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Two New Phorbol-Type Diterpene Esters from *Synadenium grantii* Hook F. Leaves

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Abstract: Two new phorbol-type diterpene esters have been isolated from the chloroform extract of the leaves of *Synadenium grantii* Hook F. and identified as 3,4,12,13-tetraacetylphorbol-20-phenylacetate and 4-deoxyphorbol-12,13-ditiglate for which the trivial names Synagrantol A & B respectively, were adopted. Furthermore, two known triterpenes were isolated. The structures of all isolated compounds were established by 1D and 2D NMR spectroscopy including ^{1}H and ^{13}C NMR, HSQC and HMBC techniques. The cytotoxicity as well as the antiparasitic activity of the chloroform extract was performed and proved to be active.

Keywords: *Synadenium grantii* Hook F.; Euphorbiaceae; phorbol-type diterpene esters; triterpenoids; cytotoxicity; antiparasitic activity.

1. Introduction

The plant family Euphorbiaceae; Genus/species (322/8900) is a complex heterogeneous family that occurs in several different habitats from arid regions to humid tropics [1]. It is well known for its diverse medicinal uses which often related to the phorbol-type diterpenoid polyols. Its ester derivatives are known with their abilities to inhibit an HIV-induced cytopathic effect (CPE) on MT-4 cells and to activate protein kinase C (PKC) associated with tumor-promoting action [2], cytotoxic agents, potent irritant and co-carcinogens [3].

Synadenium grantii Hook F. (Euphorbiaceae) is commonly called African Milk Bush. It is a succulent shrub or small tree native to East Central Africa. In nature, the plants will reach up to 12 feet in height with an equal spread. The pale green obolanceolate, slightly toothed leaves reach 6 inches; a shrub commonly found growing as hedges [4].

According to the observed folkloric uses of the family Euphorbiaceae it received a distinctive phytochemical investigation which revealed the presence of flavonoids, saponins, diterpenes, phorbol esters [5, 6], lectins [7] and glycoproteins [8]. However, from the Genus *Synadenium*; tigliane, senadenol, phorbol type diterpenoids [9, 10], triterpenoids [11] and anthocyanins [12] have been isolated.

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Furthermore, the fibrinolytic [8], immunoregulatory [7] and antitumoral [13, 14] pharmacological properties have been reported in association with its chemical constituents from the *Synadenium* Genus.

In the present study, we reported the isolation and structural elucidation of two new phorbol-type diterpene esters, along with two known terpenoidal compounds, from the $CHCl_3$ extract of the leaves of *Synadenium grantii* Hook F. In addition, the cytotoxicity of the $CHCl_3$ extract was evaluated against MRC-5 cells and the antiparasitic activity against *Trypanosoma cruzi*, *Trypanosoma brucei* and *Plasmodium falciparum* was also performed.

2. Materials and Methods

2.1. Plant Material

Synadenium grantii Hook F. leaves were collected from National Research Center garden, Cairo, Egypt, in February 2009. It was kindly identified by Mrs. Teresa Labib, Head of the Taxonomists specialists at El-Orman Botanical Garden, Giza, Egypt. A voucher specimen has been deposited at the Herbarium of the National Research Center, Cairo, Egypt (No.13866). The plant material was dried, finely powdered, and used for the successive extraction.

2.2. General experimental conditions

Column chromatograph was performed on silica gel LC 60 A (60-200 μm, Davisil). TLC was carried out using precoated silica gel 60 F-254 plates (Merck). Mass spectra were obtained by electrospray ionization (ESI) in the positive mode on a linear ion trap LXQ (Thermo Scientific) by direct infusion of the compound (5μL/min). NMR spectra were recorded on a Bruker DRX-400 spectrometer at 30 ^{o}C operating at 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR. Chemical shift values were reported as ppm units relative to tetramethylsilane (TMS). ^{1}H-, ^{13}C-NMR, DEPT-135 and DEPT-90 spectra were recorded, as well as 2D-NMR spectra (COSY, HSQC and HMBC). 2D-NMR was carried out using pulsed field gradients. Compounds 1, 3 and 4 were dissolved in $CDCl_3$ while compound 2 was dissolved in CD_2Cl_2. UV-Vis spectra were recorded on an Uvikon 931 instrument (Kontron Instruments).

