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DITERPENOIDS AND A SESQUITERPENE LACTONE FROM *VIGUIERA LADIBRACTATE*

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Key Word Index *Viguiera ladibractate*; Compositae; diterpenoids; sesquiterpene lactone; ladibranolide.

Abstract—Eight known diterpene acids, *ent*-12-oxokaur-9(11),16-dien-19-oic acid, *ent*-12β-hydroxykaur-9(11),16-dien-19-oic acid, *ent*-isokaur-15(16)-en-17,19-dioic acid, *ent*-15α,16-epoxy-17-hydroxykaura-19-oic acid, *ent*-kaura-17,19-dioic acid, *ent*-kaur-16-en-19-oic acid, grandifloric acid, angeloyloxygrandifloric acid, as well as a new sesquiterpene lactone, ladibranolide, were isolated from *Viguiera ladibractate*. The stereochemistry of the sesquiterpene lactone was established by NOE experiments.

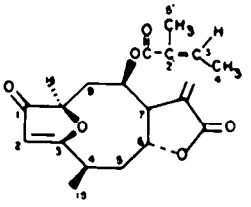
INTRODUCTION

Members of the genus *Viguiera* (tribe Heliantheae, Compositae) have previously yielded diterpenoids, sesquiterpene lactones and triterpenoids [1–6]. Here we report from another species *V. ladibractate* from Mexico eight known diterpene acids and one new sesquiterpene lactone.

RESULTS AND DISCUSSION

The concentrate from the dichloromethane extract of the aerial parts of *V. ladibractate* yielded, after column chromatography over silica gel and Sephadex LH-20, eight known diterpene acids (**2**–**9**) and one new sesquiterpene lactone (**1**).

The electron impact mass spectrum of compound **1** exhibited a strong molecular ion peak at *m/z* 360 (86%) corresponding to $C_{20}H_{24}O_6$. A characteristic IR absorption band at 1760 cm^{-1} and ^1H NMR data (δ6.36 and 5.71, doublets, J = 3.2 and 2.8 Hz, respectively) confirmed the presence of an α-methylene γ-lactone functional group. An IR ester absorption peak at 1720 cm^{-1} and the characteristic MS fragmentations at *m/z* 83 (100%) and 55 (90%), together with the ^1H NMR signals (Table 1) at δ6.11 (1H, *qq*), 1.94 (3H, *dq*), 1.81 (3H, *dq*) confirmed an angelate side chain. The ^{13}C NMR data (Table 2) indicating one ketone carbonyl group, two ester carbonyl groups and three double bonds were in accord with **1** being a furanoheliangolide with an angelate ester side chain. Spin decoupling experiments confirmed all signal assignments. The C-4 methyl signal (δ1.38) shifted further downfield than expected. But when a spectrum was recorded in C_6D_6, this signal appeared at δ0.75, thus confirming no oxygen function is attached to the C-4 (see Table 1). Comparison of the ^1H NMR data of **1** with those reported indicated that **1** differed from zexbrevin [1] only in their ester side chains. The ^1H NMR data of **1** were also different from those of zexbrevanolide [7]. The signal for H-8 in **1** appeared at δ5.21 (double doublet, J = 2.8, 4 Hz) and the signals for H-8 in zexbrevanolide appeared at δ4.53 (*ddd*, J = 2, 4, 10 Hz). Other signal differences between **1** and zexbrevanolide were also observed for H-2, H-6, H-7 etc. Since **1** and zexbrevanolide were not the same, **1** was most likely the C-4, C-8 stereoisomer of zexbrevanolide. This was finally confirmed by NOE difference spectra (500 MHz) of **1**: irradiation of the signal for H-6 increased



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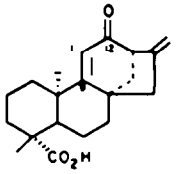

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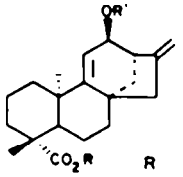
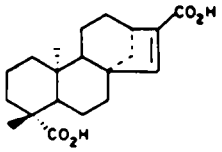


