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# SESQUITERPENE LACTONE AND DITERPENE CONSTITUENTS OF *HELIANTHUS ANNUUS*

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**Key Word Index**—*Helianthus annuus*, Asteraceae; sunflower; sesquiterpene lactones; furanoheliangolides; 4,5-dihydroniveusin A; niveusin B; argophyllin A, argophyllin B; diterpene acids.

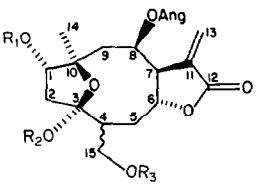
**Abstract**—One new furanoheliangolide derivative, 4,5-dihydroniveusin A, as well as the known compounds niveusin B and argophyllin A and B, were isolated from a Texas population of *Helianthus annuus*. Three previously characterized diterpene acids, grandifloric acid, ciliaric acid and 17-hydroxy-*ent*-isokaur-15(16)-en-19-oic acid were the principal constituents of this population.

## INTRODUCTION

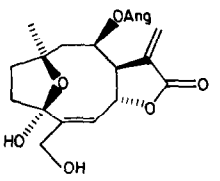
The search for a biochemical basis for pest and disease resistance in the annual sunflower (*Helianthus annuus* L.) is of great agricultural importance. Previous studies on wild and cultivated populations of this species have resulted in the isolation of kauranoic and trachylobanoic diterpene acids [1–5] and furanoheliangolide sesquiterpene lactones [6, 7]. Compounds from each of these classes have been implicated as potential resistance factors in *H. annuus* [1, 6, 7]. We here report the isolation of a new furanoheliangolide derivative, 4,5-dihydroniveusin A (**1a**), and three additional known sesquiterpene lactones niveusin B (**2**) [8], argophyllin A (**3**) and argophyllin B (**4**) [9] as minor constituents of a wild Texas population of *H. annuus*. The major terpenoid constituents of this population were found to be the known diterpenes ciliaric acid (**8**) [9], grandifloric acid (**6a**) [5] and 17-hydroxy-*ent*-isokaur-15(16)-en-19-oic acid (**7a**) [10].

## RESULTS AND DISCUSSION

Compound **1a**, mp 94–95°, $C_{20}H_{28}O_8$ (HRMS) was a furanoheliangolide sesquiterpene lactone that had an $^1H$ NMR spectrum nearly identical (Table 1) to that of the previously characterized compound tagitinin A (**5**) [11]. Differences were observed in the signals for the ester side-chain and for the H-15 methyl group. Resonances for the isobutyrate side-chain of tagitinin A were replaced by signals for an angelate side chain in **1a** (broadened quartet at $\delta$6.06, broadened methyl singlet at 1.78 and broadened methyl doublet at 1.91). The base peak in the mass spectrum (*m/z* 83) and the IR absorption at 1710 $cm^{-1}$ also supported the presence of an angelate moiety. In place of the H-15 methyl doublet of tagitinin A, **1a** contained a two-proton broadened singlet at 3.78 ($CDCl_3$). This signal was part of an ABX pattern (3.45 *dd*, $J$ = 6.3, 11 Hz; 3.59 *dd*, $J$ = 5, 11 Hz) in the $CD_3CN$



**1a** $R_1 = R_2 = R_3 = H$
**1b** $R_1 = R_3 = Ac$, $R_2 = H$
**1c** $R_1 = R_2 = R_3 = Ac$



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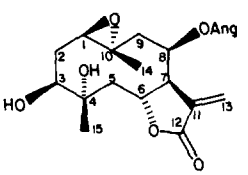

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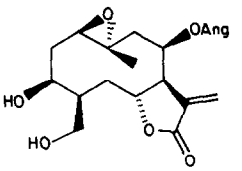

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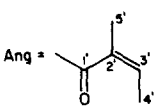

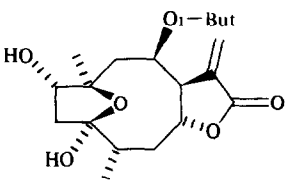

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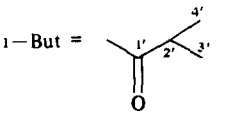

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Table 1. $^1$H NMR spectra of compounds **1a**, **1b**, **1c** and **5** [10]

