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SESQUITERPENE LACTONES AND DITERPENOIDS FROM *HELIANTHUS ARGOPHYLLUS**

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Key Word Index—*Helianthus argophyllus*; Compositae; germacranolides; argophyllin-A; argophyllin-B; eupatolide; ciliaric acid; (−)-16-α-hydroxy-kaur-11-en-19-oic acid; (−)-16-α-hydroxykaurane; nevadensin.

Abstract—Three germacranolide sesquitepene lactones (argophyllin-A and -B and eupatolide), three diterpenoids (ciliaric acid, (−)-16-α-hydroxy-kaur-11-en-19-oic acid and (−)-16-α-hydroxykaurane) and one flavonoid (nevadensin) were isolated and characterized from a chloroform extract of *Helianthus argophyllus*. Argophyllin-A and -B are both described here for the first time. Their structures were deduced by ^{1}H NMR and ^{13}C NMR. Argophyllin-A and -B were found to show anti-auxin effects while eupatolide exhibited weak insecticidal activity.

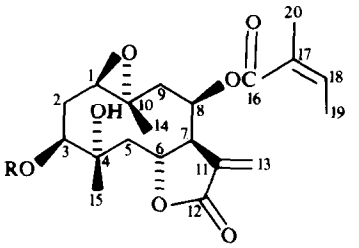
INTRODUCTION

As a part of continuing biochemical systematic investigation of *Helianthus* (tribe Heliantheae) [1–4], we analysed a $CHCl_3$ extract of *H. argophyllus* T. and G. (the silverleaf sunflower), an annual species native to south Texas and Florida. This species is closely related to the commercially important *H. annuus*. In accord with previous work on the terpenoid constituents of this genus, which has been reviewed elsewhere [5] (see also refs. [6–8] for even more recent results), sesquiterpene lactones and diterpene carboxylic acids were found to be abundant secondary metabolites in this species. In this paper, we report the isolation and structural elucidation of two new sesquiterpene lactones, argophyllin-A and -B, and five previously described compounds including another germacranolide sesquiterpene lactone, eupatolide [9], a trachylobane-type diterpene carboxylic acid, ciliaric acid [10], two kauranoid diterpenes, (−) - 16 - α - hydroxykaur - 12 - en - 19 - oic acid [4], and (−) - 16 - α - hydroxykaurane [11], and a flavonoid, nevadensin [12], from *H. argophyllus*. Some biological activities of the isolated compounds are also described.

RESULTS AND DISCUSSION

Air-dried and ground leaves and stems of *H. argophyllus* were extracted with $CHCl_3$. The extract was purified by standard procedures [13] and then subjected to Si gel column chromatography with a toluene–EtOAc gradient solvent system initiated with toluene. The most abundant compound **4a**, eluted first (toluene–EtOAc, 5 : 1) and crystallized upon removal of the solvent. The ^{1}H and ^{13}C NMR and IR data indicated that it was eupatolide (8-β-hydroxycostunolide). The spectral data and mps for **4a** and its acetate **4b** were identical to those previously published [9].

Argophyllin-A (**1a**) was eluted with toluene–EtOAc (1 : 3). On evaporation of the solvent, **1a**, $C_{20}H_{28}O_7$ (high resolution), was obtained and recrystallized from MeOH (mp 190–192°). The presence of an α-methylene-γ-lactone was indicated by the IR (1755 1650 cm^{-1}) and the ^{1}H NMR (a pair of one-proton doublets at δ 6.33 and 5.74, J = 2.0, 2.5 Hz) spectra. A five carbon α,β-unsaturated ester side-chain attached to the skeleton of the sesquiterpene lactone was indicated by the IR spectra (1720 cm^{-1}) and the MS (*m/z* 83, base peak). The side-chain was shown to be an angelic ester by typical ^{1}H NMR signals at δ 6.09 [1H, *q(br)*, J = ~2, 7.5 Hz), 1.95 [3H, *d(br)*, J = ~1, 7.5 Hz] and 1.83 [3H, *s(br)*, J = ~1, 2 Hz]. Two sharp three-proton singlets at δ 1.42 and 1.39 showed that the methyl groups of the basic skeleton, C-14 and C-15, both had geminal oxygen functions and no vicinal protons, indicating that positions 4 and 10 were both fully substituted. The presence of hydroxyl groups was clear from the IR spectrum (3400 cm^{-1}).