2.3. Extraction and isolation

The leaves of *Synadenium grantii* Hook F. (700 g) were extracted with $CHCl_3$ (5×1 L) at room temperature. The extracts were combined and the solvent was removed under vacuum to yield 56.5 g. The $CHCl_3$ extract was dissolved in 400 ml of $CHCl_3$-MeOH (1:1 v/v), and then mixed with 30 g charcoal at 40 $^{\circ}$C overnight for removing the chlorophyll pigments, then filtered. The filtrate was evaporated to dryness and weighted to afford 13 g which was chromatographed over silica gel column chromatography (120×3 cm) and eluted with mixtures of *n*-hexane and EtOAc of increasing polarity. Fractions (20 mL, each) were collected and combined based on TLC to afford seven sub-fractions (I-VII). Sub-fraction VI was further purified with PTLC using solvent system of Toluene-EtOAc (10:4.5 v/v) to afford compounds **1** and **2** (17 and 11 mg, respectively). Sub-fraction III contained compound **3** (45 mg), while, sub-fraction V contained compound **4** (13.5 mg).

2.4. Biological Activity

2.4.1. In Vitro Activity against Plasmodium falciparum

For the determination of the antiplasmodial activity, the parasite lactate dehydrogenase assay [15] was used. The assay was based on the observation that the lactate dehydrogenase (LDH) enzyme of *P. falciparum* has the ability to rapidly use 3-acetyl pyridine NAD (APAD) as a coenzyme in the reaction leading to the formation of pyruvate from lactate. Tested extracts were added in 4-fold serial

dilutions in 96-well multiwell plates to *P. falciparum* (chloroquine-sensitive Ghana strain) cultures (1% parasitemia, 2% HCT). After 48 h at 37 ^{o}C, 20 µL of the lysed culture was added to 100 µL of Malstat reagent (Flow Inc.). Adding 40 µg of nitroblue tetrazolium (NBT) and 2 µg of phenazine ethosulfate (PES) to the Malstat reagent enabled spectrophotometric (650 nm) assessment of LDH levels, and IC_{50} values were calculated.

2.4.2. In Vitro Activity against Trypanosoma brucei Trypomastigotes

IC_{50} values against *T. brucei* were determined as described [15]. Briefly, bloodstream forms of *T. brucei* were cultivated in HMI-9 medium. In a 96-well microplate, 10000 haemoflagellates were incubated at different concentrations of the test extract for 4 days. Parasite multiplication was measured fluorimetrically after addition of resazurin (excitation 530 nm, emission 590 nm).

2.4.3. In Vitro Activity against Intracellular Trypanosoma cruzi Amastigotes

IC_{50} values against *T. cruzi* were determined as described [15]. In brief, primary mouse peritoneal macrophages were seeded in 96-well microplates at 30000 cells/well. After 24 h, about 10000 trypomastigotes of *T. cruzi* were added per well together with 4-fold dilutions of the extract. The cultures were incubated at 37 °C in 5% CO_2-95% air for 7 days, and parasite growth is assessed after adding 50 µL/well CPRG (chlorophenol red *β*-D-galactopyranoside) as substrate. Color change was measured spectrophotometrically at 580 nm after 4 h incubation at 37 °C. The results were expressed as % reduction in parasite burden compared to control wells, and an IC_{50} was calculated.

2.4.4. Cytotoxicity on MRC-5 Cells

A human diploid embryonic lung cell line (MRC-5) was used to assess the cytotoxicity of the tested compounds. MRC-5 cells were seeded at 5000 cells/well in 96-well microtiter plates. After 24 h, the cells were washed and 4-fold dilutions of the extract were added in 200 µL of standard culture medium (RPMI+ 5% FCS). The final DMSO concentration in the culture remained below 0.5%. The cultures were incubated with different concentrations of test extract at 37 °C in 5% CO_2-95% air for 7 days. Untreated cultures were included as controls. Cell viability was assessed after addition of Alamar-Blue (5 µL of a 1/10 solution/well), and fluorescence was measured (550 nm excitation, 590 nm emission) after 4 h incubation at 37 °C. The results were expressed as % reduction in cell viability compared to untreated control wells [15].

