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1,2-SECOGERMACRANOLIDES FROM *HELIANTHUS GIGANTEUS* AND *H. HIRSUTUS*

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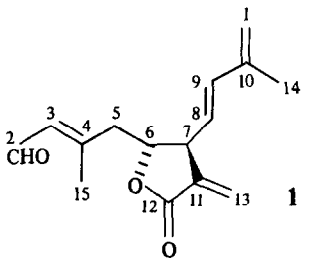
Key Word Index—*Helianthus giganteus, H hirsutus*, Asteraceae, sunflowers, sesquiterpene lactones, germacranolides, secogermacranolides, desacetyleupasserin

Abstract—Two novel 1,2-secogermacranolides were isolated from *Helianthus giganteus* and *H hirsutus* and the previously characterized 2α-hydroxy-*trans,trans*-1(10),4(5)-germacradienolide, eupasserin, was identified from *H hirsutus* These results support current ideas on both the subgeneric taxonomic disposition of these two species and the possible origin of the polyploid *H hirsutus*

INTRODUCTION

In conjunction with a long-term study of the terpenoid constituents of *Helianthus* (Asteraceae) [1–6], we have investigated two perennial species of the eastern and central United States, *H giganteus* L and *H hirsutus* Raf Previous work in the genus has resulted in the isolation of a large number of sesquiterpene lactones, principally germacranolides [6] Here we report the isolation and structural elucidation of two novel 1,2-secogermacranolides (**1** and **2**) from dichloromethane extracts of both *H giganteus* and *H hirsutus Helianthus hirsutus* also yielded the previously characterized *trans,trans*-1(10),4(5)-germacradienolide **3** (eupasserin) [7]



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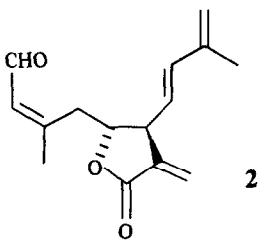

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RESULTS AND DISCUSSION

The chemical ionization mass spectrum of compound **1** showed an $[M+H]^+$ peak at *m/z* 247, consistent with a molecular formula of $C_{15}H_{18}O_3$ Spectral data established that the oxygen-containing functionalities were an α-methylene-γ-lactone (IR 1765 cm^{-1}, ^{13}C NMR δ169 *s*, ^1H NMR δ5 60 *d* and 6 33 *d*, both *J* = 3 Hz) and an α,β-unsaturated aldehyde (IR 2855, 1675 cm^{-1}, ^{13}C NMR δ189 9 *s*, ^1H NMR δ10 01 *d*, *J* = 7 5 Hz)

^1H NMR spin decoupling experiments provided further details of the structure of **1** The aldehyde proton (δ10 01, Table 1) was spin-coupled to a vinylic proton at 5 98 Irradiation at 5 98 sharpened the signal of a methyl group at 2 24 and slightly altered the shapes of two methylene signals at 2 57 and 2 68 These two methylene protons were coupled to a double doublet at 4 35, which was in turn coupled to a complex signal at 3 35 The signal at 3 35 was clearly that of H-7, since it was also coupled to the *exo*-methylene protons of the lactone ring (5 60 and 6 33, H-13a and H-13b) In addition, irradiation at 3 35 simplified a double doublet at 5 44 which was coupled to a widely-split doublet (*J* = 14 5 Hz) at 6 33 The shifts and couplings of these last two signals showed that both represented olefinic protons Thus, the signal at 4 35 was that of the proton at the point of fusion of the lactone ring These results are summarized in partial structure **A**

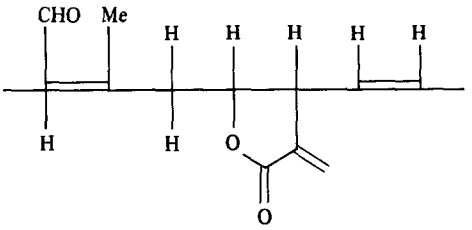
The remainder of the protons in **1** were part of a spin system **B** consisting of two terminal vinyl protons (5 04 *br s*, 5 07 *br s*) and a vinylic methyl group (1 87 *br s*), which could only be joined to **A** as shown in formula **1** The chemical shift of the vinylic proton at 6 33 confirmed the presence of a conjugated system in this portion of the molecule ^1H NMR coupling constants required the C-8 to C-9 double bond to be *trans* ($J_{8,9}$ = 14 5 Hz) and the lactone ring to be *trans*-fused ($J_{6,7}$ = 7 5 Hz), assuming that H-7 was α-oriented as in all sesquiterpene lactones of authenticated absolute stereochemistry [8]

