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SESQUITERPENE LACTONE CHEMISTRY OF *ZALUZANIA GRAYANA* FROM ON-LINE LC-NMR MEASUREMENTS

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Key Word Index—*Zaluzania grayana*; *Viguiera triloba*; Heliantheae; Asteraceae; sesquiterpene lactones; LC-NMR coupling.

Abstract—On-line LC-NMR measurements were used for the first time to elucidate the sesquiterpene lactone chemistry of a Compositae species, *Zaluzania grayana*. Structure elucidation of three compounds, visible in HPLC analysis of the constituents of glandular trichomes, was performed on a leaf extract of less than a gram of dried plant tissue by means of direct coupling of the HPLC and NMR instruments. The structures could be assigned to the guaianolide zaluzanin C, a new modified guaianolide and to the 15-hydroxy derivative of costunolide. The taxonomic relevance of the identified compounds is discussed.

INTRODUCTION

Zaluzania is a small genus of nine species in 11 taxa as currently recognized by Olsen [1]. Both the generic relationships and circumscription of *Zaluzania* have been controversial, in part because of its characteristic lack of a pappus. Stuessy [2] suggested its removal from the formerly broadly defined Helianthinae to be part of a newly created subtribe, Neurolaeninae, and further suggested possible ties to Galinsoginea. In contrast, Robinson [3] placed it in a separate subtribe, Zaluzaniinae, which he placed near subtribe Rudbeckiinae. Various workers have segregated or transferred some elements of *Zaluzania* into other genera. These include *Hybridella* (*Zaluzania* subg. *Hybridella* [4, 5]), *Greenmaniella* (*Z. resinosa* [4]), and *Kingianthus* (*Z. sodiroi* [1, 4, 5]). Most recently Olsen [1] suggested transfer of *Z. grayana* to *Viguiera*, where it goes under the name of *V. triloba*.

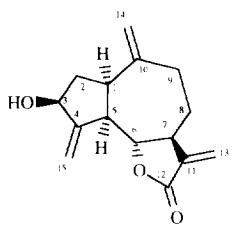
Zaluzania grayana is a perennial herb that occurs in southern Arizona (U.S.A.) and in northwestern Mexico. Olsen cited its sterile ray flowers that have showy ligules and its chromosome number of n = 17 in removing it from *Zaluzania*, species of which all have a base number of x = 18 and fertile ray flowers with short ligules [1]. Within *Viguiera* a placement in *V.* ser. *Pinnatilobatae* was suggested, although there are also features that may relate it to *V.* sect. *Heliomeris* [1]. Its sesquiterpene lactone (STL) chemistry was not considered in evaluating this transfer, although studies have reported these compounds from two species of *Zaluzania*, *Z.augusta* and *Z.triloba* [6, 7], and from various species of *Viguiera* including one of *V.* ser. *Pinnatilobatae* [8–10].

The present study shows that *Z. grayana* shares a highly distinctive STL chemistry with other species of *Zaluzania* that have been investigated and thus suggests that it should be retained within this genus. This study is notable in employing a very recently developed technique of on-line LC-NMR measurement for structure elucidation that is very suitable for chemotaxonomic applications.

RESULTS

The investigation of the STL chemistry of *Zaluzania grayana*, collected near Cuahtemoc (Chihuahua, Mexico), was guided by HPLC analysis of glandular trichomes using the microsampling technique described recently [11]. Glands were found on leaves, ray flowers and anther appendages and contained three STLs with retention times (RR_t) of 0.74, 0.78 and 1.07 relative to dimethylphenol (DMP) in the acetonitrile system that was used (see Experimental). The same compounds were obtained from methylene chloride extracts of plant tissue. One substance ($RR_t = 0.78$) accounted for 87% of the peak area compared to 8 and 5% of the two minor constituents. Quantitation by means of HPLC revealed the presence of *ca* 80 ng STLs per glandular trichome in the aforementioned ratios.

The extract from 250 mg dried leaves (containing an estimated 140 μg of the major STL and between 7 and 15 μg of the minor STLs) was used for HPLC separation (RP 18, 25% MeCN in D_2O) with direct measurement of ^1H NMR spectra. Two different ways of measurement were employed to provide data for the structure elucida-



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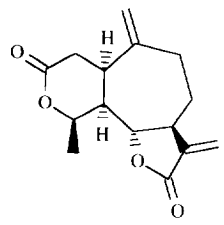

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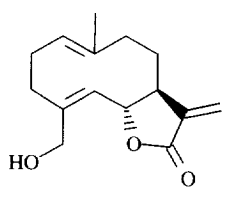

(3)

tion of all three compounds (**1**–**3**). With the on-flow technique it was possible to obtain a correlation between the HPLC separation and the occurrence of ^{1}H NMR signals (Fig. 1). This information was used to resolve compound signals from impurities and solvent resonance, respectively. Stop-flow measurements were used to determine the exact spectral positions, coupling patterns and signal intensities of the protons.

