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Antitrypanosomal alkaloids from *Polyalthia suaveolens* (Annonaceae): Their effects on three selected glycolytic enzymes of *Trypanosoma brucei*

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ABSTRACT

In continuation of our study on medicinal plants of Cameroon, stem barks of *Polyalthia suaveolens* were phytochemically studied. This investigation yielded a new indolosesquiterpene alkaloid, named polysin (**1**) and four hitherto known alkaloids (**2–5**). Polysin (**1**) appeared as a competitive reversible inhibitor (K_i = 10 μM) of phosphofructo kinase (PFK) of *Trypanosoma brucei* with respect to fructose-6-phosphate (K_i/K_M = 0.05) and could be used in the design of new trypanocidal drugs. The other isolated compounds (**2–5**) also exhibited interesting inhibitory effects on selected glycolytic enzymes (PFK, glyceraldehyde-3-phosphate dehydrogenase and aldolase).

Human African Trypanosomiasis (HAT) or sleeping sickness, due to subspecies of *Trypanosoma brucei*, is one of the world's major tropical diseases with at least 60 million people in Africa at risk of developing it.[1] Drugs used to treat trypanosomiases are hampered by some problems such as side effects, costs, toxicity and resistance of parasites.[2–7] Considering that there are over 30 million people worldwide infected with species of *Trypanosoma*,[8] there is a great need for new efficient and cost-effective drugs to treat the diseases caused by these organisms. To achieve this goal, certain features in the biology of these organisms, such as the dependence of their bloodstream form on glycolysis as the sole source of ATP production, was explored as it could be exploited for the design of new antitrypanosomal drugs.[9] Indeed, compounds which inhibit glycolysis have been shown to have trypanocidal activity.[10–13]

In search for more effective trypanocidal drugs and as a continuation of a program on this purpose,[14–16] special attention was devoted to *Polyalthia suaveolens* (Annonaceae), a West African rainforest tree found from Nigeria to Angola and also in Cameroon.[17] This plant is used in Cameroonian folk medicine to treat rheumatic pains[18] and it shows filaricidal activities.[19,20] Stem barks of *Polyalthia suaveolens* (Annonaceae) were collected at Mount Eloumden in Yaoundé (Centre Province, Cameroon) in December 2001. They were identified by Mr. Koufani Anaclet from the National Herbarium Yaoundé, where a Voucher specimen has been deposited (1227/SRFK).

Herein, we report on the isolation and structural elucidation (Supplementary data) of polysin (**1**), a new epimer of greenwayodendrin-3-one (**2**). Compound **1** was obtained alongside with the known compounds **2**, 3-*O*-acetyl greenwayodendrin (**3**), *N*-acetyl polyveoline (**4**), and polyveoline (**5**). The trypanocidal activities of these compounds on *T. brucei* cells and on three glycolytic enzymes (GAPDH, PFK and aldolase) of *T. brucei* are reported together with the corresponding kinetic studies.

A mixture of compounds **1** and **2** (Fig. 1) was first obtained as a brown powder (mp 170–171 °C) from the *n*-hexane bark extract of *Polyalthia suaveolens* upon eluting the silica gel column with *n*-hexane/ethyl acetate mixtures of increasing polarity. Solutions of this mixture in CH_2Cl_2 showed a strong fluorescence under UV light (254 and 366 nm) and a positive reaction to the Dragendorffs' reagent, an alkaloid-staining reagent. This mixture was further resolved into its two components by HPLC (Supplementary data). Isolated isomers were then analysed spectroscopically.

The ^{13}C NMR and DEPT spectra of compound **1** showed signals of 23 carbon atoms comprising four methyl groups (δ 22.3, 22.7, 26.2 and 26.3), five methylenes (δ 20.2, 26.7, 34.6, 36.3 and

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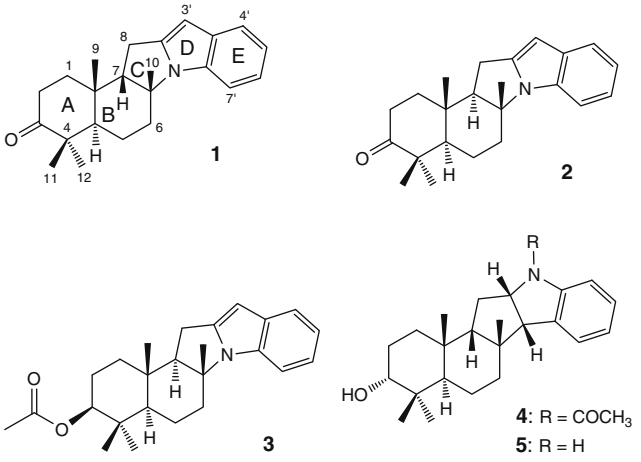

Figure 1. Chemical structures of isolated compounds polysin (**1**), greenwayodendrin-3-one (**2**), 3-*O*-acetyl greenwayodendrin (**3**), *N*-acetyl polyveoline (**4**) and polyveoline (**5**).

