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SESQUITERPENE LACTONES OF THREE *HELIOMERIS* SPECIES (HELIANTHEAE; ASTERACEAE)

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Key Word Index—*Heliomeris longifolia*; *H. multiflora*; *H. obscura*; Asteraceae; Helianthinae; sesquiterpene lactones; germacolides; heliangolides; chemotaxonomy.

Abstract—Investigation of the sesquiterpene lactone chemistry of three *Heliomeris* species resulted in the purification of 15 known and four new compounds. *H. longifolia* var. *annua* (M. E. Jones) Yates and *H. multiflora* var. *multiflora* Yates contained germacrolides in combination with heliangolides of the furano- and 1,10-epoxy-type, a very common feature of subtribe Helianthinae. In contrast *H. obscura* (Blake) Ckll. showed a distinctive pattern of four heliangolides that could not be detected in the two other species. © 1997 Elsevier Science Ltd

INTRODUCTION

Heliomeris as previously delineated by Yates and Heiser [1] is a small genus of five species within the subtribe Helianthinae (Asteraceae). Its distribution reaches from the southwestern states of the U.S.A. into Mexico. In the past there has been some controversy on the systematic relationship of *Heliomeris*. The species were combined in a separate genus by Nuttall in 1848 [2], before Bentham and Hooker [3] incorporated them in *Gymnolomia*. Blake [4] transferred them into *Viguiera* where they obtained the status of a section. Due to the distinctive involucre and phyllary type and to the lack of a pappus, Yates and Heiser [1, 5] separated them from the paraphyletic *Viguiera* and re-established the genus *Heliomeris*. Besides morphological features, caryological characters clearly distinguish *Heliomeris* from the rest of *Viguiera*. While the basic chromosome number is $x = 16$ or 17 [6–8] for the latter, it is only $x = 8$ for most of the *Heliomeris* species. Exceptions are *H. obscura* with counts of $x = 14$ [19] and three specimens of *H. multiflora* with $x = 9$ [10] and $x = 16$ [11].

In contrast to numerous publications on morphological and caryological characters of *Heliomeris*, only two have focused on its chemistry. They deal with the colour-reaction of the ray flowers (which upon treatment with caustic potash turns red, while they stay yellow in *Viguiera* species) [12] and with the isolation of chalcones and aurones [13]. Sesquiterpene lactones of *Heliomeris* have not yet been investigated.

In this study we will focus on the sesquiterpene lactone (STL) chemistry of three *Heliomeris* species (*Heliomeris longifolia* var. *annua* (M. E. Jones) Yates, *Heliomeris multiflora* var. *multiflora* Yates, *Heliomeris obscura* (Blake) Ckll.). Implications on the taxonomic relationships of the genus will be discussed.

RESULTS AND DISCUSSION

The extract of *Heliomeris longifolia* var. *annua* afforded the known germacrolides 2α,2β-dihydroxygermacra-1(10),4,11(13)-germacratrienolide (**1**) [14] and salonitenolide (**3**) [15], the heliangolides tirotundin (**4**) [16], 8-desacyl-8-isobuturylniveusin B (**11**) [17], 2′,3′-dihydroniveusin B (**12**) [18] and 14-hydroxy-2′,3′-dihydroleptocarpin (**17**) [19]. In addition, four new heliangolides (**13**, **16**, **18**, **19**) were isolated. Compounds **16**, **18** and **19** showed the typical ^{1}H NMR features of 1,10-epoxyheliangolides indicated by the chemical shift of the H-6 signal at around δ 6,6 (*dd*) (Table 1). The structure of **16** was identical with 15-hydroxy-2′,3′-dihydroleptocarpin, previously obtained by Perez *et al.* [20] by saponification of the 3-acetate, hence this is the first report of **16** as a naturally occurring substance. The ^{1}H NMR data of **19** indicated the same basic skeleton as **16**, but with an isobutyrate side chain. Due to the close structural similarity to tagitinin E [21] we suggest the name 15-hydroxytagitinin E. The ^{1}H NMR pattern of **18** resembled in general those of leptocarpin derivatives, but showed significant differences in the chemical shifts of H-1 up to H-6. This was most obvious for H-3 (δ = 5,3). Mass spectra indicated the presence of a methoxyfunction.

