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A systematic study on the chemical stability of the novel indoloquinone antitumour agent EO9

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Summary

The chemical stability of the new anticancer drug EO9 in aqueous solution has been investigated utilizing a stability-indicating reversed-phase high-performance liquid chromatographic assay with ultraviolet detection and ultraviolet spectrophotometry. The degradation kinetics of EO9 have been studied as a function of pH, buffer composition, ionic strength and temperature. A pH-rate profile, using rate constants extrapolated to zero buffer concentration, was constructed demonstrating that EO9 is most stable in the pH region 8–9. The degradation mechanism of EO9 in aqueous solution is discussed.

Introduction

Hypoxia in solid tumour tissue may represent a problem by limiting response to radiotherapy and chemotherapy (Bailey et al., 1992b). It may, however, enhance the activity of bioreductive anticancer agents. EO9 (3-hydroxymethyl-5-aziridinyl-1-methyl-2-(1H-indole-4,7-dione) propenol; Fig. 1) is the lead compound in a series of novel and fully synthetic bioreductive alkylating indoloquinones. These agents were designed to readily undergo redox cycling and formation of alkylating intermediates under bioreductive conditions (Oostveen and Speckamp, 1987). They are inactive in their own right, but are able to undergo metabolism to species which can damage biomolecules upon metabolic reduction. Although structurally related to the bioreductive agent mitomycin C (MMC; Fig. 1), EO9 demonstrates a distinct profile of antitumour activity (Winograd et al., 1989).

Studies have been conducted dealing with the bioanalysis (Binger and Workman, 1990), bioactivation (Bailey et al., 1992a,b; Robertson et al., 1992; Walton et al., 1992) and pharmacokinetics, distribution and metabolism of EO9 (Workman et al., 1992). However, no systematic kinetic study has been reported, so far, on the degradation of EO9 in aqueous solution.

Knowledge on the acid degradation of EO9 is of considerable interest, since acid-catalyzed

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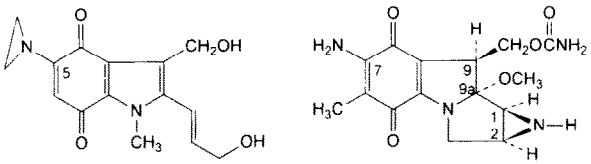

Fig. 1. Structures of EO9 and mitomycin C (MMC).

degradation may be an activation step of EO9, as for MMC (Beijnen et al., 1986c), in the process of binding to deoxyribonucleic acid (DNA), next to the bioreductive activation. Kennedy et al. (1985) showed that MMC-induced interstrand cross-linking of DNA is highly dependent upon the intracellular micro-environmental pH: a lower pH enhances the alkylation. Moreover, much evidence has suggested that environmental pH conditions may vary considerably in experimental tumours (Guillino et al., 1965; Guillino, 1975; Kennedy et al., 1981; Vaupel et al., 1981). Studies in experimental tumours have shown that the interstitial fluid pH varied from 5.8 to 7.2, whereas a more limited distribution in pH values (7.1–7.4) for normal tissue was found (Guillino et al., 1965; Guillino, 1975; Kennedy et al., 1981; Vaupel et al., 1981).

This study was initiated with the objective to obtain detailed knowledge on the degradation kinetics of EO9 in aqueous solution, including the effects of several parameters (pH, buffers, temperature and ionic strength) on the degradation process.

Materials and Methods

Chemicals

EO9 was synthesized by the University of Amsterdam and provided by the New Drug Development Office (NDDO) of the European Organisation for Research and Treatment of Cancer (EORTC; Amsterdam, The Netherlands). All other chemicals used were of analytical grade and deionized water was used throughout.

Buffer solutions

For the kinetic studies the following aqueous buffer solutions were used: pH 3.5–6, acetate; pH 6–9, phosphate; pH 9–11, carbonate; and pH > 11, sodium hydroxide. The pH values between 3.5 and 12 were measured at 25°C, using an Ingold Lot-401 combined glass-reference electrode (Ingold Electrodes Inc., Wilmington, MA, U.S.A.) and a Consort P514 pH meter (Consort, Turnhout, Belgium). Extension of the acidity scale, above pH 12, was accomplished with the Hammett acidity function (Bates, 1973).

A constant ionic strength (μ) of 0.3 was maintained for each solution by addition of the appropriate amount of sodium chloride, except with the experiments where the effect of the ionic strength on the degradation of EO9 was investigated and where the OH^- or H^+ concentrations were higher than 0.3 M.

