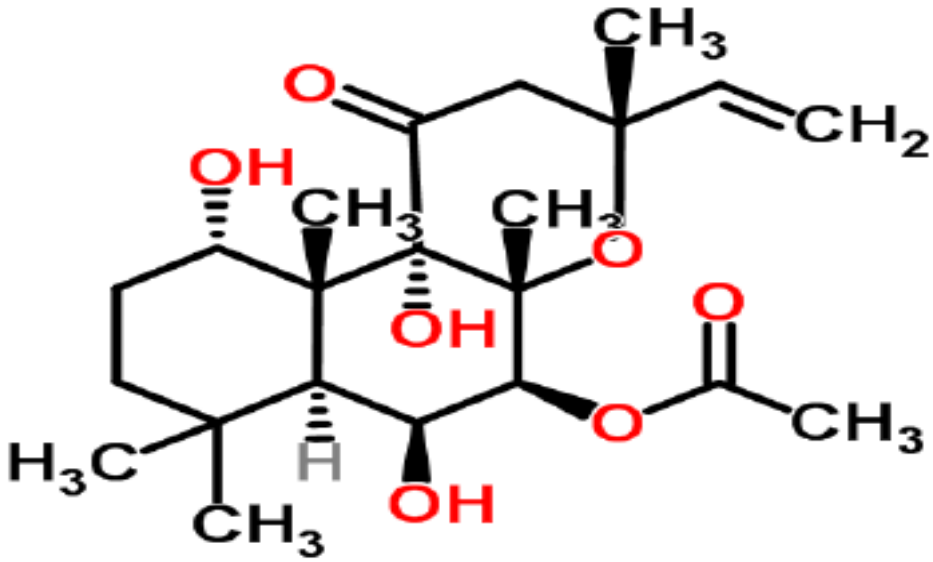
INDUSTRIAL PRODUCTION, ESTIMATION AND UTILIZATION OF PHYTOCONSTITUENTS

1. FORSKOLIN:

forskolin is a labdanediterpene that is extracted from pulverised dried plant material of Coleus forskohli.

Family : Labiatae.

Structure:



FORSKOLIN

Industrial Production:

1) Tubers of C. forskohliiare collected, washed, dried, and pulverised into granules.
2) The whole forskolin is extracted (utilising methanol as solvent) in crude form by the traditional methods.

3) The obtained methanol extract is concentrated, and the resultant concentrate is added with chloroform.
4) Equal volume of water is added to the separating funnel.
5) The resulting mixture is shaken thoroughly, then allowed to settle, after which the chloroform layer is separated.
6) The mixture is treated with water for 2 -3 times and the chloroform layer is separated and concentrated.
7) A precipitate of forskolin is obtained by using ice cold n-hexane.
8) Forskolin is obtained as a reddish brown to brown coloured powder.

Estimation:

1)HPLC is used to estimate forskol in content in the raw material, which shouldcontain more than 1% of forskolin on a dry basis. The raw material is analysed by isocratic liquid chr omatography using aHPLC system, equipped with a dual/quaternary pump, a manual/auto injector, and a photodiode array or UV detector supported by a suitable software.(Ed.by.Dr.k.prabhupageno.232)

2) Mob. Phase- toluene:ethyl acetate(8.5:1.5v/v)

3) Stationary phase- silica gel F254.

Utilization:

1. Antidepressants.
2. Vasodilating.
3. Antiobesity.

REFERENCES:

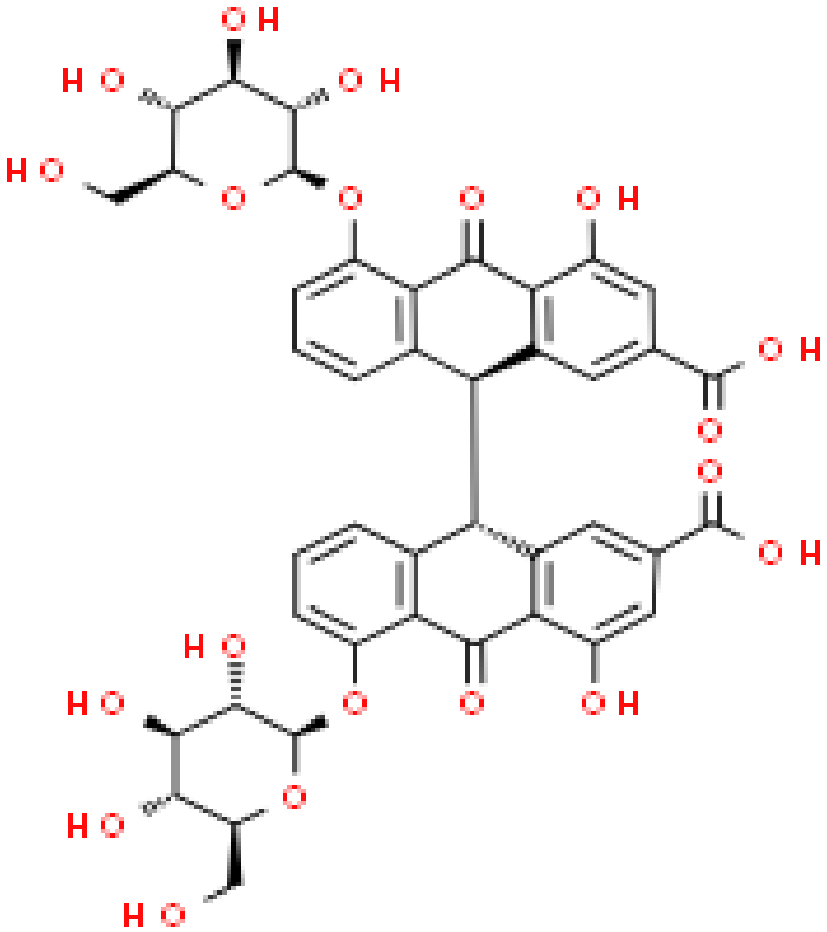
1) Pharmacognosy and phytochemistry-ll Ed.by.Dr.k.prabhupageno.232.

