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DITERPENE CARBOXYLIC ACIDS AND A HELIANGOLIDE FROM *HELIANTHUS ANGUSTIFOLIUS**

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Key Word Index—*Helianthus angustifolius*; *H. ciliaris*; *H. salicifolius*; Compositae; sesquiterpene lactone; heliangolide; budlein A; diterpene carboxylic acids; (−)-*cis*-ozic acid; flavonoid; hymenoxin.

Abstract—Two diterpene carboxylic acids, one a new kaurenoid derivative and one the previously characterized labdane, (−)-*cis*-ozic acid, as well as a known heliangolide, budlein A, and a known flavonoid, hymenoxin, were obtained from a chloroform extract of *Helianthus angustifolius*. The new kaurenoid-type carboxylic acid was also isolated from *H. ciliaris* and *H. salicifolius*.

INTRODUCTION

In the course of our systematic investigation of the chemistry of *Helianthus* (tribe Heliantheae, family Compositae), we previously reported the major terpenoid constituents in *H. mollis* Lam. [1], *H. debilis* subsp. *debilis* Nutt. [2] and *H. niveus* subsp. *canescens* (A. Gray) Heiser [3]. Sesquiterpene lactones (germacranolides and heliangolides) and diterpenoids (mainly kaurane, trachylobane and labdane type carboxylic acids) have been found to be the major terpenoid constituents in *Helianthus* so far. In this paper, we report the structural determination of a new kaurenoid carboxylic acid and the isolation and identification of three known compounds, a flavonoid, hymenoxin, a diterpene carboxylic acid, (−)-*cis*-ozic acid, and a heliangolide, budlein A, from *H. angustifolius* L. The new kaurenoid carboxylic acid was also found in *H. ciliaris* DC. and *H. salicifolius* A. Dietr.

RESULTS AND DISCUSSION

A chloroform extract [4] of dried leaves of *Helianthus angustifolius* collected near Baton Rouge, LA, was chromatographed through a Si gel column and each fraction was monitored by TLC. Further purification of the material on small columns and preparative TLC (Si gel) gave a sesquiterpene lactone, two diterpene carboxylic acids and a flavonoid. The flavonoid, which was shown to be hymenoxin (**1**) by means of UV, ^{1}H NMR and mp, was previously isolated from *Hymenoxys scaposa* [5] and *Helianthus angustifolius* from Tennessee [6].

One of the diterpene carboxylic acids (**2**) showed a characteristic *cis*-1,3-diene UV absorption (236 and 232 nm) as well as three methyl signals and six olefinic protons in its ^{1}H NMR spectrum. The molecular ion (M^+ at m/z 316 as its methyl ester) and the spectroscopic data suggested a labdatriene carboxylic acid-type skeleton. The acid was finally identified as *cis*-ozic acid (**2**) by comparison of the mp, IR and ^{1}H NMR and ^{13}C NMR spectra of the acid and its methyl ester with those of an authentic specimen. *Cis*-ozic acid has been previously isolated from *Daniellia ogea* [7], *Hermas villosa* [8] and *Helianthus occidentalis* [9]. Both dextrorotatory [8] and levorotatory [7,9] forms are known, with that from *H. angustifolius* being the (−)-antipode, $[\alpha]_D = -53.5°$ ($CHCl_3$ $c = 0.3$).

The second diterpene acid (**3**) had the formula $C_{20}H_{30}O_3$ based on the HRMS of its methyl ester **4** ($C_{21}H_{32}O_3$) M^+ at m/z 332.236; calc. 332.235). The diterpene acid (mp 244–246°, $[\alpha]_D$ −234.6°) appeared to be a hydroxycarboxylic acid by IR (3400 and 1700) and by the presence of a strong M^+-H_2O fragment in the MS of its methyl ester (**4**). The ^{1}H NMR of **3** showed three methyl singlets at δ 1.08, 1.32 and 1.48 as well as two olefinic protons at 5.58 (*dd*, J = 10 and 4.0 Hz) and 6.00 (*ddd*, J = 10, 7.0 and 1 Hz), but no signal which could be assigned to a proton geminal to a hydroxyl group; these data indicated the tertiary nature of the hydroxyl group. Although **3** could be hydrogenated with Pd–C (in EtOH) to give **5**, the methyl ester **4** was only slowly reduced under these conditions to give **6** and not at all with Pd–C in EtOAc. These results suggested some steric hindrance around the disubstituted double bond. The ^{13}C NMR of **4** indicated the presence of only two olefinic carbons, showing that **4** has four rings, as expected for a kaurane skeleton. The skeleton was confirmed to be (−)-kaurane when dehydration of **6** with $SOCl_2$–pyridine gave a mixture of (−)-methyl kaurenoate and its Δ^{15} isomer; the product was analysed by GLC and compared with an authentic specimen.