Kinetic measurements

The degradation reactions were initiated by adding a 25 μl sample of a 2 mg/ml stock solution of EO9 in methanol to 3 ml of the pre-heated buffered solution to obtain an initial EO9 concentration of 16.7 μg/ml (5.8×10^{-5} M). The solutions were kept in screw-capped polypropylene test tubes in a thermostatically controlled water bath at 25 ± 0.2°C in the dark.

Ultraviolet (UV) spectrophotometry Degradation reactions at pH values between 3.5–6 and > 11 were followed spectrophotometrically by continuously monitoring the increase in absorbance at 340 and 290 nm, respectively.

High-performance liquid chromatography (HPLC) Degradation reactions at pH values between 6 and 11 were followed by withdrawal of 25 μl samples from the reaction solutions at appropriate time intervals, which were analyzed directly for undegraded EO9 by means of a stability-indicating HPLC assay.

All kinetic experiments have been performed in duplicate.

Apparatus and experimental conditions

UV UV/Vis spectra of EO9 were recorded on a Lambda 5 UV/Vis spectrophotometer equipped with a Lambda computer and a printer/plotter (all from Perkin-Elmer, Gouda, The Netherlands). Kinetic studies were performed with a UV-140 double-beam absorption

spectrophotometer (Shimadzu, 's-Hertogenbosch, The Netherlands) equipped with a Kipp BD40 recorder. The 1 cm quartz cells were kept in a thermostatically controlled cell compartment at 25°C.

HPLC The liquid chromatographic system consisted of a Model M6000 solvent delivery system, a Model U6K septumless injection device and a Model 440 dual-wavelength UV detector (all from Waters Associates Inc., Milford, MA, U.S.A.) with a fixed wavelength filter for detection at 254 nm and a sensitivity of 0.05 AUFS. The pre-column (2 cm × 4 mm i.d.) was packed with Lichrosorb 10 μm RP-8 material and the stainless-steel analytical column (12.5 cm × 4 mm i.d.) was packed with Lichrosorb 5 μm RP-8 material (both materials were obtained from Merck, Darmstadt, Germany). The mobile phase consisted of methanol/water (30:70 w/w) to which 1% (v/w) 0.5 M sodium phosphate buffer (pH 7.0) was added. The flow rate was 1.0 ml/min and the column was at ambient temperature. The column pressure was approx. 3000 lb/inch^2. Quantitation of EO9 was based on peak height measurements using a Model BD40 recorder (Kipp & Zonen B.V., Delft, The Netherlands).

Calibration curves of standard EO9 solutions in water were linear ($r > 0.999$) in the concentration range of interest.

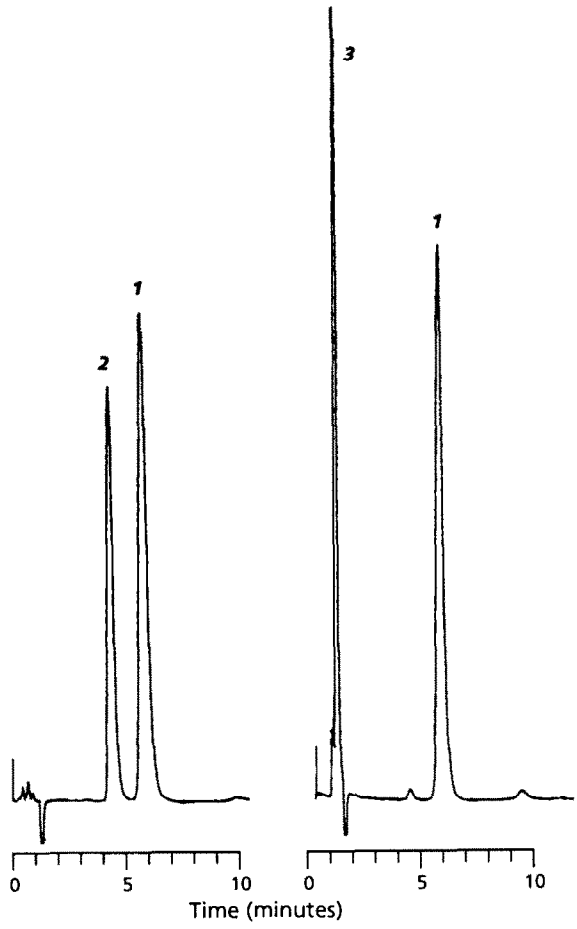

Fig. 2. HPLC chromatograms of partly degraded EO9 in acidic (left) and alkaline medium (right). Peak 1, EO9; peaks 2 and 3, degradation products.