2. SENNOSIDE:

Sennoside is obtaine d from the dried leaves of Cassia angustifoliaVahl.

family: Leguminosae.

Structure:



*SENNOSIDE *

Industrial Production:

Method 1

1) The leaves are dried, powdered, and extracted with benzene for 2 hours on an electronic shaker.
2) The extract is filtered and the solvent is distilled off.
3) The resultant mark is dried, extracted with 70% methanol for 4 -6 hours, and separated by filtration.
4) The extraction process is repeated using 70% methanol for 2 hours, and the extract is filtered again.
5) The metabolic extracts are combined and concentrated till 1/8th of its original volume remains.
6) The resultant concentrate is acidified with hydrochloric acid to obtain pH 3.2.
7) The extract is filtered and alcoholic anhydrou s calcium chloride is added with continuous stirring.
8) Ammonia is added to maintain the pH at 8, and then the mixture is left undisturbed for 2 hours.

Method 2

1) The leaves of senna are dried, powdered , and extracted with chloroform-ethanol (93:7) mixture for 30 minutes.
2) The obtained extract is filtered.
3) The resultant mark is again extracted using acidic methanol.
4) Both the extracts are combined and concentrated.
5) The resultant mixture is left undisturbed at room temperature for 12 hours.
6) The precipitate of sennoside A obtained is recrystallised using triethylamine.
7) The sennoside B present in the solution is precipitated b y 10% methanolic calcium chloride solution.
8) The p recipitate of sennoside B is purified using a mixture of ammonia -methanol (40:60).
9) The obtained precipitate is dried, washed with water, kept for a day, and recrystallised with glycomonoethylether

Estimation:

1. Columbia - C18.
2. Flow rate - 1ml/min
3. Detection - 350nm
4. Various spectrophotometric, spectrofluorimetric and chromatographic techniques like HPLC, HPTLC, etc. may be used for the estimation of sennoside s. Calcium sennosides are ext racted with boiling water and oxidised by treating with ferric chloride

Utilization:

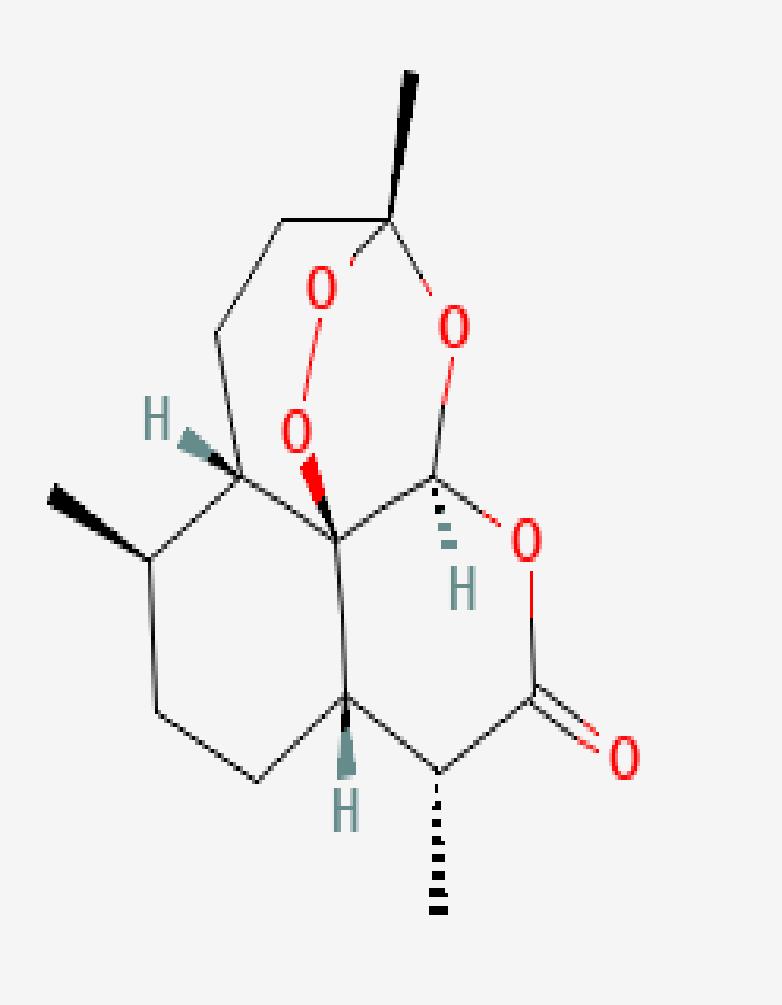
1. Treatment of constipation.
2. In skin disease.
3. As an anthelmintic.

