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SESQUITERPENE LACTONES AND DITERPENE CARBOXYLIC ACIDS IN *HELIANTHUS NIVEUS* SUBSPECIES *CANESCENS**

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Key Word Index—*Helianthus niveus*; Compositae; heliangolides; niveusin-A; niveusin-B; niveusin-C; diterpene carboxylic acids; kaurenoic acid; grandifloric acid; ciliaric acid.

Abstract—Three new heliangolides (niveusin-A, -B and -C) and three known diterpene carboxylic acids were isolated and characterized from a $CHCl_3$ extract of *Helianthus niveus*. The structures of the three new lactones were deduced by ^1H and ^{13}C NMR as well as extensive decoupling experiments and derivatizations.

INTRODUCTION

Previous studies on sesquiterpene lactones from the genus *Helianthus* have yielded several germacranolides and heliangolides. For example, we previously found four germacranolides, mollisorin-A and -B, desacetyleupaserrin and eupaserrin, in *H. mollis* [1]. The latter compound was also isolated from *H. pumilus* [2]. In addition, two heliangolides, heliangine [3, 4] and ciliarin [5], were isolated from *H. tuberosus* and *H. ciliaris*, respectively. Diterpenoids known from *Helianthus* include four kaurenoid carboxylic acids (−)-kaur-16-en-19-oic acid ([6]; O'Brien, D. H. and Hanlon, K.; Stipanovic R. D., personal communications), grandifloric acid (McCrindle, R., personal communication), angeloylgrandifloric acid [7] and tetrachryin, a rearranged kaurenoid lactone [7], as well as three trachylobanoid carboxylic acids: trachyloban-19-oic acid [8], 15-α-hydroxytrachyloban-19-oic acid (McCrindle, R., personal communication) and ciliaric acid [9]. The isolation of two labdanoid carboxylic acids (*cis*- and *trans*-ozic acids) from *H. occidentalis* has also been reported (O'Brien, D. H., personal communication).

Both sesquiterpene lactones and diterpene carboxylic acids appear to be abundant secondary metabolites in *Helianthus* and preliminary field observations indicate that species containing large amounts of these substances have enhanced protection against insect predation. Until the present report, all the annual *Helianthus* species investigated yielded only diterpene carboxylics acids while perennials afforded various sesquiterpene lactones, mainly germacranolides and heliangolides.

In this paper, the isolation and structure determination of three new heliangolides as well as the isolation and characterization of three known diterpene carboxylic acids from the annual subspecies *H. niveus* subspecies *canescens* (A. Gray) Heisev (Tribe Heliantheae, Fam. Compositae) are described.

RESULTS AND DISCUSSION

Air-dried and ground leaves and stems of *Helianthus niveus* were extracted with $CHCl_3$. The extract was purified by standard procedures [10], then subjected to Si gel CC separation. Lactone **1** was isolated as a main component (*ca* 0.44% yield from dry plant material), mp 127–128° (EtOAc), $C_{20}H_{26}O_8$ (HRMS and elemental analysis). Presence of an α-methylene-γ-lactone was indicated by IR (1760, 1650 cm^{-1}) and ^1H NMR (a pair of doublets at δ 6.20 and 5.37). A C-5 α,β-unsaturated ester side chain on the skeleton of sequiterpene lactone **1** was indicated by IR (1720 cm^{-1}) and MS (*m/e* 83 as a base peak). The C_5-side chain was shown to be an angelic ester by ^1H NMR δ 5.28 1H, *bq*; 1.91 3H *bd* and 1.75 3H *bs* signals (typical for angelic esters). The other ^1H NMR signals of **1** either in $CDCl_3$ or pyridine-d_5 were difficult to interpret at 100 MHz; however, the signals were well resolved when the spectrum was recorded in a mixture of C_6D_6–$CDCl_3$ at 200 MHz. Extensive decoupling experiments were therefore conducted at 200 MHz. On irradiation at 4.06 (*m*), two doublets for H-13a and H-13b changed into sharp singlets, thus confirming that this signal could be assigned to H-7; at the same time the multiplet at 5.60 changed into a double doublet ($J = 12.0$ and 5.0) and a broadened triplet at 5.14 changed into a doublet ($J = 4.0$). This unusually low chemical shift for H-7 is typical for germacranolides with a tetrahydrofuran system in which H-7 is in close proximity to the furan oxygen atom [11]. The two multiplets at 5.60 and 5.14 were assigned to H-8 and H-6 respectively and since irradiation on the 5.14 triplet changed a doublet at 5.62 into a singlet, this latter signal can be assigned to the olefinic proton at C-5. The small coupling constant between H-5 and H-6 ($J = 4.0$) indicated a *cis*-configuration for the