Results and Discussion

Analytical procedures

Degradation of EO9 in the pH range 6–11 can be followed using the presented HPLC method. At pH values > 10, EO9 degrades principally into one degradation product, which elutes with the solvent front. At pH values < 7, EO9 degrades principally into one degradation product with a retention time of 4.4 min. For both pH ranges, EO9 is well separated from the degradation products. Fig. 2 shows typical HPLC chromatograms for partly degraded solutions of EO9 in acidic and alkaline media. They demonstrate the stability-indicating capability of the HPLC assay.

Degradation of EO9 in strong alkaline solution (pH > 11) is accompanied by marked spectral changes, indicating that the degradation product(s) of EO9 possess(es) different chromophores in this medium from those of the parent drug. The spectral changes are most profound at 290 nm, whereby continuously monitoring the increase in absorbance at this wavelength can be used for the quantitation of the degradation reactions of EO9. In acidic medium (pH 3.5–6) the observed spectral changes are also significant. The absorption maximum of EO9 at 269 nm decreases and an increase in absorbance is observed at 340 nm, which can be used for the quantitation of the degradation reactions of EO9

in acidic medium. Thus, UV spectrophotometry seems to be an adequate technique for the quantification of the degradation reactions of EO9 in both alkaline and acidic media, by continuously monitoring the increase in absorbance at 290 and 340 nm, respectively.

Degradation kinetics

Order of reactions The disappearance of EO9 in buffered media follows (pseudo) first-order kinetics over several half-lives. This is indicated by the linearity of plots of the natural logarithm of residual EO9 concentration against time at various pH values. The observed (pseudo) first-order rate constants (k_{obs}) for the overall degradation have been extracted from the slopes of these plots.

Standard deviation of k_{obs} The standard deviation (SD) of the overall rate constant, k_{obs}, was determined for the HPLC and the UV spectrophotometric assays. These statistical experiments were performed at 25°C. The value of k_{obs} ($\pm$SD) for the spectrophotometric assay was determined at pH 4.0 (0.1 M acetate buffer; μ = 0.3); the value of k_{obs} was $7.6 \pm 0.1 \times 10^{-3}$ s^{-1} ($n = 6$) and the relative SD is equal to 1.3%. For the HPLC assay, the values of k_{obs} were determined at pH 5.0 (0.25 M acetate buffer; $\mu = 0.3$); the value of k_{obs} was $4.9 \pm 0.1 \times 10^{-5}$ s^{-1} ($n = 6$) and the relative SD is equal to 2.9%.

Influence of pH It is hypothesized that the degradation mechanism of EO9 in both alkaline and acidic media is similar to that of other aziridinylquinones (Kusai et al., 1981, 1982). Since the degradation of EO9 is strongly influenced by pH, it is important to keep the pH constant during the experiments. The kinetic experiments were, therefore, performed in buffer solutions. The overall observed rate constant for the degradation of EO9 in buffer solutions is:

$$k_{obs} = k_o + k_H[H^+] + k_{OH}[OH^-] + k_{buffer}[buffer] \quad (1)$$

where k_o is the (pseudo) first-order rate constant for degradation in water only, and k_H and k_{OH} the second-order rate constants for proton- and hydroxyl-catalyzed degradation, respectively. The term k_{buffer}[buffer] represents the sum of the second-order rate constants for the degradation catalysed by each of the buffer components multiplied by its concentration. For each pH the (pseudo) first-order rate constant for [buffer] = 0, $k'_{obs} = k_o + k_H[H^+] + k_{OH}[OH^-]$, was calculated as the intercept from the linear part of the plot of k'_{obs} vs [buffer] at a fixed pH.

