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Dithranol and Active Oxygen Species, II[1)]

$^1O_2$-Oxidation of Dithranol to Chrysazin

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Raab's biochemically active species, derived from dithranol **(1)**, is the anion **1a** (Sect. A), which is a photosensitizer. Its sensitizing potency is quantitatively compared with that of rose bengal, tetraphenylporphyrine and other sensitizers (Sect. B). In Sect. C the mechanism of the conversion of **1a** with $^1\Delta g^{18}O_2$ to chrysazin **(2)** is elucidated.

Dithranol und aktive Sauerstoffspezies, 2. Mitt.: $^1O_2$-Oxidation des Dithranols zu Chrysazin

Raab's biochemisch aktives Dithranol(1)-Derivat ist dessen Anion **1a**, das zugleich ein Photosensibilisator ist (Abschn. A). Die Sensibilisatorstärke von **1a** wird quantitativ mit der von Bengalrosa und Tetraphenylporphyrin u. a. verglichen (Abschn. B). In Abschn. C wird der Reaktionsmechanismus der Umsetzung von **1** zu Chrysazin **(2)** mit Hilfe von $^1\Delta g^{18}O_2$ geklärt.

In our first communication[1)] we described the photosensitizing properties of dithranol **(1)** which lead to chrysazin **(2)** and to allylhydroperoxides, resp., if a pertinent $^1O_2$-acceptor is available. 2,3-Dimethyl-2-butene was converted to 3-hydroperoxy-2,3-dimethyl-1-butene, whereas methyloleate was oxygenated at C-9 or C-10, pointing towards a possible interference of **1** with the arachidonic acid cascade. In addition deleterious effects such as lipid peroxidation have to be considered.

This paper is concerned with three problems, resulting from these experiments: the nature of the photoactive **1**-derivative (sect. A), its potency as a photosensitizer (sect. B), and the conversion of **1** to **2** (sect. C).

Herrn Prof. Dr. *H. Oelschläger* zum 65. Geburtstag in herzlicher Verbundenheit gewidmet.

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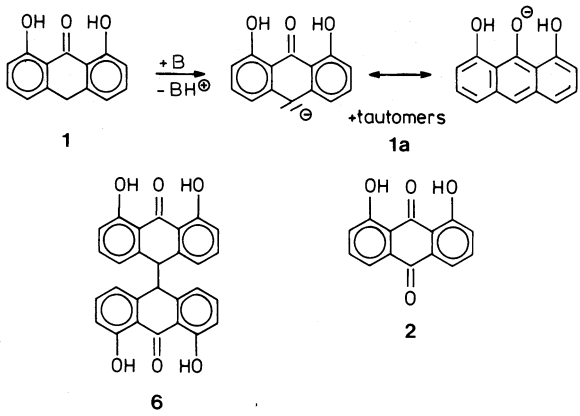
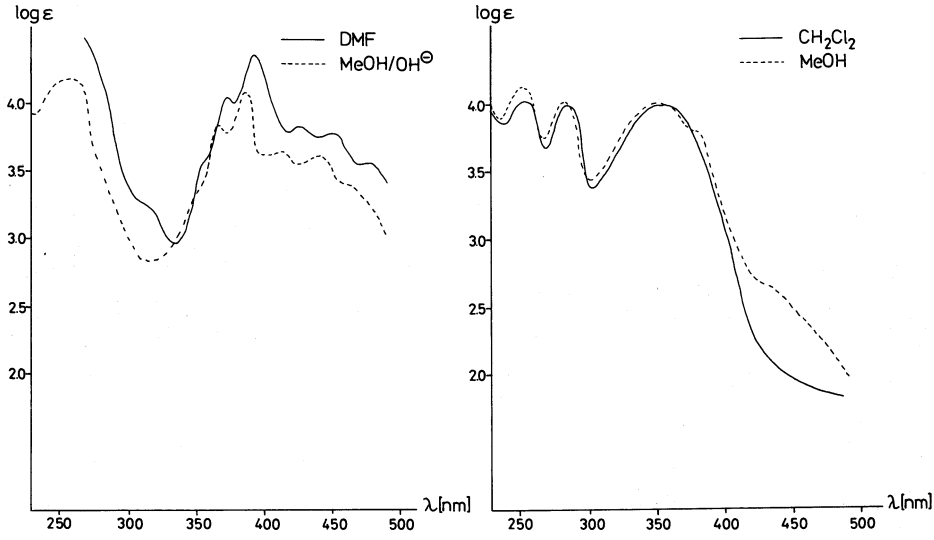




