with a Hitachi IR 270-50 machine. Mass spectra were measured with a Hitachi M-80B mass spectrometer, a 0101 control system, and a M8061 SIMS apparatus. All compounds were analyzed for C, H, N, and values were within ±0.4% of theoretical values.

In Vitro Antibacterial Activity. According to the method of Goto et al.,[13] the MICs of compounds tested in this study were determined by the serial 2-fold dilution technique, using Mueller-Hinton agar. The inoculum size was approximately 10^6 cfu/mL. The concentrations of compounds in the plates ranged from 0.006 to 100 μg/mL. MIC was defined as the lowest concentration of a compound that prevented visible growth of bacteria after incubation at 37 °C for 18 h.

In Vivo Efficacy on Systemic Infections. In vivo assays were carried out according to the general method already published.[14] Groups of five male ddY mice (20 ± 2 g) were infected with bacteria. A 0.5-mL volume of a bacterial dilution, corresponding to 10 or 100 times the 50% lethal dose, was inoculated intraperitoneally. The test compounds were suspended in 0.5% sodium (carboxymethyl)cellulose and administered orally at 1 h postinfection. Survival rates were evaluated after 1 week.

Oral Absorbability Test. The serum concentration of NFLX in mice treated with compounds **2**, **4**, and NFLX itself was determined by HPLC. Test compounds were suspended in 0.5% sodium (carboxymethyl)cellulose and administered orally at a dose of 50 mg/kg. After 0.25, 0.5, 1.0, 2.0, 3.0, and 4.0 h, the mice were killed by bleeding. The collected blood was centrifuged, and the test serum was adjusted. A HPLC machine was equipped with a Model 6000A pump, a Model U6K universal injector, a Shimadzu Model SPD-2A spectrophotometric detector (at 280 nm), and a YMC A-312 column. The mobile phase consisted of 5% acetic acid–methanol (80:20 v/v), and a flow rate was 2.0 mL/min.

(A) *N*-[(4-Methyl-5-methylene-2-oxo-1,3-dioxolan-4-yl)oxy]norfloxacin (4). To a solution of *N*-[(5-methyl-2-oxo-1,3-dioxol-4-yl)methyl]norfloxacin (**2**)[3] (1.0 g, 2.3 mmol) in $CHCl_3$ was added MCPBA (400 mg, 2.3 mmol) in small portions, and the mixture was heated at 50 °C under an argon atmosphere for 10 min. After the mixture cooled, the solvent was removed under reduced pressure. The residue was washed with ether to give **4** (730 mg, 70%) as a white powder: mp 162–168 °C dec; IR (KBr) cm^{-1} 1845 (five-membered carbonyl); ^1H NMR (DMSO-d_6) δ 1.42 (3 H, t, J = 7.0 Hz), 1.76 (3 H, s), 2.80–3.90 (8 H, m), 4.60 (2 H, q, J = 7.0 Hz), 4.98 (1 H, d, J = 4.0 Hz), 5.10 (1 H, d, J = 4.0 Hz), 7.18 (1 H, d, J = 8.0 Hz), 7.84 (1 H, d, J = 14 Hz), 8.86 (1 H, s), 15.06 (1 H, s); MS, m/e 448 (MH^+, 30), 336 (21), 317 (26), 201 (81), 130 (83), 106 (100). Anal. ($C_{21}H_{22}N_3O_7F$) C, H, N.

