[CANCER RESEARCH 47, 1036–1039, February 15, 1987]

Loss of Fluorescence by Anthracycline Antibiotics: Effects of Xanthine Oxidase and Identification of the Nonfluorescent Metabolites[1]

Pierre Dodion,[2] Amy L. Bernstein, Bonnie M. Fox, and Nicholas R. Bachur

Laboratory of Medicinal Chemistry and Pharmacology, National Cancer Institute, Bethesda, Maryland 20205 [P. D., N. R. B.], and Division of Developmental Therapeutics, University of Maryland Cancer Center, Baltimore, Maryland 21201 [A. L. B., B. M. F.]

ABSTRACT

Rat liver cytosol and buttermilk xanthine oxidase both converted 7-deoxypyrromycinone, the 7-deoxyaglycone of marcellomycin, a new anthracycline antibiotic, to a nonfluorescent compound under anaerobic conditions and in the presence of an electron donor. Reduced nicotinamide adenine dinucleotide and reduced nicotinamide adenine dinucleotide phosphate were equally effective electron donors for liver cytosol, and xanthine was the best cofactor for xanthine oxidase. However, xanthine was inactive with liver cytosol. Reactions with xanthine oxidase obeyed Menten-Michaelis kinetics and were inhibited by allopurinol. No xanthine oxidase activity was detected in liver cytosol. Xanthine oxidase also induced a loss of fluorescence when incubated with 7-deoxydaunorubicin aglycone. The nonfluorescent metabolite of 7-deoxypyrromycinone was tentatively identified as the dihydroquinonic derivative of the parent deoxyaglycone on the basis of its spectrophotometric, fluorescent, thin layer chromatographic, and mass spectral characteristics. Our data demonstrate that more than one enzymatic activity, xanthine oxidase, and an unidentified rat liver cytosolic enzyme convert the 7-deoxyaglycones of anthracycline antibiotics to nonfluorescent metabolites.

INTRODUCTION

The anthracycline antibiotics are among the most commonly used antineoplastic agents. Doxorubicin and daunorubicin are the two major representatives of this class of compounds. Their pharmacology in humans has been investigated by studies which rely essentially on fluorescence assays (1, 2). Fluorescence assay studies have shown that the recovery of the administered dose of anthracycline antibiotics is not total, despite prolonged urinary and biliary collection (1, 3). Total recovery of drug is higher when assessed by radioactive assays in patients or animals treated with radiolabeled anthracycline antibiotics (1, 4). This may indicate that nonfluorescent metabolites are produced physiologically and are missed by the techniques commonly used to assay the anthracycline antibiotics.

In a previous report, our laboratory demonstrated that marcellomycin, a new anthracycline antibiotic (Fig. 1) recently studied in phase I trials (5, 6), is converted to nonfluorescent metabolite(s) by rat liver *in vitro* (7). We showed that under anaerobic conditions and in the presence of an electron donor, rat liver homogenate, microsomes, and cytosol metabolize marcellomycin to its 7-deoxyaglycone, dPo[3] (Fig. 1). This aglycone is subsequently converted to a nonfluorescent compound(s) in a reaction also requiring anaerobic conditions and an electron donor. These similar requirements suggested that the production of the nonfluorescent metabolite(s) might be the result of the interaction of the 7-deoxyaglycone with a flavoprotein, similar to the flavoproteins involved in the conversion of the anthracycline antibiotics to 7-deoxyaglycones. However, no loss of fluorescence occurred when dPo was incubated in the presence of purified NADPH cytochrome P-450 reductase (7), the main enzyme responsible for the formation of 7-deoxyaglycones (1, 8).

Xanthine oxidase is a flavoprotein capable of converting anthracycline antibiotics to their 7-deoxyaglycones (9). In this report, we demonstrate that dPo loses its fluorescence when incubated with xanthine oxidase. In addition, we extend our observations to the 7-deoxyaglycone of daunorubicin and propose a structure for the nonfluorescent metabolites. Finally, we present evidence that xanthine oxidase and the proposed rat liver enzyme(s) are distinct.

