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Vernoguinosterol and vernoguinoside, trypanocidal stigmastane derivatives from *Vernonia guineensis* (Asteraceae)

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Abstract

Two bitter stigmastane derivatives, vernoguinosterol (**1**) and vernoguinoside (**2**), have been isolated from the stem bark of *Vernonia guineensis* and their structures eludicated using spectroscopic methods. The new compounds exhibit trypanocidal activity

Keywords: *Vernonia guineensis*; Asteraceae; Stigmastane derivatives; Vernoguinosterol; Vernoguinoside; Trypanocidal activity

1. Introduction

Vernonia guineensis Benth. (Asteraceae) is a small tree that grows in the savannah region. The plant is used in Cameroon as an anthelmintic, an aphrodisiac, an antidote to poison, and to treat malaria and jaundice (Iwu, 1993). No previous studies have been carried out on this species, although several bitter and non-bitter stigmastane-type steroids, the vernoniosides (Jisaka et al., 1992, 1993; Ponglux et al., 1992; Igile et al., 1995; Sanogo et al., 1998) have been isolated and characterized from the genus. In the course of a search for anti-parasitic principles from Cameroonian medicinal plants (Tchuendem et al., 1999) the stem bark of *V. guineensis* was examined and the isolation and structural elucidation of two new stigmastane-type steroids is now reported.

Compound **1** has the molecular formula $C_{29}H_{42}O_6$ as deduced from microanalysis, EIMS, ^{13}C NMR data, and DEPT analysis. The ^1H NMR spectrum of **1** was typical of a sterol structure, displaying two angular methyl groups, each a singlet at δ 0.92 and 0.83, a characteristic multiplet for H-3 at δ 3.35 (m) and two secondary methyls of an isopropyl group, each a doublet (J 6.8 Hz) at δ 0.91 and 0.63. The ^{13}C NMR spectrum (Table 2) showed the presence of 29 carbon atoms, including a ketonic carbonyl group, a hemiacetal function, four sp^2, and five oxygenated carbon atoms. Examination of the DEPT and HMQC spectra led to the assignment of a tetrasubstituted carbon–carbon double bond at δ 144.0 (*s*) and 122.7 (*s*), and a trisubstituted one at δ 155.7 (*s*) and 116.3 (*d*). The hemiacetal carbon atom appeared at δ 103.5 (CH), while in the ^1H NMR spectrum the hydroxyl proton of the hemiacetal was present at δ 6.29 (*d*, $J=4.6$ Hz) with a subsequent correlation in the COSY spectrum with H-21 at δ 5.19 (*d*, $J=4.6$ Hz). The presence of a tertiary hydroxyl group was characterized by signals at δ_H 4.58 (*s*) and δ_C 81.4 (*s*). The skeletal framework of the molecule was constructed by analysis of correlations present in the HMBC spectrum of **1**. Important correlations were observed between H-15 (δ 5.29), C-8 (δ 122.7), C-13 (δ 44.7), C-14 (δ 155.7), C-16 (δ 88.7) and C-17 (δ 59.5). Further correlations were observed between H-22, C-16, C-17, C-20 (δ 53.6), C-21 (δ 103.5) and C-24 (δ 81.4) and between Me-26, Me-27 and Me-29 and C-24. Structure **1** was assigned to this novel compound, named vernoguinosterol, on the basis of the above data.

Treatment of **1** with a mixture of pyridine–Ac_2O (1:1) gave a diacetylated derivative **1a**. This result also confirmed the presence of a tertiary hydroxyl group in the compound since the IR spectrum of **1a** showed bands at ν_{max} 3425 (OH) and 1724 cm^{-1} (C=O).

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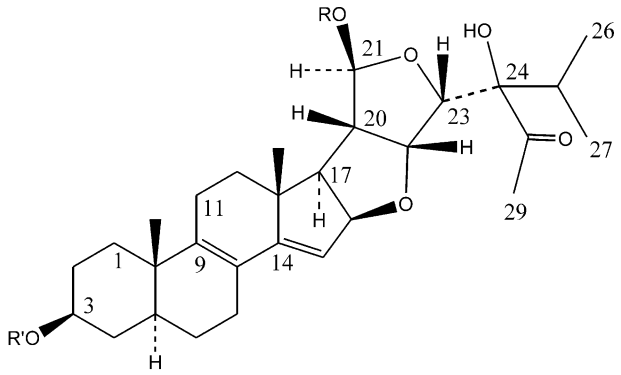

1 R = R' = H
1a R = R' = Ac
2 R = H, R' = Glc
2a R = Ac, R' = Glc-(Ac)$_4$

Table 1
^{1}H NMR spectral data for compounds **1** (DMSO-d_6), **1a** and **2a** ($CDCl_3$) at 500 MHz (J in Hz)

