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Pulse radiolysis studies of several analogues of 1,8-dihydroxy-9-anthrone (anthralin) under various conditions

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Abstract

Pulse radiolysis of solutions of 10-methylanthralin and 10, 10-dimethylanthralin under various conditions including air saturated benzene, O_2 saturated benzene and with sodium azide in N_2O saturated acetonitrile: phosphate buffer (pH=7) were investigated, respectively. 10, 10-Dimethylanthralin triplet observed in all cases and found to decay with first order kinetics (K = 4. 96 E+06 sec^{-1} , K = 1.53 E+07 sec^{-1}). As expected, only 10-methylanthralin free radicals were formed at wavelengths of 400-760 nm. Also, pulse radiolysis of 1,8-Dihydroxy-9-anthrone (Anthralin) solutions at concentration of 1×10^{-4}M with sodium azide (NaN_3) (1×10^{-2}M) in N_2O saturated CH_3CN: Phosphate buffer (1:1 v/v) (pH7) was investigated and the corresponding free radical observed at wavelength of 720 nm.

Keywords

Pulse radiolysis, anthralin, 1, 8-dihydroxy-9-anthrone, triplet excited state, 10-methylanthralin**,** 10, 10-dimethylanthralin.

Introduction

Anthralin (1,8-dihydroxy-9,10-dihydroanthracen-9-one, dithranol) (I A and IB), is a synthetic compound which is medically applied to the skin of people with psoriasis but the precise mechanism of its anti-psoriatic action is not yet fully understood. However, numerous studies have demonstrated anti-proliferative and anti-inflammatory effects of anthralin on psoriatic and normal skin. The anti-proliferative effects of anthralin appear to result from both an inhibition of DNA synthesis as well as from its strong reducing properties. Recently, anthralin's effectiveness as an anti-psoriatic agent has also been in part attributed to its abilities to induce lipid peroxidation and reduce levels of endothelial adhesion molecules which are markedly elevated in psoriatic patients. Anthralin accumulates in mitochondria where it interferes with the supply of energy to the cell, probably by the oxidation of dithranol releasing free radicals (II). This impedes DNA replication and so slows the excessive cell division that occurs in psoriatic plaques.[1]

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Anthrlin is available as creams, ointment or pastes in 0.1 to 2% strengths under various commercial names (Drithocreme, Dithrocream, Zithranol-RR, Micanol, Psorlin, Dritho-Scalp, Anthraforte, Anthranol and Anthrascalp). In this study, pulse radiolysis technique was used to investigate the transition states and free radicals produced from anthralin derivatives, 10-methylanthralin and 10, 10-dimethylanthralin. Therefore a brief introduction about this technique is given. Often the end result of a chemical reaction is only the last in a series of very fast reactions that cannot be observed by conventional chemistry. Pulse radiolysis is a technique which enables a researcher to measure the fast reactions. The principle is very simple. The chemicals to be studied (in solution) are bombarded with a very short pulse of radiation which causes chemical changes to occur then the short lived chemical changes in the irradiation cell are detected. This is done while a steady beam of ordinary light is passed through the solution. By measuring how much of this light is absorbed on a very short time scale (a few millionths of a second) deductions can be made about the chemical changes that have

occurred. This technique was explained in more detail in authors' recent manuscript and the references therein[2]. The technique also allows direct evaluation of energy transfer rate constants for quenching of triplet sensitizers produced in the pulse and for the appearance of various solute triplets resulting from the initially formed sensitizer triplet by energy transfer.

Experimental

The solvent used in this work was mainly benzene. Absorption of electrons by this solvent is known to cause ionization followed by recombination and relaxation to give high yields of excited benzene triplet. The singlet and triplet energies for benzene are high; 110 and 84.3 kcal mol-1, respectively, and hence a wide range of solute singlets and triplets can be produced by energy transfer. The linear accelerator was used as the source of radiation for this work which was operated at Paterson Laboratories at Christie Hospital and Holt Radium Institute Manchester in UK. It was manufactured by Radiation Dynamics Ltd (Swindon). It is a 12 million Volt microwave machine which can produce intense pulses of radiation ranging from 5 μs down 5 thousandths of a microsecond in duration. The machine consists of a cylindrical waveguide 2 meters long from which air has been pumped out carrying a stream of microwaves. The microwaves are produced by a high power radar instrument called a Klystron. Each pulse of microwaves lasts for 5 μs and they are produced at the rate of 50 per minute. Electrons are produced and fired by an electron "gun" into one end of the wavelength tube. The firing of the gun is organized to coincide with the production of the microwave pulses so that the electrons are accelerated by the microwaves to very high speeds. The very fast electron beams which are thus produced can be used directly as a means of radiating the material being studied. The radiations produced from this machine are lethal and will penetrate normal buildings. It is therefore shielded by very thick concrete walls and roof and experiments are carried out by remote control.3

