Pergamon

0031-9422(95)00905-1

Phytochemistry, Vol. 42, No. 2, pp. 479–484, 1996

0031-9422/96 $15.00 + 0.00

KAURANES AND RELATED DITERPENES FROM *ADENOSTEMMA BRASILIANUM*

ALICIA BARDÓN, SUSANA MONTANARO, CÉSAR A. N. CATALÁN, JESÚS G. DÍAZ† and WERNER HERZ*†

Instituto de Química Organica, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Ayacucho 491, 4000 S.M. de Tucumán, Argentina; †Department of Chemistry, The Florida State University, Tallahassee, FL 32306, U.S.A.

(*Received in revised form* 25 *October* 1995)

Key Word Index—*Adenostemma brasilianum*; Eupatorieae; Compositae; kauranes; modified kauranes; diterpenes.

Abstract—Aerial parts of *Adenostemma brasilianum* provided a number of new ent-kauranes oxygenated at C-1 and a modified abietane.

INTRODUCTION

Adenostemma is a pantropical genus of 24 species [1]. Previously studied members are South African *A. caffrum* [2] and *A. lavenia* from Taiwan [3] and Shizuoka, Japan [4] all of which furnished *ent*-11-α-hydroxy-19-kauranoic acids and their glycosides. As according to [1] *A. lavenia* is limited to Ceylon, the authors of [3] and [4] were possibly dealing with *A. viscosum* Forster and Forster which has the widest range in the paleotropics. In the present article, we describe the results of our study of *A. brasilianum* Cass. Our collection from northern Argentina contained the *ent*-kauranes **1–8a** and the modified abietane **9**. The eudesmane **10** was also found.

RESULTS AND DISCUSSION

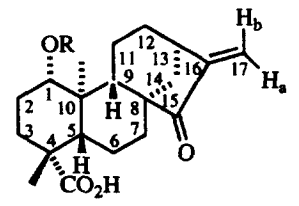
Hydroxy acids **1a** ($C_{20}H_{28}O_4$) and **2a** ($C_{20}H_{30}O_4$) were obtained in the form of a mixture which was converted to a mixture of acetates **1b** and **2b**. The ^{1}H NMR spectra of the two mixtures (see Experimental) exhibited the typical low field signals of **1a** and **1b**, respectively, owing to conjugated H-17a,b at δ 5.92 and 5.23 allylically coupled to H-13 at δ 3.05, whereas the presence of **2a** and **2b**, respectively, was indicated by another H-13 signal at higher field (δ 2.43) coupled to H-16 at δ 2.24 which was, in turn, coupled to the methyl doublet of H-17 at δ 1.07. Signals of appropriate multiplicity were also seen in the ^{13}C NMR spectrum of the **1b/2b** mixture; thus, the signals of C-13, C-15, C-16 and C-17 of **1b** appeared at δ 38.2, 210.2, 149.5 and 117.4 whereas those of **2b** were at δ 34.9, 224.2, 47.9 and 10.0. The chemical shift of C-17 in **2b** indicated that the methyl group on C-16 was *endo* to the 3, 2, 1 system of rings C and D, i.e. β in the configuration shown in the formulae.

The ^{1}H NMR spectrum of the **1a/2a** mixture also exhibited *dd*'s at δ 3.41 and 3.43 (J = 11, 4.5 Hz), respectively, which moved downfield to δ 4.59 and 4.61, respectively, in the spectrum of the **1b/2b** mixture and were characteristic of axial H under equatorial -OH at C-1, C-3 or C-7. Its location at C-1 was deduced as follows. In the ^{1}H NMR spectra of the two mixtures the H-18 signals appeared at the usual frequency (approximately δ 1.25) of *ent*-19-kauranoic acids [5] while the H-20 signals were shifted downfield to approximately δ 1.13 in the **1a/2a** mixture and δ 1.22 in the **1b/2b** mixture compared with the usual frequency of H-20 at δ 0.85–0.95, thus suggesting substitution on C-1. In the ^{13}C NMR spectrum of the **1b**, **2b** mixture the signal of the carbon under the acetate appeared at δ 83.8, more appropriate for equatorially acylated C-1 than for C-3 or C-7, and there was no triplet near δ 41 as required for C-6 if the acetate had been attached to C-7. Finally, while C-18 exhibited the usual shift near δ 28, the upfield shift of C-20 from the usual δ 15–16 to δ 12.2 confirmed attachment of the acetate function to C-1.

