Determination of Aldehydes in Mixtures of Mono- and Dicarboxylic Acids

E. P. CROWELL and B. B. BURNETT, Union Camp Corporation, P.O. Box 412, Princeton, New Jersey 08540

Abstract

Determination of total aldehydes in fatty acid oxidation products was accomplished by using a colorimetric procedure based on 2,4-dinitrophenylhydrazine derivatives. To overcome problems of high reagent blanks and poor reproducibility the carbonyl derivatives were separated from excess reagent by extraction. Solubility and phase interface problems were avoided by using a sample dispersing solvent. Potential interferences were explored. The final procedure was demonstrated to be applicable from 25 μmoles to 2 mmoles/g carbonyl with a relative standard deviation of 3.5%.

Introduction

A need developed in our laboratory for a method to determine small amounts of aldehydes in samples resulting from the ozonolysis-oxidation of unsaturated fatty acid mixtures. The product samples were primarily mixtures of mono- and dicarboxylic acids and were expected to contain 1% to 5% aldehydes. The technical problems associated with this determination were interference of carboxylic acids, low solubility of the sample in aqueous media, and the fact that some of the aldehydes present could be bifunctional aldehydic acids. Since all of our products were prepared from oleic and linoleic acid feed stocks, we were primarily concerned with nonanal and aldehydononanoic acid.

The general approaches to the determination of aldehydes involve volumetric or gravimetric analysis employing addition, oxidation or reduction (1). Colorimetric methods have been reported based on 2,4-dinitrophenylhydrazones (2,4-DNPH) and Schiff's base formation. Most of the titrimetric methods could not be considered because they were applicable only to aqueous systems, suffered from interferences by the fatty acids, and lacked sensitivity.

A colorimetric method based on the 2,4-DNPH derivative appeared to offer the best possibility for success. However, unreacted reagent interfered with the absorptivity of the derivatives. Henick et al. (2) attempted to circumvent this problem by working with the longer wavelength absorbing complex which forms in alkaline solutions. It has been our experience that the alkaline method is troublesome because of high reagent blanks, poor reproducibility and instability of the color complex in dicarboxylic acid systems. The approach of Toren and Heinrich (3) to reagent interference was to extract the derivative from the excess reagent.

Heistand (4) expanded the extraction approach and showed the need for heat to bring the reaction to completion. This method appeared to offer a good solution to our problem, and was the starting point for our investigation. It is our intention to describe a method that permits quantitative determination of carbonyl compounds in samples insoluble in water or aliphatic hydrocarbons, to demonstrate further the advantage of the extraction methods over the alkaline 2,4-DNPH methods, and finally, to explore interferences that may be encountered with fatty acid samples where aldehyde content can be a significant quality factor.

Experimental Procedures

Reagents

Carbonyl Free Methanol. Reagent grade methanol was further purified by refluxing 1 gal with 17 g of 2,4-dinitrophenylhydrazine and 5 ml of concentrated hydrochloric acid for 4 hr. The methanol was then distilled through a Vigreaux column, discarding the first 100 ml, then collecting distillate until the condensate at the top of the condenser appeared yellow.

2,4-DNPH Reagent. A saturated solution of 2,4-dinitrophenylhydrazine (Eastman Organic Chemicals) in 20% v/v phosphoric acid was prepared fresh daily.

Cyclohexane. Nanograde cyclohexane (Mallinckrodt) was found to be sufficiently pure for this application.

Method of Analysis

About 40 mg samples were weighed into 125 ml polyethylene stoppered Erlenmeyer flasks and then dissolved in exactly 5 ml of purified methanol. Exactly 25 ml of 2,4-DNPH reagent was added, and the flasks were stoppered tightly and then heated in a 60 C water bath for 30 min. When the samples had cooled to room temperature, 25 ml of cyclohexane was pipetted into the flasks and the flasks were shaken for 90 min on a mechanical shaker. The phases were then allowed to separate and a 5 ml aliquot of the cyclohexane solution was transferred to a 25 ml volumetric flask and diluted to volume with additional cyclohexane. The optical absorbance of the final solution was then determined at 339 nm versus a suitable reagent blank.

