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POLYPRENOLS AND ACYLPHLOROGLUCINOLS FROM *ESENBECKIA NESIOTICA**

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Key Word Index—*Esenbeckia nesiotica*; Rutaceae; polyprenols; acylphloroglucinols.

Abstract—β-Sitosterol, caryophyllene β-oxide, friedelin, a mixture of polyprenols, lupeol, clovandiol and a mixture of phloroglucinols are biosynthesized by *Esenbeckia nesiotica*; 3-geranyl-1(3-methylbutanoyl)-phloroglucinol is a new natural product. This is the first report of the occurrence of polyprenols in the Rutaceae. Polyprenols did not display toxicity in the *Artemia salina* bioassay but the phloroglucinols showed moderate activity.

INTRODUCTION

The occurrence of flavonoids, lignans, coumarins, alkaloids and limonoids in the Rutaceae has been established [1]. In particular, the three last types of compound are considered as the main constituents of *Esenbeckia* [2–5], a genus comprising *ca* 30 species [6]. In connection with our chemical investigation of Mexican plants [7], herein we report the chemical constituents of *Esenbeckia nesiotica*.

RESULTS AND DISCUSSION

A hexane-soluble fraction of the leaves of *E. nesiotica* was chromatographed on silica gel to give β-sitosterol, caryophyllene β-oxide [8], friedelin [9], lupeol [10] and a polyprenol fraction which included [3*E*,9*Z*]-13-prenol (**1**) [11] and [3*E*,6*Z*]-10-prenol (**2**) [12–14]. The previously unpublished ^{13}C NMR data of **1** are recorded in the Experimental and were assigned according to Tanaka [15, 16].

Although long-chain polyisoprenoid alcohols have been isolated from several plant families [17–21], animal sources [15, 22, 23], and micro-organisms [24–26], this is the first report of the occurrence of such substances in the Rutaceae. The biological role of polyprenoids has been studied by several groups [27]. It is known that these substances are constituents of some plants eaten by some insects [12, 13] or infected by certain organisms [28]. Accordingly, **1** and **2** were not toxic in *Artemia salina* bioassays ($LC_{50} > 1000$) [29].

From an acetone extract of this species were isolated the tricyclic sesquiterpene clovandiol [30] and a mixture of phloroglucinols, **3a**, **4a** and **5a**. EI mass spectrometry of this mixture gave $[M]^+$ of *m/z* 346 and 332, which corresponded to $C_{21}H_{30}O_4$ and $C_{20}H_{28}O_4$, respectively. The IR of the mixture exhibited bands at 3570, 3380–3340 and 1620 cm^{-1} due to free hydroxyl, chelated hydroxyl and chelated carbonyl groups, respectively, while UV absorptions at 223 and 290 nm indicated the presence of a benzenoid ring. ^{1}H NMR data (Table 1) of the mixture (**3a–5a**) showed the presence of a geranyl residue. Treatment with Ac_2O and pyridine afforded initially a mixture of diacetylated phenols **3b**, **4b** and **5b**, which finally gave triacetylated phenols **3c**, **4c** and **5c**, establishing that the natural products were phloroglucinol derivatives acylated with different acyl residues.

Careful analysis of the 2D ^{1}H NMR spectrum of the phloroglucinol mixture established the presence of the acyl fragments. The 3-methylbutanoyl residue of **3a** was characterized by the methine heptuplet at δ2.26 (H-3′) that exhibited correlation with the doublets at δ2.94 (H-2′) and δ0.97 (H-4′ and H-5′). Analogous correlations indicated the presence of 2-metylbutanoyl and 2-methylpropanoyl residues in **4a** and **5a**, respectively. The area under the H-2′ signal of each compound established a 3:3:1 ratio of **3a**, **4a** and **5a** in the mixture. The structures **3a–5a** were in agreement with (a) the upfield shifts of H-7 and H-8 and the downfield shift of H-5 observed in the diacetyl and triacetyl derivatives **3b–5b** and **3c–5c**, respectively (Table 1), and (b) the mass spectral fragmentation pattern of the mixture (Scheme 1), in particular, the presence of the fragments *m/z* 346 $[M]^+$, 277, 223 for **3a** and **4a**, and the fragments *m/z* 332 $[M]^+$, 263, 209 for **5a**. ^{13}C NMR of the mixture of **3a–5a** confirmed the structures and are reported in the Experimental. The assignments were made by comparative analysis with published data [31, 32]. 3-Geranyl-1(3-methylbutanoyl)-phloroglucinol (**3a**) represents a new natural product, but **4a** and **5a** have been previously reported [32]. The mixture (**3a–5a**) displayed toxicity in the brine shrimp bioassay [29] ($LC_{50} = 307$ ppm). The occurrence of acylphloroglucinols in *Esenbeckia* is unprecedented, although this type of compound has been reported from other Rutaceae [31].

