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Investigación

Sesqui- and Tri- Terpenoids from *Esenbeckia* species (Rutaceae)

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In memoriam to Dr. Jacobo Gómez-Lara

Abstract. Three *Esenbeckia* species (Rutaceae) were chemically analyzed by means of conventional chromatographic, spectroscopic and spectrometric techniques. From the aerial parts of *E. berlandieri* ssp *berlandieri* were obtained friedelin, β-sitosterol, and β-sitosteryl-β-D-glucoside. Limonin was characterized as the main constituent from the seeds of *Esenbeckia ovata*. From the aerial parts of *E. velutinosa* friedelin, caryophyllene β-oxide, lupeol, lupenone, cryptomeridiol and β-sitosterol were isolated and characterized.
Key Words: *Esenbeckia berlandieri* ssp. *berlandieri, E. ovata, E. velutinosa*, Rutaceae, sesquiterpenes, triterpenes, limonin.

Resumen. Fueron analizadas químicamente tres especies del género *Esenbeckia* (Rutaceae) por medio de técnicas cromatográficas, espectroscópicas y espectrométricas convencionales. De las partes aéreas de *E. berlandieri* ssp *berlandieri* se aisló fridelina, β-sitosterol y el β-D-glucósido de β-sitosterilo. La limonina fue caracterizada como el principal constituyente de las semillas de *E. ovata*. De las partes aéreas de *E. velutinosa* se aisló e identificó a la fridelina, β-óxido de cariofileno, lupeol, lupenona, criptomeridiol y β-sitosterol.
Palabras clave: *Esenbeckia berlandieri* ssp. *berlandieri*, *E. ovata*, *E. velutinosa*, Rutaceae, sesquiterpenos, triterpenos, limonina.

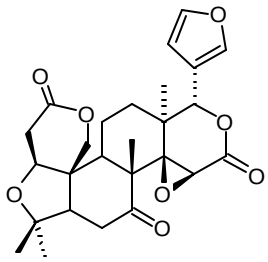
Introduction

Esenbeckia is an American genus of *ca*. 30 species, 17 of which are located mainly in the tropical regions of the states of Veracruz, Guerrero, Michoacán, Oaxaca and Chiapas in Mexico [1-2]. Previous chemical studies from the aerial parts of some species indicate that limonoids, acridone and furoquinoline alkaloids, and furanocoumarines are the main secondary metabolites [3-8]. In addition, phloroglucinols and polyprenols have been characterized from *E. nesiotica* [9], alkaloids from *E. almawilla* [10], indole alkaloids, triterpenes, lignanes and amides from the roots of *E. leiocarpa* [11-12], furanocoumarines and quinoline alkaloids from *E. glandiflora* [13], and terpenes and coumarins from *E. stephani* and *E. ovata* [14].

Results and Discussion

From the aerial parts of *E. berlandieri* ssp *berlandieri* were isolated and identified friedelin [15], β-sitosterol and β-sitosteryl-β-D-glucoside. From the seeds of *E. ovata* was isolated a white solid that exhibited positive reaction in the Ehrlich test for furanes [16], whose presence was according to the signals at δ 7.42 (2H, s, H-21, H-23) in the ^{1}H NMR spectrum, and was confirmed by the fragment at *m*/*z* 95 in the EIMS. The presence of an AB system centered at δ 4.92 (1H, J = 13 Hz) and δ 4.48 (1H, J = 13 Hz) in the ^{1}H NMR spectrum is characteristic for an oxygenated function at C-19 of polycyclic modified triterpenes, according to the molecular formula $C_{26}H_{30}O_8$ determined by EIMS. The presence of an α, β-epoxy-δ-lactone in the D-ring was established by the signals at δ 5.47 (H-17) y δ 4.04 (H-15), suggesting a limonoid-type substance. COSY experiments allowed the assignments of the signals at δ 2.68 (dd, J = 16.8, 2.1) and δ 2.98 (dd, J = 16.8, 3.9 Hz) for the hydrogens α to the carbonyl group in the A-ring, while those at δ 2.86 (t, J = 15) and δ 2.47 (dd, J = 15, 3.5 Hz) corresponded to the hydrogens at C(6). The methyl signals at δ 1.30, 1.18, 1.17 and 1.08 were assigned to CH_3-18, CH_3-25, CH_3-26 and CH_3-24, respectively, confirming the structure of limonin A (**1**) for this secondary metabolite [17]. Friedelin, β-sitosterol, lupeol [18], cryptomeridiol [19], lupenone and caryophyllene epoxide [20] were isolated from the aerial parts of *E. velutinosa* and identified by direct comparison with authentic samples.

These results along with those previously reported [3-14] indicate that *Esenbeckia* species biosynthesize different structural types of secondary metabolites. Additional chemical studies of other species are necessary in order to establish the chemotaxonomic significance of the isolated substances.



1 Limonin

Experimental

Esenbeckia berlandieri ssp *berlandieri* ex Hemsley was collected in the state of Veracruz. A voucher specimen is deposited at the National Herbarium, (MEXU, CHR-30). The organic extract was obtained by maceration with ethanol of the air-dried aerial plant material (1.9 kg). This extract was chromatographed at reduced pressure (VLC) [21] using *n*-hexane and mixtures of *n*-hexane-ethyl acetate as elution system. This procedure allowed to isolate friedelin (240 mg), β-sitosterol (100 mg), and β-sitosteryl-β-D-glucoside (390 mg). **Friedelin.** White crystals, mp 245-247°C [15]; IR ($CHCl_3$) ν_{max} 2943, 2867, 1703, 1455, 1389, 1361, 1192, 1139, 1110 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.72 (3H, s, CH_3-27), 0.85 (3H, d, CH_3-23), 0.87 (3H, s, CH_3-29), 0.95 (3H, s, CH_3-24), 1.0, 1.18 y 1.25 (9H, s, CH_3-25, CH_3-26, CH_3-28), 2.25 (1H, c, J = 7 Hz, H-4), 2.42 (1H, dd, J = 5 Hz, H-2), 2.38 (1H, dd, J = 5, 2 Hz, H-2); EMIE *m*/*z* (int. rel.): 426 [M^+] (29), 302 8169, 274 (22), 273 8379, 246 (25), 218 8299, 205 824), 191 830), 179 (32), 164 (36), 125 (59), 123 (60), 121 (40), 109 (77), 96 852), 95 (100), 81 (60), 69 (62), 55(93).

