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THREE *ENT*-KAURENE DITERPENES FROM *VELLOZIA CAPUT-ARDEAE*

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Key Word Index—*Vellozia caput-ardeae*; Velloziaceae; *ent*-kaurene diterpenes.

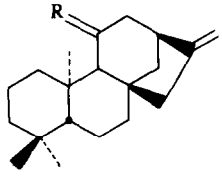
Abstract—Three new *ent*-kaurene diterpenes have been isolated from the roots and stem of *Vellozia caput-ardeae*. Their structures were elucidated by spectroscopic methods as *ent*-9β-hydroxy kaur-16-ene, *ent*-11α-hydroxy kaur-16-ene and *ent*-9β,11α-dihydroxy kaur-16-ene.

INTRODUCTION

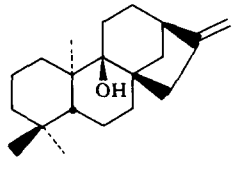
In previous papers [1, 2] we described two kaurene diterpenes and one *seco*-kaurene from *Vellozia caput-ardeae*. In the present communication we report the isolation and structural determination of three hydroxylated kaurenes from the same species.

RESULTS AND DISCUSSION

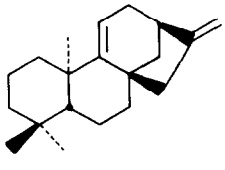
The molecular formulae of the isomeric alcohols **1a** and **2a** ($C_{20}H_{32}O$) and the diol **3a** ($C_{20}H_{32}O_2$) were determined by high-resolution mass spectrometry. The ^{1}H NMR spectra of each of the three compounds contained resonances assigned to three tertiary methyl groups and an exocyclic methylene which suggested that they were tetracyclic diterpenes, probably of the kaurene class.



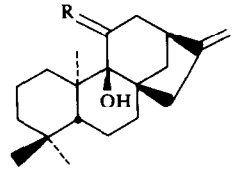
1a R = H, αOH
1b R = H, αOAc
1c R = O



2a



2b



3a R = H, αOH
3b R = H, αOAc
3c R = O

The oxygen-containing functional group of **1a** was shown to be a secondary hydroxyl group by IR (ν_{max}^{KBr} cm^{-1}: 3450 and 1060) and by the presence of a triple doublet at δ 4.28 (1H, J = 10, 8 and 8 Hz) in the ^{1}H NMR spectrum. This signal was shifted downfield to δ 5.37 upon acetylation of **1a** to **1b**. The multiplicity of this signal (*ddd*) indicated the vicinity of methine and methylene units. This limited the possible positions for the hydroxyl group to C-6 or C-11. The C-6 position was eliminated by analysis of the ^{13}C NMR spectra (Table 1). In the spectrum for kaurene, the triplets for C-2, C-6 and C-11 are found near δ 20 while the triplets for C-7 and C-12 appear near δ 40 and 30, respectively [3]. As the ^{13}C NMR spectrum of **1a** exhibited only two triplets near δ 20 and absence of a triplet near 30, it was clear that C-11 was oxygenated. This was further corroborated by the spectroscopic properties of the ketone **1c** derived from **1a**. The ^{13}C NMR signals for C-9 and C-12 showed the expected downfield shifts (Table 1) for **1c** compared to **1a**. Compound **1a** was, thus, identified as *ent*-11α-hydroxykaur-16-ene.

Compound **2a** was found to be a tertiary alcohol: IR ν_{max} cm^{-1}: 3500; ^{13}C NMR singlet at δ 77.6, and the absence in the ^{1}H NMR spectrum of signals which could be assigned to a carbinolic proton.

Further evidence for the location of the hydroxyl group at C-9 was the dehydration product **2b** of **2a**, whose ^{1}H NMR spectrum exhibited a triplet at δ 5.14 (1H, J = 4 Hz) [4]. Comparison of the ^{13}C NMR spectra of **2a** and kaurene [3] confirmed the above deductions. The C-9 hydroxyl group in **2a** caused an up-field shift in the resonances assigned to C-1, C-5, C-7 and C-15 through the strong γ-effects within the cyclic system [5, 6]. The chemical shift of C-20, as expected, was unaltered in comparison to the model compound. This data also confirmed the stereochemistry assigned to **2a**. Compound **2a** was, therefore, identified as *ent*-9β-hydroxy-kaur-16-ene.

