MA. The ascitic sarcoma 180 cells were originally obtained from Frederic A. French, Chemotherapy Laboratory, Mount Zion Hospital, San Francisco, CA. The details of the protocol are given in Table I. Although each compound was administered at several dose levels, only the dose that gave the maximum T/C value is recorded in Table I. The daily dose that would increase survival times by 25% was obtained by interpolation and extrapolation from the results of other doses in the effective range of the compound.

Statistical Methods. Multiple linear regression studies were performed with the statistical analysis system (SAS)[27] on an IBM 370/168 computer. The RSQUARE procedure was used to determine the independent variable that was most significant for each dependent examined. This procedure determines the correlation coefficient for the dependent variable and each of the independent variables and lists these in order of their correlation coefficients. The REGRESSION procedure may then be used to obtain the equation that relates the index to any variable or variables and other statistical information.

All variables were examined by the CORRELATION procedure of the Statistical Analysis System to determine if any collinearity exits between the variables. A high degree of correlation between variables ($r = 1$) would give a biased result when they are used together.

(27) J. Service, "A User's Guide to the Statistical Analysis System", North Carolina State University, Raleigh, NC, 1972.

Acknowledgment. We appreciate the expert assistance of Janie Youles who managed our animal colony and Norman Perreira who obtained our mass spectrographic data. We are particular grateful for the many helpful discussions with Dr. Jeffrey S. McKennis.

Registry No. 1, 130-15-4; 2, 84-79-7; 3, 1090-16-0; 4, 117-80-6; 5, 2797-51-5; 6, 15707-32-1; 7, 59641-32-6; 8, 5397-78-4; 9, 83-72-7; 10, 58-27-5; 11, 2348-82-5; 12, 84369-93-7; aniline, 62-53-3.

o-Quinone Formation in the Biochemical Oxidation of the Antitumor Drug N^2-Methyl-9-hydroxyellipticinium Acetate

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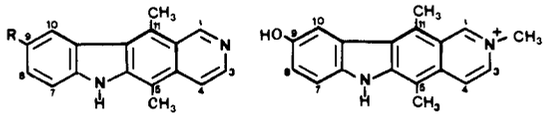
The activation of N^2-methyl-9-hydroxyellipticinium acetate (4) by a peroxidase–H_2O_2 system leads to the formation of an *o*-quinone (**7a**). This *o*-quinone is not directly generated from the starting material but through a quinone imine intermediate (**6**) which is subsequently oxidized. This reaction is highly dependent on pH values. The *o*-quinone **7a** is easily protonated (**7b**), gives an addition product with methanol (**9**), and is reduced by cysteine. The *o*-quinone **7b** has a rather low inhibitory effect against L1210 leukemia cell multiplication but acts as an electron carrier and dramatically augments the oxygen consumption in xanthine oxidase–NADH and rat liver microsomes–NADPH systems.

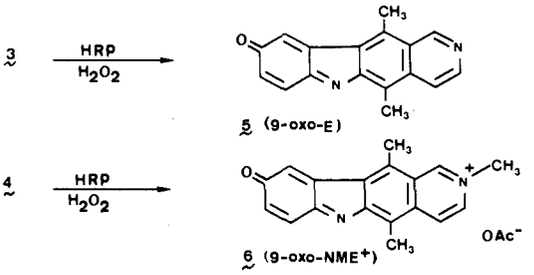
Among the molecules derived from ellipticine (**1**, 5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole), a cytotoxic alkaloid extracted from plants of the Apocynacae family, those with an alkoxy or an hydroxy group in position 9 have recently been considered for clinical trials.[1] 9-Methoxyellipticine (**2**), a natural derivative of ellipticine, has been reported to be active in acute myeloblastic leukemia;[1b] 9-hydroxyellipticine (**3**) and the N^2-methyl-9-hydroxyellipticinium (**4**, 9-OH-NME$^+$) present a wide range of antitumor activity against experimental tumors.[1c] The latter compound, **4**, exhibits an appreciable activity against osteolytic breast cancer metastasis and anaplastic thyroid carcinoma.[1c] The high DNA affinity for these molecules (**1**–**4**) by intercalation between base pairs was first suggested to explain the antitumor activity of these drugs.[2] More recently, preliminary investigations on the oxidation of **3** and **4** by a peroxidase/hydrogen peroxide system support the hypothesis of an activation in vivo of the hydroxylated ellipticines.[3]



1 R = H
2 R = OCH_3
3 R = OH (9-OH E)

4 (9-OH-NME$^+$)

So the enhanced antitumor activity of **3** and **4** might be related to the DNA intercalation phenomena and the reactivity of oxidized products obtained in vivo from **3** and **4**. For example, the two-electron oxidation of **3** and **4** by horseradish peroxidase (HRP) and hydrogen peroxide, respectively, lead to the formation of the two quinone imines **5** and **6**.[3] Compound **5** is obtained as a well-defined stable solid, but **6** has not yet been isolated and fully characterized due to its high reactivity in solution.



5 (9-oxo-E)

6 (9-oxo-NME$^+$)

† Laboratoire de Pharmacologie et Toxicologie Fondamentales.
‡ Laboratoire de Chimie de Coordination.

