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Dihydronaphtalenone and Chromone from *Cassia petersiana* Bolle and the Antisalmonellal Activity of its Crude Extract

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Abstract: Phytochemical investigation of the leaves of *C. petersiana* afforded two new compounds (4 α-Acetyl-3,7-dihydroxy-3,6-dimethyldihydronaphtalenone and 5-Hydroxy-7-acetonyl-2-methylenechromone), in addition to common terpenes as sitosterol, stigmasterol glucoside. The crude extract and the new compounds were tested for antisalmonellal activity. The structures of the compounds were determined from comprehensive NMR studies, including ^{1}H, ^{13}C, DEPT, COSY, HMQC, HMBC, MS, IR and comparison with literature data.

Key words: *Cassia petersiana*, caesalpiniaceae, naphtalenone, antisalmonellal activity

INTRODUCTION

Cassia is the major genus of the family Caesalpiniaceae and possesses about 600 species distributed worldwide (Viega *et al*., 2004). This genus constitutes a possible lead for the search of Aromatic compounds (Rao *et al*., 1999; Ingkaninan *et al*., 2000; Moriyama *et al*., 2003; Li *et al*., 2001), meanwhile terpenes and alkaloids could also be found (Msonti, 1984; Ingkaninan *et al*., 2000). Significant biological activities have been reported from member of this genus (Moriyama *et al*., 2003; Villasenor *et al*., 2002). *Cassia petersiana* Bolle is a tree generally distributed in equatorial countries from Sierra Leone to D. R. Congo. This plants species is commonly growing on sandy soils up to 12 m high and at altitude of up to 1050 m above sea level. It has pinnate leaves and yellow flowers. The compressed, hairy pods are eaten either raw or cooked as gruel. The roots of the plant are used as a treatment for coughs, colds, syphilis and stomachache. It is also used as an anthelmentic. The roots are mixed with those of *Fagara nitens* (Rutaceae) and *Stegmanotaenia araliacea* (Umbellifereae) and burnt to charcoal, which is pulverized and rubbed on incisions cut in the ankles and between the forefinger against snake bite. In Southern Africa, the leaf is used as a febrifuge and as a cure for skin diseases (Msonti, 1984). Previous phytochemical investigation on this plant species led to the

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isolation of diterpenes (Msonti, 1984) and flavonoids (Coetzee *et al*., 1999). As part of our contribution to the phytochemical and chemotaxonomic survey of the genus *Cassia* and in a continuation of our search for therapeutic agents from natural sources with potential for the treatment of typhoid and paratyphoid fevers (Gatsing *et al*., 2003, 2006), we carried out the investigation of the CH_2Cl_2-MeOH (1:1) extract of the leaves of *C. petersiana*, a species from Cameroon. We report herein the isolation and structure elucidation of two new compounds from this natural source. The extract and the pure compounds were assessed for their antisalmonellal activity. The compounds were characterised from comprehensive NMR interpretation including 1D and 2D.

MATERIALS AND METHODS

General

^1H, ^{13}C NMR and 2D spectra were recorded on JEOL 500 MHZ, Lambda spectrometer with TMS as an internal standard. EIMS spectra were recorded on TSQ-70-Triple Stage Quadruple mass spectrometer (70 ev). The IR spectra ($CHCl_3$) were recorded on a Perkin-Elmer FT-IR-spectrometer.

Plant Material

The leaves of *Cassia petersiana* Bolle were collected in Bafia, Centre province of Cameroon in July 2003. Plant material was identified by Dr. Onana, National Herbarium, Yaoundé, Cameroon, where a voucher specimen (N° 6494/SFR/Cam) was deposited.

Test Bacteria and Culture Media

The test microorganisms, *Salmonella typhi*, *Salmonella paratyphi* A and *Salmonella paratyphi* B, were obtained from the Medical Bacteriology Laboratory of the Pasteur Centre, Yaounde, Cameroon. The culture media used namely Salmonella-Shigella agar (SS agar) and Selenite Broth, were supplied by International Diagnostics Group PLC, Topley House, 52 Wash Lane, Bury, Lancashire BL96AU, UK.