Proton	1	1a	2a
1	1.17 ov.[a]	1.30 *dd* (3.5, 13.5)	1.21 *dd* (3.6, 13.5)
	1.75 *dd* (5.1, 14.9)	1.85 *dd* (6.2, 13.5)	1.81 *dd* (6.1, 13.5)
2	1.50 *m*	1.55 *m*	1.54 *m*
	1.95 *m*	1.90 *m*	1.91 *m*
3	3.35 *m*	4.69 *m*	3.55 *m*
4	1.21	1.42 ov.	1.29 *bd* (12.3)
	1.50 *bd* (12.7)	1.75 *bd* (12.5)	1.65 *bd* (12.3)
5	1.48 *m*	1.53 *m*	1.39 *m*
6	2.10 *m*	2.10 *m*	2.08 ov.
	2.12 *m*	2.12 *m*	2.11 ov.
7	1.48 ov.	1.47 ov.	1.44 ov.
	1.52 ov.	1.53 ov.	1.53 ov.
11	0.94 ov.	0.96 ov.	0.95 ov.
	2.25 ov.	2.30 ov.	2.30 ov.
12	1.22 ov.	1.38 ov.	1.35 ov.
	1.77 *dd* (5.0, 14.9)	1.87 *dd* (6.2, 12.9)	1.86 *dd* (6.0, 12.8)
15	5.29 *d* (2.4)	5.40 *d* (2.2)	5.38 *d* (2.5)
16	4.58 *dd* (2.7, 6.8)	4.82 *dd* (2.2, 6.3)	4.80 *dd* (2.5, 6.3)
17	2.43 *d* (6.3)	2.58 *d* (6.4)	2.56 *d* (6.4)
18	0.83 *s*	0.93 *s*	0.91 *s*
19	0.92 *s*	1.00 *s*	0.95 *s*
20	2.59 (5.4)	2.88 *d* (6.4)	2.86 *d* (5.4)
21	5.19 *d* (4.6)	6.10 *s*	6.10 *s*
22	4.59 *dd* (2.4, 9.6)	4.85 *dd* (2.1, 5.5)	4.84 *dd* (2.3, 5.4)
23	4.04 *d* (2.3)	4.28 *d* (2.1)	4.26 *d* (2.3)
25	2.12 *m*	2.21 *m*	2.20 *m*
26	0.91 *d* (6.8)	1.02 *d* (7.0)	1.00 *d* (7.0)
27	0.63 *d* (6.8)	0.73 *d* (7.0)	0.71 *d* (7.0)
29	2.13 *s*	2.28 *s*	2.26 *s*
3-OH	4.48 *d* (4.7)		
21-OH	6.29 *d* (4.6)		
24-OH	4.58 *s*	4.35 *s*	
1′			4.59 *d* (8.0)
2′			4.93 *t* (8.1)
3′			5.18 *t* (9.5)
4′			5.05 *t* (9.3)
5′			3.67 *ddd* (2.5, 4.8, 10.0)
6′			4.10 *dd* (2.3, 12.2)
			4.13 *dd* (4.8, 12.2)

[a] ov., Overlapping.

The stereochemistry of **1** was established from the NOESY spectrum. Important correlations were observed between H-17 (δ 2.43), H-16 (δ 4.58) and H-21 (δ 5.19) suggesting that they were all on the α face of the molecule. Further correlations were observed between H-22 (δ 4.59), H-20 (δ 2.59) and H-23 (δ 4.04), as well as between H-3 (δ 3.35), H-1α (δ 1.17), and H-5 (δ 1.48). The configuration at C-24 remains undetermined.

Compound **2** was characterised as its acetate derivative **2a**. The structure elucidation was carried out with **2a** which was obtained as a white powder from MeOH, mp 112–114 °C, $[\alpha]_D$−15.3. FABMS analysis showed a pseudomolecular ion $[M–H]^-$ at m/z 857 corresponding to a molecular weight of 858. Microanalysis and analysis of the FABMS and the ^{13}C NMR spectrum (Table 1), which revealed the presence of 45 carbon atoms, indicated the molecular formula $C_{45}H_{62}O_{16}$. Strong IR absorptions at ν_{max} 3447 and 1755 cm^{-1} were characteristic of tertiary hydroxyl and acetate groups. The ^{1}H NMR spectrum showed inter alia the anomeric proton and the C-6 methylene group of a glucose moiety at δ 4.59 (*d*, J = 8.0 Hz) and at δ 4.10 (*dd*, J = 2.3, 12.2 Hz) and 4.23 (*dd*, J = 4.8, 12.2 Hz), respectively. The size of the coupling constant (J = 8.0 Hz) of the anomeric proton was indicative of a β-linkage to the aglycone. The ^{1}H and ^{13}C NMR spectra of **2a** closely resembled those of **1**, with the exception that H-3 and C-3 were shifted downfield to δ 3.55 and 78.9 in the ^{1}H and ^{13}C NMR spectra of **2a**, respectively, due to glucosidation. The connectivity of the glucose protons was readily revealed in the ^{1}H–^{1}H COSY spectrum. The placement of the glucose unit at C-3 was supported by the HMBC correlation cross peak observed between H-1′ (δ 4.59) and C-3 (δ 78.9). The complete assignment of the structure and stereochemistry was carried out using a combination of COSY, HMBC, HMQC, and NOESY spectra and led to structure **2a** for the acetate and hence structure **2** for vernoguinoside.