*Part III in the series "Chemistry of *Helianthus*". For Parts I and II, see refs [1] and [17].

Table 1. ^{1}H NMR data for compounds **1**–**9***

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
H-1	3.53 *t*	4.08 *m*	5.42 *bt*	7.09 *d*	†	†	4.16 *bt*	4.02 *dt*	7.39 *d*
2	2.30 *d*	†	2.65 *d*	6.28 *d*	†	†	†	2.50 *d*	5.55 *d*
5	5.62 *d*	5.92 *bd*	5.96 *bd*	6.03 *bd*	5.84 *bd*	5.90 *dt*	5.54 *bs*	6.82 *d*	5.82 *dt*
6	5.14 *bt*	5.44 *bt*	5.18 *bt*	5.42 *bdd*	5.52 *bt*	5.54 *dt*	5.54 *m*	5.55 *t*	6.06 *dd*
7	4.06 *m*	4.30 *m*	4.40 *m*	3.38 *m*	4.20 *m*	4.20 *m*	4.28 *m*	4.47 *m*	3.35 *m*
8	5.60 *m*	5.72 *m*	5.68 *m*	5.50 *m*	5.66 *m*	5.56 *m*	5.74 *m*	5.78 *m*	5.54 *m*
9a	1.73 *dd*	2.59 *dd*	†	2.86 *dd*	†	†	†	†	3.42 *dd*
9b	1.44 *dd*	2.38 *dd*	†	†	†	†	†	†	2.98 *dd*
13a	6.20 *d*	6.31 *d*	6.30 *d*	6.41 *d*	6.29 *d*	6.31 *d*	6.15 *d*	6.37 *d*	6.42 *d*
13b	5.37 *d*	5.70 *d*	5.64 *d*	5.82 *d*	5.66 *d*	5.68 *d*	5.77 *d*	5.75 *d*	5.88 *d*
14	1.33 *s*	1.57 *s*	1.44 *s*	1.72 *s*	1.54 *s*	1.54 *s*	1.51 *s*	1.62 *s*	2.19 *s*
15a	3.91 *d*	4.82 *bd*	4.68 *bd*	4.95 *bd*	4.20 *bs*	4.78 *bd*	1.85 *bs*‡	9.43 *s*§	4.90 *bs*
15b	3.75 *d*	4.57 *bd*	4.30 *bd*	4.68 *bd*		4.57 *bd*			
18	5.88 *bq*	6.10 *bq*	6.08 *bq*	6.13 *bq*	6.07 *bq*	6.09 *bq*	6.08 *bq*	6.11 *bq*	6.10 *bq*
19‡	1.91 *bd*	1.92 *bd*	1.91 *bd*	1.96 *bd*	1.93 *bd*	1.93 *bd*	1.73 *bd*	1.93 *bd*	1.97 *bd*
20‡	1.75 *m*	1.75 *m*	1.75 *m*	1.77 *m*	1.87 *m*	1.76 *m*	1.73 *m*	1.76 *m*	1.83 *m*
O ‖ —OC—Me		2.11 *s*	2.04 *s* 2.07 *s* 2.13 *s*	2.05 *s* 2.14 *s*		2.10 *s*			