Extraction and Isolation

The air-dried and pulverized leaves of *C. petersiana* (800 g) were extracted by maceration with CH_2Cl_2-MeOH (1:1), (11 L, 72 h) and evaporated under reduced pressure to give 80 g of crude extract. Part of this extract, 30 g was fractionated on silica gel column chromatography (CC) eluted successively with *n*-hexane-EtOAc and EtOAc-MeOH in a step gradient by using different ratios. Three fractions A, B and C were recorded. Fraction A was purified on a Sephadex LH-20 column (*n*-hexane-CH_2Cl_2-MeOH, 7:4:1) and preparative TLC to give 4α-Acetyl-3,7-dihydroxy-3,6-dimethyldihydro-naphtalenone (1, 11 mg). Fraction B was purified on a silica gel column (*n*-hexane-EtOAc 9:1) and Sephadex LH-20 to give 5-Hydroxy-7-acetonyl-2-methylenechromone (2, 6 mg). Sitosterol and stigmasterol-3-O-β-D-glucoside were identified from cochromatography of fraction C with authentic sample available from our laboratory.

4α-Acetyl-3,7-dihydroxy-3,6-dimethyldihydronaphtalenone (1): Yellow powder, mp 117.1°C (uncorrected), IR 3370, 3100, 1710, 1290, 980; ^1H NMR ($CDCl_3$, 500 MHZ), (Table 1), ^{13}C NMR ($CDCl_3$, 125 MHZ), (Table 1) EIMS, m/z 248 (10), 247 (30) 233 (56), 231 (40), 206 (50), 190 (100), HREIMS 248.2753 [calcd. for $C_{14}H_{16}O_4$, 248.2764].

5-Hydroxy-7-acetonyl-2-hydroxymethylenechromone (2): Yellow powder, mp more than 300°C, IR 3300, 1740, 1631, 926. ^1H NMR ($CDCl_3$, 500 MHZ), (Table 1), ^{13}C NMR ($CDCl_3$, 125 MHZ), (Table 1) EIMS, m/z 248 (15), 220 (60), 217 (35), 212 (100), HREIMS 248.2431 [calcd. for $C_{13}H_{12}O_5$, 248.2428].

Table 1: ^{1}H and ^{13}C NMR data of compound 1 and 2 in $CDCl_3$, at 500 and 125 MHZ

Compound 1			Compound 2		
Position	^{13}C	^{1}H, δ, m, J (Hz)	Position	^{13}C	^{1}H, δ, m, J (Hz)
1	203.1		2	165.0	-
2	48.4	3.15, dd, 17.0, 1.0			
		2.60, dd, 17.0, 1.0	3	110.1	6.11, s
3	69.5		4	182.0	-
4	62.3	4.20, s	4a	114.0	-
4a	140.5		5	164.3	-
5	111.6	6.31, s	6	117.2	6.21, d, 2.4
6	105.0		7	143.2	-
7	166.2		8	104.0	6.35, d, 2.4
8	110.1	7.40, s	8a	160.6	-
8a	109.1		1'	52.6	3.80, s
9	206.6		2'	208.1	-
10	32.0	2.35, s	3'	30.5	2.31, s
11	25.4	1.40, s	1''	60.8	4.51, s
12	37.0	2.40, s	5-OH	-	12.44, s

Antimicrobial Assay

The antibacterial activity was determined using both agar diffusion and broth dilution techniques as previously described (Cheesbrough, 1991; Gatsing *et al*., 2006).

Agar diffusion susceptibility testing was done using the method of wells. On each plate containing Salmonella-Shigella agar (SS agar) medium already inoculated with the test organism (100 µL of the bacteria suspension in Selenite broth, at the concentration of 5.10^5 cfu mL^{-1}) wells (of 6 mm diameter) were bored using a cork borer. The bottom of each well was sealed with a drop of molten agar. The compounds and the extract were dissolved in dimethylsulfoxide (DMSO). The wells were filled with 150 µL of the solution (of known concentration) of various compounds and extract to be tested. Chloramphenicol (Sigma) was used as the standard drug. The extract, compounds and chloramphenicol were tested at the concentration of 50, 0.5 and 0.1 mg mL^{-1}, respectively. The petridishes were left at room temperature for 45 min to allow the compounds and extract to diffuse from the wells into the medium. They were then incubated at 37°C for 24 h, after which the zones of no growth were noted and their diameters recorded as the zones of inhibition.

