Operative control of dioxin xenobiotics (perchloration reaction).

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Abstract

For carrying out the operative control of dioxin xenobiotics it is necessary to accomplish the sorting of the samples, contaning dioxins. This procedure may be simplified considerably by perchloration of the organic extracts of the natural matrices samples. In this case, determining of the presence or the absense of octachlorodibenzo-p-dioxin, octachlorodibenzofuran and decachlorobiphenyl by GLC with ECD, it is possible to reduce essentially the quantity of analyzed samples by throwing out the samples non-contaning dioxins xenobiotics from the look. The reaction of perchlorination for dangerous xenobiotics - polychlotinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzofurans (PCDF) by reagent **BMC** (the mixture of SO_2Cl_2, $AlCl_3$ and S_2Cl_2) was investigated. It is shown, that in this case the contents of perchlorinated components ashives >90%.

Introduction

The detection of dioxine-like compounds (PCDD, PCDF) in the environment demands exceptionally sensitive and specific methods of analysis with high selectivity. In present only high-resolution chromato-mass spectrometry (HR CMS) ((EPA US #613, 1613, 8280 and 8290) allows toreliable detection and identification of these xenobiotics[1]. The high cost of the analitical determination of PCDD, PCDF congeners prevents wide scale studies on contaminated areas and monitoring the pollution.

The simplification of the analytical work can be accomplished by using bioindication and biotesting[2], immunochemistry[3] and analytical chemistry methods with simplified procedures. In the latter case considerable savings in time and cost of the analysis can be achieved by the choice of the integral matrix characterizing the extent of contamination in the area. This in turn leads to a reduction in analyzed samples and general improvement of of sampling procedures. The achieved breakthroughs in the preparation of lipophylic matrices for isomer-specific analysis of dioxins using the new coal microcolumn[4], abortion of an evaporation stage and increase in distribution coefficient between aqueus and organic phase by salting out[5] do not solve the problems of detection and speed of dioxin analysis[6].

The perchloration was suggesteed to simplify the detection of PCDD, PCDF congeners. The extracts from the natural sources such as soil, water, air, biota, etc. are perchlorated to the formation of the three easily detected components : octachlordibenzo-p-dioxin (OCDD), octachlordibenzofuran (OCDF). In this case the analysis can be done by gas-liquid chromatography with the ion-trap detector (GLC-ITD) by integrating and determinating the amounts of above mentioned substanses using an internal standard, e.g. 4,4'-dibrombiphenyl. In these condition the sensitivity of the method matches the required level of detection and the noise created by the other components 'disappears' because of the long retention times of the compounds of interest.

The evaluation of the OCDD, OCDF in the sample verifyis only the presence of the the xenobiotics. Such way of analysis allows to differentiate the contaminated ones from the whole selection of probes describing the ecological condition of the studied territory. The contaminated samples undergo an additional analysis according to the procedures #613, 1613, 8280, 8290, etc.

The complete direct chlorination of dioxine-like xenobiotics can be achieved theoretically by several methods:

1. Chlorination in the presence of Lewis acids ($AlCl_3$, $FeCl_3$, $SbCl_5$, $ZrCl_4$,etc) or Bronsted Acids (glacial acetic or trifluoroacetic acids). The degree of chlorination increases along with the amount of chlorine and $ALCl_3$ is the most active catalyzator$^{7)}$.
2. Electrofilyc substitution with SO_2Cl_2 at elevated temperatures (70-90 C)$^{8,9)}$.
3. Chlorination with **BMC** reagent - the mixture of SO_2Cl_2, $AlCl_3$ and S_2Cl_2. The exact structure of the active complex is unknown$^{10)}$.
4. Reaction with hypohalites obtained from halides and nitrobenzenesulfonylperoxide$^{11)}$.

All above mentioned reactions the perchloration yield reached no more trhan 90%. Out of the four chlorination methods we concider the procedure using **BMC** reagent to be more perspective since it gives the highest degree of perchloration. Presently, there is no data concerning chlorination of heterocycles with an ether bond like PCDD and PCDF.

We investigate the reaction of perchlorination for mentioned above xenobiotics by reagent **BMC** with the purpose of the solving this problem.

Experimental Methods

All analitical measurement were conducted on gas -liquid chromatograph Hewlett-Packard A-5890 equipped with ITD (^{63}Ni, β-particles ionization), carrier gas - nitrogen with speed 25 ml/min.

Gas chromatographic separaion of samples was carried out on 6-m capillary column of 0.32 mm i.d., coated with a 0.52 µm film of polymethysiloxane with 5% polyphenylsiloxane (HP-5). Nitrogen was used as carrier gas at a linear velocity 3 ml/min. The injector port temperature was 280°C, the detector temperature - 280°C. The GC was programmed as followed: 100°C maintained isotermal for 1 min, heated to 280°C at the ramp of 10°/min and maintained isotermal for 1 min.

For perchloration studies the following solutions were prepared:

1) standard solution of dibenzo-p-dioxine in methylene chloride with C = 500 ng/ml;
2) calibrating solutions of DCB with C = 1 mg/ml, 500 , 100, 10 ng/ml in toluene;
3) calibrating solutions of OCDD in toluene with C = 500, 200, 10, 1 ng/ml.

The linear dependance was obtained for the concentration range c = 1 mkg/ml-1ng/ml for both OCDD and DCB in given conditions (sample volume - 2 µl). The measurements were repeated three times.

