QUINOLINE ALKALOIDS AND FRIEDELANE-TYPE TRITERPENES ISOLATED FROM LEAVES AND WOOD OF *Esenbeckia alata* KUNT (Rutaceae)

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This work describes the phytochemical exploration of the ethanol extract from leaves and wood of *Esenbeckia alata*, leading to the isolation and identification of quinoline alkaloids 4-methoxy-3-(3’-methyl-but-2'-enyl)-N-methyl-quinolin-2(1H)-one, N-methylflindersine, dictamine, kokusaginine, γ-fagarine, flindersiamine, as well as the fridelane-type triterpenes, frideline, fridelanol and its acetate derivative. Identification of these compounds was based on full analyses of spectroscopic data (1H, 13C, ID, 2D, IR, MS) and comparison with data reported in literature. Compound 4-methoxy-3-(3’-methyl-but-2'-enyl)-N-methyl-quinolin-2(1H)-one is reported for the first time for the genus *Esenbeckia*.

Keywords: *Esenbeckia alata*; quinoline alkaloids.

INTRODUCTION

Rutaceae family is gathered in 140 genera including ca 1600 species, which are distributed in temperate and tropical zones on both hemispheres involving biological forms such as trees, shrubs and herbs. One of the most abundant taxa for Rutaceae family is the *Esenbeckia* genus involving a number of 30 species of the family. There are several reports to this taxon indicating their uses in traditional medicine and its biological activity. In Mexico, leaves and roots of *E. yaxhoob* are used by local people for the treatment of gastrointestinal diseases, epilepsy, headaches and as anti-inflammatory agent. Metabolites isolated from *E. letocarpa* exhibited antioxidant activity against worm Pectinophora gossypella. In addition, a geranylcoumarin has been isolated from *E. febrifuga*, which significantly inhibited the growth of tropical parasite *Leishmania major*. Phytochemical studies on this genus has allowed the isolation of several secondary metabolites such as flavonoids from *E. yaxhoob*, *E. grandiflora* subsp. brevipetiolata, *E. almawillia* and *E. berlandieri* ssp. Acapulcensis; terpenoids from *E. conspecta*, *E. ovata*, *E. stephani*, *E. yaxhokob*, *E. almawillia*, and *E. nesiotica*; limonoids from *E. litoralis*, *E. flava* and *E. berlandieri*; cinnamic acid derivatives from *E. almawillia*; alkaloids from *E. pentaphylla*, *E. grandiflora*, *E. litoralis*, *E. almawillia*, and *E. belizensis*; coumarins from *E. grandiflora*, *E. litoralis*, *E. febrifuga*, and *E. pentaphylla*. From the former group of metabolites, quinoline alkaloids are considered as taxonomic markers for *Esenbeckia* genus and they have been identified in various species of the genus such as *E. belizensis*, *E. pentaphylla*, *E. flava*, *E. grandiflora* and *E. litoralis*. *E. alata* is a medicinal shrub whose ecology is diverse being identified in different colombian areas. On the Atlantic coast of Colombia, its aerial parts are used as febrifuge and insecticide. This fact has prompted that many studies had been particularly focused on this plant. In a previous work, phytochemical examination of the ethanol extract of the bark from *E. alata* led to the isolation of four metabolites which were identified as 5-hydroxy-2-methylchromanone, the lignan (-)-episesamin, the amide pellitonin and sitosterol. In that study it was evaluated the antimicrobial activity of the obtained lignan, showing significant results against the microorganisms *Bacillus subtilis*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. On the other hand, from the ethanol extract of the leaves of this species were isolated furanocoumarins, pyranocoumarins, lignans and furuquinoline alkaloids. The present work aims to contribute to the chemotaxonomic board of genus through chemical study of the ethanol extract of both leaves and wood of *E. alata*, consisting the first phytochemical report for the ethanol extract from wood of *E. alata*, herein described therefore is the isolation and identification of quinoline alkaloids and friedelan-type triterpenes.

EXPERIMENTAL

General procedures

Silica gel 60 (0.063-0.200 mm, 70-230 mesh ASTM) (Merck) was used for column chromatography (CC) and silica gel 60 F254 chromatoplates Merck, (20 x 20 and 0.30 mm thickness) for thin layer chromatography (TLC). Preparative TLC was held on plate coated with Merck silica gel 60G F254 (1.0 and 2.0 mm thickness). TLC was revealed in UV lamp (254 nm), iodine vapor and ceric ammonium sulfate solution in sulfuric acid with subsequent heating at 100 °C. Vacuum column chromatography (VCC) was developed with silica gel 60G, Merck: 1H NMR (400 MHz) and 13C NMR (100 MHz) spectra were recorded on a Bruker Avance 400 using TMS as internal standard, in deuterated chloroform (CDCl3) as solvent. High Resolution Mass spectra (HRMS) were determined on a Shimadzu IT-ToF spectrometer (with an ESI source and in the positive ion mode), and electron impact
mass spectra (IEMS) were recorded in a Jeol JMS-SX102A spectrometer. Infrared spectra (IR) were taken on film in a KBr window, in a Perkin Elmer 500 series FTIR Panagon 1000. Optical rotations were measured on a polarographic-E Schmidt-Hänisch polarimeter in CHCl₃ at 20 °C. Acetylation was carried out by conventional procedure, by refluxing with pyridine and acetic anhydride for 2 h.

