Zinagrandinolides A−C, Cytotoxic δ-Elemanolide-Type Sesquiterpene Lactones from *Zinnia grandiflora*[1]

Bharat P. Bashyal, Steven P. McLaughlin, and A. A. Leslie Gunatilaka*

SW Center for Natural Products Research and Commercialization, Office of Arid Lands Studies, College of Agriculture and Life Sciences, The University of Arizona, 250 E. Valencia Road, Tucson, Arizona 85706-6800

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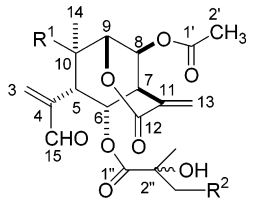
Three new δ-elemanolide-type sesquiterpene lactones, zinagrandinolides A−C (**1**−**3**), and the known δ-elemanolide **4** have been isolated by a bioassay-guided fractionation of a cytotoxic hexane extract of the aerial parts of *Zinnia grandiflora*. The structures of **1**−**3** were determined on the basis of high-resolution mass and NMR data. All compounds exhibited strong cytotoxicity against the cancer cell lines NCI-H460, MCF-7, SF-268, and MIA Pa Ca-2 and the normal human fibroblast cell type WI-38, but none showed significant selectivity.

In continuing our search for novel natural product-based anticancer agents from arid land plants and their associated microorganisms,[1] a hexane extract of *Zinnia grandiflora* Nuttall (Asteraceae) was selected for further investigation on the basis of its cytotoxic activity against a panel of four cancer cell lines. Bioactivity-guided fractionation of this extract involving solvent−solvent partitioning followed by Sephadex size-exclusion and repeated silica gel and reversed-phase chromatography furnished three new δ-elemanolides, named zinagrandinolides A−C (**1**−**3**), and the known δ-elemanolide 6*R**-(2′′-hydroxyisobutanoyloxy)-8*S**-acetoxy-15-oxo-1*S**,2-epoxy-3,11(13)-elemandien-12,9-olide (**4**). All compounds were found to exhibit strong cytotoxicity. *Zinnia* is a genus of 11 New World species ranging from the United States to Argentina. *Z. grandiflora* is a relatively widespread species found primarily in the southern Great Plains and the adjacent Southwest, including Arizona, New Mexico, Colorado, Kansas, Oklahoma, and Texas and the Mexican states Chihuahua, Coahuila, Sonora, and Tamaulipas. Previous studies of *Zinnia* species,[2−10] including *Z. grandiflora*,[2] have resulted in the isolation of eudesmanolides,[3] guaianolides,[3,4] germacranolides,[4] γ-elemanolides,[2−8] and δ-elemanolides.[2,9,10]



1 R^1 =  R^2 = Me

2 R^1 =  R^2 = Me

3 R^1 =  R^2 = H

4 R^1 =  R^2 = H

Elemanolides represent a small group of sesquiterpene lactones biogenetically derived from germacranolides.[11] Structurally, all elemanolides consist of six-membered carbocyclic rings bearing at least one five-membered lactone ring (γ-elemanolides) or a six-membered lactone ring (δ-elemanolides). Invariably, the carbocyclic ring in elemanolides carries an alcohol function esterified with a C_2, C_4, or C_5 aliphatic carboxylic acid sometimes bearing a hydroxyl function. Although other groups of sesquiterpene lactones including γ-elemanolides are widely distributed in various genera of the plant family Asteraceae (Compositae), δ-elemanolides are restricted to the genus *Zinnia* of this family; thus far only five natural δ-elemanolides are known, and these have been encountered in *Z. grandiflora*,[2] *Z. juniperifolia*,[9] and *Z. citrea*.[10] Although δ-elemanolides from *Zinnia* species have not been evaluated for their biological activity, three γ-elemanolide dilactones, zinaflavins B, D, and F, from *Z. flavicoma* have been shown to be cytotoxic toward HEp-2c (human laryngeal carcinoma cell line) and L929 (fibroblast cells from normal murine connective tissue, NCTC clone 929).[12] Herein we report the isolation and structure elucidation of zinagrandinolides A−C (**1**−**3**) and the cytotoxic activity of **1**−**4** in a panel of four cancer cell lines and normal human fibroblast cells.

