Active Oxygen Species and Structure Specificity of Antipsoriatic Anthrones

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Twenty-one analogues of the antipsoriatic compound dithranol were tested for their capability to produce $^1O_2$ and $O_2^{\cdot-}$. A correlation was found between the production of active oxygen species and the minimum structure for the antipsoriatic activity of anthrone derivatives. The ability of the anthrones to sensitize the formation of $^1O_2$ is a function of the deprotonated hydroxyl group at C-1 associated with the carbonyl group at C-9 of the anthracene molecule; the CH_2-moiety at C-10 initiates the formation of free radicals and is responsible for the production of $O_2^{\cdot-}$, which is prevented by substitution of the hydrogen atoms at C-10. Since only the active compounds dithranol, chrysarobin, frangulaemodin-anthrone and aloeemodin-anthrone produce $^1O_2$ as well as $O_2^{\cdot-}$, the therapeutic effectiveness of the anthrones appears to be due to both active oxygen species.

Aktive Sauerstoffspezies und Strukturspezifität antipsoriatischer Anthrone

Einundzwanzig Analoge des Antipsoriatikums Dithranol wurden auf Bildung von $^1O_2$ und $O_2^{\cdot-}$ untersucht, wobei ein eindeutiger Zusammenhang zwischen der Bildung aktiver Sauerstoffspezies und der Minimalstruktur für antipsoriatische Wirksamkeit von Anthronen festgestellt wurde. Die Sensibilisatoreigenschaft der Anthrone für die Bildung von $^1O_2$ ist an die deprotonierte Hydroxylgruppe an C-1 und die benachbarte Carbonylfunktion an C-9 des Anthracenmoleküls gebunden, die C-10-CH_2-Gruppe ist für die Entstehung freier Radikale verantwortlich und somit für die Bildung von $O_2^{\cdot-}$, die durch Substitution der H-Atome an C-10 verhindert wird. Da nur die wirksamen Verbindungen Dithranol, Chrysarobin, Frangulaemodinanthron und Aloeemodinanthron sowohl $^1O_2$ als auch $O_2^{\cdot-}$ bilden, scheint die therapeutische Wirksamkeit der Anthrone auf beide aktive Sauerstoffspezies zurückzuführen zu sein.

Introduction

In spite of extensive research over seventy years only a few anthrone derivatives were found to have antipsoriatic properties, dithranol, chrysarobin and 1-hydroxy-9-anthrone[1]; in addition some phytogenic anthrones like various *Cassia* species are used in folkloric medicine[2], just as the fresh bark of *Rhamnus frangula*, which is characterized by its high content of anthrones (Fig. 1).

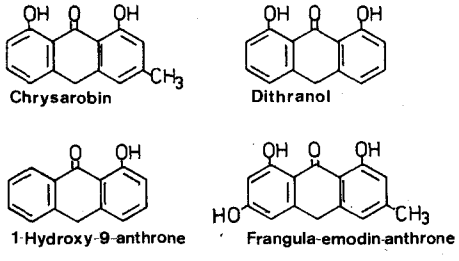

Fig. 1: Anthrones with antipsoriatic activity

The antipsoriatic anthrones show high structure specificity and the very structure common to all the active compounds was called the "minimum structure for antipsoriatic activity" by *Krebs* and *Schaltegger*[1, 3], comprising a hydroxyl group at C-1 of the anthracene molecule, a carbonyl group at C-9 and two free hydrogen atoms at C-10 (Fig. 2).

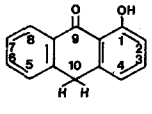

Fig. 2: Minimum structure for antipsoriatic activity

If the formation of active oxygen species by dithranol[4-6] is governed by certain structure elements, further hints to the mode of action of these drugs are given.

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Formation of $^1O_2$

According to *Gollnick*[7] there seems to be a correlation between the structure of a photosensitizer and its capability to produce $^1O_2$. In contrast to 2-hydroxyanthraquinones, 1-hydroxyanthraquinones act as sensitizers in alkaline methanol or in pyridine[8]. Therefore, investigations on the corresponding anthrone derivatives have to show in as much the conjugated anion of the assemble built up by the hydroxyl- and the carbonyl-group is essential for sensitizing property and formation of $^1O_2$.

