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The effectiveness of various scavengers on the γ-irradiated, methanolic solution of medazepam

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Summary

A methanolic solution of 2% medazepam was protected from radiolysis caused by γ-irradiation with a dose of 25 kGy. Scavengers used were: argon, nitrous oxide, ascorbic acid, thiourea and potassium thiocyanate at concentrations of 0.1 and 0.01 M or less, according to their solubility. Ascorbic acid was shown to provide the best protection. Gas and liquid chromatography were used for the analysis.

Introduction

With the increased awareness of the toxic problems associated with ethylene oxide, greater attention is being paid to γ-irradiation, (Gopal et al., 1988; Jacobs and Wills, 1988). Several projects at our department have focused on various pharmaceuticals in solid state and in solutions in order to study the feasibility of radiation sterilization and to elucidate the reaction mechanism for different chemical structures (Altorfer, 1974; Trost, 1982; Barghash, 1983). More recently, medazepam, a 1,4-benzodiazepine, in methanolic solution has been studied after γ-irradiation (Werner et al., 1989).

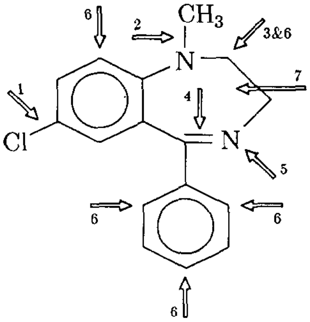

Fig. 1. Possible attacks of radicals of methanol on medazepam (7-chloro-2,3-dihydro-1-methyl-5-phenyl-1H-1,4-benzodizaepine): (1) dechlorination by $e^-_{solv.}$ or $\dot{O}H$; (2) demethylation by $e^-_{solv.}$ or $\dot{H}$; (3) hydroxylation and oxidation in position 2 by $\dot{O}H$; (4) azomethine reduction by $e^-_{solv.}$, $\dot{H}$, H_2; (5) hydrolysis to the benzophenone by H^+; (6) hydroxylation in position 9, at the phenyl ring in position 2′, 4′ and 6′ by $\dot{O}H$; (7) ring contraction to a 6-ring; or combinations.

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In an irradiated methanolic solution, the following radicals and molecules must be considered:

reducing species: $e^-_{solv.}$, $\dot{H}$, H_2, $H\dot{O}_2/\dot{O}_2^-$, $\dot{C}H_2OH$, HCOH
oxidizing species: $\dot{O}H$, H_2O_2, $H\dot{O}_2/\dot{O}_2^-$
hydrolyzing species: H^+, OH^-

$H\dot{O}_2/\dot{O}_2^-$ can react as an oxidizing or reducing species according to the pH of the solution. HCOH is ignored as it is produced in a secondary or tertiary step. Details on the reactions in methanolic solutions and in water of those species are given elsewhere (Werner et al., 1989). Fig. 1 shows the most likely attacks of radicals on the molecule

