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SESQUITERPENE LACTONES AS A RESULT OF INTERSPECIFIC HYBRIDIZATION IN *HELIANTHUS* SPECIES

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Key Word Index—*Helianthus annuus*; *H. debilis*; Heliantheae; Asteraceae; interspecific hybridization; sesquiterpene lactones; synergistic compounds.

Abstract—The effect of hybridization on the sesquiterpene lactone chemistry of F1-progenies from two different *Helianthus* species, *H. annuus* and *H. debilis*, was investigated. As in morphological traits the hybrid plants showed intermediacy in the sesquiterpene lactone chemistry owing to the additivity of the parental patterns. Moreover, new sesquiterpene lactones were found in the F1-generation, in addition to known compounds of both parents. Chemical analysis of two new compounds by means of ^{1}H NMR and mass spectrometry revealed them as biosynthetic chimeras formed by a combination of the basic skeleton of the one parent with the side chain of the other parental species.

INTRODUCTION

The chemistry of Asteraceae, in addition to pharmacological reasons, has successfully been employed in recent years to clarify taxonomic aspects [1–3]. The sesquiterpene lactone content of glandular trichomes was used for comprehensive reinvestigations of various genera in the Heliantheae [4–6]. As with morphological investigations, the chemotaxonomic classification is significantly impeded by the tendency of many species to hybridize. For *Helianthus*, this readiness was impressively demonstrated by various crossing experiments [7, 8]. The high number of allopolyploid species, as well as the existence of two natural hybrid taxa, *H.* × *multiflorus* and *H.* × *laetiflorus*, shows that interspecific crossing may play an important role in the speciation of the genus.

The object of this study was to investigate the influence of interspecific hybridization on the sesquiterpene lactone chemistry of two *Helianthus* species. This should not only help to provide more information about the inheritance and the synthesis of sesquiterpene lactones, but also about the usefulness of these compounds for the identification of possible progenitors of natural hybrids in Asterceae [9].

RESULTS

Two commercially available cultivars of *Helianthus* were used in the experiments, *H. annuus* cv. *giganteus* and a cultivar form of *H. debilis* ssp. *cucumerifolius*. Both species were chosen because of their distinctive morphology in combination with clear differences of their sesquiterpene lactone patterns [10, 11]. The sesquiterpene lactone profiles of the cultivars were virtually indistinguishable from those of their wild ancestors [12]. Two crossing experiments were performed by using *H. annuus* and *H. debilis* alternatively as pollen- or egg-donor.

In both experiments the number of seeds obtained was relatively low, reaching only 1% (with *H. annuus* as maternal parent) and 15% (with *H. debilis* as maternal parent) of the possible output. This corresponds with the results of Heiser [13] who described a similarly reduced fertility and reduced seed viability in an experiment with the same combination of species. According to his observation natural hybrids of *H. annuus* and *H. debilis* ssp. *cucumerifolius* occur in southwestern parts of Texas, where both species are sympatric.

As with Heiser's plants [13], our F1-hybrids showed morphological intermediance compared to their parents which is summarized in Table 1. Successful hybridization, in contrast to self-pollinated offspring, was most obvious in the height of the F1 progenies, in their shape of leaves and phyllaries, and in the number of inflorescences and in the blooming time. Caryologic investigation of meiotic stages during pollen formation revealed a chromosome number of $X = 17$ in all hybrid plants. In addition molecular markers were used to assure the hybrid nature. Restriction site analysis of rDNA, carried out by E. E. Schilling (personal communication), with three enzymes (BamHI, SpeI, DraI) afforded the additive fragment patterns of the two parental species as expected according to the data reported by Rieseberg [14].

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Table 1. Morphologic and caryologic features of the parental plants, *Helianthus annuus* cv *giganteus* and *Helianthus debilis* ssp. *cucumerifolius*, and their F1-hybrids

	Helianthus annuus	*Helianthus debilis*	Hybrid
Average height	2.5 m	1.3 m	1.5–2 m
Stem	unbranched	branched	branched
Leaves	large, heart-shaped	smaller, lanceolate	intermediate
Glandular trichomes	on both leaf sides	on lower leaf side	on lower leaf side
Blooming after germination	14 weeks	9 weeks	11 weeks
Inflorescence	one, large capitulum	8–10 smaller heads	few heads of variable size
Phyllaries	broad, hispid, ovate in shape	narrower, glabrous, lanceolate in shape	intermediate
Ratio of phyllaries (width:length)	0.40	0.34	0.37
chromosome number	$X = 17$	$X = 17$	$X = 17$