Formation of $O_2^{\cdot-}$

One-electron-oxidation of dithranol anion connected to the formation of $O_2^{\cdot-}$ takes place at the methylene group at C-10 (2 H-atoms). Substitution abolishes the antipsoriatic properties, moreover, no dimerization or polymerization products are known from C-10-substituted compounds[1, 9]. In case of mono-substituted 10-acyl analogues the generation of free radicals is complicated by bulky 10-acyl substituents owing to increased steric hindrance[10a]. By ESR-measurements *Bruce* has found that C-10-monosubstituted dithranol derivatives also produce $O_2^{\cdot-}$[10b].

Results and Discussion

Formation of $^1O_2$

The production of $^1O_2$ was determined as described[4] using 2,3-dimethyl-2-butene as an acceptor of $^1O_2$ (*Schenck*-reaction) (Fig. 3).

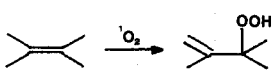

Fig. 3: *Schenck*-reaction

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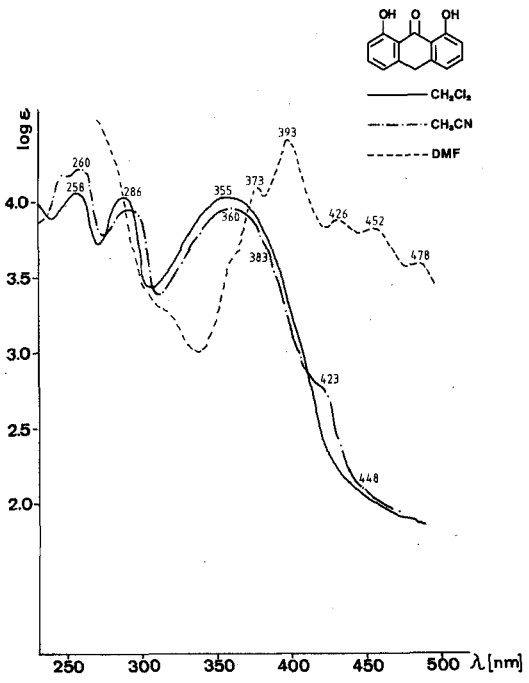

Fig. 4: UV/Vis spectra of dithranol

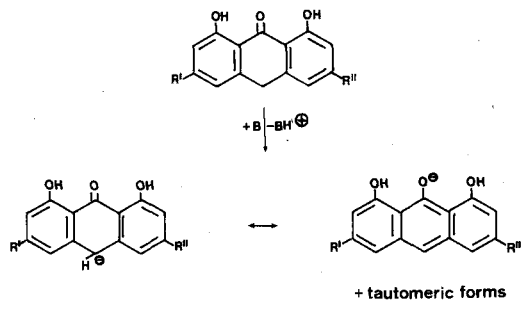

Fig. 5: 1,8-Dihydroxy-9-anthrone tautomerism in highly polar and basic solvents

All experiments were carried out under standard conditions, the sensitizer properties were examined in three different solvents (Fig. 4): the highly polar solvent CH_3CN (where dihydroxyanthrones are deprotonated at least partially to the 1,8,9-trihydroxyanthracene-anions[11]), the less polar CH_2Cl_2 (here the anthrone-tautomer exists exclusively[5]) and the basic solvent CH_2Cl_2/DMF/pyridine (20:2:1) leading to the trihydroxyanthracene-anion[5]. Results are given in Table 1.

Whereas basic or polar solvents, respectively, are required on the one hand to deprotonate the anthrones to their photosensitising species[5] (the corresponding hydroxyanthracene anions) they have the disadvantage to solvatize ionpair intermediates on the other hand and so give rise to electron transfer photooxygenation reactions[12]. The characteristic allylic hydroperoxide of the "*Schenck*-reaction" may also be formed by electron transfer, for example, according to the following reaction sequence (Fig. 6):

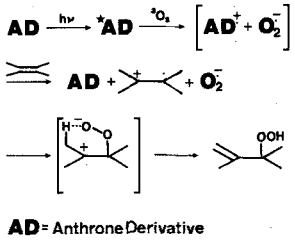

Fig. 6: Electron transfer photooxygenation of 2,3-dimethyl-2-butene

As we expected for the anthrone derivatives no products were detected in CH_2Cl_2, indicating that the anthrone tautomer does not possess any sensitizing property*), whereas un-

*) In this case lack of sensitization may be due to the formation of intramolecular hydrogen bonds. In molecules with an internal H-bond very fast radiationless deactivation of the singlet state takes place[13].

