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# TETRACHYRIN, A NEW REARRANGED KAURENOID LACTONE, AND DITERPENE ACIDS FROM *TETRACHYRON ORIZABAENSIS* AND *HELIANTHUS DEBILIS*

NOBUO OHNO*, TOM J. MABRY*, VOLKER ZABEL† and WILLIAM H. WATSON†

*The Department of Botany, The University of Texas at Austin, Austin, TX 78712, U.S.A.; †Department of Chemistry, Texas Christian University, Fort Worth, TX 76129, U.S.A.

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**Key Word Index**—*Tetrachyron orizabaensis* var. *websteri*; *Helianthus debilis* ssp. *debilis*; *Calea*; Heliantheae; Compositae; tetrachyrin; kaurenoid acids; kaurenoid lactones.

**Abstract**—(−)-Kaur-16-en-19-oic acid and tetrachyrin, a new rearranged kaurenoid lactone, were isolated from *Tetrachyron orizabaensis* var. *websteri* and *Helianthus debilis* ssp. *debilis*. The latter species also afforded angeloylgrandifloric acid.

## INTRODUCTION

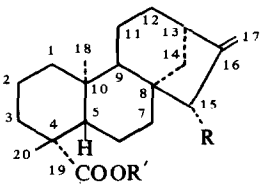
In connection with a broad biochemical systematic investigation of the tribe Heliantheae (family Compositae), we describe here the isolation and characterization of three kaurenoid diterpenes from *Helianthus debilis* ssp. *debilis* Nutt, and *Tetrachyron orizabaensis* Sch. Bip ex Klatt var. *websteri* Wussow & Urbatsch. The latter taxon was previously recognized as a *Calea* species (see Acknowledgement). Two of the compounds were identified as (−)-kaur-16-en-19-oic acid (**1**) and (−)-angeloylgrandifloric acid (**2**) by comparison with authentic specimens. Compound **1** has been previously isolated from various species of *Helianthus* including *H. annuus* [1], *H. ciliaris* (O'Brien, D. H. and Hanlon, K., personal communication) and *H. occidentalis* (Stipanovic, R. D., personal communication). Other diterpene acids are also known from various *Helianthus* species; grandifloric acid [4], trachyloban-19-oic acid [1], 15-α-hydroxytrachyloban-19-oic acid (McCrindle, R., personal communication), *cis*- and *trans*-ozic acid (O'Brien, D. H., personal communication) and ciliaric acid [2].

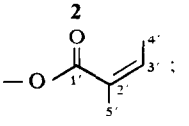
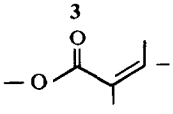
Structure **6** was proposed for the third compound (named here tetrachyrin) on the basis of spectral and X-ray data as well as biogenetic analogy. The lactone **6** represents a new type of rearranged kaurenoid lactone.

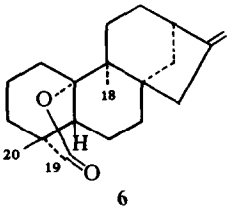
## RESULTS AND DISCUSSION

Chromatographic (Si gel) separation of a $CHCl_3$ extract of *Tetrachyron orizabaensis* gave two diterpenoid compounds, the more abundant of which was (−)-kaur-16-en-19-oic acid (**1**), identified by comparison with an authentic specimen [3].

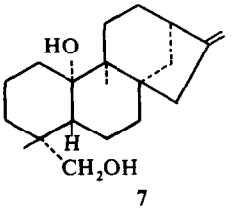
The second diterpenoid, tetrachyrin (**6**) (mp 170.5–171.5°; $C_{20}H_{28}O_2$: HRMS, obs. 330.229, calc. 330.229) was isolated in 0.03% yield. It was a new γ-lactone (IR $cm^{-1}$: 1770) with an exocyclic methylene group (1660, 882 $cm^{-1}$). The $^1H$ NMR spectrum showed two methyl signals at δ 1.08 (*s*) and 1.18 (*s*) as well as a signal for olefinic protons at 4.79 (*bs*, 2H) characteristic for kaurenoid diterpenes. A broadened singlet for the protons at C-15 (2.53, 2H) was also typical for kaurenoids. The absence of a signal for a proton geminal to the lactone oxygen indicated that compound **6** did not belong to the known kauren-19,7-olides [4]. The $^{13}C$ NMR spectrum of **6** had signals similar to those observed for **1** (cf. 103.5 (*t*) and 155.8 (*s*) for two olefinic carbons of an exomethylene group). The signal at δ 88.6, which remained as a singlet in an off-resonance decoupled experiment, was assigned to a carbon atom attached to an oxygen function. The spectral data for the new lactone as well as its co-occurrence with **1** and **2** suggested structure **6** except




| | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| R = H; | |  |  | − OH; | − OH |
| R' = H; | | H; | Me; | H; | Me |