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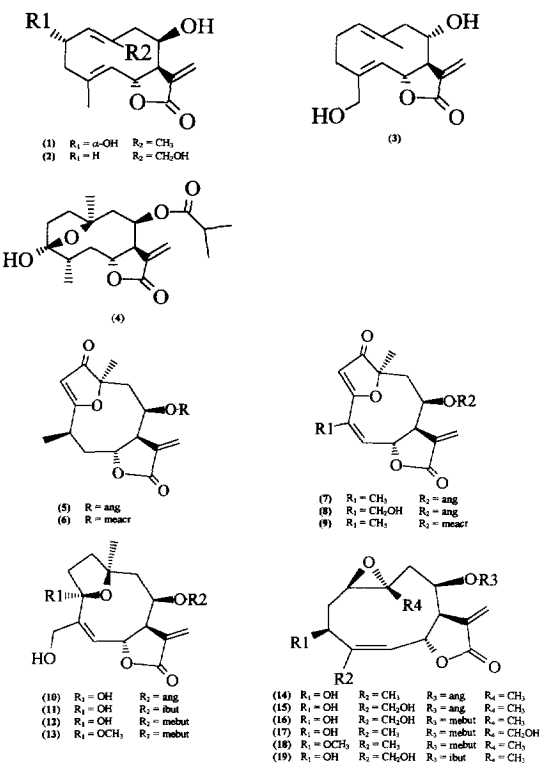

According to structure modelling calculations we suggest methoxylation at C-3. This parallels similar substitutions of 1,10-epoxy-heliangolides that had been previously isolated from other Helianthinae [22]. Additional extraction of plant material and chromatographic separation in the absence of methanol verified the natural occurrence of this methoxygroup in **18**. Due to the 2-methylbutyrate side chain the structure was named 3-*O*-methyl-2′,3′-dihydroleptocarpin. Compound **13** showed the typical structural elements of 3-hydroxy-furanoheliangolides (H-7 at δ 4,2; Table 1). The ^{1}H NMR data were identical with those of **12** [18] except for the presence of an additional three proton signal at δ 3,5. As a result of the low amount of substance material Nuclear Overhauser Experiments could not clarify the exact position of the methoxy function in C-3 or C-15, respectively. However, comparison with other methoxylated sesquiterpene lactones reported in the literature provide evidence that due to biogenetic reasons the methoxylation preferentially affects secondary hydroxyl groups instead of primary alcohols. Since the mass spectroscopic investigation did not show a fragmentation pattern that would be in accordance with a C-15-methoxyl-group, we tentatively assigned structure **13** as 3-*O*-methyl-2′,3′-dihydroveusin B. Extraction and HPLC separation in non-methanolic solvents established the natural occurrence of **13**.

The plant extract of *H. multiflora* var. *multiflora* afforded five sesquiterpene lactones of known structure. Besides the germacrolide 8β,14-dihydroxycostunolide (**2**) [23, 24] the 1,10-epoxyheliangolides leptocarpin (**14**) [25], and 15-hydroxyleptocarpin (**15**) [22] as well as the furanoheliangolides budlein A (**8**) [24], and niveusin B (**10**) [26] were identified.

H. obscura, in contrast to the previous two species, is a very distinctive taxon that has been collected from

Table 1. ^{1}H NMR signals of sesquiterpene lactones from *Heliomeris longifolia* var. *annua*