Preparation of the *N*-Oxide of Compound 2 (3). According to the method of Craig et al.,[7] to an ice-cooled, stirred solution of *N*-[(5-methyl-2-oxo-1,3-dioxol-4-yl)methyl]norfloxacin (**2**)[3] (500 mg, 1.15 mmol) in $CHCl_3$ was added MCPBA (200 mg, 1.15 mmol) in small portions at 0–5 °C. The mixture was stirred for 2.5 h at 0–5 °C and the resulting precipitate was separated. The crude solid was purified by washing with cold $CHCl_3$ to give **3** (460 mg, 88%) as a white powder: mp 138–144 °C dec; ^1H NMR (DMSO-d_6) δ 1.42 (3 H, t, J = 7.2 Hz), 2.13 (3 H, s), 3.5–3.96 (8 H, m), 4.57 (2 H, q, J = 7.2 Hz), 4.62 (2 H, s), 7.30 (1 H, d, J = 7.0 Hz), 8.0 (1 H, d, J = 13 Hz), 8.98 (1 H, s); MS m/e 448 (MH^+, 27), 432 (MH − 16, 10), 414 (25), 335 (44), 318 (72), 245 (80), 130 (100).

Procedure for the Rearrangement of the *N*-Oxide. A suspension of the amine oxide **3** (400 mg) in dry $CHCl_3$ was heated at 50 °C under an argon atmosphere for 20 min. After the mixture cooled, the solvent was removed under reduced pressure. The residue was washed with ether to give **4** (390 mg, 97.5%) as a white powder.

General Procedure for the Thermolysis of *N*-Oxide in the Presence of 1-Butanethiol. A suspension of the amine oxide and varying concentrations of 1-butanethiol in dry $CHCl_3$ was stirred at 5 °C for 5 min, followed by heating at 50 °C for 20 min. After the mixture cooled, the solvent was removed under reduced pressure, and the residue was washed with ether. The results are shown in Table II.

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In Vitro Metabolic Transformations of Vinblastine: Oxidations Catalyzed by Peroxidase

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Vinblastine is converted to a single major metabolite during in vitro enzymatic oxidations catalyzed by horseradish peroxidase in the presence of hydrogen peroxide. Preparative-scale enzyme incubations permitted the isolation of sufficient amount of the transformation product for complete structural identification and biological evaluation. The metabolite was identified as catharinine (also known as vinamidine) by ^1H and ^{13}C NMR and by mass spectrometry. Incubations conducted in $H_2{}^{18}O$-enriched water gave catharinine in which a single atom of ^{18}O was incorporated into the metabolite structure. The labeling experiment provided evidence for an unusual ring-fission pathway by which peroxidase transforms vinblastine to catharinine. Catharinine is 77 times less active than vinblastine when tested in vitro against the human T-cell leukemic cell line (CRFF-CEM).

The dimeric Vinca alkaloids vinblastine (VLB) (1) and vincristine (VCR) have been used extensively in the treatment of human cancers for nearly three decades.[1] Structural analogues vindesine,[2] vizolidine,[3] and navelbine[4] have been developed in the hope of reducing the dose-limiting neurotoxicities and myelosuppressive effects exhibited by VCR[5,6] and VLB,[2] respectively. Clinically observed differences in neurotoxicities among Vinca alkaloids may be due to differences in compartmentalization as the drugs are distributed after administration, differences in

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tubulin binding, or contact with nervous tissue. The differences in Vinca alkaloid toxicities and clinical utility may also be due to metabolic alterations of the alkaloid structures.[7]

Surprisingly little is known of the possible role of drug metabolism in the mechanism(s) of action and/or dose-limiting side effects of the Vinca alkaloids. Ample evidence indicates that the Vinca alkaloids are extensively metabolized in mammals.[3,7,8-16] Six VLB metabolites were reported as spots on chromatograms in the first mammalian metabolism study in 1964.[9] Since then, numerous other Vinca alkaloid metabolites have been observed by thin-layer or high-performance liquid chromatography.[3,7,9,11-16] To date, however, the only identified VLB metabolite is desacetylvinblastine (**1a**) found in human[16] and mouse tissue.[7] Aside from the simple hydrolytic product which was easily identified by comparison with chemically prepared **1a**,[17] the structures of other metabolites remain unknown.

