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Bancoensone, a new antioxidant caffeoyl derivative from *Cussonia bancoensis*

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ABSTRACT

A new caffeoyl derivative *p*-caffeoylphenol has been isolated from the *n*-butanol-soluble fraction of stem bark of *Cussonia bancoensis* (Araliaceae) together with the known liriodendrin. The structure elucidation of the new compound was carried out with the help of modern spectroscopic techniques (^{1}H and ^{13}C NMR, COSY ^{1}H-^{1}H, HMBC and HMQC) and mass spectrometry. This compound was further screened for its potential antioxidant properties by using three different assays: DPPH (1,1-Diphenyl-2-picrylhydrazyl) radical assay model, non-enzymatic superoxide anion generating system NADH/PMS (β-nicotineadeninenucleotide/phenazinemethosulphate) and towards inhibition of xanthine oxidase (XO) enzyme, and revealed potent antioxidant activities.

Key words: *Cussonia bancoensis*, Araliaceae, *p*-caffeoylphenol, antioxidant.

RESUME

Un dérivé nouveau du groupement caffeoyl (*p*-caffeoylphenol) auquel nous avons attribué le nom trivial bancoensone a été isolé de l'extrait au *n*-butanol des écorces du tronc de *Cussonia bancoensis* (Araliaceae) avec la liriodendrin. La structure de ce nouveau composé a été élucidée à l'aide des techniques spectroscopiques modernes (RMN ^{1}H et ^{13}C, COSY ^{1}H-^{1}H, HMBC et HMQC) et la spectrométrie de masse. Ses propriétés anti-oxydantes ont été évaluées par trois méthodes différentes et les résultats obtenus indiquent que ce composé est un anti-oxydant potentiel. Ces résultats de bioassay couplés à ceux précédemment obtenus d'autres composés de cette même plante, en l'occurrence des saponines triterpéniques pourraient expliquer ses diverses utilisations en médecine traditionnelle.

Mots clés : *Cussonia bancoensis*, Araliaceae, *p*-caffeoylphenol, anti-oxydant

INTRODUCTION

Cussonia bancoensis Aurev. & Pellegr; (Araliaceae) is a medium size tree of 5-7 m in height. It is mostly found in dense humid forest and widespread from Ivory Coast to Nigeria. It is used in Nigerian folk medicine for the treatment of dizziness and infertility in women [1]. Previous work on this species has revealed the presence of flavonoids [2] and triterpene saponins [3-5]. The human organism permanently produces reactive oxygen species (ROS) which cause many cellular disorders because of their high reactivity with proteins, DNA and membrane fatty acids resulting to different inflammatory and cardiovascular diseases [6, 7], rheumatoid arthritis, neurodegenerative diseases and the aging process [8, 9]. The physiological production of ROS is regulated by enzymes and low molecular weight compounds with antioxidant properties.

In the course of our continuing search for secondary metabolites of biological importance from Cameroonian medicinal plants, we investigated the *n*-butanol extract of stem bark of *C. bancoensis*. In the present paper we report the isolation and structural elucidation of a new caffeoyl derrivative, *p*-caffeoylphenol (**1**) and the known liriodendrin (**2**) isolated from this plant. The antioxidant activities of these compounds have also been tested through three different assays: DPPH radical assay model, non-enzymatic superoxide anion generating system and towards inhibition of xanthine oxidase (XO) enzyme.

MATERIAL AND METHODS

General

Melting points were determined on a Yanaco micro-melting point apparatus. IR spectra were recorded on the JASCO A-302 spectrophotometer and the UV spectrum recorded in methanol on Hitachi U-3200 spectrophotometer. The ^{1}H NMR spectra (δ ppm, *J* in Hz) were recorded in C_5D_5N on Bruker AM-400 spectrometer (400 MHz) while ^{13}C NMR spectra were recorded in the same solvents on an AM-400 instrument at 100 MHz with tetramethylsilane (TMS) as internal standard; EIMS and FABMS were taken on the mass spectrometer MAT 311A and mass spectrometer JMS HX-110, respectively. Isolations were carried out using Column Chromatography (CC) [silica gel 60 (Merck, 70-200 µm) and the purity of the samples was checked on TLC (Si gel, precoated plates, Merck, PF_{254}, 20x20, 0.25 mm). Solvents used for chromatography were distilled prior to use.

