RADIATION RESEARCH **162**, 616–622 (2004)
0033-7587/04 $15.00

Attempts at Correlation of the Radiolytic Species of Irradiated Solid-State Captopril Studied by Multi-frequency EPR and HPLC

Alix Engalytcheff,[a,1] René Debuyst,[b] Gauthier C. A. M. Vanhaelewyn,[c] Freddy J. Callens[c] and Bernard Tilquin[a]

[a] *Laboratory of Chemical and Physicochemical Analysis of Drugs (CHAM), UCL, B.1200 Brussels, Belgium;* [b] *Laboratory of Biomedical Magnetic Resonance (REMA), UCL, B.1200 Brussels, Belgium; and* [c] *Department of Solid State Sciences, UGent, B.9000 Ghent, Belgium*

Engalytcheff, A., Debuyst, R., Vanhaelewyn, G. C. A. M., Callens, F. J. and Tilquin, B. Attempts at Correlation of the Radiolytic Species of Irradiated Solid-State Captopril Studied by Multi-frequency EPR and HPLC. *Radiat. Res.* 162, 616–622 (2004).

The purpose of this study was to provide insight into the processes that occur after the irradiation of solid-state drugs. Electron paramagnetic resonance (EPR) experiments were performed at two different frequencies, X-band (about 9.5 GHz) and Q-band (about 34 GHz), to identify the radicals present in irradiated captopril. The results confirmed that an irradiated drug can trap several main radicals. Moreover, the radical composition varied as a function of the treatment. In addition, non-volatile final products were studied by liquid chromatography coupled to UV and to mass spectrometry (LC-MS). The variation of the radical composition did not influence the profile of the final products; this appears to indicate that, in the case of captopril, the trapped radicals observed by EPR are not the main precursors of the final products. Finally, high-performance liquid chromatography data appear to indicate that radiosterilization of captopril is feasible. © 2004 by Radiation Research Society

INTRODUCTION

Radiosterilization has been investigated widely since this technique may be able to sterilize drugs with an efficiency as good as that of the present reference method (steam) (*1*, *2*). Since irradiation does not induce a significant rise in the temperature of the drug (*3*), thermosensitive drugs that currently require alternative sterilizing methods (e.g. filtration) could be sterilized efficiently using this technique. In addition, it would have the advantage of being terminal, which means the drug could be sterilized in its packaging, a quality now requested by the European Agency for the Evaluation of Medicinal Products (EMEA) (*4*). However, the damage produced by irradiation of solid-state drugs is not clearly defined, and the general lack of knowledge of the mechanisms and effects of radiosterilization prevents its widespread use.

The most studied phenomenon in irradiated solid-state substances is the trapping of radicals in the matrix (*5*). Electron paramagnetic resonance (EPR) is the analytical tool of reference for work on radicals. It is very sensitive and allows paramagnetic species to be detected down to 10^{-9} *M* (*6*). The literature focuses on the detection of irradiated drugs (*7*) or the decay (*8*) or quantification (*9*) of radicals, and very few articles deal with the identification of the radicals (*10*, *11*).

The identification of radicals is indeed a real challenge in the case of irradiated solids. The resulting EPR spectrum is often composite since several types of radicals can be trapped (*12*, *13*). Moreover, polycrystalline or amorphous solids such as drugs give powder EPR spectra, which means that the spectrum is usually poorly resolved with broad features, and therefore qualitative information is much more difficult to extract. Nevertheless, their identification could provide a better understanding of the mechanisms of radiolysis by enabling a qualitative assessment of the radicals and the products of radiolysis (i.e. the final products). Such comparisons have been attempted previously from a quantitative point of view (*14*).

In this study, we investigated the radical effects after the irradiation of captopril, an anti-hypertensive drug. First, we attempted to identify the radicals, using a higher frequency (Q-band: ~34 GHz) in addition to the classical X-band (~9.5 GHz). High-field EPR increases the spectrum resolution and hence allows more information to be retrieved (*15*, *16*). Its efficiency for identifying radicals in drugs has already been shown (*17*). Additional changes in the EPR spectra are obtained by varying the experimental parameters such as the microwave power or the acquisition temperature (*18*).

The second part of the study dealt with the final products. After dissolution of irradiated drugs in water, which is a necessary step for their administration, radicals disappear and final products can be observed by high-performance liquid chromatography (HPLC). To determine whether there was a correlation between the radicals and the final products, the powders studied by EPR were dissolved and

[1] Address for correspondence: Unité CHAM, School of Pharmacy—UCL, Avenue Mounier, 72.30, B-1200 Bruxelles, Belgium; e-mail: alix.engalytcheff@cham.ucl.ac.be.

analyzed further by LC-UV-MS. The variations in the EPR profiles were compared with the HPLC data.

Finally, some quantitative data are reported to give an idea about the feasibility of the radiosterilization of captopril.

MATERIAL AND METHODS

Captopril was purchased from Sigma. It meets the U.S. Pharmacopoeia specifications. The water content is low (<1%).

Treatment of the Sample (Irradiation and Photobleaching)

All irradiations were performed at room temperature in air without vacuum treatment.

