Isolation, Identification, and Biological Activity of Isopersin, a New Compound from Avocado Idioblast Oil Cells

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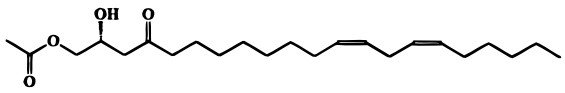
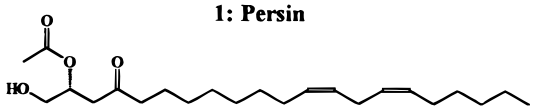
A new compound, (12*Z*,15*Z*)-1-hydroxy-4-oxo-heneicosa-12,15-dien-2-yl acetate, isopersin (**2**), has been isolated from avocado idioblast oil cells. In artificial diet bioassays, **2** showed no effects on either larval survivorship or growth of early-instar beet armyworm *Spodoptera exigua*. In contrast, the isomeric persin (**1**), (12*Z*,15*Z*)-1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene, reduces larval growth at equivalent concentrations (500 μg g^{-1}). Compound **2** is not very stable and isomerizes readily to **1**. Both compounds are acid-labile, rearranging rapidly to alkylfuran **3** in the presence of traces of acid.

Avocado fruit, *Persea americana* Mill. (Lauraceae), contains idioblast oil cells that differentiate from surrounding cells of the same tissue.[1] The oil from these cells deters feeding by the beet armyworm, *Spodoptera exigua* Hübner (Noctuidae),[2] a generalist phytophagous insect and a major pest in many agricultural crops. Feeding deterrent effects to *S. exigua* have been attributed to the presence of unique compounds present in these cells, including persin (12*Z*,15*Z*)-1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene (**1**), and several 2-alkylfurans.[3,4] These compounds were isolated from two of eight fractions collected from an initial low-pressure liquid chromatographic fractionation of the crude idioblast oil. Persin (**1**) was isolated from the third of eight fractions, while the avocadofurans were identified in the least polar fraction. Surprisingly, larvae fed a diet containing an intermediate fraction showed an increase in larval weight compared to control larvae.[3] Herein we report the identification of the major compound (**2**) present in this fraction and its effect on *S. exigua* larval mortality and growth.

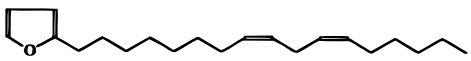
The new compound **2** (isopersin) was identified as follows. On silica TLC, eluting with EtOAc−hexane (2:3) the compound was more polar than **1**.



1: Persin



2: Isopersin



3

It was acetylated with acetic anhydride and pyridine, suggesting the presence of one or more primary or secondary hydroxyls or amines. The compound was destroyed by hydrolysis with aqueous base and by $NaBH_4$ reduction, suggesting the presence of ester and ketone (or aldehyde) functionalities, respectively. Exact mass chemical ionization mass spectrometry gave a molecular formula of $C_{23}H_{40}O_4$, identical to **1**.

Our first attempt at obtaining a ^{1}H NMR of the compound in $CDCl_3$ resulted in complete and clean rearrangement of the unknown to 2-(8*Z*,11*Z*-heptadecadienyl)furan **3**, another component of avocado idioblast cell oil.[4] Comparison of the NMR spectrum of the rearranged material with that of an authentic standard of the furan[4] showed that the spectra differed primarily in a single peak; the spectrum of the rearranged compound contained an extra singlet at δ 2.11 from the acetic acid generated during the rearrangement. Furthermore, the GC retention times and electron impact mass spectra of the rearranged compound and the furan standard matched exactly. This clean rearrangement, presumably catalyzed by traces of DCl in the $CDCl_3$, also provided strong evidence for the structure of the hydrocarbon-chain portion of the molecule, with a (*Z*,*Z*)-1,4-diene structure. Persin (**1**) has also been demonstrated to undergo a similar acid-catalyzed rearrangement.[5] This process, however, appears to be even more facile in the new compound, presumably due to the free primary hydroxyl group on C-1, which can readily form a hemiketal with the ketone on C-4 (see below) to initiate the rearrangement.

