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Secondary Metabolites from *Asclepias otarioides*

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Abstract. Chemical study of the aerial parts of *Asclepias otarioides* led to the isolation of four pentacyclic triterpenes and one cardenolide glycoside. This is the first report on the occurrence of the triterpenes **1**, **3**, and **4** in the genus *Asclepias*.
Key words: *Asclepias otarioides*; Apocynaceae; Asclepiadoideae; Triterpenes; Cardenolide Glycoside.

Resumen. El estudio químico de las partes aéreas de *Asclepias otarioides* condujo al aislamiento de cuatro triterpenos pentacíclicos y de un glicósido de cardenólida. Este es el primer informe sobre la presencia de los triterpenos **1**, **3** y **4** en el género *Asclepias*.
Palabras clave: *Asclepias otarioides*; Apocynaceae; Asclepiadoideae; triterpenos; glicósido de cardenólida.

Introduction

The American genus *Asclepias* (fam. Apocynaceae, subfamily Asclepiadoideae) includes about 150 species, 68 of which grow in Mexico, and nearly half of them, are endemic [1-3]. Previous chemical investigations of *Asclepias* species have shown that different types of steroidal compounds such as cardenolides, pregnanes and androstanes, usually as glycosides, are the main metabolites of these plants [4-6]. However, flavonoid glycosides [7], megastigmane glycosides [8], triterpenes [4, 9], conduritols, and conduritol glycosides [10] have been also isolated from these plants. *Asclepias* species have ecological significance by their relationship with the monarch butterfly, *Danaus plexippus*; an insect that sequestrates cardenolides from *Asclepias* plants as a chemical defense mechanism against predators [11]. Although *Asclepias* species are considered toxic, some of them are used in folk medicine as anthelmintic, analgesic, cardiotonic, and for the treatment of dermatological problems [3], cancer [4], pleuresy, bronchitis [6], and asthma [9]. This paper describes the isolation and the structure elucidation of the major constituents of the aerial parts of *Asclepias otarioides* E. Fourn., an herbaceous plant, endemic to Mexico [12].

Results and Discussion

As result of the chemical study of the aerial parts of *A. otarioides*, three oleanane-type triterpenes (**1**-**3**), one lupane-type triterpene (**4**), one cardenolide glycoside (**5**), *β*-sitosterol glucoside and a mixture of *β*-sitosterol/stigmasterol were isolated. The oleanane-type triterpenes were identified as oleanonic acid (**1**) [13-15], oleanolic acid (**2**) [16, 17], and 3,4-*seco*-olean-12-en-3,28-dioic acid (**3**) [18], while the structure of the lupane-type triterpene corresponded to betulinic acid (**4**) [19, 20] (Fig. 1). Structures of compounds **1**-**4** were determined by analyses of their IR, MS and NMR spectra and comparison of these data with those reported in the literature. *β*-Sitosterol glucoside and the mixture of *β*-sitosterol/stigmasterol were identified by comparison of their ^{1}H NMR spectra and physical constants with those of authentic samples.

Compound **5** was part of a complex mixture from which it could not be isolated. So, the mixture was esterified (Ac_2O-pyridine) and compound **5** was isolated as the pentaacetyl derivative **6**. The molecular formula of this derivative was assigned as $C_{39}H_{54}O_{15}$ by the pseudomolecular ion at *m/z* 785.3356 [M + Na] $^+$ observed in its HRESIMS (calcd. for, 785.3355). The ^{13}C NMR spectrum of compound **6** showed 39 signals; 10 of them correspond to 5 acetyl groups, another 6 signals were assigned to a monosaccharide, and the remaining 23 signals were atributted to a cardenolide. The ^{1}H NMR spectrum showed the signals for the sugar moiety at *δ* 4.84 (d, H-1'), 4.79 (dd, H-2'), 5.60 (t, H-3'), 4.66 (dd, H-4'), 3.94 (dq, H-5'), and 1.21 (d, H-6'). The coupling constants of these signals ($J_{1\text{-}2}$ = 8.0 Hz, $J_{2\text{-}3}$ = $J_{3\text{-}4}$ = 3.0 Hz, $J_{4\text{-}5}$ = 10.0 Hz) led to the identification of this sugar as *β*-allomethylose [21]. The presence of an *α*,*β*-unsaturated-*γ*-lactone in the aglycone was deduced from ^{1}H NMR signal at *δ* 5.85 for the vinyl proton H-22 and those

