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Isolation and caracterisation of sodium monocarboxylate ixoside salt from the stem bark of *Oxyanthus pallidus*

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ABSTRACT

A sodium monocarboxylate ixoside salt(1) and four known compounds, ixoside (2), mannitol (3), uncargenine C (4) and oleanolic acid (5) have been isolated from the stem bark of *Oxyanthus pallidus*. Their structures were established on the basis of spectroscopic techniques.

Keywords: *Oxyanthus pallidus*, *Oxyanthus sankuruensis*, *Oxyanthus schubotzianus*, Rubiaceae, iridoids, sodium monocarboxylate ixoside salt.

INTRODUCTION

Oxyanthus pallidus Hiern/*Oxyanthus sankuruensis* De Wild/*Oxyanthus schubotzianus* K. Krause (Rubiaceae) is a small shrub widespread from Senegal to Nigeria, and extending from Sudan to Ethiopia (Hallé, 1970). Plants of the genus *Oxyanthus*, occupy a prominent position in traditional African medicine (Watt and Breyer-Brandwijk, 1962; Bouquet, 1969; Adjonohoun et al., 1988; Kawukpa and Angoyo, 1994; Obijiofor, 2002; Chaaib, 2004). Our previous contribution reported the isolation from the leaves of three new cycloartane glycosides (Tigoufack et al., 2010). In an extension of our studies, the methanol extract of the stem bark of *Oxyanthus pallidus* was examined for minor concentrations of sodium monocarboxylate ixoside salt (**1**).

MATERIALS AND METHODS

General experimental procedures

All melting points were recorded with a Reichter microscope and are uncorrected. IR spectra were recorded with a Shimadzu FTIR-8400 spectrometer. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded in CD_3OD on a Bruker Avance DRX-500 spectrometer. Chemical shifts (δ) are reported in parts per million with solvent signals δ_H 3.31 and δ_C 49.1 as references, while the

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coupling constants (*J*) are given in Hertz. HR-ESI-MS experiments were performed using a Micromass Q-TOF micro instrument (Manchester, UK) with an electrospray source. Column chromatography was run on Merck silica gel 60 and Sephadex LH-20 while TLC was carried out on silica gel GF_{254} precoated plates with detection accomplished by spraying with 50% H_2SO_4 followed by heating at 100 °C.

Plant material

Stem barks of *Oxyanthus pallidus* were collected in the city of Dschang, Cameroon, in March 2010 and identified by Mr. Victor Nana, a botanist of the National Herbarium of Cameroon (Yaoundé) where a voucher specimen (n° 7335/SFR/CAM) was deposited.

Extraction and isolation

The air-dried and finely powdered material (4.5 kg) was extracted with MeOH (10 l) in a glass tank at room temperature. After complete removal of solvent by vacuum evaporation, a dark residue (132 g) was obtained. Part of this, (52 g) was fractionated, on silica gel (40-63 µm, 98 g) column chromatography, eluted with ethyl acetate, followed by a mixture of ethyl acetate and 10-70% of MeOH, yielded five main fractions (A-E). Silica gel column chromatography of the ethyl acetate fractions, A (2 g), and B (5.1 g) using hexane-EtOAc (1:1) for A and hexane-EtOAc (2:8) for B yielded oleanolic acid (1.1 g) (**5**) and uncargenine C (10 mg) (**4**) respectively. Fraction C (357.3 mg), was obtained with EtOAc-MeOH (8:2); when the mixture of solvents was distilled off, a precipitate was formed that crystallized in ethyl acetate affording 43.7 mg of mannitol (**3**) after vacuum filtration. Fraction D (2.4 g) obtained with a mixture of EtOAc-MeOH (7:3) was purified over Sephadex LH-20 gel using MeOH followed by silica gel column chromatography using the mixture of EtOAc-MeOH-H_2O (8:1:1) to yield compound **2** (30.8 mg). Sub-fraction D-3 (80 mg), was purified over silica gel column chromatography, using the mixture of EtOAc-MeOH-H_2O (8:1:1) to yield compound **1** (50 mg).