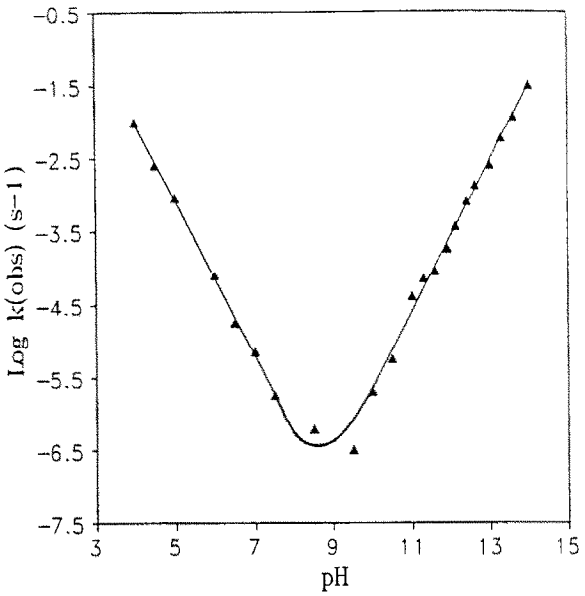

Fig. 3. pH-rate profile of EO9 at 25°C.

The calculated values for k'_{obs} were used in the construction of the pH-rate profile (Fig. 3). The profile shows that no prototropic equilibria are involved. The V-shaped curve has a slope of -1.0 in the weakly acidic pH region and a slope of $+1.0$ in the alkaline pH region, indicating the occurrence of specific proton and hydroxyl catalysis, respectively.

With the use of Eqn 2,

$$k'_{obs} = k_o + k_H[H^+] + k_{OH}[OH^-] \quad (2)$$

the specific rate constants for the degradation of EO9 were calculated. These values are shown in Table 1. The contribution of k_o to k'_{obs} is negligible. The pH at which the minimum occurs in the pH-rate profile can be determined graphically or

TABLE 1

Rate constants for catalyzed degradation reactions of EO9 at 25°C

k_H	99.6	$M^{-1} s^{-1}$
k_o	≈ 0	s^{-1}
k_{OH}	3.19×10^{-2}	$M^{-1} s^{-1}$

can be calculated from the first derivative of Eqn 2, set to zero,

$$\frac{dk'_{obs}}{d[H^+]} = k_H - \frac{k_{OH} K_w}{[H^+]^2} = 0 \qquad (3)$$

or:

$$pH_{min} = \tfrac{1}{2} pK_w + \tfrac{1}{2} \log \frac{k_H}{k_{OH}} \qquad (4)$$

At 25°C $pK_w = 14.00$. So pH_{min} is calculated to be 8.75, which is in agreement with the graphically derived value.

Influence of buffers The degradation of EO9 is strongly affected by phosphate buffer components. This appears from experiments where k_{obs} was measured at constant pH, ionic strength ($\mu = 0.3$) and temperature (25°C), but at different buffer concentrations. A linear relationship exists between k_{obs} and the phosphate buffer concentration. At pH 7.0 ($\mu = 0.3$ and $T = 25$°C), this relationship between k_{obs} and the phosphate buffer concentration can be described by the equation:

$$k_{obs} = 6.96 \times 10^{-6} + 6.71 \times 10^{-5} \cdot [\text{phosphate}]$$

$$(r > 0.9999;\ p < 0.001) \qquad (5)$$

Acetate and carbonate buffers seem to have no significant effect on the degradation rate of EO9. Table 2 shows the influence of buffer ions on the degradation of EO9.

Influence of ionic strength The influence of the ionic strength (μ) on the degradation of EO9 was investigated by adding various amounts of sodium chloride to buffer solutions of fixed pH. The influence of the ionic strength on the degradation of EO9 at pH 11.3 and 13.0 is negligible. However, at pH 4.0, a linear relationship exists between log k_{obs} and $\sqrt{\mu}$. This relationship can be described by the equation:

$$\log k_{obs} = 0.56 + 1.84 \cdot \sqrt{\mu}$$

$$(r > 0.9930;\ p = 0.0007) \qquad (6)$$

The results from these experiments are listed in Table 3.

TABLE 2

Influence of acetate, phosphate and carbonate buffer concentration on k_{obs} of the degradation reactions of EO9

