Glycosides
Anthracenes
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Glycosides

• more important in medicine than a lot of drugs
• occur in higher plant tissues in very small amounts
• also fungal and bacterial cells (exuded in medium) and animals
• formed by a biochemical reaction that makes a water insoluble compound more polar than a water soluble molecule
• hence can be removed from an organic system
• man forms them in the liver as part of the process of detoxification and they are excreted via urine
• mammalian glycosides are simple compounds whereas plant glycosides are much larger and chemically more complex
• higher plant glycosides used therapeutically
• have a bio-action
  – therapeutic in low doses, toxic in excess
  – ie have a narrow therapeutic index

• Glycosides =
  – aglycone / ‘genin’ - hydrocarbon part
  – + glycone - sugar part (water solubility)

• Ether linked:
  – X-OH + R-OH $\leftrightarrow$ X-O-R + H$_2$O (glycosidic bond)
  – unstable
  – susceptible to hydrolysis (dilute acid, enzymes)
• important to determine which isomer has the activity
  - α or β glycosidal bond from an α or β pyranose sugar ring

- natural glycosides tend to have β-linkage
- acid hydrolysis to cleave α or β glycosides
- identify component part of molecule
- check stereochemistry with β-glucosidase
• Sugars vary
  – glucose, rhamnose, xylose, etc
  – simple mono- to 2-12 unit polysaccharides
  – can be branched

• (To determine non-linear linkages)
  – acetylate or methylate the sugar
  – above taken up by all free –OH groups
  – hydrolyse – determine by NMR technique

• Other possible linkages
  – direct C-C eg aloes of cascara
    • resistant to hydrolysis
    • oxidise C link with ferric chloride and split bond
  – S-linked eg in spices giving hotness, mustards
    • aglycones must have S-H in it to link up
    • very unstable – breakdown and liberate oil of mustard (pungent)
  – N-linked eg antitumour drugs (can straddle DNA strands)
    • sugar OH + NH aglycone -> R-N-X -> the nucleic acid
    • (ribose based link is N-glycosidal bond)
Classification

• On the basis of aglycone structure

• [1] Saponins (soaps)
  • aglycone = trans-linked steroid

• [2] Cardiac glycosides (poisons)
  • from squill, digitalis, lily of the valley
  • used as crow poisons through history
  • aglycone = cis-linked steroid

• [3] Anthracene derivatives (purgatives)
  • also poisons, cause inconvenience not death

• [4] Flavenoids and coumarins
  • yellow or orange coloured
  • phenolic compounds with aromatic rings
– (a) Flavenoids
  • mainly anti-inflammatory drugs, cyclooxygenase inhibitors
  • inhibit inflammatory mediators (prostaglandins)
– (b) Coumarins
  • eg from clover - basis of anticoagulants

• [5] Simple phenols
  • from willow and poplar bark
  • analgesics – aspirin

  • S-linked compounds

• [7] Cyanogenic compounds
  • breakdown liberating CN
  • found in ‘cherry’ bark and kernel
  • also liberate benzaldehyde on breakdown (almond smell)
Preparation & extraction

• Polar substances – soluble in polar solvents

• Extraction:
  – starting material should be well dried and carefully stored
    • enzymes will decompose glycosides if >10% water content remaining
  – cold extraction procedure (room temp)
    • with percolation and maceration
  – water, water/alcohol mixture or alcohol
    • depending on mol wt
• **Purification:**
  - solvent/solvent partition
    - $\text{H}_2\text{O}$/hexane or $\text{CH}_3\text{Cl}$ to remove pigments in the non-polar phase
  - or adsorption methods
    - make column and do chromatography
    - or mix with adsorbants (Celite, Fuller’s Earth, graphite)
    - or use heavy metal to precipitate out impurities
  - should end up with clear (or coloured) alcoholic extract
  - crystallisation – final stage
Anthracene glycosides

- purgative principles
- found in several plant drugs
- occur in glycoside form
  - and less commonly in aglycone form
  - free aglycones have to be removed in assay because inactive
- 2-3% w/w (both forms)
- based on anthracene molecule
• 3 oxygenated or substituted forms of the anthracene molecule exist

- all flat, planar structures
  • has to be free rotation at dimer join for potency
  • flat molecule can get into gut mucosa and irritate eventually causing peristalsis
• 4 aglycone structures
  – all existing in any of the 3 forms
  – phenolic group is the irritant principle

Rhein anthraquinone

Chrysophanol

Aloe-emodin

Emodin
• biologically active part is the glycoside
  • tend to have simple sugars attached

[1] monoglucoside at C8
  • O-linked

[2] diglucoside at C1 and C8
[3] ‘C’- glycosides
  - have a direct C linkage – aloins