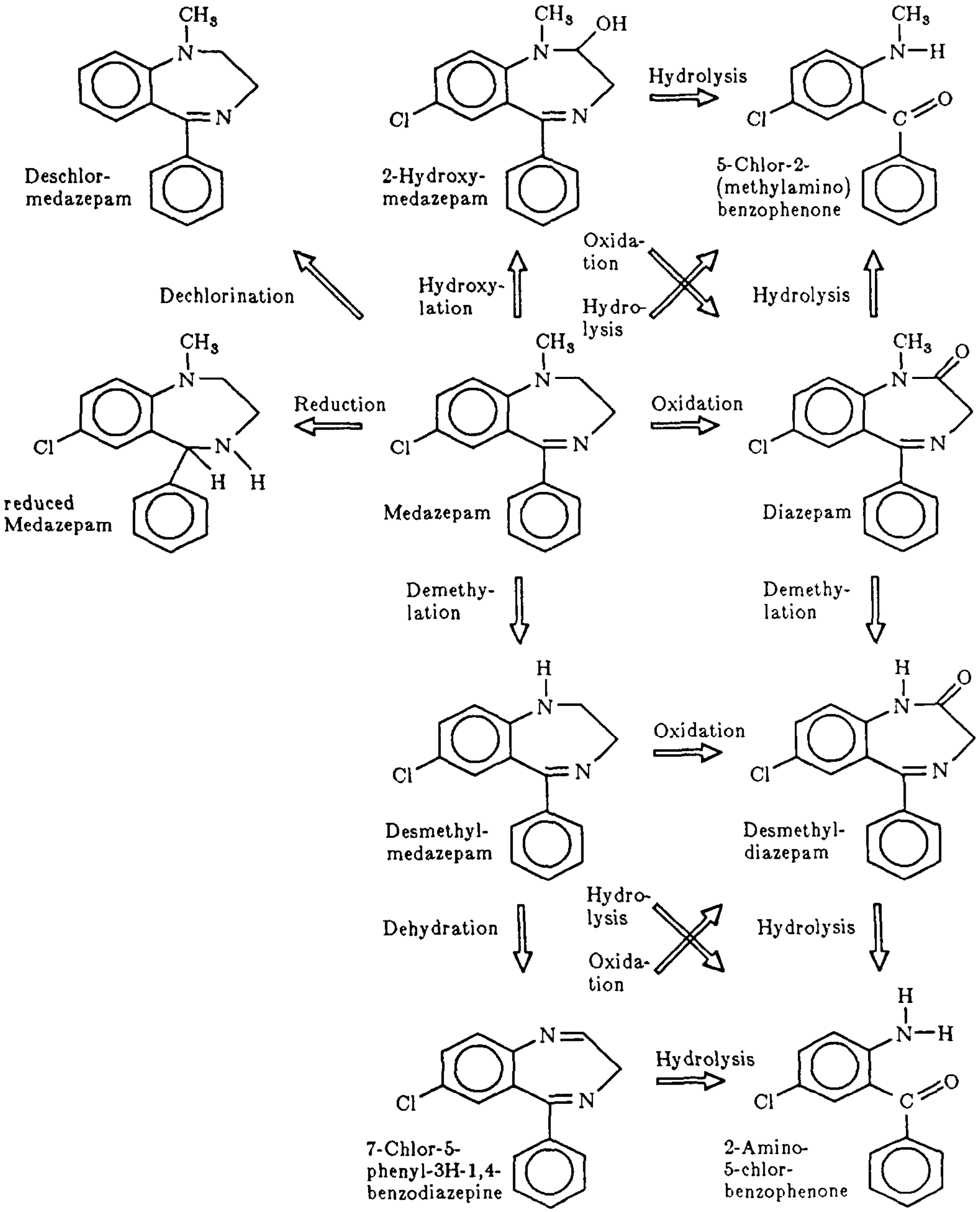


Fig. 2. Radiolytic reaction scheme of medazepam.

examined. Using different analytical methods, a reaction scheme has been established (Fig. 2). With this knowledge scavengers can be found to prevent radiolytic decomposition. Furthermore, they can be corroborated to provide a better explanation for the reaction scheme.

Definition and action of scavengers

A scavenger is preferably a simple chemical substance which has a positive influence on the chemical action during irradiation. This protective influence can be one of the following:

(i) The scavenger absorbs the exciting energy of another molecule (energy transfer). The exciting energy of the scavenger should therefore be lower than that of the molecule to be protected. The liberated energy can be fluorescence or caloric energy (Altorfer, 1974). This protection mechanism is relevant for aromatic compounds and hydrocarbons (Blackburn et al., 1975).

(ii) The scavenger prevents the undesirable reaction at the target molecule by competing with the radicals, before they can interact with the target molecule. Charge transfers or electron exchanges occur. The remaining species are non-reacting (Blackburn et al., 1975), or only one species is left, as in the following example in an aqueous solution:

$$e^-_{aq.} + N_2O \rightarrow N_2 + OH^- + \dot{O}H$$

(iii) The scavenger forms a complex with the molecule to be protected and makes it less sensitive to irradiation or to attack by radicals (Schulman, 1973).

(iv) The scavenger reacts with the target molecule after it has been attacked by a radical, but before it has undergone chemical changes leading to irreversible damage. The target molecule resumes its original state and only the scavenger is changed. This process is called a repair mechanism (Alexander, 1962). Example $\dot{R}_1$ = radical, RSH = sulphur containing compound

$$\dot{R}_1 + RSH \rightarrow R_1H + R\dot{S}$$

$$2R\dot{S} \rightarrow RSSR$$

These reactions take place in solutions in what are known as reaction centers (spurs).

(v) Another possibility of protection is the exchange of oxygen for a non-reactive gas. All radicals stemming from oxygen, such as $H\dot{O}_2/\dot{O}_2^-$, H_2O_2 and HCOH, are eliminated. Example: Degassing of a solution with argon or nitrogen.

(vi) Neutralization reactions of acids and alkalies and the redox processes with hydrogen peroxide should also be mentioned, because they contribute to the stability of a molecule in solution.

Requirements for ideal scavengers

An ideal scavenger: (i) is more sensitive to the direct influence of irradiation than the molecule to be protected; (ii) reacts faster with the radicals than the molecule to be protected; (iii) does not react directly with the molecule to be protected itself; (iv) in case the molecule to be protected is attacked, the scavenger undoes all the damage; (v) is not toxic nor does it yield toxic reaction products; (vi) has no therapeutical influence; (vii) is protective in very small amounts.

Materials and Methods

The $^{60}_{27}$Co-γ-irradiation source was a Gammacell 220 (AECC), PSI, Würenlingen. The dose delivered was 25 kGy ± 10%, based on an approximate dose rate of 3 kGy/h. The temperature during irradiation was 25 ° C. The containers were 10 ml polyethylene vessels (Lupolen 1810 D) containing 5 ml solution. The outer container was a baccelite vessel to stabilize degassing conditions.

