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Dithranol, Singlet Oxygen and Unsaturated Fatty Acids

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Chemically or photochemically generated $^1O_2$ ($^1\Delta g\ O_2$) transforms the anion of dithranol **(1)** into chrysazine **(2)**, probably *via* an *endo*-peroxide. Under basic conditions, $^3O_2$ converts **1** to the bisanthrone **3** and to dithranol brown. The anion of dithranol is a photosensitizer. $^1O_2$ was trapped by 2,3-dimethyl-2-butene and by methyl oleate which was converted to the C-9- and C-10-hydroperoxides. From this point of view interference of the antipsoriatic dithranol with the metabolism of arachidonic acid appears reasonable.

Dithranol, Singulett-Sauerstoff und ungesättigte Fettsäuren

Chemisch bzw. photochemisch erzeugter $^1O_2$ ($^1\Delta g\ O_2$) überführt das Dithranol **(1)**-Anion in Chrysazin **(2)**, vermutlich über ein Endoperoxid. – Unter basischen Bedingungen entstehen aus **1** mit $^3O_2$ das Bianthron **3** und sog. Dithranolbraun. Dithranol **(1)** –Anion ist selbst ein Photosensibilisator, $^1O_2$ wurde mit 2,3-Dimethyl-2-buten bzw. mit Ölsäuremethylester abgefangen, der zu den entspr. C-9- bzw. C-10-Hydroperoxiden umgesetzt wird. Von hier aus sind Eingriffe des Antipsoriatikums Dithranol **(1)** in den Arachidonsäurestoffwechsel denkbar.

The lability of the antipsoriatic compound dithranol **(1)** is well known[1]. Especially under basic conditions chrysazine **(2)** and 1,8,1′,8′-tetrahydroxy-10,10′-bisanthrone **(3)** and the so-called dithranol brown arise very quickly. These components are ineffective against psoriasis. According to *Mustakallio*[2] **3** is formed *via* a radical intermediate whose structure was elucidated by *Davies*[3]. Therefore, we regard **3** to be a $^3O_2$-oxidation product. On the other hand, *Schultz* and *Frey*[4] have synthesized *Diels-Alder*-adducts using dithranol as a diene under basic conditions. In addition, *Retzow*[5] obtained the same compounds even in a slightly acidic medium. From this point of view a reaction of **1**-anion with $^1O_2$ is conceivable.

In dipolar, aprotic solvents **1** is deprotonated at least partially[5] to the 1,8,9-trihydroxyanthracene-anion which is converted to **2** by $^1O_2$. $^1O_2$ is generated by

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$NaOCl/H_2O_2$, by thermal decomposition of 1,4-dimethylnaphthalene-1,4-endoperoxide or by photosensibilisation (tetraphenylporphyrine – TPP). No reaction occurs between **1** and $^1O_2$ in CH_2Cl_2* where **1** exists as the pertinent anthrone ($\lambda max = 355$ nm). Under basic conditions **3** is converted rapidly to the corresponding bisanthranole[6], which is also transformed to **2** by $^1O_2$. On the other hand, **1** is converted only to **3** and dithranol brown using NaOCl, H_2O_2 and *Fenton*'s reagent. Therefore, two oxidation pathways can be differentiated: with $^1O_2$ there arises **2**, a typical $^1O_2$ product; whilst **3** and dithranol brown are formed by $^3O_2$ under basic conditions. Table 1 shows the percentage of **2** and **3** after 4 h. Fig. 1 indicates the formation of **2** correlated to the sensitizing conditions for O_2.

