

VOLATILE PRODUCTS OF APPLES

III. IDENTIFICATION OF ALDEHYDES AND KETONES

By F. E. HUELIN*

[Manuscript received March 3, 1952]

Summary

The volatile aldehydes and ketones produced by whole Granny Smith apples at 30°C. were identified by paper chromatography and spectral absorption of the dinitrophenylhydrazones, and by conversion of the aldehydes to hydroxamic acids. Acetaldehyde was found to be the major constituent, with smaller amounts of propionaldehyde and acetone.

I. INTRODUCTION

A systematic study of the volatile substances produced by apples is being made in relation to the problem of superficial scald, a functional disorder of cold-stored fruit. A study of the volatile acids and alcohols has recently been published (Thompson 1951*b*). This paper is concerned with the identification of aldehydes and ketones.

Power and Chesnut (1920) obtained the volatile carbonyl compounds by passing air over whole apples and then through bisulphite solution. They identified acetaldehyde by the Rimini test.

White (1950) examined a volatile fraction of apple juice, and found acetaldehyde, acetone, caproaldehyde, and 2-hexenal. The dinitrophenylhydrazones were separated and identified by melting points and elementary analysis. One cannot be certain that all these substances are present in whole apples, as they may be produced during extraction and distillation of the juice.

This paper describes a further study of the volatile carbonyl compounds produced by whole apples. The methods of White were not applicable to the comparatively small quantity of material available, and use was made of paper chromatography and spectral absorption.

II. COLLECTION OF SAMPLE FROM AIR STREAM

Air, purified by combustion of organic matter and absorption of carbon dioxide, was passed over 10 kg. of Granny Smith apples at 30°C. The apples had been picked commercially mature and subsequently stored for 4-7 months at 0°C. The volatile carbonyl compounds were absorbed in 30 ml. of concentrated metabisulphite solution (25 g. $K_2S_2O_5$ per 100 ml.) contained in a spiral absorber cooled in ice. After 4 days the absorbing solution was transferred to a stoppered bottle, and the absorber was washed with a small amount of water.

* Division of Food Preservation and Transport, C.S.I.R.O., Homebush, N.S.W.

After each collection the apples were examined for rots and other lesions. In the course of 18 collections only one rot, 1 cm. in diameter, and no other lesions were found. Hence the substances identified were definitely produced by sound tissue.

The absorbing solutions and washings from several collections were bulked in the bottle and stored at 1°C. This bulk solution was finally made alkaline with sodium carbonate (to liberate the free carbonyl compounds) and distilled to about one-quarter of the original volume. The distillate was collected in an ice-cooled receiver and stored at 1°C. It was free from volatile acids but contained alcohols and a trace of esters in addition to carbonyl compounds.

III. CONVERSION TO DINITROPHENYLHYDRAZONES

A solution of the dinitrophenylhydrazones of the carbonyl compounds was prepared as follows. To 40 ml. of the aqueous distillate was added 25 ml. of 2,4-dinitrophenylhydrazine solution (0.1 per cent. in 1N HCl), and the mixture was allowed to stand for 1 hour at 25°C. Longer periods of standing up to 24 hours made no difference to the final result. The dinitrophenylhydrazones were extracted with 25 ml. of a mixture of light petroleum and benzene in equal proportions. The solution of dinitrophenylhydrazones was washed with small amounts of water, filtered, and evaporated to about 20 ml. It was then washed into a 25 ml. volumetric flask and made to volume with light petroleum.

The volatile ketones were converted separately to dinitrophenylhydrazones after removal of the aldehydes by oxidation. The most suitable oxidizing agent was found to be freshly precipitated silver oxide in an alkaline solution. This oxidized dilute solutions of aldehydes very readily with little effect on ketones or alcohols. In the procedure adopted, 40 ml. of the aqueous distillate was mixed with 5 ml. of 1N silver nitrate followed by 5 ml. of 2N sodium hydroxide. All solutions were cooled to 0°C. before mixing. The mixed solution was shaken for 1 hour at 0°C. The precipitated silver oxide was then filtered and washed at 0°C. The filtrate was acidified with 5 ml. of 2N hydrochloric acid. The derivatives of the ketones were prepared from the filtrate by the procedure already described, and the solution of the dinitrophenylhydrazones was made to 25 ml.