3. ARTEMISININ:

Artemisinin is obtained from the plant Artemisia annua.

family: Asteraceae.

Structure:



*** ARTEMISININ***

Industrial Productions:

1) Artemisia annua is mainly grown in the temperate regions.

2) Artemisinin is present in the leaves and flowering tops of the plant.

3) The yield of art emisinin may be enhanced by 30% using the growth regulator (e.g., chlormequat) before harvesting.

4) For in vitro production, Artemisia annua is grown and propagated in a hormone-free medium by the process of micro cutting.

5} If used for research purposes, the plant is dried by lyophi lisation before extraction, thus the moisture content of samples can be controlled easily.

6) Artemisinin is extracted from air dried plants using ethyl ether, petroleum ether ,and even gasoline as a solvent.

7) Petroleum ether (30-60□C) is the most satisfactory solvent among various non -protic solvents.

8) Extraction with hexane at room temperature.

Estimation:

1. Artemisinin is electrochemically active, therefore HPLC with r eductive electrochemical detection (HPLCFC) is used to estimate the concentration of artemisinin in plane extracts.(Ed.by.Dr.k.prabhupageno.237)
2. Stationary phase - silica gel F254.

Utilization:

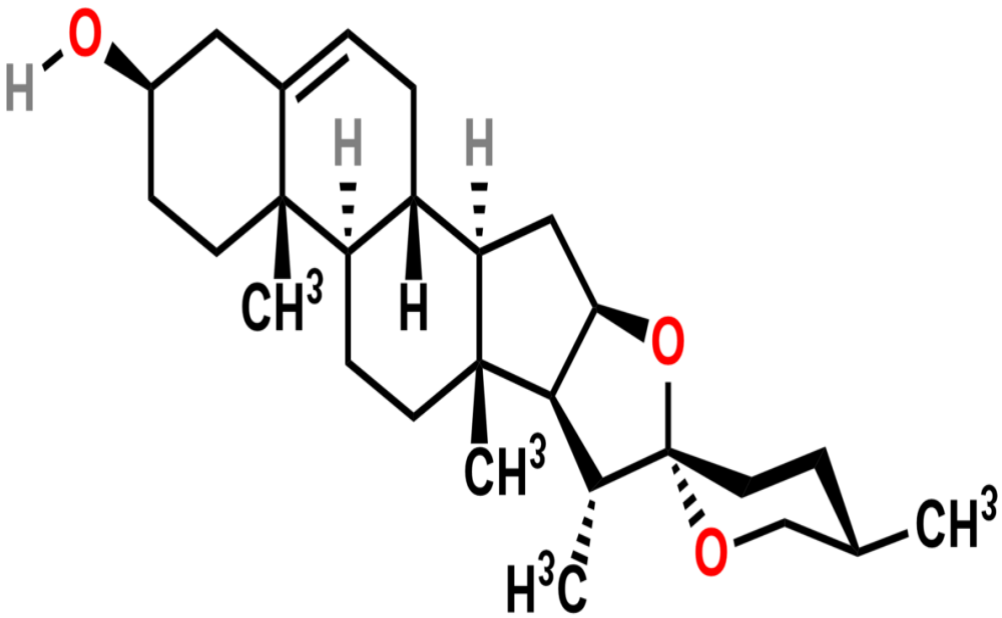
1. Antimalarial.
2. Anticancer.

4. DIOSGENIN:

diosgenin is extracted fro m the dried tubers of the plants Dioscoreaeltoidea, D. composita.

family: Dioscoreaceae.

Structure:



DIOSGENIN

Industrial Production:

1) Alcoholic Extraction Method

Dioscorea tubers are cut into small pieces and dried under sunDried tubers are powdered, extracted with ethanol/methanol twice for 6-8 hoursFiltered and filtrate

is concentrated to a syrupy liquidConcentrated liquid is hydrolysed using an acid (HCl or H_2SO_4) for 2-12 hours85% of diosgenin is precipitatedPrecipitate is filtered and washed with waterPurification with alcohol.

2) Acid Hydrolysis Method

Dried rhizomes are powdered and subjected to hydrolysis by refluxing with 5% HCl for 2 hoursThe hydrolysed mass is filtered, washed twice with water and then twice with 5% sodium bicarbonate solutionFinally washed with water till the washing are neutral. The residue obtained is dried and extracted with toluene for 8 hoursToluene extract is concentrated during which diosgenin getsprecipitatedDiosgenin is filtered, washed with little hexane and dried (40-60°C) to yield about 95% pure product.

