A Furanoheliangolide in *Helianthus debilis*; Implications for a Chemotaxonomy of the Genus *Helianthus*

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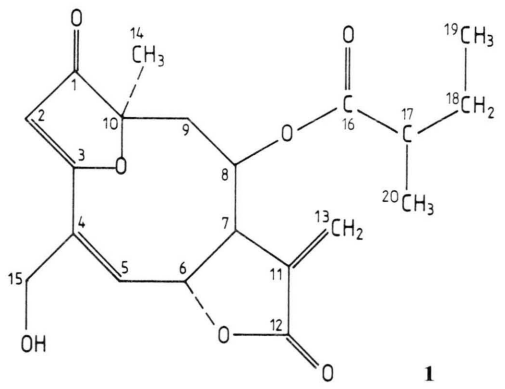
Leaves from *Helianthus debilis* subsp. *cucumerifolius* yielded the furanoheliangolide 17,18-dihydrobudlein A. The same compound has been previously isolated from *Viguiera hemsleyana, V. procumbens* and *Helianthus strumosus*. 17,18-dihydrobudlein A revealed strong antimicrobial, insecticidal and plant growth inhibiting activity. Its distribution within the plant is restricted to the leaves. Implications for the chemotaxonomy of the genus *Helianthus* are discussed.

Introduction

The hitherto studied species of the genus *Helianthus* are mostly characterized by the occurrence of sesquiterpene lactones (SQL), which were shown to be antifeedant and antimicrobial acting substances. Recently, it was reported that *H. debilis* (subsp. *debilis* as well as subsp. *cucumerifolius*) would be an exception having no SQL [1, 2]. In spite of this account we observed a strong inhibitory activity of leaf extracts from *H. debilis* subsp. *cucumerifolius* in bioassays comparable to those of *H. annuus,* a SQL-rich species [3–6]. In this paper, we report on the isolation and identification of 17,18-dihydrobudlein A (**1**) from *H. debilis* subsp. *cucumerifolius,* a furanoheliangolide which has previously been characterized from *Viguiera hemsleyana* [7], *V. procumbens* [8] and *H. strumosus* [9]. This compound seems to be responsible for the above mentioned inhibitory activities of leaf extracts from *H. debilis*.

Results and Discussion

Aceton extracts from fresh leaves of *H. debilis* (grown in the greenhouse) were chromatographed on TLC and screened for antibiotic activity on agar diffusion tests with *Bacillus brevis*. An active compound could be identified and the further purification was performed on preparative HPLC. 17,18-dihydrobudlein A (**1**) (content in primary leaves of 3 to 4 week old plants: 120 ± 35 µg · g fresh weight^{-1}) was identified by spectroscopical measurements (MS; ^{1}H and ^{13}C NMR) and spin decoupling experiments. Comparison with recently published data for the same compound [7, 9] revealed a definite concurrence. Slight differences of the NMR data may have been due to the use of different solvents. Vacuum-dried samples of (**1**) from HPLC purifications could not be resolved sufficiently in $CDCl_3$ and were therefore resuspended and measured in CD_3OD/D_2O (3:1, v/v). In our NMR-measurements the ^{1}H and ^{13}C NMR signals of the 2-methylbutanoate side chain were doubled. This is in agreement with the proposed existence of two different epimers of (**1**) [9]. According to the intensity of the signals, we suggest a ratio of about 2:1 of the two epimers in our sample.



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Concerning the distribution of (**1**) within plants of *H. debilis*, we found very similar results as we reported for the SQL of *H. annuus* [6]. Only leaves (except cotyledones) contained compound (**1**) and increasing amounts could be extracted from leaf to leaf towards the apex (Table I). Primary leaves from about 3 week old plants (120 μg **1**·g fresh weight^{-1}) showed complete protection against insect predation in choice experiments with larvae from *Locusta migratoria* (Spring *et al.*, in prep.). 17,18-dihydrobudlein A shows strong antimicrobial activity (MIC: 16 μg/ml on *Bacillus brevis*) and is able to inhibit auxin-induced plant growth at a concentration of 10 μM and more.

So far 5 from 11 species, placed in the section *Helianthus* [10], were examinated for SQL. All of them contained germacrolides or mainly heliangolides except *H. debilis* (subsp. *cucumerifolius* and subsp. *debilis*). With the identification of a furanoheliangolide in *H. debilis* subsp. *cucumerifolius*, this species can again be seen to have a closer relationship to the other members of this section (Table II).