These morphological and genetic hints correlated with the results of the chemical investigation. The sesquiterpene lactone pattern of *Helianthus debilis* was dominated by compounds of the so-called budlein type (1-keto-2,3-unsaturated-furanoheliangolide) with an attached 2-methylbutyrate side-chain and various other ester substituents at C-8 (isobutyrate, tiglate, angelate). *Helianthus annuus*, in contrast, was characterized by furanoheliangolides of the niveusin, tifruticin and argophyllin type and produced exclusively angelate side-chains. The difference in budlein type compounds between the two species allowed us to distinguish them easily in HPLC-analysis by means of simultaneous doublewavelength UV-detection. Most of the sesquiterpene lactones (**5**–**7**, **9**, **10**) in *Helianthus annuus*, except for tifruticin, desoxy-3-dehydro-15-hydroxy (**8**), possess only one chromophore region which leads to a strong end absorption at around 210 nm, while the budleins (**1**–**4**) of *Helianthus debilis* have a second chromophore region that can be detected at 265 nm.

Glandular trichome extracts of the F1-hybrid plants were analysed on HPLC and compared with similar extracts of their parents. Most of the detected peaks in the chromatograms could be assigned to known parental sesquiterpene lactones according to retention times relative to dimethylphenol (DMP; internal standard) and UV-absorption ratios (225 nm:265 nm). Besides the additivity of parental compounds, three new peaks were found that were not present in either of the parents. Interestingly the patterns of both crossing experiments (*H. annuus* used alternatively as male and female parent; details of plants in Table 1) were most similar to each other with respect to the additivity and were identical in the occurrence of new compounds (Table 2). Analysis of F2-hybrids indicated a segregation into parental and intermediate sesquiterpene lactone patterns. However, the low amount of viable seeds from F1 crosses did not allow further statistical interpretation.

To verify the correct assignment of known structures, the compounds were isolated from hybrid plants and analysed by spectroscopic methods. ^{1}H NMR and mass spectroscopic data confirmed the assumed identity with parental structures **1**–**5** and **7**–**10**.

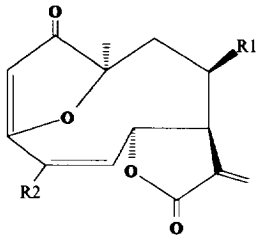
Two of the three new compounds could be purified in sufficient amounts for complete structure elucidation. The ^{1}H NMR signals of **11** (retention time $RR_t = 0.79$ relative to DMP in 50% MeOH) were basically identical with those of the tifruticin derivative **8**, a major constituent in *H. annuus* (Table 3). However, the typical angelate signals were replaced by three aliphatic multiplets of single proton intensity and two methyl signals at $\delta 0.83\ t$ and $1.04\ d$ indicating a 2-methylbutyrate side chain cleaved to C-8. This was confirmed by mass spectroscopic data that showed a molecular ion at m/z 378 and a side-chain fragment of 85 (in contrast to $[M]^+$ at m/z 376 and 83 for **8**).

The same side-chain characteristics were observed for **12** ($RR_t = 0.98$) indicating again a 2-methylbutyrate substitution at C-8. The ^{1}H NMR signals of the basic skeleton were identical with those of argophyllone B (**13**), a constituent previously reported from *H. argophyllus* [15] and closely related to the structure of argophyllin B (**6**) in *H. annuus*.

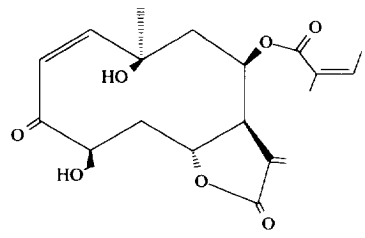
A third new constituent ($RR_t = 0.57$) was only obtained in trace amounts and decomposed before complete structure elucidation. ^{1}H NMR signals indicated the presence of a heliangolide with an isobutyrate side chain.

