Experimenteller Teil

1. Allgemeine Angaben und Geräte [3]

2. 1,4-Thymochinon-4-dibromarylimine 11, 12

Die Lösung von 5 mmol 2,6-Dibromsulfanilamid (**9**) und 5 mmol Thymol (**7**) in 70 ml 3 M wässriger Natronlauge wird bei Raumtemperatur und unter Rühren tropfenweise mit 2,5 ml 13%iger Natriumhypochloritlösung versetzt. Nach einer Stunde Rühren wird das jeweilige rotviolette Farbprodukt erschöpfend mit Essigsäureethylester extrahiert, die organische Phase mit Wasser neutral gewaschen, mit Natriumsulfat getrocknet, im Vakuum eingeengt und sc aufgearbeitet. Sc-/Dc-Fließmittel: Dichlormethan/Acetonitril/Cyclohexan (25 + 1 + 1).

2.1. 5-Methyl-2-(1-methylethyl)-1,4-benzochinon-4-[(4-amino-3,5-dibromphenyl)imin] (**11**)

Ausbeute: 120 mg (6%) rotviolette Kristalle vom Schmp. 149 °C. Dc: $R_f = 0{,}45$. MS (EI, 70 eV) m/z (rel. Int.): 414 (M^+, ^{81}Br, 22), 412 (M^+, ^{81}Br, ^{79}Br, 38), 410 (M^+, ^{79}Br, 18), 399 (7), 397 (16), 331 (21), 252 (53), 120 (63), 92 (100). IR (KBr, cm^{-1}): 3500, 3420, 3070, 2940, 1660, 1595, 1470, 1395. ^{1}H NMR ($CDCl_3$, δ, ppm): 1,44 (d, $^3J = 6.8$ Hz, 6 H, $CH(CH_3)_2$); 2,11 (s, 3 H, CH_3); 3,04 (sept., $^3J = 6.8$ Hz, $CH(CH_3)_2$); 3,73 (s breit, 2 H, NH_2); 6,47 (s, 1 H, Thymochinon-6-H); 6,95 (s, 2 H, Aryl-H); 7,15 (s, 1 H, Thymochinon-3-H). UV/Vis (CH_2Cl_2, nm): λ_{max} (log ε) = 275 (4,20), 325 (sh), 514 (3,55).
$C_{16}H_{16}N_2OBr_2$ (414,1; 412,1; 410,1).

2.2. 5-Methyl-2-(1-methylethyl)-1,4-benzochinon-4-[(4-amino-2,6-dibromphenyl)imin] (**12**)

Ausbeute: 75 mg (4%) magenta Kristalle vom Schmp. 158 °C. Dc: $R_f = 0{,}35$. MS (EI, 70 eV) m/z (rel. Int.): 414 (M^+, ^{81}Br, 18), 412 (M^+, ^{81}Br, ^{79}Br, 38), 410 (M^+, ^{79}Br, 58), 399 (9), 397 (21), 331 (21), 252 (57), 120 (67), 92 (100). IR (KBr, cm^{-1}): 3520, 3440, 3060, 2950, 1662, 1636, 1602, 1470, 1395. ^{1}H NMR ($CDCl_3$, δ, ppm): 1,52 (d, $^3J = 6{,}5$ Hz, 6 H, $CH(CH_3)_2$); 2,19 (s, 3 H, CH_3); 3,12 (sept., $^3J = 6{,}5$ Hz, $CH(CH_3)_2$); 3,85 (s breit, 2 H, NH_2); 6,42 (s, 1 H, Thymochinon-6-H); 7,08 (s, 2 H, Aryl-H); 7,19 (s, 1 H, Thymochinon-3-H). UV/Vis (CH_2Cl_2, nm): λ_{max} (log ε) = 270 (4,15), 328 (sh), 529 (3,85).
$C_{16}H_{16}N_2OBr_2$ (414,1; 412,1; 410,1).

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Eingegangen am 6. März 2000
Angenommen am 12. April 2000

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Formation of hydrogen peroxide during the UVA induced disintegration of ketoprofen

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Ketoprofen is an antiinflammatoric, antipyretic and analgesic drug, that is used for therapy of rheumatic diseases. To avoid gastro-intestinal side effects some efforts have been made during the last years to administer ketoprofen topically [1–4]. On the other hand some publications on photodynamic investigations have shown that ketoprofen can in connection with UV-irradiation provoke contact dermatitis [5], DNA-damages [6–8], peroxidation of unsaturated fatty acids and damages of membranes [8]. These effects are commonly attributed to both the benzophenone moiety and the photochemical metabolites of ketoprofen.
A new method for the rapid determination of various peroxides, developed in our group and based on a combination of capillary electrophoresis with electrochemical detection [9] allows the direct separation and determination of the photoproduced peroxides. In contrast to the methods used up to now (e.g., measurement of the conjugated diene hydroperoxides of linoleic acid at 234 nm, the thiobarbituric acid assay, chemical derivatization into 1-naphthyldiphenylphosphin oxide [10] or fluorescent dichlorofluorescein assay [11]) misleading results through side reactions and following reaction can be nearly excluded.
Applying this method we were able to detect the production of hydrogen peroxide during UVA-irradiation of aqueous solutions of ketoprofen. The concentration of hydrogen peroxide increases with increasing total UVA dose (Fig. 1). The steep initial increase of the concentration lowers in the range between 4 and 8 J/cm^2 and changes to an almost linear ascent at higher doses. After the application of the maximal dose of 14 J/cm^2 a concentration of 0.22 mM hydrogen peroxide could be detected in the reaction mixture.
Additional capillary electrophoresis with UV-detection was carried out to monitor the photochemical induced decomposition of ketoprofen (Fig. 2). At irradiation doses exceeding 4 J/cm^2 no more ketoprofen was detectable. Simultaneously the formation of metabolites could be observed. By applying a microemulsion as separating buffer it could be proved that actually a variety of uncharged metabolites was generated. This finding is in accordance with the general reaction scheme of the photochemical decomposition of ketoprofen [8].

