8) Applications of Nuclear Radiation in Science and Technique (1)

Radioactive labeling of organic compounds

Where is radioactive labeling used

- chemical science (reactions mechanisms)
- phamacy (pharmacokinetics)
- life science (metabolisms)

Why labeling with radioactive compounds ?

- high sensitivity even in highest dilutions
- high selectivity
- The „original“ can be labeled (identical chemical substance)

Comparison ^{15}N (non-radioactive) vs. ^{3}H or ^{14}C)

- ^{15}N (natural abundance 0.37%)
- even with 100% ^{15}N-enriched indicator (very expensive) one will not be able to observe a change of 0.01 % at a dilution by a factor of 10,000
- this, however, is a normal dilution factor during the observation of metabolic processes
- radioactively labeled compounds, however, allow the detection in a femtomolar range (^{3}H) or in the picomolare range (^{14}C)

8) Applications of Nuclear Radiation in Science and Technique (2)

Radioactive labeling of organic compounds

Criteria for radioactive labeling procedures:

- radioactive nuclide (radiation, detectability)
- labeling position in the molecule
- specific activity of the labeled compound
- chemical purity
- radiochemical purity

Frequently used isotopes

Isotope	Radiation	Half-life
^{11}C	$ß^{+}$	20 min
^{14}C	$ß^{-}$	5730 a
^{3}H	$ß^{-}$	12.3 a
^{32}P	$ß^{-}$	14.25 d
^{35}S	$ß^{-}$	87.5 d

Labeling methods

- **chemical synthesis**
- biochemical procedure
- isotope exchange
- Recoil labeling
- radiation-induced labeling and self-labeling

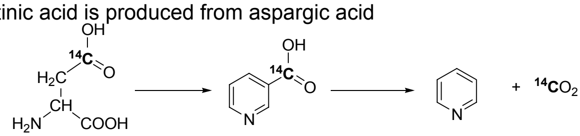
8) Applications of Nuclear Radiation in Science and Technique (3)
Radioactive labeling of organic compounds

Example: The Formation of Nicotinic Acid in Plants

- Animals and some microorganisms form nicotinic acid from tryptophane
- This is not the case with higher plants

- **Hypothesis**: Nicotinic acid is produced from aspargic acid

Chemical proof:



- Application of monolabeled aspargic acid to the plant and isolation and analysis of the metabolic products
- Isolation of the formed nicotinic acid and decarboxylation
- The formed $^{14}CO_2$ has the same specific activity as the formed nicotinic acid
- **Conclusion:** all ^{14}C was incorporated into the carboxylic group
- Double labeling with ^{14}C and (non-radioactive) ^{15}N gives more information using the $^{14}C/^{15}N$ ratio

-Trick: at constant $^{14}C/^{15}N$ ratio: the radioactive tracer can readily be used to detect the non-radioactive label ^{15}N

8) Applications of Nuclear Radiation in Science and Technique (4)
Radioactive labeling of organic compounds

a) Labeling by chemical synthesis:

Specific problems

- Only distinct starting materials are available at a reasonable price (e.g. $Ba^{14}CO_3$, T_2O, $H_3{}^{32}PO_4$)
- Frequently new routes of synthesis must be found to optimize the yield for the isotopic label
- Maintenance of the specific activity should be checked throughout the synthesis (loss by isotopic exchange must be excluded)
- Radiation protection (particularly with gaseous intermediates)

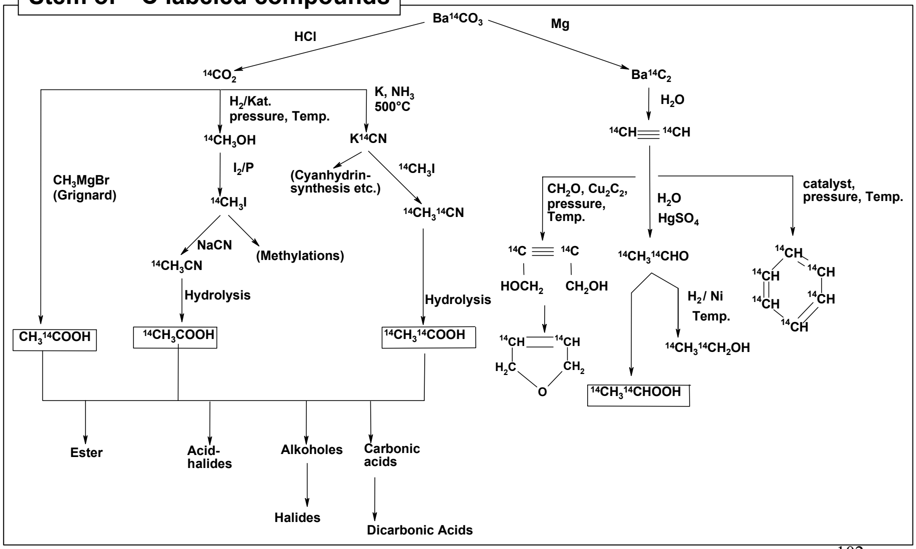
Frequently used isotopes

Isotope	Radiation	Half-life
^{11}C	ß$^{+}$	20 min
^{14}C	ß$^{-}$	5730 a
^{3}H	ß$^{-}$	12.3 a
^{32}P	ß$^{-}$	14.25 d
^{35}S	ß$^{-}$	87.5 d

8) Applications of Nuclear Radiation in Science and Technique (5)

Radioactive labeling of organic compounds

Stem of ^{14}C labeled compounds



8) Applications of Nuclear Radiation in Science and Technique (6)

Radioactive labeling of organic compounds

b) Biochemical Labeling:

- Bases on $^{14}CO_2$ or T_2O assimilation by plants
- Isolation of natural products (glucose, proteins, hormones, alkaloids) from the metabolism of the plants
- Practical realization in so-called „isotope farms“ (frequently chlorella algaes are grown in an atmosphere of $^{14}CO_2$ or in T_2O enriched water
- Strict radiation protection is required throughout the procedure

Frequently used isotopes

Isotope	Radiation	Half-life
^{14}C	ß$^-$	5730 a
^{3}H	ß$^-$	12.3 a

8) Applications of Nuclear Radiation in Science and Technique (7)

Radioactive labeling of organic compounds

c) Isotopic exchange Labeling:

- Is frequently applied when direct synthesis gives low yields
- Advantage: the target structure must not be synthesised „radiochemically“
- Frequently used between organic and inorganic substances
  e.g.

$$\mathbf{Li^{36}Cl\ (in\ solution)\ +\ RCl \longrightarrow LiCl\ (in\ solution)\ +\ R^{36}Cl}$$

- Heterogeneous exchange reactions are possible at exchange columns (e.g. tritium labeling of acidic H-atoms in columns of a gaschromatograph)
- Exchange at columns is frequently a multiple-step reaction (mobile phase can reach the same specific activity, which is supplied by the stationary phase)
- Additionally ionising radiation may increase the exchange rate by bond cleavage reactions (radiation-induced exchange)

8) Applications of Nuclear Radiation in Science and Technique (8)

Radioactive labeling of organic compounds

d) Isotopic Labeling by Back Scattering, Self Labeling

- Rarely used for the labeling of biochemical samples
- Uses that part of the energy that is released during nuclear reactions, that is transfered to the product atom
- This results not exclusively in bond cleavages, but also in bond formations
- **Disadvantage:** non-specific reaction, purification is required

e) Labeling by Radiation Chemistry

- Rarely used for the labeling of organic samples
- **Example**: Addition of halogenes or HX on C-C double bonds