CYTOSTATICS (ANTINEOPLASTICS, CANCEROSTATICS)

- Cancer – main cause of premature death
- Developed countries – each fifth death caused by cancer
- Incidence depends on sex, age, race, genetic predisposition and on presence of cancerogens in environment
- Costs on treatment extraordinary high (surgery, radiotherapy, price of drugs)

History:

1914 verification of chemical cancerogens in environment (one is usually not enough to trigger cancer)

1947 Berenblum model (on mice):

- cancerogen in subliminal dose --- no cancer
- Repeated effect of cancer promotors --- no cancer
- cancerogen + promotor --- always cancer

- Syncancerogenesis – exposition to several cancerogens
- Cocancerogenesis – cancerogen + promotor
- Cancerogenesis: 1) Initiation, 2) promotion effect (phorbol acetate, phenobarbital) --- manifested cancer process

CYTOSTATICS

- Research prograsms: USA (1951 NCI, Maryland, J. Hartwel); GB; Japan; China; O.E.R.T.C. (l'Organisation européenne pour la recherge et la traitment de cancer)

Cancerogens – most important factor of cancer development

- In present time different branches of human activity use approx. 70 000 chemical substances
- Each year plus approx. 800 new substances
- Genotoxicity (mutagenity, cancerogenity) is tested from 1986 (5 % of used compounds are cancerogens
- Some compounds are activated by usage, for example herbicides aminotriazols are mutagenic after activation via effect of plant enzymes
- Derivatives of nitrofuran are mutagenic after activation via anaerobic processes in guts

Corelation mutagenity : cancerogenity = 92 : 8

Methodically defect, because from 26 cancerogens only 19 showed mutagenity on mice

In present time it is known more than V 30 ultimate cancerogens

Etaps of programs for development of plant cytostatic compound

1. Harvest (NAPRALERT, empirie – USDA, ARD)
2. Extraction
3. Primary screening (PS –lymphocytic leukemia, KB – carcinom of larynx) ED50 ≤ 4 μg/ml
4. Repeated harvest of species showing activity
5. Activity-guided fractionation
6. Isolation, structure elucidation
7. Testing on a pannel of cancer cells
8. Further extraction → obtaining of large amount of active compound
9. Preklinical pharmacology
10. Clinical trials

- Basic point of program is screening, affecting majority of development levels
- Cytotoxic compounds (ED50 ≤ 4 μg/ml) trigger the cell lysis
- Cytostatics trigger the stasis of division of fast reproducing cells

Panel of cancer cells for testing of extracts

- B1 = B16 melanocarcinom — cell kinetic and further characteristics
- LL = Lewis lung carcinom — similar to main types of human cancer cells
- LE = L 1210 leukemia
- PS = P388 lymphocytic leukemia
- WA = Walker carcinosarcom 256
- CA = Adenocarcinom 755
- DL = Dunning leukemia
- FV = Friend viral leukemia
- PX = Plasmocytom NCS 38280
- P1 = Plasmocytom n. 1
- SP = P1798 Lymphosarcom
- SA = Sarcom 180

Future

1. Prevention and improvement of methods
2. Studies of biologic characteristics of tumors
3. Gen therapy
4. Discovery and development of further cytostatics
   - synthetic
   - semisynthetic
   - natural

   which would be used for:
   - direct therapeutic usage
   - model or lead compounds
   - starting material for preparation of more effective derivatives
   - knowledge of biochemical mechanisms of activity (in correspondence to structural heterogeneity of used compounds)

- Aim of chemotherapy: selective devitalization of cancer cells

Character of natural cytotoxic substances and their occurrence in plant kingdom

- Character of natural cytotoxics:
  - simple sugars and „small" molecules of secondary metabolites (most numbered)
  - polymer peptides
  - polymeric sugars
  - glycoproteins

- Occurrence in plant kingdom:
  - Apocynaceae
  - Asteraceae
  - Rutaceae
  - Ranunculaceae
  - Celastraceae
  - Liliaceae (relatively at minimum)

Systematic screening of plant extracts from 1959
Till present time examined approx. 250 000 of extracts from 3500 genera

CYTOSTATICS

1. Alkylating compounds (Cyclophosphamid, Busulphan, Chlorambucil …)
2. Antimetabolites (Cytarabin, Florouracil, Metotrexate, analogues of folic acid, purines …)
3. Enzymes (L-asparaginase)
4. Metabolites from higher plants

   inhibitors of mitosis, blocking metaphase by dissolving of microtubules
   - Colchicine
   - Vincaleucoblastin (VLB)
   - Vincaleurocristin (VCR)
   - Vindesin, Vinorelbin, Vinflunin

   accelerate formation of microtubules, stabilizing them and prevent depolymerization
   - Taxans

CYTOSTATIKA

5. Intercalation substances (intercalate into neighborring nucleotide pairs and produce frameshift mutations) and inhibitors of topoisomerases
   - Amsacrine, Doxorubicine, Mitramycine, Actinomycines
   - Podophyllotoxins
   - Camptothecins
6. Hormons
   - glucocorticoids
   - estrogens, antiestrogens
   - antiandrogens
7. Other
   - Cisplatin, Procarbazine
   - Interferon α