Compound 1

$[\alpha]_D^{25}$ +24° (CH_3OH, *c 0.308 x* 10^{-2}); IR ν_{max} (NaCl) cm^{-1}: 1726 (C=O); 1271 (C-O); ^1H and ^{13}C (CD_3OD) : Table 1; HR-ESI-MS (negative-ion mode) *m/z* 387.0927 $[M-Na]^-$ (calculated for $C_{16}H_{16}O_{11}$: 387.0931).

Determination of the absolute configuration of the glucose moiety in 1

20 mg of compound **1** were refluxed with 10% HCl (10 ml) at 80 °C for 3 h. The reaction mixture was extracted with EtOAc, and the aqueous phase was concentrated under reduced pressure to yield D-glucose (4.8 mg), identified on TLC by comparison with an authentic sample. Its relative configuration was determined by measurement of the optical rotation value, $[\alpha]^{23}_D$ +56.2° (H_2O, *c 0.973*).

RESULTS AND DISCUSSION

Compound 1, $C_{16}H_{19}O_{11}Na$, which carbonized to a cinder without melting, was obtained as a black powder with optical rotation $[\alpha]_D^{25}$+24° (CH_3OH, *c 0.308 x*10^{-2}). HR-ESI-MS, in negative-ion mode, showed a molecular ion peak at *m/z* 387.0927 $[M-Na]^-$ in agreement with the molecular formula $C_{16}H_{19}O_{11}$. The presence of the sodium ion in this molecular formula was deduced from the HR-ESI-MS in positive-ion mode. The IR spectrum indicated the presence of hydroxyl group (3500-3360 cm^{-1}), carbonyl (1726 cm^{-1}), and carbon carbon double bond (1645 cm^{-1}). The ^1H NMR spectrum (Table 1) showed characteristic signals of iridoid glycoside type of ixoside (Guarnaccia et al., 1972; Yoshio et al., 1975; Luciano et al., 2010). Indeed, two allylic protons were observed at δ 2.35 (1H, ddd, *J* = 17.5, 8.2 and 2.5 Hz, H-6β), 2.87

(1H, ddd, *J* = 17.5, 8.2 and 2.5 Hz, H-6α)] and two vinylic protons at δ 6.52 (1H, brd, *J* = 1.6 Hz, H-7), 7.34 (1H, s, H-3). Signals observed at δ 3.17 (1H, m, H-9) and δ 3.32 (1H, m, H-5)] were attributed to the iridan methine groups. The signal observed at δ 5.62 (1H, d, *J* = 4.7 Hz, H-1) was attributed to the iridan acetal group due to the downfield shift. Remaining protons observed between δ 4.63-3.30 are those of a β-glucopyranosyl moiety after analysing its Cosy spectrum (Figure 1). Acid hydrolysis of **1** afforded D-glucose, which was identified on TLC by comparison with an authentic sample, and the configuration was determined by measurement of the optical rotation value. The 1,2 diaxial coupling of anomeric proton (H-1') at δ 4.63 (d, *J* = 7.8 Hz) indicated the β configuration of the glucose unit. Compound **1** was clarified by HMBC experiments. Indeed, the connectivity of the β-D-glucopyranosyl moiety was elucidated on the basis of HMBC correlations, observed between H-1' (δ 4.63, d, *J* = 7.8 Hz) and C-1 (δ 95.1), and between H-1 (δ 5.62) and C-1' (δ 98.6), as shown on Figure 2. The ^{13}C NMR spectrum of **1** was almost superimposable on that of ixoside (**2**) (Table 1), except for the NMR data of carbons C-6, C-7, C-8, C-9 and C-10. Specifically, the significant upfield shift (δ 138.8 from 146.6 of C-7), and downfield shift (δ 38.7 from 33.6 of C-6, 141.0 from 135.0 of C-8 and 171.9 from 166.7 of C-10) indicated that the sodium carboxylate group was located at the C-8 position. This assumption was confirmed by an HMBC correlation observed between H-7 and C-10 (Figure 2). The two carbonyl groups, C-10 and C-11 of **1** which both appeared at the same value of δ 171.9 ppm, were clearly observed at δ 166.7 and 169.2 for C-10 and C-11 respectively, after adding a drop of TFA in the nmr tube. The relative stereostructure of **1** was established by NOESY experiment; the strong NOE correlation observed between H-9 at δ 3.17 and H-5 at δ 3.32 (Figure 3) suggested their β-orientation (Guarnaccia et al., 1972; Yoshio et al., 1975; Kanchanapoom et al., 2002; Luciano et al., 2010). The absence of NOE correlation between H-1 at δ 5.62 and H-9 at δ 3.17; H-5 at δ 3.32, confirmed the α-orientation of H-1. All these information allowed us to suggest that, compound **1** was elucidated to be a sodium monocarboxylate ixoside salt.