The structure of isopersin (**2**) (12*Z*,15*Z*)-1-hydroxy-4-oxo-heneicosa-12,15-dien-2-yl acetate, was determined from the 1D proton and ^{1}H−^{1}H COSY NMR spectra in C_6D_6, a solvent in which the molecule proved to be stable. The key features included a doublet of doublets at δ 3.42 from the protons on C-1 bearing the primary alcohol, a multiplet at 5.30 due to the single proton on C-2 and bearing the acetate group, two doublets of doublets at 2.35 and 2.27 with a large geminal coupling of 16.8 Hz due to the methylene group at C-3, and a two-proton multiplet at 1.90 from the methylene (C-5) on the other side of the carbonyl group at C-4. The presence of the carbonyl group at C-4 and the carboxyl group of the acetate were confirmed by peaks in the ^{13}C NMR spectrum at δ 205.99 and 169.58, respectively. The

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Table 1. Biological Activity of Isopersin, (12*Z*,15*Z*)-1-Hydroxy-4-oxo-heneicosa-12,15-dien-2-yl Acetate (**2**), Against Early Instar *Spodoptera exigua* at a Concentration of 500 μg g^{-1} in Artificial Diet

treatment	n^a	% mortality^b	larval weight (mg ± SE)^c
isopersin **2**	88	12 n.s.	26.45 ± 1.76 n.s.
control	89	10	30.02 ± 2.43

^{a} Total number of larvae used in bioassays. ^{b} $F = 2.74$, $df = 1.79$, $p > 0.05$. ^{c} $\chi^2 = 0.56$, $df = 1$, $p > 0.05$.

absolute configuration is probably (*R*), by analogy with **1**, for which the (*R*) configuration has been determined.[6]

A final, indirect piece of evidence of the structure of **2** was obtained from reexamination of a sample stored in acetone for a period of several weeks at −20 °C. The sample was found to have equilibrated to a mixture of **2** and its isomer, persin (**1**), further indicating the lability of **2**. This lability may also explain why compound **2** has not been reported previously, despite several studies on the components of avocado oil.[5,7−9]

S. exigua larvae fed **2** at a concentration of 500 μg g^{-1} in artificial diet did not differ significantly in larval weight or survivorship compared to control larvae (Table 1). In contrast, **1** at the same concentration of 500 μg g^{-1} in the diet inhibited larval growth by more than 80% compared to controls.[3] We estimated that **2** represents about 0.15 wt % of fresh tissue. This concentration, however, may be an underestimate due to its lability. Persin (**1**) is reported to occur in avocado fruit at concentrations ranging from 760 to 2280 μg/g and 1500−5800 μg/g of fresh weight in the pericarp and mesocarp, respectively.[8]

Isopersin **2**, like **1**, is a structural mimic of a linoleic acid monoglyceride;[10] however, it does not appear to cause the same inhibitory effects as **1**. In fact, in preliminary bioassays, larvae fed **2** appeared to be heavier than larvae fed artificial diet alone.[3] Those results, which we were not able to reproduce, may have been due to the small sample size (24 neonates per treatment) used in that bioassay.

Experimental Section

General Experimental Procedures. NMR spectra were taken using a GE−300 instrument (General Electric, Fremont, CA) operated at 300 MHz for protons. LRCIMS and HRCIMS (NH_3) were obtained with direct insertion probe on a VG-7070 instrument (VG Instruments, Manchester, England).

Plant Material. Avocados, *P. americana* var. Hass, were harvested from trees grown at the South Coast Research and Extension Center, University of California, in Santa Ana, CA. The oil was isolated from idioblast cells as described by Rodriguez-Saona and Trumble.[2]

Extraction and Isolation. The crude idioblast cell oil (5 g) was fractionated by flash chromatography on silica (5 cm × 25 cm), eluting with toluene−ethyl ether−HOAc, to give eight fractions as previously described.[3] The fourth fraction (0.34 g) was purified further by flash chromatography on silica, eluting with EtOAc−hexane 2:3. A compound (**2**) with an R_f value (0.31) slightly less than **1** [R_f = 0.42; Si gel TLC; eluting with toluene−ethyl ether−HOAc (60:40:1 v/v/v) and visualized by spraying with H_2SO_4, charring with a hot-air blower] was isolated (40 mg) and stored in dilute solution in Me_2CO (10 mg/mL) until required for bioassay or analysis.