Although it still seems too early for a comprehensive chemotaxonomic consideration of the genus *Helianthus* (about 40% of the species have not yet been examined), some trends can be discerned:

1) All SQL-containing species of the genus contain germacrolides and/or heliangolides, with the latter dominant. Only three members of the section *Divaricati* series *Corona-solis* are reported to possess other skeletal types (eudesmanolides in *H. grosseserratus* [24] and *H. nuttallii* [21]; a melampolide and guaianolides in *H. maximiliani* [33, 36].
2) C_6–C_7 lactones are usual, but additional C_7–C_8 lactones may occur in different sections, thus indicating no further chemotaxonomic significance.
3) Mostly ester side chains of 5 C-atoms occurred and angilic acid is the preferred one.
4) *H. occidentalis* and *H. rigidus*, both investigated members of the series *Atrorubentes* (4 species total) did not contain SQL. Since the position of the hexaploid species *H. rigidus* is still controversial [10, 40], the lack of SQL may indicate a closer relationship to the series *Atrorubentes* [10] than to the former series *Divaricati* [40].
5) In the Wagner network of *Helianthus* species reported by Schilling and Heiser [10] a close connection is shown between *H. radula* as well as *H. heterophyllus* (series *Angustifolius*) and the 4 members of the series *Atrorubentes*. Interestingly *H. radula* contains no SQL [29] in contrast to the other species of its series [2, 26]. Thus *H. radula* is more corresponding to the series *Atrorubentes (e.g. H. occidentalis, H. rigidus)* than to the series *Angustifolius (e.g. H. angustifolius, H. simulans)*.

Table I. SQL content (μg **1**·g fresh weight^{-1}) in different plant organs of an 8 week old *H. debilis* plant grown under 70 W·m^{-2} white light (14 h per day), 80% rel. humidity, 20 °C.

	SQL (**1**) (μg·g fresh weight^{-1})
Roots	–
Hypocotyl	–
Cotyledones	–
Epicotyl	–
Leaves from:	
1st node	154
2nd node	255
3rd node	338
4th node	363
5th node	461
6th node	677

Experimental

Seedlings of *H. debilis* subsp. *cucumerifolius* (Fa. Benary, Hann. Münden, FRG) were grown in a greenhouse for 3 to 4 weeks. For some experiments, plants were kept under constant conditions in a climate chamber (VKZPH 5/200/S, Fa. Heraeus-Vötsch, Balingen; 20 °C, 80% rel. humidity, 70 W/m^2 white light 14 h per day).

The extraction, purification and determination of SQL was performed following the previously described method [6]. The homogenized plant material was incubated for 1 h in aceton/water (3:1, v:v; 4 ml per g fresh weight). Aceton was removed in vacuo and the aqueous residue was extracted with peroxide-free diethylether. The dried ether phase was resuspended in MeOH and diluted for HPLC analysis with water (1:1, v:v). HPLC separation was performed on Shandon Hypersil ODS (5 μm, 250 mm × 8 mm column, solvent 50% MeOH in water).

17,18-dihydrobudlein A (**1**): Fractions of several separations were combined (peak detection at 225 nm) and dried under reduced pressure to give

Table II. Sesquiterpene lactones in species of the genus *Helianthus*.

	SQL	Germacrolides	Heliangolides	Other skeletal types	C_3–C_{10} Furanes	C_7–C_8 Lactones	Budlein derivatives	Side chain: Ang	Epoxyang	Tig	Sar	Sarac	i-Val	i-But	2-Mebut	Mac	2–OH–Et–Acr	i–But–4–OH	Without side chain	Section/Series[d]	References
H. annuus[a]	+	+	+		+			+													4; 12; 13
H. argophyllus	+	+				+		+													14; 15; 37
H. debilis (ssp. *cucumerif.*)	−/+		+		+		+								+					I	2/this report
(ssp. *debilis*)	−																			(11)	1
H. niveus (ssp. *niveus*)	+	+	+											+							16
(ssp. *canescens*)	+		+		+	+		+													17; 37
H. petiolaris	+		+		+		+	+													9
H. ciliaris	+		+				+							+		+				III/1 (3)	18
H. pumilus	+	+	+			+		+	+			+					+		+	III/2	19; 20
H. gracilentus	+	+	+		+			+	+		+		+							(3)	39
H. californicus	+	+				+		+													21
H. decapetalus	+	+									+										22
H. divaricatus	+	+									+	+									38
H. giganteus	−																				22; 23
H. grosseserratus	+	+	+	Eu	+	+	+						+						+		24
H. hirsutus	+	+	+		+		+	+										+			9; 23
H. maximiliani	+		+	Me/Gu	+			+	+	+	+						+		+	IV/1	24; 33–36
H. mollis	+	+							+	+		+								(15)	25
H. nuttallii[b]	(+)		(+)	(Eu)	(+)																(21)
H. resinosus	+	+						+	+		+						+		[e]		38
H. salicifolius	−/+	+									+						+				26–27/38
H. schweinitzii	+		+		+		+	+		+											28
H. strumosus	+		+		+		+								+						9
H. tuberosus	+/−		+							+											12/22
H. occidentalis	−																			IV/3	2
H. rigidus	−																			(4)	27
H. angustifolius	+		+		+		+	+													26
H. radula	−																			IV/4 (7)	29
H. simulans	+		+					+													2
H. lehmannii[c]	+		+		+		+								+						30