6

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for the orientation of the C-9 methyl group. This is the first report of a kaurenolide with the C-10 methyl group migrated to C-9. The relative stereochemistry shown in **6** was confirmed by X-ray diffraction. The absolute structure as shown in **6** is proposed on the basis of biogenetic analogy to the compounds of known absolute structure with which it co-occurs, namely **1** and **2**. $LiAlH_4$ reduction of **6** gave the diol **7** (mp 177–179°; IR $cm^{-1}$: 3200, 1065 and 879; $M^+ = m/e$ 304) which was characterized by a typical $^1H$ NMR AB coupling pattern: two doublets at $\delta$ 3.27 and 2.74 ($J_{AB} = 12$ Hz) due to the C-19 hydroxymethylene group. The latter signal exhibited an additional coupling ($J = 1.5$ Hz) presumably involving a W-type long-range coupling either with H-4 or H-6.

The concentrate from the $CHCl_3$ extraction of *Helianthus debilis* ssp. *debilis* was partitioned between 50% aq. MeOH and hexane. The hexane-soluble material yielded **1**, **2** and **6** after repeated CC and PLC (Si gel). The $^1H$ NMR and $^{13}C$ NMR spectra for compound **2** (mp 197–199°, $C_{26}H_{38}O_4$; for the methyl ester **3**: $M^+ = m/e$ 414.276, calc. 414.277) indicated the presence of an angelic ester side chain on a kaurenoid skeleton. The base ion at $m/e$ 83 in the MS of the methyl ester **3** was also diagnostic for an angelic ester. Final identification of **2** was made by comparison with authentic specimens of its methyl ester [5] and the deacylated methyl ester **5** [6].

Elliger *et al.* [7] reported larval growth inhibitory activity for (−)-kaurenoic acid and trachyloban-19-oic acid against the sunflower moth (*Homosoma electellum*).

Table 1. $^{13}C$ NMR data for kaur-16-en-19-oic acid (**1**), angeloylgrandifloric acid (**2**) and tetrachyrin (**6**)*

| Carbon Nos. | 1 | 2 | 6† |
|---|---|---|---|
| 1 | 40.8 *t* | 40.6 *t* | 43.1 *t* |
| 2 | 19.2 *t* | 19.0 *t* | 18.6 *t* |
| 3 | 37.8 *t* | 35.1 *t* | 31.8 *t* |
| 4 | 43.9 *s* | 43.8 *s* | 42.3 *s* |
| 5 | 57.2 *d* | 56.6 *d* | 51.4 *d* |
| 6 | 21.9 *t* | 20.8 *t* | 20.5 *t* |
| 7 | 41.4 *t* | 37.5 *t* | 35.4 *t* |
| 8 | 44.3 *s* | 47.6 *s* | 44.5 *s* |
| 9 | 55.3 *d* | 53.0 *d* | 48.3 *s* |
| 10 | 39.8 *s* | 39.9 *s* | 88.6 *s* |
| 11 | 18.5 *t* | 20.7 *t* | 30.1 *t* |
| 12 | 33.2 *t* | 32.7 *t* | 30.4 *t* |
| 13 | 43.9 *d* | 42.6 *d* | 42.3 *d* |
| 14 | 39.8 *t* | 37.7 *t* | 33.5 *t* |
| 15 | 49.1 *t* | 82.6 *d* | 47.1 *t* |
| 16 | 155.9 *s* | 155.5 *s* | 155.8 *s* |
| 17 | 103.1 *t* | 110.0 *t* | 103.5 *t* |
| 18 | 29.0 *q* | 28.9 *q* | 19.1 *q* |
| 19 | 185.1 *s* | 184.9 *s* | 180.5 *s* |
| 20 | 15.6 *q* | 15.8 *q* | 17.2 *q* |
| 1′ | | 168.2 *s* | |
| 2′ | | 128.4 *s* | |
| 3′ | | 137.5 *d* | |
| 4′ | | 15.8 *q* | |
| 5′ | | 18.5 *q* | |

*Run on a Bruker WH-90 $CDCl_3$ with TMS as an internal standard. Signals were assigned by means of off-resonance decoupled spectra and by comparison with published data for (−)-kaurenoic acid [8], methyl grandiflorate [8], kaurenolides [9] and cinnamoylgrandifloric acid [10].

†Assignments are tentative.

However, preliminary antifeedant tests of **1** and **6** against larvae of the southern armyworm (*Spodoptera eridania*) did not show any significant antifeedant or antigrowth activity (up to 1000 ppm level).