Preparative perchloration of dibenzo-p-dioxine with **BMC:**

To a solution of 25-30 mg of $AlCl_3$ in 7 ml of SO_2Cl_2 refluxing in a 3-neck flask, equipped a water condenser and a dropping funnel, solution of dibenzo-p-dioxin and 30 mcL S_2Cl_2 in 2-3 ml of SO_2Cl_2 was added dropwise over 20 min.

After the removal of the condenser mixture was allowed to evaporated approximately to it's half. Then the reflux was continued for 4-5 hrs more with addition of fresh SO2Cl2 to maintain constant volume.

After reaction was completed , the solvent was evaporated and 15% aq. K_2CO_3 (potash) was added until no gas evolution was observed. The contents of the flask was transferred into an erlenmayer flask (vial) and was sonicated with organic solvent (CH_2Cl_2, Benzene) and water for 10 min. To ensure the completeness of the transfer the original flask was repeatedly washed and sonicated. The organic layer was removed in the funnel and combined fractions were dried over Na_2SO_4. Extract was separated by flash chromatography in the the following system (down-up): 2 g Na_2SO_4, 1 g K_2SiO_2, 2 g Na_2SO_4, 3 g H_2SO_4/SiO_2 40/60, 2 g Na_2SO_4, 3 g H_2SO_4/SiO_2, 2 g Na_2SO_4, 3 g H_2SO_4/SiO_2, 2 g Na_2SO_4. The column was washed 3-fold volume of methylene chloride. The organic extract was flushed through high activated silicagel, washed with 3-fold amount of solvent and evaporated. The resulting residue was dissolved in toluene and analyzed by GLC.

A. 100 mg of dibenzo-p-dioxin was chlorinated with reagent **BMC** for 5 hrs. The resulting mixture (105mg) contained 88.4% of OCDD. The rest of the mixture (11.6%) consisted of the chlorinated derivatives with 4-7 chlorine atoms. The isomers were found in the following amounts (%): 1,3,6,8-TCDD (0.14); 2,3,7,8-TCDD (7.6); 1,2,3,7,8-PeCDD (0.8); 1,2,3,4,6-PeCDD (0.8); 1,2,3,4,6,8-HexCDD (0.16); 1,2,3,4,6,7-HexCDD (1.5); 1,2,3,6,7,8-HexCDD (0.05); 1,2,3,7,8,9-HexCDD (0.2); 1,2,3,4,6,8,9-HepCDD (0.3); 1,2,3,4,6,7,8-HepCDD (0.05); OCDD (88.4).

B. 100 mg of dibenzo-p-dioxin was chlorinated with reagent **BMC** for 4 hrs. 232 mg of OCDD was obtained (yield 93%). The purity of the material was >99.5%; m.p. 332°C.

Using this procedure OCDF was synthesized (Yield 94%; purity > 99.9%, m.p. 260 C).

Results and Discussion.

The reacion of chlorination was carried out with varied temperature (70-90 C) and ratio between $AlCl_3$ and S_2Cl_2 (from 1:2 to 2:1 respectively. The isolation of the final product was undertaken by the procedure described in the experimental section.

During the investigation we found out that if multilayer column (see Experimental section) is substituted for a high activated silica gel column and the solvent methylene chloride is changed to benzene the amount of the solvent used is significantly reduced.

The higher yield of the perchlorated products correspond to the ratio of $AlCl_3$:S_2Cl_2 =1:1 and 2:1 at the b.p. of sulfuryl chloride (68-70°C). At higher temperatures or two-fold excess of S_2Cl_2 the yield decreases. The reduction of PCDD and PCDF yields is also connected with $AlCl_3$ hydrolysis (precipitat appearance). It is important to use AlCl3 immediately after ampule opening.

After taking into account all above mentioned factors OCDD was obtained with the yield of 93% and purity 99.6%.

Perchloration of dioxin and it's analogs has theoretical as well as practical interest as other tricyclic heteroaromatic compounds are considered dioxin-like xenobiotics. For example, several reports discussed high toxicity of sulfur analogs of PCDD and PCDF[12)], which were discoverd in the municipal waste incinerations. Since natural matrices are presently tested for the sulfur containing compounds we also studied **BMC** reagent interaction with dibenzo-p-dithian, dibenzothiophen and phenazine.

Chlorination of dibenzothiophen at 70 C gives 93% of OCDT but at similar conditions DBT do not give octachlorinated compound.

Although high selectivity of **BMC** in aromatics suggests an electrophylic nature of the reagent it's structure and the exact mechanism are still a mystery.

As we noted before during the analysis of the reaction products other chlorinated isomers were found. The study of the isomer ratios also points to the electrophylic mechanism of **BMC** action on DD:

- 2,3,7,8- TCDD content is 40 times higher than other TCDD in procedure A;
- the material isolated in procedure B contains only one hepta and hexachlorinated substances.

Quantamechanical calculation of the π-electronic density in DD(13) and experimental data(14) from Cl_2 chlorination in glacial acetic acid of DD the lateral hydrogens (β-position) are substituted leading to the formation of 2378-TCDD, further chlorination turns it into 12378-penta, then 123678-hexa, then 1234678-hepta and ,finally, octachloroDD.

These agree with our data and can serve as an argument for the SE-Ar mechanism of **BMC** chlorination of DD.

Conclusion

1. The total chlorination of dangerous xenobiotics (PCDDs, PCDFs) was investigated.
2. The possibility of using **BMC** reagent for perchloration of dioxin-like xenobiotics was shown (with exeption of dithian).
3. A sensitive and selective procedure of dioxin screening based on the perchlorination by the **BMC** reagent, can be created.

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