[4] ‘CO’-glycosides
  - O-linked at 1 and 8
  - C linked as in aloins
  - all types combined to give complex mixture in the plant
  - assays different since each compound has different purgative potency

* resistant to hydrolysis (need to use ferric chloride)
Extraction

- most quite polar
  - due to phenols and sugars
- water|alcohol or mixtures of them used
- dried plant material percolation in industrial columns with dilute alcohol
- tincture produced
- partitioned with chloroform|ether to clean up (remove green pigment, fats, lipids)
- clean yellow tincture subjected to column chromatography
- gradual elution of individual glycosides
- crystallised for purity
• pure glycoside makes expensive products
• cheaper to
  – use a clean tincture to make a dry extract
  – used for granules in tablets
  – standardise final tablet

• Identification:
  – easy – coloured orange-yellow
  – chemical test: Borntrager’s test
  – in alkali (KOH, NH$_3$) phenolic groups -> phenate complex (bright red)
  – TLC using silica gel – plates do not have to be sprayed since yellow but can confirm with KOH (red spot)
  – mass spectrometry
Mechanism of action

• Molecules have to possess certain features for activity:
  – [1] glycosides
  – [2] carbonyl keto function on centre ring
  – [3] 1,-8- positions have to have –OH

• Potency:
  – anthrone > anthraquinone> dianthrone

• Aglycones not therapeutically active in animals – lipid soluble – absorbed in stomach and never reach colon to produce a local effect
• Highly active phenolic group irritant to mucosa

• Glycosides very water soluble – reach large intestine where they are hydrolysed by *E. coli* enzymes – become lipid soluble – absorbed into circulation – on way through gut wall disturb Aubach nerve plexus causing smooth muscle to contract – peristalsis

• 5-8 hours to act
  – take night before
  – in low doses – drug metabolised by liver and recirculated via bile to give more effect
  – people esp elderly can become reliant on them needing higher dose to produce an effect
  – carcinogenic – melanosis coli
Assay

• Isolating each active component too expensive
  – powdered plant material (tablets or capsules)
  – or aqueous (fluid) extracts used

• Difficult – each component in mixture has different potency

• Safest assay is:

[i] biological assay of dry material

  – wet faeces method – cage full of mice or rats on a grid with collecting tray below – feed eg senna in food
  – collect faeces and weigh – calculate ED_{50} – oral dose in food correlating to faeces produced
[ii] chemical assay
  – spectroscopy – quick and cheap, more accurate but gives same emphasis to each compound

- To remove aglycones
  – make an extract, shake with ether
    • discard ether phase containing free aglycones
  – then acid hydrolyse aqueous phase containing glycosides
    • with ferric chloride for direct C- bonds
    • and with dilute HCl
  – extract in CHCl₃
    • gives aglycones from glycosides
  – colour with magnesium acetate
    • then measure on spectrophotometer peak 515nm
  – OR do colourimetric assay – red in alkali - 250nm
Senna

- **Cassia angustifolia**
  - Tinnevelly (India)
- **Cassia acutifolia**
  - Alexandria (Egypt)
- (Leguminosae)
- dry pods, leaves or mixture used
- tablet form
  - eg sennakot
  - (isolation of anthraquinone too expensive)
- kinder action - use
  - pregnant women
  - iron constipation
- activity & content same
Chemical constituents:

(i) 1 and 1,8 ‘O’ glucosides
   = 1\textsuperscript{st} series glycosides
   aglycones: rhein, aloe emodin

(ii) dimeric dianthrones
    = 2\textsuperscript{nd} series
    reduced products

dimer can be split into two parts with FeCl\textsubscript{3}
hydrolysis and monomer aglycones assayed for
Cascara

- *Rhamnus pershiana* (Rhamnaceae)
- bark extract
  - collected, dried and stored for 12 months (↓ anthraquinone content -> less toxic)
- modern substance
  - discovered 100 years ago
  - Rocky Mtns, W.Coast, US
- more violent purgative
  - griping action
  - harder to eliminate
- **Use:** night before to clear bowels for x-rays and barium meal
Chemical constituents:

(i) 4 primary glycosides
   - O- and C- linkages

To get aglycones FeCl$_3$
To get aloins oxidise with acid

(ii) C-glycosides - two aloins
    - barbaloin - derived from aloe-EMODIN
    - chrysaloin - derived from chrysophanol

(iii) a number of O-glycosides
    - derived from emodin oxanthrone, aloe-EMODIN, chrysophanol

(iv) various dianthrones
    - incl. emodin, aloe-EMODIN, chrysophanol, herterodianthrone
      palmidin A B C

(v) aloe-EMODIN, chrysophanol, emodin in free state