(1) (a) G. M. Svoboda, G. A. Poore, and M. L. Montfort, *J. Pharm. Sci.*, **57**, 1720 (1968). (b) G. Mathé, M. Hayat, F. de Vassal, L. Schwarzenberg, M. Schneider, J. R. Schlumberger, C. Jasmin, and C. Rosenfeld, *Rev. Eur. Etud. Clin. Biol.*, **15**, 541 (1970), and references therein. (c) C. Paoletti, J. B. Le Pecq, N. Dat-Xuong, P. Juret, H. Garnier, J. L. Amiel, and J. Rouesse, *Recent Results Cancer Res.*, **74**, 107 (1980), and references therein.
(2) (a) B. Festy, J. Poisson, and C. Paoletti, *FEBS Lett.*, **17**, 321 (1971). (b) J. B. Le Pecq, N. Dat-Xuong, C. Gosse, and C. Paoletti, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 5078 (1974).
(3) C. Auclair and C. Paoletti, *J. Med. Chem.*, **24**, 289 (1981).
(4) G. Meunier, Ph.D. Thesis, Toulouse University, June 1982.

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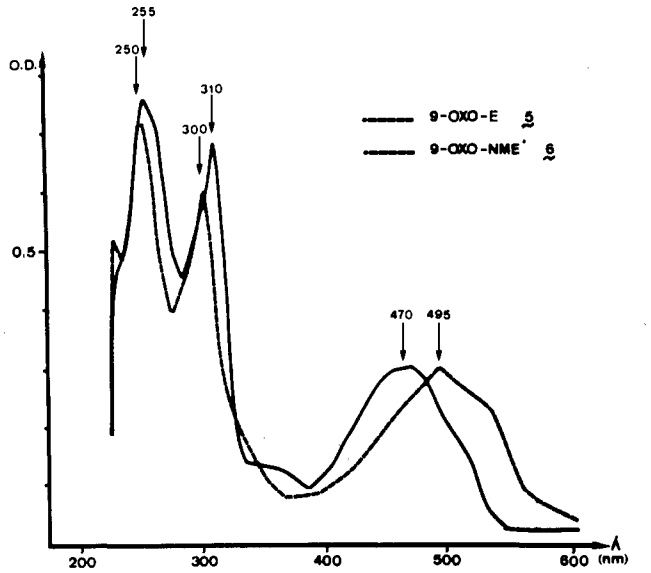
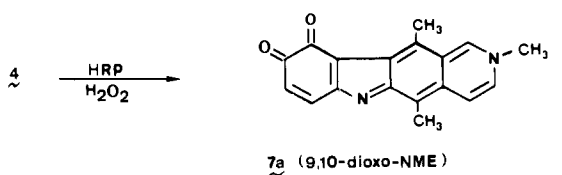


Figure 1. UV–visible spectrum of **5** and **6** in CH_2Cl_2. Experimental conditions: phosphate buffer, pH 5, 5×10^{-2} M; [9-OH-E (**3**)] = [9-OH-NME$^+$ (**4**)] = 2.5×10^{-5} M; [HRP] = 10^{-8} M; [H_2O_2] = 5×10^{-5} M; extraction with CH_2Cl_2 at t = 2 min in the presence of NH_4PF_6, 2.5×10^{-4} M.

We report here the formation of the *o*-quinone **7a** obtained in the bioactivation of **4** by the HRP–H_2O_2 system.



7a (9,10-dioxo-NME)

We also report some chemical and biological properties of **7a**, keeping in mind the electrophilic reactivity of *o*-quinone derivatives (for some examples, see ref 5–7) and the presence of quinonoid groups in some antitumor drugs (see ref 8 and references therein).

Stability of the Quinone Imine 6, and Formation of the *o*-Quinone 7a. After several unsuccessful trials, we have not been able to isolate as a pure well-defined solid the quinone imine **6** generated from **4** by the HRP–H_2O_2 system. However, we have other evidence to confirm the quinone imine structure previously proposed for **6**.[3] It is possible to obtain a solution of the hexafluorophosphate salt of **6** by extraction of the aqueous phase with methylene chloride in the presence of NH_4PF_6. This solution is stable at low temperature (below −20 °C), but a slow degradation (polymers?) is observed at room temperature. Unfortunately, the solutions of **6** in deuterated methylene chloride are too diluted to record ^1H NMR spectra with well-defined peaks.

However, the electron-impact mass spectra of this organic solution of **6** show the expected molecular peak (M^+) at m/e 275 (in the same conditions the molecular peak of the starting material appears at m/e 277). The UV–visible spectrum of **6** in CH_2Cl_2 exhibits three maxima at λ 255, 310, and 470 nm and is in good correlation with the spectrum of **5** (Figure 1).

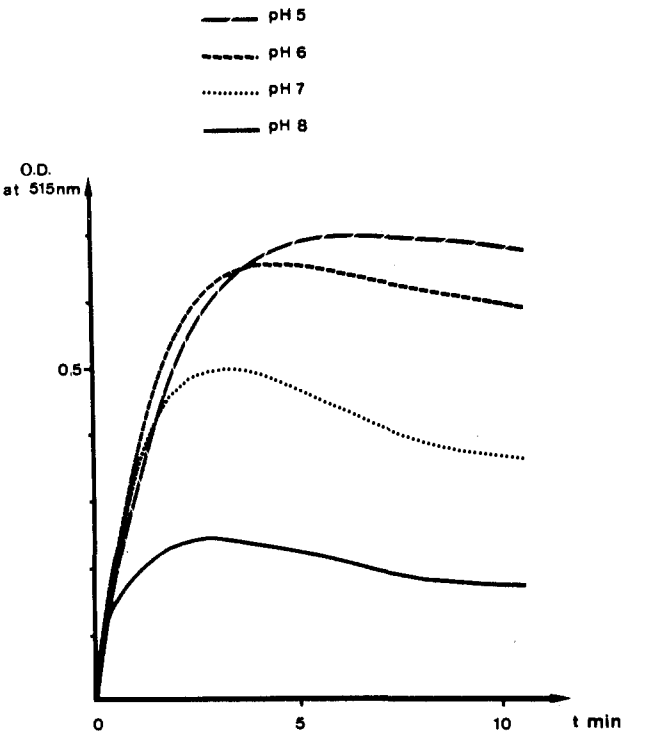
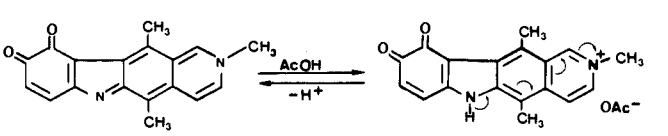