For the broth dilution susceptibility testing, the solution (maximum concentration) of the extract (that induced a zone of inhibition) was prepared in DMSO, serially (2-fold) diluted and 0.5 mL of each dilution was introduced into a test tube containing 4.4 mL of Selenite broth; then 0.1 mL of bacteria suspension (5.10^5 cfu mL^{-1}) was added and the mixture was homogenised. The total volume of the mixture was 5 mL, with the extract concentrations in the tube ranging from 48 to 0.75 mg mL^{-1} and those of chloramphenicol ranging from 40 to 0.625 µg mL^{-1}. After 24 h of incubation at 37°C, the Minimum Inhibitory Concentration (MIC) was reported as the lowest concentration of antimicrobial that prevented visible growth. The Minimum Bactericidal Concentration (MBC) was determined by subculturing the last tube to show visible growth and all the tubes in which there was no growth on already prepared plates containing SS agar medium. The plates were then incubated at 37°C for 24 h and the lowest concentration showing no growth was taken as the MBC.

RESULTS AND DISCUSSION

The air-dried and pulverized leaves of *C. petersiana* Bolle were extracted with CH_2Cl_2/MeOH (1/1), to give an organic extract. Part of this extract was fractionated by silica gel column chromatography (CC) eluted successively with *n*-hexane-EtOAc and EtOAc-MeOH in a step gradient by using different ratios. Three fractions A, B and C were recorded and purified to give two new compounds, 1 and 2 (Fig. 1).

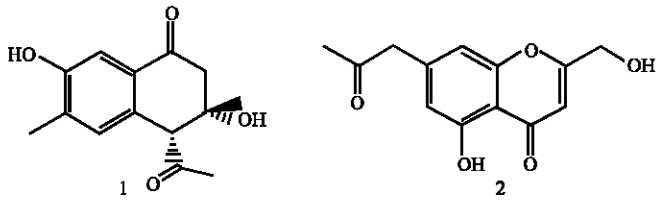

Fig. 1: Structures of the isolated compounds

Compound 1, was isolated as a yellow powder, mp 117.1°C (uncorrected). The IR spectrum showed bands at 3370 (hydroxyl group), 1710 (carbonyl), 1290 (hydroxyl bond) and 980 (substituted aromatic ring) cm^{-1}; the cross formula was deduced to be $C_{14}H_{16}O_4$ on the basis of a molecular ion peak at m/z 248.2753 on HRMS. On EIMS a fragment was observed at m/z 247 $[M-H]^+$, suggesting the structure to possess hydroxyl group. Further fragments could also be observed at, m/z 233 $[M-CH_3]^+$, 231 $[M-OH]^+$, 206 $[M-Ac]^+$, 190 $[M-OAc]^+$. The ^1H NMR and the ^{13}C NMR data are listed in Table 1. The structure was established from the following observations on the spectra. (I) From the ^1H NMR we could observed three singlets each integrating for three protons attributed to the methyl's H-10, H-11 and H-12. (ii) From the ^{13}C the structure was suggested to possess two carbonyls (C-1 and C-9) and two carbons link to hydroxyl group (C-3 and C-7). (iii) H-5 and H-8 were placed *para* to each other, as they were both singlet. (iv) The positions of H-5 and H-8 were distinguished from a correlation on HMBC between H-8 (δ 7.40) and the carbonyl at (δ 203.1, C-1). (v) Me-12 was placed at C-6 based on the HMBC where H-5 (δ 6.31) was correlated to C-12 (37.0). (iv) The acetyl moiety was deduced from an intense correlation of H-10 to C-9 on HMBC. Used of HMQC help to assign carbon multiplicities. The stereochemistry was deduced from comparison of our data to those of a closely related compound (Ingkaninan *et al*., 2000). Based on our data here described and compared to those of similar compound (Ingkaninan *et al*., 2000), structure 1 was attributed to the molecule which is a new derivative trivially named petersone A.