Medazepam (7-chloro-2,3-dihydro-1-methyl-5-phenyl-1H-1,4-benzodiazepine) was obtained from Hoffman-La Roche (Basle, Switzerland) and used without any further purification in this study. The solution to be irradiated was 2% in methanol (methanol-Merck zur Rückstandsanalyse with max. 0.1% water).

The scavengers were argon (Carbagas > 99.997%), ascorbic acid (Fluka, > 99.5%), nitrous oxide (Carbagas > 99.99%), potassium thiocyanate

(Fluka, > 99.0%), and thiourea (Fluka, > 99.0%). All chemicals were used without any further purification. Degassing conditions: 1–2 ml/min for 10 min through the solution and 10 min between the two vessels. Scavenger concentration: 0.01, 0.1 and 1 M according to solubility in methanol.

Analytical conditions

High-performance liquid chromatography was carried out with a Perkin Elmer liquid chromatograph series 3 B, LC-system Sigma 1 (integration control), UV-detector LC 75, autosampler 420 PE 10 μl (Rheodyn 7010), N_2 pressure 0.5 kg/cm^2 with a sample volume of 1 ml.

The conditions were: Column—Manufix-system (Merck) with Lichrospher®, RP-18, 5 μm, length 25 cm, diameter 4 mm, with precolumns PVDF Lichrospher®, RP-18, 5 μm, length 4 mm, diameter 4 mm. Mobile phase—methanol (Romil Chemie) containing 0.005 M nonylamine (Fluka)/water = (6 : 4) (degassed 10 min at 14.66 Pa), flow rate 1.4 ml/min, pressure (25–30 MPa according to the state of the column).

The conditions were: UV-detector, $\lambda = 230$ nm; sensitivity, 200; range, 0.04 A; injection volume, 10 μl medazepam 0.4%; chart speed, 5 mm/min with attenuation 0; integration method, area percent.

Gas chromatography was carried out on a Perkin Elmer gas chromatography system Sigma 1 (analyzer and integration control) with one wide-bore column (Permaphase CPMS 1701, length 25 m, inner diameter 0.53 mm, film thickness 1 μm. The carrier gas was nitrogen 45 (Carbagas) with a flow rate of 3 ml/min, and the detector was an FID.

The conditions were: Injector temperature, 270 ° C; detector temperature, 300 ° C; temperature program; 210 ° C, 10 ° C/min; 235 ° C, 18 min; 27 ° C/min; 260 ° C, 30 min; sensitivity, 200; chart speed, 5 mm/min with attenuation 1; injection volume, 1 μl medazepam 2% in methanol; integration method, area percent.

Thin-layer chromatography turned out to be insufficiently sensitive for the comparison of protected solutions. However, it was useful for the isolation and identification of the radiolytic products (Werner et al., 1989).

Results and Discussion

Effectiveness of the different scavengers

Table 1 lists the percentage recoveries of medazepam of the various solutions containing scavengers. There may be peaks which remained undetected due to the fact that they do not absorb in the UV region of 230 nm or are not separated by gas chromatography.

Thiourea seems to provide good protection, but the *G* value described does not take into consideration that the unirradiated solution with the scavenger is already decomposed. Thiourea (0.1 M) does not satisfy the requirement of having no direct influence on the molecule to be protected.

Table 2 presents the influence of a scavenger on the different radiolytic products of Fig. 2. In this context, 'scavenged' means that under the chosen

TABLE 1

G values (– medazepam) of the gas chromatography and high-performance liquid chromatography analysis

Scavenger	Concentration	*G* value	
		HPLC	GC
None	–	28.19	23.49
Argon	2×10^{-3} mol/l	8.34	13.69
Nitrous oxide	2×10^{-2} mol/l	9.94	16.32
Ascorbic acid	0.1 M	7.27	9.16
	0.01 M	10.37	12.37
Thiourea	0.1 M	8.09	7.52
	0.01 M	12.87	8.05
Potassium thiocyanate	0.1 M	8.48	13.19
	0.01 M	13.47	16.75

The *G* values are calculated according to Jacobs (1980):

$$\%\ \text{medazepam}_{\text{non-irradiated}} - \%\ \text{medazepam}_{\text{irradiated}}$$

$$= \Delta\ \text{medazepam}$$

$$G(-\text{medazepam})\ (\text{in molecules}/100\ \text{eV})$$

$$= \frac{\Delta\ \text{medazepam} \times 6.023 \times 10^{23} \times 100\ \text{molecules}/100\ \text{eV}}{100 \times 270.8 \times 2.5 \times 10^{6} \times 6.24 \times 10^{13}}$$

where 6.023×10^{23} molecules/mol^{-1} is Avogadro's number, 270.8 is the molecular weight of medazepam, and 25 kGy = 2.5 Mrad = $2.5 \times 10^{6} \times 6.24 \times 10^{13}$ eV g^{-1}.