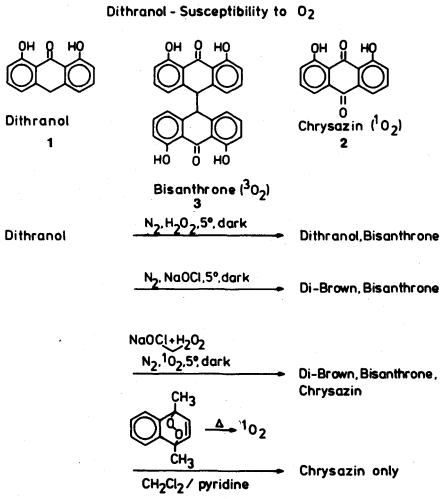

Table 1: *Percentage of* **2** *and* **3** *after 4 h*

	a	b	c
2	67 %	32 %	–
3	19 %	14 %	42 %

a: TTP/irradiation; b: no TPP/irradiation; c: no TPP/no irradition. – differences to 100 %: **1** and/or dithranol brown

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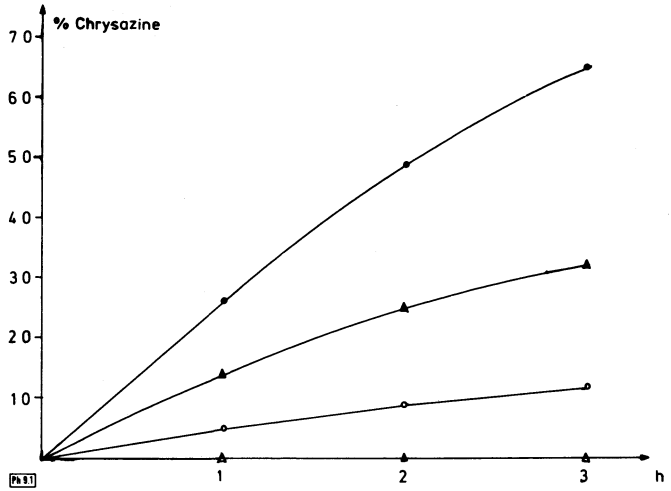
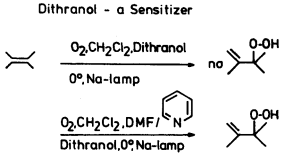


Fig. 1: Time course for the conversion of **1** to **2**
• TPP/100 W/O_2/ ▲ no TPP/100 W/O_2/ ○ no TPP/daylight/air/ △ TPP/light protection/O_2

According to Table 1 and Figure 1, **2** is formed from the **1**-anion even without addition of a photosensitizer. Therefore, **1**-anion was irradiated together with 2,3-dimethyl-2-buten leading to 2,3-dimethyl-1-buten-3-hydroperoxide, a typical $^1O_2$-product (*Schenck*-reaction[7]). Contrary to their behaviour in biological experiments[8], **2** and **3**-enol do show photosensitizing properties under our more vigorous conditions.



If the $^1O_2$-quencher β-carotene[9] is added to experiments with 2,3-dimethyl-2-butene, no hydroperoxide is formed; the photooxidation of **1**-anion to **2** was inhibited significantly (Fig. 2) by β-carotene.

The necessity of increasing concentrations of β-carotene in order to prevent the oxidation of **1** to **2** is explained by the degradation of β-carotene by $^1O_2$[10] (Fig. 3): 50 % of the β-carotene are already destroyed after 20 h.

As indicated in the introduction, **1** acts as a diene. Therefore, the formation of **2** was assumed to proceed *via* a [4+2]-cycloaddition of $^1O_2$ to **1**-anion leading to the pertinent 9,10-endoperoxide. In order to support this hypothesis, 1,8,9-trimethoxyanthracene **(4)** was used as a model compound. With $^1O_2$ **4** reacts to **5** (TPP, irradiation) and to methanol.