MATERIALS AND METHODS

Drug Supply and Purity. Marcellomycin was kindly supplied by Bristol-Myers Laboratories (Syracuse, NY). Daunorubicin was obtained from the Division of Cancer Treatment (National Cancer Institute, Bethesda, MD). DHAQ was supplied by the Drug Synthesis and Chemistry Branch (Division of Cancer Treatment, National Cancer Institute, Bethesda, MD). 7-Deoxydaunorubicin aglycone was prepared by reducing daunorubicin in the presence of sodium dithionite as described by Smith *et al.* (10). The purity of 7-deoxydaunorubicin aglycone was confirmed by TLC. We utilized Silica Gel 60 plates (E. Merck, Darmstadt, Germany) that were developed in an ascending fashion in chloroform:methanol:acetic acid:water, 80:20:14:6 (v/v/v/v) (system 1). The structure of 7-deoxydaunorubicin aglycone was confirmed by mass spectral analysis.

dPo was prepared from marcellomycin in the presence of purified NADPH cytochrome P-450 reductase as described by Dodion *et al.* (7) and was partially purified on a silicic acid column. The final preparation contained approximately 75% of dPo and 25% of bisanhydropyrromycinone. Small amounts of dPo and of bisanhydropyrromycinone were purified by TLC. The solvent system used for this purpose was a combination of chloroform:methanol:acetic acid, 100:2:2.5 (v/v/v) (system 2). After identification under 254-nm UV (UVS-54 Mineralight; Ultraviolet Products, San Gabriel, CA), dPo and bisanhydropyrromycinone were eluted from the TLC plate in chloroform. The organic phase was separated by centrifugation and evaporated to dryness. The structures of dPo and bisanhydropyrromycinone were confirmed by mass spectral analyses. Finally, DHAQ proved to be more than 99% pure by TLC in system 2.

Mass spectral analyses were done on a VG Micromass 30F mass spectrometer (VG Analytical, Altrincham, England) operated under VG Data Systems 2040 computer control. Spectra were obtained every 14 s over the range of 600–20 atomic mass units at a scan rate of 3 s/decade. Source conditions were 200°C, 70 eV ionizing voltage, 170 μA trap current, and 4 kV accelerating voltage. Selected compounds were silylated with *N,O*-bis(trimethylsilyl)trifluoroacetamide at 60°C for 2 h in pyridine.

Chemicals and Reagents. NADPH, NADH, xanthine, allopurinol, and commercial xanthine oxidase (from buttermilk) were purchased from Sigma Chemicals Co (St. Louis, MO). Bovine serum albumin was obtained from Reheis Chemical Co. (Phoenix, AZ) and Bio-Rad reagent was obtained from Bio-Rad Laboratories (Richmond, CA). *N,O*-bis(trimethylsilyl)trifluoroacetamide was obtained from Pierce Chemi-

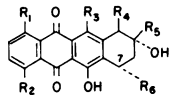
Received 8/11/86; revised 11/3/86; accepted 11/5/86.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported in part by a grant from the Fondation Rose et Jean Hoguet (Brussels, Belgium) and by a grant from the Scientific Committee of NATO (Brussels, Belgium).

[2] International Visiting Fellow at NIH (Bethesda, MD) and an International Fulbright Scholar (Washington, DC). Present address: Institut Jules Bordet, 1, rue Héger Bordet, B1000 Brussels, Belgium. To whom requests for reprints should be addressed.

[3] The abbreviations used are: dPo, 7-deoxypyrromycinone; DHAQ, 1,4-dihydroxyanthraquinone; TLC, thin layer chromatography.



COMPOUND	R_1	R_2	R_3	R_4	R_5	R_6
Daunorubicin	H	OCH_3	OH	H	$C-CH_3$ (=O)	Daunosamine
Doxorubicin	H	OCH_3	OH	H	$C-CH_2-OH$ (=O)	Daunosamine
7-Deoxydaunorubicin Aglycone	H	OCH_3	OH	H	$C-CH_3$ (=O)	H
Marcellomycin	OH	OH	H	$C-OCH_3$ (=O)	CH_2-CH_3	Rhodosamine–Deoxyfucose-Deoxyfucose
7-Deoxy pyrromycinone	OH	OH	H	$C-OCH_3$ (=O)	CH_2-CH_3	H

Fig. 1. Structures of doxorubicin, daunorubicin, marcellomycin, 7-deoxydaunorubicin aglycone, and 7-deoxypyrromycinone.

cal Co. (Rockford, IL). Sodium dithionite was obtained from J. T. Baker Chemical Co. (Philipsburg, NJ). Tetrahydroxyanthracene was purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI).

