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EPR study of anion radicals of various N-quinonyl amino acids

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Summary. Since peptide quinones possess great clinical potential in targeted chemotherapy, several series of novel N-quinonyl amino acids have been synthesized and their first products of reduction were studied by EPR spectroscopy. EPR spectra of the corresponding radical adducts were identified by computer simulation. The dependence between the splitting constants and the chemical structure of the N-quinonyl amino acids anion radicals was examined.

Keywords: Amino acids – EPR – Semiquinone radical – N-Quinonyl amino acids – Hyperfine splittings

Introduction

Natural (Thompson, 1997) and synthetic (Gutierrez, 1989; Powis, 1989) quinones are widely used as drugs for the treatment of human cancer. Their antitumour activity stems from their ability to undergo a reversible one electron reduction followed by formation of the corresponding semiquinone radicals. Under aerobic conditions the semiquinone proceeds through an intricate cascade of electron transfers (redox cycling) to form reactive oxygen species (ROS), which are literally the main reactive deleterious species to the cell (Rowley and Halliwell, 1983; Mimnaugh et al., 1983; Goodman and Hochstein, 1977). These ROS react with DNA, proteins, lipids and other cell structures. The highly reactive superoxide anion radicals are formed in this process, but they exert their reactivity only in hydrophobic media. The hydrogen peroxide formed can cross membranes, but is poorly reactive in aqueous medium. The main highly reactive and toxic species are the short lived $OH^{\cdot}$ radicals, which cause non-specific biological damage (Sugioka et al., 1984; Bates and Winterbourn, 1982; Gutteridge and Quinlan, 1985; Halliwell and Gutteridge, 1984). To avoid toxic side effects, the quinonic compound should be delivered directly to the tumour area. Once on the spot it can undergo reductive transformation, either chemically or enzymatically and yield the reactive oxygen species. The reversibility of the oxidation-reduction assures a

high concentration of the ROS in the vicinity of the tumour targeted. Thus, the delivery of the quinone derivatives directly to the tumour becomes the main issue.

It was shown that peptides which have specific biological receptors might act as carriers of drugs and target them to cancer damaged area (Payne et al., 1984; Shen, 1990). Filicori (1994) showed that many tumours possess receptors for the luteinizing hormone-releasing hormone (LH-RH). Thus, the direct delivery of a quinonic drug might be performed by covalently binding it to this decapeptide. Indeed, Schally (Janaky et al., 1992) showed that the cytotoxicity of such a peptide-drug hybride is markedly augmented in vitro.

The attachment of several free and blocked amino acids to quinone moieties and the generation of free radicals were described by us previously (Rahimipour et al., 1996; 1998). Stegmann and Hewgill (Hewgill et al., 1996) also synthesized several butyl esters of N-quinonyl alanine and N-quinonyl leucine. Reduction of these modified quinones gave EPR spectra of which splitting constants were assigned.

Lately, we have synthesized several new N-quinonyl amino acids in which 1,4-naphthoquinone, 2-chloro-1,4-naphthoquinone or 2-phenylthio-1,4-benzoquinone are attached to different natural and synthetic α-amino acids and also to β-aminoethane sulphonic acid (taurine).

In this paper we present the synthesis of several novel N-quinonyl amino acids (Fig. 1) and EPR study of their appropriate semiquinones formed by chemical reduction.

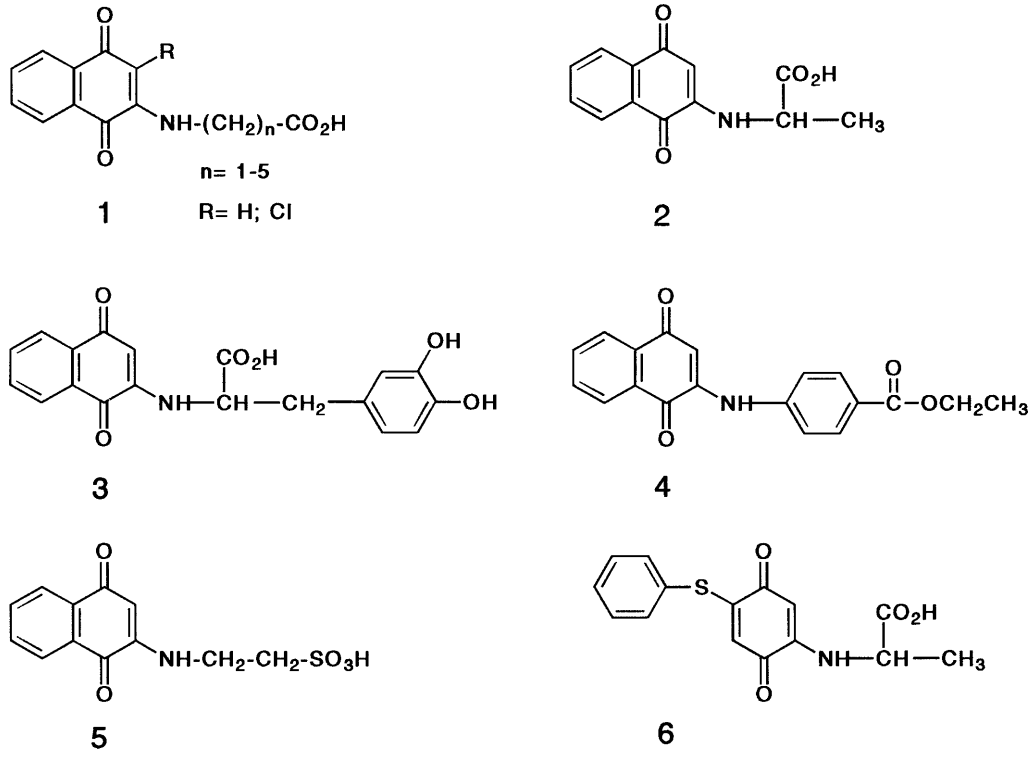

Fig. 1. Several N-quinonyl amino acids and esters synthesized and used in our study