Compound 2 was isolated as a yellow powder, which did not melt before 300°C. The HREIMS spectrum showed a molecular ion peak at m/z 248.2431 $[M]^+$, compatible with the molecular formula $C_{13}H_{12}O_5$. The chromone nature was attributed to the structure from a peak at m/z 220 (M-CO) on EIMS, characteristic for γ-pyrone system (Wagner *et al*., 1978). Fragments could also be observed at m/z 247 $[M-OH]^+$, 220 $[M-CO]^+$, 217 $[M-OCH_3]^+$ and 202 $[217-CH_3]^+$. The IR spectrum showed absorption bands at 3300 (OH), 1740 (C = O), 1631 (dienone) and 926 (substituted aromatic ring) cm^{-1}. The ^1H and ^{13}C NMR data are listed in Table 1. The structure was established from deep studies of the spectra, thus the following items could be made: (I) A 2,5,7-trisubstituted chromone skeleton was deduced from the ^1H spectra. (ii) The location of the hydroxyl group at C-5 was determined from the downfield signal at δ 12.44, hydrogen bond with the ketone at C-4. From the ^{13}C NMR it was evident that there are two carbonyls (C-4, C-2') and four carbon connected to oxygen atom (C-2, C-5, C-8a and C-1). Our data were very close and even identical to those previously reported by Ingkaninan *et al*. (2000); the difference came to be the 5-OH. From comparison of our data to those of related compounds previously reported (Ingkaninan *et al*., 2000; Wagner *et al*., 1978), structure 2 was attributed to the molecule characterised as 7-Acetonyl-5-hydroxy-2-hydroxymethylenchromone which is a new chromone derivative trivially named petersone B.

The crude CH_2Cl_2/MeOH (1:1) leaf extract of *Cassia petersiana* showed antibacterial activity against all the three bacteria species used and the diameter of inhibition were 14-16 mm against *S. typhi*, 15-18 mm against *S. paratyphi* A and 17-18 mm against *S. paratyphi* B. Two compounds, namely 4α-Acetyl-3,7-dihydroxy-3,6-dimethyldihydronaphtal-enone and 5-Hydroxy-7-acetonyl-2-hydroxymethylenechromone, were isolated from the above crude extract and were tested for their

Table 2: Diameters of inhibition of *S. typhi*, *S. paratyphi* A and *S. paratyphi* B by the CH_2Cl_2/MeOH (1:1) extract and compounds isolated from the leaves of *Cassia petersiana*

Extract/compound	Concentration (mg mL^{-1})	Bacteria and diameters of zones of inhibition (mm)		
		S. typhi	*S. paratyphi* A	*S. paratyphi* B
CH_2Cl_2/MeOH (1:1) extract	50	14-16	15-18	17-18
1	0.5	NA	NA	NA
2	0.5	NA	NA	NA
Chloramphenicol (standard)	0.1	27-28	25-27	26-28

Key: NA: Not Active, 1: 4α-Acetyl-3,7-dihydroxy-3,6-dimethyldihydronaphtalenone, 2: 5-Hydroxy-7-acetonyl-2-hydroxymethylenechromone

Table 3: MIC and MBC values of the CH_2Cl_2/MeOH (1:1) extract of the leaves of Cassia *petersiana*

Extract/compound	Parameters	Bacteria strains		
		S. typhi	*S. paratyphi* A	*S. paratyphi* B
CH_2Cl_2/MeOH (1:1) extract	MIC (mg mL^{-1})	1.5	1.5	1.5
	MBC (mg mL^{-1})	12.0	12.0	12.0
	MBC/MIC	8	8	8
Chloramphenicol (standard)	MIC (μg mL^{-1})	2.0	2.0	2.0
	MBC (μg mL^{-1})	16.0	16.0	16.0
	MBC/MIC	8	8	8

Key: MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration

antisalmonellal activities. The results obtained showed that none of these compounds was active against the bacteria strains used. Chloramphenicol, used as the standard, showed the diameters of 27-28, 25-27 and 26-28 mm against *S. typhi*, *S. paratyphi A* and *S. paratyphi* B, respectively (Table 2).

The crude extract, which showed antibacterial activity against all the three bacteria species used, was further studied using broth dilution technique and the following results were obtained: the MIC and MBC values were 1.5 and 12.0 mg mL^{-1}, respectively, against all the three bacteria tested. For chloramphenicol the MIC and MBC values were 2.0 and 16.0 μg mL^{-1}, respectively, against the same bacteria species (Table 3).

Antimicrobial substances are considered as bactericidal agents when the ratio MBC/MIC ≤ 4 and bacteriostatic agents when the ratio MBC/MIC > 4 (Carbonnelle *et al*., 1987). For the crude extract, the ratio MBC/MIC > 4, suggesting that it may be classified as bacteriostatic agent.

CONCLUSION

Chromones are regularly isolated from various species of the genus *Cassia* (Lee *et al*., 2001; Lu *et al*., 2001; Biswas and Malik, 1986; Kuo *et al*., 2002). Petersone B is a new derivative here reported for the first time. Its presence in our results is of great interest for the investigation of members of this genus where it constitutes a possible chemotaxonomic marker.

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