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CHEMICAL CONSTITUENTS OF THE ANTIDIABETIC PLANT *RAVENALA MADAGASCARIENSIS*

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ABSTRACT: *Ravenala madagascariensis* Sonn. is one of the most popular medicinal plants in Madagascar, India and several African countries. It is used in the treatment of many diseases, and particularly in those of diabetes. The duration of treatment, often for life, and its relatively high cost for certain social categories, constrain more and more patients to use this plant. Its chemical constituents were still little studied and this problem could create significant damage in case of intolerance or abuse during its use. The present study undertaken on the leaves of *Ravenala madagascariensis*, led to the isolation and structural elucidation of two new compounds (**1** and **2**), together with the known cycloartanol triterpene (**3**) which was isolated for the first time from this plant. Their structures were elucidated as (2E, 7R, 11R) phytyl-3, 7, 11, 15-tetramethylhexadec-2-enyl pentadecanoate (**1**) and (24S, 31S)-cycloartan-31, 32-diol (**2**). Structural determinations were made on the basis of analysis of 1D and 2D-NMR spectroscopic data. The complete ¹H and ¹³C resonance assignments were also carried out by using ¹H-¹H COSY, HSQC, NOESY and HMBC experiments.

INTRODUCTION: *Ravenala madagascariensis* Sonn. belongs to the family Musaceae. Commonly known as Traveler's tree, it is endemic to Madagascar and often found cultivated anywhere in the world as an ornamental plant. It is widely used in traditional medicine in the treatment of diarrhea, hypertension¹, edema², kidney stone and diabetes^{3,4}. The seeds are also reported to be antiseptic⁴.

The ethanolic and aqueous extracts of the leaves were proven to have *In vitro* and *In vivo* antidiabetic activity on alloxan induced diabetic rats⁵. In a previous paper, we reported the occurrence of the β-sitosteryl-D-glucoside in the leaves, and its complete ¹H and ¹³C resonance assignments⁶.

In this report, we investigated the hexane extract of the leaves of *Ravenala madagascariensis*. It led to the isolation and structural elucidation of two new compounds named (2E, 7R, 11R)-phytyl-3, 7, 11, 15-tetramethylhexadec-2-enyl pentadecanoate (**1**), (24S, 31S)-cycloartan-31, 32-diol (**2**), along with the known triterpene cycloartanol (**3**). Their complete ¹H and ¹³C resonance assignments were

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carried out. Cycloartanol was reported in the literature as having antidiabetic activity^{7,8}.

MATERIALS AND METHODS:

General:

The separation and isolation process were made using column chromatography. Silica gel 60 (230-400 mesh, Merck), neutral alumina (150 mesh, Sigma-Aldrich) and Sephadex LH 20 (Pharmacia LKB) were used as stationary phase. Analytical thin layer (TLC) chromatography aluminium sheets of silica gel 60 F₂₅₄ (Merck) were used to control the fraction's content. Compounds' revelation was made before heating at 120°C by spraying the TLC with sulfuric vanillin after detection under an UV lamp. NMR spectra were recorded on a spectrometer Bruker Avance DRX-500 at 500 MHz (¹H) and 125 MHz (¹³C); chemical shifts are in ppm with TMS as internal standard. The melting points were determined with a Kofler block and were uncorrected. The MS were recorded on a spectrometer Micromass Q-TOF Micro-Instrument (Manchester). Optical rotation was measured with an electronic polarimeter Polartronic-D (Schmidt + Haensch).

Plant material:

The leaves of *Ravenala madagascariensis* Sonn. were collected in Abidjan, Ivory Coast, during June 2010 and authenticated by Pr. L. Aké Assi of the University Félix Houphouët-Boigny, Cocody, Abidjan. Voucher specimens (n°21052) are deposited at the Herbarium of the "Centre National de Floristique" of this University.