10-Methyl-1,8-dihydroxy-9-anthrone(10-methyl anthralin) in air saturated benzene:

10-Methyl-1,8-dihydroxy-9-anthrone(10-methyl anthralin) solutions at concentration of 1×10 -2M in air saturated benzene were pulsed in order to observe triplet decay and formation of 10-methylanthralin radical. 10-methylanthralin triplet observed in all cases and found to decay with first order kinetics (K = 4. 96 E+06 sec-1). Time constant resolved spectrum recovered and triplet found to peak at 560 nm. 10-Methylanthralin radical observed at 600-760 nm wavelength.

10-Methyl-1,8-dihydroxy-9-anthrone(10-methyl anthralin) in O2 saturated benzene:

10-Methyl-1,8-dihydroxy-9-anthrone(10-methyl anthralin) solutions at concentration of 1×10 -2M in O2 saturated benzene were pulsed at wavelength 500 – 720 nm in order to observe triplet decay and formation of 10-methylanthralin radical. The radical was observed at wavelength ca. 500-720 nm.

10,10-Dimethyl-1,8-dihydroxy-9-anthrone (10,10-dimethyl anthralin) in O2 saturated benzene:

10,10-Dimethyl-1,8-dihydroxy-9-anthrone (10,10-dimethylanthralin) solutions at concentration of 1×10 -2M in O2 saturated benzene were pulsed in order to observe triplet decay and formation of any possible corresponding radical. 10,10-Dimethylanthralin triplet observed in all cases and found to decay with first order kinetics (K = 1.53 E+07 sec-1). Time constant resolved spectrum recovered and triplet found to peak at 560 nm. However, no such corresponding radical (as expected) was formed.

10,10-Dimethyl-1,8-dihydroxy-9-anthrone (10,10-dimethylanthralin) in air saturated benzene:

10,10-Dimethyl-1,8-dihydroxy-9-anthrone (10,10-dimethylanthralin) solutions at concentration of 1×10 -2M in air saturated benzene were pulsed in order to observe triplet decay and formation of any possible corresponding radical. 10,10-Dimethylanthralin triplet observed in all cases and found to decay with first order kinetics (K = 4. 96 E+06 sec-1). Time constant resolved spectrum recovered and triplet found to peak at 560 nm. However, no such corresponding radical (as expected) was formed.

1,8-Dihydroxy-9-anthrone (Anthralin) in N2O saturated CH3CN:Phosphate buffer:

1,8-Dihydroxy-9-anthrone (Anthralin) solutions at a concentration of 1×10 -4M with sodium azide (NaN3) (1×10 -2M) in N2O saturated CH3CN:Phosphate buffer (1:1 v/v) (pH7) were

pulsed. The growing of anthralin radical was observed at wavelength of 720 nm.

10-Methyl-1,8-dihydroxy-9-anthrone (10-methyl anthralin) in N2O saturated CH3CN:Phosphate buffer:

10-Methyl-1,8-dihydroxy-9-anthrone(10-methyl anthralin) solutions at concentration of 1×10 -4M with sodium azide (NaN3) (1×10 -2M) in N2O saturated CH3CN:Phosphate buffer (1:1 v/v) (pH7) were pulsed at varying wavelengths (400-760 nm).The corresponding radical was observed at wavelength of 400-760 nm.

10,10-Dimethyl-1,8-dihydroxy-9-anthrone (10,10-dimethyl anthralin) in N2O saturated CH3CN:Phosphate buffer:

10,10-Dimethyl-1,8-dihydroxy-9-anthrone (10,10-dimethylanthralin) solutions at concentration of 1×10 -4M with sodium azide (NaN3) (1×10 -2M) in N2O saturated CH3CN:Phosphate buffer (1:1 v/v) (pH7) were pulsed at varying wavelengths (400-760 nm). As expected, no radical was observed.

10-Methyl-1,8-dihydroxy-9-anthrone (10-methyl anthralin) in N2O saturated CH3CN:Phosphate buffer:

10-Methyl-1,8-dihydroxy-9-anthrone (10-methylanthralin) solutions at concentration of 2×10 -4M with sodium azide (NaN3) (1×10 -2M) in N2O saturated CH3CN:Phosphate buffer (1:1 v/v) (pH7) were pulsed at varying wavelengths (400-760 nm).The corresponding radical was observed at wavelength of 400-760 nm.