1a R = H Argophyllin — A
1b R = Ac

*Part 5 in the series "*Chemistry of Helianthus*". For Part 4, see Ohno, N., Gershenzon, J., Neuman, P. and Mabry, T. J. (1981) *Phytochemistry* **20**, 2393.

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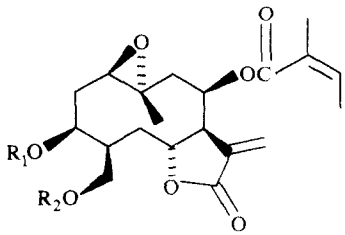
Extensive decoupling experiments were conducted to determine the location of these different functional groups. When a multiplet at δ 2.86 was irradiated, two doublets for H-13a and H-13b changed into sharp singlets, suggesting that this signal should be assigned to H-7; at the same time two one-proton multiplets at δ 5.20 and 4.78 were sharpened. In view of these chemical shifts and the decoupling data, the signal at δ 5.20 was assigned to a proton with a geminal acyloxy group and the signal at δ 4.78 was assigned to the proton attached to the carbon bearing the lactone oxygen function. At this point lactone **1a** was considered to be a C-6, C-7 *trans*-fused lactone, since almost all of the sesquiterpene lactones isolated from this genus so far are lactonized in this direction. The co-occurrence of eupatolide (**4a**) in this species also supported this assumption (which was later confirmed by X-ray analysis). Accordingly, the two multiplets at δ 5.20 and 4.78 were assigned to H-8 and H-6, respectively. The complexity of the H-6 signal requires a methylene group adjacent to C-6. On irradiation at δ 5.20 (H-8), double doublets at δ 2.81 and 1.38 became doublets (J = 15 Hz). Therefore, these two signals are assignable to H-9a and H-9b. Moreover, position 10 must be fully substituted in accord with the presence of a sharp three-proton singlet for the C-14 methyl group. The values of the coupling constants between H-9a, b and H-8 as well as between H-7 and H-8 (J = 4.0 Hz) indicated an α-orientation for H-8 [14].

The ^{13}C NMR spectrum of **1a** exhibited only four signals in the carbon–carbon double bond region, which accounted for the carbon–carbon double bonds in the angelic side-chain ester and the α-methylene-γ-lactone (Table 2). The above data suggested partial structure **A**, which accounted for six of the seven oxygen functions and six of the seven degrees of unsaturation. The nature of the remaining oxygen function in **1a** was established as follows. Lactone **1a** gave a monoacetate, **1b**, on acetylation with Ac_2O-pyridine. This monoacetate still showed the presence of a hydroxyl group in its IR spectrum (3400 cm^{-1}). Since this hydroxyl group was not acetylated under the usual reaction conditions, it had to be tertiary and therefore was located at C-4 or C-10. In the monoacetate **1b**, the double doublet at δ 3.73 (J = 10.0, 4.0 Hz) in **1a** was shifted downfield to δ 5.07. This signal was spin-coupled to a two-proton multiplet at δ 2.10 in **1a**, suggesting the presence of a secondary hydroxyl group at C-2 or C-3 with a methylene group at the other position. A C-1,10-epoxide accounted for the last unsaturated function and was supported by ^{13}C NMR signals at δ 60.6 (*d*) and 59.2 (*s*), which are typical for 1,10-epoxy ring carbons [14]. A ^1H NMR signal at δ 2.86 (*m*) also indicated the presence of an epoxy ring and was assigned to H-1. Since this signal overlapped those for H-7 and H-9a, the magnitude of the spin coupling between H-1 and H-2a, b could not be fully analysed making it difficult to ascertain the configurations at C-1 and C-10. (The ^1H NMR data for **2a**, however, discussed below, strongly suggested a 1-β,10-α-orientation for the epoxide.) The complexity of the H-1 signal, even without exact information on the coupling constants, indicated an adjacent methylene group at C-2, thus placing the secondary hydroxyl at C-3. The tertiary alcohol must then be at C-4. These data completed the structure of argophyllin-A (**1a**) except for the configuration of the 1,10-epoxy ring and the orientation of the C-3 and C-4 hydroxyl groups. An X-ray diffraction analysis carried out by V. Zabel and W. H. Watson indicated that **1a** is a 1-β,10-α-epoxygermacranolide and the C-3 hydroxyl group is on the β-side of the molecule and the C-4 hydroxyl group on the α-side. (The X-ray analysis will be the subject of a separate paper.)