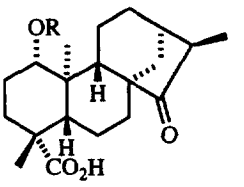
In the case of **3**, chemical shifts of H-1, H-18 and H-20 and the presence of an acetate singlet demonstrated that ring A was identical with that of **2b**. The ^{1}H NMR spectrum also showed the presence of an unconjugated methylene group as multiplets at δ 5.21 and 5.09 (H-17a,b) both of which were allylically coupled (J = 2.5 Hz) to a broadened multiplet at δ 4.53 (H-15 α under hydroxy; for a comparison see *inter alia* [6]) and to a *brdd* at δ 3.20 (H-12) whose coupling constants (J = 14.5 and J = 5 Hz) indicated that it was axially orientated and α.

Kauranes **4**, **5** and **6** exhibited very similar spectro-

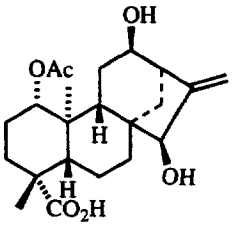
*Author to whom correspondence should be addressed.



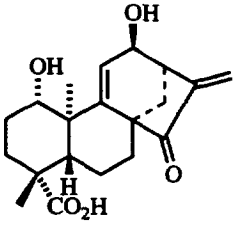
1a R=H
b R=Ac



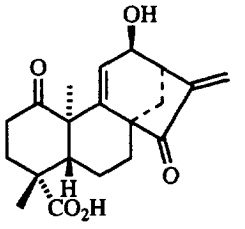
2a R=H
b R=Ac



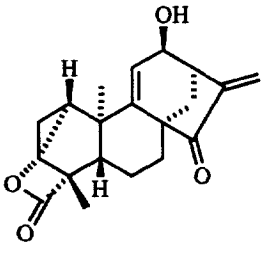
3



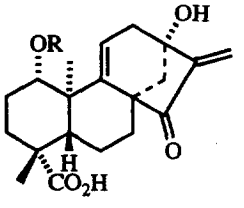
4



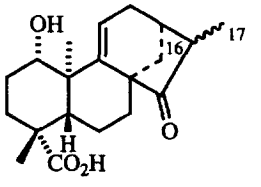
5



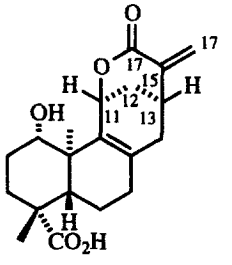
6



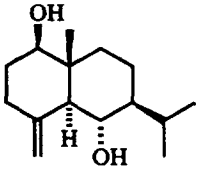
7a R=H
b R=Ac



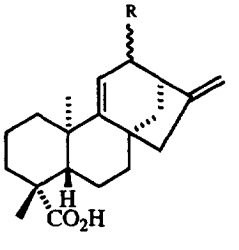
8a H-16β
b H-16α



9



10



11a R=β-OH
b R=α-OH
c R=α-OMe

scopic properties except for changes in ring A (Table 1). The α-orientated C-1 hydroxyl earlier found in **1a** and **2a** was also present in **4**, $C_{20}H_{26}O_5$, but was oxidized to a ketone group in **5**, $C_{20}H_{24}O_5$, thus producing changes in the chemical shifts and coupling constants involving H-2a,b and H-3a,b. Conversely, the empirical formula $C_{20}H_{24}O_4$ of **6**, together with the pronounced paramagnetic shift of H-1, now a doublet ($J = 5.5$ Hz) at δ 4.59, indicated the presence of a lactone function involving C-1 and C-18. In the somewhat strained Dreiding model of **6** the 1βH,2βH dihedral angle approximates 90°, thus accounting for the appearance of the H-1 signal as a doublet. Rings C and D were common to all three substances with $J_{11,12} = 2.5$ Hz, $J_{11,13} = 1$ Hz (W coupling), $J_{12,13} = J_{13,14b} = 5$ Hz and $J_{13,17a} = J_{13,17b} = 1$ Hz. In the case of **6**, a significant *gem* coupling (1 Hz) between H-17a and H-17b could also be observed.