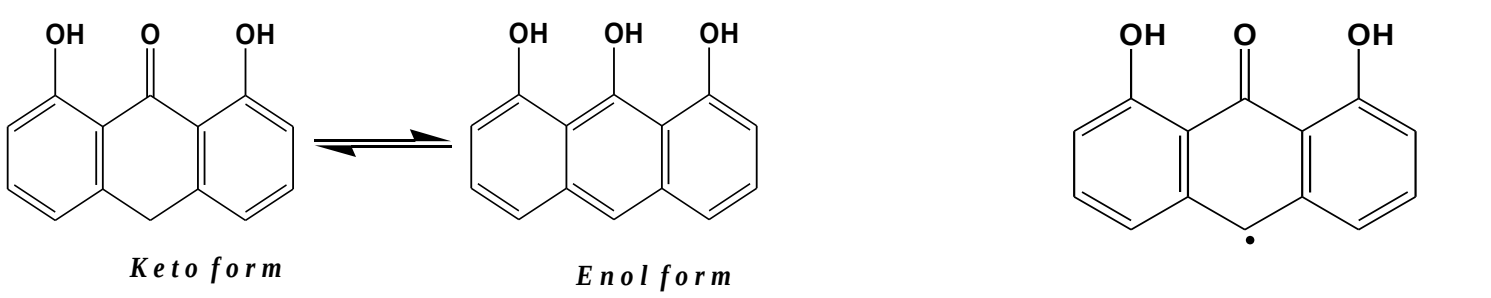
Results and Discussion

It is well documented that the structure of AnH2 (anthralin) in apolar, aprotic solvent4 and in the crystal5 is that of the dihydroxyanthrone tautomer (1A). The solvent employed in this work was mainly benzene. Since the spectra of anthralin in this solvent and in aliphatic hydrocarbons are essentially identical there is no doubt that the electronic excitation experiments to be described all involve deposition of energy into anthralin molecules which possess the ketone structure (IA). Also, in this work, we employed aqueous CH3CN: Phosphate buffer (1:1 v/v). In the latter solvent, anthralin exists as a tautomer of keto (1A) enol (1B) form (scheme 1). From the results obtained in this work, we concluded that the keto form of anthralin (1A) is responsible for the formation of free radical (AnH, II) and the triplet excitation state. Pulse radiolysis of solution of 10-methylanthralin (AnHMe) under three different conditions i.e. air, O2 in benzene and in N2O saturated CH3CN:Phosphate buffer were carried out in order to observe the triplet decay and formation of the corresponding free radical at various wavelength (400 – 760 nm). The results indicated the formation of an immediate transient absorption of 10-methylanthralin (3AnMeH∗) whose spectral decay characteristics indicated the presence of two species. The absorption profile showed one species (λ max 560 nm) decaying on a short time scale (microseconds) and another species (λ max 720 nm) decaying on a longer time scale (Figs. 1 and 2). Pulse radiolysis of 10, 10-dimethylanthralin (AnMe2) under three different conditions i.e. air, O2 in benzene and in N2O saturated CH3CN:Phosphate buffer were carried out in order to observe the triplet decay and formation of any possible free radical at various wavelength (400 – 760 nm) (Fig. 3). 10,10-Dimethylanthralin triplet observed in all cases and found to decay with first order kinetics (K = 4. 96 E+06 sec-1, K = 1.53 E+07 sec-1) (Figs. 4A and 4B). Time constant resolved spectrum recovered and triplet found to peak at 560 nm. However, no such corresponding radical (as expected) was formed. Pulse radiolysis of 1,8-Dihydroxy-9-anthrone (Anthralin) solutions at concentration of 1×10 -4M with sodium azide (NaN3) (1×10 -2M) in N2O saturated CH3CN:Phosphate buffer (1:1 v/v) (pH7) was carried out in order to observe the triplet decay and formation of any possible free radical at various wavelength (400 – 760 nm). The growing of anthralin radical was observed at wavelength of 720 nm. Based upon these results, the following mechanism is proposed (scheme 2).

