



## Chemical constituents of *Cordia platythyrsa* and evaluation of their glycation and urease inhibition activities

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### ABSTRACT

A new xanthone derivative, Cordixanthone (1-hydroxy-3,6-dimethoxy-7-methylxanthen-9-one) (1) and seven known compounds, were isolated from *Cordia platythyrsa*. This is the first time, xanthones are reported from the genus *Cordia*. Methyl orsellinate and lichexanthone were tested against the urease enzyme and methyl orsellinate was evaluated for *in vitro* antiglycation activity. Methyl orsellinate was found active for both of the test whereas lichexanthone showed no activity.

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### KEYWORDS

Antiglycation;  
*Cordia platythyrsa*;  
Cytotoxicity;  
Urease;  
Cordixanthone.

### INTRODUCTION

The genus *Cordia* belongs to the Boraginaceae family and comprises trees and shrubs, and is distributed worldwide in tropical regions<sup>[1]</sup>. Nine *Cordia* species have been identified in Cameroon<sup>[2]</sup>. Maceration of the leaves *Cordia platythyrsa* is used for the treatment of sleeping sickness and convulsion<sup>[3]</sup>. The wood of *C. platythyrsa* has an attractive colour and is used to produce drums of varied shapes, spoons, kitchen utensils and the keys of native xylophones. The trunks of plants of this genus are used in the construction of huts because of their resistance to termite<sup>[4]</sup>. The decoction of various parts of *Cordia* have been used in folk medicines to treat influenza, fever, pneumonia, coughs, insomnia, stomachache and parasitic<sup>[5]</sup>. It has been found that some leaves and roots extract of this genus present a pronounced oral and topical anti-inflammatory activity. They are active against the phytopathogenic fungus

and the larvae of the yellow fever transmitting mosquito<sup>[6]</sup>. Sphingolipids and cordiachromes A-F have been isolated from *Cordia platythyrsa*<sup>[7,8]</sup>. Chemical investigations of different parts of *Cordia* species have led to the isolation of various secondary metabolites, terpenoid benzoquinone derivatives, flavonoids, polyphenols<sup>[9]</sup>, epoxidammaranes<sup>[10]</sup>, cordianols A-I and cordialin<sup>[11]</sup>, cordiaquinone, naphthoquinone<sup>[12]</sup> and triterpenoids<sup>[13]</sup>. Some of these compounds exhibit anti-androgenic activity<sup>[12]</sup> and antileishmanial<sup>[13]</sup>.

### EXPERIMENTAL

#### General experimental procedures

Melting points were determined on a Büchi 434 melting point apparatus and were uncorrected. <sup>1</sup>H-NMR experiments were performed on a Bruker AM400 and AMX 500 NMR (Avance) instruments using the UNIX data system at 400 and 500 MHz,

respectively. The  $^{13}\text{C}$ -NMR spectrum was recorded at 300 and 400 MHz, respectively, using  $\text{CDCl}_3$  and  $\text{CD}_3\text{OD}$  as solvents.  $^1\text{H}$ - $^{13}\text{C}$  HMBC and HMQC spectra were recorded as mentioned above. Electron impact mass spectra (EI-MS) were recorded on a Finnigan MAT 312. FAB MS measurements were made on Jeol JMS HX 110 mass spectrometer. Column chromatography was carried out on silica gel, 70-230 and 230-400 meshes. Compounds on the thin layer chromatography (TLC) were employed to detect compounds at 254 and 366 nm, using ceric sulphate as spraying reagent. Optical rotations are measured on P-2000, Model P-2000 series A060061232. The infrared (IR) spectra were recorded on VECTOR22 while Ultraviolet (UV) spectra were recorded on THERMO ELECTRON~VISIONpro SOFTWARE V4.10.

### Plant material

The stem barks of *Cordia platythyrsa* (Boraginaceae) were collected from Nsimeyong, Yaounde, Cameroon, in May 2010, and identified by Dr. N. Tsabang, Centre For Study of Medicinal Plants, Yaoundé, Cameroon. The voucher specimen (No43625/HNC) was deposited at the National Herbarium of Cameroon, Yaoundé, Cameroon.