The chemical shift of α-orientated H-12 in **4–6** (δ 4.55) differs considerably from the chemical shift of

Table 1. ^{1}H NMR spectra of compounds **4–6**, **7b**, **9** and **10a,b**

H	**4**(C_5D_5N)	**5**($CDCl_3$)	**6**($CDCl_3$)	**7b**(C_6D_6)	**9**($CDCl_3$)
1ax	3.74 *dd* (12, 4, 5)	–	4.59 *d* (5.5)	4.77 *dd* (11.5, 5)	3.74 *dd* (12, 5)
2ax	2.55 *c*	2.68 *ddd* (13.5, 8.5, 7)	1.95 *m*	1.96 *dddd* (14, 13, 11.5, 4)	1.98 *dddd* (14, 14, 12, 4)
2eq	1.95 *dddd* (14, 3, 3, 3)	2.37 *ddd* (14, 7, 7)	in 1.5–1.65 *m*	1.73 *dddd* (14, 5, 4.5, 4)	1.70 *dddd* (14, 5, 3, 3)
3ax	1.23 *ddd* (14, 14, 3)	1.59 *ddd* (14, 8, 8)	1.67 *ddd* (13.5, 9.5, 7)	0.83 *ddd* (14, 14, 4)	1.10 *ddd* (14, 14, 4)
3eq	2.45 *ddd* (13.5, 3, 3)	2.48 *ddd* (13.5, 6.5, 6.5)	in 1.5–165 *m*	2.01 *ddd* (13.5, 4.5, 4)	2.17 *ddd* (14, 4, 3)
5ax	2.06 *dd* (12, 4)	2.25 *dd* (12.5, 4)	2.19 *dd* (14.5, 5, 5)	1.89 *dd* (11.5, 2)	1.25 *dd* (11.5, 2)
6ax	2.55 *c*	1.86 *ddd* (13, 8, 5)	1.46 *dddd* (14, 12, 11, 2)	1.85 *dddd* (13, 13, 11.5, 5)	in 1.99–1.84 *c*
6eq	2.55 *c*	2.17 *dddd* (14, 9, 4, 4)	2.00 *m*	2.11 *m*	in 1.99–1.84 *c*
7a	2.23 *ddd* (14, 8, 8)	2.02 *ddd* (14, 8.5, 8.5)	172 *ddd* (14.5, 11, 5)	2.20 *ddd* (14, 10, 8)	in 1.99–1.84 *c*
7b	1.80 *ddd* (14, 6, 6)	1.63 *ddd* (14, 9, 4.5)	1.91 *m*	1.45 *ddd* (14, 6, 3)	in 1.99–1.84 *c*
11	7.16 *brd* (2.5, 1)	5.46 *brd* (2.5, 1)	5.20 *dq* (2.5, 1)	5.88 *dd* (4, 2.5)	5.44 *ddd* (3.5, 2, 1)
12α	4.94 *dd* (5, 2.5)	5.54 *dd* (5, 2.5)	4.56 *dd* (5.5, 2.5)	2.08 *ddd* (17.5, 5, 2)	1.81 *ddd* (13.5, 2.5, 2)
12β	–	–	–	2.50 *dd* (17.5, 2.5)	2.32 *dddd* (13.5, 3.5, 3.5, 1)
13	3.21 *brt* (5)	3.13 *brt* (5)	3.19 *ddq* (5, 2, 1)	–	3.04 *ddddq* (5.5, 3.5, 2.5, 2, 1)
14a	2.06 *d* (12)	1.97 *d* (12)	1.97 *d* (14)	1.66 *d* (11)	2.47† (18.1, 5.5)
14b	1.86 *dd* (12, 5)	1.91 *dd* (12, 5.5)	1.98 *dd* (14, 2)	1.18 *dd* (11, 2)	2.01‡ (18, 2, 1)
17a	6.31 *brs*	6.15 *brs*	6.19 *t* (1)	6.09 *brs*	6.52 *brt* (1)
17b	5.72 *brs*	5.58 *brs*	5.59 *t* (1)	5.31 *brs*	5.36 *t* (1)
18*	1.61 *s*	1.38 *s*	1.13 *s*	1.10 *s*	1.24 *s*
20*	1.47 *s*	1.26 *s*	1.10 *s*	1.24 *s*	0.98 *s*
Ac*				1.57 *s*	

*Intensity three protons; assignments by NOE spectrometry.

†H-14α.

‡H-14β.

H-12 (δ 3.95, $J_{11,12} = J_{12,13} = 4$ Hz) in a kaurane from *Montanoa pteropoda* originally assigned formula **11a** [7] and subsequently revised to **11b** because of an NOE between H-12 and one of the exomethylene protons in the corresponding methyl ether **12c** [8]. The same substance seems to have been isolated from *Stevia eupatoria* [9], although this was not recognized. Allowing for the differences in substitution in rings A and D, the ^{13}C NMR spectra of **4** and **5** on the one hand (Table 2), and **12b** and **12c** on the other [8, 9] also differ significantly. The partial NOE data listed for **5** in Table 3 which show the absence of an NOE between H-12 and H-17a,b further support the conclusion that the orientation of the 12-hydroxyl of **4–6** differs from that of **12b** and is β.

