A Sensitive Method for the Determination of Carbonyl Compounds[1]

D.C. JOHNSON and E.G. HAMMOND,
Department of Food Technology, Iowa State University, Ames, Iowa 50010

Abstract

2,4,6-Trichlorophenylhydrazine was tested as a reagent for carbonyl compounds. As little as 0.1 of the 2,4,6-trichlorophenylhydrazones (2,4,6-TCPH) could be measured with an electron capture detector, so this reagent should be useful in measuring the carbonyl compounds in oxidized fats at levels near their flavor thresholds. Mixtures of 2,4,6-TCPHs were separated by thin layer chromatography. Alkan-2-one-2,4,6-TCPHs were separated from aldehyde-2,4,6-TCPHs on alumina plates. The alkanal, alk-2-enal and alk-2,4-dienal-2,4,6-TCPHs were separated from each other either on silica gel plates or silica gel-silver ion plates. The derivatives within each carbonyl class were separated by chain length on chromatography media impregnated with phenoxyethanol. The 2,4,6-TCPHs eluted from thin layer plates were determined with an electron capture detector after gas chromatography on a 30 cm column of freeze-dried detergent base coated with a silicone oil.

Introduction

In the oxidation of lipids, carbonyl compounds are usually among the final degradation products formed. Considerable work has been done on the isolation and identification of carbonyl and other flavor compounds (1–4). Some of these carbonyl compounds are very potent flavors and are organoleptically detectable at about 1 ppb. This work was undertaken to find a procedure to separate and analyze carbonyl compounds at this low level.

Methods

2,4,6-Trichlorophenylhydrazine

2,4,6-Trichlorophenylhydrazine (2,4,6-TCPH) Eastman Organic Chemicals) was twice recrystallized from 1N HCl before use.

Hexane, benzene and diethyl ether were purified according to Schwartz and Parks (5) and then refluxed over silver nitrate and sodium carbonate before distillation. This procedure gave solvents that gave little or no response in the electron capture detector.

Carbonyls

Alkanals and alkan-2-ones were purchased from commercial sources and distilled through a Widmer column. The distillate gave only one peak on gas chromatography on butanediol succinate. The alk-2-enals were synthesized following Bedoukian's (6) directions. Pippen and Nonaka's (7) procedure for preparing the alk-2,4-dienals was used.

Reaction Column

The reaction column was prepared as follows: 0.40 g of 2,4,6-TCPH or other chlorinated phenylhydrazine was dissolved in 40 ml of 1 N HCl with heating and mixed with 40 g of Celite 545. Hexane was added to the damp Celite to form a slurry and packed with slight tamping into columns (30 × 2 cm). To prepare derivatives, half the theoretical column capacity of carbonyl compound was added to the column and eluted with 75 to 100 ml of hexane. The hexane was removed under reduced pressure, and the viscous derivative was stored in 10 ml of hexane at −27 C. As acetone and formaldehyde formed crystalline derivatives, they were also prepared by Chattaway and Irving's (8) procedure.

Thin Layer Chromatography

Plates of alumina, Silica Gel G (Brinkmann Instruments, Inc.) and Silica Gel G impregnated with silver nitrate were used. Samples were applied as solutions in hexane, or for preparative plates, with a streak applicator (Applied Science Laboratories, Inc.). Development was done in closed jars lined with solvent-soaked filter paper. After development, the plates were sprayed with 2,7-dichlorofluorescein and viewed under an UV light to facilitate location of the spots.

Urbach's (9) procedure was adapted to separate the alkan-2-ones from alkanals. Diethyl ether in hexane (2:98 v/v) was used to develop the alumina plates.

Both Silica Gel G and Silica Gel G impregnated with 25% silver nitrate were used to separate the saturated carbonyl 2,4,6-TCPHs from those of the alk-2-enals and the alk-2,4-dienals. The solvent system was diethyl ether-hexane (16:84 v/v).

Preparative Silica Gel G plates for purification were developed in diethyl ether-hexane (20:80 v/v).

Urbach's (9) partition system for chain length separation was adopted. Gelman Chromatography Media (Gelman Inst. Co., Michigan) were soaked in 10% phenoxyethanol in acetone and air-dried. Several ascents in pure hexane were needed for resolution of the 2,4,6-TCPHs.

Gas Chromatography

The TCPHs were analyzed on a Varian Aerograph Hy-Fi 600 gas chromatograph, equipped with an electron capture detector. Columns were glass with an ⅛ in. outside diameter. Columns of different lengths were used. The freeze-dried gas chromatographic support was prepared by Harper and Hammond's (10) procedure, using a 51% solids mixture.

When necessary, the tritium foil was cleaned by Holden and Wheatly's (11) procedure. Jewelers rouge was used to polish the foil.