36.6), seven methines (δ 48.2, 62.9, 93.1, 110.0, 119.1, 120.0 and 120.7) and seven quaternary carbon atoms (δ 36.4, 47.7, 63.0, 131.8, 133.5, 140.8 and 216.0). ^{1}H NMR, ^{1}H–^{1}H COSY and 2D heterocorrelation experiments (HSQC, HMBC) were used to identify the structure of compound **1**. Two geminal methyl groups (δ_C 22.3/δ_H 1.08; δ_C 26.2/δ_H 1.10) were readily assigned to CH_3-11 and CH_3-12 by means of their mutual correlations in the HMBC spectrum. Each of the two methyl proton signals correlated with a carbonyl (δ_C 216.0, C-3) and a methine carbon (δ_C 48.2, C-4a). Another quaternary methyl (δ_C 22.7/δ_H 1.43) was assignable to CH_3-9 by means of its HMBC cross peaks with the two methine carbons C-4a and C-7 (δ_C 62.9) and the methylene carbon C-1 (δ_C 36.3). One of the diastereotopic ^{1}H signals of H_2-1 (δ_H 1.86) was linked through a ^{1}H–^{1}H COSY cross peak with another methylene proton δ_H 2.75, which therefore was assigned to H_2-2. Moreover, both the axial oriented H_2-2 (δ_H 2.75) and the equatorial H_2-1 (δ_H 1.72) showed strong HMBC correlations with the carbonyl C-3, together with the above data indicating a 4,4-dimethylcyclohexan-3-one ring A, which is typical of a terpenoid partial structure. HMBC correlations of H-4a (δ_H 1.65) with the methylene carbons δ_C 20.2 and δ_C 36.6 (respectively assignable to C-5 and C-6) plus three HMBC correlations of the remaining quaternary methyl of CH_3-10 (δ_H 2.04/δ_C 26.3) with the already assigned C-7, with C-6 and with C-6a (δ_C 63.0), completed the assignment of ring B. HMBC cross signals of H-7 (δ_H 2.48) with C-4a, C-6, C-6a, and CH_3-10 confirmed the structure of the sesquiterpene decalin moiety. Another, relatively weak but significant HMBC correlation with the remaining methylene carbon of C-8 (δ_C 26.7) suggested a key position of H-7 in the structure elucidation of compound **1**. In turn, H_2-8 (δ_H 2.95/2.85) correlated with C-6a and/or C-7 and, most important, also with the low-field quaternary carbon (δ_C 140.8), attributable to C-2′, defining a link to the non-terpenoid part of the molecule. The appearance of an AMRX spin system (δ_H 7.51, 7.44, 7.04, 7.01) of four aromatic methines in the ^{1}H NMR spectrum suggested a disubstituted aromatic ring. Together with the olefinic H-3′, resonating at δ_H 6.09, and its HMBC correlation with δ_C 131.8 (C-7′a), 133.5 (C-3′a), and δ_C 140.8 (C-2′) these AMRX methine signals suggested the presence of an indole unit, and the cross signal of H-3′ with C-8 confirmed the connection to the terpenoid unit. The appearance of the quaternary carbon C-7′a at a low-field indicated deshielding due to an electronegative substituent attached to this position. According to the uneven molecular mass and the positive Dragendorff staining, this substituent must be nitrogen, thereby confirming the indole moiety. From these data, the planar structure of compound **1** was elucidated as an indolosequiterpene alkaloid.