Kaurane **7a** was obtained only in admixture with an isomer subsequently shown to be **10a**. Acetylation of the mixture followed by TLC furnished a very small amount of **7b** and also a somewhat larger quantity of **10b**. The structure of **7b** was clear from the MS, the ^{1}H NMR data (Table 1) and extensive decoupling. The partial NOE data listed for **7b** in Table 4 identified the H-18 and H-20 signals and showed that the hydroxyl group in ring A was again on C-1 and α. Similarly, **8a** was contaminated with **9**; chemical shifts and coupling constants of H-1 and H-11 resembled those of **7a** but the signals of H-17a and H-17b were replaced by a three-proton doublet at δ 1.37. On standing in $CDCl_3$, **8a** was partially isomerized to **8b**, as shown by the appearance of additional signals emanating from H-1, H-12a,b and H-17. The chemical shift of the new C-17 doublet was close to that of C-17 in **2b**; consequently, we assume that C-17 of **8a** is α- and that of **8b** is β-orientated.

Table 2. ^{13}C NMR spectra of compounds **4**, **5** and **9** (67.89 MHz)

C	**4** (C_5D_5N)	**5**($CDCl_3$)	**9**($CDCl_3$)
1	77.6 *d*	210.2 *s*	74.9 *d*
2	28.6 *t*	34.6 *t*[a]	29.0 *t*
3	31.8 *t*	26.1 *t*	32.8 *t*
4	44.6 *s*	42.9 *s*	43.1 *s*[a]
5	45.1 *d*	43.5*d*	51.6 *d*
6	20.9 *t*	19.5 *t*	20.1 *t*
7	37.2 *t*	35.5 *t*[a]	30.1 *t*
8	51.7 *s*	52.9 *s*[b]	133.1 *s*
9	148.4 *s*	140.9 *s*	139.8 *s*
10	46.8*s*	50.7 *s*[b]	44.2 *s*[a]
11	131.1 *d*	129.7 *d*	71.5 *d*
12	71.5 *d*	70.0 *d*	41.4 *t*
13	48.8 *d*	46.8 *d*	31.8 *d*
14	43.5 *t*	41.9 *t*	35.5 *t*
15	205.2 *s*	203.9 *s*	136.8 *s*
16	146.6 *s*	143.3 *s*	165.9 *s*
17	120.5 *t*	121.7 *t*	129.6 *t*
18	29.4 *q*	26.5 *q*	28.2 *q*
19	180.4 *s*	187.1 *s*	182.1 *s*
20	17.6 *q*	20.9 *q*	13.4 *q*

[a,b]Assignments in same column with same superscript may be interchanged.

Table 3. Partial NOE difference spectrum of **5**

Irradiated	Observed (%)
H-11	H-12 (6.3), H-20 (5.6)
H-12	H-11 (13.7), H-13 (11.4), H-14a (5.8)
H-13	H-12 (11.4), H-14a,b (4.3), H-17b (7.8)
H-17a	H-17b (23)
H-17b	H-13 (6.5), H-17a (30.2)

Table 4. Partial NOE difference spectrum of **7b**

Irradiated	Observed (%)
H-1	H-2eq (2.4), H-5ax (5.0), H-11 (1.2)
H-2ax	H-2eq (10.3), H-20 (2.2)
H-2eq	H-1 (3.9), H-3eq + H-2ax (7.2)
H-3ax	H-1 (2.5), H-3eq (8.2), H-5 (1.2)
H-6eq + H-12α	H-5 + H-6ax (16.2), H-11 (2.0), H-12β (7.2), H-18 (2.3)
H-12β	H-11 (1.9), H-12α (22.9), H-14a (1.5)
H-18	H-6β (1.6)
H-20	H-11 (1.0), H-2ax (1.2), H-6ax (1.1)
Ac	H-11 (1.1)

