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ENT-KAURANES AND OTHER CONSTITUENTS OF THREE *HELIANTHUS* SPECIES

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Key Word Index—*Helianthus debilis* subsp. *cucumerifolius*; *Helianthus occidentalis*; *Helianthus simulans*; Compositae; Heliantheae; *ent*-kaurenes; ciliaric acid; occidentalic acid; heliangolide; flavone; larval growth inhibitors.

Abstract—Aerial parts of *Helianthus debilis* subsp. *cucumerifolius*, *H. occidentalis* and *H. simulans* afforded a variety of known and some unknown *ent*-kauranoic acids. *H. occidentalis* gave, in addition, ciliaric acid and a new biogenetically related atisane derivative occidentalic acid. *H. simulans* gave the heliangolide leptocarpin and the flavone hymenoxin in addition to *ent*-kauranes. Several of the diterpenoids from these species inhibited larval growth of the sunflower moth.

INTRODUCTION

In continuation of our earlier work on *Helianthus* species [1–6] we have examined *H. debilis* subsp. *cucumerifolius*, *H. occidentalis* and *H. simulans*. All three species contained various *ent*-kauranoic acids and related diterpenes as do many previously studied *Helianthus* species [3, 5–15] but only one of the three, *H. simulans*, also furnished a sesquiterpene lactone of the heliangolide type found in some of these and in other representatives of the genus [1–4, 6, 7, 10, 14, 16–23].

RESULTS AND DISCUSSION

H. debilis Nutt. subsp. *cucumerifolius* (T and G) Heiser gave relatively large amounts of *ent*-kaur-16-en-19-oic acid (**1a**), and angelylgrandifloric acid (**1d**). The following compounds were obtained in smaller quantity: grandifloric acid (**1b**) as its methyl ester **1c**, **2a** (previously isolated only in the form of the methyl ester **2b** [24]), **3a**, and **2c** and **2e** as their methyl esters **2d** and **2f**. A methyl ester $C_{26}H_{38}O_5$ derived from another naturally occurring kauranoic acid had spectral properties which tallied with structure **1g**; the ^{1}H NMR spectrum (see Experimental) differed somewhat from that reported for a substance of like structure from *Wedelia trilobata* [25] and it is possible that the two substances differ in the stereochemistry of the 5-carbon ester side chain*. The structure of the remaining constituent, the epoxyalcohol **4a**, was apparent from its ^{1}H NMR spectrum which exhibited the AB system of H-17a, b at δ 4.05 and 3.81 and the H-15 singlet at δ 2.96 and was supported by the ^{13}C NMR spectrum (Table 1). The stereochemistry was established by epoxidation of **2d** which involves attack on the 15,16-double bond from the α-face [27, 28] and gave material identical with methyl ester **4b**. Qualitatively, the nature of the constituents of *H. debilis* subsp. *cucumerifolius* does not differ significantly from that of subsp. *debilis* which reportedly gives rise to **1a**, **1d** and the rearranged *ent*-kaurane tetrachyrin [12].

Table 1. ^{13}C NMR spectrum of compound **4a**†

C-1	40.78 *t*	C-11	18.25 *t*
C-2	19.00 *t*	C-12	26.51 *t*
C-3	37.81 *t*	C-13	35.59 *d*
C-4	43.78	C-14	31.96 *t*
C-5	56.69 *d*	C-15	65.30 *d*
C-6	20.60 *t*	C-16	65.57
C-7	35.68 *t*	C-17	58.98 *t*
C-8	43.23	C-18	28.95 *q*
C-9	49.31 *d*	C-19	183.50
C-10	39.47	C-20	15.37 *q*

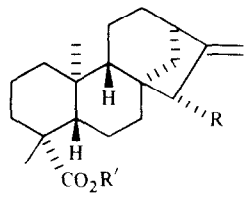
† Run in $CDCl_3$ at 67.98 MHz with TMS as internal standard. Unmarked signals are singlets. Assignments by comparison with spectra of **1a** [12], **1b** [14], **1d** [12] and spectra in our files. In ref. [12] the multiplicities of the signals at δ 20.7 and 18.5 are given incorrectly. The δ 20.7 signal is a quartet and corresponds to a C-5′, that at δ 18.5 is a triplet and corresponds to C-11.

