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SESQUITERPENE LACTONES FROM TWO NEWLY-DESCRIBED SPECIES OF *VERNONIA: V. JONESII* AND *V. POOLEAE*

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Key Word Index—*Vernonia jonesii*; *V. pooleae*; Asteraceae; chemotaxonomy; sesquiterpene lactones; germacrolides; glaucolides.

Abstract—Two novel, non-glaucolide, germacrolide sesquiterpene lactones, 8α-methacryloyloxycostunolide-1(10),4(5)-diepoxide and 1-oxo-10α-OH-8α-methacryloyloxycostunolide (not fully characterized), were isolated from *Vernonia jonesii* and the previously described glaucolide B was identified from *V. pooleae*. This is one of the few reports of non-glaucolide type germacrolides in a New World species of *Vernonia*, although members of this class of sesquiterpene lactones are common constituents of Old World species of the genus. The chemical evidence supports the suggestion that *V. jonesii* may be part of a relictual, isolated group of the New World *Vernonia*, closely related to some of the Old World taxa.

INTRODUCTION

Considerable phytochemical investigation of the large genus *Vernonia* (Asteraceae) has established the widespread occurrence of sesquiterpene lactones. The distinctive glaucolide-type germacranolide sesquiterpene lactones, which contain an endocyclic $\Delta^{7(11)}$-α,β-unsaturated lactone in which C-13 is oxidized and usually bears an acetate group (**1**), are characteristic of a large number of species of *Vernonia*. Among the New World species examined to date, there are many reports of glaucolide-type germacranolides; both glaucolide germacrolides (Δ^4*trans*) and hirsutinolides (glaucolide-type compounds with Δ^5 and a 1,4-ether bridge) are known [1–6]. Guaianolides are also found in many New World species [2–5], while non-glaucolide-type germacranolides are rather rare, found in only six of 53 New World species producing sesquiterpene lactones [2–5, 7]. By contrast, both glaucolide and non-glaucolide-type germacranolides have been reported frequently from Old World species of *Vernonia*, in addition to eudesmanolides, guaianolides and elemanolides [1–3, 8, 9].

We recently had the opportunity to chemically investigate collections of two newly-described species of *Vernonia*, *V. jonesii* B. L. Turner of section *Lepidonia* and *V. pooleae* B. L. Turner of section *Leiboldia*. Until now, no members of these sections, which are considered to be relatively primitive groups in the genus as a whole [10], had been chemically studied. Section *Lepidonia* is of particular interest because it appears to be a relictual element among the New World *Vernonia* and may be closely related to some of the Old World sections of the genus (e.g. *Cyanopsis*) [10].

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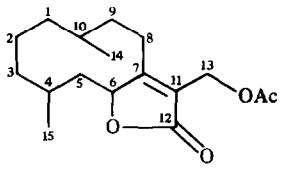
RESULTS AND DISCUSSION

Two non-glaucolide germacrolides, one 8α-methacryloyloxycostunolide-1(10),4(5)-diepoxide (**2**), and the other, tentatively characterized as 1-oxo-10α-OH-8α-methacryloyloxycostunolide (**3**), were isolated from *Vernonia jonesii* of section *Lepidonia* while only glaucolide B (**4**) was isolated from *V. pooleae* of section *Leiboldia*. The structure of **2** was recently confirmed by X-ray crystallography [11].

Air-dried leaves of *V. jonesii* were washed with dichloromethane and the resulting extract was worked up in the usual manner [12] to give 4.3 g of dark syrup. When this syrup was dissolved in toluene, crystals of **2** formed. Compound **2**, $C_{19}H_{24}O_6$ (HRMS: found 348.1559, calc. 348.1573), was shown to be an α-methylene-γ-lactone by the typical IR (1757 cm^{-1}) and ^1H NMR (δ 6.32, *d*, *J* = 3.5 Hz and 5.68 *d*, *J* = 3.0 Hz) signals. The presence of a methacrylate side chain was evident from the mass spectrum (*m/z* (rel. int.): 279 (2) $[M - C_4H_5O]^+$, 69 (100) $[C_4H_5O]^+$), IR (1711 cm^{-1}) and ^1H NMR data (6.12 *dq*, *J* = 1, 1.5 Hz; 5.65 *dq*, *J* = 1.5, 1.5 Hz; 1.92 (3H) *dd*, *J* = 1, 1.5 Hz).