The location of the disubstituted double bond was determined by ^{1}H NMR decoupling experiments on the methyl ester **4**. On irradiation of a signal for an allylic proton at δ 2.20, the broadened double doublet at 6.00 changed into a broad doublet (J = 10 and 1 Hz) while the other olefinic signal at 5.58 remained unchanged. Furthermore, irradiation of the signal at 1.48 which is assigned to the other allylic proton changed the double doublet at 5.58 into a sharp doublet (J = 10 Hz) while the

* Part IV in the series "Chemistry of *Helianthus*". For Part III see ref. [3].

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broadened double doublet at 6.00 lost its long-range coupling, appearing as a sharp double doublet ($J = 10$ and 7.0 Hz). This evidence indicates an ABXY system for the double bond, that is CH_x–CH=CH–CH_y–. This ABXY system can only be located at C-11 and C-12 on the kaurane skeleton. Examination of a Dreiding model of **4** indicated some steric hindrance of the C-11,C-12 double bond by the C-10 methyl group on the α-side of the molecule and by the C-14,C-15 ethylene bridge on the β-side. Such hindrance could account for the difficulty in the hydrogenation of **4** in EtOAc.

Compounds **5** and **6** gave IR and NMR spectra and mps similar to those reported in ref. [10]. The dihydro acid **5** was first isolated from a fermentation broth of *Fusarium moniforme*. The mp of **6** was 154–155° (155–157° reported [10]; an authentic specimen was not available). The new carboxylic acid **3** was also obtained from *H. ciliaris* and *H. salicifolius* (Gershenzon, J., Neuman, P., Ohno, N. and Mabry, T. J., unpublished results); other terpenoids in these species are presently under investigation.

A heliangolide-type sesquiterpene lactone **7**, isolated after repeated column chromatography and preparative TLC, appeared to have the same structure as budlein A, previously isolated from *Viguiera buddleiaeformis* [11] and *V. angustifolia* [12]. MS, ^{1}H NMR, and optical rotation data for **7** were in accord with published data for budlein A, and ^{13}C NMR spectra of **7** (Table 1) were superimposable on those obtained from an authentic sample of budlein A. However, IR spectra of the two substances showed minor differences in the intensity of a few small signals between 900 and 1000 cm^{-1} and similar mps were not obtained under all sets of recrystallization conditions. The small sample of budlein A available always contained a minor impurity detectable but not cleanly separable by TLC. When all these data are considered together, compound **7** can be assigned identity with budlein A. Under the same acetylation conditions (Et_3N, Ac_2O, pyridine) as those used by Romo de Vivar *et al.* [11] to convert budlein A into its acetate **8** with 74% yield, we obtained the rearranged product **9** in 83% yield, a product which had been previously produced on acetylation of budlein A with Ac_2O and pyridine [12]. As Herz has pointed out recently [13], the ^{1}H NMR spectrum of the rearranged product shows that the 5-OAc function is α-oriented (C-5 has *R*-configuration) in contrast to the previous report [12].

Table 1. ^{13}C NMR spectral data* for budlein A (**7**). Multiplicities were determined by partial decoupling experiments

Carbon No.	$CDCl_3$	DMSO-d_6	Me_2CO-d_6
1	205.6 *s*	209.1	205.0
2	105.0 *d*	108.3	104.3
3	182.9 *s*	187.1	183.0
4	136.2 *s*	141.1	136.7
5	134.2 *d*	136.2	132.6
6	75.6 *d*†	78.9†	75.0†
7	48.5 *d*	51.6	47.8
8	74.3 *d*†	78.6†	74.8†
9	42.2 *t*	45.3	41.4
10	88.0 *s*	91.3	87.4
11	138.8 *s*	142.9	139.7
12	169.2 *s*	172.5	168.3
13	124.0 *t*	127.8	122.7
14	20.1 *q*	23.5	19.1
15	62.4 *t*	64.7	61.2
16	166.0 *s*	169.5	165.4
17	126.5 *s*	130.6	126.7
18	141.7 *d*	143.7	139.3
19	15.9 *q*	19.3	14.7
20	21.3 *q*	24.8	20.4

*The ^{13}C NMR spectra of budlein A from *Helianthus angustifolius* were superimposable on those obtained from the sample of budlein A from *Viguiera* sp.

†Assignments interchangeable.

