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Biologically active bisbenzylisoquinoline alkaloids from the root bark of *Epinetrum villosum*

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Abstract

Methanol and water extracts of the root of *Epinetrum villosum* (Exell) Troupin (Menispermaceae) were found to exhibit antimicrobial and antiplasmodial activities. Investigation of the active methanol fraction led to the isolation of four bisbenzylisoquinoline alkaloids, i.e., cycleanine, cycleanine N-oxide, isochondodendrine and cocsoline. Structures were established by spectroscopic methods. Cocsoline displayed antibacterial and antifungal activities (MIC values of 1000–15.62 and 31.25 μg/ml, respectively). Isochondodendrine was found to have the most potent antiplasmodial activity (IC_{50} = 0.10 μg/ml), whereas the IC_{50} on HCT-116 human colon carcinoma cells was 17.5 μg/ml (selectivity index 175). Cycleanine acted against HIV-2 (EC_{50} = 1.83 μg/ml) but was at least 10-fold less active against HIV-1. Cycleanine N-oxide showed no activity towards all tested microorganisms.

Keywords: *Epinetrum villosum*; Menispermaceae; Bisbenzylisoquinoline alkaloids; Antibacterial activity; Antifungal activity; Antiplasmodial activity; Anti-HIV activity

1. Introduction

Epinetrum villosum (Exell) Troupin (Menispermaceae) is a twining liana, growing in secondary forests in the coastal areas of the Democratic Republic of Congo (DRC) and Angola (Troupin, 1951). The crushed leaves are used as a wound dressing for fast healing. The plant is also used for the treatment of pains and mental strain (Parvez et al., 1994). In the Lomela area, the root decoction of this plant is taken orally for the treatment of fever, malaria, diarrhea and dysentery (Longanga et al., 2000). Bisbenzylisoquinoline alkaloids (BBIQ) have been characterized in the bark of the plant (Parvez et al., 1994) but not in the root, and no biological studies have been carried out so far. As part of our search for bioactive natural alkaloids, we found that the methanol and water extracts from *Epinetrum villosum* root showed antimicrobial (Longanga et al., 1999) and antiplasmodial activities.

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These results prompted us to isolate and characterize the alkaloids, expected to be present, and to investigate their biological potential.

2. Materials and methods

2.1. Plant material

Root bark of *Epinetrum villosum* (Exell) Troupin was collected in the area of Lomela (DRC) in March 1996. The plant was first identified by the Institut National d'Etudes et de Recherches Agronomiques (INERA) of the University of Kinshasa. This identification was further confirmed by Prof. Dr. E. Robbrecht of the National Botanic Garden of Belgium (Meise) where a voucher specimen has been deposited under No. BR-SP 841595. Roots were air-dried.

2.2. General experimental procedures

All solvents used were of HPLC grade and only pro analysi reagents (if commercially available) were used. Silica gel (230–400 mesh), analytical silica gel 60 F_{254} (20 × 20) (0.25 mm) plates and preparative silica gel 60 F_{254} (20 × 20) (0.5 mm) plates were purchased from Merck (Darmstadt, Germany). For the detection of the alkaloids, Dragendorff and iodoplatinate spray reagents were used. 1D NMR (^{1}H, ^{13}C, DEPT-135 and DEPT-90) and 2D NMR spectra, including ^{1}H–^{1}H DQF-COSY, HSQC and HMBC, were recorded in $CDC1_3$ (99.8%D) on a Bruker DRX-400 instrument operating at 400 MHz for ^{1}H. Chemical shifts are quoted in Δ units relative to TMS (0 ppm). Mass spectra were recorded on an Autospec-oa-Tof mass spectrometer (Micromass) employing electrospray ionization (ESI).

2.3. Preparation of the extracts

Air-dried root material (10 g) was ground to a fine powder and percolated after maceration for 24 h, with solvents having an increasing polarity (diethyl ether, 70% methanol, H_2O; 200 ml each). The resulting extracts were evaporated to dryness in vacuum at 40 °C, yielding three crude extracts: F_1 (0.8 g), F_2 (2.1 g) and F_3 (1.7 g), respectively. In order to reproduce the traditional method of extraction, another 10 g of dried plant material was boiled in distilled water (200 ml) for 15 min. After cooling, the mixture was filtered and evaporated to dryness in vacuum at 40 °C, yielding the fourth crude extract (F_4; 3.3 g).