A) The photoactive derivative of dithranol (1)

1 does not exert its photosensitizing properties in CH_2Cl_2, but so in basic solutions, so pointing towards **1**-anion as the active species. In this context *Raab*'s[2] UV-spectra of **1** in dipolar aprotic solvents (e. g. DMSO, DMF) had to be reconsidered: from profound alterations between the spectra of **1** in MeOH and in DMF *Raab*[2] concluded that a new species had been formed which he considered to be the biochemically active form of **1**. *Retzow*[3] and *Sa e Melo*[4] have published results supporting the hypothesis that *Raab*'s active species is dithranol-anion **(1a)**. Obviously the mesomeric formula

with an anthracene chromophore contributes much to the description of this species as shown by its UV-spectrum (fig. 1). It remained to be proved, however, that this species under consideration is indeed the **1**-anion **1a** and not the **1**-tautomer 1,8,9-trihydroxyanthracene. Up to now pertinent experiments failed on account of the lability of **1** in aqueous or alcoholic basic solutions (rapid formation of so called dithranol brown)[5].

A 10^{-4} M solution of **1** in DMF is stable for at least 30 min under argon at 0° in the dark. Its UV-spectrum is influenced neither by LiOH nor by $(CH_3)_4N^{\oplus}\ OH^{\ominus}$ which cannot form a chelate on account of its bulky cation. These findings support *Retzow*'s[3] assumption that **1** is deprotonated to its anion even by DMF. – The solution mentioned above is decomposed significantly by air and light within 15 min at 0°: the long-wave prebands ($\lambda = 452$ and 478 nm) disappear, and a new maximum at 428 nm belonging to **2** arises. After 45 min **1** was degraded completely, only maxima at 428 and 528 nm (precursor of dithranol brown?) are recognizable. This is in accordance with our earlier results[1].

Comparing the spectrum of **1** in DMF with those of **1** in CH_2Cl_2, MeOH and $MeOH/OH^{\ominus}$ (fig. 1) indicates that **1** in CH_2Cl_2 (λ max 355 nm) exists as an anthrone. In MeOH (λ max 360 nm, sh at 382 and 438 nm) **1** is partially converted[4] to *Raab*'s species[2]. Addition of 1.2 mol eq. NaOH to **1** in MeOH under argon at 0° leads to a yellow greenish fluorescence, indicating an anthracene derivative[3, 4]. Measuring the electron excitation of this solution quickly by a diodes array spectrophotometer (the procedure has to be terminated not later than 15 sec after the first contact with $OH^{\ominus}$), the spectrum is free of deterioration by dithranol brown and corresponds to that of **1** in DMF, but the maxima are shifted bathochromically in DMF on account of solvatochromic effects: this experiment proves not only that **1** is deprotonated to its monoanion in DMF but also that *Raab*'s species[2] is dithranol anion **(1a)**. This apprehension had become likely by *Retzow*'s[3] experiments for the first time. – When **1** is dissolved in the basic solvent which we use for photooxygenation[1], it exhibits the same type of UV-spectrum as in DMF. Therefore, we conclude that **1a** is the photosensitizing species derived from dithranol **(1)**.

B) Sensitizing potency of dithranol anion

Is dithranol anion **(1a)** a weak or a strong photosensitizer in comparison with "typical" $^1O_2$-photosensitizers as rose bengal or tetraphenylporphyrine? Quantitative kinetic measurements make use of the β-values of *Schenck* and *Gollnick*[6], which characterize the reactivity of a given acceptor "A" with $^1O_2$ independently from the sensitizer. β is defined as that concentration of A, at which 50 % of $^1O_2$ are trapped by A, forming AO_2, and 50 % decay to $^3O_2$[7].

$$^1O_2 \xrightarrow{k_D} {}^3O_2$$

$$^1O_2 + A \xrightarrow{k_A} AO_2$$

$$\frac{k_D}{k_A} = \beta\ [\text{mol/l}] \qquad \text{(equation 1)}$$

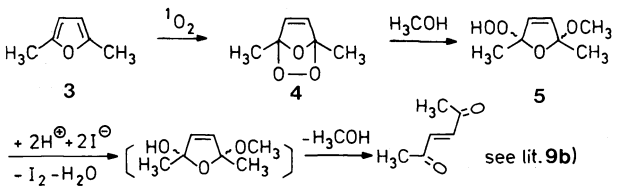
The lower the β-value of A, the higher its reactivity. β is determined by measuring the quantum yield Φ AO_2 as a function of the concentration of A. Φ $^1O_2$ is connected with Φ AO_2 by equation 2:

$$\Phi\ AO_2 = \Phi\ {}^1O_2 \cdot \frac{k_A\,[A]}{k_D + k_A\,[A]} \qquad \text{(equation 2)}$$

