

Traditional gas chromatography of complex mixtures of compounds requires precision on the part of the chromatography equipment and considerable operator skill. Variations in oven temperature, carrier gas flow rates, column lengths and liquid phase conditions cause run-to-run and machine-to-machine variations, which limit precision and productivity. After analysis, the data transfer and data handling for anything other than simple reports may require interfacing the system with generic software packages to obtain the complete data analysis you desire.

The Sherlock MIS is designed to not only improve the quality of chromatographic results, but is also integrated all the way through indexing of samples analyzed, cluster analysis and pattern recognition searches of databases. The Agilent ChemStation is the analytical heart of the MIDI Sherlock system. The ChemStation provides automated sample analysis and stores the data file. The Sherlock software provides greater flexibility by organizing information in name fields. It uses the external calibration analysis to improve precision of peak naming. It also contains diagnostic features which monitor the chromatographic performance and standardize the results. The ChemStation data are used for peak naming by the Sherlock software and the results are stored in a Sherlock data file. These data files may be sorted by the Sherlock indexing program and are then used by the principal component and multivariate analysis clustering programs and by the pattern recognition database search program.

Sherlock uses a simple sample preparation procedure and gas chromatography (GC) to yield qualitatively and quantitatively reproducible fatty acid composition profiles. Following are some terms and explanations to help you navigate those profile reports.

### Equivalent Chain Lengths (ECL) Values

Sherlock's peak naming methodology uses the composition of the calibration standard to continually monitor the health of the system. The Microbial ID calibration mix for non-bacterial or eukaryotic analysis, for example, is composed of a series of saturated fatty acids of chain lengths from 9:0 to 30:0 as well as other diagnostic compounds. The entry into the peak naming table of the Equivalent Chain Lengths (ECL) values for all of the peaks in the calibration mix permits the software to automatically calculate a "nominal retention time" for each peak. The calibration mixture is composed of compounds having the same general chromatographic properties. The saturated fatty acids are assigned an ECL value corresponding to their length (e.g. 11:0 = ECL 11.000). Compounds that elute from the column at ECL values below those of known compounds would be assigned an interpolated ECL value.

### ECL Deviation Report

Each printout of an analysis indicates the ECL value of each peak and the deviation from the expected value. This is shown in the partial report below under "Comment 1".

ECL	Name	%	Comment 1	Comment 2
15.821	16:1 w7c	11.02	ECL deviates -0.001	
15.865	16:1 w6c	1.11	ECL deviates 0.003	
15.910	16:1 w5c	0.23	ECL deviates 0.001	
16.003	16:0	27.04	ECL deviates 0.003	
16.479	Sum in Feature 4	2.31	ECL deviates 0.003	17:1 ISO I / ANTEI B

For the peak 16:1 w7c (above), an ECL of 15.821 is reported, as is "ECL Deviates -0.001". The minus sign and value indicates that the peak is emerging faster than expected by one thousandth of an ECL value (expected = 15.822). Peaks in the early part of the analysis are more affected by GC oven temperatures and those later in the analysis are more severely impacted by carrier gas flow rates. The use of the Agilent programmable electronic pressure controller to achieve constant flow minimizes the latter type of error.

## Peak Shape Rejection

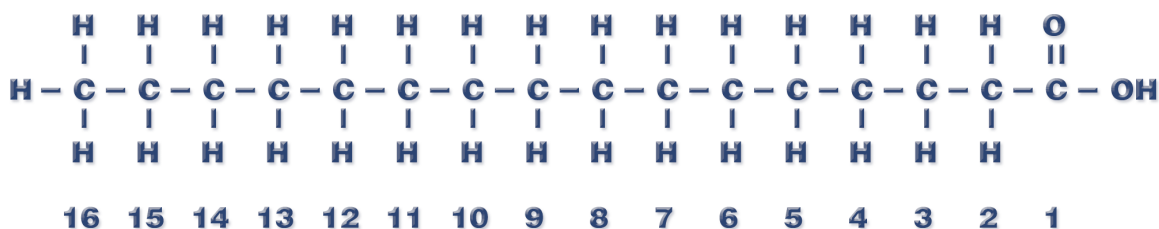
In analysis of materials such as fatty acids, the extraction procedure may carry over sterols and other non-fatty acid materials. Additionally, electronic noise may result in transient spikes, which might interfere with the chromatography. Fatty acid peaks always have area/height ratios greater than 0.017 and less than 0.070, making it possible to set exclusionary parameters at these levels. Electronic noise spikes are typically less than 0.017 and non-fatty acid peaks (carryover, sterols, etc.) are usually greater than 0.070, allowing rejection of these artifacts.