The principal constituent of *H. occidentalis*‡ was again **1a**; other kauranoids found in this species were **1b, 1d, 1e** (previously isolated only in the form of ester **1f** from *W. trilobata* [25] and *H. decapetalus* [6]), **2a**, **2c** and **2e**. (isolated as the methyls esters **2d** and **2f**), **3c**, **3d** and **5a**. Ciliaric acid (**6a**), previously isolated from several other *Helianthus* species [3, 5, 11, 14], was also found; it was admixed with a small amount of an isomeric unsaturated hydroxyacid **7a** which could be separated only in the form of its methyl ester **7b**.

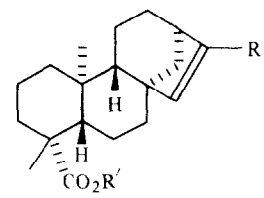
That **7b** was a 16-atisene rather than a 16-kaurene was

*A comparison with the spectral trace of the substance from *W. trilobata* kindly supplied by Professor Bohlmann was inconclusive due to instrumental differences.

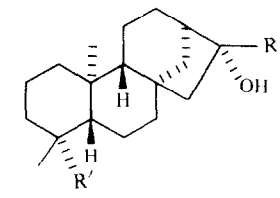
‡Our collection of *H. occidentalis* came from near Newton, Texas, which suggests that it may represent subsp. *plantagineus* [15]. An earlier report describes the isolation of *cis*- and *trans*-(−)-ozic acid from this subspecies.



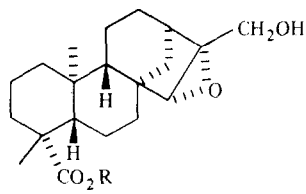
1a R, R′=H
1b R=OH, R′=H
1c R=OH, R′=Me
1d R=Ang, R′=H
1e R=Tigl, R′=H
1f R=EpoxAng, R′=H
1g R=EpoxAng, R′=Me



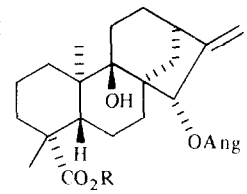
2a R=CHO, R′=H
2b R=CHO, R′=Me
2c R=CH_2OH, R′=H
2d R=CH_2OH, R′=Me
2e R=CO_2H, R′=H
2f R=CO_2Me, R′=Me



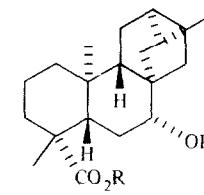
3a R=Me, R′=CO_2H
3b R=Me, R′=CO_2Me
3c R, R′=Me
3d R=CH_2OH, R′=CO_2H
3e R=CH_2OH, R′=CO_2Me



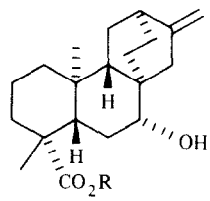
4a R=H
4b R=Me



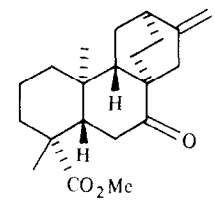
5a R=H
5b R=Me



6a R=H
6b R=Me



7a R=H
7b R=Me



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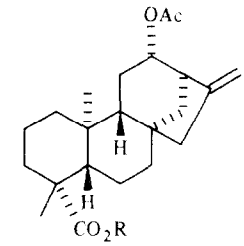
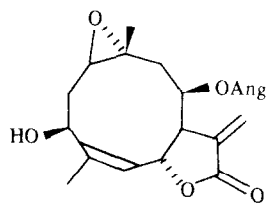


9a R=H
9b R=Me



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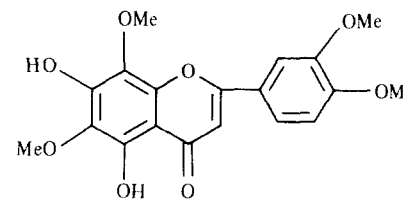