## EXPERIMENTAL

$^1H$ NMR and $^{13}C$ NMR spectra were measured at 100 and 22.6 MHz, respectively. Mps were determined on a Fischer–Johns block and are uncorr. Analytical TLC and PLC (1.0 mm) were done on Si gel 60 GF254. Si gel 60 (70–230 mesh, Merck) was used for CC. Ms were recorded by direct inlet at 70 eV.

*(−)-Kaur-16-en-19-oic acid* (**1**) *and the new lactone tetrachyrin* (**6**) *from* Tetrachyron orizabaensis *var.* websteri. Air-dried and ground leaves and stems of *T. orizabaensis* (930 g) (collected January, 1977, near Pinal de Amoles, Queretaro Mexico; Urbatsch and Pridgeon 3042, LSU Herbarium, Baton Rouge, La.) were extracted 2× with $CHCl_3$ (2 × 3.5 l.). Removal of the solvent *in vacuo* gave 34.2 g of a dark green syrup which, when purified by standard procedures [15], gave 5.3 g of a dark yellow syrup. The syrup (3.33 g) was charged on a Si gel column (100 g) and the column was eluted with a $CCl_4$–$CHCl_3$ gradient solvent system.

*Tetrachyrin* (**6**). Fractions which eluted with $CCl_4$–$CHCl_3$ (2:3) were combined and concd; the material obtained (0.56 g) was purified on PLC ($CHCl_3$–$Me_2CO$, 20:1). From the main band ($R_f$ 0.6 ~ 0.7), 380 mg of semicrystalline lactone **6** were obtained, which when triturated with *iso*-$Pr_2O$ afforded 195 mg of crystals, mp 170–172°. The prisms for X-ray analysis were prepared by a slow recrystallization from a $C_6H_6$–hexane mixture, mp 170.5–171.5°, $[\alpha]_D$-19.2° (*c* 0.9, $CHCl_3$); MS *m/e*: 300.2095 (calc. for $C_{20}H_{28}O_2$, 300.2089, 54.7%), 285, (24), 258 (51), 257 (100), 241 (40), 148 (53), 109 (66), 107 (53, 105 (58), 91 (64) and 79 (55); IR $\nu_{max}^{nujol}$ $cm^{-1}$: 1770, 1660, 1145, 945, 930, 882; $^1H$ NMR $CDCl_3$; $\delta$ 1.08 (*s*, 3H), 1.18 (*s*, 3H), 2.53 (*s*, 2H), 4.79 (*bs*, 2H).

*(−)-Kaur-16-en-19-oic acid* (**1**). The fractions which eluted with $CCl_4$–$CHCl_3$ (3:7) were combined and concd to give 400 mg of crude crystals of (−)-kaur-16-en-19-oic acid which were recrystallized from hexane to give 110 mg of colorless prisms, mp 179–180° (lit. 179–181° [3]); IR and $^1H$ and $^{13}C$ NMR spectra were identical to those of an authentic specimen of (−)-kaur-16-en-19-oic acid [3].

*Diol* **7** *from* **6**. 15 mg of **6** were reduced with $LiAlH_4$ (18 mg) in 7 ml $Et_2O$ at room temp. for 24 hr. EtOAc (0.5 ml) was added followed by 1 ml satd $NH_4Cl$ soln. The mixture was filtered through a celite pad and the filtrate was partitioned between satd $NH_4Cl$ soln and $Et_2O$. The etherial extract was dried over dry $Na_2SO_4$. Removal of the solvent gave 12.5 mg of crystalline material which was recrystallized from $Et_2O$ to give 10.5 mg of colorless needles, mp 177–179°, IR $\nu_{max}^{nujol}$ $cm^{-1}$: 3100, 1670, 1065, 830. MS *m/e* (rel. int.): 304 (3), 303 (10), 286 (5), 273 (100), 255 (50), 243 (27), 162 (36), 147 (32), 111 (55), 105 (55). $^1H$ NMR $CDCl_3$: $\delta$ 0.82 (*s*, 3H), 1,08 (*s*, 3H), 2.50 (*m*, 1H), 2.70 (*m*, 1H), 2.90 (*m*, 1H), 3.27 (*d*, $J = 12$ Hz), 3.74 (*dd*, $J = 12$, 1.5 Hz) and 4.73 (*bs*, 2H).