Animals and Tissue Preparation. Male Sprague-Dawley rats (weighing 250–300 g) obtained from Charles River Breeding Laboratories, Inc. (Wilmington, MA), were used in all experiments. The animals were fed a standard chow diet (NIH Rat and Mouse Ration, Ralston-Purina, St. Louis, MO) and water *ad libitum*. The animals were housed 3–5/cage, and maintained in a controlled environment with light and dark cycles of 12 h each. Rat liver cytosol was prepared as previously described (7).

Protein Assay. Protein concentrations were assayed according to the method described by Bradford using the Bio-Rad dye assay (11). Bovine serum albumin was used as a standard.

Xanthine Oxidase Purification and Assay. Xanthine oxidase was purified from bovine milk as described by Nelson and Handler (12). The enzyme activity was assayed spectrophotometrically at 25°C by the conversion of xanthine to uric acid (12) on a Beckman Acta CIII spectrophotometer (Beckman Instruments, Inc., Irvine, CA). One unit was defined as the amount of enzyme which changed the absorbance of 0.05 mM xanthine in 0.05 M potassium phosphate (pH 7.8) containing 0.2 mM EDTA at 25°C by 1 absorbance unit at 295 nm in 1 min.

Xanthine oxidase was also assayed by the consumption of oxygen at room temperature using a YSI Model 53 Biological Oxygen Monitor (Yellowsprings Instrument Co., Inc., Yellowsprings, OH). The procedure described by Pan was used with some modifications (9). Reaction mixtures (total volume, 3 ml) contained 40 mM potassium phosphate buffer (pH 7.4) and commercial xanthine oxidase (protein concentration, 0.48 mg/ml) or rat liver cytosol (protein concentration, 1–5 mg/ml) and were allowed to equilibrate with air for 3 min. The reaction vessel was then sealed with the oxygen electrode and a baseline was recorded for 1 min. The reaction was initiated by the addition of xanthine (1 mM). The consumption of oxygen was calculated from the initial, linear portion of the curve of saturation of oxygen *versus* time, assuming that 100% saturation corresponded to 0.186 μmol of oxygen/ml of reaction mixture (13).

Incubations. Incubations were carried out anaerobically at 37°C in a Dubnoff metabolic shaker (120 oscillations/min). Anaerobic conditions were obtained by a continuous flow of nitrogen. The reaction mixture (total volume, 2 ml) contained 40 mM potassium phosphate (pH 7.4), 1.54 mM magnesium sulfate, 1 mg/ml bovine serum albumin, xanthine oxidase (protein concentration, 0.48 mg/ml for the commercial preparation and 0.05 mg/ml for the purified enzyme) or rat liver cytosol (protein concentration, 1 mg/ml), and an electron donor (xanthine or NADPH, 1 mM). In preliminary experiments, albumin was shown to be necessary to keep DHAQ and the 7-deoxyaglycones in solution. Reactions were initiated by the addition of 5 μM DHAQ, dPo, or 7-deoxydaunorubicin aglycone.

Assay of Parent Drug and Metabolites. At appropriate times, duplicate 50-μl aliquots were removed from the incubation medium and mixed with 2 ml of 2.16 N sulfuric acid:isopropanol, 25:75 (v/v). Total fluorescence content was measured on an Aminco-Bowman spectrofluorometer (SLM Instruments, Inc., Urbana, IL) at an excitation wavelength of 470 nm and an emission wavelength of 550 nm. For some experiments, fluorescence was measured continuously by performing the incubations directly in an Aminco SPF 500 Fluorometer (SLM Instruments). Standard curves were prepared by the addition of known amounts of DHAQ, dPo, or 7-deoxydaunorubicin aglycone to 2 ml of 2.16 N sulfuric acid:isopropanol, 25:75 (v/v). Metabolites were quantified by TLC and fluorescence assay as previously described (7).

Fluorescence and Absorbance Spectra. Fluorescence spectra were obtained on an Aminco SPF 500 spectrofluorometer. Absorbance spectra were recorded on a Beckman Acta CIII spectrophotometer.