Although compound **2** was isolated from *H giganteus* only as a 1 1 mixture with **1**, its ^1H NMR spectrum could nevertheless be interpreted The ^1H NMR spectrum of **2** was very similar to that of **1** Small differences in the chemical shifts of several protons (Table 1) indicated that

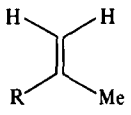
Table 1 ^{1}H NMR data for the secogermacranolides 1 and 2*

H	1	2
1a	5 07 *br*	5 07
1b	5 04 *br s*	5 04
2	10 01 *d*	9 89
3	5 98 *br d*	6 06
5a	2 68 *br dd*	3 07
5b	2 57 *br dd*	2 94
6	4 35 *dd*	4 35
7	3 35 *dddd*	3 35
8	5 44 *dd*	5 46
9	6 33 *d*	6 35
13a	6 33 *d*	6 33
13b	5 60 *d*	5 60
14	1 87 (3H) *br s*	1 87
15	2 24 (3H) *d*	2 06

*Run at 200 MHz in $CDCl_3$ with TMS as an internal standard Multiplicities and coupling constants for **1** and **2** were virtually identical Coupling constants, J (Hz) 1a, 1b = 2, 1a, 14 = 1b, 14 = 1 5, 2, 3 = 7 5, 3, 5a = 3, 5b = 1 5, 3, 15 = 1 5, 5a, 5b = 14, 5a, 6 = 4 5, 5b, 6 = 7 5, 6, 7 = 7 5, 7, 8 = 8 5, 7, 13a = 7, 13b = 3, 8, 9 = 14 5



A



B

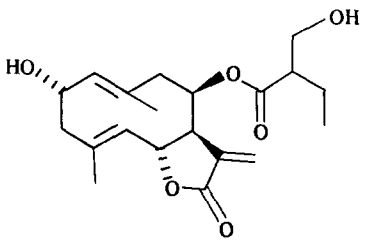
2 differed from **1** only in the geometry of its C-3 to C-4 double bond The C-4 methyl group of **2** appeared upfield from that of **1**, but the H-5 methylene protons of **2** were downfield to those of **1** Therefore, the C-3 to C-4 double bond in **2** was assigned a *Z* (*cis*)-configuration, in which the aldehyde function was on the same side of the double bond as the H-5 protons Compound **1**, then, was the 3*E*-isomer The chemical shifts of the aldehyde protons (10 0 in **1**, 9 89 in **2**) were in accord with these observations Aldehyde protons in *trans*-α,β-unsaturated systems (such as **1**) generally appear at lower field (≥ 10 ppm) that those in corresponding *cis*-α,β-unsaturated systems (such as **2**) [9]

Since the absolute configurations of **1** and **2** could not be determined from the spectral data obtained, these compounds can in principle be formulated as the corresponding 2,3-secogermacranolides with 12,8-lactonization, enantiomeric to formulae **1** and **2** However, the occurrence of the 12,6-lactonized germacranolide **3** (whose absolute configuration is known [10]) in the extract of *H hirsutus* suggests that the structures of **1** and **2** may be correct as drawn (12,6-lactonized), since both of these 1,2-secogermacranolides can be biosynthetically derived in a straightforward manner from precursors like the sesquiterpene portion of **3** A 1,2-cleavage of a 2-hydroxygermacra-12,6-olide accompanied by oxidation at C-2, a shift of the 4,5-double bond and dehydration at C-8 and C-9 would give **1** and **2**

There is only one other report of a naturally-occurring secogermacranolide [11, 12] This compound, which is a 2,3-secogermacranolide, is biosynthesized from a precursor with a different oxygenation pattern than the precursor of **1** and **2**

The presence of 1,2-secogermacranolides in both *H giganteus* and *H hirsutus* suggests that these species are closely related to each other Both are placed in section *Divaricati* series *Corona-solis* in a recent taxonomic revision of the genus [13], although they were earlier [14] considered to be members of separate series The series *Corona-solis* contains several species which have been shown to produce 2α-hydroxy-8β-acyloxy-*trans,trans*-1(10),4(5)-germacradienolides (2-OH-8-ACGs) [1, 15, Pearce, J, Gershenzon, J and Mabry, T J, unpublished results, Stewart, E, Gershenzon, J and Mabry, T J, submitted for publication] The fact that *H hirsutus* was also shown to contain 2-OH-8-ACGs and the fact that the 1,2-secogermacranolides isolated from *H giganteus* and *H hirsutus* can be considered as biosynthetic derivatives of 2-OH-8-ACGs support the current taxonomic disposition of these two species

