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Colchicine Red-Ox Chemistry Revisited: Cathodic Behavior and EPR Observation of an Intermediate Radical Anion

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Abstract: Colchicine (**1**), a potently antimitotic alkaloid and useful laboratory tool in cancer research, undergoes cathodic reduction in DMF forming an ESR-observable radical anion (**1r**) which is characterized by the isotropic hyperfine coupling constants 8.9, 4.3, 0.75, 0.49 and 0.48 G for H-8, H-12, OCH_3, H-11 and H-4, respectively, and a much flattened troponoid ring. Assignments are aided by selective deuteriation of colchicine at C-8, C-11 and $COCH_3$, as well as by spectral simulation and *ab initio* calculations of electron spin densities. Whether the colchicine radical anion may exist in nature is also discussed.

Colchicine (**1**), an alkaloid isolated from the herb *Colchicum autumnale*, has raised much interest for its strong antimitotic action.[1] Although prevented from therapeutic use by its toxicity, colchicine has remained an important tool in cancer research and a template for the synthesis of non toxic analogues. Much attention has been paid to the ground state and electronically excited states of colchicine in relation to its reactivity and antimitotic action;[2] in particular, only one atropisomer helicity (*aR*,7*S*)[3] has been found and proven to be required for binding to tubulin.[4]

In contrast, very little is known about the red-ox behavior of colchicine, limited to polarographic studies in aqueous media,[5a] which describe colchiceine (**2**) as product,[5b] or hypothesize benzyl- or pinacol-type dimers as cathodic products.[5c] More recently electroanalytical determinations of colchicine have appeared.[5d]

We report here on a re-investigation of the electrochemistry of colchicine employing both voltammetric and coulombometric techniques in either dry organic or aqueous media, as well as EPR techniques which reveal a stable colchicine radical anion as an intermediate, while contradicting much older literature.

Cathodic behavior of colchicine (1)

Polarographic reduction and coulombometric analysis of colchicine (**1**) are first carried out in aqueous medium at a mercury cathode, observing, according to the conditions , an adsorption pre-wave, besides two waves related to two distinct processes; the second wave does not appear at acidic pH, while the pre-wave is observed until pH 8. In dry DMF no pre-wave is observed. The first wave is typical of rapid

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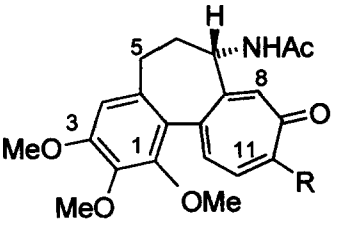
electron donation processes, followed by slow protonation.

Electrolysis of colchicine in a buffered aqueous medium at pH values ranging from 1 to 7 leads to intractable red gummy materials. The only defined material isolated from experiments at acidic pH, a small amount of colchiceine (**2**), probably is the result of hydrolysis of colchicine in the acidic medium.



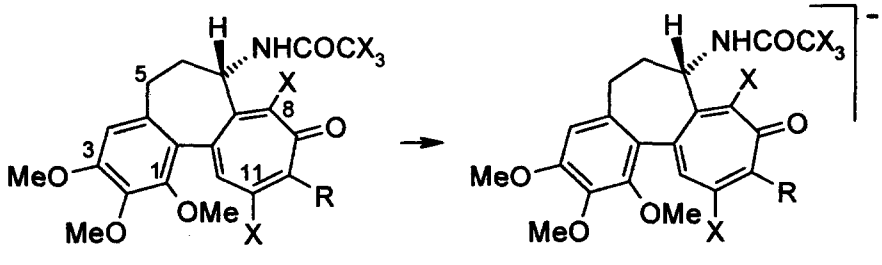
1 R=OCH_3
2 R=H
3 R=$NHCH_3$

Cathodic reduction of colchicine at a platinum electrode in freshly-distilled, dry DMF proves simpler and cleaner, involving two electrons until *ca.* 90% reaction completion. However, from the electrolysis mixture only a small amount of 10-methylaminocolchiceine (**3**)[6] (15%) is isolated, besides unreacted colchicine (**1**) (9%); very minor products, of mass range in the order of colchicine, can only be detected by LC-ESI-MS, which is an insufficient basis for reliable structural assignments. In any event, all evidence contradicts previous assumptions as to the formation of dimeric cathodic products.[5] Blank experiments show that, under the conditions used, no 10-methylaminocolchiceine (**3**) is formed without applied potential, thus showing that this product is not an artifact.

