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ISOLATION AND IDENTIFICATION OF A PLANT GROWTH INHIBITOR FROM AVOCADO

SHMUEL BITTNER
Negev Institute for Arid Zone Research, Beer-Sheva, Israel
SHMUEL GAZIT
Hebrew University, Faculty of Agriculture, Rehovot, Israel
and
AMOS BLUMENFELD
Volcani Institute of Agricultural Research, Bet-Dagan, Israel

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Abstract—A compound was isolated from avocado mesocarp and identified as 1-acetoxy-2,4-dihydroxy-*n*-heptadeca-16-ene. The new compound inhibits soybean callus growth and elongation of wheat coleoptiles.

INTRODUCTION

DURING a comprehensive study of growth and development in the avocado fruit, the presence of a new kind of inhibitor was observed.[1] The inhibitory zone was detected at R_f 0·80–1·0 when a methanolic extract of avocado mesocarp was chromatographed on paper in propan-2-ol–ammonia–water (10:1:1, by vol.). This zone differs from the so-called 'β-inhibitor' zone (R_f 0·6–0·75) which is attributed to abscisic acid.[2,3] Preliminary investigations showed the new inhibitory compound to be neutral and extractable with petroleum from a basic aqueous solution, thus separating it from acidic inhibitors. The new inhibitory zone showed very high activity, and the quantity extracted from 100 mg fr. mesocarp (equivalent to 10 mg dry wt.) caused growth inhibition in two bioassays, namely, the soybean callus bioassay[4] and wheat coleoptile elongation.[5,6] Chromatography of various plant tissues such as tobacco leaf, mango seed, and annona fruit,[1] showed a similar inhibitor of the same R_f with activity in coleoptile elongation. It was, therefore, of great interest to isolate this compound since it appears to be of wide occurrence.

RESULTS

Isolation and Structure Determination of the Inhibitor

Extraction of avocado mesocarp with methanol, followed by continuous fractional crystallization of the crude product from petroleum and acetonitrile furnished the new inhibitor (I) m.p. 55–56°. The mass spectrum and elementary analysis corresponded to the formula $C_{19}H_{36}O_4$. The overal yield was 2–3 mg (I)/g of mature *Fuerte* avocado mesocarp.

[1] A. BLUMENFELD and S. GAZIT, *Israel. J. Botany* **18**, 217 (1969).
[2] O. M. K. GARB and C. G. GUTTRIDGE, *Planta* **78**, 305 (1968).
[3] B. V. MILBORROW, *Planta* **76**, 93 (1967).
[4] C. O. MILLER, in *Modern Methods of Plant Analysis*, Springer-Verlag, Berlin (1963).
[5] J. P. NITSCH and C. NITSCH, *Plant Physiol.* **31**, 94 (1956).
[6] C. R. HANCOCK, H. W. B. BARLOW and H. J. LACEY, *J. Exptl. Botany* **15**, 166 (1964).

The identity of (I) was established by observation of its chemical behavior and spectroscopic data. The i.r. spectrum shows the presence of three functional groups: an hydroxyl absorption at 3440 cm^{-1}, a terminal double bond absorption at 1645 and 910 cm^{-1}, and an *O*-acyl group absorption at 1145, 1045 (νc-o) 1730, 1260 (νo-Ac) cm^{-1}. The NMR spectrum of (I), displayed the following signals:* a multiplet at *ca.* δ 5·8 (1H), a doublet at δ 4·98 (1H) and a singlet at δ 4·75 (1H) typical of the three vinylic hydrogens of a terminal double bond CH_2=CH—. Two singlets at δ 4·25 (1H) and δ 3·75 (1H) which disappeared on addition of D_2O indicated the presence of two hydroxyl groups in the molecule. One acetyl group was observed at δ 1·98 (3H) and integration showed the presence of 22 methylenic hydrogens at δ 1·22. An additional signal at δ 3·95 was attributed to the presence of two hydrogens adjacent to the acetoxy group thus proving the acetylated OH to be primary. No terminal methyl group was observed.

Acetylation of (I) with acetic anhydride in pyridine gave the oily triacetate (II) which could not be crystallized. It shows a molecular ion peak at *m/e* 412, and elementary analysis agree with a molecular formula of $C_{23}H_{40}O_6$. The i.r. spectrum lacks the OH bands at 3440 cm^{-1} but shows the terminal double bond bands (1635 and 910 cm^{-1}) and the carbonyl band (1740 cm^{-1}). The NMR spectrum of this compound is in agreement with structure (II) revealing one nine proton triplet for the acetyl methyl groups at δ 1·96, a multiplet (1H) and a double doublet (2H) at δ 5·45, δ 4·95 and δ 4·75 respectively, of the terminal olefinic hydrogens, and the twenty-two proton broad singlet at δ 1·22.

