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A new cardiac glycoside from *Alstonia boonei* (Apocynaceae)

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Abstract
The ethyl acetate extract from the stem bark of *Alstonia boonei* (Apocynaceae) were investigated for highlight its cardiac glycosides components. This has leaded to the isolation and identification of two digitoxigenin derivative. The first one, Evomonoside, was known and the second one, 3b-O-[5(3,4-dihydroxy-5-methyltetrahydrofuran-2-yloxy)digitoxopyranosyl-2-yloxy]-5-en-14b -hydroxy-16b -acryloxydigitoxigenin, *was* new. Both compounds were isolated for the first time from *A. boonei* and their structures were established thanks to their spectral data (NMR, MS, IR and UV).

Keywords: *Alstonia boonei*, isolation, cardiac glycoside, digitoxigenin

1. Introduction
The genus *Alstonia* (Apocynaceae) is widely distributed throughout the tropical regions of Africa and Asia. The phytochemical constituents of *Alstonia* species have been extensively investigated; nearly 400 compounds have been isolated and characterized [1]. Most of the compounds identified so far are indole and quinoline alkaloids [1, 2, 3, 4, 5]. The species of our interest, *Alstonia boonei* De Wild, is a common plant employed for different ailments in Africa [6]. It is a large tree commonly found in the rain-forests of Côte d'Ivoire. The stem bark is used as anti-malaria drug [7, 8]. An infusion of the bark is indicated as anti-venom for snake bites [9]. The stem bark is also astringent, alternative tonic and febrifuge for relapsing fevers [9]. The leaves and the latex are used topically to reduce swellings for the treatment of rheumatic pains, muscular pains and hypertension [10, 11]. Concerning its chemical composition, many studies have reported the presence of alkaloids, tannins, saponins, flavonoids, cardiac glycosides and ascorbic acid in this species [12, 13, 14]. Among these compounds, triterpenes, saponins, flavonoids and alkaloids have been isolated from its stem bark [15, 16]; but not cardiac glycoside. We report here, the isolation and structure determination of a new cardiac glycoside from the stem back of *A. boonei*.

2. Material and Methods
2.1. General
Column chromatography method was used for the fractionation process, along with Silica gel 60 (230–400 mesh, Merck) as the stationary phase. Analytical TLC (thin layer chromatography) was performed on percolated silica gel 60 F254 plates (Merck). KEDDE's regent and KELLER KILIANI's regent were used for cardiac glycosides detection. The infrared (IR) spectra were taken on a BRUKER spectrometer, methanol as solvent. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded in deuterated methanol (MeOD) on a BRUKER-Avance DRX-400 spectrometer at 400 MHz (^{1}H) and 100 MHz (^{13}C); chemical shifts (δ) are in ppm rel. to Me_4Si (internal standard). The electro-spray ionization mass spectroscopy (ESIMS) at 70ev by direct inlet probe,

2.2. Plant material
The stem barks from *A. boonei* were collected in July 2009 in Côte d'Ivoire, in the forest region near Abidjan (Southern Côte d'Ivoire). Botanical determination was performed by Pr. L. Aké Assi (Centre National de Floristique, Université Félix Houphouët Boigny). Voucher

Specimens are deposited at the Herbarium of the Centre National de Floristique (CNF).

2.3. Preparation of crude extract

Air-dried and powdered stem barks (1.8 kg) from *A. boonei* were extracted exhaustively over-night (17 hours), tree times, at room temperature, with methanol (MeOH). The concentrated extract was completed with distillated water to 500 mL. Then, this extract was washed four times (by liquid-liquid extraction method) with cyclohexane (4x500 mL). The insoluble phase in cyclohexane was extracted six times by the same method with chloroform (6x4500 mL). The insoluble phase in chloroform was also extracted seven times with ethyl acetate (EtOAc) (7x350 mL). These extracts (cyclohexane, chloroform and ethyl acetate) and the aqueous residual phase have been respectively taken to dryness under vacuum. This has leaded to four crud extracts namely: CY (cyclohexane extract) 3.8 g, CF (chloroform extract) 9.5 g, d EA (ethyl acetate extract) 13.2 g and AQ (aqueous residual) 30.5 g.