Zinagrandinolide A (**1**) was isolated as a colorless oil that analyzed for $C_{22}H_{28}O_9$ by a combination of HRFABMS and ^{13}C NMR data and indicated nine degrees of unsaturation. The IR spectrum had bands due to OH (3456 cm^{-1}), ester/δ-lactone carbonyl (1735 cm^{-1}), α,β-unsaturated ester/δ-lactone carbonyl (1693 cm^{-1}), and α,β-unsaturated aldehyde/ketone carbonyl (1631 cm^{-1}) functionalities. The ^1H NMR spectrum of **1** (Table 1) consisted of three 3H singlets, four olefinic 1H singlets at δ 5.84, 6.22, 6.65, and 6.76, a 1H singlet at δ 9.33, due to an aldehyde group, and three independent spin systems, as indicated by its COSY spectrum. On the basis of their chemical shifts, the 3H singlets were assigned to an $OCOCH_3$ (δ 2.05) and two CH_3 groups (δ 1.47 and 1.11) on quaternary carbons, of which one is oxygenated. Three 1H double doublets at δ 3.04 (J = 3.5 and 3.0 Hz), 2.50 (J = 3.5 Hz), and 2.43 (J = 3.5 and 3.0 Hz) suggested the presence of an ABC spin system due to a monosubstituted oxirane ring. The presence of an ABX_3 spin system in **1** was apparent from a 3H triplet at δ 0.92 (J = 7.5 Hz) and two 1H double quartets (J = 15.0 and 7.5 Hz) at δ 1.85 and 1.76. The remaining spin system contained five protons in a complex pattern, and on the basis of their chemical shifts and coupling constants these protons were assigned to a −CH(O)CH(O)CHCH(O)CH− moiety. The ^{13}C NMR spectrum of **1** (Table 1) when analyzed with the help of HSQC showed the presence of four methyls, four methylenes (two of which are olefinic and one oxygenated), seven methines (one due to an aldehyde carbonyl and four due to oxygenated carbons), and seven quaternary carbons (three of which are due to ester/lactone carbonyl and two due to olefinic carbons). On the basis of the HMBC correlations (Figure 1) the carbon skeleton of **1** was determined to be that of a bicyclic sesquiterpene related to **4**.[2,3] Comparison of

* To whom correspondence should be addressed. Tel: (520) 741-1691. Fax: (520) 741-1468. E-mail: leslieg@ag.arizona.edu.

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Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data for **1**−**3** in $CDCl_3$

	zinagrandinolide A (**1**)		zinagrandinolide B (**2**)		zinagrandinolide C (**3**)	
position	δ_H[a]	δ_C[b]	δ_H[a]	δ_C[b]	δ_H[a]	δ_C[b]
1	3.04 dd (3.0, 3.5)	55.1 d	5.83 dd (10.5, 17.5)	141.0 d	5.84 dd (10.5, 17.5)	141.0 d
2a	2.50 dd (3.5)	44.2 t	5.05 d (10.5)	115.7 t	5.05 d (10.5)	115.6 t
2b	2.43 dd (3.0, 3.5)		4.93 d (17.5)		4.92 d (17.5)	
3a	6.22 s	139.3 t	6.18 s	139.2 t	6.18 s	139.3 t
3b	6.65 s		6.62 s		6.63 s	
4		145.5 s		145.2 s		145.3 s
5	3.37 d (3.0)	29.6 d	3.42 d (3.0)	32.3 d	3.42 d (3.0)	32.3 d
6	4.92 dd (4.3)	64.1 d	4.95 dd (2.7)	64.5 d	4.93 dd (3.0)	64.6 d
7	3.27 ddd (3.0)	43.4 d	3.26 ddd (3.0)	43.3 d	3.28 ddd (2.5)	43.1 d
8	5.42 dd (2.5)	78.1 d	5.42 dd (2.5)	77.9 d	5.44 dd (2.3)	77.9 d
9	4.58 dd (2.0)	82.3 d	4.33 dd (2.0)	84.8 d	4.33 dd (2.0)	84.8 d
10		43.3 s		45.1 s		45.2 s
11		131.0 s		131.3 s		131.2 s
12		162.3 s		162.6 s		162.6 s
13a	6.76 s	134.4 t	6.76 s	134.1 t	6.75 s	134.2 t
13b	5.84 s		5.83 s		5.83 s	
14	1.11 s	13.9 q	1.43 s	18.1 q	1.40 s	18.1 q
15	9.33 s	193.0 d	9.27 s	193.1 d	9.27 s	193.1 d
1′		170.1 s		170.2 s		170.3 s
2′	2.05 s	21.1 q	2.04 s	21.1 q	2.04 s	21.1 q
1″		176.1 s		176.3 s		176.4 s
2″		75.2 s		75.2 s		72.4 s
3″a	1.85 dq (7.5, 15.0)	33.5 t	1.85 dq (7.5, 14.0)	33.6 t	1.50 s	27.2 q
3″b	1.76 dq (7.5, 15.0)		1.76 dq (7.5, 14.0)			
4″	0.92 t (7.5)	8.1 q	0.92 t (7.5)	8.1 q	1.53 s	27.8 q
5″	1.47 s	25.6 q	1.46 s	25.7 q		

[a] Multiplicities deduced from HSQC; coupling constants (*J* values in Hz) are in parentheses. [b]Multiplicities deduced from DEPT.