Extraction and isolation:

Air dried and powdered leaves of *Ravenala madagascariensis* (1090 g) were extracted three times with EtOH – H₂O (8 : 2) at room temperature for 48 hours, each. After filtration and evaporation of the solvent, the residue (90 g) was partitioned between n-hexane and H₂O. After decantation and evaporation of the solvent, the hexane extract (24 g) was obtained and aqueous phase was still extracted three times with EtOAc for other investigation.

The hexane extract of the leaves of *Ravenala madagascariensis* was fractionated by dry flash silica gel chromatography using a gradient of

methylene chloride in hexane at 10% increment as eluent. The fractions (20 ml each) obtained on elution with methylene chloride pure were separated by silica gel column chromatography eluted with 20% methylene chloride in hexane. The purification of the compound **1** was finally made with a neutral alumina column chromatography using 5% dichloromethane in hexane.

It was isolated as an oily compound (7 mg). The 50-60% methylene chloride in hexane fractions presented crystals. They were collected and purified on a silica gel column chromatography using 40% methylene chloride in hexane. The compound **2** was isolated as a white powder (5 mg). The 30-40% methylene chloride in hexane fractions were rechromatographed on a neutral alumina column eluted with 30% methylene chloride in hexane to afford compound **3** (5 mg).

(2E, 7R, 11R) – Phytyl - 3, 7, 11, 15 –tetra methylhexadec-2-enyl pentadecanoate (1):

Pale yellow oil, $[\alpha]_D^{20} +21.37$ (c 0.23, CHCl₃), APCI MS m/z 521.9245 [M+H]⁺, 379, 213, 155. TOF MS (EI⁺) m/z(%) 113 (24), 156 (22), 184 (37), 212 (36), 256 (100), 310 (40). ¹H (500 MHz, CDCl₃) and ¹³C (125 MHz, CDCl₃) (see table 1).

(24S, 31S)-Cycloartan-31, 32-diol (2):

White amorphous powder, mp.193-195, $[\alpha]_D^{20} -32.7$ (c 0.16, CHCl₃). TOF MS (EI⁺): m/z 473.7912 [M+H]⁺, 429 (45), 355 (100), 341 (28), 327 (16), 175 (22), 124 (41). ¹H (500 MHz, CDCl₃) and ¹³C (125 MHz, CDCl₃) NMR data, (see Table 2).

Cycloartanol (3):

White amorphous powder: TOF MS (EI⁺): m/z 428.4120 [M]⁺. ¹H (500 MHz, CDCl₃): δ 3.29 (1H, dd, J_{aa} = 11.3 Hz and J_{ae} = 4.5 Hz, H-3), 1.31 (1H, m, H-5), 1.52 (2H, m, H-8 and H-17), 1.37 (1H, m, H-20), 1.53 (1H, m, H-25), 1.57 (1H, m, ½ H₂-1), 1.25 (1H, m, ½ H₂-1), 1.76 (1H, m, ½ H₂-2), 1.59 (1H, m, ½ H₂), 1.60 (1H, m, ½ H₂-6), 0.80 (1H, m, ½ H₂-6), 1.90 (1H, m, ½ H₂-7), 1.30 (3H, m, ½ H₂-7 and H₂-23), 2.00(1H, m, ½ H₂-11), 1.11 (1H, m, ½ H₂-11), 1.63 (2H, m, H₂-12), 1.37 (2H, m, ½ H₂-15 and ½ H₂-16), 0.99 (1H, , m, ½ H₂-15), 1.15 (3H, m, ½ H₂-16 and H₂-24), 1.28 (2H, m, H₂-22), 0.97 (3H, s, H₃-18), 0.90 (3H, d, J =5.6 Hz, H₃-21), 0.87 (6H, d, J = 7 Hz, H₃-26 and H₃-27), 0.9 (3H, s,

