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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(1*H*)-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 (^{1}H, ^{13}C, 1D, 2D, IR, MS) and comparison with data reported in literature. Compound 4-methoxy-3-(3'-methyl-but-2'-enyl)-*N*-methyl-quinolin-2(1*H*)-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[1] involving biological forms such as trees, shrubs and herbs.[2] One of the most abundant taxa for Rutaceae family is the *Esenbeckia* genus involving a number of 30 species of the family.[3] 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 antidiuretic agent.[4,5] Metabolites isolated from *E. leiocarpa* exhibited antifeedant activity against worm *Pectinophora gossypiella*.[6] In addition, a geranylcoumarin has been isolated from *E. febrifuga*, which significantly inhibited the growth of tropical parasite *Leishmania major*.[7] Phytochemical studies on this genus has allowed the isolation of several secondary metabolites such as flavonoids from *E. yaxhoob*,[4] *E. grandiflora* subsp. *brevipetiolata*, *E. almawillia*, and *E. berlandieri* ssp. *Acapulcensis*;[8] terpenoids from *E. conspecta*, *E. ovata*, *E. stephani*, *E. yaaxhokob*, *E. almawillia*, and *E. nesiotica*;[9] limonoids from *E. litoralis*, *E. flava* and *E. berlandieri;*[10] cinnamic acid derivatives from *E. almawillia*;[11] alkaloids from *E. pentaphylla*,[12] *E. grandiflora*, *E. litoralis*,[13] *E. almawillia*,[11,14] and *E. belizencis*;[15] coumarins from *E. grandiflora*, *E. litoralis*,[13] *E. febrífuga*,[7] and *E. pentaphylla*.[12] 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. belizencis*,[15] *E. pentaphylla*,[12] *E. flava*,[10] *E. grandiflora* and *E. litoralis.*[13]

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.[16] 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*.[16] On the other hand, from the ethanol extract of the leaves of this species were isolated furanocoumarins, pyranocoumarins, lignans and furoquinoline alkaloids.[17] The present work aims to contribute to the chemotaxonomic hoard 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 friedelane-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 F_{254} 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 F_{254} (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 ºC. Vacuum column chromatography (VCC) was developed with silica gel 60 G, Merck. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker Avance 400 using TMS as internal standard, in deuterated chloroform ($CDCl_3$) 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

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mass spectra (IEMS) were recorded in a Jeol JMS-SX102A spectrometer. Infrared spectra (IR) were taken on film on a KBr window, in a Perkin Elmer 500 series FTIR Panagon 1000. Optical rotations were measured on a polartronic-E Schmidt-Hänsch polarimeter in $CHCl_3$ 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. Carbonó 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 sample 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/*i*PrOAc 9:1 to 1:1 (14 L) as elution system, collecting 138 fractions (100 mL each one), 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) (647 mg) and a solid (EaM6S) (421 mg). EaM6L and EaM6S 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 **1** (30 mg), and **3** (8 mg), respectively. EaM7 fraction (793 mg) was subjected to CC (40 x 3.5 cm) on silica gel eluting with toluene/EtOAc 7:3 (0.8 L) obtaining **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/EtOAc 8:2 (3 L) as eluting 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 **6** (102 mg). A similar procedure was carried out on EaH6 fraction (2330 mg), which was subjected to VCC (28 x 5 cm) on silica gel with hexane/acetone (4L) as mobile phase obtaining EaH6.4 subfraction (410 mg), which was then purified by VCC (20 x 1.5 cm) eluted with dichloromethane/acetone 9:1 (0.7 L) to yield **4** (76 mg). EaH10 fraction (1367 mg) was subjected to VCC (20 x 2.5 cm) on silica gel using hexane/EtOAc (2.5 L) by increasing polarity as eluent, collecting 25 subfractions (EaH10.1-EaH10.25). From EaH10.15 (92 mg) and EaH10.21 (146 mg) subfractions resulted into two solids, which were recrystallized from chloroform/methanol 2:8 (35 mL), affording **7** (76 mg) and **8** (130 mg), respectively. In order to increase the solubility in chloroform and thus facilitate spectra recording, **8** was acetylated to yield **9** (56 mg), which was recrystallized from chloroform-methanol 9:1.