The ^{1}H NMR spectrum of **9**, $C_{20}H_{26}O_5$, departed from the kaurane pattern of the previous compounds, although ring A again contained an α-orientated hydroxyl group on C-1 (Table 1). The presence in **9** of a δ-lactone carbonyl conjugated with an exocyclic methylene group and the presence of a tetrasubstituted double bond was indicated by an IR band at 1710 cm^{-1} superimposed on the carboxyl frequency and, in the ^{13}C NMR spectrum (Table 2), by singlets at δ 165.9 (C=O), 139.8, 136.8, 133.1 (C-8, C-9, C-15) and a triplet at δ 129.6 (C-17). Spin decoupling established the usual sequence H-1 through H-3 (H-5 through H-7 were bunched near δ 1.90) and an additional sequence involving a signal at δ 5.44 representing the proton under the lactone oxygen (H-11), H-12α at δ 1.81 and H-12β at δ 2.32, H-13 at δ 3.04 allylically coupled to H-17a,b and vicinally coupled to two geminally coupled protons at δ 2.47 (H-14α) and δ 2.01 (H-14β). The magnitude of $J_{14\alpha,14\beta}$ (18 Hz) supported the conclusion that the two protons were allylic with respect to the tetrasubstituted double bond and led to formula **9** for the new substance. W coupling between H-11 and H-13 and between H-12β and H-14β (1 Hz each) and a significant NOE between H-11 and H-20 (8.1%) were in accordance with a Dreiding model in which the lactone ring joining C-11 and C-13 was β. As abietanes are relatively rare in the Eupatorieae the oxygenation pattern of **9** and its co-occurrence with $\Delta^{9(11),16}$-kauran-15-ones suggests a possible biogenetic route to **9** which involves cleavage of the five-membered ring followed by or concomitant with an allylic rearrangement leading to a lactone ring closed toward C-11.

Although the absolute configurations of the kauranes isolated from *A. brasilianum* was not established we assume that they belong to the *ent*-series like other kauranes from species within Eupatorieae.

EXPERIMENTAL

General. For sepn of mixts, HPLC with monitoring by means of a differential refractometer was used. The columns employed were (A) Phenomenex Maxsil 10C8 (10 μm, 10 × 500 mm) and (B) Phenomenex Ultremex C18 (5 μm, 10 × 250 mm). R_t values were measured from the solvent peak.

Plant material. Aerial parts of *A. brasilianum* (Pers.) Cass. were collected at the flowering stage in May 1991 near Orán, Salta province, Argentina. A voucher specimen LIL #596531 is on deposit in the herbarium of the Instituto Miguel Lillo, Tucumán.

Extraction and isolation. Flowers and leaves (390 g) were extracted with $CHCl_3$ (2 × 2 l) at rt for 7 days. Removal of solvent *in vacuo* yielded 16 g of residue which was suspended in 142 ml EtOH at 55°, diluted with 106 ml H_2O and extracted successively with hexane (3 × 200 ml) and $CHCl_3$ (3 × 20 ml). Evapn of the $CHCl_3$ extract at red. pres. furnished 6.2 g residue which was chromatographed over silica gel using $CHCl_3$ and increasing amounts of EtOAc (0–100%) and finally with Me_2CO to give 78 frs. Frs 58–67 (combined wt 117 mg) were processed by HPLC using column A (MeOH–H_2O, 7:4, 2 ml min^{-1}) to give 5 mg of **11** (R_t 16 min), identified by MS and ^1H NMR spectrometry and comparison with authentic material, and unidentified mixtures. A 30 mg portion of frs 68–72 (combined wt. 707 mg) was processed by repeated HPLC (Column A, MeOH–H_2O, 4:3, 2 ml min^{-1}) to give 2.1 mg **6**. The remaining 677 mg were acetylated (Ac_2O–Py, overnight) and subjected to HPLC (column Al, MeOH–H_2O, 4:3, 2 ml min^{-1}) to give 15 mg of the mixture of **1a** and **2a** (R_t 26 min) and 36 mg of the mixture of **1b** and **2b** (R_t 51 min).

A 250 mg portion of frs 73–74 (total wt 1.50 g) was processed by HPLC (column B, MeOH–H_2O, 1:1, 2.5 ml/min) to give 47 mg of a mixture (R_t 25 min) containing **9** as minor and **8a** as major component and a peak (R_t 67 min) further purified by repeated HPLC (column A, MeOH–H_2O, 10:11, 2 ml min^{-1}) to give 4.6 mg of **9**. HPLC of fr. 75 (50 mg) using column A (MeOH–H_2O, 10:11, 2 ml min^{-1}) gave 1.5 mg of **3** (R_t 11 min) and complex mixtures.

CC of frs 76–77 (1.02 g) over silica gel using $CHCl_3$–EtOAc mixtures (0–100%) gave 24 frs HPLC (column A, MeOH–H_2O, 10:11, 1.7 ml min^{-1}) of frs 8–12 gave undefined material and 31 mg of **9** (R_t 35 min). HPLC of frs 13–19 (column A, MeOH–H_2O, 10:11, 2 ml min^{-1}) gave mixts followed by 9.1 mg of **5** (R_t 36 min). Frs 20–22 (56.8 mg), although showing a single spot on TLC, were a mixture of **7a** (minor constituent, significant signals at δ 6.30 (H-11, *dd*, J = 4, 2.5 Hz), 6.03 (*brs*, H-17a), 5.65 *brs*, H-17b) 3.24 (H-1, *dd*, J = 12.5) and a major constituent whose structure requires further study. Acetylation of the mixture (10 mg, 1 ml Ac_2O, 1 ml pyridine) for 1 hr followed by the usual work-up and TLC (C_6H_6–Me_2CO 7:3) yielded 1.5 mg of **7b** and 5 mg of the acetate of the unknown. Frs 23. HPLC (column A, MeOH–H_2O 10:11, 1.2 ml min^{-1}) of frs 23–24 (117 mg) provided a mixture of **7a** (major constituent) and the unknown (minor constituent). Elution of the main column with Me_2CO yielded fr. 78. Removal of solvent followed by trituration with $CHCl_3$ yielded 12 mg of $CDCl_3$-insoluble solid **4**.