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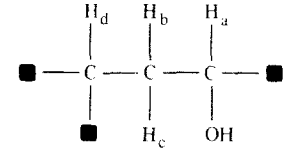
apparent from the characteristic splitting [6] of the C-17 protons (quartets, $J = 2$ Hz, at δ4.75 and 4.60). The location and orientation of the hydroxyl on the atisane skeleton was revealed by spin-decoupling. This established the presence of partial structure **A** (■ represents a quaternary center) in which axial H_a, a *dd* at δ3.26, was vicinally coupled ($J = 11, 4$ Hz) to H_b and H_c at 1.99 and 1.82 ($J_{b,c} = 12$ Hz) and each of the latter to H_d at 1.12 ($J_{b,d} = 2$ Hz, $J_{c,d} = 11$ Hz), a structure which could be accommodated only by placing an equatorial hydroxyl on C-7. In the spectrum of the oxidation product **8**, the H_a signal, was absent and the signals of H_b and H_c had moved to predictably lower field at δ2.91 and 2.61, with $J_{b,c} = 15$ Hz, $J_{b,d} = 3$ Hz and $J_{c,d} = 14$ Hz.



A

Dreiding models indicate that the equatorial hydroxyl of **7a** should deshield H-14 and/or H-15α and that H-15α should be deshielded by the carbonyl group of **8**. In fact a *td* (J = 2, 16 Hz) is found at δ 2.62 of **7b** (and at 2.64 of **8**) which is coupled (J = 16 Hz) to a signal at 1.82 partially merged with that of H_c of **A**. Irradiation at either of these frequencies, which must be those of H-15a, b, collapses the quartets of H-17a, b to triplets; conversely, irradiation at the frequencies of H-17a or H-17b collapses the δ 2.62 signal to a *dd* (J = 2, 16 Hz). Consequently, the splitting of the H-17 signals arises from gem-coupling ($J_{17a,17b}$ = 2 Hz) and from allylic coupling to $H_{15a,b}$ (J = 2 Hz) and not to coupling to H-7 and H-9 as suggested earlier [6].

Interestingly enough, **7a** which we have named occidentalic acid can be formally derived from ciliaric acid (**6a**) by a process reversing the 1,3-elimination involved in the biogenesis of the trachylobanes [29–31]. That **6a** and **7a** are biogenetically related and have the same absolute configuration was shown by the CD curve of **8** which exhibited the strongly positive Cotton effect (Δε = + 2.18) characteristic of *ent*-7-ketoditerpenes [32].

H. simulans contained somewhat smaller amounts of kaurenes. Isolated were **1a**, **1b**, **1d**, **2a** (in the form of its methyl ester), **3d** and **9a** (previously isolated only in the form of its methyl ester from *H. decapetalus* and *H. tuberosus* [6]). A sesquiterpene lactone, $C_{25}H_{26}O_6$, mp 198–200°, was identified as the heliangolide leptocarpin (**10**) which has been isolated from *Leptocarpha rivularis* [33] and *Tithonia rotundifolia* [34]. Lastly, we identified the flavone hymenoxin (**11**) which was originally isolated from *Hymenoxys scaposa* [35] and *Hymenoxys linearifolia* [36], but has more recently been found in *Helianthus angustifolius* [7, 37].

In a recent revision [38] of Heiser's infrageneric classification of *Helianthus* [28], *H. occidentalis* was placed in series Atrorubentes of section Divaricati. Its chemistry resembles that of *H. rigidus*, the only previously studied member of this four-membered series [5], in producing *ent*-kauranoic acids, ciliaric acid and no lactones. Likewise, *H. angustifolius*, the only previously studied member of series Angustifolii of section Divaricati resembles *H. simulans*, also assigned to this series, in yielding *ent*-kauranoic acids, hymenoxin and a heliangolide, although the latter was budlein A and not leptocarpin [7]. On the other hand, *H. debilis*, unlike several other members of section *Helianthus* [10, 14], furnishes no sesquiterpene lactones, a result which parallels the non-uniformity of lactone content in series Corona-solis pointed out earlier [5].

