# Learning Organic Chemistry Through Natural Products

## 5. A Practical Approach

***N R Krishnaswamy***

**N R Krishnaswamy was initiated into the world of natural products by T R Seshadri at University of Delhi and has carried on the glorious tradition of his mentor. He has taught at Bangalore University, Calicut University and Sri Sathya Sai Institute of Higher Learning. Generations of students would vouch for the fact that he has the uncanny ability to present the chemistry of natural products logically and with feeling.**

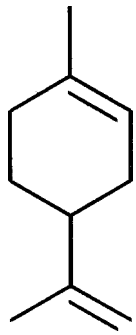
**In this article details for the isolation of natural products are described.**

In parts 1-4 of this series, an approach to the chemistry of natural products was delineated covering a fairly wide range of aspects. The learning process could be made more interesting through demonstrations and experimentation by the students themselves. The former could be incorporated within the classroom lectures whereas the latter could form part of the laboratory curriculum. In part 1 of the series, a simple thin-layer chromatographic experiment was demonstrated. In this article we describe the procedures for the isolation of some natural products. Many simple experiments using the isolated molecules that can be demonstrated in the classroom are also discussed.

Students should be encouraged to analyse the chemical components of a specimen like a seed, bark, flower, root or a lichen (*Box 1*) which may draw their attention because of a distinctive colour or smell or some other characteristic. I recall the advice given to students of natural products chemistry by the New Zealand chemist L H Briggs, "Carry a pair of scissors, a pen-knife and a few polythene bags so that you can collect interesting

Box 1

Lichens ( *pron.*liken) are symbiotic organisms consisting of tiny fungi and equally tiny algae. The two live together so closely and intimately that they appear as a single organism. Lichens can be found on trees, rocks and ancient monuments such as the Angkor Vat of Kampuchea. A species of the lichen *Parmelia* is commonly seen in Bangalore as pale green patches on trees and rocks. It is believed that the Biblical manna was a lichen.



**Limonene**

species when you go for a walk or are on a tour." The teacher should also build up a collection of the natural products, included in the syllabus, to be shown to the students. New additions could be continuously made and these can ultimately go into a permanent collection. It is easy to procure samples of terpenoids like geraniol, linalool, limonene, menthol, pinene, camphene and camphor, the alkaloids such as nicotine, piperine, codeine and quinine, and flavonoids like quercetin, kaempferol, their glucosides and cyanidin chloride. Similarly, samples of some of the carotenoid pigments like lycopene, beta-carotene and bixin can also be readily obtained. Some of these compounds can be isolated in the laboratory without any sophisticated instrument or apparatus.

## Isolation of Limonene and Hesperidin from Orange Peels

Dry orange peels in the shade by spreading them out on a table. Cut them into small bits and pack them in a Soxhlet extractor. Extract the material with light petrol (petroleum ether boiling at 40 – 60°C) till the siphoned material is colourless. Remove the solvent by distillation on a water-bath and distil the residue under reduced pressure. Limonene distils over as a colourless liquid at 75°C at 27 mm pressure. Alternatively, subject fresh orange peels to steam distillation and separate the essential oil distilling over. Dry the oil over anhydrous sodium sulphate and distil to obtain pure limonene (*Box 2*). 100 grams of the crude essential oil yields on the average, 75 grams of pure limonene.

Box 2

(+) Limonene occurs in orange and lemon oils. The (-) form is present in the oil of peppermint whereas the racemic form, also known as dipentene, can be obtained from turpentine oil. (+) Limonene can be converted via its nitroso-chloride into (-) carvone.

After the orange peels have been completely de-fatted, extract the material remaining in the Soxhlet extractor with methanol till the siphonings are colourless. Concentrate the extract under reduced pressure and re-crystallise the syrupy residue from aqueous acetic acid. The flavonoid glycoside, hesperidin, separates out as colourless needles, m.p. 252 – 254°C. The compound gives a wine-red colour with alcoholic ferric chloride and bright violet colour in the Shinoda test (*Box 3*). To perform the Shinoda test

add a pinch of magnesium powder to a solution of the compound in alcohol. Add concentrated HCl in drops and observe the colour formed.