Figure 2. pH-dependent formation and stability of 9-oxo-NME$^+$ (**6**) (OD variations at 515 nm): phosphate buffer 5×10^{-2} M; [**4**] = 10^{-4} M; [H_2O_2] = 2×10^{-4} M; [HRP] = 5×10^{-8} M; t = 20 °C.

During our investigations on the stability of the quinone imine **6** we observed that this stability is highly pH dependent and appears to be optimal at pH 5.0 (Figure 2). The formation of **6** in the HRP–H_2O_2 oxidation of **4** is monitored by visible spectroscopy at 515 nm, where the absorbance of **4** is very weak.[3] Above pH 5.0, the quinone imine absorption rapidly decreases (see Figure 2). The UV–visible spectra in the range 200–600 nm exhibit the initial formation of the quinone imine **6** (λ_{max} 260, 310, and 490 nm) and the pH-dependent conversion to a new compound, **7** (λ_{max} 230 and 300 nm at pH 8.0, 222 and 285 nm at pH 5.0; see Figure 3a,b). This interconversion of products in the HRP–H_2O_2 oxidation of **4** can also be monitored by high-performance liquid chromatography (HPLC). At pH 5.0, the rapid formation of 9-oxo-NME$^+$ (**6**; Rt = 0.88, as defined under Experimental Section) and the slow formation of a new compound (**7**; Rt = 0.58) are observed within 3–4 h time (see Figure 4a). As we increase the pH of the solution, the formation of **7** is accelerated: for example, at pH 8.0, **6** is rapidly converted to **7** in 15–20 min (see Figure 4b).

Isolation and Identification of the *o*-Quinones 7a,b. Under the conditions described in detail under Experimental Section, a dark-green precipitate is isolated from the peroxidase oxidation of **4** with HRP and H_2O_2 at pH 8.0. The crude product is washed with water to eliminate the residues of starting materials. From spectroscopic data, the structure **7a** has been attributed to this water-insoluble compound. The protonated form **7b** is easily prepared



7a (9,10-dioxo-NME) 7b (9,10-dioxo-NME$^+$)

(5) W. M. Horspool, P. I. Smith, and J. M. Tedder, *J. Chem. Soc. C*, 138 (1971).
(6) R. Davies and J. L. Frahn, *J. Chem. Soc., Perkin Trans. 1*, 2295 (1977).
(7) (a) D. J. Liberato, V. S. Byers, R. G. Dennick, and N. Castagnoli, *J. Med. Chem.*, **24**, 28 (1981). (b) S. Ito, S. Indue, Y. Yamamoto, and K. Fujita, *J. Med. Chem.*, **24**, 673 (1981).
(8) (a) H. W. Moore, *Science*, **197**, 527 (1977). (b) L. R. Bachur, S. L. Gordon, and M. V. Gee, *Cancer Res.*, **38**, 1745 (1978).

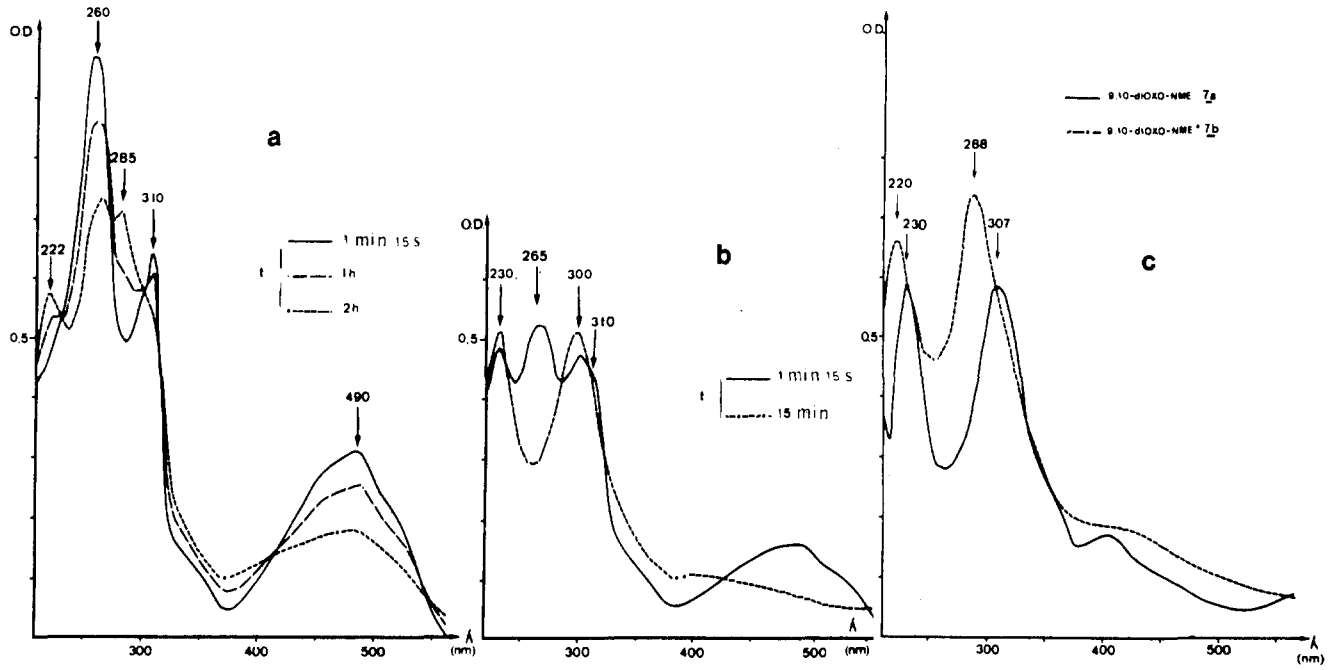
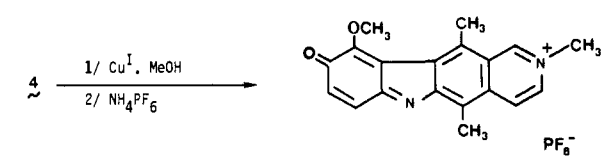