DISCUSSION

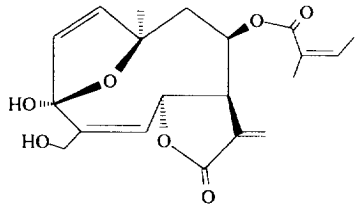
The additivity of chemical characters in hybridization has been described several times in the past. Thus, flavonoid patterns were used to identify the fern *Asplenium kentuckiense* as an allopolyploid hybrid of three diploid *Asplenium* species [16]. For sesquiterpene lactones in Asteraceae, McMillan *et al.* [17] observed additivity of isomeric xanthanolides in F1-progenies of *Xanthium strumarium* obtained from crosses of distinctive chemical races. In polyploid plants of *Ambrosia dumosa* Seaman and Mabry [18] found additive sesquiterpene lactone patterns of diploid chemotypes. The



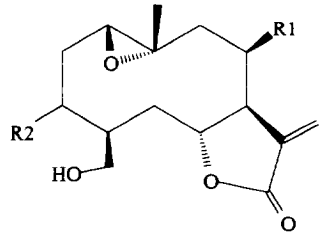
	R1	R2
(1) Budlein A, isobutyrate-4, 5-iso	ibut	CH_2
(2) Budlein, tiglate	tig	CH_2OH
(3) Budlein A	ang	CH_2OH
(4) Budlein A, 2-methylbutyrate	2-mebut	CH_2OH



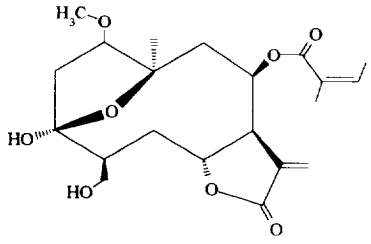
(5) Niveusin A, 1, 2-andihydro-4, 5- dihydro



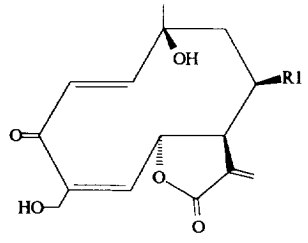
(7) Niveusin A, 1, 2 anhydrido

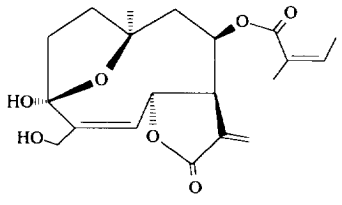


	R1	R2
(6) Argophyllin B	ang	OH
(12) Argophyllon 2-methylbutyrate	2-mebut	OH
(13) Argophyllon B	ang	O =



(9) Niveusin A, 1-methoxy-4, 5-dihydro





(10) Niveusin B

	R1
(8) Tifruticin, desoxy-3-dehydro-15-hydroxy	ang
(11) Tifruticin, desoxy-3-dehydro-15-hydroxy-2', 3'-dihydro	2-mebut

combined inheritance of sesquiterpene lactones in interspecific hybridization was recently described for a natural F1-hybrid of *Helianthus angustifolius* × *H. porteri* [19]. On the basis of such results, conclusions were drawn for the possible progenitors of the hybrid taxa *H.* × *multiflorus* and *H.* × *laetiflorus* [9]. However, specific investigations to elucidate the inheritance of sesquiterpene lactones were still lacking.

For our hybridization experiments we have chosen two readily crossable annual species of the genus *Helianthus* with clear chemical distinctiveness (1-keto-2,3-unsaturated furanoheliangolides with various ester side

Table 2. Distribution of sesquiterpene lactones in the parental plants and in their hybrids. Compounds are listed in the ranking of their retention time in HPLC-analysis (50% MeOH)

H. debilis	*H. annuus* × *H. debilis*	*H. debilis* × *H. annuus*	*H. annuus*
(1) +	+	+	
		+	+ **(5)**
(2) +	+		
			+ **(6)**
(3) +	+	+	
(4) +	+		
	+	+	
	new compound	new compound	
	+	+	+ **(7)**
	+	+	+ **(8)**
	+	+	
	new compound **(11)**	new compound **(11)**	
	+	+	+ **(9)**
	+	+	
	new compound **(12)**	new compound **(12)**	
	+	+	+ **(10)**

Structure assignment given in parentheses.