Structures (**2-5**) (Figure 4) were determined by means of *Co* TLC, spectroscopic data, and by comparative analysis of physical and spectral data with those in the literature (Takeda et al., 1975; Pouchet et al., 1970; Yang et al., 1995; Meicai, 2008). TLC retention time, obtained for compounds **1** and **2** in the mixture EtOAc-MeOH-H_2O (7:2:1) are 0.43 and 0.28 respectively.

Table 1: ^{1}H and ^{13}C NMR data of compounds **1** and **2** in CD_3OD.

H	**1**	**2**	C	**1**	**2**
position	δ ^{1}H (mult., *J*(Hz))	δ ^{1}H (mult., *J*(Hz))	position	δ ^{13}C	δ ^{13}C
1	5.62 (1H, d, 4.7)	5.70 (1H, d, 5.0)	1	95.1	94.9
3	7.34 (1H, s)	7.51 (1H, s)	3	149.4	152.1
			4	114.7	111.1
5	3.32 (1H, m)	3.33 (1H, m)	5	34.3	33.6
6α 2.87 (1H, ddd, 17.5, 8.2, 2.5)		2.94 (1H, ddd, 17.6, 8.0, 2.3)	6	38.7	33.8
6β 2.35 (1H, ddd, 17.5, 8.2, 2.5)		2.47 (1H, ddd, 17.6, 8.0, 2.3)			
7	6.52 (1H, brd, 1.6)	6.92 (1H, brd, 1.7)	7	138.8	146.6

			8	141.0	135.0
9	3.17 (1H, m)	3.22 (1H, m)	9	46.7	46.0
			10	171.9	166.7
			11	171.9	169.1
Glc-1’	4.63 (1H, d, 7.8)	4.65 (1H, d, 7.9)	1’	98.6	98.9
2’	3.23 (1H, dd, 8.6, 7.8)	3.22 (1H, dd, 8.5, 7.9)	2’	73.2	73.2
3’	3.38 (1H, dd, 8.9, 8.6)	3.36 (1H, dd, 8.9, 8.5)	3’	76.3	76.4
4’	3.31 (1H, dd, 8.9, 8.5)	3.32 (1H, dd, 8.9, 8.6)	4’	70.1	70.0
5’	3.30 (1H, m)	3.29 (1H, m)	5’	76.7	76.8
6’α	3.67 (1H, dd, 12.2, 2.2)	3.70 (1H, dd, 12.1, 2.1)	6’	61.3	61.2
6’β	3.85 (1H, dd, 12.2, 2.2)	3.88 (1H, dd, 12.1, 2.1)			

