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CHROMATOGRAPHIC ANALYSIS, ISOLATION AND PARTIAL CHARACTERIZATION OF TWO PHOTOCATALYSED REACTION PRODUCTS OF HAEM AND QUINONE

K. PELTONEN*

Department of Industrial Hygiene and Toxicology, Institute of Occupational Health, SF-00250 Helsinki (Finland)

R. KOSTIAINEN

Finnish Research Project of Verification of Chemical Disarmament, University of Helsinki, Helsinki (Finland)

and

H. SAVOLAINEN

Institute of Occupational Health Sciences, University of Lausanne, Lausanne (Switzerland)

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SUMMARY

Quinone was reacted in vitro with free haem under ultraviolet light Two principal products were detected by liquid chromatography and purified by column chromatography. Mass spectral and Fourier transform infrared analyses showed that both products contained pyrroles and that the first product was probably an ether formed after reaction with the haem vinyl moieties. This product was entirely different from the known N-alkyl intermediates in the haem-catalysed epoxidation reactions of porphyrin dimerization by quinone.

INTRODUCTION

Photoallergic reactions are a topic frequently discussed in the medical literature. Although primarily associated with errors in porphyrin metabolism [1], allergic interactions with drugs and chemicals at the workplace are also known without a prior defect in the porphyrin metabolism. The interesting aspect of the latter reactions is that they tend to be triggered even by UV light, once sensitization has taken place.

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It may be presumed that sensitization includes reactions involving a natural constituent in the skin, catalysed by UV quanta. Because of the findings in studies of porphyric skin disease, haem molecules have been suggested to play a role [2]. Experiments with isolated skin microsomes show that their contents of cytochrome P-450, an oxidizing enzyme complex with a prosthetic haem molecule, decreases rapidly on exposure to UV light and a haematoporphyrin derivative [2].

As haem and its precursor or derivative porphyrins are probably involved, we decided to simulate in vitro photocatalysed reactions between haem and quinone. Good analytical techniques available for a series of metalloporphyrins [3–5] were applied to the analysis of products [4,6].

EXPERIMENTAL

Chemicals

Phenol, *p*-methoxyphenol, pyrrole, hydroquinone, quinone, protoporphyrin IX dimethyl ester, haematin and zinc protoporphyrin IX were obtained from Aldrich (Steinheim, F.R.G.) and were used as standards in the Fourier transform infrared (FTIR) analysis. The stable haem was purchased as its arginate complex (250 mg per protohaem, 38.3 m*M* in 10 ml of 153 m*M* arginine) dissolved in a 4:1.9 (v/v) mixture of 1,2-propanediol, ethanol and water (Normosang®, Medica, Helsinki, Finland).

Irradation

The photoreactions with haem (1 m*M*) and quinone (1 m*M*) were carried out in water at pH 7.0 in quartz cells. The UV irradiation lasts for 30 min and the total volume was 5 ml. Water was added in blank tests instead of quinone. The UV source was a naked Airam 250 W mercury lamp in a quartz casing (Airam, Helsinki, Finland). The UV-B range flux rate was 116 $\mu W/cm^2$ at a distance of 1 cm [7].

Instrumentation

Chromatography. The reaction mixtures were sampled for the analysis as follows: 1 ml of the reaction supernatant was directly scanned with a Shimadzu spectrophotometer between 200 and 600 nm. Then 10 μl of the same sample were injected into an LKB liquid chromatograph (2152 LC controller with 2150 pump) coupled to a Shimadzu SPD-6A UV detector. The analytical column used was a Supelcosil LC-18-DB (5 μm, 250 mm$\times$4.6 mm I.D., Supelco, Bellefonte, PA, U.S.A.). An isocratic mode was used with an eluent mixture found experimentally by using variable mixtures of two eluent solutions A and B (30:70, v/v). Solution A was methanol–acetic acid–water (5·4:1, v/v) and solution B was methanol–acetic acid (9:1, v/v). The flow-rate was 0.8 ml/min.

A Sephadex LH-20 column (450 mm$\times$30 mm I.D.) was used to isolate the

detected products, and the fractions were collected. The gel was preconditioned and eluted with pure methanol. The purity of the fractions was confirmed by liquid chromatography (LC) as above.

Mass spectrometry. All mass spectra were obtained with a Finnigan MAT triple-stage quadrupole (TSQ) 45 A instrument with an Incos data system. The electron-impact (EI) spectra were recorded at 70 eV and the positive-ion chemical ionization (CI) spectra at 150 eV. An emission current of 0.3 mA was used for both modes. The ammonia CI spectra were recorded at an ion source pressure of 0.6 Torr. The ion source temperature was 100°C. The desorption probe was heated between 100 and 350°C at a rate of 25°C/min.