Standardization of Method

For our purposes we considered it appropriate to calibrate the method using nonanal as the standard. Commercially available nonanal was assayed using the oxime titration procedure given by Siggia (1). This material was used to prepare methanol solutions in the concentration range of 0.5 to 2.5 mmole/liter. Five milliliter aliquots of these solutions were then carried through the procedure described above.

Results and Discussion

Initially we attempted to apply the Heistand (4) procedure to our samples. We found, however, that in the presence of suberic, azelaic and sebacic acids, low recoveries of nonanal were obtained from synthetic samples. It was concluded that the difficulty encountered in the presence of these acids was due to poor sample dispersion and reagent-sample contact. Derivative formation from aldehydes that are water-insoluble and hydrocarbon-soluble involves an interfacial reaction between the cyclohexane and water phases. The presence of a relatively large amount of azelaic acid type compounds which are insoluble in

TABLE I

Duplicate Analyses for Aldehydes

Results mmoles		Relative difference, %
0.023	0.027	16
0.028	0.029	4
0.062	0.063	2
0.076	0.072	5
0.083	0.082	1
0.085	0.089	4
0.090	0.088	2
0.092	0.094	2
0.105	0.109	4
0.124	0.113	9
0.151	0.165	9
0.171	0.166	3
0.176	0.188	7
0.200	0.198	1
0.283	0.276	2
0.276	0.289	5
0.288	0.290	1
0.354	0.354	0.7
0.653	0.651	0.3
0.822	0.837	2
1.07	1.13	5
1.77	1.77	0

both phases causes a physical interference to reactions at the interface.

To overcome this problem two corrective actions were taken. First, the extraction solvent was added to the system after the incubation step rather than before as in the Heistand (4) method. This would keep the aldehyde closer to the reagent interface. Secondly, a sample solvent was sought that would promote dissolution of the sample in the aqueous reagent phase. Initially, acetic acid was thought to be a good sample solvent. In this solvent, 100% of the nonanal added was recovered from a synthetic sample containing a large excess of azelaic acid. However, when this solvent was used to analyze a sample of oxidized fatty acid, lower than expected values were obtained. Subsequent study showed that samples run without acetic acid gave aldehyde results three to four times higher than aliquots analyzed in acetic acid.

A consideration of the components present in these samples and their influence on the chemistry of aldehydes in acetic acid led to the suspicion that a condensation could be taking place between the aldehydes and malonic acid present in the samples. Further evidence for this possibility was obtained by the analysis of synthetic samples of nonanal and malonic acid in acetic acid. Only 40% of the aldehyde was recovered in the presence of a threefold excess of malonic acid. When the solvent was changed to dimethylsulfoxide only 9% of the aldehyde was recovered.

Other sample solvents studied included propionic acid, pelargonic acid and methanol. Methanol appeared to be the best solvent from the standpoint of sample dispersion, but requires clean-up to remove residual carbonyl compounds in order to achieve a satisfactory reagent blank. Efficacy of the methanol purification was demonstrated by the fact that a batch of methanol that was found to give a reagent blank absorbance of 1.690 before treatment gave an absorbance of 0.108 after treatment.

Studies were conducted to determine if any of the components known or suspected of being present in tall oil fatty acid oxidation products interfered with the method. Peroxides, formic acid, representative monobasic fatty acids and low molecular weight dibasic acids did not affect recoveries.

As indication of the precision that can be expected for this method, replicate analyses of a single sample over a period of 12 days gave a standard deviation (SD) of 0.0056 and a relative SD of 3.4%. The SD shows reasonable precision from this procedure without significant day-to-day variation. Table I presents duplicate results observed for actual samples containing a wide concentration range of aldehydes. These results show that good duplication can be obtained over the concentration range from as little as 0.025 mmole/g to as much as 1.8 mmole/g.

REFERENCES

1. Siggia, S. "Quantitative Organic Analysis Via Functional Groups," John Wiley and Sons, Inc., New York, 1963.
2. Henick, A. S., M. F. Benca, J. H. Mitchell, JAOCS, *31* 88 (1954).
3. Toren, R. E., B. J. Heinrich, Anal. Chem. *22*, 1986 (1955).
4. Heistand, R. N., Anal. Chem. Acta. *39*, 258 (1967).

[Received March 20, 1970]