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SESQUITERPENE LACTONES FROM *HELIANTHUS NIVEUS* SUBSP. *NIVEUS*

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Key Word Index—*Helianthus niveus*; Compositae; sesquiterpene lactones; germacranolides; germacrolides; heliangolides; tirotundin; tagitinin A; orizabin.

Abstract—Six germacranolides, tirotundin, tagitinin A, orizabin, 8β,14-dihydroxycostunolide and its 14-isobutyrate, and 4β,5-dihydrotagitinin C, were isolated from aboveground parts of *Helianthus niveus* subsp. *niveus*. The last two compounds are new.

INTRODUCTION

As part of our continuing phytochemical investigation of the genus *Helianthus*, we have examined *H. niveus* (Benth.) Brandeg. subsp. *niveus*, a perennial taxon of sect. *Helianthus* [1]. The annual *H. niveus* subsp. *canescens* (A. Gray) Heiser has already yielded several terpenoids, including 3,10-hemiketal bridged heliangolides [2]. We report here the isolation of six germacranolides, including two new compounds. Only one of these six (**5**) is known from other species of *Helianthus*.

RESULTS AND DISCUSSION

Three of the compounds isolated were readily identified by their spectral characteristics as a group of closely related sesquiterpene lactones known from *Tithonia*, tirotundin (**1**) [3–5], tagitinin A (**2**) [5], and orizabin (**3**) [6]. High field ^{1}H NMR and ^{13}C NMR data for **3** have never been published and are reported here (Tables 1 and 2). As suspected by Herz and Kumar [7] extensive spin decoupling at 200 MHz has revealed some errors in the original assignments [6]. In view of the fact that the stereochemistry of **3** has been revised twice since its structure was first proposed [5, 8] it is reassuring to report that the ^{1}H NMR signals of the main skeleton of orizabin correspond closely to those of niveusin C-2′,3′-epoxide, an analogous compound with a different side chain whose structure has been confirmed by X-ray diffraction [9].

The fourth compound (**4**) seemed to be the 1,2-anhydro derivative of the open form of **2**. The most striking feature of the ^{1}H NMR spectrum of this apparently new compound was a two proton AB pattern ($J = 16$ Hz) centred at δ6.5, representing an isolated *trans* double bond. Also evident were the characteristic signals of an isobutyrate side chain (δ2.44 *sept.*, δ1.06 *d*, δ1.09 *d*), an isolated methyl group adjacent to an oxygen atom (δ1.48 *s*) and a ketone (IR: 1711 cm^{-1}, ^{13}C NMR: δ202.5). Decoupling allowed the assignment of all remaining ^{1}H NMR signals to give structure **4** (without stereochemistry). The similar chemical shifts of H-1 and H-2 are in accord with several known compounds containing α,β-unsaturated ketones where steric constraints prevent coplanarity and thus allow only partial conjugation [10, 11]. The correspondence of chemical shifts and coupling constants with the ^{1}H NMR spectrum of an analogous lactone (compound **4** in ref.

Table 1. ^{1}H NMR data for compounds **3**–**7** (200 MHz, $CDCl_3$)