Table 1: Photooxygenation of 2,3-dimethyl-2-butene by various dithranol analogues

	CH_3CN	CH_2Cl_2	CH_2Cl_2/DMF/Pyridine
Dithranol	+	–	+
Chrysarobin	+	–	+
Frangulaemodin-anthrone	+	–	+
Aloeemodin-anthrone	+	–	+
Juglone	+	+	+
Plumbagin	+	+	+
1-Hydroxy-9-anthrone	–	–	+
1,2-Dihydroxy-9-anthrone	–	–	–
1,4-Dihydroxy-9-anthrone	–	–	+
1,5-Dihydroxy-9-anthrone	–	–	+
3-Hydroxy-9-anthrone	–	–	+
4-Hydroxy-9-anthrone	–	–	+
1-Amino-9-anthrone	–	–	+
1,8-Dichloro-9-anthrone	+	–	+
1,8,9-Trimethoxy-anthracene	–	–	(+)
1,8,9-Triacetyl-dithranol	–	–	(+)
10-Acetyl-dithranol	–	–	(+)
10-Myristoyl-dithranol	–	–	(+)
9-Anthrone	+	–	+
Aloin	–	–	(+)
10,10'-Dipropyl-dithranol	–	–	–
10,10'-Diallyl-dithranol	–	–	–
2,2'-Dihydroxybenzophenone	–	–	(+)
Menadione	+	+	+

der basic conditions with the exception of the C-10-disubstituted analogues (electron transfer via the C-10 radical is not possible) the desired $^1O_2$-products were formed in each case. 1,2-Dihydroxy-9-anthrone decomposes immediately under these reaction conditions. All the other anthrones effective against psoriasis show sensitizing properties in CH_3CN; among the clinically ineffective compounds only 9-anthrone and 1,8-dichloro-9-anthrone give positive results. Here, however, as a result of a change in effects of the substituents a photooxygenation pathway by electron transfer analogous to the electron-deficient sensitizer 9,10-dicyanoanthracene[12] (DCA) becomes likely. Since [4 + 2]- as well as [2 + 2]- cycloaddition products are no longer an unambiguous proof for the participation of $^1O_2$[14], we were looking for a so-called "fingerprint-reaction"[15] for $^1O_2$. A suitable probe for such a reaction is D-limonene**) **(1)**. Irradiation of **1** in the presence of oxygen and typical $^1O_2$-sensitizers leads to a mixture of six characteristic alcohols **(2–7)** obtained after reduction of the corresponding hydroperoxides with $NaBH_4$ (Fig. 7). Products and product distributions are a specific indication for $^1O_2$, they are independent of both the sensitizer and the polarity of the solvent, but completely different from those mixtures of compounds obtained in autoxidation reactions[14].

On account of the lower ionization potential **1** is attacked at the higher substituted C=C-bond[16]. The results are summarized in Table 2.

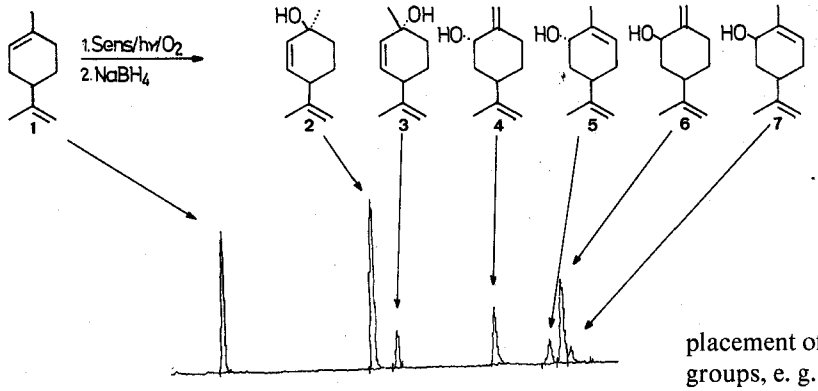

Fig. 7: Alcohols from D-limonene **(1)** obtained after reduction of the corresponding hydroperoxides: a $^1O_2$ fingerprint