pH	[Buffer]	k_{obs}	pH	[Buffer]	k_{obs}
3.5	0.05	3.1×10^{-2}	7.0	0.05	1.0×10^{-5}
	0.10	3.1×10^{-2}		0.10	1.4×10^{-5}
	0.25	1.7×10^{-2}		0.25	2.4×10^{-5}
	0.50	1.3×10^{-2}		0.50	4.0×10^{-5}
				0.80	6.1×10^{-5}
				1.00	7.4×10^{-5}
4.0	0.05	9.3×10^{-3}	7.5	0.05	3.8×10^{-6}
	0.10	8.6×10^{-3}		0.10	5.5×10^{-6}
	0.25	5.7×10^{-3}		0.25	9.8×10^{-6}
	0.50	5.6×10^{-3}		0.50	1.6×10^{-5}
	0.80	6.1×10^{-3}			
	1.00	7.5×10^{-3}			
4.5	0.05	2.0×10^{-3}	8.0	0.05	2.1×10^{-6}
	0.10	1.7×10^{-3}		0.10	1.2×10^{-6}
	0.25	1.7×10^{-3}		0.25	5.7×10^{-6}
				0.50	7.4×10^{-6}
5.0	0.05	8.5×10^{-5}	8.5	0.05	1.7×10^{-6}
	0.10	8.6×10^{-4}		0.10	1.4×10^{-6}
	0.25	6.6×10^{-4}		0.25	2.8×10^{-6}
	0.50	8.1×10^{-4}		0.50	4.0×10^{-6}
5.5	0.05	2.2×10^{-4}	9.5	0.05	4.0×10^{-7}
	0.10	2.6×10^{-4}		0.10	5.9×10^{-7}
	0.25	1.4×10^{-4}			
	0.50	2.1×10^{-4}			
6.0	0.05	8.7×10^{-5}	10.0	0.05	2.8×10^{-6}
	0.10	9.3×10^{-5}		0.10	6.2×10^{-6}
	0.25	9.5×10^{-5}		0.25	6.3×10^{-6}
	0.50	1.5×10^{-4}			
6.5	0.05	2.6×10^{-5}	10.5	0.05	6.4×10^{-6}
	0.10	3.4×10^{-5}		0.10	6.2×10^{-6}
	0.25	7.1×10^{-5}		0.25	6.3×10^{-6}

pH 3.5–6.0, acetate; pH 6.5–8.5, phosphate; pH 9.5–10.5, carbonate; [buffer] in M; k_{obs} in s^{-1}.

TABLE 3

Influence of the ionic strength on k_{obs} of the degradation reactions of EO9 at 25°C

pH	$\sqrt{\mu}$	log k_{obs}	pH	$\sqrt{\mu}$	log k_{obs}
4.0	0.32	−2.50	13.0	0.32	−2.53
	0.50	−2.10		0.40	−2.55
	0.56	−2.07		0.50	−2.57
	0.65	−1.83		0.65	−2.64
	0.80	−1.62		0.80	−2.65
11.3	0.04	−4.35			
	0.30	−4.38			
	0.45	−4.27			
	0.60	−4.27			
	0.70	−4.71			
	0.80	−4.92			
	1.00	−4.75			

pH 4.0, 0.1 M acetate buffer solution; pH 11.3 and 13.0, NaOH solutions.

Influence of temperature The influence of temperature on the degradation of EO9 was studied at pH 4.0 (0.1 M acetate buffer; $\mu = 0.3$), pH 7.0 (0.1 M phosphate buffer; $\mu = 0.3$) and pH 12.1 (sodium hydroxide solution; $\mu = 0.3$) in the temperature range 15–50°C. The Arrhenius relationship between the natural logarithm of k_{obs} and the reciprocal of the absolute temperature holds.

TABLE 4

Activation energies (E_a) *and frequency factors* (A) *for the degradation of EO9 in buffer as a function of pH*

pH	E_a (kJ mol^{-1})	A (s^{-1})
4.0	40.6	1.1×10^5
12.1	67.0	1.6×10^8

Activation energies (E_a) and frequency factors (A) are listed in Table 4 and are in accordance with the formation of different degradation products in acidic and alkaline solutions.

Degradation mechanism As for MMC (Beijnen et al., 1986a,b), two pathways have been proposed for the in vivo activation of aziridinylquinones, such as EO9 (Driebergen, 1987). The first pathway involves reduction of the quinone function of EO9 to its semiquinone or hydroquinone, generating a species with an electrophilic aziridine ring system, which serves as a target for nucleophilic DNA moieties.