H	**13**	**16**	**18**	**19**
1	2,3 *m*	2,81 *dd* (12.5, 5.0)	2,62 *dd* (10, 4.5)	2,75 *m*
2a	2,3 *m*	2,48 *dq* (14.6, 4.6)	2,43 *dd* (15, 4.5)	2,49 *dd* (14.1, 4.7)
2b	2,05 *dd* (11.1, 4.1)	1,73 *dq* (14, 10)	1,82 *ddd* (15, 10.1, 2.6)	1,81 *ddd* (15.7, 11, 2.2)
3	4,61 *m*	5,3 *m* 4,63 *dd* (5.3, 2.2)		
5	5,96 *m*	5,55 *d* (12.5)	6,36 *d* (10.7)	5,58 *dt* (12.5, 1.6)
6	5,44 *m*	6,61 *dd* (2.8, 11.1)	6,75 *dd* (10.7, 2.3)	6,60 *dd* (13.2, 2.8)
7	4,20 *m*	2,94 *m*	3,0 *m*	2,91 *m*
8	5,59 *m*	5,21 *m*	5,3 *m*	5,21 *m*
9a	2,21 *dd*	2,70 *dd* (16.7, 4.2)	2,75 *dd* (15, 4.3)	2,75 *m*
9b	1,87 *dd*	1,35 *dd* (15.6, 3.1)	1,4 *dd*	1,37 *dd* (3.1)
13a	6,27 *d* (2.6)	6,38 *d* (2.5)	6,49 *d* (2.3)	6,40 *d* (2.5)
13b	5,63 *d* (2.4)	5,80 *d* (2.5)	5,89 *d* (2.2)	5,78 *d* (2.2)
14	1,53 *s**	1,52 *s**		1,53 *s**
15a	4,21 *br d* (13.7)	4,09 *s*		4,13 *br s*
15b	3,99 *br d* (13.9)			4,13 *br s*
2′	2,28 *m*	2,29 *m*	2,35 *m*	2,56 *m*
3′a	1,61 *m*	1,62 *m*	+	1,13 *d*
3′b	1,44 *m*	1,43 *m*	+	
4′	0,85 *t**	0,88 *t**	0,85 *t**	1,16 *d*
5′	1,03 *d**	1,10 *d**	1,13 *d**	
O-Me	3,5 *s**		3,5 *s**	

Spectra were run in $CDCl_3$ at 250 MHz with TMS as internal standard. Coupling constants given in (Hz).
* = Three proton intensity.
+ = Signal obscured.

Table 2. Distribution of sesquiterpene lactones in three *Heliomeris* species

	Germacrolide	3-OH-Furano-heliangolide	1-keto-Furano-heliangolide	1,10-epoxy-Heliangolide
H. longifolia var. *annua*	+	+		+
H. multiflora var. *multiflora*	+	+	+	+
H. obscura			+	

few places in Mexico. Chemical analysis resulted in the identification of four compounds, although only two grams of leaf material were available for extraction. Its chemistry differed from the other two species by the lack of germacrolides, 3-hydroxy-furanoheliangolides and 1,10-epoxyheliangolides. Instead we detected the four 1-keto-furanoheliangolides ladibranolide (**5**) [27], zexbrevin (**6**) [28], atripliciolide, angelate (**7**) [18] and calaxin (**9**) [29].

From the chemotaxonomic point of view it is remarkable that the three investigated *Heliomeris* species differed significantly in their sesquiterpene lactone patterns. Nevertheless, consideration of the skeletal types revealed a closer relationship among them (Table 2). Thus, *H. longifolia* var. *annua* and *H. multiflora* var. *multiflora* shared the presence of germacrolides and various subtypes of heliangolides, a typical combination in Helianthinae [30]. The presence of only 1-keto-furanoheliangolides in *H. obscura* underlines the exceptional status of this species and coincides with its distinctiveness in other taxonomic characters like the chromosome number.

EXPERIMENTAL

Plant material. Heliomeris longifolia var. *annua* was collected in Luna Co., New Mexico, U.S.A., and *H. multiflora* var. *multiflora* in Boulder Co., Colorado, U.S.A., by E. E. Schilling and O. Spring (voucher #OS 309 and #OS 254 at the Universität Hohenheim). *H. obscura* was collected in the state of Puebla, Mexico, by J. L. Panero (voucher #Panero 2312 at Michigan State University and Universidad Autonoma Nacional de Mexico).