Compounds **1** and **2** exhibited significant inhibitory activity against four strains of bloodstream trypomastigotes *Trypanosoma brucei rhodesiense* with IC_{50} values in the range 3–5 μg/ml. The tests were carried out by Professor Cyrus Bacchi, Pace University, Haskins Laboratory, New York, NY 10038 (see Experimental).

2. Experimental

2.1. General experimental procedures

All mps were recorded with a Reichert microscope and are uncorrected. The optical rotations were measured with a Perkin-Elmer 141 polarimeter at 22 °C, while the UV and the IR spectra were recorded with Varian Cary 2290 and Perkin-Elmer 298 spectrometers, respectively. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were recorded in $CDCl_3$ or in DMSO-d_6 using a

Brüker ARX500 spectrometer with an inverse multinuclear 5 mm probe head equipped with a shielded gradient coil. The chemical shifts (δ) are reported in ppm with the solvent signals, (δ_H 7.26 and δ_C 77.0 for $CDCl_3$ or δ_H 2.49 and δ_C 39.5 for DMSO-d_6) as reference, while the coupling constants (J) are given in Hz. COSY, HMQC and HMBC experiments were recorded with gradient enhancements using sine shaped gradient pulses. For 2D heteronuclear correlation spectroscopy the refocusing delays were optimized for $^1J_{CH} = 145$ Hz and $^nJ_{CH} = 10$ Hz. The raw data were transformed and the spectra evaluated with the standard Bruker UXNMR software (ref. 941001). Mass spectra (EI and FAB) were recorded with a Jeol SX102 spectrometer at 70 eV. Elemental analysis was performed at Lund Institute of Technology. Column chromatography was run on Merck Si gel 60 and Sephadex LH-20, while TLC analyses were carried out on Si gel GF_{254} pre-coated plates with detection accomplished by spraying with 50% H_2SO_4 followed by heating at 100 °C.

2.2. Plant material

The stem bark of *V. guineensis* was collected from Bafoussam, West Province, Cameroon, in July 1998. Mr. Paul Mezili, a retired botanist of the Cameroon National Herbarium, authenticated the voucher specimens (BUD 301) that have been deposited at the Herbarium of the Botany Department of the University of Dschang.

2.3. Extraction and isolation

The crushed dried and ground stem bark (3 kg) was extracted overnight at room temperature by percolation with EtOH (8 l). The solvent was concentrated in vacuo to yield the crude extract (250 g) which was then suspended in H_2O and extracted three times with 500 ml each of hexane, EtOAc, and *n*-BuOH to give a hexane-soluble fraction, a EtOAc-soluble fraction, and an *n*-BuOH-soluble fraction, respectively. The hexane extract (20 g), after column chromatography, yielded mostly fats and mixtures of phytosterols. The EtOAc (38 g) and the EtOH (18 g) extracts were qualitatively very similar on TLC analysis. They were thus combined and a portion (25 g) was subjected to column chromatography on Si gel using mixtures of CH_2Cl_2–MeOH to furnish four major fractions: A (4.1 g, pure CH_2Cl_2), B (1.3 g, CH_2Cl_2–MeOH 9:1), C (2.5 g, CH_2Cl_2–MeOH 9:2) and D (6.0 g, CH_2Cl_2–MeOH 1:1).

Fraction A was further purified by gel permeation through a Sephadex LH-20 column using MeOH as eluent to afford compound **1** (200 mg) as a white powder, while sitosterol (150 mg) was obtained from fraction C by crystallization from MeOH. Due to difficulties in separation fraction B was treated with a mixture of pyridine (20 ml) and acetic anhydride (20 ml) for 24 h at room temperature. The reaction mixture was worked-up and subjected to Si gel column chromatography (eluting with CH_2Cl_2–Me_2CO 95:5) followed by preparative TLC with the same mixture of solvents to yield compound **2a** (100 mg). Finally, a mixture of non-separable compounds was obtained from fraction D.