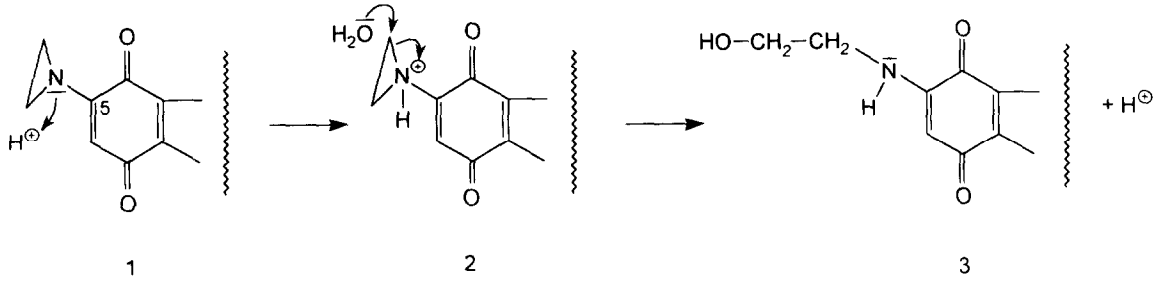

Fig. 4. Proposed overall degradation scheme of EO9 in acidic medium.

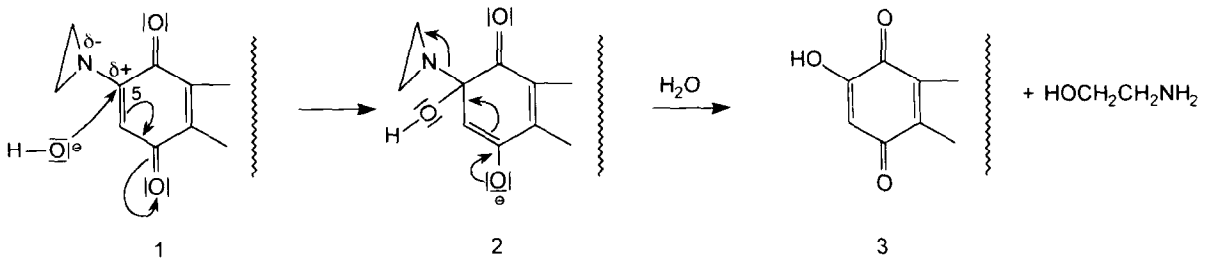

Fig. 5. Proposed overall degradation scheme of EO9 in alkaline medium.

The second activation pathway involves acid degradation of EO9. In acidic medium EO9 decomposes principally into one compound with a retention time of 4.4 min. In analogy with other aziridinylquinones (Kusai et al., 1981, 1982; Driebergen, 1987), the following overall degradation scheme for EO9 in acidic medium is proposed (Fig. 4). The initial step of the reaction in acidic medium is thought to be protonation of the trivalent nitrogen in the aziridine ring, yielding an iminium ion. This electrophilic center is then attacked by nucleophilic particles, i.e., water molecules, resulting in a degradation product with an ethanolamine group at C5.

In analogy with MMC (Beijnen et al., 1985) and other aziridinylquinones (Kusai et al., 1981, 1982; Driebergen, 1987), the degradation of EO9 in alkaline medium may proceed through the substitution of the aziridine ring by an hydroxyl ion at C5, as outlined in Fig. 5.

Conclusions

Degradation of EO9 follows (pseudo) first-order kinetics. The pH-rate profile of EO9 shows the occurrence of specific proton and hydroxyl catalysis. The degradation rate and mechanism of EO9 are strongly pH-dependent. EO9 is most stable in the pH region 8–9.

The degradation rate of EO9 is strongly affected by phosphate buffer components. Acetate and carbonate buffer components have no significant effect on the degradation rate of EO9. The same holds for the ionic strength.

References

Bailey, S.M., Friedlos, F., Knox, R.J. and Workman, P., Bioreductive activation of indoloquinone EO9: involvement of DT-diaphorase and DNA-crosslinking. *Ann. Oncol.*, 3 (1992a) 185.

Bailey, S.M., Sugget, N., Walton, M.I. and Workman, P., Structure-activity relationships for DT-diaphorase reduction of hypoxic cell directed agents: indoloquinones and diaziridinyl benzoquinones. *Int. J. Radiat. Oncol. Biol. Phys.*, 22 (1992b) 649–653.

Bates, R.G., *Determination of pH; Theory and Practice*, Wiley, New York, 1973, pp. 165–169.

Beijnen, J.H., Den Hartigh, J. and Underberg, W.J.M., Quantitative aspects of the degradation of mitomycin C in alkaline solution. *J. Pharm. Biomed. Anal.*, 3 (1985) 59–69.

