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GERMACRANOLIDES FROM *EUPATORIASTRUM NELSONII*

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Key Word Index—*Eupatoriastrum nelsonii*; Compositae; sesquiterpene lactones; germacranolides; liacylindrolide derivatives.

Abstract—The aerial parts of *Eupatoriastrum nelsonii* afforded, in addition to known sesquiterpene lactones, four new lactones all closely related to the germacranolides isolated previously from other genera of the tribe Eupatorieae. The structures were elucidated by spectroscopic methods. Chemotaxonomic aspects are discussed briefly.

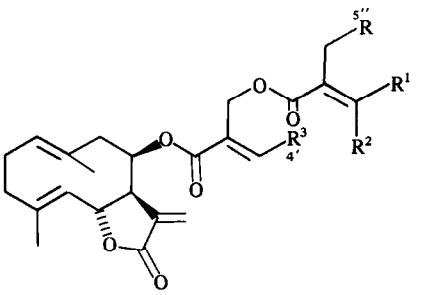
INTRODUCTION

The genus *Eupatoriastrum*, with the exclusion of *E. opadoclinium* Blake (Compositae, tribe Eupatorieae), contains four species [1], all of which are native to Mexico and Central America. So far, nothing is known about the chemistry of this genus. We have now studied the constituents of *E. nelsonii* Greenman. The results are discussed in this paper.

RESULTS AND DISCUSSION

The aerial parts of *E. nelsonii* afforded, in addition to large amounts of polyisoprene, a known eudesmane derivative [2], hex-2*E*-enoic acid and hex-3*Z*-enoic acid, mollisorin A [3], eupatolide [4], the corresponding tiglate [5] and liacylindrolide [6], four esters of eupatolide, all closely related to the latter, and the germacranolides **1**, **2**, **3** and **4**.

The main constituent was **1**, which afforded a diacetate. The molecular formulae and the fragmentation pattern indicated that mono-substituted germacranolides were present. Accordingly, the base peak in the spectrum of the acetate was at *m/z* 230 ($C_{15}H_{18}O_2$), formed by loss of $C_{14}H_{18}O_8$. However, elimination of $C_7H_{10}O_4$ was also visible (*m/z* 386) and a strong fragment at *m/z* 141 ($C_7H_9O_3$) could be observed, while the base peak in the spectrum of **1** was at *m/z* 99 ($C_5H_7O_2$). These observations indicated that an ester residue was present, which was a dihydroxy-angelate or -tiglate esterified with hydroxy-angelate or -tiglate. Inspection of the ^{1}H NMR spectrum (Table 1) supported this assumption and spin decoupling together with the chemical shifts clearly showed that a 4,5-dihydroxytiglate esterified at C-5 with 5-hydroxyangelate was present. Accordingly, the H-3′ signal was a low-field triplet at δ7.06 and the H-3″ signal was a slightly broadened quartet at δ6.37. While the signals of H-4′ were split into pairs of double-doublets, those of H-5′ and H-5″ were broadened signals at δ4.96 and 4.13, respectively, and that of H-4′ was a doublet at δ2.00. All the other signals were nearly identical with those of liacylindrolide and eupatolide tiglate, indicating the same stereochemistry and the same position of the ester residue. Also, the ^{13}C NMR spectrum (Table 2) agreed well with this structure. Thus **1** was the 2″*Z*-isomer of 4′-hydroxyliacylindrolide.



	1	2	3	4
R	OH	OH	OH	H
R^1	H	Me	H	Me
R^2	Me	H	Me	H
R^3	OH	OH	H	OH

The molecular formula of **2** indicated that this lactone was an isomer of **1**. The ^{1}H NMR spectrum (Table 1) differed from that of **1**, especially by the downfield shift of H-3′ (δ6.89), indicating that in **2** the $\Delta^{2''}$-double bond had the *E*-configuration. Accordingly, also the chemical shifts of H-4″ differed characteristically. Furthermore, the H-5′ signals were now a pair of doublets.

The mass spectrum as well as the ^{1}H NMR spectrum of **3** (Table 1) showed that we were dealing with an isomer of liacylindrolide. Accordingly, the ^{1}H NMR spectra differed in the signals of the ester side-chain. While H-3″ showed a quartet at δ6.39, this signal was shifted to δ6.86 in the spectrum of liacylindrolide and thus **3** was the $\Delta^{2''}$*Z*-isomer of the latter.