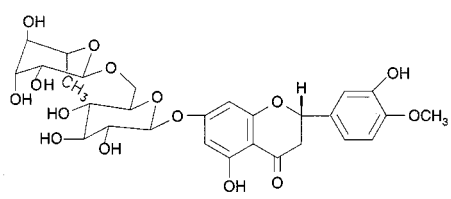
## Hydrolysis of Hesperidin: Paper Chromatographic Identification of the Sugars and Isolation of the Aglycone (hesperetin)

Dissolve 1 g of hesperidin in 20 ml of ethylene glycol containing 1 ml of concentrated sulfuric acid and heat the mixture on a boiling water-bath for 40 – 45 minutes. Pour the clear yellow solution into 50 ml of water and cool. Collect the precipitated hesperetin (aglycone) on a Büchner funnel, wash it free of acid using ice-cold water, and recrystallise from ethanol. The pure aglycone separates out as crystals melting at 224 – 226°C (yield: 0.35 g). Observe the colours given by the compound with alcoholic $FeCl_3$ and Mg-HCl (Shinoda test).

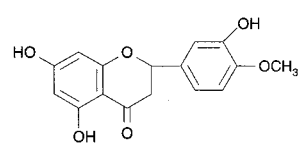
Neutralise the filtrate remaining after the separation of hesperetin and concentrate it preferably under reduced pressure or in a porcelain dish on a water bath. Examine the syrupy liquid thus obtained by circular paper chromatography (*Box 4*) using n-butanol-acetic acid-water (4:1:5) as the developing solvent. Spray the developed paper with a solution made by mixing equal volumes of 0.5 N aqueous silver nitrate and 5 N aqueous ammonia. Dry the chromatogram at 100°C for ten minutes when you will notice two brown coloured rings appearing. Measure the $R_f$ values and compare them with $R_f$ values of common

Box 3

Colour reactions, though largely empirical in nature, are very useful in structural elucidations. They are widely used in studies on alkaloids, steroids and triterpenoids and flavonoids. The Shinoda test involves a reductive transformation of colourless or pale yellow coloured flavones and flavonols into deeply coloured products among which are anthocyanidins. Try to think of a possible mechanism for this reaction.



Hesperidin



Hesperetin

Box 4

Circular paper chromatography, also known as horizontal chromatography is a quick and convenient method of separation and analysis of mixtures of compounds such as the amino acids, reducing sugars and polyphenolics. No elaborate equipment is needed (a Petri dish with a glass plate as a cover will do) and the compounds appear as concentric rings which can be detected with the help of appropriate spraying agents.

monosaccharides for the same solvent system. The sugars in this case are glucose ($R_f$ 0.29) and rhamnose ($R_f$ 0.48).

## Related Experiments

Make ethanolic extracts of the flowers listed below, concentrate the extracts and examine the residue by paper chromatography (circular and ascending or descending). View the developed chromatograms under ultraviolet light with and without exposure to ammonia. Spray the chromatograms separately with alcoholic ferric chloride and ethanolic aluminium chloride, and observe the number of rings or spots and the colour in each case. View the paper under UV light after spraying, and note if any fluorescent rings or spots are seen (*Box 5*). With each crude extract carry out the following colour reactions:

1. Colour with alcoholic ferric chloride.
2. Colour and fluorescence, if any, in concentrated sulfuric acid.
3. Play of colours, if any, with aqueous sodium hydroxide.
4. Colour with magnesium and HCl.
5. Colour with zinc dust and HCl
6. Colour with sodium amalgam before and after addition of HCl.

Make a careful record of the colours observed and draw appropriate conclusions in consultation with your laboratory instructor.