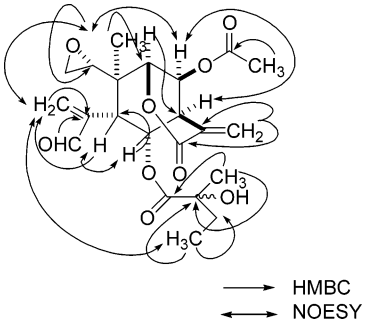

Figure 1. Selected 2D NMR data of zinagrandinolide A (**1**).

^{1}H and ^{13}C NMR spectroscopic data of **1** with those of **4**, which was found to co-occur in the same extract (see below), suggested that they are identical except for the ester group at C−6; in **4** attached to C-6 is the 2″-hydroxyisobutyrate ester function, whereas in **1**, C-6 bears a 2″-hydroxy-2″-methylbutyrate ester function. The stereochemical disposition of groups on the bicyclic skeleton of **1** was deduced from ^{1}H−^{1}H coupling constants[2] and NOESY data (Figure 1). However, these data were not useful in the determination of the stereochemistry of the OH group in the 2″-hydroxy-2″-methylbutyryl side chain. Furthermore, the failure of **1** to yield an MTPA ester under a variety of conditions precluded stereochemical assignment of the OH group of the side chain. On the basis of the foregoing evidence, the structure of zinagrandinolide A was determined as 6*R**-(2″-hydroxy-2″-methylbutanoyloxy)-8*S**-acetoxy-15-oxo-1*S**,2-epoxy-3,11(13)-elemandien-12,9-olide (**1**).

Zinagrandinolide B (**2**), isolated as a colorless oil, analyzed for $C_{22}H_{28}O_8$ by a combination of HRFABMS and ^{13}C NMR and indicated nine degrees of unsaturation. The ^{1}H NMR and ^{13}C NMR data of **2** (Table 1) closely resembled those of zinagrandinolide A (**1**) except for the signals due to the C_2 substituent at C−10. In **2** the ^{1}H signals of this substituent appeared at δ 5.83 (dd, J = 10.5 and 17.5 Hz), 5.05 (d, J = 10.5 Hz), and 4.93 (d, J = 17.5 Hz), and the ^{13}C signals at δ 141.0 (d) and 115.7 (t), suggesting that in **2** the C_2 substituent at C-10 is −CH=CH_2. These data combined with 2D (COSY, HMBC, and NOESY) NMR analysis identified zinagrandinolide B as 6*R**-(2″-hydroxy-2″-methylbutanoyloxy)-8*S**-acetoxy-15-oxo-1(2),3,11(13)-elemantrien-12,9-olide (**2**). Zinagrandinolide C (**3**), obtained as a colorless oil, analyzed for $C_{21}H_{26}O_8$ by a combination of HRFABMS and ^{13}C NMR and indicated nine degrees of unsaturation and one carbon atom less than in **1** and **2**. Comparison of ^{1}H and ^{13}C NMR data of **3** with those of **2** suggested that these are almost superimposable except that in **3** the −CH_2CH_3 substituent at C−2″ is replaced with a −CH_3 group (Table 1). The presence of strong correlations from protons to both CH_3 groups at C-2″ and to the C-1″ carbonyl carbon in the HMBC spectrum confirmed the presence of a 2-hydroxyisobutyroyl substituent at C-6 in **3**. The structure of zinagrandinolide C was thus elucidated as 6*R**-(2″-hydroxybutanoyloxy)-8*S**-acetoxy-15-oxo-1(2),3,11(13)-elemantrien-12,9-olide (**3**).

Table 2. Cytotoxicities of the Compounds **1**−**4** against a Panel of Human Tumor Cell Lines and Human Normal Fibroblast Cells[a]

	cell type[b]				
compound	NCI-H460	MCF-7	SF-268	MIA Pa Ca-2	WI-38
1	0.45	0.51	0.29	0.22	0.69
2	0.25	0.38	0.30	0.21	0.43
3	0.27	0.55	0.44	0.32	0.64
4	0.97	0.52	0.64	0.44	0.76
doxorubicin	0.01	0.07	0.04	0.05	0.30

[a] Results are expressed as IC_{50} values in μM. [b]Key: NCI-H460 = human non-small-cell lung cancer; MCF-7 = human breast cancer; SF-268 = human CNS cancer (glioma); MIA Pa Ca-2 = human pancreatic carcinoma; WI-38 = human normal fibroblast cells.