TABLE 2

Influence of the scavengers on each radiolysis product

Radiolysis product	Argon	Nitrous oxide	Ascorbic acid (0.1 M)	Thiourea (0.1 M)	Potassium thiocyanate (0.1 M)
7-Chloro-5-phenyl-3H-1,4-benzodiazepine	decreases	decreases	scavenged	decreases	not affected
Reduced medazepam	increases	increases	scavenged	scavenged	decreases
2-Hydroxy-medazepam	decreases	decreases	decreases considerably	decreases	decreases
Diazepam	scavenged	scavenged	decreases	decreases	decreases
Desmethyldiazepam	scavenged	scavenged	scavenged	scavenged	decreases
5-Chloro-2-(methyl-amino)benzophenone	scavenged	not affected	scavenged	scavenged	decreased

analytical conditions no radiolytic product was detectable.

Figs 3–6 show ascorbic acid to be the best scavenger on the GC and HPLC chromatograms.

Protection mechanism of the scavengers

Argon

Solubility in water or methanol: approx. 2×10^{-3} mol/l (Scholes and Simic, 1964).

Reaction mechanism: this rare gas displaces oxygen when bubbled through a solution. The formation of $H\dot{O}_2$, $\dot{O}_2^-$, H_2O_2 and peroxides of methanol is prevented. (Jacob and Leupin (1974) used argon to protect chlordiazepoxide.)

Nitrous oxide

Solubility in water or methanol: $1.6–2.8 \times 10^{-2}$ mol/l at 25 °C according to the authors (Allen and Beck, 1964; Buxton, 1967; Loman and Blok, 1968; Behar et al., 1972).

Reaction mechanism: nitrous oxide is the most commonly used $e^-_{solv.}$ scavenger. It also displaces oxygen.

Reaction with $e^-_{solv.}$:

$$N_2O + e^-_{solv.} \rightarrow N_2\dot{O}^- \tag{1}$$

$$N_2\dot{O}^- + CH_3OH \rightarrow N_2 + \dot{O}H + CH_3OH^-$$

$$\big(k = 8.7 \times 10^9\ M^{-1}\ s^{-1} \text{ at pH} = 7 \quad \text{(Spinks and Woods, 1976)}\big) \tag{2}$$

98% of all $e^-_{solv.}$ are transformed to $\dot{O}H$ radicals (Hayon et al., 1970) in this way. In acid solution of water reaction 3 occurs

$$N_2\dot{O}^- + H_3O^+ \rightarrow N_2O + \dot{H}_3O \rightarrow N_2O + H_2O + \dot{H}$$

$$\text{(Hart and Anbar, 1970)} \tag{3}$$

The reaction with $\dot{H}$ is practically negligible

$$N_2O + \dot{H} \rightarrow N_2 + \dot{O}H$$

$$\big(k = 1 \times 10^5\ M^{-1}\ s^{-1} \quad \text{(Dainton and Logan, 1965)}\big) \tag{4}$$

Reaction with $\dot{C}H_2OH$

$$N_2O + \dot{C}H_2OH \rightarrow N_2 + HCHO + \dot{O}H \tag{5}$$

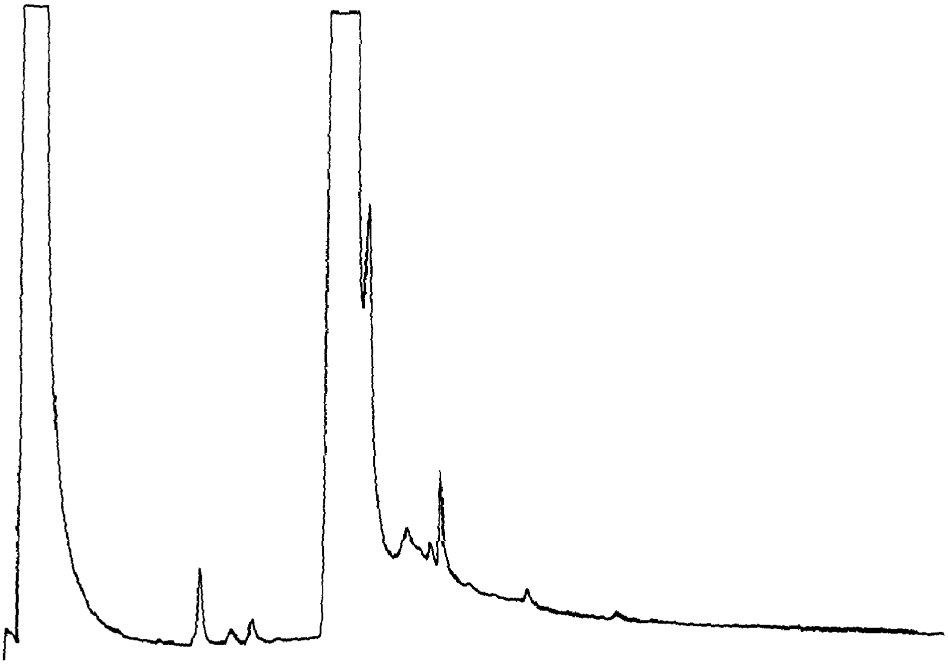
(according to Seki and Imamura, 1967; Teply and Habersbergerova, 1967). There is no reaction with $\dot{O}H$ (Nakken and Pihl, 1965).