	3	**4**	**5**	**6**	**7**
H-1	4.03 *m*	6.55 *d*	5.03 *br dd*	5.12 *dd*	5.15 *dd*
H-2	2.47 *dd*	6.41 *d*	2.1–2.35	2.2–2.4	2.2–2.4
	2.36 *d*				
H-3	—	—	2.1–2.35	2.2–2.4	2.2–2.4
H-4	—	3.26 *m*	—	—	—
H-5	5.61 *m*	1.97 *br dd*	4.86 *br d*	4.86 *d*	4.82 *d*
		1.61 *ddd*			
H-6	5.31 *tq*	4.75 *br d*	5.13 *dd*	5.24 *dd*	5.08 *dd*
H-7	4.17 *m*	3.34 *m*	2.79 *dddd*	2.76 *m*	2.91 *m*
H-8	5.61 *m*	5.28 *ddd*	4.49 *ddd*	4.59 *br d*	5.73 *br d*
H-9	1.87 *dd*	2.28 *dd*	2.89 *dd*	2.93 *dd*	3.16 *dd*
	1.75 *d*	1.97 *dd*	2.41 *dd*	2.2–2.4	2.2–2.4
H-13	6.26 *d*	6.24 *d*	6.36 *d*	6.35 *d*	6.31 *d*
	5.61 *d*	5.71 *d*	5.62 *d*	5.59 *d*	5.60 *d*
H-14	1.53 *s*	1.48 *s*	4.15 *d*	4.80 *d*	4.74 *d*
			3.84 *d*	4.58 *d*	4.28 *d*
H-15	1.85 *br s*	1.15 *d*	1.68 *d*	1.64 *d*	1.70 *br s*
H-2′	2.40 *sept*	2.44 *sept*	—	2.56 *sept*	2.51 *sept*
H-3′,4′	1.03 *d*	1.06 *d*	—	1.17 *d*	1.14 *d*
	1.05 *d*	1.09 *d*		1.17 *d*	1.14 *d*
OH	3.73	—	—	—	—
	3.24	—	—	—	—
OAc	—	—	—	—	2.03 *s*

Coupling constants (Hz). Compound **3**: 1,2a = 4; 2a,b = 14; 5,6 = 4; 6,15 = 2; 6,7 = 4; 7,13 = 2; 8,9b = 6; 9a,b = 14; 1,OH = 6; 2′,3′ = 6. Compound **4**: 1,2 = 16; 4,5a = 9; 4,5b = 11; 4,15 = 6; 5a,b = 14; 5b,6 = 9; 5a,6 and 6,7 = small; 7,8 = 4; 8,9a = 5; 8,9b = 10; 9a,b = 14; 7,13a = 2; 7,13b = 1.5; 2′,3′ = 7. Compound **5**: 1,2a = 5; 1,2b = 10.5; 1,14 < 1.5; 5,15 = 1.5; 5,6 = 9.5; 6,7 = 8.5; 7,8 < 1.5; 7,13a = 4; 7,13b = 3.5; 8,9a = 5; 8,9b = 2.5, 9a,b = 15; 14a,b = 12.5. Compounds **6** and **7**: 2′,3′ = 7, otherwise similar to compound **5**.

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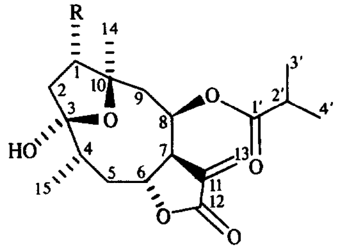
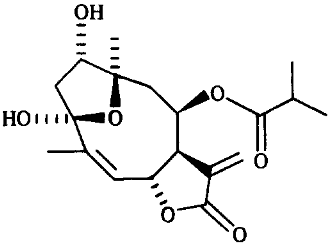
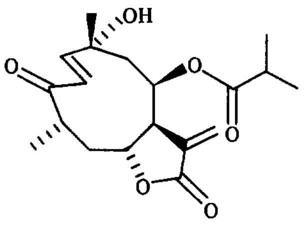


1 R=H
2 R=OH



3



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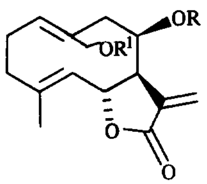
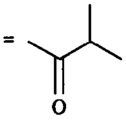
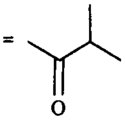


5 R=R¹=H

6 R=H R¹=



7 R=Ac R¹=



[12]) indicates that the stereochemistry is as depicted for C-6 through C-10, and comparison of the coupling patterns around C-4 through C-6 with Dreiding models shows that the C-4 methyl group must be α. This compound thus has the same stereochemistry at all corresponding centres as compounds **1**–**3** (although the formal depiction in the two-dimensional representation must be reversed at C-10 [13, pp. 59–61]).