HPLC Analyses. Nonfluorescent metabolites were purified by high pressure liquid chromatography on Spectra Physics 3500 B high pressure liquid chromatography (Spectra Physics, Santa Clara, CA) equipped with a C_{18}-μBondapak column (Waters Associate, Milford, MA). The mobile phase consisted of acetonitrile:water, 48:52 (v/v), pumped at flow rate of 2 ml/min. Detection was accomplished by fluorescence with an Aminco-Bowman Fluoromonitor (SLM Instruments) fitted with a 470-nm excitation filter and a 500-nm cutoff emission filter, and spectrophotometrically with a Schoeffel Model 770 Spectrophotometric Detector (Kratos Analytical Instruments, Ramsey, NJ) set at 420 nm.

RESULTS

Effect of Xanthine Oxidase on dPo, 7-Deoxydaunorubicin Aglycone, and DHAQ. DPo lost its fluorescence at a rate of 4.7 ± 0.1 (SE) nmol/min/mg protein when incubated with xanthine and purified xanthine oxidase. Commercial xanthine oxidase (protein concentration, 0.48 mg/ml) was also active with a rate of 0.64 ± 0.04 nmol/min/mg protein. Because of a limited supply of purified xanthine oxidase, further tests were done with the commercial preparation. NADH was less effective than xanthine as electron donor (Table 1) and NADPH was ineffective. No activity was detected in the absence of cofactor, under aerobic conditions, or without enzyme. When xanthine was used as cofactor, the reaction was almost totally inhibited by 1 mM allopurinol (rate, 0.03 ± 0.03 nmol/min/mg protein). The inspection of the TLC plates did not reveal the formation of any new fluorescent compound during the dPo disappearance.

Xanthine oxidase at 0.48 mg/ml did not induce any loss of fluorescence when incubated with 7-deoxydaunorubicin aglycone and xanthine under anaerobic conditions. At a higher protein concentration (4.8 mg/ml), however, a loss of fluorescence was observed (rate, 0.039 ± 0.004 nmol/min/mg protein). No decrease of fluorescence was observed in the absence of xanthine oxidase. In the presence of 1 mM allopurinol, the reaction rate became 0.001 ± 0.001 nmol/min/mg protein (statistically lower than without inhibitor, $P \leq 0.001$; Student's

Table 1 *Conversion of dPo and DHAQ to nonfluorescent metabolites by xanthine oxidase*

dPo or DHAQ (5 μM) was incubated in the presence of xanthine oxidase (protein concentration, 0.48 mg/ml) and the specified cofactors (1 mM) under anaerobic or aerobic conditions. The fluorescence was measured at specified times, and the rate of fluorescence loss was calculated from the initial, linear portion of the curve of fluorescence *versus* time.

Cofactor	Conditions	Activity (nmol/min/mg/protein) dPo	DHAQ
	Anaerobic	0 ± 0[a, b]	0 ± 0[b]
NADPH	Anaerobic	0 ± 0[b]	Not done
NADH	Anaerobic	0.15 ± 0.01[b]	0.89 ± 0.05
Xanthine	Anaerobic	0.64 ± 0.04	1.02 ± 0.05
Xanthine	Aerobic	0 ± 0[b]	0 ± 0[b]
Xanthine + allopurinol	Anaerobic	0.03 ± 0.03[b]	Not done

[a] Mean ± SE.
[b] Statistically lower than with xanthine under anaerobic conditions ($P < 0.001$; Student's *t* test).

t test). The inspection of the TLC plates did not reveal the formation of any new fluorescent compound while 7-deoxydaunorubicin aglycone was disappearing.

Xanthine oxidase (protein concentration, 0.48 mg/ml) was extremely active in inducing a loss of fluorescence from DHAQ (Table 1). In the presence of xanthine and under anaerobic conditions, the reaction rate was 1.02 ± 0.05 nmol/min/mg protein. The rate was slightly lower in the presence of NADH. No loss of fluorescence occurred without enzyme, cofactor, or under aerobic conditions. On TLC, the disappearance of DHAQ was accompanied by the formation of another compound which had a blue fluorescence and was identified as tetrahydroxyanthracene (Fig. 2) (see below).

The specific fluorescent properties (see below) of tetrahydroxyanthracene allowed a continuous monitoring of the reaction in order to determine the reaction rates at various concentrations of DHAQ. The system obeyed Menten-Michaelis kinetics (correlation coefficient, 0.98), with a V_{max} of 23.1 ± 2.6 nmol/min/mg protein and a K_m of 4.2 ± 0.6 μM.