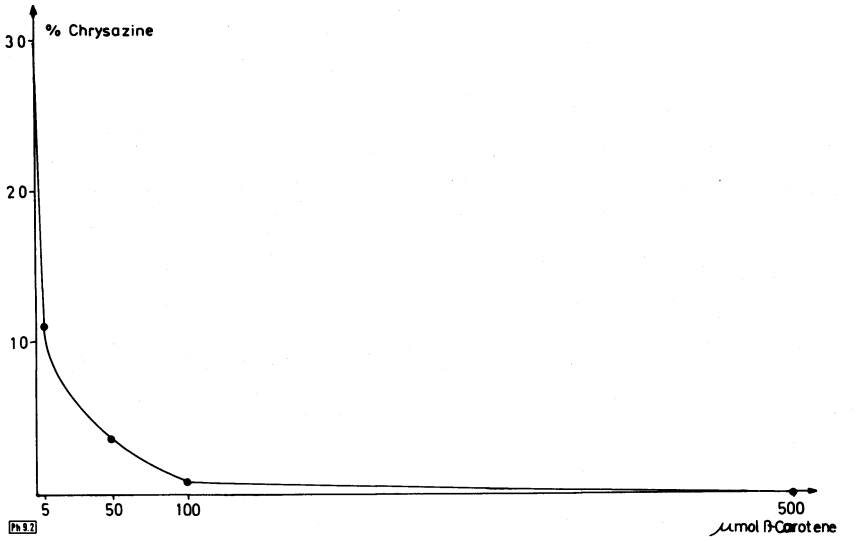


Fig. 2: Photooxygenation of dithranol: inhibition by β-carotene

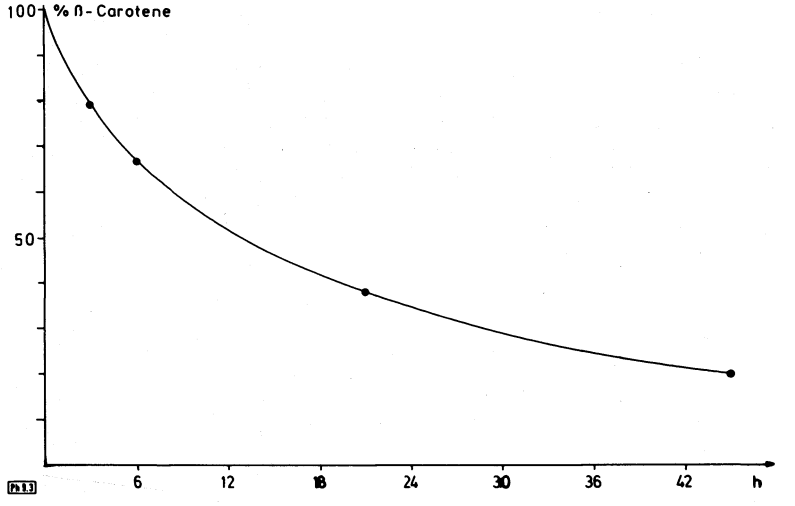
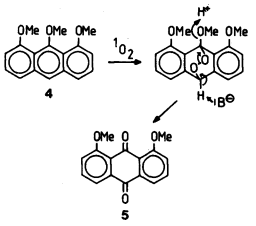


Fig. 3: Time course of the decomposition of β-carotene ($5 \cdot 10^{-3}$ M)

The corresponding 9,10-endoperoxide was found as the pertinent intermediate by nmr-spectroscopy at 0 °C: the singulet of C-10-H in **4** (δ = 8.02 ppm) was shifted to 5.74 ppm (endoperoxide); when the sample was allowed to warm up to 35° in the nmr spectrometer, the intensity of the singulet at 5.74 ppm decreased, whilst the MeOH signal increased.



The bisanthrone **3** was cleaved by $^1O_2$ to **2**, moreover dithranol brown was formed. **2** was resistant to $^1O_2$.

The reactivity of **1** as a photosensitizer and the conversion of 2,3-dimethyl-2-butene into the pertinent hydroperoxide point towards a conceivable interference of dithranol **(1)** and unsatured fatty acids under antipsoriatic therapy. In psoriatic epidermis, the concentrations of arachidonic acid and 12-hydroxy-5,8,10,14-eicosatetraenic acid are increased 25- and 81-fold, resp., as compared to unaffected skin, whilst the prostaglandines E_2 and $F_{2\alpha}$ are increased by only 40 % and 86 %[11]. This indicates that the cyclooxygenase system is slightly increased while the lipoxygenase activity is greatly so[12]. Other groups demonstrated a significant reduction in prostaglandine E and F syntheses in psoriatic skin to about 50 % of the normal rate[13, 14]. An inhibitor of the cyclooxygenase system was found in psoriatic plaques[15]. A participation of $^1O_2$ in the prostaglandine synthesis was assumed, because the activity of the prostaglandine synthetase system was diminished by $^1O_2$-trapping or quenching. Moreover, low concentrations of these trappers and quenchers are supposed to protect the prostaglandine synthetase and the pertinent peroxidase from autocatalysed degradation or inactivation by peroxides[16]. Although there are many reports of $^1O_2$ involvement in biological systems, there has not been a direct detection of $^1O_2$ in a biological reaction up to now. None of the techniques which have been used appear to be completely specific for $^1O_2$ and a new type of electron-transfer oxygenation gives rise to products which are identical of those obtained from $^1O_2$ reactions[17].