The failure of traditional drug metabolism approaches to solve the problem of the metabolic fates of Vinca alkaloid dimers may be attributed to several factors which include: the very low doses of compounds used in therapy; the need to obtain relatively large amounts of Vinca alkaloid metabolites for complete structural analysis; the presumed instabilities of metabolites; and the difficulties encountered in using total synthetic approaches for the synthesis of the presumed metabolites.

Previous investigations in our laboratories have focused on elaborating biochemical and chemical mechanisms by which dimeric and monomeric Vinca alkaloids are oxidized by different types of enzymes. Initial studies were conducted with the monomeric Vinca alkaloid vindoline as a model Vinca alkaloid substrate. Vindoline is found structurally intact as the "lower half" of the clinically active Vinca dimers. Vindoline is transformed to the same series of metabolites by peroxidase,[18] copper oxidase,[19,20] cytochrome P-450,[21] and enzyme systems of a bacterium.[22] Detailed investigations clearly established that the mechanism of vindoline biotransformation involved initial one-electron oxidation[18,19] leading to the formation of reactive enamine and iminium intermediates. Relatively stable carbinolamine products were isolated when 14,15-dihydrovindoline[23] and the active dimer leurosine[24] were oxidized by copper oxidases. Surprisingly, leurosine undergoes a completely different type of ring-fission reaction when subjected to oxidations with peroxidase.[25]

This report is concerned with the in vitro metabolic transformation of VLB catalyzed by peroxidase. A new VLB metabolite was isolated from preparative enzymatic incubations, its structure has been completely determined by NMR, IR, UV, and mass spectral analysis, and its biological activity was evaluated.

Results and Discussion

Although the Vinca alkaloids are important, clinically active antineoplastic agents, little success has been achieved in elucidating pathways of metabolism. Because metabolic alterations in the structures of Vinca dimers may result in their bioactivation, bioinactivation, or increased neurotoxicity, it is important to identify metabolites to elaborate the biochemical mechanisms by which they are formed and to establish their biological activities.

In our laboratories, initial metabolic investigations focused on in vitro transformations of monomeric Vinca alkaloid vindoline. All of the intermediates involved in a complex transformation pathway catalyzed by peroxidase and copper oxidases were trapped and isolated, and their structures were rigorously proved by chemical derivatization and spectral analyses.[18-22] This work confirmed the involvement of highly reactive iminium and enamine chemical intermediates in the oxidation pathway. It was possible to observe the initial vindoline oxidation step spectrophotometrically as the powerful oxidant peroxidase compound I abstracted an electron and was reduced to peroxidase compound II.[18] Spectral analysis of the events occurring at the enzyme active site also permitted the establishment of the stoichiometry of the reaction as vindoline was oxidized to a dimeric structure.[22] These detailed chemical and biochemical investigations established the basis for biotransformation experiments with VLB, a nitrogen heterocyclic compound of much more complex structure.

Peroxidase has served as a useful tool in elaborating the mechanism of action and drug-induced toxicities of other antitumor agents such as etoposide[26] and the ellipticines.[27] In addition to reactions described in our laboratories with Vinca alkaloids, peroxidases are also known to catalyze the wide range of metabolic reactions of possible pertinence to the Vinca alkaloids including O-demethylation,[28] N-demethylation,[29] hydroxylations,[30] and others.[31]

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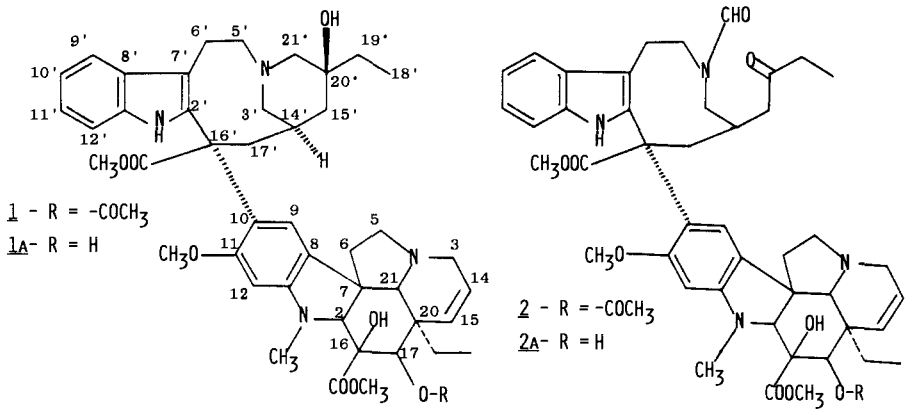