EXPERIMENTAL

Plant material. Leaves of *E. nesiotica* Stand. were collected in the Hwy Playa Azul to Tecomán, State of Michoacán, México.

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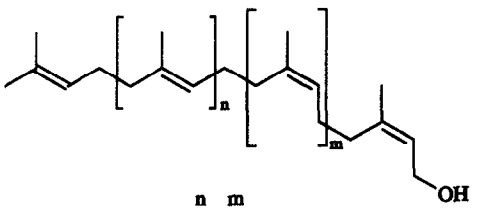
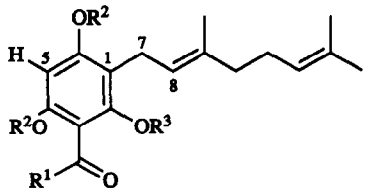


	n	m
1	3	8
2	3	5

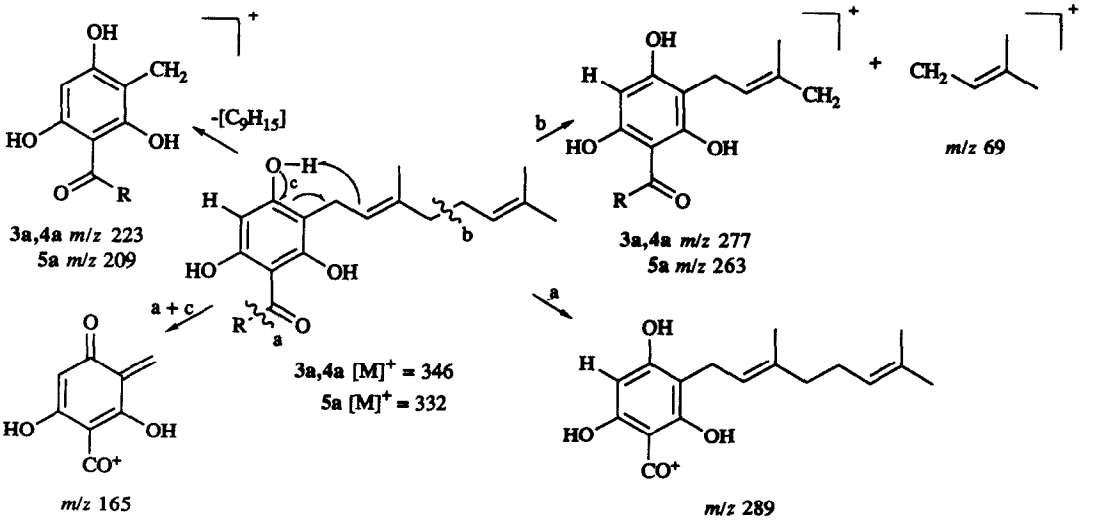


	3a	4a	5a	3b	4b	5b	3c	4c	5c
R^1	α-MeBu	β-MeBu	*i*-Bu	α-MeBu	β-MeBu	*i*-Bu	α-MeBu	β-MeBu	*i*-Bu
R^2	H	H	H	Ac	Ac	Ac	Ac	Ac	Ac
R^3	H	H	H	H	H	H	Ac	Ac	Ac

A voucher specimen is deposited at the National Herbarium, Instituto de Biología [UNAM (MEXU, CHR-84)].

Extraction and isolation. Air-dried plant material (1.9 kg) was extracted with *n*-hexane at room temp. (×2) affording 37 g of residue. The defatted plant material was then extracted with Me_2CO to obtain 108 g of residues. The crude hexane extract was adsorbed on to silica gel and carefully chromatographed on a column of silica gel via vacuum CC (VLC) [33] eluting with *n*-hexane and *n*-hexane containing increasing proportions of EtOAc. This procedure allowed the isolation of friedelin (204 mg), caryophyllene β-oxide (117 mg), a mixt. of polyprenols (1.2 g), lupeol (148 mg) and β-sitosterol (190 mg).

