for testing. This area contains components with polarities consistent with drug-like properties, having eliminated the early-eluting highly polar compounds such as saccharides and amino acids and the highly retained lipophilic materials that elute at the end of the run. The area is divided into 10 fractions that are concentrated and plated for use in high-throughput screening.

Fractionated screening samples derived from chromatographic separations such as indicated in Figure 7.8 offer several advantages over crude extracts. The major one is the ability to independently evaluate diverse components produced by a particular source organism. In cases where one component in an extract is toxic, its adverse effect on the test organism in a phenotypic assay may obscure the positive response of a second component. Actinomycetes are particularly notable in their ability to produce numerous families of compounds. For example, it has been well documented that *Streptomyces* species that produce the milbemycin class of macrolides, such as the previously mentioned nemadectin, generally also produce oligomycins. The oligomycin macrolides are known respiration inhibitors that are highly toxic to eukaryotic cells. Therefore, if one were screening in a rodent model for antiparasitic activity, a crude extract containing both the milbemycin and oligomycin would likely only show the toxicity. Whereas if the components were resolved chromatographically prior to screening, the cryptic antiparasitic effect of the milbemycin would be observed.

Apart from unmasking activities, there are benefits that accrue from enhancing the concentration of minor components present in a complex mixture. This is particularly true if in the preparation of fractionated screening samples the effort is made to normalize the concentration of the samples. The benefits of obtaining the maximum positive responses from these samples, often representing precious material collected under unique conditions, argue for expending the extra effort required.

In the course of resolving "hits" from natural product screening through bioassay-guided fractionation, as shown in Figure 7.6, it was emphasized that this is an empirical and highly experimental process, each new extract requiring an individual strategy for the isolation of its biologically active principles. If one has prefractionated the extract prior to initial screening, and the activity falls into a neat cluster of fractions, then one has valuable information on how to begin the purification process. This information will facilitate the resolution of the hit and lead to greater efficiency of the entire isolation and purification process.

7.5 OPTIMIZATION OF NATURAL PRODUCT LEADS

Nature has preserved the ability of a given organism to make these fascinating secondary metabolites, although their inherent biological roles remain obscure. As scientists seek to co-opt these metabolites as medicinal agents, attempts are typically made to enhance their pharmaceutical effectiveness. Such enhancements may be to improve the spectrum of activity against a range of targets, as in the case of antibiotics where broad-spectrum activity in inhibiting the growth of both gram-positive and gram-negative bacteria is important. In other cases, it may be crucial to enhance the specificity to a narrower range of targets, such as the ability to selectively inhibit a particular kinase reaction in a signaling cascade. Furthermore, it may be necessary to improve the drug-like properties of a natural product lead. Here, improvements in solubility, chemical stability in biological matrices or metabolic stability may be crucial. These and a host of other reasons drive the process to make structural modifications of the core natural product, which may be effected by chemical or biosynthetic means.

7.5.1 Semisynthesis

Semisynthesis refers to the process of performing synthetic chemical transformations starting with a natural product, for the purpose of enhancing the pharmaceutical performance of the natural product. This approach has been most effectively used with complex microbial products, owing to the ready availability of the starting material through fermentation of highly productive variants of the parent organism. The challenge in these experiments is twofold: one, to achieving adequate selectivity in the chemical process and two, the subsequent purification of reaction mixtures. A good example