2.4. Phytochemical analysis of the crud extracts

In view to determine the presence of cardiac glycosides, every crud extracts (CY, CF, EA, AQ) have been analysis using Kellar Kiliani's test [17]. Standard protocols were used for this phytochemical analysis [18, 19]. The concentrated and dried of each extract (0.5 g) was dissolved in acetic acid 98% (2 mL) containing one drop of ferric chloride. Then, sulfuric acid (H_2SO_4) was added carefully until the formation of a brown red ring between superior phase and lower phase. Positive reaction indicates the presence of cardiac glycosides in the analysis extract [20].

2.4. Isolation and purification

Phytochemical analysis of the crude extracts (CY, CF, AE, AQ) has reveled the presence of cardiac glycoside in AE and AQ; but the best reaction was observed with EA (ethyl acetate extract). Therefore, only this extract has been fractioned. The first fractionation was carried out on silica gel column chromatography. The column's size was: 3.5 cm (diameter) and 15 cm (height). The elusion was performed with methylene chloride (CH_2Cl_2), ethyl acetate (EtOAc) and methanol (MeOH). Three fractions were obtained according to their TLC profile. The Fraction F1 (3.25 g) was carried out with the mixture CH_2Cl_2/EtOAc (6:4 to 3:7 v/v). The Fraction F2 (2.5 g) was obtained with the mixture CH_2Cl_2/EtOAc (2:8 to 0:10 v/v) and the fraction F3 (1.69 g) with the mixture EtOAc/MeOH (10:0 to 9:1 v/v). Only fractions F1 and F2 gave positive reaction to Kellar Kiliani's test. Those, they were purified over silica gel column chromatography, using the same column (1.5 cm of diameter). F1 was purified by elution with CH_2Cl_2/EtOAc (4:6 v/v) to yield compound 1, after recristallization in cyclohexane. F2 was also purified in the same condition. The elution with CH_2Cl_2/EtOAc (2:8 v/v) yielded compound 2 after recristallization in cyclohexane.

Digitoxigenin 3-Rhamnoside (**1**)

It was isolated as light brown needles; mp: 189-191°C; UV max (MeOH) l_{max}: 266 nm; IR bands in MeOH: 3683, 3436, 2965, 2931; 1781; 1740 and 1665 cm^{-1}; ^{1}H-NMR (400 MHz, MeOD) d_H:1.26 (1H, *m*, H-1a), 1.49 (1H, *m*, H-1b), 1.37 (1H, *m*, H-2 a), 1.70 (1H, *m*, H-2b), 2.80 (1H, *m*, H-3), 1.56 (1H, *m*, H-4a), 1.68 (1H, *m*, H-4b), 1.44 (1H, *m*, H-5), 1.30 (1H, *m*, H-6a), 1.48 (1H, *m*, H-6b), 1.26 (1H, *m*, H-7a), 1.52 (1H, *m*, H-7b), 1.51 (1H, *m*, H-8), 1.39 (1H, *m*, H-9), 1.26 (1H, *m*, H-11a), 1.52 (1H, *m*, H-11b), 1.26 (1H, *m*, H-12a), 1.49 (1H, *m*, H-12b), 1.48 (1H, *m*, H-15a), 1.75 (1H, *m*, H-15b), 1.37 (1H, *m*, H-16a), 1.61 (1H, *m*, H-16b), 2.17 (1H, *dd*, *J*=5.2, 6.1Hz, H-17), 1.15 (6H, *br s*, H-18, H-19), 5.56 (1H, *br s*, H-21), 4.90 (2H, *br s*, H-23). The rhamnopyranoside unit: The d_H shifts at: 5.02 (1H, *m*, H-1'), 3.73 (1H, *m*, H-2'), 3.48 (1H, *m*, H-3'), 3.41 (1H, *m*, H-4'), 3.85 (1H, *m*, H-5'), 1.22 (3H, d, xx, H-6'). ^{13}C-NMR (100 MHz, MeOD) d_C: 26.5 (C-1), 29.1 (C-2), 75.9 (C-3), 37.5 (C-4), 40.1 (C-5), 28.1 (C-6), 26.1 (C-7), 40.5 (C-8), 43.0 (C-9), 36.7 (C-10), 21.1 (C-11), 35.1 (C-12), 50.1 (C-13), 86.2 (C-14), 34.1 (C-15), 27.6 (C-16), 51.2 (C-17), 14.4 (C-18), 14.6 (C-19), 176.5 (C-20), 108.7 (C-21), 177.4 (C-22), 68.5 (C-23). d_C shifts at: 104.2 (C-1'), 76.2 (C-2'), 74.1 (C-3'), 77.1 (C-4'), 71.4 (C-5'), 20.5 (C-6'). ESI-MS *m/z*: 521.2 [M+H], molecular weight 520.2 g/mol, molecular formula $C_{29}H_{44}O_8$ (cal. 520.2968).

