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A NEW GERMACRANOLIDE FROM *ZINNIA HAAGEANA*

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Key Word Index—*Zinnia haageana*; Compositae; Heliantheae; structure elucidation; sesquiterpene lactone; germacranolide; haageanolide.

INTRODUCTION

From two species of *Zinnia* (*Z. acerosa* DC. Gray and *Z. pauciflora* L.) several elemanolides have been isolated [1, 2]. The guaianolides: zaluzanin C and D have also been found as the constituents of *Z. acerosa* [2]. No previous work has been reported on the sesquiterpene lactones from *Z. haageana* Regel.

RESULTS AND DISCUSSION

The alcoholic extract of dried leaves of *Z. haageana* furnished in about 0.06% yield a new substance, named haageanolide (**1**) which could not be obtained in crystalline form and was very unstable on storage. The empirical formula $C_{15}H_{20}O_3$ for **1** was assigned on the basis of elemental analysis and MS (M^+ 248).

IR absorption bands at 1765, 1665 cm^{-1} and NMR signals (Table 1) at δ 5.53 and δ 6.32 (doublets, J = 3.1 Hz) suggested the presence of an exocyclic methylene γ-lactone. Since two oxygen atoms were accounted for by the γ-lactone moiety, only one oxygen atom remained unassigned.

The IR spectrum of **1** showed a sharp peak at 3470 cm^{-1} indicating the presence of a hydroxyl group which was readily acetylated. Acetylation afforded an acetate (**2**), $C_{17}H_{22}O_4$, mp 196–197°, a stable crystalline compound. MS ions of m/e 290 (M^+), $M^+ - 42$ and $M^+ - 60$ established the presence of the acetoxyl group.

The NMR spectrum of **2** (in Py-D_5) was very similar to that of **1** except for the presence of a new acetyl methyl at δ 2.07 and a one proton doublet of doublets at δ 5.36 (J = 10 and 3 Hz) which in the case of **1** had appeared at δ 4.40. This result indicated that the remaining oxygen was present as a secondary alcohol and the C<u>H</u>OH proton was adjacent to two other protons.

Other features of the NMR spectrum of **2** included a broad singlet at δ 1.51 and a sharp singlet at δ 1.67 indicated the presence of two tertiary vinyl methyl groups. At low field (δ 4.45–5.45) the 4-proton signals were observed and were assigned to two vinylic protons, the lactone proton and the proton under the hydroxyl group. The combined data suggested that **1** was a member of the germacranolide diene class of sesquiterpenes.

The NMR spectral data of **1** and its acetate were very similar to that of costunolide (**3**) [3–5] except for features associated with the hydroxyl or acetoxyl group, and it appeared that **1** exhibited the same structural, configurational and conformational relationships around the medium ring. Thus a doublet of doublets at δ 4.60 in the NMR spectrum of **2** (in $CDCl_3$) could be assigned to the C-6 proton coupled with H-5 (10 Hz) and H-7 (8.5 Hz). The C-5 proton coupled (10 Hz) with H-6 gave a doublet at δ 4.72. The vinylic H-1 produced a broad signal at δ 5.20.

Consideration of all above data indicated that **1** should be a new hydroxycostunolide since its properties differed from the literature values for known hydroxycostunolides viz. hanphyllin [6], tamaulipin B [7], tamaulipin A [8] and eupatolide [9].

The assignment of the hydroxyl group to C-9 as well as its orientation were established in the following manner. The signal of C<u>H</u>OH (*dd*, J = 10 and 3 Hz) in the NMR spectrum of **1** was that expected for the disposition of this proton in a *trans*-diaxial *cis*-axial-equatorial relationship to the vicinal protons. It was compatible with either a 3β or a 9β oxygen function, if the conformation of 1,5-*trans*, *trans* germacranolides, such as costunolide (**3**), is considered [10].

Hydrogenation of **1** with $NaBH_4$ followed by acetylation afforded a crystalline compound, whose physical and spectral properties were identical with those of herbolide A (**4**) and a mmp of the two was undepressed. Herbolide A was isolated more recently from *Artemisia herba alba* and its structure was established [10].

As the herbolide A and **1** are structurally related the stereochemical assignment at all the assymetric centers and the position of the oxygen function at C-9 must be identical for them. The evidence discussed above establishes that haageanolide is 9β-hydroxycostunolide (**1**).

EXPERIMENTAL

Mps were determined on a Boetius hot stage microscope and are uncorr. ^{1}H-NMR: $CDCl_3$ or Py-D_5, δ units relative to TMS, at 100 MHz; MS (70 eV) direct insertion; IR: KBr discs, UV: EtOH. Si gel for CC refers to Schuchardt 100/200 mesh. *Zinnia haageana* was cultivated in the Botanical Garden of Institute of Pharmacology Polish Academy of Sciences in Kraków where the voucher specimen is deposited.

