# ANALYSIS OF FATTY ACID DERIVATIVES BY GAS–LIQUID CHROMATOGRAPHY*

R. SIVARAMA KRISHNAN, L. A. WITTING AND M. K. HORWITT

*L. B. Mendel Research Laboratory, Elgin State Hospital, Elgin, Ill., and Department of Biological Chemistry, University of Illinois College of Medicine, Chicago, Ill. (U.S.A.)*

(Received May 21st, 1963)

## INTRODUCTION

During the course of our work on brain lipids[1] several oxygenated compounds of unknown structure were detected by gas–liquid chromatographic analysis. To characterize these compounds an effort was made to evaluate the influence of various structural groups, with particular reference to positional isomers, on relative retention times. It has been suggested that carbon number[2], carbon chain equivalent[3], $R_{x0}$ units[4], and relative retention volumes[5] are useful for identification. With unknown mixtures containing a number of heterofunctional or isomeric components, the use of retention volume data alone for the identification of components by gas chromatography has serious limitations. The application of auxiliary chemical methods to the determination of the compound type, after collecting eluted chromatographic peaks, by means of functional group analysis[6] is limited by sample size and also is not always reliable. Wherever possible, other instrumental methods[7–10] have been employed for subsequent identification of the eluted peaks, but the supplementary instrumentation is expensive and the procedures time-consuming.

The systematic use of retention data from two or more columns having different liquid phases does provide a method for functional group classification[10], but it remains difficult to apply this in the identification of structural isomers. The purpose of this investigation is to show how conventional "log plots" and retention data from appropriately chosen columns can be used in a systematic way to provide a method for the identification of functional groups and also structural isomers.

## EXPERIMENTAL

A F & M Model 500 gas chromatograph equipped with Model 1609 flame ionization attachment was used. The columns were 4 in. coils of borosilicate glass tube, 6 mm diameter and 8 ft. long packed with SE-30 siloxane polymer (2 parts) on acid–base washed Gas-Chrom P (80–100 mesh), or Apiezon M (APM) (20 parts) on acid–base washed Chromosorb W (80–100 mesh), or Carbowax 6000 (CW) (15 parts) on acid–base washed Chromosorb W treated with dimethyldichlorosilane (60–80 mesh), or a commercial (Applied Science Laboratories, Inc.) preparation of ethylene glycol succinate (EGS) (14 parts) on Chromosorb W (80–100 mesh). The column temperatures

* Supported in part by U.S.P.H.S. NB 03523-02.

were 160°, 165°, 175° and 190°, and the corresponding argon flow rates were 23, 20, 23 and 25 ml/min with EGS, Carbowax 6000, SE-30 and Apiezon M columns, respectively.

The compounds used in this work were either purified commercial materials or were prepared in this laboratory. These were either predominantly pure or had a component which was clearly identifiable (*e.g.* from a homologous series gas–liquid chromatography plot). At least three and usually five members of each homologous series were used in obtaining log plots.

Retention times were measured from the time of injection of sample to the time of appearance of the peak maxima on the recorder chart, and were corrected for calculated carrier gas front. Methyl esters of *n*-acids were chosen as standards for the determination of relative retention times. All the values included in this paper are relative to theoretical methyl *n*-octadecanoate. The relative retention times were plotted against carbon numbers on a two-cycle semi-log paper to obtain the log plots and the equivalent carbon numbers were calculated from these plots.

## RESULTS AND DISCUSSION

All data presented in this study refer to the operating conditions stated above, and under these conditions the slopes of the log retention time *versus* carbon number plots of a given homologous series for the different columns were found to be unequal. Obviously, therefore, retention time ratios could not be used for the classification of the compound type. The slopes of retention time–carbon number graphs of various homologous series of compounds were determined relative to that of methyl *n*-esters as standard for the columns used. The relative slope and equivalent carbon number data for the different columns and compound types are given in Table I. It can be readily seen that, with a single column, even when the relative slope is unity, the differences in the calculated equivalent carbon numbers for many series of homologous compounds are not sufficiently large as to enable characterization of the compound type. However, a careful study of the data obtained from the different columns (Fig. 1 and Table I) would indicate that it is possible to identify the type of the compound. For example, both *n*-$C_{15}H_{31}CH(OH)C_2H_5$ and *n*-$C_{14}H_{29}CH(OH)C_3H_7$ have theoretical equivalent carbon numbers of 17.35 on the EGS column. They are readily distinguished on Carbowax 6000, however, by the values of 17.55 and 17.30, respectively.