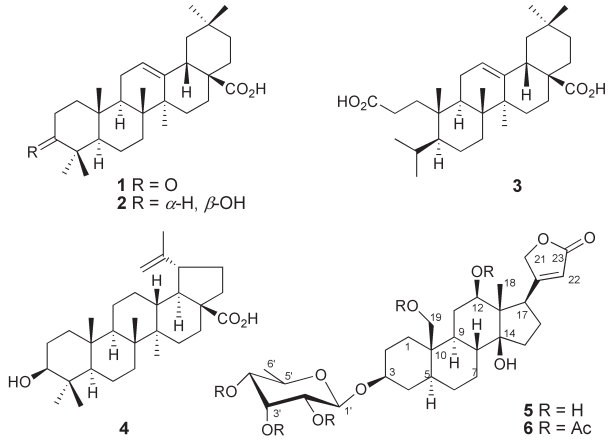

Fig. 1. Structures of compounds **1**-**6**.

for the γ-methylene protons (H_2-21) at δ 4.85 and 4.77; and confirmed by the ^{13}C NMR signals at δ 173.0 (C-20), 73.2 (CH_2-21), 118.1 (CH-22), and 174.1 (C-23). The aglycone also presents signals for one oxymethylene (δ_H 4.32 d, J = 12.5 Hz, δ_H 4.12 d, J = 12.5 Hz; δ_C 61.7, CH_2-19), two oxymethines (δ_H 4.53 dd, J = 12.0, 4.0 Hz, δ_C 77.1, CH-12; δ_H 3.65 tt, J = 11.0, 5.0 Hz; δ_C 77.6, CH-3), a non protonated carbon bonded to oxygen (δ_C 85.6, C-14), and one methyl group (δ_H 0.88 s; δ_C 10.4, CH_3-18). These assignments were based on analysis of the 2D NMR spectra, establishing the structure of the cardenolide as that of 12β-hydroxycoroglaucigenine. The HMBC correlation of H-1' to C-3 showed that the allomethylose was bonded to the oxygen at C-3. In the same manner was established that the acetyl groups were bonded to the oxygens at C-12, C-19, C-2', C-3', C-4' and C-6'. The derivative **6** was identified as 12-*O*,19-*O*,2'-*O*,3'-*O*,4'-*O*,6'-*O*-pentaacetyl-12β-hydroxycoroglaucigenine-3-*O*-β-D-allomethyloside, which has not been described previously. Thus, the cardenolide present in *A. otariodes* was identified as 12β-hydroxycoroglaucigenine-3-*O*-β-D-allomethyloside (**5**) [21].

To our knowledge, this is the first report on the ocurrence of oleanonic acid (**1**), 3,4-*seco*-olean-12-en-3,28-dioic acid (**3**), and betulinic acid (**4**) in *Asclepias* genus. Oleanolic acid (**2**) and other pentacyclic triterpenes have been isolated from *A. syriaca*, *A. linaria* and *A. speciosa* [9,22,23]. 12β-Hydroxycoroglaucigenin-3-*O*- β-D-allomethyloside (**5**) and its aglycon were isolated from *A. curassavica* [21,24]. Thus, the chemical composition found in *A. otarioides* was consistent with those found in another species of *Asclepias* genus, in which the same type of compounds were present. This indicates that not only cardiac glycosides, but pentacyclic triterpenes are relevant constituents of *Asclepias* species.

Experimental

General experimental procedures. Melting points (uncorrected) were determined on a Fisher Jones melting point apparatus. Optical rotations were measured on a Perkin Elmer 343 polarimeter. The IR spectra were recorded on a FTIR-Magna 750 spectrophotometer. NMR spectra were recorded on a Varian Unity Plus 500 or on Varian XR-300 spectrometers, using TMS as internal standard. EIMS were measured on a JEOL JMS-AX505HA mass spectrometer. HRESIMS was recorded on a Bruker microTOF II ESI mass spectrometer. Column chromatographies operated with vacuum (CC) were performed on silica gel 60 (Merck G). Thin layer chromatographies (TLC) were carried out on precoated Macherey-Nagel Sil G/UV_{254} plates with thicknesses of 0.25 mm.