Materials and methods

Materials

All chemicals and reagents were of analytical grade. DMSO was purchased from Sigma Chemical Co. $NaBH_4$ was obtained from Aldrich and used as the reducing agent for production of the semiquinone radicals. Stock solutions of 10 mM $NaBH_4$ and 10 mM N-quinonyl amino acids were prepared by dissolving in a mixture of DMSO and water (1:1). Presence of DMSO is essential because of the low solubility of the N-quinonyl amino acids in water. Deionized and triple distilled water (EASY pure LC system) were used in all experiments. 2-Phenylthio-1,4-benzoquinone was prepared according to the procedure of Dimroth (Dimroth et al., 1940).

Methods

EPR spectra of semiquinone radicals were recorded on a Bruker EMX-220 digital X-band (ν = 9.4 GHz) spectrometer equipped with a Bruker EP 4241VT temperature control system at room temperature (T = 297 K). Final solutions under analysis were prepared from stock solutions of N-quinonyl amino acids and $NaBH_4$ by dilution in DMSO-water (1:1). Final concentrations of both reagents were varied within the range of 1–9 mM depending on quinone redox potential. Liquid samples were drawn into 1 mM i.d. glass Pasteur pipettes (ESR silent within the region of g = 2.00) sealed at the bottom. All spectra were recorded under the following parameters: 2.012 mW non-saturating microwave power, 12.5 kHz magnetic field modulation of 0.02 mT amplitude. EPR spectra were processed using Bruker WIN-EPR and simulated with Bruker SimFonia and NIEHS PEST WINsim simulation software.

IR spectra were recorded on a Nicolet 5ZDX FT-IR spectrometer. ^{1}H-NMR were determined on a Bruker WP 200 SY spectrometer and mass spectra (CI in methane) were obtained using a Finnigan 4020 quadropole spectrometer. Melting points were determined using a Thomas-Hoover capillary apparatus and are incorrected. The optical specific rotation was determined in absolute MeOH on 341 Perkin Elmer polarimeter. These measurements have been performed with very dilute solutions due to the dark coloure of the compounds.

Synthesis

N-(3-chloro-1,4-naphthoquinon-2-yl)-β-alanine (**1**, **n** = **2**, **R** = **Cl**): A solution of β-alanine (0.89 gr, 10 mmol) in 20 ml of water was added to a suspension of 2,3-dichloro-1,4-naphthoquinone (2.27 gr, 10 mmol) in 150 ml of methanol. The reaction mixture was refluxed for 24 h and the solvent evaporated under reduced pressure. The red product was recrystallised from EtOH/H_2O (1:1) and yielded 2.4 gr (86%) of red needles melting at 158–160°C, litt. 160–161°C (Okamoto and Ohta, 1980). ^{1}H-NMR (d6-DMSO) δ (ppm) J (Hz)): 12.30 (br s, 1H), 7.90 (d, 2H, 7.36), 7.63–7.79 (m, 2H), 7.28 (br s, 1H), 3.90 (q, 2H, 6.78), 2.55 (t, 2H, 6.98). IR (KBr) λ (cm^{-1}): 3462, 3288, 1743, 1696, 1642, 1608. UV-Vis (MeOH) λmax (nm) (log (ε)): 464 (3.45), 330 (3.30), 2.74 (4.31), 236 (4.23). MS (m/z): 281 $(M + 3)^+$, 280 (MH^+), 245, 231, 201, 173.

N-(1,4-naphthoquinon-2-yl)-L-alanine (**2**): A solution of L-alanine (0.89 gr, 10 mmol) in 20 ml of water was added dropwise to a heated solution of 1,4-naphthoquinone (3.16 gr, 20 mmol) in 150 ml of EtOH (95%). The reaction mixture was refluxed for 4 h, and the solvent evaporated under reduced pressure, the orange product was purified by silica gel column chromatography using CH_2Cl_2/MeOH (9:1) as eluant and recrystallized from MeOH/acetone. Yield: 1.59 gr (65%). m.p. = 139–142°C. ^{1}H-NMR (d6-DMSO) δ (ppm) J (Hz)): 7.94 (td, 2H, 7.38, 1.05), 7.80 (td, 1H, 7.29, 1.40), 7.72 (td, 7.40, 1.40) 7.39 (d, 1H, 6.06), 5.57 (s, 1H), 3.70–3.80 (m, 1H), 1.33 (d, 3H, 6.81). IR (KBr) λ (cm^{-1}): 3459, 3331,

1718, 1682, 1639, 1614. UV-Vis (MeOH) λmax (nm) (log (ε)): 452 (3.70), 330 (3.57), 270 (4.49), 234 (4.35). MS (m/z): 248 ($M + 3H^+$), 227, 199, 186, 173. Specific rotation $[\alpha]^{25}$ + 56.0° (c = 0.089, CH_3OH).