### Extraction and isolation

The powdered stem barks (6.5 Kg) were extracted with  $\text{CH}_2\text{Cl}_2$ -MeOH [1:1] at room temperature for (72 h). The extract was evaporated to dryness. Evaporation of solvent yielded a dark residue (200 g), which was submitted to vacuum liquid chromatography (VLC) on silica gel with hexane, then EtOAc and finally MeOH. The fraction eluted with 100% EtOAc (98 g) was rechromatographed over silica gel. Gradient elution using hexane-EtOAc [9:1] yielded compounds (1) and (2), hexane-EtOAc [8.5:1.5] yielded compounds (4) and (5), hexane-EtOAc [8:2] yielded compound (3), hexane-EtOAc [6:4] afforded compound (6) and AcOEt-MeOH [7:3] yielded compounds (7) and (8).

### Chemicals

Human Serum Albumin (HSA; free from essential fatty acids) and rutin were purchased from Sigma. Fructose and sodium azide were obtained from Merck, Germany. All solutions were prepared in a sterile environment in deionized water<sup>[14]</sup>.

### Antiglycation assay

HSA (10 mg/mL) was glyated with fructose (500 mM) in a  $\text{NaPO}_4$  buffer (100 mM, pH 7.4) and incubated at 37° C for 7 days in the presence of various test compounds (1 mM) under sterile conditions by using 0.2  $\mu\text{m}$  pore size filter and sodium azide (0.1 mM) to prevent microbial growth. The reaction mixture (200  $\mu\text{L}$ ) of each test compound was incubated in a 96-well microtiter fluorescence plate and each test was run in a triplicate. Rutin was used as a reference compound. At the end of the incubation, fluorescence was measured at 340 nm excitation and 440 nm emission on a microplate spectrophotometer (Spectra Max, molecular Devices, USA).

The percent inhibition of AGEs formation by the test compounds verses positive control was calculated by using the following formula<sup>[15]</sup>:

$$\% \text{ inhibition} = (1 - \text{fluorescence of test compound} / \text{fluorescence of the positive control}) \times 100$$

### Urease inhibition assay

Reaction mixture s comprising 25 $\mu\text{L}$  of enzyme (jack bean urease) solution and 55  $\mu\text{L}$  of buffers containing 100mM urea were incubated with 5  $\mu\text{L}$  of test compounds (0.5 mM concentration) at 30 °C for 15 min in 96-well plates. Urease activity was determined by measuring ammonia production using the indophenol method as described by weather burn. Briefly, 45  $\mu\text{L}$  each phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 70  $\mu\text{L}$  of alkali reagent (0.5% w/v NaOH and 0.1% active chloride NaOCl) were added to each well. The increasing absorbance at 630 nm was measured after 50 min, m using a microplate reader (Molecular Devices, USA). All reactions were performed in triplicate in a final volume of 200  $\mu\text{L}$ . The results (change in absorbance per min) were processed by using softMax Pro software (Molecular Devices, USA). The entire assays were performed at pH 6.8. Percentage inhibitions were calculated from the formula  $100 - (\text{OD}_{\text{testwell}} / \text{OD}_{\text{control}}) \times 100$ . Thiourea was used as the standard inhibitor of urease<sup>[16]</sup>.

### Cytotoxicity assay

Cytotoxicity of compounds was evaluated in 96-well flat-bottomed microplates by using the standard MTT (3-[4, 5-dimethylthiazole-2-yl]-2,5-diphenyl-tet-

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razolium bromide) colorimetric assay<sup>1</sup>. For this purpose, PC-3 cells (Prostrate Cancer) / 3T3(Mouse fibroblast) were cultured in Dulbecco's Modified Eagle's Medium, supplemented with 5% of foetal bovine serum (FBS), 100 IU/mL of penicillin and 100 µg/mL of streptomycin in 25 cm<sup>3</sup> flask, and kept in 5% CO<sub>2</sub> incubator at 37 °C. Exponentially growing cells were harvested, counted with haemocytometer, and diluted with a particular medium. Cell culture with the concentration of 1 x 10<sup>5</sup> cells/mL was prepared and introduced (100 µL/well) into 96-well plates. After overnight incubation, medium was removed and 200 µL of fresh medium was added with different concentrations of compounds (1-100 µM). After 72 h, 50 µL MTT (2 mg/mL) was added to each well and incubated for 4 hrs. Subsequently, 100 µL of DMSO was added to each well. The extent of MTT reduction to formazan within cells was calculated by measuring the absorbance at 570 nm, using a microplate ELISA reader (Spectra Max plus, Molecular Devices, CA, USA). The cytotoxicity was recorded as concentration causing 50% growth inhibition for PC-3/ 3T3 cells<sup>[17]</sup>.