^1H NMR decoupling experiments were undertaken to assign the remainder of the signals. Irradiation at H-13a and H-13b (δ 6.32 and 5.68) established the location of the H-7 signal (3.24) which was shown to be coupled to two double-doublets at 4.58 (*J* = 8.5, 9.5 Hz) and 4.73 (*J* = 2.5, 10.5 Hz). One of these must be the signal for the proton at the position of the lactone ring attachment and the other, being the only remaining signal downfield from 3.5 (Table 1), must be the proton at the point where the side chain is attached. We assumed that **2** was lactonized from position C-6 to C-12 rather than to C-8 because almost all of the sesquiterpene lactones previously isolated from *Vernonia* are 6,12-lactones. This supposition puts the methacrylate side chain at C-8.

The multiplicity of the signal at δ 4.58 (*dd*, *J* = 8.5, 9.5 Hz) is quite characteristic of H-6 in *trans*-fused-germacra-6,12-olides with unsaturation or a substituent at



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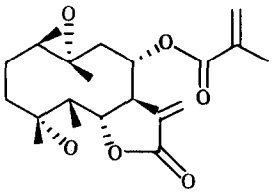

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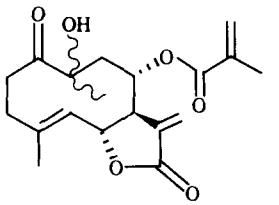

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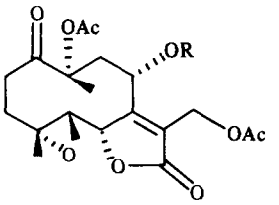
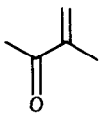


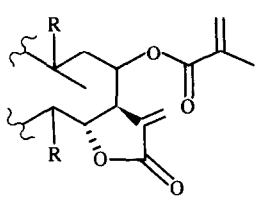
4 R = Ac

5 R = 

C-5 [13, 14]. Since $J_{7,13}$ values $\geqslant 3$ are generally taken to indicate that the lactone ring is *trans*-fused (Samek's rule [15, 16]), the 4.58 signal was provisionally assigned to H-6. Irradiation here simplified a sharp doublet at 3.85 (H-5) as well as the signal for H-7.

Irradiation of the other double doublet coupled to H-7, the signal at δ 4.73, which must now be assigned to H-8, showed that it was spin-coupled to both parts of an AB pattern at 2.55 (*br d*, J = 14 Hz) and 1.80 (*dd*, J = 10.5, 14 Hz). Clearly, position C-9 was unsubstituted. The absence of any further coupling to the C-9 protons showed that C-10, by contrast, was fully substituted.

At this point, the partial structure of the molecule **A**,



A

Table 1. ^{1}H NMR spectra of compounds **2** and **3**

	2*	3†
H-1	3.02 *br d* (10)	—
H-5	2.85 *d* (9.5)	5.47 *d* (10.5)
H-6	4.58 *dd* (8.5, 9.5)	5.02 *t* (10.5)
H-7	3.24 *ddd* (2.5, 3.0, 3.5, 8.5)	3.36 *m*
H-8	4.73 *br dd* (2.5, 10.5)	4.75 *m*
H-9β	2.55 *br d* (14)	‡
H-9α	1.80 *dd* (10.5, 14)	‡
H-13a	6.32 *d* (3.5)	6.01 *d* (3.5)
H-13b	5.68 *d* (3.0)	5.57 *d* (3.0)
H-14	1.45 (3H) *s*§	1.23 (3H) *s*
H-15	1.47 (3H) *s*§	1.86 (3H) *s*)
H-3′	6.12 *dq* (1, 1.5)	6.16 *br s*
	5.65 *dq* (1.5, 1.5)	5.75 *br s*
H-4′	1.92 (3H) *dd* (1, 1.5)	1.94 (3H) *s*

*Run at 200 MHz in $CDCl_3$ with TMS as an internal standard. Coupling constants (Hz) in parentheses.

