ISOLATION TECHNIQUES AND IDENTIFICATION METHODS IN NATURAL PRODUCT CHEMISTRY

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Analyses of some complex mixtures (plant extracts) are possible only after separation of the mixture into its components. There is no single technique more effective and generally applicable than the chromatographic methods. **Chromatography** was introduced by Tswett in 1906 and more advanced separation techniques are available today. Chromatography was first used for the separation of coloured substances (colour-chroma).

In chromatography one of the two phase system is almost always stationary with respect to the other. Therefore discussion of chromatography often speak of a stationary (immobile) and a mobile phase.

Kinds of Chromatography.

Thin layer chromatography
Paper chromatography
Column chromatography
High performance liquid chromatography
Gas liquid chromatography
Flash chromatography
Gel chromatography

Identification

Once a pure compound is isolated from a plant extract the next step is the structure elucidation. There is no single method that gives complete results except x-ray analysis. Many spectroscopic techniques and chemical methods are available for this purpose.

Determination of molecular weight and molecular formula
Detection of the presence or absence of certain functional groups
Degradation to simple compounds
Conversion to known compounds
Synthesis by an unambiguous route

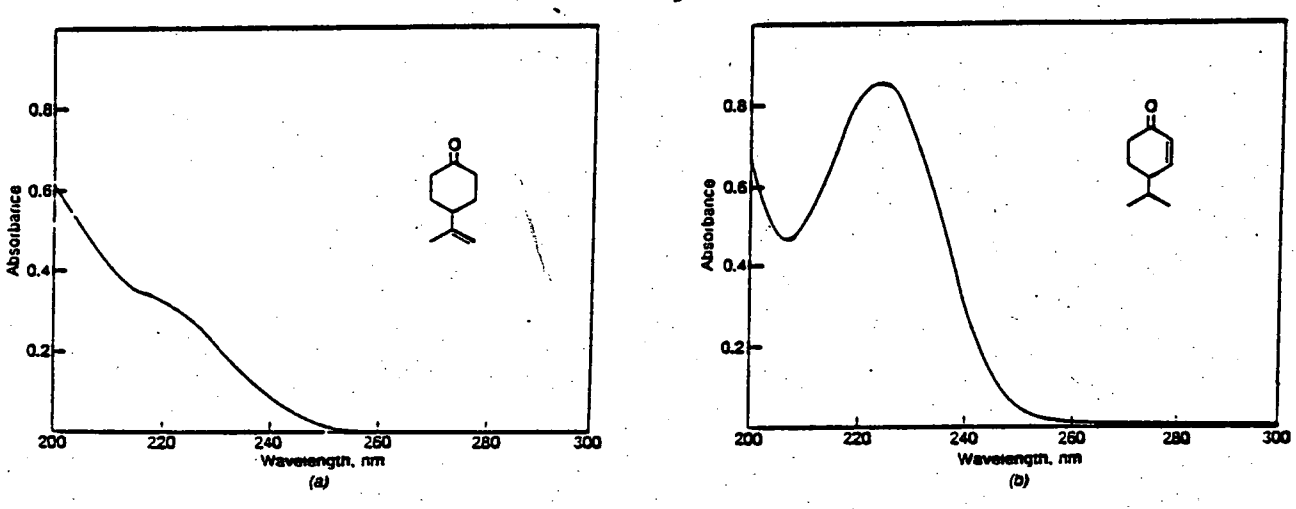
Spectroscopy

Spectroscopy is the interaction of electro magnetic radiation with matter.

Ultraviolet spectroscopy (UV)

UV spectroscopy is based on the detection of electronic transitions. (ie. the promotion of electrons from one energy level to another).