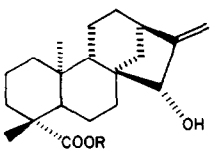
| | **1a** $CDCl_3$ | **1a** $CD_3CN$ | **1b** $CDCl_3$ | **1c** $CDCl_3$ | **5** [10] $CDCl_3$ |
|---|---|---|---|---|---|
| H-1 | 4.27 *dd* ($J$ = 9.0, 8.8 Hz) | 4.10 | 5.07 | 5.51 | 4 23 *m** |
| H-2a | 2.56 *dd* (14.5, 8.8) | 2.08 | 2.67 | 3.22 | 2.44 *m* |
| H-2b | 2.18 *dd* (14.5, 9.0) | 2.38 | 2 25 | 2.15† | 2.1 *m* |
| H-4 | 2.1 *m*† | 2 1 | † | 2 25† | 2.1 *m* |
| H-5a | 2.1 *m*† | 2.1 | † | 2 1† | 2.1 *m* |
| H-5b | 2.1 *m*† | 2.1 | † | 2 1† | 2.1 *m* |
| H-6 | 4.59 *ddd* (6.5, 4, 7) | 4.53 | 4.51 | 4.50 | 4.55 *ddd* (9, 3, 7) |
| H-7 | 4.05 *m* | 4.03 | 4.01 | 4.00 | 3.99 *m* |
| H-8 | 5.67 *ddd* (2, 8, 6) | 5.64 | 5.67 | 5.69 | 5.59 *ddd* (1.5, 8, 5) |
| H-9a | 1.71† | † | † | 1.65 | 1.81 *dd* (13, 8) |
| H-9b | 2 12† | † | † | † | 1.95 *dd* (13, 5) |
| H-13a | 6.29 *d* (3.5) | 6.13 | 6.31 | 6 31 | 6.25 *d* (3 5) |
| H-13b | 5.60 *d* (3.0) | 5.62 | 5.59 | 5.59 | 5.53 *d* (3) |
| H-14 | 1.47 *s* | 1 37 | 1.50 | 1.53 | 1.43 *brs* |
| H-15a | 3.78 *br s* | 3.45 *dd* (6.3, 11) | 4.25 | 4.21 | 1.11 *d* (6.5) |
| H-15b | | 3.59 *dd* (5, 11) | 3.96 | 3.93 | — |
| H-2′ | — | — | — | — | 2.44 *m* |
| H-3′ | 6.06 *br q* (7.5, 1.5) | 6.07 *br q* | 6.08 | 6.08 | 1 07 *d* (7) |
| H-4′ | 1.91 *br d* (7.5) | 1.83 *br d* | 1 92 | 1 93 | 1.04 *d* (4) |
| H-5′ | 1.78 *br s* (1.5) | 1.75 *br s* | 1.79 | 1.80 | — |
| Acetate methyls | | | 2.08<br>2.08 | 2.09<br>2.06<br>2.02 | |

Run at 200 MHz with TMS as an int. standard. Multiplicities are similar to previous column unless otherwise noted.

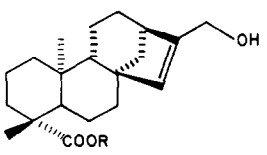
*The H-1 signal in an authentic sample of tagitinin A was seen as a *dd* with $J$ = 8.0 and 9.0 Hz (A. Whittemore, personal communication).

†Signal obscured

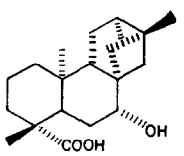
spectrum where $H_x$ was an obscured proton in the vicinity of 2.1 (spin decoupling). In the $CDCl_3$ spectra of both the diacetate (**1b**) and the triacetate (**1c**), this pattern shifted downfield to 4.10 and 4.07, respectively (Table 1). These data indicate that the C-4 methyl group had been oxidized to a hydroxymethylene in **1a**. Additional support for the presence of this functionality was the $^{13}$C NMR triplet at 64.2 ppm (Table 2). The stereochemistry at C-4 could not be determined since the overlap of signals for H-5a, H-5b and H-4 in the $^1$H NMR spectrum did not allow analysis of coupling constants for this spin system. As for the other chiral centers in **1a**, the coincidence of chemical shifts and coupling constants between tagitinin A (**5**) and **1a** for all but the signals mentioned suggested that the stereochemistry of the new compound was identical to **5** with the possible exception of the configuration at C-4. Thus, the new lactone is formulated as **1a**, the 4,5-dihydro derivative of niveusin A [8].




**6a** R = H
**6b** R = Me




**7a** R = H
**7b** R = Me




**8**

Three less polar sesquiterpene lactones were also isolated in this investigation, the known compounds niveusin B (**2**) [8], argophyllin A (**3**) and argophyllin B (**4**) [9]. Their structures were determined by comparison of $^1$H NMR and mass spectral data with those in the literature. All these lactones were first isolated from other annual species of *Helianthus*. The presence of **3** and **4** in both *H. annuus* and *H. argophyllus* supports the close relationship between these two species previously proposed on morphological grounds [12].

Three known diterpene acids were isolated in far greater quantities than the sesquiterpene lactones. These were identified as grandifloric acid (**6a**) [5], ciliaric acid (**8**) [9] and 17-hydroxy-*ent*-isokaur-15(16)-en-19-oic acid (**7a**) [11] by spectral data and by transformation to the corresponding methyl esters.

