tographed with methanol as solvent, brucine (−)-usnate travelled with the methanol front about 3 cm and brucine (+)-usnate together with brucine about 2 cm. These two solutions were then used as references.

A mixture of A (0.1 ml), B (0.1 ml), and C (0.4 ml) was warmed on the steam bath until half of the solvent had evaporated and some brucine (+)-usnate had been precipitated. The filtrate was chromatographed in methanol with brucine (+)-usnate and brucine (−)-usnate as markers. Two spots were obtained, one at the methanol front identified as brucine (−)-usnate and the other, with the lower R_F-value, as brucine (+)-usnate.

The "filter paper" method was performed according to Santesson.[2] The lichen specimen pressed down on a filter paper was treated dropwise with chloroform. Each drop of chloroform was allowed to evaporate leaving the extracted substances in a ring round the lichen fragment. When tested with an aqueous solution of titanium trichloride (8 %), a green colour was formed if usnic acid was present.

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A Rapid and Sensitive Method for the Measurement of Biological Oxidation of an Aromatic Hydrocarbon Catalyzed by Liver Microsomes

ROBERT NILSSON and GUNNAR AHNSTRÖM

Department of Radiobiology, Institute of Biochemistry, Royal University of Stockholm, Stockholm, Sweden

In a previous communication [1] we have described the radiation-induced formation of polar oxidation products from aromatic hydrocarbons used for the measurements of low doses of ionizing radiations. In these experiments an aqueous solution of tritium-labelled naphthalene was irradiated and the activity of the polar compounds formed was measured in a window-less proportional counter.

In higher animals naphthalene is mainly converted to dihydronaphthalene-1,2-diol and naphthols which are excreted as conjugates of glucuronic and sulphuric acid.[2] In view of the high degree of accuracy by which small amounts of radioactive compounds may be measured, it would be of interest if the "naphthalene-dosimeter" could be adapted and modified for biological use. Preliminary experiments with rat liver microsomes confirmed previous observations [3,4] that naphthalene in fact, is oxidized *in vitro* in the presence of oxygen and NADPH by this subcellular fraction and that the formation of radioactive oxidation products could readily be determined.[5] The experimental procedures used for extraction in the "naphthalene-dosimeter" were, however, disturbed by the presence of proteins, salts etc., resulting in high background values and lack of reproducibility. Incidently, during the development of the "naphthalene-dosimeter" it was found that most of the activity vanished completely from the water phase when aqueous solutions of labelled naphthalene were stored in vials made of polyethene.[6] Polyethene was found to function as a very rapid extraction medium for aromatic hydrocarbons in water solutions. Based on these findings it has been possible to work out a simple and rapid method for the separation of the parent hydrocarbon from its oxidation products which, thus, could be utilized for the following convenient modification of the "naphthalene-dosimeter" for the estimation of hydroxylation activity in biological systems.

Experimental. Naphthalene-1-T * (specific activity 17 C/mole, Radiochemical Centre, Amersham) was dissolved in distilled water at 20°C (magnetic stirring for 12 h) and stored at 5°C. Activities were measured in a liquid scintillation counter. As seen from Table 1, shaking for 20 min followed by transfer into a new vial gives satisfactory results. The naph-

* Naphthalene-1-T is no longer produced by the Radiochemical Centre, Amersham, but ^{14}C labelled naphthalene or methylnaphthalene available from several sources is equally suitable.

Table 1. Influence of shaking time and change of polyethene vial on the separation efficiency.

Shaking time min	Activity, cpm control	Activity, cpm irradiated (15 000 rad)
0	10 545	10 060
5	248	6 000
12	155	6 070
20	121	6 100
20 + 20 [a]	62	—
20 + 20 [a] + 20 [a]	60	—
background	45	45

[a] Transfer into new vial.

thalene adsorbed by the polyethene may easily by re-extracted by toluene.

The shaking reduces the activity of the aqueous solution approaching asymptotically a low background value which, according to previous investigations, is due to the presence of small amounts of polar compounds formed from naphthalene by autoxidation, and constitutes only around 0.15 % of the total activity of the original solution. Microsomes from the livers of starved rats were prepared according to Ernster *et al.*[7] and for the assay of hydroxylating activity, a reaction mixture was used containing microsomes, 0.05 M tris buffer (pH 7.5), 50 mM nicotinamide, 5 mM $MgCl_2$ and an NADPH-generating system consisting of 5 mM DL-isocitrate, 0.01 mM $MnCl_2$, 0.5 mM NADP, and enough isocitric dehydrogenese to reduce 0.32 μmole NADP per minute, in a final volume of 2 ml. The reactions were carried out at 37°C. The reaction was stopped by the addition of 0.3 ml saturated aqueous solution of diethylpyrocarbonate. This compound rapidly and specifically inactivates proteins without affecting nucleic acids.[8]

In Fig. 1 the formation of polar hydroxylation products from naphthalene as a function of time is depicted for two different concentrations of rat microsomal protein. The background values caused by NADPH-independent autoxidation of the naphthalene in the presence of microsomes were found to lie well below 10 % of the enzymic formation rate over the whole concentration range. The linear part of curve (A) corresponds to a formation rate of hydrophilic reaction products of 0.6×10^{-10} mole·mg protein^{-1} min^{-1}.