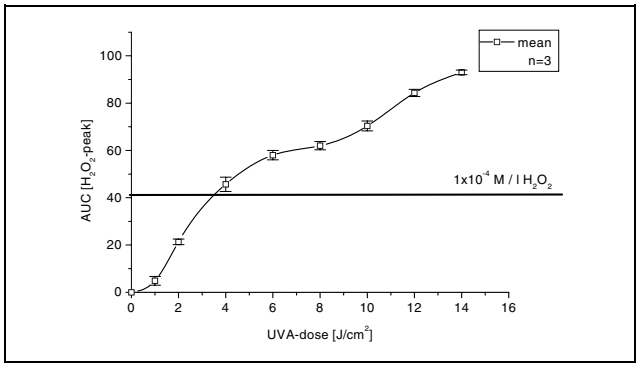

Fig. 1: Formation of hydrogen peroxide in a 0.5 mM ketoprofen-solution in buffer (pH 7.4) at different UVA-doses

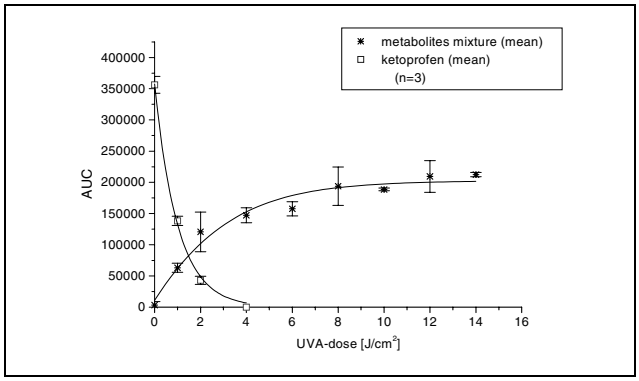

Fig. 2: Decomposition of ketoprofen and formation of ketoprofen metabolites during UVA-irradiation of 0.5 mM ketoprofen-solution in buffer (pH 7.4)

A remarkable detail consists in the fact, that the formation of hydrogen peroxide diminished, but not finished at the same dose that caused the ketoprofen to disintegrate completely. This may be considered as a hint at the major role of metabolites in the formation of hydrogen peroxide. At a dose of 8 J/cm^2 the concentration of the metabolites reaches a saturation, whereas the formation of hydrogen peroxide starts to increase linearly with the irradiation dose. The underlying reaction mechanism is not yet fully understood and will be the object of a forthcoming publication.

Preliminary investigations of micellar solution of linoleic acid containing ketoprofen showed significantly higher concentrations of hydroperoxyoctadecadienoic acid (HPODE) compared to pure linoleic acid solution.

Taking into account the presented results a topical application of ketoprofen should be critically and carefully examined. Further *in vitro* investigations referring to the catalytic effects of Fe (II) as well as photodynamic effects on human keratinocytes are under investigation.

Experimental

1. Materials

Chemicals and reagents were obtained from the following commercial sources: ketoprofen (Bayer-Leverkusen, Germany), sodium borate, Na_2HPO_4, KH_2PO_4 and H_2O_2 from Merck (Darmstadt, Germany); linoleic acid and 13(*S*)-hydroperoxy-octadeca-9*Z*,11*E*-dienoic acid (13-HPODE) solution in ethanol from Sigma (Deisenhofen, Germany). All samples, solutions and buffers were prepared from bidest. water.

2. Lamps

Irradiation was performed with CLEO Performance R UVA-Lamps from Veith Import-Export (Westerau, Germany) emitting in the range of 305 nm–420 nm ($\Lambda_{max} = 355$ nm) at a distance of 18 cm. The UVA-doses were measured with an UVA-sensor from Kühnast Strahlungstechnik (Wächtersbach, Germany).

3. Equipment

3.1. CE with electrochemical detection

Capillary electrophoresis (CE) was performed with a 40 cm long fused silica capillary with an inner diameter of 25 µm (from Supelco, Deisenhofen, Germany). The detection cell was filled with 10 mM sodium borate buffer. The sample was injected for 30 s by application of hydrostatic pressure (gravity injection). The detection of hydrogen peroxide and the peroxides of the fatty acids was performed at −600 mV and at a voltage of 17.5 kV. Before each sample injection the electrode was cleaned by applying a positive potential of 1200 mV, followed by a negative potential of −800 mV. Standard solutions were freshly prepared solutions of 0.1 mM H_2O_2 and 1 mM HPODE [9].

3.2. CE with UV-detection

A capillary electrophoresis system from Dionex (Dionex GmbH Idstein, Germany) was used for the detection of UVA induced destruction of ketoprofen. Fused silica capillaries (45 cm × 75 µm I.D., 40 cm to detector window) were purchased from Supelco. Samples were introduced by gravity injection from 20 cm height for 20 s.

The detection of ketoprofen and metabolites was performed at 254 nm and at a voltage of 17.5 kV. The ground electrolyte buffer was 0.01 M sodium borate.

4. Irradiation procedure

Ketoprofen (6.35 mg) was dissolved in 100 µl 0.1 M NaOH and further diluted in 10 mM phosphate buffer (PB, pH 7.4) leading to a final concentration of 0.5 mM.

This drug solution (10 ml) was irradiated in glass beakers covered with fused silica plates under permanent stirring with different UVA-doses (0; 1; 2; 4; 6; 8; 10; 12; and 14 J/cm^2). All shown results are mean values of a triple determination.

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Received December 3, 1999
Accepted March 23, 2000

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