N-(1,4-naphthoquinon-2-yl)-L-DOPA (**3**): A solution of L-dihydroxy phenylalanine (L-DOPA) (1.97 gr, 10 mmol) in 50 ml of water was added dropwise to a heated solution of 1,4-naphthoquinone (3.16 gr, 20 mmol) in 150 ml of EtOH (95%), and the reaction mixture refluxed for 3 h. The solvent was evaporated under reduced pressure and the brown product purified by silica gel column chromatography using CH_2Cl_2/MeOH (9:1) as eluant. Yield: 1.78 gr (52%). m.p. = 145–147°C. ^{1}H-NMR (d6-DMSO) δ (ppm) J (Hz)): 8.90 (br s, 1H), 8.70 (br s, 1H), 7.97 (t, 2H, 7.33), 7.85 (t, 1H, 7.32), 7.77 (t, 1H, 7.46), 7.17 (d, 1H, 6.56), 6.59 (d, 2H, 9.22), 6.44 (d, 1H, 8.04), 5.59 (s, 1H), 3.87–3.90 (m, 1H), 2.98–3.30 (m, 2H). IR (KBr) λ (cm^{-1}): 3420, 1687, 1611. UV-Vis (MeOH) λmax (nm) (log (ε)): 462 (3.38), 274 (4.21), 206 (4.38). MS (m/z): 354 (MH^+), 230, 178. Specific rotation $[\alpha]^{25}$ + 21.0° (c = 0.095, CH_3OH).

Ethyl N-(1,4-naphthoquinon-2-yl)-p-amino benzoate (**4**): A solution of ethyl p-amino benzoate (1.65 gr, 10 mmol) in 50 ml of EtOH (95%) was added dropwise to a heated solution of 1,4-naphthoquinone (3.16 gr, 20 mmol) in 150 ml of EtOH (95%). The mixture was stirred for 48 h at room temperature and the deep-red product which precipitated was filtered and recrystallised from EtOH (95%). Yield: 1.89 gr (59%). m.p. = 202–204°C, litt. 185–189°C (Beilstein, 1928). ^{1}H-NMR (d6-DMSO) δ (ppm) J (Hz)): 8.14 (t, 2H, 7.27), 8.10 (d, 2H, 8.69), 7.78 (td, 1H, 6.62, 0.84), 7.73 (br s, 1H), 7.70 (td, 1H, 6.57, 1.33), 7.33 (d, 2H, 9.00), 6.59 (s, 1H), 4.39 (q, 2H, 7.13), 1.41 (t, 3H, 7.12). IR (KBr) λ (cm^{-1}): 3207, 1702, 1682, 1629, 1608. UV-Vis (MeOH) λmax (nm) (log (ε)): 458 (3.57), 294 (4.25), 252 (4.06), 226 (4.10). MS (m/z): 322 (MH^+).

N-(1,4-naphthoquinon-2-yl)-β-aminoethanesulphonic acid (taurine) (**5**): A solution of β-aminoethanesulphonic acid (taurine) (0.62 gr, 5 mmol) in 20 ml of water was added dropwise to a heated solution of 1,4-naphthoquinone (1.58 gr, 10 mmol) in 100 ml of EtOH (95%). The reaction mixture was refluxed for 3 h, the solvent removed under reduced pressure and the orange product purified by silica gel column chromatography using CH_2Cl_2/ MeOH (9:1) as eluant. Yield: 0.36 gr (26%). m.p. = 285–287°C. ^{1}H-NMR (d6-DMSO) δ (ppm) J (Hz)): 7.95 (d, 1H, 7.63), 7.92 (d, 1H, 7.62), 7.81 (t, 1H, 7.43), 7.71 (t, 1H, 7.47), 7.74 (t, 1H, 5.29), 5.62 (s, 1H), 3.36–3.39 (m, 2H), 2.74 (t, 2H, 6.63). IR (KBr) λ (cm^{-1}): 3455, 3355, 1696, 1635, 1602. UV-Vis (MeOH) λmax (nm) (log (ε)): 446 (3.04), 332 (2.94), 270 (3.84), 222 (3.77). MS (m/z): 200 (MH^+-SO_3H), 174.

N-(5-phenylthio-1,4-benzoquinon-2-yl)-L-alanine (**6**): A solution of L-alanine (0.2 gr, 2.3 mmol) was added dropwise to a solution of 2-phenylthio-1,4-benzoquinone (0.5 gr, 2.3 mmol) and the mixture stirred at room temperature for 24 h. Evaporation of the solvent left the crude deep-red product which was purified by silica gel column chromatography using CH_2Cl_2/MeOH (95:5) as eluant. Yield: 0.28 gr (40%). m.p. = 157–160°C. ^{1}H-NMR (d6-DMSO) δ (ppm) J (Hz)): 7.65 (s, 5H), 7.58 (d, 1H, 6.67), 5.51 (s, 1H), 5.44 (s, 1H), 3.62–3.69 (m, 1H), 1.40 (d, 3H, 7.61). IR (KBr) λ (cm^{-1}): 3450, 3354, 1670, 1615. UV-Vis (MeOH) λmax (nm) (log (ε)): 516 (2.93), 354 (4.07), 220 (4.32). MS (m/z): 260 (MH^+-CO_2H), 244, 218, 180, 110. Specific rotation $[\alpha]^{25}$ − 18.7° (c = 0.107, CH_3OH).

Results and discussion

Simulations of all EPR spectra obtained were successfully performed without taking into account the hyperfine splitting from the hydroxy group proton of the semiquinone radicals. That is why these EPR spectra have been assigned to the deprotonated form, e.g. semiquinone anion radicals. Hyperfine splitting parameters for all samples under study are summerized in Table 1.