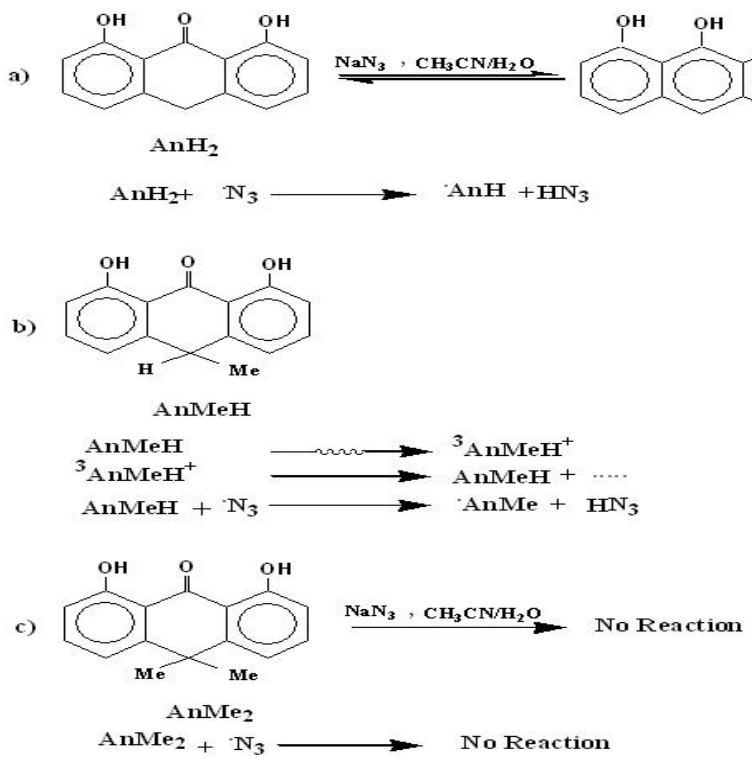
Results and Discussion

It is well documented that the structure of AnH_2 (anthralin) in apolar, aprotic solvent[4] and in the crystal[5] is that of the dihydroxyanthrone tautomer (1A). The solvent employed in this work was mainly benzene. Since the spectra of anthralin in this solvent and in aliphatic hydrocarbons are essentially identical there is no doubt that the electronic excitation experiments to be described all involve deposition of energy into anthralin molecules which possess the ketone structure (IA). Also, in this work, we employed aqueous CH_3CN: Phosphate buffer

(1:1 v/v). In the latter solvent, anthralin exists as a tautomer of keto (1A) enol (1B) form (scheme 1). From the results obtained in this work, we concluded that the keto form of anthralin (1A) is responsible for the formation of free radical ($^{\cdot}$AnH, II) and the triplet excitation state. Pulse radiolysis of solution of 10-methylanthralin (AnHMe) under three different conditions i.e. air, O_2 in benzene and in N_2O saturated CH_3CN:Phosphate buffer were carried out in order to observe the triplet decay and formation of the corresponding free radical at various wavelength (400 – 760 nm). The results indicated the formation of an immediate transient absorption of 10-methylanthralin (^{3}AnMeH$*$) whose spectral decay characteristics indicated the presence of two species. The absorption profile showed one species (λ_{max} 560 nm) decaying on a short time scale (microseconds) and another species (λ_{max} 720 nm) decaying on a longer time scale (Figs. 1 and 2). Pulse radiolysis of 10, 10-dimethylanthralin ($AnMe_2$) under three different conditions i.e. air, O_2 in benzene and in N_2O saturated CH_3CN:Phosphate buffer were carried out in order to observe the triplet decay and formation of any possible free radical at various wavelength (400 – 760 nm) (Fig. 3). 10,10-Dimethylanthralin triplet observed in all cases and found to decay with first order kinetics (K = 4. 96 E+06 sec^{-1}, K = 1.53 E+07 sec^{-1}) (Figs. 4A and 4B). Time constant resolved spectrum recovered and triplet found to peak at 560 nm. However, no such corresponding radical (as expected) was formed. Pulse radiolysis of 1,8-Dihydroxy-9-anthrone (Anthralin) solutions at concentration of 1×10^{-4}M with sodium azide (NaN_3) (1×10^{-2}M) in N_2O saturated CH_3CN:Phosphate buffer (1:1 v/v) (pH7) was carried out in order to observe the triplet decay and formation of any possible free radical at various wavelength (400 – 760 nm). The growing of anthralin radical was observed at wavelength of 720 nm. Based upon these results, the following mechanism is proposed (scheme 2).



