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Lanostane-type triterpenoid and steroid from the stem bark of *Klainedoxa gabonensis*

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ABSTRACT

A new lanostane triterpenoid, 2-hydroxy-24-methylenelanostan-1,8-dien-3-one named klainedoxalanostenone (**1**) with one new steroid, 6-*O*-acyl-β-D-glucosyl-β-sitosterol named klainedoxasterol (**2**) together with ten known compounds including six triterpenoids (**3**–**8**), two steroids (**9**, **10**) and two tanins (**11**, **12**) were isolated from the stem bark of *Klainedoxa gabonensis*. To the best of our knowledge, this is the first report of lanostane-type triterpenoids from this genus. Their structures were determined by extensive analysis of spectroscopic data (1D and 2D NMR, MS) and by comparison with literature data. The xanthine oxidase inhibitory activity of nine compounds (**1**–**6**, **8**, **10** and **11**) were evaluated. Compound **5** showed a good xanthine oxidase inhibitory activity; the other tested compounds were moderately active.

1. Introduction

Klainedoxa gabonensis Nan. (Irvingiaceae) is one of the largest trees occurring in the humid rain-forest from Senegal to West Cameroon, and extending to Uganda and Tanganyika. Different parts of this plant are used in the treatment of rheumatism, bucal infection, small pox, chickenpox, sterility and impotence [1]. Previous chemical investigation on the stem bark of *K. gabonensis* resulted in the isolation of several triterpenoids, steroids, tannins and phenolic compounds [2–4]. It is known that hypoxanthine and xanthine can be oxidized to uric acid and produce reactive oxygen species (ROS) by catalysis of xanthine oxidoreductase (XOD). Excessive accumulation of uric acid *in vivo* can cause gout; free radicals are involved in the pathologic process of inflammation, mutagenesis and aging [5]. The ROS produced by XOD can activate the 5-lipoxygenase (5-LOX) in B-lymphocytes and the 5-LOX can affect B-cell function [6]. Inhibitors of XOD decrease the tissue damage from free radicals and could be used in curing gout and diseases caused by ROS [7].

In this paper, we report the isolation and structures elucidation of a new triterpenoid, 2-hydroxy-24-methylenelanostan-1,8-dien-3-one (**1**), and a new steroid, 6-*O*-acyl-β-D-glucosyl-β-sitosterol (**2**) together with ten known compounds. The xanthine oxidase inhibitory activity of nine of the isolated compounds was evaluated.

2. Results and discussion

The CH_2Cl_2/MeOH (1:1) extract of the stem bark of *K. gabonensis* was fractionated by silica gel column chromatography to give several fractions. These fractions were further purified by repeated column chromatography to afford one

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new triterpenoid, 2-hydroxy-24-methylenelanostan-1,8-dien-3-one, klainedoxalanostenone (**1**), and one new steroid 6-*O*-acyl-β-D-glucosyl-β-sitosterol, klainedoxasterol (**2**) as well as ten known compounds (**3**–**12**) (Fig. 1).

Compound **1** was obtained as white amorphous powder. The molecular formula was determined as $C_{31}H_{48}O_2$ by HREIMS which showed a molecular ion peak at *m/z* 452.3633 $[M]^+$ (calcd. for $C_{31}H_{48}O_2$ 452.3654), in conjunction with NMR data.

Its ^1H and ^{13}C NMR spectra (Table 1) were typical of lanostane triterpenoid [8]. This hypothesis was confirmed by comparison of its NMR data with those of known lanostane triterpenoids [8]. The ^1H NMR spectrum showed singlets for five tertiary methyl groups at δ_H 0.70 (3H, s, Me-18), 0.86 (3H, s, Me-30), 1.09 (3H, s, Me-28), 1.20 (3H, s, Me-29) and 1.23 (3H, s, Me-19); three secondary methyl groups at δ_H 0.92 (3H, d, J = 5.0 Hz, Me-21), 1.00 (3H, d, J = 6.5 Hz, Me-26/27) and 1.01 (3H, d, J = 7.0 Hz, Me-27/26). The two signals at δ_H 4.64 (1H, brs, H_a-24[1]) and 4.70 (1H, brs, H_b-24[1]) were characteristic of a terminal methylene group in the side chain of a lanostane skeleton [9]. This spectrum also showed signals