Table 2. ^{13}C NMR data for compounds **3**, **4** and **6** (22.6 MHz, $CDCl_3$)

	3	4	6
C-1	77.8 *d*	151.7 *d*	134.6 *d*
C-2	44.9 *t*	129.2 *d*	25.1 *t*
C-3	106.7 *s*	202.5 *s*	38.3 *t*
C-4	140.1 *s**	41.7 *d*	141.3 *s*
C-5	128.9 *d*	40.9 *t**	127.1 *d*
C-6	75.1 *d*	77.5 *d*†	74.7 *d*
C-7	50.0 *d*	47.0 *d*	52.9 *d*
C-8	71.3 *d*	72.7 *d*†	70.4 *d*
C-9	39.5 *t*	43.2 *t**	41.9 *t*
C-10	86.5 *s*	70.8 *s*	137.9 *s*
C-11	136.2 *s**	135.1 *s*	133.4 *s*
C-12	170.1 *s*	168.8 *s*	170.0 *s*
C-13	123.1 *t*	123.1 *t*	119.9 *t*
C-14	22.0 *q*†	28.7 *q*	62.5 *t*
C-15	22.2 *q*†	16.0 *q*	16.1 *q*
C-1'	176.2 *s*	175.3 *s*	176.7 *s*
C-2'	34.1 *d*	33.2 *d*	33.3 *d*
C-3', 4'	19.1 *q*	17.8 *q*	18.2 *q*
	18.7 *q*	17.8 *q*	18.2 *q*

*,†Assignments are interchangeable.

Two additional compounds isolated proved to have germacrolide skeletons. One was identified as 8β,14-dihydroxycostunolide (desacetylovatifolin) (**5**), a compound already known from two species of *Helianthus* [14, 15]. The ^{1}H NMR spectrum of compound **6** was similar to that of **5**, but also had the characteristic signals of an isobutyrate side-chain. Thus, **6** represents an isobutyrate ester of **5**. The ^{1}H NMR data for **6** suggested that the side-chain was attached at C-14, since the signal assigned to H-8 was only 0.05 ppm downfield from the corresponding signal in **5** while the H-14 protons shifted 0.7 and 0.8 ppm downfield. This was confirmed by the acetylation of **6** to **7**, which shifted the H-8 signal downfield by 1.14 ppm, while the H-14 protons experienced a slight upfield shift. Compound **6** is of particular interest because none of the many sesquiterpene lactones previously isolated from *Helianthus* and its close relatives *Viguiera* and *Tithonia* has had a side-chain at a position other than C-8.

Although only one of the six compounds isolated in this study has been previously reported from *Helianthus*, the overall terpenoid profile of *H. niveus* subsp. *niveus* fits well with that of the genus as a whole. The prevalence of isobutyrate side-chains is unusual, as most species of *Helianthus* elaborate unsaturated or oxygenated five-carbon side-chains [16]. However, the isobutyrate side-chain is known from two other compounds isolated from *Helianthus* species [17, 18] and several species of the related genera *Tithonia* [19] and *Viguiera* [20 and refs. therein] elaborate comparable series of isobutyrate esters. *Helianthus niveus* subsp. *canescens*, sometimes treated as *H. petiolaris* Nutt. var. *canescens* A. Gray [21] contains a similar series of 3,10-hemiketal bridged heliangolides with angelate side-chains [2], including the angelate analog of orizabin. It would be interesting to see whether the profile of typical *H. petiolaris* is also similar.

EXPERIMENTAL

Air-dried leaves and heads (2.7 kg) of *Helianthus niveus* subsp. *niveus*, collected 15 miles north of Rosario, Baja California, Mexico (voucher Gershenzon #75, herb. TEX), were washed with CH_2Cl_2 and worked up in the usual manner [22]. Crude syrup (56.2 g) was loaded onto a silica gel column (1.2 kg) and eluted with a CH_2Cl_2–*iso*-PrOH gradient with increasing amounts of *iso*-PrOH. Fifty-four fractions of 0.5 to 1.0 g were collected and their composition monitored by TLC (compounds were detected on TLC plates by UV absorption (254 nm) and by color reactions after spraying with acidified vanillin [23] and heating). All TLC was performed on silica gel using either toluene–EtOAc 5:6 (TEA) or CH_2Cl_2–*iso*-PrOH 15:1 (CIPA).

