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Secondary Metabolites from *Heliotropium angiospermum*

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Abstract. Blumenol A (**1**), blumenol B (**2**) and loliolide (**3**), together with the ubiquitous β-sitosterol, α-amyrin and β-amyrin, were isolated from the organic extract of the leaves of *Heliotropium angiospermum*. Structural elucidation of the metabolites was carried out by analysis of their spectroscopic data and/or by comparison with those reported in the literature.
Key words: *Heliotropium angiospermum*, Boraginaceae, blumenol A, blumenol B, loliolide.

Resumen. Blumenol A, blumenol B y loliólido, además de β-sitosterol, α-amirina y β-amirina, fueron aislados del extracto orgánico de las hojas de *Heliotropium angiospermum*. La elucidación estructural de los metabolitos se llevó a cabo mediante la interpretación de sus datos espectroscópicos y/o por comparación de los mismos con los reportados en la literatura.
Palabras clave: *Heliotropium angiospermum*, Boraginaceae, blumenol A, blumenol B y loliólido.

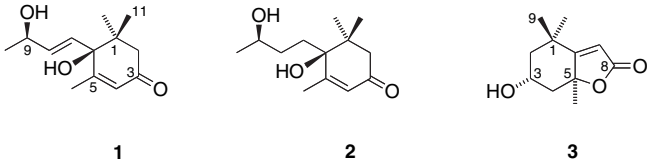
Introduction

Heliotropium angiospermum (Boraginaceae) is a shrub that grows in areas of southeastern Mexico, particularly Yucatán and Quintana Roo, where is commonly known as "cat tail" or "nemax". In Yucatecan traditional medicine, the leaves of *H. angiospermum*, administered as an infusion or a poultice, are used as an anti-inflammatory and wound-healing agent, as well as for the treatment of dysentery and diarrhea [1]. Previous phytochemical studies carried out on plants belonging to the genus *Heliotropium* have reported the presence of pyrrolizidine, pyrrolidine and indole alkaloids; similar studies on the inflorescences and leaves of *H. angiospermum*, have resulted in the isolation of putrescine, spermidine and spermine [2]. Recently, as part of a project directed towards detecting bioactive metabolites from the native flora of the Yucatan peninsula, the leaf extract of *H. angiospermum* showed DNA-interacting activity when tested using the DNA-methyl green assay [3]. We wish to report herein on the secondary metabolites isolated from the bioactive extract of *H. angiospermum*.

Results and Discussion

The dry-ground leaves of *H. angiospermum* were extracted with ethanol at room temperature; initial fractionation of the ethanol extract using liquid-liquid solvent partition with petroleum ether and ethyl acetate, produced the corresponding low and medium-polarity fractions. Purification of the low polarity fraction yielded three components in pure form, which were identified as α-amyrin, β-amyrin and β-sitosterol, by comparing their spectroscopic data with those previously reported in the literature [4,5]. β-Amyrin and β-sitosterol have previously been reported from *H. ellipticum* and *H. marifolium*, as well as from a large variety of plant species [6,7]. A number of interesting biological activities have been reported for these ubiquitous metabolites, including antimicrobial, antioxidant and anti-inflammatory [8-11].

