Phytochemistry, Vol. 21, No. 10, pp. 2551–2553, 1982.
Printed in Great Britain.

0031-9422/82/102551-03$03.00/0

THREE BIOLOGICALLY ACTIVE HELIANGOLIDES FROM *HELIANTHUS ANNUUS*

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(*Revised received* 25 *March* 1982)

Key Word Index—*Helianthus annuus*; Compositae; ^{1}H- and ^{13}C NMR; sesquiterpene lactones; germacranolides; heliangolides; biological activities.

Abstract—Growth inhibiting substances in *Helianthus annuus* have been investigated. From the ethanolic extract a new germacranolide with an α-methylene-γ-lactone moiety, the heliangolide niveusin B and its ethoxy derivative were isolated and their structures elucidated by spectroscopic methods. The biological activity of each was determined by inhibition in *Avena* coleoptile tests and antimicrobial tests.

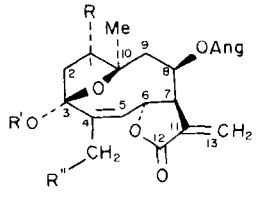
INTRODUCTION

From the sunflower, *Helianthus annuus* we previously reported the isolation of a biologically active furanoheliangolide (**1**) [1], identical with niveusin C from *H. niveus* [2], a compound which was also found in *H. maximiliani* [3]. Further investigations on growth inhibiting substances from young leaves and the apical part of the stem of *H. annuus* resulted in the extraction of three additional sesquiterpene lactones: the known compound niveusin B [2], a new germacranolide of the tifruticin-type, and 3-ethoxyniveusin B. The latter ethoxyheliangolide was shown to be formed from niveusin B during the ethanolic extraction.

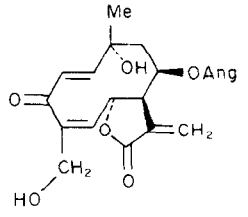
RESULTS AND DISCUSSION

The extraction of young leaves and the upper part of the stem of *H. annuus* yielded, apart from **1** [1], compound **4**, which was identical in its spectroscopic data to niveusin B, a heliangolide previously reported from *H. niveus* [2]. In addition we were able to isolate a new germacranolide (**2**) and an ethoxyheliangolide (**3**). The structures proposed are based on *IR*, ^{1}H, ^{13}C NMR, and mass spectral measurements.

The ^{1}H NMR spectroscopic data of **2** indicated a close relationship to deoxyfruticin [4]. Analysis of the ^{13}C NMR spectrum revealed an opening of the hemiketal linkage in structure **1**, and also the existence of a carbonyl function at C-3 is indicated by the ^{13}C NMR spectrum. The location of the second hydroxy group at C-15 was proved by single frequency decoupling experiments connecting the two protons at δ 4.44 and 4.30 to the ^{13}C resonance at 63.0.



	1	3	4
R =	OH	H	H
R'=	H	Et	H
R''=	H	OH	OH



2

The structure of the ethoxy derivative (**3**) was deduced from the appearance of two complex signals at δ 3.62 and 3.24 together with a triplet at 1.18. By decoupling experiments it was shown that these signals arise from an ethoxy function at which the two protons of the CH_2-group are not equivalent. In the ^{13}C NMR spectrum of **3** two new signals at δ 58.4 and 15.2 also appeared. These signals could be assigned to the O$\underline{C}$H$_2$ and the $\underline{C}$H$_3$ carbons of the ethoxy group, respectively. The lack of the signal at δ77.3 present in compound **1**, together with a new $\underline{C}$H$_2$ signal at δ 37.5 indicated the loss of the hydroxy group at C-1. Since an ether function should induce a downfield shift of the carbon atom attached, and this was not observed in the signal of C-15, only structure **3** is possible.

