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SESQUITERPENE LACTONES FROM *VIGUIERA* SPECIES*

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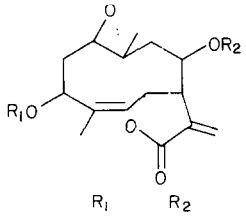
Key Word Index—*Viguiera eriophora*; *V. hemsleyana*; *V. hypochlora*; *V. schultzii*; Compositae; Heliantheae; sesquiterpene lactones; 17,18-dehydroviguiepinin; 17,18-dihydrobudlein A.

Abstract—*Viguiera eriophora* yielded the sesquiterpene lactones erioflorin, acetylerioflorin and 17,18-dehydroviguiepinin, which was correlated with tetrahydrozexbrevin and was identical with a lactone previously isolated from *Calea zacatechichi*. A new lactone 17,18-dihydrobudlein A was isolated from *V. hemsleyana*. Budlein A was a constituent of both *V. hypochlora* and *V. schultzii*.

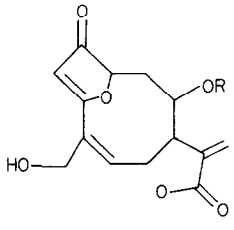
INTRODUCTION

The genus *Viguiera* is rich in sesquiterpene lactones (heliangolides and germacrolides) [1–8]. We previously found in *V. stenoloba* two heliangolides, viguiestenin (**1a**) and desacetylviguiestenin (**1b**) [1, 2]. The latter compound co-occurs in *V. pinnatilobata* [2] with viguiepinin (**2a**). Budlein A (**2b**) and B (**4**) are constituents of *V. buddleiaeformis* [3]. Budlein A, which displays strong cytotoxic activity, is also present in *V. angustifolia* [4, 5]. Erioflorin (**1c**) and sphaerocephalin (**5**) were recently reported as constituents of *V. sphaerocephala* [6], whereas very small amounts of two lactones presumed to be **1e** and **1f** were isolated from *V. procumbens* [7]. In addition, viguilenin (**6a**) was found to be a constituent of *V. linearis* [8].

In connection with a systematic chemical investigation of this and related genera, we describe here the isolation and characterization of the sesquiterpene lactones of *V. eriophora* Greenm., *V. hemsleyana* Blake, *V. hypochlora* (Blake) Blake and *V. scultzii* Blake.

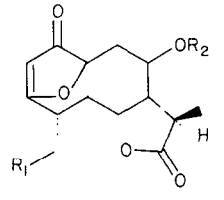


	R_1	R_2
1a	Ac	iBu
1b	H	iBu
1c	H	Meacr
1d	Ac	Meacr
1e	Ac	α-Mebu
1f	Ac	iVal



2a	R=iBu
2b	R=Ang
2c	R=Meacr
2d	R=α-Mebu

*Contribution No. 571 of the Instituto de Química U.N.A.M.



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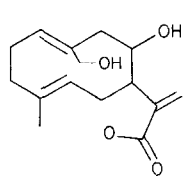
3d	H	α-Mebu



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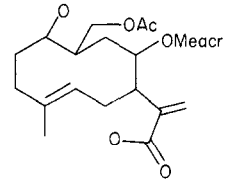

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RESULTS AND DISCUSSION

From the chloroform extract of *V. eriophora*, three sesquiterpene lactones were isolated by extensive chromatography. Erioflorin (**1c**), one of the two minor components (0.005% of the dry wt) was identified by direct comparison (IR, mmp) with an authentic sample. Acetylerioflorin (**1d**), $C_{21}H_{26}O_8$, $[M]^+$ 390, was obtained in 0.011% yield and was identical with the acetylation product of erioflorin. The third and most polar compound, 17,18-dehydroviguiepinin, $C_{19}H_{20}O_7$ (elemental analysis and mass spectrometry), mp 164°, displayed spectral properties typical of other 3(2H)-furanone heliangolides isolated from *Viguiera* [2, 3] in agreement with the proposed structure (**2c**) (Tables 1 and 2). In particular, the presence of an allylic pri-

Table 1. ^{1}H NMR data of *Viguiera* constituents and related compounds (100 MHz, $CDCl_3$)*

