Acridone Alkaloids and Coumarins from the Stem Bark of *Citropsis articulata* (Rutaceae)

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A new acridone alkaloid, citropsine A (**1**), and thirteen known compounds (**2** – **14**) were isolated from the MeOH extract of the stem bark of *Citropsis articulata*. Structures of all compounds were determined by detailed analyses of their 1D and 2D NMR spectra, mass spectrometric data and by comparison with previously known analogs.

Key words: Citropsis articulata, Rutaceae, Acridone Alkaloids, Coumarins

Introduction

As a part of our continuing search for bioactive molecules from Cameroonian rainforest medicinal plants, *Citropsis articulata* Harms (Rutaceae), a cherry orange, was studied [1]. This plant is used in folk medicine in Africa for the treatment of tumors, sexual impotence and erectile dysfunction [2]. To the best of our knowledge no phytochemical investigation has been reported on this genus. In this report, we describe the isolation and structural elucidation of one new acridone alkaloid (**1**) and thirteen known compounds (**2** – **14**) from the stem bark of *C. articulata*.

Results and Discussion

The air-dried stem bark of *C. articulata* was powdered and extracted with MeOH. The crude extract was separated by repeated column chromatography and preparative TLC (PTLC) to afford one new acridone alkaloid (**1**), and thirteen known compounds identified as 1,3-dimethoxy-*N*-methylacridone (**2**), arborinine (**3**), evoxanthine (**4**), tecleanone (**5**), (*R*)-byakangelicin (**6**), imperatorine (**7**), scopoletin (**8**), lupeol, stigmasterol, sitosterol, two fatty acids (hexacosanoic acid, decanoic acid) and stigmasterol-3-*O*-β-D-glucopyranoside [3 – 8] (Fig. 1).

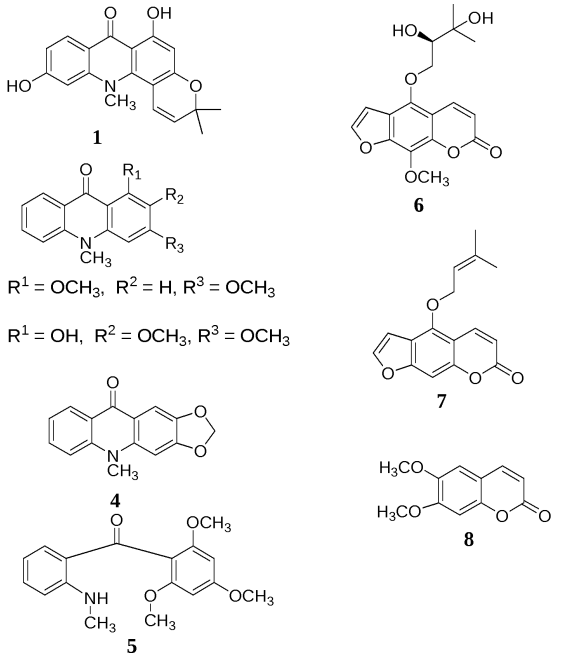

Fig. 1. Structures of compounds isolated from *C. articulata*.

Citropsine A (**1**), m. p. 261 – 263 °C, was obtained as an orange powder. The molecular composition was found to be $C_{19}H_{17}O_4N$ by HR-ESI-MS ($[M+Na]^+$ at *m/z* = 346.3409, calcd. 346.3511). The UV spectrum showed a highly conjugated system with absorption bands characteristic of the 1-hydroxy-9-acridone skeleton in the molecule [9]. A positive reaction

with ferric chloride ($FeCl_3$), an infrared (IR) band at 3300 cm^{-1}, and ^1H NMR peaks at δ = 14.48 (s) and at δ = 10.48 (s) which disappeared with deuterium oxide (D_2O), indicated the presence of phenolic hydroxyl groups. The lower-field signal at δ = 14.48 together with an IR band at 1650 cm^{-1} was indicative of a chelated C-1 hydroxyl group in a 9-acridone alkaloid [9].

The ^1H NMR spectrum of **1** also showed signals due to ABX-type aromatic protons at δ = 7.68 (1H, d, J = 7.5 Hz), 7.27 (1H, d, J = 1.3 Hz) and 7.24 (1H, dd, J = 7.5, 1.3 Hz), an isolated aromatic proton at δ = 6.14 (1H, s), an N-CH_3 unit at δ = 3.75 (3H, s) and a dimethylchromene ring at δ = 6.70 (1H, d, J = 10.0 Hz), 5.68 (1H, d, J = 10.0 Hz), and 1.47 (6H, s, CH_3-4′ and CH_3-5′). The lower-field signal at δ = 7.68 of the ABX-type aromatic protons was deshielded by a carbonyl group, which revealed the presence of the protons H-8, H-7, and H-5 of the acridone skeleton [10]. The presence of the dimethylchromene moiety was further confirmed by the ^{13}C NMR spectrum with characteristic signals at δ = 27.2 (C-4′ and C-5′), 77.1 (C-3′), 120.9 (C-1′) and 124.5 (C-2′), and the EI-MS which showed the base peak at m/z = 308 $[M–15]^+$ [10].