	R	R'
3	H	H
3a	Me	H
3b	H	Ac



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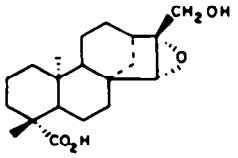

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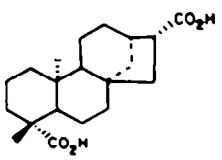

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signals for H-15, H-2 and H-5β. Irradiation of the signal for H-15 increased greatly the signal for H-2 and also the signals for H-6 and H-5β. Thus, a C-4β methyl orientation was confirmed. Another irradiation of the signal for H-2 increased signals for H-4', H-15 and H-6. A Dreiding model indicated the spatial proximity between H-2 and H-4' when the angelate is β-substituted at C-8. Therefore, the structure of **1** was established and we name it ladibranolide.

Table 1. ^{1}H NMR data of ladibranolide (200 MHz, TMS)

H	$CDCl_3$	C_6D_6*
2	5.55 *br s*	5.24
4	3.05 *br dq* (7, 7)	2.22
5a	2.61 *dd d* (7, 9, 14)	2.00
5b	2.06 *br d* (14)	1.60
6	4.54 *dd* (5, 9)	4.45
7	3.27 *m*	2.63
8	5.21 *br dd* (2.8, 5)	5.00
9a	2.72 *dd* (5, 15)	2.57
9b	2.25 *dd* (2.6, 15)	1.48
13a	6.36 *d* (3.20)	6.25
13b	5.71 *d* (2.8)	5.10
14	1.40 *s*	1.10
15	1.38 *d* (7)	0.75
3'	6.11 *qq* (2, 7)	5.03
4'	1.94 *dq* (1.5, 7)	1.90
5'	1.81 *dq* (2, 1.5)	1.78

*Coupling pattern and coupling constants are the same as those in the preceding column.

Compound **2** was previously reported [8,9] Identification of its structure was supported by com parison of ^{1}H NMR, IR, and MS data with thos previously reported [8–10], as well as by the ^{13}C NMF data first reported here.

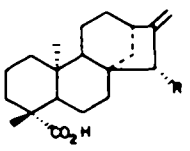
Compound **3** could be easily identified by its character istic signals at δ5.17, 5.07 (each one proton, broadene singlet, H-17) and 4.40 (one proton, broadened triplet, H 12α) and a broadened vinylic proton doublet signal a δ5.72. Compound **3** was reported from several origin [8, 11–13] and all of them referred to the first isolation o **3a** [13]. However, we found that the signal for H-13 wa reported at a lower field than expected [13]. Therefore, was methylated to give **3a**. In the ^{1}H NMR spectrum of **3a** the signal for H-13 appeared at δ2.83 (multiplet) and th signal for H-12α appeared at δ3.94 (*br t*, J = 4 Hz). Thes assignments were confirmed by double resonance experi ments: irradiation of the signal for H-11 (δ5.36, *br d* collapsed the signal at δ3.94 into a doublet and als collapsed a little bit of the signal at δ2.83. Irradiation of th signal at δ3.94 collapsed the signal at δ5.36 into broadened singlet and also collapsed the signal for H-13 a δ2.83 into a broadened doublet. Another irradiation of th signal for H-13 at δ2.83 collapsed the signal at δ3.94 into doublet and the signal at δ5.36 into a sharp double (models indicated a *W*-coupling between H-11 and H-1 for the *ent*-kaurene skeleton). The well-separated signal of H-17a and H-17b indicated most likely a 12β-hydroxy group which was easily acetylated to give **3b**. The pre viously unreported ^{13}C NMR data presented here were i accord with the structure of **3**.