Gamma rays were produced by a ^{60}Co panoramic chamber [UCL (CHIM), Louvain-La-Neuve]. This source was calibrated by alanine dosimetry; alanine pellets were supplied and analyzed by Risø National Laboratory (Denmark), which enabled the dose rate to be estimated as 7 Gy min^{-1}.

The X-ray source was a Philips tungsten anti-cathode X-ray tube (UGent, Ghent, Belgium). The maximum voltage that can be applied to the tube is 100 kV, and the maximum anode current is 75 mA. The X-ray tube was usually operated at 60 kV and 40 mA. The dose rate under these conditions was ~1300 Gy min^{-1}.

The electron beam facility used was a double-beam linear electron accelerator (LINAC) (Mölnlycke, Waremme, Belgium). The beam power of each electron generator is ~20 kW. The accelerated electrons were delivered in pulses of 474 and 478 Hz, respectively. The dose of 45 kGy was given in a few seconds; the dose rate of the double LINAC is of the order of 10^6 Gy min^{-1}. An internal standard, a polymethylmethacrylate (PMMA) film, was used to monitor the dose delivered by being irradiated together with the sample. Its absorbency was measured afterward.

The photobleaching treatment was performed with an Allen type A 409 system, whose lamp has an emission spectrum in the ultraviolet and the visible region.

EPR Spectrometers and Settings

The X-band EPR spectra were taken with a Bruker EMX-8/2.7 spectrometer. The magnetic field was measured by a Bruker ER 036 TM NMR gaussmeter and the microwave frequency by a Bruker EMX 040-1161.8A frequency counter. All spectra were recorded at room temperature. The EPR spectrometer settings were as follows: modulation frequency, 100 kHz; time constant, 20.43 ms; conversion time, 10.24 ms; resolution, 4096; number of scans, 1; modulation amplitude, 0.1 mT.

The Q-band EPR spectra were taken with a Bruker Elexsys E500 spectrometer. The magnetic field was measured by a Bruker ER035M NMR gaussmeter and the microwave frequency by an EIP 548B frequency counter. Two sets of experiments were performed: one at room temperature and the other at 100 K. The EPR spectrometer settings were as follows: modulation frequency, 87.5 kHz; time constant, 81.92 ms; conversion time, 81.92 ms; resolution, 1024; number of scans, 3; modulation amplitude, 0.2 mT; sweep width 50 mT.

The receiver gain was adapted for each spectrum. The absolute g values were determined by comparison with a Bruker reference: 2,2-diphenyl-1-picrylhydrazyl (DPPH) powder sample (g = 2.0036). Data were treated using Bruker WinEPR® and simulations were performed with Bruker SimFonia®.

LC-UV

The HPLC system consisted of a Merck-Hitachi D-6000 equipped with a Rheodyne manual injector with a loop of 20 μl, two pumps (L-6200), an oven (T-6300), and a UV-visible diode array detector (L-4500).

The chromatographic separation was performed on a 250 × 4-mm LiChrospher® RP-8 Select B column, 5-μm particle size (Merck, Darmstadt, Germany), at a flow rate of 1 ml/min. The mobile phase consisted of a mixture of HPLC-grade acetonitrile and a 0.1% (v/v) formic acid solution (20:80 v/v).

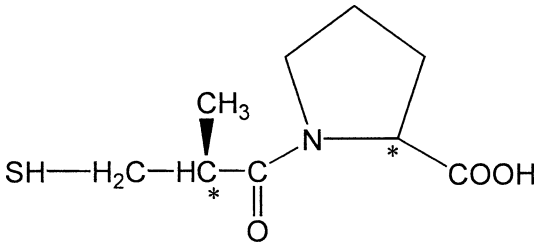

FIG. 1. Molecular structure of captopril. The asterisks show the tertiary carbons.

LC-MS

The solutions analyzed were composed of 20,000 ppm of irradiated captopril. The chromatograph consisted of a Merck Hitachi L-6200 pump equipped with a MIDAS autosampler. The mass detector was an LCQ® Advantage instrument with an electrospray ion source controlled by the Xcalibur software (Finnigan MAT). The system also included an HPLC 332 UV-visible detector from Kontron.

The chromatographic separation was performed on a 250 × 2-mm LiChrospher® RP Select B column, 5-μm particle size (Merck), at a flow rate of 250 μl min^{-1}. The mobile phase consisted of a mixture of HPLC-grade acetonitrile and a 0.1% (v/v) formic acid solution (10:90 v/v).

X-Ray Diffraction

X-ray diffraction data were collected using a Siemens D500 X-ray diffractometer [UCL (CEAN), Louvain-La-Neuve] with the following instrument parameters: radiation CuK_2 (λ = 1.5418 Å) with a nickel filter, power 45 kV, 35 mA; curved graphite monochromator on diffracted beam; detector: scintillation, scan range 4–90° (2θ); step size: 0.02°, count time, 2.4 s/step. The external standard was silicon (standard reference material SRM640b). The samples were sprinkled onto a zero-background quartz plate (Gem Dugout). During data collection, the sample was rotated using a sample spinner. The data were collected and processed by Socabin-Siemens Diffract-AT®.