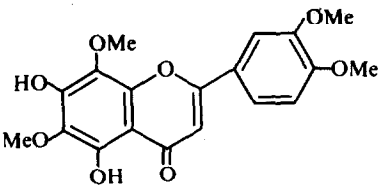
The co-occurrence of budlein A in species of *Helianthus* and *Viguiera* supports the close relationship of these two genera [14]. Other heliangolides with 3-(2H)-furanone moieties have been isolated from *Helianthus ciliaris* [15], *H. lehmannii* [16] and *H. grosseserratus* ([13]; Gershenzon, J. and Mabry, T. J., unpublished results).

Since the preparation of this manuscript, another report on the constituents of *Helianthus angustifolius* has appeared [17]. Eight compounds were isolated including four diterpene acids, one of which, (−)-*cis*-ozic acid, was obtained in the course of the present investigation. No sesquiterpene lactones were reported.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were measured at 100 and 22.6 MHz, respectively, with TMS as an int. standard. Mps were determined on a Fischer–Johns mp block and are uncorr. Si gel 60 (70–230 mesh) was used for CC and Si gel 60 GF-254 for TLC (0.25 mm) and prep. TLC (1.0 mm). Analytical TLC were visualized by UV and H_2SO_4 spray. Mass spectra were recorded by direct inlet at 70 eV.

Extraction and separation. Air-dried and ground leaves of *Helianthus angustifolius* (1.2 kg collected in October, 1978 in east Baton Rouge Parish, LA, on Perkins Road 1 mile west of Kenilworth entrance; voucher, Fischer-Ohmstede No. 1, deposited in the University of Texas Herbarium) were extrd with $CHCl_3$ (3.6 and 2.0 l.). After removal of the solvent, the crude extract (73.3 g) was purified by standard procedures [4] to afford 17.5 g of yellow syrup. The syrup was chromatographed over a Si gel column (820 g) which was eluted with a C_6H_6–EtOAc gradient (10:1–6:4) followed by $CHCl_3$–Me_2CO (8:2–1:1). Sixty 250-ml fractions were collected. Fractions eluted with C_6H_6–EtOAc (10:1) gave a major spot on TLC and were combined for a second column. The major fractions from the second column were further purified on prep. TLC to give 240 mg of *cis*-ozic acid (**2**) as colourless crystals. Fractions eluted with C_6H_6–EtOAc (7:3) showed another major spot on TLC, and gave crystals of **3** on concn and standing. The crystals (430 mg) were collected by trituration with an *iso*-Pr_2O–EtOAc mixture and filtration. The heliangolide **7** was eluted with $CHCl_3$–Me_2CO (8:2) from the first column as a partially crystalline material. The crystals (133 mg) were collected by removing the syrup with *iso*-Pr_2O–EtOAc. The material (235 mg of **7**) in the washings was purified on a smaller column and by prep. TLC. The residue from the fractions which eluted with C_6H_6–EtOAc (3:2) afforded another 152 mg of **7** after chromatography over a small Si gel column. The first fraction from this column showed a flavonoid on TLC; purification of the concentrate through a Sephadex LH-20 column (MeOH) gave 11 mg of **1**.



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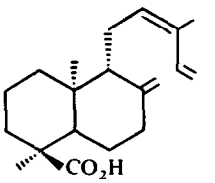
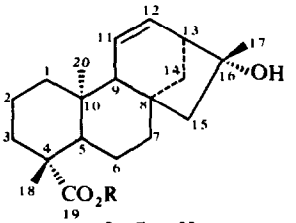


2



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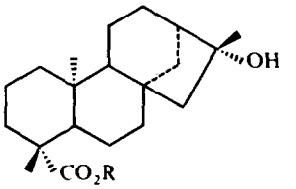
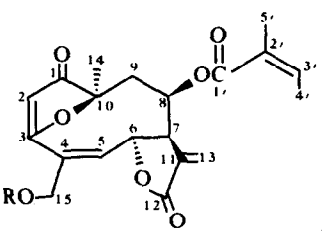
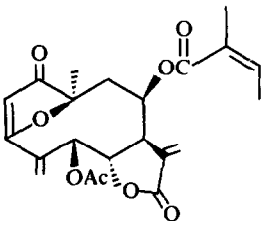
4 R = Me



5 R = H
6 R = Me



7 R = H
8 R = Ac



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Hymenoxin (**1**). Mp 210–211° (lit. 211–213° [5]), $C_{19}H_{18}O_8$ (M^+ at *m/z* 374). UV and ^1H NMR spectra were identical to published data [6].

(−)-Cis-*ozic acid* (**2**). Mp 141–142° (lit. 142° [7]), $C_{20}H_{30}O_2$ (M^+ at *m/z* 302), $[\alpha]_D$ −53.5° ($CHCl_3$, *c* = 0.3). ^1H and ^{13}C NMR spectra were identical to those of an authentic specimen [9].