Table 3. ^{1}H NMR spectral data of compounds **4**, **8**, **11**–**13**

H	4*	8†	11	12	13‡
1		7.04 *d*	7.03 *d*	3.25 *dd*	3.39 *dd*
2a	5.86 *s*	6.27 *d*	6.25 *d*	2.40 *dd*	2.35 *dd*
2b				3.25 *dd*	3.29 *dd*
4				3.20 *m*	3.21 *m*
5a	6.21 *dt*	6.07 *dt*	6.03 *dt*	1.50 *m*	1.53 *ddd*
5b				2.21 *m*	2.23 *td*
6	5.34 *m*	5.46 *dd*	5.42 *dd*	4.54 *dd*	4.56 *dd*
7	3.79 *m*	3.61 *m*	3.56 *m*	3.02 *s* (*br*)	3.06 *s* (*br*)
8	5.08 *bs*	5.43 *ddd*	5.35 *m*	5.18 *t*	5.22 *t*
9a	2.59 *dd*	2.02 *dd*	2.00 *dd*	2.64 *dd*	2.72 *dd*
9b	2.44 *dd*	2.57 *dd*	2.52 *dd*	1.40 *dd*	1.45 *dd*
13a	6.30 *d*	6.37 *d*	6.35 *d*	6.37 *d*	6.37 *d*
13b	5.93 *d*	5.84 *d*	5.81 *d*	5.82 *d*	5.84 *d*
14§	1.47 *s*	1.54 *s*	1.53 *s*	1.40 *s*	1.40 *s*
15a	4.34 *s*	4.44 *dd*	4.43 *dd*	3.88 *dd*	3.87 *dd*
15b	4.34 *s*	4.30 *dd*	4.28 *dd*	3.70 *dd*	3.69 *dd*
2'	2.32 *qt*		2.24 *qt*	2.35 *qt*	
3'a	1.40 *m*	6.08 *qq*	‖	1.45 *m*	6.13 *qq*
3'b	1.57 *m*		‖	1.55 *m*	
4'§	0.79 *t*	1.93 *dq*	0.83 *t*	0.85 *t*	1.97 *dq*
5'§	1.05 *d*	1.75 *dq*	1.04 *d*	1.10 *d*	1.84 *pentet*

Compounds **11** and **12** run at 250 MHz with TMS as int. standard. All data obtained in $CDCl_3$.

*Data from ref. [10], 400 MHz.

†Data from ref. [23], 400 MHz.

‡Data from ref. [15], 360 MHz.

§Three proton intensity.

‖Signal obscured.

Coupling constants given in (Hz).

J (Hz): compound **11**: 1, 2 = 16.9; 5, 6 = 9.8; 5, 15a = 5, 15b ≈ 1; 13a, 7 = 1.9; 13b, 7 = 1.7; 15a, 15b = 12.5; compound **12**: 4, 15a = 8.4; 4, 15b = 5.0; 5a, 6 = 11.0; 5b, 6 = 4.0; 8, 9a = 3.8; 9a, 9b = 15.1; 13a, 7 ≈ 1; 13b, 7 = 1.5; 15a, 15b = 9.9.

chains in *H. debilis* [10, 12]; tifruticin-like heliangolides and 1,10-epoxidized compounds with exclusively angelate ester substitution in *H. annuus* [11, 20]). The indistinguishable occurrence of compounds from all basic skeleton types (budleins, niveusins, tifruticins and argophyllins) in F1-hybrids deriving from both, *H. debilis* as well as *H. annuus* maternal plants underlines the genome-bound rather than the plasmon-bound inheritance of sesquiterpene lactones and the activation of both parental alleles. Moreover, the formation of the tifruticin derivative (**11**) and the argophyllone isomer (**12**) with 2-methylbutyrate substitution illustrates the independent biosynthesis of the major skeleton and the side chain. The enzymes responsible for the substitution at C-8 obviously do not differentiate between basic skeletons. In this context it would be interesting to investigate hybrids of plants combining sesquiterpene lactones or greater difference in biosynthetic complexity (e.g. guaianolides, eudesmanolides, germacranolides).

The results of these experiments affect future considerations for the use of sesquiterpene lactones in chemotaxonomy and may be an important clue for the elucidation of the origin of many allopolyploid species in the Asteraceae. The assumption of simple additivity of parental sesquiterpene lactones in F1-progenies must be extended by the possibility of the occurrence of new compounds with synergistic structures. This coincides with similar findings for flavonoids in Hawaiian tarweeds [21].

EXPERIMENTAL

Plant material and hybridization. Helianthus annuus cv. *giganteus* and *Helianthus debilis* ssp. *cucumerifolius* (Fa. Benary, Han.-Münden, Germany), two basically self-incompatible species, were used for hybridization and were alternatively used as pollen- and egg-donors in two independent experiments. Plants were grown in the greenhouse. To keep self-pollination to a minimum the pollen was exhausted by a vacuum-pump and pollinating insects were kept away from the plants.