The relative stereochemistry of chiral centers of **1** was inferred from a 2D ROESY experiment. The observation of ROESY correlations of H_3-9 with H-2$_{ax}$, H_3-10 and H-7, and between H_3-10 and H-7 indicated that these groups were in β-orientation. Moreover, ROESY correlations of H-4 with H-5$_{ax}$ and H-8 were observed, indicating α-orientation of these groups. The opposite orientation of H-4a and H_3C-9 implied *trans*-fusion and the 3D drawing (Fig. 2) of the energy-minimized structure was consistent with a chair–chair conformation of the decalin system. Ring C seems to have envelope configuration with a least square plane formed by C-6a, N-1′, C-2′, and C-8. The 3D structure also shows that the indole moiety is nearly planar. The above NMR-based structure elucidation was confirmed by EIMS analysis (*m/z* 335 [M]$^+$, rel. int. 100). The constitution of **1** suggested a structural relationship with indolosesquiterpene alkaloids previously isolated from the same species.[17,21–25] Indeed, the molecular mass of **1** was consistent with that of greenwayodendrin-3-one (**2**)[17] and identical with that of the isolated compound **2**. Comparing ^{13}C NMR data of **2** isolated in this study (Table 1) with those reported by Hasan et al. 1982[17] identified compound **2** as the known greenwayodendrin-3-one. In addition, a NOESY spectrum of **2** displayed a strong ROE between H-7 and H-4a but no cross signal between H-7 and the two methyl groups H_3-9 and H_3-10 was observed. These data confirm an α-configuration of H-7 in **2** as established by Hasan et al. 1982[17] by means of X-ray crystallography. In conclusion, compound **2** was established as greenwayodendrin-3-one, its 7β-epimer **1** is therefore a new compound and was named polysin.

NMR spectroscopic and mass spectrometric data of further compounds (Fig. 1) matched those of previously reported 3-*O*-acetyl greenwayodendrin (**3**), *N*-acetyl polyveoline (**4**) and polyveoline (**5**).[17,21–25]

Isolated compounds were tested for their antiparasitic activities against *Trypanosoma brucei* cells and for their cytotoxicity on MRC-5 fibroblast cell lines (Supplementary data). Results of the assay with *T. brucei* are shown in Table 2. The mixture of **1** and **2** was 2 to 3-fold more effective than compounds **4** and **5**. These results are similar to that published[26–29] for some alkaloids on the bloodstream form of *T. brucei*. A general analysis of these results indicated that the isolated compounds are not too harmful to the parasite. However, they exhibited significant cytotoxicity towards the human MRC-5 cell lines.

Despite the moderate inhibitory activities of isolated compounds on parasite growth, their effects were tested on some selected enzymes of the parasite glycolysis such as GAPDH of *T. brucei* and its homologue from rabbit muscle, which was chosen as mammalian reference. Results are presented in Table 3. *N*-acetyl polyveoline (**4**) was slightly active on GAPDH, especially on the

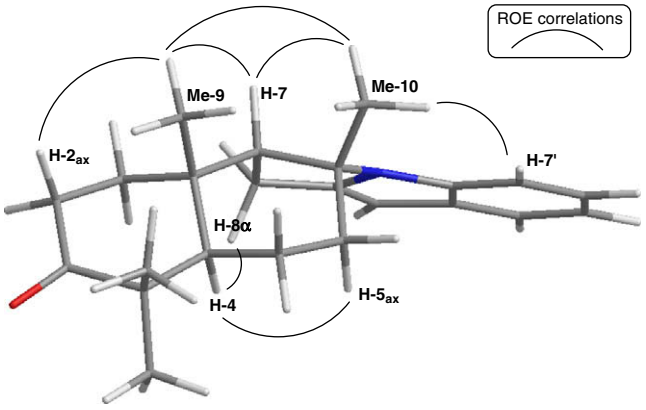

Figure 2. 3D Structure drawing of compound **1** showing important ROE correlations. The ChemDraw structure has been energy-minimized using MOPAC.

Table 1
^{1}H and ^{13}C NMR data of compounds **1** and **2**. Chemical shifts (500/125 MHz, $CDCl_3$) of compounds **1** and **2**