Following is the list of plants, the flowers of which can be easily procured and examined for their flavonoid compositions:

Box 5

5-Hydroxyflavones form chelate complexes with aluminium chloride which exhibit bright yellow fluorescence under uv light. Several types of aromatic and hetero-aromatic compounds such as the polythienyls and coumarins exhibit fluorescence under uv light and can thus be readily detected on paper and thin-layer chromatograms. Synthetic coumarins are used as optical brighteners on account of this property.

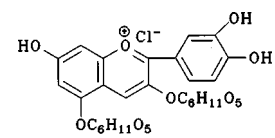
- *Gossypium indicum* (Cotton flower).
- *Argemone mexicana* (A plant with prickly leaves and bright yellow flowers) (*Box 6*).
- *Tagetes erecta* (marigold).
- *Chrysanthemum coronarium* (Samanthi).
- *Hibiscus esculentus* (Bindi) and other species of Hibiscus.
- *Butea frondosa* (flame of the forest).

Box 6

Most yellow and orange coloured flowers contain flavonols and their glycosides. Yellow and orange floral colours may also be due to the presence of chalcones, aurones, carotenoids and quinones.

## Isolation of Cyanin Chloride from Red Roses

Collect fresh, deep-red coloured roses, cut them into small bits and soak them in methanol containing 10% HCl. After the petals are completely bleached, decant the clear solution and concentrate it, preferably under reduced pressure. Examine the concentrated solution of the anthocyanin pigment thus obtained by paper chromatography using butanol-acetic acid-water (4:1:5) (use both layers in separate experiments) and phenol-water as the developing solvents. Observe the number of rings (spots) and measure their $R_f$ values. The major pigment is cyanin chloride. Keep the concentrated solution in a vacuum desiccator over concentrated sulfuric acid. Collect the crystals formed.




Cyanin chloride

Crude anthocyanin pigments of the following plant materials can also be similarly extracted and examined (*Box 7*).

- Flowers of Jacaranda, *Hibiscus Rosa sinensis* (Jabakusum), and other red, blue and purple coloured garden flowers.
- Berries of jasmine, coffee, black grapes and other dark coloured fruits.

Box 7

Deep orange, red, purple and blue coloured flowers are likely to contain anthocyanin pigments, many of which occur in nature as complexes with proteins and metals. During their isolation these complexes often break up and what are obtained are the simpler anthocyanins with different tinctorial properties.

Box 8

Natural bixin or labile bixin is a polyene in which one double bond has the *cis* configuration. It is a non-toxic, fat soluble pigment and is, therefore, used as a food colouring agent.

- Poinsettia leaves.

In each case, observe the colour on changing the pH of the solution from acidic to alkaline.

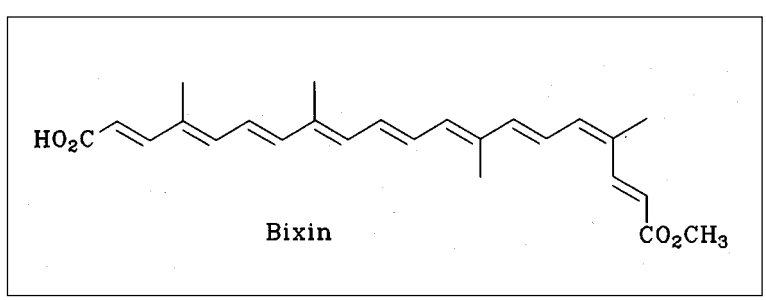
## Isolation of a Carotenoid Pigment — Bixin from *Bixa orellana* (Annatto Seeds)

Boil whole seeds of *Bixa orellana* (do not powder!) with ethyl acetate. Decant the extract and concentrate it to less than half its volume. Collect the pure crystalline bixin which separates out on cooling the concentrated extract on a Büchner funnel (*Box 8*). The average yield is 1.1 g from 100 g of the seed. Pour the filtrate into light petrol when a deep-red coloured solid separates out. This is impure bixin. Purer bixin can be obtained by re-dissolving it in a minimum quantity of ethyl acetate and precipitating it by careful addition of light petrol. By this method another 0.7 to 0.8 g of pure bixin can be obtained. Pure bixin separates out from ethyl acetate – light petrol as deep red needles. It gives a blue colour with concentrated sulfuric acid. Its purity can be checked by TLC on silica gel using chloroform-methanol (94:6) as the developing solvent.