4-methoxy-3-(3'-methyl-but-2'-enyl)-*N*-methyl-quinolin-2(1*H*)-one (**1**): Needles, mp 199-200 °C; HRESIMS $[M+H]^+$ *m/z* 258.1472, calcd for $C_{16}H_{20}NO_2$ 258.1494; IR (KBr, cm^{-1}) 3272, 1731, 1633, 1467, 756; ^1H RMN ($CDCl_3$, 400 MHz): δ 7.5 (ddd, *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* =6.8Hz, H-1'), 1.8 (s, H-4'), 1.6 (s, H-5'), 3.7 (s, *N*-Me), 3.9 (s, *O*-Me); ^{13}C RMN ($CDCl_3$,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 quinolone-type alkaloid 4-methoxy-3-(3'-methyl-but-2'-enyl)-*N*-methyl-quinolin-2(1*H*)-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 ^{13}C NMR and by comparison with published data in the literature (Figure 1).

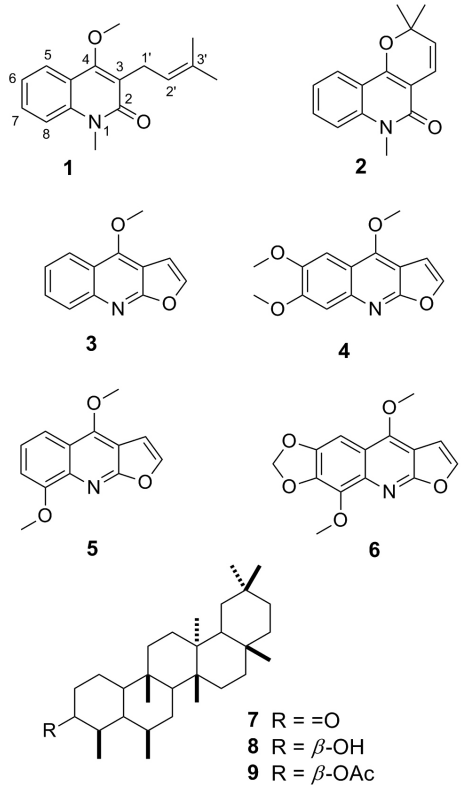

Figure 1. *Structures of secondary metabolites isolated from E. alata*

Compound **1** is a crystalline solid (needles, mp 199-200 °C, MeOH), possessing a molecular formula $C_{16}H_{19}NO_2$, as deduced from HRESIMS analysis ($[M+H]^+$ *m/z* 258.1472, calcd for $C_{16}H_{20}NO_2$, 258.1494), whose IR spectrum showed tension bands at 1633 and 1467 cm^{-1} characteristic of C=C stretching of aromatic ring, as well as flexion bands in 756 and 1363 cm^{-1} for the aromatic group =CH and $-CH_3$, respectively. Other bands were also identified at 1102 and 1730 cm^{-1} characteristic of C-O and C=O stretchings, respectively.[18] In ^1H NMR spectrum were observed signals of four aromatic hydrogens at δ_H 7.5 (1H, ddd, *J* = 1.5, 7.2, 8.6 Hz), δ_H 7.8 (1H, dd, *J* = 1.5, 7.2 Hz), δ_H 7.3 (1H, d, *J* = 8.6 Hz), and δ_H 7.22-7.28 (1H, m), whose chemical