*1,6 and angeloylgrandifloric acid* (**2**) *from* Helianthus debilis *ssp.* debilis. Air-dried and ground leaves of *H. debilis* ssp. *debilis* (314 g), cultivated and collected at the U.S.D.A. Southwestern Great Plains Research Center, Bushland, Texas (voucher No. Deb-841 is deposited at the Center), was extracted with $CHCl_3$ twice (2 × 2.1 l.). Removal of the solvent under red. pres. gave 23.3 g of a dark syrup. The syrup was dissolved in 200 ml 50% aq. MeOH and the soln was extracted with hexane (3 × 150 ml). The hexane layers were combined and washed once with 50 ml

50% aq. MeOH, then concd *in vacuo* to give 17.5 g of dark syrup. The hexane-soluble part was chromatographed over a Si gel column (320 g) using a $C_6H_6$–hexane–EtOAc gradient solvent system, initiated with $C_6H_6$–hexane (1:1). The fractions which eluted with $C_6H_6$–EtOAc (15:1) gave 2.2 g of crystals which after recrystallization from MeOH gave colorless needles identical to **1** by IR, NMR and TLC. Later fractions which eluted with the same solvent showed a second spot on TLC in addition to that of **1**. These fractions gave 6 g of gummy material which were chromatographed over a Si gel column (205 g) with $C_6H_6$–hexane (2:3). The first five fractions (each 120 ml) gave an additional quantity (2.0 g) of **1**. The main fraction, which eluted with $C_6H_6$–EtOAc (15:1), afforded 2.5 g of material which was purified further by a smaller Si gel column (100 g) developed with $C_6H_6$; yield: 390 mg of **2** after recrystallization from EtOAc. A portion of the crystals was recrystallized further from MeOH to give colorless needles. The fractions following those which afforded **2** showed another spot on TLC close to that for **2**. The material from these fractions was purified on PLC ($CH_2Cl_2$–EtOAc, 17:3), and ultimately afforded 26.1 mg **6** and 40 mg **2**.

*Angeloylgrandifloric acid* (**2**). Mp 197–199° (lit. 193–195° [12]); IR $\nu_{max}^{nujol}$ $cm^{-1}$: 3200–2500, 1702, 1250, 1040, 1005 and 896. $^1H$ NMR $CDCl_3$: $\delta$ 0.97 (*s*, 3H), 1.23 (*s*, 3H), 1.88 (*q*, 3H, $J = 1.0$ Hz), 1.98 (*dq*, 1H, $J = 8$, 1.0 Hz), 2.80 (*m*, 1H), 5.40 (*m*, 1H), 5.10 (*bs*, 1H), 5.18 (*bs*, 1H) and 6.07 (*qq*, 1H, $J = 8$, 1.0 Hz).

*Methyl angeloylgrandiflorate* (**3**). 20 mg of **2** were methylated with etherial $CH_2N_2$. The crude product was purified on PLC ($C_6H_6$–EtOAc, 5:6) to give 14 mg of needles of **3**, mp 135–137°; IR $\nu_{max}^{nujol}$ $cm^{-1}$: 1730, 1675, 1240, 1165, 916; MS *m/e* (rel. int.): 414 (11), 399 (2), 314 (45), 299 (34), 255 (29) and 83 (100). $^1H$ NMR $CCl_4$: $\delta$ 0.84 (*s*, 3H), 1.23 (*s*, 3H), 1.89 (*q*, 3H, $J = 1.0$ Hz) 2.00 (*dq*, 3H, $J = 8$, 1.0 Hz), 2.80 (*m*, 1H), 3.63 (*s*, 3H), 5.30 (*m*, 1H), 5.06 (*bs*, 1H), 5.16 (*bs*, 1H) and 6.00 (*dq*, 1H, $J = 8$, 1.0 Hz). These data were identical to those recorded for an authentic specimen of methyl angeloylgrandiflorate (**3**) [5].

*Grandifloric acid* (**4**) *from* **2**. A soln of 40 mg **2** in 2 ml 3N aq. NaOH and 1 ml 95% EtOH was refluxed for 2 hr. The soln was concd to $H_2O$ and acidified with 20% $H_2SO_4$ to pH 1, then extracted with $Et_2O$ (2 × 20 ml). The $Et_2O$ extract gave 36.5 mg of oily crystals, which when recrystallized from EtOAc, gave 11 mg of colorless needles, mp 230–232° (lit. 228–230° [16]); IR $\nu_{max}^{nujol}$ $cm^{-1}$: 3300, 3200–2500, 1700, 1240, 1025 and 894.

*Methyl grandiflorate* (**5**). The crude crystals (16 mg) obtained from the mother liquor of the above recrystallization step were methylated with etherial $CH_2N_2$ and the resultant derivative was purified on PLC ($C_6H_6$–EtOAc, 5:6) to give 15.1 mg **5** as colorless prisms, mp 110–111° (lit. 111–112° [3]). The compound was identical by mp, IR, NMR and TLC with an authentic specimen; IR $\nu_{max}^{nujol}$ $cm^{-1}$: 3400, 1735, 1240, 1150, 1030 and 950.

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