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IDENTIFICATION OF NATURAL AND SEMISYNTHETIC ω-CYCLOALKYL FATTY ACIDS

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Abstract—The experimental criteria, principally GLC behaviour and spectroscopic data, by which ω-cycloalkyl fatty acids (cyclobutyl to cycloheptyl, C_{14} to C_{21}) can be identified, are described.

INTRODUCTION

As previously reported, *Bacillus acidocaldarius* is an acidophilic thermophilic bacterium the lipids of which normally contain a high proportion of 11-cyclohexylundecanoic and 13-cyclohexyltridecanoic acids [1, 2]. From exogenous precursors, $R.CO_2H$ (R = cyclopentyl, cyclohexyl, cyclohexenyl, or cycloheptyl) or $R.CH_2CO_2H$ (R = cyclobutyl to cycloheptyl), it can also synthesize a corresponding range of other C_{16} to C_{21} [3] ω-cycloalkyl fatty acids. The ω-cyclohexyl fatty acids have also been detected in trace amounts in the depot fat of ruminants and in the rumen flora [4], while 13-cyclopentenyltridecanoic acid and its homologues are well-known as components of chaulmoogra oil. It is possible, particularly in bacteria, that some of these ω-cycloalkyl acids occur more commonly but have not been recognized, since compiled GLC data are usually related to "expected" acids. In our own work their initial recognition was materially assisted by the fact that *B. acidocaldarius* only produces saturated fatty acids with a consequent simplification of the GLC pattern.

In this paper we report a sufficient range of GLC data to allow the identification of ω-cycloalkyl fatty acids (as methyl esters) in mixtures, together with such spectroscopic data as we have found particularly characteristic for pure compounds.

RESULTS AND DISCUSSION

Over the range C_{16}–C_{21} the log GLC R_t of individual methyl esters is directly proportional to the carbon number for each type of ester studied so that the data, presented in Table 1, can be satisfactorily quoted as differences in the "effective chain length" from the actual carbon number. On both polar and non-polar stationary phases the presence of the ω-cycloalkyl structure substantially increases the R_t. This is quite different from the effect of simple branching (*iso*- or *anteiso*-) which slightly decreases the retention time on both polar and non-polar columns (Table 1), and also from the effect of unsaturation which selectively increases

Table 1.

Effective Chain Length of Methyl Esters by GLC

Fatty acid type	Column type		
	DEGS*	SE-30†	GAL‡
n	*x*	*x*	*x*
iso	*x* − 0·43	*x* − 0·40	*x* − 0·44
anteiso	*x* − 0·27	*x* − 0·40	*x* − 0·44
ω-cyclobutyl	*x* + 1·22	*x* + 0·40	*x* + 0·64
ω-cyclopentyl	*x* + 1·65	*x* + 0·60	*x* + 1·04
ω-cyclohexyl	*x* + 1·90	*x* + 0·80	*x* + 1·32
ω-cycloheptyl	*x* + 2·60	*x* + 0·92	*x* + 1·68

* DEGS: 2 m × 5 mm column packed with 10% DEGS on silanized Chromosorb P100–120 mesh, operated at 200°. R_t of methyl palmitate and methyl stearate 8·21 and 14·2 min respectively.

† SE-30: 2 m × 5 mm column packed with 5% SE-30 on silanized Chromosorb P100–120 mesh, operated at 170°. R_t of methyl palmitate and methyl stearate 8·40 and 19·20 min respectively.

‡ GAL: 2 m × 5 mm column packed with 10% GAL on silanized Chromosorb P100–120 mesh, operated at 235°. R_t of methyl palmitate and methyl stearate 6·20 and 12·40 min respectively.

the retention time on polar columns. The magnitude of the effect of ω-cycloalkyl groups increases with the ring size but is quite marked even for the ω-cyclobutyl esters.

For individual ω-cycloalkyl esters the most informative region of the IR spectra is the "methylene" region, 1430–1470 cm^{-1}. This region also includes the band at 1468 cm^{-1} due to aliphatic CH_2 groups, but those in the ring absorb somewhat differently: 1444 cm^{-1} for ω-cyclobutyl, 1450 cm^{-1} for ω-cyclopentyl, 1455 cm^{-1} for ω-cyclohexyl, and 1453 + 1464 cm^{-1} for ω-cycloheptyl. For any but the simplest mixtures the diagnostic value of the IR spectra is limited.

The MS of ω-cyclopentyl, ω-cyclohexyl, and ω-cycloheptyl esters show many features seen in normal fatty acid methyl ester spectra *viz.* peaks at *m/e* 74, 87, 143 and M^+-43, but α-cleavage adjacent to the cyclic moiety gives additional strong peaks at M^+-69, M^+-83, or M^+-97 respectively, each of which is distinctively accompanied by H-abstraction fragments at +1 and +2 mass units (as in the corresponding cleavage of branched-chain esters [5]). The MS of ω-cyclobutyl esters show a strong M + 1 peak (as for cyclobutylalkanes [6]) and fragmentations similar to monounsaturated esters (M^+-31 and M^+-32). However, for the general diagnosis of cycloalkyl fatty acids in mixtures, the most characteristic MS feature is, of course the M^+ seen in saturated fractions, obtained either by a separation method or after hydrogenation.

In the NMR spectra of ω-cycloalkyl methyl esters there is no C–Me signal but the ring–CH_2 groups give signals in the region δ = 1·5 to 2·2 (cyclobutyl, *ca.* 2·1; cyclopentyl, 1·61; cyclohexyl, 1·65; cycloheptyl, 1·54) but these overlap with signals from the α–CH_2 and β–CH_2 groups, centred at δ = 2·23 and *ca.* 1·6 respectively, making precise integration difficult. Neither of these features is therefore particularly diagnostic for distinguishing mixtures of esters.

The most useful diagnostic features for identifying ω-cycloalkyl fatty acid methyl esters in mixtures are their GLC and MS behaviour, particularly if the two types of data are obtained simultaneously by GC–MS.

EXPERIMENTAL

Precursor acids were purchased commercially except for cyclobutyl- and cycloheptyl-acetic acids which were prepared by Arndt-Eistert homologation from cyclobutane- and cycloheptane-carboxylic acids respectively; after purification the products gave satisfactory MS and NMR spectra.

Large-scale recovery of acids. B. acidocaldarius was grown at 60° in a 30 l. fermentor with 12·5 l./min air sparging, containing 25 l. of medium (0·1% glucose, 0·1% yeast extract, ammonium and other salts, H_2SO_4 to pH 3·5) plus 0·5 or 0·1 mM precursor acid [3]. Some 30 hr from the end of the log phase the cells were centrifuged down, washed, lyophilized and saponified; the recovered acids were methylated and after preliminary purification on a silica gel column the major components were recovered, 97–98% pure, by preparative GLC (2 m × 10 mm column with 25% DEGS on Chromosorb P30–60 at 180° with N_2 at 130 ml/min). Free fatty acids were obtained by treating the esters with 10% KOH in MeOH under reflux. GC–MS were determined at 70 eV. 100 MHz NMR spectra in CCl_4 soln with TMS as internal standard and IR spectra in $CHCl_3$ soln.

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