H₃-28), 0.82 (3H, s, H-29), 0.97 (3H, s, H₃-30), 0.55 (1H, d, J = 5 Hz, ½ H₂-19), 0.33 (1H, d, J = 5 Hz, ½ H₂-19). ¹³C (125 MHz, CDCl₃): δ 31.9 (C-1), 30.3(C-2), 78.8(C-3), 40.4 (C-4), 47;1 (C-5), 21.1 (C-6), 28.1 (C-7), 48.0 (C-8), 20.0 (C-9), 26.0 (C-10), 26.4 (C-11), 32.8 (C-12), 45.2 (C-13), 48.8 (C-14), 36.4 (C-15), 24.1 (C-16), 52.4 (C-17), 18.0 (C-18), 29.9 (C-19), 36.1 (C-20), 18.3 (C-21), 35.5 (C-22), 24.7 (C-23), 39.5 (C-24), 28.0 (C-25), 22.6 (C-26), 22.8 (C-27), 19.3 (C-28), 14.0 (C-29), 25.4 (C-30).

RESULTS AND DISCUSSION:

The ¹H NMR spectrum of **1** showed signals of four secondary methyl groups at δ 0.85 (d, 3H), 0.86 (d, 3H), 0.87 (d, 6H); a triplet of a primary methyl group at δ 0.89; resonances for an allylic methyl at 1.65 ppm, a vinyl proton at δ 5.34 and two protons of a -CH₂-O- group at δ 4.59 (d). Furthermore, many overlapping methylene protons at δ 1.28 characteristics of long chain fatty acids were observed. The ¹³C NMR spectrum of **1** exhibited

resonances of many methylene carbons between δ 29.1 and 29.7 ppm which matched well with the presence of a long chain fatty acids deduced from the analysis of the ¹H NMR data.

The resonances of a carbonyl carbon of an ester at δ 173.9; those of olefinic carbons at δ 142.3 and 118.1; and finally the signal of an oxymethylene carbon at δ 61.2 indicated that **1** could be an ester of the linear diterpenephytol (2E, 7R, 11R)-3, 7, 11, 15 - tetramethyl - 2 - hexadecen - 1 - ol⁹. The structure of the ester was deduced from the observed fragments of the mass spectrum. Its positive APCI MS showed a quasi-molecular ion peak at m/z 521.9158 [M+H]⁺, which in accordance with NMR data enable the molecular formula C₃₅H₆₈O₂, accounting for two degrees of unsaturation. As the phytol has already the molecular formula C₂₀H₄₀O, the other moiety of the phytylester might be the radical C₁₅H₂₉. So, the compound **1** should have the following structure. (Figure 1)

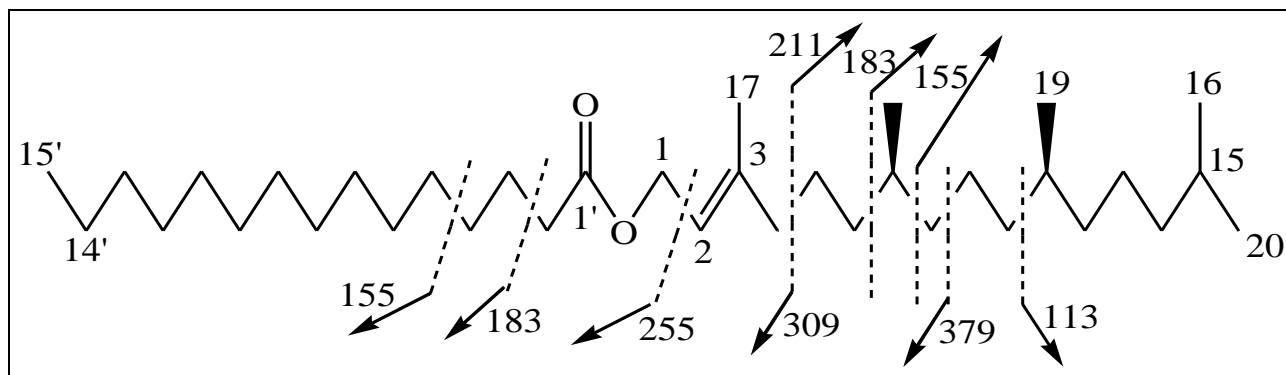


FIGURE 1: CHEMICAL STRUCTURE AND MASS FRAGMENTATIONS OF **1**.