Compound **4** was previously isolated as its methyl este [14, 15]. The downfield shifted vinylic proton signal a δ6.81 (singlet) made it unambiguously assignable to th β-proton of a conjugated carbonyl group. The previousl

Table 2. ^{13}C NMR data of compounds **1**–**4** (**1**, **2** and **4** were recorded at 125.7 MHz and **3** was recorded at 22.6 MHz, TMS)*

Compound	**1** ($CDCl_3$)	**2** ($CDCl_3$)	**3** (C_5D_5N)	**4** (C_5D_5N)
C-1	205.13 *n*	40.35 *n*	41.17 *t*	41.02 *n*
2	103.30 *p*	19.81 *n*	20.68 *t*	19.04 *n*
3	192.00 *n*	39.68 *n*	39.08 *t*	38.88 *n*
4	31.51 *p*	45.40 *n*	43.51 *s*	43.95 *n*
5	43.01 *n*	58.04 *p*	49.62 *d*	56.63 *p*
6	74.90 *p*	18.33 *n*	18.86 *t*	21.17 *n*
7	51.86 *p*	48.48 *n*	47.41 *t*	43.63 *n*
8	73.68 *p*	44.78 *n*	44.74 *s*	50.64 *n*
9	41.11 *n*	181.20 *n*	158.81 *s*	46.34 *p*
10	88.30 *n*	37.89 *n*	39.08 *s*	38.68 *n*
11	139.39 *n*	120.08 *p*	118.82 *d*	19.80 *n*
12	168.62 *n*	200.78 *n*	72.71 *d*	25.91 *n*
13	123.48 *n*	45.25 *p*	46.24 *d*	41.16 *p*
14	22.92 *p*	44.20 *n*	40.78 *t*	40.30 *n*
15	16.06 *p*	28.94 *n*	29.72 *t*	152.19 *p*
1'	165.60 *n*	(16) 146.46 *n*	153.87 *s*	140.07 *n*
2'	126.41 *n*	(17) 111.31 *n*	108.09 *t*	167.72 *n*
3'	140.96 *p*	(18) 28.10 *p*	28.68 *q*	29.31 *p*
4'	15.77 *p*	(19) 183.18 *n*	180.01 *s*	180.04 *n*
5'	20.08 *p*	(20) 22.71 *p*	23.67 *q*	15.91 *p*

*n and p are attached proton test results and s, d, t and q are single frequency off-resonance decoupled results.



7 R = H

8 R = OH

9 R = OAng

unreported ^{13}C NMR data we present here also supported the structure of **4**.

The structures of **5–9** were confirmed by comparison of their spectral properties with the reported data: **5** [14], **6–8** [4], **9** [5] and for those of **6–9** also by direct comparison of authentic samples.

EXPERIMENTAL

Viguiera ladibractate (Hemsl.) Blake was collected by Fred Barrie and Douglas Gage in Nov. 1984 along the Durango-Mazatlan Road in the state of Sinaloa, Mexico. It was identified by B. L. Turner in this department. A voucher specimen (Barrie & Gage 1251) is deposited in the Herbarium of the University of Texas at Austin.

Leaves of *V. ladibractate* (1075 g) were extracted with CH_2Cl_2. The usual work-up procedure [3] gave 53 g residue which was charged onto a silica gel column (1.2 kg, packed in hexane). The column was eluted with a hexane–EtOAc gradient solvent system with increasing the amount of EtOAc. Further purifications were achieved by a Sephadex LH-20 column (cyclohexane–CH_2Cl_2–MeOH, 7:4:1). Compounds **1** (94 mg), **2** (13 mg), **3** (535 mg), **4** 15 mg), **5** (91 mg), **6** (38 mg), **7** (5.8 g), **8** (75 mg), **9** (990 mg) were obtained.