A voltammetric analysis at a Pt cathode in dry N_2-flushed DMF shows that colchicine (**1**) undergoes reduction at -1.79 V (vs. SHE) in a single voltammetric wave. Cyclic voltammetry suggests the involvement of an intermediate species of low-stability: signs of reversibility only begin to appear at scan rates ≥ 1 V/s.

EPR spectra of colchicine (1r) and deuteriated colchicine (4r) radical anions

Following the above indications, reduction of colchicine (**1**) is carried out at room temperature in



1 R=OCH_3, X=H
4 R=OCH_3, X=D

1r R=OCH_3, X=H
4r R=OCH_3, X=D

Scheme 1

N_2-flushed, dry DMF containing *ca.* 0.1 M $(n\text{-Bu})_4NClO_4$ directly in the EPR spectrometer cavity at a platinum cathodic grid, under an applied potential of -1.9 V. EPR signals thus emerge and persist for *ca.* 10 min. (Fig. 1a), revealing a spectral structure of hyperfine interactions that consists of a quartet of

sextets. This may be interpreted in terms of two major proton hyperfine coupling constants (hfcc), one twice as large as the other one. The spectrum can be nicely simulated[7] (Fig. 1b) by best fitting to the experimental spectrum, with optimization of both the hfcc and the linewidth. This confirms the above qualitative indications, assigning hfcc 8.9 and 4.3 G to the two major hfcc, while minor hfcc are also revealed. These suggest couplings of the unpaired electron with three sets of ½ spin atoms, one constituted of three magnetically equivalent protons (0.75 G), and the other two of a single proton each (0.49 and 0.48 G).

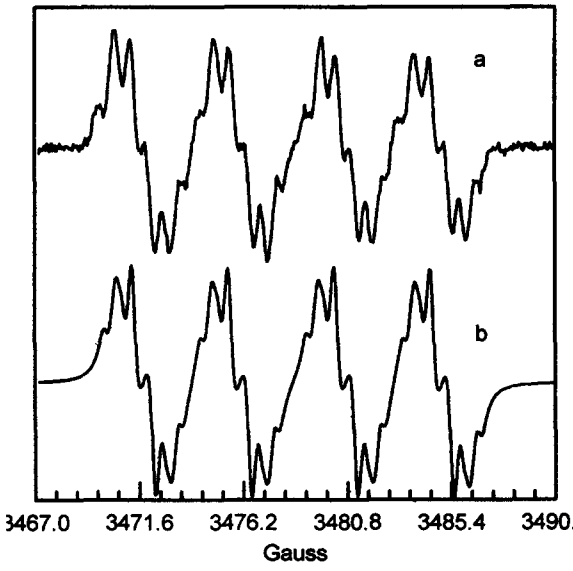


Figure 1. EPR spectrum of colchicine radical anion **1r** obtained from cathodic reduction of colchicine (**1**) in DMF solution at r.t.: a) experimental spectrum, b) simulated spectrum

Specific H-atom assignments (Table) are based on a comparative examination of the radical anion **4r** obtained from cathodic reduction of C-8, C-11 and $COCH_3$ deuteriated colchicine (**4**) (obtained by deuterium exchange with colchiceine[8,9]). The EPR spectrum of **4r** consists of two structured lines for a single major hfcc (Fig. 2a), approximately corresponding to the second major hfcc of the light radical anion **1r**. Simulation[7] of this spectrum (Fig. 2b) reveals a hyperfine structure resulting from hfcc values: 4.2 G and 0.44 G for single protons each, 0.66 G for the three protons of a methyl group, and 1.3 G for a single deuteron. Deuterium substitution at positions 8 and 11 is responsible for the large decreases in hfcc 8.9 → 1.3 G and 0.49 → < 0.2 G, respectively on changing from **1r** to **4r**, while the substantial invariance 4.3 / 4.2 fits for position 12. Thus, it is only the 0.48 G hfcc (which is substantially unaltered in the deuteriated radical) that remains to be assigned. Likely positions are NH, H-7 and H-4.