Plant material

Plant sample corresponding to the wood and leaves of *Esenbeckia alata* was collected in Los Montes de María (9°39’58.77”N and 75°20’3.45”O), Department of Bolívar, Colombia, on October 2005. Specimen was identified by the botanist E. Carboné and a voucher was deposited in the Herbarium of the University of Magdalena with the collection number 001(UTMC).

Extraction and fractionation

Isolation of metabolites from wood

Wood of *E. alata* was air dried at room temperature for 8 days. A total of 1200 g were extracted by percolation using 96% EtOH (15 L) for 10 days. Resulting ethanol extract (called EaM) was concentrated under reduced pressure to obtain 16.3 g crude extract. EaM was subjected to CC (80 x 5 cm) on silica gel using toluene/PrOAc 9:1 as elution system yielding 114.8 g crude extract. A sample of this extract (65 g) was subjected to successive washing with methanol (4 x 3 mL) to obtain a liquid subfraction (EaM-L) (647 mg) and a solid (EaM-S) (421 mg). EaM-L and EaM-S subfractions were separately purified by CC (40 x 3.5 and 50 x 2.5 cm, respectively) on silica gel eluting with petroleum ether/EtOAc 7:3 (0.6 L) affording one (1), combined into 16 fractions by their CCD profiles (EaM1-EaM16). EaM6 fraction (1088 mg) was subjected to successive washing with methanol (4 x 3 mL) to obtain a liquid subfraction (EaM6L) (421 mg) and a solid (EaM6S) (421 mg). EaM6L and EaM6S subfractions were separately purified by CC (70 x 7 cm) on silica gel using hexane/acetone 9:1 (2.5 L) by increasing polarity as eluent, collecting 25 subfractions (EaH1-EaH25). EaH5 fraction (3220 mg) was subjected to successive washing with methanol (4 x 3 mL) on silica gel using toluene/PrOAc 8:2 (3 L) as elution system yielding 5 (15 mg).

Isolation of metabolites from leaves

Dried and powdered leaves (2875 g) of the specimen were extracted by percolation using 96% EtOH (25 L) for 10 days. Resulting ethanol extract (called EaH) was concentrated under reduced pressure to obtain 113.8 g crude extract. A sample of this extract (65 g) was fractionated by VCC (70 x 7 cm) on silica gel using hexane/acetone (18 L) as mobile phase by increasing polarity producing 23 fractions (EaH1-EaH23). EaH5 fraction (3220 mg) was subjected to VCC (28 x 5 cm) on silica gel using dichloromethane/acetone (2.5 L) as eluent by increasing polarity to collect ten subfractions (EaH5.1-EaH5.10). EaH5.3 subfraction (987 mg) was purified by VCC (20 x 2.5 cm) eluting with dichloromethane/acetone 9:1 (2 L) to obtain 2 (33 mg) and 4 (3 mg). EaM9 fraction (1682 mg) was purified by CC (60 x 3.5 cm) on silica gel using toluene/PrOAc 8:2 (3 L) as eluting system yielding 5 (15 mg). One (1): needles, mp 199-200 °C; HRESIMS [M+H]+ m/z 258.1472, calcd for C₁₅H₁₈NO₅ 258.1494; IR (KBr, cm⁻¹) 3272, 1731, 1633, 1467, 756; ¹H RMN (CDCl₃, 400 MHz): δ 7.5 (dd, J = 1.5, 7.2, 8.6 Hz, H-7), 7.8 (dd, J = 1.5, 7.2 Hz, H-5), 7.3 (d, J = 8.6 Hz, H-8), 7.22-7.28 (m, H-6), 5.2 (m, H-2'), 3.4 (d, J = 6.8 Hz, H-1'), 1.8 (s, H-4'), 1.6 (s, H-5'), 3.7 (s, N-Me), 3.9 (s, O-Me); ¹³C RMN (CDCl₃,100 MHz) δ 17.9 (C-4'), 24.3 (C-1'), 25.7 (C-5'), 29.7 (N-Me), 61.7 (O-Me), 114 (C-8), 117.8 (C-4a), 121.5 (C-2'), 121.8 (C-6), 122.6 (C-3), 123.4 (C-5), 130 (C-7), 132.5 (C-3'), 139 (C-8a), 160.1 (C-4), 163.9 (C-2).