FTIR analysis. FTIR analysis was performed with a Nicolet 20 SXC FTIR spectrometer using the Nicolet 660 data system. All samples were analysed with the diffuse reflectance technique (DRIFT). They were evaporated to dryness and 500 μg of [^{2}H]methanol were added; 50 μl of the diluted sample were placed on KBr in the DRIFT sample cup. The scan range was 400–4000 cm^{-1} and the resolution was 2 cm^{-1}; 320 scans were carried out in each case.

RESULTS AND DISCUSSION

Two products of photocatalysed reactions between the haem and the quinone were analysed by liquid chromatography (Fig. 1). Unchanged haem was eluted at 6 min and unchanged quinone at 1 min (Fig. 1).

When the two reaction products were isolated with the Sephadex column,

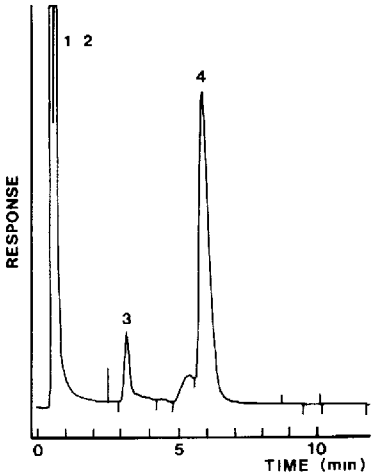

Fig. 1. HPLC profile of products from photocatalysed reactions between haem arginate and quinone. Free quinone (peak 1) elutes immediately after the solvent front at 1 min and the free haem (peak 4) at 6 min. Two products are noted at 2.6 and 3.2 min, respectively (peaks 2 and 3). The UV detector was set at 380 nm.

the first product (peak 2 in Fig. 1) was eluted in 145–165 min and the second product in 170–195 min. The free haem in this system was eluted in 80–90 min. When the isolated fractions were reanalysed with the LC technique they were found to be virtually pure, with an elution pattern identical with that found for the original reaction mixture.

The isolated products had the same absorption maximum as the haem (398 nm), in addition to new maxima at 484 and 518 nm, respectively (Fig. 2). The haem peak is discernible in the CI-mode mass spectrum (Fig. 3). It produces the typical fragmentation due to the known tri-, di- and monopyrrole cleavage [6]. The ion *m/z* 322 is discernible in both products (Fig. 3), with new lighter fragments that were not identical for the two products. The results of the EI measurements are in Table I.

The FTIR analysis confirmed the pyrrolic nature of both products (Fig. 4). On closer examination, it showed that product **1** did not have the out-of-plane C–H deformation typical of the vinyl groups in free haem or in product **2** (Fig. 4). C–O stretching typical for alkyl–aryl ethers is also discernible in the spectrum for product **1**. Thus, it seems that the photocatalysed reaction of the haem with quinone produces two main products. One contains the hydroquinone probably in an ether conjugation to replace the vinyl groups of the haem. The second product also has the porphyrin structure. It could very well correspond to the recently discovered porphyrin dimer from quinone–porphyrin reactions [8].

Quinone was chosen as a model for the photocatalysed reaction because it is

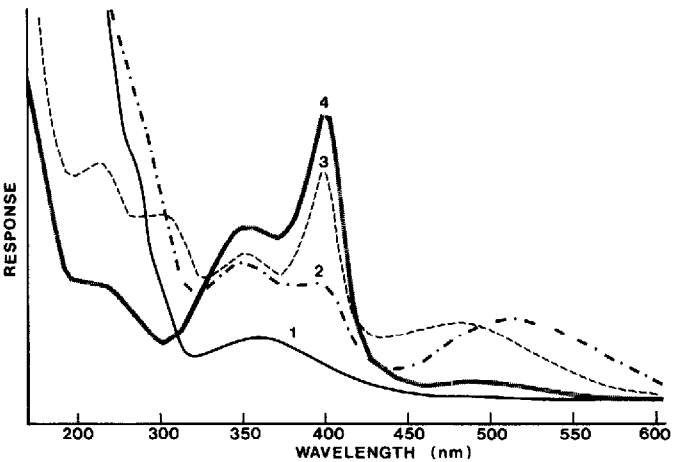

Fig. 2. UV absorption spectra of the two isolated haem–quinone reaction products (curves 2 and 3) and of quinone (curve 1) and haem (curve 4). Haem and the products have absorption maxima at 398 nm, while pure quinone has no major absorption at this wavelength. The spectral characteristics of the two products are clearly distinct.