3. Results and Discussion

The $CHCl_3$ extract of *Synadenium grantii* Hook F. leaves was subjected to silica gel column chromatography eluted with *n*-hexane and EtOAc. Further separation was achieved by subsequent TLC on silica gel, which resulted in the isolation of the four terpenoidal compounds.

Compound 1, It was isolated as brown needles. Its molecular weight was determined to be $C_{36}H_{44}O_{11}$ by HRESIMS (+ve), which showed a quasi-molecular ion peak at *m/z* 653.2956 $[M+H]^+$ (calcd 653.2950 for $C_{36}H_{45}O_{11}$). ^{1}H-, ^{13}C-NMR, DEPT & HSQC data (Table 1) for this compound gave an overall indication that the compound was a diterpenoid ester with a phorbol nucleus [16], with eight methyl, three methylene, twelve methine, and thirteen quaternary groups. ^{1}H- and ^{13}C-NMR ($CDCl_3$) spectra showed the presence of two doublet methyls at δ_H 0.86 (3H, *d*, *J* = 7.2 Hz, CH_3-18) and δ_H 1.53 (3H, *d*, *J* = 1.2 Hz, CH_3-19), and two singlet methyls at δ_H 0.97 and 1.08 (each 3H, *s*, CH_3-17 & -16), which were confirmed by the DEPT and HSQC data at δ_C 15.60, 15.10, 17.25, and 28.65 respectively. Furthermore, four acetyloxy methyls were assigned at δ_H 2.00, 2.01, 2.10 and 2.14 (each 3H, *s*, CH_3OCO-3, -4, -12, & -13), and at δ_C 20.59, 20.72, 20.97 and 21.09, this was in agreement with the DEPT data. HSQC spectrum showed four cross-peaks corresponding to the correlation of the four

methyls with its four carbonyl groups appeared at δ_C 169.74, 170.14, 170.74 and 170.88. Two doublets appeared at δ_H 4.38 (1H, br*d*, *J* = 12.4 Hz, H-20a) & 4.64 (1H, br*d*, *J* = 12.4 Hz, H-20b), corresponding to the geminal methylene CH_2-20 which appeared at δ_C 60.61 and confirmed by the cross-peak in HSQC. A doublet of doublet signal appeared at 4.66 (1H, *dd*, *J* = 1.2 & 11.2 Hz) corresponding to a methene group CH-1 at δ_C 129.21 as indicated by the cross-peak appeared in HSQC. This group (CH-1) correlated through a cross-peak with a methine proton at δ_H 1.11 – 1.16 (1H, *m*, H-10), and allylicly coupled to CH_3-19 at δ_H 1.53. The absence of the characteristic carbonyl carbon at position-3 which usually appeared around at δ_C 209.70 for all the previously isolated phorbol-type diterpene ester [16] suggesting the possible acylation instead. This was confirmed by the presence of a singlet signal at δ_H 5.36 (1H, *s*), which correlated with the methine carbon at δ_C 70.82 confirming the presence of a methine group (CH) at position-3. This singlet signal at δ_H 5.36 (1H, *s*, H-3) in turn showed a long range correlation with the carbonyl carbon at δ_C 169.74 of the acetyloxy group (Figure 1), this confirming the presence of acetyloxy group at the methine group CH-3. ^{1}H-^{1}H COSY spectrum, revealed the presence of a number of cross-peaks represents the correlations between H-7 at δ_H 5.16 (1H, br*d*, *J* = 11.6 Hz) & H-8 at δ_H 1.39 (1H, br*d*, *J* = 9.2) which in turn correlated with H-14 at 1.11 – 1.16 (1H, *m*). Moreover, two cross-peaks correlated H-11 at δ_H 2.29 (1H, *dd*, *J* = 7.2 & 8.4 Hz) with CH_3-18 at δ_H 0.86 (3H, *d*, *J* = 7.2 Hz) and H-12 at δ_H 5.11 (1H, *d*, *J* = 8.4 Hz), a cross-peak corresponding to the geminal coupling between the methylene protons at δ_H 2.12 – 2.19 (2H, br*d*, *J* = 12.8 Hz, CH_2-5). The ESI/MS/MS spectrum suggested the presence of a phenylacetyl group (C_6H_5-CH_2-CO) at *m/*z = 136 corresponding to the loss of the corresponding acid (C_6H_5-CH_2-COOH). This was confirmed by ^{1}H-NMR spectrum at δ_H 7.24 – 7.35 (5H, overlapped, H-2`, -3`, -4`, -5`, -6`) and characterized by $^{13}$C-NMR and DEPT as two quaternary carbons at $\delta_C$ 169.94 (CO) and $\delta_C$ 127.22 (C-4`) and two methine carbons at δ_C 128.59 (C-3` & C-5`) and 129.48 (C-2` & C-6`). A singlet signal at δ_H 3.72 (2H, *s*) correlated with a methylene carbon at δ_C 41.64 corresponding to the methylene group of the phenylacetyl as confirmed by DEPT and HSQC. The attachment position of the phenylacetyl group was assigned at OCH_2-20 as indicated by the HMBC, which revealed the presence of a long range correlation between the geminal methylene CH_2-20 protons at δ_H 4.38 & 4.64 (2H, br*d*, *J* = 12.4 Hz) and the carbonyl carbon of the phenylacetyl at δ_C 169.94 (Figure 1). Hence, according to the above mentioned data, compound **1** was established as depicted in (Figure 1) and assigned to 3,4,12,13-tetraacetylphorbol-20-phenylacetate.