IV. PAPER CHROMATOGRAPHY OF DINITROPHENYLHYDRAZONES

The separation of the dinitrophenylhydrazones of simple aldehydes and ketones by paper chromatography was investigated in order to identify the volatile products of the apple. The system water-butanol, recommended by Cavallini, Frontali, and Toschi (1949) for the derivatives of keto acids, was found unsuitable for simple aldehydes and ketones, whose derivatives travelled practically with the front. Workers of the Food Investigation Board of Great Britain have proposed the system methanol-heptane, and this system was found suitable for separating simple aldehydes and ketones containing up to four

carbon atoms. A petroleum fraction boiling between 95 and 105°C. was used in place of pure heptane.

The procedure involved upward movement of solvent in a relatively simple apparatus. A battery jar, 8 in. square and 20 in. high, contained a flat, circular dish of diameter 6 in. Spots containing approximately 10^{-7} mole of each derivative were placed about 1.5 in. from the bottom of a sheet of Whatman No. 1 paper 18 in. high. Spots should be not less than 1.5 in. apart. The sheet was formed into a cylinder and placed in the inner dish.

Methanol (50 ml.) and "heptane" (100 ml.) were shaken together in a separating funnel. The mixture separated into approximately 75 ml. of each phase. The lower, methanol-rich phase was transferred to the bottom of the battery jar outside the dish carrying the paper. The jar was covered by a glass plate sealed with grease and the system was left to equilibrate overnight. A square of filter paper pasted to the bottom of the glass plate was found to assist equilibration and prevent any condensate dripping back. In the morning the "heptane" phase was transferred to the inner dish and the glass cover replaced. The mobile phase travelled fairly rapidly up the paper, which was usually removed after 6-8 hours.

On drying the paper in a current of air the dinitrophenylhydrazones were visible as faint yellow spots. Spraying with 10 per cent. aqueous sodium hydroxide gave much more intense colours—dark orange with simple aldehydes and ketones, and red to blue with some dicarbonyl compounds.

R_F values of the same derivative varied somewhat in different runs, but were in good agreement when taken from the same paper. Hence derivatives from the apple could be readily identified by running them alongside known derivatives in the same chromatogram. The usual values for simple aldehydes and ketones were as follows: formaldehyde 0.35-0.40, acetaldehyde 0.50-0.55, propionaldehyde and acetone 0.70-0.75, butyraldehyde and methyl ethyl ketone 0.85-0.90. The R_F values of the aldehyde and ketone of the same carbon number did not differ significantly. Diacetyl travelled level with formaldehyde and furfural just behind, while glyoxal remained at the origin.

The derivatives from the apple were identified by the following procedure. Spots containing derivatives of (a) aldehydes with one to four carbon atoms, (b) apple aldehydes and ketones, (c) apple ketones, and (d) ketones with three and four carbon atoms were placed from left to right on the same paper. Equivalent amounts of the apple derivatives (0.1 ml. of each solution) were contained in spots b and c. A photograph of the chromatogram (using pan-chromatic film with a green filter) is shown in Plate 1.

Among the apple derivatives acetaldehyde predominated with smaller amounts of propionaldehyde and acetone. The presence of both propionaldehyde and acetone was indicated by the fact that the spot containing both derivatives (3b) was larger than the spot containing acetone alone (3c). This conclusion was confirmed by tests to be described in subsequent sections. Faint spots, which gave a similar orange colour on spraying, remained at the origin. The spots at the origin were not obtained if the solution of the derivatives was first poured through a column of anhydrous magnesium sulphate.

V. SPECTRAL ABSORPTION OF DINITROPHENYLHYDRAZONES

Solutions of the dinitrophenylhydrazones in ethanol gave characteristic colours on addition of ethanolic potassium hydroxide. The colour faded slightly after mixing but became relatively stable after the first hour. An aliquot of a dilute ethanolic solution of the derivative was measured into a 10 ml. volumetric flask, followed by 1 ml. of 0.5N ethanolic potassium hydroxide and sufficient ethanol to bring to volume. After standing for an hour the absorption was measured between 400 and 700 $m\mu$ with a Beckman DU spectrophotometer.