Results and Discussion

To test the relative sensitivity of the electron capture detector to various chlorinated phenylhydrazones, acetone derivatives were prepared, and

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TABLE I

Electron Capture Response of Acetone Chlorinated Phenylhydrazones[a]

Reagent	Response of recorder range, %[b]
o-chlorophenylhydrazine	2
p-chlorophenylhydrazine	3
N-*o*-chlorophenylhydrazine	0
3,5-dichlorophenylhydrazine	20
2,4,6-trichlorophenylhydrazine	70

[a] Six-foot 10% SF 961 chromosorb W column at 185 C injection port 205 C, and 90 ml/min of purified nitrogen.
[b] Full recorder response was 1 mv.

equal concentrations were injected into the gas chromatograph. Table I shows the various responses of the hydrazones. These results indicated that the greatest sensitivity could be achieved with 2,4,6-TCPH.

2,4,6-TCPHs may be formed by adding carbonyl compounds to acidified solutions of 2,4,6-trichlorophenylhydrazine (8). Except for acetone and a few aromatic aldehydes, however, none of the carbonyls were reported to form crystalline derivatives. With 2,4-dinitrophenylhydrazine, similar methods do not give quantitative yields of derivatives, and better yields have been obtained with reaction columns (12). Therefore, a reaction column modeled after those used to make 2,4-dinitrophenylhydrazones was used in these experiments. With this method, only a few carbonyls (acetone, formaldehyde and the diketones) formed crystalline 2,4,6-TCPHs. The others formed viscous oils.

The yield of 2,4,6-TCPH derivative formed by passage of a carbonyl compound through a reaction column was checked with acetone, since it forms a derivative that can be purified by crystallization. A measured amount of reagent grade acetone was introduced onto the column. The derivative was diluted in hexane to the proper concentration (5×10^{-10} g, assuming 100% yield) and injected onto a 30 cm column containing 10% Dow Corning silicone oil 200 on freeze-dried support at 145 C. The area under the peak was compared with that obtained from the injection of a solution containing 5×10^{-10} g of the crystalline derivative. There was a recovery of 97.9% of the acetone added to the column as the 2,4,6-TCPH. Recent work by Badings (13) and Craske and Edwards (14) indicates that carbonyl with a vinyl group alpha to the carbonyl group do not react quantitatively on 2,4-dinitrophenylhydrazine columns. The same would probably be true with 2,4,6-TCPH columns.

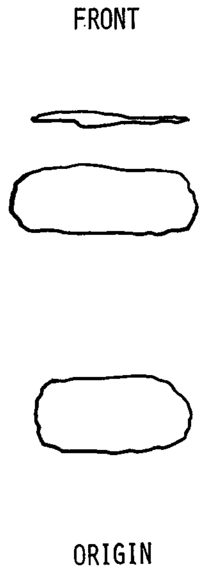

FIG. 1. Preparative thin layer plate of a one-week-old 2,4,6-TCPH. The hydrazone is the middle spot and the lowest spot is 2,4,6-trichlorophenylhydrazine.

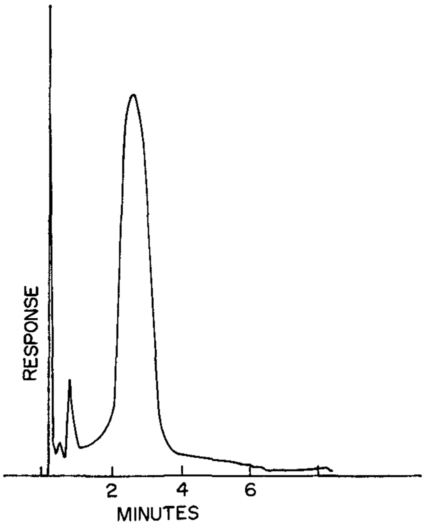

FIG. 2. Gas chromatogram of acetone-2,4,6-TCPH. The oven was 200 C, injection port was 225 C and the flow rate of purified nitrogen was 115 ml/min.

The stability of the 2,4,6-TCPHs was very poor, possibly because they are not crystalline. Immediately after coming off the reaction column, the derivatives showed only trace amounts of impurity by thin layer chromatography (TLC). Usually within a week, the thin layer plates resembled those in Figure 1. The faster-moving spot appeared only on heavily inoculated plates of aged derivatives. It was a very minute impurity. The middle spot showed up as a blue shadow. It had most, if not all, of the electron capture response. The lowest spot was usually highly colored and sometimes smelled aldehydic. It sometimes gave a slight response on the electron capture detector. The free base form of the 2,4,6-TCPH reagent migrated similarly to the lowest spot on the thin layer plates and gave the same appearance. Evidently the 2,4,6-TCPHs are hydrolyzed during storage. Preparative TLC was used to purify aged 2,4,6-TCPH derivatives before use. After elution from the plates, the 2,4,6-TCPHs (middle spot)