The ^{1}H NMR spectrum of **4** (Table 1), which also had the same molecular formula as **3**, showed that here the hydroxyl group at C-5″ was missing while a triplet at δ7.06 indicated a 4′-hydroxyl group in a 4′,5′-dihydroxytiglate residue. Accordingly, the H-4′ signals were broadened double-doublets (δ4.53 and 4.44), but in this case again the H-5′ signals were split into a pair of doublets. Thus **4** is 4′-

Table 1. ^{1}H NMR spectral data of 1–4 (400 MHz, $CDCl_3$, TMS as internal standard)

	1	Diacetate of 1	2	3	4
H-1	4.89 *br d*	4.89 *br dd*	4.89 *br dd*	4.87 *br d*	4.88 *br d*
H-2 H-2' H-3	2.35 *m* 2.22 *m*	2.35 *m* 2.23 *m*	2.35 *m* 2.23 *m*	2.35 *m* 2.22 *m*	2.36 *m* 2.24 *m*
H-3'	2.08 *ddd*	2.08 *ddd*	2.09 *ddd*	2.08 *ddd*	2.08 *ddd*
H-5	4.76 *br d*	4.77 *br d*	4.75 *br d*	4.74 *br d*	4.75 *br d*
H-6	5.14 *dd*	5.11 *dd*	5.13 *dd*	5.10 *dd*	5.12 *dd*
H-7	2.95 *br ddd*	2.94 *br ddd*	2.95 *br ddd*	2.93 *br ddd*	2.93 *br ddd*
H-8	5.84 *br d*	5.82 *br d*	5.85 *br d*	5.81 *br d*	5.84 *br d*
H-9	2.84 *br dd*	2.86 *br dd*	2.86 *br dd*	2.85 *br dd*	2.86 *br dd*
H-9'	2.36 *br d*	2.35 *br d*	2.35 *br d*	2.34 *br d*	2.35 *br d*
H-13	6.24 *d*	6.26 *d*	6.25 *d*	6.24 *d*	6.22 *d*
H-13'	5.59 *d*	5.58 *d*	5.59 *d*	5.59 *d*	5.56 *d*
H-14	1.44 *br s*	1.42 *br s*	1.44 *br s*	1.43 *br s*	1.44 *br s*
H-15	1.76 *br s*	1.75 *br s*	1.76 *d*	1.76 *d*	1.75 *br s*
OCOR	7.06 *t*	6.96 *t*	7.07 *t*	7.14 *q*	7.06 *t*
	4.49 *dd*	4.89 *d*	4.52 *dd*	1.99 *d*	4.53 *br dd*
	4.43 *dd*	5.00 *d*	4.44 *dd*	4.98 *d*	4.44 *br dd*
	4.96 *br s*	4.87 *d*	5.01 *d*	4.87 *d*	5.01 *d*
	6.37 *br q*	6.47 *br q*	4.92 *d*	6.39 *br q*	4.86 *d*
	2.00 *d*	2.03 *d*	6.89 *qq*	1.98 *d*	6.77 *qq*
	4.13 *br s*	4.64 *br s*	1.90 *d*	4.16 *br s*	1.77 *dq*
			4.31 *br s*		1.75 *br s*

J (Hz): 1, 2 = 10; 1, 2' = 4; 5, 6 = 10; 5, 15 = 1.5; 6, 7 = 9; 7, 8 ~ 1; 7, 13 = 3.5; 7, 13' = 3; 8, 9 = 4; 9, 9' = 15; compound **1**: 3', 4' = 6; $4_1'$, $4_2'$ = 16; 3", 4" = 7; diacetate of **1**: 3', 4' = 6; $5_1'$, $5_2'$ = 12.5; 3", 4" = 7; compound **2**: 3', 4' = 6; $4_1'$, $4_2'$ = 16.5; $5_1'$, $5_2'$ = 12.5; 3", 4" = 7; compound **3**: 3', 4' = 7; $5_1'$, $5_2'$ = 12; 3", 4" = 7.5; 4", 5" = 0.5; compound **4**: 3', 4' = 6; $4_1'$, $4_2'$ = 15; 3", 4" = 7; 3", 5" = 4", 5" = 1.

Table 2. ^{13}C NMR signals of **1** ($CDCl_3$, TMS as internal standard)

C-1	127.2 *d*	C-14	19.0 *q*
C-2	26.2 *t*	C-15	17.4 *q*
C-3	39.4 *t*	C-1'	164.9 *s*
C-4	142.6 *s*	C-2'	127.0 *s*
C-5	130.9 *d*	C-3'	147.7 *d*
C-6	75.7 *d*	C-4'	64.5 *t*
C-7	52.7 *d*	C-5'	57.7 *t*
C-8	72.5 *d*	C-1"	166.5 *s*
C-9	43.9 *t*	C-2"	131.1 *s*
C-10	133.9 *s*	C-3"	142.0 *d*
C-11	136.5 *s*	C-4"	15.6 *q*
C-12	169.7 *s*	C-5"	59.3 *t*
C-13	121.2 *t*		

hydroxy-5"-desoxyliacylindrolide.

