

THE IDENTIFICATION OF ETHYLENE AS A VOLATILE PRODUCT OF RIPENING AVOCADOS¹

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(WITH ONE FIGURE)

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Introduction

The production of a physiologically active emanation by ripening fruits has frequently been demonstrated by biological tests, including leaf epinasty, respiration effects, potato sprout abnormalities, and the triple response of etiolated legume seedlings (11). HUELIN (8) first noted the analogy between these effects and those produced by small concentrations of ethylene, and GANE (4, 5) first showed by chemical means that the active volatile substance produced by apples was actually ethylene gas. An inferential proof, partly chemical and partly biological, that ethylene is produced by pears was achieved by HANSEN and HARTMAN (7), and NIEDERL, BRENNER, and KELLEY (10) demonstrated ethylene production by bananas. PRATT and BIALE (11) found that the climacteric rise in carbon dioxide production by ripening avocado fruits was accompanied by a cycle of production of a gas, presumably ethylene, which caused the triple response of etiolated pea seedlings. The purpose of the present study was to identify chemically this active emanation and to develop a technique which would simplify the isolation and identification of ethylene as a probable volatile product of other fruits.

Apparatus for absorbing ethylene

GAS SCRUBBING APPARATUS

The most effective reagent for the removal of minute quantities of ethylene from a gas stream is bromine, which is difficult to manage being decidedly volatile and noxious. The apparatus of NIEDERL, BRENNER, and KELLEY (10) was tried, but the charge of bromine was lost from the apparatus within a few hours, even with temperatures lower than they specified. This problem was solved as follows² (Fig. 1): The efficient spiral absorber (H) of MARTIN and GREEN (9) was charged with liquid bromine (30% by volume) dissolved in a solvent (methylene chloride), and above the absorber was a "cold finger" trap (J) chilled with pulverized dry ice in acetone supplied from a large vacuum-jacketed reservoir (K). As the moving gas stream evaporated the bromine, it was carried to this trap on which it con-

¹ A cooperative project of the Division of Subtropical Horticulture and the Division of Truck Crops, College of Agriculture, University of California, carried out in the laboratories of the former division.

² The operating principle suggested by Dr. John D. Roberts, Massachusetts Institute of Technology.

densed by freezing. The volatile solvent, also carried to this surface, was condensed to liquid and redissolved the bromine, washing it back into the absorber. This apparatus permitted continuous operation with no loss of absorbent solution and no bromine odor nuisance in the laboratory. The