## Summed Features

In an ideal world, all peaks would be clearly resolved and no data would ever be lost due to inability of the chromatographic separation process. Practicality constraints like limited run time force acceptance of less than perfect chromatography. It is essential to not misname peaks. The Sherlock approach is to use a "Summed Feature" wherever imperfect peak separation occurs. Both compounds will be named in the comment field to the right of the report (see Comment 2 in the partial report on page 1). The one closest to the observed ECL will be listed first. In the majority of cases this is the correct name for the compound, but both names are always included as a single feature. This avoids using incorrect peak names in the cluster analysis or pattern recognition programs of Sherlock.

## Nomenclature of Fatty Acids

### Straight Chain

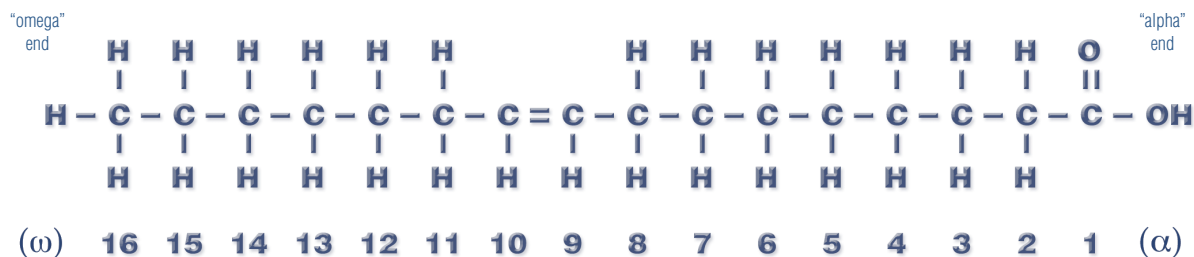


The figure above represents the straight chain fatty acid palmitic acid, written as 16:0. The "16" represents the number of carbons in the compound. The number after the colon indicates the number of double bonds in the carbon chain, in this case none. The carboxyl group (COOH) is at the right.

These compounds may also be written with the letter "C" in front of the number. For example 16:0 can be equivalently written C16:0. The letter "C" stands for carbons in the compound.

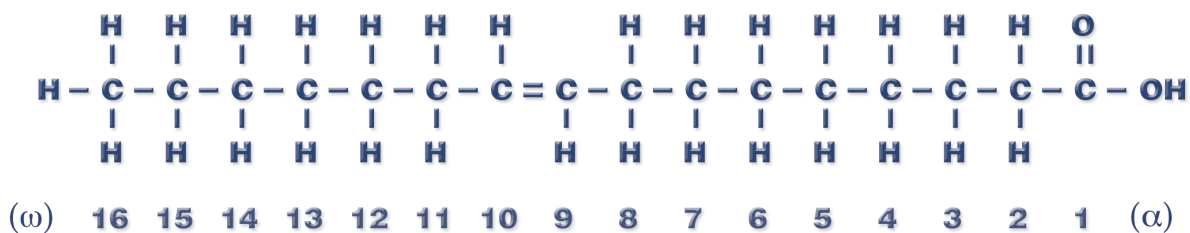
## Unsaturated

### 1. *cis* conformation



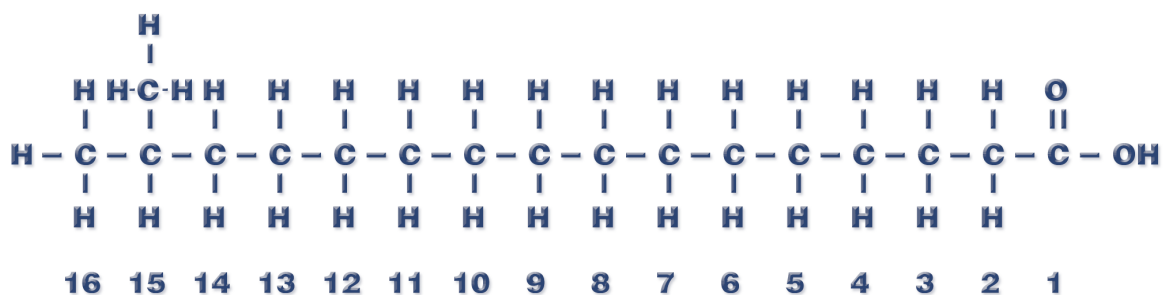
The designation 16:1 indicates that the compound has 16 carbons and 1 double bond. The figure above represents the unsaturated fatty acid 16:1 w7c. Note that both hydrogens at the double bond are on the same side in *cis* conformation. The "w7c" notation refers to the 7th carbon from the "omega" or "ω" end of the chain; the carboxyl group is located at the "alpha" end. This compound may have been represented as 16:1 *cis* 9 in other literature.