Scheme 1: Keto-enol tatuomerisation of anthranlin



Scheme 2

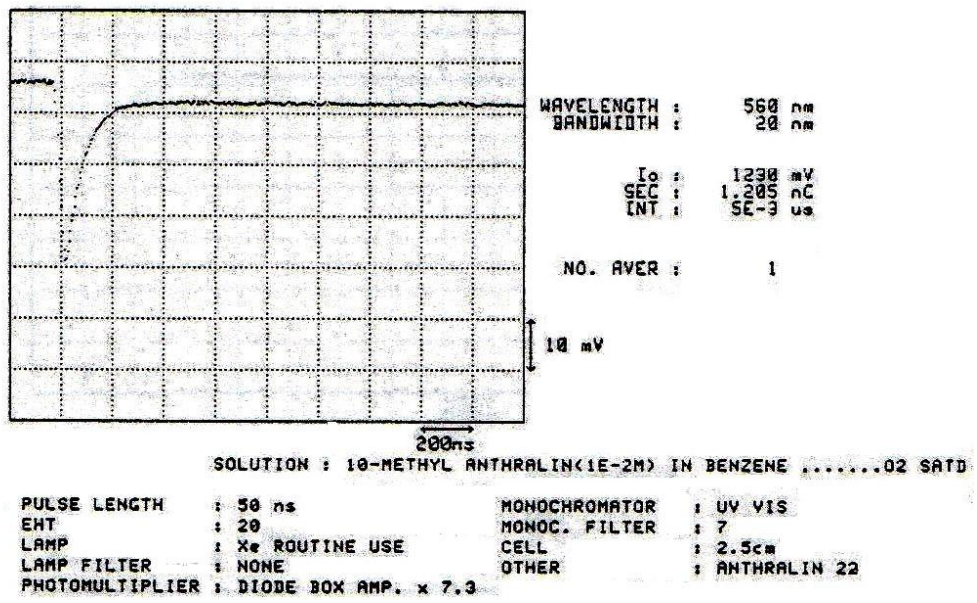


Fig. 1: Transient Absorption of (^{3}AnMeH*) in O_2 purged Benzene (1×10^{-2}M)

SOLUTION : AnMeH(2E-4M) IN CH3CN/PHOS BUFFER(pH=7) 1:1 .....N2O SATD

ABSORPTION NORMALISED TO SEC = 1

WAVELENGTH (nm)	SEC (nC)	660 ns	2.22 u	4.35 u	8.58 u
420	1.020	-4.63E-3	-9.07E-3	-1.15E-2	-1.22E-2
430	.990	-4.45E-3	-9.67E-3	-1.16E-2	-1.18E-2
440	.975	-4.02E-3	-8.08E-3	-1.08E-2	-1.16E-2
450	.990	-3.59E-3	-7.75E-3	-1.03E-2	-1.09E-2
460	1.000	-3.11E-3	-6.67E-3	-8.37E-3	-8.79E-3
470	.985	-2.05E-3	-4.26E-3	-5.30E-3	-5.88E-3
480	.995	-1.89E-3	-2.35E-3	-3.09E-3	-3.19E-3
490	1.020	-1.21E-4	-4.44E-4	-8.48E-4	-2.82E-4
500	.995	8.97E-4	1.79E-3	2.15E-3	2.74E-3
520	1.015	2.34E-3	4.11E-3	5.25E-3	5.69E-3
540	1.005	2.06E-3	3.86E-3	5.16E-3	5.80E-3
560	1.010	2.61E-3	4.91E-3	5.95E-3	6.93E-3
580	1.025	2.39E-3	4.58E-3	5.83E-3	6.79E-3
600	1.015	2.52E-3	4.29E-3	5.59E-3	6.87E-3
620	1.050	3.07E-3	5.76E-3	6.79E-3	7.82E-3
640	1.000	3.86E-3	7.38E-3	9.59E-3	1.05E-2
660	1.020	3.79E-3	9.05E-3	1.10E-2	1.21E-2
680	.995	4.94E-3	9.50E-3	1.16E-2	1.25E-2
700	1.035	4.85E-3	9.32E-3	1.16E-2	1.23E-2
720	.995	4.10E-3	7.78E-3	9.63E-3	1.09E-2
740	1.010	2.06E-3	5.32E-3	6.10E-3	6.39E-3
760	1.035	1.50E-3	2.89E-3	3.14E-3	3.63E-3

