Fatty acids are the basic components of the lipids of all microorganisms, except the group of Archaeabacteria. Frequently unusual fatty acids are detected in bacterial lipids. Due to the easy volatilization of their methyl esters, fatty acids are conveniently analyzed by gas chromatography GC, but precise assignment of structure to a fatty acid detected as a peak in GC investigations is not always an easy task, and mass spectrometry MS has an important role to play. Much work has been performed on the analysis of fatty acids by GC/MS, mainly in the field of bacterial lipids, so the part devoted to fatty acids in this review is much larger than that concerning unvolatile complex lipids.

1. FATTY ACIDS

GC and MS, working separately or on tandem, have provided a major contribution to structure determination of the fatty acids obtained after saponification of the lipids of microorganisms. At the present time, many fatty acids are known, so that, when standard samples are available, the
use of GC can allow the identification of every component of a mixture of fatty acids, if the resolving power of the apparatus under the conditions chosen for the analysis is high enough. However, mass spectrometry is a helpful and sometimes indispensable method for choosing between hypothetical structures compatible with the results of the GC analysis. MS is indispensable in the case of unusual fatty acids with new structural features, and allows their structure determination by using a minimum amount of sample. The sequential use of GC and MS is often due to material reasons, such as the lack of suitable apparatus.

At first we shall consider the kind of information that can be expected on the structures of fatty acids, from the use of GC and MS, working independently or in line.

### 1.1. Preparation of the Samples

Many works have been performed by direct transesterification of fatty acids from the complex lipids in the cells with methanol, followed by GC/MS analysis of the resulting fatty acid methyl esters.

Transesterification in alkaline medium (sodium methanolate) can produce artefacts by epimerization when the mixture to be analyzed contains α-substituted fatty acids. Transesterification catalyzed by boron trihalides more or less destroys cyclopropane fatty acids; unsaturated fatty acids can also be partially modified. Moreover, the recovery of some fatty acids, particularly hydroxy acids, is less than with classical saponification (Moss and Dees, 1975). For these reasons, saponification has to be preferred. It must be noted that some very long chain fatty acids can be partly extracted with ether because of the dissociation of their potassium salts. The presence of nonacidic lipid components in the mixture to be analyzed by GC can be an advantage (Asselineau et al., 1979).

Methylation of the free fatty acids can be performed by using an ether solution of diazomethane. It must be noted that α,β-unsaturated fatty esters can add diazomethane on their double bond to give pyrazolines (Eistert, 1948).

The methylation step can be performed by addition of a few milliliters of commercial boron-trichloride-methanol reagent (Marshall et al., 1970). This procedure gives artefacts as mentioned above. Practical details on the preparation of samples for GC/MS can be found in Chapter 1.

If the amount of sample is large enough, preliminary chromatographic fractioning on a microcolumn or by thin-layer chromatography can be useful to separate polar and non-polar fatty esters. In every case, the mixture of methyl esters is dissolved in about 0.1 ml of ether or hexane, and analyzed as soon as possible. When necessary the sample solutions are