

New Antibacterial Agent Isolated from the Avocado Pear

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A group of eight new long-chain aliphatic compounds recently isolated from the avocado and some derivatives thereof were tested for antibacterial activity on 13 different species of bacteria and a yeast. Some of these compounds inhibited the growth of microorganisms. 1,2,4-Trihydroxy-n-hepadeca-16-en was found to be the most active, inhibiting certain gram-positive bacteria at 4 $\mu\text{g}/\text{ml}$.

L. B. Jensen (U.S. Patent 2,550,254, 1951) reported that acetone extract of avocado seeds exhibited antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Aspergillus glaucus*, *Penicillium notatum*, and *Achromobacter perolens*. This extract was found to be inactive against *Esherichia coli*, *Pseudomonas fluorescens* and *Penicillium camemberti*. Valeri and Gimeno (3) extracted avocado seeds with petrol ether and reported that the resulting crude wax inhibited growth of *Micrococcus pyogenes* and *Sarcina lutea*, but not growth of *B. subtilis* or of *E. coli*. The avocado extracts tested in these experiments were crude, and the compound causing the inhibition was not isolated.

Recently, we succeeded in isolating from avocado seeds and fruits a family of natural compounds, all having a long aliphatic chain with one end being unsaturated and the other end highly oxygenated. The structure of these eight compounds was elucidated and a number of derivatives thereof were prepared. It was of interest to find out which of these compounds were responsible for the antibacterial activity of the avocado seed and whether any of the derivatives were also active.

MATERIALS AND METHODS

Compounds. The compounds reported here were isolated and prepared according to the methods described by Kashman, Néeman, and Lifshitz (1). All the compounds were further refined before the bacteriological assay by subsequent passage through silica-gel columns and by recrystallization to fully free them from possible traces of impurity. The purity of each compound was ascertained by thin-layer chromatography and by nuclear magnetic resonance.

Organisms. Bacteria were grown on beef extract-dextrose agar or broth (0.5% peptone, 0.3% beef

extract, 0.5% dextrose). The yeasts were grown on peptone-yeast extract-dextrose agar or broth (0.5% peptone, 0.3% yeast extract, and 2% dextrose). Assays were carried out in the same media.

P. fluorescens was grown at 22 C, *B. subtilis* at 30 C, and all other organisms at 37 C.

Disc inhibition zone. Each compound was dissolved in CHCl_3 (10 mg/ml), and 5 μl iters of the solution was transferred to a sterile 6-mm diameter disc made from Whatman no. 3 filter paper, so that each disc contained 0.05 mg of the compound. To the blank discs, 5 μl iters of CHCl_3 was added. The discs were left for about 3 hr to evaporate the solvent.

A suspension containing ca. 3,000 cells per ml was prepared by washing 3-day-old slants. One-tenth milliliter of this suspension was transferred to a petri dish containing 10 ml of solid medium and spread evenly with a glass rod. Three discs, each containing 0.05 mg of the test compound, and one blank disc were put on each dish and incubated at the appropriate temperature. The diameter of the inhibition zone around the disc was measured after 48 hr. Each experiment was repeated four times.

Minimal inhibitory concentration. A serial dilution assay was made to determine the minimal inhibitory concentration as described by Kavanagh (2). As the compounds do not readily dissolve in water, 20 mg of each compound was dissolved in 1 ml of absolute ethyl alcohol, and 0.05 ml (containing 1 mg of the compound) was added to the first test tube. The first test tube of the blank series contained 0.05 ml of ethyl alcohol.

Growth curves. Growth curves were made as follows. Two milligrams of the compound tested was dissolved in 0.1 ml of absolute ethyl alcohol and was added to 25 ml of sterile broth in a 250-ml Erlenmeyer flask with a side arm. One milliliter of 1-day-old culture grown in slants containing ca. 10^7 cells was then added to the Erlenmeyer flask and shaken continuously at 37 C (30 C for *B. subtilis*). Plate counts were made, and the optical density of the culture was measured at predetermined intervals, by using a Spec-