Successive purifications of the medium-polarity fraction using silica gel column chromatography, HPLC and prep-TLC, yielded three components (**1**, **2**, **3**, see Figure 1 for structures) in pure form. The ESI-HRMS of **1** showed a molecular ion peak at *m/z* 224.1412, corresponding to the formula $C_{13}H_{20}O_3$. The ^{1}H NMR spectrum of **1** showed a one proton singlet at 5.89 ppm and a three proton broad-singlet at 1.88 ppm, suggesting a methyl-substituted α,β-unsaturated carbonyl system. Additionally, the spectrum showed the two signals of an AB system at 5.75 and 5.77 ppm, having the characteristic *J* value (15 Hz) of protons in a *trans* double bond, together with those corresponding to the non-equivalent protons of a methylene group at 2.20 and 2.40 ppm (e.a. *J* = 12 Hz). The carbonyl carbon signal at 198.7 ppm, and the presence of two oxygenated carbon signals in the ^{13}C NMR of **1**, one at 77.2 (s) and the other at 67.9 (d) ppm, allowed identification of the three oxygen atoms in the molecular formula as a ketone and a tertiary and secondary alcohols, respectively. Since an unsaturated ketone and a double bond accounted for only three of the four unsaturation sites implied by the molecular formula, the remaining unsaturation site was identified as a carbocyclic ring. A search of the literature showed that the spectroscopic data of the isolated metabolite **1** was identical to those reported for blumenol A, a norisoprenoid isolated from the



1 **2** **3**

Fig. 1. Structures of metabolites **1**, **2** and **3** isolated from *Heliotropium angiospermum* in this investigation.

leaves of *Brassica fruticulosa* (Brassicaceae) [12,13,16]. As there has been some discussion about the relative configuration of C-9 of **1**, a NOESY experiment was carried out in order to determine this. 2-Hα gives NOESY correlations to 12-H_3 and 7-H while 2-Hβ gives NOESY correlations to 11-H_3 and 12-H_3. The examination of a Dreiding model shows that the six-membered ring must have the 3-hydroxy-1-butenyl and 11-methyl (C-11) substituents axial, while the 12-methyl (C-12) group is equatorial, in order for the observed correlations to make sense. The observed correlations between 11-H_3 and 4-H as well as 13-H_3 confirms this. 12-H_3 and 13-H_3 give NOESY correlations to both 7-H and 8-H, indicating that the exocyclic double bond is extended perpendicular to the ring. 9-H gives major correlations to 7-H/8-H and 10-H_3, but weak correlations can also be observed to 2-Ha and 12-H_3. That would place 9-H closer to 2-Hα and 12-H_3 in the most stable confirmation, and the observed strong correlation from 10-H_3 to 8-H suggests that C-9 of **1** has the configuration shown in Figure 1.

The parent ion peak at m/z 227 $[M+H]^+$ in the ESI-HRMS of the second component (**2**), corresponded to a molecular formula of $C_{13}H_{22}O_3$ which indicated a structure similar to **1**, but with one less unsaturation site. Accordingly, the spectroscopic data (^1H and ^{13}C NMR) of **2** was very similar to that of **1**; however, the absence of the trans–vinylic protons in the ^1H NMR spectrum of **2**, when compared to that of **1**, suggested that the side chain in the new metabolite was fully saturated. Final identification of **2** as blumenol B was confirmed when its spectroscopic data proved to be identical to those reported in the literature [12,13]. It was not possible to determine the configuration of C-9 in **2** by a NOESY experiment, but it can be assumed to be the same as in **1**.

The third metabolite (**3**) was assigned a molecular formula of $C_{11}H_{16}O_3$, on the basis of the parent ion peak observed at *m/z* 197.1178 $[M+H]^+$ in its ESI-HRMS. The ^1H-NMR spectrum of **3** exhibited signals for three methyl groups (1.27, 1.46 and 1.78 ppm) attached to quaternary carbons, a vinylic proton (5.69 ppm) in a trisubstituted double bond, a carbinol proton (4.33 ppm), and two methylene groups (2.45 and 1.78; 1.97 and 1.53 ppm). Similarly, the ^{13}C-NMR spectrum of **3** revealed the presence of three oxygen-bearing carbons at 171.8, 86.6 and 66.8 ppm; this indicated the presence of an ester, or lactone, and a secondary alcohol in the structure of **3**. On the basis of this data, the four unsaturation sites implied by the molecular formula could be explained by a bicyclic structure having an α,β-unsaturated-γ-lactone ring. The spectroscopic data of **3** coincided with those reported for loliolide, a metabolite previously isolated from *Eucommia ulmoides* (Eucommiaceae) and *Hydrilla verticillata* (Hydrocharitaceae) [14,15].