Trachyloban-19-oic acid and *ent*-kaur-16-en-19-oic acid (**1a**) found in florets of *Helianthus* annuus inhibit larval development of the sunflower moth (*Homeosoma electellum* L.) and of several *Lepidoptera* species and have been implicated in imparting resistance to insect pests of cultivated varieties of sunflower [39]. Substance **1a** has also been reported from *H. angustifolius* [6], *H. decapetalus* [6], *H. debilis* subsp. *debilis* [12], *H. giganteus* [6], *H. niveus* [14] and is found in the three species studied in the present work. Bioassays of two other diterpenes widely-distributed in *Helianthus* species have now been carried out, namely ciliaric acid (**6a**), reported from *H. argophyllus* [10], *H. ciliaris* [11], *H. grosseserratus* [3], *H. laciniatus* [13], *H. niveus* [14], *H. rigidus* [5], *H. salicifolius* [5] and *H. occidentalis* of the present work, and angelylgrandifloric acid (**1d**) reported from *H. annuus* [9], *H. debilis* subsp. *debilis* [12], *H. giganteus* [6] and the three species studied in the present work*. Sunflower moth larvae feeding on a diet containing 1% ciliaric acid and angelylgrandifloric acid experienced a significantly higher mortality throughout the development period than larvae feeding on the diet alone. The treatment appeared to have no significant effect on the developmental period, but 7-day-old larvae feeding on **1d** and **6a** were significantly smaller by day 7 than larvae feeding on diet alone. Thus, it appears that kauranoic and trachylobanoic acids may exert a protective function in *Helianthus* and related species in which they occur.

*Bioassays were carried out by Dr. Charlie E. Rogers, Southern Region Conservation and Production Research Laboratory, Bushland, Texas. We are indebted to Dr. Rogers for communicating these results.

EXPERIMENTAL

Extraction of Helianthus debilis *subsp.* cucumerifolius. $CHCl_3$ extraction of 9.8 kg of above ground parts of *H. debilis* Nutt. subsp. *cucumerifolius* (T. and G.) Heiser (voucher RKG 77197 collected by Dr. R. K. Godfrey and Mr. D. Gage on 31 August 1979, in the vicinity of St. Augustine, Texas, on deposit in the Herbarium of Florida State University) and work-up in the usual fashion [40] gave 215 g of crude gum. A 102 g portion of this was extracted with petrol (7 × 250 ml). The soluble fraction (61 g) was adsorbed on 150 g Si gel 60 (Merck, particle size 0.063–0.200 mm) and chromatographed over 1 kg of the same adsorbent packed in *n*-hexane. Fractions were collected as follows: 1–3 (hexane, 1.5 l.), 4–7 (hexane–EtOAc, 97:3, 4 l.), 8–19 (hexane–EtOAc, 93:7, 6 l.), 20–31 (hexane–EtOAc, 17:3, 6 l.), 32–39 (hexane–EtOAc, 3:1, 4 l.), 40–43 (hexane–EtOAc, 1:1, 4 l.) and 44–49 (EtOAc, 3 l.)

Fractions 13–18 (18 g) were combined and recrystallized to give 10.6 g **1a**, mp 176.5–178°, $[\alpha]_D^{25}$ − 108° (EtOH; *c* 0.99); IR, ^{1}H NMR and ^{13}C NMR and MS identical with authentic material [12]. Fractions 19 and 20 (2.5 g) contained **1a** and **1d** (TLC). Fractions 20–28 (15.5 g) were combined. The major constituent **1d** was isolated by trituration with hexane and recrystallization from MeOH, yield 9.0 g, mp 199–201°, $[\alpha]_D^{25}$ − 79.0° (EtOH; *c* 1.00); IR, MS, ^{1}H NMR and ^{13}C NMR identical with authentic material [12].

Fractions 35–42 (5.6 g) were combined. Rechromatography of a 3.5 g portion over Si gel and elution with C_6H_6–EtOAc (17:3) gave 0.3 g of crude **1f** and 0.44 g of non-crystalline **2a**; each substance was further purified by TLC (C_6H_6–EtOAc, 4:1). Epoxyangeloxygrandifloric acid (**1f**) had IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2950, 2800–2550, 1735, 1700; ^{1}H NMR ($CDCl_3$): δ 2.78 (*mm*, H-13), 2.16 (*br d*, J = 14 Hz, H-14a), 5.09 (*br*, 2H) and 5.26 (*br*, H-15 and H-17), 0.96 (H-20), 1.27 (H-18), 3.28 (*q*, J = 7 Hz, H-3'), 1.35 (*d*, J = 7 Hz, H-4') and 1.50 (H-5'). The gummy methyl ester **1g** had IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2950, 1735; NMR ($CDCl_3$): δ 2.77 (*m*, H-13), 2.17 (*br d*, J = 13 Hz, H-14a), 5.07 (*br*), 5.08 and 5.24 (*br*, H-15 and H-17), 0.84 (H-18), 1.17 (H-20), 3.27 (*q*, H-3'), 1.35 (*d*, H-4') and 1.50 (H-5'), 3.63 (OMe). *ent*-17-Oxokaur-15(16)-en-19-oic acid (**2a**) had IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2950, 2800–2550, 1730, 1700 and 1690; ^{1}H NMR ($CDCl_3$): δ 3.05 (*m*, H-13), 6.60 (*br*, H-15), 9.74 (H-17), 1.02 (H-20) and 1.28 (H-18); MS *m/z*: 316, 301, 298, 271, 255. [Calcd for $C_{20}H_{28}O_3$: MW, 316.2038. Found: MW(MS), 316.2019.] IR and ^{1}H NMR spectra of the methyl ester were identical with spectra reported for **2b** [24].