Table 2 $^{13}C$ NMR spectra of compounds **1a*** and **5** [5]

| | **1a** | **5** [10] |
|---|---|---|
| C-1 | 78.4 *d* | 78.46 *d* |
| C-2 | 47.5 *t* | 46.94 *t* |
| C-3 | 104.2 *s* | 105.69 *s* |
| C-4 | 47.8 *d*† | 44.38 *d* |
| C-5 | 33.8 *t*‡ | 37.81 *t* |
| C-6 | 82.2 *d* | 81.86 *d* |
| C-7 | 47.9 *d*† | 47.84 *d* |
| C-8 | 69.9 *d* | 69.90 *d* |
| C-9 | 34.5 *t*‡ | 34.65 *t* |
| C-10 | 81.8 *s* | 81.69 *s* |
| C-11 | 136.9 *s* | 137.01 *s* |
| C-12 | 169.9 *s* | 169.75 *s* |
| C-13 | 122.2 *t* | 121.73 *t* |
| C-14 | 24.6 *q* | 24.96 *q* |
| C-15 | 64.2 *t* | 19.18 *q*§ |
| C-1′ | 167.1 *s* | 176.45 *s* |
| C-2′ | 127.3 *s* | 34.11 *d* |
| C-3′ | 139.1 *d* | 18.76 *q*§ |
| C-4′ | 15.7 *q* | 18.42 *q* |
| C-5′ | 20.4 *q* | |

*Run at 22.6 MHz in $CDCl_3$ with TMS as an int. standard.

†, ‡, §Assignments interchangeable.

## EXPERIMENTAL

Leaves of *Helianthus annuus* (15.2 kg) collected by J. Gershenzon in July 1981 in eastern Travis Coutry, TX, along F. M. 969, 9.5 miles east of U.S. Hwy 183 (No. 194, voucher on deposit in the Herbarium of the University of Texas) were washed with $CH_2Cl_2$ and worked up in the usual manner [13]. Approximately 75% of the crude extract (79.2 g) was dissolved in a minimum amount of $CH_2Cl_2$ and applied to a silica gel column (2 kg) packed in the same solvent. The column was eluted with a $CH_2Cl_2$–*iso*-PrOH gradient, with increasing amounts of *iso*-PrOH. Two hundred fractions of 1 l. each were collected. Most of the material chromatographed was eluted from the column before fraction 50. The first nine fractions (100% $CH_2Cl_2$) contained waxy material (*ca* 18 g), while the main components of fractions 10–50 (1–5% *iso*-PrOH) were compounds **6a**, **7a** and **8** (estimated total yield of each 5–10 g). Fractions 29–30 (5% *iso*-PrOH) were combined and triturated with $Et_2O$ to give a mixture of diterpenes **5a** and **6a** (450 mg). Methylation of 40 mg of this mixture with $CH_2N_2$ produced a mixture of two products which were separated by prep. TLC on silica gel (1 mm, $CH_2Cl_2$–MeOH, 15:1) to give 8 mg of the methyl ester **6b** and 5 mg of the methyl ester **7b**, whose spectral data were identical to those previously reported [5, 10]. Fraction 42 (5% *iso*-PrOH) contained ciliaric acid (**8**) (40 mg) obtained as crystals on trituration with $Et_2O$. The $^1H$ NMR spectrum and the mp data for its methyl ester [mp 131–134° (lit. 136–137°)] were found to be identical to those of authentic specimens isolated from other species of *Helianthus* [8]. Fraction 56 (5% *iso*-PrOH) showed one major and three less polar minor spots on TLC. The material from the major spot was purified by prep. TLC ($CH_2Cl_2$–MeOH, 8:1, 2 mm) to give 250 mg of argophyllin A [mp 189–190° (lit. 190–192°)] The $^1H$ NMR spectrum was identical to that previously reported for the compound from *H. argophyllus* [9]. The material from the least polar spot was purified on TLC ($CH_2Cl_2$–MeOH, 8:1, 2 mm) to give 6 mg of niveusin B, whose $^1H$ NMR spectral data were identical to those of an authentic specimen previously isolated from *H. niveus* [8]. Fraction 80 (10% *iso*-PrOH) showed two bands on prep. TLC. The material from the upper band gave 6 mg of argophyllin B, which exhibited spectral data identical to those previously reported. The lower band yielded the new lactone (**1a**) 4,5-dihydroniveusin A: mp 95–96° (needles from $CHCl_3$), $C_{20}H_{28}O_8$, HRMS for $M^+$, found *m/z* 396.1796, calc *m/z* 396.1784, MS 70 eV, *m/z* (rel. int.): 396 (1), 378 (1), 361 (1), 295 (1), 278 (2), 261 (20), 83 (81), 55 (100). IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3415 (*br*), 1745, 1708, 1645, 1258.

*Acetylation of* **1a**. Compound **1a** (50 mg) was acetylated by standard procedures [8]. After the usual work-up the crude product was purified by repeated TLC ($CH_2Cl_2$–MeOH; 9:1) to give two minor products, the triacetate **1c** (6 mg) and the diacetate **1b** (21 mg), MS *m/z* (rel. int.): $M^+$ 480 (not seen) 432 (1), 261 (13), 243 (11), 83 (100), 55 (94), IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$: 3450, 2960, 1755, 1740, 1720, 1705. See Table 1 for $^1H$ NMR data. Triacetate **1c**, MS *m/z* (rel. int.): $M^+$, 522 (not seen), 480 (2), 462 (1), 402 (4), 337 (8), 319 (11), 277 (12), 260 (20), 243 (20), 217 (20), 83 (100). IR $\nu_{max}^{CHCl_3}$ $cm^{-1}$. 2970, 2915, 1760–1705 (board absorption)

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