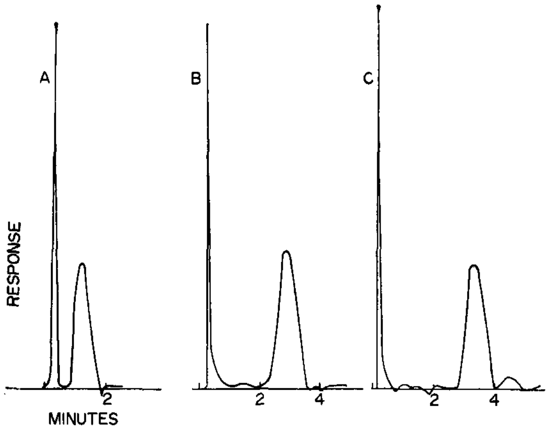

FIG. 3. Gas chromatograms of (A) propanal-2,4,6-TCPH, (B) hexanal-2,4,6-TCPH, and (C) nonanal-2,4,6-TCPH. A 1 ft column of 10% Dow-Corning silicone oil 200 on freeze-dried support was used. The oven was 145 C, injection port 170 C and a flow rate of 115 ml/min was used.

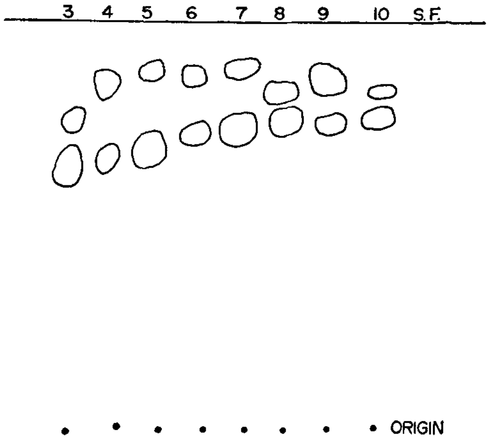

FIG. 4. Thin layer separation of 2,4,6-TCPHs of homologous series of alkan-2-ones and alkanals on Silica Gel G. The solvent system was hexane-diethyl ether (98:2 v/v). The alkan-2-ones are the faster moving spots.

seemed more stable and did not decompose very rapidly.

Many supports and stationary phases were tried in attempts to separate the 2,4,6-TCPHs by gas chromatography. None of the systems tried were very good. Most gave peaks that tailed badly and had very poor plate efficiencies. Metal columns contributed to the tailing, while a glass column eliminated much of the tailing.

The best column had a support of freeze-dried detergent base coated with a stationary phase of 10% Dow Corning silicone oil 200. This packing gave symmetrical peaks and convenient retention times with the 2,4,6-TCPHs. Figure 2 shows a gas chromatogram of acetone-2,4,6-TCPH. The plate efficiency is low (only 70–80 theoretical plates). Chlorotoluene and chlorobenzene, which are detectable by electron capture, were tested on the same detergent columns and gave a much higher number of theoretical plates (800). Possibly the reason for the poor plate efficiencies of the 2,4,6-TCPHs is association of the derivative in the silicone oil, in which case, high column efficiency may not be attainable on nonpolar columns. If polar stationary phases are used, the retention times are very long and low sensitivity results. The electron capture detector used here was based on tritium foil and had a temperature limit of 225 C. If a different types of electron capture detector had been available, higher temperatures might have allowed the use of more polar phases and cut down on the association.

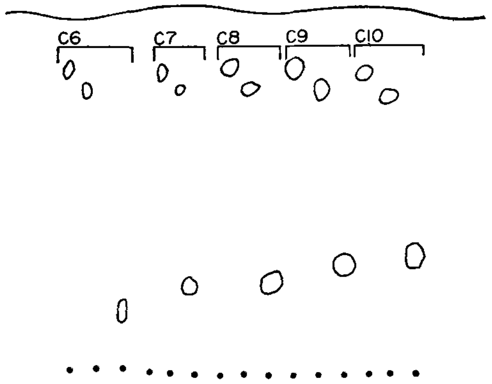

FIG. 5. Thin layer separation of 2,4,6-TCPHs of homologous series of alkanals, alk-2-enals, and alk-2,4-dienals on Silica Gel G. The solvent system was hexane-diethyl ether (84:16 v/v). The alkanals are the fastest moving spots, the alk-2-enals are the middle spots, and the alk-2,4-dienals are the slowest moving spots.

