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TERPENOIDS FROM *WEDELIA BUPHTHALMIFLORA*

CLAUDIO D. SCHTEINGART and ALICIA B. POMILIO

Departamento de Química Orgánica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Pabellón 2, Ciudad Universitaria, 1428 Buenos Aires, Argentina

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Key Word Index—*Wedelia buphthalmiflora*; Compositae; Heliantheae; Ecliptinae; kaurene derivatives; (−)-kaur-16-en-19-oic acid; (−)-kaur-9(11),16-dien-19-oic acid; 15α-angeloyloxykaur-16-en-19-oic acid; 15α-isobutyroyloxykaur-16-en-19-oic acid; 15α-isovaleroyloxykaur-16-en-19-oic acid; 15α-tigloyloxykaur-16-en-19-oic acid; β-amyrin; β-amyrin acetate; ursolic acid.

Abstract—A series of known di- and triterpenoids were characterized in the whole plant of *Wedelia buphthalmiflora*.

INTRODUCTION

In continuation [1] of a chemical study on the genus *Wedelia* we now report the isolation and characterization of di- and triterpenoids from *Wedelia buphthalmiflora* Lorentz. This suffruticose species is native to the central region of Argentina and is known for its toxicity to cattle. As far as we know no chemical work has been reported on this plant. Other species of the genus *Wedelia* have previously been examined. Wedelolactone was isolated from *W. calendulaceae* [2], kaurenic acid derivatives from *W. trilobata*, *W. helianthoides*, *W. grandiflora* [3] and *W. scaberrima* [4], a eudesmanolide from *W. rugosa* [5] and recently pseudoguaianolides from *W. grandiflora* [6].

RESULTS AND DISCUSSION

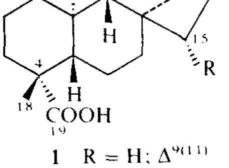
Column chromatography of the petrol extract of aerial parts of *W. buphthalmiflora* provided fractions rich in kaurene derivatives. One of these fractions contained acidic compounds which were separated as methyl esters yielding (−)-kaur-9(11), 16-dien-19-oic acid (grandiflorenic acid) (**1**) and (−)-kaur-16-en-19-oic acid (**2**). These compounds were identified by spectroscopic methods and by comparison with authentic samples. Other fractions contained diterpenic esters. Upon alkaline hydrolysis of the corresponding methyl derivatives, all esters provided methyl 15α-hydroxykaur-16-en-19-oate. The size of the acyl group was determined by MS. According to the spectral data (IR, MS, ^{1}H NMR and ^{13}C NMR) these esters were identified as: 15α-angeloyloxykaur-16-en-19-oic acid (**3**), 15α-isobutyroyloxykaur-16-en-19-oic acid (**4**), 15α-isovaleroyloxykaur-16-en-19-oic acid (**5**) and 15α-tigloyloxykaur-16-en-19-oic acid (**6**). Structures of **4** and **5** were confirmed by partial synthesis.

Labdane-type diterpenes are typically found in the tribes Eupatorieae [7], Inuleae [8] and Astereae [9]. Kaur-16-ene derivatives with an oxygen function at C-19 have been isolated from the tribes Heliantheae [10], Eupatorieae [11] and Inuleae [8], but esters of 15α-hydroxykaur-16-en-19-oic acid seem to be characteristic of Heliantheae. As far as we know these esters have only twice been reported in members (*Campovassouria bupleurifolia* [12] and *Mikania cordata* [13]) of another tribe (Eupatorieae). However, with regard to the esterifying moieties, no appreciable differences have been detected amongst the numerous subtribes of the Heliantheae. Thus, the angelate ester has been isolated from plants of the subtribes Ecliptinae [3, 14], Melampodiinae [15], Verbesininae [16] and Helianthinae [17]. The tiglate ester has been isolated from the subtribes Ecliptinae [3, 4], Helianthinae [17] and Verbesininae [18] and the isovalerate ester from Ecliptinae [14], Verbesininae [16] and Espeletiinae [19]. The isobutyrate ester has only been identified in roots of *Mikania cordata* [13] (tribe Eupatorieae) and recently from stems of *Espeletiopsis purpurascens* [19] (tribe Heliantheae, subtribe Espeletiinae). Further investigations are required to determine whether these esters of 15α-hydroxykaur-16-en-19-oic acid are really typical of the Heliantheae only.

EXPERIMENTAL

Plant material. W. buphthalmiflora was collected in Padre Buodo (Utracán, Province of La Pampa, Argentina). Voucher specimen Steibel 6105 is deposited in University of La Pampa, Faculty of Agronomy.

Extraction, isolation and identification of the compounds. Dried, powdered aerial parts (3 kg) were successively extracted with petrol (69 g of extract; 2.4% of dry plant) and EtOH (151 g of extract; 5.2% of dry plant). Upon concn of the petrol extract a solid separated (3.2 g) which was filtered. A portion of the petrol



1 R = H; $\Delta^{9(11)}$
2 R = H
3 R = MeCH=CMeCOO—(*Z*)
4 R = Me_2CHCOO—
5 R = Me_2CHCH_2COO—
6 R = MeCH=CMeCOO—(*E*)

extract (21 g) was chromatographed on a Si gel H column using petrol, petrol–$CHCl_3$, $CHCl_3$ and $CHCl_3$–EtOH as solvents. Nine main fractions were obtained.

Hydrocarbons were detected in fraction 1, as well as waxes in fraction 2. Fraction 3 was repeatedly recryst. from Me_2CO–MeOH to give a solid identified as β-amyrin acetate by comparison with a standard. Normal alcohols were obtained from fraction 4.

Fraction 5 was chromatographed on a column. On elution with petrol–EtOAc–HOAc (50:3.7:2) a mixture (600 mg) of **1** and **2** was obtained followed by small amounts of β-amyrin contaminated with **2**. The separation of **1** and **2** was performed as methyl esters over a column with petrol–C_6H_6 (50:3). β-Amyrin was purified by CC using gradients of petrol–EtOAc (4–15%).

Fraction 6 was methylated and chromatographed on a column using petrol–C_6H_6 (2:1) as eluant. **3**, a mixture of **4** and **5** and finally **6** were obtained as methyl derivatives. The methyl ester of **3** showed spectral data (IR, ^1H NMR, ^{13}C NMR and MS) in accordance with methyl 15α-angeloyloxykaur-16-en-19-oate [3, 14, 20]. The mixture of the methyl esters of **4** and **5** was analyzed by GC/MS.

Synthesis of methyl 15α-isobutyroyloxykaur-16-en-19-oate (methyl ester of **4**) was carried out by treatment of methyl 15α-hydroxykaur-16-en-19-oate (5.6 mg) with isobutyroyl chloride (0.2 ml) in dry CH_2Cl_2 (5 ml) and pyridine (0.2 ml) under reflux for 2 hr. Yield: 70%.

Synthesis of methyl 15α-isovaleroyloxykaur-16-en-19-oate (methyl ester of **5**) was performed by the method of ref. [14].

Spectral data (IR, ^1H NMR, ^{13}C NMR and MS) of the methyl ester of **6** were coincident with those of methyl 15α-tigloyloxykaur-16-en-19-oate [3, 4].

Alkaline hydrolysis of the esters was carried out with 3 N KOH in 95% EtOH for 3 hr under reflux and work-up in the usual manner. Fraction 7 contained ursolic acid accompanied by unidentified substances.

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