The polyprenyl fr. was subjected to repeated CC on silica gel using hexane. Two sets of frs were subjected to repeated HPLC analysis using a Hypersil ODS reverse-phase column, using *n*-PrOH. This procedure allowed the isolation of [3*E*,9*Z*]-13-prenol (**1**, 40 mg) [11] and [3*E*,6*Z*]-10-prenol (**2**, 25 mg) [12] as oils. Compound **1**: UV λ_{max} nm (log ε): 197 (5.047). IR $\nu_{max}^{CDCl_3}$ cm^{-1}: 3330, 2960, 2920, 2850, 1665, 1445, 1378, 1000, 830. ^{1}H NMR (300 MHz, $CDCl_3$): δ5.45 (1H, *d*, *J* = 7 Hz, =C*H*–CH_2OH), 5.12 (12H, *br s*, $W_{1/2}$ = 8 Hz, =C*H*–CH_2), 4.20 (2H, *d*, *J* = 7 Hz, –CH_2–OH), 2.03 (48H, *br s*, =CH–CH_2–), 1.75 (3H, *s*, –C(*Me*)=CH–CH_2–OH), 1.67 (27H, *s*, –C(*Me*)=CH–*cis*), 1.58 (12H, *s*, –C(*Me*)=CH– *trans*). ^{13}C NMR (75 MHz, $CDCl_3$): δ139.8 (*s*, C-2α), 136.0 (*s*, C-2 *trans-cis*), 135.3 (*s*, C-2 *trans-trans*), 135.2, (*s*, C-2 *cis*), 134.9 (*s*, C-2 *cis*-α), 134.8 (*s*, C-2 *trans-trans*), 131.2 (*s*, C-2ω-*trans*), 125.0, 124.9 (*d*, C-3 *cis*) 124.9, 124.8, 124.5, 124.4, 124.4, 124.3, 124.2, 124.1 (*d*, C-3ω, C-3 *trans*, and C-3α), 59.0 (*t*, C-4α), 39.7 (*t*, C-1 *trans-trans* and C-1ω-*trans*), 32.2, 32.0 (*t*, C-1 *cis-cis*), 32.0 (*t*, C-1 *trans-cis*), 26.7 (*t*, C-4ω), 26.7,



Scheme 1. Mass spectral fragmentation pattern of compounds **3a**–**5a**.

Table 1. ^{1}H NMR (300 MHz, $CDCl_3$) of phloroglucinols (**3–5**) and their derivatives

H	3a	4a	5a	3b	4b	5b	3c	4c	5c
5	5.82 (*s*)	5.82 (*s*)	5.82 (*s*)	6.45 (*s*)[a]	6.47 (*s*)[a]	6.47 (*s*)[a]	6.99 (*s*)[b]	6.95 (*s*)[b]	6.85 (*s*)[b]
7	3.39 (*d*)	3.39 (*d*)	3.39 (*d*)	3.30 (*d*)	3.30 (*d*)	3.30 (*d*)	3.14 (*d*)	3.14 (*d*)	3.07 (*d*)
8	5.24 (*t*)	5.24 (*t*)	5.24 (*t*)	5.12 (*t*)	5.12 (*t*)	5.12 (*t*)	5.06 (*t*)	5.06 (*t*)	5.06 (*t*)
10	2.10 (*s*)	2.10 (*s*)	2.10 (*s*)	2.04 (*m*)	2.04 (*m*)	2.04 (*m*)	2.03 (*m*)	2.03 (*m*)	2.03 (*m*)
11	2.10 (*s*)	2.10 (*s*)	2.10 (*s*)	1.97 (*m*)	1.97 (*m*)	1.97 (*m*)	1.97 (*m*)	1.97 (*m*)	1.97 (*m*)
12	5.06 (*t*)	5.06 (*t*)	5.06 (*t*)	5.06 (*t*)	5.06 (*t*)	5.06 (*t*)	5.01 (*t*)	5.01 (*t*)	5.01 (*t*)
14	1.68 (*s*)	1.68 (*s*)	1.68 (*s*)	1.64 (*s*)	1.64 (*s*)	1.64 (*s*)	1.64 (*s*)	1.64 (*s*)	1.64 (*s*)
15	1.59 (*s*)	1.59 (*s*)	1.59 (*s*)	1.57 (*s*)	1.57 (*s*)	1.57 (*s*)	1.57 (*s*)	1.57 (*s*)	1.57 (*s*)
16	1.81 (*s*)	1.81 (*s*)	1.81 (*s*)	1.74 (*s*)	1.74 (*s*)	1.74 (*s*)	1.69 (*s*)	1.69 (*s*)	1.69 (*s*)
2′	2.94 (*d*)	3.75 (*q*)	3.88 (*h*)	2.78 (*d*)	3.32 (*q*)	3.65 (*h*)	2.62 (*d*)	2.80 (*q*)	3.41 (*h*)
3′	2.26 (*h*)	1.40 (*m*)	1.19 (*d*)	2.29 (*h*)	1.45 (*m*)	1.18 (*d*)	2.20 (*h*)	1.40 (*m*)	1.17 (*d*)
4′	0.97 (*d*)	0.82 (*t*)	1.19 (*d*)	0.97 (*d*)	0.90 (*t*)	1.18 (*d*)	0.96 (*d*)	0.92 (*t*)	1.17 (*d*)
5′	0.97 (*d*)	1.15 (*d*)	—	0.97 (*d*)	1.18 (*d*)	—	0.96 (*d*)	1.12 (*d*)	—
OH on C-2	11.62 (*s*)	11.62 (*s*)	11.62 (*s*)	13.35 (*s*)[a]	13.19 (*s*)[a]	13.35 (*s*)[a]	—	—	—
OH on C-4	8.50 (*s*)	8.50 (*s*)	8.50 (*s*)	—	—	—	—	—	—
OH on C-6	6.08 (*s*)	6.08 (*s*)	6.08 (*s*)	—	—	—	—	—	—
OCOMe	—	—	—	2.20–2.37 (*s*)	2.20–2.37 (*s*)	2.20–2.37 (*s*)	2.20–2.37 (*s*)	2.20–2.37 (*s*)	2.20–2.37 (*s*)