Compounds **1**−**4** were evaluated for in vitro cytotoxicity against a panel of four sentinel cancer cell lines, NCI-H460 (non-small-cell lung), MCF-7 (breast), SF-268 (CNS glioma), and MIA Pa Ca-2 (pancreatic carcinoma), and normal human fibroblast cells, WI-38. Cells were exposed to serial dilutions of test compounds for 48 h in RPMI 1640 media supplemented with 10% fetal bovine serum, and the cell viability was evaluated by the MTT assay.[13] As shown in Table 2, all compounds were found to be strongly cytotoxic. The concentrations resulting in 50% inhibition of cell proliferation/survival as measured by an MTT assay (IC_{50} values) were found to range between 0.21 and 0.97 μM. Although no significant selectivity was observed toward any of the cell lines,

these compounds merit further biological evaluation, as several naturally occurring sesquiterpenes bearing an α,β-unsaturated-δ-lactone moiety have been reported to possess anticancer, antifungal, insect antifeedant, and plant growth inhibitory properties.[14] Further work to evaluate the mechanism of action of the novel sesquiterpene lactones encountered in this study is currently in progress.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Jasco DIP-370 polarimeter using $CHCl_3$ as solvent. For IR spectroscopic determinations, samples were dissolved in CH_2Cl_2 and adsorbed in KBr, dried in vacuum, and KBr disks were made and spectra recorded on a Shimadzu FTIR-8300 spectrometer. 1D and 2D NMR spectra were recorded in $CDCl_3$ with a Bruker DRX-500 instrument at 500 MHz for ^1H NMR and 125 MHz for ^{13}C NMR using residual solvent as internal standard. The chemical shift values (δ) are given in parts per million (ppm), and the coupling constants are in Hz. High-resolution MS were recorded in a JEOL HX110A spectrometer.

Plant Material. The aerial parts of *Z. grandiflora* were collected and identified by Dr. S. P. McLaughlin in Chiricahua Mountains in Cochise County, AZ, on September 6, 1999, and a voucher sample is deposited at the University of Arizona Herbarium (ARIZ) under the accession number 376627.

Extraction and Isolation. The dried and powdered aerial parts of *Z. grandiflora* (166.0 g) were extracted (× 5) with hexane, and the resulting extract was evaporated under reduced pressure to afford a dark yellow liquid residue (3.34 g). A portion (3.23 g) of this extract was partitioned between hexane and 80% aqueous MeOH, and the cytotoxic 80% aqueous MeOH fraction was diluted to 50% aqueous MeOH by the addition of H_2O and extracted with $CHCl_3$. Evaporation of $CHCl_3$ under reduced pressure yielded a yellow liquid (450 mg), which was subjected to gel permeation chromatography on a column of Sephadex LH-20 (20 g) in hexane−CH_2Cl_2 (1:4) and eluted with hexane−CH_2Cl_2 (1:4) (200 mL), CH_2Cl_2−acetone (3:2) (50 mL), CH_2Cl_2−acetone (1:4) (50 mL), CH_2Cl_2−MeOH (1:4) (50 mL), and MeOH (50 mL). Nine fractions were collected and combined on the basis of their TLC profiles to yield a major fraction (282.2 mg), which was shown to be cytotoxic. Separation of this fraction (265.0 mg) on reversed-phase Si gel column chromatography by elution with increasing amounts of MeCN in H_2O afforded two cytotoxic subfractions, F1 (55.0 mg) and F2 (45.7 mg). A portion (45.0 mg) of F1 was further purified by preparative TLC on silica gel (CH_2Cl_2−MeOH, 95:4) followed by preparative TLC on RP-18 (MeCN−H_2O, 40:60) and preparative TLC on silica gel (EtOAc−CH_2Cl_2, 20:80) to afford **1** (7.9 mg), **3** (15.8 mg), and **4** (7.8 mg). A portion (37.3 mg) of the fraction F2 was further purified by preparative TLC on silica gel (EtOAc−CH_2Cl_2, 30:70) followed by preparative TLC on RP-18 (H_2O−MeCN−MeOH, 4:3:3) to obtain **2** (8.7 mg).

Zinagrandinolide A (1): colorless oil; $[\alpha]_D^{25}$ +68.5 (*c* 0.16, $CHCl_3$); IR (KBr) ν_{max} 3452, 1735, 1693, 1631, 1377, 1234, 1137, 1041, 802, cm^{-1}; ^1H and ^{13}C NMR data, see (Table 1); HRFABMS *m*/*z* 437.1800 $[M + 1]^+$ (calcd for $C_{22}H_{29}O_9$, 437.1811).