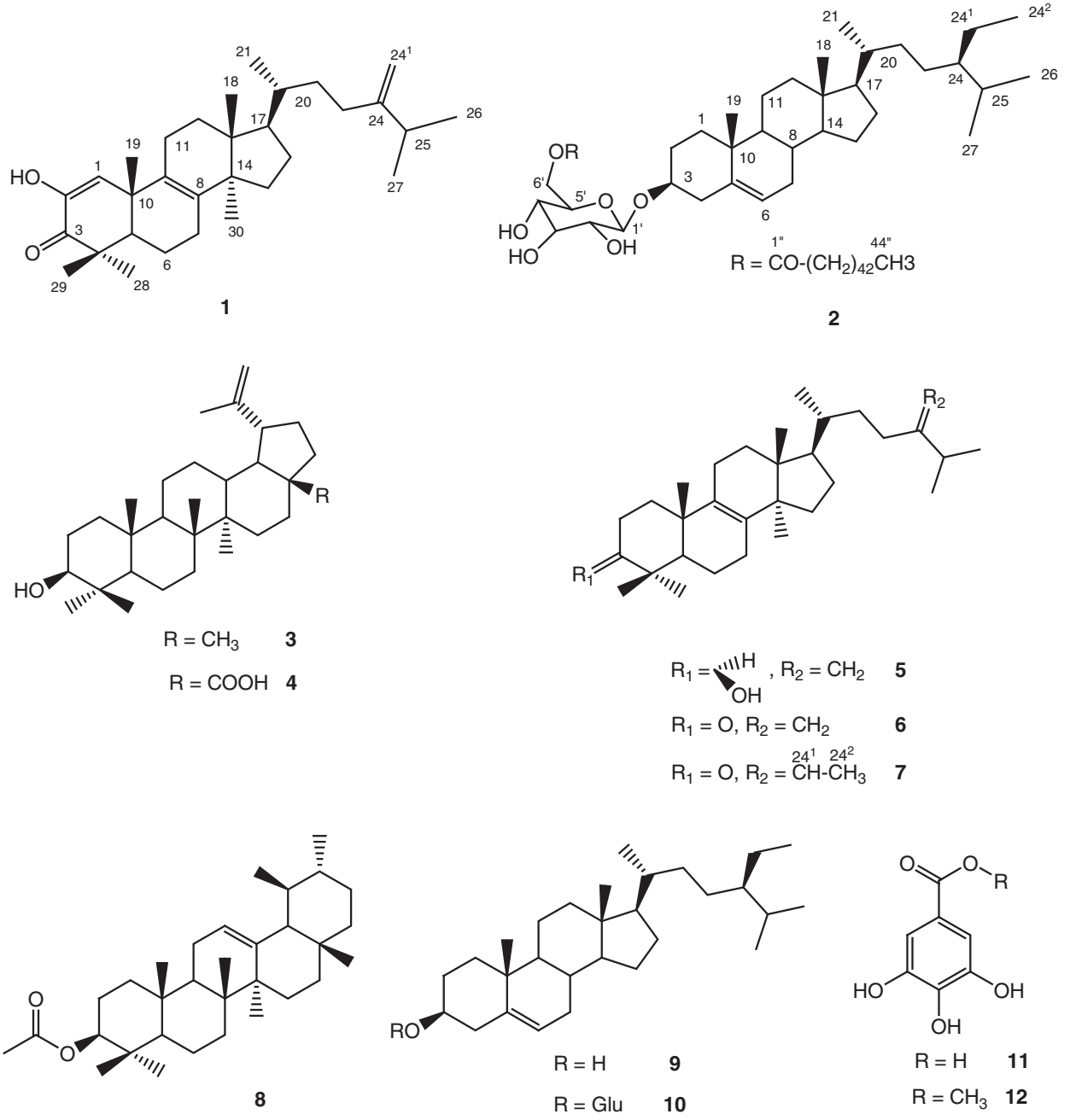

Fig. 1. Structures of compounds **1**–**12**.

Table 1
^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$) data of compound **1** (δ in ppm, J in Hz)[a].