Argophyllin-B (**2a**) proved to be an isomer of argophyllin-A, $C_{20}H_{28}O_7$ (high resolution MS), and its ^1H and ^{13}C NMR spectra showed close relationships to those of **1a**. A double doublet at δ 2.98 (J = 10.0, 5.0 Hz) was assigned to H-1, and corresponded to the signal at δ 2.86 (*m*) in **1a**. The coupling constants between H-2a, b and H-1, which were very similar to those of heliangine (**3**), whose structure was established by an X-ray diffraction analysis [15, 16] indicated an α-orientation of H-1 and a β-orientation of the C-14 methyl group. Thus, the stereochemistry of **2a** at C-1 and C-10 was equivalent to that of **1a**. A one-proton multiplet appeared at δ 4.23, instead of the double doublet for H-3 at δ 3.73 in **1a**, indicating that C-4 was not fully substituted. Further evidence for this was the lack of a signal corresponding to the sharp three-proton singlet observed at δ 1.39 in **1a**. Instead, two double doublets appeared at δ 3.98 and 3.90, the AB part of an ABX system.

Lactone **2a** gave the diacetate **2b** on acetylation with Ac_2O–pyridine. The IR spectrum of **2b** indicated the absence of a hydroxyl group, and in the ^1H NMR spectrum the multiplet at δ 4.23 and the double doublets centered at δ 3.94 in **2a** shifted downfield to δ 5.20 and 4.15, respectively. The former signal was assigned to H-3, while the latter were assigned to the two protons of a hydroxymethyl group attached to C-4. These data provided the structure of **2a**, except for the configuration at C-4. Since the ^1H NMR spectrum of **2a** in $CDCl_3$ (90 MHz) was poorly resolved in the region of the signals for H-3 and H-4, it was difficult to analyse the stereochemistry at C-4. In a 200 MHz ^1H NMR spectrum of **2a** in C_6D_6, however, the signals were resolved well and the spin coupling constant between H-3 and H-4 appeared to be *ca* 7 Hz (see Table 1). The configuration for H-4 should therefore be α on the basis of the dihedral angle between H-3 and H-4 predicted by the Karplus equation. Hence the most probable structure for argophyllin-B must be as depicted in formula **2a**. The structure of argophyllin-B is of rare occurrence, since it lacks any unsaturated function at C-4. Almost all the germacranolides found so far have either a C–C



2a $R_1 = R_2 = H$ Argophyllin — B
2b $R_1 = R_2 = Ac$

Table 1. ^{1}H NMR data for compounds **1a–2b** and **4a** (90 MHz, $CDCl_3$, TMS as internal standard)