As seen from Fig. 1, the rate of formation of hydrophilic products from naphthalene is only linear during the first 10 min for the higher concentration. In presence of the low naphthalene concentration used in the experiment mentioned above (10^{-5} M) this factor was found to be rate limiting at protein concentrations above 1—1.5 mg protein per ml of the reaction mixture and the formation rate of hydrophilic oxidation products was not proportional to the protein concentration above these levels. It must be kept in mind that,

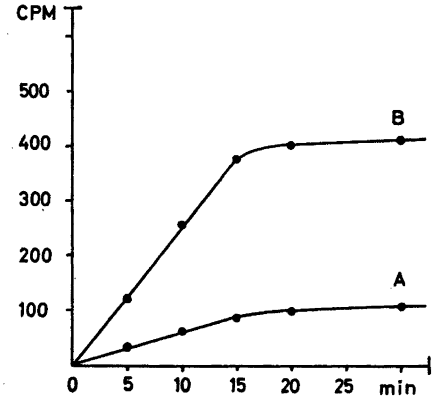

Fig. 1. The NADPH-dependent formation of oxidation products from naphthalene catalyzed by rat liver microsomes as a function of time. (A) = 0.5 mg, (B) = 3.0 mg of microsomal protein present in reaction mixture. Background values due to autoxidation subtracted.

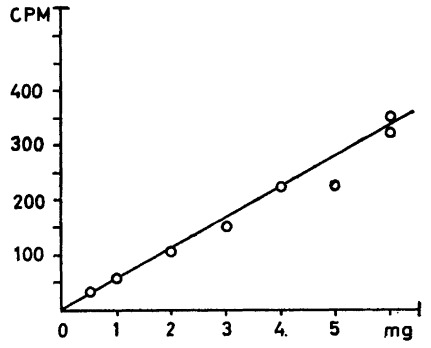

Fig. 2. The NADPH-dependent formation of oxidation products from naphthalene catalyzed by rat liver microsomes as a function of protein concentration. The activity of the reaction products were measured 10 min after initiation of the reaction. Background values due to autoxidation subtracted.

although only around 10 % of the total naphthalene present is converted at higher protein concentrations, an appreciable fraction of the very small amount of hydrocarbon added may rapidly dissolve into microsomal structures rich in lipids where it is not immediately accessible to oxidation during the limited time period during which the reaction was followed. By adding 15–20 μl of a 4×10^{-2} M unlabelled naphthalene solution in ethanol the range of linearity was extended as shown in Fig. 2. A more detailed analysis of the microsomal oxidation of hydrocarbons will be published elsewhere.[5] The Fenton reagent as expected,[9] efficiently oxidized naphthalene.

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The Molecular Structure of Oxalyl Chloride. An Electron Diffraction Study

K. E. HJORTAAS

Kjemisk institutt, Norges lærerhøgskole, Trondheim, Norway

Several investigations have been performed on the molecular structure of oxalyl chloride, $C_2O_2Cl_2$. Luvalle [1] obtained from electron diffraction data the bond distances and angles listed in Table 1. Groth and Hassel suggested that the molecule in the solid phase is nearly in the planar *trans* form.[2] The X-ray diffraction method had been used. Contrary to what had been reported earlier,[3] Hencher and King claimed that a satisfactory analysis of the infrared and Raman spectra of oxalyl chloride can be made by assuming the molecules to be in the planar *trans* form only.[4] A dipole moment for oxalyl chloride in the benzene solution has been reported.[5] However, microwave investigations on oxalyl chloride in the gas phase did not indicate any dipole moment.[6] Because of the conflicting opinions which are found in the literature as to the structure of oxalyl chloride, it was decided to make further investigations by the electron diffraction method.

Experimental. The electron diffraction data were obtained at the University of Oslo. The nozzle-to-plate distances were about 48 and 19 (cm). A modified s^3 sector was used. The data were treated according to the procedure described by Almenningen *et al.*[7] An experimental background was first drawn and later on

Table 1. Least-squares results. Distances $r_g(1)$[8] and root-mean-squares amplitudes of vibration (u) both with standard deviations (σ) in Å. Uncertainty of wavelength is also included in σ_r.

	$r_g(1)$	σ_r	u	σ_u
C–C	1.534 (1.50 [1])	0.003	0.033	0.003
C–O	1.189 (1.20 *ass.*[1])	0.002	0.045	0.001
C–Cl	1.749 (1.72 [1])	0.003	0.058	0.001
C–O	2.410	0.005	0.058	0.003
O–Cl	2.602	0.004	0.061	0.002
C–Cl	2.732		0.070	0.004

$\angle$ CCO: 124.1° (123° [1]), $\angle$ ClCO: 123.5° (123° [1]).