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Application of I.R. Spectroscopy & Mass Spectrometry in Structural Elucidation of Drugs

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Abstract: *The use of IR and Mass spectroscopy in the R&D of natural drugs and pharmaceutical products has been reviewed highlighting the advantages of the techniques. IR spectroscopy has been applied to natural product composition analysis, product quality assessment and production control. Its disadvantages include the difficulty in quantification and interpreting the data, although recently developed instrumentation and computer algorithms have made this a lot easier. Mass spectrometry has both qualitative and quantitative uses. These include identifying unknown compounds, determining the isotopic composition of elements in a molecule, and determining the structure of a compound by observing its fragmentation.*

1. Introduction

Infrared spectroscopy (IR spectroscopy) is the spectroscopy that deals with the infrared region of the electromagnetic spectrum, that is light with a longer wavelength and lower frequency than visible light. It covers a range of techniques, mostly based on absorption spectroscopy. As with all spectroscopic techniques, it can be used to identify and study chemicals. Infrared spectroscopy exploits the fact that molecules absorb specific frequencies that are characteristic of their structure. These absorptions are resonant frequencies, i.e. the frequency of the absorbed radiation matches the transition energy of the bond or group that vibrates. The energies are determined by the shape of the molecular potential energy surfaces, the masses of the atoms, and the associated vibronic coupling.

In particular, in the Born–Oppenheimer and harmonic approximations, i.e. when the molecular Hamiltonian corresponding to the electronic ground state can be approximated by a harmonic oscillator in the neighborhood of the equilibrium molecular geometry, the resonant frequencies are associated with the normal modes corresponding to the molecular electronic ground state potential energy surface. The resonant frequencies are also related to the strength of the bond and the mass of the atoms at either end of it. Thus, the frequency of the vibrations is associated with a particular normal mode of motion and a particular bond type.

Infrared radiation is largely thermal energy. It induces stronger molecular vibrations in covalent bonds, which can be viewed as springs holding, together two masses, or atoms.

The complexity of infrared spectra in the 1450 to 600 cm^{-1} region makes it difficult to assign all the absorption bands, and because of the unique patterns found there, it is often called the fingerprint region. Absorption bands in the 4000 to 1450 cm^{-1}region are usually due to stretching vibrations of diatomic units, and this is sometimes called the group frequency region.

Fourier transform infrared (FTIR) spectroscopy is a measurement technique that allows one to record infrared spectra. Infrared light is guided through an interferometer and then through the sample (or vice versa). A moving mirror inside the apparatus alters the distribution of infrared light that passes through the interferometer. The signal directly recorded, called an "interferogram", represents light output as a function of mirror position. A data-processing technique called Fourier transform turns this raw data into the desired result (the sample's spectrum): Light output as a function of infrared wavelength (or equivalently, wave number). As described above, the sample's spectrum is always compared to a reference.

A beam of infrared light is produced, passed through an interferometer and then split into two separate beams. One is passed through the sample, the other passed through a reference. The beams are both

reflected back towards a detector, however first they pass through a splitter, which quickly alternates which of the two beams enters the detector. The two signals are then compared and a printout is obtained. This "two-beam" setup gives accurate spectra even if the intensity of the light source drifts over time.

Mass spectrometry (MS) is an analytical chemistry technique that helps identify the amount and type of chemicals present in a sample by measuring the mass-to-charge ratio and abundance of gas-phase ions.[2] The information contained in a single mass spectrum, recorded using very small amounts of sample, will often allow the unambiguous identification or structural elucidation of an unknown compound. The development of ionization techniques which remove the requirement that the sample be vaporized before ionization, have made a whole range of compounds of biomedical and biochemical importance amenable to mass spectrometric analysis. The high sensitivity and specificity of the mass spectrometer are enhanced by using a gas chromatograph as the mass spectrometer inlet, and" combined gas chromatography-mass spectrometry (GCMS) is one of the most powerful analytical techniques available today. The combination of the very high sensitivity and specificity of GCMS is unobtainable even with highly sensitive immunological techniques. A number of major developments have occurred in recent years in GCMS methodology. These include the direct coupling of capillary gas chromatographic columns to mass spectrometers, the widespread availability of ion sources for chemical ionization, and the increasing use of isotopic ally labeled internal standards in assays using quantitative selected ion monitoring (QSIM).