### RESULTS AND DISCUSSION

Compound (1) [ $\alpha$ ]<sub>D</sub><sup>29</sup> -0.005 (c. 0.31 CHCl<sub>3</sub>), was obtained as a white, powder. Its molecular formula was established as C<sub>16</sub>H<sub>14</sub>O<sub>5</sub> by HREI MS (observed m/z 286.0901, calculated 286.0841); UV (MeOH) showed  $\lambda$  max ( $\epsilon$ ) at 243, 308 nm; IR (KBr) vcm<sup>-1</sup> spectra showed absorptions at 3625 (OH), 2922 (CH), 1654 (conjugated carbonyl) cm<sup>-1</sup>. The 16 carbon signals present in the <sup>13</sup>C NMR (TMS) spectrum were characterized by DEPT-90 and 135 spectra as one carbonyl [ $\delta$ ppm 175.53 (C-9)], four aromatic methines [ $\delta$ ppm 96.54(C-2), 91.89 (C-4), 98.86 (C-5), 115.27 (C-8)] and eight aromatic quaternary carbons [ $\delta$ ppm 162.87 (C-1), 165.63 (C-3), 163.66 (C-6), 143.15 (C-7), 156.79 (C-4a), 110.84 (C-8a), 104 (C-9a), 159.29 (C-10a)], as well as two methoxy methyl carbons [ $\delta$ ppm 55.36 (3-OCH<sub>3</sub>), 55.41 (6-OCH<sub>3</sub>)] and one methyl carbon substituted to an aromatic ring [ $\delta$ ppm 23.00 (7-CH<sub>3</sub>)]. The <sup>13</sup>C NMR (TMS) chemical shifts of all hydrogenated carbons were assigned unambiguously by using the HSQC spectrum. The <sup>1</sup>H NMR (TMS) spectrum of compound (1) (CDCl<sub>3</sub>) showed

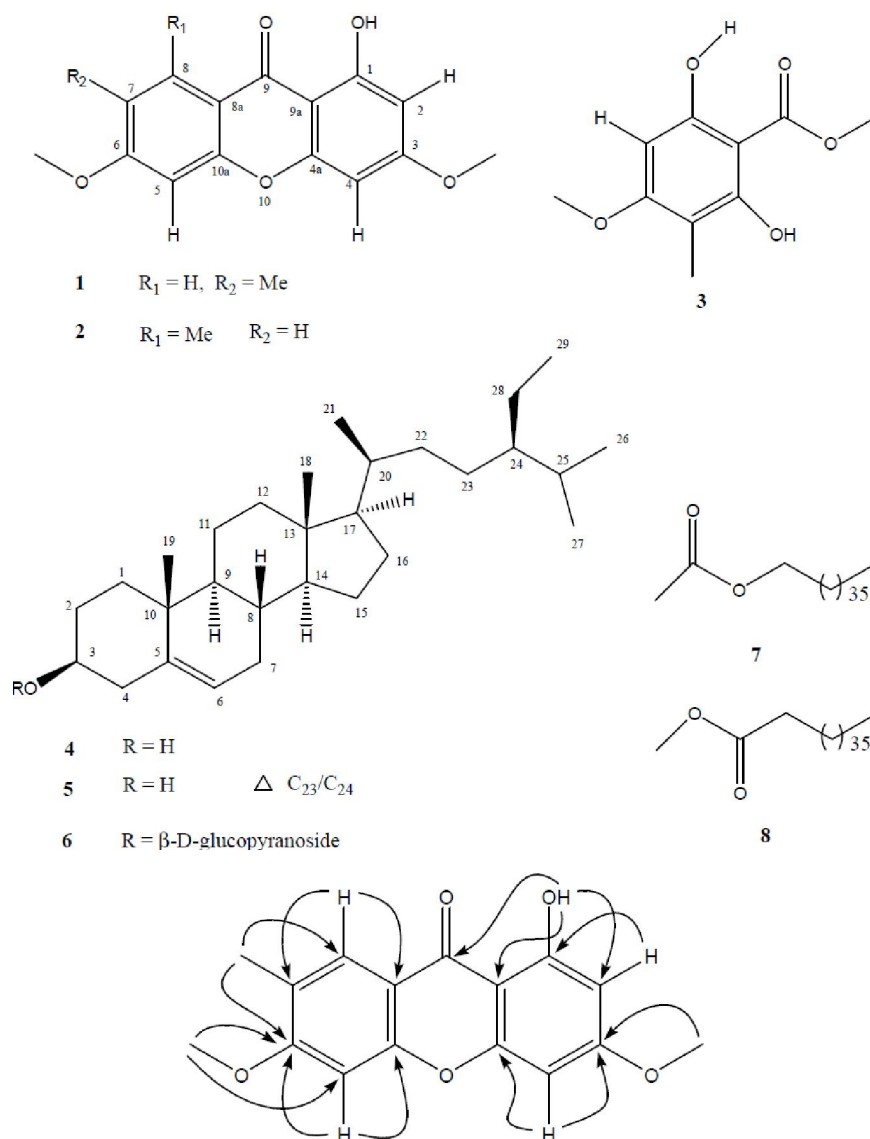
signals for one methyl group at  $\delta$ ppm 2.83 (s, 3H, H-CH<sub>3</sub>), four aromatic proton signals at  $\delta$ ppm 6.28 (d, 1H, *J*=2.5 Hz, H-2), 6.31ppm (d, 1H, *J*=2.5 Hz, H-4), 6.66ppm (s, 1H, H-8) and 6.64ppm (s, 1H, H-5), two methoxy protons at  $\delta$ ppm 3.85 (s, 3H, H-3/OCH<sub>3</sub>) and 3.87 (s,3H, H-6/OCH<sub>3</sub>), one hydroxyl-group ( $\delta$ ppm 13.35 br). COSY correlations between the proton signals at H-8 ( $\delta$ ppm 6.66), 7-CH<sub>3</sub> (3H,  $\delta$ ppm 2.83) allowed partial elucidation of the structure. Complete elucidation of the structure of compound (1) was achieved on the basis of HMBC correlations. <sup>1</sup>H/<sup>13</sup>C: H-CH<sub>3</sub>/C-7, C-8, C-8a and C-5; H-6-OCH<sub>3</sub>/ C-6; 3-OCH<sub>3</sub>/ C-3; H-8/C-8 and C-6; H-5/C-5, C-6 and C10a; H4/C-4 and C-4a; C-2/C-2,C-1 and C-9a; 1-OH/C-1, C-9a, C-2 and C-3. These data suggest structure of compound (1) as 1-hydroxy-3,6-dimethoxy-7-methylxanthen-9-one. The spectral data of compound (1) are shown on (TABLE 1)