According to *Mustakallio*[18] an activation of the cyclooxygenase pathway leads to an increased level of inflammatory mediators, e. g. endoperoxides or prostaglandines, which are responsible for the unpleasant side effects – erythema and irritation of the uneffected skin – under dithranol **(1)** therapy. Latest results, however, indicate that **1** inhibits the lipoxygenase dependent production of 12-HETE, whilst cyclooxygenase dependent processes are not significantly affected[19]: these are only a few of many (partially) contradictory statements.

$^1O_2$ converts unsaturated fatty acids to hydroperoxides, bearing the -OOH-group at those C-atoms which were part of the former C=C-bond[20, 21]. Radicalic autoxidation ($^3O_2$) attacks the allylic positions[22]. *Unna*[23] has already performed experiments so as to elucidate an interaction of chrysarobin (**1**, with an additional CH_3-group at C-3) and oleic acid. We photooxygenated methyloleate as a model compound, using **1**-anion as a sensitizer. The positions of the OOH-groups were determined by reduction to the corresponding saturated hydroxy acids which on the other hand were reoxidized to the ketones. These molecules were separated from stearinic acid and identified by GC-MS, making use of α-cleavage and *McLafferty*-rearrangement ions: we obtained the $^1O_2$-products selectively, bearing the HOO-groups at C-9 and C-10, resp. No regioselectivity was observed. Therefore, we did not extend our experiments to manifold unsaturated fatty acids.

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Experimental Part

Apparatus: MP: (uncorr.): apparatus according to Dr. Tottoli; *IR*: Beckman Acculab III; *^{1}H-NMR*: Varian EM 390 (90 MHz), $CDCl_3$, 35°, TMS as int. stand. *UV*: Shimadzu 210, MeOH Uvasol „Merck", 1 cm sample thickness; *MS*: Varian MAT CH 5, excitation energy 70 eV; densitometer: Shimadzu TLC-Scanner CS 910 with Servogor two-channel recorder, model 2 S.

Materials: 1,8-dihydroxy-9-anthrone (dithranol, **1**)[24] was purified by column chromatography (SiO_2/CH_2Cl_2). – 1,8-dihydroxy-9,10-anthraquinone (chrysazine, **2**): commercially available (Synochem); 1,8,1′,8′-tetrahydroxybisanthrone **(3)**[24]; 1,8,9-trimethoxy-anthracene **(4)**[25]; 1,8-dimethoxy-9,10-anthraquinone **(5)**[26]. Solvents for photochemical reactions and densitometric determinations were of analytical grade („Merck").

General remarks

a) *Photochemical reactions*: photosensitized oxidations of **1, 3** and **4** were performed in test tubes, closed by serum caps, which were placed in a glass cylinder (12 cm diameter) filled with MeOH. The methanol was cooled by a Haake cryostat. O_2 was introduced into the reaction mixture via a frit D_4, the test tubes were irradiated by a cooled halogen lamp (Osram Halostar, 100 W) fixed at a distance of 5 cm from the reaction vessel. Irradiation conditions: 4 h at −10 °C, unless otherwise stated.

b) *Quantitative determination of the reaction products*[27]: 2 × 2 µl of the sample and 2 × 2; 4; 6 and 8 µl of the standard solutions (2 mg of the authentic subst. in 10.00 ml CH_2Cl_2) were spotted by a Hamilton-syringe on a tlc plate (Silica F_{254}). Development with toluene/glacial acetic acid (97:3, -vol) for **2** and **3**, with toluene for **5**. Densitometric determination by measuring the reflected part of the incident monochromatic light. λ max of the absorption spectra, taken in reflection technique: **2**: 430 nm; **3**: 365 nm; **5**: 380 nm.