shift, multiplicity and coupling constants indicated the presence of a disubstituted aromatic ring.[19] There were also signals at δ_H 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 δ_H 3.7 (3H) and δ_H 3.9 (3H), whose assignment was defined to be *N*-methyl and *O*-methyl groups.[6] ^{13}C NMR spectrum and DEPT experiments showed a signal at δ_C 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 δ_C 29.7 and δ_C 61.7, respectively.[21] Above-mentioned spectral data allowed identifing signals of quaternary and methylene carbons for the isoprenyl moiety at δ_C 121.4 and δ_C 132.5, respectively.[20] HMBC and HMQC spectra confirmed the location of the isoprenyl and carbonyl groups. Information provided by ^{1}H and ^{13}C 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(1*H*)-one.[19-21] Compound **1** is reported for the first time for the genus *Esenbeckia*. Similarly, full-analyses of ^{1}H and ^{13}C NMR (one- and two-dimensional) spectra of compound **2** having a condensed formula $C_{15}H_{15}NO_2$ [HRESIMS analysis ([M+H]$^+$ *m/z* 242,1171, calcd for $C_{15}H_{16}NO_2$, 242,1181) allowed identifying it as another pyranoquinolone-type alkaloid, *N*-methylflindersine (**2**). This metabolite was previously isolated from species belonging to the genus *Esenbeckia*.[12]

Compounds **3-6** have molecular formulas assigned by HRESIMS analyses as $C_{12}H_9NO_2$ ([M+H]$^+$ *m/z* 200.0707, calcd for $C_{12}H_{10}NO_2$, 200.0712), $C_{14}H_{13}NO_4$ ([M+H]$^+$ *m/z* 260.0912, calcd for $C_{14}H_{14}NO_4$, 260.0923), $C_{13}H_{11}NO_3$ ([M+H]$^+$ *m/z* 230.0808, calcd for $C_{13}H_{12}NO_3$, 230.0817), and $C_{14}H_{11}NO_5$ ([M+H]$^+$ *m/z* 274.0715, calcd for $C_{14}H_{12}NO_5$, 274.0715), respectively. ^{1}H NMR spectra of those compounds showed similar profiles including signals at δ_H *ca* 7.5 (d, $J \approx$ 2.5 Hz, 1H) and δ_H *ca* 6.9 (d, $J \approx$ 2.5 Hz, 1H), for vinyl protons at furan ring.[22] ^{13}C NMR spectra of **3**-**6** revealed signals for oxygenated quaternary carbons at δ_C *ca* 165-150 range, and nitrogen atom-bonded carbon at δ_C *ca* 145. Differences among them were stablished 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 ^{1}H and ^{13}C NMR (one- and two-dimensional), on comparing spectroscopic data with the literature,[23] compounds **3**-**6** were identified as furoquinoline alkaloids dictamine, kokusaginine, γ-fagarine, and flindersiamine, respectively, which had been previously isolated from Rutaceae specimens such as *Boronia pinnata, Dictamnus angustifolius,Teclea ouabanguiensis*, *Haplophyllum vulcanicum*, *Melicope lunu-ankenda*, *Acronychia laurifolia*, *Melicope ptelefolia*,[23] and particularly from genus Esenbeckia from the species *E. pentaphylla*,[12] *E. grandiflora*, *E. litoralis*,[13] *E. almawillia*,[15] *E. belizencis*,[16] and *E. febrífuga*.[24]

Compounds **7**, **8** and **9** were characterized by analyses of ^{1}H and ^{13}C NMR and MS spectroscopic data and optical rotation values, thereby identifying them as friedelane-type triterpenes, friedeline (**7**) ($[\alpha]_D^{25}$ -75.2, *c* 0.1, $CHCl_3$; EIMS M$^+$ *m/z* 426), friedelanol ($[\alpha]_D^{25}$ 16.2, *c* 0.1, $CHCl_3$; EIMS M$^+$ *m/z* 428) (**8**), and its acetate derivative (friedelanyl acetate) ($[\alpha]_D^{25}$ -12.5, *c* 0.1, $CHCl_3$; EIMS M$^+$ *m/z* 470) (**9**), whose analyses of both NMR and optical rotation data, in comparison with reported data in literature,[14] allowed establishing the configuration showed in Figure 1 for **7**-**9**. Compounds **7** and **8** have been previously identified in *E. litoralis*.[14]

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