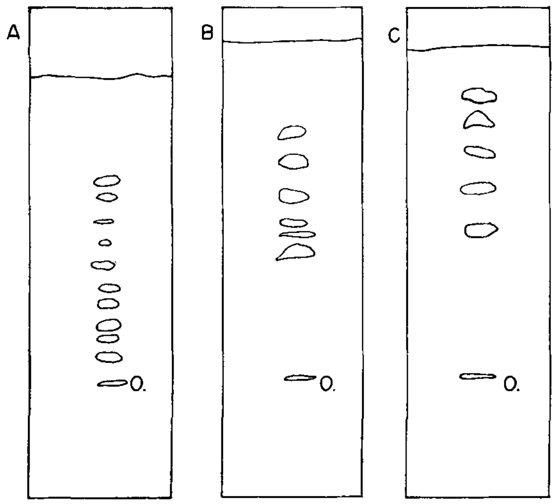

FIG. 6. Chain length separation of 2,4,6-TCPHs of (A) alkanals, (B) alk-2-enals, and (C) alk-2,4-dienals on phenoxyethanol impregnated silica gel media. Hexane was used to develop the plates.

There was no way to separate classes of 2,4,6-TCPHs such as alkanals, alk-2-enals, alkan-2-ones and alk-2,4-dienals from each other on the gas chromatograph. Even chain length separation was difficult, as most of the longer derivatives tended to overlap.

On the 6 ft silicone-oil column, 10^{-9} to 10^{-10} g of the 2,4,6-TCPH derivatives of carbonyls up to six carbons long gave peaks as high as 50% of the full recorder scale. Because of the poor plate efficiency of the column, the longer chain carbonyl compounds require 10^{-6} g to 10^{-7} g to give similar deflections.

Tripp et al. (15), in a paper at the American Oil Chemists' Society Meeting in April 1969 reported that they also used 2,4,6-TCPH derivatives as one method to identify carbonyl compounds. They mentioned that double peaks were obtained on gas chromatography of 2,4,6-TCPH derivatives. Double peaks were found in our early work, but with purification of the solvents and the use of glass columns, only single peaks were seen. Tripp et al. (15) attributed the double peaks to syn and anti isomers, but our results tend to discount this possibility.

In contrast to the poor resolution achieved by gas chromatography, chain length and class separation of the 2,4,6-TCPHs were both possible by TLC, so attempts were made to use the electron capture detector simply as a quantitation device for derivatives eluted from thin layer plates. A short column of 10% Dow Corning silicone oil 200 on freeze-dried support resulted in symmetrical peaks and acceptable plate efficiency. Figure 3 shows the gas chromatograms of the 2,4,6-TCPH derivatives of propanal, hexanal and nonanal. Injection of 5×10^{-10} g gave recorder responses of 22%, 24% and 22% of full scale.

TLC was modeled after methods used with 2,4-dinitrophenylhydrazones. Alumina plates separated the alkan-2-one-TCPHs from the aldehyde-TCPHs (Fig. 4). The alkan-2-ones were the faster-moving compounds. These plates were developed twice with hexane-diethyl ether (98:2 v/v).

The TCPHs of the alkanals, alk-2-enals and the alk-2,4-dienals were separated in two ways. The first and best method was on plain silica gel developed in hexane-diethyl ether (84:16 v/v) solvent systems. These results are illustrated in Figure 5. The resolution of the alkanals and alk-2-enals-2,4,6-TCPHs was not as good as the corresponding separations achieved with 2,4-dinitrophenylhydrazones. The resolution was not appreciably improved on silica gel impregnated with 25% silver nitrate. In both systems, the alk-2,4-dienals were easily separated from both the alkanals and alk-2-enals. The plain silica gel plate was preferred, as the silver nitrate interfered with the 2,7-dichlorofluorescein indicator.

Strips of silica gel chromatography media impregnated with phenoxyethanol were used for chain length separations (Fig. 6). The separation was very good, but streaking occasionally occurred if the media strips were overloaded. The spots were detected by holding the strips over an incandescent lamp.

On thin layer plates, about 10^{-6} or 10^{-7} g of 2,4,6-TCPH derivative could be visualized. For more sensitive work, guide spots could be run on the side of the plate, and the corresponding zones could be eluted from the plate and quantified with the electron capture detector.

The problem of quantitative isolation of carbonyl compounds from oxidized fat remains an incompletely solved problem; however, this method will increase the sensitivity of detection of the carbonyl that are isolated. A general scheme for the analysis of carbonyl compounds from oxidized fat might be: after distillation of the volatile carbonyl compounds from the fat, a proper concentration of the distillate would be introduced onto a reaction column. The hexane elutant would be concentrated, then purified on preparative thin layer plates. The first class separation would be on silica gel to isolate the saturated carbonyl compounds (alkanals and alkan-2-ones) from the alk-2-enals and the alk-2,4-dienals. The alkanals and alkan-2-ones would be separated on alumina plates. Each class would be run on phenoxyethanol impregnated silica gel media to make chain length separation. The individual compound would be injected into the gas chromatograph with the electron capture detector for the quantitative determination at the nanogram level.

ACKNOWLEDGMENTS

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