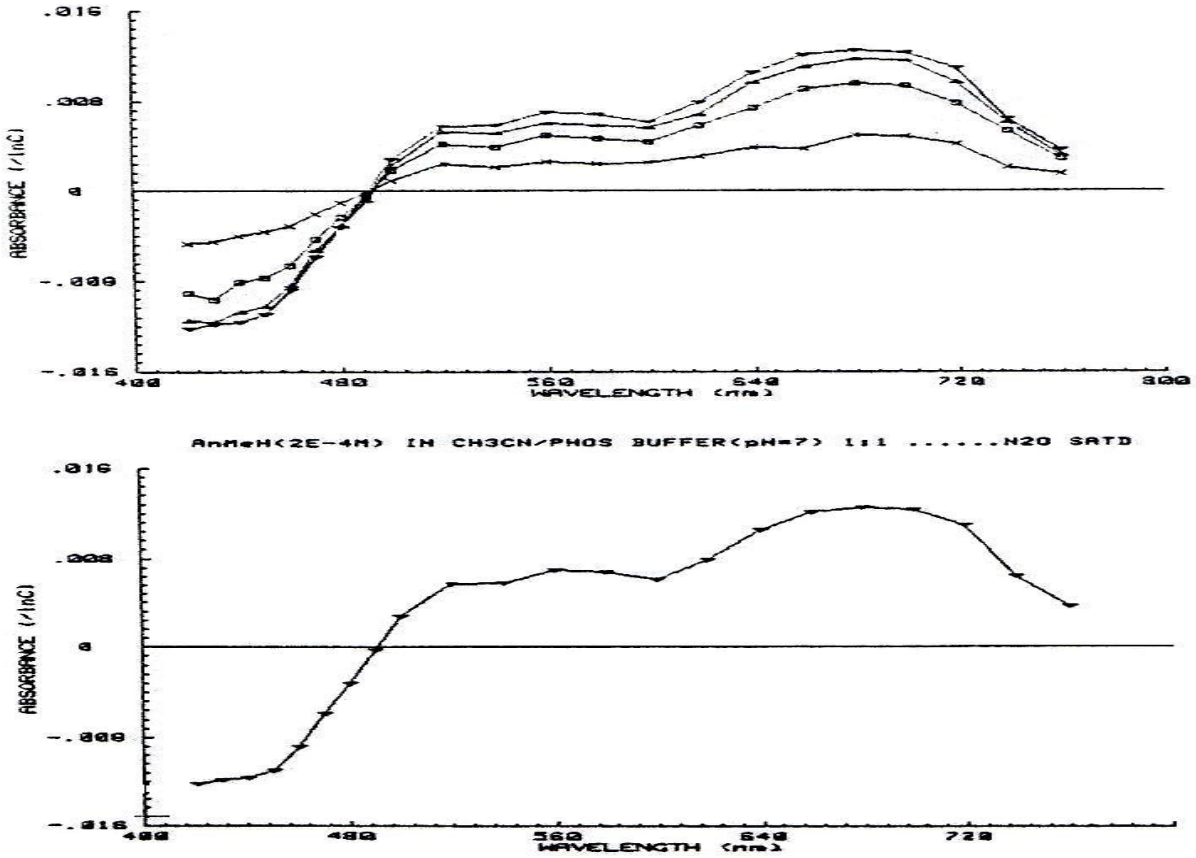


Fig.2: Transient Absorption of (^{3}AnMeH*) in N_2O saturated CH_3CN:Phosphate buffer