Position	**1**		
	δ_C	δ_H (mult., J)	HMBC (C→H)
1	126.1	6.51, 1H, s	C-2, C-3, C-5, C-9, C-10, C-19
2	143.7	–	
3	200.9	–	
4	43.6	–	
5	47.6	1.92, 1H, br t (7.5)	C-1, C-4, C-9, C-10, C-29, C-6, C-19, C-28
6	18.2	1.68, 1H, m 1.54, 1H, m	C-4, C-5, C-8, C-10, C-29
7	28.1	1.23, 2H, ov. m	
8	135.8	–	
9	131.5	–	
10	38.9	–	
11	22.6	2.18, 2H, dd (6.5; 4.5)	C-8, C-9
12	25.2	2.12, 1H, m 1.20, 1H, ov. m	C-8, C-9, C-11
13	44.5	–	
14	50.0	–	
15	30.7	2.19, 1H, m 1.76, 1H, m	C-13, C-14, C-30
16	30.6	1.16, 1H, m 1.57, 1H, m	C-13, C-14, C-21, C-30
17	50.3	1.49, 1H, m	C-13, C-18
18	15.7	0.70, 3H, s	C-13, C-14, C-17
19	23.9	1.23, 3H, s	C-1, C-5, C-9, C-10
20	36.4	1.38, 1H, m	C-17
21	18.7	0.92, 3H, d (5.0)	C-17, C-20, C-22
22	34.8	1.08, 1H, m 1.49, 1H, m	C-17, C-20
23	31.2	1.86, 1H, m 2.09, 1H, m	C-24, C-24[1]
24	156.8	–	
25	33.8	2.21, 1H, m	C-23, C-24, C-24[1], C-26, C-27
26	22.0	1.00, 3H, d (6.5) [b]	C-24, C-25
27	21.8	1.01, 3H, d (7.0) [b]	C-24, C-25
28	21.6	1.09, 3H, s	C-3, C-4, C-5
29	25.9	1.20, 3H, s	C-3, C-4, C-5
30	23.9	0.86, 3H, s	C-8, C-14, C-13, C-15
24[1]	105.9	4.70, 1H, brs 4.64, 1H, brs	C-23, C-24, C-25
2-OH	–	5.90, 1H, s	C-1, C-2, C-3

[a] Assignments were based on the DEPT, HSQC and HMBC experiments.
[b] Signals may be interchanged.

of an olefinic proton at δ_H 6.51 (1H, s, H-1) and a proton of a hydroxyl group at δ_H 5.90 (1H, s). The ^{13}C NMR spectrum of compound **1** (Table 1) displayed 31 carbon resonances, which were assigned by DEPT and HSQC experiments as eight methyls, nine methylenes, five methines and nine quaternary carbons. In this spectrum, signals at δ_C 126.1, 143.7 and 200.9 were attributed to an α,β-unsaturated keto group bearing an enolic function at C-2. This spectrum also showed resonances of one terminal methylene at δ_C 105.9 (C-24[1]) and 156.8 (C-24), and two additional olefinic carbons at δ_C 131.5 (C-9) and 135.8 (C-8). The ^{1}H–^{1}H COSY spectrum (Fig. 2a) together with the HSQC data revealed several proton spin systems, among which a –CH–CH_2–CH_2– unit, a CH_3–CH–CH_3 unit, a –CH_2–CH_2– unit and a –CH_2–CH_2–CH–CH–CH_2–CH_2– unit were evident. In the HMBC spectrum (Fig. 2a), pertinent correlations observed between H-1 (δ_H 6.51) and carbon C-2 (δ_C 143.7) and C-3 (δ_C 200.9) as well as between the proton of the hydroxyl group (δ_H 5.90) and carbons C-1 (δ_C 126.1), C-2 and C-3 confirmed the presence of the C-2