Box 9

The orange stalks of the flower heads of *Nyctanthes arbortrystis*, known in Sanskrit as the Parijatha flowers, contain carotenoid glucosides which are closely related to the pigments of *Crocus sativa* (saffron). Therefore, thinly sliced and dried parijatha flower stalks have been used to adulterate saffron. The parijatha flowers do not have the flavouring principles of saffron.

The carotenoid glycosides of the flowers of *Nyctanthes arbortrystis* (Parijatha) can be similarly extracted and examined. These compounds are derivatives of crocetin which is closely related to bixin (*Box 9*).



Bixin

## Betanins from Bougainvillea

Immerse air dried bracts of coloured *Bougainvilleae glabra* in 1% methanolic HCl. After allowing the mixture to stand for a day, decant the coloured solution and add a second lot of fresh solvent to the once-extracted material. Concentrate the combined extract under reduced pressure. To the concentrated solution add a mixture of diethyl ether and light petrol (2:1 ratio) when the colouring matter separates. After a couple of hours, decant the supernatant liquid, re-dissolve the residue in 1% methanolic HCl and re-precipitate the pigments with ether-petrol. Repeat the process 5 to 8 times. Finally, dry the deep blue-red coloured viscous residue in a vacuum desiccator over calcium chloride. The dark purple coloured solid thus obtained is a mixture of betacyanins and betaxanthines.

Box 10

The colouring principles of Bougainvilleae resemble the anthocyanins and the flavonoids but they belong to a different chemical type. They are nitrogenous compounds biosynthetically produced from the amino acid proline. These compounds known as the betacyanins and betaxanthins are confined to the plant order Centrospermae and have considerable value as taxonomic markers. They are also present in beet root and Amaranthus.

## Mandelonitrile from a Millipede – Comparison with a Synthetic Sample

With the help of an entomologist collect a few polydesmid millipedes (*Harpaphe haydeniana*) which are found in large numbers in areas dominated by deciduous trees (there are a large number of them in the Calicut University campus). Immobilise the millipedes by chilling them to 4°C (this can be done by keeping them in a petri dish inside the freezer compartment of a refrigerator). Then place them under boiling ethanol and crush them. Filter the extract, if necessary with the aid of Celite, and extract the residue a second time with boiling ethanol. Concentrate the combined extract under reduced pressure, add water and extract with ether in a separatory funnel. Use light petrol-methanol-carbon tetrachloride (125:50:50) as the developing solvent for the TLC examination of the crude mandelonitrile present in the ether extract. The compound has an $R_f$ value of 0.5 in this solvent system and can be detected by spraying the TLC plates with 5% NaOH solution followed by a saturated solution of 2,4-dinitrophenylhydrazine in 6 M HCl (*Box 10*).

Compare the compound on TLC, with a sample of synthetic mandelonitrile prepared as follows :

In an 125 ml Erlenmeyer flask, dissolve 11 g of sodium hydrogen sulphite in 30 ml of water, add 10 ml of benzaldehyde, swirl and stir vigorously until the oily aldehyde is completely converted into the crystalline bisulphite adduct. Cool to room temperature, add a solution of 14 g of KCN in 25 ml of water (CAUTION: Since KCN is poisonous wear gloves and do this in a fume hood with proper ventilation) swirl and stir for about 10 minutes until all but a trace of the solid has dissolved. Mandelonitrile separates out as a thick oil. Pour the mixture into a separatory funnel, rinse the flask with small amounts of ether and water and shake the mixture vigorously for a minute to complete the reaction. Add 20 ml of ether, shake, pour off the aqueous layer down the drain (CAUTION: make sure that the sink does not have any acid, as this may cause the formation of poisonous HCN gas) and wash the ether layer with 25 ml of water. Dry the ether solution over anhydrous sodium sulphate and remove the solvent. Examine the residue by TLC.

