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## Note

# Gas chromatographic retention characteristics of ω-alicyclic fatty acids*

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Gas–liquid chromatography has been used extensively for the tentative identification of fatty acids prior to their confirmatory identification by mass spectrometry. In most instances the retention time can conveniently be measured, but the net retention volume adjusted for the air peak is the basic parameter. Although the net retention can be expressed in several ways, the equivalent chain length is perhaps the most useful parameter to use for identification purposes. Identification is usually accomplished by comparing the equivalent chain lengths of the unknown fatty acid obtained on two columns of different polarities with those of an authentic sample. Even without an authentic sample, the differences between the two equivalent chain lengths of the unknown compound permit the prediction of the presence or absence of unsaturation and, if present, the number of unsaturated bonds in the fatty acid molecules[1,2]. This approach has been used routinely in this laboratory for the identification of bacterial fatty acids with 13–18 carbon atoms.

It recently became necessary to identify various ω-alicyclic fatty acids synthesized by a bacterium. The behavior of these acids in gas chromatographic analysis is significantly different from that of normal or methyl-substituted acids. This paper reports findings obtained during the identification of alicyclic acids on the basis of their equivalent chain lengths.

## EXPERIMENTAL

### *Fatty acids*

Methyl esters of lauric, myristic, palmitic and stearic acids were purchased from Applied Science Labs. (State College, Pa., U.S.A.). Bacterial fatty acids were prepared from cells of *Bacillus subtilis* (ATCC 7059) grown on a culture medium containing glucose (1%), yeast extract (0.1%) and inorganic nutrients as described previously[3]. The fatty acids have previously been identified belonging to the *iso* series (*iso*-$C_{14}$, -$C_{15}$, -$C_{16}$ and -$C_{17}$), *anteiso* series (*anteiso*-$C_{15}$ and -$C_{17}$) and normal series (*n*-$C_{14}$ and -$C_{16}$) by their physical and chemical properties, as well as by gas–liquid chromatography and mass spectrometry.

ω-Alicyclic fatty acids were isolated from the cells of *B. subtilis* grown on glucose–yeast extract medium to which one of four cyclic acid substrates (cyclopropyl,

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cyclobutyl, cyclopentyl and cyclohexyl acids) was added. The $\omega$-alicyclic fatty acids with 14–18 carbon atoms cyclo-3-$C_{14}$ ($\omega$-cyclopropylundecanoic acid), cyclo-3-$C_{16}$, cyclo-4-$C_{15}$, cyclo-4-$C_{17}$, cyclo-5-$C_{16}$, cyclo-5-$C_{18}$, cyclo-6-$C_{15}$, and cyclo-6-$C_{17}$, were identified by gas–liquid chromatography and mass spectrometry[4].

*Esterification*

Fatty acids were esterified by diazomethane in diethyl ether[5].

*Gas–liquid chromatography*

The gas chromatograph used was a Hewlett-Packard Model 5830A equipped with a dual hydrogen flame-ionization detector. The reproducibility of temperature programming judged by the measured retention time of a given fatty acid sample, was found to be within 2%.

Three columns were used: one was a support-coated open-tubular (SCOT) column with an ethylene glycol adipate (EGA) polymer coating (50 ft. × 0.02 in.) purchased from Perkin-Elmer (Norwalk, Conn., U.S.A.) and the others were 6 ft. × $\frac{1}{8}$ in. stainless-steel tubes filled with either 2% Silar 5CP or 2.5% SE-30 on Gas-Chrom G AW DMCS (Applied Science Labs.).

The SCOT column was operated isothermally at 180° or 190° with a carrier gas (helium) flow-rate of 3 ml/min. The other packed columns were maintained at 120° for 2 min and then the temperature was increased at the rate of 2°/min up to 240°. The flow-rate of the carrier gas (helium) was 13 ml/min.