Extraction. Prep. purification of sesquiterpene lactones was carried out according to ref. [31] and started with a CH_2Cl_2 extract of air-dried plant material. Compounds were separated by reversed-phase HPLC (Hypersil ODS, 5 μm) using gradients of MeOH–H_2O or CH_3CN–H_2O. To establish the natural occurrence of compounds **13** and **18**, an additional extraction of plant material was carried out using CH_3CN, and HPLC sepn was performed in CH_3CN–H_2O (in order to avoid methoxylation by MeOH). Dried plant material of *Heliomeris longifolia* var. *annua* (73 g) yielded 0.7 mg of 2α,8β-dihydroxygermacra-1(10),4,11(13)-germacratrienolide (eupasserin, 8β-hydroxy-8β-desacyl-desacetyl) (**1**), 0.5 mg of salonitenolide (**3**), 1.2 mg of tirotundin (**4**), 1.2 mg of 8-desacyl-8-isobutyrylniveusin B (**11**), 5.6 mg of 2′, 3′-dihydroniveusin B (**12**), 1.2 mg of 3-O-methyl-2′,3′-dihydroniveusin B (**13**), 9.4 mg of 15-hydroxy-2′,3′-dihydroleptocarpin (**16**), 2 mg of 14-hydroxy-2′,3′-dihydroleptocarpin (**17**), 0.5 mg of 3-O-methyl-2′,3′-dihydroleptocarpin (**18**) and 1.4 mg of 15-hydroxy-tagitinin E (**19**).

3-O-methyl-2′,3′-dihydroniveusin B (**13**). $C_{21}H_{30}O_7$ APCI+Q1MS; grad. 20% MeOH/40 min/100% (rel. int.): *m/z* 395 $[M+H]^+$ (100), 363 $[395\text{-}CH_3OH]^+$ (90), 293 $[395\text{-}C_5H_{10}O_2]^+$ (20), 261 $[363\text{-}C_5H_{10}O_2]^+$ (40), 243 (20), 85 $[C_5H_9O]^+$ (1).

15-hydroxy-2′,3′-dihydroleptocarpin (**16**). $C_{20}H_{28}O_7$. APCI+Q1MS; grad. 45% MeOH/40 min/100% (rel. int.): *m/z* 381 $[M+H]^+$ (100), 361 $[381\text{-}H_2O]^+$ (35), 349 $[381\text{-}CH_3OH]^+$ (24), 279 $[381\text{-}C_5H_{10}O_2^+$ (10), 261 $[279\text{-}H_2O]^+$ (60), 243 $[279\text{-}CH_3OH]^+$ (58), 85 $[C_5H_9O]^+$ (2).

3-O-methyl-2′,3′-dihydroleptocarpin (**18**). $C_{21}H_{30}O_6$· APCI+Q1MS; grad. 20% MeOH/40 min/100% (rel. int.): *m/z* 379 $[M+H]^+$ (100), 277 $[379\text{-}C_5H_{10}O_2]^+$ (30), 259 (40), 241 (30), 85 $[C_5H_9O]^+$ (3).

15-hydroxytagitinin E (**19**). $C_{19}H_{26}O_7$·APCI loop (rel. int.): *m/z* 367 $[M+H]^+$ (100), 349 $[367\text{-}H_2O]^+$ (10), 331 $[349\text{-}H_2O]^+$ (18), 279 $[367\text{-}C_4H_8O_2]^+$ (40), 261 $[279\text{-}H_2O]^+$ (90), 243 $[261\text{-}H_2O]^+$ (65), 71 $[C_4H_7O]^+$ (3)

Air dried plant material of *Heliomeris muiltiflora* var. *multiflora* (32, 1 g) was extracted as described previously [31]. Isolation by means of prep. HPLC yielded 0.8 mg of 8β,14-hydroxycostunolide (**2**), 0.6 mg of leptocarpin (**14**), 1 mg of 15-hydroxyleptocarpin (**15**), 1.8 mg of budlein A (**8**) and 1.6 mg of niveusin B (**10**).

Air dried plant material of *Heliomeris obscura* (2 g) was extracted as previously described [31]. Prep. HPLC of the extract yielded 0.7 mg ladibranolide (**5**), 1 mg zexbrevin (**6**), 1.3 mg atripliciolide, angelate (**7**) and 0.9 mg calaxin (**9**).

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