In the preliminary phytochemical screening of crude extracts from *Epinetrum villosum,* the diethyl ether, MeOH and H_2O extracts were found to contain alkaloids (Longanga et al., 2000). TLC profiles of the methanol extract (F_2) and the total aqueous extract (F_4) in ethyl acetate/isopropanol/25% NH_4OH (80:15:5, v/v/v, top layer), were found to be comparable after development of the plates and visualization of the alkaloid spots with Dragendorff and iodoplatinate reagents. This suggests the presence of the same alkaloids in both extracts.

2.4. Isolation and purification of bisbenzylisoquinoline alkaloids

Powdered and dry roots (500 g) of *Epinetrum villosum* were extracted exhaustively with 70% MeOH (1.5 l) by percolation after 24 h of maceration. After filtration, the MeOH extract was evaporated to dryness under reduced pressure to yield a syrupy residue (95 g). The MeOH residue (95 g) was moistened with aqueous 25% NH_4OH (50 ml), and extracted with $CHCl_3$ (6 × 150 ml). The $CHCl_3$ extracts were combined and evaporated to dryness under reduced pressure at 40 °C. The resulting residue was dissolved in 3% aqueous HCl (300 ml). After filtration, the solution was extracted with petroleum ether (3 × 100 ml), and then the aqueous acid phase was alkalinized with aqueous 25% NH_4OH before extraction with $CHCl_3$ (6 × 150 ml). The combined $CHC1_3$ extracts were washed with distilled H_2O, dried over anhydrous Na_2SO_4, and evaporated to dryness, yielding 8.2 g of total alkaloid fraction (1.6%). This total alkaloid fraction (8.2 g) was dissolved in $CHCl_3$ (10 ml) and chromatographed over a silica gel (230–400 mesh ASTM) column (3.5 × 80 cm) packed in $CHCl_3$. The elution started with $CHCl_3$, and the polarity was increased gradually by addition of MeOH. Fractions were collected and monitored by TLC (silica gel 60 F_{254}, layer thickness 0.20 mm) in ethyl acetate/isopropanol/25% NH_4OH (80:15:5, v/v/v, top layer). Alkaloids were detected with Dragendorff and iodoplatinate reagents. Fractions showing a positive reaction were pooled in sub-fractions 1–5 according to their chromatographic pattern. Final separation was achieved by means of repeated preparative TLC (silica gel 60 F_{254} Merck, thickness layer 0.50 mm) using as mobile phase: acetone/toluene/methanol/25% NH_4OH (40:40:8:3, v/v/v/v, top layer). This resulted in the isolation of compound **1** (R_f: 0.71, 740 mg) from sub-fraction 1, compounds **2** (R_f: 0.71, 270 mg) and **3** (R_f: 0.50, 8.8 mg) from sub-fraction 2, compounds **4** (R_f: 0.71, 112 mg) and **5** (R_f: 0.45, 14 mg) from sub-fraction 3, compound **6** (R_f: 0.40, 16 mg) from sub-fraction 4 and compound **7** (R_f: 0.21, 11 mg) from sub-fraction 5. Compound **5** was not identified. Compounds **1**, **2** and **4** were identified by spectroscopic methods and comparison with published data as cycleanine (denoted compound **A**) (Kashiwaba et al., 1998; Kanyinda et al., 1997), compound **3** as cocsoline (**D**) (Ohiri et al., 1983), compound **6** as isochondodendrine (**C**) (Dwuma et al., 1975; Debray et al., 1966; Singh et al., 1981; Kanyinda et al., 1997; Mambu et al., 2000), and **7** as cycleanine N-oxide (**B**) (Kashiwaba et al., 1998; Ohiri et al., 1983). Since ^{13}C NMR data for cocsoline have not been reported yet, the assignment of ^{13}C signals was based on correlations observed in the 2D-NMR spectra (Table 1) and based on the ^{13}C assignments of (+)-2-*N*-methyltelobine, a structurally related compound (Likhitwitayawuid et al., 1993).