Mixture of ent-1β-*hydroxykaur*-16-*en*-15-*one*-19-*oic acid and* ent-1β-*hydroxykaur*-16β(*H*)-15-*one*-19-*oic acid* (**1a** *and* **2a**). Gum; MS PCI (isobutane) *m/z* (rel. int.): 335 (34.1, $[M + H]^+$ of **2a**) 333 (74.4, $[M + H]^+$ of **1a**), 317 (100), 315 (78.2); IR ν^{film} cm^{-1}: 3400, 3100, 1720, 1690, 1640; ^1H NMR (500 MHz, $CDCl_3$): δ 5.92 and 5.23 (both *t*, J = 1 Hz, H-17a,b of **1a**), 3.41 and 3.39 (both *dd*, J = 15.5, 5 Hz, H-1 of **1a** and **2a**), 3.05 (*brq*, J = 1 Hz, H-13 of **1a**), 2.43 (*m*, H-13 of **2a**), 2.43 and 2.40 (both *d*, J = 12 Hz, H-14a of **1a** and **2a**), 2.23 (*quint*, J = 7 Hz, H-16 of **2a**), 1.92*m* (H-2ax of **1a** and **2a**), 1.61*m* (H-2eq of **1a** and **2a**), 1.43 and 1.39 (both *dd*, J = 12, 3 Hz, H-14b of **1a** and **2b**), 1.25*s* and 1.24*s* (each 3p, H-18 of **1a** and **2a**), 1.13*s* and 1.12*s* (each 3p, H-20 of **1a** and **2a**), 1.09 (*d*, J = 7 Hz, H-17 of **2a**).

Mixture of ent-1β-*acetoxykaur*-16-*en*-15-*one*-19-*oic acid and* ent-1β-*acetoxykaur*-16β(*H*)-15-*one*-19-*oic acid* (**1b** *and* **2b**). Gum; MS PCI (NH_3) *m/z* (rel. int.): 394 (100, $[M + NH_4]^+$ of **2b**), 392 (54.8, $[M + NH_4]^+$ of **1b**); IR ν^{film} cm^{-1}: 3200, 3030, 1720, 1695, 1645, 1240, 1030; ^1H NMR (500 MHz, $CDCl_3$): δ 5.92 and 5.24 (both *t*, J = 1 Hz, H-17a,b of **1b**), 4.61 and 4.59 (both *dd*, J = 11, 4.5 Hz, H-1 of **1b** and **2b**), 3.05 (*brs*, H-13 of **1b**), 2.42 (*m*, H-13 of **2b**), 2.41 (*d*, J = 11.5 Hz) and 2.37 (*d*, J = 12 Hz, H-14a of **1b** and **2b**), 2.24 (*quint*, J = 6.5 Hz, H-16 of **2b**), 1.99 and 1.98 (both *s*, 3p, Ac), 1.91 (*m*, H-2ax of **1b** and **2b**), 1.69 (*m*, H-2eq of **1b** and **2b**), 1.43 (*dd*, J = 12, 3 Hz, H-14b of **1b**), 1.39 (*dd*, J = 12, 3 Hz, H-14b of **2b**), 1.27 and 1.25 (both *s*, 3p, H-18), 1.22 and 1.20 (both *s*, 3p, H-20), 1.07 (3p *d*, J = 7 Hz, H-17 of **2b**); ^{13}C NMR spectrum (67.89 MHz $CDCl_3$) δ 224.2*s* (C-15 of **2b**), 210.2*s* (C-15 of **1b**), 181.8*s* (C-19 of both), 169.9*s* (C-21 of both), 149.5*t* (C-16 of **1b**), 114.4*t* (C-17 of **1b**), 83.8*d* (C-1 of both), 55.0 and 54.9 (both *d*, C-5 of both), 52.9 and 52.7 (both *s*, C-8 of both), 51.1*d* (C-9 of both), 47.9*d* (C-16 of **2b**), 44.3 and 44.0 (both *s*, C-4 and C-10 of both), 38.2*d* (C-13 of **1b**), 38.0, 37.2, 35.1, 34.8, 34.3 (all *t*, C-3, C-7, C-14 of both) 34.9*d* (C-13 of **2b**), 32.5*t* (C-12 of both), 28.7*q* (C-18 of both), 25.4 and 25.3 (both *t*, C-2 of both), 21.7*q* (C-22 of both), 20.1 and 20.0 (both *t*, C-11 of both 19.9 and 19.8 (both *t*, C-6 of both), 12.3*q* (C-20 of both), 10.0*q* (C-17 of **2b**).

