 32 carbon atoms of Broadband spectrum the triterpene structure was confirmed and by collecting all the NMR spectral data of the compound **1**, we deduced to the conclusion that compound **1** is the 3-acetoxy-olean-9(11)-en-28-oic acid. It is a new pentacyclic triterpene.

Compound **(2)** was obtained as a white solid and its molecular formula was assigned as C29H48O by the molecular ion peak at m/z 412.37 in the HREIMS.

The mass spectral data of the compound gave a molecular formula C29H48O, which was supported by the 13C NMR spectral data. In the 1H NMR spectrum of compound **2** varied between 0.71 to 5.30 ppm, This spectrum showed the presence of 6 high intensity peaks indicating the presence of six methyl groups at δ 0.71 , 0.86, 0.92, 1.00, 1.18 and 1.36 ppm. The proton corresponding to the H-3 of a sterol moiety was appeared as a triplet of doublet of doublet at δ

3.17 ppm. At δ 5.24 ppm and at δ 5.30 ppm corresponds to a peak in the form of a single in the region of the ethylene protons suggesting the presence of three protons corresponding to that of a trisubstituted and a disubstituted olefin bond. The signal at δ 0.68 ppm and δ 1.18 ppm corresponds to H-18 and H-19 protons respectively of Stigmasterol.

In the NMR DEPT 135 ° spectrum showed 29 carbon atoms which is a steroid including quaternary carbon. In the twenty nine carbon atoms including 6 methyl carbons, 9 methylene carbons, 11 methynique carbons and three quaternary carbons .

Table 1: 1H and 13C NMR spectral dataa for compound 1

Position	**δ 1H (ppm)**	**δ 13C (ppm) Experimental Value**	**δ 13C (ppm) [6] Literature Value**
1	1.53 ; 1.85	37.93	39.0
2	1.53 ; 1.85	23.47	28.2
3	3.85	80.72	78.1
4	-	39.03	39.4
5	1.37	55.59	55.8
6	1.18 ; 1.53	17.31	18.8
7	1.18 ; 1.53	33.32	33.3
8	-	37.32	39.8
9	-	160.56	48.2
10	-	51.46	37.4
11	5.44	116.82	23.7
12	1.85;2.10	27.96	122.6
13	-	37.68	144.8
14	1.37	40.76	42.2
15	1.37 ; 1.53	30.71	28.4
16	1.85 ; 2.28	29.30	23.8
17	-	49.07	46.7
18	1.37 ; 1.53	37.39	42.0
19	1.18 ; 1.53	41.42	46.5
20	-	30.71	31.0
21	1.37 ; 1.53	35.34	34.3
22	1.85, 2.28	33.78	33.2
23	0.83	15.64	28.8
24	0.85	16.60	16.6
25	0.87	26.19	15.6
26	0.88	27.96	17.5
27	0.88	22.45	26.2
28	-	183.86	180.2
29	0.80	28.66	33.3
30	0.76	28.66	23.8
1’	-	171.00	
2’	2.00	21.34	

Table 2: 1H and 13C NMR spectral data for compound 2a

Position	δ 1H (ppm)	δ 13C(ppm) Experimental Value	δ 13C(ppm) [7] Literature Value
1	1.32 ; 1.56	38.81	37.3
2	0.80 ; 2.10	33.06	31.6
3	3.17 m	79.07	71.8
4	1.84 ; 2.76	41.08	42.3
5	-	143.41	140.8
6	5.30 br s	122.75	121.7
7	1.62 ; 2.34	32.43	31.9
8	1.34	32.93	31.9
9	2.10	52.44	51.2
10	-	38.41	36.5
11	0.84 ; 1.84	23.29	21.1
12	0.95 ; 1.34	38.60	39.7
13	-	47.87	42.3
14	1.07	55.21	56.9
15	153 ; 2.18	24.15	24.4
16	1.53 ; 2.18	30.60	28.4
17	1.56	53.91	56.1
18	0.68 s	39.04	40.5
19	1.18 s	28.00	21.2
20	5.24	138.37	138.3
21	5.24	125.05	129.3
22	1,53 (d,7.5)	47.54	51.2
23	1.44	27.17	31.9
24	1.00	16.95	12.1
25	1.84	28.18	31.9
26	0.92 (d,6.5)	23.57	25.4
27	0.86 (d,6.5)	21.17	19
28	0 .71 (t,7.5)	17.05	11
29	1.36	28.66	21.2

The spectrum showed some recognizable signals at δ 143.41 ppm, δ 138.37 ppm, δ 125.05 ppm and δ 122.75 ppm which is assignable to the double bond at C-5, C-20, C-21 and C-6 [1]. The carbon at δ 143.41 ppm C-5 is a ethylene quaternary carbon. The δ value observed at 79.07 ppm is due to C-3 is a carbon hydroxyl group [8]. Additional, the signal observed at 28.00 ppm correspond to angular carbon at C-19.

