METHODOLOGY OF PHARMACOGNOSY

BASED ON METHODOLOGY OF BASIC SCIENTIFIC DISCIPLINES

1. Corrected botanic determination (each expedition should „hire" systematic botanist)
2. Macroscopic analysis of drug
3. Microscopic analysis of drug
4. Chemical control of drug
   - proof of content compounds
     (physical methods – fluorescence, microsublimation, chromatography)
     (biological methods – haemolytic activity, agglutination effect)
     (chemical methods – colour and precipitation reaction after previously carried out extraction of tested compounds, histochemistry)
   - determination of compounds content (quantification)
     methods derived from characteristic of quantified compound
5. Study of mechanisms of biosynthesis of effective compounds

METHODOLOGY OF PHARMACOGNOSY

6. Preliminary assays of biological activity (selection of material for further studies)
   (effect antibacterial, antimycotic, antiviral, cytotoxic, antihypertensive, antiphlogistic, spasmolytic ….)
   (Selection based on relations of species and genera, based on folk medicine, random selection - intuition)
7. Separation and isolation of content compounds from experimental material
   (distillation, crystallization, liquid-liquid extraction, preparative chromatography)
8. Detection of isolátes and proof of purity
   physical: melting point, index lomu, optical rotation, CD, GC, HPLC)
   chemical: creation of color products with specific reagents, degradation
   biologic: haemolysis, agglutination of erythrocytes, determination of bitterness
9. Characterization and identification of isolates

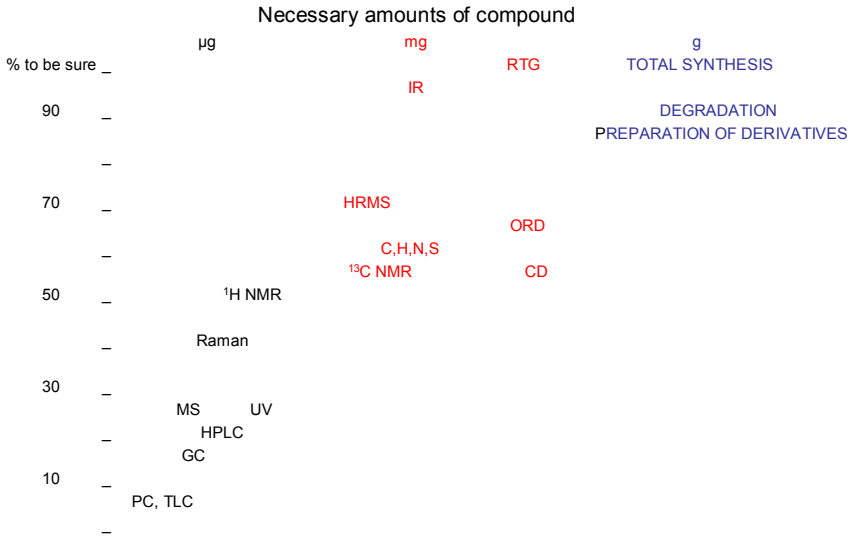
STUDIES OF BIOLOGIC ACTIVITIES

CHARAKTERISATION AND IDENTIFICATION

Chemical structure

- Mass spectrometry
- Electron spectrometry
- Infrared spectrometry
- Raman spectrometry
- ^{1}H NMR, ^{13}C NMR
- ORD
- CD
- RTG
- Degradation
- Derivatives
- Synthesis

Parameters



ISOLATION OF NATURAL COMPOUNDS

1. EXTRACTION: (series of solvents with different polarity – eluotropic series)
   solid compound with liquid
   - periodical (maceration, digestion)
   - repeated
   - continual (percolation, Soxhlet)

   liquid with liquid (perforation, shaking)

2. DISTILLATION
   - at normal pressure (with capillary, with boiling stones)
   - vacuum (RVO)
   - molecular
   - with rectification column (rectification)

3. SUBLIMATION (purines, quinones)

4. CRYSTALLISATION

5. PRECIPITATION
   - change of solvent polarity, change of pH (purification of alkaloids)
   - salting

ISOLATION OF NATURAL COMPOUNDS

6. FILTRATION, ULTRACENTRIFUGATION, DIALYSIS
7. CHROMATOGRAPHIC METHODS (Cvět, Reichstein, Mistrujakov, Stahl, Hostetmann)
   - **Classification according principles**: adsorption, partitioning, liquid, gas, ion exchange, gel filtration, affinity
   - **Classification according to the process**: column, paper, thin layer, high performance, droplet counter-current
   - **Classification according to the polarity**: at polar stationary phases – normal, at non-polar stationary phases - reversed
8. ELECTROPHORETIC METHODS
   - without bearer
   - paper (mobility is caused by charge, molecule size…)
   - in gels (starch, agarose, polyacrylamid)
   - isotachoforesis (separates anions or cations only)
   - isoelectric focusation (pH gradient)
9. COMBINATION OF SEPARATION METHODS
10. SEPARATION BASED ON DIFFERENT PRINCIPLES
    - counter-current shaking (Creig)
    - centrifugation (separation according to the density – high-molecular compounds, in gradient of sacharose)
    - flotation (enrichment of surface active compounds in interface of gas-liquid system

ISOLATION OF NATURAL COMPOUNDS

11. CHEMICAL METHODS

- derivatisation (acetylation, etherification)
- Preparation of molecular compounds - adducts (for example sterols + digitonin)
- clathrates

12. FERMENTATION METHODS

(purification of plant extracts from sugars with help of yeast)