Plant Material

The stem bark of *C. bancoensis* was collected in Bafou village (Menoua Division, Western province of

Cameroon) in April 2000. The plant material was identified by Dr Zapfack Louis of the Department of Plant Biology of the University of Yaoundé I, Cameroon. Specimens documenting the collection have been deposited at the Cameroon National Herbarium (ref. N° 16896/SRF/CAM).

Extraction and Isolation

The dried and pulverized stem bark of *C. bancoensis* (2 kg) was extracted with MeOH at room temperature for three days, and the extract concentrated to dryness under reduced pressure. Part of the residue obtained (95 g) was suspended in water and successively extracted with ethyl acetate and *n*-butanol to obtain after evaporation of solvents 38 g and 32 g respectively. Part of the butanol-soluble extract (27 g) was subjected to silica gel column chromatography and eluted with CH_2Cl_2 containing increasing amounts of MeOH. Four main fractions were obtained, A (2.8 g), B (2.0 g), C (3.0 g) and D (4.0 g). Fractions A, B and D have been previously investigated and mainly afforded triterpenoid saponins [4, 5]. Fraction C was purified over silica gel column eluted with the mixture CH_2Cl_2/MeOH/H_2O (65:35:10, lower phase) to give compounds **1** (60 mg) and **2** (80 mg).

Bancoensone (1): Pale yellow crystal in MeOH/CH_2Cl_2 (MP. 185-187°C), $[\alpha]_D^{31}$ + 47.6° (c 0.042, MeOH); ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD): see Table 1. IR (KBr) cm^{-1}: 3250 (phenolic OH), 1660 (conjugate C=O), 1600, 1520 and 1250. UV λmax (MeOH) nm (log ε): 322.0 (3.806), 295.4 (3.998), 256.6 (4.028), 217.8 (4.340), 205.6 (4.376), 194.5 (4.911) and 189.8 (4.911). EI-MS m/z: 272 (M^+), 180, 163, 136.

Liriodendrin (2): Pale yellow needles in MeOH/CH_2Cl_2, MP. 264-266 °C, $[\alpha]_D^{31}$ -15.9° (c 0.32, MeOH). All its spectral data are in conformity with reported literature values [10-12].

DPPH free radical scavenging activity

The reaction mixture containing 5 µL of test sample was dissolved in DMSO and 95 µL of DPPH in ethanol. Different concentrations of test sample were taken in reaction mixture, while the concentration of DPPH was maintained at 300 µM. These reaction mixtures were transferred in 96-well microtitre plates (Molecular Devices, spectramax 340 USA) and incubated at 37 °C for 30 min. The absorbance was measured at 515 nm [13].

Superoxide scavenging activity

The reaction mixture contained 280 µM β-nicotinamide adenine dinucleotide reduced form (NADH), 80 µM nitroble tetrazolium (NBT), 8 µM phenazine methosulphate (PMS) and the sample ranging from 10-1000 µM in 200µL of 0.1 M phosphate buffer (pH 7.5). The NBT, NADH and PMS were prepared in the same buffer. Tested compounds were dissolved in DMSO. The reaction was performed in 96-well microtitre plates (Molecular Devices, spectramax 340 USA) at room temperature and absorbance was measured at 560 nm [14].

Xanthine oxidase inhibition activity

The XO inhibition activity was assayed in phosphate buffer (0.1 M, pH 7.5) 200 µL, XO (0.003 unit/well), 20 µL and test sample in 10 µL DMSO was diluted to the desired range of concentrations, were mixed in 96-well microplate and pre-incubated for 10 minutes at room temperature. The reaction was initiated by adding 20 µL of 0.1 mM of xanthine as substrate. The uric acid formation was measured spectrophotometrically at 295 nm by using Molecular Devices, spectramax 340 USA [13].

3-*t*-Butyl-4-hydroxyanisole (BHA) and propyl gallate (PG) were used as positive controls. All the chemicals used were of analytical grade (Sigma, USA).