In the TOF-MS (EI⁺) spectrum of **1**, the base peak at m/z 255 resulted from the loss of the radical C₁₉H₃₇ from the molecular ion at m/z 520. An allylic fragmentation gave peaks at m/z 310 and 212 after proton transfer and. fragments at m/z 379, 212, 184, 156, 113 confirmed the above structure. The COSY spectrum led to the determination of different partial structures of **1**; most of them were shown in Figure 2.

The HMBC spectrum showed a long range correlation between the carbon of the ester carbonyl at δ 173.9 (C-1') and both methylene carbonyl protons at δ 4.59 (H-1) and methylene protons at α position of the carbonyl δ 2.29 (H-2'). These correlations justified the attachment of the

fatty ester to the position 1 of the phytol. The position of the double bond was given from the correlations between the protons of the methylene oxide -CH₂-O- and those of the olefinic carbons at δ 118.2 (C-2) and δ 142.6 ppm (C-3). Other significant HMBC correlations were shown in Figure 2.

The configuration of the double bond and those of the asymmetric carbons C-7 and C-11 were deduced from the comparison of the chemical shift values of C-2, C-3, C-7 and C-11 with those of phytol and other phytol fatty esters reported in the literature⁹⁻¹¹. The complete ¹H and ¹³C resonance assignments were achieved from the analysis of ¹H-¹H COSY, HSQC and HMBC NMR correlations.

The results are recorded in the Table I. Therefore, compound **1** was identified as (2E, 7R, 11R) - phytol - 3, 7, 11, 15 - tetramethyl hexadec-2-enyl

pentadecanoate. The NMR spectral data were listed in **Table 1**.

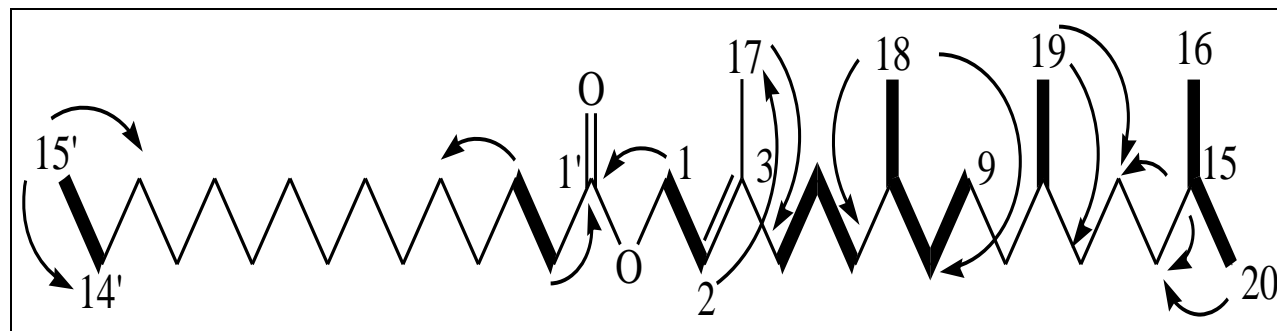


FIGURE 2: SELECTED ^1H - ^1H COSY (BOLD LINE) AND HMBC CORRELATIONS (ARROW) OF **1**.