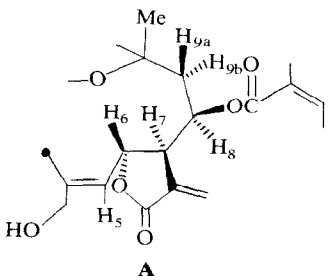
* Run in $CDCl_3$ with TMS as internal standard on a 100 MHz instrument, except for **5** (in acetone-d_6) and **1** (in 1:1 mixture of $CDCl_3$ and C_6D_6 on a 200 MHz instrument). Coupling constants were virtually identical for **1** through **9**. Those for **1** are given as representatives; values for those which changed significantly for **4** and **9** are also given: *J* values in Hz for compound **1**: 1,2a = 2.5; 1,2b = 2.5; 5,6 = 4.0; 6,7 = 4.0; 7,8 = 5.5; 8,9a = 5.0; 8,9b = 12.0; 9a,9b = 14.0; 15a,15b = 13.0; 7,13a = 7,13b = 3.0; 18,19 = 7.5; 18,20 = ~2; and 19,20 = ~1. For compound **4**: 1,2 = 17.0; 5,6 = 9.0. For compound **9**: 1,2 = 6.0; 5,6 = 8.0. Additional coupling constant data are available on request to the authors.

† Could not be observed because of overlapping.

‡ Three protons.

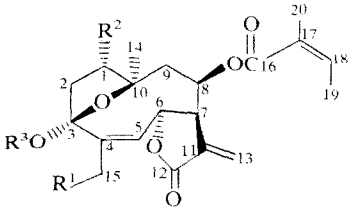
§ One proton.

double bond [8, 12]. When the overlapping signals for H-8 and H-5 were irradiated simultaneously, the AB part of the complex ABX pattern at 1.73 (*dd*) and 1.44 (*dd*) changed into a clear AB system, indicating that the latter signals are assignable to H-9a and H-9b and that C-10 is fully substituted. Coupling constants between H-9a,b and H-8 as well as H-7 and H-8 (J = 5.5) indicated an α-orientation for H-8 [13]. The other AB doublet pattern at 3.91 and 3.75 with apparent long range couplings could be assigned to the C-15 hydroxymethyl group on the following basis. On irradiation at 3.91 the signal for H-6 as well as the broadened doublet for H-5 was sharpened indicating homoallylic and allylic coupling between the C-15 hydroxymethyl group and H-5 and H-6. A sharp three-proton singlet at 1.33 was in accord with the C-14 methyl group having a geminal oxygen function. These decoupling experiments suggested partial structure **A**.



A

The nature of the other two oxygen functions in **1** was characterized as follows. **1** gave the monoacetate **2** on acetylation with Ac_2O–K_2CO_3 and a mixture containing the diacetate **4** and the triacetate **3** when treated with Ac_2O–Py, indicating three hydroxyl groups in **1**. In the monoacetate **2** the AB doublets for H-15a,b shifted downfield to 4.82 and 4.57 showing that acetylation had occurred only on the primary hydroxyl group. In the triacetate **3** a narrowly split triplet at 3.53 in **1** shifted downfield to 5.42; in addition, downfield shifts were observed for H-15a,b to 4.68 and 4.30. Coupling between the triplet at 3.53 and a two-proton doublet at 2.30 was established by a decoupling experiment. While the secondary hydroxyl group could be assigned to C-1 or C-2, the third hydroxyl group appeared to be part of a hemiketal function at C-3 in order to account for a tetrahydrofuran ring and the lack of any other signals for **3**



	1	**2**	**3**	**5**	**6**	**7**
R^1 =	OH	OAc	OAc	OH	OAc	H
R^2 =	OH	OH	OAc	H	H	OH
R^3 =	H	H	Ac	H	H	H

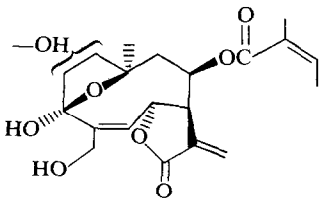
Table 2. ^{13}C NMR data for compounds **1–8***