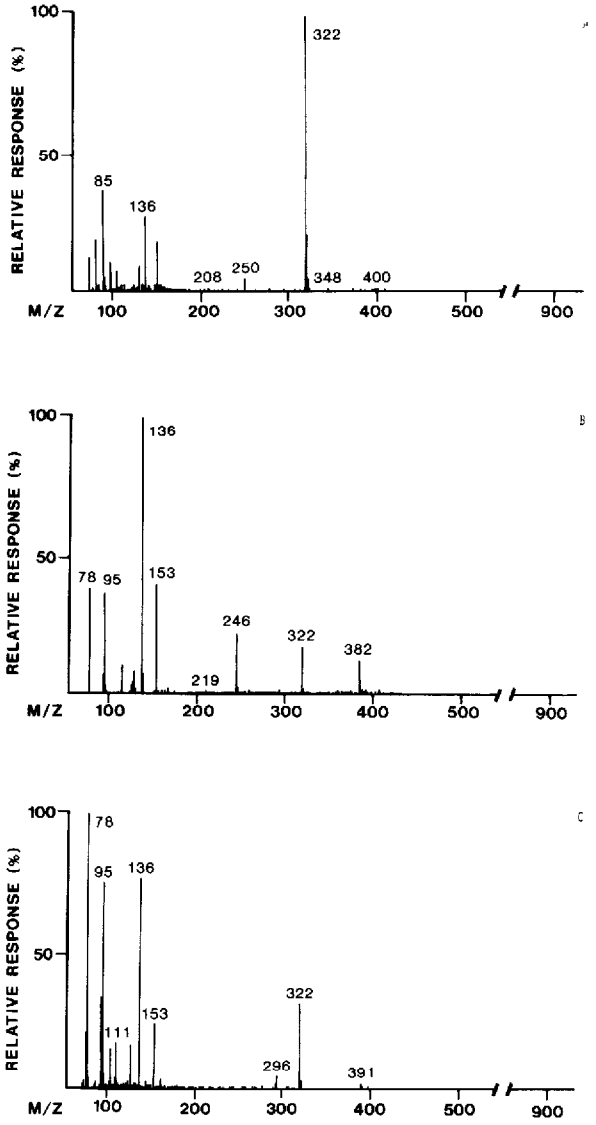

Fig. 3. CI mass spectra of free haem (A) and of product **1** (B) and product **2** (C). The ion m/z 322 is typical of the haem cleavage products and is also found in both products.

a confirmed photoallergen with important clinical significance [9]. UV irradiation can directly produce reactive intermediates from phototoxins. They often have a radical character [10–12]. The simultaneously available free oxygen often enhanced the reaction rates significantly [12,13]. The role of molecular oxygen is not clear though: it can be assumed that it may act as a ligand to the iron coordinated in the porphyrin structure. This is the requirement in the haem-catalyzed in vitro epoxidation of norbornene [14]. In this case, a transient N-alkyl haemin is formed.

The first product, i.e. the aryl ether replacing a vinyl moiety, has not been

TABLE I

ELECTRON IONIZATION (70 eV) MASS SPECTRA OF HAEM AND QUINONE REACTION PRODUCTS

Ion (*m/z*)	Relative intensity (%)		
	Haem	Product **1**	Product **2**
43	–	81.2	86.8
44	–	73.2	100.0
45	–	100.0	76.8
46	–	17.4	13.6
53	–	12.8	5.6
54	–	6.6	2.1
55	–	25.8	14.5
69	–	23.0	8.1
70	–	6.7	3.4
73	23.4	8.8	4.7
74	2.2	3.2	3.3
79	–	7.3	8.9
80	–	11.1	5.6
81	2.0	11.1	5.6
96	16.0	3.0	–
97	1.5	1 0	–
119	2.3	3.1	1.3
120	–	4.7	1.1
133	10.9	4.64	–
134	1.2	3.3	–
191	12.0	–	–
192	2.4	–	–
193	5.1	–	–
207	100.0	–	–
208	19.2	–	–
209	11.9	–	–
245	–	21.5	–
246	–	3.2	–
267	6.0		
268	1.2		
281	26.7		
282	6.9		
283	4.2		
341	7.9		
342	2.2		
355	6.3		
356	2.0		
429	3.7		
430	1.3		