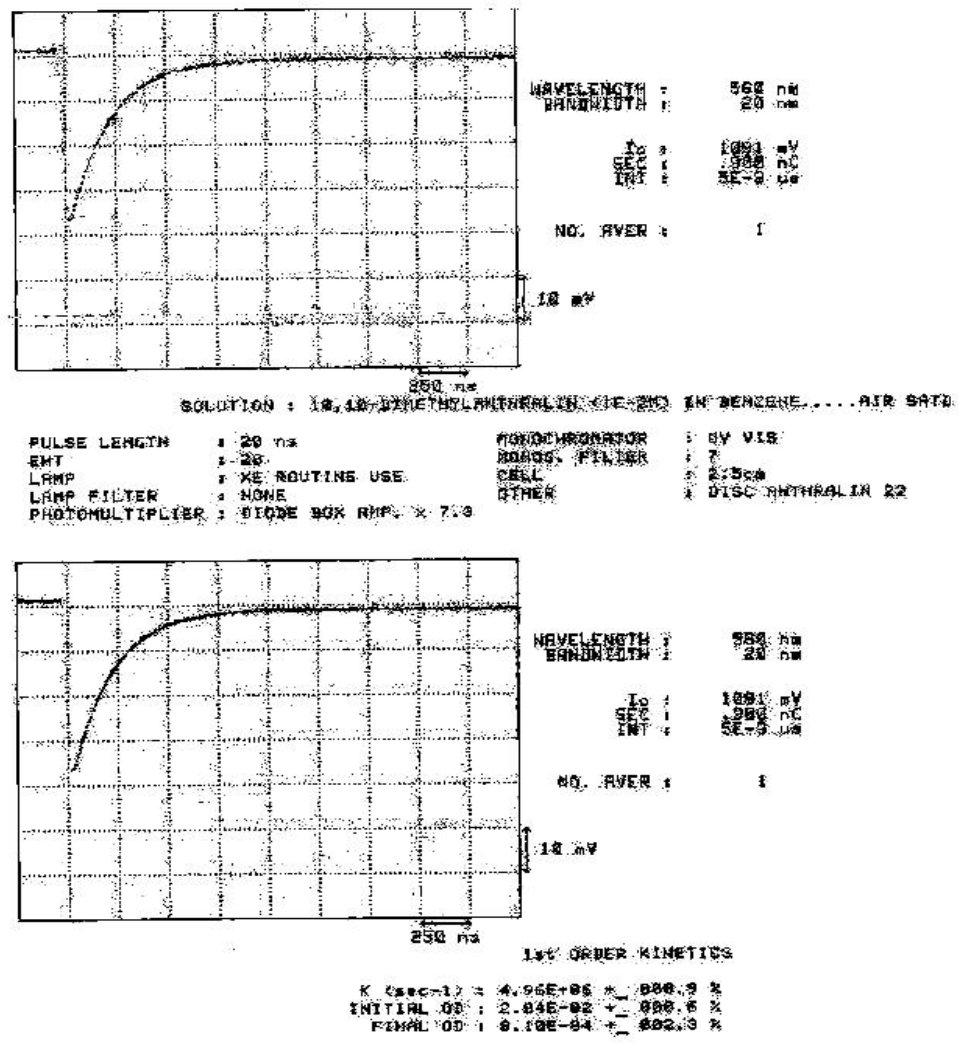


Fig 3: First order kinetics of ($^3AnMe_2*$) in O_2 purged Benzene (1×10^{-2}M)

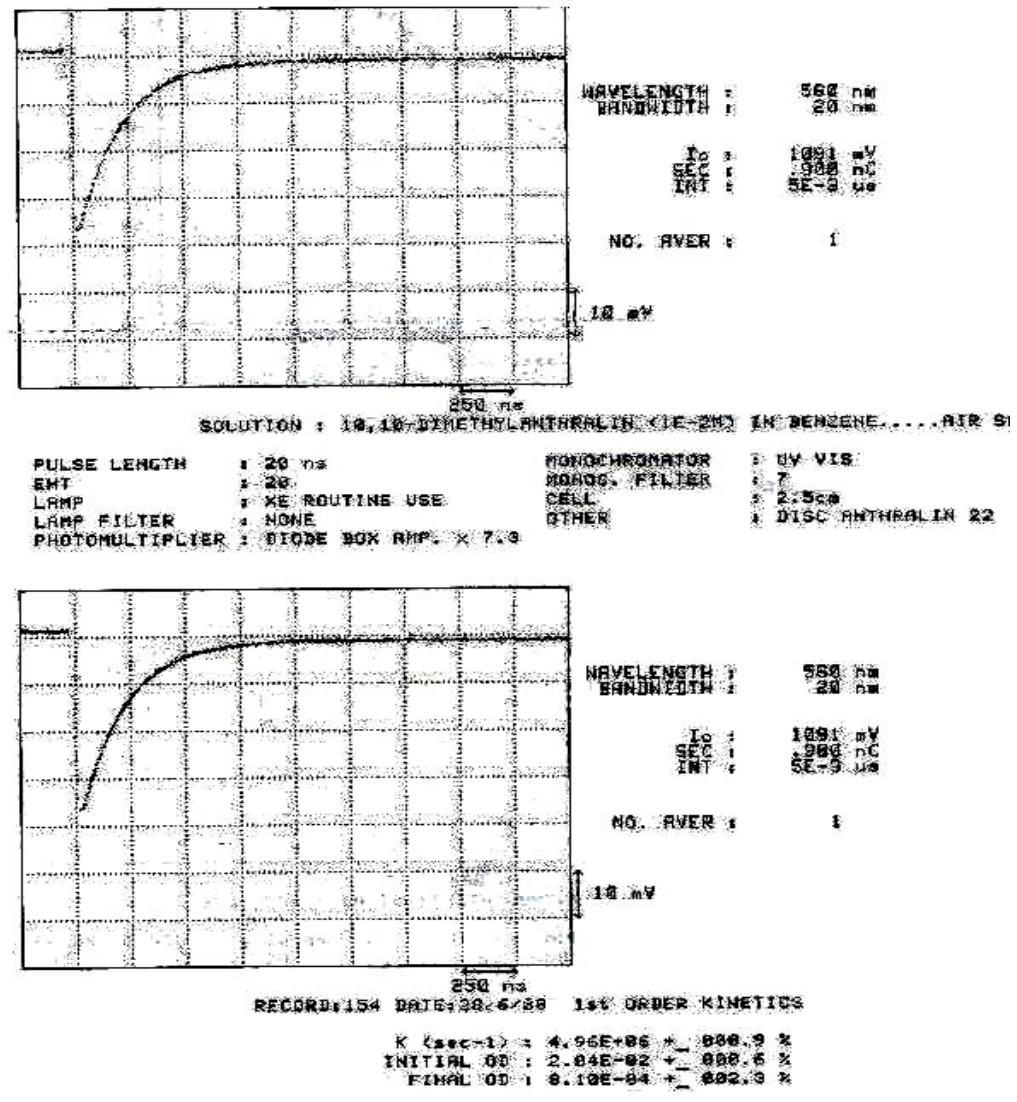


Fig. 4A: Transient Absorption of ($^3AnMe_2*$) in air saturated Benzene (1×10^{-2}M)