RESULTS

The maceration of the stem bark of *C. bancoensis* in MeOH yielded after evaporation of solvent a crude extract which was suspended in water and successively partitionated between EtOAc and *n*-BuOH. Evaporation of the *n*-BuOH layer yielded a crude mixture which was fractionated over silica gel column to afford different fractions that further purification yielded compound **1** and **2**. Their structures were elucidated mainly by 1D and 2D NMR spectroscopy (COSY, HMBC and HMQC) and by EIMS and HREIMS as well as by comparison with literature data.

The HREIMS (High Resolution Electron Impact Mass Spectrometry) spectrum of **1** showed the molecular ion peak at m/z 272.2493 corresponding to the molecular formula of $C_{15}H_{12}O_5$ (calc. 272.2521) indicating ten degrees of unsaturation in the molecule. Significant ion peaks at m/z 180, 163, and 136 were due respectively to the caffeoyl moiety and the losses of hydroxyl group (-OH) and carboxyl group (-CO_2) from the parent caffeoyl group [15].

The UV (Ultra Violet) spectrum exhibited absorptions at 322, 218 and 206 nm, which indicated the presence of an aromatic conjugated system. The IR (Infrared) spectrum of **1** exhibited strong absorption bands at 3320 (OH), 1660 (C=C-CO), 1605 (aromatic C=C), 1295, 1250, 1210 and 1190 cm^{-1}.

Analysis of the ^{1}H-NMR spectrum of **1** (Table 1) indicated the presence of two olefinic protons at δ_H 6.20 (H-α) and 7.52 (H-β) with a mutual coupling (J=15.6 Hz) characteristic of a *trans* configuration, and three aromatic protons at δ_H 7.03 (H-2, d, J=2.0 Hz), 6.93 (H-6, dd, J =2.0, 8.2 Hz) ascribed to the caffeoyl group. Four extra aromatic protons of a *para*-disubstituted benzene ring were also depicted at δ_H 6.78, 6.80, 7.41, 7.44 ppm.

Table 1: ^{1}H and ^{13}C-NMR data of bancoensone **1** (400 MHz ^{1}H, 100 MHz ^{13}C in CD_3OD)

Position	^{1}H (mult, *J* in Hz)	^{13}C shift	^{1}H –^{13}C HMBC(^{2}J and ^{3}J)
C=O	-	170.2	-
α	6.20 (d, *J*= 15.6 Hz)	115.0	170.2 (C=O), 127.8 (C-1)
β	7.52 (d, *J* =15.6 Hz)	147.0	115.0 (C-2), 122.8 (C-6)
1	-	127.8	-
2	7.03 (d, *J* = 2.0 Hz)	115.0	149.4 (C-4), 122.8 (C-6)
3	-	146.4	-
4	-	149.4	-
5	6.77 (d, *J* = 8.2 Hz)	116.5	146.7 (C-3), 127.8 (C-1)
6	6.93 (dd, *J* = 2.0, 8.2 Hz)	122.8	146.7 (C-3), 149.4 (C-4)
1'	-	146.0	-
2', 6'	7.44 (dd, *J* = 2.2, 8.2 Hz)	117.7, 116.5	151.5 (C-4)
3', 5'	6.78 (dd, *J* = 2.4, 8.2 Hz)	115.7, 115.5	146.0 (C-1')
4'	-	151.5	-

Table 2: *In vitro* free radical % scavenging activity and XO inhibitory activities of bancoensone **1**.

Compound	IC_{50} (μM)*		
	DPPH Scavenging activity	Superoxide scavenging activity	XO enzyme inhibition Activity
1	50 ± 0.031	274 ± 2	207 ± 4
PG**	30 ± 0.27	106 ± 2	628 ± 5
BHA**	44 ± 2.00	96 ± 2	591 ± 8

* IC_{50} values are the mean ± *standard error of the mean* (SEM) of three assays.
** Standard compounds (BHA = 3-*t*-Butyl-4-hydroxyanisole, PG = propyl gallate)