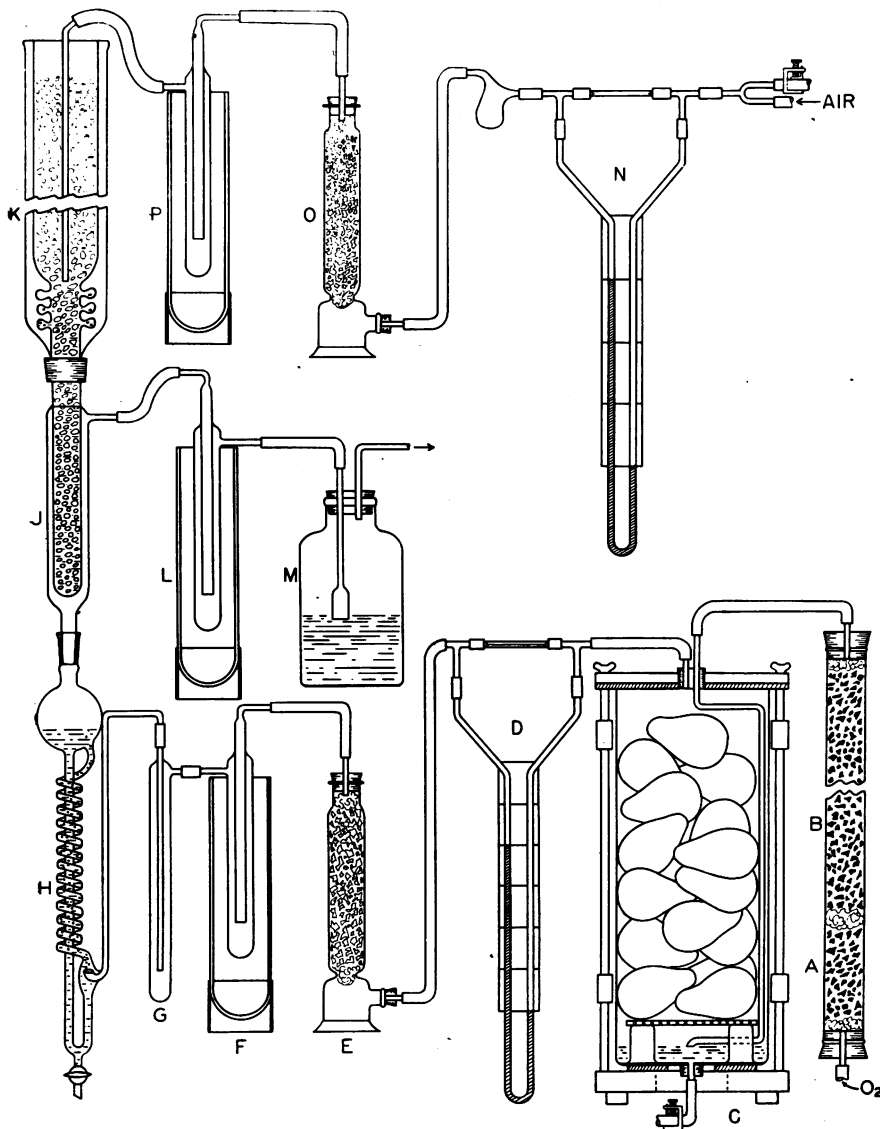


FIG. 1. Absorption train for the collection of ethylene from avocados.

efficiency of the absorption apparatus was verified with an ethylene concentration of 1000 p.p.m., by passing the scrubbed gas stream over etiolated pea seedlings; no response was obtained, showing removal of ethylene to less than one-tenth part per million.

Important dimensions of the absorption apparatus were as follows: The spiral absorber consisted of twenty-seven turns of six millimeter tubing and had a capacity of 100 ml. of solution. The "cold finger" of the trap was 24 cm. long with a diameter of 4 cm. enclosed in a six cm. outer jacket. The vacuum-jacketed reservoir had an overall height of 80 cm. with an outside diameter of 10 cm., an inside diameter of eight cm., and a lower outlet diameter of four cm., the lower outlet connecting through a rubber sleeve to the cold finger of the trap. The reservoir was charged with approximately one liter of acetone and seven pounds of crushed dry ice. Although acetone is superior to ethyl alcohol as a vehicle since it prevents caking of the dry ice in the reservoir, stirring was required to move a steady supply of refrigerant into the trap. To this end, three liters of dry air per minute were passed into the reservoir at the constriction above the expansion bellows, via the flowmeter (N), the calcium chloride drying tower (O), and the freezing trap (P). This large reservoir permitted overnight operation without attention. The entire apparatus was kept in a room at 15° C. throughout the experiment. All critical connections were made with Pyrex tubing held end to end with "Tygon."

The choice of methylene chloride as the solvent was important. Not only must the solvent be inert, but its physical properties must be in appropriate relation to those of bromine at room temperature and at the temperature of dry ice to secure the washing and return action. The inertness and freedom from olefin contamination of the absorbent solution were verified before use by allowing the mixture to stand two days, followed by distillation at 40° C. and a pressure of 350 mm. of mercury—no residue remained.

ABSORPTION TRAIN

The absorption train used for the collection of the active emanation of avocados is diagrammed in figure 1. Oxygen from high pressure cylinders was passed through a tower of brominated activated charcoal (Columbia 4ACW, size 6/14) to remove possible active contaminants by the method of SOUTHWICK and SMOCK (12, 13). This tower consisted of a tube 50 mm. in diameter and 80 cm. long filled one-third full of brominated carbon (A): (24 ml. of bromine on 200 gm. of carbon), followed by twice this volume of unbrominated carbon (B). The efficiency of this method of removal of possible contamination from the gas stream was verified by the etiolated pea test.

After purification, the gas was passed over the fruit, contained in nine jars (C) (connected in series) of the type described by BIALE and SHEPHERD (2). In the bottom of each jar, except the first, was placed 500 ml. of 6-normal sodium hydroxide to absorb the carbon dioxide of respiration, and this was replenished as needed via the drain in the bottom of each jar. The net flow of gas through the absorber, after the appreciable absorption of oxygen by the fruit, was held at 350 ml. per minute through the flowmeter (D). After passing the fruit, the gas stream was dried by a tower of anhydrous calcium chloride (E) and a trap in dry ice-acetone

(F) and then entered the gas scrubbing apparatus described above, consisting of a safety trap (G), a spiral absorber (H), and a "cold finger" dry ice trap (J). Following the absorber was another dry ice-acetone trap (L) and a trap (M) containing sodium hydroxide to prevent any possible escape of bromine vapors.

Experimental procedure and results

HANDLING OF THE FRUIT.—During February, 1947, mature preclimacteric Fuerte avocados were harvested from the experimental orchard of the College of Agriculture, Los Angeles, and twenty-five were placed in each of nine respiration jars (figure 1, C), giving a total fruit weight of 57.0 kg. The fruit was kept at 15° C. throughout the experiment. Since the rate of respiration of avocados is very high, a stream of air at a rate low enough to permit efficient absorption could not provide enough oxygen for the normal metabolism of such a large sample. BIALE (1) showed that the behavior of avocados in pure oxygen was not significantly different from that in air. Therefore, a stream of oxygen (instead of air) was passed over the fruit throughout the experiment. All fruits in composite fruit samples usually seem to enter the respiration climacteric simultaneously, presumably due to its stimulation in all fruits by the production of ethylene in the first fruit to ripen. To expedite the experiment, this effect was simulated by subjecting the jars of experimental fruit to the emanations of ripe avocados for twenty hours before starting the run.