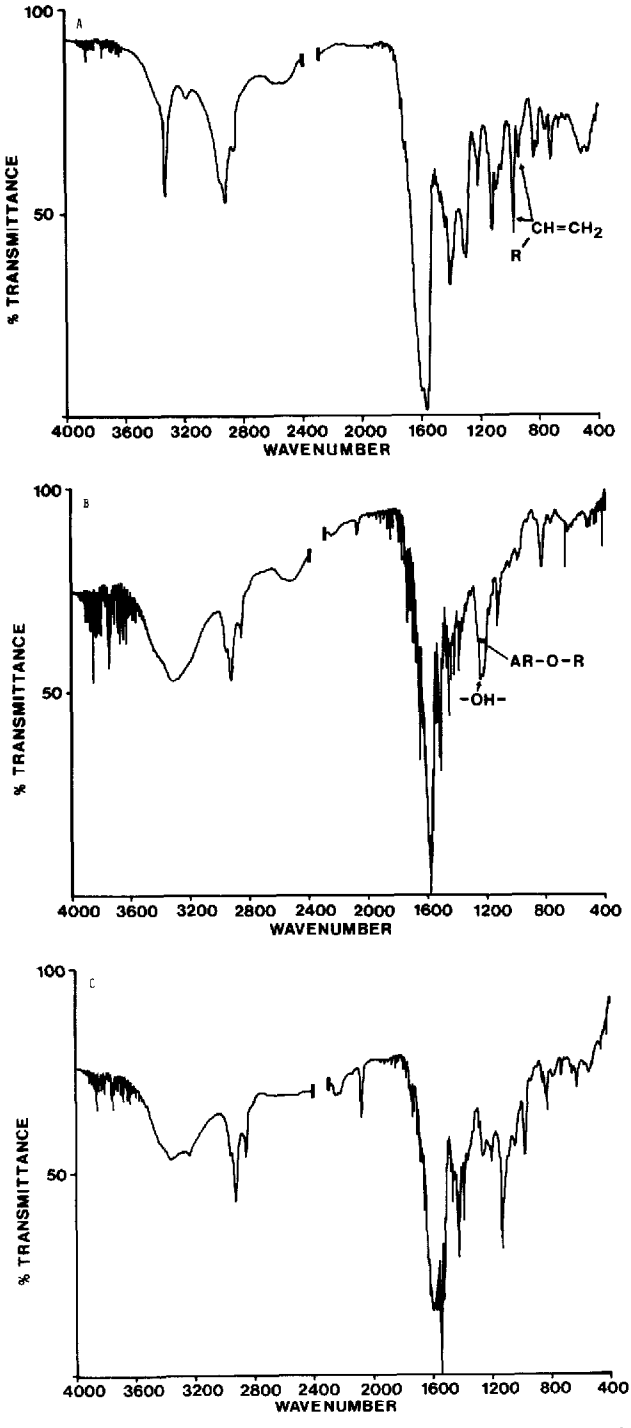

Fig. 4. FTIR spectra of free haem (A), product **1** (B) and product **2** (C). The vinyl groups in haem cause the typical out-of-plane C–H deformation at wavenumber 900 cm (A). This is replaced by a typical alkyl-aryl C–O stretching in product **1** (B), although no such effect is observed in product **2** (C).

previously reported, and it is distinct from the N-alkyl haemins [14] and the quinone adducts in the interpyrrolic methylene bridges [8]. It is not a major reaction product, but it does account for the unexplained UV absorption maxima in the reaction mixture. We have not presented exact structures of our model products, in the absence of the relevant NMR data. However, the presence of covalently bound quinone is certain and the IR spectra indicate that no major changes took place in the haem moiety. It seems that the analytical techniques described are powerful enough for the elucidation of the reaction products in more complex biological matrices, e.g. in the skin. This would shed light on the possible roles of quinone–haem adducts in quinone-induced photoallergy.

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REFERENCES

1 L.C. Harber and D.R. Bickers, Photosensitivity Diseases: Principles of Diagnosis and Treatment, Saunders, New York, 1981.
2 D.R. Bickers, R. Dixit and H. Mukhtar, Biochem. Biophys. Res. Commun., 108 (1982) 1032.
3 R. Bonnett, P. Burke and A. Reszka, Fuel, 66 (1986) 515
4 M. Gastagnola, R. Landalfi, D. Rossetti, F. Angelis and S. Ceccarelli, Anal. Lett., 19 (1986) 1793.
5 M. Salmi and R. Tenhunen, Clin. Chem., 26 (1980) 1832.
6 R. Evershed, G. Wolff, J. Shaw and G. Eglinton, Org. Mass Spectrom., 20 (1985) 445.
7 K. Peltonen, A. Zitting, H. Koskinen and A. Itkonen, Photochem. Photobiol., 43 (1986) 481.
8 J.L. Sessler, M.R. Johnson, T.-Y. Lin and S.E. Creager, J Am. Chem. Soc., 110 (1988) 3659.
9 C.R. Denton, A.B. Lerner and T.B. Fitzpatrick, J. Invest. Dermatol., 18 (1952) 119.
10 G.R. Buettner, A.G. Motten, R.D. Hall and C.F. Chignell, Photochem. Photobiol., 46 (1987) 161.
11 W.H. Koch and M.R. Chedekel, Biochim. Biophys. Acta, 924 (1987) 458.
12 J.P. Martin and N. Logsdon, Arch. Biochem. Biophys., 256 (1987) 39.
13 S.T. Mosley, Y.-L. Lang, J.R. Falck and R.G.W. Anderson, Exp. Cell Res., 155 (1984) 389.
14 T.G. Traylor, T. Nakano, A.R. Miksztal and B.E. Dunlap, J. Am. Chem. Soc., 109 (1987) 3625.