Carbon	1	2	3	4	5	6	7	8
1	77.9 *d*	77.4 *d*	76.8 *d*	159.6 *d*	37.4 *t*	37.5 *t*	77.7 *d*	77.5 *d*
2	46.9 *t*	45.8 *t*	45.6 *t*	128.6 *d*	40.9 *t*	40.9 *t*	44.9 *t*	47.1 *t*
3	107.0 *s*	105.6 *s*	108.5 *s*	193.8 *s*	106.2 *s*	105.7 *s*	106.6 *s*	104.7 *s*
4	138.3 *s*	135.9 *s*	136.2 *s*	135.4 *s*	136.6 *s*	136.4 *s*	136.4 *s*	134.9 *s*
5	129.3 *d*	132.4 *d*	132.3 *d*	141.1 *d*	131.2 *d*	133.0 *d*	128.7 *d*	156.1 *d*
6	76.0 *d*	75.2 *d*	75.4 *d*	75.0 *d*	75.4 *d*	75.1 *d*	75.6 *d*	74.9 *d*
7	50.9 *d*	49.8 *d*	49.0 *d*	47.6 *d*	49.9 *d*	49.8 *d*	50.0 *d*	49.4 *d*
8	73.2 *d*	71.6 *d*	71.4 *d*	72.5 *d*	72.1 *d*	71.9 *d*	71.7 *d*	71.4 *d*
9	40.2 *t*	39.5 *t*	40.1 *t*	47.3 *t*	39.3 *t*	39.0 *t*	39.7 *t*	39.8 *t*
10	87.1 *s*	86.7 *s*	87.5 *s*	79.5 *s*	83.3 *s*	83.7 *s*	86.6 *s*	87.9 *s*
11	144.8 *s*	138.5 *s*	137.2 *s*	137.4 *s*	143.6 *s*	139.9 *s*	140.3 *s*	142.0 *s*
12	169.8 *s*	170.0 *s*‡	170.2 *s*‡	170.0 *s*‡	170.3 *s*	169.9 *s*‡	170.3 *s*	169.0 *s*
13	122.5 *t*	123.5 *t*	123.5 *t*	125.2 *t*	123.4 *t*	123.3 *s*	123.2 *s*	124.3 *t*
14	20.5 *q*	20.3 *q*	20.4 *q*	24.4 *q*	28.2 *q*	28.2 *q*	20.4 *q*	20.4 *q*
15	64.5 *t*	65.5 *t*	66.1 *t*	64.1 *t*	65.9 *t*	66.0 *t*	22.0 *q*	195.2 *d*
16	166.8 *s*	166.7 *s*	166.6 *s*	166.2 *s*	166.7 *s*	166.8 *s*	166.8 *s*	166.5 *s*
17	128.6 *s*	127.1 *s*	127.1 *s*	126.6 *s*	127.3 *s*	127.3 *s*	127.3 *s*	126.9 *s*
18	138.8 *d*	139.9 *d*	140.0 *d*	137.7 *d*	139.6 *d*	139.6 *d*	139.8 *d*	140.3 *d*
19	15.7 *q*	15.7 *q*	15.8 *q*	15.8 *q*	15.7 *q*	15.7 *q*	15.7 *q*	15.8 *q*
20	22.3 *q*	21.9 *q*‡	21.9 *q*†	21.6 *q*†	20.4 *q*	20.4 *q*†	22.3 *q*	21.6 *q*
CH_3—C(=O)—		21.1 *q*†	20.9 *q*†	20.1 *q*†		21.1 *q*†		
			20.9 *q*†					
			21.2 q†	20.8 q†				
Me—C(=O)—		170.9 *s*‡	169.2 *s*‡	169.2 *s*‡		170.8 *s*‡		
			169.3 *s*‡					
			170.3 s‡	170.8 s‡				

* Run in $CDCl_3$, except for **1** which was run in acetone-d_6, on a 22.6 MHz instrument. Signals were assigned by means of partially decoupled off-resonance spectra and comparison with reported data for goyazensolide [20] and euperfolitin [21],

†,‡ Assignments are interchangeable.

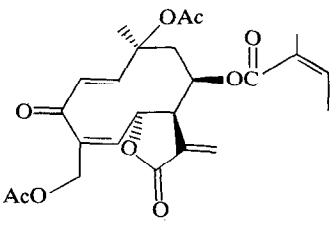
(relative to the signals for **1**) shifting downfield thus indicating the tertiary nature of the third hydroxyl group. The presence of a hemiketal function in **1** was supported further by a typical low field ^{13}C NMR signal at 107.0 for a hemiketal carbon.