3) Incubation cum Acid Hydrolysis Method

Fresh plant material is incubated in water at 37°C for few days. It is later subjected to acid hydrolysisThe hydrolysed liquid is concentrated and extracted with hydrocarbon solvent to obtain diosgenin

Estimation:

1. By HPTLC Method: A stock solution of diosgenin (100µg/ml concentration)is prepared by dissolving 1mg of diosgenin1ml of chloroform. A calibration curve from 1000 - 6000ng/spot is prepared and analysed for reproducibility(Ed.by.Dr.k.prabhupageno.238)
2. Mob. Phase - toluene:ethylacetate:formic acid (5:4:1).

3. Stationary phase- silica gel F254.

Utilization:

1. in treatment of rheumatism.
2. In preparation of oral contraceptives.

REFERENCES:

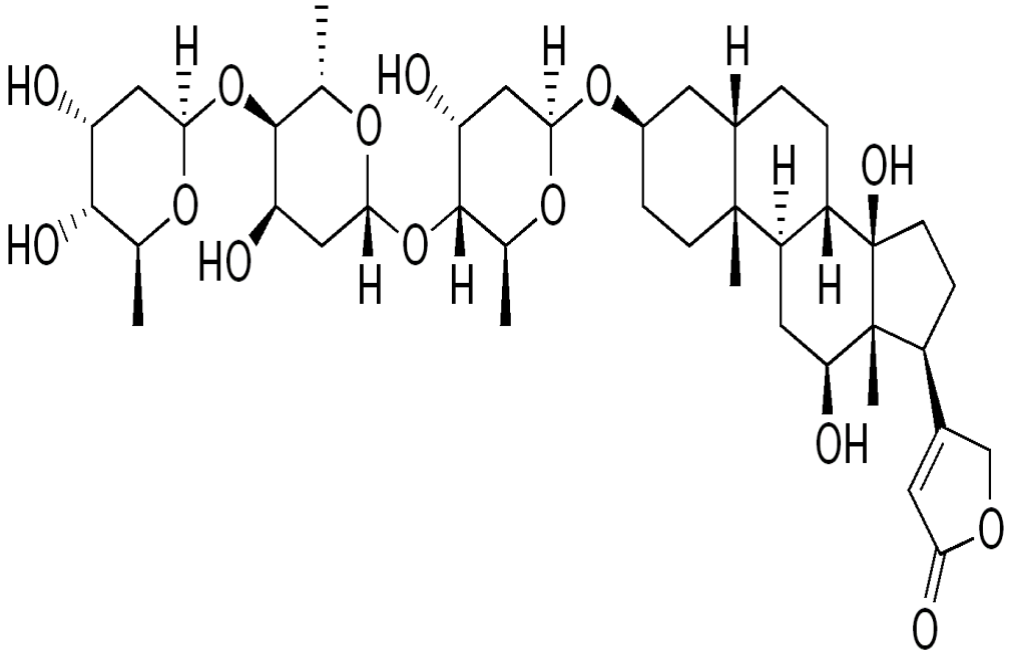
Pharmacognosy and phytochemistry-ll
Ed.by.Dr.k.prabhupageno.238)

5. DIGOXIN:

Digoxin is obtained from Digitalis lanata and is u sed a Digoxin is obtained from Digitalis lanatas.

family: Plantaginaceae.

Structure:



DIGOXIN

Industrial Production:

1) Initial Extraction and Removal of Phenolics by Precipitation

   i) The powdered drug (20ml) is extracted with 100ml 70% ethanol and heated on a hot plate for 20 minutes.

ii) The extract is cool ed and filtered or centrifuged to remove solid plant debris.

iii) 150ml of water and 20ml of strong lead sub -acetate solution is added to the filtrate or supernatant.

iv) A precipitate of phenolic compounds is obtained in the form of insoluble lead complexes.
v) The mixture is centrifuged, the supernatant is pipette d out, and 10% H_2SO_4 is added drop wise till no precipitate forms.
vi) This treatment removes excess amount of lead ions in the form of insoluble lead sulphate.
2) Solvent Partition to Remove Cardenolides from Aqueous Layer
i) The mixture is centrifuged, the supernatant is pipetted out , and further extracted with 50ml of chloroform for four times.
ii) The chloroform extract is combined and washed with 20 ml of water to remove any residual lead ions.
iii) The chloroform extract is separated and dried u sing anhydrous sodium sulphate.
iv) The obtained chloroform solution is filtered into a round bottom flask.
v) 5ml aliquots of chloroform are evaporated to dryness in separa te evaporating dishes.

vi) In the final step , two tests are performed on the residue; first is Kedde test for cardenolides and the second is iron (III) chloride test for phenolic compounds.

Estimation:

1. Concentration of digoxin in plant extracts, therapeutic preparation s, body fluids, etc.
2. may be measured by various methods based on the principal of colorimetry, fluorimetry, gas liquid chromatography, HPLC, and radioimmunoassay.