3-b-O-[5(3,4-dihydroxy-5-methyltetrahydrofuran-2-yloxy)digitoxopyranosyl-2-yloxy]-5-en-14-hydroxy-16-acryloxydigitoxigenin ***(2)***.

It was isolated as light brown, mp: 187-189°C; UV (MeOH) l_{max}:268 nm; IR bands in MeOH: 3436, 2968, 2954, 1781, 1741 and 1625 cm^{-1}; ^{1}H-NMR (400 MHz, MeOD) d_H :1. 16 (1H, *m*, H-1a), 1.35 (1H, *m*, H-1b), 1.31 (1H, *m*, H-2a), 1.55 (1H, *m*, H-2b), 2.98 (1H, *m*, H-3), 1.96 (1H, *m*, H-4a), 2.20 (1H, *m*, H-4b), 5.38 (1H, *dd*, *J=7.5 and 5.2Hz*, H-6), 1.76 (1H, *m*, H-7a), 2.02 (1H, *m*, H-7b), 1.52 (1H, *m*, H-8), 1.41 (1H, *d*, H-9), 1.22 (1H, *m*, H-11a), 1.51 (1H, *m*, H-11b)), 1.22 (1H, *m*, H-12a), 1.43 (1H, *m*, H-12b), 1.71 (1H, *m*, H-15a), 1.95 (1H, *m*, H-15b), 4.01 (1H, *m*, H-16), 2.76 (1H, *d*, *J=6.5Hz*, H-17), 1.25 (3H, *br s*, H-18), 1.15 (1H, *br s*, H-19), 6.43 (1H, *dd*, *J=7.5, 15Hz*, H-21), 5.06 (1H, *dd*, *J=7.5* and *3.2Hz*, H-22a),,6.40 (1H, *dd*, *J=15.6* and *3.2Hz*, H-22b), 5.93 (1H, *br s*, H-24), 4.90 (2H, *br,s*, H-26). ^{13}C-NMR (100 MHz, MeOD) d_C: 31.0 (C-1), 29.7 (C-2), 77.9 (C-3), 40.2 (C-4), 140.8 (C-5), 133.9 (C-6), 24.4 (C-7), 38.9 (C-8), 44.6 (C-9), 39.1 (C-10), 23.7 (C-11), 33.2 (C-12), 46.1 (C-13), 84.5 (C-14), 38.5 (C-15), 74.5 (C-16), 56.6 (C-17), 14.5 (C-18), 23.2 (C-19), 169.8 (C-20), 133.9 (C-21), 119.9 (C-22), 177.1 (C-23), 117.1 (C-24), 178.1(C-25), 73.4 (C-26).