## 2. trans conformation



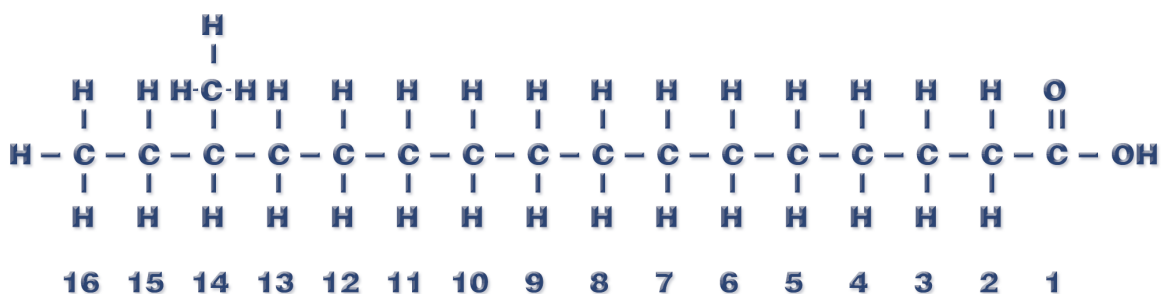
This figure above represents the unsaturated fatty acid 16:1 w7t. Note that the hydrogens at the double bond are on opposite sides of the compound in trans conformation.

### Iso



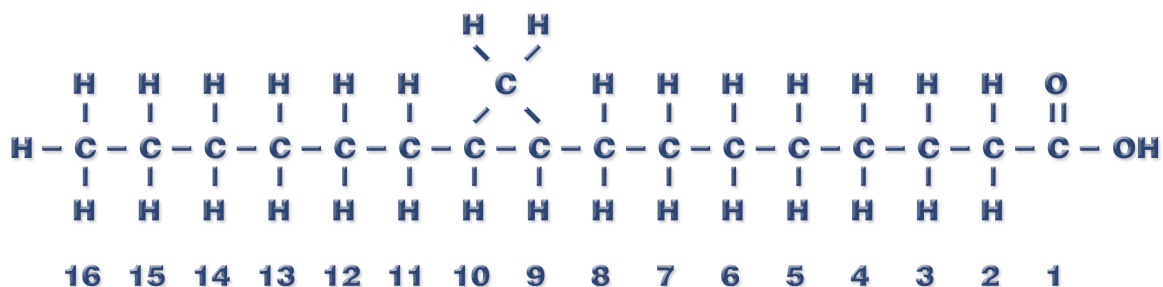
The figure above represents the fatty acid 17:0 ISO. A methyl group occurs at the second to the last carbon in the chain.

### Anteiso



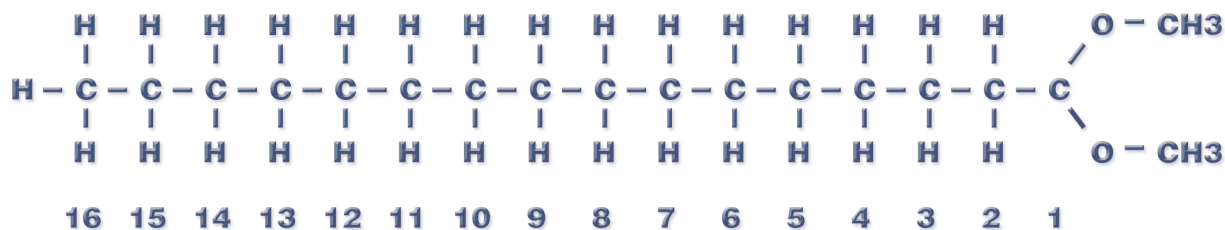
The figure above represents the fatty acid 17:0 ANTE ISO. A methyl group occurs at the third to the last carbon in the chain.