	2c	2d	2c + TAI	2d + TAI	3c	3d	7
H-2	5.68 *s*	5.64 *s*	5.78 *s*	5.74 *s*	5.67 *s*	5.53 *s*	5.65 *s*
H-5	6.21 *dt* (4,2)	6.20 *dt* (4,2)	6.39 *dt* (4,2)	6.31 *dt* (4,2)			5.62 *s*
H-6	5.29 *m*	5.36 *m*	†	†	4.67 *m*	4.55 *m*	4.52 *d* (5)
H-7	3.78 *m*	3.75 *m*	†	†			4.20 *m*
H-8	5.18 *m*	5.23 *m*	†	†	5.13 *m*	5.10 *m*	5.15 *m*
H-9a	2.58 *dd* (15,5)	2.52 *dd* (15,5)	†	†	—	—	2.63 *dd* (15,5)
H-9b	2.31 *dd* (15,3)	2.26 *dd* (15,3)	†	†	—	—	2.28 *dd* (15,3)
H-13a	6.33 *d* (2.8)	6.32 *d* (2.8)	†	†	—	—	6.34 *d* (2.8)
H-13b	5.66 *d* (2.8)	5.72 *d* (2.8)	†	†	—	—	5.67 *d* (2.8)
H-14	1.48 *s*	1.48 *s*	†	†	1.40 *s*	1.38 *s*	1.50 *s*
H-15	4.38 *dt* (3,2)	4.35 *dt* (3,2)	4.99 *dt* (3,2)	4.92 *dt* (3,2)	4.00 *c*	—	6.09 *s* 5.97 *s*
H-18a	6.10 *dq*	—	†	†	—	—	5.92 *dq*
H-18b	5.60 *dq*	—	†	†	—	—	5.56 *dq*
H-19	1.85	0.80 *t* (7)	†	†	0.80 *t* (7)	0.80 *t* (7)	1.83
H-20	—	1.07 *d* (7)	—	†	1.07 *d* (7)	1.07 *d* (7)	2.06 *s* ($\underline{CH_3}CO$)

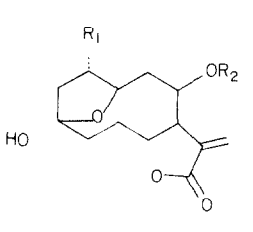
*Coupling constants in Hz.
†No change in chemical shifts.

mary alcohol was confirmed by the downfield shift in the ^{1}H NMR spectrum (δ 4.33 to 4.99) of the methylene to which the hydroxyl is attached after formation of the ester *in situ* on treatment with trichloroacetyl isocyanate (TAI). Additional proof of the structure and stereochemistry of **2c** was obtained by correlation with tetrahydrozexbrevin (**3a**) [9], the structure of which has recently been established [10, 11] unambiguously by correlation with tirotundin (**6b**). Catalytic hydrogenation of **2c** afforded two products. The more polar was a hexahydro-derivative identical in all respects with tetrahydroviguiepinin (**3b**), previously obtained by catalytic reduction of viguiepinin (**2a**) [2]. The less polar substance was a hydrogenolysis product whose spectroscopic and physical data tallied exactly with those reported for tetrahydrozexbrevin (**3a**). The identity was confirmed by direct comparison. This correlation also established the stereochemical course of the reduction. The hydrogens entered by the β-face of the molecule, since the C-4 methyl group in tetrahydrozexbrevin is α-orientated.

Structure **2c** deduced for 17,18-dehydroviguiepinin has recently been assigned to one of two lactones from *Calea zacatechichi* which could not be induced to crystallize [12]. Direct comparison of the IR, ^{1}H and ^{13}C NMR spectra of 17,18-dehydroviguiepinin (**2c**) and the substance from *C. zacatechichi* established their identity in spite of the differences in physical states.

Acetylation of 17,18-dehydroviguiepinin afforded **7**, due to an allylic rearrangement which has been discussed previously in similar instances [2, 4].