Figure 1. Structures of vinblastine (1), desacetylvinblastine (1a), catharinine (2) and desacetylcatharinine (2a).

With peroxidase, VLB (1) was reproducibly converted into a major, less polar metabolite. The reaction required hydrogen peroxide and did not occur in the absence of enzyme. The new metabolite was not desacetylvinblastine (1a) as shown by TLC. A preparative-scale incubation reaction yielded 29 mg of the new VLB (1) derivative for complete structure elucidation and biological evaluation. The high-resolution mass spectrum of the metabolite exhibited a molecular ion 14 mass units higher than that of VLB (1). The chemical-ionization mass spectrum indicated a protonated molecular ion (M + 1) at 825.4090, consistent for a compound with the empirical formula $C_{46}H_{57}N_4O_{10}$, and derived from VLB (1) by the introduction of an oxygen atom and the cleavage of a carbon–carbon bond.

The ^1H NMR spectrum of the metabolite was very similar to that of VLB (1). All of the signals for the Aspidosperma ring (lower half of dimer) and those for the indole ring and carbomethoxyl group of the Iboga ring (upper half of the dimer) were clearly observed. This evidence indicated that the structural change had occurred in the heterocyclic ring portion of VLB (1). A new signal appeared at 7.93 ppm, typical for the resonance of an *N*-formyl group. The IR spectrum confirmed the presence of the *N*-formyl group (1664 cm^{-1}), and it clearly demonstrated the presence of a ketone functional group by the absorption band at 1718 cm^{-1}. Neither of these functional groups are present in the structure of VLB (1). The ^{13}C NMR spectrum (Table I) confirmed that the Aspidosperma portion of the metabolite structure was the same as in VLB (1). Noticeably absent from the Iboga half of the metabolite were signals at 63.1 and 68.6 ppm (C-21′ and C-20′ in VLB (1)), and the appearance of new signals at 163.45 ppm (NCHO) and 210.38 (C=O). These data suggested that the metabolite consisted of a structure in which the six-membered heterocyclic ring underwent peroxidase-catalyzed carbon–carbon bond fission. That these structure changes took place at positions 20′ and 21′ was supported by the observed downfield shifts of carbons 15′, 18′, and 19′. All of the other carbon signals are in complete agreement with the structure of the metabolite as shown in 2. The metabolite is spectrally and structurally identical with a natural product known as catharinine (or vinamidine), which was previously isolated from *Catharanthus* plant species.[32,33] Catharinine has also been obtained synthetically.[34] Biosynthetic processes leading to catharinine in plants are unknown. However, it has been

Table I.[a] ^{13}C NMR Chemical Shift Assignments of VLB (1), Isolated Metabolite, and Catharinine (2)