Zinagrandinolide B (2): colorless oil; [α] +75.7 (*c* 0.87, $CHCl_3$); IR (KBr) ν_{max} 3456, 1735, 1693, 1631, 1377, 1230, 1137, 1045, 806, cm^{-1}; ^1H and ^{13}C NMR data, see (Table 1); HRFABMS *m*/*z* 421.1842 $[M + 1]^+$ (calcd for $C_{22}H_{29}O_8$, 421.1862).

Zinagrandinolide C (3): colorless oil; $[\alpha]_D^{25}$ +58.7 (*c* 0.79, $CHCl_3$); IR (KBr) ν_{max} 3456, 1735, 1693, 1631. 1380, 1230, 1137, 1045, 802, cm^{-1}; ^1H and ^{13}C NMR data, see (Table 1); HRFABMS *m*/*z* 407.1711 $[M + 1]^+$ (calcd for $C_{21}H_{27}O_8$, 407.1706)

6*R-(2″-Hydroxyisobutanoyloxy)-8*S**-acetoxy-15-oxo-1*S**,2-epoxy-3,11(13)-elemandien-12,9-olide (4):** colorless oil; $[\alpha]_D^{25}$ +47.7 (*c* 0.71, $CHCl_3$); IR (KBr) ν_{max} 3452, 1735, 1693, 1631, 1377, 1230, 1137, 1045, 802, cm^{-1}; ^1H and ^{13}C NMR data identical to those reported.[2]

Cytotoxicity Bioassays. The tetrazolium-based colorimetric assay (MTT assay)[13] was used for the in vitro assay of cytotoxicity to human non-small-cell lung carcinoma (NCI-H460), human breast carcinoma (MCF-7), human glioma (SF-268), and pancreatic carcinoma (MIA Pa Ca-2) cell lines and normal human fibroblast (WI-38) cells.

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References and Notes

(1) Studies on Arid Land Plants and Microorganisms, Part 12. For Part 11, see: Zhan, J.; Gunaherath, G. M. K. B.; Wijeratne, E. M. K.; Gunatilaka, A. A. L. *Phytochemistry* **2006**, in press.
(2) Herz, W.; Govindan, S. V. *Phytochemistry* **1982**, *21*, 787−788.
(3) Bohlmann, F.; Zdero, C.; King, R. M.; Robinson, H. *Phytochemistry* **1979**, *18*, 1343−1348.
(4) Bohlmann, F.; Ziesche, J.; King, R. M.; Robinson, H. *Phytochemistry* **1981**, *20*, 1623−1630.
(5) Herz, W.; Govindan, S. V. *Phytochemistry* **1981**, *20*, 2229−2231.
(6) Ortega, A.; De Vivar, A. R.; Toscano, R. A.; Maldonado, E. *Chem. Lett.* **1983**, 1607−1610.
(7) Maldonado, E.; Soriano-Garcia, M.; Guerrero, C.; De Vivar, A. R.; Ortega, A. *Phytochemistry* **1985**, *24*, 991−994.
(8) Ortega, A.; Maldonado, E. *Phytochemistry* **1985**, *24*, 2635−2639.
(9) Ortega, A.; Maldonado, E. *Phytochemistry* **1982**, *21*, 785−786.
(10) Ortega, A.; Vazquez, A.; Gavino, R.; Maldonado, E. *Phytochemistry* **1995**, *39*, 1479−1481.
(11) Fischer, N. H.; Olivier, E. J.; Fischer, H. D. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Grisebach, H., Kirby, G. W., Eds.; Springer-Verlag: Vienna, 1979; Vol. 38, pp 47−390.
(12) Tellez, J. M.; Ortega, A.; Maldonado, E.; Wong, L.; Vidales, G.; Tello, J.; Calzada, L.; Salazar, L.; Taboada, J. *Adv. Contraceptive Delivery Syst.* **1995**, *11*, 57−63.
(13) Rubinstein, L. V.; Shoemaker, R. H.; Paul, K. D.; Simon, R. M.; Tosini, S.; Skehan, P.; Scudiero, D. A.; Monks, A.; Boyd, M. R. *J. Nat. Cancer Inst.* **1990**, *82*, 1113−1118.
(14) Davies-Coleman, M. T.; Rivett, D. E. A. In *Progress in the Chemistry of Organic Natural Products 55*; Herz, W., Grisebach, H., Kirby, G. W., Tamm, Ch., Eds.; Springer-Verlag: Vienna, 1989; pp 1−35.

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