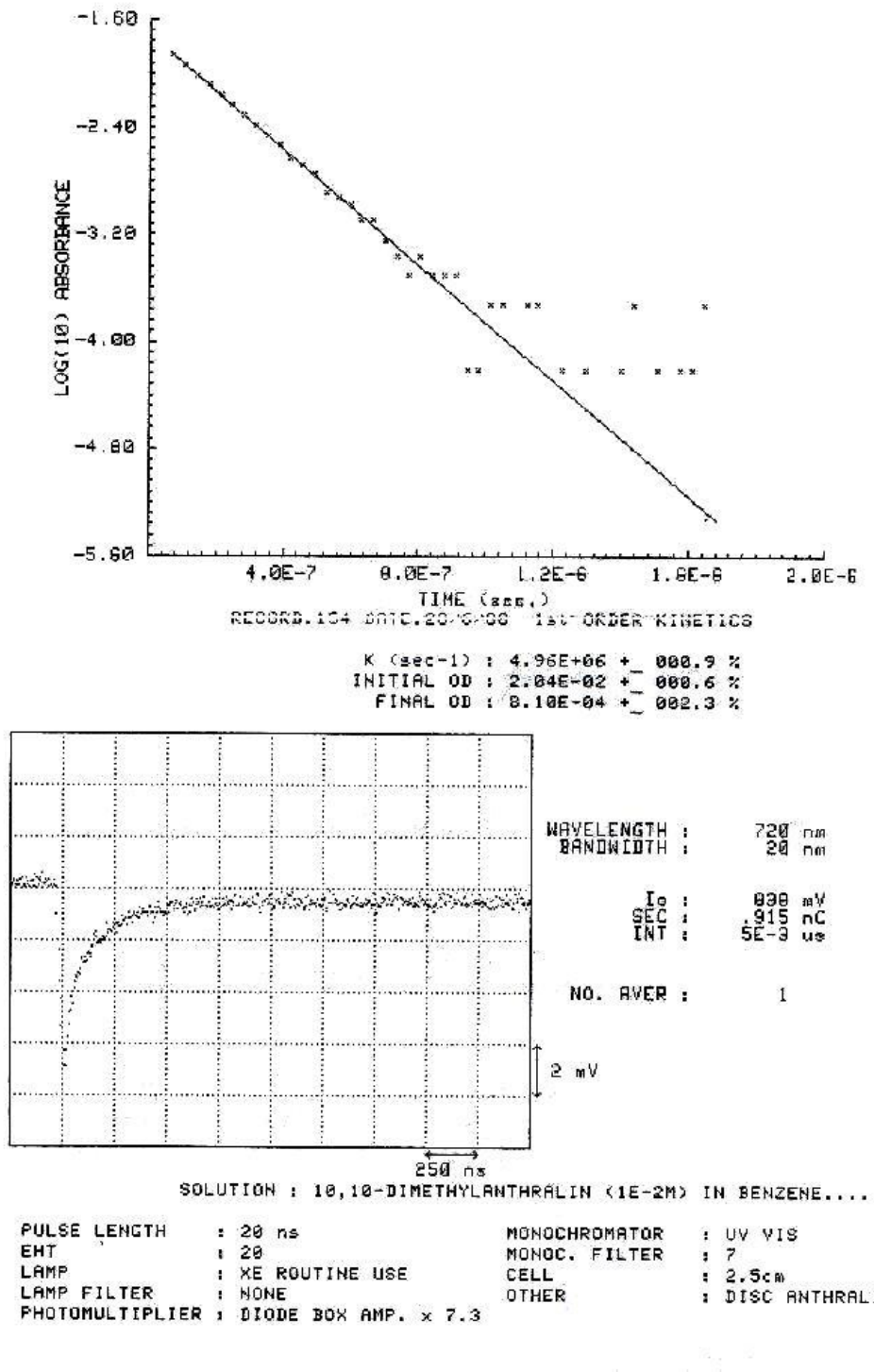


Fig.4B: Transient Absorption of ($^{3}AnMe_2*$) in air saturated Benzene ($1\times10^{-2}M$)

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