Liver Cytosolic Activity on dPo and DHAQ. Liver cytosol induced a loss of fluorescence of dPo in the presence of an electron donor (Table 2). NADH and NADPH were equally active as cofactors, but no activity occurred with xanthine. As with xanthine oxidase, the disappearance of dPo was not accompanied by the formation of any new fluorescent compound. Liver cytosol also metabolized DHAQ (Table 2). In this case, as with xanthine oxidase, the decrease of fluorescence was accompanied by the formation of tetrahydroxyanthracene.

The cytosol was tested for xanthine oxidase activity and no activity was detected in cytosol by the assay based on the formation of uric acid. In contrast, activities of 9.4 ± 0.9 units/mg protein and 1.4 ± 0.6 unit/mg protein were obtained for the purified and commercial enzymes, respectively. Similarly, in the presence of xanthine, there was no consumption of oxygen by cytosol, whereas, under similar conditions, commercial xanthine oxidase induced a consumption of 0.16 ± 0.02 μmol of oxygen/min/mg of protein.

Characterization of the Nonfluorescent Metabolites. DHAQ was metabolized by both xanthine oxidase and liver cytosol to a compound which had the same TLC mobility as tetrahydroxyanthracene (R_f = 0.66 in system 2). The R_f for DHAQ was 0.69 in the same system. Both the reaction product and tetrahydroxyanthracene had maximal absorbance at 401 and 420 nm in the visible range and at 246 nm in the UV range. The maximal absorbances occurred at 483, 280, and 251 nm for DHAQ. The reaction product and tetrahydroxyanthracene had identical fluorescence characteristics with a wavelength of maximal excitation at 400 nm and a wavelength of maximal emission at 460 nm. For DHAQ, the highest excitation occurred at 470 nm and the highest emission at 532 nm. The reaction product and tetrahydroxyanthracene gave similar mass spectral data: the major peak (reference peak) was a molecular ion of 242 and the main fragment was a molecular ion of 224, corresponding to the loss of a molecule of water (intensity of 15 and 30% of that of the reference peak for the reaction product and tetrahydroxyanthracene, respectively). Another major peak was observed at 240, corresponding probably to the neutral fragmentation loss of two hydrogen atoms. After silylation, the ion of the highest mass in the spectrum was 530, which is consistent with the addition of four silyl groups. Finally, the reaction product as well as tetrahydroxyanthracene could be reversed to DHAQ by oxygenation at alkaline pH.

The product resulting from the effect of xanthine oxidase on dPo was a yellow compound with a R_f of 0.44 in TLC system 2. The R_f for dPo was 0.68 in the same TLC system. The product of the reaction showed maximal absorbance at 420 and 443 nm in the visible range and at 253 nm in the UV range. dPo was absorbing maximally at 493, 259, and 240 nm. The reaction product was not fluorescent. These characteristics are identical to those previously reported for the product resulting from the action of rat liver fractions on dPo (7). In mass spectral analysis, the reaction product gave a molecular ion of 414 (intensity of 68% of that of the reference peak). The major fragment was a molecular ion of 396 (intensity of 12% of that of the reference peak) and corresponded to the loss of a molecule of water. Another major peak was observed at 412, corresponding probably to the neutral fragmentation loss of two hydrogen atoms. Finally, the reaction product could be reversed to dPo by oxygenation at alkaline pH.

Fig. 2. Structures of 1,4-dihydroxyanthraquinone (*left*) and tetrahydroxyanthracene (*right*).

Table 2 *Conversion of dPo and DHAQ to nonfluorescent metabolites by rat liver cytosol*

DPo or DHAQ (5 μM) was incubated in the presence of liver cytosol (protein concentration, 1 mg/ml) and the indicated cofactors (1 mM) under anaerobic conditions. The fluorescence was measured at specified times, and the rate of fluorescence loss was calculated from the initial, linear portion of the curve of fluorescence *versus* time.

	Activity (nmol/min/mg/protein)	
Cofactor	dPo	DHAQ
Xanthine	0 ± 0[a]	Not done
NADPH	0.34 ± 0.15	0.28 ± 0.06
NADH	0.31 ± 0.17	0.09 ± 0.05

[a] Mean ± SE.