carbon	VLB (1) (ref 42)	metabolite 2	catharinine 2 (ref 32)
2	83.0	83.63	83.6
3	50.0	50.01	50.4
5	50.0	50.45	50.4
6	44.3	43.87	44.1
7	52.9	53.30	53.3
8	122.6	123.59	123.4
9	123.1	124.93	124.7
10	120.4	120.12	120.2
11	157.8	157.92	157.9
12	93.9	93.68	93.6
13	152.5	153.05	153.1
14	124.3	124.58	124.7
15	129.7	129.88	129.9
16	79.3	79.69	79.7
17	76.1	76.51	76.4
18	8.1	8.40	8.3
19	30.4	30.83	30.7
20	42.3	42.72	42.6
21	65.2	66.13	65.8
COO	170.6	170.89	170.9
OMe	51.8	52.19	53.2
OCO	171.4	171.72	171.7
AcMe	20.7	21.11	21.3
ArOMe	55.3	55.52	55.8
NMe	38.0	38.31	38.5
2′	130.9	132.71	132.6
3′	47.5	49.46	49.5
5′	55.5	55.73	50.4
6′	28.7	25.28	25.2
7′	115.9	111.53	111.5
8′	129.0	128.37	128.4
9′	118.1	117.68	117.7
10′	122.2	122.50	122.5
11′	118.8	119.31	119.4
12′	110.2	110.78	110.9
13′	134.7	135.29	135.4
14′	29.2	29.59	29.6
15′	40.0	51.09	50.4
16′	55.3	55.52	55.8
17′	34.1	34.88	34.8
18′	6.7	7.89	7.9
19′	34.1	37.44	37.5
20′	68.6	210.38	210.4
21′	63.1	163.45	163.6
CO	174.6	174.19	174.2
OMe	52.0	52.35	53.2

[a] Multiplicities were determined by a modified INEPT technique and assignments were confirmed by C–H correlation experiment (CHORTLE).[43]

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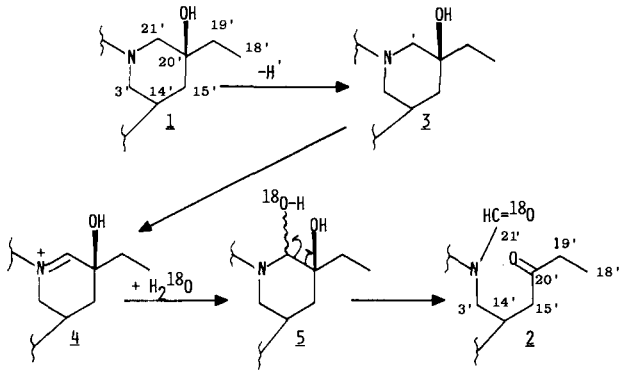

Figure 2. Proposed pathway for the peroxidase-catalyzed transformation of vinblastine (1) to catharinine (2).

Table II. IC_{50} Values of Catharinine (2), Vinblastine (1), and Other Dimeric Vinca Alkaloids vs the CRFF-CEM, T-Cell Leukemic Cell Line

compound tested	IC_{50}, μg/mL
catharinine (2)	7.700
vinblastine (1)	0.100
vincristine	0.025
vinrosidine	1.000
vinleurosine	0.400

speculated that catharinine is derived from leurosine and VLB.[32,35]

Peroxidases are well known to catalyze oxidations of nitrogen heterocyclic compounds. Strong evidence indicates that the first step in the oxidation of molecules such as VLB involves abstraction of hydrogen from a carbon atom adjacent to nitrogen.[18,36] A scheme which can account for the peroxidative transformation of VLB to catharinine begins with the initial abstraction of hydrogen from position 21′ with the subsequent loss of an electron from the Nb′-nitrogen atom to yield the iminium derivative **4** as shown in Figure 2. Addition of water to **4** affords the carbinolamine **5**, which undergoes the biochemical equivalent of a periodate oxidation reaction to cleave the vicinal diol **5** to give catharinine. Recently, we have reported on a similar reaction with the Vinca alkaloid leurosine.[25] This is analogous to the cytochrome P-450 mediated diol cleavage which occurs in cholesterol side-chain degradation.[37] The cleavage of substituted 1,2-diols by iron–porphyrin catalysts is also reported to involve a similar mechanism.[38] This proposed pathway was tested by conducting reactions in the presence of the $H_2{}^{18}O$-enriched buffer, isolating labeled catharinine, and determining the amount of ^{18}O incorporated into the metabolite structure by mass spectrometry. Exactly one atom of heavy oxygen was incorporated into the metabolite structure, as evidenced by the M + 2 fragment in the mass spectrum of the labeled metabolite. The amount of label was identical with that present in the incubation water as measured by mass spectrometry (Figure 3). This result supports the proposed pathway, which is completely consistent with our earlier findings with other Vinca alkaloid monomers and dimers.[17,24,25]