The ^{1}H NMR spectrum of compound **3a** showed two signals at δ 1.62 and 2.10 (both exchangeable with D_2O). This data determined that **3a** was a diol. One of the hydroxyl groups was secondary, as shown by a double doublet at δ 3.96 (1H, J = 10 and 8 Hz) which moved downfield to 4.94 upon acetylation of **3a** to **3b**. The

Table 1. ^{13}C NMR chemical shifts of kaurene diterpenoids*

	1a	1b	1c	2a	3a	3b	3c
C-1	42.3†	40.5	40.2	36.5	37.0	36.9	35.5
C-2	18.5	18.7	18.2	18.6	18.7	18.8	18.3
C-3	42.2†	41.4	41.6	41.6	42.2	41.0	41.4
C-4	33.6	33.5	33.4	33.3	33.8	33.9	33.7
C-5	56.9	56.3	55.5	48.0	50.1	48.3	46.6
C-6	20.2	20.2	20.0	20.1	20.1	20.0	20.1
C-7	42.0†	38.6	39.1	34.6	35.3	36.9	35.5
C-8	45.9	46.1	44.7	49.0	50.6	51.6	50.7
C-9	60.4	57.8	72.4	77.6	80.9	78.2	84.6
C-10	40.9	40.9	38.6	43.8	45.4	45.7	43.2
C-11	70.7	72.2	212.2	29.1	78.6	81.9	214.5
C-12	43.2	41.7	52.9	32.3	42.7	40.2†	52.2
C-13	39.6	43.1	43.1	42.3	40.7	41.9	43.2
C-14	41.8†	39.5	39.1	40.5	41.5	40.7†	42.8†
C-15	48.3	47.9	48.6	43.8	43.3	43.3	42.9†
C-16	154.8	153.9	152.2	155.0	154.4	154.1	152.3
C-17	103.1	104.1	106.6	102.8	102.9	103.7	106.6
C-18	34.1	33.9	33.6	33.7	34.2	33.5	33.4
C-19	21.8	21.7	21.7	21.8	22.0	21.8	21.9
C-20	18.7	18.8	18.6	19.2	19.3	19.3	20.5
(OAc)CO	—	170.3	—	—	—	173.7	—
(OAc)Me	—	20.2	—	—	—	21.6	—

*Values are in δ (ppm) downfield from TMS in $CDCl_3$.
†Signals may be reversed.

presence of a tertiary hydroxyl group was confirmed by the ^1H NMR spectra of **3b** and the ketone **3c**, obtained from **3a** by oxidation.

The position of the tertiary hydroxyl group was determined to be at C-9, from the ^{13}C NMR chemical shifts of C-1, C-5, C-7 and C-15 in comparison with the spectrum of **2a**.

The multiplicity of the signal assigned to the carbinolic proton, a double doublet, limited the location of the secondary hydroxyl group to C-1, C-7 or C-11. Positions C-1 and C-7 were eliminated by analysis of the ^{13}C NMR spectra of compounds **3a**–**3c**.

As regards the stereochemistry of two substances, **1a** and **3a**, the magnitude of the coupling constants for the carbinolic proton at C-11 in these substances and their derivatives (**1b** and **3b**) made it obvious that this hydroxyl group was equatorial. Compound **3a** was, thus, shown to be *ent*-9β,11α-dihydroxy-kaur-16-ene.

EXPERIMENTAL

Mps are uncorr. IR spectra were recorded on a Perkin-Elmer 137B. ^1H and ^{13}C NMR spectra at 100 and 25.2 MHz, respectively, and chemical shifts [δ (ppm)] measured from TMS as int. standard. Optical rotation was determined in $CHCl_3$ on a Perkin-Elmer 241.