16α-*Hydroxykaur*-11-*enoic acid* (**3**). Mp 244–246° $[\alpha]_D$ −234.6° (EtOH, *c* = 0.4), $C_{20}H_{30}O_3$ (M^+ of the Me-ester **4**, $C_{21}H_{32}O_3$ at *m/z* 332). IR ν_{max}^{Nujol} cm^{-1}: 3500, 3200–2600, 1700, 1660, 1250, 1240, 1102 and 940. ^1H NMR: $\delta^{pyridine\text{-}d_5}$ 1.08 (3 H, *s*), 1.32 (3 H, *s*), 1.48 (3 H, *s*), 5.58 (1 H, *dd*, *J* = 10, 4 Hz) and 5.98 (1 H, *br dd*, *J* = 10, 6 and 1).

Budlein A (**7**). mp 154–155°, lit. 106–108° [11] (crystals from *iso*-Pr_2O–Me_2CO); mp 134–136°, authentic sample 133–135° (crystals from *iso*-Pr_2O–MeOH). $[\alpha]_D$ −83.1° (MeOH, *c* = 1.27), lit. −82.3° (MeOH) [11] IR ν_{max}^{Nujol} cm^{-1}: 3440 (sh), 3300, 1755, 1725, 1705, 1680, 1660, 1580, 1140 and 816. MS *m/z* (rel. int.): 374.1355 ($C_{20}H_{22}O_7$, calc. 374.1365, 25), 356 (4), 292 (20), 274 (42), 248 (50), 187 (16), 138 (50), 83 (80), 55 (85), 43 (100). ^1H NMR: δ^{CDCl_3} 1.48 (3 H, *s*, 10-Me), 1.80 (3 H, *bs*, 2′-Me), 1.94 (3 H, *dq*, *J* = 7, 1.5, 3′-Me), 2.32 (1 H, *dd*, *J* = 15, 4, H-9b), 2.57 (1 H, *dd*, *J* = 15, 6, H-9a), 3.79 (1 H, *m*, H-7), 4.40 (2 H, *bs*, C-4-CH_2OH), 5.27 (1 H, *m*, H-8), 5.38 (1 H, *m*, H-6), 5.70 (1 H, *s*, H-2), 5.74 (1 H, *d*, *J* = 2, H-13b), 6.14 (1 H, *qq*, *J* = 7, 1.5, H-2′), 6.22 (1 H, *dt*, *J* = 4, 1.5, H-5), 6.37 (1 H, *d*, *J* = 3, H-13a).

Methyl 16α-*hydroxykaur*-11-*enoate* (**4**). The hydroxy acid **3** (40 mg) was methylated with ethereal CH_2N_2 in the usual manner to give 41 mg of the methyl ester **4** after prep. TLC purification (C_6H_6–*iso*-PrOH, 10:1). A part of the sample was recryst from 80% aq. MeOH, mp 131–133°. MS *m/z* (rel. int.): no M^+ observed on low resolution EIMS, 314 (22), 274 (28), 255 (33), 210 (17), 180 (22), 131 (56), 121 (61), 105 (67), 91 (83) and 43 (100). IR ν_{max}^{Nujol} cm^{-1}: 3280, 1730, 1660, 1140 and 930. ^1H NMR ($CDCl_3$): δ 0.72 (3 H, *s*), 1.18 (3 H, *s*), 1.22 (3 H, *s*), 3.64 (3 H, *s*), 5.54 (1 H, *dd*, *J* = 10 and 4) and 5.92 (1 H, *br dd*, *J* = 10, 6 and 1). ^{13}C NMR ($CDCl_3$): 15.3 (*q*), 19.1 (*t*), 21.9 (*t*), 25.8 (*q*), 28.7 (*q*), 34.5 (*t*), 38.0 (*t*), 38.8 (*s*), 39.9 (*t*), 41.4 (*t*), 43.3 (*s*), 44.0 (*s*), 50.1 (*d*), 51.2 (*d*), 55.9 (*q*), 58.6 (*t*), 61.3 (*d*), 83.8 (*s*), 127.1 (*d*), 132.7 (*d*) and 178.1 (*s*).

16α-*Hydroxykauranoic acid* (**5**). 15α-Hydroxykaur-11-enoic acid (**3**) (20 mg) was reduced with 5% Pd–C (70 mg) in EtOH at room temp. for 2.5 hr. The catalyst was filtered off through a celite bed and the filtrate was taken to dryness to give 28 mg of the crude acid **5**, mp 281–283°. IR ν_{max}^{Nujol} cm^{-1}: 3450, 3200–2800, 1705 and 1250. ^1H NMR (pyridine-d_5): δ 1.22 (3 H, *s*), 1.35 (3 H, *s*), 1.56 (3 H, *s*).