The Digitoxopyranoside unit at d_H: 4.98 (1H, *m*, H-1'), 1.75 (1H, *m*, H-2'a), 2.01 (1H, *m*, H-2'b), 3.64 (1H, *m*, H-3'), 3.02 (1H, *m*, H-4'), 4.06 (1H, *m*, H-5'), 1.22 (3H, *d*, *J= 6.8Hz*, H-6') and the d_C: 98.3 (C-1'), 38.7 (C-2'), 65.1 (C-3'), 88.5 (C-4'), 67.6 (C-5'), 17.1 (C-6'). The tetrahydrofuranoside unit at d_H: 5.15 (1H, *m*, H-1"), 3.95 (1H, *m*, H-2"), 3.64 (1H, *m*, H-3"), 3.98 (1H, *m*, H-4"), 1.22 (3H, *d*, *J= 6.8Hz*, H-5") and the d_C: 107.5 (C-1"), 77.9 (C-2"), 80.2 (C-3"), 80.1 (C-4"), 16.9 (C-5"). ESI-MS *m/z*: 689.3 [M+H], molecular weight 688.3 g/mol, molecular formula $C_{37}H_{52}O_{12}$ (cal. 688.33744).

3. Results and Discussion

The phytochemical screening with Kellar Kiliani's test**[17]** of extract from the stem bark of *A. boonei* has revealed the presence of cardiac glycoside in two fractions (EA and AQ). The best positive reaction was obtained with the ethyl acetate extract (EA). This means that fraction EA contains the highness quantity of cardiac glycoside, in comparison with AQ. Therefore, EA (6.2 g) was subjected to column chromatography. This fractionation leaded to tree fractions F1 (2.25 g), F2 (1.45 g) and F3 (1.69 g). Only F1 and F2 contain cardiac glycoside, because they gave positive reaction to Kellar Killiani's test. Their purification by crystallization method yielded compounds 1 (149.51 mg) and 2 (35.8 mg) respectively. Their structures were established thanks to their spectral data. Both compounds are cardiac glycosides.

Digitoxigenin 3-Rhamnoside (**1**)

This compound was isolated as light brown needles. Its ESI-MS spectrum gave fragment of the pseudo-molecular ion $[M+H]^+$ at *m/z*: 521.2. The molecular weight was deducted as 520.2 g/mol, corresponding to the molecular formula $C_{29}H_{44}O_8$ (cal. 520.2968 g/mol). The fragments $[M+2H]^+$ and $[M+3H]^+$ are observed at *m/z*: 522.2 and 523.2 respectively. The spectroscopic UV analyses in MeOH gave maximum absorption band of unsaturated carbon–carbon (C=C) at l_{max}: 266 nm. The IR spectrum, recorded in MeOH displayed absorption band of hydroxyl group (OH) at $\upsilon_{max:}$ 3683 and 3436 cm^{-1}. The methylene (-CH_2-) and methyl (CH_3-) groups gave elongation and distortion bands of C-H at $\upsilon_{max:}$ 2965 and 2931 cm^{-1}. The carbonyl group (C=O) gave strong absorption band at $\upsilon_{max:}$ 1740 cm^{-1}. The small absorption band observed at $\upsilon_{max:}$ 1781 cm^{-1} and the strong one at $\upsilon_{max:}$ 1740 cm^{-1} indicated the presence of cardenolide[21]. The double bond carbon-carbon (C=C) gave absorption band at $\upsilon_{max:}$ 1665 cm^{-1}. The ^1H and ^{13}C NMR spectra of 1 (Table 1) were recorded in CD_3OD. The ^1H NMR spectrum showed chemical shifts between d_H 0.98 and 2.02 ppm, characteristic of a saturated tetracyclic. Two broad singlet at d_H 5.56 and 4.90ppm belong to the cardenolide group. The signal at d_H 5.56 ppm was attributed to proton H-21 and the other one at d_H 4.90 ppm (2H, H-23) for protons of the ester function. The ^{13}C NMR spectrum displayed signal of one cardenolid**e** unit at d_C 177.4 ppm (C-22; C=O of ester function), d_C 176.5 ppm (C-20 or C_b unsaturated), d_C 108.7 ppm (C-21 or C_a unsaturated) and d_C 68.5 ppm (C-23, -CH_2-). The chemical shifts of one α-L-rhamnosyl unit are observed at d_H 5.02 ppm (H-1'; anomeric proton) and d_H 1.22 ppm (H-6'; CH_3-). Carbon signals are observed at d_C 104.2 ppm (C-1'; anomeric carbon), d_C 20.5 ppm (C-6'; CH_3-). Additionally, HSQC and HMBC spectral data gave important information on the structure of compound 1. The HMBC spectrum displayed $^2J_{C-H}$ and $^3J_{C-H}$ correlations like this: C-22 with H-21/H-23; C-21 with H-17/ H-23; C-23 with H-21/H17; C-3 with H-1' (anomeric proton of L-rhamnosyl unit). All these data were in confirming with those reported by the literature[22]. Therefore, compound 1 was identified as *Digitoxigenin 3-Rhamnoside* or *Digitoxigenin-3β-O-α-L-rhamnopyranoside* (Fig.1). It is a digitoxigenin derivative and its common name is Evomonoside. It was previously isolated and identified from the seeds of *Lepidium apetalum* and *Carissa spinarum* [22].