Hydrolysis of (I) in basic conditions yielded acetic acid (as proved by formation of its *S*-benzyl-*iso*-thiuronium salt), and the trihydroxy compound (III). The latter compound, recrystallized from acetonitrile, melted at 65–66°. It showed a molecular ion peak at m/e 286 in the mass spectrum, and analyzed for $C_{17}H_{34}O_3$. The i.r. spectrum lacks the ester bands at 1730 and 1260 cm^{-1} but shows the presence of hydroxyl group (3310 cm^{-1}) and a terminal double bond (3090, 1640 and 900 cm^{-1}). The NMR spectrum showed that the singlet corresponding to the acetyl methyl protons (δ 1·92) had disappeared but the triplet at δ 1·86 (2H) could clearly be discerned. It was assigned to the two methylene protons between the two CHOH groups. The presence of three hydroxyl groups was shown by the

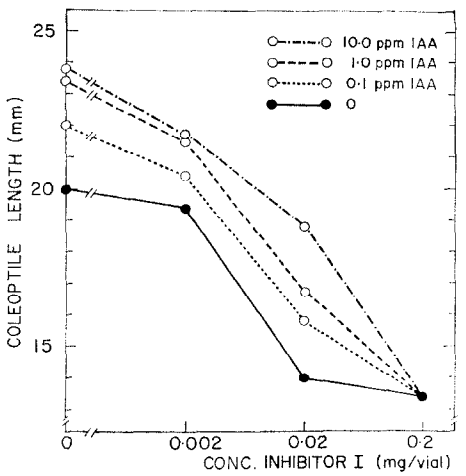
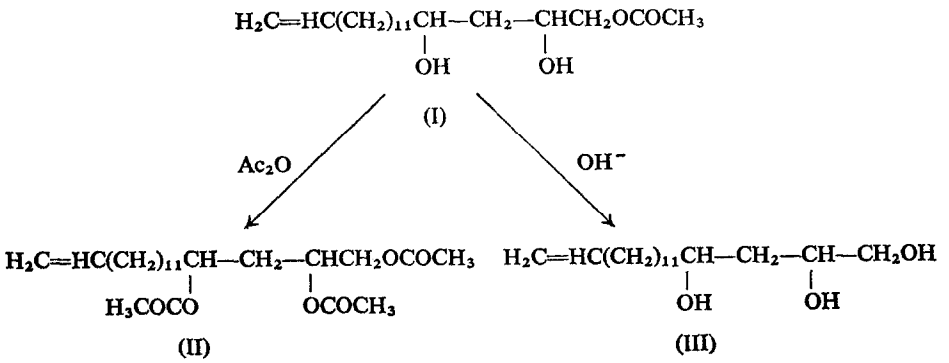


FIG. 1. RESPONSE OF WHEAT COLEOPTILES TO VARIOUS COMBINATIONS OF KINETIN AND INHIBITOR (I).

* δ Values are given in ppm relative to internal tetramethylsilane as reference.

disappearance of the signals at δ 3·41 (2H) and δ 3·7 (1H) on addition of D_2O. No terminal methyl group was observed.

Based on the above evidence, I was assigned the structure 1-acetoxy-2,4-dihydroxy-*n*-heptadeca-16-ene*



Biological Activity

Paper chromatography of the petroleum fraction of *Fuerte* avocado mesocarp extract, showed the presence of a new endogenous inhibitor.[1] It inhibited wheat coleoptile elongation in the presence or absence of indoleacetic acid. The pure compound (I) showed similar activity (see Fig. 1).

Growth inhibition of soybean callus in the presence of kinetin, was the second bioassay in which (I) resembled the crude petroleum fraction.[1] This inhibition was proportional to the concentration of the inhibitory fraction added to the medium and callus growth was inhibited completely. When the inhibitor level is constant, higher kinetin concentrations reduced the inhibition.[8] Pure (I) has a similar effect on the growth of soybean callus in the presence of kinetin (see Table 1).

TABLE 1. GROWTH RESPONSE OF SOYBEAN CALLUS TO VARIOUS COMBINATIONS OF KINETIN AND INHIBITOR I

	Kinetin (mg/l.)		
Inhibitor I (mg/flask)	0·005	0·05	0·5
0	0·45	1·15	1·90
0·1	0·40	1·08	1·60
0·33	0·09	0·35	1·12
1·0	0	0·25	0·70

Results are given as average increase in yield, g/flask.

* While this investigation was in progress, Kashaman *et al.*[7] published a study on the isolation of eight compounds from avocado. One of those compounds, according to its analytical and spectral behaviour, is identical to (I).

[7] Y. KASHMAN, I. NEEMAN and A. LIFSHITZ, *Tetrahedron* **25**, 4617 (1969).
[8] S. GAZIT and A. BLUMENFELD, *Plant Physiol.* (in press).

DISCUSSION

Compound (I) is a growth regulating substance occurring naturally in avocado mesocarp. It inhibits elongation of wheat coleoptiles and growth of soybean callus in the presence of cytokinins. It is not a toxic agent since the inhibited tissue shows renewed growth when transferred to an inhibitor-free medium.