Methyl 16α-*hydroxykauranoate* (**6**). Compound **4** (75 mg) was hydrogenated in 10 ml of EtOH with 5% Pd–C (120 mg) at room temp. for 21 hr. The catalyst was filtered through a celite bed and the filtrate was concd to dryness to leave 85 mg of gummy crystals. The crude crystals were recryst. 2 × from 70% aq. MeOH to give 24 mg of colourless crystals, mp 154–155° (lit. 155–157° [9]): $[\alpha]_D$ −110.2° ($CHCl_3$, *c* = 0.01). IR ν_{max}^{Nujol} cm^{-1}: 3350, 1730, 1235, 1155, 1145, 930 and 875. ^1H NMR ($CDCl_3$): δ 0.84 (3 H, *s*), 1.16 (3 H, *s*), 1.87 (3 H, *s*) and 3.66 (3 H, *s*). MS *m/z* (rel. int.): 334 (4), 316 (60), 301 (27), 273 (40), 257 (61), 241 (56), 187 (54), 121 (81), 94 (98) and 43 (100).

Attempted hydrogenation of **4** *in* EtOAc. Compound **4** (28 mg) was hydrogenated in 5 ml of EtOAc in the presence of 5% Pd–C (100 mg) for 3 hr at room temp. The catalyst was filtered off through a celite bed and the filtrate was taken to dryness and analysed by ^1H NMR. Spectra did not show the presence of any hydrogenated product, only the starting material.

Dehydration of methyl 16α-hydroxykauranoate (**6**) *into a mixture of methyl kaurenoate and its* Δ^{15}*-isomer.* Compound (7.5 mg) **6** in 1 ml of pyridine was treated with $SOCl_2$ (0.1 ml) at ice-bath temp for 30 min. GLC analysis of a portion of the reaction mixture indicated the absence of starting material. The mixture was poured into ice-water and partitioned with Et_2O. The Et_2O layer was washed with 10% HCl, H_2O and satd NaCl successively and finally dried over dry $MgSO_4$. Removal of the solvent gave 6.9 mg of oily material. GLC analysis of the oil indicated that it consisted of (−)-methylkaur-16-en-19-oate (34.2%) and (−)-methylkaur-15-en-19-oate (65.8%) (column: 5% OV-17 on Chromosorb W-AW, 1.0 m, at 200°, N_2: 0.6 kg/cm^2, R_t Δ^{15}-isomer, 15 min; Δ^{16}-isomer, 16.5 min).

Acetylation of budlein A (**7**). Compound **7** (30 mg) from *H. angustifolius* was acetylated following the procedure of Romo de Vivar [11] (400 μl of CH_2Cl_2, 85 μl of NEt_3, 2.5 μl of Ac_2O at room temp. for 1.5 hr). The reaction mixture was taken to dryness and the crude product was purified by prep. TLC (C_6H_6–EtOAc, 5:6) to give 24.6 mg of the acetate **8**, mp 157–158°. MS *m/z* (rel. int.): 416 (20), 398 (1), 375 (5), 339 (6), 284 (18), 152 (30), 83 (100), 57 (56), 55 (69) and 43 (86). IR ν_{max}^{Nujol} cm^{-1}: 1770, 1750 (sh), 1720, 1670, 1595, 1210, 1130, 1040 and 948. ^{1}H NMR ($CDCl_3$): δ 1.51 (3 H, *s*, 10-Me), 1.76 (3 H, *bs*, 2′-Me), 1.93 (3 H, *dq*, *J* = 7, 1, 3′-Me), 2.09 (3 H, *s*, –OAc), 2.20 (1 H, *dd*, *J* = 15.5, 3.0, H-9b), 2.61 (1 H, *dd*, *J* = 15.5, 5.5, H-9a), 4.23 (1 H, *m*, H-7), 4.63 (1 H, *d*, *J* = 5.0, H-6), 5.25 (1 H, *m*, H-8), 5.72, (1 H, *s*, H-5), 5.74 (1 H, *d*, *J* = 2.5, H-13b) 5.81 (1 H, *s*, H-2), 6.02 (1 H, *bs*, H-14b), 6.12 (1 H, *qq*, *J* = 7, 1, H-3′), 6.13 (1 H, *bs*, H-14a), and 6.37 (1 H, *d*, *J* = 3, H-13a). All the couplings were confirmed by double resonance expts.

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