Table 1: ^1H and ^{13}C NMR spectral data of compounds 1 and 2.

	1		**2**	
N° C	**d_C (ppm)**	**d_H (ppm), J (Hz)**	**d_C (ppm)**	**d_H (ppm), J (Hz)**
1	26.5	1.49 (*m*); 1.26 (*m*)	31.0	1.35 (*m*); 1.16 (*m*)
2	29.1	1.70 (*m*); 1.37(*m*).	29.7	1.55 (*m*); 1.31 (*m*).
3	75.9	2.80 (*m*)	77.9	2.98 (*m*)
4	37.5	1.68 (*m*); 1.56 (*m*)	40.2	2.20 (*m*); 1.96 (*m*)
5	40.1	1.44 (*m*)	140.8	-
6	28.1	1.48 (*m*), 1.30 (*m*)	133.9	5.38 (*dd*, 7.5; 5.2Hz)
7	26.1	1.52(*m*); 1.26 (*m*)	24.4	2.02 (*m*); 1.76 (*m*)
8	40.5	1.51 (*m*)	38.9	1.52 (*m*)
9	43.0	1.39 (*m*)	46.6	1.41 (*m*)
10	36.7	-	39.1	-
11	21.1	1.52 (*m*); 1.26 (*m*)	23.7	1.51 (*m*), 1.22 (*m*)-
12	35.1	1.49 (*m*); 1.26 (m)	33.2	1.43 (*m*), 1.22 (*m*)
13	50.1	-	46.1	-
14	86.2	- OH	84.5	- OH
15	34.1	1.75(*m*); 1.48(*m*)	38.5	1.95 (*m*); 1.71 (*m*)
16	27.6	1.61(*m*); 1.37(*m*)	74.5	4.01 (*m*)
17	51.2	2.17 (*dd*, 5.2; 6.1)	56.6	2.76 (*d*, 6.5Hz)
18	14.4	1.15 (*br*, *s*)	14.5	1.25 (*br*, *s*)
19	14.6	1.15 (*br*, *s*)	23.2	1.15 (*br*, *s*)
20	176.5	-	169.8	-
21	108.7	5.56 (*br*, *s*)	133.9	6.43 (*dd*, 7.5; 15.6 Hz)
22	177.4	-	119.9	5.06 (*dd*; 7.5; 3.2 Hz), 6.40 (*dd*,15.6; 3.2 Hz)
23	68.5	4.90 (*br*, *s*)	177.1	-
24			117.0	5.93 (*br*, *s*)
25			178.1	-
26			73.4	4.90 (*br*,*s*)
	Rha*	Rha*	Dipy*	Dipy
1'	104.2	5.02 (*m*)	98.3	4.98 (*m*)
2'	76.2	3.73 (*m*)	38.7	2.01 (*m*); 1.75 (*m*)
3'	74.1	3.48 (*m*)	65.1	3.64 (*m*)
4'	77.1	3.41 (*m*)	88.5	3.02 (*m*)
5'	71.4	3.87 (*m*)	67.6	4.06 (*m*)
6'	20.5	1.22 (*m*)	17.1	1.22 (*d*; 6.8Hz)
			Teh	Teh*
1''			107.5	5.15 (*m*)
2''			77.9	3.95 (*m*)
3''			80.2	3.64 (*m*)
4''			80.1	3.98 (*m*)
5''			16.9	1.22 (*d*; 6.8Hz)

* Rha : rhamnopyranoside; Dipy : digitoxopyranoside; Teh: trahydrofuranoside

3b-O-[5(3,4-dihydroxy-5-methyltetrahydrofuran-2-yloxy)digitoxopyranosyl-2-yloxy]-5-en-14b -hydroxy-16b -acryloxydigitoxigenin (2).