Figure 3. UV–visible data. (a) Evolution of **6** at pH 5: phosphate buffer, pH 5, 5×10^{-2} M; [4] = 10^{-4} M; [H_2O_2] = 2×10^{-4} M; [HRP] = 5×10^{-8} M. (b) Evolution of **6** at pH 8: phosphate buffer, pH 8, 5×10^{-2} M; [4] = 10^{-4} M; [H_2O_2] = 2×10^{-4} M; [HRP] = 5×10^{-8} M. (c) UV–visible spectrum of **7a** (2.68×10^{-5} M in CH_3OH) and **7b** (3.09×10^{-5} M in H_2O).

from **7a** by the addition of acetic acid in a methanolic solution of **7a**, followed by a precipitation with diethyl ether. The brown water-soluble quinone **7b** is reversibly deprotonated in basic medium.

The structure of **7a** has been established by ^1H NMR, IR, mass spectra, and microanalysis. The absence of both a H_{10} NMR resonance and a $^4J_{H_8-H_{10}}$ coupling constant indicates the absence of the hydrogen atom in position 10. Furthermore, the H_7 and H_8 protons appear as an AB quartet with a chemical-shift difference of 1.25 ppm. This same feature for H_7 and H_8 is also observed in the NMR spectrum of a methoxylated quinone imine **8**. This compound is obtained by oxidation of **4** with molecular oxygen in the presence of copper complexes in methanol. The

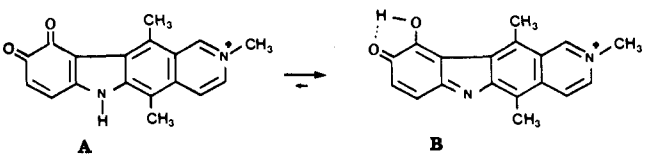


8 9-oxo-10-MeO-NME$^+$

complete description of the preparation and the properties of **8** will be published elsewhere.[9] However, the ^1H NMR of **8** is reported under Experimental Section to support the proton assignment for **7a**. The resonance at 6.34 ppm has been attributed to the proton H_8. A similar shielding is observed for H_8 in the quinone imines **5**[3] and **8** (see Experimental Section). The infrared spectrum of **7a** presents two bands at 1660 and 1650 cm^{-1}, compatible with two carbonyl stretching vibrations of an *o*-quinone. A weak band at 1630 cm^{-1} is also observed and attributed to a C=N vibration. The electron-impact mass spectrum shows the molecular peak (M^+) at *m/e* 290, and by using the chemical-ionization method, two ions are detected at *m/e* 292 (MH_2^+) and 293 (MH_3^+) for the protonated molecules. This type of protonation has also been observed for other quinone molecules.[10] All these data support the assignment of *o*-quinone structure **7a**. Unusual features are observed for the ^1H NMR spectra of the protonated *o*-quinone **7b**. Attempts to record the NMR data in solvents like D_2O, CD_3COOD, or CD_3OD lead to poorly resolved spectra, possibly due to stacking of the molecules. In these protic solvents, the solutions have the deep-red color usually observed for solutions of **7b**. In contrast, the solutions of **7b** in Me_2SO are green and present a spectrum similar to that of **7a** in the same solvent, except for the presence of an acetate peak at 1.94 ppm. Apparently, **7b** (a weak acid, pK_a = 5.1; see below) is deprotonated by dimethyl sulfoxide.

In fact, upon addition of deuterated acetic acid, the solution turns to a red color, and a well-defined spectrum of the protonated form of the *o*-quinone can be recorded. The protons H_7 and H_8 and also the methyl groups at position 5 and 11 are affected by the protonation of the molecule. The chemical shift (4.44 ppm) for the *N*-Me group is the same in Me_2SO for both forms, indicating that the protonation of the indolic nitrogen atom modifies only the electronic density of the two contiguous cycles of the five-membered ring. So the chemical shifts of the elements of the pyridine ring are nearly the same for both forms.