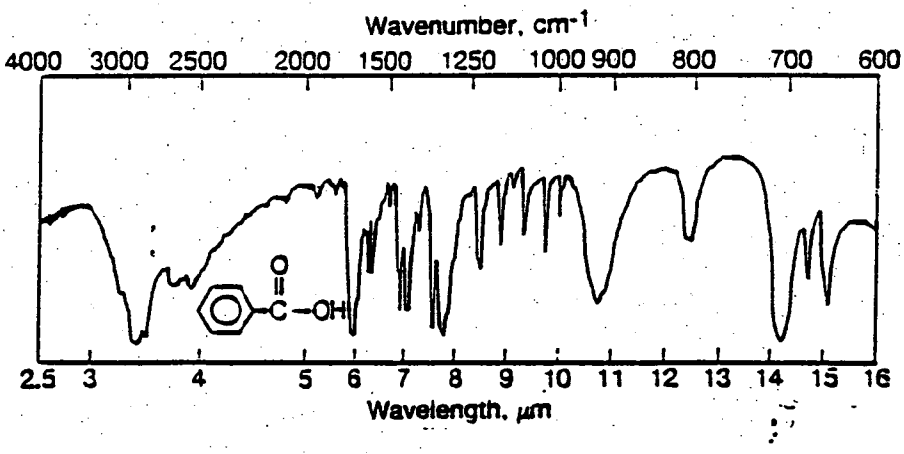
UV spectroscopy is useful for detection of chromaphores and conjugated systems.
Compounds which are structurally close but have different arrangements of chromaphores may often be distinguished by UV spectroscopy.



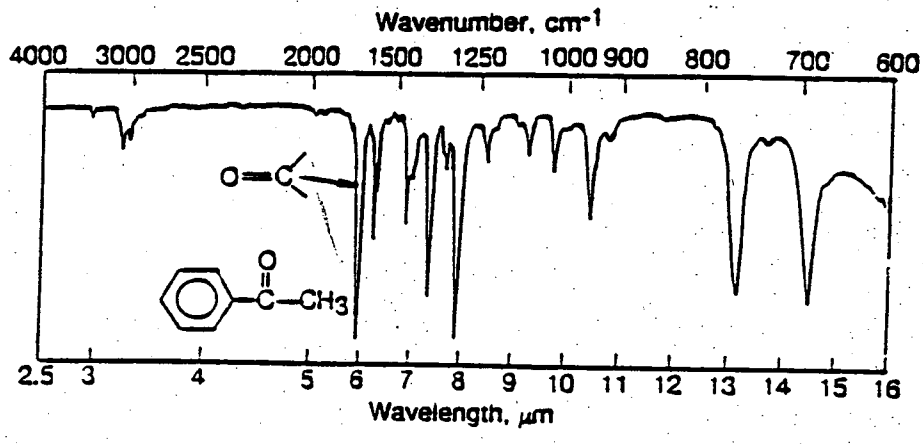
The UV spectra of **(a) 4-isopropylcyclohexanone**
(b) 4-isopropylcyclohex-2-en-one

Infrared spectroscopy (IR)

Atoms in molecules constantly vibrate. The bonds stretch, bend and the frequencies of these motions correspond to the frequencies of IR radiation. When IR radiation passes through a molecule, those frequencies that correspond to the molecular motions are absorbed. Hence IR spectroscopy is useful to determine the bond types present in a molecule.



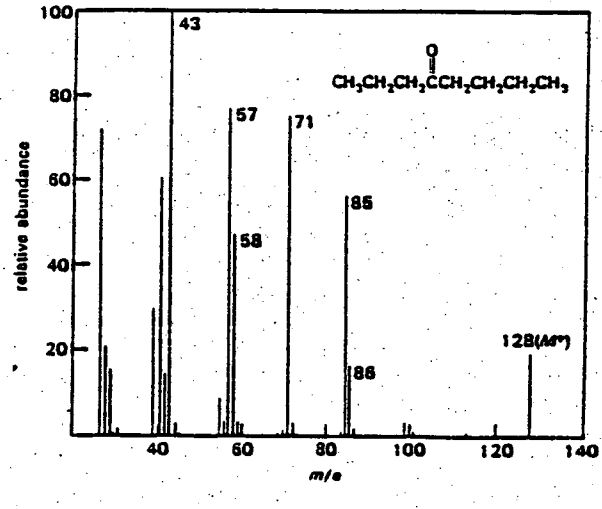
The IR spectrum of benzoic acid, a typical aromatic acid.