The derivatives of the simple aldehydes and ketones all gave a maximum absorption at 420-430 $m\mu$, and the absorption curves did not differ sufficiently to distinguish readily between aldehydes or ketones of different carbon number. There was, however, a slight but characteristic difference between the aldehyde and ketone curves, which was used to confirm the presence of acetone in the apple volatiles. The absorption of the ketone derivatives gave a slight rise from 490 to 520 $m\mu$ with a subsidiary maximum at 520 $m\mu$, while the aldehyde curves only gave inflections in this region.

A solution of acetone derivative from the apple was obtained by the following procedure. The solution of apple volatiles (200 ml.) was oxidized with silver oxide and the derivatives prepared. The solution of dinitrophenylhydrazones in equal parts of light petroleum and benzene was poured through a column of anhydrous magnesium sulphate and washed through with further solvent mixture. The solution was evaporated to dryness and the residue crystallized twice from light petroleum. The purified derivative was dissolved in benzene and applied as a series of spots on sheets of filter paper. Chromatograms were run, and the spots corresponding to acetone were cut out and extracted with ether. The ether was evaporated and the derivative made to 10 ml. with ethanol. An aliquot of this solution was used for obtaining the spectral absorption curve.

For comparison of the apple derivative with known derivatives, the function $\log D_{\lambda} - \log D_{400}$ was plotted against wavelength (D_{λ} = optical density at wavelength λ and D_{400} = density at 400 $m\mu$). This function is independent of concentration where Beer's law is obeyed, i.e. up to $2 \times 10^{-4}M$ for the dinitrophenylhydrazones concerned. The curves of the derivatives of pure propionaldehyde and acetone, and of the apple derivative, are given in Figure 1. The curve of the apple derivative corresponds closely to that of the acetone, and is different from that of the propionaldehyde derivative.

The concentration of mixed aldehyde and ketone derivatives from the apple was calculated approximately from the absorption at 420 $m\mu$, taking acetaldehyde as standard. The production of total carbonyl compounds by the apples was estimated at about 7 μ moles/kg./24 hr. This figure is only approximate, as the method has not been adapted for quantitative work.

VI. CONVERSION OF ALDEHYDES TO HYDROXAMIC ACIDS

The presence of acetaldehyde and propionaldehyde was confirmed by conversion to hydroxamic acids which were identified by paper chromatography

(Thompson 1951a). The solution of apple volatiles (40 ml.) was oxidized with silver oxide as previously described. The filtrate was acidified with sulphuric instead of hydrochloric acid. The oxidized solution was then distilled to about one-fifth of the original volume. The distillate, which contained the acids from oxidation of aldehydes, was neutralized to pH 8 with dilute sodium

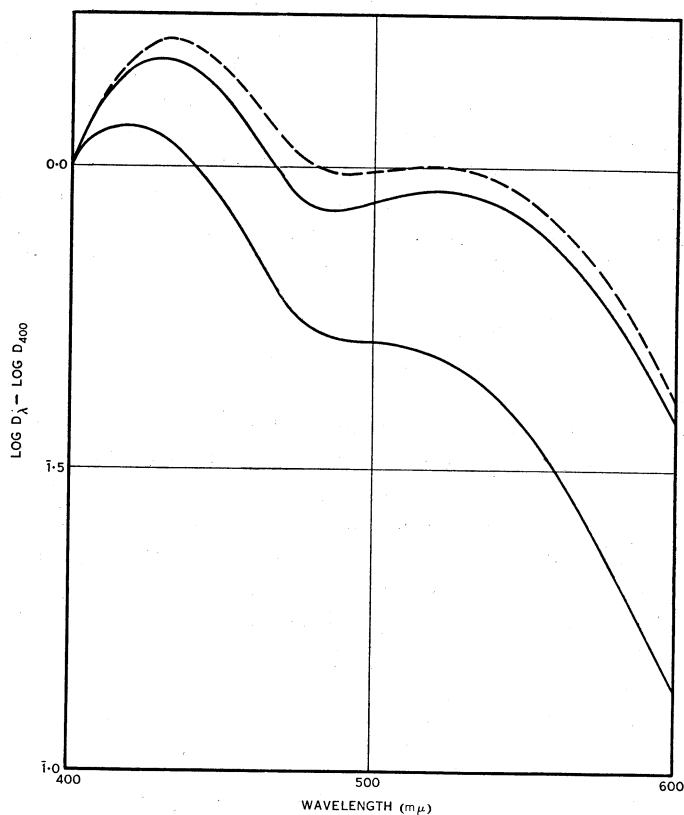


Fig. 1.—Spectral curves of dinitrophenylhydrazones. Propionaldehyde (pure): lower continuous curve. Acetone (pure): upper continuous curve. Acetone (apple): broken curve.