†Run at 90 MHz in DMSO-d_6. A multiplet centered at 3.78 integrating for *ca* 2 protons was not assignable to any proton in **3** and may be due to an impurity.

‡Signals obscured.

§Assignments interchangeable.

including the α-methylene-γ-lactone and the methacrylate ester side chain accounted for six of the eight degrees of unsaturation and four of the six oxygen atoms required by the molecular formula. The presence of ^{1}H NMR signals at δ 3.02 and 2.85 and ^{13}C NMR resonances at 58.5, 59.6, 60.7 and 66.1 (Table 2) along with the lack of OH

Table 2. ^{13}CNMR spectra of compounds **2** and **3***

	2($CDCl_3$)	3(DMSO-d_6)
C-1	60.7 *d*†	217.9 *s*
C-2	23.2 *t*†	32.7 *t*‖
C-3	35.1 *t*†	34.2 *t*‖
C-4	58.5 *s*‡	143.9 *s*
C-5	66.1 *d*†	124.4 *d*
C-6	79.9 *d*†	77.3 *d*
C-7	50.5 *d*	48.3 *d*
C-8	70.0 *d*†	70.2 *d*
C-9	47.6 *t*†	40.8 *t*
C-10	59.6 *s*‡	78.0 *s*
C-11	132.7 *s*	136.2 *s*
C-12	168.9 *s*	169.5 *s*
C-13	125.1 *t*	120.7 *t*
C-14	18.1 *q*§	29.2 *q*
C-15	17.0 *q*§	20.0 *q*
C-1′	166.8 *s*	166.0 *s*
C-2′	135.8 *s*	135.6 *s*
C-3′	126.9 *t*	126.6 *t*
C-4′	18.1 *q*	17.9 *q*

*Run at 22.6 MHz with TMS as internal standard.

†Assignments confirmed by single frequency off-resonance decoupling experiments.

‡, §, ‖Assignment interchangeable.

stretching in the IR, strongly suggested that **2** had two epoxide functions. These would account for both the remaining oxygen atoms and degrees of unsaturation. The ^{1}H NMR chemical shifts of the C-4 and C-10 methyl groups (δ 1.45 and 1.47) indicated that the epoxide rings had to be attached at these positions. The fact that C-9 was unsubstituted and that H-5 was a simple doublet at 2.85 showed that the structure should be formulated with epoxide rings attached to C-1, C-10 and C-4, C-5. From a biogenetic standpoint, these are the most likely positions for epoxidation.

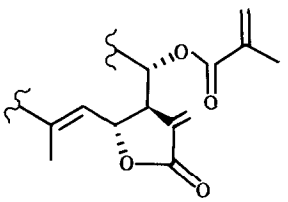
Compound **2** had seven chiral centers, carbons 1, 4, 5, 6, 7, 8 and 10, whose configurations remained to be established. As mentioned above, based on Samek's rule [15, 16], the lactone ring is clearly *trans*-fused because of the magnitudes of $J_{7,13}$ ($\geq$ 3 Hz) and $J_{6,7}$ (= 8.5 Hz). From the Karplus relationship, the protons at C-6 and C-7 must have nearly an anti-periplanar relationship. Assuming that H-7 is α-oriented, as in almost all sesquiterpene lactones of established absolute stereochemistry [17], H-6 must be β and the five-membered lactone ring is attached 6α,7β ($6S_a$, $7R_a$)*.