RESULTS

X-Ray Diffraction

The molecular structure of captopril is shown in Fig. 1. A preliminary study of the crystalline structure of captopril was performed by X-ray diffraction. Comparison with the X-ray diffraction spectra of the two polymorphic forms of reference described by Kadin (*19*) indicated that it was the high-melting polymorph. Concerning the intensities of the peaks, the peaks representing the l plane are more intense, demonstrating that the sample was not isotropic but was needle-shaped. The study of the full widths at half maximum (FWHM) gave a mean value of about 0.15, which suggests that the powder was rather crystalline.

X- and Q-Band EPR

Captopril powder was irradiated with different types of radiation: X rays, electron beams and γ rays. Figure 2

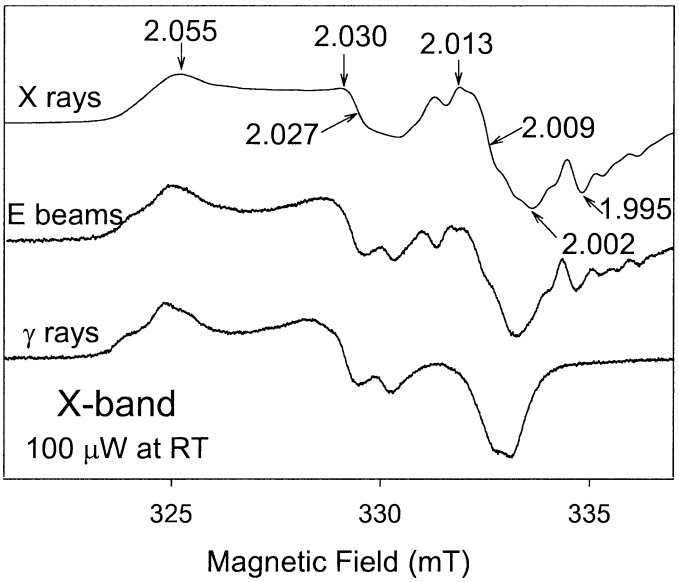

FIG. 2. X-band EPR spectra of captopril irradiated with different types of radiation (X rays, electron beams and γ rays) at 40 kGy. The spectra were recorded at room temperature (RT) with a low microwave power (100 μW).

shows the resulting X-band EPR spectra of the captopril irradiated with a dose of 40 kGy. All the spectra were recorded at room temperature at a microwave power of 100 μW. They are very wide spectra, extending to a sweep width of 15 mT, resulting in *g* values that are rather high for organic radicals (up to 2.055). The lines are broad and superimposed, leading to composite, badly resolved spectra that are difficult to interpret completely. Therefore, EPR measurements were undertaken at a higher frequency to better separate the different components and to distinguish between possible *g* anisotropy and hyperfine structures. Magnetic-field splittings between the *g* components are known to increase linearly with the microwave frequency, whereas hyperfine splittings remain approximately constant. Figure 3 shows the Q-band EPR spectrum of captopril recorded at room temperature. The sample was irradiated with X rays at 120 kGy, a dose far higher than the reference dose of radiation (25 kGy) that ensures drug sterility (*1, 2*). In Q-band, the signal heights are reduced as the spectrum is spread over a larger magnetic-field range. Therefore, the dose had to be increased to 120 kGy to obtain a good signal-to-noise ratio. For X-band, a reasonable spectrum could be recorded with smaller doses. No significant changes in the spectra were observed with different doses (from 40 to 120 kGy). Figure 3A shows the Q-band spectrum for a microwave power of 10 μW, which allows comparison with the upper X-band spectrum of Fig. 2. It shows that the spectrum is qualitatively modified in Q-band. It now extends over more than 35 mT. This enables one to observe the lines more distinctly, especially between $g = 2.025$ and $g = 2.060$.

Another spectrum was taken at a higher microwave power (160 mW) and is shown in Fig. 3B. The left part of the