Compound 2, It was isolated as brown needles. Its molecular weight was determined to be $C_{30}H_{40}O_7$ by HRESIMS (+ve), which showed a quasi-molecular ion peak at *m/z* 512.2852 $[M+H]^+$ (calcd 513.2846 for $C_{30}H_{41}O_7$). Pseudo-molecular ion $[M+18]^+$ appeared at *m/z* 530.31068 corresponding to $[M+H+NH_3]^+$. ^{1}H-, ^{13}C-NMR, DEPT & HSQC data (Table 1) for compound **2** gave an overall indication that the compound was a diterpenoid ester with a phorbol nucleus [16], with nine methyl, one methylene, nine methine, and eleven quaternary groups. A characteristic signal appeared at δ_C 209.68 stipulated for the carbonyl carbon of the phorbol nucleus at C-3 [16]. ^{1}H- and ^{13}C-NMR (CD_2Cl_2) spectra showed the presence of four singlet methyls appeared at δ_H 1.15, 1.19, 1.75 and 1.85 (each 3H, *s*, CH_3-17, CH_3-16, CH_3-19 & CH_3-20), one more doublet methyl appeared at δ_H 0.85 (3H, *d*, *J* = 8 Hz, CH_3-18) and were confirmed by the DEPT and HSQC data at δ_C 18.79, 17.19, 10.05, 22.21 and 14.98 respectively. Furthermore, a multiplet signals appeared at δ_H 1.76 – 1.86 (12H, *m*, four methyls of tiglate-A & -B), and confirmed by DEPT and HSQC at δ_C 12.34 & 14.53 for tiglate-A, and δ_C 14.27 & 14.57 for tiglate-B. A multiplet signal appeared at δ_H 6.80 – 6.82 (2H, *m*) characteristic for the methine CH-group of tiglate-A &-B. The attachment positions of the tiglate-A & -B groups were assigned at C-12 and C-13 respectively, this was confirmed by the HMBC long range correlations between the multiplet H-12 appeared at δ_H 5.38 – 5.47 (1H, *m*) and the carbonyl group at δ_C 168.05 of tiglate-A, while, for tiglate-B was assigned at C-13 depending on the down-field shift of the C-13 at δ_C 66.16 as compared with un esterified phorbol [16]. ^{1}H-^{1}H COSY spectrum revealed the presence of a cross-peak, represented the correlation of the broad singlet signals at δ_H 7.76 (1H, brs, H-1) with δ_H 3.47 (1H, brs, H-10). Two characteristic cross-peaks revealed the correlations of the broad singlet signal at δ_H 3.35 (1H, brs, H-8) together with δ_H 5.38 – 5.47 (1H, *m*, H-7) and δ_H 1.02 (1H, brs, H-14). Furthermore, two cross-peaks correlates the H-11 at δ_H 2.01 (1H, *m*) with H-12 at δ_H 5.38 – 5.47 (1H, *m*), and H-1 at δ_H 7.76 (1H, brs) with H-10 at δ_H 3.47 (1H, brs). Finally, a broad doublet appeared at δ_H 3.66 (2H, br*d*, *J* = 8 Hz, CH_2-5). Hence, according to the above mentioned data compound **2** was formulated as shown in (Figure 1) and assigned to 4-deoxyphorbol-12,13-ditiglate.