Combination of fractions 43–47 (7.5 g) methylation with CH_2N_2 and repeated purification by TLC (C_6H_6–EtOAc, 9:1 and 17:3) gave methyl grandifolate (**1c**, 105 mg from 0.9 g of

crude gum) [12, 41] which was identical with authentic material prepared by hydrolysis of **1d** and subsequent methylation.

The petrol insoluble material (63 g) was adsorbed on 130 g silicic acid (Mallinckrodt 100 mesh) and chromatographed over 1000 g of the same adsorbent packed in toluene–$CHCl_3$ (1:1). Fractions (500 ml) were collected as follows: 1–4 (toluene–$CHCl_3$, 1:1), 5–11 ($CHCl_3$), 12–26 ($CHCl_3$–MeOH, 49:1), 27–39 ($CHCl_3$–MeOH, 19:1), 40–45 (MeOH). Fractions 10–16 (2.0 g) contained primarily **1a**. Rechromatography of fraction 23 (6.6 g) (Si gel, *n*-hexane with increasing proportions of EtOAc) and further purification by TLC (C_6H_6–EtOAc, 17:3 or 4:1 and $CHCl_3$–Me_2CO, 9:1) gave 2.6 g **1d**, 0.4 g **1f** and 0.5 g **2a**.

Fractions 24 and 25 (15.6 g) were combined. A 4 g portion was rechromatographed over Si gel; fractions which were eluted by hexane–EtOAc (7:3 and 1:1) were combined, methylated with CH_2N_2 and purified by TLC (C_6H_6–EtOAc, 9:1, 17:3 and 4:1). This gave 0.3 g **1c**, 0.2 g **2b**, 0.2 g **2d**, 0.3 g **2f** and 0.2 g **3b**. Methyl *ent*-17-hydroxykauran-19-oate (**2d**), mp 131–132° (from hexane) had properties identical with those reported previously [42] and was identical with material prepared by methylation of **2c**, or $NaBH_4$ reduction of **2b**. Dimethyl *ent*-kaur-15(16)-en-17, 19-dioate (**2f**), a gum, had IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2950, 1720–1730, 1620; ^1H NMR ($CDCl_3$): δ 2.92 (*m*, H-13), 2.16 (*br d*, J = 13 Hz, H-14a), 6.49 (*br*, H-15), 0.87 (H-20), 1.17 (H-18), 3.64, 3.72 (OMe). MS *m/z*: 360 $[M]^+$, 345, 330, 328, 303, 302, 301, 300, 285, 253, 241, 207, 205, 193, 192. [Calcd for $C_{22}H_{32}O_4$: MW, 360.2300. Found: MW(MS), 360.2294.] Authentic **2f** was prepared from partially oxidized **2a**, which has been stored for 3 months and showed two spots on TLC, by prep. TLC (C_6H_6–EtOAc, 4:1) and methylation of the more polar fraction. Methyl *ent*-16β-hydroxykaur-15-(16)en-19-oate (**3b**), mp 155–157°, from hexane, had spectral properties identical with those reported previously [43, 44].