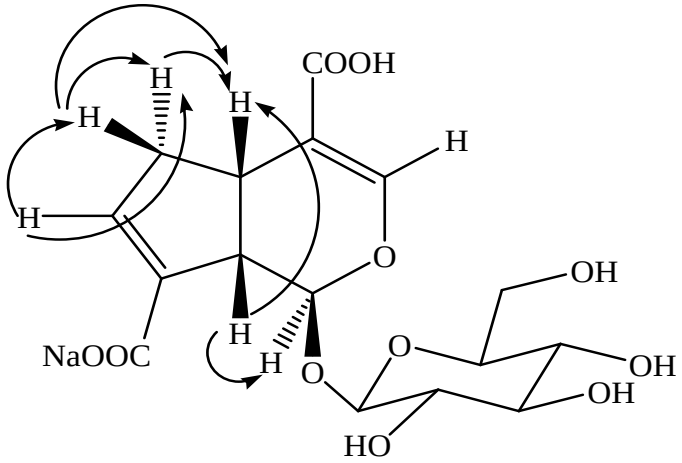


Figure 1: ^{1}H-^{1}H COSY Correlations of compound **1**

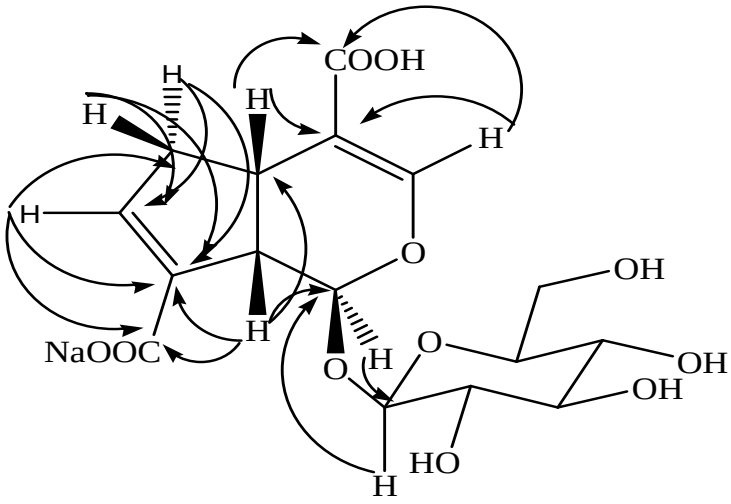


Figure 2: HMBC Correlations of compound **1**

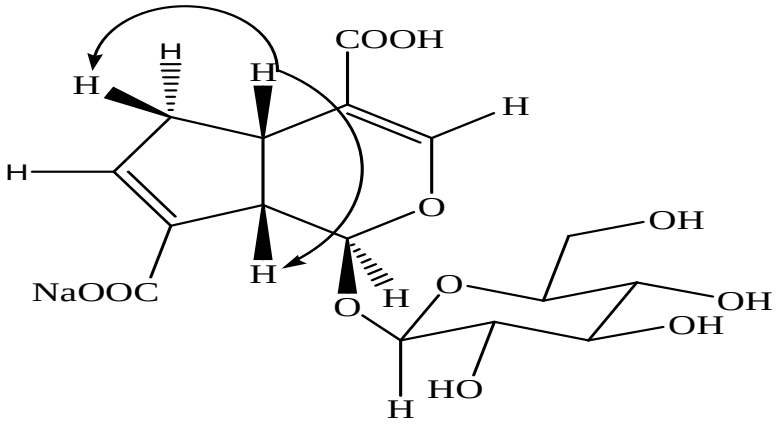
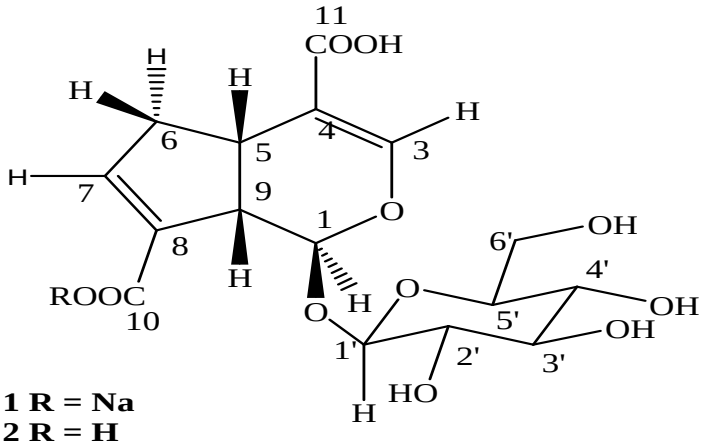
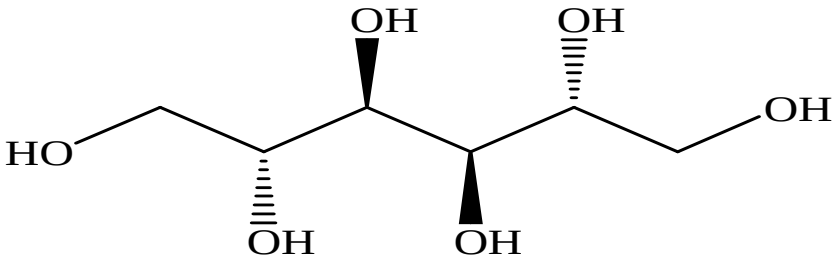