Another reaction of nitrous oxide ions is

$$N_2\dot{O}^- + N_2O \rightarrow N_2\dot{O}_2^-$$

$$\text{(Hatano et al., 1973)} \tag{6}$$

However, the $N_2\dot{O}_2^-$ ion exists only $10^{-13}–10^{-9}$ s (Spinks and Woods, 1976). At an alkaline pH it decomposes rapidly into $\dot{O}H$ and N_2 (Nakken and Pihl, 1965).



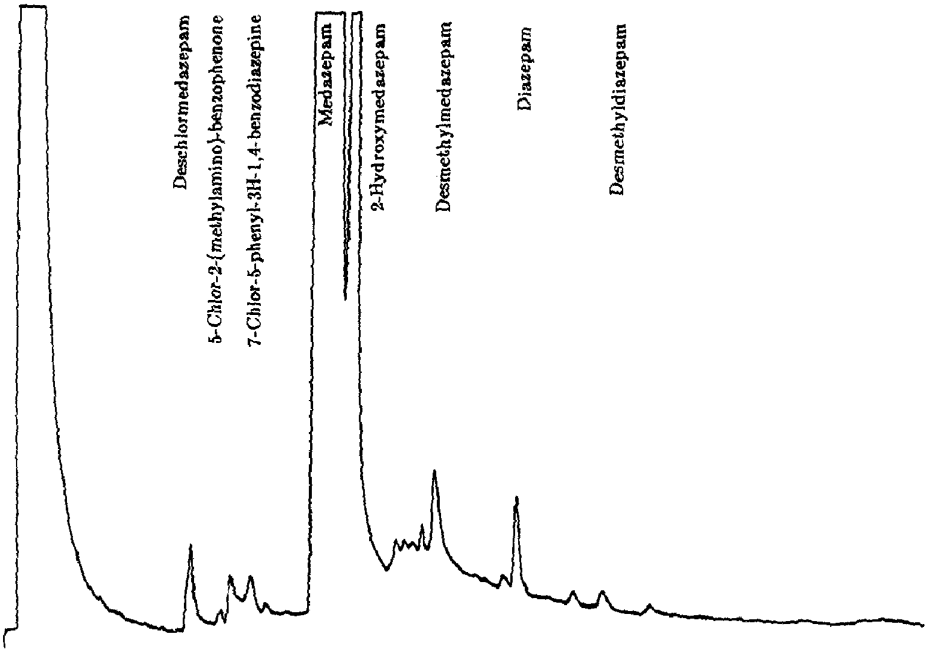
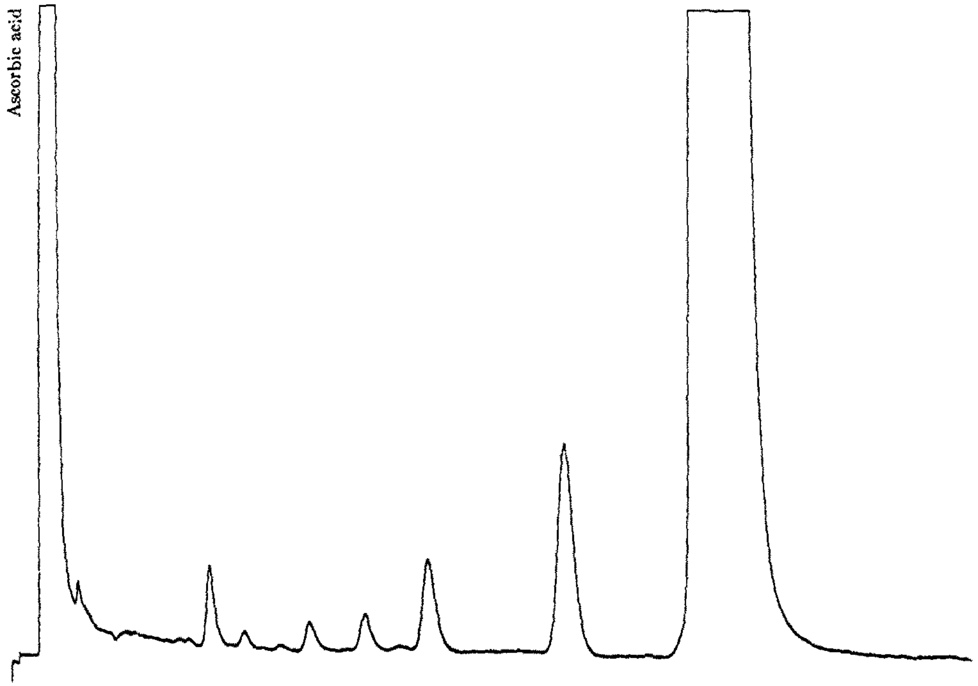


Fig. 3. GC chromatogram of an irradiated solution with ascorbic acid (0.1 M).