Oxidation of **1** *with chemically generated $^1O_2$*

a) with H_2O_2/NaOCl[28]: 22.6 mg (0.1 mmol) **1** in 10 ml MeOH were cooled to 5 °C under N_2 in the dark; after addition of 0.15 ml H_2O_2 (30 %), 1 ml NaOCl-solution (15 %) was added under stirring over 10 min. The originally yellow solution became deep red. After 1 h the oxidation products were identified by tlc; comparision with authentic materials was made.

b) With 1,4-dihydro-1,4-dimethylnaphthalene-1,4-endoperoxide[29]: 1 g 1,4-dimethylnaphthalene and 5 mg methylene blue in 25 ml CH_2Cl_2 were irradiated under O_2 at −10 °C for 3 h. After evaporation of CH_2Cl_2, the endoperoxide crystallized. Half of the reaction product and 50 mg **1** in 20 ml "solvent" (CH_2Cl_2/DMF/pyridine 20:2:1 – vol) were refluxed under N_2 for 4 h in the dark. The oxidation products were identified by tlc. – 1,4-dihydro-1,4-dimethylnaphthalene-endoperoxide: ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.85 (s; 6H, -CH_3); 6.65 (s; 2H, C-2-H, C-3-H); 7.15–7.35 (m; aromatic H's).

Oxidation of **1** *with $^1O_2$ from photosensitization*

22.6 mg (0.1 mmol) **1** and 2 mg TPP were irradiated in 10 ml "solvent" under O_2. At hourly intervals 2 × 2 µl samples were removed for densitometric analysis.

Proof of photosensitizing properties in **1, 2, 3** *and* **4**

0.1 mmol of the compounds mentioned above and 100 mg 2,3-dimethyl-2-butene in 5 ml "solvent" were irradiated under O_2. After evaporation of the solvents i. vac. the residues were dissolved in 5 ml $CDCl_3$. 2,3-dimethyl-1-butene-3-hydroperoxide[7, 30] was characterized by nmr-spectroscopy. ^{1}H-NMR ($CDCl_3$): δ (ppm) = 1.0 (s; 6H, -CH_3), 1.50 (s; 3H, H_3C-C=C), 4.57–4.74 (m; 2H, =CH_2).

Quenching of **1***-photooxygenation by β-carotene*

22.6 mg (0.1 mmol) **1** and varying quantities of β-carotene (Fig. 3) in 20 ml "solvent" were irradiated under O_2; **2** was determined densitometrically.

Determination of the decrease of β-carotene during irradiation

27 mg (0.05 mmol) β-carotene in 20 ml "solvent" were irradiated at −10 °C under 0_2. 20 µl samples taken at time zero and after 3; 6; 20 and 44 h were diluted to 5.00 ml by MeOH (Uvasol, Merck) and their extinctions determined at 448 nm (λmax of β-carotene) in 1 cm cuvets, using 20 µl "solvent" filled up to 5.00 ml with MeOH as a blank.

Photosensitized oxidation of **4** *to* 5

270 mg **4** and 10 mg TPP in 20 ml CH_2Cl_2 were irradiated under O_2. **5** was separated from **4** and TPP by column chromatography (SiO_2/CH_2Cl_2), the solution was evaporated to dryness i. vac.: **5**, mp. 223° (glacial acetic acid). Analytical data correspond to those of an authentic sample[26].