The C-8 ester side chain appears to have an α-orientation ($8S_a$) based on the chemical shift of H-8 (δ 4.73), which is at a relatively high field. In C-6 *trans*-fused germacrolides with C-8α-ester side chains, the H-8 signal is usually found between δ 4.5 and 5.2; in contrast, H-8 appears between 5.7 and 5.9 when the C-8 ester side chain is β-oriented [19]. The only major difference in the ^{1}H NMR spectrum between **2** and *epi*-tulipinolide diepoxide [20], which has an 8β-acetyl function, is the position of the H-8 signal: 4.73 in **2** (C-8 ester α) and 5.7 in *epi*-tulipinolide diepoxide (C-8 ester β). However, the $J_{7,8}$ value for **2** (2.5) is much less than that reported for most C-6-*trans*-fused germacrolides with 8α-ester side chains.

The epoxide rings were assumed to have *trans*-configurations and to be attached 10α,1β and 4α,5β(10R_a, 1R_a, 4R_a and 5R_a) since all previously described germacra-6,12-olides with 1,10- or 4,5-epoxides have this pattern [2]. For compound **2**, this conclusion is supported by the similarity of its H-1 and H-5 coupling constants to those given in the literature for C-6-lactonized germacrolide-1,10- or 4,5-epoxides [e.g. 14, 21].

The structure of **2** was recently confirmed by X-ray crystallography [11]. The epoxide rings have a crossed orientation with both skeletal methyl groups protruding above the plane of the ten-membered ring, the usual condition for *trans*-fused germacra-6,12-olides [22].

A second non-glaucolide germacrolide (**3**) was also isolated from the *V. jonesii* extract by column chromatography and preparative TLC. By its ^{1}H NMR, ^{13}C NMR and mass spectral similarities to **2**, compound **3** appeared to have a 6,12-*trans*-fused α-methylene-γ-lactone and an 8α-methacryloyl ester side chain. However, there was no indication of any epoxide functions. The ^{1}H NMR signal for H-6 (δ 5.02 *t*, J = 10.5 Hz) was spin coupled to a broadened one proton doublet at 5.47 (J = 10.5 Hz), which was in turn coupled to a vinylic methyl group at 1.86. These results indicated the presence of a 4,5-double bond which must have a *trans* geometry from the magnitudes of $J_{6,7}$ (10.5 Hz) and $J_{7,13}$ (3.0 and 3.5 Hz) [23]. The ^{1}H NMR shift of the C-10 methyl group (1.23) indicated an oxygen substituent at that position. Consistent with this was the presence of a ^{13}C NMR singlet at 78.0. The presence of a D_2O-exchangeable proton in the ^{1}H NMR spectrum and the lack of other signals in the ^{13}C NMR spectrum for carbons adjacent to sp^3 oxygen atoms suggested that the substituent at C-10 was a hydroxyl group rather than a cyclic ether.



B

The remaining oxygen function in the molecule was a ketone (^{13}C NMR signal at 217.9) which was assigned to C-1 both for biogenetic reasons and because of the co-occurrence of **3** with **2**. By analogy with compound **2**, the C-10 methyl group might be assumed to have a β-orientation (axial) and the hydroxyl group an α-orientation (10R_a).

Unfortunately, the characterization of **3** must remain incomplete, since the sample decomposed before its IR spectrum could be obtained. In addition, the molecular ion was not clear from the EIMS, although a number of peaks corresponding to logical losses from the molecular ion were present in the high mass region.

The species studied in this investigation, *V. jonesii* of section *Lepidonia* and *V. pooleae* of section *Leiboldia*, are the first representatives of their sections to have been chemically investigated. Both of these sections seem to be relatively primitive groups in the genus as a whole, as judged by characters of the capitulescence, involucre and pollen grains [10]. Section *Lepidonia*, in particular, has been suggested to be an ancient relictual element among the New World *Vernonia* on the basis of the chaffy receptacle of one of its members and may be closely related to some of the Old World sections [10]. The presence of two non-glaucolide germacrolides (**2** and **3**) in *V. jonesii* and the lack of any glaucolides supports this proposal. Non-glaucolide germacrolides occur rather frequently among Old World species of *Vernonia*, but are rare in New World species where glaucolides are common constituents. However, glaucolide germacrolides with oxygenation patterns very similar to **3**, such as **4** and **5**, are known from New World species of *Vernonia* [24].