In order to clarify whether **3** was a naturally occurring product or an artefact caused by the extraction conditions, a modified extraction proce-

Table 1. ^{1}H NMR spectral data of compounds **2** and **3** (400.1 MHz, $CDCl_3$, TMS as int. standard)

	2	3
H – 1α	7.04 *d*	1.85 *m**
H – 1β		2.30 *m**
H – 2α	6.27 *d*	2.07 *m**
H – 2β		2.30 *m**
H – 5	6.07 *dt*	5.93 *dt*
H – 6	5.46 *dd*	5.46 *dd*
H – 7	3.61 *m*	4.27 *m*
H – 8	5.43 *ddd*	5.64 *ddd*
H – 9α	2.02 *dd*	2.01 *dd*
H – 9β	2.57 *dd*	2.20 *dd*
H – 13a	6.37 *d*	6.27 *d*
H – 13b	5.84 *d*	5.62 *d*
H – 14	1.54 *s*	1.53 *s*
OH – 14	2.40 *br*	—
H – 15a	4.44 *dd*	4.20 *d*
H – 15b	4.30 *dd*	3.95 *dd*
OH – 15	1.82 *br*	2.22 *br*
H – 3′	6.08 *qq*	6.04 *qq*
H – 4′	1.93 *dq*	1.91 *dq*
H – 5′	1.75 *dq*	1.74 *dq*
H – 1″α	—	3.62 *dt*
H – 1″β	—	3.24 *dt*
H – 2″	—	1.18 *t*

J (Hz): compound **2**: 1,2 = 17.0; 5,6 = 9.1; 5,15a = 1.1; 5,15b = 1.2; 6,7 = 1.7; 7,8 = 3.3; 7,13a = 7,13b = 1.7; 8,9α = 9.6; 8,9β = 6.3; 9α,9β = 14.0; 15a,15b = 14.0; 3′,4′ = 7.2; 3′,5′ = 1.5; 4′,5′ = 1.5; compound **3**: 5,6 = 9.1; 6,7 = 2.1; 7,8 = 4.3; 7,13a = 2.9; 7,13b = 2.4; 8,9α = 6.8; 8,9β = 5.3; 9α,9β = 12.3; 15a,15b = 14.3; 3′,4′ = 7.2; 3′,5′ = 1.4; 4′,5′ = 1.7; 1″α,1″β = 9.7; 1″α,2″ = 1″β,2″ = 7.0; 15 – OH, 15b = 6.3.

*Overlapping signals, interchangeable.

Table 2. ^{13}C NMR spectral data of **2** of **3** (100.6 MHz, $CDCl_3$, TMS as int. standard)

Carbon no.	2	3
1	161.3 *d*	37.5 *t*
2	129.8 *d*	40.8 *d*
3	196.3 *s*	108.6 *q*
4	135.9 *s*	136.6 *s*
5	140.5 *d*	130.5 *d*
6	75.7 *d*	75.9 *d*
7	48.7 *d*	49.6 *d*
8	73.9 *d*	71.4 *d*
9	47.1 *t*	40.0 *t*
10	72.1 *s*	83.7 *s*
11	141.7 *s*	143.4 *s*
12	169.6 *s*	169.5 *s*
13	124.9 *t*	122.5 *t*
14	28.9 *q*	26.8 *q*
15	63.0 *dd*	65.4 *dd*
1′	166.5 *s*	166.7 *s*
2′	126.8 *s*	127.3 *s*
3′	137.4 *s*	139.1 *s*
4′	15.8 *q*	15.7 *q*
5′	20.1 *q*	20.3 *q*
1″	—	58.4 *dd*
2″	—	15.2 *q*

dure with water and chloroform, thus avoiding the use of alcohol, which is known to react with trifruticin- and tirotundin-like sesquiterpene lactones [4, 5], was employed. TLC experiments as well as spectroscopic investigations and bioassays clearly indicated that **3** was formed during alcoholic extraction whereas **1**, **2** and **4** could be identified in all extracts. The ethoxyheliangolide is formed by reaction of ethanol with the 3-OH group of **4**.

A further compound [characterized by its R_f 0.3 on TLC in methylene chloride–acetone–ethyl acetate (5:4:1)] always changed partly into **2** during purification, thus rendering exact NMR spectroscopic measurements impossible. Because of the higher polarity on TLC (R_f of **2** is *ca* 0.42) and the lack of a shoulder in the UV spectrum (typical for a carbonyl group) it might be the 3-hydroxy form of compound **2**.