### Cyclopropane



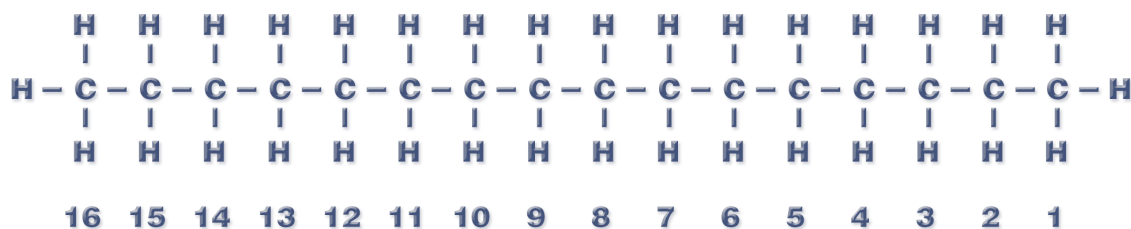
This represents the fatty acid 17:0 CYCLO w7c. In other literature it may be named as 17:0 CYCLO 9-10. This compound is made from 16:1 w7c with the addition of the carbon group at the double bond position.

### Dimethyl acetal



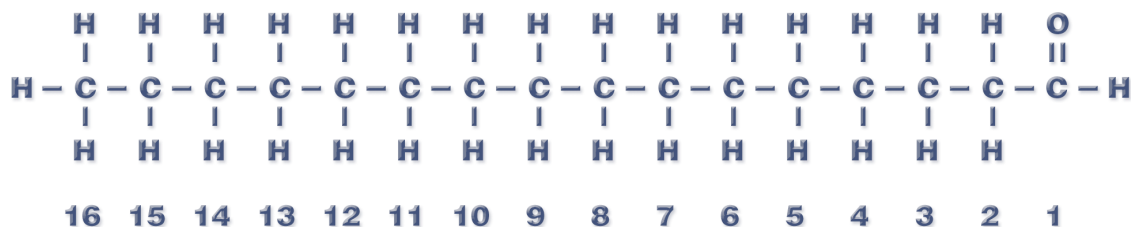
This figure above represents dimethyl aldehyde 16:0, written as 16:0 DMA on the MIS printed reports. Dimethyl acetals occur as analogs of the fatty acids present in anaerobic bacteria, and can contain any of the above functional groups. They result from the ether-linked lipids in plasmalogens.

### Normal hydrocarbon



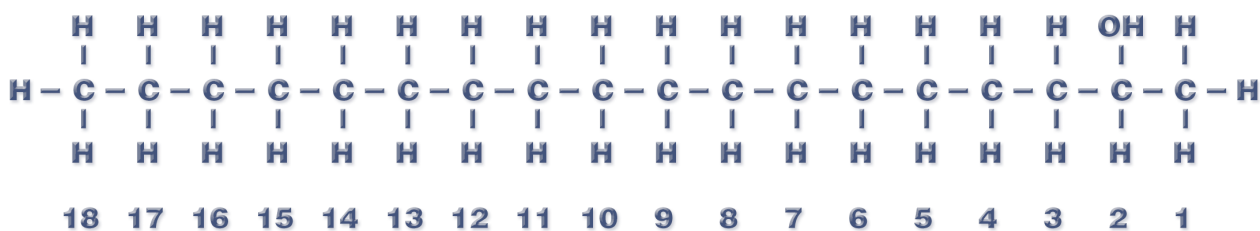
The figure above represents normal hydrocarbon 16:0, also written as n 16:0.

### Aldehyde



The figure above represents aldehyde 16:0.

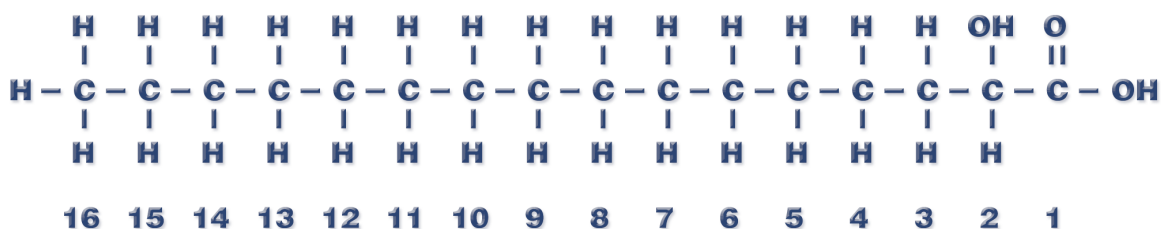
### Alcohols



The figure above represents the alcohol **2-octadecanol**. This compound, as well as 2-eicosanol (20 carbons long), occurs in some species of *Mycobacterium*.

