Partial hydrolysis of **2** *and* **3**. To 10 mg **2** and **3** in 0.5 ml MeOH, 20 mg K_2CO_3 in H_2O were added. After 1 hr H_2O and dil H_2SO_4 were added. Extraction with $CHCl_3$ yielded a mixture which was separated by TLC ($CHCl_3$–Et_2O–MeOH, 13:6:1). In addition to unchanged **2** and **3**, 3 mg of **6** and **7** were isolated, colourless gum, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH); 1775 (lactone); 1725 (CO_2R); 1650 (C=C), ^1H NMR: see Table 1. MS *m/e* (rel. int.): 412.210 (M^+, 1%) and 414.225 (M^+, 0.3) ($C_{21}H_{32}O_8$ and $C_{21}H_{34}O_8$) 312 ($M - RCO_2H$, 31); 294 ($312 - H_2O$, 20); 85 ($C_4H_9CO^+$, 43); 83 ($C_4H_7CO^+$, 100); 57 ($85 - CO$, 66); 55 ($83 - CO$, 80).

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DITERPENES AND STEROLS FROM *WEDELIA GLAUCA*

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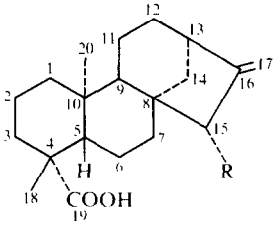
INTRODUCTION

Wedelia glauca (Ort.) Hoffmann ex Hicken (Compositae) is a perennial shrub widely distributed in Argentina, Brazil and Uruguay, and well known for its toxicity to cattle. Previous work on this species has not led to the isolation of any pure constituent, although Dominguez [1] and Burkart *et al.* [2] have reported the presence of a resin which could be responsible for the toxicity of the plant. Other species of the genus *Wedelia* have been studied previously, notably *W. calendulaceae* (L.) Less which has yielded wedelolactone [3].

The present work deals with the isolation and identification of higher alcohols, sterols, and the tetracyclic diterpenoids kaur-16-en-19-oic acid (**1**) and 15α-cinnamoyloxy-kaur-16-en-19-oic acid (**2**). **2** has been previously isolated from *Mikania oblongofolia* [4] and also reported as its methyl ester in *W. trilobata* [5].

RESULTS AND DISCUSSION

Chromatography of the petrol extract yielded fractions rich in higher alcohols, sterols, and tetracyclic diterpenes. One of the latter was characterized as (−)kaur-16-en-19-oic acid (**1**), from the ^1H NMR spectrum of the corresponding methyl ester [6]. From the same extract a diterpene ester was isolated and identified as the 15α-cinnamoyloxy-kaur-16-en-19-oic acid (**2**). The presence of the cinnamoyl group was evidenced by the ^1H NMR spectrum which showed an AB system at δ 6.45 and 7.70 (J_{AB} = 15 Hz) and a phenyl group at δ 7.38. The structure



1 R = H
2 R = OCOCH=CH–Ph

of **2** was confirmed by analysis of its alkaline hydrolysis products which were identified as 15α-hydroxy-kaur-16-en-19-oic acid [7] and cinnamic acid.

EXPERIMENTAL

Plant material. Whole plants of *W. glauca* were collected in Balcarce (province of Buenos Aires) during the flowering period. Voucher specimens were deposited in INTA (Balcarce) under No. 1768. Dried and milled plants (3 kg) were successively extracted with petrol (72 g of extract; 2.42% of dry plant) and EtOH (242 g

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of extract; 8.30% of dry plant). Large amounts of KCl (32 g) pptd spontaneously from the ethanolic extract and were filtered off. A portion of the petrol extract (30 g) was chromatographed on a Si gel column using CH_2Cl_2 and CH_2Cl_2–EtOH. Seven main fractions were collected which were purified by column chromatography and PLC.

Fraction 2. It was analysed by GC-MS (OV-101, 230 → 265°, 8°/min) detecting hexacosanol (C-26), *n*-octacosanol (C-28) and triacontanol (C-30) whose MS were coincident with those from authentic samples.