## RESULTS AND DISCUSSION

Fig. 1 shows retention times on the EGA column at 180°, plotted on a logarithmic scale against total carbon number for seven series of fatty acids. All of these plots have identical slopes. The equivalent chain length (ECL) of an acid is determined by finding the point on the normal series line corresponding to the measured retention time for that acid. The corresponding ECL are listed in Table I. The $\Delta$ECL value is the difference between the actual total number of carbon atoms of the fatty acid and its ECL. Because the lines in Fig. 1 are parallel, $\Delta$ECL is constant for a given series and, once known for one member, can be assumed for any other member. For example, $\Delta$ECL for cyclo-3-$C_{14}$ acid is +0.97. Hence the equivalent chain length of cyclo-3-$C_{16}$ acid is expected to be 16.97, which is almost identical with the measured value of 17.00. ECLs measured at 190° were identical with the listed values obtained at 180° (maximum deviation 0.03, except for *iso*-$C_{14}$, which gave a deviation of 0.09).

The retention times of the fatty acids were also measured under programmed temperature conditions. Plots of retention time (on a linear scale) against total number of carbon atoms for the seven series of fatty acids gave parallel straight lines for the SE-30 column. On the Silar 5CP column under the same conditions, however, the normal, *iso*, and *anteiso* series gave parallel straight lines, whereas the plots of the four $\omega$-alicyclic series showed a larger and parallel slope. Under the conditions used here, the difference in slopes between the two groups corresponded to 0.17 ECL unit per 10°. This difference is small enough that, to a first approximation and over the limited range of carbon numbers of interest, $\Delta$ECL can be considered to be constant (see Table I). (Table I also includes literature values for three alicyclic $C_{15}$

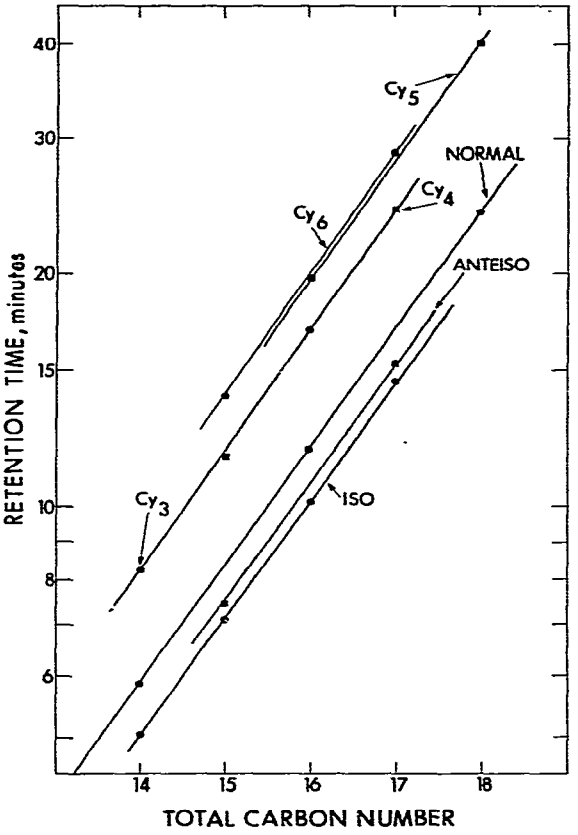


Fig. 1. Retention times of methyl esters of six series of fatty acids in relation to number of carbon atoms in the fatty acids (including the carboxyl carbon). The abbreviations used are: NORMAL, ANTEISO, ISO, $Cy_3$, $Cy_4$, $Cy_5$ and $Cy_6$ for normal, *anteiso*, *iso*, ω-cyclopropyl, ω-cyclobutyl, ω-cyclopentyl and ω-cyclohexyl acids, respectively.

acids from isothermal runs on columns of similar characteristics[6] and which agree fairly well with the values found in the present work). Attempts were made to measure ECLs on a 6-ft. packed EGA (7%) column under the same conditions of temperature programming but the maximum operational temperature of the column (190°) was not high enough for such analyses to be completed.