Mitotic poisons of plant origin

Effect is observed in process of mitosis (prometaphase)

- Alkaloids from *Catharanthus roseus*, their semisynthetic derivatives and colchicine (or colcemide) inhibit polymeration of tubuline, avoid the formation of microtubules and therefore block process of mitosis in metaphasis
- Taxans inhibit depolymeration of tubuline, stimulate formation of microtubules and prevent their disintegration. Mitosis is prolonged from usual 30 min up to 15 hours; taxans possess radiopotentiation effect, induce apoptosis

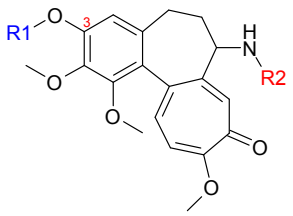
Functional effects of both groups are to some extend similar

At normal condition is polymeration and depolymeration of tubiline balanced.

Colchicum autumnale – autumn crocus (Liliaceae)

Colchicine

- Present in testa and tubers
- Cytotoxic activity known from 40ies of 20th century, blocks cell division, do not prevent division of chromosomes, causes polyploidization
- Usage of desacetyl-N-methylcolchicine = demecolcine during therapy of myeloid leukemia
- Today prevalently antiuratic, affects the chemotaxis of inflammatory cells into place of inflammation
- Series of side effects, toxic



	R1	R2
Colchicine	CH_3	$COCH_3$
Demecolcine	CH_3	CH_3
3-demethylcolchicine	H	$COCH_3$

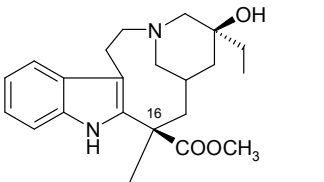
Colchicum autumnale L. – autumn crocus (Liliaceae)

VINBLASTINE, VINCRISTIN
Catharanthus roseus (L.) G. Don. – Madagascar periwinkle (Apocynaceae)

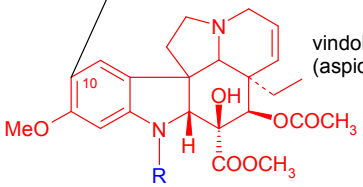
- Native to Madagascar, today large cultivation in tropics and subtropics
- Originally antidiabetic, experimentally not proved, but discovered leucopenia as a result of lowered activiy of bone marrow – antimitotic substances
- Eli Lilly Indianolopis, USA. Gordon H. Svoboda
- Contains more than 60 alkaloids, from that approx. 20 dimeric.
- From that number is used vinblastin and vincristin and their semisynthetic derivatives
- To obtain 1 g VCR it is necessary to process 500 kg of drug
- Development of VLB: 10,7 mil. USD, protocols about 32 000 pages (weight 14,5 kg)
- Usage: Hodgkin disease, lymphocytic leukemia, testicular tumors, component of highly active and aggressive chemotherapy combinations
- Main side effect of VCR: neurotoxicity
- Semisynthetic derivatives: Vindesin, Vinorelbin, Vinflunine

Catharanthus roseus (L.) G. Don. – Madagascar periwinkle

Structure of VLB and VCR



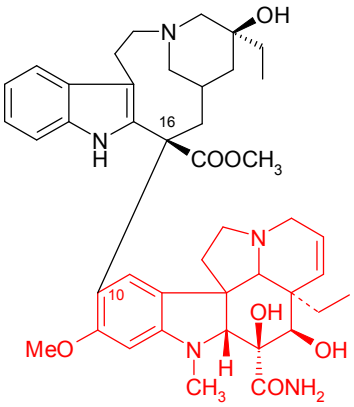
catharanthine
(ibogaine type)



vindoline
(aspidospermine type)

	R
Vincaleucoblastin VLB	CH_3
Vincaleurocristin VCR	CHO

Structure of vindesine



(- Ac)

(- OMe + NH_2)

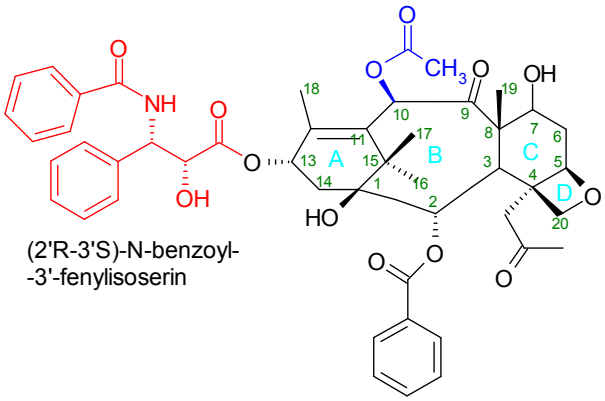
Vindesine

TAXOL
Taxus brevifolia Nutt. – pacific yew (Taxaceae)

- Taxol is one of the structurally related diterpenes
- From the bark of Pacific yew (*Taxus brevifolia* Nutt., - Taxaceae) was firstly isolated and described by M.C. Wani, et al.: J. Am. Chem. Soc. 93, 2325 (1971)
- Pacific yew is a tree native to west coast of North America, today widely cultivated.
- During screening extract showed effect against different types of leukemia and carcinosarcoms. In clinical assays (started 1983 and still continue), taxol showed activity against cancer of ovarias, lungs, breast and malignant melanoma. Valuable drug for treatment of metastatic carcinomas of ovaria and metastating breast cancer.
- Rhone-Poulenc prepared Taxotere® (docetaxel), which is probably more effect and more tolerated than original paclitaxel.
- To obtain 1 kg of paclitaxel it is necessary to process bark from 2500 adult trees. Approximated consumption of paclitaxel for treatment of ovarian cancer is 20-25 kg per year.