Fraction 7 (1% *iso*-PrOH) showed a vanillin–purple band, which was purified by prep. TLC in TEA, then CIPA to yield 23 mg **6** (as oil). Fractions 9–16 (1% *iso*-PrOH) were chiefly composed of a vanillin–orange compound which crystallized from these fractions; recrystallization from $(iso\text{-}Pr)_2O$–MeOH gave 1.15 g of tirotundin (**1**). Fractions 19–21 (1% *iso*-PrOH) gave a vanillin–orange compound which crystallized from the fractions; recrystallization from Me_2CO–CH_2Cl_2 gave 0.2 g of orizabin (**3**). Fraction 30 (2% *iso*-PrOH) showed a vanillin–purple band, purified by prep. TLC in TEA (double-developed) and recrystallized from MeOH to yield 66 mg **5**. Fractions 32–34 (2% *iso*-PrOH) were combined and run through a silica gel column eluted with a toluene–EtOAc gradient (increasing EtOAc). Five fractions were collected; the third (10% EtOAc) contained a vanillin-colorless band; repeated prep. TLC with TEA and CIPA yielded 9 mg of **4**, as an oil. Fractions 32 through 36 (2% *iso*-PrOH) contained a vanillin–green band; prep. TLC of fract. 35 with TEA, then CIPA gave 27 mg tagitinin A (**2**), as oil.

Tirotundin (**1**). Colorless needles, mp and spectral properties as reported [3, 5].

Tagitinin A (**2**). Spectral properties as reported [5].

Orizabin (**3**). Colorless thin triangular prisms, mp and spectral properties as reported [6].

4,5-*Dihydrotagitinin C* (**4**). IR $\nu_{max}^{CHCl_3}$: 3447 (OH), 1764 (lactone C=O), 1731 (side-chain ester C=O), 1711 (ketone), 1681, 1155, 1124, 1170, 990, 872, 819, MS (EI, probe) 70 eV *m/z* (rel. int.): 350 (6) $[M]^+$, 332 (23) $[M-H_2O]^+$, 279 (10) $[M-C_4H_7O]^+$ α-cleavage of ester side-chain, 262 (34) $[M-C_4H_8O_2]^+$ McLafferty rearrangement and loss of ester side-chain, 261 (74) $[M-C_4H_7O\text{–}H_2O]^+$, 220 (58), 123 (77), 97 (88), 71 (87) $[C_4H_7O]^+$ side chain acylium ion, 43 (100) $[C_3H_7]^+$ loss of CO from $C_4H_7O^+$. UV λ_{max}^{MeOH} nm. (log ε): 212 (4.01), 224 sh (3.91).

8β,14-*Dihydroxycostunolide* (**5**). White crystals, mp and spectral properties as reported [14] except for minor discrepancies in ^1H NMR data; our data are presented in Table 1.

8β,14-*Dihydroxycostunolide*-14-*isobutyrate* (**6**). IR $\nu_{max}^{CHCl_3}$: 3480 (OH), 1755 (lactone C=O), 1728 (side-chain ester C=O), 1664, 1289, 1227, 1146, 973, 949, 887. MS: CI (*iso*butane) *m/z* (rel. int.): 335 (41) $[M+1]^+$ EI: 332 (6), 107 (41), 91 (47), 71 (86) side-chain acylium ion, 43 (100) loss of CO from 71.

Acetylation of **6**. Compound **6** (23 mg) was dissolved in 0.5 ml pyridine, mixed with 1.0 ml Ac_2O and stirred for 14 hr at room temp. Examination of the crude product by ^1H NMR showed that the reaction had been quantitative; yield 10 mg oil. IR $\nu_{max}^{CHCl_3}$: 2900, 1755 (lactone C=O), 1745 (acetate C=O), 1730 (side-chain C=O), 1360, 1148. MS (EI, probe) 70 eV *m/z* (rel. int.): 228 (28), 71 (73), 43 (100).

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