The sesquiterpene lactones from sunflower were biologically active. The linear reduction of growth in the *Avena* coleoptile test at a concentration of 100 μM was 80% (±6) for **2**, 57% (±9) for **3** and 61% (±6) for **4**. Anti-microbial activity was tested against bacteria and fungi. Compound **2** was the strongest inhibitor against bacteria (MIC: 15 μg/ml on *Bacillus brevis*; 50 μg/ml on *Proteus vulgaris*; 95 μg/ml on *Eremothecium ashbyi*) whereas **3** was more active against fungi (MIC: 40 μg/ml on *B. brevis*; 85 μg/ml on *P. vulgaris*; 65 μg/ml on *E. ashbyi*) than compound **4** (MIC: 35 μg/ml on *B. brevis*; 87 μg/ml on *P. vulgaris*; 98 μg/ml on *E. ashbyi*).

EXPERIMENTAL

The plants of *Helianthus annuus* var. *giganteus* were grown in the greenhouse for 3–4 weeks. Leaves and the upper part of the stem were harvested and extracted in the usual manner [1]. The crude extract was chromatographed by CC (Polygosil 60–4063), eluted with petrol (fractions 1–5), petrol–$CHCl_3$ (1:1) (6–10), $CHCl_3$ (11–15), $CHCl_3$–EtOH (49:1) (16–20), $CHCl_3$–EtOH (19:1) (21–25), and $CHCl_3$–EtOH (9:1) (26–28).

Fractions 16–20 afforded the known heliangolides **1** and **4**. Compound **2** could be purified from fractions 21–25 by TLC (CH_2Cl_2–Ac–EtOAc, 5:4:1). Yield: 45 ± 2.6 (s.e., n = 12) μg/g fr. wt. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3570 (OH), 1750 (OCOR), 1700 (ester), 1640 C=C). MS 70 eV *m/z* (rel. int.): 376 $[M]^+$ (2.5), 358 $[M\text{-}H_2O]^+$ (0.5), 276 $[M\text{-}Ang]^+$ (5.0), 258 $[276 - H_2O]^+$ (5.7), 100 ($C_5H_8O_2$) (8.6), 83 ($C_5H\text{-}O$) (100), 55 (C_4H_7) (82.1). (Calc. for $C_{20}H_{24}O_5$: 376.405. Found: (MS) 376.) UV λ_{max}^{EtOH}: end absorption, ε = 25 200 at 215 nm and a shoulder at 250 nm (ε = 14 700), typical for a carbonyl function.

Fractions 11–15 of the ethanolic extraction contained **3**. Further purification was performed by HPLC (Nucleosil RP 18, 10 μm, 250 × 4.8 mm, Waters M 6000, Reodyne 7125) in H_2O–MeOH (20:80). Yield: 11 ± 1.7 (s.e., n = 4) μg/g fr. wt.

IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3570 (OH), 1755 (OCOR), 1720 (ester), 1650 (C=C). MS 70 eV *m/z* (rel. int.): 406 $[M]^+$ (8.9), 388 $[M-H_2O]^+$ (0.5), 375 $[M\text{-}CH_2OH]^+$ (0.3), 360 $[388-C_2H_4]^+$ 2.1), 306 $[M-Ang]^+$ (1.4), 288 $[388-Ang]^+$ (3.6), 99 (Ang) (6.4) 83 (C_5H_7O) (100), 55 (C_4H_7) (67.4), (Calc. for $C_{22}H_{30}O_7$: 406.475. found: 406). UV λ_{max}^{EtOH}: strong end absorption, ϵ = 18 500 at 210 nm.

Modified extraction. The plants were homogenized in H_2O, filtered and extracted by $CHCl_3$. The crude extract was purified by TLC (CH_2Cl_2–Ac–EtOAc, 5:4:1). The inhibitors were analysed by bioassay or by colouring with Ehrlich's reagens on TLC.

Bioassay. Avena coleoptile tests and anti-microbial testing were performed as described earlier [1].

Acknowledgements—We thank Dr. J. Kupka and Dr. T. Anke, University of Tübingen, for supplying and for assistance with antimicrobial tests.

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