Table Hyperfine coupling constants (G) for the radical anions of colchicine (**1r**) and deuteriated colchicine (**4r**)

Position	**1r**		**4r**	
	exper	calcd	exper	calcd
8	8.9	-9.2	1.3	-1.4
12	4.3	-5.0	4.2	-5.0
OCH_3	0.75	0.84	0.66	0.84
11	0.49	0.59	[a]	0.09
4	0.48	0.48	0.44	0.48

[a]<0.2 G, i.e. smaller than the computed linewidth

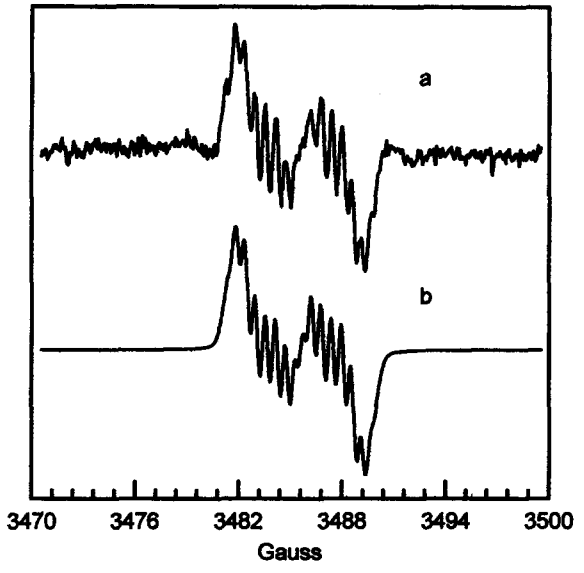


Figure 2. EPR spectrum of deuteriated colchicine (4) in DMF solution at r.t.: a) experimental spectrum, b) simulated spectrum

Ab initio calculations on colchicine (1) and its radical anion 1r

In order to complete the above hfcc assignments, open shell, *ab initio*[10] calculations are carried out for

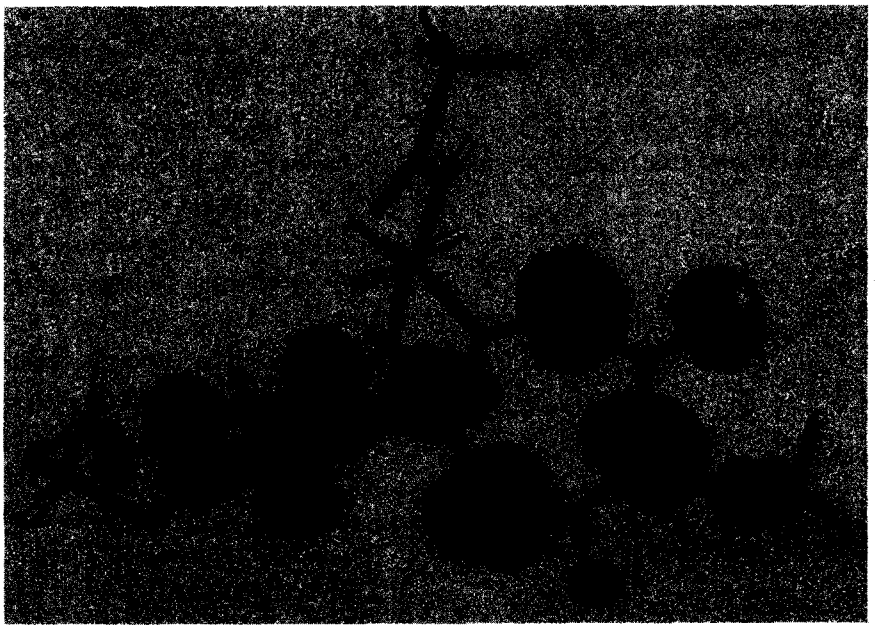


Figure 3. Electron spin density surfaces at 0.002 e/au^3 for the colchicine radical anion (1r)

the colchicine radical anion (**1r**). The process involves calculating the electron spin densities at the hydrogen atoms and, through the Fermi contact interaction, obtaining the isotropic hfcc, which can be compared with the experimental values. Thus, geometry optimization of the free radical species at the unrestricted Hartree-Fock (UHF) level is first carried out, followed by an unrestricted second-order Moeller-Plesset perturbative expansion (UMP2) using a 6-31** basis set,[11] which is appropriate for hfcc calculations of split-valence-plus-polarization quality.[12] Spin contamination effects[12,13] and the influence of the solvent are corrected through semi-empirical extrapolations,[14,15] as it is very hard to single out any such effect. The calculated electron spin density contours are shown for the radical anion **1r** in Fig. 3.