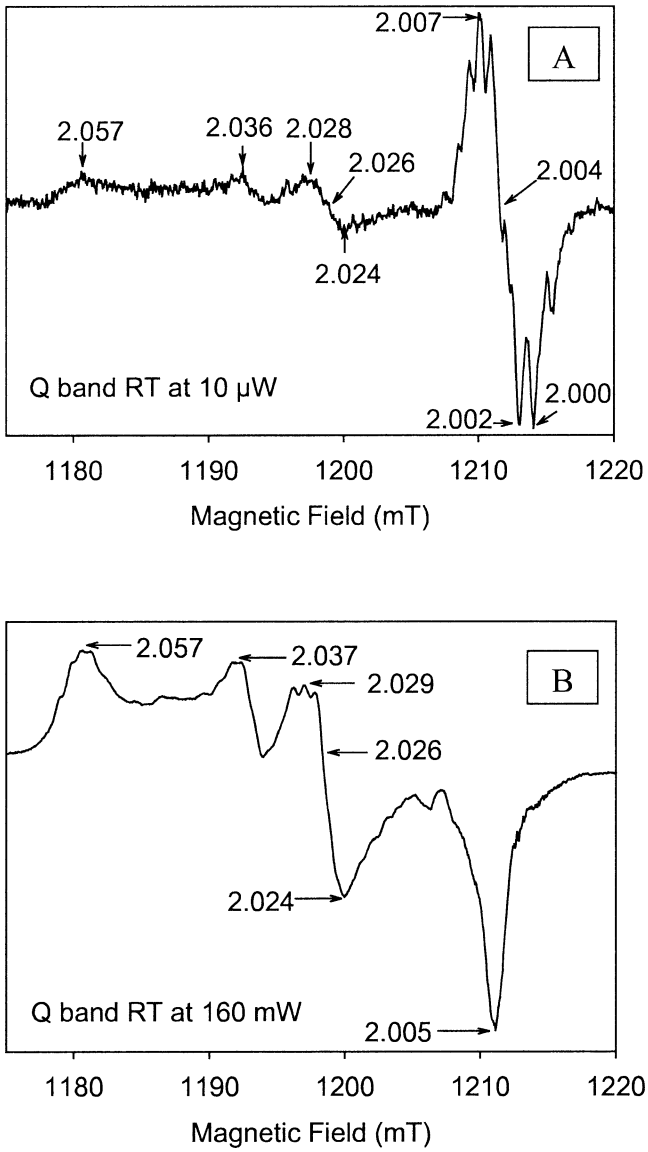

FIG. 3. Q-band EPR spectra of captopril irradiated with X rays at 120 kGy. Spectra were recorded at room temperature (RT) at low (10 μW) and high (160 mW) microwave power (panels A and B, respectively).

spectrum responded differently to the increase in microwave power when compared with the right part (1205–1220 mT); it increased with the microwave power, in contrast to the right part. This indicates that these parts of the spectrum belong to different radicals. The spectrum contributions between 1205 and 1220 mT saturated more rapidly with microwave power than in the field region between 1175 and 1205 mT.

This attribution of the right part of the spectrum to at least one radical was confirmed by two other experiments. The first was the comparison of the X-band spectra of Fig. 2, which were all recorded under the same conditions, the only difference being the type of irradiation. The aforementioned easily saturated species, around $g = 2.004$, was obviously missing after γ irradiation but not after X-ray or electron-beam treatment. The major difference between

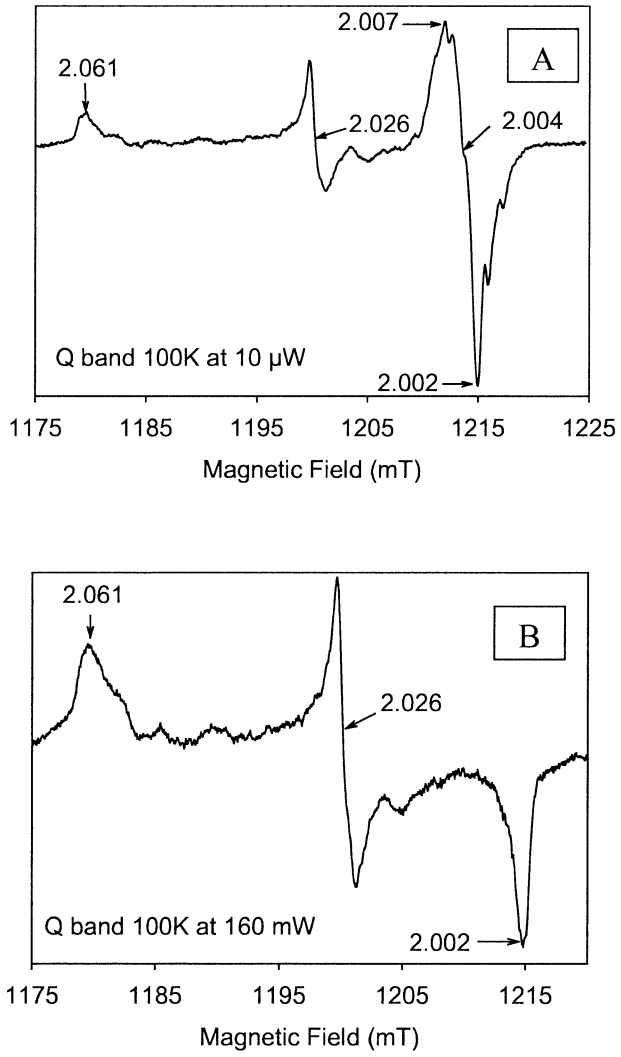

FIG. 4. Q-band EPR spectra of captopril irradiated with X rays at 120 kGy at 100 K. The experimental spectrum (panel A) was recorded at 100 K with a low microwave power (10 μW). The other experimental spectrum (panel B) was recorded at 100 K with a high microwave power (160 mW). It corresponds to a spectrum typical for perthiyl radicals (RSS•).

these types of radiation is the dose rate; it was far lower for γ rays. Finally, a last test enabled us to make this species disappear. A simple heat treatment to 50°C for 2 h transformed the X-band spectrum obtained after X irradiation into that obtained for γ rays. No other visible changes occurred in the rest of the EPR spectrum. Hence it is evident from these results that an additional radical was present in the right part of the spectrum. Moreover, two of its properties were characterized: its thermosensitivity and its easy saturation at moderate microwave powers (few mW). Since this could therefore be eliminated at least in part, it allowed us to focus on the rest of the spectrum.