Position	Compound **1**		Compound **2**	
	^{13}C (δ)	^{1}H (δ; mult.; J Hz)	^{13}C (δ)	^{1}H (δ; mult.; J Hz)
1	36.3	1.86, ddd, 13.5, 13.2, 5.6 1.72, ddd, 13.5, 6.4, 2.6	37.9	1.89; m 1.69; m
2	34.6	2.75, ddd, 16.3, 13.2, 6.4 2.31, ddd, 16.3, 5.6, 2.6	33.6	2.65; ddd; 16.3, 10.3, 2.4 2.53; ddd; 16.3, 7.7, 3.5
3	216.0		216.5	
4	47.7		47.2	
4a	48.2	1.65, dd, 11.6, 1.6	54.9	1.66; dd; 12.4, 3.0
5	20.2	1.62, m 1.55, m	21.0	1.91; m 1.79; ddd; 12.7, 12.4, 3.8
6	36.6	1.36, m 2.32, dddd, 13.4, 3.5, 3.3, 1.3	37.5	2.76; m 2.07; ddd; 12.7, 12.5; 3.8
6a	63.0		62.8	
7	62.9	2.48, dd, 11.8, 8.2	64.3	2.28; dd; 12.8, 6.2
7a	36.4		36.1	
8	26.7	2.95, dd, 15.6, 8.2 2.85, dd, 15.6, 11.8	22.6	2.86; dd; 14.9, 6.2 2.74; dd; 14.9, 12.8
9	22.7	1.43, s	15.2	1.16; s
10	26.3	2.04, s	19.6	1.25; s
11	22.3	1.08, s	20.9	1.13; s
12	26.2	1.10, s	26.7	1.17; s
2′	140.8		142.4	
3′	93.1	6.09, br s	94.3	6.08, br s
3′a	133.5		131.6	
4′	110.0	7.51; dd; 7.8, 1.3	120.5	7.55; d; 7.8
5’	119.1	7.01; ddd; 7.8, 7.2, 1.3	118.8	7.04; ddd; 7.8, 7.2, 1.3
6’	120.0	7.04; ddd; 8.0, 7.2, 1.3	120.1	7.09; ddd; 7.8, 7.2, 1.3
7’	120.7	7.44; dd; 8.0, 1.2	109.4	7.33; d; 7.8
7’a	131.8		132.6	

Table 2
Antiparasitic activities (μM) of compounds **1–5** on *T. brucei* cells

Compound	ED_{50} (μM)	Reference drug
Mixture of **1** and **2**	18	Melarsoprol (0.00395 μM)
3	No effect	
4	32	
5	54	

Table 3
Inhibitory activities of compounds **1–5** on GAPDHs

Compound	IC_{50} (μM or % of inhibition at given concentration) on GAPDHs	
	Rabbit muscle	*T. brucei*
Mixture of **1** and **2**	No inhibition at 3000 μM	1050 ± 50
3	2050 ± 100	110 ± 4
4	10 ± 2% at 69 μM	10 ± 2% at 278 μM
5	620 ± 40	110 ± 20

Table 4
Inhibitory activities of compounds **1–5** on PFKs

Compound	IC_{50} (μM or % of inhibition at given concentration) on PFKs	
	Rabbit muscle	*T. brucei*
Mixture of **1** and **2**	18 ± 3% at 170 μM	20 ± 5
3	16 ± 3% at 94 μM	170 ± 10
4	12 ± 3% at 690 μM	28 ± 4% at 690 μM
5	No inhibition at 500 μM	30 ± 5

Table 5
Inhibitory activities of compounds **1–5** on aldolases

Compound	IC_{50} (μM or % of inhibition at given concentration) on aldolases	
	Rabbit muscle	*T. brucei*
Mixture of **1** and **2**	87 ± 2	8 ± 2% at 100 μM
3	150 ± 10	0.5 ± 0.1
4	No inhibition at 280 μM	11 ± 3% at 140 μM
5	270 ± 12	80 ± 2

mammals' homologue. The mixture of compounds **1** and **2** can be considered as a moderate inhibitor but it is specific of the parasites enzyme. Better results were obtained with 3-*O*-acetyl greenwayodendrin (**3**) and polyveoline (**5**), which were respectively 19 times and twofold more active on *T. brucei* GAPDH (IC_{50} = 110 and 310 μM, respectively) than on its rabbit muscle homologue (IC_{50} = 2050 and 620 μM, respectively). The acetyl group on *N*-acetyl polyveoline (**4**) may be responsible for the inactivity of compound **4** compared to polyveoline (**5**).

Results of inhibitory activities of isolated compounds on PFK of *T. brucei* (Table 4) globally indicate that, compounds **1–3** and **5** are very interesting inhibitors of the enzyme PFK, since they show a noticeable selectivity to the enzyme from *T. brucei*. The mixture of **1** and **2** inhibits the PFK of the parasite significantly (IC_{50} ~20 μM).