Combination of fractions 26 and 27 (3.6 g) prep. TLC (hexane–EtOAc, 1:1 developed × 3) and recrystallization from MeOH–H_2O gave 0.2 g *ent*-15β,16β-epoxy-17-hydroxykauran-19-oic acid (**4a**), mp 203.5–205.5°; IR ν_{max}^{KBr} cm^{-1}: 3350, 2950, 2800–2600, 1720, 855, 795; ^1H NMR ($CDCl_3$): δ 2.29 (*m*, H-13), 2.17 (*br d*, J = 14 Hz, H-14a), 2.96 (H-15), 4.05 (*d*) and 3.81 (*d*, J = 13 Hz, H-17a, b), 0.95 (H-20), 1.26 (H-18). MS *m/z*: 334 $[M]^+$, 316, 301, 298, 270, 255; ^{13}C NMR: see Table 1. [Calcd for $C_{20}H_{30}O_4$: MW, 334.2145. Found: MW(MS), 334.2153.] Methylation of the mother liquor with CH_2N_2, purification by TLC (C_6H_6–EtOAc, 3:2) and recrystallization from hexane–EtOAc gave **4b**, mp 177–179°; IR ν_{max}^{KBr} cm^{-1}: 3420, 2940, 1735; ^1H NMR ($CDCl_3$): δ 2.29 (*m*, H-13), 2.19 (*br d*, J = 14 Hz, H-14a), 2.94 (H-15), 4.04 (*d*) and 3.80 (*d*, J = 13 Hz, H-17a, b), 0.83 (H-20), 1.19 (H-18), 3.64 (OMe). Methyl ester **4b** was identical with **4b** prepared by epoxidation of 35 mg **2d** with 20 mg *m*-chloroperbenzoic acid in 3 ml $CHCl_3$ for 2 hr at room temp. followed by the usual work-up and purification by prep. TLC (C_6H_6–EtOAc, 3:2) to give 20 mg of product.

Fractions 32–34, (3.0 g) partially solidified on being combined. The solid **3d**, (0.5 g) had mp 266.5–268.5° (MeOH); IR ν_{max}^{KBr} cm^{-1}: 3430, 3260, 2950, 2800–2500, 1700; ^1H NMR (DMSO-d_6): δ 1.87 (*m*, H-13), 2.00 (*br d*, J = 13 Hz, H-14a), 3.50 (*d*) and 3.39 (*d*, J = 11 Hz, H-17a, b) 0.88 (H-20), 1.10 (H-18) and its methyl ester **3e**, mp 153.5–154.5° (hexane–EtOAc); IR ν_{max}^{KBr} cm^{-1}: 3420, 3330, 2940, 1725; ^1H NMR ($CDCl_3$): δ 2.02 (*m*, H-13), 2.15 (*br d*, J = 13 Hz, H-14a), 3.78 (*d*) and 3.66 (*d*, J = 11 Hz, H-17a, b), 0.83 (H-20), 1.16 (H-18), 3.64 (OMe). These properties are identical with those reported [45] for *ent*-16β, 17 dihydroxykauran-19-oic acid and its methyl ester.

Extraction of Helianthus occidentalis. $CHCl_3$ extraction of 12 kg of above ground parts of *H. occidentalis* Riddell (voucher RKG 77196 collected by R. K. Godfrey and D. Gage on 31 August 1979, in the vicinity of Newton, Texas) and work-up in the usual fashion gave 73 g of crude gum which was adsorbed on 100 g silicic acid (Mallinckrodt 100 mesh) and chromatographed over 1 kg of the same adsorbent packed in hexane, 500 ml fractions being collected as follows: 1–6 (hexane), 7–14 (hexane–EtOAc, 19:1), 15–22 (hexane–EtOAc, 9:1), 23–30 (hexane–EtOAc, 4:1), 31–38 (hexane–EtOAc, 4:5), 39–46 (hexane–EtOAc, 1:4), 47–50 (EtOAc), 51–54 (EtOAc–MeOH, 99:1), 55–58 (EtOAc–MeOH, 49:1), 59–62 (EtOAc–MeOH, 19:1) and 63–70 (EtOAc–MeOH, 19:1).