TABLE 1. Organism tested^a

Compounds tested		<i>Bacillus subtilis</i> NCTC 3610	<i>Bacillus cereus</i> NCTC 1587	<i>Salmonella typhi</i> NCTC 8393	<i>Shigella dysenteriae</i> NCTC 4837	<i>Staphylococcus aureus</i> oxford	<i>Candida albicans</i>	<i>Saccharomyces cerevisiae</i> ATCC 7152	<i>Saccharomyces cerevisiae</i> S 288C
I	$\text{HC}\equiv\text{C}-(\text{CH}_2)_{11}-\text{CH}(\text{OH})-\text{CH}_2-\text{CH}-$ $(\text{OH})-\text{CH}_2\text{OAc}$	- ^b	-	-	-	-	-	-	-
II	$\text{H}_2\text{C}=\text{CH}-(\text{CH}_2)_{11}-\text{CH}(\text{OH})-\text{CH}_2-\text{CH}_2-$ $(\text{OH})-\text{CH}_2\text{OAc}$	-	-	-	-	12	10	18	9
III	$\text{HC}\equiv\text{C}-(\text{CH}_2)_{11}-\text{CH}(\text{OH})-\text{CH}_2-\text{CH}-$ $(\text{OH})-\text{CH}_2\text{OH}$	-	-	10	-	9	-	14	-
IV	$\text{H}_2\text{C}=\text{CH}-(\text{CH}_2)_{11}-\text{CH}(\text{OH})-\text{CH}_2-\text{CH}-$ $(\text{OH})-\text{CH}_2\text{OH}$	35 ^b	26	36	30	40	20	16	10
V	$\text{HC}\equiv\text{C}-(\text{CH}_2)_{11}-\text{CH}(\text{OAc})-\text{CH}_2-\text{CH}-$ $(\text{OAc})-\text{CH}_2\text{OAc}$	-	-	-	-	-	-	-	-
VI	$\text{H}_2\text{C}=\text{CH}-(\text{CH}_2)_{11}-\text{CH}(\text{OAc})-\text{CH}_2-\text{CH}-$ $(\text{OAc})-\text{CH}_2\text{OAc}$	-	-	-	-	-	-	-	-
VII	$\text{HC}\equiv\text{C}-(\text{CH}_2)_{11}-\text{C}(=\text{O})-\text{CH}_2-\text{CH}-$ $(\text{OH})-\text{CH}_2\text{OAc}$	12	15	-	-	14	-	-	-
VIII	$\text{H}_2\text{C}=\text{CH}-(\text{CH}_2)_{11}-\text{C}(=\text{O})-\text{CH}_2-\text{CH}-$ $(\text{OH})-\text{CH}_2\text{OAc}$	-	15	-	-	16	-	-	-
IX	$\text{H}_3\text{C}-\text{CH}_2-(\text{CH}_2)_{11}-\text{CH}(\text{OH})-\text{CH}_2-\text{CH}-$ $(\text{OH})-\text{CH}_2\text{OH}$	-	-	-	-	-	-	-	-
X	$\text{H}_3\text{C}-\text{CH}_2-(\text{CH}_2)_{11}-\text{CH}(\text{OH})-\text{CH}_2-\text{CH}-$ $(\text{OH})-\text{CH}_2\text{OAc}$	-	-	-	-	-	-	-	-
XI	$\text{HC}\equiv\text{C}-(\text{CH}_2)_{11}-\text{C}=\text{CH}-\text{CH}=\text{CH}$ O	-	-	-	-	-	-	-	-
XII	$\text{H}_2\text{C}=\text{CH}-(\text{CH}_2)_{11}-\text{C}=\text{CH}-\text{CH}=\text{CH}$ O	10	-	-	-	11	-	-	-

^a *Salmonella typhimurium* NCTC 5710, *Pseudomonas fluorescens* NCTC 10338, *P. aeruginosa* NCTC 6750, *Salmonella manhattan*, and *E. coli* were not affected by any of the compounds.

^b Disc inhibition zone (in millimeters) of compounds isolated from avocado and derivatives thereof and tested on various organisms. (-) No inhibition.

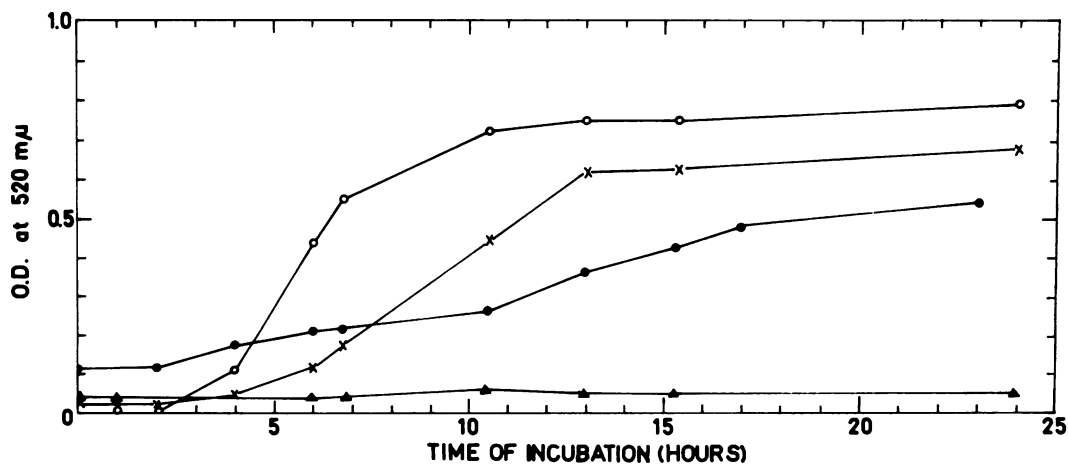


FIG. 1. Growth curves of *Staphylococcus aureus* oxford grown in the presence of 80 μg/ml of (▲) compound IV, 1,2,4, trihydroxy-n-heptadeca-16-en; (●) compound IX, 1,2,4, trihydroxy-n-heptadecan (reduced analogue); (×) compound III, 1,2,4, trihydroxy-n-heptadeca-16-yn (acetylinic analogue); (○) blank.