Dividing the nominator and the denominator in eq. 2 by k_A followed by insertion of eq. 1 leads to:

$$\Phi\ AO_2 = \Phi\ {}^1O_2 \cdot \frac{[A]}{\beta + [A]} \qquad \text{(equation 3)}$$

When [A] ≫ β, the fraction in eq. 3 is approximately 1, so Φ AO_2 ≈ Φ $^1O_2$[8].
We used 2,5-dimethylfuran[9] as an acceptor A; its β-value is 0.001 in MeOH[7, 10], a concentration ≫ 10^{-3} mol/l should fulfil Φ AO_2 ≈ Φ $^1O_2$, so leading to nearly quantitative consumption of $^1O_2$ by A, the decay to $^3O_2$ will be negligible. We made use of 10^{-1} mol/l of the furan A and 10^{-4} mol/l of the sensitizers shown in table 1. Lower concentrations might cause insufficient absorption of light, whilst higher concentrations may lead to side reactions, such as sensitizer bleaching[11].



2,5-dimethylfuran **(3)** is converted to the ozonide **4**, which in turn is solvolized to the hydroperoxide **5**. Instead of gravimetric determination[12], we developed an iodometric titration of **5**, modifying the prescription "peroxide value" of Pharm. Eur. III[13]. Results are given in table 1.

Table 1: *Formation of* $^1O_2$ *by various photosensitizers under standard conditions in* MeOH (*: MeOH/$OH^{\ominus}$); *mean values of 3 determinations and standard deviations.*

Sensitizer	$^1O_2$ [mmol]
Rose bengal	7.66 ± 0.16
Methylene blue	6.53 ± 0.15
Tetraphenylporphyrine	5.90 ± 0.06
Dithranol-anion*)	1.66 ± 0.15
Chrysazin-anion*)	1.51 ± 0.08
1,8,1′,8′-Tetrahydroxy-bianthrone-anion*)	0.67 ± 0.18
Chrysazin	0.44 ± 0.02
Fluorescein	0.22 ± 0.02
Trimethoxyanthracene	0.16
Dithranol	0.12
1,8,1′,8′-Tetrahydroxy-bianthrone	0.09

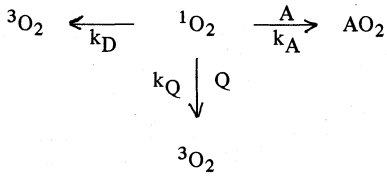
Schenck and *Gollnick* have already shown[14] that α-hydroxyanthraquinones produce $^1O_2$ (called moloxide at that time) in alkaline alcoholic solutions or in pyridine.

According to table 1 the efficacy of **1**, **2** and 1,8,1′,8′-tetrahydroxybianthrone **(6)** (scheme 1) is increased significantly in basic solution. The absorption maxima correspond to **1**-anion (fig. 1), to **2**-anion (**2** in MeOH: λ max = 430 nm; **2**-anion in MeOH: λ max = 499 nm) and to (twofold?) deprotonated **6** (**6** in MeOH: λ max = 366 and 380 nm; **6**-anion in MeOH: λ max = 385 and 441 nm).

As dithranol-anion has about 25 % of the efficiency of usual photosensitizers, we conclude that this feature may be of some importance in antipsoriatic therapy with dithranol **(1)**.

Formation of what are considered "typical" $^1O_2$-acceptor products does not prove the existence of $^1O_2$ in this system. *Foote* et al.[15] have shown that electron transfer oxidation of e. g. 9,10-dicyanoanthracene leads to the same product as with $^1O_2$. Therefore, a specific identification of $^1O_2$ requires the combination of a $^1O_2$-acceptor with a $^1O_2$-quencher and quantitative determinations[10, 16]. Qualitative aspects have been reported[1].