The infrared spectrum of **7b** presents two broad bands at 1640 and 1620 cm^{-1}, suggesting that the tautomer B is



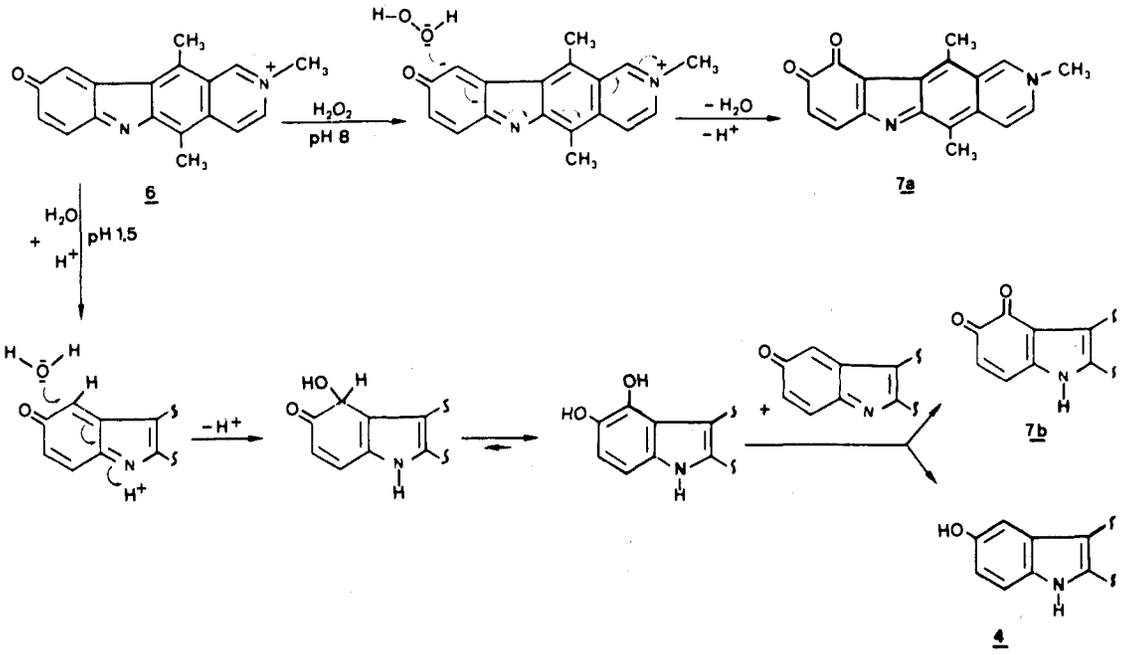
A B

the predominant form for the protonated *o*-quinone **7b**. The intramolecular hydrogen bond would explain the displacement of the CO band to lower frequency than in **7a**. In comparison, **8** shows two bands at 1670 and 1640 cm^{-1} compatible with the C=O and C=N of the quinone imine structure, but for this molecule the presence of a methoxy group in position 10 eliminates the shift to lower frequency of the CO band by hydrogen bonding.

The maxima in UV–visible spectra are close together for both forms **7a** and **7b**: 230 and 307 nm for **7a**; 220 and

(9) B. Meunier, unpublished results.
(10) D. Issachar and J. Yinon, *Anal. Chem.*, **52**, 49 (1980).

Scheme I. Proposed Mechanisms for the Formation of the o-Quinone 7



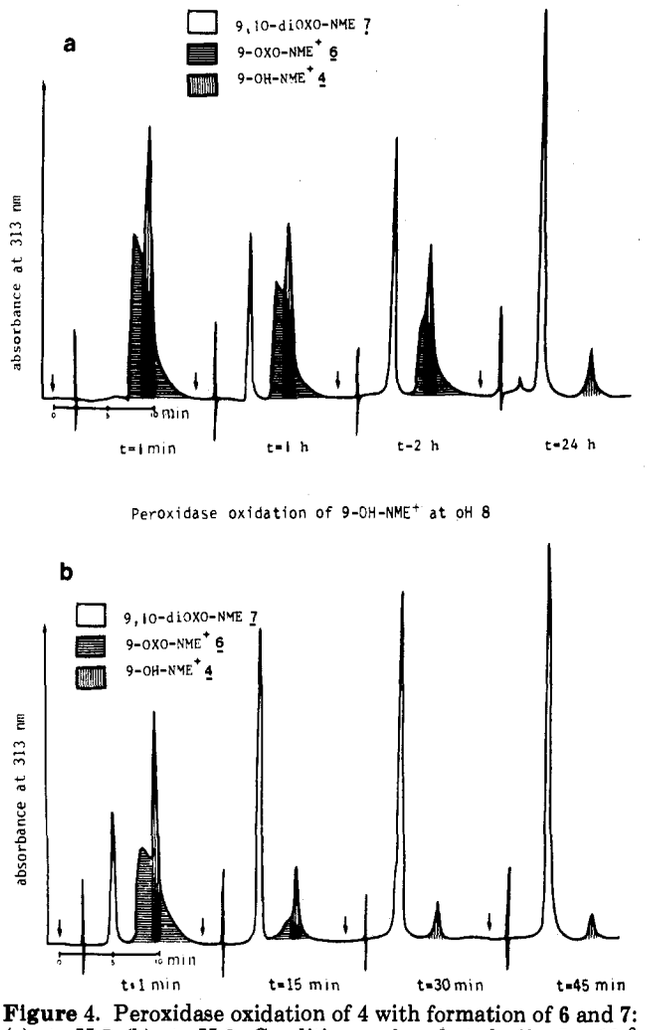
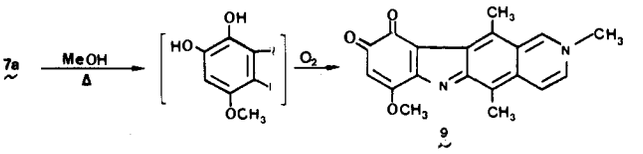


Figure 4. Peroxidase oxidation of 4 with formation of 6 and 7: (a) at pH 5, (b) at pH 8. Conditions: phosphate buffer 5×10^{-2} M; [4] = 10^{-4} M; [H_2O_2] = 2×10^{-4} M; [HRP] = 5×10^{-8} M. The different products evolution is monitored by HPLC with a spectrophotometric detector (λ 313 nm).