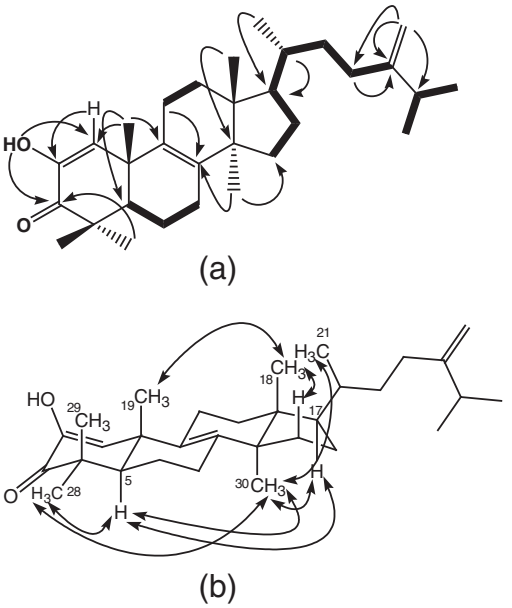

Fig. 2. (a) Selected HMBC (H→C) and ^{1}H–^{1}H COSY (▬) correlations of compound **1**. (b) Selected NOESY correlations of **1**.

hydroxylated α,β-unsaturated keto group. Further HMBC correlations between H_3-19 (δ_H 1.23) and carbons C-1, C-5 (δ_C 47.6), C-9 (δ_C 131.5) and C-10 (δ_C 38.9); between H_3-18 (δ_H 0.70) and carbons C-13 (δ_C 44.5), C-14 (δ_C 50.0), C-15 (δ_C 30.7) and C-17 (δ_C 50.3); and between proton H_3-30 (δ_H 0.86) and carbons C-8 (δ_C 135.8), C-13 (δ_C 44.5), C-14 (δ_C 50.0) and C-15 (δ_C 30.7) confirmed the C-10, C-13 and C-14 positions of the three angular methyl groups on the lanostane skeleton. Correlations between H_3-19 and C-9, between H_3-30 and C-8 as well as between H-11 (δ_H 2.18) and carbons C-8 (δ_C 135.8) and C-9 indicated the C-8/C-9 location of one of the double bonds [10]. This spectrum also exhibited correlations between the terminal methylene protons and carbons C-23 (δ_C 31.2), C-24 and C-25; between H-25 (δ_H 2.21) and C-24, C-24[1]; between H_2-23 (δ_H 1.86, 2.09) and C-24, C-24[1], enabled the positioning of the terminal methylene at C-24 of the side chain. This side chain was located at C-17 of the lanostane ring from the cross peak observed in the HMBC spectrum between H-20 (δ_H 1.38), H-21 (δ_H 0.92) and C-17 (δ_C 50.3). The relative configuration of compound **1** was determined by analysis of the NOESY spectrum (Fig. 2b). Cross peaks were observed between H_3-18 and H_3-19, between H_3-28, H-5 and H_3-30, between H_3-30 and H-5, H_3-21 and H-17, as well as between H-5 and H-17. Therefore, compound **1** was thus characterized as 2-hydroxy-24-methylenelanostan-1,8-dien-3-one, named klainedoxalanostenone. To the best of our knowledge, this is the first report of lanostane-type triterpenoid from the *Klainedoxa* genus. Sublateriol A, a C-2 hydroxylated α,β-unsaturated 3-keto lanostane-type triterpenoid was previously isolated from *Naematoloma sublateritium*, an edible mushroom [11].

Compound **2** was isolated as white amorphous powder. Its molecular formula was determined as $C_{79}H_{146}O_7$ as suggested by HRFABMS which showed a pseudomolecular ion peak at m/z 1208.1158 $[M+H]^+$ (calcd. for $C_{79}H_{147}O_7$ 1208.1147) in conjunction with NMR data. On the basis of its ^{1}H and ^{13}C NMR spectral data (Table 2), compound **2** was

Table 2
^{1}H NMR (600 MHz, $CDCl_3$/MeOD) and ^{13}C NMR (150 MHz, $CDCl_3$/MeOD) data of compound **2** (δ in ppm, J in Hz)[a].