A mass spectrum is a plot of the ion signal as a function of the mass-to-charge ratio. The spectra are used to determine the elemental or isotopic signature of a sample, the masses of particles and of molecules, and to elucidate the chemical structures of molecules, such as peptides and other chemical compounds. Mass spectrometry works by ionizing chemical compounds to generate charged molecules or molecule fragments and measuring their mass-to-charge ratios.

In a typical MS procedure, a sample, which may be solid, liquid, or gas, is ionized, for example by bombarding it with electrons. This may cause some of the sample's molecules to break into charged fragments. These ions are then separated according to their mass-to-charge ratio, typically by accelerating them and subjecting them to an electric or magnetic field: ions of the same mass-to-charge ratio will undergo the same amount of deflection.[1] The ions are detected by a mechanism capable of detecting charged particles, such as an electron multiplier. Results are displayed as spectra of the relative abundance of detected ions as a function of the mass-to-charge ratio. The atoms or molecules in the sample can be identified by correlating known masses to the identified masses or through a characteristic fragmentation pattern.

A mass spectrometer consists of three components: an ion source, a mass analyzer, and a detector. The ionizer converts a portion of the sample into ions. There is a wide variety of ionization techniques, depending on the phase (solid, liquid, gas) of the sample and the efficiency of various ionization mechanisms for the unknown species. An extraction system removes ions from the sample, which are then trajected through the mass analyzer and onto the detector. The differences in masses of the fragments allow the mass analyzer to sort the ions by their mass-to-charge ratio. The detector measures the value of an indicator quantity and thus provides data for calculating the abundances of each ion present. Some detectors also give spatial information, e.g., a multichannel plate. A mass spectrometer ionizes molecules in a high vacuum, and sorts the ions according to their masses, and records the abundance of the ions of each mass

Mass spectrometry data analysis is specific to the type of experiment producing the data. General subdivisions of data are fundamental to understanding any data.[3]

Many mass spectrometers work in either negative ion mode or positive ion mode. It is very important to know whether the observed ions are negatively or positively charged. This is often important in determining the neutral mass but it also indicates something about the nature of the molecules.

Different types of ion source result in different arrays of fragments produced from the original molecules. An electron ionization source produces many fragments and mostly single-charged (1-) radicals (odd number of electrons), whereas an electro spray source usually produces non-radical quasi molecular ions that are frequently multiply charged. Tandem mass spectrometry purposely produces fragment ions post-source and can drastically change the sort of data achieved by an experiment.

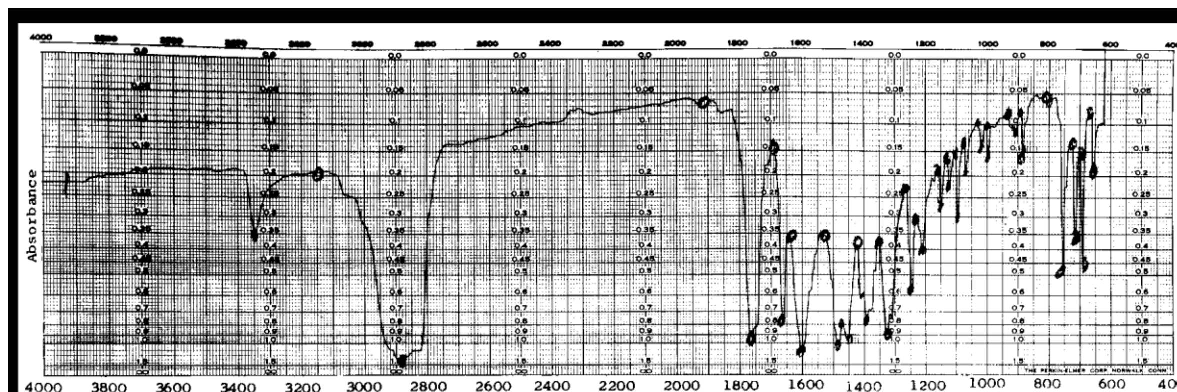
2. Structural Elucidation of Drugs

In drug Analysis, IR spectroscopy and mass spectrometry are powerful instrumental methods for chemical analysis.