Taxus brevifolia Nutt. – Pacific yew
Taxus baccata L. – English yew, European yew
(Taxaceae)

Taxol structure



(2'R-3'S)-N-benzoyl-
-3'-fenylisoserin

Paclitaxel (Taxol)

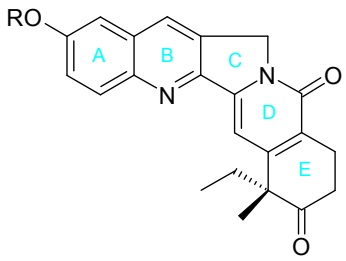
Camptothecines

Camptotheca acuminata (Nyssaceae), *Ervatamia hyeneana* (Apocynaceae), *Opiorriza mungos* (Rubiaceae)

- *Camptotheca* species – trees native in China, 0,012 % of mixture of basis in bark
- *Ervatamia* species – trees native to south-east Asia
- Camptothecin inhibits DNA-topoisomerase I
- Usage: colorectal carcinomas (CRC)
- High price – approximation of Czech Republic patients is 1 000 000 000 Kč

Camptothecine structure

- Monoterpenic chinoline „alkaloids"
- Molecule is not basic
- Do not form stable salts with mineral acids
- Do not react with Mayer nor Dragendorff reagent



Camptothecine, R = H

10-hydroxycamptothecine, R = OH

10-methoxykamptothecine, R = OCH_3

Glycosides of epipodophyllotoxin

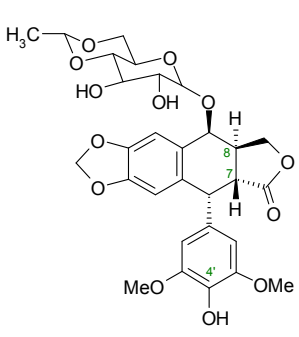
Podophyllum peltatum – mayapple (Berberidaceae)

- Podophyllotoxin is not used as cytostatic, but its semi-synthetic derivatives
  - Glycoside of C-9-β–hydroxy isomer
- Belongs to coniferylalcohol derived lignans
- Inhibition of topoisomerase II
- Indications:

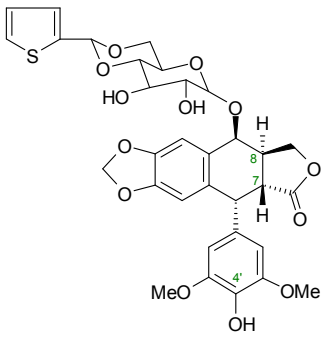
  - Teniposide – urinary bladder cancer

  - Etoposide – small cellular lung carcinoma, several leukemia and Hodgkin disease
- These compounds possess nno affinity to tubuline

Structure of etoposide and teniposide

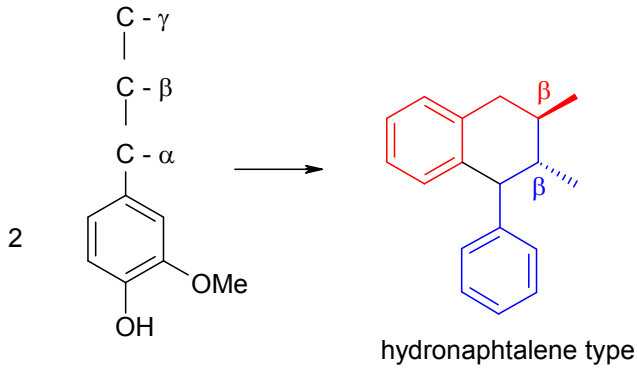


Etoposid, Vepesid



Teniposid

Biosynthesis of podophylline lignans



hydronaphtalene type

Cancerogens

Some drugs:
Metronidazol
Cimetidin (Tagamet)
Nitrofurans
Nitromack
Phenacetine → nitrosoderivatives
p-nitrosophenol
INH
Cyclophosphamid

Chemicals:
Benzidine
2-naphtylamin
azo-pigments
α-halogenated ethers
polychlorinated biphenyls

Product of *Aspergillus flavus* - aflatoxins

CA INHIBITORS

- Flavones
- α-Angelicolactone
- Benzylisothiocyanates (broccoli, cauliflower, savoy cabage)
- Indol derivatives - betalains
- Vitamin C
- α-Tocoferol, β-sitosterol
- Microelements: Selenium
- Drugs: Indomethacin