**TABLE 1:** <sup>1</sup>H- and <sup>13</sup>C NMR(TMS) (CDCl<sub>3</sub>, 500 and 125 MHz), data for compound (1)

Position	<sup>1</sup> H-NMR (TMS) $\delta$ (ppm)	<sup>13</sup> C-NMR (TMS) $\delta$ (ppm)	Number of hydrogens; multiplicity; frequency
1-OH	13.36	-	(S,OH)
1	-	162.87	-
2	6.28	96.8	(d,1H, J=2.5Hz)
3	-	165.63	-
4	6.31	91.89	(d,1H, J=2Hz)
5	6.64	98.26	(s,1H)
6	-	163.66	-
7	-	143.15	-
8	6.66	115.27	(s,1H)
9	-	175.33	-
4a	-	156.79	-
8a	-	110.84	-
9a	-	104	-
10a	-	159.29	-
3-OCH <sub>3</sub>	3.85	55.36	(s,3H)
6-OCH <sub>3</sub>	3.87	55.41	(s,3H)
7-CH <sub>3</sub>	2.83	23.00	(s,3H)

Structures of the compounds (2-8) were also elucidated by 1D- and 2D NMR spectroscopy. All the physical and spectral data were unambiguously identical to the reported values in literature<sup>[18-22]</sup>. The compounds were identified as lichexanthone (2), methyl orsellinate (3),  $\beta$ -sitosterol (4), stigmasterol (5), 3-O-

$\beta$ -D-glucopyranosyl- $\beta$ -sitosterol (6), heptatriacountanyl ethanote (7) and methyl octatriacountanoate (8).



**TABLE 2 : IC<sub>50</sub> values of compounds (3), (4), (5) and doxorubicin towards PC3 and 3T3 line cells at 24 hours incubation in the MTT assay**

Treatments	Concentration ( $\mu\text{g/ml}$ )	PC3 line cells	3T3 line cells
		IC <sub>50</sub>	IC <sub>50</sub>
Vehicle	1-100	>20	>20
3	1-100	>20	>20
4	1-100	>20	>20
5	1-100	>20	>20
Doxorubicin	1-100	0.912 $\pm$ 0.12	3.1 $\pm$ 0.20

The cytotoxicity of  $\beta$ -sitosterol (4), stigmasterol (5) and methyl orsellinate (3) were evaluated by using PC-3/3T3 cells and a standard MTT bioassay (TABLE 2).