Isolation of ent-11α-*hydroxy kaur*-16-*ene* (**1a**) ent-9β-*hydroxy kaur*-16-*ene* (**2a**) and ent-9β,11α-*dihydroxy kaur*-16-*ene* (**3a**). Chromatography of the hexane extract (51 g) of roots, stems and leaf sheaths of *Vellozia caput-ardeae*, collected in Diamantina, State of Minas Gerais, Brazil, yielded **1a**, mp 106–108°, 0.29% of dry plant wt; IR ν_{max}^{KBr} cm^{-1}: 3450, 2940, 1650, 1060 and 880. ^1H NMR (100 MHz, $CDCl_3$): δ 0.84 (3H, *s*), 0.86 (3H, *s*), 1.22 (3H, *s*), 1.46 (1H, *br s*, exchangeable with D_2O), 4.28 (1H, *ddd*, J = 10, 8 and 8 Hz), 4.70 (1H, *br s*) and 4.82 (1H, *br s*). ^{13}C NMR (25.2 MHz, $CDCl_3$): δ 18.5 (*t*), 18.7 (*q*), 20.2 (*t*), 21.8 (*q*), 33.6 (*s*), 34.1 (*q*), 39.6 (*d*), 40.9 (*s*), 41.8 (*t*), 42.0 (*t*), 42.0 (*t*), 42.3 (*t*), 43.2 (*t*), 45.9 (*s*), 48.3 (*t*), 56.9 (*d*), 60.4 (*d*), 70.7 (*d*), 103.1 (*t*) and 154.8 (*s*). MS *m/z* (rel. int.): 228 $[M]^+$ (46), 273 (30), 270 (14), 255 (17), 163 (12), 146 (9), 137 (32), 123 (100), 109 (31), 107 (40), 91 (51), 69 (59), 55 (47) and 41 (86). Found *m/z* 288.2462, $C_{20}H_{32}O$ requires 288.2445.

Acetylation of **1a**. Ac_2O (2 ml) was added to a soln of **1a** (60 mg) in C_5H_5N (2 ml). The mixture was left for 12 hr at room temp., followed by the usual work-up. Recrystallization of the crude product from hexane and EtOAc yielded **1b** (50 mg), mp 96–98°. IR ν_{max}^{KBr} cm^{-1}: 1725, 1235 and 890. ^1H NMR (100 MHz, $CDCl_3$): δ 0.84 (3H, *s*), 0.86 (3H, *s*), 1.26 (3H, *s*), 2.02 (3H, *s*), 4.76 (1H, *br s*), 4.86 (1H, *br s*) and 5.37 (1H, *ddd*, J = 12, 6 and 6 Hz). ^{13}C NMR (25.2 MHz, $CDCl_3$): δ 18.7 (*t*), 18.8 (*q*), 20.2 (*t*), 20.2 (*q*), 21.7 (*t*), 33.5 (*s*), 33.9 (*q*), 38.6 (*t*), 39.5 (*t*), 40.5 (*t*), 40.9 (*s*), 41.4 (*t*), 41.7 (*t*), 43.1 (*d*), 46.1 (*s*), 47.9 (*t*), 56.3 (*d*), 57.8 (*d*), 72.2 (*d*), 104.1 (*t*), 153.9 (*s*) and 170.3 (*s*). MS *m/z* (rel. int.): 330 $[M]^+$ (2), 315 (4), 288 (18), 270 (23), 255 (28), 163 (8), 146 (30), 136 (52), 109 (29), 91 (41), 81 (43), 69 (60), 55 (50), 43 (100) and 41 (62).