The chloroform extract of *V. hemsleyana* afforded the new 3(2H)-furanone heliangolide 17,18-dihydrobudlein A (**2d**), $C_{20}H_{24}O_7$ (elemental analysis and mass spectrometry). Like **2c**, it contained a hydroxyl group, a δ-lactone and a conjugated furanone moiety, as shown by IR bands at 3440, 1768 and 1705 cm^{-1} and by ^{1}H and ^{13}C NMR signals typical of this skeleton (Tables 1 and 2 and UV data). The signals of the ester residue were a doublet (3H) at δ 1.07 (J = 7.0 Hz) and a triplet at δ 0.80 (3H, J = 7.0 Hz) suggestive of a methyl butyrate residue; this was confirmed by its mass spectrum, m/z 85 $[C_5H_9O]^+$ and 57$[C_4H_9]^+$ (100%), and by carbon signals in the ^{13}C NMR at δ 173.03 (*s*), 40.95 (*d*), 26.45 (*t*), 11.37 (*q*) and 16.02 (*q*) due to C-16, C-17, C-18, C-19 and C-20 respectively. Catalytic hydrogenation of **2d** yielded



	R_1	R_2
6a	OH	α-Mebu
6b	H	iBu

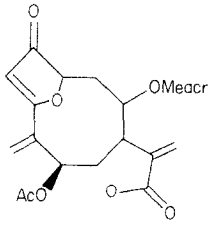


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Table 2. ^{13}C NMR data of *Viguiera* constituents (20.1 MHz, $CDCl_3$)

	2a	2b	2c	2d
C-1	205.65 *s*	205.19 *s*	205.12 *s*	205.32 *s*
C-2	104.72 *d*	104.87 *d*	105.03 *d*	104.71 *d*
C-3	183.05 *s*	182.75 *s*	182.64 *s*	182.90 *s*
C-4	138.45 *s*	138.64 *s*	138.88 *s*	138.44 *s*
C-5	133.54 *d*	134.24 *d*	134.41 *d*	133.69 *d*
C-6	74.26 *d*	74.19 *d*	75.14 *d*	74.05 *d*
C-7	48.25 *d*	48.56 *d*	48.47 *d*	48.42 *d*
C-8	75.34 *d*	75.50 *d*	75.25 *d*	75.29 *d*
C-9	41.99 *t*	42.14 *t*	42.58 *t*	42.01 *t*
C-10	87.73 *s*	87.86 *s*	87.94 *s*	87.60 *s*
C-11	136.26 *s*	136.17 *s*	136.07 *s*	136.43 *s*
C-12	168.97 *s*	168.91 *s*	168.66 *s*	168.71 *s*
C-13	123.98 *t*	123.75 *t*	123.44 *t*	123.82 *t*
C-14	21.11 *q*	21.21 *q*	21.40 *q*	21.11 *q*
C-15	62.06 *t*	62.41 *t*	62.57 *t*	62.11 *t*
C-16	175.43 *s*	156.92 *s*	165.89 *s*	175.03 *s*
C-17	21.11 *d*	126.54 *s*	135.21 *s*	40.95 *d*
C-18	18.77 *q*	141.11 *d*	127.33 *t*	26.47 *t*
C-19	18.39 *q*	15.74 *q*	18.05 *q*	11.37 *q*
C-20	—	19.99 *q*	—	16.02 *q*

Signals assigned by mean of partially decoupled off-resonance spectra and comparison with reported data for dehydrotagitinin A[12] and niveusin A, B and C[16].

two substances. The more polar product corresponded to a tetrahydro-derivative whose structure (**3c**) was consistent with the ^1H NMR spectrum (Table 1). The second and less polar substance, $C_{20}H_{28}O_6$ (elemental analysis and mass spectrometry), corresponded to a hydrogenolysis product (**3d**) whose spectroscopic data were consistent with the structure proposed. These products are analagous to those obtained in the catalytic reduction of 17,18-dehydroviguiepinin (**2c**) (see above).

Budlein A (**2b**) was the major constituent of both *V. hypochlora* and *V. schultzii* as shown by direct comparison with an authentic sample (IR, ^1H NMR and mmp).

The limited results based on the examination of several taxa of this large genus suggest similarities in the sesquiterpene lactone composition of *Viguiera* [1–7] and sections of *Helianthus* [13–15] and *Tithonia* [10, 16, 17] in partially confirming the generally accepted phylogenetic relationships of these genera [18]. All *Vigueira* species of the subgenus *Amphilepsis* examined so far (*V. buddleiaeformis*, *V. angustifolia*, *V. hemsleyana*, *V. hypochlora* and *V. schultzii*) show homogeneity in their lactone constituents which are 3(2H)-furanone heliangolides. Additional studies on this genus and related genera are in progress.