Table 1. Hyperfine splitting parameters for chemically reduced N-quinonyl amino acids obtained by simulation of EPR spectra

Quinones	Hyperfine splitting parameters (mT) \ ± 0.002 mT
1	0.138 (4H), 0.106 (1H), 0.192 (1N), 0.014 (^{35}Cl), 0.012 (^{37}Cl)
2	0.203 (5H), 0.148 (1H), 0.191 (1N)
3	0.194 (4H), 0.147 (1H), 0.217 (1H), 0.201 (1N)
4	0.037 (4H), 0.028 (5H), 0.163 (1H), 0.122 (1N)
5	0.224 (4H), 0.171 (1H), 0.023 (1H), 0.011 (2H), 0.173 (1N)
6	0.236 (1H), 0.207 (1H), 0.066 (1H), 0.032 (1H), 0.172 (1N)

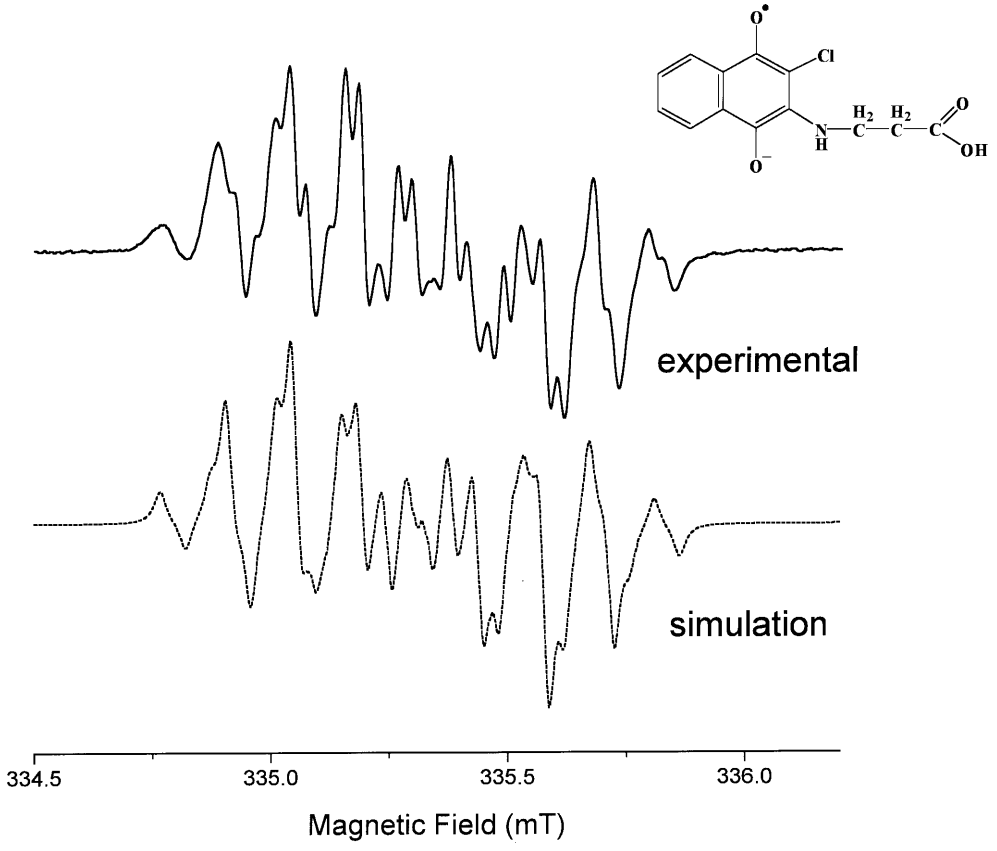

Fig. 2. Experimental and simulated EPR spectra of 2-N-(3-chloro-1,4-naphthoquinonyl) β-alanine (**1**, **n** = **2**, **R** = **Cl**). Spectrum was recorded at T = 297 K and γ = 9.399 GHz

EPR spectra of ω-*N-naphthoquinonyl amino acids*

The series of ω-N-naphthoquinonyl amino acids (**1**) was synthesized aiming to check whether the length of the fatty acid residue affects hyperfine parameters of the corresponding EPR spectra. It was found that the number of -CH_2- groups does not significantly affect the shape of the EPR spectrum. Within that given series all EPR spectra look very similar. In our previous EPR investigations of the ω-2-N-(3-chloro-1,4-naphthoquinonyl) amino acids