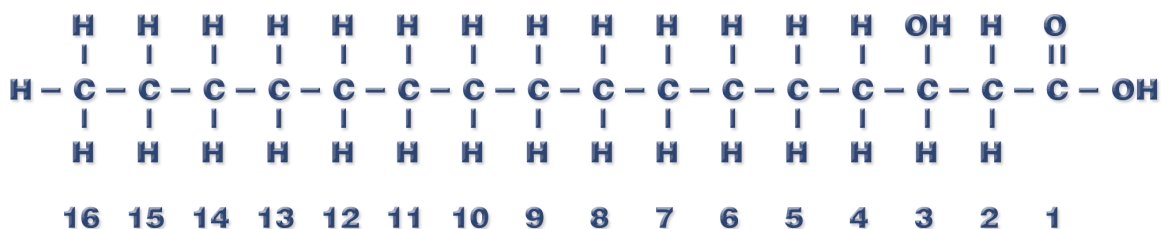
## Hydroxy

### 1. The 2-hydroxy



This figure represents the fatty acid 16:0 2OH. A hydroxyl group was added at the 2 (alpha) position.

### 2. The 3-hydroxy

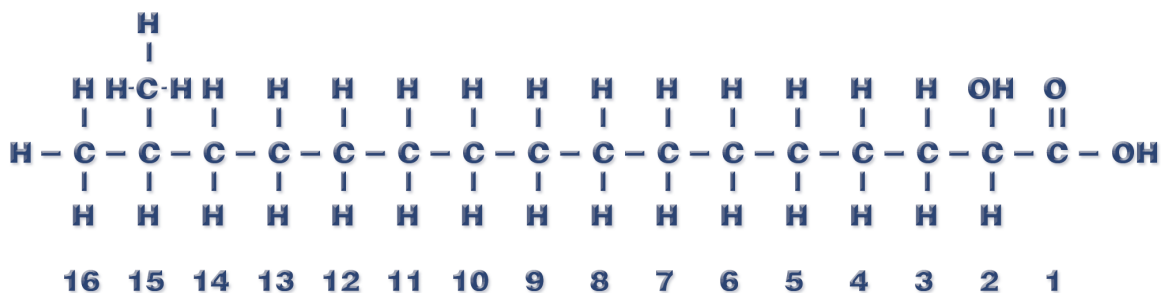


The above represents the fatty acid 16:0 3OH. A hydroxyl group is added at the 3 (beta) position

### 3. Other Hydroxys

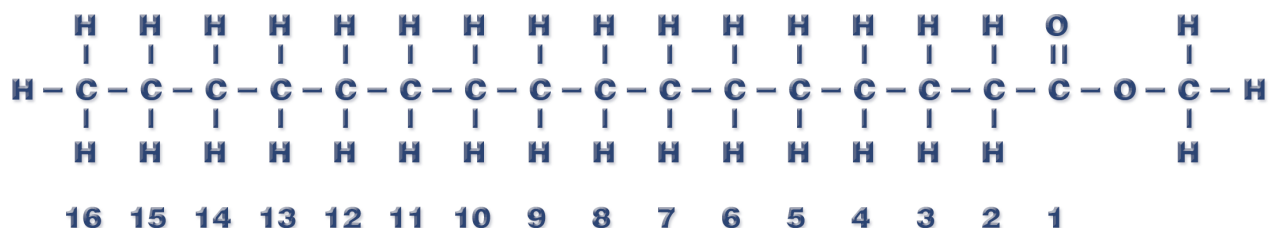
Hydroxyl functional groups may occur at other positions besides the second and third carbons. However, these are not common across the numerous species of bacteria which have been analyzed to-date.

## Mixed functional groups



Combinations of the various functional groups also occur. The above represents the fatty acid 17:0 ISO 2OH

## Fatty acid methyl ester



The above represents the fatty acid methyl ester 16:0. This compound is written as 16:0 FAME on some MIS printed reports. A methyl group is added to the carboxyl group to increase volatility for GC analysis.