Oxidation of **1a**. Compound **1a** (120 mg) was treated with pyridinium chlorochromate (140 mg) in dry CH_2Cl_2 (2 ml) at room temp. for 2 hr followed by the usual work-up, to yield **1c** (100 mg), mp 112–113°. IR ν_{max}^{KBr} cm^{-1}: 2940, 1700, 1650 and 890. ^1H NMR (100 MHz, $CDCl_3$): δ 0.84 (3H, *s*), 0.90 (3H, *s*), 1.08 (3H, *s*), 2.46 (3H, *m*), 2.92 (1H, *br s*), 4.82 (1H, *br s*) and 4.92 (1H, *br s*) ^{13}C NMR (25.2 MHz, $CDCl_3$): δ 18.2 (*t*), 18.6 (*q*), 20.0 (*t*), 21.7 (*q*), 33.4 (*s*), 33.6 (*q*), 38.6 (*s*), 39.1 (*t*), 39.1 (*t*), 40.2 (*t*), 41.6 (*t*), 43.1 (*d*), 44.7 (*s*), 48.6 (*t*), 52.9 (*t*), 55.5 (*d*), 72.4 (*d*), 106.6 (*t*), 152.2 (*s*) and 212.2 (*s*). MS *m/z* (rel. int.): 286 $[M]^+$ (56), 271 (28), 149 (81), 123 (56), 105 (41), 91 (63), 55 (55) and 41 (100). CD (*c* 2.0 $\times 10^{-4}$ g/ml, cyclohexane): $[\theta]_{350}$ 0, $[\theta]_{333}$ +2994, $[\theta]_{321}$ +6273, $[\theta]_{310}$ +6986, $[\theta]_{300}$ +5845, $[\theta]_{260}$ 0.

ent-9β-Hydroxy-kaurene (**2a**). Mp 84–85°, 0.3% dry plant wt. IR ν_{max}^{KBr} cm^{-1}: 3500, 2940, 1640 and 880. ^1H NMR (100 MHz,

$CDCl_3$): δ 0.85 (3H, *s*), 0.90 (3H, *s*), 1.17 (3H, *s*), 3.50 (1H, *s*, exchangeable with D_2O) and 4.80 (2H, *br s*). ^{13}C NMR (25.2 MHz, $CDCl_3$): δ 18.6 (*t*), 19.2 (*q*), 20.1 (*t*), 21.8 (*q*), 29.1 (*t*), 32.3 (*t*), 33.3 (*s*), 33.7 (*q*), 34.6 (*t*), 40.5 (*t*), 41.6 (*t*), 42.3 (*d*), 43.8 (*s*), 43.8 (*t*), 48.0 (*d*), 49.0 (*s*), 77.6 (*s*), 102.8 (*t*) and 155.0 (*s*). MS *m/z* (rel. int.): 288 $[M]^+$ (60), 270 (36), 255 (48), 163 (36), 146 (56), 136 (66), 123 (84), 109 (62), 91 (70), 69 (88), 55 (76) and 41 (100). Found *m/z* 288.2456, $C_{20}H_{32}O$ requires 288.2445.

Dehydration of **2a**. To a soln of **2a** (50 mg) in C_5H_5N (3 ml) was added $POCl_3$ (1 ml). The reaction mixture was allowed to stand overnight, and work-up gave a colourless oil of **2b** (10 mg). ^1H NMR (60 MHz, $CDCl_3$): δ 0.84 (3H, *s*), 0.92 (3H, *s*), 1.06 (3H, *s*), 4.76 (1H, *br s*), 4.88 (1H, *br s*) and 5.14 (1H, *t*, $J = 4$ Hz). MS *m/z* (rel. int.): 270 $[M]^+$ (14), 255 (55), 136 (24), 105 (32), 91 (53), 81 (26), 69 (54), 55 (63) and 41 (100).