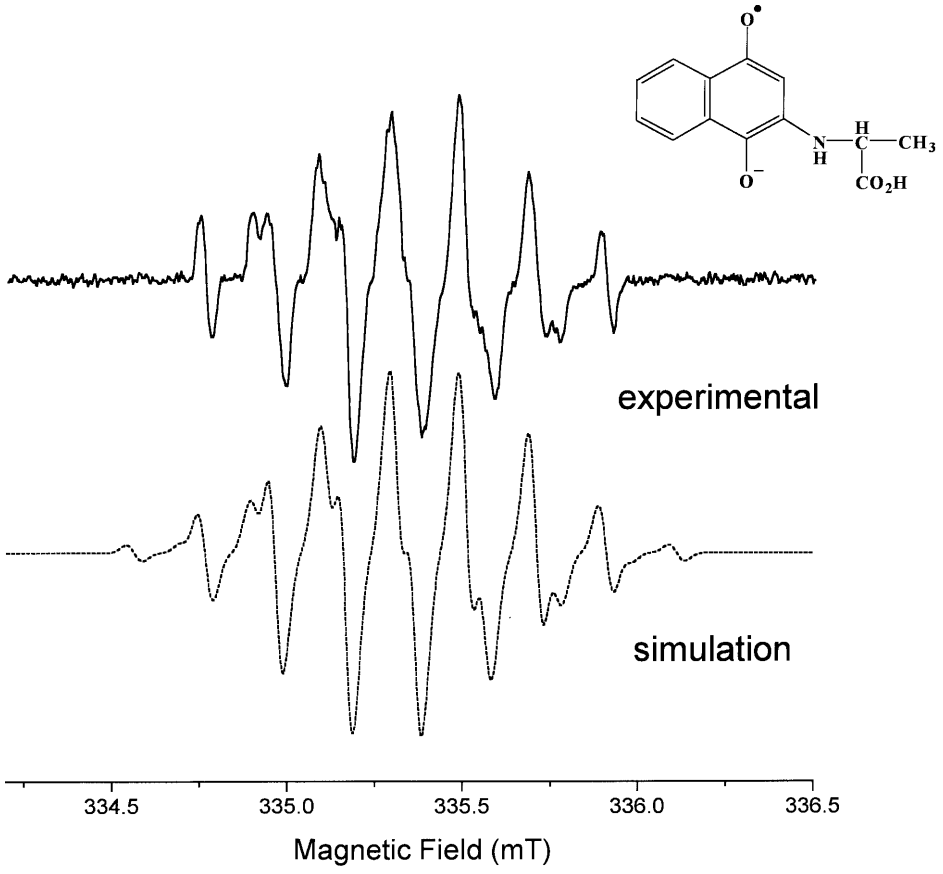

Fig. 3. Experimental and simulated EPR spectra of 2-N-(1,4-naphthoquinonyl)-L alanine (**2**). Spectrum was recorded at T = 297 K and γ = 9.395 GHz

(Rahimipour et al., 1996) no hyperfine splittings belonging to chlorine nuclei were reported. In the present case all splittings observed in the EPR spectrum (Fig. 2) may be successfully described as involving non-vanishing hyperfine interactions with the two chlorine isotopes possessing nuclear spin (^{35}Cl and ^{37}Cl) (Table 1). Since gyromagnetic ratio of chlorine nuclei (both ^{35}Cl and ^{37}Cl) is about 10 times smaller than the gyromagnetic ratio of a proton, the relatively small chlorine coupling constant found (about 0.014 mT for ^{35}Cl) points to similar overlap electron density on chlorine atom and ring protons. It can be concluded that the unpaired electron is localized mainly on nitrogen and equally distributed over the naphthalenic ring.

EPR spectra of 2-N-(1,4-naphthoquinonyl)-α-amino acids

Typical EPR spectrum may be represented by the one of the simplest amino acid derivative of this series, namely 2-N-(1,4-naphthoquinonyl)-L-alanine (**2**). Parameters, obtained by simulation (Table 1), successfully describe the experimental spectrum (Fig. 3). Relatively broad individual lines and

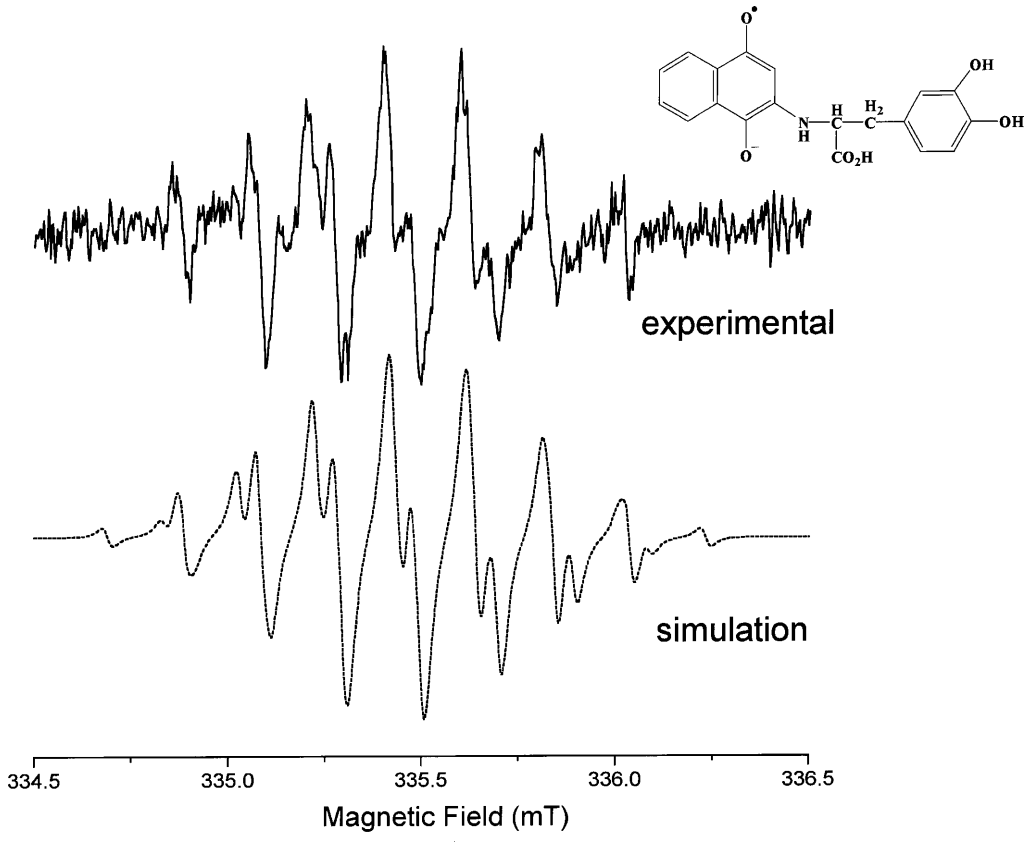

Fig. 4. Experimental and simulated EPR spectra of 2-N-(1,4-naphthoquinonyl)-L-DOPA (**3**). Spectrum was recorded at T = 297 K and γ = 9.404 GHz

unoptimal signal-to-noise ratio influence the small amplitude low- and high-field peaks which are not clearly observable. In some cases, one naphthalenic proton differs from the other four.