Utilization:

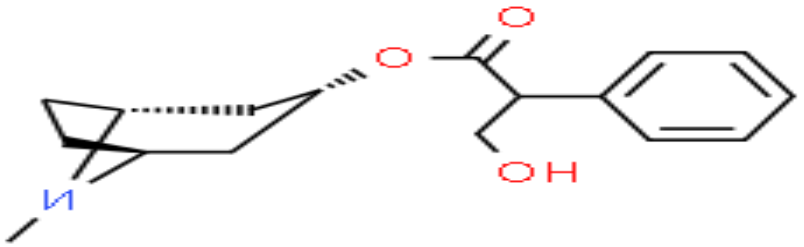
1. Digoxin is used along with other medications for treating heart failure.

6. ATROPINE:

Atropine is obtained from the dried leaves and flowering tops of plant Atropa belladonna.

family: Solanaceae.

Structure:



ATROPINE

Industrial Production:

Method 1

1) The powdered crude drug is moistened by adding sodium carbonate solution.
2) Then the powder is extracted with benzene or ether.
3) The alkaloidal bases are extracted with acidified water.
4) The acidified extract is treated with solvent ether to remove the colouring matter.
5) Alkaloids are precipitated by sodium carbonate.
6) The obtained residue is filtered, washed, and dried.

7) The dry mass is dissolved in solvent ether and dehydrated with sodium sulphate.
8) The obtained extract is filtered.

9) The filtrate is concentrated and cooled to obtain a mixture of closely related alkaloids (majorly atropine and hyoscyamine).

10) After filtration , the crude crystalline mass is separated and dissolved in alcohol.

11) To convert hyoscyamine into atropine, sodium hydroxide solution is added to the above mixture.
12) Acetone is added to crude atropine for re-crystallisation.

.Method 2

1) The plant leaves are powdered and extracted with 95% ethanol.
2) Then the ethanol is distilled off.
3) The resinous matter is removed by treating the obtained syrupy mass with 1% hydrochloric acid.
4) This acidified solution is tre ated with light petroleum ether, followed by the addition of ammonia to make it alkaline.
5) The alkaline solution is then treated with chloroform.
6) The chloroform layer is treated with dilute acid.
7) The chloroform layer is separated, made alkaline, and re - extracted with chloroform.
8) This chloroform layer is evaporated, and oxalic acid is added to get oxalates of atropine and hyoscyamine.

Method 3

1) Isolation of atropine is done from roots of belladonna plant or juice of datura plant.
2) 95% alcohol is used to extract atropine from the powdered drug.
3) To convert hyoscyamine to atropine, the alcoholic extract obtained in the above step is heated with potassium carbonate.
4) The obtained extract is re-extracted with chloroform.
5) The chloroform layer is evaporated, and the resi due is treated with dilute sulphuric acid.
6) Atropine is precipitated by treating the above solution with po tassium carbonate.
7) This atropine is further extracted with ether, and purified by adding oxalic acid to yield crystals of atropine oxalate.

Estimation:

1. Assay - sulphate salts of Atropine titrated against 0.1N Perchloric acid.

Utilization:

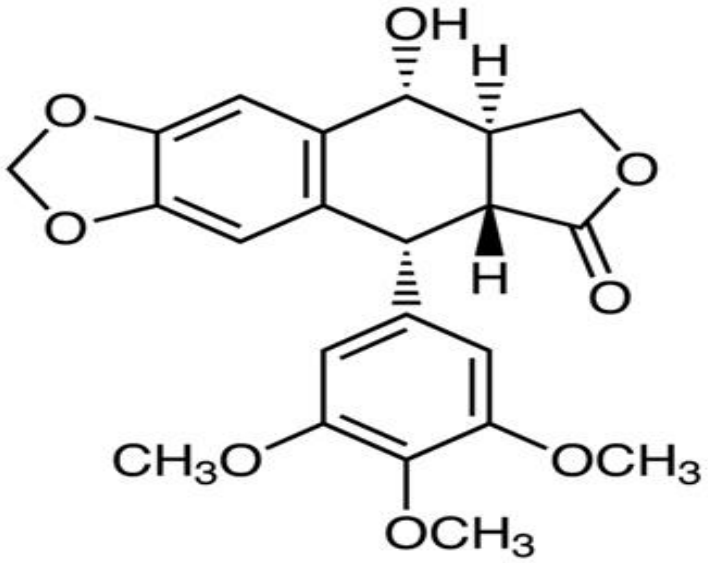
1. As preanesthetic medication.

7. PODOPHYLLOTOXIN:

Podophyllotoxin occurs naturally and is obtained from Podophyllumpeltatum.

family: Berberidaceae.

Structure:



PODOPHYLLOTOXIN

Industrial Production:

Method 1

1) Accurately weighed powdered drug is extracted with methanol.

2) The extract is filtered and concentrated.

3) The obtained semisolid concentrate is dissolved in acidified water.