This compound was isolated as light brown with the melting point: 187-189°C. Its ESI-MS spectrum gave fragment of the pseudo-molecular ion $[M+H]^+$ at *m/z* 689.3. Thus, its molecular weight was deducted as 688.3 g/mol, corresponding to the molecular formula $C_{37}H_{52}O_{12}$ (cal. 688.33744 g/mol). The fragments $[M+2H]^+$ and $[M+3H]^+$ are observed at *m/z*: 690.2 and 691.2 respectively. Spectroscopic UV analyses in MeOH gave maximum absorption at l_{max}: 296 nm; corresponding to the unsaturated linkages C=C and C=O. Its IR spectrum also recorded in MeOH, displayed absorption bands of hydroxyl group (OH) at $\upsilon_{max:}$ 3436 cm^{-1}; the elongation and distortion bands of methylene (-CH_2-) and methyl (CH_3-) groups at$\upsilon_{max:}$ 2968 and 2954 cm^{-1}. Absorption bands at $\upsilon_{max:}$ 1741 cm^{-1} belong to carbonyl groups (C=O) and that at $\upsilon_{max:}$ 1625 cm^{-1} to the double bond carbon-carbon (C=C). As in the case of compound **1**, the small absorption band observed at $\upsilon_{max:}$ 1781 cm^{-1} and the strong one at $\upsilon_{max:}$ 1741 cm^{-1} are characteristic of lactones group; which supposes the presence of a cardenolide[21]. The ^1H and ^{13}C NMR spectra of 2 were recorded in CD_3OD and they were very similar to those of 1. Therefore, compound 2 was also identified as a digitoxigenin derivative. Differences in NMR spectral data were observed as follow. The Dept-135 spectrum gave signal of a vinylic carbon at d_C 119.9 ppm (C-22; =CH_2). This signal belongs to the acryloyloxy group whose chemical shifts are observed at d_C 169.8 ppm (C-20; O-C=O), d_C 133.9 ppm (C-21; -CH=) and d_C 119.9 ppm (C-22; =CH_2). The digitoxopyranoside unit gave chemical shifts at d_H 4.98 ppm (*m*, H-1', anomeric proton), d_H 1.22 ppm (*d*, *J= 6.8Hz*, H-6', CH_3), d_C 98.3 ppm (C-1'; anomeric carbon) and d_C 17.1 ppm (C-6'; CH_3). The tetrahydrofuranoside unit displayed peaks at d_H: 5.15 (*m*, H-1''; anomeric proton), d_H 1.22 ppm (d, *J= 6.8Hz*, H-6'; CH_3), d_C 107.5 ppm (C-1''; anomeric carbon) and d_C:16.9 ppm (C-5"; CH_3). Thanks to the HMBC spectrum of 2, positions of digitoxopyranoside, tetrahydrofuranoside and acryloyloxy units were clarified. Correlations ($^2J_{C-H}$, and $^3J_{C-H}$) were observed between C-20 and H-16/H-21/H-22 (=CH_2); which indicated the position of the acryloyloxy group on carbon C-16. The $^3J_{C-H}$ correlation between C-3 and H-1' shown that the digitoxopyranoside unit was fisted on carbon C-3. The tetrahydrofuranoside unit was attached on the digitoxopyranoside unit in position C-4'; because of the correlation between C-4' and H-1'' (anomeric proton of the tetrahydrofuranoside unit). Position of the double bond C=C on the ring A of the digitoxigenin was clarified thanks to the $^3J_{C-H}$ and $^2J_{C-H}$ correlations between C-5 and H-3/H-4/H-6/H-7/H-19.