Ladibranolide (**1**). Colourless prisms from EtOAc. IR ν_{max}^{KBr} cm^{-1}: 3100, 1640, 1590 (C=C), 1760 (γ-lactone), 1700 (COOR), 1690 (C=O), 1230, 1040, 830. EIMS (probe) 70 eV, *m/z* (rel. int.): 360 $[M]^+$ ($C_{20}H_{24}O_6$) (86), 342 $[M-H_2O]^+$ (5), 316 $[M-44]^+$ (24), 261 $[M-C_5H_7O_2]^+$ (57), 83 $[C_5H_7O]^+$ (100), 55 $[C_4H_7]^+$ (90).

ent-12-*Oxokaur*-9(11),16-*dien*-19-*oic acid* (**2**). Its IR, ^1H NMR and MS data were the same as those reported [8–10]. The ^{13}C NMR data are summarized in Table 1.

ent-12β-*Hydroxykaur*-9(11),16-*dien*-19-*oic acid* (**3**). Colourless prisms from EtOAc, mp 178–180°. EIMS (probe) 70 eV, *m/z* (rel. int.): 316 $[M]^+$ ($C_{20}H_{28}O_3$) (43), 298 $[M-H_2O]^+$ (100), 283 $[298-Me]^+$ (96), 237 $[283-HCOOH]^+$ (83). ^1H NMR (200 MHz, C_5D_5N): δ5.72 (1H, *br d*, *J* = 4 Hz, H-11), 5.17, 5.07 (each 1H, *br s*, H-17), 4.40 (1H, *t*, *J* = 4 Hz, H-12), 3.18 (1H, *m*, H-13), 1.32 (3H, *s*, H-18), 1.41 (3H, *s*, H-20). ^{13}C NMR see Table 1.

Methylation of **3**. Compound **3** (86 mg) was methylated with CH_2N_2 to give 74 mg **3a** (purified by Sephadex LH-20 column). EIMS (probe) 70 eV, *m/z* (rel. int.): 330 $[M]^+$ ($C_{21}H_{30}O_3$) (5), 312 $[M-H_2O]^+$ (46), 297 $[312-Me]^+$ (53), 253 $[312-COOMe]^+$ (28), 237 $[297-HCOOMe]^+$ (100). ^1H NMR (200 MHz, $CDCl_3$): δ0.98 (3H, *s*, H-20), 1.19 (3H, *s*, H-18), 2.83 (1H, *m*, H-13), 3.66 (3H, *s*, OMe), 3.94 (1H, *t*, *J* = 4 Hz, H-12), 4.90, 5.04 (each 1H, *br s*, H-17), 5.36 (1H, *br d*, *J* = 4 Hz, H-11).

Acetylation of **3**. Compound **3** (15 mg) was acetylated with Ac_2O–pyridine in the usual manner to give 14 mg **3b**. EIMS (probe) 70 eV, *m/z* (rel. int.): 358 $[M]^+$ ($C_{22}H_{30}O_4$) (16), 343 $[M-Me]^+$ (9), 316 $[M-CH_2CO]^+$ (100), 298 $[M-HOAc]^+$ (45), 283 $[298-Me]^+$ (48). ^1H NMR (200 MHz, $CDCl_3$): δ1.08 (3H, *s*, H-20), 1.25 (3H, *s*, H-18), 2.05 (3H, *s*, Ac), 2.87 (1H, *m*, H-13), 4.97 (1H, *t*, *J* = 4 Hz, H-12), 4.95, 5.14 (each 1H, *br s*, H-17), 5.31 (1H, *br d*, *J* = 4 Hz, H-11).

ent-*Isokaur*-15(16)-*en*-17,19-*dioic acid* (**4**). IR ν_{max}^{KBr} cm^{-1}: 3200–2500, 1690 (COOH), 1620 (C=C). EIMS (probe) 70 eV, *m/z* (rel. int.): 332 $[M]^+$ ($C_{20}H_{28}O_4$) (40), 317 $[M-Me]^+$ (45), 288 $[M-CO_2]^+$ (95), 109 (100). ^1H NMR (200 MHz, C_5D_5N): δ6.80 (1H, *s*, H-15), 3.19 (1H, *m*, H-13), 1.36 (3H, *s*, H-18), 1.20 (3H, *s*, H-20). ^{13}C NMR see Table 1.

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