Caryology. Analysis of chromosomes was carried out using young disk flowers in the meiotic stage of pollen formation. Chromosome staining was performed with carmin acetic acid according to ref. [22].

Analysis of glands. Sesquiterpene lactones were analysed using a recently described technique for trichome microsampling [4] and separated by reversed phase HPLC (Hypersil ODS, 5 μm) in independent runs with two different isocratic mobile phases (50% MeOH; 30% MeCN). Peak detection was carried out simultaneously at 225 and 265 nm and compound assignment was performed by means of authentic reference samples.

Extraction and isolation. Preparative purification of sesquiterpene lactones was carried out as given in ref. [23] starting with CH_2Cl_2 extracts of air-dried leaves (11 g) of hybrid plants. Vouchers are deposited at Universität Hohenheim (OS # 86, OS # 243, OS # 247, OS # 248).

Tifruticin, desoxy-3-dehydro-15-hydroxy-2',3'-dihydro (**11**). $C_{20}H_{26}O_7$, EI-MS 70 eV 378 *mz* (rel. int.): $[M]^+$ (2), 377 $[M - H]^+$ (4), 85 $[C_5H_9O]^+$ (60).

Argophyllone 2-methylbutyrate (**12**). $C_{20}H_{28}O_7$, EI-MS 70 *mz* (rel. int.): 380 ($[M]^+$ not observed), 362 $[M - H_2O]^+$ (1.2), 85 $[C_5H_9O]^+$ (70).

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REFERENCES

1. Heywood, V. H., Harborne, J. B. and Turner, B. L. (eds) (1977) *The Biology and Chemistry of the Compositae*. Academic Press, London.
2. Seaman, F. C. (1982) *Bot. Rev.* **48**, 121.
3. Emerenciano, V. de P., Ferreira, Z. S., Kaplan, M. A. C. and Gottlieb, O. R. (1987) *Phytochemistry* **26**, 3103.
4. Spring, O. (1991) in *Modern Phytochemical Methods*, (Fischer, N. H., Isman, M. B. and Stafford, A. H., eds), p. 319. Plenum Press, New York.
5. Spring, O., Panero, J. L. and Schilling, E. E. (1992) *Biochem. Syst. Ecol.* **20**, 671.
6. Spring, O., Zitterell-Haid, B., Bierner, M. W. and Mabry, T. J. (1994) *Biochem. Syst. Ecol.* **22**, 171.
7. Heiser, C. B., Smith, D. M., Clevenger, S. B. and Martin Jr., W. C. (1969) *Mem. Torrey Bot. Club* **22**, 1.
8. Rogers, C. E., Thompson, T. E. and Seiler, G. J. (1982) *Sunflower Species of the United States*. National Sunflower Association.
9. Spring, O. and Schilling, E. E. (1990) *Biochem. Syst. Ecol.* **18**, 19.
10. Spring, O., Klemt, V., Albert, K. and Hager, A. (1986) *Z. Naturforsch.* **41c**, 695.
11. Spring, O., Benz, T. and Ilg, M. (1989) *Phytochemistry* **28**, 745.
12. Spring, O. and Schilling, E. E. (1989) *Biochem. Syst. Ecol.* **17**, 519.
13. Heiser, C. B. (1951) *Evolution* **5**, 42.
14. Rieseberg, L. H. (1991) *Am. J. Botany* **78**, 1218.
15. Stipanovic, R. D., Miller, R. B. and Hope, H. (1985) *Phytochemistry* **24**, 358.
16. Smith, D. M. and Levin, D. A. (1963) *Am. J. Botany* **50**, 952.
17. McMillan, C., Chavez, P. I. and Mabry, T. J. (1975) *Biochem. Syst. Ecol.* **3**, 137.
18. Seaman, F. C. and Mabry, T. J. (1979) *Biochem. Syst. Ecol.* **7**, 7.
19. Spring, O. (1989) *Biochem. Syst. Ecol.* **17**, 509.
20. Melek, F. R., Gage, D. A., Gershenzon, J. and Mabry, T. J. (1985) *Phytochemistry* **24**, 1537.
21. Crins, B. J., Bohm, B. A. and Carr, G. D. (1988) *Syst. Botany* **13**, 567.
22. Hill, S. R. (1982) *Rhodora* **84**, 1.
23. Spring, O. (1991) *Phytochemistry* **30**, 519.
24. Spring, O., Albert, K. and Hager A. (1982) *Phytochemistry* **21**, 2551.