Toxic Nitrophenols from the Liquid Phase Nitration of Phenols

A contribution to subproject CMD

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Aromatics with polar substituents such as phenol, benzoic acid, nitrophenols, nitrocresols and others have been found in concentrations in the μg L^{-1} range in the Rhein-Ruhr area in western Germany and in urban Los Angeles rain (Hansen *et al*., 1991). Aqueous phase nitration of aromatics may lead to the production of harmful products. Several reports are available related to the toxicity of nitrophenols to organisms. While there is considerable evidence for the occurrence of aromatic and nitroaromatic compounds in cloud and rainwater, different possibilities exist with regard to the origin of the latter compounds. Aromatic nitroderivatives may either be directly emitted from anthropogenic sources, they may be formed by tropospheric gas phase reactions and finally they may be formed within the tropospheric aqueous phase. Whereas the assessment of emissions of harmful compounds such as nitroaromatics represents an environmental research issue by itself, some gas phase studies of nitrate radical reactions with aromatics indicate that aromatic nitroderivatives are formed. Due to their polar character, these molecules may subsequently undergo phase transfer and contribute to the levels of nitroaromatic compounds detected in rain as outlined earlier. The third possibility of the origin of nitroaromatics in clouds and rainwater is the occurrence of nitration reaction within the tropospheric aqueous phase. These processes may be driven by reactions of the nitrate radical with dissolved aromatics or by the reaction of the nitronium cation (NO_2^+) formed from the dissociation of its gas-phase precursor N_2O_5 on aqueous surfaces within the troposphere.

In this work we are studying the reactions of phenol with NO_2^+ in aqueous solution (HNO_3 1M, A in Table 1) and the reaction with NO_3 radical formed by

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photolysis of cerium(IV) ammonium nitrate in organic solvent (CH_3CN, B in Table 1). Product studies will be performed for the reactions of NO_3 by direct injection in analytical HPLC and are summarised in Table 1: 4-nitrophenol, 2-nitrophenol, 2,6-dinitrophenol, 2,4-dinitrophenol and *p*-benzoquinone were identified and quantified. This preliminary work is important for future mechanistic studies.

The separation procedure used a RP-C18 column with a Kromasil stationary phase 25 cm × 4.6 mm I.D., 5 μm, 100 Å, (RESTEK Corporation, Bellefonte,PA). The analytical conditions were: flow 1 mL/min,
detector UV (DAD) 230 nm; isocratic eluent A 55 % and B 45 %;
(A) KH_2PO_4: 0.05 M, pH 4.5 + 10 % CH_3CN,
(B) KH_2PO_4: 0.05 M, pH 4.5 + 75 % CH_3CN.

Table 1: Per cent product yields of the nitration reaction of phenol.

Moles phenol	2,6-dinitro phenol	2,4-dinitro phenol	*p*-benzo quinone	phenol	4-nitro phenol	2-nitro phenol
2.69×10^{-2} A	0.19	0.83	6.07	0	59.21	33.7
2.51×10^{-4} B	8.13	13.15	15.06	0	41.98	21.68

Molecular structures of nitro- and dinitro-phenols (9 + phenol), cresols, nitro- and dinitro-cresols (29 compounds) have been represented by 145 molecular descriptors: 1D-structural and empirical (19), 2-D topological (35) and 3-D WHIM descriptors (91), recently successfully applied to the QSAR modelling in many environmental problems (Todeschini and Gramatica, 1997).

Principal component analysis (PCA) of these molecular representations allows us to distinguish the structural similarity and dissimilarity of these compounds in a ten dimensional space.

Multidimensional scaling (MDS) of the first 10 principal components (explaining the 96.7 % of the structural variance of the studied compounds) (Fig. 1) highlights the most representative bidimensional scatter of the compounds studied: the complete structural information is summarised from a multidimensional representation into two dimensions. This allows an immediate view of molecules more similar or dissimilar, depending on their distance in this plane.

Hierarchical clustering analysis (using all the molecular descriptors for 13 compounds: phenol, 9 nitrophenols and 3 cresols) (Fig. 2) highlights simple clusters of similar compounds. These different approaches allows us to find similar compounds (or dissimilar) with the aim of a better planning for the following toxicological experiments.

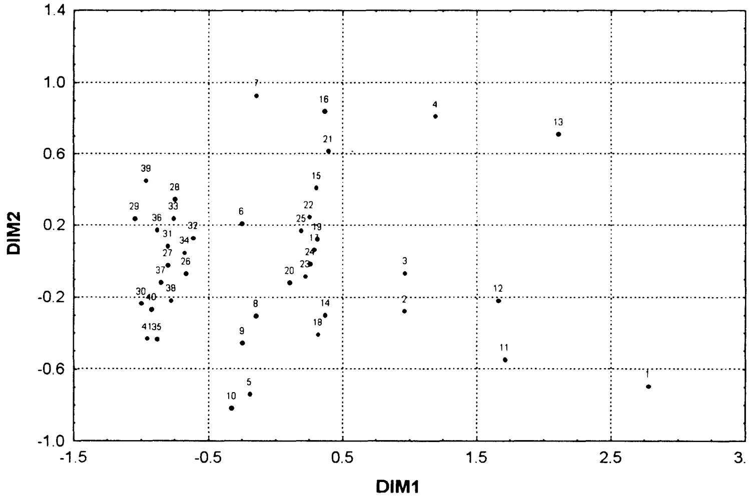

Fig.1: Multidimensional scaling on PC1-PC10 (E.V.= 96.7 %). Phenol; cresol; mono and di-NO_2-phenols/cresols.