2.1. The Penicillins

Penicillin is the name given to the mixture of natural compounds having the molecular formula $C_9H_{11}N_2O_4SR$ and differing only in the R.[4] The penicillins are all strong mono basic acids e.g. they form salts. They are hydrolysed by hot dilute inorganic acids; one carbon atom is eliminated as carbon dioxide, and two products are obtained in equimolecular amounts, one being an amine, penicillamine, and the other an aldehyde, penilloaldehyde. All the pencillins give the same amines but different aldehydes; it is the latter which contain the R group.

2.2. IR Spectrum of Pencillin

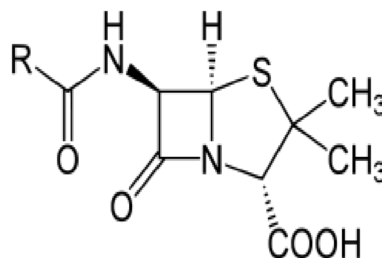


The Infrared spectra of many pencillins were examined and correlation between various bands and functional groups was carried out by examining the spectra of synthetic modal compounds which contained different parts of structures that had been proposed on chemical evidence. Those may be illustrated with methyl ester and sodium salt of benzylpencillin, which showed the following maxima

Methyl ester: 3333, 1770, 1748, 1684, 1506cm^{-1}

Sodium salt: 3333, 1770, 1613, 1681, 1515cm^{-1}

The Band at 3333cm^{-1}in both compounds was assigned to NH group(str.), and the 1748cm-1 band of the ester and the 1613cm-1 band of the salts were assigned to carbonyl group(str.) in the carboxyl group (as ester or salt). Then model oxazolones were studied; these showed two characteristic bands one at 1825$cm^{-1}$for the carbonyl group and one at 1675cm^{-1}or the C= N group. The absence of the first but possible presence of the secondary bezylpencillin derivatives would not permit a decision to be reached between (III). When a large number of thiazolidines were examined in the double bond region down to 1470cm^{-1}, only the carbonyl band was found to be present(~1748 and 1613cm^{-1}). A large no. of amides were examined. All three types had a band close 1670cm-1, which can be attributed to carbonyl group, but with the primary amides, there was a band near 1613$cm^{-1}$and with secondary amides the band was close to 1515cm^{-1}. These results suggests that pencillins have secondary amide structure (i.e.(IV)), since the secondary amide band at 1670cm-1 1684 and 1681cm^{-1}, and the band at 1515cm^{-1}1506 and 1515cm^{-1}. Thus, four of the five bands have been accounted for. Finally, a number of simple β-lactans and fused thiazolidine- β-lactans were examined. The former did not show a band near 1770cm^{-1}, but all the latter were found to have a band at 1770cm^{-1}. This accounts for the fifth band, and so it follows the structure of pencillins is:

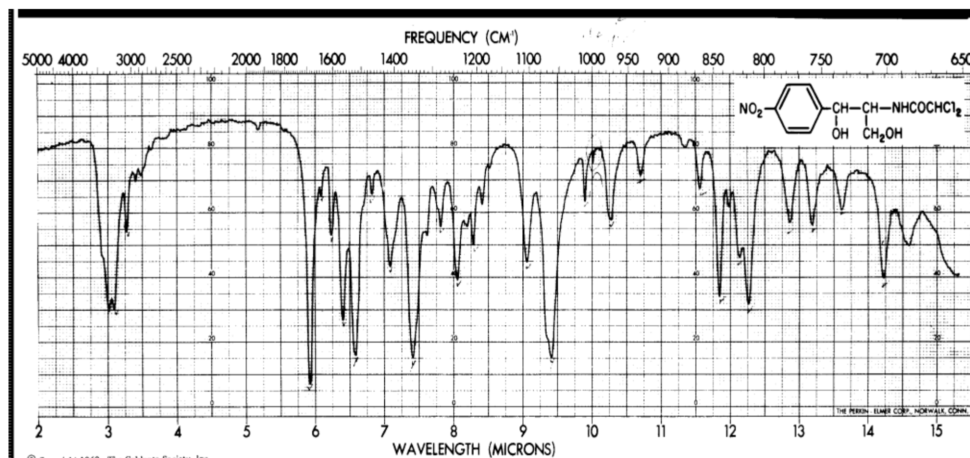


Penicillin core structure, where "R" is the variable group

2.3. Chloramphenicol (Chloromycetin)

It is laevorotatory compound that is produced by *Streptomyces vinezuelae.* It is very effective in the treatment of Typhoid fever, etc. The molecular formula of chloramphenicol is $C_{11}H_{13}Cl_2N_2O_5$andmolecular weight 323.129.Chloramphenicol is a synthetic antibiotic. It is often used for bacterial selection in molecular biology applications at 10-20 µg/mL and as a selection agent for transformed cells containing chloramphenicol resistance genes.[5]