ent-9β,11α-*Dihydroxy-kaurene* (**3a**). Mp 162–163°, 0.36% dry plant wt. IR ν_{max}^{KBr} cm^{-1}: 3450, 2940, 1655 and 880. ^1H NMR (100 MHz, $CDCl_3$): δ 0.86 (3H, *s*), 0.90 (3H, *s*), 1.34 (3H, *s*), 1.62 (1H, exchangeable with D_2O), 2.10 (1H, exchangeable with D_2O), 3.96 (1H, *dd*, $J = 10$ and 8 Hz), 4.72 (1H, *br s*) and 4.82 (1H, *br s*). ^{13}C NMR (25.2 MHz, $CDCl_3$): δ 18.7 (*t*), 19.3 (*q*), 20.1 (*t*), 22.0 (*q*), 33.8 (*s*), 34.2 (*q*), 35.3 (*t*), 37.0 (*t*), 40.7 (*d*), 41.5 (*t*), 42.2 (*t*), 42.7 (*t*), 43.3 (*t*), 45.4 (*s*), 50.2 (*d*), 50.6 (*s*), 78.6 (*d*), 80.9 (*s*), 102.9 (*t*) and 154.4 (*s*). MS *m/z* (rel. int.): 304 $[M]^+$ (4), 286 (42), 271 (30), 163 (23), 151 (22), 136 (40), 123 (98), 109 (31), 91 (51), 81 (59), 69 (73), 55 (63) and 41 (100).

$$[\alpha]_{24°}^{\lambda} = \frac{\begin{matrix}589 & 578 & 546 & 436 & 365\end{matrix}}{\begin{matrix}-19.8 & -20.5 & -23.1 & -35 & -37\end{matrix}}\ \text{nm}\ (c\ 0.80).$$

Found *m/z* 304.2382, $C_{20}H_{32}O_2$ requires 304.2394.

Acetylation of (**3a**). Compound **3a** was acetylated using the same procedure described for **2** above. Compound **3b** was recrystallized from a mixture of hexane and EtOAc, mp 140–142°. IR ν_{max}^{KBr} cm^{-1}: 3510, 2940, 1720, 1230 and 885. ^1H NMR (100 MHz, $CDCl_3$): δ 0.85 (3H, *s*), 0.88 (3H, *s*), 1.32 (3H, *s*), 2.08 (3H, *s*), 3.98 (1H, *s*, exchangeable with D_2O), 4.78 (1H, *br s*), 4.86 (1H, *br s*) and 4.94 (1H, *dd*, $J = 12$ and 8 Hz). ^{13}C NMR (25.2 MHz, $CDCl_3$): δ 18.8 (*t*), 19.3 (*q*), 20.0 (*t*), 21.6 (*q*), 21.8 (*q*), 33.5 (*q*), 33.9 (*s*), 36.9 (*t*), 36.9 (*t*), 40.2 (*t*), 40.7 (*t*), 41.0 (*t*), 41.9 (*d*), 43.3 (*t*), 45.7 (*s*), 48.3 (*d*), 51.6 (*s*), 78.2 (*s*), 81.9 (*d*), 103.7 (*t*), 154.1 (*s*) and 173.7 (*s*). MS *m/z* (rel. int.): 346 $[M]^+$ (2), 331 (1), 286 (26), 271 (7), 253 (12), 215 (7), 189 (13), 163 (12), 149 (25), 136 (32), 123 (71), 109 (31), 91 (29), 81 (41), 69 (63), 55 (5), 43 (100) and 41 (63).

Oxidation of **3a**. This was carried out using the same procedure described for **2a**. Compound **3c**, mp 144–145°, IR ν_{max}^{KBr} cm^{-1}: 3570, 2940, 1695, 1650 and 880. ^1H NMR (100 MHz, $CDCl_3$): δ 0.86 (3H, *s*), 0.92 (3H, *s*), 1.24 (3H, *s*), 3.82 (1H, *s*, exchangeable with D_2O) and 4.86 (2H, *br d*). ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.3 (*t*), 20.1 (*t*), 20.5 (*q*), 21.9 (*q*), 33.4 (*q*), 33.7 (*s*), 35.5 (*t*), 41.4 (*t*), 42.8 (*t*), 42.9 (*t*), 43.2 (*s*), 43.2 (*d*), 46.6 (*d*), 50.7 (*s*), 52.2 (*t*), 84.6 (*s*), 106.6 (*t*), 152.3 (*s*) and 214.5 (*s*). MS *m/z* (rel. int.): 302 $[M]^+$ (100), 220 (15), 206 (9), 178 (15), 165 (35), 146 (29), 123 (26), 109 (15), 91 (20), 69 (26), 55 (21) and 41 (35).

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