Fraction 3. It was rechromatographed on a Si gel column using C_6H_6 as eluent. Main fractions were crystallized from EtOH yielding (−)kaur-16-en-19-oic acid (**1**), mp 177–179°, $[\alpha]_D$ − 91° (*c* 2, CH_2Cl_2); MS (70 eV) *m/e* (%): 302 (M^+, 68), 287 (M − Me, 55), 284 (M − H_2O, 10), 259 (M − C_3H_7, 75), 257 (M − CO_2H, 25), 256 (M − HCO_2H, 10), 241 (M − HCO_2H − Me, 52), 121 (48), 109 (49), 91 (100); ^{1}H NMR ($CDCl_3$, 100 MHz): δ 0.98 (3 H, *s*, Me-20), 1.25 (3 H, *s*, Me-18), 1.58 (2 H, *m*, H-3), 2.04 (2 H, *br s*, H-15), 2.20 (1 H, *br s*, H-5), 2.64 (1 H, *br s*, H-13), 4.76 (1 H, *br s*, H-17), 4.81 (1 H, *br s*, H-17′); on irradiation of H-15, allylic partition disappears and the signals of H-17 appear as a doublet; ^{13}C NMR ($CDCl_3$, 25.2 MHz): ppm 15.61 (*q*, C-20), 18.47 (*t*, C-2), 19.13 (*t*, C-11), 21.88 (*t*, C-6), 28.93 (*q*, C-18), 29.69 (*t*, C-12), 33.10 (*t*, C-7), 37.83 (*t*, C-3), 39.73 (*s*, C-10), 40.81 (*t*, C-1), 41.36 (*d*, C-13), 43.89 (*s*, C-4), 44.32 (*t*, C-14), 49.06 (*t*, C-15), 55.23 (*d*, C-9), 57.19 (*d*, C-5), 64.90 (*s*, C-8), 103.04 (*t*, C-17), 155.83 (*s*, C-16), 184.54 (*s*, C-19).

Methyl kaur-16-en-19-oate. **1** was methylated with CH_2N_2 following the usual procedure. The methyl ester had mp 88–89° (MeOH–H_2O); $[\alpha]_D$ − 107° (*c* 2, CH_2Cl_2); IR (KBr) cm^{-1}: 1733 (C=O ester); MS (70 eV) *m/e* (%): 316 (M^+, 37), 301 (M − Me, 30), 286 (M − 2 × Me, 15), 273 (M − C_3H_7, 58), 257 (M − CO_2 Me, 61), 241 (55), 121 (68), 109 (63), 91 (100); ^{1}H NMR ($CDCl_3$): δ 0.84 (3 H, *s*, Me-20), 1.15 (3 H, *s*, Me-18), 2.09 (2 H, *br s*, H-15), 2.65 (1 H, *br s*, H-13), 3.64 (3 H, *s*, MeO . CO), 4.75 (2 H, *br s*, H-17).

Fraction 4. It yielded a yellow oil that was purified by PLC on Si gel GF_{254} using CH_2Cl_2 0.5% MeOH as solvent. Two main bands were obtained which were eluted with Me_2CO. The minor R_f band showed to be the 15α-cinnamoyloxy-kaur-16-en-19-oic acid (**2**) which could not be induced to crystallize; ^{1}H NMR ($CDCl_3$); δ 0.94 (3 H, *s*, Me-20), 1.19 (3 H, *s*, Me-18), 1.59 (2 H, *br s*, H-3), 2.80 (1 H, *br s*, H-13), 5.16 (2 H, *br s*, H-17), 5.80 (1 H, *br s*, H-15β), 6.45 and 7.70 (2 H, *dd*, *J* = 15 Hz, AB system), 7.38 (5 H, *m*, Ph).

Alkaline hydrolysis of **2**. **2** was hydrolysed with 5% KOH in EtOH at 100° for 1 hr. Upon acidification and the usual work up, cinnamic acid and 15α-hydroxy-kaur-16-en-19-oic acid were isolated and identified.

Fraction 5. It yielded a ppt of mp 144–145° (EtOH) that was identified as a mixture of stigmasterol and sitosterol by GC–MS (OV-17, 270°).

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