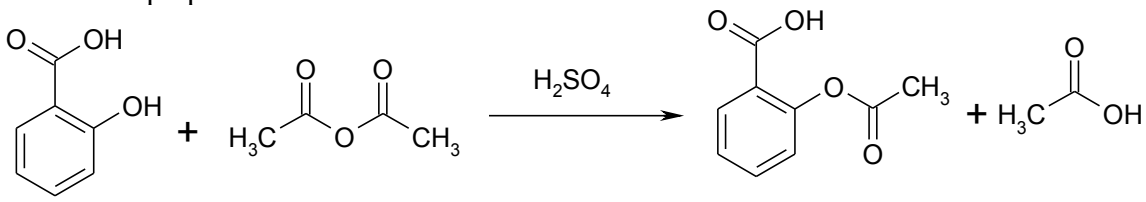
3. Acetylsalicylic acid

Systematic names: 2-acetoxybenzoic acid, 2-acetoxybenzenecarboxylic acid
It is prepared by a simple acetylation of salicylic acid with acetic anhydride.

Scheme of preparation:



Chemicals:
salicylic acid 6.9 g (0.05 mol)
acetic anhydride 14 g (0.13 mol)
(*Calculate and measure **volume** of acetic anhydride; density data can be found on bottle label, if not, see a laboratory chemicals catalogue. Ask a lecturer for checking of your calculated volume before you start the preparation.)*
concentrated sulfuric acid 10 drops

Procedure:
6.9 g of salicylic acid is mixed with 14 g of acetic anhydride in an Erlenmayer flask and shaken well (*but carefully !*) until the solid is completely dissolved. Then 10 drops of sulfuric acid are added and the mixture is well shaken again. Its temperature should spontaneously increase to approx. 45°C and crystals of acetylsalicylic acid should start to form. If not, the forming of crystals can be initiated by rubbing of the flask wall with a glass rod. The content of the flask is then left to crystallize for the additional 30 minutes and then poured into 140 ml of water in a beaker. Then it is shortly heated on an electric hotplate and stirred with a glass rod until dissolution. If some solid remains undissolved the mixture must be filtered. The solution (or filtrate) is then allowed to cool down to laboratory temperature until crystals of acetylsalicylic acid are formed again. After reaching laboratory temperature, the mixture can be refrigerated for several minutes. Crystals of acetylsalicylic acid are then isolated by suction and washed with water on the filter until pH of the filtrate is greater than 3. Acetylsalicylic acid can be recrystallized from aqueous ethanol. Yield is approx. 90 % of theory. It needs to be well dried in a hot-air dryer before identity confirmation by its **melting point** determination on a capillary melting point apparatus. Its purity or, more accurately, absence of starting salicylic acid, is then confirmed by **thin layer chromatography (TLC)** on a silica gel sheet and reversed-phase **high performance liquid chromatography (HPLC)**. Salicylic acid is used as a comparison compound in both chromatography procedures. *Try the mixture ethyl acetate/petroleum ether 1:1 as a mobile phase for TLC.*

Confirmation of purity of acetylsalicylic acid by HPLC

Chromatography conditions:

Mobile phase: *probably* methanol : water : acetic acid 50 : 49 : 1 (*is prepared in advance by the technician)*

Flow 0.6 $ml.min^{-1}$

Detection UV, λ=254 nm

Column: a reversed-phase column (*probably C18 or C16 with inserted amide moiety, ask a lecturer)*

Sample loop volume: 20 µl

A suitable integration hardware and software

Switching the chromatograph on/off and adjusting of parameters and mobile phase is performed by a lecturer or a laboratory technician.

Chromatography procedure:
The solution of the proper sample of acetylsalicylic acid of concentration 0.1 $mg.ml^{-1}$ in the actual mobile phase is prepared in a volumetric flask. The comparison solution of salicylic acid of the same concentration is prepared similarly. Approximately 0.3 ml of an analysed solution is aspired into a syringe equipped with a suitable needle, the syringe piston is pulled down and whole the inner surface of the syringe is washed with the solution. Then the syringe is emptied into a waste bottle. This procedure is at least three times repeated. Approx. 0.3 ml of the solution is then aspired again, the syringe is oriented with needle to top and the air bubble is sprayed out. The solution is then injected to the needle port of the manual sample injector. At least 2 drops which drop out from the waste capillary indicate that the sample loop is full (the handle is turned to **Load**). Then the handle is turned to **Inject** and the sample solution begins to stream into the column and, simultaneously, the observing of the analysis starts. The handle can be returned to **Load** after 2 minutes. The analysis proceeds about 10 minutes, then it is stopped. The chromatogram is automatically evaluated and peaks are assigned with retention times and areas. Ideally, a chromatogram could contain only one peak of acetylsalicylic or salicylic acid respectively. If not, per cents of area of the particular peak in the total area are expressed. Every sample is analysed three times, chromatograms are printed and mean retention times and per cents of total area are calculated.
The report must contain in addition to record of synthesis:

- actual chromatographic conditions (composition of mobile phase, flow, wavelength of detection, specification of column
- mean retention times of acetylsalicylic and salicylic acids
- mean per cents of total area which belong to acetylsalicylic and salicylic acids respectively (the area of eventual solvent peak near the start of analysis is not taken into account)

Properties:

Acetylsalicylic acid forms white needle-shaped crystals of m.p. 135-137°C, odourless, of weakly acid taste. The compound is slightly soluble in water, freely soluble in ethanol 96%.

Usage:

Acetylsalicylic acid has been used as analgesic, antipyretic and anti-inflammatory drug for more than 110 years. More recently, its anti-platelet and thus antithrombotic effect is employed. All its effects are mediated by cyclooxygenases inhibition.