	1a	**1b**	**2a** ($CDCl_3$)	**2a** (200 MHz, C_6D_6)	**2b**	**4a** (Pyridine-d_5)
H-1	2.86 *m*	*	2.98 *dd*	2.55 *dd*	3.27 *dd*	4.75 *dd*
2	2.10 *m*	*	*	*	*	*
3	3.73 *dd*†	5.07 *dd*	4.23 *m*	3.84 *ddd*	5.20 *m*	*
6	4.78 *m*	5.30 *m*	4.88 *m*	4.64 *m*	4.95 *m*	5.65 *t*
7	2.86 *m*	2.90 *m*	2.91 *m*	2.10 *m*	3.37 *m*	*
8	5.20 *m*	5.30 *m*	5.28 *m*	5.02 *m*	5.30 *m*	5.25
9a	2.81 *dd*	2.83 *dd*	2.75 *dd*	2.59 *dd*	2.60 *dd*	*
9b	1.38 *dd*	*	1.40 *dd*	*	*	*
13a	6.33 *d*	6.10 *d*	6.40 *d*	6.33 *d*	6.12 *d*	6.40 *d*
13b	5.84 *d*	5.08 *d*	5.80 *d*	5.75 *d*	5.10 *d*	5.87 *d*
14	1.42 *s*‡	1.46 *d*‡	1.47 *s*‡	1.22 *s*‡	1.55 *s*‡	1.65 *s*(*br*)‡
15	1.39 *s*‡	1.42 *s*‡	a 3.98 *dd*	3.60 *dd*	4.23 *dd*	1.80 *s*(*br*)‡
			b 3.90 *dd*	3.50 *dd*	4.07 *dd*	
18	6.09 *q*(*br*)	6.16 *q*(*br*)	6.09 *q*(*br*)	5.64 *q*(*br*)	6.10 *q*(*br*)	
19	1.95 *d*(*br*)‡	1.97 *d*(*br*)‡	1.95 *d*(*br*)‡	1.86 *d*(*br*)‡	1.95 *d*(*br*)‡	
20	1.83 *m*‡	1.86 *m*‡	1.86 *m*‡	1.75 *m*‡	1.85 *m*‡	
O || —C—Me		2.10 *s*‡			2.00 *s*‡ 2.05 *s*‡	

*Could not be observed because of overlap of signals.

†Coupling constants were virtually identical for **1a–2b**. Those for **1a** are given as representatives; values for those which are characteristic for **2a** and **2b**, are also given; *J* values in Hz for compound **1a**: 2a,3 = 10.0: 2b,3 = 4.0; 7,8 = 4.0; 8,9a = 5.0; 8,9b = 3.0; 9a,b, = 15.0; 7,13a = 2.5; 7,13b = 2.0; 18,19 = 7.5; 18,20 = ~2; 19,20 = ~1; compound **2a**: 1,2a = 10.0; 1,2b = 5.0; 3,4 = 7.0.

‡Three protons.

double bond or an oxygen function at this position. Argophyllin-B belongs to the rare class of C-15-oxygenated 4,5-dihydrogermacranolides represented by melnerines A, B [17] and 9-acetoxymelnerins A, B [18].

Four previously known compounds were also isolated from this plant: a kauranoid diterpene alcohol (**5**), a hydroxykaurenoic acid (**6**), a trachylobane-type diterpene acid, ciliaric acid (**7**) and the flavonoid nevadensin (**8**). The spectral data and mps of these compounds were identical to those previously reported.

A number of reports have been published concerning the anti-auxin activity of heliangine (**3**), isolated from *H. tuberosus* [19, 20]. Since **1a** and **2a** are closely related to **3**, the inhibitory effect of **1a** and **2b** on the IAA-induced elongation of Azuki (*Azukia angularis*) hypocotyl sections was examined (Table 3). The potencies of **1a** and **2a** were almost comparable to that reported for **3** [21]. The biochemical or ecological significance of this kind of activity, however, is currently unclear.