TABLE 1: NMR SPECTRAL DATA OF COMPOUND **1** IN CDCl_3

Position	Type	$\delta^1\text{H}$ (m, J in Hz)	$\delta^{13}\text{C}$	HMBC (H-C)
1	$\text{CH}_2\text{-O}$	4.59 (d, 7.1)	61.2	C2, C3, C1'
2	CH=	5.34 (t, 7.1)	118.1	C4, C17
3	C		142.3	
4	CH_2	2.00 (m)	39.8	C5, C17
5	CH_2	1.42 (m)	24.8	C4, C6
6	CH_2	1.27 (m); 1.08 (m)	36.6	
7	CH	1.39 (m)	32.8	C6, C8, C18
8	CH_2	1.39 (m); 1.27 (m)	37.3	
9	CH_2	1.20 (m)	24.4	
10	CH_2	1.39 (m); 1.27 (m)	37.4	
11	CH	1.39 (m)	32.7	C10, C12, C13, C19
12	CH_2	1.39 (m); 1.27 (m)	37.3	
13	CH_2	1.34 (m)	24.4	
14	CH_2	1.15 (m)	39.3	
15	CH	1.52 (m)	27.9	C13, C14, C16/20
16	CH_3	0.87 (d, 3.4)	22.7	C14, C15
17	CH_3	1.65 (s)	16.3	C4
18	CH_3	0.86 (d, 2.4)	19.7	C6, C7, C8
19	CH_3	0.85 (d, 2.7)	19.7	C10, C11, C12
20	CH_3	0.87 (d, 3.4)	22.6	C14; C15
1'	C=O		173.9	
2'	CH_2	2.29 (t, 7.5)	34.4	C1', C3', C4'
3'	CH_2	1.59 (m)	25.0	C1', C2', C5'
4'	CH_2	1.28 (m)	29.5	
5'-12'	CH_2	1.28 (m)	29.1-29.7	
13'	CH_2	1.28 (m)	31.9	
14'	CH_2	1.30 (m)	22.7	C15'
15'	CH_3	0.88 (t, 3.6)	14.1	C13', C14'

Compound **2** was isolated as white powder, Mp. 193-195. Its TOF MS (EI^+) showed a pseudo molecular ion $[\text{M}+\text{H}]^+$ at m/z 473.7912 compatible with the molecular formula $\text{C}_{32}\text{H}_{56}\text{O}_2$. The ^1H -NMR spectrum displayed signals of a methine (δ 3.29, m) and methylene protons (δ 3.65, dd, $J=6.5$ and 6.7 Hz) attached to carbons bearing hydroxyl groups, four tertiary methyls at δ 0.82 (3H), 0.90 (3H) and 0.97 (6H), three secondary methyls at δ 0.81 (3H) and 0.87 (6H) and a pair of doublets

indicative of a cyclopropane ring bearing two non-equivalent protons (δ 0.33, d, $J=5$ Hz and δ 0.55, d, $J=5$ Hz).

The ^{13}C -NMR spectrum presented 32 signals and its analysis corroborated the above ^1H -NMR data. Furthermore it showed the resonances of five quaternary carbons, six methines and eleven methylenes. These analyses suggested that compound **2** should be a cycloartan-dioltriterpene

with 32 carbons. The ^1H - ^1H COSY spectrum revealed a correlation between the methine proton signals at δ 1.56, δ 3.29 and that of the methylene protons at δ 3.65, suggesting the presence of the partial structure $-\text{CH}-\text{CHOH}-\text{CH}_2\text{OH}$. The compound **2** had already seven methyl groups as in a cycloartane triterpene skeleton, so this partial structure might be located on the side chain and the compound should derive from 24-ethyl cycloartane type triterpenoids^{12,13}. This hypothesis agreed with the observed EISM fragmentations: the peak at m/z 429 was obtained by the loss of the isopropyl radical of the side chain from the molecular ion at m/z 472; the base peak at m/z 355 derived from the loss of the radical $\text{C}_6\text{H}_{13}\text{O}_2$ by cleavage between C-23 and C-24.

The fragment peak at m/z 124 resulting from a characteristic fragmentation of cycloartane type triterpene^{14,15}, indicated the absence of hydroxyl group on the A ring of the compound (**Figure 3**). The fragment at m/z 175 obtained from the successive loss of the fragment at m/z 124 and the side chain ($\text{C}_{10}\text{H}_{21}\text{O}_2$) from the molecular ion peak at m/z 472, supported the presence of the hydroxyl groups in the side chain.