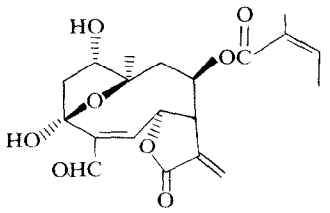
B

The ^1H and ^{13}C NMR patterns (Tables 1 and 2) for the diacetate **4** compared to those for **1**, **2** and **3** suggested a major skeletal change as observed previously when tagitinin-B was acetylated [14]. ^1H NMR of **4** showed a new pair of doublets at 6.28 and 7.09 ($J = 17.0$) together with H-5 at 6.03 in accord with a C-4,5 double bond conjugated with a carbonyl function. The ^{13}C NMR spectrum of **4** exhibited a signal for a ketone carbonyl carbon at 193.8 and new olefinic carbon signals at 159.6 (C-1), and 128.6 (C-2). Moreover, the UV spectrum of **4** gave a cross-conjugated dienone absorption at 245 nm. These spectral data provided partial structure **B** for **1**.

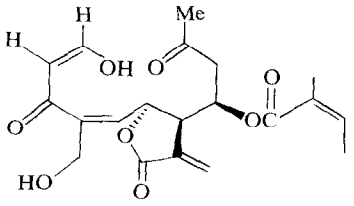
The secondary hydroxyl group in **1** could be assigned to C-1 since HIO_4 fission of **1** gave **9** whose ^1H NMR spectrum showed a sharp acetyl methyl singlet at 2.19 as well as a typical pair of doublets for an α-hydroxymethylene ketone system at 7.39 and 5.55 ($J = 6.0$). The small coupling constant between H-1 and H-2 indicated a *cis*-geometry of the two olefinic protons. An α-orientation of the hydroxyl group is proposed on the basis of coupling constants between H-1 and H-2 (2.5 and 2.5). Bohlmann *et al.* [15] proposed a β-orientation of an acetoxyl group in 1-β-acetoxyzacatechinolide on the basis of $Eu(fod)_3$ induced shifts in its ^1H NMR spectrum. The reported coupling constants were 6.0 and 1.0, respectively. The aldehyde proton of **8**, the MnO_2-oxidized product of **1**, appeared at 9.43 in accord with a *cis*-geometry of the C-4,5 double bond [16].



4



8



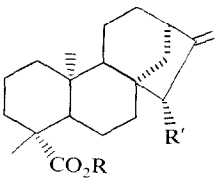
9

An X-ray diffraction analysis by V. Zabel completed after preparation of this manuscript confirmed the proposed structure for **1**.

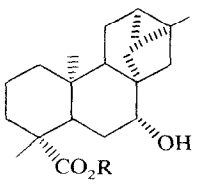
Niveusin-B (**5**) was isolated in 0.02% yield, $C_{20}H_{26}O_7$ (HRMS) as a colourless oil and gave the monoacetate **6** on treatment with Ac_2O–K_2CO_3, $C_{22}H_{28}O_8$ (M^+, *m/e* 420), colourless oil. ^1H and ^{13}C NMR spectra of **5** and **6** indicated **5** was identical to **1** except that it lacked a C-1 hydroxyl group (C-1 at 37.4 (*t*) instead of 77.9 (*d*) in **1**).

Niveusin-C (**7**), isolated in 0.005% yield, showed an isomeric molecular formula $C_{20}H_{26}O_7$ (HRMS) to that of **5**, mp 88–89° (EtOAc). Presence of an angeloyl side chain (MS: *m/e* 295.119 ($M^+ - C_5H_7O$), 278.116 ($M^+ - C_5H_8O_2$) and 83 (base peak), as well as consistent ^1H NMR signals), a hemiketal carbon (^{13}C NMR at 106.6) and an α-methylene-γ-lactone (IR: 1760, 1660 cm^{-1}; ^1H NMR: 6.31 (*d*) 5.68 (*d*)) were indicated. It had a broadened triplet at 4.16 for H-1 with a hydroxyl group and a broad three-proton singlet at 1.85 instead of the typical AB quartet for the C-15 hydroxylmethyl group as observed in the spectra of **1** and **5**. In the ^{13}C NMR spectrum of **7** the 64.5 (*t*) signal observed for C-15 in **1** was not present; instead a new methyl carbon signal at 22.0 (*q*) appeared. In addition, a signal appeared at 77.7 (*d*) in accord with C-1 having a hydroxyl group in **7**.