Position	**2**		
	δ_C	δ_H (mult., J)	HMBC (C→H)
1	37.2	1.79, 1H, m 0.98, 1H, m	C-3, C-5
2	31.9	1.91, 1H, ov. m 1.46[b], 1H, m	
3	79.6	3.48, 1H, m	C-1′, C-2
4	38.7	2.30, 1H, brd (13.0) 2.21, 1H, brt (13.0)	C-3, C-5, C-6
5	140.3	–	
6	122.1	5.30, 1H, brs	C-7, C-8, C-10
7	31.8	1.38[b], 1H, m 1.46[b], 1H, m	C-9, C-14
8	31.8	1.38[b], 1H, m	C-9, C-14
9	50.1	0.85, 1H, m	C-10
10	36.6	–	
11	21.0	1.41, 1H, m 1.47, 1H, m	C-8, C-9, C-13
12	39.7	1.62, 1H, ov. m 1.06, 1H, m	C-9, C-14
13	42.2	–	
14	56.7	0.93, 1H, ov. m	C-18
15	24.2	1.02, 1H, m 0.93, 1H, ov. m	C-13, C-14
16	28.2	1. 61, 1H, m 1.19, 1H, ov. m	C-15, C-17
17	56.0	1.03, 1H, m	C-16
18	11.8	0.62, 3H, s	C-12, C-13, C-14
19	19.3	0.95, 3H, s	C-5, C-9, C-10
20	36.1	1.28, 1H, m	C-16, C-17, C-21
21	18.7	0.86, 3H, d (7.5)	C-17, C-20, C-22,
22	33.9	0.94, 1H, m 1.26, 1H, m	C-17
23	25.9	1.54, 1H, m 1.09, 1H, ov. m	C-22, C-24, C-25
24	45.7	0,86, 1H, m	C-23, C-24^1, C-25
25	29.0	1.54, 1H, ov. m	C-26, C-27
26	18.9″	0.75, 3H, d (6.6)[c]	C-25
27	19.8″	0.77, 3H, d (6.6)[c]	C-25
24^1	22.9	1.20, 1H, ov. m 1.15, 1H, m	C-24, C-24^2
24^2	11.9	0.78, 1H, brt (7.5)	C-24, C-24^1
Glucopyranosyl			
1′	101.2	4.30, 1H,d (7.6)	C-3, C-2′, C-3′
2′	76.2	3.38, 1H, m	C-1′, C-3′
3′	73.7	3.23, 1H, m	C-2′, C-4′
4′	70.1	3.27, 1H, m	C-3′, C-5′
5′	73.3	3.39, 1H, m	C-4′, C-6′
6′	63.6	4.23, 1H, dd (12.0, 6.4) 4.28, 1H, brd (12.0)	C-5′, C-1″
Acyl			
1″	174.5	–	
2″	34.3	2.27, 2H, t (7.6)	C-1″
3″	24.9	1.53, 2H, ov. m	C-1″
4″- 41″	29.7	1.19, n CH_2 brs	C-44″
42″	31.9	1.19, 2H brs	C-41″, C-43″, C-44″
43″	22.7	1.19, 2H brs	C-41″, C-42″, C-44″
44″	14.1	0,82, 3H, t (6.6)	C-42″, C-43″

ov. overlapped.
[a] Assignments were based on the DEPT, HSQC and HMBC experiments.
[b] Signals may be interchanged.
[c] Signals may be interchanged.