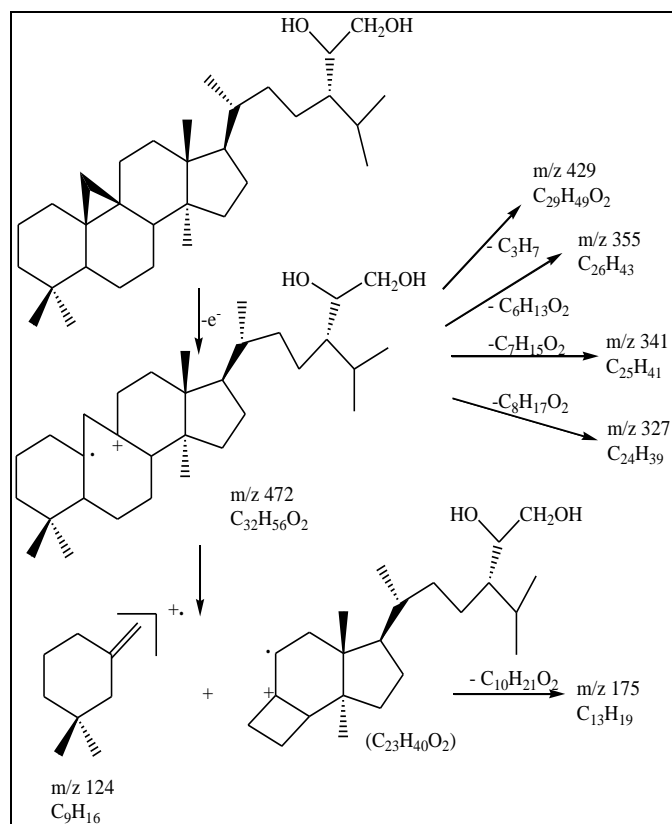


FIGURE 3: CHEMICAL STRUCTURE AND MASS FRAGMENTATIONS OF COMPOUND 2

The determination of the relative stereochemistry of **2** was established by analysis of the NOESY spectrum. The cross-peaks between H_3 -28 and H-8; H-8 and H-19 β (δ 0.55); H-8 and H_3 -18; H_3 -18 and H-20; H-20 and H-24; H-24 and H-31, suggested the β -orientation of these protons. Further correlations observed between H_3 -29 and H-5; H-5 and H_3 -30; H_3 -30 and H-17; H-17 and H_3 -21, indicated their α -orientation. So, the configurations at C-24 and C-31 were determined to be 24S and 31S on the basis of the NOESY correlations (**Figure 4**). Compound **2** was then finally identified as (24S, 31S)-cycloartan-31, 32-diol (**2**) (**Figure 5**).