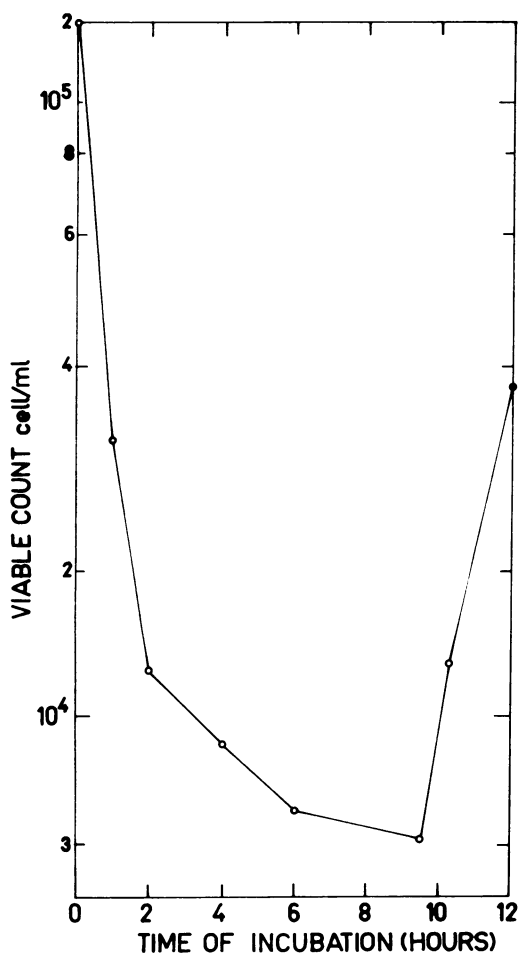


FIG. 2. Viable cell count of *C. albicans* grown in presence of 80 µg/ml of compound IV.

tronic 20 (Bausch & Lomb, Inc., Rochester, N.Y.) at 520 nm.

RESULTS AND DISCUSSION

Results of the disc inhibition zone test are tabulated in Table 1. The values indicate the diameter of the inhibition zone in millimeters after 48 hr of incubation. The gram-negative organisms were slightly inhibited by the compounds, whereas the gram-positive organisms were strongly inhibited by some of the compounds, especially by 1,2,4-trihydroxy-n-heptadeca-16-en (compound IV). It is notable that, when the olefinic bond in this compound is fully reduced, the product (compound IX) is no longer inhibitory. Furthermore, when the hydroxyl groups on the oxidized part of compound IV are totally, or partially, acetylated, the anti-

TABLE 2. Sensitivity of various microorganisms to compound IV

Organism	Minimum inhibitory concn (µg/ml)	
	After 18 hr	After 40 hr
<i>Bacillus subtilis</i> NCTC 3610	2	7.8
<i>Bacillus cereus</i> NCTC 7587	7.8	7.8
<i>Staphylococcus aureus</i> oxford	16	31
<i>Saccharomyces cerevisiae</i> ATCC 7752	31	125
<i>S. cerevisiae</i> S 288C	31	125
<i>Candida albicans</i>	>500	>500
<i>Shigella dysenteriae</i> NCTC 4837	>500	>500
<i>Salmonella typhi</i> NCTC 8393	>500	>500

bacterial activity is greatly weakened. This can also be seen in Fig. 1, showing the effect of a concentration of 80 µg/ml of three compounds on *S. aureus*.

Whereas compound IV totally inhibited the growth of the organism, the acetylenic analogue (compound III) had a limited bacteriostatic effect, if any, and the fully reduced compound IX had no effect. Similar results were obtained with *B. subtilis* and *Saccharomyces cerevisiae*.

The organisms found to be affected in the first experiment performed on solid media were tested for their sensitivity to compound IV in liquid media (Table 2). The growth of five organisms was inhibited in this experiment as it was in the first one. However, three organisms, *Candida albicans*, *Shigella dysenteriae*, and *Salmonella typhi*, whose growth was inhibited on solid media, gave satisfactory growth on liquid media. To investigate further the action of compound IV on these three organisms, their growth curves in the presence of the inhibitor were analyzed. The results of such an experiment with *C. albicans* are shown in Fig. 2. The number of the cells dropped markedly during the first 10 hr but started to increase afterward. Similar results were obtained with the other two organisms, *S. dysenteriae* and *S. typhi*. The large decrease in the number of viable cells immediately after exposure to the inhibitory compound shows that its action is bactericidal. The subsequent increase in cell count is probably due to the emergence of resistant cells. This did not occur, however, when *B. cereus*, *B. subtilis*, *Saccharomyces cerevisiae* and *Staphylococcus aureus* were tested. These microorganisms were inhibited by compound IV, which was found to

be heat-resistant since its antibacterial efficiency did not decrease after sterilization.

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