Table 2: Corresponding ID number for each compound.

ID	Molecule Name	ID	Molecule Name	ID	Molecule Name
1	phenol	14	3-nitro-*o*-cresol	27	4,5-dinitro-*o*-cresol
2	*o*-nitrophenol	15	4-nitro-*o*-cresol	28	4,6-dinitro-*o*-cresol
3	*m*-nitrophenol	16	5-nitro-*o*-cresol	29	5,6-dinitro-*o*-cresol
4	*p*-nitrophenol	17	6-nitro-*o*-cresol	30	2,4-dinitro-*m*-cresol
5	2,3-dinitrophenol	18	2-nitro-*m*-cresol	31	2,5-dinitro-*m*-cresol
6	2,4-dinitrophenol	19	4-nitro-*m*-cresol	32	2,6-dinitro-*m*-cresol
7	2,5-dinitrophenol	20	5-nitro-*m*-cresol	33	4,5-dinitro-*m*-cresol
8	2,6-dinitrophenol	21	6-nitro-*m*-cresol	34	4,6-dinitro-*m*-cresol
9	3,4-dinitrophenol	22	2-nitro-*p*-cresol	35	5,6-dinitro-*m*-cresol
10	3,5-dinitrophenol	23	3-nitro-*p*-cresol	36	2,3-dinitro-*p*-cresol
11	*o*-cresol	24	3,4-dinitro-*o*-cresol	37	2,5-dinitro-*p*-cresol
12	*m*-cresol	25	3,5-dinitro-*o*-cresol	38	2,6-dinitro-*p*-cresol
13	*p*-cresol	26	3,6-dinitro-*o*-cresol	39	3,5-dinitro-*p*-cresol

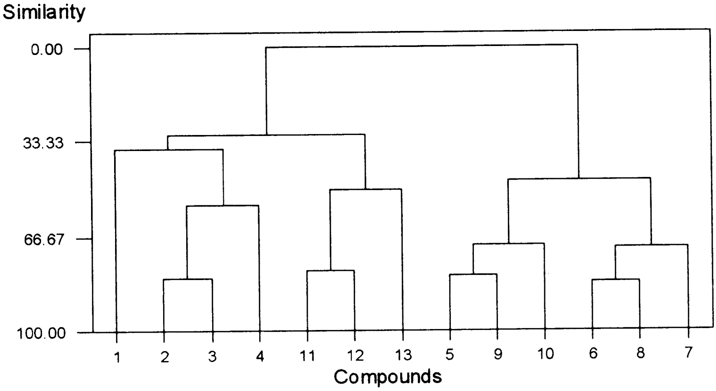

Fig. 2: Hierarchical cluster of molecular descriptors for nitro-phenols and cresols (complete linkage – euclidean distance).

The phytotoxicity of phenol and its mono- and dinitroderivates was investigated. This simple and rapid test is designed to determine possible toxic effects of liquid substances on the emergence of seedlings. Soy seeds were grown on filter paper disks imbibed with 7 mL of aqueous solution containing the nitroderivates at concentrations ranging from 11 to 180 mg/L. Control seeds were grown in *aqua fontis*. Seeds were kept in the dark, at constant humidity and temperature conditions. After 5 days of exposure, the reduction in germination was determined measuring the root length of seedlings and calculating the "germination index" (GI) as follows:

$$GI = \frac{R \times L}{Rr \times Lr}$$

where: R = % rooting of treated seeds; L = average root length of treated seeds
Rr = % rooting of control seeds; Lr = average root length of control seeds

The IC50 values for the "germination index" and root length of seedlings were calculated by the method of Finney (Finney, 1991), see Table 3.

The different effect of various mono- and dinitrophenols seems to reflect the uncoupling potency on oxidative phosphorylation.

Table 3: IC50 values (95 % confidence limits).

Compounds	Germination index	Root length
2,4-dinitrophenol	19.54 *(15.45-24.71)*	18.43 *(14.43-23.54)*
2,6-dinitrophenol	27.04 *(19.25-37.97)*	27.77 *(19.44-39.66)*
2,5-dinitrophenol	56.41 *(43.57-73.02)*	62.14 *(47.10-81.98)*
2,3-dinitrophenol	74.89 *(67.69-82.85)*	78.39 *(70.60-88.25)*
3,4-dinitrophenol	78.04 *(57.42-106.05)*	107.99 *(74.81-155.88)*
4-nitrophenol	87.90 *(76.72-100.70)*	80.08 *(67.90-94.46)*
2-nitrophenol	95.11 *(78.74-114.89)*	100.75 *(85.08-119-29)*
3-nitrophenol	126.65 *(95.73-167.55)*	137.33 *(105.64-178.52)*
Phenol	no effect	no effect

Conclusions

Producs for the reactions of the phenol with NO_2^+ and NO_3 radicals were nitrophenols and dinitrophenols. QSAR modelling approaches allows us to find compounds more similar (or dissimilar) with the aim of a better planning of following toxicological experiments.

These preliminary results show that dinitrophenol activity is higher than nitrophenols'. In particular the substituent in 2,4 and 2,6 position are more potent in inducing phytotoxicity. The different effect of various mono- and dinitroderivates seems to reflect the uncoupling potency on oxidative phosphorylation.

Acknowledgements

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