Absorption from the experimental lot was started on the third day and continued for a total of 139 hours. Twice during this period the alkali in the jars was replaced. The fully ripe stage (fruit soft and edible) was reached on the fifth day of the run (the eighth day in storage).

RECOVERY OF THE PRODUCT.—At the end of the absorption period, the solvent and most of the excess bromine were removed from the reaction mixture by distillation at 40° C., starting at a pressure of 600 mm. of mercury and gradually reducing to 300 mm. When approximately five ml. of liquid (mostly bromine) remained, the still was dismantled, and the remaining free bromine removed by cautious addition, with cooling, of sodium bisulfite solution. A small amount of yellow oil separated from the bisulfite solution. This mixture was extracted three times with ether which was in turn washed with half-normal hydrochloric acid and dried over anhydrous calcium chloride. The ether was removed by slow aeration, leaving 650 mg. of light yellow oil.

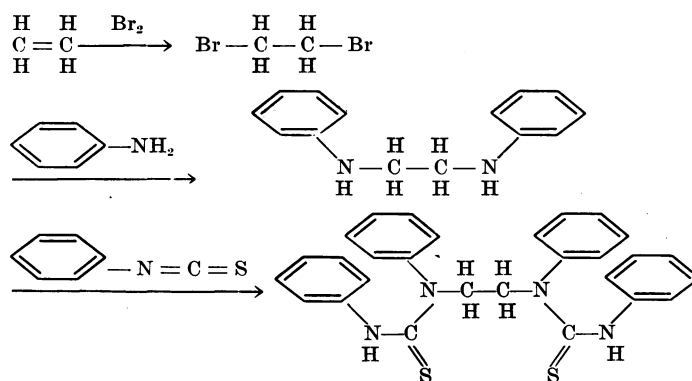
PREPARATION OF DERIVATIVES.—Following the method of GANE (4, 5), 6 ml. of redistilled aniline were added to the entire sample of unknown oil, and the mixture heated for four hours on a boiling water bath. The excess aniline was then driven off by steam distillation, leaving a dark red gummy phase which was separated by chloroform extraction. Removal of the chloroform by aeration left a brown crystalline deposit. This was recrystallized from 50% ethyl alcohol, giving white lustrous flakes melting at 64.4–65.3° C. (uncorrected). Recrystallized N,N'-diphenylethylenedia-

mine (Eastman White Label) melted at 64.3–65.3° C., and the known and unknown mixed at 64.2–65.0° C. (uncorrected).

The above derivatives were converted to *N,N'*-diphenyl-*N,N'*-dianilinothioformyl ethylenediamine by the procedure of DAVIS (3). Approximately 75 mg. of each of the aniline derivatives were mixed in test tubes with 2 ml. of phenylisothiocyanate and heated to boiling for eight minutes. After standing overnight, the reaction mixtures were completely solid. The products were then washed three times with petroleum ether, followed by three washings with 95% ethyl alcohol, and were recrystallized from benzene. Melting point data (uncorrected): From Fuerte avocado derivative, 187.4–188.0° C.; from *N,N'*-diphenylethylenediamine (Eastman White Label), 187.2–188.0° C.; mixture of these, 187.4–188.0° C.

Discussion

The melting point and mixed melting point data of the derivatives described above show that the expected sequence of reactions has indeed occurred, and that ethylene was therefore present as a volatile product of the ripening fruit:



It is interesting to estimate roughly the quantity produced. If the 650 mg. of oil may be assumed to be pure ethylene dibromide, we have the equivalent of 77.5 ml. of ethylene which was recovered from 2920 liters of gas, an average concentration of 26.5 parts per million. This also represents a total production of 0.34 ml. per fruit of 253 gm. average weight, or 1.4 ml. per kg. This may be contrasted with the estimates of 0.0033 ml. per kg. of bananas (10) and with 5 ml. per kg. of apples (4, 5). PRATT and BIALE (11) showed that the peak of ethylene production coincided with the respiratory peak. This presumably occurred during the third day of absorption, at which time determination of ethylene by the microbromination method of HANSEN (6) on a sample of the gas stream from the jars of fruit gave about 135 parts per million. Similar results were obtained in an experiment with Nabal, the leading summer variety of avocados.

Summary

An efficient and convenient method was devised for absorbing and identifying ethylene from low concentrations in gas streams. Using this tech-

nique, ethylene was identified as a volatile product of ripening avocado fruits.

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