The isolation of glaucolide B (**4**) from *V. pooleae* of section *Leiboldia*, on the other hand, indicates that this species group may be closely related to other New World elements of the genus. Chemical investigation of other members of both of these sections is necessary to substantiate these conclusions.

*The R_a and S_a notation for describing relative configuration follows the recommendation (rule 2) of Rogers, Moss and Neidle [18] where C-7 is assumed to have an *R* configuration as in almost all sesquiterpene lactones of known absolute configuration.

EXPERIMENTAL

Isolation of **2** *and* **3** *from* Vernonia jonesii. Dried leaves (165 g) of *V. jonesii* collected in Oaxaca, Mexico on 13 March, 1980 along Highway 175, 21 miles south of Valle Nacional (J. M. Poole #2246, voucher on deposit in the Herbarium of the University of

Texas) were washed with CH_2Cl_2 and worked up by standard procedures [12]. When the crude syrup (4 g) was dissolved in toluene in preparation for column chromatography, a ppt. formed which upon recrystallization from petrol–EtOAc yielded large colorless plates of **2** (2 g).

The remainder of the crude extract was chromatographed on a silica gel column (40 g) eluted with a toluene–EtOAc gradient. Fraction 4 (190 mg), which eluted with toluene–EtOAc (10:1), was rechromatographed on a second silica gel column using hexane–EtOAc (5:6) as an eluting solvent. The first fraction (66 mg) was purified by preparative TLC, $CHCl_3$–MeOH (19:1), to give 39 mg of **3**.

8α-Methacryloyloxycostunolide-1(10),4(5)-diepoxide (**2**). Colorless plates, mp 206–208° (petrol–EtOAc); IR ν_{max}^{Nujol} cm^{-1}: 1757 (lactone C=O), 1711 (ester C=O), 1658, 1631, 1274, 1155, 1042, 1024, 944, 898; CIMS (*i*-butane, probe) *m/z* (rel. int.): 349 $[M+H]^+$ (32), 331 $[M+H-H_2O]^+$ (21); EIMS (probe) 70 eV *m/z* (rel. int.): 320 $[M-CO]^+$ (0.02), 305 $[M-Me-CO]^+$ (0.2), 279 $[M-C_4H_5O]^+$ (2), 263 $[M-C_4H_5O_2]^+$ (2), 262 $[M-C_4H_6O_2]^+$ (2), 69 $[C_4H_5O]^+$ (100), 41 $[69-CO]^+$ (90).

1,Oxo-10α-OH-8α-methacryloyloxycostunolide (**3**). Pale yellow gum; MS (probe) 70 eV *m/z* (rel. int.): 348 $[M]^+$ (< 1), 333 $[M-Me]^+$ (< 1), 320 $[M-CO]^+$ (3), 305 $[M-Me-CO]^+$ (2), 247 (20), 69 $[C_4H_5O]^+$ (60).

Isolation of **4** *from* Vernonia pooleae. Dried leaves (50 g) of *V. pooleae* collected in Oaxaca, Mexico, on 13 March, 1980 along Highway 175, 24 miles south of Valle Nacional (J. M. Poole # 2232) were extracted with CH_2Cl_2 following standard procedures [12]. A portion of the crude extract (1 g) was separated by repeated preparative TLC, Et_2O–$CHCl_3$–EtOAc (3:1:1), to give 15 mg of **4** (glaucolide B), which was shown to be identical to an authentic specimen by comparison of its IR and ^1H NMR spectra [24] and co-chromatography.

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