The positions of the dimethylchromene moiety and the free hydroxyl group were determined by 2D NMR techniques (HMBC and NOESY) (Figs. 2, 3). In the HMBC spectrum, correlations between the aromatic singlet signal at δ = 6.14 and the carbon signals at C-3 (δ = 161.1), C-9a (δ = 106.7), C-4 (δ = 102.4) and C-1 (δ = 164.2) suggested this aromatic proton to be located at C-2. The HMBC spectrum also showed correlations between the protons H-1′ (δ = 6.70) and the carbons C-4a (δ = 147.7), C-3 (δ = 161.1) and C-3′ (δ = 77.1) while the NOESY spectrum showed interaction between this proton (H-1′) and the N-CH_3 group (δ = 3.75). These findings allowed the assignment of an angular orientation of the dimethylchromene ring [10]. On the other hand, the HMBC spectrum showed correlations between the proton H-8 (δ = 7.68) and the carbon signals at C-6 (δ = 149.4) and C-10a (δ = 137.1), while the NOESY spectrum showed a successive interactions between the proton H-7 (δ = 7.24) and the free hydroxyl group (δ = 10.48), between the free hydroxyl group and the proton H-5 (δ = 7.27), and finally between H-5 and the N-CH_3 group (δ = 3.75). These correlations indicated that the second hydroxyl group was located at the C-6 position. From the above spectroscopic data, the structure of citropsine A (**1**) was assigned as 6-hydroxynoracronycine.

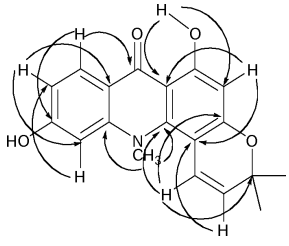

Fig. 2. Selected HMBC correlations for compound **1**.

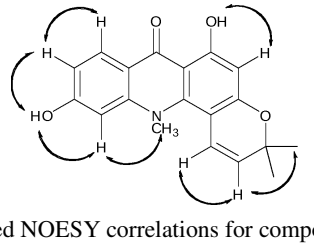

Fig. 3. Selected NOESY correlations for compound **1**.

Experimental Section

General

Infrared spectra were recorded on a JASCO FT/IR-410 spectrophotometer. UV spectra were determined on a Spectronic Unicam spectrophotometer. HR-ESI-MS were recorded on an APEX III (Bruker Daltonik) 7 Tesla (ESI-FT-ICR-MS) instrument. EI-MS spectra were recorded on a Finnigan MAT 95 spectrometer (70 eV) with perfluorokerosene as reference substance for HR-EI-MS. The ^1H- and ^{13}C NMR spectra were recorded at 500 MHz and 125 MHz, respectively, on a Bruker AMX 500 NMR spectrometer. Methyl, methylene and methine carbons were distinguished by DEPT experiments. Homonuclear ^1H connectivities were determined by using the COSY experiment. One-bond ^1H-^{13}C connectivities were determined with HMQC gradient pulse factor selection. Two- and three-bond ^1H-^{13}C connectivities were determined by HMBC experiments. Chemical shifts were reported in δ (ppm) using TMS as internal standard, and coupling constants J were measured in Hz. Column chromatography was carried out on silica gel (70 – 230 mesh, Merck) and flash silica gel (230 – 400 mesh, Merck). TLC was performed on Merck precoated silica gel 60 (F_{254}, Merck), and spots were visualized by using ceric sulfate spray reagent. All reagents used were of analytical grade.

Collection and identification

The stem bark of *C. articulata* was collected at the Batouri locality, Eastern Cameroon in April 2006 and identified by Mr. Nana Victor of the National Herbarium, Yaounde, Cameroon, where the voucher specimen (Ref. 20009SRF/CAM) has been deposited.