[a,b] Signals may be interchanged.

26.6 (*t*, C-4 *trans*), 26.5, 26.4, 26.3, 26.2 (*t*, C-4 *cis*), 25.7 (*q*, C-5ω-*trans*), 23.4 (*q*, C-5 *cis*), 23.3 (*q*, C-5α), 17.7 (*q*, C-5ω), 16.0 (*q*, C-5 *trans*).

The Me_2CO extract was adsorbed on to silica gel and chromatographed on silica gel using VLC [33] and *n*-hexane–EtOAc (20:1). Increasing proportions of EtOAc were used for elution. This procedure allowed the isolation of 2 g of residue which was further chromatographed to afford 1.6 g of a mixt. of **3a**, **4a**, **5a** in a ratio of 3:3:1. Mp 128–130°. UV λ_{max}^{EtOH} nm (log ε): 207 (4.45), 223 (4.27), 290 (4.35). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3570, 3360, 2960, 2920, 2870, 1630, 1610, 1430, 1365, 1060. ^{1}H NMR (300 MHz, $CDCl_3$) in Table 1. ^{13}C NMR (75 MHz, $CDCl_3$): δ 211.8, 207.3 (*s*, C-1′), 163.8, 163.7, 162.1, 162.0, 161.3, 161.1 (*s*, C-2, C-4 and C-6), 141.3 (*s*, C-9 and C-13), 133.4 (*s*, C-1), 124.8 (*d*, C-8), 122.7 (*d*, C-12), 106.9, 106.8, 106.6 (*s*, C-3), 96.8, 96.7 (*d*, C-5), 54.1 (*t*, C-2′ of **3a**), 47.2 (*d*, C-2′ of **4a**), 41.0 (*t*, C-10), 40.8 (*d*, C-2′ of **5a**), 28.3 (*t*, C_7), 27.6 (*t*, C_{11}), 27.0 (*d*, C-3′ of **3a**), 24.1 (*t*, C-3′ of **4a**), 22.9, 22.9 (*q*, C-4′ of **4a** and C-5′ of **3a**), 20.6 (*q*, C-14), 19.0 (*q*, C-15), 18.0 (*q*, C-4′ of **3a**), 17.5 (*q*, C-16), 13.2 (*q*, C-5′ of **4a**). EIMS 70 eV *m/z* (rel. int.): 346 $[M]^+$ of **3a** and **4a** (21), 289 (39), 277 (15), 261 (18), 259 (17), 223 (86), 205 (33), 165 (100), 69 (51), 41 (76); 332 $[M]^+$ of **5a** (6), 263 (9), 209 (14). From the polar frs of the initial CC of the Me_2CO residue was isolated 17 mg of clovandiol [30].

Acetylation of mixture **3a–5a**. The mixt. (100 mg) was treated with Ac_2O (1 ml) and pyridine (1 ml) at room temp. for 3 min. After usual work-up 80 mg of organic residue was obtained. This residue was chromatographed on an open silica gel column (10 g) using *n*-hexane and increasing proportions of EtOAc. This procedure allowed the isolation of the mixt. **3b–5b** as an oil (45 mg). UV λ_{max}^{EtOH} nm (log ε): 213 (4.35), 250 (3.64), 295 (3.38). IR $\nu_{max}^{CDCl_3}$ cm^{-1}: 3540–3100, 2960, 2920, 1780, 1640, 1370, 1180, 1135, 1112, 1045, 892. ^{1}H NMR (300 MHz, $CDCl_3$) in Table 1. EIMS 70 eV *m/z* (rel. int.): 430 $[M]^+$ (0.2), 387 (2), 345 (3), 289 (2), 277 (6), 223 (10), 165 (6), 69 (44), 43 (100), 41 (53), and the relatively more polar mixt. **3c–5c** (15 mg): IR $\nu_{max}^{CDCl_3}$ cm^{-1}: 2965, 2925, 1780, 1370, 1185, 1045. ^{1}H NMR (300 MHz, $CDCl_3$) in Table 1.

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