Table 2: Photooxygenation of D-limonene **(1)** by various dithranol analogues

Sensitizer	Products in %					
	2	3	4	5	6	7
Autoxidation[17]	17	18	–	34	–	31
RB*)[18]	34	10	20	10	21	5
DCA**)[14]	43	10	17	7	20	3
TPP***)	40	9	16	7	23	5
Dithranol	32	11	15	11	16	7
Chrysarobin	34	9	16	8	18	7
Frangulaemodin-anthrone	37	10	15	9	16	5
Aloeemodinanthrone	34	10	15	10	18	7
Juglone	33	10	14	9	19	7
Plumbagin	32	9	16	10	19	7

*) Rose Bengale
) 9,10-Dicyanoanthracene; *) Tetraphenylporphyrine

**) I thank Prof. Dr. *K. Gollnick* for a helpful discussion.

Of the 21 analogues of dithranol tested only the four active compounds with 1,8-dihydroxy-9-anthrone structure show this characteristic fingerprint. As a matter of fact, contrary to pure $^1O_2$-sensitizers as rose bengale (RB) or tetraphenylporphyrine (TPP) in this case as many as five additional products arise at a low rate (<10 %). These findings are explained by the fact that in CH_3CN the $^1O_2$-pathway and photooxygenation reactions via electron transfer compete with each other. In addition to the energy transfer to oxygen (formation of $^1O_2$) the excited trihydroxyanthracene anion is capable to transfer an electron (Fig. 8), whereby the second reaction pathway occurs with a ratio <1 % in most cases[19].

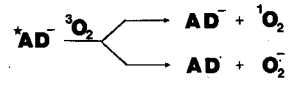

Fig. 8: Energy transfer and electron transfer from the excited 1,8,9-trihydroxyanthracene-anion to oxygen

Likewise this characteristic fingerprint was received by the naphthoquinones juglone and plumbagin which had been used for the treatment of psoriasis in former times. All other test compounds were not able to convert **1** to hydroperoxides at any appreciable extent. Also 1-hydroxy-9-anthrone which is described to be active fails to do so. Substitution by one hydroxy group in another part of the dithranol molecule or replacement of the two 1,8-hydroxy groups by other functional groups, e. g. $-NH_2$, $-Cl$, $-OCH_3$ or $-H$, results in loss of the sensitizing properties. The same holds true for the 10-acyl-derivatives, which are obviously compounds with "prodrug"-character[9]. Though 1,4- or 1,5-dihydroxy-9-anthrone contain *Schaltegger's* "minimum structure" they are inactive against psoriasis. This was related to insufficient penetration through the epidermis since the hydrophilic-lipophilic balance is increased[3]. As it turns out now this might just as well be due to the lack of $^1O_2$-production.

Finally substitution of a H-atom at C-10 or omission of the methylene group as in the case of 2,2′-dihydroxybenzophenone prevent the formation of $^1O_2$ by a sensitizing process.

Formation of $O_2^{\cdot-}$

The formation of $O_2^{\cdot-}$ was measured spectrophotometrically as described[6] by evaluating the reduction of nitro blue tetrazolium (NBT) which leads to the formation of nitro blue diformazan. Its absorption maximum at 560 nm does not interfere with the absorption of the anthrone derivatives. Superoxide dismutase (SOD) was used as a probe for the involvement of $O_2^{\cdot-}$ in this reaction.

The results obtained with C-10-substituted anthrones confirm their stability to dimerization or polymerization. They all failed to produce $O_2^{\cdot -}$. In addition 1,8,9-triacetoxyanthracene and 1,8,9-trimethoxyanthracene did not produce $O_2^{\cdot -}$. The four active compounds with 1,8-dihydroxy-9-anthrone structure were found to produce $O_2^{\cdot -}$ to the same extent as dithranol.

In all cases the maximum inhibition of NBT reduction that could be achieved by SOD under aerobic conditions was approximately 60 %, indicating that nearly 40 % of the reduction was accomplished by a direct electron transfer[6] from the anthrones to NBT (for example dithranol + SOD, see Fig. 9). Similar results were obtained with the remaining anthrones tested, but 1-hydroxy-9-anthrone and 1-amino-9-anthrone turned out to be even stronger producers of $O_2^{\cdot -}$ (Fig. 9). There is strong evidence for a correlation between skin irritating properties and the production of $O_2^{\cdot -}$ since 1-hydroxy-9-anthrone causes more powerful irritation or inflammation of normal skin than dithranol, a problem which prevents this compound from being used as an antipsoriatic agent[1]. Likewise 1-amino-9-anthrone provokes skin inflammation though it has no therapeutic effectivness.