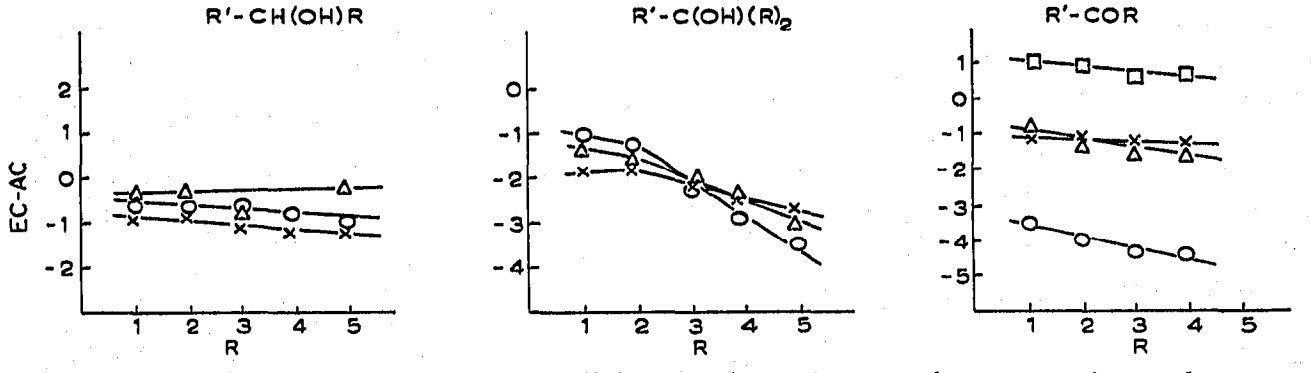


Fig. 1. Relationship between the observed differences in carbon numbers on various columns and the position of the functional group along the carbon chain. ○—○ EGS; △—△ CW 6000; ×—× SE-30; □—□ APM. EC = equivalent carbon number; AC = actual carbon number.

TABLE I

RELATIVE SLOPE AND EQUIVALENT CARBON NUMBER ON DIFFERENT COLUMNS
(Each compound has a basic 18-carbon chain)*