Box 11

Mandelonitrile is detected on TLC plates as benzaldehyde as it is easily hydrolysed being a cyanohydrin. As a matter of fact, millipedes use it as a precursor for generating the toxic hydrogen cyanide.

## Piperine from Black Pepper

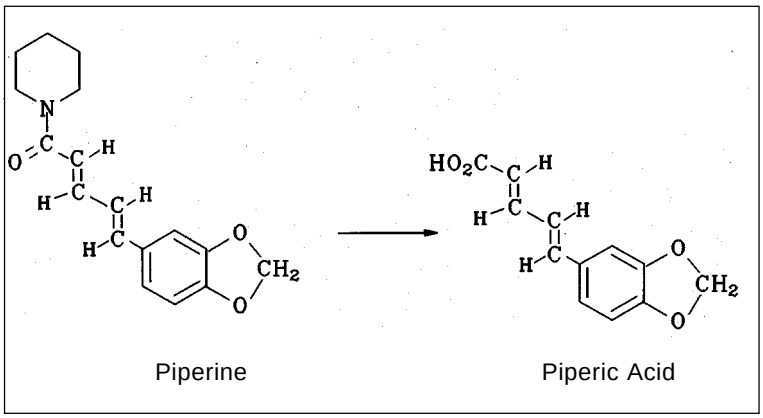
Grind 10 g of black pepper to a coarse powder and extract with 95% ethanol in a Soxhlet extractor. Concentrate the extract under reduced pressure on a water-bath at 60°C. Add 10 ml of 10% alcoholic KOH to the residue and allow the mixture to stand for a few minutes (*Box 12*). Carefully decant the supernatant solution from the solid deposit. When the alcoholic solution is left overnight, yellow-coloured needles separate out. The yield is 0.3g. Record the melting point (125–126°C) and the NMR spectrum of the compound.

Box 12

Piperine, being an amide, is a non-basic alkaloid and is the compound responsible for the pungency of black pepper.

## Hydrolysis of Piperine – Isolation of Piperic Acid

Reflux a solution of 1 g of piperine in 10 ml of 10% ethanolic KOH for 90 minutes. Evaporate the solution to dryness by




distillation under reduced pressure, the receiver being cooled in an ice-salt bath. Suspend the solid potassium piperinate remaining in the distillation flask in hot water and add concentrated HCl. Collect the yellow precipitate, wash it with ice-cold water and re-crystallise it from ethanol. Pure piperic acid is obtained as yellow needles, m.p. 216 – 217°C. Saturate the distillate (in the receiver) with HCl and evaporate it to dryness when piperidine hydrochloride separates out. Re-crystallise it from ethanol. Pure piperidine hydrochloride has a m.p. 244°C.

## Suggested Reading


- ***Natural Products - A laboratory guide*** **by R Ikan. Academic Press. 2nd Edition.**
- **Fieser and Fieser .** ***Organic Experiments.*** **p.110, 1965.**


*Address for Correspondence*
N R Krishnaswamy
No.12, 9th Main Road
Banashankari 2nd Stage
Bangalore 560 070, India.

**Max Planck on a New Scientific Truth...** "Max Planck surveying his own career in his 'Scientific Autobiography' sadly remarked that a new scientific truth does not triumph by convincing its opponents and making them see the light, but rather because its opponents eventually die, and a new generation grows up, that is familiar with it." (From *The Structure of Scientific Revolutions* by Thomas S Kuhn)