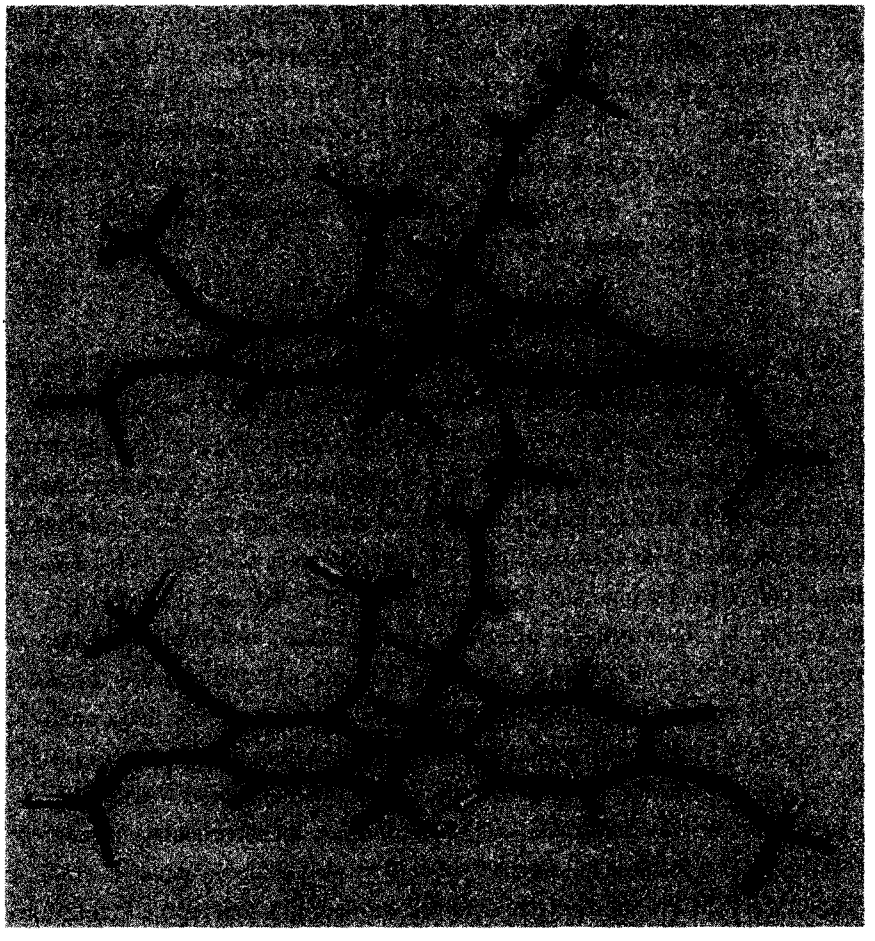


Figure 4. Calculated least-energy conformation for colchicine (top) and its radical anion (bottom)

The least-energy conformation of the colchicine radical anion (Fig. 4, bottom) can be compared with the neutral molecule (Fig. 4, top).[16] It is seen that, according to these calculations, which fit EPR spectra, formation of the radical anion substantially involves a flattening of the troponoid ring only.

The calculated spin density distribution (Fig. 3) suggests that the unpaired electron resides in a p orbital with maximum values for p_z atomic orbitals. As to the interpretation of the EPR spectrum, spin densities at the H-atoms are more relevant: the isotropic hfcc values, $a_1 = -9.2$, $a_2 = -5.0$, $a_3 = 0.59$, $a_4 = 0.48$, and $a_5 = 0.84$ G for the H-8 , H-12 , H-11, H-4 protons and OCH_3 protons, respectively, substantially agree with the experimental values. The calculations allow also $a_4 = 0.48$ to be assigned as 4-H. This pattern of hfcc agrees with previous observations for radical anions of simple troponoids.[17]

In conclusion, we offer here a complete picture of a key intermediate - the radical anion **1r** - in the cathodic reduction of colchicine (1), for which alleged reaction courses[5] are also disproved. The reduction potential of colchicine in water at pH 7 is lower, -1.32V, than in partially aqueous/organic media, though not low enough to assume that colchicine - as such - can uptake electrons in living bodies. It may be expected, however, that the reduction potential of colchicine is lowered enough on contact with proteins, particularly in tumor cells,[18] to accept an electron. The lowest energy conformation for the colchicine radical anion (Fig. 4, bottom) would geometrically allow the same array of contacts with tubulin that are deemed important for colchicine.[3] This suggests experiments with colchicine in the growing area of electron-transfer processes in biological systems.[19]