Three known diterpene carboxylic acids were also isolated from *H. niveus*; grandifloric acid (**12**), (−)-kaur-16-en-19-oic acid (**10**), and ciliaric acid (**13**). ^{13}C NMR data are given in Table **3** for both **12** and **13**; the data for **13** (in part by comparison with published results for trachylobanic acids [17]) supported its identification.



10 R = H, R′ = H
11 R = Me, R′ = H
12 R = H, R′ = OH



13 R = H
14 R = Me

Table 3. ^{13}C NMR data for compounds **12** and **13***

Carbon No.	**12**	**13**
1	38.7 *t*	39.7 *t*
2	19.8 *t*	19.4 *t*
3	36.4 *t*	38.5 *t*
4	43.9 *s*	43.6 *s*
5	57.2 *d*	54.0 *d*
6	22.0 *t*	24.9 *t*
7	41.2 *t*	74.2 *d*
8	48.4 *s*	47.4 *s*
9	54.0 *d*	52.2 *d*
10	40.2 *s*	39.3 *s*
11	18.7 *t*	22.3 *t*
12	33.1 *t*	24.0 *d*
13	42.9 *d*	21.0 *d*
14	36.7 *t*	32.2 *t*
15	82.7 *d*	45.4 *t*
16	161.3 *s*	20.1 *s*
17	107.8 *t*	20.9 *q*
18	29.3 *q*	29.2 *q*
19	180.3 *s*	179.9 *s*
20	16.3 *q*	13.1 *q*

* Run in pyridine-d_5 on a 22.6 MHz instrument with TMS as internal standard.

EXPERIMENTAL

^1H and ^{13}C NMR spectra were measured at 100 and 22.6 MHz, respectively except for one ^1H spectrum of niveusin-A which was measured at 200 MHz. Mps were determined on a Fischer–Johns mp block and are uncorr. Si gel 60 (70–230 mesh) was used for CC separations. Analytical TLC and PLC (1.0 mm) were done on Si gel 60 GF 254. C_6H_6–EtOAc (5:6 and 7:3), $CHCl_3$–Me_2CO (15:1), C_6H_6–*i*-PrOH (9:1) and $CHCl_3$–MeOH (15:1) solvent systems were used for TLC analyses throughout the separations. MS were recorded by direct inlet at 70 eV ionization.

Extraction and isolation. Air-dried and ground leaves and stems, including some flower heads (3.14 kg) of *Helianthus niveus* (collected near Interstate 8 at Fortuna Road turnoff, *ca* 3 miles east of Yuma, Arizona, by W. D. and P. L. Clark #1250, 22 Oct. 1978; voucher is deposited in the Arizona State University Herbarium) were extracted 2× with $CHCl_3$ (11.5 and 7.0 l.) at room temp. The extract was filtered and concd *in vacuo* giving 282 g dark syrup. The syrup was purified by standard procedures [10] to give 84.2 g of yellow syrup. The latter syrup was charged on top of a Si gel column (1.7 kg) which was eluted with a C_6H_6–EtOAc gradient solvent system, initiated with a 7:3 mixture; 1 l. of each eluent was collected for one fraction. All the fractions were monitored by TLC with a C_6H_6–EtOAc (5:6) solvent system. Fractions 3 and 4 showed one major spot on TLC and were combined; the material (2.35 g) from these fractions was purified on a smaller Si gel column (80 g) using C_6H_6 as solvent. The main fractions gave crystalline (−)-kaurenoic acid (**10**), 0.15 g. Fractions 6–11 yielded 3.5 g of crystals (0.11% yield vs dry plant) which when recrystallized from 80% aq. MeOH gave pure grandifloric acid (**12**) as colourless needles. Fractions 16–26 (eluted with C_6H_6–EtOAc, 3:2)