Fig. 4. GC chromatogram of an irradiated solution without scavengers.



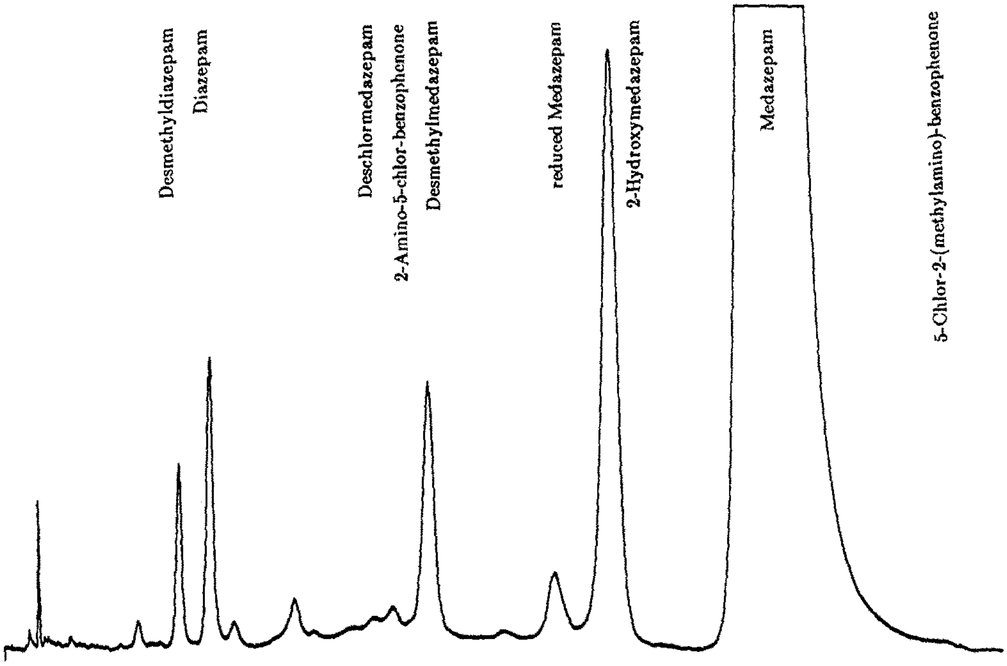


Fig. 5. HPLC chromatogram of an iradiated solution with ascorbic acid (0.1 M).

Fig. 6. HPLC chromatogram of an irradiated solution without scavengers.

Ascorbic acid

Solubility: 1 g dissolves in 30 ml alcohol (Merck Index, 1983).

Reaction mechanism: Switek and Modrzejewski (1976) and Pandula et al. (1970) examined ascorbic acid after γ-irradiation. They found that it undergoes decomposition into dehydroascorbic acid and diketogulene acid. (Abbreviations: AH_2, ascorbic acid; AH^-, ascorbate; A, dehydroascorbic acid.).

Reaction with $e^-_{\text{solv.}}$

$AH_2 + e^+_{\text{solv.}} \rightarrow AH^- + \dot{H}$

$(k = 10^9\ M^{-1}\ s^{-1})$ (7)

Reaction with $\dot{H}$

$AH_2 + \dot{H} \rightarrow \dot{A}H + H_2$

$(k = 3.6 \times 10^8\ M^{-1}\ s^{-1}$ (Anbar et al., 1975)) (8)

Reaction with $\dot{C}H_2OH$

$AH_2 + \dot{C}H_2OH \rightarrow \dot{A}H + CH_3OH$

$(k < 10^6\ M^{-1}\ s^{-1}$ (Swallow, 1981)) (9)

Reaction with $\dot{O}H$

$AH_2 + \dot{O}H \rightarrow \dot{A}H + H_2O$

$(k = 7.2 \times 10^9\ M^{-1}\ s^{-1}$ (Tobback, 1983)) (10)

Reactions in the presence of oxygen

$AH_2 + H\dot{O}_2 \rightarrow \dot{A}H^- + H_2O_2$

$(k = 1.6 \times 10^4\ M^{-1}\ s^{-1}$

(Cabelli and Bielski, 1983)) (11)

$AH_2 + \dot{O}_2^- \rightarrow \dot{A}^- + H_2O_2$

$(k = 10^{-7}\ M^{-1}\ s^{-1}$ (Cabelli and Bielski, 1983)) (12)

Reactions of ascorbic acid as acid

$AH_2 + OH^- \rightarrow AH^- + H_2O$

(neutralization reaction) (13)

$H^+ + e^-_{\text{solv.}} \rightarrow \dot{H}$

$(k = 2.2 \times 10^{10}\ M^{-1}\ s^{-1}$

(Spinks and Woods, 1976)) (14)