The competing system of 2,5-dimethylfuran **(3)** (A), of the $^1O_2$-quencher β-carotene (Q) and of the spin conversion to $^3O_2$ is described as follows:



As a consequence, eq. 2 is extended to eq. 4[17]:

$$\Phi\, AO_2 = \Phi\, {}^1O_2 \cdot \frac{k_A\,[A]}{k_D + k_A\,[A] + k_Q\,[Q]} \qquad \text{(equation 4)}$$

If less than 10 % of A are converted to AO_2 at constant time of irradiation, the twofold reciprocal presentation yields a straight line[18, 19]:

$$\frac{1}{\Phi\, AO_2} = \frac{1}{\Phi\, {}^1O_2} + \frac{1}{\Phi\, {}^1O_2} \cdot \frac{k_D + k_Q\,[Q]}{k_A} \cdot \frac{1}{[A]}$$

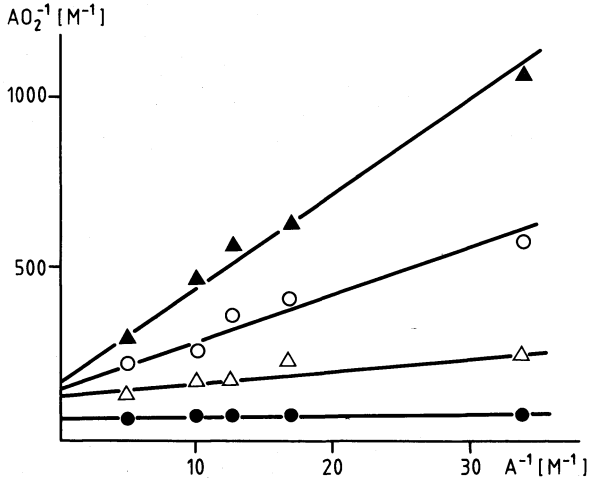
The following experiments were performed with solutions containing 10^{-4} M of **1** as a sensitizer, various concentrations of β-carotene as a quencher (table 2) and varying concentrations of the acceptor **3** in MeOH/$OH^{\ominus}$. The solutions were irradiated for 1 h, the hydroperoxide **5** was determined iodometrically as described above.

Table 2: *Inhibition of* **1a**-*sensitized photooxygenation of 2,5-dimethylfuran* (A) *by various concentrations* (a–d) *of β-carotene* (Q): a: –; b = 10^{-4} M; c = $3 \cdot 10^{-4}$ M; d = $6 \cdot 10^{-4}$ M.

A [M]	AO_2 [M · 10^2]			
	a	b	c	d
$2 \cdot 10^{-1}$	1.84	0.74	0.45	0.34
10^{-1}	1.66	0.60	0.38	0.22
$8 \cdot 10^{-2}$	1.60	0.59	0.28	0.18
$6 \cdot 10^{-2}$	1.59	0.45	0.25	0.16
$3 \cdot 10^{-2}$	1.43	0.40	0.17	0.09

The reciprocal values of table 2 are presented in fig. 2.

Fig. 2: Reciprocal values of table 2

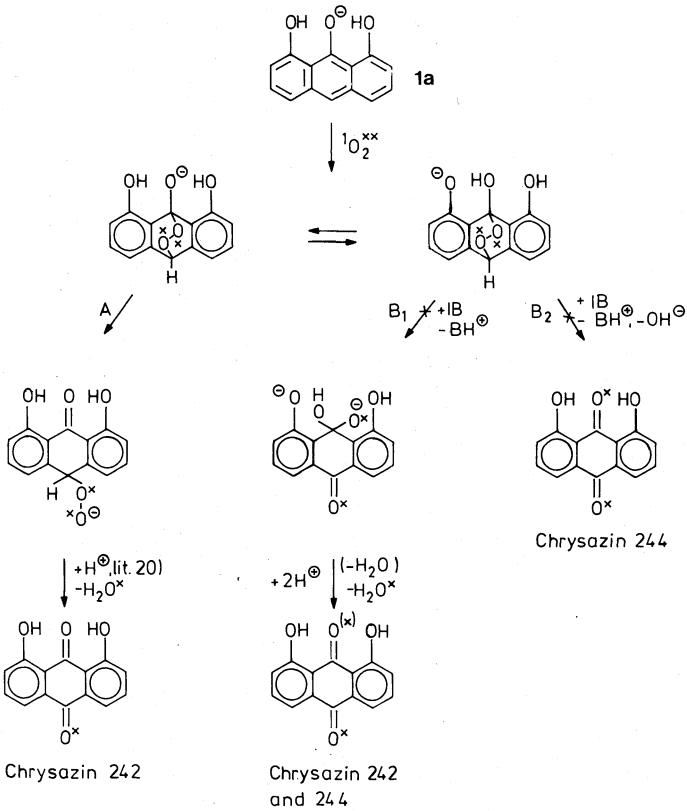


Photooxygenation of 2,5-dimethylfuran **(3)** *with dithranol as a sensitizer and β-carotene as a quencher. Concentrations of β-carotene:* ● –; Δ 10^{-4} M; ○ 3 · 10^{-4} M; ▲ 6 · 10^{-4} M