ent-1β-*Acetoxy*-12α,15α-*dihydroxykaur*-16-*en*-19-*oic acid* (**3**). Gum; MS PCI (isobutane) *m/z* (rel. int.): 333 (100) $[M + H - C_2H_4O_2]^+$, 315 (74.8); ^1H NMR (500 MHz, $CDCl_3$): δ 5.21 (*br*, H-17a), 5.01 (*br*, H-17b), 4.53 (*brt*, J = 2.5 Hz, H-15β), 4.23 (*dd*, J = 11.5, 5 Hz, H-1ax), 3.20 (*brdd*, J = 14.5, 5 Hz, H-12), 2.73 (*m*, H-13), 2.38–2.29 (*c*, contains H-11a), 2.17 (*s*, 3p, Ac), 2.14 (*ddd*, J = 14, 3.5, 3 Hz, H-3eq), 2.09 (*brd*, J = 13 Hz, H-14a), 1.97 (*dddd*, J = 13.5, 13.5, 11.5, 3.5 Hz, H-2ax), 1.91–1.87 (*c*, contains H-9), 1.72

(*dddd*, $J = 13$, 13, 5, 3 Hz, H-11b coupled to H-13 by 3 Hz), 1.65 (*m*, H-2eq), 1.25 (*s*, 3p, H-18), 1.18 (*s*, 3p, H-20).

ent-1β,12α-*Dihydroxykaur*-9(11),16-*dien*-15-*one*-19-*oic acid* (**4**). Mp 203–205°, MS PCI (isobutane) *m/z* (rel. int.): 347 (13.4) $[M + H]^+$, 329 (100), 311 (36.3); IR (KBr) ν_{max} cm^{-1}; 3400, 1745, 1730, 1645, 1045; ^1H NMR (C_5D_5N): Table 1; ^1H NMR ($CDCl_3$ 500 MHz): δ 6.13 (*brs*, H-11), 6.11 (*brs*, H-17a), 5.54 (*brs*, H-17b), 4.55 (*dd*, $J = 5$, 2 Hz, H-12), 3.22 (*dd*, $J = 12$, 5 Hz, H-1ax), 3.06 (*brt*, $J = 5$ Hz, H-13), 2.16 (*ddd*, $J = 14$, 3, 3 Hz, H-3eq), 1.99 (*d*, $J = 12$ Hz, H-14a), 1.86 (*dd*, $J = 12$, 5.5 Hz, H-14b), 1.65 (*dddd*, $J = 13.5$, 3, 3, 3 Hz, H-2eq), 1.29 (*s*, 3p, H-18), 1.02 (*s*, 3p, H-20); ^{13}C NMR: Table 2.

ent-12α-*Hydroxykaur*-9(11),16-*dien*-1,5-*dione*-19-*oic acid* (**5**). Gum; PCI MS (NH_3) *m/z* (rel. int.): 362 (100) $[M + NH_4]^+$, 346 (20.6), 327 (39.9); IR ν^{film} cm^{-1}: 3350, 1705, 1640; ^1H NMR: Table 1; ^{13}C NMR: Table 2.

ent-12α-*Hydroxykaur*-9(11),16-*dien*-15-*one*-1β,19-*olide* (**6**). Gum; MS PCI (isobutane *m/z* (rel. int.): 329 (100) $[M + H]^+$, 311 (12.9), 283 (5.5), 267 (10.3), 259 (20.6); IR ν^{film} cm^{-1}: 3350, 1710; ^1H NMR: Table 1.