identified as a β-sitosterol derivative. This was confirmed by comparison of its NMR data (Table 2) with those of known sitosterol derivatives [12,13]. The EIMS showed ion peaks at *m/z* 71, 73, 129, 229, and 241, characteristic of steroids and a peak at *m/z* 396, typical of β-sitosterol [14]. Moreover, several fragments were observed exhibiting a uniform difference of 14 mass units, revealing the presence of an aliphatic long chain in the molecule [15,16]. The peak at *m/z* 161 was characteristic of glucose moiety [17]. This suggested that compound **2** was a β-sitosterol derivative with a glucose unit and an aliphatic side chain. The IR spectrum of compound **2** showed ester carbonyl absorption at 1723 cm^{-1} and hydroxyl groups at 3431 cm^{-1}. The ^{1}H NMR spectrum (Table 2) displayed two characteristic angular methyl groups at δ_H 0.62 (Me-18) and 0.95 (Me-19), an oxymethine proton at δ_H 3.48 (m, H-3) and an olefinic proton at δ_H 5.30 (1H, brs, H-6) characteristic of cholest-5-ene-3αH [18,19]. Signals of the side chain of the β-sitosterol included a doublet of a secondary methyl group at δ_H 0.86 (J=7.5 Hz, Me-21), signals of an isopropyl unit with two methyl doublets at δ_H 0.75 (J=6.6 Hz, Me-26/27), 0.77 (J=6.6 Hz, Me-27/26) and a multiplet of a methine proton at δ_H 1.54 (H-25) and one ethyl group with signals at δ_H 0.78 (3H, brt, J=7.5 Hz, Me-24^2), 1.15 (1H, m, H-24^{1}a) and 1.20 (1H, m, H-24^{1}b). Furthermore, the presence of an anomeric proton signal at δ_H 4.30 (d, J=7.6 Hz, H-1′), in addition with several signals between δ_H 3.28 and 4.28 in the ^{1}H NMR spectrum suggested the presence of a glucose unit [17]. The relative stereochemistry of this sugar moiety was determined as β on the basis of the characteristic $J_{1,2}$ coupling constant (7.6 Hz) of the anomeric proton and the typical ^{1}H and ^{13}C NMR shifts [20]. The methyl triplet at δ_H 0.82 (t, J= 6.6 Hz, H_3-44″), the broad singlet of several methylene protons at δ_H 1.19 and the triplet at δ_H 2.27 (2H, t, J=7.6 Hz, H-2″) indicated the presence of an aliphatic long chain linked to a carbonyl group. The ^{13}C NMR spectrum of compound **2** displayed signals of an exocyclic double bond at δ_C 122.1 (C-6) and 140.3 (C-5), an ester carbonyl at δ_C 174.5 (C-1″) and an oxygenated methine carbon of the steroid nucleus at δ_C 79.6 (C-3). In this spectrum, the six signals at δ_C 101.2 (C-1′), 76.2 (C-2′), 73.7 (C-3′), 70.1 (C-4′), 73.3 (C-5′) and 63.6 (C-6′) confirmed the presence of a glucose unit. The size of the long aliphatic chain linked to the ester carbonyl group was determined on the basis of EIMS which showed peak at *m/z* 397 [M − $C_6H_{10}O_6$–$COC_{43}H_{87}$] and the FABMS with the peak at *m/z* 808 [M − $C_{29}H_{50}$]. In the HMBC spectrum (Fig. 3), pertinent correlations observed between H-3 (δ_H 3.48) and carbon C-1′ as well as between H-1′ and C-3 showed the linkage of the sugar moiety at C-3. Further HMBC correlations observed between proton H-6′ and C-1″ and between H-2″ and C-1″ confirmed the attachment of the long aliphatic chain on the ester carbonyl linked to the sugar unit at C-6′ through the oxygen atom. Acid hydrolysis of compound **2** yielded free sugar that was identified as D-glucose by measurement of the optical rotation $[\alpha]_D^{28}$ + 40.5. Compound **2** was thus characterized as a new β-sistosterol glucoside derivative 6-*O*-acyl-β-D-glucosyl-β-sistosterol, named klainedoxasterol.

Additionally, ten known compounds, including six triterpenoids, lupeol (**3**) [2,4], betulinic acid (**4**) [2,4], 24-methylenelanost-8-en-3β-ol (**5**) [8], 24-methylenelanost-8-en-3-one (**6**) [8], 24*Z*-ethylidenelanost-8-en-3-one (**7**) [8], and

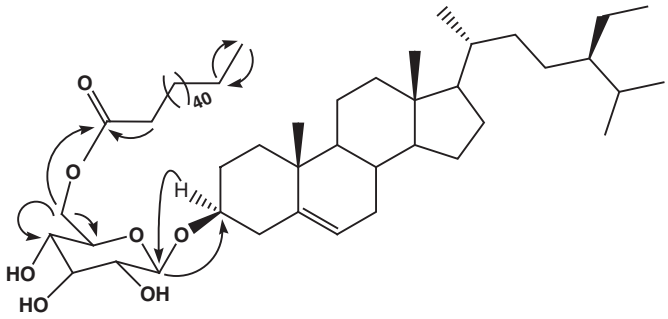

Fig. 3. Selected HMBC (H→C) correlations of compound **2**.

3-acetyl ursolic acid (**8**) [21], two known sterols, β-sitosterol (**9**) [2], and β-D-glucopyranosylsitosterol (**10**) [22] and two tanins, gallic acid (**11**) [3,4] and methyl gallate (**12**) [4] were isolated. Their structures were confirmed by comparison of their spectral data with those reported in the literature.