EXPERIMENTAL

^1H NMR: 100 MHz; ^{13}C NMR: 20.1 MHz; MS: direct inlet, 70 eV. Chemical analyses were performed by Dr. A. Bernhardt, Elbach, West Germany. Mps (Fischer–Jones apparatus) are uncorr. Si gel 60 (70–230 mesh) was used for CC. TLC and prep. TLC were done on Si gel 60 GF 254 (Merck).

Extraction of V. eriophora. Air-dried and ground leaves (3.48 kg) of *V. eriophora* (collected on 12 October 1978; *ca* 4 km NNE. of Huajuapan de León, Oaxaca, Hwy 125; voucher deposited in the National Herbarium, MEXU-ARV0038, Reg. No. 282634) were extracted with hexane (twice) and then with $CHCl_3$ (twice). The latter extract was concd *in vacuo*, leaving 119.2 g syrup. This syrup was chromatographed on a Si gel column (2.5 kg) using a C_6H_6–EtOAc gradient elution system. The fractions which eluted with C_6H_6–EtOAc (19:1) showed a major spot on TLC, were combined and the residue (0.643 g) purified on a Si gel column (19 g) using a $CHCl_3$–Me_2CO gradient system. The fractions eluted in this system (9:1) gave 376 mg (0.011% of the dry wt) acetylerioflorin (**1d**), mp and mmp 203–205°. The C_6H_6–EtOAc (9:1) fractions of the initial CC which showed the same spot on TLC were combined, yielding 328 mg residue. This was purified by prep. TLC (C_6H_6–EtOAc, 1:1; two developments) to give 167 mg (0.005% of the dry wt) erioflorin (**1c**), mp and mmp 237–238°, identical in all respects with an authentic sample. The fractions eluted with C_6H_6–EtOAc (4:1) gave gummy crystals when triturated with *iso*-Pr_2O–Me_2CO which, when purified by prep. TLC ($CHCl_3$–Me_2CO, 1:1) afforded 246 mg (0.007% of the dry wt) 17,18-dehydroviguiepinin (**2c**), mp 162–163°. Successive recrystallizations from *iso*-Pr_2O–EtOAc raised the mp to 164°; $[\alpha]_D^{25}$ −70.12 (MeOH; *c* 0.241); UV λ_{max}^{MeOH} nm (log ϵ): 215 and 266 (4.26 and 4.02); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3430, 1770, 1720, 1709, 1650; MS: *m/z* (rel. int.): 360 $[M]^+$ (4.3), 274(3.4), 291(1.0), 248(9.4), 85(1.7), 69(100), 43(28.4). (Found: C, 63.10; H, 5.68. $C_{19}H_{20}O_7$ requires: C, 63.33; H, 5.59%.)

Acetylation of erioflorin **1c**. Acetylation of 82.4 mg **1c** with pyridine–Ac_2O followed by the usual work-up afforded 64.9 mg **1d** identical in all respects with the natural product obtained from *V. eriophora*.

Tetrahydroviguiepinin (**3b**) *and tetrahydrozexbrevin* (**3a**). Hydrogenation of a soln of 17,18-dehydroviguiepinin (**2c**) (110.7 mg) in EtOH (6 ml) containing Pd/C-10% (20.2 mg) afforded a mixture of two products which was separated by prep. TLC ($CHCl_3$–Me_2CO, 4:1). The less mobile component, 50.8 mg, mp 190–191° was identical in all respects (mmp, IR, ^1H NMR) with **3b**. The more mobile component (32.7 mg), mp 156–157°, was identical by direct comparison (mmp, IR and ^1H NMR) with an authetic sample of **3a**.

Acetylation of 17,18-*dehydroviguiepinin*. To a soln of 50.3 mg **2c** in 2 ml pyridine was added 2 ml Ac_2O. The reaction mixture was kept for 5 hr at room temp. After the usual work-up, 39.8 mg **7** were obtained. Mp 203–205°. UV λ_{max}^{MeOH} nm (log ϵ): 216 and 278 (4.05 and 3.9); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760, 1730, 1720, 1710; MS: *m/z* (rel. int.): 402 $[M]^+$ (1.2), 360(2.6), 342(2.1), 316(2.0), 69(100), 43 (56.1).