EXPERIMENTAL

General. TLC was performed on either analytical or preparative, 2 mm thick, Merck Kieselgel 60 F_{254} plates. Reverse-phase HPLC was done on a Machery-Nagel Nucleosil 18, 25 x 0.5 cm, column. UV spectra were taken with a Perkin-Elmer Hitachi 200 spectrophotometer. Cyclic voltammetry was performed on a platinum micro electrode placed into a three-electrode conventional cell connected to a model-563 AMEL Electrochemlab. NMR spectra were recorded on a Varian Gemini BB spectrometer operating at 200 MHz on ^1H. ESR spectra were taken with a Bruker 200D-SRC spectrometer equipped with a TE_{102} rectangular cavity, operating at 10 GHz. Commercial colchicine (Aldrich 95%) was used as such. Deuteriated colchicine was secured by diazomethane treatment of deuteriated colchiceine[8,9] followed by TLC separation on silica gel with 9:1 $CHCl_3$/MeOH. Cathodic reduction of colchicine was carried out directly in the spectrometer cavity on a 5 x 25 mm platinum grid placed into a flat, 0.5 mm deep, quartz rectangular container. An Ag/AgCl electrode, used as reference, was insulated from the bulk electrolysis solution by means of a glass frit and a salt bridge. As a counter electrode a platinum spiral was used. The quartz cell was connected to a reservoir containing the solution to analyze, an intermediate three-way stopcock allowing rapid discharge and refill of the electrolytic cell, as well as deoxygenation of the mixture by means of a N_2 stream. LC-ESI-MS spectra were obtained with a triple-quadrupole Perkin-Elmer SCIEX API III PLUS mass spectrometer operating at nebulizer voltage 5.5 KV, orifice potential 60 V, scan range 380-2000 Da, scan rate 32 msec/amu, with resolution > 1 amu; reaction mixtures (*ca.* 10^{-4} M) in MeOH were subjected to flow injection analysis using a HPLC system.

Electrolysis of colchicine 1. (a) In aqueous acidic medium. Coulombometric data were obtained for reduction of colchicine (1) (100 mg) at a mercury cathode in 25 mL of aqueous medium at pH = 1. During the process a copious precipitation of a gummy, red material was observed. The electrolysis was

interrupted at 80% conversion of **1**, the precipitate was separated from the mother liquor by centrifugation, and both the precipitate and the mother liquor were $CHCl_3$ extracted. The organic layer was evaporated and TLC of the residue revealed a complex array of colored spots, while reverse-phase HPLC only allowed to obtain from the precipitate in very minute amounts unreacted **1**, colchiceine (**2**) and a new product that was preliminarily identified as a ring-contracted benzenoid aldehyde derivative of colchicine. LC-ESI-MS analysis of the precipitate confirmed the presence of minute amounts of colchicine and colchiceine, whereas peaks at *m/z* 721, 1121, and 1458, likely for protonated molecules, could not be assigned. No evidence for dimeric colchicinoids could be obtained. *(b) In a semiaqueous medium.* Reduction of colchicine (**1**), 100 mg, was carried out in CH_3CN/0.1 M aq. $HClO_4$ 1:1 at a mercury cathode, by which the mixture turned to a red color while no precipitate was observed. After *ca.* 90% conversion of colchicine, the electrolysis was interrupted and the mixture was $CHCl_3$ extracted. TLC analysis revealed a complex array of products similar to that observed for the electrolysis in aqueous medium. Extraction of the mixture with CH_3CN and slow evaporation of the solvent led to the separation of colorless crystals in very minute amount. These were revealed by HPLC analysis to be a mixture of at least three difficultly separable products. The mixture was fractionally recrystallized from CH_3CN, no fraction revealing ^1H NMR signals at lower field than $\delta = 5$ ppm. *(c) In non aqueous medium.* Colchicine (**1**), *ca.* 100 mg, was reduced at a platinum cathode in 25 mL of freshly distilled, dry DMF, observing no precipitate, while the solution turned to red in color. The electrolysis was interrupted at *ca.* 90% conversion of colchicine and the mixture was evaporated at reduced pressure. Reverse-phase HPLC with CH_3CN/H_2O 2:3, monitoring at $\lambda = 254$ nm, afforded 10-aminomethylcolchiceine (**3**) (15%) and unreacted colchicine (9%); LC-ESI-MS of the reaction mixture revealed also a signal at *m/z* 413, possibly due to dimethylaminocolchiceine, besides a signal at *m/z* 416.