The protons at δ 1.00 ppm, at δ 2.18 ppm and δ 3.17 ppm are carried by the carbon at δ 16.95 ppm, at δ 52.44 ppm and δ 79.07 ppm respectively.

In the COSY experiment, irradiation of H-8 (δ 1.34 ppm) gave use to the enchancement of the H- 9 (δ 2.10 ppm). A proton geminate H-7 at δ 1.62 ppm and at δ 2.34 ppm.

In the HMBC spectrum (Table 2), the protons resonanting at δ 0.80 ppm showed longe range heteronuclear connectivities with C-3 (79.07 ppm), C-9 (δ 52.44 ppm) and C-1 (δ 38.83 ppm). The H-18 protons, resonanting at 1.18 ppm, exhibited HMBC interactions with C-22 (δ 47.54 ppm), C-21 (125.05 ppm) and C-20 (δ 138.37 ppm).

Thus, the structure of compound 2 was assigned as the know compound Stigmasterol.

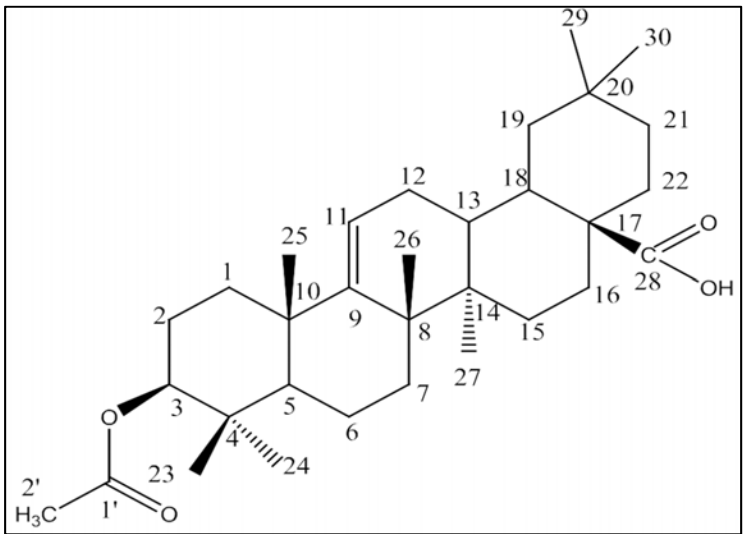


Fig 1: Structure of 3-acetoxy Olean-9(11)-en-28-oic acid

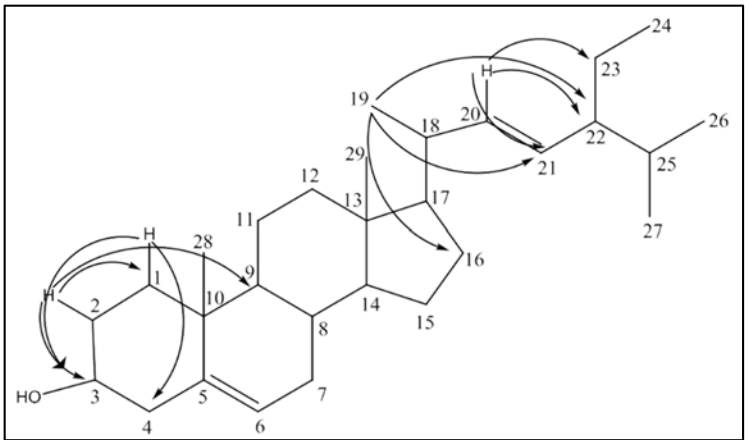