hydroxide and evaporated to dryness. The dry residue of sodium salts was converted to hydroxamic acids by the method of Thompson (1951b), and half the total quantity was applied as a spot to the paper. In a chromatogram using amyl alcohol and acetic acid, hydroxamic acids containing one to four carbon atoms were run alongside hydroxamic acids derived from the apple aldehydes. A photograph of the chromatogram is shown in Plate 2.

The presence of acethydroxamic and propionhydroxamic acids is clearly shown, confirming the presence of acetaldehyde and propionaldehyde in the original solution of volatiles. There was no interference by free acids in the original solution, as these were in negligible concentration. To investigate

interference by esters, a further 40 ml. of the original solution was extracted with ether and the esters converted to hydroxamic acids. Half the total quantity (equivalent to the hydroxamic acids from aldehydes) was applied to the paper. The chromatogram showed a comparatively faint spot in position 2 and none in position 3, indicating negligible interference.

As silver nitrate was used in the oxidation of the aldehydes, the distillate and the sodium salts obtained on evaporation may have contained nitrate. It was demonstrated that 30 mg. of sodium nitrate gave no spots on the chromatogram. As a further check, the oxidation was repeated with an equivalent amount of silver sulphate (instead of nitrate) but with no other modification. On account of its low solubility solid silver sulphate was added directly to the solution of volatiles and shaken for 30 minutes before adding sodium hydroxide. As before only acethydroxamic and propionhydroxamic acids were obtained on the chromatogram.

Another sample of sodium salts, free from nitrate, was tested for unsaturation by measuring hydrogen uptake in the presence of colloidal palladium (Milton and Waters 1949). The result indicated less than one double bond in 100 moles of aldehydes.

VII. DISCUSSION

Acetaldehyde and propionaldehyde have now been identified as the predominant volatile aldehydes produced by whole Granny Smith apples at 30°C. after 4-7 months at 0°C. In contrast White (1950) found acetaldehyde, caproaldehyde, and 2-hexenal in the volatile portion of juice derived from McIntosh and Stayman Winesap apples. It is plausible to assume that intact tissue produces caproaldehyde and 2-hexenal as well as the lower aldehydes. This assumption is supported by the identification of hexyl alcohol and caproic acid as products of whole apples (Thompson 1951*b*). On this view the difference in the aldehydes identified would be due to variety or the physiological state of the fruit rather than to changes during the preparation and distillation of the juice. It is even possible that other aldehydes may predominate in the volatile products of Granny Smith apples at lower temperatures.

Fatty acids and alcohols containing one, two, three, four, five, and six carbon atoms have been identified in the volatile products of apples (Thompson 1951*b*; White 1950). The aldehydes may be intermediates in the interconversion of acids and alcohols, and acetaldehyde probably arises also from decarboxylation of pyruvic acid, an important intermediate of respiration.

The acetone is probably a product of fatty acid metabolism. It could arise from decarboxylation of aceto-acetic acid, an intermediate in the β -oxidation of fatty acids.