Beijnen, J.H., Van der Houwen, O.A.G.J., Rosing, H. and Underberg, W.J.M., A systematic study on the chemical stability of mitomycin A and mitomycin B. *Chem. Pharm. Bull.*, 34 (1986a) 2900–2913.

Beijnen, J.H., Lingeman, H., Van Munster, H.A. and Underberg, W.J.M., Mitomycin antitumour agents: a review of their physico-chemical and analytical properties and stability. *J. Pharm. Biomed. Anal.*, 4 (1986b) 275–295.

Beijnen, J.H., Fokkens, R.H., Rosing, H. and Underberg, W.J.M., Degradation of mitomycin C in acid phosphate and acetate buffer solutions. *Int. J. Pharm.*, 32 (1986c) 111–121.

Binger, M. and Workman, P., Gradient high-performance liquid chromatographic assay for the determination of the novel indoloquinone antitumour agent EO9 in biological specimens. *J. Chromatogr.*, 532 (1990) 321–336.

Driebergen, R.J., Qualitative and quantitative aspects of structure-electrochemistry-cytotoxicity relationships of aziridinylquinones. Thesis, University of Utrecht, Utrecht, The Netherlands (1987).

Guillino, P.M., In vivo utilization of oxygen and glucose by neoplastic tissue. *Adv. Exp. Med. Biol.*, 75 (1975) 521–536.

Guillino, P.M., Grantham, F.H., Smith, S.H. and Haggerty, A.C., Modifications of the acid-base status of the internal milieu of tumors. *J. Natl. Cancer Inst.*, 34 (1965) 857–869.

Kennedy, K.A., Teicher, B.A., Rockwell, S. and Sartorelli, A.C., Chemotherapy approaches to cell populations of tumors. In Sartorelli, A.C., Bertino, J.R. and Lazo, J.S. (Eds), *Molecular Targets and Actions for Cancer Chemotherapeutic Agents*, Academic Press, New York, 1981, pp. 85–101.

Kennedy, K.A., McGurl, J.D., Leondaridis, L. and Alabaster, O., pH dependence of mitomycin C-induced cross-linking activity in EMT6 tumor cells. *Cancer Res.*, 45 (1985) 3541–3547.

Kusai, A., Tanaka, S. and Ueda S., The stability of carboquone in aqueous solution: I. Kinetics and mechanisms of degradation of 2,5-diethylenimino-1,4-benzoquinone in aqueous solution. *Chem. Pharm. Bull.*, 29 (1981) 3671–3679.

Kusai, A., Tanaka, S. and Ueda S., The stability of carboquone in aqueous solution: II. Kinetics and mechanisms of degradation of 2,5-bis(1-aziridinyl)-3,6-dimethyl-1,4-benzoquinone and 2,5-bis(1-aziridinyl)-3,6-diisopropyl-1,4-benzoquinone in aqueous solution. *Chem. Pharm. Bull.*, 30 (1982) 2534–2543.

Oostveen, E.A. and Speckamp, W.N., Mitomycin analogs: I. Indoloquinones as (potential) bisalkylating agents. *Tetrahedron*, 43 (1987) 255–262.

Robertson, N., Stratford, I.J., Houlbrook, S., Carmichael, J. and Adams, G.E., The sensitivity of human tumour cells to

quinone bioreductive drugs: What role for DT-diaphorase? *Biochem. Pharmacol.*, 44 (1992) 409–412.

Vaupel, P.W., Frinak, S. and Bicher, H.I., Heterogeneous oxygen partial pressure and pH distribution in C3H mouse mammary adenocarcinoma. *Cancer Res.*, 41 (1981) 2008–2013.

Walton, M.I., Sugget, N. and Workman, P., The role of human and rodent DT-diaphorase in the reductive metabolism of hypoxic cell cytotoxins. *Int. J. Radiat. Oncol. Biol. Phys.*, 22 (1992) 643–647.

Winograd, B., Lobbezoo, M.W., Double, J.A., Fiebig, H.H., Lelieveld, P., Workman, P. and Pinedo, H.M., Preclinical antitumor profile of EO9, a novel bioreductive alkylating indoloquinone derivative. *Proc. Am. Assoc. Cancer Res.*, 30 (1989) 582.

Workman, P., Binger, M. and Kooistra, K.L., Pharmacokinetics, distribution, and metabolism of the novel bioreductive alkylating indoloquinone EO9 in rodents. *Int. J. Radiat. Oncol. Biol. Phys.*, 22 (1992) 713–716.