Vegetal material. The aerial parts of *Asclepias otarioides* E. Fourn. were collected in the Ajusco Mountain, Southwest Mexico City, in July 2006. V. Juárez-Jaimes authenticated the vegetal material. A voucher specimen (MEXU 1 248 428) was deposited at the National Herbarium.

Extraction and isolation. Fresh aerial parts of *A. otarioides* (446 g) were extracted with MeOH and then with EtOAc. Both extracts were combined (148.7 g) and fractioned by partition between EtOAc-H_2O and BuOH-H_2O, to obtain 44.7 and 8.1 g of extract, respectively. The EtOAc-soluble extract was fractioned by silica gel column chromatography (CC) with hexane-EtOAc mixtures to obtain five combined fractions (1A to 5A). Purification of fraction 2A (eluted with hexane-EtOAc 19:1) by silica gel CC eluted with hexane-EtOAc 19:1 and 9:1 gave fractions 1B-5B. A mixture of the ubiquitous β-sitosterol/stigmasterol (299 mg) was obtained from fraction 2B. Crystallization of fraction 3B led to the isolation of oleanonic acid (**1**) (mp 167-169 °C, $[\alpha]_D$ 93.5, *c* 0.23, $CHCl_3$, lit.: mp 170-176 °C, $[\alpha]_D$ 96.6, *c* 0.23, MeOH [13], $[\alpha]_D$ 73.6, *c* 0.26, $CHCl_3$ [15]) Mother liquors of **1** were combined with fraction 4B and subjected to silica gel CC eluted with CH_2Cl_2, followed by a second CC eluted with hexane-*i*PrOH 98:2 and crystallization to obtain 41.2 mg of betulinic acid (**4**) (mp 285-287 °C, $[\alpha]_D$ 7.0, *c* 0.20, $CHCl_3$, lit.: mp 290-292 °C, $[\alpha]_D$ 7.5, *c* 0.5, pyridine [19], $[\alpha]_D$ 8.0, *c* 0.37, $CHCl_3$ [20]), together with an additional amount of **1**, to make a total of 3.87 g. Fractions 3A (eluted with hexane-EtOAc 17:3 to 7:3) and 5B were combined, decoloured with activated charcoal and subjected to CC eluted with hexane-EtOAc gradient. Fractions eluted with hexane-EtOAc 9:1 afforded 568 mg of oleanolic acid (**2**) (mp 297-299 °C, $[\alpha]_D$ 70.8, *c* 0.226, $CHCl_3$, lit.: mp 301-302.5 °C, $[\alpha]_D$ 68.9, *c* 0.21, $CHCl_3$ [16,17]). Chromatography of fraction 5A (eluted with hexane-EtOAc 1:4 to 0:1) over a silica gel column eluted with $CHCl_3$-MeOH 19:1 to17:3 gave fractions 1C-4C. Fraction 1C was purified by CC eluted with mixtures of $CHCl_3$-MeOH of increasing polarity. Fractions eluted with $CHCl_3$-MeOH 98:2 gave 21 mg of 3,4-*seco*-olean-12-en-3,28-dioic acid (**3**) (mp 268-270 °C, $[\alpha]_D$ 60.0, *c* 0.21, $CHCl_3$, lit.: mp >250 °C, $[\alpha]_D$ 54.4, *c* 0.006, MeOH [18]. Crystallization of fraction 2C gave 53.2 mg of β-sitosterol glucoside.

Silica gel CC ($CHCl_3$-MeOH) of the BuOH-soluble extract gave fractions 1D-4D. Repeated CC of fraction 2D gave a mixture (77 mg) containing 12β-hydroxycoroglaucigenin-3-*O*-β-*D*-allomethyloside (**5**), which could not be purified. A portion (64.4 mg) of this mixture was acetylated in the usual manner (pyridine/Ac_2O, room temp., 24 h), and purified by silica gel CC eluted with hexane-Me_2CO 4:1, to obtain the pentaacetyl derivative of **5** (**6**, 71.7 mg).