4) The resultant precipitate is allowed to settle for 2 hours.
5) The filtrate is decanted and washed with cold water.
6) The residue is collect ed, washed with acidified water, and dried to obtaindark brown amorphous powder of podophyllin.
7) This powder is treated with hot alcohol, filtered, and evaporated to dryness.
8) The residue is re-crystallised with benzene or mixture of alcohol and benzene followed by washing with petroleum ether.

Method 2

1) The chloroform soluble fraction of powder is dissolved in alcohol.
2) The resultant mixture is refluxed with neutral aluminium oxide to obtain light yellow coloured solution.
3) The alcoholic solution is added with benzene to obtain podophyllotoxin.

Method 3

1) This method involves extraction over a bed of neutral alumina with benzene, toluene etc. solvents for 1-4 hours.
2) Pure podophyllotox in is obtained by re-crystallisation using h ot benzene, toluene or xylene.

Estimation:

1. HPLC Determination of Podophyllotoxin Column cis-symmetry 4.6 × 150mm of 5□.(Ed.by.Dr.k.prabhupageno.245)

2. Mob. Phase- methanol:water (62:38v/v)

3.Detector wavelength - 280n

Utilization:

1. Antitumour.
2. Purgative.
3. Emetic.

8. CAFFEINE:

Caffeine is obtained from the dried ripe seeds of Coffea a rabicaLinne or C. liberica.

family: Rubiaceae

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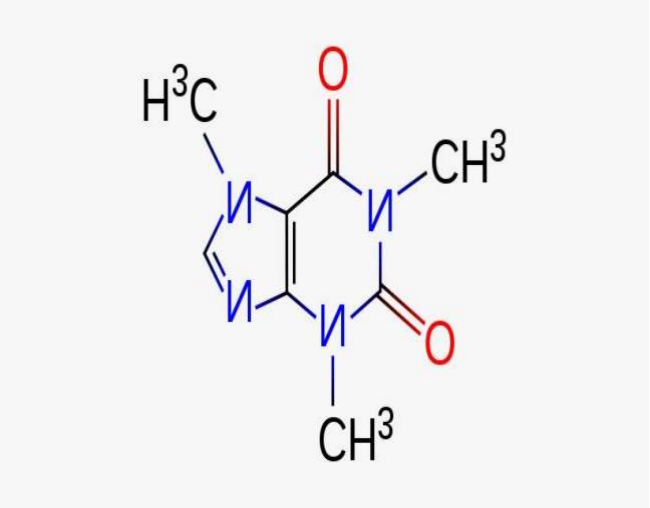
Structure:



CAFFEINE

Industrial Production:

Method 1

1) The moistened coffee beans are fed to the extractor. For 10 hours, CO 2 from CO2 holding tank is passed through the bed of beans.

2) This process extracts around 97% of caffeine from the beans. The CO 2-containing caffeine is stored in the holding tank till the extraction process ends. Thereafter, the exhausted material is removed from the extractor and fresh material is fed.

3) Then the caffeine -containing CO2 stored in the holding tank is passed at a steady rate through the water wash column.

4) Here the supercritical CO 2 comes in contact with a steam of water. This removes 99.5% of caffeine from the CO2.

5) The regenerated supercritical CO 2 leaves the water wash column. At this point, CO2 is at a lower pressure due to pressure drop through the system.

6) The regenerated CO2 accumulates in the holding tank after 2 hours CO2 is fed back to the extractor, to start decaffeination of a new batch of coffee.

7) The caffeine-rich water in the process vessel is added with water.

8) Caffeine is extracted from green tea by supercritical fluid extraction under the following conditions:

i) 15ml ethanol is used as co-solvent, and

ii) Extraction is carried out at 80°C temperature.

9) The flow rate and pressure of SC -CO2 are maintained at 1.5 l/min and 300 bars, respectively. The extraction duration is 2 hours. This method produce s70.2% caffeine evaporatand 6.2% catechins.

Method 2

1) Caffeine isisolated from instant coffee granules by supercritical CO2 methanol.

2) The apparatus has two reciprocating pumps, column oven, back -pressure regulator, a circulating water bath with ethylene glycol mixture, an extraction cell, and a collection cell.

3) SFE is done under the conditions stated below:

i) The flow rate of liquid carbon dioxide and methanol is maintained at 1.8ml/min and 0.02ml/min, respectively.

ii) The oven temperature is set at 60°C and pressure is maintained at 250kg/cm2

Estimation:

1. Mob. Phase- methanol:Acetonitrile (65:35v/v).
2. Column -C18.

Utilization:

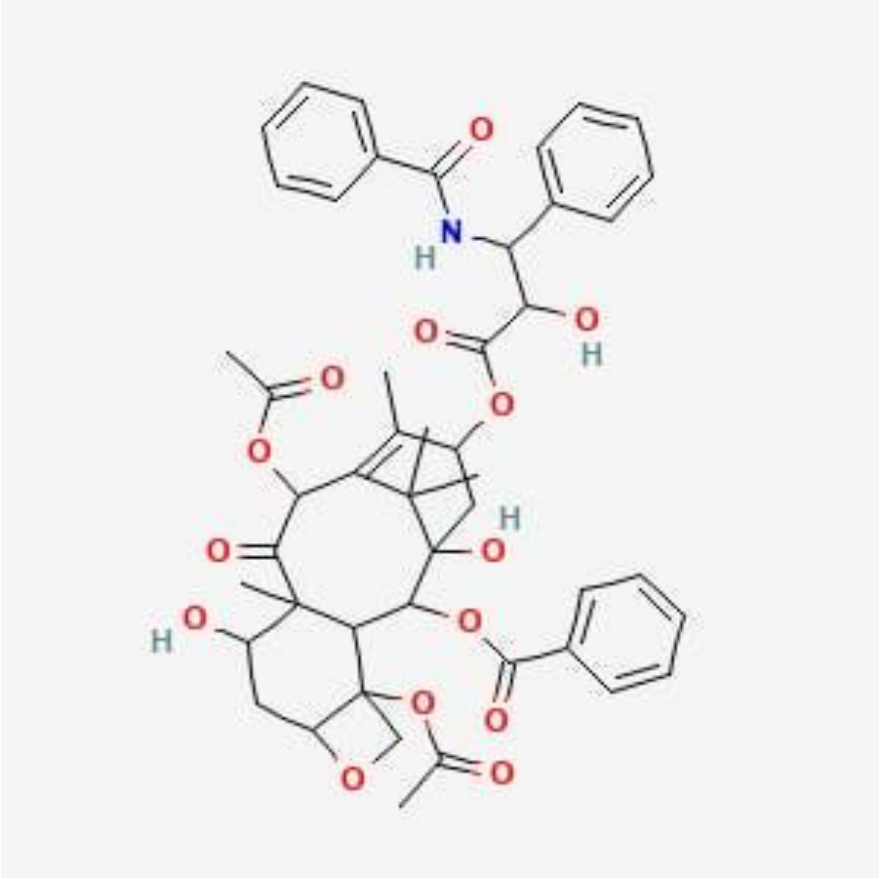
1. CNS stimulant.

9. TAXOL:

Taxol (or paclitaxel) is a natural diterpenoid.

family: Taxaceae.

Structure:



TAXOL

Industrial Production:

1) The dried ground bark is extracted with methanol or ethanol , and alcohol is removed by concentrating the combined extract.
2) The obtained concentrate is re -extracted with dichloromethane and the solvent extract is concentrated to a powder.
3) This powder is stirred with a mixture of acetone and ligroin (1:1) and filtered to remove the insoluble matter.
4) The filtrate containing taxol is concentrated, dissolved in 30% acetone in ligroin, and applied to a column of Florisil.

5) The taxol fraction from the column is twice purified by crystallisation.
6) The crystalline taxol is subjected to chromatography on a silica column. The closely related analogue, cephalomannine, separates out from taxol.
7) The purified taxol obtained from the column is crystallised twice.
8) The unseparated mixtures and mother liquors are recycled through the silica column to obtain more pure taxol.

Estimation:

1. HPTLC method. (Ed.by.Dr.k.prabhupageno.251)
2. Mob. Phase - chloroform:Methanol(7:1v/v).
3. Visualizing agent- vanillin sulphuric acid.

Utilization:

1. Treatment of ovarian, lungs, bladder, esophageal and other type of cancer.
2. Antiproliferative agent.

REFERENCES:

Pharmacognosy and phytochemistry-ll Ed. by.Dr.k.prabhupageno.251.

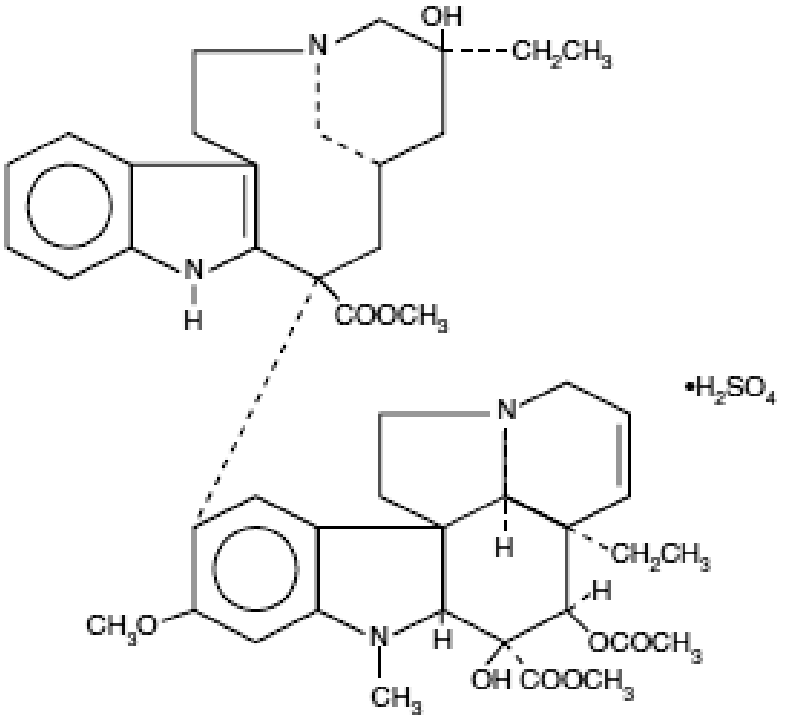
10. VINCRISTINE AND

VINBLASTINE:

Biological source indolealkaloid,dried leaves of Catharanthusrosea.

family: Apocynaceae.