Observation of a number of wild *Helianthus* species has indicated that they are resistant to some of the serious insect pests that feed on the cultivated varieties [22, 23]. Since it is thought that chemical factors might be important in this resistance, and several diterpene carboxylic acids from *Helianthus* have been previously demonstrated to inhibit insect growth [22], we tested two of the isolated compounds, a diterpene carboxylic acid and a sesquiterpene lactone, for insecticidal activity. Eupatolide (**4a**), showed weak activity against tobacco cutworm larvae

Table 2. ^{13}C NMR data for compounds **1a**, **2a** and **4a** (22.6 MHz, $CDCl_3$)

Carbon	**1a**	**2a**	**4a** (pyridine-d_5)
1‡	60.6 *d*§	60.0 *d*	124.0 *d*
2	31.0 *t*	36.6 *t**	26.5 *t*
3	74.8 *d*	70.8 *d*	39.6 *t*
4	74.2 *s*	43.9 *d*†	141.8 *s*
5	42.5 *t*	36.9 *t**	123.8 *d*
6	78.1 *d*	74.9 *d*	75.7 *d*
7	50.5 *d*	49.1 *d*	54.3 *d*
8	77.5 *d*	75.3 *d*	71.4 *d*
9	49.2 *t*	43.3 *t*†	48.4 *t*
10	59.2 *s*	58.1 *s*	140.3 *s*
11	136.6 *s*	137.1 *s*	136.8 *s*
12	169.7 *s*	169.1 *s*	170.9 *s*
13	125.1 *t*	124.9 *t*	120.0 *t*
14	19.7 *q*	19.5 *q*	19.9 *q*
15	18.0 *q*	66.0 *t*	17.3 *q*
16	166.3 *s*	166.4 *s*	
17	126.6 *s*	127.0 *s*	
18	141.0 *d*	140.2 *d*	
19	15.8 *q*	15.7 *q*	
20	20.3 *q*	20.2 *q*	

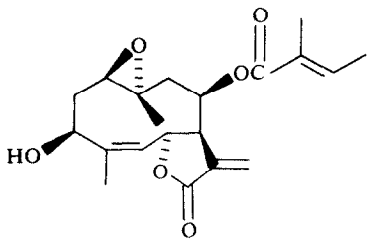
*,†Assignments are interchangeable.

‡Signals were assigned by means of partially decoupled off-resonance spectra and comparison with those reported for heliangine **3** and eupalinin-A [25].

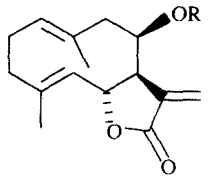
§Indicates multiplicities on partially decoupled spectra.

Table 3. Inhibitory effect of argophyllin-A and -B on the IAA-induced elongation of (*A. angularis*) hypocotyl sections. The concentration of **1a** and **2a** was 10^{-4} M (38 ppm)

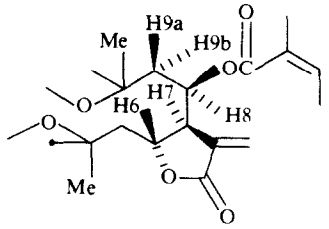
Conc of IAA (M)	Average elongation of (mm) of 12 sections		
	Without **1a** or **2a**	With **1a**	With **2a**
0	13.0	12.0	11.9
10^{-5}	15.5	12.6	12.2
10^{-4}	18.2	12.0	12.1