Skeletal types: Eu (eudesmanolides); Me (melampolides); Gu (guaianolides); Ang (angelate); Epoxyang (epoxyangelate); Tig (tiglate); Sar (sarracinate); i-Val (isovalerate); i-But (isobutyrate); 2-Mebut (2-mythylbutanoate); Mac (methylacrylate); 2–OH–Et–Acr (2-hydroxyethylacrylate); i–But–4–OH (4-hydroxyisobutyrate).

[a] *H. annuus* was reported to possess the tiglic ester compound heliangine [11]. But the structure of heliangine was determined with leaf extracts from *H. tuberosus* [12]. Although Shibaoka [31] suggested the identity of a so far unknown growth inhibitor from *H. annuus* with heliangine due to similar R_f-values in TLC, heliangine has never been found in *H. annuus*.

[b] Unpublished results, for ref. see [21].

[c] Previously placed in the genus *Helianthopsis* [32].

[d] Classification according to [10]; I = *Helianthus*; III/1 = *Ciliares/Ciliares*; III/2 = *Ciliares/Pumili*; IV/1 = *Divaricati/Corona-solis*; IV/3 = *Divaricati/Atrorubentes*; IV/4 = *Divaricati/Angustifolii*.
() = Number of species.

[e] Seldom side chains: epoxysarracinate; 2′,5′-epoxy-,3′-hydroxyangelate; 2′,3′,5′-trihydroxyangelate.

colorless crystalls of (**1**). ^{1}H NMR (400 MHz, CD_3OD/D_2O 3/1): δ 6.30 (d, J = 2.5, H-13a), 6.21 (dt, J = 4.3, 1.6, H-5), 5.93 (d, J = 2.5, H-13b), 5.86 (s, H-2), 5.34 (m, H-6), 5.08 (bs, H-8), 4.34 (s, H-15a, H-15b), 3.79 (m, H-7), 2.59 (dd, J = 15.5, 3.2, H-9a), 2.44 (dd, J = 15.5, 4.7, H-9b), 2.32 (qt, J = 7.0, 6.9, H-17, H-17$^+$), 1.48 (m, J = 7.0, H-18, H-18$^+$), 1.47 (s, H-14), 1.05 (d, J = 7.0, H-20$^+$), 1.04 (d, J = 7.0, H-20), 0.79 (t, J = 7.3, H-19), 0.76 (t, J = 7.3, H-19$^+$); signals of the second epimer are indicated by $^+$.

^{13}C NMR (100.6 MHz, CD_3OD/D_2O 3/1): δ 209.9 (s, C-1), 186.1 (s, C-3), 177.8 (s, C-16$^+$), 177.7 (s, C-16), 171.8 (s, C-12), 140.0 (s, C-4), 137.5 (s, C-11), 134.4 (d, C-5), 125.9 (t, C-13), 105.9 (d, C-2), 89.8 (s, C-10), 77.1 (d, C-6), 76.8 (d, C-8), 62.4 (t, C-15), 49.0 (d, C-7), 43.4 (t, C-9), 42.4 (d, C-17), 42.2 (d, C-17$^+$), 27.4 (t, C-18$^+$), 27.3 (t, C-18), 21.8 (q C-14), 16.8 (q, C-20), 16.6 (q, C-20$^+$), 11.8 (q, C-19), 11.7 (q, C-19$^+$); signals of the epimer are indicated by $^+$.

$C_{20}H_{24}O_7$; MS, 70 eV m/z (rel. int.): 376 [M$^+$] (3.3), 292 (3.6), 274 (12.1), 187 (100) and 85 (100).

IR ν_{max}^{MeOH} cm^{-1}: 3450, 1760, 1720, 1700 and 1640. UV λ_{max}^{MeOH} nm: end absorption 208 (ε = 11300) and a shoulder at 267 (ε = 7500).

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