Table 2 indicates increasing inhibition of the AO_2-production with increasing quantities of β-carotene: 6 · 10^{-4} M Q inhibit 87 % of the photooxygenation (0.22 · 10^{-2} M AO_2 instead of 1.66 · 10^{-2} M AO_2). According to *Foote*[17] the twofold reciprocal presentation allows a differentiation between quenching of $^1O_2$ or quenching of the sensitizer in its triplet- or singlet-excitation state. If plotting of AO_2^{-1} against A^{-1} at various [Q] leads to a constant intercept of the ordinate, this result points towards $^1O_2$-quenching. If, however, this intercept of the ordinate varies, and the straight lines of various [Q] are parallel, this finding indicates that A and Q do not compete for a common intermediate ($^1O_2$), demonstrating sensitizer quenching. Fig. 3 resembles very much typical presentations for $^1O_2$-quenching, although the control ([Q] = O) does not intersect the ordinate at the same point as do the other lines. The straight lines, however, are not at all parallel. A mixed type of quenching may be conceivable.

C) Conversion of dithranol to chrysazin

Dithranol **(1)** is converted to chrysazin **(2)** by $^1O_2$[1], the reaction mechanism, however, is unknown. In model experiments with 1,8,9-trimethoxyanthracene we found an endoperoxide intermediate[1]. Therefore, routes A, B_1 and B_2 (or similar presentations) are conceivable for the transformation of an analogous endoperoxide derived from **1**-anion. Our experiment with $^1\Delta g\,^{18}O_2$ decided unequivocally between these possibilities. Normal chrysazin (4 × ^{16}O) has the mol. weight 240; according to route A one ^{18}O is incorporated (→ mol. weight 242); incorporation of oxygen following route B_1 should yield nearly equal amounts of chrysazin 242 and 244, and that as per route B_2 only chrysazin 244 (scheme 3).



Photooxygenation of a basic solution of **1** with $^{18}O_2$ (98.5 %) was followed by mass sepctrometric determination of ^{18}O incorporation which indicated molecular ions at m/z 242 (97 %, high resolution $C_{14}H_{18}{}^{16}O_3{}^{18}O$) and m/z 240 (3 %, $C_{14}H_{18}{}^{16}O_4$). No molecular ion at m/z 244 was observed. These results agree with route A and rule out routes B_1 and B_2. – In addition, the MS shows a $M^{+\cdot}$ at m/z = 260 (7 %), indicating a hydroxylation (^{18}OH) of chrysazin 242 at an unknown position. Furthermore, the MS of the ^{18}O-labelled chrysazin 242 reveals that the H-bridged CO-group at C-9 is lost preferantially (m/z 214; 13 %, $M^{+\cdot} - C^{16}O$) as compared with the elimination of the CO-moiety at C-10 (m/z 212; 5 %, $M^{+\cdot} - C^{18}O$).

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Experimental Part

Devices: see[1]. –

Section A: solvents for UV/Vis-spectroscopy were of analytical grade (E. Merck).

Section B:

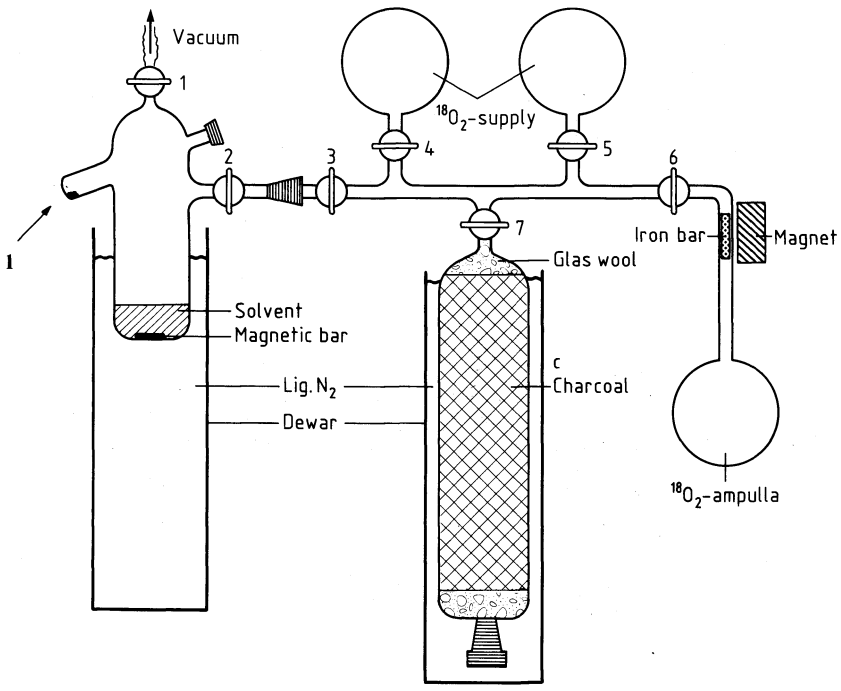
Determination of photooxygenating potency

960 mg (0.01 mol) 2,5-dimethylfuran **(3)** and 0.01 mmol of each sensitizer (table 1) in 100 ml MeOH (*: plus 5 drops N-NaOH) were irradiated with 200 W (2 × Osram halostar, 100 W) at −10° under O_2 for 60 min. The solvent was evaporated (*: after neutralization with 5 drops N-HCl) and the residue was dissolved in 30 ml glacial acetic acid/$CHCl_3$ (3:2-vol.). 20 ml 1M-KI were added, the mixture was kept in the dark for exactly 60 min and then titrated with 0.1 N $Na_2S_2O_3$ under permanent stirring. 1 ml 0.1 N-$Na_2S_2O_3$ ≈ 0.05 mmol hydroperoxide **5** ≈ 0.05 mmol $^1O_2$. – Results: table 1.

Quenching of 3-photooxygenation by β-carotene

192 mg (2 mmol); 288 mg (3 mmol); 576 mg (6 mmol); 768 mg (8 mmol); 960 mg (10 mmol); and 1920 mg (20 mmol) 2,5-dimethylfuran **(3)**, 2.26 mg (0.01 mmol) **1** and various conc. of β-carotene (5.36 mg – 0.01 mmol; 16.08 mg – 0.03 mmol; 32.16 mg – 0.06 mmol) were dissolved in 100 ml MeOH + 5 drops N-NaOH and irradiated (200 W) at −10° for 60 min. – Hydroperoxide **5** was determined as described above.

Section C:
Reaction of dithranol with $^1\Delta g\,^{18}O_2$.



A special device (see above) was constructed for distribution of the $^{18}O_2$-supply (98.5 %; MSD Isotopes, Merck Canada) and for photooxygenation.

Distribution: the charcoal in the stoppered glastube was desorbed at 10^{-1} Torr for 3 h by heating with a Bunsen-burner; then tab 7 was closed. 23 mg (0.1 mmol) **1** were put into the protrusion of the reaction vessel, avoiding contact with the solvent. 10 ml CH_2Cl_2/DMF/pyridine (20:2:1-vol.) were degased in the

reaction vessel by the freeze-thaw-method. The $^{18}O_2$ ampulla was fused on and the whole apparatus was evacuated (taps 1–6 open, tap 7 closed). The charcoal was cooled with liquid N_2, tap 3 was closed and tap 7 was opened in order to improve the vac. After closing tap 7, the valve at the oxygen ampulla was broken by a magnet and an iron bar so distributing $^{18}O_2$ into two further bulbs. *Reaction*: Now taps 4–6 were closed, taps 7, 3 and 2 were opened to evacuate the reaction vessel by the degased charcoal. After closing tap 7, $^{18}O_2$ flew into the reaction vessel by opening taps 6, 3 and 2. After closing taps 2 and 3, **1** was dissolved in the degased solvent, the mixture was irradiated by 100 W at –5° for 9 h under stirring. After removing the solvent the residue was identified by high resolution MS (table 3):