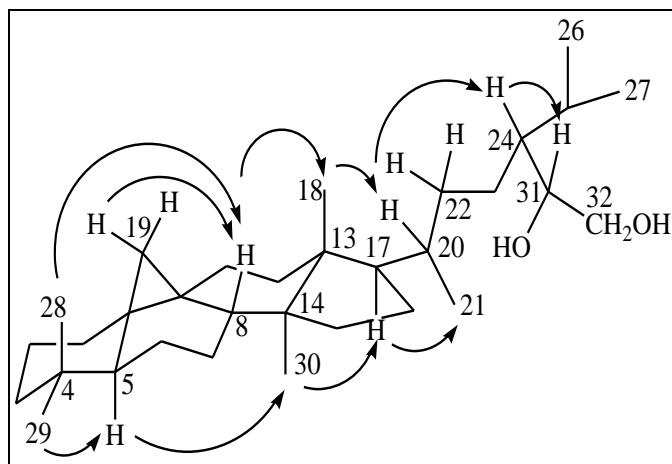


FIGURE 4: SELECTED NOESY CORRELATIONS OF 2

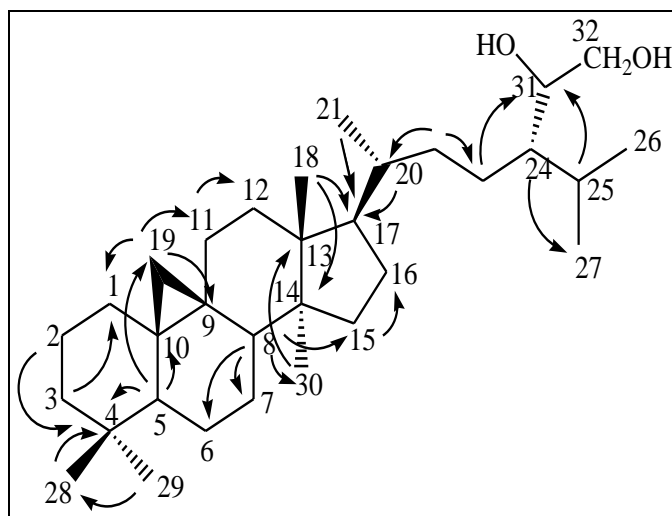


FIGURE 5: CHEMICAL STRUCTURE AND MAIN HMBC CORRELATIONS OF 2.

Regarding the NMR resonance assignments, we analyzed the 2D-NMR data spectra. In the HMBC spectrum were observed correlations between the signals of H-19 and those of two methylene carbons at 31.9 and 26.4 ppm (C-1 or C-11), as

well as to those of two methine carbons at 47.1 and 48.0 ppm (C-5 or C-8). They also correlated with the signal of a quaternary carbon situated at δ 19.9 which could correspond to C-9 or C-10. The chemical shift of the methine proton at 1.31 ppm was assigned to H-5 on the basis of its signal correlation with that of C-1. This differentiated C-5 to C-8 and C-1 to C-11. The methylene carbon resonating at 22.7 ppm was assigned to C-2 because of the correlation of its proton signal (δ 1.29) to that of C-1 and those of two quaternary carbons attributed to C-4 and C-10.

This removed the ambiguity between the assignments of C-9 and C-10. The signal of a methylene carbon at δ 30.3 was assigned to C-3 on the basis of its proton correlations (δ 1.77) to C-1 and C-4. The signals of the methyl protons at 0.82 and 0.97 ppm were assigned respectively to H-28 and H-29 because of their correlations with both C-4 and C-5. Furthermore, the correlation between the signal of H-29 and that of a methyl carbon at δ 22.8 attributed to C-28 confirmed the location of the two methyl groups at C-4. The β position of the methyl 28 was determined by its NOESY correlation with H-8 (**Figure 4**) and this facilitated the assignments of H-28 and H-29.

The HMBC spectrum showed correlations of both H-5 and H-8 signals to that of a methylene carbon located at δ 21.1, assigned to C-6. The signal of H-

8 correlated with that of a methyl carbon attributed to C-30. The methylene proton signals at δ 1.10 and 1.07 correlating with C-6 and C-8 were assigned to H-7. The signal of H-8 correlated also with that of a methylene carbon (δ 35.5), assigned to C-15.

Its corresponding proton signal (δ 1.29) correlated with that of another methylene carbon (δ 29.7), attributed to C-16. The signals of two angular methyl protons at δ 0.90 (H-30) and δ 0.97 assigned to H-18 because of their correlations with two quaternary carbon peaks located at 45.2 and 48.8 ppm, were attributed respectively to C-13 and C-14.

Furthermore, the correlations between H-18 and both a methylene carbon signal at 32.8 ppm and that of a methine carbon at 52.4 ppm were assigned respectively to C-12 and C-17. Concerning the side chain, the HMBC correlations between the methine proton signal at δ 1.37 and that of C-16 on one side and that of a methyl proton at δ 0.87 and C-17 on the other side, allowed to assign them respectively to H-20 and H-21. The methylene proton at δ 1.30 was attributed to H-22 on the basis of its correlation with C-20. It was also connected with a methylene carbon signal at δ 26.0 assigned to C-23 because of its correlations with both H-22 and the oxymethine proton at δ 3.29 corresponding to H-31.