The seeds of *E. ovata* Brandegee (940 g; collected in the state of Veracruz, provided by Prof. C.H. Ramos, key: CHR 32) were extracted with *n*-hexane and then with $CHCl_3$ at room temp. affording 63.4 g of residue. The crude $CHCl_3$ was adsorbed onto silica gel and analyzed *via* vacuum liquid chromatography (VLC) [21], the cromatographic fractions were monitored using the Ehrlich´s test. Limonin (**1** [17], 123 mg) was isolated after purification by recristallization from EtOH-*i*PrOH. White crystals, mp: 232-233°C, orange with Ehrlich's test; IR ($CHCl_3$) ν_{max} 3064, 2958, 2876, 1753, 1284, 1028 cm^{-1}; NMR ^1H ($CDCl_3$, 300 MHz): δ1.30, 1.18, 1.78 and 1.08 (s, 12H), 2.23 (dd, J = 15.9 0, 3 Hz, 1H, H-5), 2.47 (dd, J = 14.7, 3.5 Hz, 1H, H-6a), 2.56(dd, J = 12.2, 3 Hz, 1H, H-9), 2.68 (dd, J = 16.7, 2.1 Hz, 1H, H-2a), 2.86 (t, J = 15.5 Hz, 1H, H-6b), 2.98 (dd, J = 16.8, 3.9 Hz, 1H, H-2b), 4.04 (s, 2H, H-1 and H-15), 4.47 (d, J = 13.2 Hz, 1H, H-19a), 4.77 (d, J = 13.2 Hz, 1H, H-19b), 5.47 (s, 1H, H-17), 6.34 (dd, J = 1.35, 0.9, 1H, H-22), 7.41 (s, 2H, H-21 and H-23). ^1H NMR (DMSO-d_6, 500 MHz): δ 1.18, 1.11, 1.03 and 1.00 (s, 12H), 2.28 (dd, J = 15, 3 Hz, 1H, H-5), 2.46 (dd, 15.8, 3.5 Hz, 1H, H-6a), 2.58 (dd, J = 19.5, 3 Hz, 1H, H-9), 2.62 (dd, J = 16.5 Hz, 1H, H-2a), 2.77 (t, J = 16 Hz, 1H, H-2b), 3.12 (dd, J = 15 Hz, 1H, H-6a), 4.11 (d, J = 15 Hz, 2H, H-1 and H-15), 4.92 (d, J = 13.2 Hz, 1H, H-19a), 4.48 (d, J = 13 Hz, 1H, H-19b), 5.48 (s, 1H, H-17), 6.51 (dd, J = 1.5, 1.0, 1H, H-22), 7.68 (t, J = 2 Hz, 2H, H-23), 7.72 (d, J=0.5 Hz, 1H. H-21). ^{13}C NMR ($CDCl_3$, 75 MHz, assignments by APT): δ77.8 (t, C-2), 36.4 (C-2), 166.6 (C-3), 80.3 (C-4), 53.9 (C-5), 35.7 (C-6), 206.1 (C-7), 45.9 (C-8), 48.1 (C-9), 51.3 (C-10), 18.9 (C-11), 30.8 (C-12), 37.9 (C-13), 65.7 (C-14), 60.6 (C-15), 169.1 (C-16), 79.2 (C-17), 21.4 (C-18), 65.3 (C-19), 119.9 (C-20), 141.1 (C-21), 109.6 (C-22), 143.2 (C-23), 17.6 (C-24), 20.7 (C-25), 30.2 (C-26). EMIE *m*/*z* (rel. int.). 348 (100), 347 (28.2), 329 (12.8), 147 (20.5), 121 (28.2), 119 (20.5), 97 (33.3), 95 (100), 93 (35.9), 91 (56.4), 69 (59), 43 (100), 41 (61).

After drying, the aerial parts of *E. velutinosa* C. H. Ramos (sp. nov.) (1.8 kg, provided by C. H. Ramos and deposited at the National Herbarium) were macerated with acetone. The crude extract (62 g) was adsorbed onto silica gel and carefully chromatographed on a silica gel column at reduced pressure (VLC) [21] eluting with *n*-hexane and *n*-hexane containing increasing proportions of EtOAc. This procedure allowed the isolation of friedelin (250 mg) [15], β-sitosterol (220 mg), lupeol (22 mg) [18]. Elution with *n*-hexane-EtOAc (3:2), afforded 3.45 g of a residue, which was rechromatographed at reduced pressure (VCC), and some fractions (TLC control) were further purified by prep. TLC (silica gel, *n*-hexane-AcOEt, 5:9), to give cryptomeridiol (19 mg) [19]. The mother liquors from the isolation of friedelin, were rechromatographed via VLC using *n*-hexane as eluent, to give lupenone (20 mg) and caryophyllene β-oxide (40 mg) [20]. All the substances were identifed by direct comparison with authentic samples.

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