Complementary data were obtained by lowering the recording temperature. Q-band spectra of irradiated captopril were recorded at 100 K (Fig. 4). The EPR spectrum recorded at 10 μW is given in Fig. 4A and can be compared to that shown in Fig. 3A. The measurements at low temperature allowed us to simplify the spectrum. The part between $g = 2.025$ and $g = 2.060$, which was present in Q-band at room temperature, disappeared. Figure 4B, which shows the spectrum recorded at 160 mW, is very interesting. By eliminating one spectral component, thanks to the low temperature and the easy saturation of the radical, this new experiment enabled us to isolate another radical. This spectrum can be described satisfactorily in terms of rhombic g anisotropy without hyperfine structure.

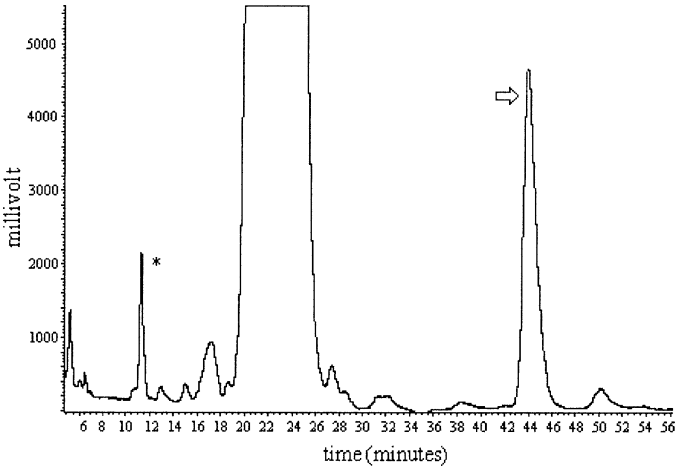

FIG. 5. HPLC separation of the radiolytic products of captopril irradiated with γ rays at 25 kGy. The chromatogram was taken at 220 nm. The asterisk shows the peak that is diminished in the photobleached captopril. The arrow shows the major impurity.

LC-MS

The analysis of the impurities of captopril was performed by liquid chromatography. No impurity was detected in the nonirradiated drug. After γ irradiation at 25 kGy, the final products were observed. Their chromatographic separation is shown in Fig. 5. They were detected by both UV spectrometry and by mass spectrometry (MS), allowing a complete qualitative study. The methods are indeed complementary. Some final products could very easily be observed with UV spectrometry and not with MS and vice versa. Moreover, co-eluting peaks, which are not separated with UV spectrometry, can be distinguished by MS. The chromatogram obtained for electron-beam-irradiated captopril was the same, and several peaks were detected. Only one subtle variation was observed for the irradiated captopril that was photobleached; the peak (marked with an asterisk) is smaller in this case, corresponding to an *m/z* value of 184. Nevertheless, it was only diminished and not absent.

LC-UV

To evaluate the degradation of the product after irradiation at 25 kGy simply and rapidly, we studied the concentration of the main compound (test of content). The analysis was performed by HPLC coupled to a UV-light detector to determine whether there was a loss of the irradiated sample

TABLE 1
Non-exhaustive List of *g* Values of Perthiyl and Peroxyl Thiyl Radicals Found in the Literature[a]

	g_z	g_y	g_x	Reference	Radical
RSS·					
	2.05–2.06	2.02–2.03	2.00	*20*	in general
	2.060	2.025	2.002	*21*	in BSA[b] film
	2.058	2.025	2.002	*21*	in lyophilized BSA[b]
	2.061	2.026	2.002	*21*	in cysteine polycryistalline powder
	2.056	2.026	2.0019	*22*	$HOOC\text{-}CH(NH_2)\text{-}CH_2\text{-}SS^{\cdot}$
	2.058	2.026	2.0014	*22*	glutathione perthiyl
	2.056	2.025	2.0022	*22*	$HOOC\text{-}CH(NH_2)\text{-}C(CH_3)_2\text{-}SS^{\cdot}$
	2.061	2.026	2.002	this work	R1
RSOO·					
	2.034	2.006 ($g_\perp$)		*21*	in lyophilized BSA[b]
	2.035	2.0025 ($g_\perp$)		*24*	$CH_3COSOO^{\cdot}$
	2.034	—	—	*25*	$(CH_3)_3CSOO^{\cdot}$
RC·					
		2.004 (isotropic *g* value)		this work	R2 radicals

Note. Data found for the radicals in this work are included.
[a] The recording temperature and the matrix are not the same.
[b] Bovine serum albumin.

by comparing the area under the curve of the peak of the active compound for the nonirradiated and the irradiated drug. The area under the curve of the peak of the nonirradiated drug was considered to be 100%. The standard deviation obtained was 0.5%. The area under the curve of the peak of the irradiated drug was then compared to that of the nonirradiated drug and a comparative percentage was obtained. The area under the curve for captopril irradiated with γ rays corresponded to 100.5% (± 0.2) and was 100.3% (± 0.9) for irradiation with electron beams.