Neither compound (II), the triacetate nor the deacetylated compound (III), show any inhibitory activity. Chemically, (I) bears no close structural relationship to other known natural growth inhibitors, most of which are acidic or have a potential acidic function.[9]

A closely related series of C_{17} compounds, some of acetylenic character, were recently isolated[7] and found to act as antibiotics against gram negative bacteria.[10] It was found that although compound (II) was active against bacteria, it was completely inactive as an inhibitor of plant tissue growth. Thus, the two types of activity are different.

Whether (I) has a role as an endogenous inhibitor and regulator, is uncertain. It does accumulate in the avocado fruit during growth, and a consistently negative correlation was found between fruit growth rate and level of inhibitory activity,[8] which may point towards a growth regulating action.

EXPERIMENTAL

M.ps were taken on a Thomas–Hoover Capillary apparatus and are uncorrected. I.r. spectra were run as Nujol mulls or as KBr discs. NMR spectra were recorded in $CDCl_3$ or CCl_4 with TMS as internal standard. Mass spectra were obtained on a Matt-Atlas CH-4 spectrometer, by direct introduction of samples. Kieselgel G (Woelm) was used for analytical TLC.

Isolation Procedure

Fresh or frozen avocado mesocarp tissue (var. *Fuerte*) was macerated and extracted twice with absolute methanol. The brei was filtered through two layers of muslin and then through filter paper (Whatman No. 1). The clear solution was concentrated *in vacuo* (40°) to an aqueous solution which was brought to pH 9·0 with 10% aq. NaOH. The basic solution was extracted with three equal portions of petroleum (60–80°) and the combined petroleum fractions were dried. The precipitate which separated after storage overnight at 0° was collected and recrystallized twice from petroleum (60–80°) and twice from acetonitrile. An additional portion was recovered from the mother liquors. It melted at 58–59°, and migrated as a single spot when run on TLC plates in MeOH, MeOH–hexane (3:97) and $CHCl_3$–acetonitrile (10:1). The yield was 2–3 mg/g of fresh avocado mesocarp; I.r. bands (Nujol): 3440 (OH), 3080 and 1645 ($H_2C{=}C$), 1145 and 1045 (C—O) 1730 and 1260 cm^{-1} (ester carbonyl). (Found: C, 69·6; H, 10·8%: mol. wt. (mass spectrum) 328, calcd for $C_{19}H_{36}O_4$ C, 69·5; H, 10·9%.)

Hydrolysis of (I)

0·5 g of (I) were suspended in 25 ml N NaOH, and the mixture stirred at room temp. for 24 hr. Conc. HCl was dripped into the cooled solution (0°) to pH 6·0, and the aq. solution extracted twice with petroleum (60–80°). The combined petroleum fractions were dried, filtered and evaporated to dryness. The residue was recrystallized twice from acetonitrile to give needles, melting at 65–66°. I.r. bands (Nujol): 3310 (OH), 3090 and 1640 (terminal double bond), 1138 (C—O), 1108, 1070, 1015, 845 cm^{-1}. (Found: C, 71·2; H, 12·0%, mol. wt. (mass spectrum): 286, Calcd for $C_{17}H_{34}O_3$; C, 71·3; H, 11·9%.)

Acetylation of (I)

A solution of 0·25 g of (I) in 10 ml Ac_2O and 8 ml pyridine was allowed to stand at room temp. for 20 hr. The product was a colourless syrup which could not be crystallized. I.r. bands (in CCl_4) 1740 (C=O), 1635 (C=C), 1450, 1425, 1345, 1215, 1040, 1018 cm^{-1}. (Found: C, 66·7; H, 9·7%; mol. wt. (m.s.) 412 Calc. for $C_{23}H_{40}O_6$; C, 66·9; H, 9·7%.)

Bioassay Procedures

The wheat coleoptile bioassay was similar to that of Nitsch and Nitsch[5] and of Hancock *et al.*[6] Varying amounts of inhibitor were put into small vials. Three 10 mm coleoptile sections were placed in each vial with

[9] A. CARL. LEOPOLD, *Plant Growth and Development*, p. 148, McGraw-Hill, New York (1970).
[10] I. NEEMAN, A. LIFSHITZ and Y. KASHMAN, *Israel. J. Chem.* 7, 151 (1969).

0·75 ml phosphate–citrate buffer pH 5·7+2% sucrose. The vials were rotated in a horizontal rotator (1 rpm). After incubation for 22 hr at 23°, the sections were measured to the nearest 0·5 mm. The 10 mm sections usually grew to 20 mm when neither IAA nor inhibitor was included in the buffer (see Fig. 1).

The kinetin inhibition was bioassayed on soybean cotyledon callus (var. *Acme*),[4] according to the procedure previously described.[1]

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