gave gummy crystals, which when triturated with an *i*-Pr_2O-EtOAc mixture gave 0.25 g of ciliaric acid (**13**). The mother liquors from the separation of ciliaric acid which showed one major spot on TLC, afforded 3.4 g of syrup. This syrup was purified through a Si gel column (140 g) which was developed with a $CHCl_3$–Me_2CO gradient solvent system, starting with $CHCl_3$. Fractions 11–14 (eluted with $CHCl_3$–Me_2CO, 15:1) gave 147 mg of crystals: niveusin-C (**7**). After removal of crystalline **13** (2.0 g) the syrup (2.0 g) from fractions 23–26 was purified through a Si gel column, developed with C_6H_6–EtOAc (8:2). The last three fractions from this latter column showed one main spot on TLC in accord with a less polar compound than niveusin-A (**1**). The material from these three fractions (0.51 g) was purified several times on PLC plates (C_6H_6–EtOAc (5:6); EtOAc; $CHCl_3$–*i*-PrOH (15:1)) to give pure niveusin-B (**5**) as a colourless oil, 135 mg. Similarly, the material from fractions 31–35 (2.6 g) gave 370 mg of **5**. Fractions 31–51, which were eluted with C_6H_6–EtOAc (1:1), gave 13.8 g of crystalline niveusin-A (**1**); colourless prisms were obtained by recrystallization from boiling EtOAc.

Niveusin-A (**1**). Mp 127–128° (EtOAc), $C_{20}H_{26}O_8$, HRMS for M^+ found *m/e* 394.1645, calc. *m/e* 394.1627. (Found: C, 59.96; H, 6.69. calc: C, 60.90; H, 6.65%) MS *m/e* (rel. int.): 394 (2), 376 (3), 359 (1), 293 (5), 276 (6), 259 (8), 83 (100). IR ν_{max}^{Nujol} cm^{-1}: 3350, 3150, 1760, 1720, 1670, 1650, 1150, 1100.

Niveusin-B (**5**). Colourless oil. $C_{20}H_{26}O_7$ HRMS for M^+ found *m/e* 378.1684, calc. *m/e* 378.1678. MS *m/e* (rel. int.): 378 (1), 377 (3), 359 (4), 277 (3), 242 (8), 83 (100), 55 (93). IR ν_{max}^{Film} cm^{-1}: 3300, 1760, 1720, 1655, 1220, 1170, 1070.

Niveusin-C (**7**). Mp 88–89° (EtOAc). HRMS for M^+ found *m/e* 378.1677, calc. *m/e* 378.1678; for $M^+ - C_5H_7O$, found *m/e* 295.1186, calc. *m/e* 295.1181; for $M^+ - C_5H_8O_2$, found *m/e* 278.1161, calc. *m/e* 278.1154. MS *m/e* (rel. int.): 378 (1), 295 (1), 278 (3), 205 (8), 83 (100), 55 (73). IR ν_{max}^{Nujol} cm^{-1}: 3400, 3100, 1760, 1715, 1670, 1660, 1130, 1100, 980.

(−)-*Kaurenoic acid* (**10**). Mp 179–180° (lit. 179–180° [18]) IR and ^1H NMR spectra were identical to those of an authentic specimen. The methyl ester **11**, which was prepared by the usual method (CH_2N_2), mp 87–88° (lit. 88–90° [18]), showed IR and ^1H NMR identical to those of an authentic specimen.

Grandifloric acid (**12**). Mp 230–232° (lit. 228–230° [19]). The IR and ^1H NMR spectra were identical to those of an authentic specimen obtained by hydrolysis of angeloylgrandifloric acid [7].

Ciliaric acid (**13**). Mp (Me ester) 132–133° (lit. 136–137° [9]). The IR and ^1H NMR spectra were identical to those of an authentic specimen isolated from *H. ciliaris* from which the acid was originally isolated. The methyl ester **14** which was prepared by the usual method (CH_2N_2) exhibited IR and ^1H NMR spectra identical to those previously reported [9].