TABLE 2: NMR SPECTRAL DATA OF THE COMPOUND 2 IN CDCl₃

Position	Type	$\delta^1\text{H}$ (m, J in Hz)	$\delta^{13}\text{C}$	HMBC (H→C)
1	CH ₂	1.56 (m)	31.9	C10, C19
		1.26 (m)		C1, C19
2	CH ₂	1.29(m)	22.7	C1, C4, C10
		1.77 (m)		C1, C4, C10
3	CH ₂	1.58 (m)	30.3	C1, C4, C10
		1.58 (m)		C1, C10
4	C		40.4	
5	CH	1.31 (m)	47.1	C1, C4, C6, C10, C19
6	CH ₂	1.60 (m)	21.1	C5, C10
		0.82 (m)		C4, C10
7	CH ₂	1.10 (m)	26.0	C5, C8, C10, C19
		1.07 (m)		C6
8	CH	1.52 (m)	48.0	C6, C9, C13, C15, C19, C30
9	C		20.0	
10	C		25.7	
11	CH ₂	2.00(m)	26.4	C9, C10, C12, C19
		1.14 (m)		C12, C19
12	CH ₂	1.63(m)	32.8	C9, C11, C13, C14, C17, C18
13	C		45.2	
14	C		48.8	
15	CH ₂	1.29 (m)	35.5	C8, C13, C16, C30

16	CH ₂	1.27 (m)	29.7	C14, C17, C20
17	CH	1.57 (m)	52.4	C22
18	CH ₃	0.97 (s)	18.0	C12, C13, 14, C17
19	CH ₂	0.55 (d, 5)	29.9	C1, C5, C8, C9, C11
		0.33 (d, 5)		
20	CH	1.37 (m)	36.1	C16
21	CH ₃	0.87 (d, 6.6)	18.3	C17
22	CH ₂	1.89 (m)	28.1	C13
		1.30 (m)		C13, C20, C23, C24, C31
23	CH ₂	1.33 (m)	26.0	C22, C31
24	CH	1.56 (m)	28.0	C23, C31
25	CH	0.98 (m)	25.4	C27, C31
26	CH ₃	0.87 (d, 2.0)	18.0	C27, C31
27	CH ₃	0.81 (d, 2.0)	14.0	C25, C31
28	CH ₃	0.82 (s)	14.0	C4, C5
29	CH ₃	0.97 (s)	22.8	C4, C5, C28
30	CH ₃	0.90 (s)	19.3	C8, C13, C14, C15, C17
31	CHOH	3.29 (m)	78.8	C23, C24, C25, C26, C27
32	CH ₂ OH	3.65 (dd, 6.5 and 6.7)	63.1	C23

The correlation of C-31 and the signals of two methine protons at δ 1.56 and δ 0.98, gave the assignments of H-24 and H-25. The signal of C-25 showed connectivity with that of a methyl protons at δ 0.81 attributed to H-27.

The correlation between the signal of other methyl protons at δ 0.87 and C-27 led to the assignment of H-26. The observed ¹H-¹H COSY correlations between the signals at δ 1.56 (H-24), δ 3.29 (H-31) and δ 3.65 corresponding to H-32, completed the assignments of the side chain. The NMR spectral data of **2** were given in **Table 2**.

In addition to **1** and **2**, cycloartanol **3** was also isolated for the first time from *Ravenala madagascariensis*. It was identified from its spectral data which were in agreement with those of the literature^{7, 16}. It was reported to be an antidiabetic compound^{7, 8}. Its presence in the leaves of the plant justifies its use in traditional medicine in the treatment of diabetes.

CONCLUSION: A phytylester and two triterpenes cycloartane type were isolated from the hexane extract of the leaves of *Ravenala madagascariensis*. One of the triterpenes has the particularity of having two hydroxyl groups at positions 31 and 32 of its side chain. The other was identified as cycloartanol; its presence in the plant could justify the use in traditional medicine of *Ravenala madagascariensis* in the treatment of diabetes.

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