The nor-isoprenoids blumenol A (**1**) and blumenol B (**2**), are important components to the flavor of tobacco, tea, and some fruits [16]. A number of different nor-isoprenoids have also been identified in *Lawsonia inermis* (Lythraceae), *Podocarpus blumei* (Podocarpaceae), *Macaranga tanarius* (Euphorbiaceae) and *Perrottetia multiflora* (Celastraceae) [16,17-19]; although their biogenetic origin is as yet uncertain, it has been proposed that these metabolites might be biosynthesized from (+)-abscisic acid, through the oxidative removal of the two terminal carbon atoms, or that they might result from the degradation of higher terpenoids, e.g. carotenoids, in plant tissue [20-23]. Loliolide (**3**), a C_{11}-terpene lactone that arises from biological or oxidative degradation of carotenoids, has been isolated from both land plants and marine algae. This metabolite is well-known to have immunosuppressive, inhibition of germination, and insect-repellent activities [24-27].

This is the first report on the occurrence of this type of nor-isoprenoids in a *Heliotropium* sp.

Experimental section

General experimental procedures

Vacuum liquid chromatography (VLC) separations were carried out using TLC-grade silica gel (Merck), while flash and open-column chromatography separations were run using silica gel 60 (230-400 mesh, Merck). Sephadex LH-20 (GE Healthcare) was used for gel permeation column chromatography. Preparative TLC (PTLC) separations were performed on glass-coated (1 mm thickness) 20 x 20 cm plates (Aldrich). TLC analyses were carried out using aluminum-backed silica gel 60 F_{254} (0.20 mm thickness) plates (Merck); chromatograms were first visualized by observing under a UV lamp (254 nm) and then spraying with 10% sulfuric acid, followed by heating at 100°C. HPLC separations were conducted using a Dynamax Rainin model SD-200 HPLC, equipped with a UV-1 model Rainin absorbance detector and a Dynamax-100A normal phase column (21.4 x 250 mm); a CH_2Cl_2/acetone (9:1) isocratic system was used for elution. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) were recorded at room temperature with a Bruker DRX 400 spectrometer; the spectra were recorded in $CDCl_3$ and the solvent residual signals (7.26 and 77.0 ppm, for ^1H and ^{13}C NMR, respectively) were used as reference. The chemicals shifts (δ) are given in ppm, and the coupling constants (*J*) in Hz. ESI-HRMS spectra were recorded in a Waters Q-TOF Micro system spectrometer, using H_3PO_4 for calibration and as internal standard.

Plant material

Leaves of *Heliotropium angiospermum* Murray were collected in July 2003 in Libre Unión (Cenote Xtojil), Yucatán, Mexico. A voucher specimen (PSimá 2660) was deposited at the herbarium of the Unidad de Recursos Naturales of the Centro de Investigación Científica de Yucatán.

Extraction and isolation

Dried-ground leaves (1 kg) were extracted with ethanol, three times at room temperature for one week. After filtration, the extracts were combined and the solvent was evaporated under reduced pressure to give 37.95 g of organic extract. The extract

was suspended in a mixture of water:methanol (9:1, v/v, 500 mL) and the resulting aqueous suspension was successively partitioned between petroleum ether (three times, 2:1, v/v), ethylacetate (three times, 2:1, v/v) and butanol (three times, 1:1, v/v), to yield the corresponding low (19.51 g), medium (530 mg) and high polarity (1.20 g) fractions, respectively.