Proton signals of the major constituent (**1**) were in agreement with the structure of zaluzanin C, a *cis* guaianolide, previously reported from *Z. augusta* and *Z. triloba* [7] (Table 1). The stereochemistry of the hydroxyl group at C-3 was determined from the position of the triplet signal of H-6 at δ 4.10. In the 3α-OH-isomer of zaluzanin C H-6 is less deshielded and appears at around δ 3.90 [12].

The ^{1}H NMR data of the preceeding compound (**2**) ($RR_t = 0.74$) were very similar to those of zaluzanin C thus indicating a closely related structure. However, the methylene signal of H-15a/b was replaced by a doublet at δ1.43 (J = 6.4 Hz) with a three-proton intensity. A doublet of quartets at δ4.58 showed coupling to the methyl signal as well as to H-5 (δ2.24 *ddd*). This allowed the assignment of H-4 and indicated that the methyl group was attached to C-4. The stereochemistry at this position was deduced from a comparison of the coupling constants ($J_{4,5}$ = 8.9 Hz; $J_{5,6}$ = 9.2 Hz) with those calculated for model structures (PCMODEL 4.0 and GMMX

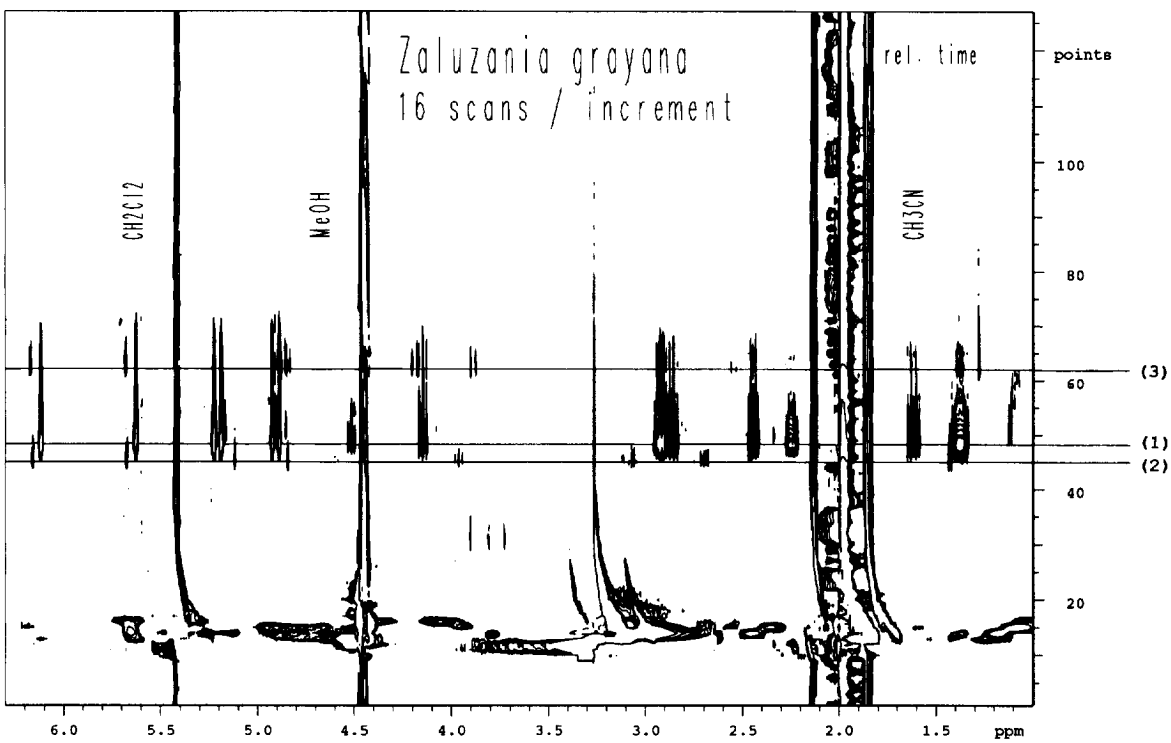

Fig. 1. On-flow ^{1}H NMR spectrum of *Z. grayana*. A series of 128 consecutive spectra (y-values as given in datapoints relative to the start of the HPLC analysis) with 16 scans per spectrum was performed using the following parameters: SF = 500 MHz, SW = 10000 Hz, TD = 8 k, FIDRES = 1.2 Hz, AQ = 0.4 sec, solvent suppression by NEOPRESAT;for processing a QSINE-window was used.