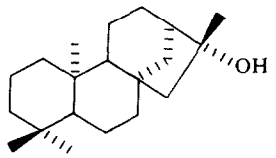
3 Heliangine



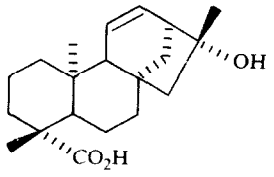
4a R = H Eupatolide
4b R = Ac



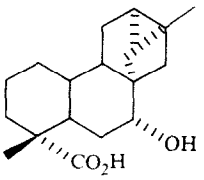
A



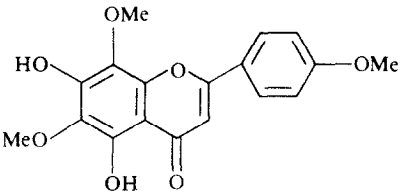
5 (—)-16-α-Hydroxykaurane



6 (—)-16-α-Hydroxy-kaur-11-en-19-oic acid



7 Ciliaric acid



8 Nevadensin

(*Spodoptera litura*, 63% kill after 48 hr, 2000 ppm in an artificial diet) and mosquito larvae (*Culex pipens*, 60% kill after 24 hr in 10 ppm solution). Ciliaric acid (**7**), however, did not show either of these activities. Eupatolide could thus be involved in the natural resistance of *H. argophyllus* to insect predation.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were measured at 90 and 22.6 MHz, respectively, with TMS as an int. standard, except for one ^{1}H NMR spectrum of argophyllin-B which was measured at 200 MHz. Si gel 60 (Merck, 70–230 mesh) was used for column chromatography separations. Analyt. TLC and prep. TLC (0.5 and 2.0 mm) were done on precoated Si gel 60 GF_{254}. MS were recorded by direct inlet at 70 eV ionization.

Extraction and isolation. Air-dried and ground leaves and stems (1.63 kg) of *H. argophyllus* (collected along Texas Highway 80, 7.4 miles south of U.S. Highway 90, along the edges of a cultivated field, Gonzalez Co., TX, 25 Aug. 1979, voucher deposited in the Herbarium at the University of Texas at Austin), were extracted with $CHCl_3$ (×2, 4.0 and 6.0 l.) at room temp. The extract was filtered and concd *in vacuo* to give 256 g of a dark green syrup. The syrup was purified by standard procedures [13] to give 88.0 g of yellow syrup which was charged on the top of a Si gel column (1.76 kg). The column was eluted with a toluene–EtOAc gradient solvent system initiated with toluene; 1 l. of eluent was collected for each fraction. All the fractions were monitored by TLC with a toluene–EtOAc, solvent system. Fractions 23 and 24 (eluted with toluene–EtOAc, 15:1) yielded 0.4 g (−) - 16 - α - hydroxykaurane (**5**) as white needles. Fractions 51–55, which were eluted with toluene–EtOAc (5:1), gave 1.5 g of crystalline eupatolide (**4a**) (0.09% yield from the dried plant). Colourless prisms were obtained by recrystallization from MeOH. Fractions 61–63 yielded 30 mg of yellow needles of nevadensin (**8**). Fraction 64 yielded 20 mg of crystalline (−) - 16 - α - hydroxy - kaur - 11 - en - 19 - oic acid (**6**). Fractions 65–75 (eluted with toluene–EtOAc, 3 : 1–1 : 1) gave 2.0 g of white needles of ciliaric acid (**7**). Fractions 96–102 (eluted with toluene–EtOAc, 1 : 3) showed several spots of TLC and were combined; the material (9.0 g) from these fractions was purified on a smaller Si gel column (300 g) eluted with $CHCl_3$–Me_2CO (1 : 1). The material from fractions 77–89 (1.30 g) was purified ×2 by prep. TLC ($CHCl_3$–Me_2CO, 1 : 2, 2 mm;

$CHCl_3$–*iso*-PrOH, 10 : 1, 0.5 mm) to give 400 mg of crystalline argophyllin-A (**1a**). Colourless prisms were obtained by recrystallization from MeOH. These crystals were used for X-ray diffraction analysis. Fractions 110–114 (eluted with EtOAc) gave 4.5 g yellow syrup. The syrup was purified through a smaller Si gel column (250 g) which was developed with $CHCl_3$–MeOH (10:1). Fractions 38–43 were combined and gave 300 mg syrup which showed one main spot. The material from these fractions was purified by prep. TLC (2 mm; $CHCl_3$-iso-PrOH, 10:1 and 0.5 mm; $CHCl_3$–*iso*-PrOH, 8:1) to give 250 mg argophyllin-B (**2a**) as white needles.