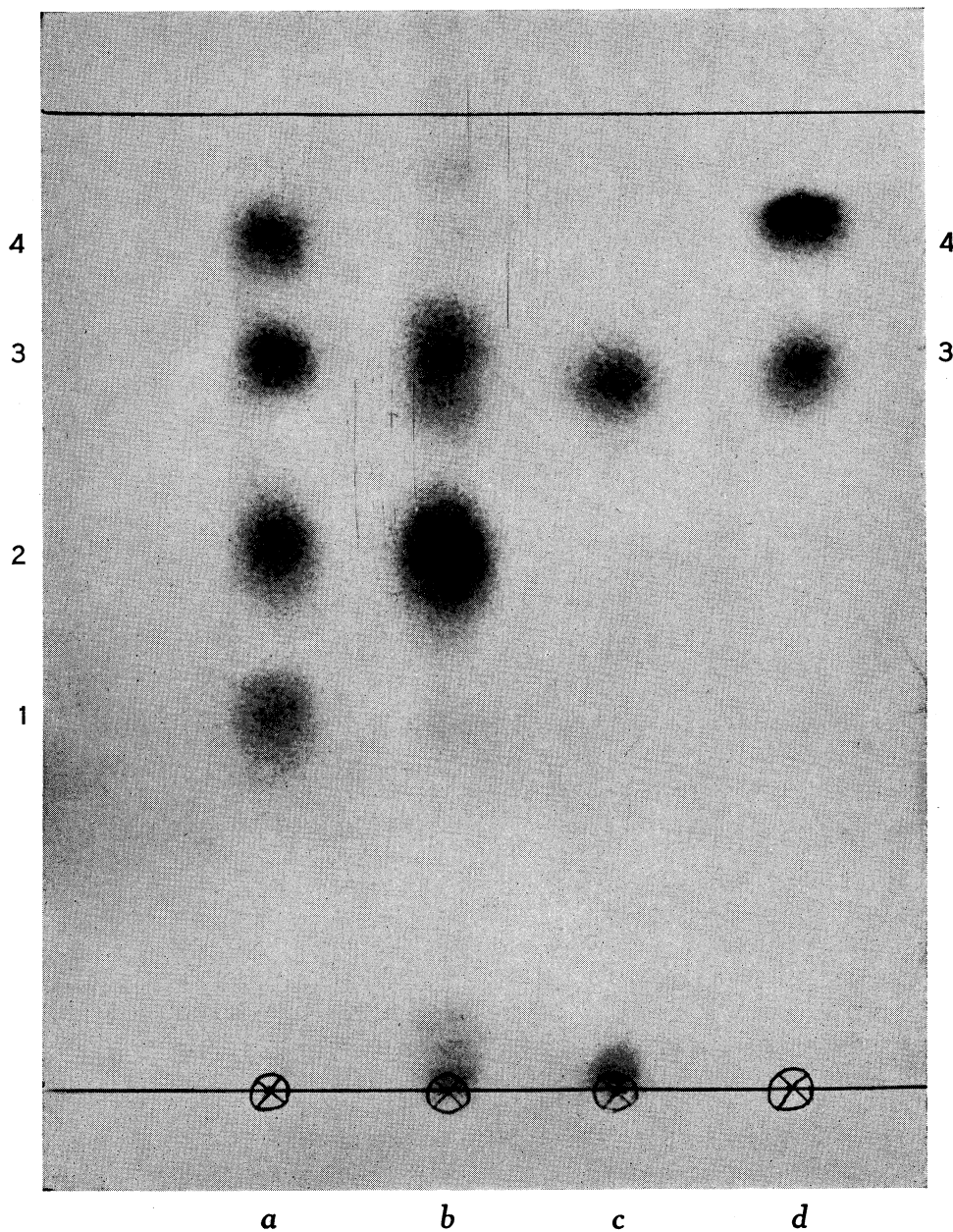
VIII. ACKNOWLEDGMENT

The author is indebted to Mr. Bruce Kennett for technical assistance.

IX. REFERENCES

- CAVALLINI, D., FRONTALI, N., and TOSCHI, G. (1949).—*Nature* **163**: 568.
- MILTON, R. F., and WATERS, W. A. (1949).—"Methods of Quantitative Micro-analysis."
(Edward Arnold & Co.: London.)
- POWER, F. B., and CHESNUT, V. K. (1920).—*J. Amer. Chem. Soc.* **42**: 1509.
- THOMPSON, ADRIENNE R. (1951*a*).—*Aust. J. Sci. Res. B* **4**: 180.
- THOMPSON, ADRIENNE R. (1951*b*).—*Aust. J. Sci. Res. B* **4**: 283.
- WHITE, J. W. (1950).—*Food Res.* **15**: 68.