The ^{13}C-NMR spectrum of **1** (Table 1) showed resonances at δ_c 170.2 (ester carbonyl), 127.8, 146.4, 149.4, 146.0 and 151.5 corresponding respectively to the five substituted carbon atoms of two aromatic rings C-1, C-3, C-4, C-1' and C-4'. HMQC (Heteronuclear Multiple Quantum Coherence) data were used to assign carbon resonances to their attached protons. The cross peak correlation observed in the HMBC (Heteronuclear Multiple Bond Correlation) spectrum between the signal of the carbonyl at δ_c 170.2 and the two olefinic protons at δ_H 6.20 and 7.52 were important in determining the structure of **1** (Figure 1). Further HMBC cross peak correlations were also observed between the proton signal at δ_H 7.52 (H-β) and the carbons at δ_c 115.0 (C-2) and 122.8 (C-6), between the proton at δ_H 6.93 (H-6) and C-2 (115.0). Other correlations were observed between H-2 (δ_H 7.03) and C-4 (δ_c 149.4) and C-6 (δ_c 122.8), between H-3' and H-5'(δ_H 6.78) and C-1'(δ_c 146.0) and between H-2' and H-6' (δ_H 7.44) and C-4' (δ_c 151.5).

The structure of **1** was further supported by a COSY ^{1}H-^{1}H experiment which revealed cross peak correlations between Hα (δ_H 6.20) and Hβ (δ_H 7.52), between H-2 (δ_H 7.03) and H-6 (δ_H 6.93), between H-2'(δ_H 7.44) and H-3'(δ_H 6.78) and between H-5'(δ_H 6.78) and H-6'(δ_H 7.44). On the basis of the above spectroscopic studies it was concluded that compound **1** is a *p*-caffeoylphenol derivative. This previously unreported compound has been assigned the trivial name bancoensone.

Compound **2** was identified as liriodendrin by comparison of its physical and spectral data with those reported in the literature [10-12].

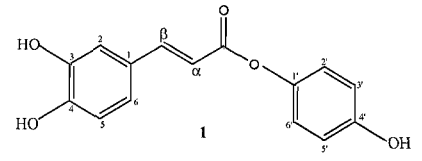
Compound **1** has shown significant antioxidant activities towards DPPH radical scavenging, superoxide scavenging and xanthine oxidase inhibition assays and table 2 presents the IC_{50} values compared to standards. Compound **2** shows no significant activity in the three assays.

DISCUSSION

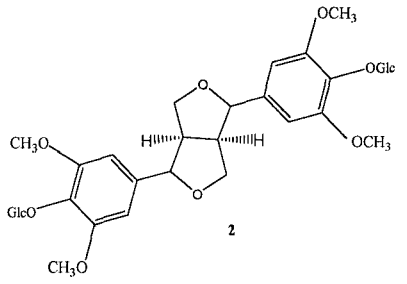
The isolation of triterpene saponins from the other polar fractions of *C. bancoensis* collected in Cameroon [4, 5] is in agreement with previous phytochemical work on the same plant from Ghana [3-5]. Although some flavonoids have been reported from the Ghanaian species [2], no flavonoid has been mentioned in the present study. It is important to notice that the isolation and characterisation of

bancoensone (**1**), a caffeoyl derivative during the present work is the first report of a caffeoyl derivative from *Cussonia* genus and so far from Araliaceae family.

It has been demonstrated that antioxidants may act by two mechanisms of action either by scavenging free radicals or removing the initiator of ROS (reactive oxygen species). Compound **1** has then been checked for its both mechanisms and has been found to have both actions to prevent attack of free radicals. In all three assays compound **1** showed significant activity. These results are in conformity with previous works relative to the structure-activity relationship of antioxidant properties of caffeic acid analogues [8, 18]. Previously, cytotoxic, analgesic and anti-inflammatory triterpenoids have also been reported from this plant [5, 16, 17].



1



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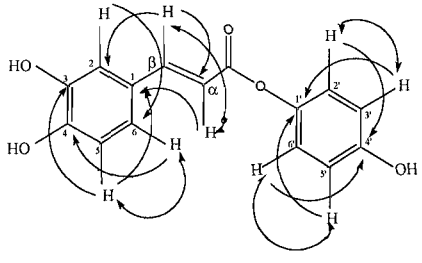

Figure 1: Important HMBC and ^{1}H-^{1}H COSY interactions in 1

All these results then show some relationship between the chemical composition of this plant, the bioactivities of the different isolates and its uses in folk medicine.

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