Helianthus hirsutus is a tetraploid taxon whose origin is not well understood Four diploid species of *Helianthus* have been proposed as possible progenitors of *H hirsutus* [14] Three of these (*H decapetalus*, *H divaricatus* and *H mollis*) also contain 2-OH-8-ACGs [1, 15] and one (*H giganteus*) produces 1,2-secogermacranolides Since *H hirsutus* contains both 2-OH-8-ACGs and 1,2-secogermacranolides, the available sesquiterpene lactone data suggest that it may have originated from hybridization between *H giganteus* and any of the other three species These systematic conclusions should not be given undue emphasis, however, until studies have been carried out to assess the extent of intraspecific variability in the sesquiterpene lactone compositions of these species For exam-



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ple, *Helianthus maximiliani*, which like *H giganteus* and *H hirsutus* has a wide distribution, has been shown to consist of three distinct sesquiterpene lactone races, with a different skeletal type predominating in each one [Gershenzon, J, Stewart, E and Mabry, T J, unpublished results]

EXPERIMENTAL

Extraction of H giganteus Leaves collected at several sites in central North Carolina during September, 1980 (J G #152, #153, #154, #159, #173, #176, #182, vouchers on deposit at the Herbarium of the University of Texas) were found to have identical TLC patterns These collections were combined (4 4 kg), washed with CH_2Cl_2 for 5 min and the extract worked up in the usual manner [16] The resulting crude syrup (20 g) was applied to a silica gel column (500 g), which was eluted with a CH_2Cl_2–*iso*-PrOH gradient Thirty fractions of 500 ml each were collected Fractions 18–21 (1% *iso*-PrOH) gave two major spots on TLC Separation by preparative TLC (silica gel, 1 mm, CH_2Cl_2–*iso*-PrOH, 15 1) gave 15 mg of an oily mixture of **1** and **2** (*ca* 1 1) as well as one other unidentified compound

Extraction of H hirsutus Leaves (960 g) collected in Bandera Co, Texas, along state highway 187, 5 miles north of Utopia near the Sabinal River, on 22 August 1982 (J G #244) were washed with CH_2Cl_2 and the extract worked up in the usual manner [16] The crude syrup (8 5 g) was applied to a silica gel column (120 g) which was eluted with a CH_2Cl_2–*iso*-PrOH gradient Forty-eight fractions of 200 ml each were collected Fraction 28 (1% *iso*-PrOH) showed one spot on TLC Induced crystallization with CH_2Cl_2–toluene gave 30 mg of crystalline **3**, mp 153–155° (lit 153–154° [7]) Fractions 44–46 (2% *iso*-PrOH) contained a mixture of **1** and **2** Repeated preparative TLC (silica gel, 1 mm, CH_2Cl_2–*iso*-PrOH, 15 1) gave 7 mg of **1** contaminated with a small amount (10%) of **2** No data other than NMR data (Table 1) were obtained for **2**

A group of sesquiterpene lactones isolated from both *H giganteus* and *H hirsutus* gave ^{1}H NMR spectra which show the presence of more than one conformer at room temp The structures of these compounds are still under investigation

(6R*)-(3E, 8E)-2-*Oxo*-1,2-*secogermacra*-1(10),3(4),8(9)-*trien*-12,6-*olide* (**1**) UV λ_{max}^{MeOH} nm (log ε) 224 (4 19) IR $\nu_{max}^{CHCl_3}$ cm^{-1} 2855 (aldehyde C–H), 1765 (lactone C=O), 1675 (aldehyde C=O), 1652, 1613, 1455, 1381, 1265, 1135, 1040, 973, 954, 901, 880, 826 CIMS (isobutane) *m/z* (rel int) 247 $[M+H]^+$ (24), 229 $[M+H-H_2O]^+$ (100), 219 (9), 211 (12), 201 (37), 183 (28), 163 (43), 149 (80), 129 (83), 119 (59), 71 (50) ^{13}C NMR ($CDCl_3$, 22 6 MHz, D_2O as external standard) δ117 3 (C-1), 189 9 (C-2), 129 0 (C-3), 157 (C-4), 43 8 (C-5), 79 3 (C-6), 49 1 (C-7), 124 0 (C-8), 137 7 (C-9), 144 (C-10), 140 (C-11), 169 (C-12), 122 6 (C-13), 17 0 and 17 5 (C-14 and C-15) Multiplicities were not determined since the small quantity of sample prevented the obtaining of partially-decoupled spectra Assignments are tentative and based on appropriate models for the various substructures Resonances at 140, 144, 157 and 169 were partially obscured by background noise and their positions are not precise

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