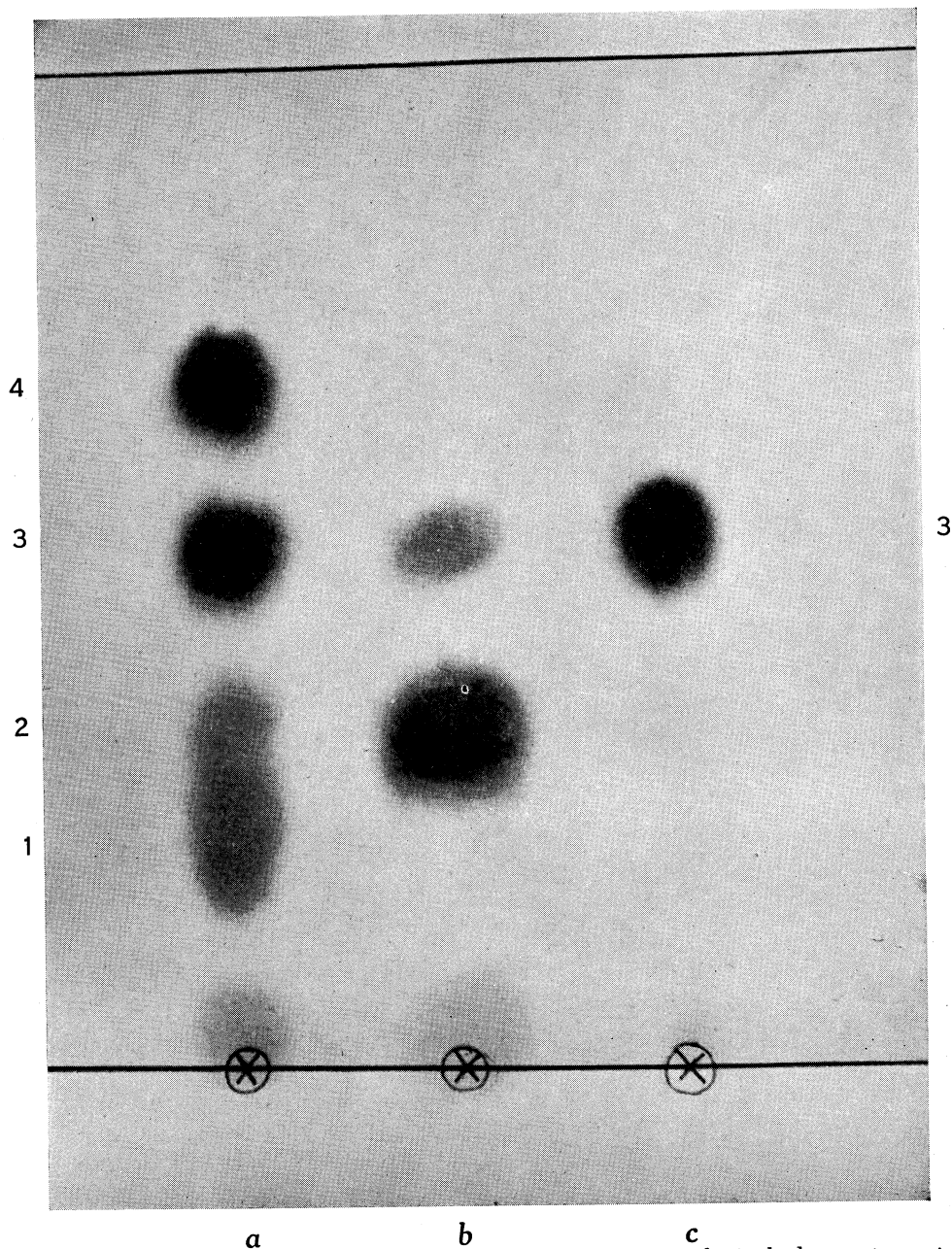
VOLATILE PRODUCTS OF APPLES. III



Paper chromatogram run with methanol-"heptane" showing dinitrophenylhydrazones derived from:

- (a) Aldehydes with one to four carbon atoms (as numbered),
- (b) Apple aldehydes and ketones,
- (c) Apple ketones, and
- (d) Ketones with three and four carbon atoms.

VOLATILE PRODUCTS OF APPLES. III



Paper chromatogram run with amyl alcohol-acetic acid showing ferric hydroxamate spots derived from:

- (a) Hydroxamic acids with one to four carbon atoms (as numbered),
- (b) Apple aldehydes, and
- (c) Propionhydroxamic acid.