| Compound type | EGS | | Carbowax 6000 | | SE-30 | | APM | |
|---|---|---|---|---|---|---|---|---|
| | Rel. slope | Eq. C. No. | Rel. slope | Eq. C. No. | Rel. slope | Eq. C. No. | Rel. slope | Eq. C. No. |
| $RCH_2OH$ | 1.094 | 19.75 | 1.226 | 19.90 | 1.000 | 16.70 | | |
| $RCH(OH)CH_3$ | 1.000 | 17.35 | 0.926 | 17.50 | 1.000 | 17.00 | | |
| $RCH(OH)C_2H_5$ | 1.000 | 17.35 | 0.959 | 17.55 | 1.000 | 17.10 | | |
| $RCH(OH)C_3H_7$ | 1.000 | 17.35 | 0.990 | 17.30 | 1.000 | 16.85 | | |
| $RCH(OH)C_4H_9$ | 1.000 | 17.20 | — | — | 1.000 | 16.75 | | |
| $RCH(OH)C_5H_{11}$ | 1.000 | 17.00 | 1.170 | 17.70 | 1.000 | 16.80 | | |
| $RC(OH)(CH_3)_2$ | 1.000 | 17.00 | 1.000 | 16.75 | 1.000 | 16.20 | | |
| $RC(OH)(C_2H_5)_2$ | 1.000 | 16.80 | 1.000 | 16.57 | 1.000 | 16.30 | | |
| $RC(OH)(C_3H_7)_2$ | 1.000 | 15.85 | 1.000 | 15.90 | 1.000 | 15.85 | | |
| $RC(OH)(C_4H_9)_2$ | 1.000 | 15.15 | 1.000 | 15.70 | 1.000 | 15.60 | | |
| $RC(OH)(C_5H_{11})_2$ | 1.000 | 14.60 | 1.000 | 15.15 | 1.000 | 15.40 | | |
| RCHO | 1.000 | 17.35 | 1.000 | 16.95 | 1.000 | 16.90 | 1.000 | 17.20 |
| $RCOCH_3$ | 1.000 | 14.50 | 1.000 | 17.05 | 1.033 | 16.75 | 1.084 | 18.90 |
| $RCOCH_2CH_3$ | 1.000 | 13.90 | 0.935 | 16.70 | 1.033 | 16.75 | 1.084 | 18.85 |
| $RCOCH_2CH_2CH_3$ | 1.000 | 13.65 | 0.989 | 16.40 | 1.033 | 16.60 | 1.084 | 18.50 |
| $RCOCH_2CH_2CH_2CH_3$ | 1.000 | 13.55 | 1.057 | 16.35 | 1.033 | 16.60 | 1.084 | 18.50 |
| $RCO_2CH_3$ | 1.000 | 18.00 | 1.000 | 18.00 | 1.000 | 18.00 | 1.000 | 18.00 |
| $MeCO_2RCO_2CH_3$ | 1.000 | 20.40 | 1.000 | 24.15 | 1.000 | 21.35 | 1.000 | 20.70 |
| $RCH(Br)CO_2CH_3$ | 1.000 | 23.15 | 1.000 | 21.20 | 1.000 | 20.60 | 1.000 | 20.35 |
| $RCH(OH)CO_2CH_3$ | 1.000 | 20.00 | 1.000 | 21.40 | 1.000 | 19.50 | 1.000 | 19.10 |
| RCN | 1.000 | 20.95 | 1.000 | 19.30 | 1.000 | 17.90 | 1.000 | 17.65 |
| RCH(Br)CN | 1.000 | 23.80 | 1.000 | 21.95 | 1.000 | 20.50 | — | — |
| Retention time of $C_{18}$ methyl ester in min | 3.65 | | 10.35 | | 39.00 | | 155 | |

* The methyl group in a methyl ester is not considered part of the basic carbon chain.

The use of many different columns is time-consuming and laborious. A few pairs of appropriately chosen columns should provide sufficient retention information to enable characterization. Combinations of column pairs were considered, and the differences in the theoretical equivalent carbon numbers of the various homologous series were calculated. The data presented in Table II show that EGS–Carbowax 6000 and EGS–SE-30 as column pairs provide, in general, the maximum numerical separation of equivalent carbon numbers, and most suitable of the combinations considered for distinguishing functional groups and isomers of the types included in this study.

Identification of important functional group types by means of retention volume constants reported by MERRITT AND WALSH[11] is rather limited in its application. The analysis described in this paper based on the equivalent carbon numbers permits not only functional group analysis but also characterization of positional isomers in the series studied.

ACKNOWLEDGEMENT

The technical assistance of Mrs. UNA BARNETT in the synthesis of many of the compounds used in this study is acknowledged.

TABLE II

COMPARISON OF COLUMNS FOR SELECTION OF COLUMN PAIRS
(Separation of equivalent carbon numbers)