Extraction of V. hemsleyana. Air-dried and ground leaves and stems (640 g) of *V. hemsleyana* (collected 28 October 1978 near Villa del Carbón, State of Mexico, *ca* 8 km SE. Villa del Carbón, Hwy E 146; voucher deposited in the National Herbarium, MEXU-ARV0004, Reg. No. 262825) were extracted as described for *V. eriophora* affording 7.3 g syrup. This material was chromatographed over Si gel (250 g) using a $CHCl_3$–Me_3CO gradient system. Several fractions which were eluted with $CHCl_3$–Me_2CO (3:1) and showed the same spot on TLC were combined and the residue (366 mg) re-chromatographed on a column of Si gel (11 g) packed with C_6H_6 and eluted with a C_6H_6–EtOAc gradient system. The C_6H_6–$CHCl_3$ (3:1) fractions were combined and concd affording crystals of 17,18-dihydrobudlein A (**2d**) (149 mg, 0.023% of dry plant), mp 180–181°, $[\alpha]_D^{25}$ −90.13° (MeOH: *c* 0.152); UV λ_{max}^{MeOH} nm (log ϵ): 213 and 217 (4.04 and 4.01); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440, 1768, 1737, 1705, 1653; MS: *m/z* (rel. int.): 376 (5.9), 292(7.7), 274(16.2), 85(20.5), 69(18.8), 57(100), 55(59.8). (Found: C, 63.72; H, 6.31. $C_{20}H_{24}O_7$ requires: C, 63.82; H, 6.43%.)

Hydrogenation of 17,18-*dihydrobudlein A* (**2d**). A soln of **2d** (91.4 mg) in EtOH (8 ml) was hydrogenated with Pd/C-10% (21.6 mg) until the H_2 uptake ceased. After the usual work-up two products were obtained which were separated by TLC (C_6H_6–EtOAc, 1:1; two developments). The more polar product was the tetrahydro-derivative **3c** (40.5 mg), mp 145–147°. UV λ_{max}^{MeOH} nm (log ϵ): 262(3.96); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440, 1770, 1730, 1703; MS: *m/z* (rel. int.): 380(1), 362(1), 278(1), 125(100), 85(26.4), 69(19.1), 57(95.5). The less polar product was 15-desoxytetrahydro-17,18-dihydrobudlein A (**3d**), (36.2 mg), mp 149–150°. UV λ_{max}^{MeOH} nm (log ϵ): 262(4.17); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440, 1770, 1730, 1705; MS: *m/z* (rel. int.): 364(1), 264(1), 125(100), 85(28.1), 69(19.1), 57(84.1), 43(26.4). (Found: C, 66.15; H, 7.76. $C_{20}H_{28}O_6$ requires: C, 65.91; H, 7.74%.)

Extraction of V. hypochlora. The plant was collected on 2 December 1979 near Cuernavaca, Morelos, Hwy 95, *ca* 2 km NE. Cuernavaca; voucher deposited in the National Herbarium, (MEXU-ARV0010, Reg. No. 262822). Dried leaves (2.93 kg) were extracted and worked-up as described above to yield 54.8 g syrup which was chromatographed over Si gel (1.3 kg) using $CHCl_3$ and mixtures of $CHCl_3$–Me_2CO. 840 mg budlein A (**2b**) (0.028% of the dry wt) was obtained in pure form by repeated recrystallization of the gummy crystals obtained on concn of the fractions eluted with $CHCl_3$–Me_2CO (4:1). Mp and mmp 106–107°.

Extraction of V. schultzii. Air-dried leaves and flowers (1.8 kg) of *V. schultzii* (collected near San Pedro, Nayarit, *ca* 26 km SE. Tepic, Nay., Hwy 15, 7 October 1980; voucher deposited in the National Herbarium (MEXU-ARV0028, Reg. No. 282553) were extracted twice with $CHCl_3$. The extract was concd *in vacuo* giving 107 g dark syrup. Part of this syrup (46.3 g) was fractionated over tonsil (400 g) developed with hexane–C_6H_6 (1:1). The $CHCl_3$ residue was chromatographed over Si gel (750 g) with a $CHCl_3$–Me_2CO gradient solvent system initiated with $CHCl_3$. Fractions eluted with this system (4:1) gave 519 mg (0.067% of dry plant) budlein A (**2b**) identified by direct comparison with an authentic sample using the standard methods.

Acknowledgement—We are greatly indebted to Dr. Arthur Cronquist, New York Botanical Garden, for the identification of the plants voucher and discussions on the taxonomy of Mexican *Viguiera* species.

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