Compound 3, colourless needles; ^{1}H-NMR ($CDCl_3$): δ 3.26 (1H, *dd*, *J* = 11.6 & 11.6 Hz, H-3), 0.80 (1H, *s*, H-l8). 0.76 (1H, *s*, H-19), 0.85 (2H, *d*, *J* = 6.0 Hz ,H-21), 1.6 (3H, *s*, CH_3-26), 1.68 (3H, *s*, CH_3-27), 0.97 (3H, *s*, CH_3-28), 5.10 (each 3H, *dd*, *J* = 7.0 and 7.0 Hz, CH_3-24 & -25), 0.86 (each 3H, *s*, CH_3-29 & -30). ^{13}C-NMR ($CDCl_3$): δ 35.3 (C-1), 28.0 (C-2), 79.1 (C-3), 39.0 (C-4), 51.2 (C-5), 19.2 (C-6), 27.8 (C-7), 134.2 (C-8), 133.6 (C-9), 37.3 (C-10), 21.6 (C-11), 90.8 (C-12), 44.3 (C-13), 50.1 (C-14), 29.8 (C-15), 28.3 (C-16), 49.8 (C-17), 15.6 (C-18), 20.4 (C-19), 35.9 (C-20), 18.9 (C-21), 35.5 (C-22), 24.9 (C-23), 125.3 (C-24), 130.9 (C-25), 17.7 (C-26), 25.9 (C-27), 82.2 (C-28), 15.5 (C-29), 24.5 (C-30). Hence, according to the above mentioned data and by comparison with the literature [17, 18] compound **3** was established as depicted in (Figure 2) and assigned to euphol.

Compound 4, brown needles; ^{1}H-NMR ($CDCl_3$): δ 0.75 (3H, *s*, H-23), 0.77 (3H, *s*, H-25). 0.88 (3H, *s*, H-24), 0.95 (6H, *s*, H-28), 1.03 (3H, *s*, H-29), 1.08 (3H, *s*, H-30), 3.96 (1H, *dd*, *J* = 11.6 & 11.6 Hz, H-3), 4.86 (3H, *s*, H-19). ^{13}C-NMR ($CDCl_3$): δ 38.5 (C-1), 27.6 (C-2), 78.9 (C-3), 38.9 (C-4), 55.4 (C-5), 18.3 (C-6), 34.5 (C-7), 40.5 (C-8), 51.2 (C-9), 37.4 (C-10), 21.3 (C-11), 26.5 (C-12), 38.9 (C-13), 43.4 (C-14), 27.8 (C-15), 37.8 (C-16), 34.6 (C-17), 142.7 (C-18), 129.5 (C-19), 32.4 (C-20), 32.3 (C-21), 37.5 (C-22), 27.9 (C-23), 15.5 (C-24), 15.9 (C-25), 16.7 (C-26), 14.5 (C-27), 25.3 (C-28), 31.3 (C-29), 29.6 (C-30). Hence, according to the above mentioned data and by comparison with the literature [19, 20] compound **4** was established as depicted in (Figure 2) and assigned to germanicol.