1,8,9-trimethoxyanthracene-9,10-dihydro-9,10-endoperoxide: an intermediate between **4** *and* **5**

a) Proof by formation of I_2: 20 mg **4** and 5 mg TPP in 10 ml MeOH were irradiated at −10 °C under O_2. After 30 min bubbling of O_2 and irradiation were stopped and after 1 further min 1 ml of KI/starch solution (Pharm. Eur.) was added: blue colour. Under identical conditions neither the solvent nor **5**, TPP or the mixture of both reacted positively.

b) Proof by nmr-spectroscopy: ^{1}H-NMR of **4**: δ (ppm) = 3.80 (s; 3H, OCH_3 at C-9), 3.90 (s; 6H, -OCH_3), 6.59–6.72 (m; 2H, arom. H at C-4 and C-5), 7.12–7.49 (m; 4H, arom. H), *8.02* (s; 1H, arom. H at C-10). – 250 mg **4** and 10 mg TPP in 5 ml $CDCl_3$ were irradiated at −15 °C under O_2. After 30 min 0.7 ml of the mixture were taken by a cooled syringe and used for nmr-spectroscopy: C-10-H was shifted to δ = 5.74 ppm. This sample was slowly heated to 75 °C in the nmr-spectrometer: the spectra were taken at 15 (35 °C); 30 (35 °C); 60 (55 °C) and 90 (75 °C) min. The signal at δ = 5.74 ppm decreased, whilst the singlet of MeOH at δ = 3.35 ppm increased at the same rate.

Oxidation of methyloleate, photosensitized by **1***-anion*

Photooxidation: 2.96 g (0.01 mol) methyloleate and 10 mg **1** in 10 ml "solvent" were irradiated under O_2 at −10 °C for 6 h. Afterwards an additional 10 mg **1** were added and the irradiation was continued for further 6 h. Then the "solvent" was evaporated i. vac., the components were separated by column chromatography (SiO_2). With petrolether (b. p. 40–60 °C) unchanged starting material was eluted; with ether/petrolether (1:5 − vol) **2** was eluted, followed by the mixture of hydroperoxides. Yield 0.74 g (23 %) of hydroperoxides.

9- and 10-oxo-methyloleates: the hydroperoxides mentioned above were dissolved in 50 ml EtOH and hydrogenated with 50 mg PtO_2 under normal pressure and room temp. until the H_2-consumption ceased. After filtration and evaporation i. vac. the residue was dissolved in 10 ml acetone. With stirring 0.4 g CrO_3 in 5 ml 5-N H_2SO_4 were added drop by drop to this solution; after completion stirring was continued for 15 min at room temp. After addition of 30 ml H_2O, the mixture was extracted with ether, the organic layer was washed with $NaHCO_3$ solution, dried over Na_2SO_4 and evaporated to dryness i. vac. The mixture of the oxo-acids was separated from stearinic acid by gc: Column: fused silica OV 101 (8 m); Carrying gas H_2, flow 2 ml/min, split 10 ml/min; column temp. 80–170 °C (5 °C/min), 170° isotherm. Retention times: methylstearinate: 18.2 min, 9-oxo- and 10-oxo-methylstearinates: 26.4 min. The mixture of the oxo-esters was identified by ms[31]: M_1 = H_3C-$(CH_2)_7$-CO-$(CH_2)_8$-CO-OCH_3, M_2 = H_3C-$(CH_2)_8$-CO-$(CH_2)_7$-CO-OCH_3 (fragments in italic

figures). MS (70 eV): m/e = 312 (7 %, $M_1^{+\bullet}$ and $M_2^{+\bullet}$), 214 (35 %, $M_1^{+\bullet}$-$C_7H^{\bullet}_{14}$), *200* (35 %, $M_2^{+\bullet}$-$C_8H^{\bullet}_{16}$), *170* (30 %, $M_2^{+\bullet}$-$C_8H_{14}O_2^{\bullet}$, 156 (42 %, $M_1^{+\bullet}$-$C_{10}H_{20}O^{\bullet}$ and $M_1^{+\bullet}$-$C_9H_{16}O_2^{\bullet}$), *142* (16 %, $M_2^{+\bullet}$-$C_{11}H_{21}O^{\bullet}$), *112* (9 %, $M_2^{+\bullet}$-$C_{11}H_{20}O_3^{\bullet}$), 98 (20 %, $M_1^{+\bullet}$-$C_{12}H_{22}O_2^{\bullet}$), 74 (100 %, $M_1^{+\bullet}$ and $M_2^{+\bullet}$-$C_{16}H_{30}O^{\bullet}$).

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