Pentaacetyl-12β-hydroxycoroglaucigenin-3-O-β-D-allomethyloside (6). Amorphous solid; $[\alpha]_D^{20}$ + 5.37 (*c* 0.28, $CHCl_3$); IR ($CHCl_3$, ν, cm^{-1}):1747, 1629, 1373, 1171, 1083, 1037; ^1H NMR (500 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 2.18 (1H, dt, J = 14.0, 3.5 Hz, H-1a), 0.91 (1H, td, J = 14.0, 3.5 Hz, H-1b), 1.93 (1H, m, H-2a), 1.38 (1H, m, H-2b), 3.65 (1H, tt, J = 11.0, 5.0 Hz, H-3), 1.92 (1H, m, H-4a), 1.76 (1H, m, H-4b), 1.25 (1H, m, H-5), 1.43 (1H, m, H-6a), 1.24 (1H, m, H-6b), 2.02 (1H, m, H-7a), 1.13 (1H, m, H-7b), 1.67 (1H, td, J = 12.0, 3.5 Hz, H-8), 1.08 (1H, td, J = 12.0, 3.0 Hz, H-9), 1.87 (1H, m, H-11a), 1.33 (1H, m, H-11b), 4.53 (1H, dd, J = 12.0, 4.0 Hz, H-12), 1.73 (1H, m, H-15a), 1.36 (1H, m, H-15b), 2.15 (1H, m, H-16a), 1.92 (1H, m, H-16b), 2.87 (1H, td, J = 6.0, 4.0 Hz, H-17), 0.88 (3H, s, H-18), 4.32 (1H, d, J = 12.5 Hz, H-19a), 4.12 (1H, d, J = 12.5 Hz, H-19b), 4.85 (1H, br dd, J = 18.0, 1.5 Hz, H-21a), 4.77 (1H,

dd, J = 18.0, 2.0 Hz, H-21b), 5.85 (1H, br s, H-22); 4.84 (1H, d, J = 8.0, Hz, H-1'), 4.79 (1H, dd, J = 8.0, 3.0 Hz, H-2'), 5.60 (1H, t, J = 3.0, Hz, H-3'), 4.66 (1H, dd, J = 10.0, 3.0 Hz, H-4'), 3.94 (1H, dq, J = 10.0, 6.5 Hz, H-5'), 1.21 (3H, d, J = 6.5, Hz, H-6'), 2.15, 2.10, 2.05, 2.02, and 2.01 (3H each, s, CH_3CO); ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 31.9 (C-1), 29.2 (C-2), 77.6 (C-3), 32.9(C-4), 44.6 (C-5'), 27.9 (C-6), 27.4 (C-7), 41.7 (C-8), 45.9 (C-9), 38.1 (C-10), 27.4 (C-11), 77.1 (C-12), 53.9 (C-13), 85.6 (C-14), 34.4 (C-15), 27.1 (C-16), 45.9 (C-17), 10.4 (C-18), 61.7 (C-19), 173.0 (C-20), 73.2 (C-21), 118.1 (C-22), 174.1 (C-23), 96.9 (C-1'), 69.5 (C-2'), 68.8 (C-3'), 71.4 (C-4'), 68.1 (C-5'), 17.5 (C-6'), 21.2, 21.1, 20.74, 20.68, and 20.6 (CH_3CO), 170.9 170.8, 169.8, 169.3, and 169.0 (CH_3CO); FABMS *m/z* 763 $[M + H]^+$ (3), 703 (1), 473 (2), 413 (2), 353 (8), 335 (8), 273 [triacetylallomethylose] $^+$ (87), 231 (4), 213 (4), 153 (38), 111 (42), 69 (73), 57 (100), 43 (72). HRESI MS *m/z* 785.3356 $[M + Na]^+$ (calcd. for $C_{39}H_{54}NaO_{15}$, 785.3355).

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