ent-1β-*Acetoxy*-13β-*hydroxy*-9(11),16-*dien*-15-*one*-19-*oic acid* (**7b**). Gum; MS PCI (NH_3) *m/z* (rel. int.): 406 (100) $[M + NH_4]^+$, 346 (11.6), 328 (11.0); ^1H NMR ($CDCl_3$, 500 MHz: δ 6.10 (*brs*, H-17a), 5.85 (*dd*, $J = 4$, 2.5 Hz, H-11), 5.66 (*brs*, H-17b), 4.47 (*dd*, $J = 11.5$, 5 Hz, H-lax), 2.70 (*dd*, $J = 17$, 2.5 Hz, H-12β), 2.00 (*d*, $J = 10.5$ hz, H-14a), 1.97 (*s*, 3p, Ac), 1.67 (*dd*, $J = 11$, 2 Hz, H-14b), 1.33 and 1.14 (each *s*, 3p, H-18 and H-20), 1.25 (*ddd*, $J = 13.5$, 13.5, 4.5 Hz, H-3ax); ^1H NMR (C_6D_6): Table 1. Prior to acetylation, the **7a**, **10a** mixture exhibited significant signals of the minor constituent **7a** at δ 6.31 (*t*, $J = 4.5$ Hz, H-11 deshielded by 1-OH), 6.03 (*brs*, H-17a), 5.65 (*brs*, H-17b), 3.24 (*dd*, $J = 11.5$, 4.5 Hz, H-1ax), 2.70 (*dd*, $J = 17$, 2.5 *z*, *H*-12β), 1.26 and 1.04 (each *s* and 3p, H-18 and H-20).

Mixture of ent-1β-*hydroxy*-15-*oxo*-16α(*H*)-*kaur*-9(11)-*en*-19-*oic acid* (**8a**) *and* **9**. Gum; MS PCI (NH_3) *m/z* (rel. int.): 366 ($[M + NH_4]^+$ of **8a**) 364 ($[M + NH_4]^+$ of **9**) (32.8), 348 (21.0), 331 (30.9); ^1H NMR: **8a** δ 5.41 (H-11), 3.72 (*dd*, $J = 12$, 5 Hz, H-1), 2.36 (*brdd*, $J = 18$, 5 Hz, H-12), 2.27 *ddd* ($J = 13.5$, 3, 3 Hz), 2.16 (*ddd* $J = 13.5$, 3, 3 Hz), 2.07-1.80 (*c*), 1.68 (*brd*, $J = 14$ Hz, H-14b), 1.37 (*d*, $J = 7$ Hz, 3p, α-orientated H-17), 1.27 (*d*, $J = 14$ Hz, H-14b), 1.23 (*s*, 3p, H 18), 1.10 (*ddd*), 14, 14, 4 Hz, H-3ax), 0.94 (*s*, 3, H-20). On standing in $CDCl_3$ new signals of **8b** appeared at δ 5.44 (*brs*, H-11), 3.85 (*dd*, $J = 11.5$, 5 Hz, H-1), 2.75 (*brdd*, $J = 18$, 12 Hz, H-12β), 1.50 (*dd*, $J = 12$, 4.5 Hz, H-14b), 1.23 (*s*, 3p, H-20), 1.17 (*d*, $J = 7$ Hz, 3p, β-orientated H-17).

ent-1β-*Hydroxyabieta*-8,15(17)-*dien*-11α,13α-*olide*-19-*oic acid* (**9**). Gum; MS PCI (NH_3) *m/z* (rel. int.): 364 $[M + NH_4]^+$ (65.2) 272 (89), 134 (100); MS PCI (isobutane): 347 $[M + H]^+$ (19.6), 329 (100); IR ν^{film} cm^{-1}: 3350, 1710, 1640; ^1H NMR spectrum in Table 1; ^{13}C NMR: Table 2.

Acknowledgement—Work in Tucumán was supported by grants from Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina and the Consejo de Investigaciones de la Universidad de Tucumán.

REFERENCES

1. King, R. M. and Robinson, H. (1987). *The Genera of the Eupatorieae (Asteraceae). Monographs in Systematic Botany, Missouri Botanical Garden*, **22**, 58.
2. Bohlmann, F. and Mahanta, P. K. (1978) *Phytochemistry* **17**, 814.
3. Cheng, P. C., Hufford, C. D. and Doorenbos, N. J. (1979) *J. Nat. Prod.* **42**, 183.
4. Shimizu, S., Miyase, T., Umehara, K. and Ueno, A. (1990). *Chem. Pharm. Bull.* **38**, 1308.
5. Herz, W. and Sharma, R. P. (1976) *J. Org. Chem.* **41**, 1021.
6. Zdero, C. and Bohlmann, F. (1989) *Phytochemistry* **28**, 2745.
7. Bohlmann, F. and Le Van, N. (1978) *Phytochemistry* **17**, 1957.
8. Ahmed, M., Jakupovic, J. and Castro, V. (1991) *Phytochemistry* **30**, 1712.
9. Ortega, A., Morales, F. J. and Salmón, M. (1985) *Phytochemistry* **24**, 1850.