2.4. IR Spectrum of Chloramphenicol



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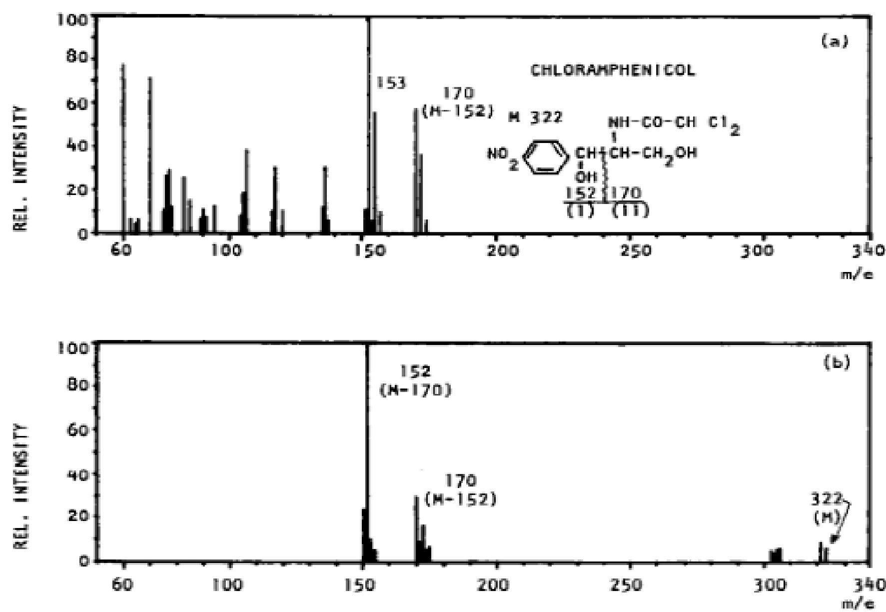

FIG. 8[23] - MASS SPECTRA OF CHLORAMPHENICOL - (a) ELECTRON IMPACT IONIZATION (b) FIELD IONIZATION

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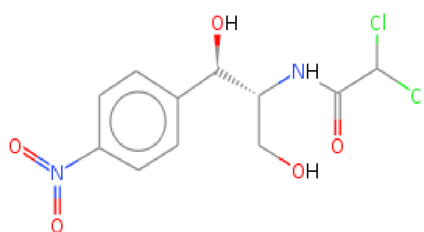
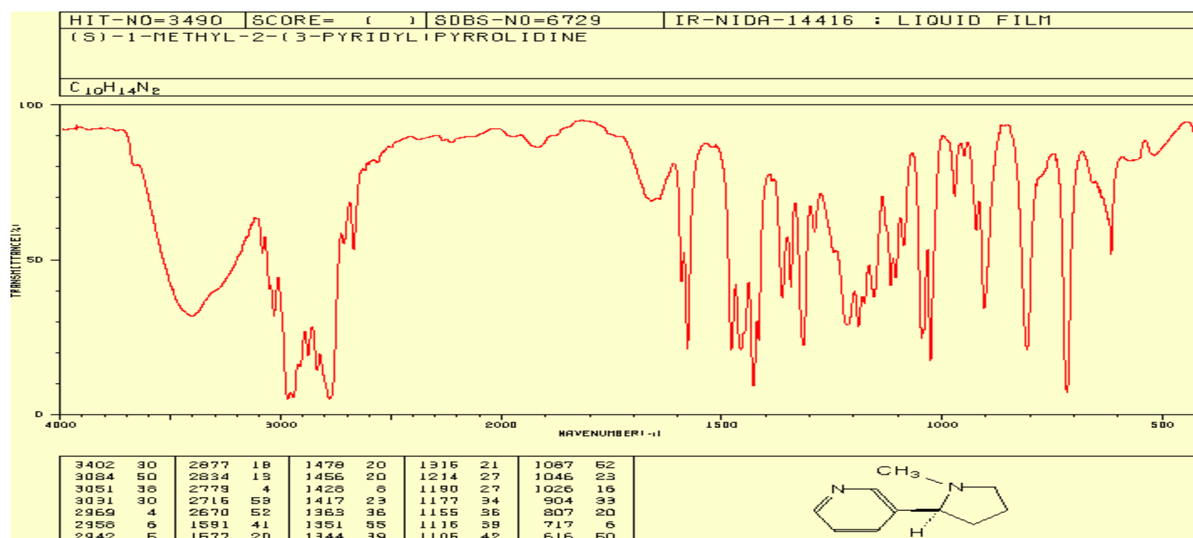