As shown, incubation for 24 hrs of these cells lines with various concentrations of  $\beta$ -sitosterol, stigmasterol or methyl orsellinate; produces no cell toxicity. The toxicity values observed were not significantly ( $p < 0.001$ ) different from baseline. However, incubation of PC-3/3T3 cells with a higher concentration of doxorubicin ( $X \mu\text{M}$ ) produced greater cell death with IC<sub>50</sub> of  $3.1 \pm 0.2 \mu\text{M}$ .

Among the compounds screened, methyl orsellinate (5) was identified as active against the urease of Jack bean, whereas lichexanthone (2) showed no activity. Compound (5) was found to be as active than the standard inhibitor thiourea (IC<sub>50</sub> =  $21 \pm 0.011$ ), with an IC<sub>50</sub> value of  $67 \pm 1.23$  and  $0.5 \text{ mM}$  for urease of Jack beam.

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Compound (1) has no ability to inhibit the urease of Jack bean (TABLE 3).

**TABLE 3 : Inhibition of jack bean urease by Lichexanthone 2 and Methyl orsellinate 3**

Compound	Conc. (mM)	% Inhibition	IC <sub>50</sub> SEM[ $\mu$ M]
2	0.5	86.8	67 $\pm$ 1.23
3	0.5	15.1	Not Active
Standard (Thiourea)	0.5	98.2	21 $\pm$ 0.011

Methyl orsellinate (3) was evaluated for its antiglycation activity. It exhibited an antiglycation activity with IC<sub>50</sub> value of 210 $\pm$  0.017 compared with standard rutin IC<sub>50</sub> 70 $\pm$ 0.5  $\mu$ M. This value showed that the methyl orsellinate (3) exhibit a very good antiglycation activity near to the standard (IC<sub>50</sub> =210 $\pm$  0.017  $\mu$ M).

**TABLE 4 : Anti-glycation activity of compound (3)**

Compound	Conc. (mM)	% Inhibition	IC <sub>50</sub> SEM[ $\mu$ M]
3	1mM	66.4%	210 $\pm$ 0.017
Standard	1mM	82%	70 $\pm$ 0.5

As seen, using the more sensitive MTT assay, no cytotoxicity has been observed on PC-3/3T3 cells lines incubated with various concentration of Compound 2 (1 - 100  $\mu$ M), 3 (1 - 100  $\mu$ M), 4 (1 - 100  $\mu$ M) or 5 (1 - 100  $\mu$ M). MTT assay measured the metabolism of 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide to form formazan precipitate by mitochondrial dehydrogenase which only present in viable cells. Formazan accumulation directly reflected mitochondrial activity, which was an indirect measure of cell viability.

The antiglycation activity of 2-hydroxy-3-methoxybenzaldehyde-N-(2-oxo-1, 2-dihydro-3-indol-3-ylidene) hydrazone and 2-hydroxy-5-methoxybenzaldehyde-N-(2-oxo-1, 2-dihydro-3H-indol-3-ylidene) hydrazone which both have aromatic ortho-hydroxyl group but the first one has a methoxy group and the second has a methyl group has been evaluated. 2-Hydroxy-3-methoxybenzaldehyde-N-(2-oxo-1, 2-dihydro-3-indol-3-ylidene) hydrazone was found to be active antiglycating agent while 2-hydroxy-5-methoxybenzaldehyde-N-(2-oxo-1, 2-dihydro-3H-indol-3-ylidene) hydrazone showed extremely low antiglycation potential or complete lack of activity. These results compared with the result of compound (5) which suggest that the benzyl methoxy group has an effect on the antiglycation activity<sup>[23]</sup>.

Compounds (2) and (3) have the similar functional group; except the methyl group on compound (1). Compound (3) which has not methyl group was identified as active against the urease of Jack bean, whereas lichexanthone (2) showed no activity. Compound (2) has a xanthone skeleton and compound (3) has only one aromatic ring with two hydroxyl groups. The ester group and the hydroxyl groups in compound 3 may therefore form a stable complex. These observations indicated that compound (3) is active against the urease, whereas lichexanthone (2) showed no activity. It is reported that the carbonyl oxygen play an important role in urease inhibition<sup>[24]</sup>.

## CONCLUSION

In conclusion, the present study demonstrated the urease inhibition and the antiglycation activity of methyl orsellinate. This finding will contribute to the new modelation and optimization of chemical drugs however further studies are needed for the purpose. Phytochemical studies on *Cordia platythyrsa* yielded to a new xanthone derivative isolated for the first time on *Cordia* genus. It is for the first time to isolate xanthone skeleton on *Cordia* genus.

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