For example, in the case of the semiquinone anion radical produced by 2-N-(1,4-naphthoquinonyl)-L-DOPA (**3**), the nonequivalent naphthalenic proton located in position 3 shows higher value of the splitting parameter 0.217 mT (1H) (Table 1). This was also demonstrated by the investigations of Clay and Murphy (1981) and of Hewgill and Stegmann (Hewgill et al., 1996). It was suggested that such quinonic derivatives behave more like o-quinones rather than p-quinones with respect to their spin density distribution.

The catecholic ring is isolated from the naphthalenic system by an aliphatic bridge, thus no splittings from its protons have been observed.

Because of the high reactivity of the semiquinone radicals formed from 2-N-(3-chloro-1,4-naphthoquinonyl)-α-amino acids, their EPR spectra undergoe changes in both line shapes and intensities during spectra recording. Such a short lifetime did not allow us to record the reliable CW EPR spectrum and to obtain hyperfine parameters by simulation. Further study of these systems will be done after finding stabilizing conditions or by pulsed FT EPR technique.

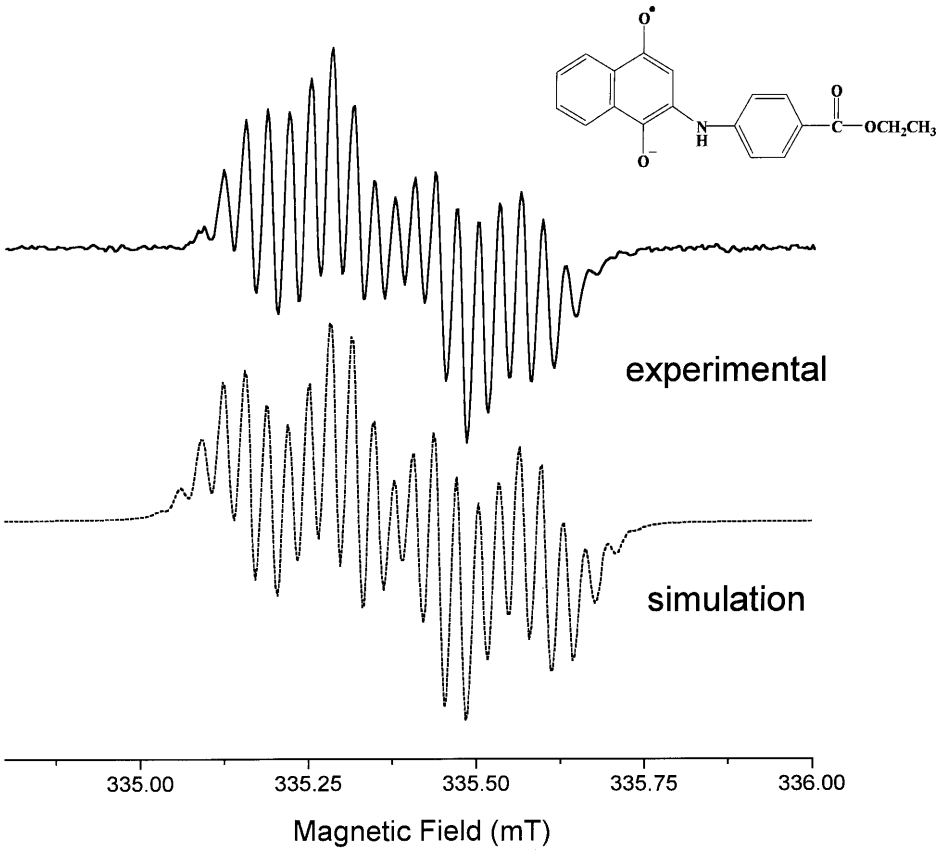

Fig. 5. Experimental and simulated EPR spectra of ethyl 2-N-(1,4-naphthoquinonyl)-p-aminobenzoate (**4**). Spectrum was recorded at T = 297 K and γ = 9.399 GHz

EPR spectra of naphthoquinonyl derivatives of p-amino benzoic acid and its esters

The chemical structure of ethyl 2-N-(1,4-naphthoquinonyl)-p-aminobenzoate (**4**) indicates that this compound possesses a conjugated π-system. Both experimental and simulated EPR spectra of **4** perfectly demonstrate the distribution of the unpaired electron within the molecule (Fig. 5). In contrast with 3, splittings from both the naphthalenic and the benzenic rings are observed. Most of the electron density is localized on the NH-group and only reduced density on the ring protons.