Computational details. Full optimization of the geometry *in vacuo* of the radical anion and calculation of the spin density surface were carried out using the UNIX version of *Spartan*[20] computer program, running on an IBM RISC 6000/595 workstation. To reduce the number of cycles required for geometry optimization, a semi-empirical model at PM3 level was initially used to provide a starting equilibrium geometry and a matrix of second energy derivatives for *ab initio* calculations.

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REFERENCES AND NOTES

1. Shin, Q.; Chen, K.; Brossi, A.; Verdier-Pinard, P.; Hamel, E.; McPhail, A.T.; Lee, K.-H. *Helv. Chim. Acta* **1998**, *81*, 1023-1037.
2. Cabin, R.M.; Feliciano, F.; Hastie, S.B. *Biochemistry* **1990**, *29*, 1869-1875.
3. Berg, U.; Bladh, H. *Helv. Chim. Acta* **1999**, *82*, 323-325.
4. Brossi, A.; Yeh, H.J.C.; Chrzanowska, M.; Wolf, J.; Hamel, E.; Lin, C.M.; Quin, F.; Suffness, M.;

Silverto, J. *Med. Res. Rev.* **1988**, *8*, 77-94.

5. (a) Brdicka, R. *Cas. ces. lékarn* **1945**, *37*, 35-47; Šantavý, F.*Coll. Czech. Chem. Commun.* **1949**, *14*, 145-155; Kirkpatrick, H.F.H. *Quart. J. Pharm. Pharmacol.* **1946**, *19*, 526-535; (b) Sartori, G.; Gaudiano, A. *Rend. Ist. Super. Sanità* **1950**, *13*, 659-669; (c) Neish, W.J.P.; Müller, O.H *Recl. Trav. Chim. Pays-Bas* **1953**, *72*, 301-313; (d) Wang, J.; Ozsoz, M. *Talanta* **1990**, *37*, 783-787.
6. Davis, P.J. *Antimicrob. Agents Chemother.* **1981**, *19*, 465-469; Fabian, J.; Delaroff, V.; Poirier, P.; Legrand, M. *Bull. Chem. Soc. Fr.*, **1955**, 1455-1463.
7. WinSim32, V 0.95, 1994, by Dave Duling, Laboratory of Molecular Biophysics, NIEHS, USA.
8. Cavazza, M.; Veracini, C.A.; Pietra F. *J. Chem. Soc., Perkin Trans 2* **1992**, 2201-2204.
9. Cavazza, M.; Pietra F. *J. Chem. Soc., Perkin Trans 1* **1995**, 2657-2661.
10. Hehre, W. J.; Radom, L.; Schleyer, P.v.R.; Pople, J.A. *Ab Initio Molecular Orbital Theory*, Wiley, New York, 1986.
11. Hehre, W. J.; Ditchfield, R.; Pople, J.A. *J. Chem. Phys.* **1972**, *56*, 2257-2261.
12. Chipman, D.M. *Theor. Chim. Acta* **1992**, *82*, 93-115.
13. Hameka, H. F.; Turner, A.G. *J. Magn. Reson.* **1985**, *64*, 66-75.
14. Fortunelli, A.; Salvetti, O. *J. Mol. Struct. (Theochem)* **1993**, *287*, 89-92.
15. Fortunelli, A. *Int. J. Quantum Chem.* **1994**, *52*, 97-108.
16. Donaldson, W.A. *Tetrahedron* **1988**, *44*, 7409-7412.
17. Cavazza, M.; Colombini, M.P.; Martinelli, M.; Nucci, L.; Pardi, L.; Pietra, F.; Santucci, S. *J. Am. Chem. Soc.* **1977**, *99*, 5997-6002, and references therein to previous work.
18. Hoganson, C.W.; Sahlin, M.; Sjöberg, B.-M.; Babcock, G.T. *J. Am. Chem. Soc.* **1996**, *118*, 4672-4679.
19. Williams, M.J. Ed., *Enzyme Mechanisms*, Royal Society of Chemistry, Burlington House, London, 1989.
20. *Spartan*, version 4.1.2, Wavefunction, Inc., Irvine, California.