3. Structure of Chloramphenicol

3.1. Nicotine

Nicotine ($C_{10}H_{14}N_2$) is also called 3-(1-methyl-2-pyrrolidinyl) pyridine according to the IUPAC nomenclature. It is most isolated from tobacco leaf. It is a bicyclic compound with a pyridine cycle and a pyrrolidine cycle. The molecule possesses an asymmetric carbon and so exists in two enantiomeric compounds. In nature, nicotine only exists in the S shape, which is levogyre.[6]

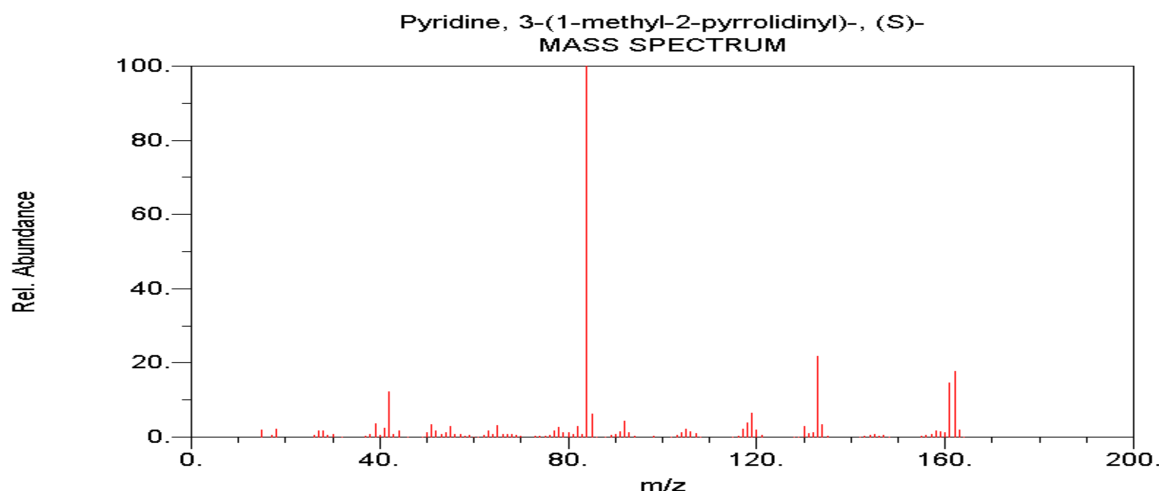
3.2. IR analysis of Nicotine



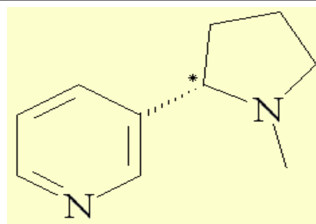
On this spectrum, we can notice several peaks, which characterise the different chemical functions of nicotine:

- Around 3400 cm^{-1}, we can see the large peak of water (it deals with a liquid film).
- Between 2970 and 2780 cm-1: C-H stretching.
- The peak at 1677 cm^{-1}: aromatic C=N double bond stretching.
- The peak at 1691 cm^{-1}: aromatic C=C double bond stretching.
- The peaks at 717 cm-1 and 904 cm-1 correspond to the out of plane bending of the C-H bond of the monosubstituted pyridinic cycle.

3.3. Mass Spectrum Analysis of Nicotine



We can notice the molecular peak at 162 m/z. However, the biggest peak is at 84 m/z. It corresponds to the fragmentation of nicotine. It deals with the pyrrolidine cycle, which has this molecular weight. During the electronic bombardment, the nicotine was split between the two cycles.