DISCUSSION

Radical R1: Perthiyl Radical

The radical responsible for the spectrum in Fig. 4B will be called R1. The *g* values read from the experimental spectrum are 2.061, 2.026 and 2.002. These values are very characteristic, because such a high g_z value is seldom observed for organic radicals. In fact, they match the values proposed in the literature for perthiyl radicals (RSS·) (*20–22*). A nonexhaustive list of these is given in Table 1. It is worth mentioning that these values were previously, and incorrectly, attributed to thiyl radicals (RS·) (*23*). They would be the result of a succession of well-established reactions (*20*), which transform the thiyl into the perthiyl radical. The irradiation of captopril would lead initially to a thiyl radical. The abstraction of a hydrogen atom is the most observable effect that can occur in an irradiated solid. The complexing of a thiyl radical with a molecule of captopril then engenders an intermediary adduct, which is finally transformed into a perthiyl radical.

Radical R2: Carbon-Centered Radicals

R1 appears to be the major component in the EPR spectra of captopril at high microwave powers. At lower powers, a second signal is observed at high fields, and the corresponding radical will be called R2. It appears to be isotropic with a *g* value around 2.004, which corresponds to values observed for carbon-centered radicals. This assignment is confirmed by its easy power saturation. Carbon-centered radicals saturate at lower powers in comparison with sulfur-centered radicals (*21*).

From a radiolytic point of view, its absence at very low dose rates can be explained by a better diffusion of oxygen. Radicals would have time to react with oxygen. In time, the resulting highly reactive oxygenated species would react further, and the radical character would be lost and therefore would no longer be observed.

The most likely carbon-centered radicals that could be formed after the irradiation of captopril powder are two radical species derived from the abstraction of a hydrogen atom on the tertiary carbons (carbons with an asterisk in Fig. 1). These radicals would be the most stable. In addition, the hydrogen can be considered as acidic since the carbon is close to a carbonyl function. R2 could therefore be the resulting spectrum of these two species. Other carbon-centered radicals could be subjacent.

Other Radicals

The part of the spectra between the components of RSS· at low field (see Fig. 3) remains unexplained. Hence it establishes the presence of other radicals. This part indeed varies from one spectrum to another, suggesting that more than one radical is involved.

The unidentified line around 2.035–2.037 possesses a rather high *g* value, which is typical of the g_{max} of peroxyl radicals (*24, 25*) (see Table 1). Even though the line shape does not look like a g_{max} singularity, this hypothesis was suggested since thiyl peroxyl radicals are species that are frequently encountered in the radiolysis of sulfur com-

pounds when a thiyl radical is produced in the presence of oxygen.

A classic test to confirm the presence of a thiyl peroxyl radical is the photobleaching of the sample, which should transform $RSOO^{\cdot}$ into $RSO_2^{\cdot}$ (*26*). This test was performed, and the resulting spectrum at room temperature indeed showed discrepancies with the initial one. The lines of $RSS^{\cdot}$ as well as those around 2.035–2.037 diminished, whereas a line appeared around 2.044. In addition, the resulting spectrum at 100 K also exhibited differences. A signal can indeed be observed at $g = 2.010$. Nevertheless, this value is normally too high for a sulfonyl radical, which should be around $g = 2.005$ (*26*). Thus changes are observed after photobleaching but neither the disappearance of $RSOO^{\cdot}$ nor the production of $RSO_2^{\cdot}$ can be proved convincingly.

In conclusion, the identification is open to doubt. The features at low field, in addition to the fact that they do not saturate as a function of the microwave power, suggest that the spin of these radicals might be carried by a hetero-atom.

Evaluation of the Degradation of the Main Compound

The percentages obtained by the test of content give an estimation of the sensitivity of captopril radiation. It appears to be rather radioresistant. The difference between the irradiated and the nonirradiated drug is not significant, which indicates that the induced changes must be weak. Hence the radiolysis of captopril produced trace products. This explains why the analyses are tricky. In addition, it appears that the type of radiation has no effect on the results. From these tests on the main compound, it appears that radiosterilization of captopril by either γ or electron-beam irradiation is conceivable. A complete specific study concerning its feasibility should nevertheless be performed.

Study of the Non-volatile Final Products and Correlation with the Trapped Radical Species

The UV chromatographic profile of the irradiated captopril shows the production of several radiolytic products. The MS coupling reveals few and weak co-elutions. In comparison with previous studies on other drugs, the number of impurities is quite low. In addition, the concentration of the major peak (indicated by an arrow in Fig. 5) is below 0.001% of the captopril peak. The quantification was performed with the same procedure used for the study of the final products from β blockers (*14*); in this case, the percentages could reach 0.1%. Hence these results confirm the test of content and support the feasibility of the radiosterilization of captopril.