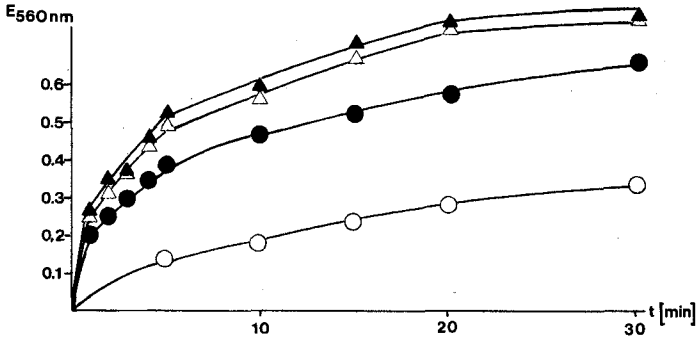

Fig. 9: $O_2^{\cdot -}$ mediated reduction of NBT by various anthrones: ▲ 1-hydroxy-9-anthrone; △ 1-amino-9-anthrone; ● dithranol; ○ dithranol + SOD

It may be concluded that only those derivatives of dithranol with 1,8-dihyroxy-9-anthrone structure are effective against psoriasis and are capable to produce $^1O_2$ as well as $O_2^{\cdot -}$. So the antipsoriatic activity may be related to combined efforts of these two active forms of oxygen. This is a common denominator for mechanisms discovered for dithranol, each explaining parts of its antipsoriatic efficacy: interaction with DNA[20-22], inhibition of enzymes[23, 24], interference with the arachidonic acid cascade[25, 26], and with polyunsaturated fatty acids[25-29]. – $^1O_2$ causes DNA damage[30, 31], inactivates enzymes[32] and destroys membranes, with PUFA and cholesterol being typical targets[32]. – $O_2^{\cdot -}$, on the other hand, reacts with chemotaxin precursors[33] causing inflammation. So the relevance of these active oxygen species becomes evident for antipsoriatic anthrones (Fig. 10).

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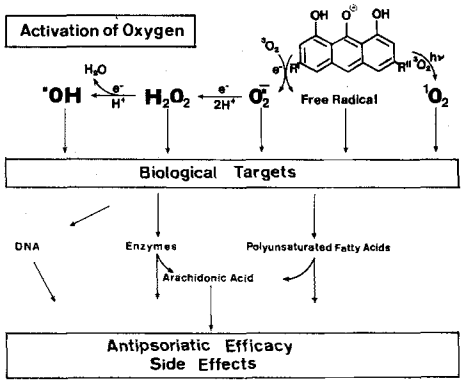

Fig. 10: Activation of oxygen and resulting effects of the antipsoriatic anthrones

Experimental Part

Chemicals

The anthrones were synthesized by reduction of the corresponding anthraquinones according to Auterhoff[34], unless otherwise stated, purification by column chromatography (CC) on silica gel or crystallization. Analytical data are in accord with those described. Pertinent solvents for CC are indicated in brackets.