The chemistry of this *Eupatoriastrum* species showed some similarities to *Eupatorium*, *Eupatoriadelphus*, *Lasiolaena*, *Mikania*, *Campovassouria*, and *Liatris*, where similar germacranolides with 8β-ester groups and in part a 2α-hydroxyl group were isolated [4–21]. However, these lactones have also been reported from *Helianthus* and some other genera, although no di-esters like **1**–**4** were isolated. The presence of these relatively unspecialized germacranolides suggests that *Eupatoriastrum* is an ancestral group from which a number of eupatorioid lines have evolved.

Eupatoriastrum was originally delineated by Greenman, who positioned the group in the subtribe Ageratinae near *Eupatorium*. McVaugh [22] notes, however, that chaffy members of the Ageratinae are unrelated to *Eupatoriastrum* and that any relationship with *Eupatorium* (*sensu stricta*) is remote.

Robinson and King [23] position *Eupatoriastrum* next to *Koanophyllon* in their critonioid grouping, largely on the basis of corolla and anther structures [7]. Using the same characters, in addition to others, *Eupatoriastrum* may be better positioned near *Decachaeta* and its immediate allies. Robinson and King include the latter genus in their subtribe Hebeclinae, remote from *Koanophyllon* itself [23].

Morphologically *Eupatoriastrum* is noteworthy in possessing well-defined receptacular bracts, a trait which most synantherologists would accept as primitive. Indeed, McVaugh [22] reckons that the paleaceous receptacle in *Eupatoriastrum* "has persisted" over evolutionary time. In any case, *Eupatoriastrum*, in spite of its small size, even with the exclusion of *E. opadoclinium* mentioned above, is remarkable for its variability in a number of characters (e.g. anther appendages), which Robinson and King [23]

use in their controversial classification of the Eupatorieae [24].

Eupatoriastrum seems to retain a number of morphological features, in addition to the receptacular bracts discussed above, that mark the group as perhaps ancestral to several phyletic lineages of the Hebeclinae and probably other groups in yet other subtribes as delineated by Robinson and King [23], including *Bartlettina*. Additional evidence for a *Decachaeta–Bartlettina* alliance comes from chromosomal data [25, 26] in which *Eupatoriastrum* is found to have a base of $x = 16$, as do the genera *Decachaeta* and *Bartlettina*.

The present chemical data corroborate these views in that *Eupatoriastrum* sequesters a wide spectrum of rather generalized germacranolides which are shared by a number of phyletic lines in the Eupatorieae as well as some in the tribe Heliantheae, from which most workers would derive the former.

Classification of the numerous, closely related, generic taxa proposed by Robinson and King are in part tenuous and any phyletic groupings will have to draw upon a wide range of data, including that from microcharacters, cytogenetics and *especially* chemistry.

EXPERIMENTAL

The air-dried aerial parts (1.2 kg) (voucher Robles 355, TEX, XAL, collected in Mexico) were worked up in the usual fashion [27] and after treatment with MeOH, the unsoluble part (10 g) consisted mainly of polyisoprene. The CC fractions of the soluble part were as follows: 1 (petrol), 2 and 3 (Et_2O–petrol, 9:1, and Et_2O–petrol, 1:1), 4 (Et_2O) and 5 (Et_2O–MeOH, 9:1). TLC of fraction 1 gave 20 mg germacrene D, fractions 2 and 3 gave nothing of interest and TLC of fraction 4 (Et_2O–petrol, 1:1) gave 5 mg eupatolide tiglate [5] (R_f 0.58). Fraction 5 (2.5 g) was separated further by medium-pressure liquid chromatography using 80 g silica gel (30–60 g) to give fractions 5/1–5 (Et_2O and Et_2O–MeOH, 20:1 to 5:1). From 5/1 after TLC (Et_2O–petrol, 1:1), only fatty acids were obtained, while TLC of 5/2 (Et_2O–petrol, 1:1) gave 5 mg of a mixture, which was separated further by GC to afford 2 mg hex-2*E*-enoic acid and 1 mg hex-3*Z*-enoic acid. TLC (Et_2O–petrol, 3:1) of 5/3 gave two fractions (5/3/1 and 5/3/2), which were separated further. TLC of 5/3/1 (Et_2O–petrol, 1:1, 3 developments) gave 2 mg of the eudesmane [2] (R_f 0.52), 4 mg eupatolide (R_f 0.28) and 5 mg **6** (R_f 0.18). TLC (Et_2O–petrol, 3:1, 2 developments) of 5/3/2 gave two bands. The first one (R_f 0.45) gave 23 mg **3** and the second one a mixture, which was separated further by TLC ($CHCl_3$–C_6H_6–Et_2O, 2:2:1, 3 developments). The band with R_f 0.45 was still a mixture of **3** and liacylindrolide and could be separated by TLC (Et_2O–petrol, 3:1, 5 developments) into 6 mg **3** (R_f 0.62) and 6 mg liacylindrolide (R_f 0.62) and the band with R_f 0.20 gave 2 mg mollisorin A. 5/4 on standing in $CHCl_3$–Et_2O gave 1.8 g of crystalline **1**, while the mother liquid showed no further signals in the ^1H NMR spectrum. TLC (Et_2O–MeOH, 200:1, 3 developments) of 5/5 gave 6 mg **1** (R_f 0.45) and 5 mg **2** (R_f 0.38). Known compounds were identified by comparing the 400 MHz ^1H NMR spectra with those of authentic material and by co-TLC in different solvent mixtures.