The chromatograms for the different types of radiation are similar, which is also in accordance with the test of content. One would expect to observe some dose-rate effect. The reactions involved in each case should indeed be different. Hence other hypotheses need to be considered such as the local thermal fusion of solid micro-zones during the high-dose-rate radiolysis.

Thus, from a qualitative point of view, no changes are observed in the chromatograms of different solutions of irradiated captopril. The profile of non-volatile final products is not modified although the EPR spectra of the samples were different. It seems, therefore, that there is no direct relationship between the EPR spectra and the liquid chromatograms. One possibility that could be considered is that the concentration of radicals seen by EPR is too small to yield a noticeable change in the peak heights of the end products in the chromatogram. Hence, in the case of captopril, the contribution of the trapped radicals to the final products is negligible. This is in accordance with the conclusion drawn after attempts at quantitative correlations for β blockers (*14*). Nevertheless, radical mechanisms cannot be totally excluded. Since the captopril was irradiated at room temperature, it is indeed possible that a large fraction of the radiation-induced radicals will have reacted and only those that have been sufficiently immobilized are visible in the EPR measurements. In fact, the fraction depends on the molecule studied. Comparisons of radical yields at 77 K and room temperature were performed on three antibiotics and showed that the fraction of radical lost can be low, as in the case of ampicillin (*27*).

To explore the relationships between radicals and final products further, a complete LC-MS study of the final products should be performed. Establishing their identification would certainly be laborious. Such a study was undertaken on cephalosporins and demonstrated the complexity of the task, but it only enabled the authors to propose some mechanistic pathways (*28*).

CONCLUSION

The use of high-frequency Q-band EPR was proven to be very helpful. It provided useful additional information for the identification of the radicals. However, Q-band-induced changes are not always sufficient, as in the case of weakly anisotropic radicals, and the use of higher frequencies may be required to enable any conclusion on drug radicals to be drawn (*17*).

Two main types of radicals can explain the recurrent lines in the EPR spectrum of irradiated captopril. The unpaired electron can be carried by either a carbon or a sulfur atom. Other radicals are present and vary as a function of the treatment. They have not been identified. Hence the resulting spectrum of an irradiated drug can be very intricate. Moreover, it may depend on the type of radiation or the treatment applied.

If the trapped radicals influence the mechanisms of production of the radiolytic compounds, the final products produced after different types of irradiation or treatment should be different. Major discrepancies were not observed in our study, which suggests that these trapped radicals do not play an important role in the formation of the final products in irradiated captopril. Other precursors could be involved, including other types of radicals (e.g. untrapped radicals).

In view of the chromatographic results, the radiosterilization of captopril appears to be feasible. Nevertheless, a complete and precise quantification of the impurities would be necessary to confirm this.

ACKNOWLEDGMENTS

We would like to thank Jean Naud and his team of CEAN in Louvain-La-Neuve (UCL, Belgium) for their work on the X-ray diffraction study of captopril as well as Volodia Naydenov (UCL, Belgium) and Henk Vrielinck (UGent, Belgium) for their excellent technical help. The Brussels team also thanks the Institut interfacultaire des sciences nucléaires (IISN-FNRS) for its financial support.