1,8-Dihydroxy-9-anthrone (dithranol) (CH_2Cl_2); 1,8-dihydroxy-3-methyl-9-anthrone (chrysarobin), isolated from commercially available powder (Caelo) (CH_2Cl_2); 1,3,8-trihydroxy-6-methyl-9-anthrone (frangulaemodin-anthrone) (Et_2O); 1,8-dihydroxy-3-hydroxymethyl-9-anthrone (aloeemodin-anthrone) by borax cleavage[35] from aloin (Caelo) (EtOAc); 1-hydroxy-9-anthrone from 1-aminoanthraquinone[36] and reduction (CH_2Cl_2); 1,2-dihydroxy-9-anthrone from alizarine[37]; 1,4-dihydroxy-9-anthrone (EtOAc); 1,5-dihydroxy-9-anthrone (EtOAc); 3-hydroxy-9-anthrone[36]; 4-hydroxy-9-anthrone from 1-acetoxy-anthraquinone[37] (CH_2Cl_2); 1-amino-9-anthrone (crystallized from EtOH/charcoal); 1,8-dichloro-9-anthrone (CH_2Cl_2); 1,8,9-trimethoxyanthracene[9]; 1,8,9-triacetyl-dithranol (Ciba-Geigy); 10-acetyl-dithranol[38] (CH_2Cl_2); 10-myristoyl-dithranol[38] (CH_2Cl_2); 9-anthrone (Janssen); capaloin (Roth); 10,10′-dipropyldithranol[9]; 10,10′-diallyldithranol[9]; 2,2′-dihydroxybenzophenone (EGA); juglone[39] was a gift of Prof. Dr. *G. Wurm*; menadione (Fluka); 2,3-dimethyl-2-butene (Janssen); D-limonene **(1)** (Riedel-deHaen AG); nitrobluetetrazolium (Merck); superoxide dismutase (EC 1.15.1.1) type I from bovine erythrocytes (Sigma).

Standard performance of photochemical reactions

Photooxygenations were carried out in special glass cylinders equipped with a side neck for sampling and a standard ground joint. They were placed in a 4 l-thermostatic reaction vessel provided with a cool jacket (Wertheim) and filled with EtOH cooled by a Haake cryostate KT 33. O_2 was introduced via frits D4 equipped with a standard ground joint. Irradiation: cooled halogen lamps (Osram Halostar, 2 × 100 W) fixed at a distance of 5 cm from the reaction mixtures, 4 h at –10 °C; three parallel experiments.

Schenck-reaction

See[4]. – 200 mg 2,3-dimethyl-2-butene and 10 mg of the sensitizer were irradiated in 30 ml solvent (Table 1) under O_2, the formation of 2,3-dimethyl-1-butene-3-hydroperoxide was followed by tlc (SiO_2/CH_2Cl_2), detection with KI/HOAc at 50 °C, the structure was identified by ^{1}H-NMR-spectroscopy[4].

Photooxygenation of D-limonene **(1)**

350 mg **1** and 20 mg of the sensitizer were irradiated in 30 ml CH_3CN under O_2. The formation of the corresponding hydroperoxides was followed by tlc as indicated above. The solvent was removed under reduced pressure and the hydroperoxides were reduced with $NaBH_4$ in methanol. The resulting alcohols **2–7** were analysed by gas chromatography and compared with authentic materials (Table 2).

Gas chromatography: Perkin Elmer F 22 equipped with an integrator. Column: quartz capillary 50 m. – Oven from 60 °C till 240 °C, 4°/min. – Detector: FID. – Air: 350 ml/min. – H_2: 35 ml/min. – Retention times: **1**: 12.35; trans-p-menthadiene-(2,8)-ol-(1) **(2)**: 15.50; cis-p-menthadiene-(2,8)-ol-(1) **(3)**: 16.02; p-menthadiene-[1(7),8]-trans-ol-(2) **(4)**: 18.05; trans-carveol **(5)**: 19.20; p-menthadiene-[1(7),8]-cis-ol-(2) **(6)**: 19.47; cis-carveol **(7)**: 19.65.

Identification of $O_2^{\cdot -}$

4 moles of $O_2^{\cdot -}$ reduce NBT to nitroblue diformazan, λ max = 560 nm[40]; addition of SOD inhibits this reaction. In order to measure the formation of $O_2^{\cdot -}$ from the anthrones, mixtures containing $1.33 \cdot 10^{-5}$ M anthrone (50 µl stock solution, 0.005 mmol in 5.00 ml EtOH, N_2, light protection), $1.67 \cdot 10^{-4}$ M NBT (100 µl stock solution, 0.02 mmol in 10.00 ml EtOH, N_2) were filled up to 3.00 ml with 0.05 M potassium phosphate buffer, pH 7.8, saturated with O_2 and monitored at 560 nm. In case of SOD experiments 6.4 – 186 µ SOD corresponding to 10 – 300 µl of stock solution (3.2 mg of enzyme – 3300 units/mg protein – in 5.00 ml phosphate buffer pH 7.8) were added before filling up. – Spectrophotometer: Shimadzu 210; Uvikon 810 (Kontron) with Uvikon recorder 21 and thermostatized cell holder, temp. 25 °C.

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