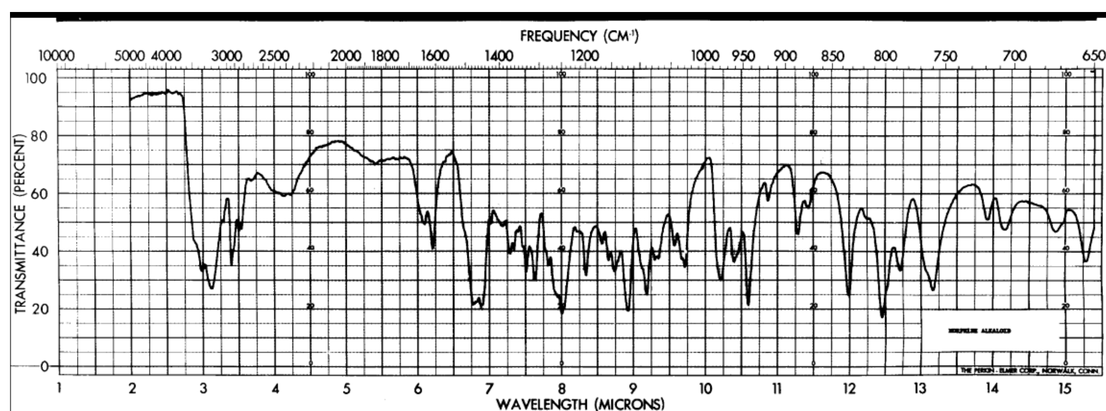


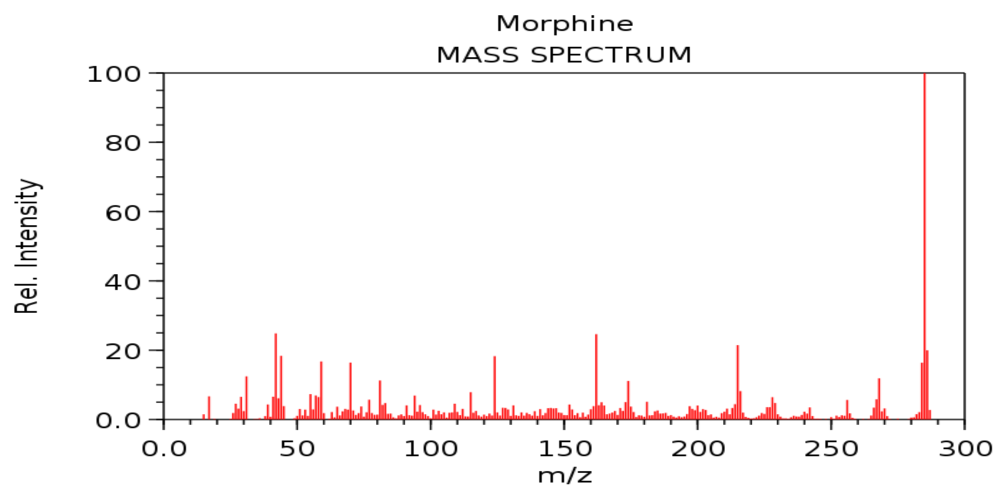
4. Structure of Nicotine

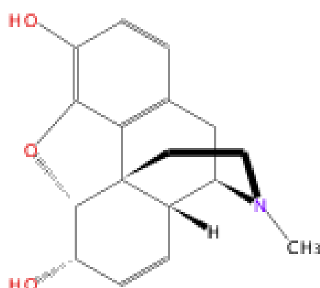
4.1. Morphine

Morphine, an alkaloid isolated from opium, is generally considered to be the most valuable of all pain relieving drugs. It is widely used to relieve moderate to severe pain associated with acute and chronic disorders, to provide analgesia during diagnostic and orthopedic procedures and as a preoperative medication before surgery. Morphine is variably absorbed from the gastro intestinal tract after oral ingestion[6].

4.2. IR Spectrum of Morphine



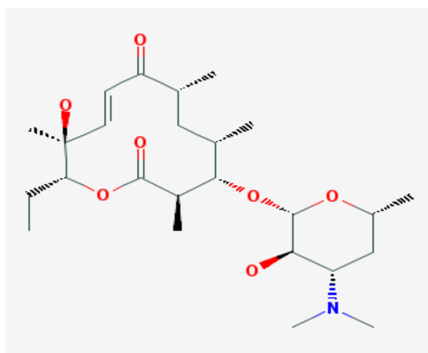




5. Structure of Morphine

5.1. Methmycin

Kinumaki et al.(1972) isolated a new antibiotic[7] m.p.68-70^{0}C, from cultures producing methmycin. The infrared spectrum data and their assignments were: 3420cm^{-1} (OH); 1730cm^{-1} (lactone and carbonyl); 1695cm^{-1} (conjugated ketone); and 1635cm^{-1} (conjugated double bond), the mass spectrum this new compound showed its molecular formula to be $C_{25}H_{46}NO_6$(M^+ 453.3048) .These spectroscopic data suggested a structure of methmycin as follows:



6. Structure of Methymycin

6.1. Patulin

This has been obtained from various moulds. It is an optically inactive solid. It has however never become important as an antibiotic because of bad side effects e.g. it slows down the healing process, although limiting the infection. The molecular formula of patulin is $C_7H_6O_4$. It is a neutral substance and forms a monoacetate. Patulin is a mycotoxin produced by several fungal species of the genera *Penicillium* and *Aspergillus*, found on several fruit species and, remarkably, in apples and apple products. Patulin has a broad spectrum of toxicity, including carcinogenicity and teratogenicity in animals. Due to the stability of the molecule, considerable amounts of patulin still remain in apple products after processing.[8]

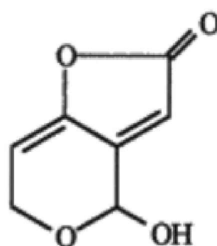


FIGURE 1 - Structure of Patulin (4-Hydroxy-4H-furol [3,2-c] pyran-2(6H)-one).

Infrared spectrum of patulin showed a band at 3660cm^{-1}, a region typical of free hydroxyl group. Furthermore, when patulin was acetylated, this band disappeared, but most of the other bands were still present, in particular, the bands at 1792cm-1(v.s.),1685cm^{-1} (s), and 1636cm^{-1}(s). Now the band corresponding to a keto group is always very strong and so the maximum at 1792cm^{-1}was assigned to a keto group. This frequency is higher than the usual range for acyclic (1725-1700cm-1) and 5- and 6-ring ketones (1750-1700cm-1), and α, β- unsaturated ketones (1690-1660cm-1). This suggests the presence of lactone (1800-1760cm^{-1}). Woodward synthesized it and found that its infrared spectrum was essentially identical with that of patulin in the 1600-1800cm-1 region.

7. Conclusion

The main benefits of IR spectroscopy are the speed and ease of use compared with traditional chemical and chromatographic methods, backed up by its non-destructive nature. In addition, the lack of formal education in IR spectroscopy and chemometrics presents problems in this area of research. The uses of mass spectrometry include quantifying the amount of a compound in a sample or studying the fundamentals of gas phase ion chemistry . MS is now in very common use in analytical laboratories that study physical, chemical, or biological properties of a great variety of compounds.

As an analytical technique mass spectrometry possesses distinct advantages such as: Increased sensitivity over most other analytical techniques because the analyzer, as a mass-charge filter, reduces background interference, Excellent specificity from characteristic fragmentation patterns to identify unknowns or confirm the presence of suspected compounds, Information about molecular weight, Information about the isotopic abundance of elements, Temporally resolved chemical data. A few of the disadvantages of the method is that often fails to distinguish between optical and geometrical isomers and the positions of substituent in o-, m- and p- positions in an aromatic ring. Also, its scope is limited in identifying hydrocarbons that produce similar fragmented ions.

REFERENCES

[1] From Wikipedia, the free encyclopedia.

[2] Sparkman, O. David (2000). Mass spectrometry desk reference. Pittsburgh: Global View Pub. ISBN 0-9660813-2-3.

[3] Sowell, Renã A.; Koeniger, Stormy L.; Valentine, Stephen J.; Moon, Myeong Hee; Clemmer, David E. (2004). "Nanoflow LC/IMS-MS and LC/IMS-CID/MS of Protein Mixtures". Journal of the American Society for Mass Spectrometry **15** (9): 1341–1353.doi:10.1016/j.jasms.2004.06.014. PMID 15337515.

[4] Finar I.L., Organic Chemistry Vol 2: Stereochemistry and the chemistry of natural products pp 865, 1995.

[5] Finar I.L., Organic Chemistry Vol 2: Stereochemistry and the chemistry of natural products pp 881, 1995

[6] http://webbook.nist.gov/chemistry.

[7] Finar I. L., Organic Chemistry Vol 2: Stereochemistry and the chemistry of natural products pp 879, 1995.

[8] Finar I.L., Organic Chemistry Vol 2: Stereochemistry and the chemistry of natural products pp 882, 1995.

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