2″Z-4′-Hydroxyliacylindrolide (**1**). Colourless crystals, mp 95° (Et_2O–$CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH), 1760 (γ-lactone), 1720, 1650 (C=CCO_2R); MS *m/z* (rel. int.): 460.210 $[M]^+$ (0.5) (calc. for $C_{25}H_{32}O_8$: 460.210), 442 $[M-H_2O]^+$ (1), 362 $[M-O{=}C{=}C(H_2OH)CH{=}CH_2]^+$ (2), 344 $[M-RCO_2H]^+$ (0.4), 230 $[M-RCO_2H]^+$ (42), 215 $[230-Me]^+$ (10), 99 $[C_4H_7(OH)CO]^+$ (100), 81 $[99-H_2O]^+$ (34), 69 $[99-CH_2O]^+$ (32).

$$[\alpha]_{22°}^{\lambda} = \frac{589 \quad 578 \quad 546 \quad 436}{+61 \quad +66 \quad +76 \quad +150} (CHCl_3;\ c\ 0.35).$$

50 mg **1** in 0.5 ml Ac_2O was heated for 1 hr at 70°. Usual work-up and TLC (C_6H_6–$CHCl_3$–Et_2O, 2:2:1) afforded 40 mg of the diacetate (R_f 0.48), colourless oil; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1770 (γ-lactone), 1745, 1230 (OAc), 1720, 1660 (C=CCO_2R); MS *m/z* (rel. int.): 544.231 $[M]^+$ (1) (calc. for $C_{29}H_{36}O_{10}$: 544.231), 484 $[M-HOAc]^+$ (5), 404 $[M-O{=}C{=}C(OAc)CH{=}CH_2]^+$ (5.5), 386 $[M-RCO_2H]^+$ (1), 344 $[404-HOAc]^+$ (1), 230 $[M-RCO_2H]^+$ (100), 215 $[230-Me]^+$ (21), 141 $[RCO]^+$ (62), 81 $[141-HOAc]^+$ (92).

4′-Hydroxyliacylindrolide (**2**). Colourless crystals, mp 92° (Et_2O–$CHCl_3$); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3420 (OH), 1755 (γ-lactone), 1710, 1645 (C=CCO_2R); MS *m/z* (rel. int.): 460.210 $[M]^+$ (0.5) (calc. for $C_{25}H_{32}O_8$: 460.210), 442 $[M-H_2O]^+$ (1), 362 $[M-O{=}C{=}C(CH_2OH)CH{=}CH_2]^+$ (2), 344 $[M-RCO_2H]^+$ (1), 230 $[M-RCO_2H]^+$ (58), 215 $[230-Me]^+$ (14), 99 $[C_4H_7(OH)CO]^+$ (100), 81 $[99-H_2O]^+$ (40), 69 $[99-CH_2O]^+$ (56).

2″Z-Liacylindrolide (**3**). Colourless oil; IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3500 (OH), 1770 (γ-lactone), 1720 (C=CCO_2R); MS *m/z* (rel. int.): 444.215 $[M]^+$ (1) (calc. for $C_{25}H_{32}O_7$: 444.215), 426 $[M-H_2O]^+$ (1), 346 $[M-O{=}C{=}C(CH_2OH)CH{=}CH_2]^+$ (3), 328 $[M-RCO_2H]^+$ (1), 230 $[M-RCO_2H]^+$ (45), 99 $[RCO]^+$ (100), 81 $[99-H_2O]^+$ (27), 69 $[99-CH_2O]^+$ (12).

4′-Hydroxy-5″-desoxyliacylindrolide (**4**). Colourless oil; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH), 1760 (γ-lactone), 1715, 1650 (C=CCO_2R); MS *m/z* (rel. int.): 444.215 $[M]^+$ (1) (calc. for $C_{25}H_{32}O_7$: 444.215), 426 $[M-H_2O]^+$ (0.5), 230 $[M-RCO_2H]^+$ (34), 215 $[230-Me]^+$ (20), 83 $[C_4H_7CO]^+$ (100), 55 $[83-CO]^+$ (54). $[\alpha]_D^{22}$ +60° ($CDCl_3$; *c* 0.94).

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