Argophyllin-A (**1a**). Mp 190–192° (MeOH), $[\alpha]_D^{22} - 157.0°$ ($CHCl_3$; *c* 0.10) $C_{20}H_{28}O_7$, for M^+ found *m/z* 380.1826 calc. *m/z* 380.1835, MS *m/z* (rel. int.): 380 (1), 362 (0.5), 178 (11), 95 (12), 83 (100), 55 (64), 43 (45); IR ν_{max}^{Nujol} cm^{-1}: 3400, 3100, 1755, 1720, 1680, 1650, 1230, 1150, 1040, 1000.

Argophyllin-B (**2a**). Mp 63–69° ($CHCl_3$), $[\alpha]_D^{22} - 137.5°$ ($CHCl_3$; *c* 0.14) $C_{20}H_{28}O_7$, for M^+ found *m/z* 380.1837 calc. 380.1835; MS *m/z* (rel. int.): 380 (0.3), 362 (0.7), 336 (0.7), 305 (1), 83 (100), 55 (75), 43 (32). IR ν_{max}^{Nujol} cm^{-1}: 3400, 1760, 1725, 1650, 1220, 1140, 1040.

Eupatolide (**4a**). Mp 185–188° (MeOH) (lit. 182–188° [9]), IR and ^1H NMR spectra and mp were identical to the previously published data. **4b**, which was prepared with Ac_2O–pyridine, exhibited IR and ^1H NMR spectra identical to those of an authentic specimen.

(−)-16-*α*-*Hydroxykaurane* (**5**). Mp 211–213° (lit. 212–214°), $[\alpha]_D^{22} - 40.0°$ ($CHCl_3$; *c* 0.10) (lit. −45.0°). The IR and NMR were identical to those previously reported [11].

(−)-16-*α*-*Hydroxy-kaur*-11-*en*-19-*oic acid* (**6**). Mp 242–244° (lit. 244–246° [4]). The IR and NMR were identical to those of an authentic specimen.

Ciliaric acid (**7**). Mp (methyl ester) 132–133° (lit. 136–137° [10]). IR and ^1H NMR spectra were identical to those of an authentic specimen. The methyl ester which was prepared by the usual method (CH_2N_2) exhibited IR and ^1H NMR spectra identical to those of previously reported [10].

Nevadensin (**8**). Mp 190–192° (lit. 193–195° [12]). ^1H NMR, UV data and mp were identical to the published data.

Acetylation of argophyllin-A (**1a**). 30 mg **1a** was acetylated with Ac_2O (1 ml) and pyridine (0.5 ml) for 12 hr at room temp. After the usual work-up, the crude product was purified by prep. TLC (EtOAc) to give 12 mg of the monoacetate **1b**, colourless oil, $[\alpha]_D^{22} - 79.8°$ ($CHCl_3$; *c* 0.14) MS *m/z* (rel. int.): 422 (0.3), 404 (0.7), 374 (0.7), 83 (100), 55 (57), 43 (90). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 2940, 1760, 1730, 1720, 1680, 1640, 1230 and 1140.

Acetylation of argophyllin-B (**2a**). 56 mg **2a** was acetylated with Ac_2O (2 ml) and pyridine (1 ml) for 12 hr at room temp. After prep. TLC (EtOAc) of the crude product, 47 mg of the diacetate **2b** was obtained as colourless oil, $[\alpha]_D^{22} - 70.2°$ ($CHCl_3$; *c* 0.05) MS *m/z*: 464 (0.5), 422 (0.4), 245 (12), 83 (100), 55 (58), 43 (66); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2940, 1760, 1740, 1720, 1700, 1690, 1650, 1230, 1140, 1040.

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