Identification of Isopersin (2). Portions of **2** were used to obtain ^{1}H, ^{13}C, and ^{1}H−^{1}H COSY NMR spectra in both $CDCl_3$ and deuteriobenzene. The sample decomposed in $CDCl_3$, so NMR data in this solvent are not reported: ^{1}H NMR (C_6D_6) δ 5.45 (m, 4H, H-12, H-13, H-15, H-16), 5.30 (m, 1H, H-2), 3.42 (dd, 2H, J = 5.6, 4.6 Hz, H-1, H-1′), 2.85 (br t, 2H, J = 5.8 Hz, H-14), 2.35 (dd, 1H, J = 16.8, 6.9 Hz, H-3), 2.27 (dd, 1H, J = 16.8, 6.1 Hz, H-3′), 2.05 (m, 4H, H-11, H-17), 1.92 (m, 2H, H-5), 1.60 (s, 3H, acetate CH_3), 1.5−1.1 (m, 16 H, H-6−H-10, H-18−H-20), 0.84 (t, 3H, J = 6.7 Hz, H-21), 0.47 (s, 1H, OH); ^{13}C NMR (C_6D_6) δ 205.99, 169.58, 130.08, 129.94, 70.92, 63.56, 42.76, 42.74, 31.46, 29.65, 29.36, 29.33, 29.12, 29.04, 27.24, 25.76, 23.42, 22.56, 20.23, 13.90). Two of the alkenyl carbons were obscured by the deuteriobenzene solvent peak cluster centered at δ 127.6. LRCIMS and HRCIMS (NH_3) found: *m/z* 398.3281; calcd for $C_{23}H_{44}NO_4$ [M + NH_4^+] 398.3270.

Aliquots of **2** (ca. 200 μg) were also subjected to microchemical tests as follows, checking the products against the starting material by TLC on silica (40% EtOAc in hexane): (a) An aliquot was treated with 10 μL of Ac_2O and 4 μL of pyridine at room temperature overnight. The mixture was then concentrated under a stream of nitrogen, and the residue taken up in hexane. A single new product was obtained (R_f = 0.61), which was less polar than the starting material (R_f = 0.33). (b) An aliquot was dissolved in 20 μL EtOH and 4 μL 5M aqueous NaOH. After 2 h at room temperature, the mixture was concentrated, and the residue was taken up in ether. Upon TLC analysis, the spot corresponding to the starting material had disappeared, and several new spots at higher and lower R_f (0.04, 0.65, 0.73) appeared, indicating that the starting material had been consumed. (c) An aliquot was treated with a few crystals of $NaBH_4$ in 20 μL EtOH for 45 min at 0 °C. The mixture was quenched by addition of HOAc and concentrated, and the residue was taken up in ether. Upon TLC analysis, there was no spot corresponding to the starting material, indicating that it had been consumed.

Bioassays. The biological activity of **2** was tested against early instar *S. exigua* in an artificial-diet bioassay test. Lima bean artificial diet was prepared as described by Patana.[11] A concentration of 500 μg g^{-1} was used because previous tests with early instars suggested that the compound, when mixed with artificial diet at 500 μg g^{-1}, may cause an increase in larval growth.[3] Treated diet was prepared by transferring 1.9 mL of the Me_2CO solution of **2** into 50-mL centrifuge tubes, evaporating the Me_2CO, adding 2 mL of 0.1% Tween-80 solution, mixing with an ultrasonic homogenizer, and finally adding prepared diet to produce a final weight of 15 g. Control diet was prepared by adding 13 g of artificial diet to 2 mL of Tween solution, to produce a final weight of 15 g. Control and treated diets were poured into 16-well bioassay trays. One neonate larva was added per well, and 25−30 neonates were tested per treatment. Bioassays were replicated three times. Mortality and larval weights were recorded after seven days. Bioassays were maintained at 28 ± 2 °C, 75% relative humidity, and 14:10 (L:D) photope-

riod with fluorescent lighting (see Rodriguez-Saona et al.[3,4] for details). Statistical comparisons were conducted with SuperAnova.[12]

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