Nine compounds (**1**–**6**, **8**, **10** and **11**) were evaluated for their xanthine oxidase inhibitory activity at the concentration of 0.25 mM (Table 3). In our study 6 out of 9 tested compounds were triterpenoids. The biological activities of triterpenoids, continue to be of interest [23]. Several triterpenoids showing XOD inhibitory effect were reported [24–26]. Klainedoxalanostenone (**1**) and klainedoxasterol (**2**) exhibited weak inhibitory activity. Lupeol (**3**) and betulinic acid (**4**) did not show significant inhibitory activity towards XOD as previously reported by de Souza et al. (2012) and Isobe et al. (2007) respectively [27,28]. Compound **5** exhibited the highest activity with $IC_{50} = 16.96 \pm 0.69$ μM, while compound **6** was completely inactive. This activity may be due to free hydroxyl group in the lanostane ring in **5**. The activity of ursolic acid, on XOD inhibition was not significant ($IC_{50} > 100$ μM) [29]. In our study the derivative of ursolic acid, 3-acetyl ursolic acid (**8**) showed weak inhibitory activity. β-Sitosterol did not show any XOD inhibition, like β-D-glucopyranosylsitosterol (**10**) which was evaluated in this study for the first time.

In the XOD molecule, the active sites of superoxide anion generation and uric acid formation are different. It is suggested that the inhibition of superoxide anion generation was caused by the binding of the inhibitor to the FAD binding site. That is, an inhibitor which binds the xanthine binding site in xanthine oxidase inhibits the uric acid formation by xanthine oxidase.

Table 3
In vitro inhibitory effect of compounds (**1**–**6**, **8**, **10** and **11**) against xanthine oxidase.

Compounds	Concentration (mM)	%Inhibition	IC_{50} value (μM)
1	0.25	19.0	ND
2	0.25	7.3	ND
3	0.25	7.8	ND
4	0.25	26.1	ND
5	0.25	90.7	16.96 ± 0.69
6	0.25	−5.2	ND
8	0.25	36.5	ND
10	0.25	1.9	ND
11	0.25	17.2	ND
Standard (allopurinol)	1	98.8	13.703 ± 0.150

ND: not determined.

The presence of these metabolites can explain, at least in part, the XOD inhibitory and antioxidant activities of *K. gabonensis* (Irvingiaceae) and support its use in traditional medicine.

3. Experimental

3.1. General experimental procedures

Melting points were obtained on a Gallenkamp melting point apparatus. UV spectra were recorded on a Hitachi UV 3200 spectrophotometer. A JASCO 320-A spectrophotometer was used for scanning IR spectroscopy using KBr pellets. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) spectra were recorded at room temperature in $CDCl_3$ or $CDCl_3$-MeOD using a Bruker spectrometer and chemical shifts are given in δ (ppm) value relative to TMS as internal standard. EIMS spectra were obtained on Varian MAT 311A mass spectrometer. FABMS spectra were measured on a JOEL-HX 110 mass spectrometer. Column chromatography was performed on silica gel 230–400 mesh (Merck). Fractions were monitored by TLC using precoated aluminum-backed silica gel 60 F_{254} sheets. Spots were visualized under UV light (254 and 365 nm) and then sprayed with ceric sulfate reagent followed by heating at 100 °C.

3.2. Plant material

The stem bark of *K. gabonensis* was collected in Kékem (Haut Nkam Division, West Region of Cameroon) in December 2008 and authenticated in the Cameroon National Herbarium, Yaoundé, where a voucher specimen was deposited under the reference SRFC/22309.