EPR spectra of naphthoquinonyl derivatives of β*-aminoethanesulphonic acid (taurine)*

EPR spectra of semiquinone anion radical produced by 2-N-(1,4-naphthoquinonyl)-β-aminoethanesulphonic acid (**5**) have been recorded (Fig. 6). This spectrum was expected to be very similar to the spectra of ω-N-1,4-naphthoquinonyl amino acids radicals. However, it was found that the -SO_3H

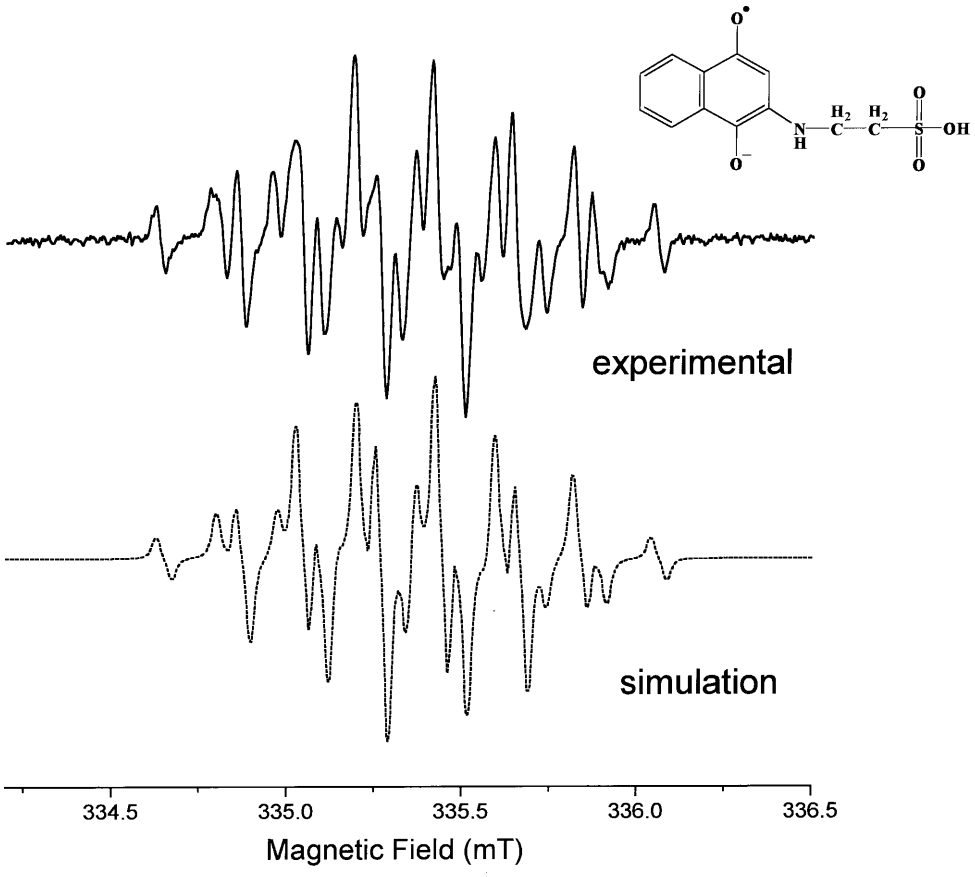

Fig. 6. Experimental and simulated EPR spectra of 2-N-(1,4-naphthoquinonyl)-β-aminoethanesulphonic acid (taurine) (**5**). Spectrum was recorded at T = 297 K and γ = 9.395 GHz

group exerts a strong negative inductive effect, and additional splitting from the two methylenic protons appears.

EPR spectra of 2-N-(5-phenylthio-1,4-benzoquinonyl)-α-amino acids

The simulated EPR spectrum of the semiquinone anion radical of 2-N-(5-phenylthio-1,4-benzoquinonyl)-L-alanine (**6**) shows good agreement with the experimental one (Fig. 7). Besides the regular splitting from the two non-equivalent protons of the quinonic ring and one proton of the NH-group, the additional hyperfine splitting from another proton is observed. The low value of this constant (0.032 mT) allows us to suggest that this splitting is due to the proton of the neighbouring α-CH- group.

Conclusions

We have synthesized several N-quinonyl amino acids in which quinones were attached to different types of natural and unnatural amino acids. Part of the