Acetylation of niveusin-A (**1**). (A): **1** (212 mg) was acetylated with Ac_2O (3 ml) and Py (1.5 ml) for 24 hr at room temp. After the usual work-up, the crude product was purified by repeated PLC (C_6H_6–EtOAc, 7:5; $CHCl_3$–Me_2CO, 10:1) to give two main products, triacetate **3** (107 mg) and diacetate **4** (41 mg). Triacetate **3**; colourless oil, MS *m/e* (rel. int.): 478 (1), 460 (1), 416 (1), 400 (25), 335 (12), 317 (14), 274 (18), 258 (50), 215 (35), 83 (98), 43 (100); it did not give an M^+ ion on EI. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760, 1740, 1715, 1660, 1280, 1180, 953. Diacetate **4**; MS *m/e* (rel. int.): 488 (1), 460 (1), 340 (15), 290 (17), 259 (48), 83 (100), 55 (90), 43 (97). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770, 1740, 1720, 1680, 1230, 1005. UV λ_{max}^{EtOH} nm (log ϵ): 217 and 245 sh (4.02 and 3.90).

(B): **1** (200 mg) was acetylated with Ac_2O (5.7 ml) in the presence of powdered dry K_2CO_3 (12 mg) for 2 hr at room temp. The mixture was poured into ice water, then stirred for 2.5 hr. The soln was extracted 3× with $CHCl_3$. The extract was washed successively with H_2O, 5% K_2CO_3 and H_2O and then dried over Na_2SO_4. Removal of the solvent gave a gummy material which was purified on PLC (C_6H_6–EtOAc, 5:6; developed 2×). The main band gave 162 mg of the monoacetate **2** as crystals. Acetate **2**: mp 137–138°. MS *m/e* (rel. int.): 436 (3), 418 (2), 358 (5), 276 (35), 259 (32), 215 (12), 83 (100), 55 (95), 43 (88). IR ν_{max}^{Nujol} cm^{-1}: 3550, 1770, 1750, 1700, 1670, 1280, 1160, 995.

H_5IO_6 *Oxidation of* **1** *into* **9**. **1** (100 mg) was oxidized with 560 mg H_5IO_6 in 5 ml dry THF for 1 hr (after 30 min separation of yellow ppt. was observed). The reaction mixture was partitioned between H_2O and Et_2O. The Et_2O layer was washed with H_2O and satd NaCl successively, and dried over $MgSO_4$. Removal of Et_2O gave 123 mg of oily material which was purified through a small Si gel column (5 g) developed with C_6H_6–EtOAc (7:3). **9** (34.1 mg) was obtained as a colourless oil; MS *m/e* (rel. int.): 292 (8; presumably for $M^+ - HOOCC(Me)=CHMe$), 274 (20, $292 - H_2O$), 231 (5), 205 (10), 83 (100), 43 (75). IR ν_{max}^{Film} cm^{-1}: 3200, 1760, 1720, 1705, 1680, 1640, 1595.

MnO_2 *oxidation of* **1** *into aldehyde* **8**. **1** (120 mg) was stirred with 300 mg MnO_2 in 2.0 ml dry Et_2O for 24 hr; at the 4th and 19th hr fresh portions of MnO_2 (100 mg each) were added. The reaction mixture was filtered through a Celite pad and the filtrate was concd *in vacuo*. The crude product was purified through a small Si gel column (5 g) developed with C_6H_6–EtOAc (1:1). The main fraction gave 64 mg **8** as colourless oil; MS *m/e* (rel. int.): 374 (1, $M^+ - H_2O$), 291 (5; presumably $374 - MeCH{=}C(Me){-}C{\equiv}O^{+}$), 83 (100), 55 (60). IR ν_{max}^{Film} cm^{-1}: 3400, 1760, 1720, 1700, 1680, 1610, 1120.

Acetylation of niveusin-B (**5**). **5** (148 mg) was acetylated by method B as described for niveusin-A. After PLC purification (C_6H_6–*i*-PrOH, 9:1) of the crude product, 65 mg **6** was obtained as colourless oil; MS *m/e* (rel. int.): 420 (4), 342 (5), 260 (15), 243 (7), 99 (15), 83 (100), 55 (90). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1770, 1740, 1720, 1680, 1285, 1180, 1070.

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