Aldolase inhibitory experiments (Table 5) indicate that, the mixture of **1** and **2** is selective to the mammalian enzyme, compound **4** is not active and compound **5** is threefold more active on the parasite enzyme than on the hosts' one. Likewise, compound **3** is 300-fold more active on *T. brucei* aldolase than on its mammal homologue and is the most active compound (IC_{50} ~0.5 μM).

From these experiments, it is obvious that the free N-functionality is essential for the activity observed for compounds **1–3, 5**. The N-acetylated compound **4** does not show any interesting activity.

For a better comprehension of the reaction mechanism of some isolated secondary metabolites, we have carried out kinetic studies on PFK and GAPDH of *T. brucei* (Table 6). Initially, the inhibitive behaviour studies of the mixture of **1** and **2** were analysed at a stationary state by measuring its effects on kinetic parameters K_M and

Table 6
Kinetic studies of reversible inhibition on PFK and GAPDH of *T. brucei*

Compound	PFK of *T. brucei*	GAPDH of *T. brucei*
Mixture of **1** and **2**	Competitive with respect to F6P $K_i = 10 \pm 2$ μM	
1	Competitive with respect to F6P $K_i = 9 \pm 1$ μM	
5		Mixed with respect to NAD^+ $K_i = 18 \pm 3$ μM $K'_i = 50 \pm 7$ μM

V_m known for the enzyme PFK. Inhibition kinetics have been done with respect to the natural substrate fructose-6-phosphate (Fru-6P) of PFK by varying the concentration of inhibitor around PFK Michaelis constant (K_M ~200 μM) for fructose-6-phosphate. Experiments resulted in a value of $K_i = 10 \pm 2$ μM for the mixture **1** and **2**. The results of these experiments show that the mixture of compounds **1** and **2** is a competitive reversible inhibitor of PFK of *T. brucei* and the low value of inhibition constant (10 μM) suggests that it tightly binds to the enzyme. The ratio $K_i/K_M = 0.05$ shows that the inhibitor (**1** and **2**) is twenty times more effective than the natural substrate Fru-6P. These interesting results need to be follow up in further research, since organism will accumulate Fru-6P, due to PFK inhibition.

In order to investigate which of the isomers **1** or **2** is responsible for the observed activities of the mixture; a few milligrams of compound **1** were separated and subjected to kinetic studies using PFK of *T. brucei*, the enzyme which had shown best results with the mixture of **1** and **2**. Results of these experiments reveal that compound **1** is a competitive reversible inhibitor of PFK of *T. brucei*, with a value of $K_i = 9 \pm 1$ μM. These results, which are much closed to those obtained with the mixture, suggest that observed activities of the mixture may be essentially due to the presence of compound **1**. Since the ratio in mixture of compounds **1** and **2** is roughly 1/1, compound **2** may be an agonist of **1** and they may have synergistic activities. This is plausible since the indole moiety seems to be essential for the activity.

Kinetic studies on GAPDH of *T. brucei* have been carried out with respect to the cofactor NAD^+ by varying concentrations around GAPDH Michaelis constant (K_M ~200 μM) for NAD^+. Polyveoline (**5**) was evaluated on *T. brucei* GAPDH and two constants K_i and K'_i could be determined. The presence of these two values showed that it likely binds to the enzyme and the enzyme-substrate complex as a mixed reversible inhibitor. It is threefold more effective on the enzyme GAPDH ($K_i = 18 \pm 3$ μM) than on the complex GAPDH/NAD^+ ($K'_i = 50 \pm 7$ μM). The natural cofactor NAD^+ is tenfold less associated than polyveoline (**5**) to the enzyme (K_M ~$6K_i$).

The isolation and characterization of a new indolosesquiterpene-type alkaloid, polysin (**1**), have been achieved by spectroscopic and chemical methods. It appears from this study that the new compound **1** is a good competitive reversible inhibitor of PFK ($K_i = 10$ μM) of *T. brucei*. 3-*O*-Acetyl greenwayodendrin (**3**) is a selective inhibitor of *T. brucei* aldolase (IC_{50} ~0.5 μM). Furthermore, polyveoline (**5**) is a selective inhibitor of *T. brucei* PFK and is a mixed reversible inhibitor of GAPDH of *T. brucei*. Compound **3**, which is ineffective on *T. brucei* cells, shows an interesting inhibitory effect on isolated enzymes, especially on *T. brucei* aldolase.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.04.145.

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