Extraction and isolation. Ground, dried leaves (2 kg) collected on 16 July, 1976 were exhaustively extracted with EtOH at room temp. and filtered. The residue after removal of the solvent was dissolved in 250 ml EtOH and treated with a soln of Pb (II) acetate (20 g) in H_2O (500 ml), left at room temp. overnight and filtered over celite. The filtrate was evapd *in vacuo* to remove most of EtOH and extracted exhaustively with $CHCl_3$. The combined $CHCl_3$ extracts were dried and the solvent was removed under red. pres. The residue (42 g) was chromatographed over a column of Si gel (1.1 kg, packed in C_6H_6) using C_6H_6 with gradually increasing proportions of EtOAc as eluent. The homogeneous (TLC) fractions of mixed solvent (9:1) afforded 1.3 g almost pure **1** as a gum. Trituration with Et_2O gave an amorphous powder. Attempts to crystallize failed and the compound showed a tendency to deteriorate on standing. Freshly purified sample had: IR ν_{max}^{KBr} cm^{-1}: 3470 (OH), 1765 (γ-lactone), 1665 (C=C), 1260, 1145 and 960; MS m/e (rel. int.): 248 (M^+, 2.1), 230 ($M^+ - 18$, 8.2), 215 ($M^+ - 18 + 15$, 15.8), 91 (48.1), 53 (100). (Found: C, 72.40; H, 8.15; O, 19.48. $C_{15}H_{20}O_3$ requires: C, 72.55; H, 8.12; O, 19.33%).

Haageanolide acetate (**2**). Acetylation of **1** under standard conditions yielded, after recrystallization from EtOH, **2**, mp 196–197°; UV, λ_{max}^{EtOH} nm (ε): 210 (18700); IR, ν_{max}^{KBr} cm^{-1}: 1760

(γ-lactone), 1725 (acetate), 1665 (C=C), 1245, 1145, 960 and 860, MS *m/e* (rel. int.): 290 (M^+, 1.5), 248 ($M^+ - 42$, 3.6), 230 ($M^+ - 60$, 38.6), 43 (100). (Found: C, 70.29; H, 7.70; O, 21.98. $C_{17}H_{22}O_4$ requires: C, 70.32; H, 7.64; O, 22.04%).

Dihydrohaageanolide acetate (**4**). To a soln of **1** (150 mg) in 2 ml MeOH, 200 mg $NaBH_4$ in 10 ml MeOH was added. After 30 min the reaction mixture was diluted with H_2O, acidified and extracted with $CHCl_3$. The washed and dried $CHCl_3$ soln was evapd to dryness and the residue acetylated in the usual way. The purified product, after recrystallization from EtOH, was shown to be identical with herbolide A (mp, mmp and IR spectrum).

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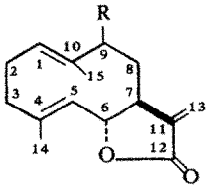

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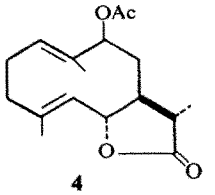

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Table 1. ^{1}H-NMR data for **1** and **2** (ppm, δ-scale, TMS as internal standard, 100 MHz)

	1	**2** (Py-D_5)	**2** ($CDCl_3$)	*J*(Hz)
1-H	5.07 (*m*)	5.15 (*m*)	5.20*	5α 6β = 10
5-H	4.70*	4.69*	4.72 (*d*)	6β 7α = 8.5
6-H	4.70*	4.69*	4.60 (*dd*)	7α 13 = 3.1
7-H	2.70 (*m*)	2.77 (*m*)	2.70 (*m*)	7α 13′ = 3.1
9-H	4.40 (*dd*)	5.36 (*dd*)	5.20*	9α 8β = 10
13-H	5.53 (*d*)	5.53 (*d*)	5.54 (*d*)	9α 8α = 3
13′-H	6.32 (*d*)	6.29 (*d*)	6.24 (*d*)	
14-H	1.68 (*s*)	1.67 (*s*)	1.71 (*s*)	
15-H	1.40 (*br s*)	1.51 (*br s*)	1.46 (*br s*)	
OAc		2.07 (*s*)	2.03 (*s*)	

* The exact chemical shift and coupling constants cannot be determined because of overlap of the signals.

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THE DITERPENE DARUTIGENOL FROM *PALAFOXIA ARIDA**

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Key Word Index—*Palafoxia arida*; Compositae; darutigenol; diterpene.

INTRODUCTION

A number of years ago we isolated from *Palafoxia arida* B. L. Turner and M. I. Morris (Compositae)† a substance $C_{20}H_{34}O_3$ whose structure could not be established satisfactorily. Re-examination of a still extant very small sample has now permitted its identification as darutigenol (**1a**), the aglycone of the glycoside darutoside from *Siegesbeckia orientalis* L. (Compositae) [2, 3]‡. To our knowledge the aglycone itself has not been reported previously as a natural product.

RESULTS

Elemental analysis of our material, mp 168–170°, lit. 168–170° [2], consistently showed retention of one molecule of water of solvation, but that the peak of

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† The collection was originally identified as *P. linearis* by the collector. Because of its provenance (Sonora desert region of Southern Arizona) we deduced that it represents *P. arida* var. *arida* as Turner and Morris [1] point out that the binomial *P. linearis* properly belongs to a taxon which occurs only in Southern Baja, California, and has been misapplied by nearly all recent taxonomists working in the desert Southwest to the relatively common and widespread species named by them *P arida*

‡The stereochemistry of darutigenol at C-15 has not been established.