DISCUSSION

The conversion of anthracycline antibiotics to 7-deoxyaglycones has been shown to occur in animals and human beings (1, 14); therefore, it is important to investigate the metabolic fate of these compounds. In the present work, we extend our previous study on the conversion of dPo, the 7-deoxyaglycone of marcellomycin, to nonfluorescent metabolites by rat liver preparations (7). A similar conversion was obtained with xanthine oxidase, a flavoprotein previously shown to be active in the reductive metabolism of the anthracycline antibiotics (9). As previously described for liver preparations, anaerobic conditions and an electron donor were required for the reaction. In addition, xanthine oxidase was active on 7-deoxydaunorubicin aglycone. We have been unable to study a possible loss of fluorescence when 7-deoxydaunorubicin aglycone is incubated with liver cytosol or microsomes because other metabolic pathways, such as the reduction of the carbonyl side chain by the aldoketoreductase, occur simultaneously (1, 15). Our results

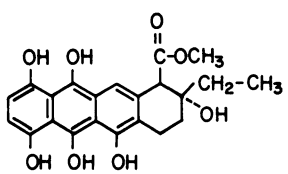


Fig. 3. Proposed structure for the nonfluorescent derivative of 7-deoxypyrromycinone.

with xanthine oxidase suggest that the loss of fluorescence could be a metabolic pathway for the anthracycline antibiotics in general. In addition, xanthine oxidase is a widely distributed enzyme in mammalian systems (8, 16). Therefore, it is likely that 7-deoxyaglycones are physiologically exposed to that enzyme in many tissues and that the formation of nonfluorescent compounds may be a widespread phenomenon.

Xanthine oxidase and liver cytosol induced a loss of fluorescence with DHAQ. DHAQ contains the basic structure of the anthracycline antibiotics, with three aromatic cycles and the quinone structure. Therefore, DHAQ can be considered as a model compound to study the pathway in which fluorescence is lost. The product of the reaction with DHAQ has been identified with certainty as tetrahydroxyanthracene on the basis of its identical chromatographic, fluorescent, spectrophotometric, and mass spectral characteristics. In addition, the conversion of DHAQ to tetrahydroxyanthracene was reversible, arguing against a major change in the molecule such as the opening of the ring structure. The spectrophotometric and mass spectral characteristics of the nonfluorescent derivative of dPo, the possibility to reverse this compound to dPo, and the structural analogy with DHAQ, strongly support the hypothesis that the nonfluorescent derivative of dPo is the corresponding dihydroquinonic compound (Fig. 3).

Xanthine oxidase and cytosolic activities responsible for the loss of fluorescence are clearly distinct. To begin with, xanthine oxidase activity could not be detected in cytosol by either of two different techniques. The absence of xanthine oxidase activity in rat liver cytosol is surprising since xanthine oxidase has been isolated from the liver of several mammalian species (16). Xanthine oxidase is known to be a highly unstable enzyme (16, 17), and its activity might have been lost during the preparation of cytosol. In addition, xanthine oxidase and cytosolic activities differ in their cofactor requirements. We previously reported that the cofactor requirements were also different for liver cytosol and microsomes (7), suggesting the presence of distinct cytosolic and microsomal enzymes capable of inducing a loss of fluorescence from anthracycline antibiotics. The formation of a dihydroquinonic derivative of 7-deoxydaunorubicin aglycone has also been observed in the presence of the enzyme ferredoxin:NADP reductase under anaerobic conditions (18). Therefore, an entire family of enzymes may be able to induce a loss of fluorescence from anthracycline antibiotics.

The difference in activity between xanthine oxidase and the NADPH cytochrome P-450 reductase is intriguing. Both enzymes are capable of reducing the anthracycline antibiotics to their semiquinone free radicals (1, 9), the intermediates in the formation of the 7-deoxyaglycones. However, only xanthine oxidase appears to be capable of reducing DHAQ and dPo to their dihydroquinonic derivatives, despite the fact that the quinone structure is the site of reduction in this pathway too. The two enzymes may differ in their capacity of transferring one *versus* two electrons. The reduction of the anthracycline antibiotics to the semiquinone free radicals requires only one electron transfer, whereas two electrons must be transferred to form the dihydroquinonic derivatives. In that regard, xanthine oxidase has been shown to be a complex enzyme, capable of accepting a variable number of electrons (16).