RESULTS AND DISCUSSION

Ethanol extract of leaves and wood of *E. alata* was fractionated and purified by conventional chromatographic methods in order to isolate eight compounds corresponding to quinoline-type alkaloid 4-methoxy-3-(3'-methyl-but-2'-enyl)-N-methyl-quinolin-2(1H)-one (1), pyranoquinolone alkaloid N-methylflindersine (2), furoquinoline alkaloids dictamine (3), kokusaginine (4), γ-fagarine (5), flindersiamine (6), as well as the friedelane-type triterpenes friedeline (7) and fridelanol (8). Metabolites were identified by spectroscopic techniques ¹H and ¹³C NMR and by comparison with published data in the literature (Figure 1).

![Figure 1. Structures of secondary metabolites isolated from *E. alata*](image-url)
shift, multiplicity and coupling constants indicated the presence of a disubstituted aromatic ring. There were also signals at δH 5.2 (1H, m), 3.4 (2H, d, J = 6.8Hz), 1.89 (s, 3H) and 1.69 (s, 3H) corresponding to an isoprenyl moiety. 20 Same spectrum exhibited two singlets at δH 3.7 (3H) and δH 3.9 (3H), whose assignment was defined to be N-methyl and O-methyl groups. 6 13C NMR spectrum and DEPT experiments showed a signal at δC 163.9 for a quaternary carbon, corresponding to a carbonyl group, 21 and it confirms the presence of N-methyl and O-methyl groups whose carbon signals were observed at δC 29.7 and δC 61.7, respectively. 22 Above-mentioned spectral data allowed identifying signals of quaternary and methylene carbons for the isoprenyl moiety at δC 121.4 and δC 132.5, respectively. 22 HMBC and HMOC spectra confirmed the location of the isoprenyl and carbonyl groups. Information provided by H and 13C NMR spectra led to determine the presence of a quinoline alkaloid, named as 4-methoxy-3-(3′-methyl-but-2′-enyl)-N-methyl-quinolin-2(1H)-one. 23-25 Compound 1 is reported for the first time for the genus Ensenbeckia. Similarly, full-analyses of H and 13C NMR (one- and two-dimensional) spectra of compound 2 having a condensed formula C19H18NO5 [HRESIMS analysis ([M+H]+ m/z 242.1171, calcd for C19H17NO5, 242.1181) allowed identifying it as another pyranooquinoline-type alkaloid, N-methylflindersine (2). This metabolite was previously isolated from species belonging to the genus Ensenbeckia. 12

Compounds 3-6 have molecular formulas assigned by HRESIMS analyses as C11H16NO5 ([M+H]+ m/z 200.0707, calcd for C11H16NO5, 200.0712), C20H20NO7 ([M+H]+ m/z 260.0912, calcd for C20H20NO7, 260.0923), C21H18NO8 ([M+H]+ m/z 230.0808, calcd for C21H18NO8, 230.0817), and C21H18NO9 ([M+H]+ m/z 274.0715, calcd for C21H18NO9, 274.0715), respectively. 13C NMR spectra of those compounds showed similar profiles including signals at δC ca 7.5 (d, J = 2.5 Hz, 1H) and δC ca 6.9 (d, J = 2.5 Hz, 1H), for vinyl protons at furan ring. 14 13C NMR spectra of 3-6 revealed signals for oxygenated quaternary carbons at δC ca 165-150 range, and nitrogen atom-bonded carbon at δC ca 145. Differences among them were stabilized through NMR spectra according to the presence of a methoxy groups signals, whose location was defined by HMBC experiments. According to the above-mentioned information obtained from H and 13C NMR (one- and two-dimensional), on comparing spectroscopic data with the above-mentioned information obtained from C NMR and MS spectroscopic data and optical rotation values, 12,13 it confirms the presence of a compound 1-4 methoxylated 13C NMR and MS spectroscopic data and optical rotation values, thereby identifying them as friedelane-type triterpenes, friedelane (7) ([α]D -75.2, c 0.1, CHCl3; EIMS M+ m/z 426), friedelanol (8) ([α]D -16.2, c 0.1, CHCl3; EIMS M+ m/z 428) and its acetate derivative (friedelanyl acetate) ([α]D -12.5, c 0.1, CHCl3; EIMS M+ m/z 470) (9), whose analyses of both NMR and optical rotation data, in comparison with reported data in literature, 20 allowed establishing the configuration showed in Figure 1 for 7-9. Compounds 7 and 8 have been previously identified in E. littoralis. 14

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