| *Compound type* | *EGS–CW* | *EGS–SE-30* | *EGS–APM* | *CW–SE-30* | *CW–APM* | *SE-30–APM* |
|---|---|---|---|---|---|---|
| $RCH_2OH$ | —0.15 | 3.05 | | 3.20 | | |
| $RCH(OH)CH_3$ | —0.15 | 0.35 | | | | |
| $RCH(OH)C_2H_5$ | —0.20 | 0.25 | | 0.45 | | |
| $RCH(OH)C_3H_7$ | 0.05 | 0.50 | | 0.45 | | |
| $RCH(OH)C_4H_9$ | — | 0.45 | | — | | |
| $RCH(OH)C_5H_{11}$ | —0.30 | 0.20 | | 0.50 | | |
| $RC(OH)(CH_3)_2$ | 0.75 | 0.80 | | 0.55 | | |
| $RC(OH)(C_2H_5)_2$ | 0.23 | 0.50 | | 0.27 | | |
| $RC(OH)(C_3H_7)_2$ | —0.05 | 0.00 | | 0.05 | | |
| $RC(OH)(C_4H_9)_2$ | —0.55 | —0.45 | | 0.10 | | |
| $RC(OH)(C_5H_{11})_2$ | —0.55 | —0.80 | | —0.25 | | |
| RCHO | 0.40 | 0.45 | 0.15 | 0.05 | —0.25 | —0.30 |
| $RCOCH_3$ | —2.55 | —2.25 | —4.40 | 0.30 | —1.85 | —2.15 |
| $RCOCH_2CH_3$ | —2.80 | —2.85 | —4.95 | —0.05 | —2.15 | —2.10 |
| $RCOCH_2CH_2CH_3$ | —2.75 | —2.95 | —4.85 | —0.20 | —2.10 | —1.90 |
| $RCOCH_2CH_2CH_2CH_3$ | —2.80 | —3.05 | —4.95 | —0.25 | —2.15 | —1.90 |
| $CH_3CO_2RCO_2CH_3$ | —3.75 | —0.95 | —0.30 | 2.80 | 3.45 | 0.65 |
| $RCH(Br)CO_2CH_3$ | 1.95 | 2.55 | 2.80 | 0.60 | 0.85 | 0.25 |
| $RCH(OH)CO_2CH_3$ | —1.40 | 0.50 | 0.90 | 1.90 | 2.30 | 0.40 |
| RCN | 1.65 | 3.05 | 3.30 | 1.40 | 1.65 | 0.25 |
| RCH(Br)CN | 1.85 | 3.30 | — | 1.45 | — | — |

SUMMARY

A method for the classification of functional groups and positional isomers based on gas chromatographic retention data and theoretical equivalent carbon numbers is described. The columns used were ethylene glycol succinate (EGS), Carbowax 6000, SE-30 and Apiezon M. Combinations of EGS–Carbowax 6000 and EGS–SE-30 as column pairs appeared most suitable for the primary, secondary and tertiary alcohols, aldehydes, ketones and methyl esters of a few fatty acids and their derivatives studied. Operating conditions for the different columns are described.

REFERENCES

1 L. A. WITTING, C. C. HARVEY, B. CENTURY AND M. K. HORWITT, *J. Lipid Res.*, 2 (1961) 412.
2 F. P. WOODFORD AND C. M. VAN GENT, *J. Lipid Res.*, 1 (1960) 188.
3 T. K. MIWA, K. L. MIKOLAJCZAK, F. R. EARLE AND I. A. WOLFF, *Anal. Chem.*, 32 (1960) 1739.
4 M. B. EVANS AND J. F. SMITH, *J. Chromatog.*, 8 (1962) 541.
5 S. EVERED AND F. H. POLLARD, *J. Chromatog.*, 4 (1960) 451.
6 J. T. WALSH AND C. MERRITT, Jr., *Anal. Chem.*, 32 (1960) 1378.
7 J. C. HOLMES AND F. A. MORRELL, *Appl. Spectry.*, 11 (1957) 86.
8 C. M. DREW, J. R. MCNESBY, S. R. SMITH AND A. S. GORDON, *Anal. Chem.*, 28 (1956) 979.
9 W. B. HEATON AND J. T. WENTWORTH, *Anal. Chem.*, 31 (1959) 349.
10 J. E. LOVELOCK AND S. R. LIPSKY, *J. Am. Chem. Soc.*, 82 (1960) 431.
11 C. MERRITT JR. AND J. T. WALSH, *Anal. Chem.*, 34 (1962) 903.