Structure:



*** VINBLASTINE***

Industrial Production:

- Plants tissue culture Techniques.
- Enhance extraction an of anticancer drug, Vinblastine, from Catharanthusroseus.

Estimation:

1) Chromatographic Method
i) Detection:a) UV = 254nm
b) DRG No. 13B = visible range
ii) Drug Sample: Vinca (fresh leaves).
iii) Reference Compounds: T1 = v incamine, T 2 = vincaminine, T 3 = vincine, T4 = vincamajine, T5 minouincine, and T6 = reserpinine.
iv) Solvent System: Ethyl acetate:methanol (90:10).
2) USP NF-1995
i) Diethyl Amine Solution: 5ml of diethylamine is mixed with 295ml of water, and the pH is adjusted to 7.5 with phosphoric acid.
ii) Mobile Phase: A filtered and degassed mixture of methanol and diethyl amine solution (70:30) is prepared.
iii) Standard Preparation: Accurately weighed quantity of USP vincristine sulphate is dissolved in water to achieve 1mg/ml concentration.

Utilization:

1. In chemotherapy regimens.
2. Childhood leukemia.

REFERENCES:

- Pharmacognosy and Phytochemistryvol - I, II, IV Ed. By Vinod. D. Rangari. .

HERBAL INDUSTRIAL MACHINE :

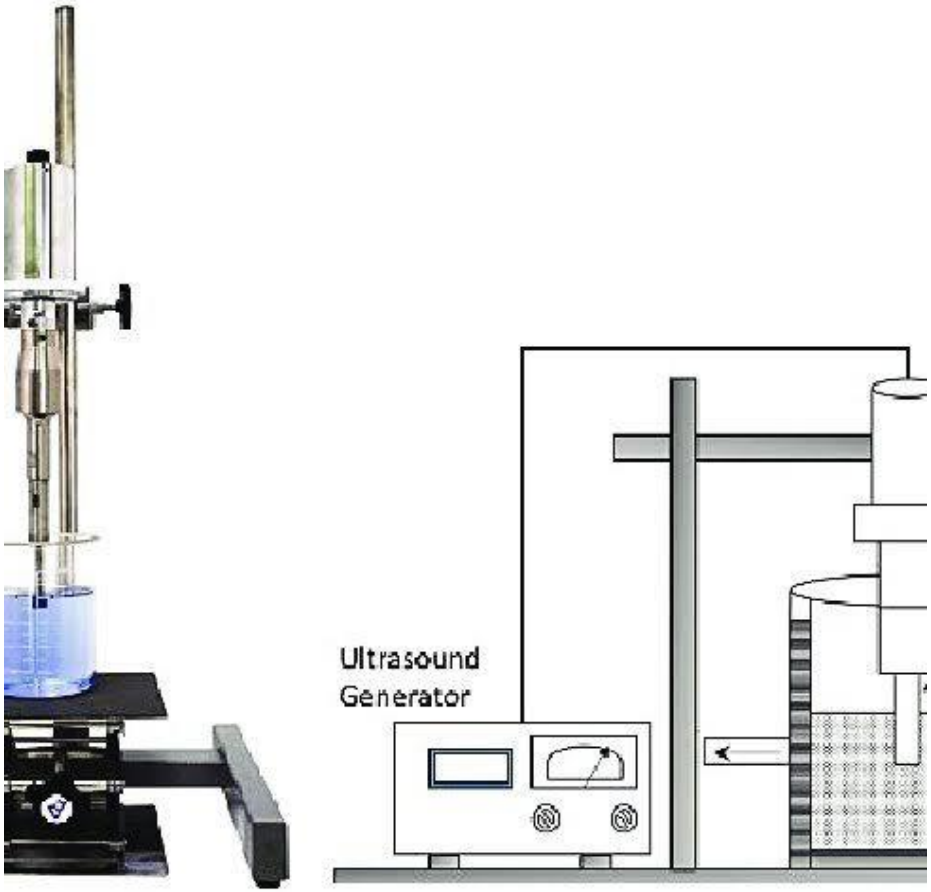
1) extraction machine:

2) super critical fluid extraction machine:

3) Heat reflux extraction machine:

4) maceration extraction machine:

5) Ultrasound assisted extraction machine:



6) Herbal extraction machine:

7) oil extraction machine :

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SUBJECT: Pharmacognosy And Phytochemistry-II

CLASS: T.Y. B.Pharmcy

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