In conclusion, we suggest that the 7-deoxyaglycones of anthracycline antibiotics can be reduced by several enzymes, including xanthine oxidase, to nonfluorescent metabolites, likely to be the dihydroquinonic derivatives of the parent 7-deoxyaglycones. The pharmacological and biochemical importance of these metabolites remains to be investigated.

ACKNOWLEDGMENTS

The authors thank D. Davin for her excellent secretarial assistance.

REFERENCES

1. Bachur, N. R. Anthracycline antibiotic pharmacology and metabolism. Cancer Treat. Rep., *63:* 817–820, 1979.
2. Myers, C. E. Anthracyclines. *In:* B. Chabner (ed.), Pharmacologic Principles of Cancer Treatment, pp. 416–434. Philadelphia: W. B. Saunders Co., 1982.
3. Riggs, C. E., Jr., Benjamin, R. S., Serpick, A. S., and Bachur, N. R. Biliary disposition of Adriamycin. Clin. Pharmacol. Ther., *22:* 234–241, 1977.
4. Liss, R. H., Yesair, D. W., Schepis, J. P., and Marenchic, I. C. Adriamycin and daunomycin pharmacokinetics in rats. Proc. Am. Assoc. Cancer Res., *18:* 221, 1977.
5. Joss, R. A., Kaplan, S., Goldhirsch, A., Varini, M., Brunner, K. W., and Cavalli, F. A phase I trial of marcellomycin with a weekly dose schedule. Eur. J. Cancer Clin. Oncol., *19:* 455–459, 1983.
6. Nicaise, C., Rozencweig, M., DeMarneffe, M., Crespeigne, N., Dodion, P., Piccart, M., Sculier, J. P., Lenaz, L., and Kenis, Y. Clinical phase I trial of marcellomycin with a single-dose schedule. Eur. J. Cancer Clin. Oncol., *19:* 449–454, 1983.
7. Dodion, P., Tamburini, J. M., Fox, B. M., Riggs, C. E., and Bachur, N. R. Comparative anthracycline metabolism: loss of marcellomycin fluorescence. Cancer Res., *46:* 2714–2719, 1986.
8. McLane, K. E., Fisher, J., and Ramarkrishnan, K. Reductive drug metabolism. Drug Metab. Rev., *14:* 741–799, 1983.
9. Pan, S. S., and Bachur, N. R. Xanthine oxidase catalyzed reductive cleavage of anthracycline antibiotics and free radical formation. Mol. Pharmacol., *17:* 95–99, 1980.
10. Smith, T. H., Fujimara, A. N., Lee, W. W., Wu, H. Y., and Henry, D. W. Synthetic approaches to Adriamycin. 2. Degradation of daunorubicin to a nonasymmetric tetracyclic ketone and refunctionalization of the A ring to Adriamycin. J. Org. Chem., *42:* 3653–3660, 1977.
11. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., *72:* 248–254, 1976.
12. Nelson, C. A., and Handler, P. Preparation of bovine xanthine oxidase and the subunit structures of some iron flavoproteins. J. Biol. Chem., *243:* 5368–5373, 1978.
13. Umbreit, W. W. Constant volume manometry. The "Warburg." *In:* W. W. Umbreit, R. H. Burris, and J. F. Stauffer (eds.), Manometric and Biochemical Techniques, pp. 1–19. Minneapolis: Burgess Publishing Company, 1972.
14. Dodion, P., Egorin, M. J., Tamburini, J. M., Riggs, C. E., and Bachur, N. R. The murine metabolism and disposition of marcellomycin. Drug Metab. Dispos., *12:* 209–216, 1984.
15. Ahmed, N. K., Felsted, R. L., and Bachur, N. R. Heterogeneity of anthracycline antibiotic carbonyl reductases in mammalian livers. Biochem. Pharmacol., *27:* 2713–2719, 1978.
16. Massey, V. Iron-sulfur flavoprotein hydroxylases. *In:* W. Lovenberg (ed.), Iron-Sulfur Proteins, Vol. 1, pp. 301–360. New York: Academic Press, Inc., 1973.
17. Edmonson, D., Massey, V., Palmer, G., Beacham, L. M., III, and Elion, G. B. The resolution of active and inactive xanthine oxidase by affinity chromatography. J. Biol. Chem., *247:* 1957–1604, 1972.
18. Fisher, J. New redox chemistry of the anthracyclines. Biochemistry, *22:* 14A, 1983.