$H^+ + \dot{H} \rightarrow H_2$

$(k = 2.6 \times 10^3\ M^{-1}\ s^{-1}$

(Spinks and Woods, 1976)) (15)

Reactions of ascorbate

$AH^- + O_2 \rightarrow \dot{A}^- + \dot{O}_2^- + H^+ (\text{or } HO_2)$

(Douzou, 1958) (16)

$AH^- + \dot{O}H \rightarrow \dot{A}^- + H_2O$

$(k = 1.3 \times 10^{10}\ M^{-1}\ s^{-1}$

(Ross and Farhataziz, 1977)) (17)

in acid solution

$AH^- + O_2 + H^+ \rightarrow A + H_2O_2$

(Cabelli and Bielski, 1983) (18)

$AH^- + H\dot{O}_2 \rightarrow \dot{A}^- + H_2O_2$

$(k = 10^7\ M^{-1}\ s^{-1}$ (Cabelli and Bielski, 1983)) (19)

Reaction of the ascorbate radical

$A\dot{H} + O_2 \rightarrow A\dot{H}O_2 (\text{or } A + H\dot{O}_2)$

(Douzou, 1958) (20)

$$\mathrm{AH\dot{O}_2 + \dot{A}H \rightarrow 2A^- + H_2O_2}$$

(Rao, 1962) (21)

$$\mathrm{A\dot{H} + H_2O_2 \rightarrow A + H_2O + \dot{O}H}$$

(Rao, 1962) (22)

Thiourea

Solubility: 1 part dissolves in 11 parts alcohol (Merck Index, 1983).

Reaction mechanism: thiorea is a scavenger which, among others, exhibits a repair mechanism by providing an $\dot{\mathrm{H}}$ for a damaged molecule. It exists in thioketo or thioenol form in solution (Cramp, 1966). Natsvlishvili et al. (1983) observed that thiourea decomposes under the influence of $\dot{\mathrm{O}}\mathrm{H}$; $\dot{\mathrm{H}}$ and e^- are split off. (Abbreviation: $\mathrm{NH_2NHC-SH}$, thiourea).

Reaction with $e^-_{\mathrm{solv.}}$

$$\mathrm{NH_2NHC-SH} + e^-_{\mathrm{solv.}} \rightarrow \mathrm{NH_2NHC-S^-} + \dot{\mathrm{H}}$$

$(k = 2.9 \times 10^9\ \mathrm{M^{-1}\ s^{-1}}$ (Hart and Anbar, 1970)) (23)

Reaction with $\dot{\mathrm{H}}$

$$\mathrm{NH_2NHC-SH} + \dot{\mathrm{H}} \rightarrow \mathrm{NH_2NHC-\dot{S}} + \mathrm{H_2}$$

$(k = 6 \times 10^9\ \mathrm{M^{-1}\ s^{-1}}$ at $\mathrm{pH} = 1$ (Anbar et al., 1975)) (24)

Reaction with $\dot{\mathrm{C}}\mathrm{H_2OH}$

$$\mathrm{NH_2NHC-SH} + \dot{\mathrm{C}}\mathrm{H_2OH} \rightarrow \mathrm{NH_2NHC-\dot{S}} + \mathrm{CH_3OH}$$

(Cohen and Lam, 1971) (25)

Reaction with $\dot{\mathrm{O}}\mathrm{H}$

$$\mathrm{NH_2NHC-SH} + \dot{\mathrm{O}}\mathrm{H} \rightarrow \mathrm{NH_2NHC-\dot{S}} + \mathrm{H_2O}$$

$(k = 4.7 \times 10^9\ \mathrm{M^{-1}\ s^{-1}}$ (Gutteridge, 1987)) (26)

Reaction of the thiourea radical

$$\mathrm{NH_2NHC-\dot{S}} + \mathrm{NH_2NHC-\dot{S}} \rightarrow \mathrm{NH_2NHC-SS-CNHNH_2} \qquad (27)$$

$\mathrm{NH_2NHC-SS-CNHNH_2}$ can continue reacting:

$$\mathrm{NH_2NHC-SS-CNHNH_2} + e^-_{\mathrm{solv.}} \rightarrow [\mathrm{NH_2NHC-\dot{S}S-CNHNH_2}]$$

(Adams, 1967) (28)

Alexander (1962) observed that a high concentration of a sulphuric scavenger is necessary for the repair mechanism to prevent the reaction of radicals with oxygen.

Potassium thiocyanate

Solubility: soluble in 12 parts alcohol (Merck Index, 1983).

Reaction mechanism: potassium thiocyanate is a classic $\dot{\mathrm{O}}\mathrm{H}$ scavenger and is widely used in dosimetry.