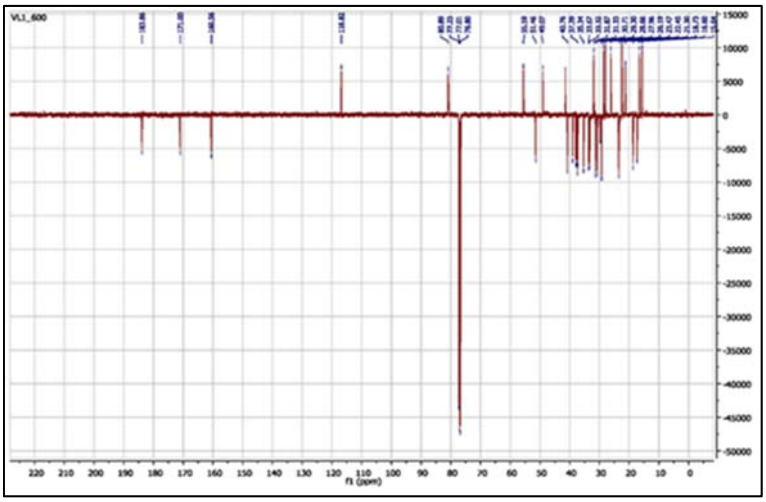
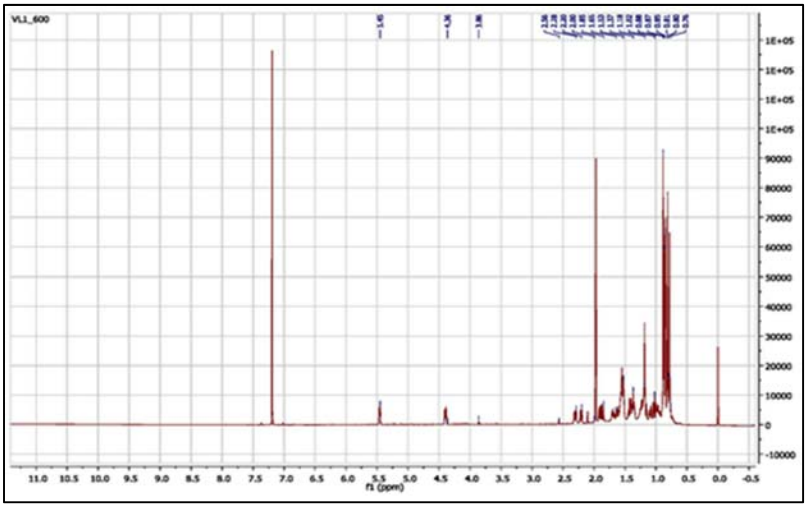
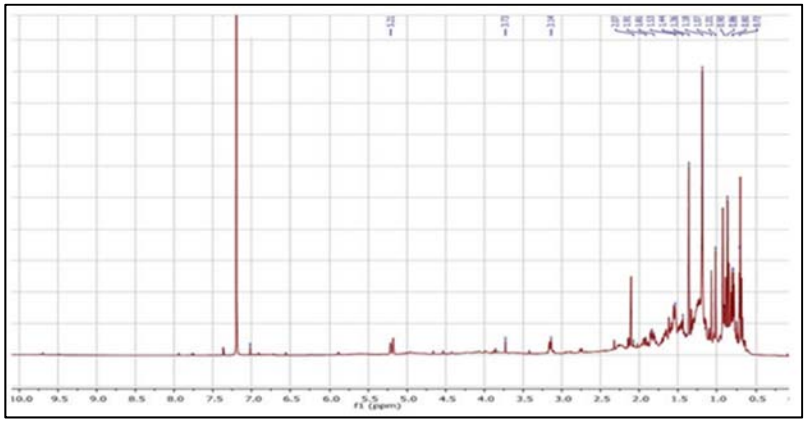
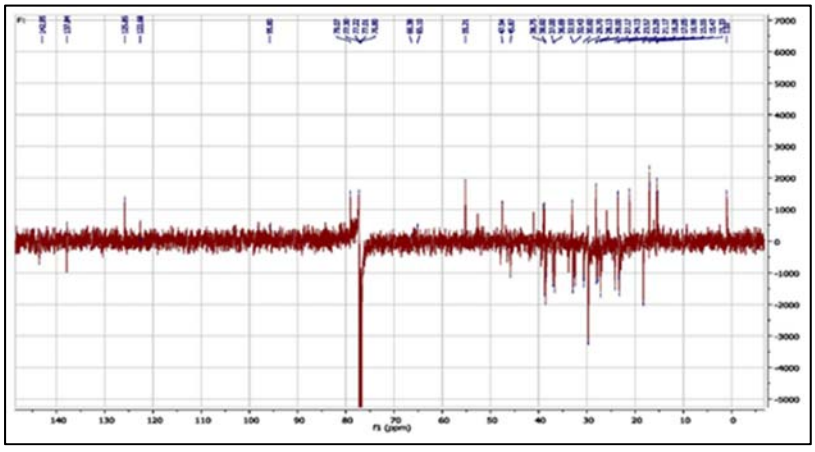


Fig 2: Long-range heteronuclear correlation observed for 2 **(**stigmasterol)









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5. Conclusion

Stigmasterol and acetoxy myrtifolic acid were isolated from the ethyl acetate extract from the stem bark of *Anthostema madagascariense.* The structures of the sterol and triterpene compounds were identified as 3-acetoxy-olean-9(11)-en-28-oic acid and stigmastérol on the basis of spectroscopic methods. The complete 1H, NMR DEPT 135° and Broadband spectral assignments of the one isolated 2 compounds were made based on HSQC, HMBC spectroscopic data.

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