Fractions 17–14 (7.2 g) contained mainly **1a**, identified by TLC purification (C_6H_6–EtOAc, 39:1) of a 225 mg portion to give 164 mg pure **1a** mp 176–178° (hexane). Purification of fraction 18 (0.44 g) by TLC (C_6H_6–EtOAc, 39:1) gave two bands. Repurification of the upper band by TLC and recrystallization gave 115 mg **1d**, mp 197–199° (hexane–EtOAc); similar treatment of the lower band furnished 53 mg *ent*-kauran-16β-ol (**3c**), mp 213–215°. TLC (C_6H_6–EtOAc, 39:1, developed twice) of fractions 22–24 (1.53 g) furnished **1e**, mp 181–183° (hexane–EtOAc); IR ν_{max}^{KBr} cm^{-1}: 3600–2400 (*br*), 1700, 1650; ^1H NMR ($CDCl_3$): δ 2.79 (*m*, H-13), 2.16 (*br d*, J = 14 Hz, H-14a), 5.30 (*br*, H-15), 5.09 (*br*) and 5.06 (*br*, H-17a, b), 0.96 (H-20), 1.22 (H-18), 6.81 (*br q*, H-3'), 1.84 (*br d*, H-4'), 1.79 (*br*, H-5'), MS *m/z*: 400 $[M]^+$, 300, 285, 272, 255, 194, 165 [Calcd for $C_{25}H_{36}O_4$: MW, 400.2611. Found: MW(MS), 400.2619.] The only substance obtained on purification of fraction 32 (335 mg) by TLC (C_6H_6–EtOAc, 9:1) was **2a** (160 mg). Fraction 35 (142 mg) was a mixture; methylation with CH_2N_2 followed by TLC (C_6H_6–EtOAc, 39:1, developed twice) gave 29 mg methyl *ent*-15β-angeloxy-9α-hydroxykaur-16-en-19-oate (**5b**), mp 191–192° (Et_2O); ^1H NMR: δ 2.77 (*m*, H-13), 2.15 (*br d*, J = 14 Hz, H-14a), 6.02 (*br*, H-15), 5.16 (*br*) and 5.10 (*br*, H-17a, b), 0.98 (H-20), 1.17 (H-18), 6.03 (*br q*, H-3'), 1.99 (*br d*, H-4'), 1.88 (*br*, H-5'), 3.64 (OMe), similar to values reported in the lit. [25]. Trituration of fractions 39 and 40 with hexane–EtOAc gave 295 mg grandifloric acid (**1b**), mp 225–227° (hexane–EtOAc).

Trituration of fraction 43 with $CHCl_3$ gave 92 mg ciliaric acid (**6a**) mp 290° ($CHCl_3$) [11, 12] identical in all respects with material previously isolated from *H. grosseserratus* and *H. rigidus* [3, 5]. Similar treatment of fractions 44–47 gave 585 mg of solid material whose NMR spectrum indicated, in addition to **6a**, the presence of a very minor constituent which could not be separated by TLC. Esterification of the mixture with CH_2N_2 and purification by TLC (C_6H_6–EtOAc, 19:1, two developments) gave **6b** and 22 mg methyl *ent*-7β-hydroxyatis-16-en-19-oate (**7b**), mp 169–170° (hexane–EtOAc); IR ν_{max}^{KBr} cm^{-1}: 3530, 1707; ^1H NMR ($CDCl_3$): δ 2.19 (*br d*, J = 13 Hz, H-3a), 1.00 (*dt*, J = 4, 13 Hz, H-3b), 1.12 (*dd*, J = 11, 2 Hz, H-5), 1.99 (*ddd*, J = 12, 4, 2 Hz, H-6a), 1.82 (*m*, H-6b and H-15b) 3.26 (*dd*, J = 11, 4 Hz, H-7), 2.62 (*td*, J = 2, 16 Hz, H-15a), 4.75 (*q*) and 4.60 (*q*, J = 2 Hz, H-17a, b), 1.19 (H-18), 0.80 (H-20), 3.63 (OMe). [Calcd for $C_{21}H_{32}O_3$: MW, 332.2351. Found: MW(MS), 332.2334.] Other significant peaks in the high resolution MS were at *m/z* (composition, %) 314 ($C_{21}H_{30}O_2$, 12.5), 272 ($C_{19}H_{28}O$, 2) 254 ($C_{19}H_{26}$, 21.6), 239 ($C_{18}H_{23}$, 23). Oxidation of 15 mg **7b** in 3 ml Me_2CO with 0.2 ml Jones' reagent for 30 min at 0° followed by the usual work-up and purification by TLC (C_6H_6–EtOAc, 9:1) gave 11 mg methyl *ent*-7-oxoatis-16-en-19-oate (**8**), mp 110–111°, IR ν_{max}^{KBr} cm^{-1}: 1725, 1695; ^1H NMR ($CDCl_3$): δ 2.99 (*dd*, J = 15, 14 Hz, H-6a), 2.61 (*dd*, J = 15, 3 Hz, H-6b), 2.64 (*td*, J = 2, 16 Hz, H-15a), 4.78 (*q*) and 4.67 (*q*, J = 2 Hz, H-17a, b), 1.17 (H-18), 0.98 (H-20), 3.67 (OMe); CD curve (MeOH) $[\theta]_{293}$ + 7200, $\Delta\varepsilon$ + 2.18. [Calcd for $C_{21}H_{30}O_3$: MW, 330.2194. Found: MW(MS), 330.2190]. Other significant peaks in the high resolution MS were at *m/z* (composition, %) 315 ($C_{20}H_{27}O_3$, 17.6) and 270 ($C_{19}H_{26}O$, 57.5).