4. Biological investigation

Despite all the efforts to eradicate malaria, this disease continues to be one of the greatest health problems facing the tropical and subtropical regions. WHO estimated that about 300–500 million clinical cases and more than 2 million deaths each year. Malaria is one of the three most deadly communicable diseases in the world [21]. The increasing prevalence of drug-resistant strains of *Plasmodium falciparum*, its most widespread etiological agent, to standard antimalarial drugs necessitates a continuous effort to search for new antimalarial drugs with new modes of action, used alone or in association. The search for new drugs can follow many directions: study of *Plasmodium* biochemical pathways, chemical synthesis and phytochemical investigation of medicinal plants.

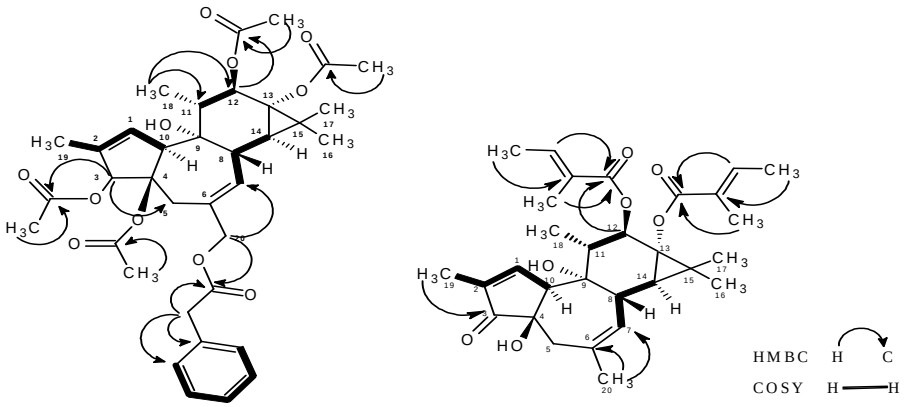
The cytotoxicity and antiparastic activity of the $CHCl_3$ extract of *Synadenium grantii* leaves were evaluated against MRC-5 cells (Secondary Human Lung Fibroblasts), *Trypanosoma cruzi*, *Trypanosoma brucei* and *Plasmodium falciparum*. As shown in (Table 2). The extract showed a marginal cytotoxicity (IC_{50} 26.49 µg/mL) against MRC-5. However, the extract was active against *Trypanosoma brucei* and *Plasmodium falciparum* (IC_{50} 8.11 and 23.70 µg/mL, respectively). While, it was highly potent against *Trypanosoma cruzi* (IC_{50} 2.21 µg/mL), this activity was mainly due to the isolated tigliane phorbol esters. These compounds have been shown to be responsible for eliciting a remarkable range of biochemical effects, although the ability of these compounds to promote tumors presents one potential limitation to their utility. It should be stressed that there are many phorbol esters that exert profound beneficial biological effects without tumorigenesis [22].

Table 1. ^{1}H-, ^{13}C-NMR, and DEPT data of the isolated new compounds
a: quaternary (C), b: tertiary (CH), c: secondary (CH_2), d: primary (CH_3)