288 for **7b** (see Figure 3c). The pK_a for **7b** determined by base titration is 5.1 ± 0.1, implying that the o-quinone 7 would be deprotonated under physiological conditions.

Some Chemical Properties of the o-Quinone 7. The o-quinone **7a** is stable at room temperature in methanol



over 5–6 h. A degradation occurs beyond this period. In boiling methanol, a substitution product (**9**) is obtained after 2 h. The ^1H NMR and IR data are consistent with an o-quinone bearing a methoxy group in position 7.

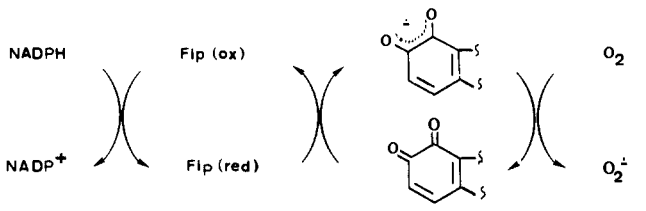
The reaction of **7a** with cysteine in methanol involves a rapid color change of the solution from green to yellow. This yellow solution is only stable in the presence of an excess of cysteine. All attempts to isolate the corresponding bisphenol have failed. The o-quinone is regenerated from this solution probably through the aerial autoxidation of the bisphenol as supposed in the formation of the methoxyquinone **9**. It has to be noted that the o-quinone **7a** cannot be reduced under aerobic conditions to the bisphenol with ammonium sulfide or sodium borohydride; the starting material is always recovered due to the extremely rapid oxidation by oxygen of the corresponding bisphenol to the o-quinone.

Proposed Mechanism for the Formation of the o-Quinone 7. To understand the chemical properties of **6**, we extracted this quinone imine, generated in aqueous medium, with dichloromethane in the presence of ammonium hexafluorophosphate and then reextracted this solution with water. Thus, it is possible to study a water solution of **6** without the presence of the horseradish peroxidase.

Such a solution leads rapidly to the o-quinone **7a** after the addition of hydrogen peroxide alone at pH 8.0. The peroxidase itself cannot do this transformation without hydrogen peroxide.

This type of oxidation by H_2O_2 is pH dependent and is favored in basic medium (see Scheme I). At low pH values, the formation of the o-quinone **7** is probably due

Scheme II. Bachur's Model for the Catalyzed Oxygen Consumption by Quinone Derivatives



to another mechanism. As matter of fact, at pH 1.5 a water solution of **6** gives without peroxidase or hydrogen peroxide a mixture of **7b** and the hydroxy derivative **4**. At such pH, the addition of a water molecule in position 10 leads to an *o*-bisphenol, which is rapidly oxidized by the quinone imine **6** to give the *o*-quinone **7b** and the reduction product **4** (see Scheme I).

So two possibilities have to be considered for the formation of the *o*-quinone **7**; the first one is probably the route for the oxidation of **6** to the *o*-quinone **7** under physiological conditions.

It should also be noted that this *o*-quinone results from the nucleophilic addition of an H_2O_2 or H_2O molecule in position 10, which is more sterically hindered than the position 8. This fact has also been observed for the addition of other various nucleophiles (amines, thiols, etc.) on the same quinone imine **6** and is currently under investigations.[4]

Biological Activities of the *o*-Quinone 7. The cytotoxicity of the hydroxy derivative 9-OH-NME^+ has been tested in vitro on experimental tumors such as murine leukemia L1210. This molecule is highly cytotoxic against that type of tumor cells. The ID_{50}, the dose that reduces the cell growth by 50% after 48 h, is close to the most cytotoxic antitumoral drugs.[1c] The ID_{50} for **6** is 0.05 μM.[1c] Under the same conditions, the ID_{50} has been determinated for two *o*-quinones, **7b** and **9**: their respective values are 9.6 and 5.8 μM. Therefore, the cytotoxicity seems to be very low in comparison with the 9-OH-NME^+ itself.

This weak cytotoxicity is not comparable with those of highly antitumoral quinonoid drugs like anthracyclines and related compounds. In these drugs the antitumoral activity is related to the alkylating properties obtained through a bioreduction of the quinone moiety of the molecule.[8a] As described above, the *o*-quinone **7** appears to be a poor electrophile (**7** reacts only with methanol at reflux), and the absence of a leaving group on the molecule does not give rise to alkylating properties after a bioreductive process.