Received: June 3, 2003; accepted: May 24, 2004

REFERENCES

1. Sterilization and sterility assurance of compendial articles. In *The United States Pharmacopoeia USP 24*, pp. 2143–2147. United States Pharmacopeial Convention, Rockville, MD, 2000.
2. Textes généraux sur la stérilité. In *Pharmacopée Européenne 4ème Edition*, pp. 439–444. Direction Européenne de la Qualité du Médicament, Strasbourg, 2000.
3. B. D. Reid and B. P. Fairand, Gamma radiation sterilization of pharmaceuticals. In *Sterilization of Drugs and Devices Technologies for the 21st Century* (F. M. Nordhauser and W. P. Olson, Eds.), pp. 311–392. Interpharm Press, Buffalo Grove, 1998.
4. Decision trees for the selection of sterilisation methods. In *CPMP/QWP/054/98 corr*. The European Agency for the Evaluation of Medicinal Products, London, 2000.
5. J. W. T. Spinks and R. J. Woods, Eds., *Introduction to Radiation Chemistry*, 3rd ed., pp. 452–474. Wiley-Interscience, New York, 1990.
6. Experimental considerations. In *Electron Paramagnetic Resonance Elementary Theory and Practical Applications* (J. A Weil, J. R Bolton and J. E. Wertz, Eds.), pp. 500–502. Wiley-Interscience, New York, 1994.
7. J. Raffi, S. Gelly, L. Barral, F. Burger, P. Piccerelle, P. Prinderre, M. Baron and A. Chamayou, Electron paramagnetic resonance of radicals induced in drugs and excipients by radiation or mechanical treatments. *Spectrochim. Acta A* **58**, 1313–1320 (2002).
8. J-P. Basly, I. Basly and M. Bernard, Electron spin resonance detection of radiosterilization of pharmaceuticals: Application to four nitrofurans. *The Analyst* **123**, 1753–1756 (1998).
9. A. Engalytcheff, V. Deridder, R. Debuyst and B. Tilquin, Determination of radical yields in solid-state drugs as one technique to identify drugs that will withstand radiosterilization: Radioresistance of beta blockers. *Radiat. Res.* **160**, 103–109 (2003).
10. R. Köseoglu, E. Köseoglu and F. Köksal, Electron paramagnetic resonance of some γ-irradiated drugs. *Appl. Radiat. Isot.* **58**, 63–68 (2003).
11. H. B. Ambroz, E. M. Kornacka, B. Marciniec, M. Ogrodowczyk and G. K. Przybytniak, EPR study of free radicals in some drugs γ-irradiated in the solid-state. *Radiat. Phys. Chem.* **58**, 357–366 (2000).
12. M. Gibella, A-S. Crucq, B. Tilquin, P. Stocker, G. Lesgards and J. Raffi, Electron spin resonance of some irradiated pharmaceuticals. *Radiat. Phys. Chem.* **58**, 69–76 (2000).
13. G. C. A. M. Vanhaelewyn, S. A. Amira, W. K. P. G. Mondelaers and F. J. Callens, Decomposition study of the electron paramagnetic spectrum of irradiated alanine. *Spectrochim. Acta A* **56**, 387–397 (2000).
14. A. Engalytcheff, J-P. Vanhelleputte and B. Tilquin, HPLC detection and quantification of radiolytic products of 8 β-blockers irradiated in the solid state and hypotheses on their origins. *Pharmaceut. Res.* **21**, 1103–1109 (2004).
15. F. Callens, G. Vanhaelewyn and P. Matthys, Some recent multi-frequency electron paramagnetic resonance results on systems relevant for dosimetry and dating. *Spectrochim. Acta A* **58**, 1321–1328 (2002).
16. A. Ivancich, P. Dorlet, D. B. Goodin and S. Un, Multifrequency high-field EPR study of the tryptophanyl and tyrosyl radical intermediates in wild-type and the W191G mutant of cytochrome c peroxidase. *J. Am. Chem. Soc.* **123**, 5050–5058 (2001).
17. A. Engalytcheff, M. Kolberg, A-L. Barra, K. K. Andersson and B. Tilquin, The use of multi-frequency EPR techniques to identify the radicals produced in irradiated β-blockers. *Free Radic. Res.* **38**, 59–66 (2004).
18. G. C. A. M. Vanhaelewyn, W. K. P. G. Mondelaers and F. J. Callens, Effect of temperature on the electron paramagnetic resonance spectrum of irradiated alanine. *Radiat. Res.* **151**, 590–594 (1999).
19. H. Kadin. Captopril. *Anal. Profiles Drug Subst.* **11**, 79–137 (1982).
20. Y. Razskazovskii and M. Mel'nikov, Reactions of thiyl radicals in the solid phase. In *S-centered Radicals* (Z. B. Alfassi, Ed.), pp. 225–244. Wiley Interscience, New York, 1999.
21. G. Lassmann, M. Kolberg, G. Bleifuss, A. Gräslund, B-M. Sjöberg and W. Lubitz, Protein thiyl radicals in disordered systems: A comparative EPR study at low temperature. *Phys. Chem. Chem. Phys.* **5**, 2442–2453 (2003).
22. D. Becker, S. Swarts, M. Champagne and M. D. Sevilla, An ESR investigation of the reaction of glutathione, cysteine and penicillamine thiyl radicals: Competitive formations of $RSO^{\bullet}$, $R^{\bullet}$, $RSSR^{-\bullet}$, and $RSS^{\bullet}$. *Int. J. Radiat. Biol.* **53**, 767–786 (1988).
23. M. Symons, Ed., *Chemical and Biochemical Aspects of Electron Spin Resonance Spectroscopy*, pp. 77–79. Van Nostrand Reinhold, Wokingham, England, 1978.
24. N. Varmenot, S. Remita, Z. Abedinzadeh, P. Wisniowski, G. Strzelczak and K. Bobrowski, Oxidation processes of N,S-diacetyl-L-cysteine ethyl ester: Influence of S-acetylation. *J. Phys. Chem. A* **105**, 6867–6875 (2001).
25. S. Swarts, D. Becker, S. DeBolt and M. D. Sevilla, An electron spin resonance investigation of the structure and formation of sulfinyl radicals: reaction of peroxyl radicals with thiols. *J. Phys. Chem.* **93**, 155–161 (1989).
26. M. D. Sevilla, M. Yan and D. Becker, Thiol peroxyl formation from the reaction of cysteine thiyl radical with molecular oxygen: An ESR investigation. *Biochem. Biophys. Res. Commun.* **155**, 405–410 (1988).
27. T. Miyazaki, J. Arai, T. Kaneko, K. Yamamoto, M. Gibella and B. Tilquin, Estimation of irradiation dose of radiosterilized antibiotics by electron spin resonance: ampicillin. *J. Pharm. Sci.* **83**, 1643–1644 (1994).
28. N. Barbarin, B. Tilquin and E. de Hoffmann, Radiosterilization of cefotaxime: Investigation of potential degradation compounds by liquid chromatography-electrospray mass spectrometry. *J. Chromatogr. A* **929**, 51–61 (2001).