The mother liquor of fractions 44–47 remaining after removal

of **6a** and **7a** was concd, esterified with CH_2N_2 and rechromatographed over Si gel (100 g). Elution with hexane–EtOAc gave in the initial fractions, 230 mg crude **2f** which was purified by TLC (C_6H_6–EtOAc, 9:1, two developments). Further elution with hexane–EtOAc gave, 1.3 g crude **2d**, purified by TLC (C_6H_6–EtOAc, 9:1). Fraction 37 on trituration with MeOH gave 72 mg **3d**.

Extraction of Helianthus simulans. Aerial parts of *H. simulans* E. E. Watson (11.7 kg, voucher RKG 76708 collected by R. K. Godfrey and D. Gage on 9 October 1978 near Chattahoochee, Gadsden Co., Florida) were extracted with $CHCl_3$ and worked-up in the usual fashion. The crude gum (24 g) was adsorbed on 40 g Si gel and chromatographed over 400 g of the same adsorbent set in hexane, 500 ml fractions being collected as follows: 1–6 (hexane), 7–12 (hexane–EtOAc, 19:1), 13–18 (hexane–EtOAc, 9:1), 19–24 (hexane–EtOAc, 4:1), 25–30 (hexane–EtOAc, 5:2), 31–36 (hexane–EtOAc, 3:2), 37–40 (hexane–EtOAc, 2:5), 41–44 (EtOAc), 45–48 (EtOAc–MeOH, 99:1), 49–52 (EtOAc–MeOH, 49:1), 53–56 (EtOAc–MeOH, 19:1), 57–60 (EtOAc–MeOH, 9:1) and 61–64 (EtOAc–MeOH, 4:1).

TLC of fraction 9 (430 mg, C_6H_6–EtOAc, 39:1) gave **1a**. TLC (same solvent mixture, two developments) of fraction 14 (170 mg) gave **1d**. Fractions 16 and 17 (375 mg) contained a mixture of sitosterol and stigmasterol. TLC (C_6H_6–EtOAc, 39:1) of fraction 25 which contained two major constituents gave in the upper band 55 mg crystalline *ent*-12β-acetoxykaur-16-en-19-oic acid (**9a**), mp 173.5–175° (hexane–pentane); IR ν_{max}^{KBr} cm^{-1}: 3600–240, 1735, 1695; ^1H NMR ($CDCl_3$): δ 4.76 (*br t*, J = 4.5 Hz, H-12), 2.76 (*br t*, J = 4 Hz, H-13), 4.96 (*br*) and 4.84 (*br*, H-17a, b), 1.26 (H-18), 1.03 (H-20), 2.02 (Ac); MS *m/z*: 360 $[M]^+$, 300, 285, 255, 254, 239. [Calcd for $C_{22}H_{32}O_4$: MW, 360.2300. Found: MW(MS), 360.2294]. The material from the lower band was esterified with CH_2N_2 and purified by TLC (C_6H_6–EtOAc, 39:1) to give 47 mg **9b** [6] and 12 mg **2b**. Trituration of fraction 32 with hexane–EtOAc gave 7 mg **1b**; similar treatment of fractions 44 and 45 gave 0.15 g leptocarpin (**10**), mp 198–200°, lit. mp 192–195° [32], IR and ^1H NMR spectra as reported [33], and trituration of fraction 48 yielded 12 mg **3d**. TLC ($CHCl_3$–MeOH–EtOAc, 18:1:1, two developments) of fraction 51 gave 7 mg hymenoxin (**10**), mp 210–216°, identical with authentic material [35–37].

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