However, the high activity of these *o*-quinones as electron carriers has to be noted. The *o*-quinone derivatives **7** and **9** augment the flow of electrons from NADPH (reduced adenine dinucleotide phosphate) to molecular oxygen as measured by enhanced oxygen consumption with rat liver microsomes as described by Bachur et al. for quinone-containing anticancer drugs.[8b,11]

The stimulation of oxygen consumption has been determinated for **7** with two systems: rat liver microsomes/NADPH[8b,11] and xanthine oxidase/NADH[12a] (see Figure 5). As assumed by Bachur, the first product in the reduction of molecular oxygen could be the superoxide anion (see Scheme II), which may influence the peroxidation of lipids directly or through the generation of hydroxide radical HO·.

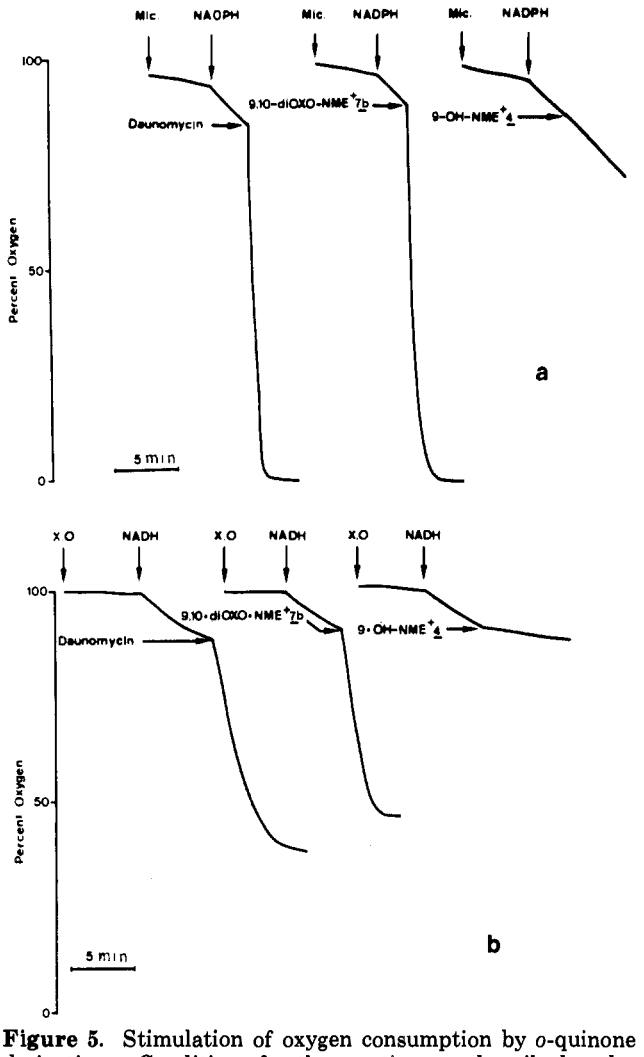


Figure 5. Stimulation of oxygen consumption by *o*-quinone derivatives. Conditions for the reaction are described under Experimental Section: (a) rat liver microsomes/NADPH system; (b) xanthine oxidase/NADH system.

This catalytic activation of oxygen consumption is probably not the whole explanation of the cytotoxicity of these *o*-quinones, but it could be a way to explain some side toxicity of these ellipticine derivatives through the decrease of oxygen concentration in cells. Studies are in progress to verify such an hypothesis.

Experimental Section

UV–visible spectra were performed on a Beckman Acta III spectrophotometer. IR data were obtained from a Perkin-Elmer 225 spectrophotometer with samples in CsBr pellets. NMR spectra were recorded on a Brucker WH 90 (90 MHz) in the FT mode. The chemical shifts are expressed in parts per million (δ) with tetramethylsilane as internal standard. A Riber R10-10 apparatus was used for the mass spectra. All the HPLC studies were performed on a Waters chromatograph with a μBondapak C_{18} column and a mixture of methanol–water (50:50, v/v) as eluent with ammonium acetate (10^{-3} M and acidified to pH 4.5 with acetic acid). A "Rt value" is assigned to each product and defined as the ratio of the retention time of the product to the retention time of the 9-OH-NME^+ (**4**), with the injection used as zero. Detection of the ellipticine derivatives is monitored by a UV spectrometer at 313 nm. Microanalyses were kindly performed by the analysis department of the SANOFI Co. (Toulouse, France).

Chemicals. Horseradish peroxidase, xanthine oxidase, NADPH, and NADH were obtained from Sigma Chemical Co. (St. Louis). The 9-OH-NME^+ (**4**) was a gift of the SANOFI Co. (Sisteron, France).

Oxidation of 4 with HRP–H_2O_2. The usual oxidations of **4** were carried out in 5×10^{-2} M phosphate buffer (pH 8.0) with

(11) N. Bachur, J. L. Gordon, and M. V. Gee, *Mol. Pharmacol.*, **13**, 901 (1977).
(12) (a) S. Pan and N. Bachur, *Mol. Pharmacol.*, **17**, 95 (1980).

10^{-4} M 9-OH-NME$^+$ (4), 2×10^{-4} M H_2O_2, and 5×10^{-8} M HRP at 25 °C.