Table 3: *Photooxygenation of* 1 *to* 2; *ms-identification of products*

MS (HR): $C_{14}H_8\ ^{16}O_3\ ^{18}O_2$, Calc. 260.04566, Found 260.04533. $C_{14}H_8\ ^{16}O_3\ ^{18}O$, Calc. 242.04650, Found 242.04699. $C_{14}H_8\ ^{16}O_4$, Calc. 240.04225, Found 240.04248. $C_{14}H_7\ ^{16}O_2\ ^{18}O$, Calc. 225.04376, Found 225.04388. $C_{13}H_8\ ^{16}O_2\ ^{18}O$, Calc. 214.05159, Found 214.05154. $C_{13}H_8\ ^{16}O_3$, Calc. 212.04734, Found 212.04745. $C_{12}H_8\ ^{16}O\ ^{18}O$, Calc. 186.05667, Found 186.05598. $C_{12}H_8\ ^{16}O_2$, Calc. 184.05243, Found 184.05189.

MS (70 eV): m/z = 260 (7 %, $M_1^{+\cdot} + {}^{18}O$), 242 (100 %, $M_1^{+\cdot}$), 240 (3 %, $M_2^{+\cdot}$), 225 (6 %, $M_1^{+} - OH$), 214 (13 %, $M_1^{+\cdot} - C^{16}O$), 212 (5 %, $M_1^{+\cdot} - C^{18}O$), 186 (2 %, 214 – $C^{16}O$), 184 (12 %, 214 – $C^{18}O$). $M_1 = C_{14}H_8\ ^{16}O_3\ ^{18}O$; $M_2 = C_{14}H_8\ ^{16}O_4$.

References

1 K. Müller, K. K. Mayer and W. Wiegrebe, Arch. Pharm. (Weinheim) *319*, 2 (1986).
2 W. P. Raab und B. M. Gmeiner, Dermatologica *150*, 267 (1975).
3 Diss. *A. Retzow*, Regensburg 1981; A. Retzow and W. Wiegrebe, Sci. Pharm. *53*, 209 (1985).
4 T. Sa e Melo, L. Dubertret, P. Prognon, A. Gond, G. Mahuzier and R. Santus, J. Invest. Dermatol. *80*, 1 (1983).
5 H. Auterhoff and J. Knabe, Lehrbuch der Pharmazeutischen Chemie, 11. Aufl. p. 496, Wissenschaftl. Verlagsges. mbH, Stuttgart 1983.
6 K. Gollnick, Adv. Photochem. *6*, 1 (1968).
7 C. S. Foote, Acc. Chem. Res. *1*, 104 (1968).
8 G. O. Schenck, K. Gollnick and A. O. Neumüller, Liebigs Ann. Chem. *603*, 46 (1957).
9a C. S. Foote, M. T. Wuesthoff, S. Wexler, I. G. Burstain, R. Denny, G. O. Schenck and K.-H. Schulte-Elte, Tetrahedron *23*, 2583 (1967).
9b K. Gollnick and A. Griesbeck, Tetrahedron *41*, 2057 (1985).
10 C. S. Foote in: Free Radicals in Biology II (W. A. Pryor, Ed.), p. 85, Academic Press, New York 1976.
11 R. W. Denny and A. Nickon, Org. React. *20*, 133 (1973).
12 B. Franck, M. Dust and A. Stange, Naturwissenschaften *69*, 401 (1982).
13 M. Arens in: Kommentar Ph. Eur. III (H. Böhme and K. Hartke, Ed.), p. 68, Wissenschaftl. Verlagsges. mbH, Stuttgart 1979.
14 G. O. Schenck and K. Gollnick, J. Chim. Phys. *55*, 892 (1958).
15 J. Eriksen, C. S. Foote and T. L. Parker, J. Am. Chem. Soc. *99*, 6455 (1977).
16 C. S. Foote in: Biochemical and Clinical Aspects of Oxygen (W. S. Caughey, Ed.), p. 603, Academic Press, New York 1979.
17 C. S. Foote in: Singlet Oxygen (H. H. Wasserman and R. W. Murray, Ed.), p. 139, Academic Press, New York 1979.
18 D. Bellus in: Singlet Oxygen – Reactions with Organic Compounds and Polymers (B. Ranby and J. F. Rabek, Ed.), p. 61, Wiley, New York 1978.

19 A. Zweig and W. A. Henderson, J. Polym. Sci. Polym. Chem. Ed. *13*, 717 (1975).
20 For comparison see: H. H. Wassermann and J. E. Pickett, Tetrahedron *41*, 2155 (1985).

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