The IR spectrum of acetophenone, a typical aryl ketone.

Mass Spectrometry

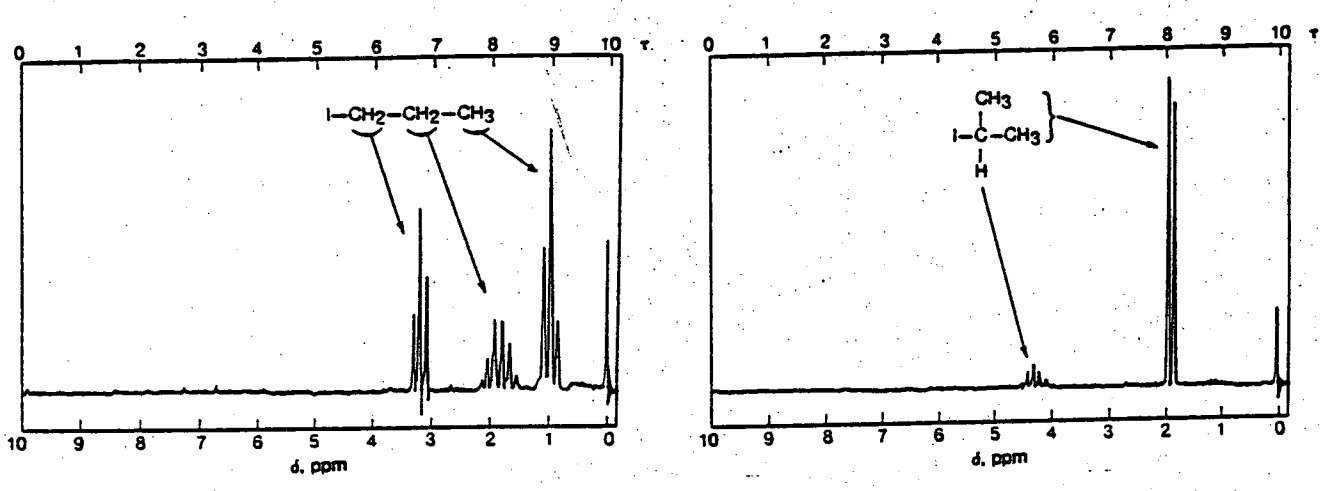
Molecules can be converted to ions by bombarding elctrons, sorts them according to their mass-to-c harge ratio (m/e), and determines the relative amounts of each ion present. This is the basic principle of mass spectrometry. Number of modern mass spectroscopic methods are available today.



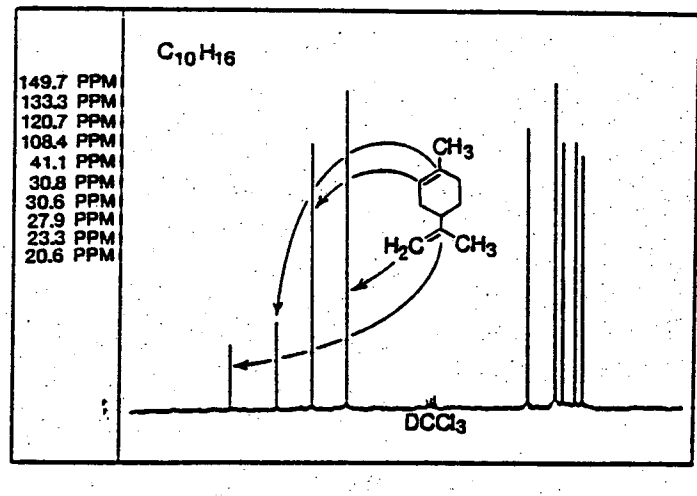
The mass spectrum of 4-octanone

Nuclear Magnetic Resonance Spectroscopy (NMR)

Radio frequency interacts with nuclei of certain atoms exposed to a strong magnetic field. NMR spectra gives information regarding different protons and carbons in a molecule.



The proton NMR spectrum of (a) 1-iodopropane (n-propyliodide)
(b) 2-iodopropane (isopropyliodide)



The carbon NMR spectrum of limonene