Table 1 ^{1}H NMR spectral data of compounds **1–3** (in D_2O at 500 MHz)

H	1*	1	2	3
1		2.92 *m*	3.08 *bq* (6, 8, < **1**)	**4.93** *bt* (6)
2a		2.25 *ddd*	2.70 *dd* (17, 6.8)	2.15 *m*
b		1.62 *ddd* (13.5,7.3)	2.61 *dd* (17,7.0)	2.15 *m*
3a	4.58 *t* (7.0)	4.52 *bt* (7.3)		2.60 *ddd* (12.5)
b				1.98 *ddd* (12.5)
4			4.58 *dq* (8.9, 6.4)	
5		2.87 *m*	2.24 (8.9, 8.9, 7.1)	4.85 *bq* (10)
6	4.14 *t* (9.0)	4.13 *t* (9.3)	3.92 *t* (9.1)	4.79 *t* (10, 9)
7		2.90 *m*	3.02 *m*	2.55 *m*
8a		2.24 *m*	2.42 *m*	2.18 *m*
b		1.40 *m*	1.32 *m*	1.65 *m*
9a		2.45 *dd* (12.6, 6)	2.52 *m*	2.48 *dd* (13.8, 5)
b		2.15 *dd* (12.6, 4.8)	2.20 *m*	2.12 *dd*
13a	6.21 *d* (3.0)	6.14 *d* (3.5)	6.12 *d* (3.4)	6.20 *d* (3.5)
b	5.52 *d* (3.0)	5.62 *d* (3.2)	5.63 *d* (3.1)	5.63 *d* (3.5)
14a	5.02 *s*	4.94 *s*	5.08 *bs* (< 1)	1.22 *s*†
b	4.96 *s*	4.91 *s*	4.80 *bs* (< 1)	
15a	5.42 *t*	5.23 *t* (2.0)	1.43 *d* § (6.4)	4.11 *brd* (13.5)
b	5.34 *t*	5.20 *t* (2.0)		3.85 *brd* (13.5)

*Data from ref. [7] measured in $CDCl_3$ at 60 MHz.
†Three proton intensity.

1.0 on IBM RS/6000 by Serena software). Further couplings established the sequence of H-1 (δ3.08 *q*) to H-2a (δ 2.70 *dd*) and H-2b (δ2.61 *dd*). The multiplicity of the H-2 signals and the COSY data suggested a quatenary centre at C-3. All other signals were in agreement with the proposed structure of the modified guaianolide (**2**). This was supported by mass spectroscopic measurements which showed M^+ at m/z 262 for $C_{15}H_{18}O_4$.

The on-flow NMR spectrum (Fig. 1) showed that the signals of the third compound (**3**) were overlapping with those of zaluzanin C because of insufficient separation due to peak tailing of the major constituent. Nevertheless, it was possible to deduce its structure from a stop-flow analysis. In addition, the correct assignment of signals was ascertained by measuring a prepurified sample of the same extract where overlap with the signals of zaluzanin C (**1**) was absent. The spectrum of **3** showed the presence of an unsaturated γ-lactone and COSY experiments revealed the sequences of H-7 to H-9 and H-7 to H-5. The carbons C-4 and C-10 appeared quatenary. A CH_2OH signal at δ4.11 (*dd*) and 3.85 (*dd*) showed long-range coupling to H-5 thus indicating H-15a,b at this position. A quaternary methyl group at δ 1.22 (*s*) showed long range coupling to the signals at H-9 as well as to an olefinic proton at δ4.96 (*brt*). This allowed the assignment of H-14 and H-1. The latter proton showed further coupling with a proton multiplet in the aliphatic part of the spectrum and COSY data established the positions of H-2a,b and H-3a,b. The ^{1}H NMR data were in agreement with the structure of the germacrolide **3**, a 15-hydroxy derivative of costunolide that was previously reported from *Platycarpha glomerata* under the name 8-desoxysalonitenolide [13].

DISCUSSION

The results show that ^{1}H NMR spectra of good quality can be obtained with less than 1 g of plant tissue which contains STLs in the μg range. This downscaling in the weight of plant material by a factor of about 1000 in comparison to previously applied techniques could make chemical data more easily accessible for taxonomic purposes and may help to solve physiological and ecological problems in many fields of biology. Of course this will not replace the standard technique of working up large amounts of plant material for special problems in structure elucidation of new compounds, but it is certainly an excellent tool to verify the structures of known constituents.

One of the biggest advantages is the speed of this new technique. It reduces the preparation steps between extraction and spectroscopic measurement to a single HPLC run so that degradation of sensitive compounds can be kept to a minimum. Modern NMR techniques like 2D and 3D experiments can be applied as well, thus providing the full range of spectroscopic information even to heteroelements. It should be pointed out that due to the signals of the LC solvent parts of the NMR spectrum remain necessarily obscured (in our case with MeCN the region of *ca* δ1.8–2.1). However, this can be compensated by running the same sample in a second solvent system to close the gap (e.g. in MeOH, covering δ3.2–3.5).