No.	**Compound 1**		**Compound 2**	
	δ_H	δ_C	δ_H	δ_C
1	4.66 (1H, *dd*, *J* = 1.2 and 11.2 Hz)	129.21 [b]	7.76 (1H, brs)	164.40 [b]
2		136.53 [a]		138.32 [a]
3	5.36 (1H, *s*)	70.82 [b]		209.68 [a]
4		66.59 [a]		76.71 [a]
5	2.12 – 2.19 (2H, br*d*, *J* = 12.8 Hz)	38.90 [c]	3.66 (2H, br*d*, *J* = 8 Hz)	41.61 [c]
6		124.69 [a]		138.29 [a]
7	5.16 (1H, br*d*, *J* = 11.6 Hz)	129.22 [b]	5.38 – 5.47 (1H, *m*)	129.62 [b]
8	1.39 (1H, br*d*, *J* = 9.2 Hz)	26.44 [b]	3.35 (1H, brs)	39.54 [b]
9		77.73 [a]		78.45 [a]
10	1.11 – 1.16 (1H, *m*)	29.70 [b]	3.47 (1H, brs)	54.11 [b]
11	2.29 (1H, *dd*, *J* = 7.2 and 8.4 Hz)	29.32 [b]	2.01 (1H, *m*)	41.61 [b]
12	5.11 (1H, *d*, *J* = 8.4 Hz)	77.49 [b]	5.38 – 5.47 (1H, *m*)	77.31 [b]
13		66.55 [a]		66.16 [a]
14	1.11 – 1.16 (1H, *m*)	29.69 [b]	1.02 (1H, brs)	34.66 [b]
15		22.88 [a]		26.52 [a]
16	1.08 (3H, *s*)	28.65 [d]	1.19 (3H, s)	17.19 [d]
17	0.97 (3H, *s*)	17.25 [d]	1.15 (3H, s)	18.79 [d]
18	0.86 (3H, *d*, *J* = 7.2 Hz)	15.60 [d]	0.85 (3H, *d*, *J* = 8 Hz)	14.98 [d]
19	1.53 (3H, *d*, *J* = 1.2 Hz, CH_3-19)	15.10 [d]	1.75 (3H, s)	10.05 [d]
20	4.38 & 4.64 (2H, br*d*, *J* = 12.4 Hz)	60.61 [c]	1.85 (3H, s)	22.21 [d]
Phenylacetyl				
C=O		169.94 [a]		
CH_2	3.72 (2H, *s*)	41.64 [c]		
1´		134.14 [a]		
2´,6´	7.24 – 7.35 (5H, overlapped)	129.48 [b]		
3´,5´		128.59 [b]		
4´		127.22 [a]		
Acetyl groups				
3-$OCOCH_3$	2.00 (3H, *s*)	169.74 [a] – 20.59 [d]		
4-$OCOCH_3$	2.01 (3H, *s*)	170.14 [a] – 20.72 [d]		
12-$OCOCH_3$	2.10 (3H, *s*)	170.74 [a] – 20.97 [d]		
13-$OCOCH_3$	2.14 (3H, *s*)	170.88 [a] – 21.09 [d]		
Tiglate-A				
C=O				168.05 [a]
C				133.81 [a]
CH_3			1.76 – 1.86 (6H, *m*)	12.34 [d]
CH_3				14.53 [d]
CH			6.80 – 6.82 (1H, *m*)	137.90 [b]
Tiglate-B				
C=O				167.62 [a]
C				134.65 [a]
CH_3			1.76 – 1.86 (6H, *m*)	14.27 [d]
CH_3				14.57 [d]
CH			6.80 – 6.82 (1H, *m*)	137.78 [b]

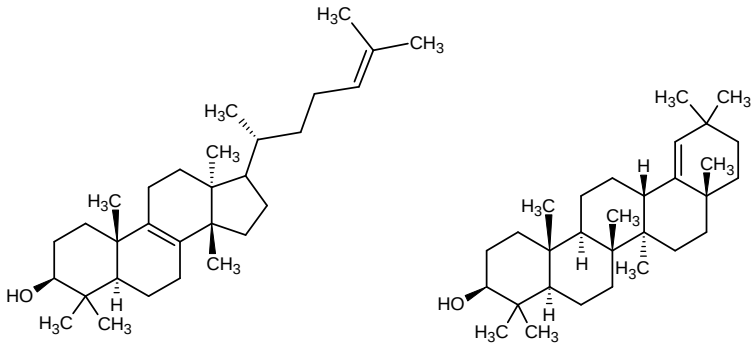
Table 2. Antiprotozoal activity and cytotoxicity of $CHCl_3$ extract of *Synadenium grantii* leaves (IC_{50}, µg/mL)

Extract	*Plasmodium falciparum*	*Trypanosoma brucei*	*Trypanosoma cruzi*	MRC-5
$CHCl_3$ extract	23.70	8.11	2.21	26.49



3,4,12,13-tetraacetylphorbol-20-phenylacetate (**1**) 4-deoxyphorbol-12,13-ditiglate (**2**)

Figure 1. ^{1}H-^{1}H COSY indicated by bold bonds and HMBC indicated by arrows of 3,4,12,13-tetraacetylphorbol-20-phenylacetate and 4-deoxyphorbol-12,13-ditiglate



Euphol (**3**) Germanicol (**4**)

Figure 2. The structures of the isolated known compounds

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