

servation possibly related to the fact that the high incidence of stomach cancer in Japan may be related to dietary factors (Marquardt et al., 1977).

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## Diterpenoid Acids, (-)-*cis*- and (-)-*trans*-Ozic Acid, in Wild Sunflower, *Helianthus occidentalis*

Two diterpenoid acids, (-)-*cis*- and (-)-*trans*-ozic acid, were isolated from the wild sunflower species, *Helianthus occidentalis*. This is the first report of the levorotatory optical enantiomorph of *trans*-ozic acid and the first isolation of both acids from *Helianthus*. Because *H. occidentalis* is resistant to sunflower insect pests and various diterpenoid acids have shown antibiotic activity to several insect species, the presence of *cis*- and *trans*-ozic acid may contribute to host plant resistance.

The wild sunflower *Helianthus occidentalis* subsp. *plantagineus* (T. & G.) Heiser is resistant to several insect pests (Thompson and Rogers, 1977; Rogers and Thompson, 1978). No feeding and complete larval mortality of the sunflower beetle [*Zygogramma exclamationis* CF.] were noted on *H. occidentalis* compared to only 22% mortality on susceptible *H. annuus* L., and total mortality of an aphid [*Masonaphis masoni* (Knowlton)] occurred on this wild species of sunflower. Early investigators further noted that individual plants of *H. occidentalis* exhibited autotoxic action (allelopathy) against other plants of the same species (Curtis and Cottam, 1950).

In an effort to identify a chemical basis for resistance to insects, we compared the composition of extracts of florets of *H. occidentalis* with those from a susceptible check, Hybrid 896 (*H. annuus*). Two diterpenoid acids were isolated from the extracts of *H. occidentalis* but not from the extracts of Hybrid 896. These diterpenoid acids were also present in extracts of *H. occidentalis* leaves and roots.

Two diterpenoid acids, 16-kauren-19-oic acid and trachyloban-19-oic acid, were isolated from florets of *H. annuus* (Pyrek, 1970) and inhibited the growth of the sunflower moth [*Homeosoma electellum* (Hulst)] (Waiss et al., 1977). These and several other diterpenoid acids also inhibited the growth of *Heliothis virescens* (Fab.), *Heliothis zea* (Boddie), and *Pectinophora gossypiella* (Saynders) (Elliger et al., 1976).

In view of the general toxicity of diterpenoid acids to insects, we investigated two diterpenoids from *Helianthus occidentalis* that were absent in Hybrid 896. These are identified as (-)-*cis*- and (-)-*trans*-ozic acids.

## EXPERIMENTAL SECTION

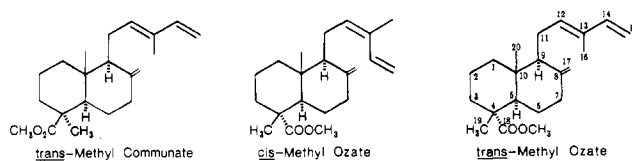
**Materials and Isolation.** *H. occidentalis* was grown at the USDA, Southwestern Great Plains Research Center, Bushland, TX. Leaves and florets from mature plants were frozen, lyophilized, and ground to a powder with a

mortar and pestle. Leaves (20 g) were stirred with a mixture of ethyl acetate/petroleum ether (bp 35-60 °C) (1:3, 120 mL) and water (2 mL) for 30 min. The slurry was poured into a glass column (2.5 cm diameter) and extracted successively with hexane (400 mL), ethyl acetate/petroleum ether (1:3, 200 mL), and ether (110 mL). Because all extracts contained the desired acids, they were combined and evaporated to dryness to give 1.0 g of crude material. The leaf residue was further extracted in a Soxhlet with refluxing ether and gave an additional 0.3 g of crude material. The combined crude material was chromatographed on thin layers of silica gel with chloroform/acetone/formic acid (94:5:1). The UV (254 nm) absorbing band ( $R_f \sim 0.7$ ) was collected. This band, which gave an acid reaction with bromocresol green, was chromatographed as above with ether/hexane (1:1; solvent 1) and gave 230 mg of material consisting primarily of *cis*- and *trans*-ozic acids.

The mixed ozic acids (75 mg) were esterified with diazomethane (see safety precautions: Fales and Jaouni, 1973) and chromatographed on thin layers of silica gel containing 3% silver nitrate (solvent 1). The top UV absorbing band was *cis*-methyl ozate (25 mg) and the lower band was *trans*-methyl ozate (23 mg).