Extraction and isolation

The air-dried and powdered stem bark of *C. articulata* (5.0 kg) was extracted at r. t. with MeOH (3 × 7 L, 24 h each). After removing the solvents by evaporation under reduced pressure, 40.3 g of crude extract was obtained and chromatographed over silica gel 60 (70 – 230 mesh, 6.5 × 50 cm^2), using hexane, CH_2Cl_2, ethyl acetate, and MeOH in increasing polarity order. A total of 115 sub-fractions (*ca.* 150 mL each) were collected and combined on the basis of TLC analysis leading to three main fractions A – C.

Fraction A (11.2 g, combined from sub-fractions 1 – 35 (150 – 5250 mL)), was chromatographed over a silica gel column (230 – 400 mesh, 3.5 × 50.0 cm^2) with a hexane-ethyl acetate gradient. A total of 25 fractions of *ca.* 100 mL each was collected and combined on the basis of TLC. Fractions 1 – 10 (100 – 1000 mL) and 10 – 15 (100 – 500 mL) were further chromatographed over silica gel (230 – 400 mesh, 2.5 × 30.0 cm^2), with a mixture of hexane-ethyl acetate (9 : 1) to yield lupeol (13.4 mg), a mixture of 2 sterols (stigmasterol + sitosterol) (23.1 mg) and a mixture of fatty acid (20.5 mg).

Fraction B (15.2 g, composed of sub-fractions 36 – 75 (100 – 4900 mL)) was chromatographed over silica gel (230 – 400 mesh, 3.5 × 50.0 cm^2) with a hexane-ethyl acetate gradient. A total of 20 fractions of *ca.* 100 mL each was collected and combined on the basis of TLC. Fractions 1 – 15 (100 – 1500 mL) were further chromatographed over silica gel (230 – 400 mesh, 2.5 × 30.0 cm^2) with a mixture of hexane-ethyl acetate (3 : 2) to yield imperatorine (**7**) (3.5 mg), scopoletin (**8**) (10.2 mg), and evoxanthine (**4**) (7.0 mg).

Fraction C (10.6 g, composed of sub-fractions 76 – 115 (100 – 3900 mL)) was chromatographed over silica gel (230 – 400 mesh, 3.5 × 50.0 cm^2) with a hexane-ethyl acetate and ethyl acetate-MeOH gradient. A total of 55 fractions of *ca.* 100 mL each was collected and combined on the basis of TLC. Fractions 5 – 30 (100 – 2500 mL) were further chromatographed over silica gel (230 – 400 mesh, 2.5 × 30.0 cm^2) with a mixture of hexane-ethyl acetate (2 : 1) to yield arborinine (**3**) (12.4 mg), (*R*)-byakangelicin (**6**) (8.3 mg), 1,3-dimethoxy-*N*-methylacridone (7.2 mg) (**2**), tecleanone (**5**) (11.2 mg), citropsine A (**1**) (18.0 mg), and stigmasterol-β-D-glucopyranoside (14.0 mg).

*Citropsine A (**1**)*

Orange powder (DMSO); m. p. 261 – 263 °C. – UV (MeOH): λ_{max} (log ε) = 255 (6.05), 279 (5.62), 291 (5.60), 295 (5.67), 300 (5.58), 346 (4.58) nm. – IR (KBr): ν_{max} = 3900, 3130, 2850, 2832, 1750, 1640, 1600, 1602, 1390, 1012, 750 cm^{-1}. – ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ_H = 1.47 (s, 6H, 4′-CH_3 and 5′-CH_3), 3.75 (s, 3H, N-CH_3), 5.68 (d, *J* = 10.0 Hz, 1H, 2′-H), 6.14 (s, 1H, 2-H), 6.70 (d, *J* = 10.0 Hz, 1H, 1′-H), 7.24 (dd, *J* = 7.5, 1.3 Hz, 1H, 7-H), 7.27 (d, *J* = 1.3 Hz, 1H, 5-H), 7.68 (d, *J* = 7.5 Hz, 1H, 8-H), 10.48 (s, 1H, 6-OH), 14.48 (s, 1H, 1-OH). – ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ_C = 27.2 (C-4′), 27.4 (C-5′), 48.9 (N-CH_3), 77.1 (C-3′), 97.4 (C-2), 102.4 (C-4), 106.7 (C-9a), 115.7 (C-8), 120.5 (C-5), 120.9 (C-1′ and C-8a), 124.0 (C-7), 124.5 (C-2′), 137.1 (C-10a), 147.0 (C-4a), 149.4 (C-6), 161.1 (C-3), 164.1 (C-1), 181.8 (C-9). – MS ((+)-ESI): *m/z* = 346 $[M+Na]^+$. – HRMS ((+)-ESI): *m/z* = 346.3409 (calcd. 346.3511 for $C_{19}H_{17}O_4NNa$, $[M+Na]^+$).

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