9,10-Dioxo-NME (7a). A solution of 84 mg (0.25 mmol) of 4 in 100 mL of water, 0.5 mmol of H_2O_2, and 100 nmol of HRP were incubated at 25 °C for 30 min. After filtration, the dark-green precipitate was washed with water and dried under vacuum: 70 mg of **7a** are obtained (73% yield); EIMS, *m/e* 290 (M^+); CIMS, *m/e* (ammonia) 292 (MH_2^+), 293 (MH_3^+); IR (CsBr) 1650 and 1660 (C=O) 1630 (weak, C=N) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.90 (s, 3 H, 5-Me), 3.46 (s, 3 H, 11-Me), 4.42 (s, 3 H, N-Me), 6.34 (d, 1 H, J = 9.8 Hz, H_8), 7.59 (d, 1 H, J = 9.8 Hz, H_7), 8.28 (2 d, 2 H, J = 7.0 Hz, H_3, H_4), 9.69 (s, 1 H, H_1); UV-visible (CH_3OH) λ_{max} 230 nm (ϵ 21 600), 307 (21 600), 405 (6600), 608 (3600). Anal. (WO_3 catalyzed) ($C_{18}H_{14}N_2O_2$) C, H, N.

9,10-Dioxo-NME$^+$OAc$^-$ (7b). To a solution of **7a** (25 mg) in 800 μL of CH_3OH and 250 μL of CH_3COOH, 25 mL of diethyl ether was added. After filtration, the brown precipitate was washed with ether and dried under vacuum: 23 mg of **7b** was obtained (88% yield). EIMS, *m/e* 291 (M^+); CIMS, *m/e* (ammonia) 292 (MH^+), 293 (MH_2^+); IR (CsBr) 1640 and 1620 (broad-band, C=O) cm^{-1}; ^1H NMR Me_2SO-d_6/CD_3COOD, 2.5:1, v/v) δ 2.79 (s, 3 H, 5-Me), 3.64 (s, 3 H, 11-Me), 4.44 (s, 3 H, N$^+$-Me), 6.97 (d, 1 H, J = 7.6 Hz, H_8), 7.22 (d, 1 H, J = 7.6 Hz, H_7), 8.31 (m, 2 H, H_3 and H_4), 9.85 (s, 1 H, H_1); UV-visible (CH_3OH) 220 nm (ϵ 20 600), 295 (23 100), 400 (7000) (sh); UV-visible (H_2O) 220 (21 400), 288 (23 900), 420 (6000). Anal. (WO_3 catalyzed) ($C_{20}H_{18}N_2O_4$) C,[13] H, N.

9-Oxo-10-OMe-NME$^+$PF$_6^-$ (8): ^1H NMR (CD_3COCD_3/D_2O, 2:1, v/v) δ 2.96 (s, 3 H, 5-Me), 3.24 (s, 3 H, OMe), 3.34 (s, 3 H, 11-Me), 4.60 (s, 3 H, N$^+$-Me), 6.48 (d, 1 H, J = 10.0 Hz, H_8), 7.91 (d, 1 H, J = 10.0 Hz, H_7), 8.47 (2 d, 2 H, J = 8.0 Hz, H_3 and H_4), 10.00 (s, 1 H, H_1).

7-OMe-9,10-dioxo-NME (9). A solution of **7a** (15 mg) in 50 mL of CH_3OH was refluxed for 2 h. After concentration, 10 mg of **9** was obtained (61% yield); EIMS, *m/e* 320 (M^+); IR (CsBr) 1660 and 1640 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.88 (s, 3 H, 11-Me), 3.94 (s, 3 H, OCH_3), 4.38 (s, 3 H, N-Me), 5.77 (s, 1 H, H_8), 8.25 (2 d, 2 H, J = 5.5 Hz, H_3 and H_4), 9.71 (s, 1 H, H_1), 5-Me is masked by the solvent. Anal. (WO_3 catalyzed) ($C_{19}H_{16}N_2O_3 \cdot 2H_2O$) C, H, N.

Oxygen Measurements. Oxygen consumption was measured with a Clark electrode in a Gilson oxygraph in a final volume of 1.3 mL with 0.067 M phosphate buffer (pH 7.5) at 37 °C.

Xanthine Oxidase/NADH System. The procedure described by Bachur[12] was used with 0.2 mM NADH, 240 μg/mL of xanthine oxidase, and 0.5 mM drugs.

Microsomes/NADPH System.[8b,11] Male Wistar rats weighing about 150 g were used. Livers were removed and microsomes were prepared according to a previously described procedure.[14] Proteins were measured as described by Lowry et al:[15] 2 mg/mL of liver microsomes, after treatment with a solution (1%) of Triton N101, was added to a mixture containing 6 mM NADPH and 0.5 mM drugs.

Cytotoxicity. Inhibition of cell growth was determined with L1210 leukemia cells in vitro as previously described.[16] The inhibitory efficiency of the drug against cell multiplication is expressed in terms of ID_{50}, the drug concentration that reduces the number of cells by 50% as compared to the control after 48 h.

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Registry No. 4, 58337-34-1; **6**, 84303-61-7; **7a**, 84303-55-9; **7b**, 84303-57-1; **8**, 84303-59-3; **9**, 84303-60-6; NADPH, 53-57-6; NADH, 58-68-4; horseradish peroxidase, 9003-99-0; xanthine oxidase, 9002-17-9.

(13) C: calcd, 68.14; found, 68.57. Without WO_3, all the analyses of the quaternarized molecules are below the calculated values due to a poor combustion of samples. In some cases, even with WO_3, the values are not in the admitted 0.4% limit.

(14) P. Lesca, P. Lecointe, C. Paoletti, and D. Mansuy, *C. R. Hebd. Seances Acad. Sci., Ser. D*, **282**, 1457 (1976).
(15) O. H. Lowry, N. J. Rosenbrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
(16) C. Paoletti, S. Cros, N. Dat-Xuong, P. Lecointe, and A. Moisand, *Chem.-Biol. Interact.*, **25**, 45 (1979).