## RESULTS AND DISCUSSION

The methyl esters of the diterpenoid acids from *H. occidentalis* gave parent ions at  $m/e$  316. High-resolution



measurement of these ions indicated that the molecular formula was  $C_{21}H_{32}O_2$  for each ester. Thus six degrees of unsaturation are indicated. The  $^1H$  and  $^{13}C$  NMR spectra

Table I.  $^{13}\text{C}$  NMR Spectra<sup>a</sup>

C no.	<i>trans</i> -methyl communate, <sup>b</sup> $\delta$	<i>trans</i> -methyl ozate, $\delta$	<i>cis</i> -methyl ozate, $\delta$
1	39.4	38.2 <sup>c</sup>	38.2 <sup>c</sup>
2	20.1	18.4	18.4
3	38.3	37.6 <sup>c</sup>	37.8 <sup>c</sup>
4	44.4	47.7	47.7
5	56.4	49.8	49.8
6	26.1	26.6	26.6
7	38.6	37.0 <sup>c</sup>	37.6 <sup>c</sup>
8	148.0	147.8	147.8
9	56.5	57.0	57.3
10	40.2	38.9	38.9
11	23.3	23.0	22.0
12	133.9	133.4	131.2
13	133.4	133.4	131.6
14	141.6	141.5	133.8
15	109.9	109.7	113.1
16	11.9	11.7	19.6
17	107.6	107.9	108.0
18	28.9	179.0	178.9
19	177.7	16.6	16.6
20	12.7	14.6	14.6
OCH <sub>3</sub>	51.1	51.7	51.7

<sup>a</sup> Spectra were taken in  $\text{CDCl}_3$ , and shifts are expressed in ppm downfield from  $\text{Me}_4\text{Si}$  using the central resonance of  $\text{CDCl}_3$  as reference ( $\delta$  76.9); shift assignments were made on the basis of comparisons with similar compounds and by proton off resonance decoupling experiments.

<sup>b</sup> Taken from the work of Braun and Breitenbach (1977).

<sup>c</sup> Shift assignments of carbons so designated may be interchanged.

showed the presence of three carbon-carbon double bonds. A carbonyl group and a bicyclic ring structure would satisfy the remaining unsaturation. The UV absorption maxima at 236 (17400) and 232 (22900) nm indicated the presence of an acyclic conjugated diene in which the substituents are *cis* and *trans*, respectively.

These data are consistent with a labdatriene type structure. The  $^1\text{H}$  NMR shifts for the protons on carbons 16 and 20 ( $\delta$  1.11 and 1.75, respectively) agree closely with the shifts reported for both methyl communates and methyl ozates (Bohlman and Zdero, 1974; Bevan et al., 1968). However, the proton chemical shift of the methyl group attached to carbon 4 in both the esters from *H. occidentalis* occurs at  $\delta$  0.74 and agrees with those for the methyl group in methyl ozate ( $\delta$  0.77) and differs from the epimeric methyl group in methyl communates ( $\delta$  0.55). The IR spectrum of authentic (+)-*trans*-methyl ozate was identical with that of the *trans* ester from *H. occidentalis*.

The optical rotations of the methyl ozates isolated from the South African plant *Hermas villosa* Thunb. was  $[\alpha]^{24}_{\text{D}} +48.1^\circ$  for *cis*-methyl ozate and  $[\alpha]^{24}_{\text{D}} +34.5^\circ$  for *trans*-methyl ozate ( $\text{CHCl}_3$ ) (Bohlman and Zdero, 1974). *trans*-Methyl ozate from *Pinus densiflora* had  $[\alpha]^{21}_{\text{D}} +33.2^\circ$  ( $\text{CHCl}_3$ ) (Zinkel, 1976). The *cis*-methyl ozate from *H. occidentalis*, however, had  $[\alpha]^{23.5}_{\text{D}} -40.4^\circ$  (*c* 0.28,  $\text{CHCl}_3$ ), and the *trans*-methyl ozate had  $[\alpha]^{23.5}_{\text{D}} -29.4^\circ$  (*c* 0.34,  $\text{CHCl}_3$ ), and the absolute value of these rotations were not increased by further chromatographic purification. The  $^{13}\text{C}$  NMR spectra indicated that the compounds were pure and thus both the (+) and (-) forms of ozic acid may be present with the majority being in the (-) antipodal form. (-)-*cis*-Ozic acid was first isolated by Bevan et al. (1966, 1968) from *Daniellia ogea*, but this is the first report of (-)-*trans*-ozic acid.

Comparison of the  $^{13}\text{C}$  NMR shifts of *cis*-methyl ozate and *trans*-methyl ozate with those published for *trans*-methyl communate verifies the relative configuration of the carbonyl and methyl groups attached to carbon 4 (Table I). The change from a 1,3-diaxial interaction between carbon 20 and the carbomethoxy in *trans*-methyl communate to a 1,3-diaxial methyl-methyl interaction in *trans*-methyl ozate produces a small downfield shift at carbon 20 ( $\Delta\delta$ , +1.9 ppm). The chemical shift change in the methyl carbon attached to carbon 4 is large ( $\Delta\delta$ , -12.3 ppm) as the methyl is changed from an equatorial position in communate to an axial position in ozate. This upfield shift is due to a 1,3-synaxial interaction between carbons 19 and 20 in methyl ozates (Wehrli and Wirthlin, 1976).

The wild sunflower *H. occidentalis* has shown strong resistance to attack by aphids and sunflower beetles. In view of the demonstrated toxicity of the diterpenoid acids to several insect species, (-)-*cis*- and (-)-*trans*-ozic acids may have an important role in insect resistance. Bioassays on these diterpenoid acids are in progress.

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**Supplementary Material Available:** Spectral data (UV,  $^1\text{H}$  NMR, and mass) for (-)-*cis*-methyl ozate and (-)-*trans*-methyl ozate (1 page). Ordering information is given on any current masthead page.

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