The absolute configuration of 2 was done according to the correlations observed on its NOESY spectrum. In generally, stereochemistry of d*igitoxigenin* is known. Herein, we plan to determined spatial position of acryloyloxy group. Thus, correlation between protons H-16 and H-17 seem like they were in the same plan; therefore acryloyloxy unit and tetrahydrofuranoside unit were in the same spatial position.

Thanks to these spectral data, 2 was identified as *3b-O-[5(3,4-dihydroxy-5-methyltetrahydrofuran-2-yloxy)digitoxopyranosyl-2-yloxy]-5-en-14b -hydroxy-16b -acryloxydigitoxigenin* (Fig.2). It is a digitoxigenin derivative and, any reference was found in the literature. In consequence, 2 had been decrypted as a new cardiac glycoside, isolated and identified for the first time here.

The isolation and identification of these cardiac glycosides are in confirming of literature's reports. Cardiac glycosides had been characterized in organic extracts from *A. boonei*[23,24]. In plants, the presence of cardiac glycosides appears to be confined for many families [12, 13, 14]. These compounds have great pharmaceutical importance because of their relationship to compounds such as the sex hormones, cortisones, diuretic steroids and vitamin D[20]. Apocynaceae appear to be one of these families, because many cardiac glycosides have been isolated or characterized in genus belonging to this family [25].

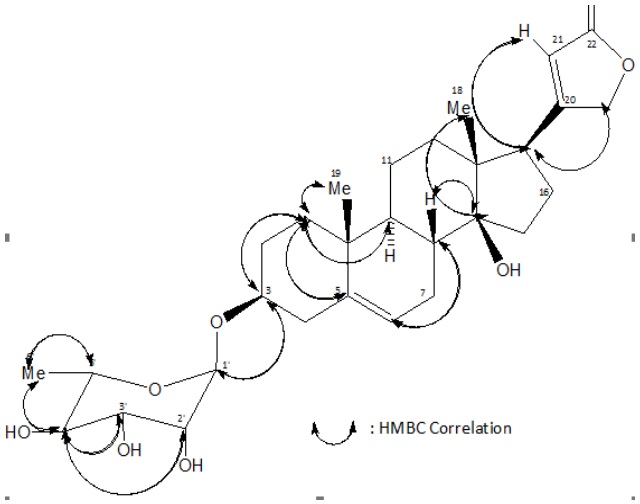

Fig 1: *Digitoxigenin 3-Rhamnoside* (1)

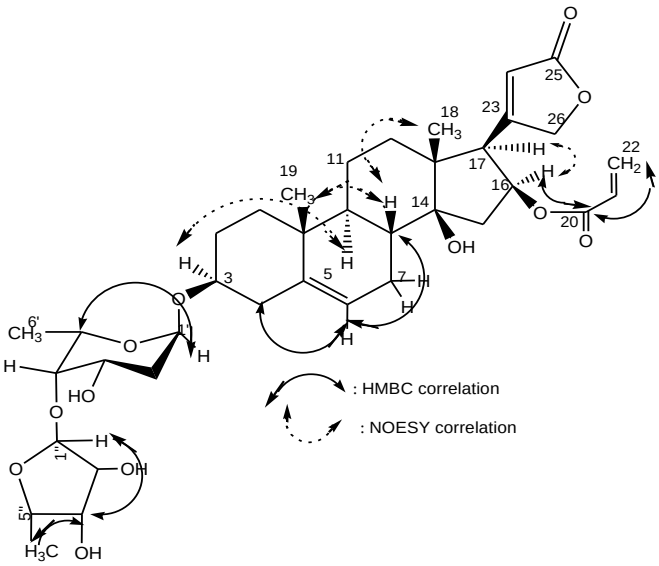

Fig 2: 3b-O-[5(3,4-dihydroxy-5-methyltetrahydrofuran-2-yloxy) digitoxopyranosyl- 2-yloxy]-5-en-14-hydroxy-16-acryloxydigitoxigenin (2).

Conclusion

Phytochemical investigation of the ethyl acetate extract from *Alstonia boonei'*s stem bark has leaded to the isolation and characterization of two cardiac glycosides derivatives. Their structures were formally established thanks to their spectral data, compared to those of the literature. One of these compounds has an original structure.

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