Zaluzanin C (**1**), the major STL of *Z. grayana* was previously detected in two other species of the genus, *Z. augusta* and *Z. triloba* [7]. It was also reported from two additional taxa, *Z. parthenoides* and *Z. robinsonii* [14], both of which were merged with *Z. triloba* by Olsen [1].

The two other compounds (**2**, **3**) were not previously reported from *Zaluzania* species. With respect to the guaianolide **2** this seems to be the first report of a modified guaianolide (while a similar modification between C-4 and C-3 is common for pseudoguaianolides). The germacrolide **3** is the 15-hydroxy derivative of costunolide. Costunolide is a relatively common STL and was previously reported from *Z. montagnifolia* [14]. A similar germacrolide was found in *Z. pringlei*, also [14]. In contrast to its related species, *Z. grayana* did not contain any of the other constituents found in the genus, among which are the unusual 7,5-lactonized guaianolides zaluzanin A and B from *Z. augusta* [6] and *Z. montagnifolia* as well as two eudesmanolides from *Z. triloba* and *Z. montagnifolia* [14].

Summarizing these results, the genus *Zaluzania*, can be characterized chemically by the formation of *cis*-guaianolides, eudesmanolides and/or simple germacrolides. The STL pattern of *Z. grayana* fits very well into this pattern. In contrast, species of the *Viguiera* series *Pinnatilobatae* are dominated by 1,10-epoxydized-heliangolides and 1-keto-2,3-unsaturated-furanoheliangolides [8–10]. No guaianolides have so far been found in the series *Pinnatilobatae* and they have to be regarded as exceptional in the whole genus. It is, therefore, obvious that Olsen's concept of transferring *Z. grayana* into *V. triloba* is not supported by the phytochemical data for the STLs. The presence of such a distinctive STL as zaluzanin C, that *Z. grayana* shares with other *Zaluzania* species supports its retention within this genus. This is also concordant with recently obtained cpDNA data that show clear distinctiveness between *Z. grayana* and species of the *Viguiera* series *Pinnatilobatae* (E. E. Schilling, unpublished results).

EXPERIMENTAL

Plant material. Zaluzania grayana was collected close to Cuahtemoc, State of Chihuahua, Mexico by E. E. Schilling and O. Spring (voucher # OS 324 at University of Hohenheim).

Extraction and structure elucidation. HPLC screening for STLs was performed in the usual manner [11] using extracts of glandular trichomes. The HPLC conditions used were as follows: Hypersil ODS (5μm; 4 × 250 mm), 50% MeOH or 30% MeCN as solvent; UV detection simultaneously at 225 and 265 nm; dimethylphenol as int. standard. Peak quantitation was performed by means of purified reference samples from a leaf extract. For on-line HPLC/NMR experiments, 1 g of dried leaves was extracted for 5 min in 5 ml CH_2Cl_2. The extract was filtered and dried in a vacuum concentrator. The residue was resuspended in MeCN and diluted with H_2O to the final concentration of the solvent used for HPLC (Spherisorb ODS 2; 4.6 × 250 mm; 30% MeCN, UV detection at 225 nm). Before injection, pptd and insoluble residues were removed by centrifugation (3000 *g*, 1 min, Eppendorf centrifuge). A portion equivalent to the extract of 250 mg leaves was applied to the HPLC for separation with on-line coupling to a microcell in a Bruker AMX 500 MHz spectrometer. For the stop-flow technique, the HPLC peaks were collected in the BPSU-12 peak-sampling unit and analysed later.

Zaluzanin C (**1**). $C_{15}H_{18}O_3$, EI-MS 70 *m/z* (rel. intensity): 246 $[M]^+$ (18), 218 $[M—CO]^+$ (20), 105 (60), 91 (96), 53 (64).

Zaluzanin E (**2**). $C_{15}H_{18}O_4$; EI-MS 70 *m/z* (rel. intensity): 262 $[M]^+$ (3), 234 $[M—CO]^+$ (5), 220 $[M—42]^+$ (12), 190 $[M—C_3H_4O_2]^+$ (8), 176 $[C_{10}H_{14}O_2]^+$ (100).

Costunolide, 15-hydroxy [*8-desoxysalonitenolide*] (**3**). $C_{15}H_{20}O_3$, EI-MS 70 *m/z* (rel. intensity): 248 $[M]^+$ (not observed), 230 $[M—H_2O]^+$ (6), 217 $[M—CH_2O]^+$ (6), 177 $[M—C_4H_6OH]^+$ (100).

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