Figure 3: NOE Correlations of compound **1**



1 R = Na
2 R = H



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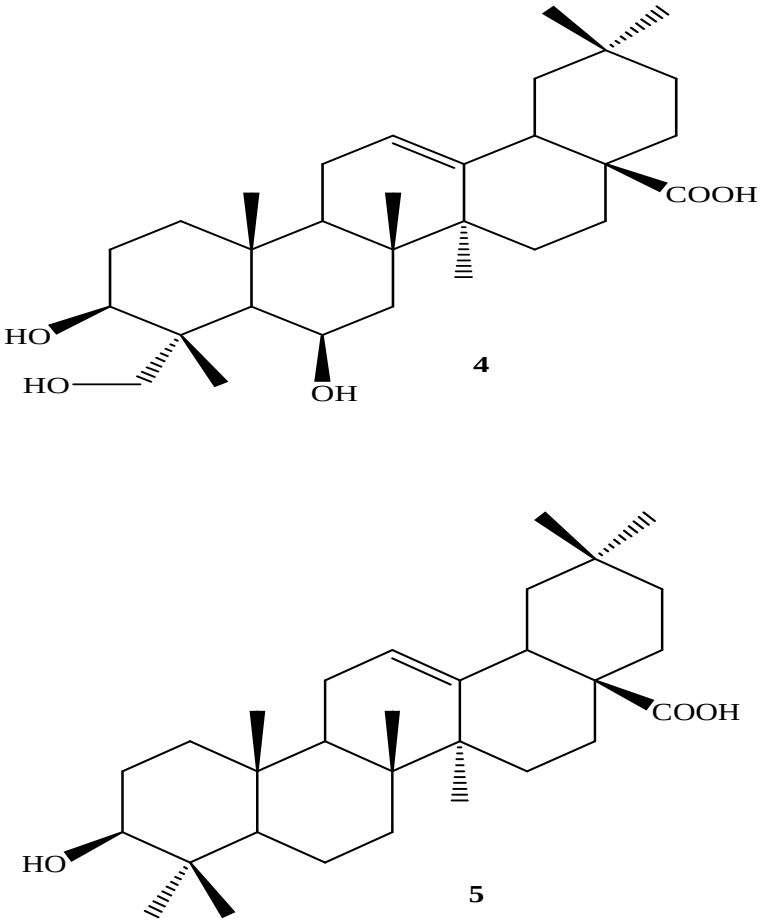

Figure 4: Structures of isolated compounds.

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