The low polarity fraction was purified by VLC using a gradient elution with mixtures of petroleum ether and ethyl acetate, to produce 17 major fractions (A-Q). Flash column chromatography purification of fractions B and C (1.64 g), eluting with petroleum ether:ethylacetate (9:1, v/v) and petroleum ether/dichloromethane (8:2, v/v), produced 15 new fractions (A1-O1). Further purification of fractions J1-K1, using flash column chromatography and dichloromethane/methanol (9:1, v/v), produced 7 mg of a mixture of α- and β-amyrin. Successive purifications of fraction H (256.3 mg), using Sephadex LH-20 (chloroform/methanol 1:1, v/v) and preparative TLC (dichloromethane/methanol 95:5, v/v), yielded 20.6 mg of b-sitosterol in pure form.

Purification of the medium polarity fraction by flash column chromatography (dichloromethane/methanol 95:5) produced 11 major fractions (A2-J2). Fractions C2-D2 (80 mg) were purified by HPLC to produce 12 new fractions (A3-L3). The metabolites in fractions G3 (5 mg) and K3 (4 mg) were identified as blumenol A (**1**) and blumenol B (**2**), respectively. Purification of fraction C3 (40 mg), using silica gel open-column chromatography and eluting with diethyl ether, produced four fractions (A4-D4). Purification of C4 (16 mg) by prep-TLC using diethyl ether furnished 3 mg of loliolide (**3**).

Blumenol A (1): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.89 (1H, s), 5.77 (1H, d, J = 15 Hz), 5.75 (1H, d, J = 15 Hz), 4.34 (1H, m), 2.41 (1H, d, J = 17 Hz), 2.20 (1H, d, J = 17 Hz), 1.88 (3H, bs), 1.26 (3H, d, J = 6.4 Hz), 1.05 (3H, s), 0.99 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 198.7 (s), 163.7 (s), 135.6 (d), 129.0 (d), 126.7 (d), 77.2 (s), 67.9 (d), 49.7 (t), 41.1 (s), 23.9 (q), 23.5 (q), 22.8 (q), 18.9 (q); *m/z* HRMS (ESI, M^+) found 224.1412, $C_{13}H_{20}O_3$ requires 224.1412; EIMS *m/z* (rel. int.): 224 $[M]^+$ (7), 207 (100), 189 (21), 149 (15), 135 (10), 123 (15), 107 (3).

Blumenol B (2): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.84 (1H, s), 3.73 (1H, m), 2.48 (1H, d, J = 18 Hz), 2.23 (1H, d, J = 18 Hz), 2.05 (3H, s), 1.80 (2H, m), 1.50 (2H, m), 1.21 (3H, d, J = 6.2 Hz), 1.08 (3H, s), 1.04 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 198.3 (s), 168.9 (s), 125.9 (d), 77.3 (s), 68.5 (d), 49.9 (t), 41.7 (s), 34.7 (t), 33.3 (t), 24.1 (q), 23.8 (q), 23.6 (q), 21.8 (q); *m/z* HRMS (ESI, $M+H^+$) found 227.1647, $C_{13}H_{22}O_3 + H^+$ requires 227.1602; EIMS *m/z* (rel. int.): 227 $[M+H]^+$ (9), 209 (100), 193 (22), 149 (24), 125 (16).

Loliolide (3): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.69 (1H, s), 4.33 (1H, qui), 2.45 (1H, ddd, J = 2.4, 2.4, 14 Hz), 1.97 (1H, ddd, J = 2.6, 2.8, 14 Hz), 1.78 (1H, dd, J = 4, 13.6 Hz), 1.53 (1H, dd, J = 3.6, 12.6 Hz), 1.78 (3H, s), 1.46 (3H, s), 1.27 (3H, s); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 182.3 (s), 171.8 (s), 113.0 (d), 86.6 (s), 66.8 (d), 47.3 (t), 35.9 (s), 30.6 (q), 27.0 (q), 26.4 (q); *m/z* HRMS (ESI, $[M+H^+]$) found 197.1178, $C_{11}H_{16}O_3 + H^+$ requires 197.1133; EIMS *m/z* (rel. int.): 197 $[M+H]^+$ (82), 145 (25), 113 (19), 83 (100).

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