3.3. Extraction and isolation

The air-dried and powder stem bark of *K. gabonensis* (4 kg) were extracted with CH_2Cl_2/MeOH (1:1, 3×10 L, 72 h) at room temperature to yield a crude extract (170 g) after evaporation under vacuum. One hundred and sixty grams of this extract was subjected to silica gel chromatography eluted with *n*-hexane–EtOAc (10:0, 9:1, 8:2, 7:3, 1:1, 2:8, 0:10) and EtOAc–MeOH (10:0, 9:1, 8:2, 7:3, 1:1, 2:8, 0:10) to give six main fractions (A–F). Fraction A (4.4 g) submitted to GC–MS analysis, revealed the presence of fatty acids and was not further investigated. Fraction B (2.1 g) was purified by column chromatography over silica gel using a gradient of *n*-hexane–EtOAc to yield compound **1** (13 mg). Fraction C (2.9 g) was rechromatographed over Sephadex gel LH-20 with CH_2Cl_2/MeOH (9:1) as eluent and

further purified over silica gel using a gradient of *n*-hexane–EtOAc to afford compounds **3** (14 mg), **5** (10 mg) and **9** (16 mg). Fraction D (3.7 g) was subjected to column chromatography over silica gel using *n*-hexane/CH_2Cl_2 (1:1) as solvent to afford compounds **4** (21 mg) and **8** (14 mg). Fraction E (4.5 g) was purified by column chromatography over silica gel using a gradient of *n*-hexane–EtOAc to yield compounds **2** (13 mg), **6** (9 mg), **7** (12 mg), **10** (8 mg), **11** (31 mg) and **12** (25 mg).

*3.3.1. Klainedoxalanostenone (**1**)*

White amorphous powder, $[\alpha]_D^{28} + 1.4$ (C 0.057, MeOH), ^{1}H NMR and ^{13}C NMR data: see Table 1; HREIMS: $m/z = 452.3633$ $[M]^+$ (calcd. for $C_{31}H_{48}O_2$ 452.3654). EIMS: *m/z* 437 (42), 339 (54), 299 (21), 109 (46), 83 (100), 69 (62) 47 (17.5).

*3.3.2. Klainedoxasterol (**2**)*

White amorphous powder, $[\alpha]_D^{28} - 5.9$ (C 0.057, MeOH); IR (KBr) υ_{max}: 3431 (OH), 1723 (CO) cm^{-1}; ^{1}H NMR and ^{13}C NMR data: see Table 2; HRFABMS: $m/z = 1208.1158$ $[M+H]^+$ (calcd. for $C_{79}H_{147}O_7$ 1208.1147). EIMS: *m/z* 397 (19), 396 (26), 256 (11), 213 (9), 161 (7), 129 (18), 85 (10) and 55 (100).

*3.4. Acid hydrolysis of compound **2***

A solution of compound **2** (3 mg) in water (2 mL) and 2 N aqueous CF_3COOH (10 mL) was refluxed at 100 °C for 2 h. The mixture was then diluted in water (10 mL) and extracted with EtOAc (3×3 mL). The combined EtOAc layers were washed with H_2O and evaporated to dryness to afford the aglycon. The aqueous residue was concentrated to dryness by adding MeOH to remove the acid. The residue was purified over ODS column to afford D-glucose ($[\alpha]_D^{28} + 40.5$ (C 0.050, MeOH), (0. 55 mg)).

3.5. Determination of xanthine oxidase inhibitory activity

The xanthine oxidase inhibitory activity of test compounds was determined by measuring the rate of hydroxylation of the substrate (xanthine) with the formation of uric acid, which is a colorless end product of the reaction and shows absorption at 295 nm [30]. Briefly, the reaction mixture containing 10 μL of 1 mmol L^{-1} pure sample was dissolved in DMSO, 150 μL of phosphate buffer (0.05 mol L^{-1}, pH 7.4), 0.003 units of xanthine oxidase dissolved in buffer (20 μL), and 20 μL of 0.1 mmol L^{-1} xanthine as substrate for enzyme. After addition of xanthine oxidase, the mixture was incubated for 10 min at room temperature and pre-read in the UV region (λ_{max} 295 nm). The substrate was added to reaction mixture, and final continuous reading for 15 min at an interval of 1 min was observed (Spectra MAX-340). The percentage inhibitory activity by the samples was determined against a DMSO blank and calculated using the following formula: Inhibition (%) = 100 − [(OD test compound/OD control) × 100]. The IC_{50} of the compounds was calculated using EZ-Fit windows-based software (Perrella Scientific Inc. Amherst, U.S.A.). To compare the inhibitory activities of the compounds, allopurinol was used as standard. The reaction for each compound was performed in triplicate [30].

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