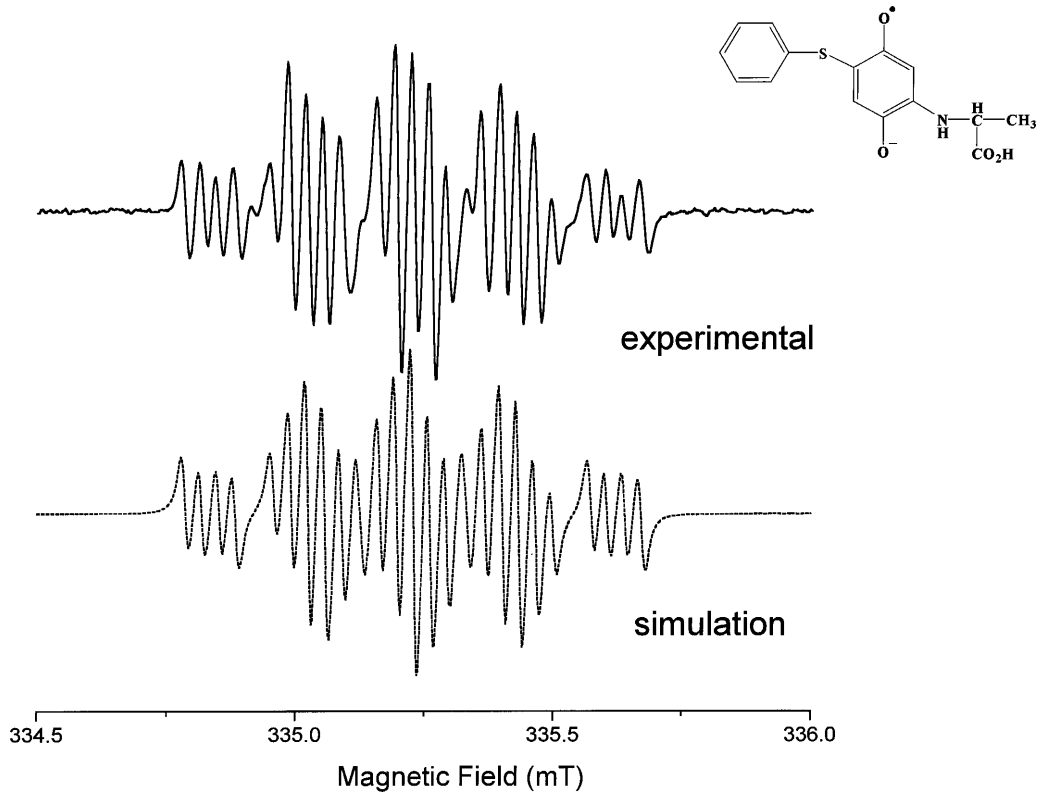

Fig. 7. Experimental and simulated EPR spectra of 2-N-(5-phenylthio-1,4-benzoquinonyl)-L-alanine (**6**). Spectrum was recorded at T = 297 K and γ = 9.395 GHz

compounds can be employed as building blocks in stepwise syntheses of cytotoxic peptides and others can be used for post-chain assembly modifications. EPR studies of several of the newly synthesized compounds confirmed the formation of semiquinone anion radicals using $NaBH_4$ as a reducing source. Generation of such reactive species might be a prerequisite for the manifestation of site-directed antitumour activity of corresponding quinone-peptide conjugates.

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References

Bates DA, Winterbourn CC (1982) Deoxyribose breakdown by the adriamycine, semiquinone and H_2O_2: evidence for hydroxyl radical participation. FEBS Lett 145: 137–142

Beilsteins Handbuch der organischen Chemie, Bd 14/432 (1928) Springer, Berlin, p 576

Clay JDR, Murphy D (1981) Electron spin resonance spectra of aminosemiquinones and diaminosemiquinones. J Chem Soc, Faraday Trans 277: 1589–1594

Dimroth O, Kraft L, Aichinger K (1940) Action of thiophenols on quinone. Ann Chem 545: 124–139
Filicori M (1994) Gonadotrophin-releasing hormone agonist. Drugs 48: 41–58
Goodman J, Hochstein P (1977) Generation of free radicals and lipid peroxidation by redox cycling of adriamycin and daunomycin. Biochem Biophys Res Commun 77: 797–803
Gutierrez PL (1989) Mechanisms of bioreductive activation. Free Radic Biol Med 6: 405–445
Gutteridge JMC, Quinlan GJ (1985) Free radical damage to doxyribose by anthracycline, aureolic acid and aminoquinone antitumour antibiotics. An essential requirement for iron, semiquinones and hydrogen peroxide. Biochem Pharm 34: 4099–4103
Halliwell B, Gutteridge JMC (1984) Oxygen toxicity, oxygen radicals, transition metals and desease. Biochem J 219: 1–14
Hewgill FR, Hofler U, Power RC, Stegmann HB (1996) ESR spectra of some amino acid derivatives of semiquinone anions. Mag Res Chem 51: 47–51
Janaky T, Juhasz A, Bajusz S, Csernus V, Srcalovic G, Bokser L, Milovanovic S, Redding TW, Rekasi Z, Nagy A, Schally AV (1992) Analogues of luteinizing hormone-releasing hormone containing cytotoxic groups. In: Bacon BA (senior ed) Proceedings Natl Acad Sci USA 89: 972–976 (For colloquium "Industrial Ecology" May 20–21, 1991. National Academy of Sciences, Washington 1992, USA)
Mimnaugh EG, Gram TE, Trush MA (1983) Stimulation of mouse heart and liver microsomal lipid peroxidation by anthracycline anticancer drugs. J Pharmacol Exper Ther 226: 806–816
Okamoto M, Ohta S (1980) Studies on the synthesis of condensed indole-4,7-diones. II. Synthesis of pyrrolo[1,2-a]indole-5,8-dione derivatives. Chem Pharm Bull 28: 1071–1076
Payne JW, Morley JS, Armitage P, Payne GM (1984) Transport and hydrolysis of antibacterial peptide analogues in Escherichia coli: backbone-modified aminoxy peptides. J Gen Microbiology 130: 2253–2265
Powis G (1989) Free radical formation by antitumour quinones. Free Radic Biol Med 6: 63–101
Rahimipour S, Weiner L, Fridkin M, Bade Shrestha-Dawadi P, Bittner S (1996) Novel naphthoquinonyl derivative(s) of luteinizing hormone-releasing hormone (LH-RH). Lett in Peptide Sci 5: 263–274
Rahimipour S, Weiner L, Bade Shrestha-Dawadi P, Bittner S, Koch I, Fridkin M (1998) Cytotoxic peptides: naphthoquinonyl derivative(s) of luteinizing hormone-releasing hormone (LH-RH). Lett in Peptide Sci 5: 421–427
Rowley DA, Halliwell B (1983) DNA damage by superoxide-generating systems in relation to the mechanism of action of the antitumour antibiotic adriamycin. Biochim Biophys Acta 761: 86–93
Shen WC (1990) Acid-sensitive dissociation between poly(lysine) and histamine-modified poly(glutamate) as a model for drug-releasing from carriers in endosomes. Biochim Biophys Acta 1034: 122–124
Sugioka K, Nakano H, Tsuchiya J, Nakano M, Sugioka Y, Kubota S, Ikegami Y (1984) Clear evidence for the participation of OH radical in λ DNA breakage induced by the enzymatic reduction of adriamycin in the presence of iron-ADP. Importance of local OH radical concentration for DNA strand cleavage. Biochem Intern 9: 237–242
Thompson RH (1997) Naturally occuring quinones, 4th edn. Academic Press, London

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