Compound **1**, 16β,22*R*; 21,23*S*-diepoxy-3β,21*S*,24-trihydroxystigmasta-8,14-dien-28-one, white powder from acetone; UV (MeOH) λ_{max} (log ϵ) 250 nm (4.75); IR (KBr) ν_{max} 3436, 1704, 1629, 1359, 1090, 1049, 1024, and 924 cm^{-1}; ^1H NMR (DMSO-d_6, 500 MHz) see Table 1; ^{13}C NMR (DMSO-d_6, 125 MHz) see Table 2; EIMS (70 eV) m/z [M]+ EIMS (70 eV) m/z $[M]^+$ 486 (2), 468 (100), 450 (31), 395 (20), 328 (35), 312 (18), 211 (5), 179 (7), 113 (67), 71 (15), 43 (20); anal. C 71.55%, H 8.71%, calc. for $C_{29}H_{42}O_6$, C 71.57%, H 8.70%.

Table 2
^{13}C NMR spectral data for compounds **1** (DMSO-d_6, 125 MHz), **1a** and **2a** ($CDCl_3$, 125 MHz)

Carbon	1	1a	2a
1	35.0	34.8	35.0
2	26.7	27.5	29.3
3	69.3	73.0	78.9
4	38.6	34.0	34.6
5	40.7	40.5	40.6
6	26.5	26.5	26.5
7	25.2	24.9	25.1
8	122.7	123.3	123.1
9	144.0	143.6	143.8
10	36.8	36.7	36.8
11	21.9	22.0	22.0
12	35.9	36.1	36.2
13	44.7	45.1	45.1
14	155.7	156.0	156.0
15	116.3	115.8	115.7
16	88.7	89.7	89.6
17	59.5	59.5	59.5
18	20.5	20.3	20.3
19	18.5	18.3	18.3
20	53.6	52.8	52.9
21	103.5	104.1	104.1
22	85.8	85.2	85.2
23	83.8	85.8	85.7
24	81.4	81.4	81.5
25	32.2	32.1	32.1
26	16.5	16.4	16.4
27	16.4	15.9	15.9
28	210.5	210.1	210.3
29	27.4	26.7	26.6
1′			99.5
2′			71.6
3′			72.9
4′			68.6
5′			71.5
6′			62.1
3-$COCH_3$		170.6, 21.4	
21-$COCH_3$		170.3, 21.2	170.5, 21.1
4x$COCH_3$			170.2–169.2
			20.6–20.5

Assignements based on HMQC and HMBC experiments.

2.4. Acetylation of **1**

A solution of vernoguinosterol (**1**) (75 mg) was treated with pyridine (4 ml), and acetic anhydride (4 ml). The reaction mixture was left at room temperature overnight and then evaporated to dryness after addition of toluene (3×10 ml). The residue was filtered through a Si gel column eluting with pure CH_2Cl_2 to yield compound **1a** (79 mg).

2.4.1. Diacetylvernoguinosterol (**1a**)

White powder (MeOH): mp 105–107 °C; $[\alpha]_D^{22}$-22.4 (*c* 2.5, $CHCl_3$); IR (KBr) ν_{max} 3425 (OH), 1724 (C=O), 1641, 1468, 1462, 1431, 1217, 1011, 945, and 762 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; EIMS (70 eV) *m*/*z* $[M]^+$ 570 (1), 527 (10), 511 (8), 467 (100), 439 (13), 395 (23), 353 (10), 328 (36), 312 (16), 251 (10), 237 (10), 211 (6), 143 (10), 113 (77), 71 (18), 43 (22); anal. C 69.43%, H 8.14%, calc. for $C_{33}H_{45}O_8$, C 69.45%, H 8.12%.

2.5. Compound **2a**

16β,22*R*;21,23*S*-diepoxy-3β-*O*-β-D-glucopyranosyloxy-21*S*,24-dihydroxystigmasta-8,14-dien-28-one, white powder (MeOH): mp 112–114 °C, $[\alpha]_D^{22}$−15.3 (c 2.3, $CHCl_3$); IR (KBr) ν_{max} 3447 (OH), 1755 (C=O), 1624, 1468, 1454, 1429, 1366, 1219, 1138, 1040, 1013, 972, 945, and 762 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 125 MHz), see Table 2; FABS *m*/*z* $[M–H]^-$ 857 (15), 815 (8), 799 (100), 755 (29), 683 (14), 616 (17), 600 (24), 511 (17), 451 (24), 407 (11), 331 (82), 269 (38); anal. C 62.90%, H 7.29%, calc. for $C_{29}H_{42}O_6$, C 62.92%, H 7.28%.

2.6. Biological tests

The compounds were tested against trypanosome isolates grown in bloodforms in an HMI-18 medium containing 20% fetal bovine serum. Coulter counts were made daily and IC50 values determined after 48h. The strains used in the tests include Lab 110 EATRO, KETRI 242, 269 and 243 As 103.

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