Synthesis and handling of volatile nitrosothiols being proposed as a new class of cancer markers in human breath

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Introduction

Four volatile nitrosothiols (vSNOs) are proposed as volatile cancer markers (details in the talk on Tuesday, 9:10 Room 269. Hints for their role as active ingredients in cancer smell have been delivered by successful sniffer dog tests showing that synthetic and malignant tissue smell seems to be identical. Volatile nitrosothiols are known to be rather unstable. They tend to decompose quickly during enrichment, are unstable to light, heat, low pH, oxygen, Cu²⁺ and Fe³⁺ ions and they have an extreme odorous potential making it difficult to handle them. So a synthesis in diluted solutions and storage in air tight diffusion devices has been developed and are presented here. To prove the success of the synthesis measurements with gas chromatography and mass spectrometry have been carried out.

Synthesis

Thiols are treated with aqueous NaN₃ in the presence of oxalic acid (analog Zolfigol 1999). The syntheses are carried out in a two phase reaction with a layer of n-decane above the reaction solution (analog Shishido 2000) which solves and conserves the vSNOs. For the synthesis of methane-SNO sodium-thiomeanolate is utilized. 160mg of sodium-thiomeanolate and 160mg of oxalic acid are overlaid with 4ml n-decane. 6ml of NaN₃ solution (40mg/ml) are added and the mixture is vigorously shaken for 2min. The decane layer becomes ruby red (see fig. 9). The water layer is removed and the decane layer is washed twice with aqueous Na₂CO₃ and twice with water to remove any acid reaction residues. The decane solution is cooled (°30°C) and stored under argon to eliminate oxygen which promotes decomposing. Ethane-, 2-propene- and allylthiol are directly used for synthesis. 4ml aqueous oxalic acid (4mg/ml) are added to 70ml of thiol solved in 4ml decane followed by the addition of 3ml of aqueous NaN₃ solution (20mg/ml). The mixture is shaken vigorously for 2min and cleaned by the same procedure as described above.

Analytical investigations

Online analysis: To record the generation of the vSNOs the headspace of the reaction vials has been monitored. This is displayed exemplarily during the synthesis of methane- and ethane-SNO investigated by online MS (Varian Triple-Quad MS 320). The reaction was carried out without decane layer and a stir bar (300 rnd/min) was used instead of shaking. During the reaction process the headspace gas was continuously sucked through a transfer line inlet capillary to the MS and spectra were recorded. The fundamental differences in the reaction course between these two syntheses are shown in fig. 1 and 2 (the other vSNOs react very similar to ethane-SNO). Additionally as a proof for the presence of the synthesized vSNOs MS/MS spectra from their molecular ions were made showing the same spectra received during the GC/MS-runs (fig. 3-6) shown at the right top of the poster.

In fig. 1 the signals during the methane-SNO synthesis are displayed. A few seconds after the addition of the NaN₃ solution the molecular ions of methane-SNO as well as the molecular ions of the decomposition product methane disulfide appear with rising intensity. The consumption of methanethiol could be seen in the decline of its main ion. After approx. 6min a sudden decomposition of the methane-SNO and the disulfide starts. After few minutes all signals have reached the detection limit of the MS. Oxygen and the low pH of the reaction solution seem to promote the decomposition.

In fig. 2 the courses of signals during ethane-SNO synthesis are displayed. After the addition of the reagent to a solution of ethanethiol the signal curves at the beginning are quite equal to those during the reaction of methane-SNO though the reaction speed is slightly lower. The signals of ethane-SNO and the decomposition product ethane disulfide are going up while that of ethanethiol descends. After the end of the reaction the products are stable for a longer period (proven for one hour, the maximum time of online detection).

vSNO-mixture storage and use for sniffer tests

To prevent the decomposition of the vSNOs and to deliver them in concentrations which are adequate for sniffer dog tests low emission diffusion devices have been constructed. 1.4ml of the odor solutions consisting of a 1:1 mixture of the four vSNOs (=1mg/ml) are filled into a PTFE hose (2) which is tightly closed by glass stoppers (3) and encased by a metal tubing (1) (see fig. 8). Metal and glass are effectively pressed together (with tools 6-8) against the PTFE tube walls by high pressure Swagelok ferrules (4, 5). The compound emission rates are <1mg/min (calculated by weighing over the time of one year) which is sufficient for sniffer dog training (see fig. 7) but far away from being recognized by human noses. For control of the decomposition process of the vSNOs the tubes could be used as cuvettes (fig. 10). Red color indicates the presence of vSNOs being stable for approx. 1 month.

Conclusion

Synthesis of diluted solutions (in the mg/ml range) in a two phase reaction and storage in air tight diffusion devices allows easy handling of vSNOs. Dog training could be carried out using these devices. Several attempts to detect vSNOs in vivo failed. The detection limit of nowadays analytical equipment seems not to be sufficient to prove the existence of vSNOs emissions from cancer. The diffusion devices can be seen as a perfect tool for optimization steps of suitable analytical procedures to finally reach sufficient detection limits enabling the measurements of this supposed new class of cancer markers in the breath of cancer suffers or as direct emissions from malignant tissues.

Literature


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Fig. 1: Synthesis of methane-SNO

Fig. 2: Synthesis of ethane-SNO

Headspace GC/MS analyses: Samples from the headspace of the reaction vials were analyzed by GC/MS. To reduce decomposition a loop injection at room temperature and fast separation on a short GC column with high gas flow and low temperature (for details see fig. 3 – 6) were carried out. First the thiols are eluting followed by the corresponding vSNOs and the runs end with the signal of the disulfides. The vSNO spectra and proposals for the structure of fragments are displayed in red inside the spectra above the GC-runs.

Fig. 3: GC/MS run of methane-SNO headspace

Fig. 4: GC/MS run of ethane-SNO headspace

Fig. 5: GC/MS run of 2-propene-SNO headspace

Fig. 6: GC/MS run of allyl-SNO headspace

Fig. 7: Sniffer dog training

Fig. 8: Diffusion device

Fig. 9: Synthesis

Fig. 10: Control