The average $\Delta$ECL values are listed at the bottom of Table I. It can be seen that all $\Delta$ECL values of the methyl branched series (*iso* and *anteiso*) are negative, whereas all $\Delta$ECL values of the ω-alicyclic series are positive. Thus, with respect to gas chromatographic retention characteristics, carbon atoms in an ω-alicyclic ring behave differently from alkyl-substituent carbons.

A fatty acid with a polar function, such as unsaturation or a hydroxyl group, can be recognized by chromatographing it on polar and non-polar columns, the resultant ECL being significantly larger on the polar than on the non-polar column. The differences between the ECLs for the ω-alicyclic series measured on the EGA and SE-30 columns, as listed in Table II, range from 0.45 to 0.94. This range can be

TABLE I

ECL AND ΔECL VALUES OF FIVE SERIES OF FATTY ACIDS MEASURED ON THREE DIFFERENT COLUMNS

| *Total carbon number of fatty acid* | *Equivalent chain length (ECL)* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *Iso series* | | | *Anteiso series* | | | *ω-Cyclopropyl series* | | |
| | *EGA** | *Silar*** | *SE-30*** | *EGA* | *Silar* | *SE-30* | *EGA* | *Silar* | *SE-30* |
| 14 | 13.53 | 13.53 | 13.52 | | | | 14.97 | | |
| 15 | 14.54 | | | 14.68 | 14.68 | 14.70 | | | |
| 16 | 15.54 | 15.53 | 15.60 | | | | 17.00 | 17.13 | 16.35 |
| 17 | 16.57 | 16.57 | | 16.72 | 16.72 | 16.62 | | | |
| 18 | | | | | | | | | |
| ΔECL | −0.45 | −0.46 | −0.44 | −0.30 | −0.30 | −0.34 | 0.98 | 1.13 | 0.35 |

TABLE II

DIFFERENCE BETWEEN ECL VALUES MEASURED ON EGA AND SE-30 COLUMNS

| *Carbon number of fatty acid* | $ECL_{EGA} - ECL_{SE\text{-}30}$ | | | |
|---|---|---|---|---|
| | *Cyclopropyl* | *Cyclobutyl* | *Cyclopentyl* | *Cyclohexyl* |
| 15 | — | 0.45 | — | 0.79 |
| 16 | 0.65 | — | 0.94 | — |
| 17 | — | 0.60 | — | 0.92 |
| 18 | — | — | 0.91 | — |

compared with values of 0.75 and 1.48 for oleic and linoleic acid, respectively, on columns of EGA and Apiezon L (Apiezon L is substantially the same as SE-30)[7]. Hence the substitution of an ω-alicyclic ring into a fatty acid appears to be similar in some respects to the introduction of unsaturation. Studies on the biochemical significance of this substitution are continuing.

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| *ω-Cyclobutyl series* | | | *ω-Cyclopentyl series* | | | *ω-Cyclohexyl series* | | |
|---|---|---|---|---|---|---|---|---|
| *EGA* | *Silar* | *SE-30* | *EGA* | *Silar* | *SE-30* | *EGA* | *Silar* | *SE-30* |
| 15.92 | 15.98 (16.1)*** | 15.47 (15.6)§ | | (16.5)*** | (15.8)§ | 16.43 | 16.42 (16.7)*** | 14.64 (15.9)§ |
| | | | 17.46 | 17.40 | 16.52 | | | |
| 18.03 | 18.15 | 17.43 | | | | 18.54 | 18.61 | 17.62 |
| | | | 19.45 | 19.56 | 18.54 | | | |
| 0.98 | 1.07 | 0.45 | 1.46 | 1.48 | 0.53 | 1.49 | 1.52 | 0.64 |

* Operated under isothermal conditions at 180° as described under Experimental.
** Operated under temperature-programmed conditions as described under Experimental.
*** Measured on Reoplex column[6].
§ Measured on Apiezon L column[6].

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