Reaction with $e^-_{\mathrm{solv.}}$

$$\mathrm{SCN^-} + e^-_{\mathrm{solv.}} \rightarrow [\mathrm{SC\dot{N}}]^{2-}$$

$(k < 10^6\ \mathrm{M^{-1}\ s^{-1}}$ (Anbar et al., 1973)) (29)

Reaction with $\dot{\mathrm{H}}$

$$\mathrm{SCN^-} + \dot{\mathrm{H}} \rightarrow \mathrm{HSC\dot{N}^-}$$

$(k = 2.7 \times 10^8\ \mathrm{M^{-1}\ s^{-1}}$ (Anbar et al., 1975)) (30)

Reaction with $\dot{\mathrm{O}}\mathrm{H}$

$$\mathrm{SCN^-} + \dot{\mathrm{O}}\mathrm{H} \rightarrow \mathrm{SC\dot{N}OH^-}$$

[fast] (Hunt et al., 1975) (31)

$$\mathrm{SC\dot{N}OH^-} \rightleftarrows \mathrm{SC\dot{N}} + \mathrm{OH^-} \qquad (32)$$

and/or

$SC\dot{N}OH^- + SCN \rightleftarrows [SC\dot{N}]_2^- + OH$

$(k = 2 \times 10^5\ M^{-1}\ s^{-1})$

$(k(\dot{O}H + SCN^-) = 1.1 \times 10^{10}\ M^{-1}\ s^{-1}$

(Spinks and Woods, 1976)) (33)

Reactions of the thiocyanate radical

$SC\dot{N} + SCN^- \rightarrow [SC\dot{N}]_2^-$ (34)

$[SC\dot{N}]_2^-$ can react further

$2[SC\dot{N}]_2^- \rightarrow 2SCN^- + [SCN]_2$

(Spinks and Woods, 1976) (35)

In conclusion, it can be said that a 2% (= 0.08 M) methanolic solution is best protected with 0.1 M ascorbic acid. This fact confirms the theoretical considerations above in the summary of the protection of the scavengers on the influenced and remaining radicals and molecules. Because slight radiolytic decomposition can still be observed in the chromatograms, reactions occur with a k value greater than $10^{10}\ M^{-1}\ s^{-1}$. Full protection is therefore possible if the scavenger reacts quickly enough with all radicals and noxious molecules.

It would be of advantage if the scavenger concentrations were lower. The scavengers examined, however, were only useful at concentrations of 0.1 M, and not 0.01 M. 1 M in methanolic solution was possible with thiourea and potassium thiocyanate, but decomposition was observed in the non-irradiated solution.

Summary of the Protection of the Scavengers Examined

Scavenger	radical or molecule influenced (scavenged)	remaining radicals or molecules
Argon	O_2, $H\dot{O}_2/\dot{O}_2^-$	$\dot{O}H$, $e^-_{solv.}$, $\dot{H}$, $\dot{C}H_2OH$
Nitrous oxide	O_2, $H\dot{O}_2/\dot{O}_2^-$, $e^-_{solv.}$	$\dot{O}H$, $\dot{H}$, $\dot{C}H_2OH$, H_2
Ascorbic acid	$\dot{O}H$, $e^-_{solv.}$, $\dot{H}$, $\dot{C}H_2OH$, $H\dot{O}_2/\dot{O}_2^-$	O_2, H^+
Thiourea	$\dot{O}H$, $e^-_{solv.}$, $\dot{H}$	O_2, $H\dot{O}_2/\dot{O}_2^-$, $\dot{C}H_2OH$
Potassium thiocyanate	$\dot{O}H$, $\dot{H}$	O_2, $H\dot{O}_2/\dot{O}_2^-$, $e^-_{solv.}$, $\dot{C}H_2OH$

TABLE 3

Formation and prevention of the radiolytic products

Radiolysis product	Formed by radical	Formation and prevention
7-Chloro-5-phenyl-3H-1,4-benzodiazepine	$e^-_{solv.}$, $\dot{H}$	appears and disappears in connection with desmethylmedazepam
Deschloromedazepam	$e^-_{solv.}$, $\dot{H}$	is not totally scavengable; increases with thiourea
Desmethylmedazepam	$e^-_{solv.}$, $\dot{H}$	difficult to detect properly
Reduced medazepam	$e^-_{solv.}$, $\dot{H}$	well scavenged by ascorbic acid and thiourea
Diazepam	$\dot{O}H$, $H\dot{O}_2^-/\dot{O}_2^-$	best protected by argon and nitrous oxide
Desmethyldiazepam	$\dot{O}H$, $H\dot{O}_2^-/\dot{O}_2^-$	reacts like diazepam; best prevented by ascorbic acid and thiourea
2-Hydroxymedazepam	$\dot{O}H$, $H\dot{O}_2^-/\dot{O}_2^-$	not totally prevented by any scavenger but decreased by all scavengers
5-Chloro-2-(methylamino)benzophenone	$\dot{O}H$, $H\dot{O}_2^-/\dot{O}_2^-$, H^+	always together with 2-hydroxymedazepam

While full protection was not achieved, the radiolytic reaction scheme could be proved (Table 3). Our ongoing research is aimed at combining two or more scavengers in order to improve the protection of medazepam.

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