The metabolite was examined for biological activity in the Vinca alkaloid sensitive, CRFF-CEM leukemic test system,[39,40] and the results are shown in Table II. The IC_{50} value for catharinine was 77 times more than that of VLB and 308 times more than that for VCR. Thus, peroxidase oxidations lead to the bioinactivation of VLB. However, it is important to regard peroxidase metabolite **2** as an end product in the proposed metabolic scheme. Since it appears to be formed through a pathway which involves free-radical iminium and carbinolamine intermediates, any one of which can chemically interact with critical macromolecules, such a pathway may be associated with side effects common to VLB.

This work provides new evidence for the oxidative transformation of VLB (1). It is likely that metabolites like catharinine and desacetylcatharinine (**2a**) will be formed in other enzymatic systems. Investigations of the metabolism of VLB by mammalian peroxidases and other mammalian metabolic systems are in progress.

Experimental Section

Vinblastine (1) sulfate was obtained from OMNICHEM, PRB, Belgium and possessed physical and spectral data consistent with those reported in the literature.[41] Desacetylvinblastine (**1a**) was obtained as a gift from Eli Lilly Co., Indianapolis, IN. Horseradish peroxidase enzyme (HRP) (Type VI, expressed activity of 300 units/mg, Lot 125F-9645) and hydrogen peroxide were purchased from Sigma Chemical Co., St Louis, MO. $H_2{}^{18}O$ (95 atom % ^{18}O) was purchased from Merck Sharp and Dohme, Canada Limited, Montreal, Canada. All other chemicals used were reagent grade and were supplied from commercial sources.

Low-resolution mass spectra were obtained using an E.I. NERMAG R 10–10 C (70-eV ionization potential). High-resolution mass spectra were recorded on ZAB-HF mass spectrometer. ^1H and ^{13}C NMR spectra were recorded in deuterated chloroform on a Bruker WH-360 FT spectrometer operating at 360.134 (^1H) and 90.556 (^{13}C) MHz. The chemical shift values are reported in ppm. IR spectra were performed in chloroform solution (0.1-mm NaCl cells) or KBr pellets with a Nicolet 5DXB FT-IR spectrophotometer. UV spectra were determined in 95% ethanol solution with a Shimadzu UV-160 spectrophotometer.

Chromatography. Thin-layer chromatographic (TLC) analyses were carried out on 0.25 mm thick silica gel GF_{254} plates (Merck). The solvent system used was benzene/methanol (5:1, v:v) in which the R_f values of desacetylvinblastine, VLB, and metabolite **2** were 0.31, 0.42, and 0.62, respectively. Spots were visualized by UV fluorescence quenching at 254 nm and by spraying the developed plates with Dragendorff's reagent. The adsorbent used for column chromatography was silica gel (60–200 mesh, Baker 3405) which was oven-activated at 110 °C for 30 min prior to use.

High-performance liquid chromatography (HPLC) was performed with a Waters Associates (Milford, MA) Model 6000 A pump, a U6K Universal injector, and an ISCO variable wavelength V^4 UV detector. Samples were analyzed on an Alltech (Deerfield, IL) 600 RP C-18 column (25 cm × 4.6 mm, 10 μm) protected with a guard column filled with the same material. Mobile phase consisted of acetonitrile/0.005 M dibasic ammonium phosphate and 0.005 M 1-pentanesulfonic acid/H_2O (7:3, v:v). Concentrations

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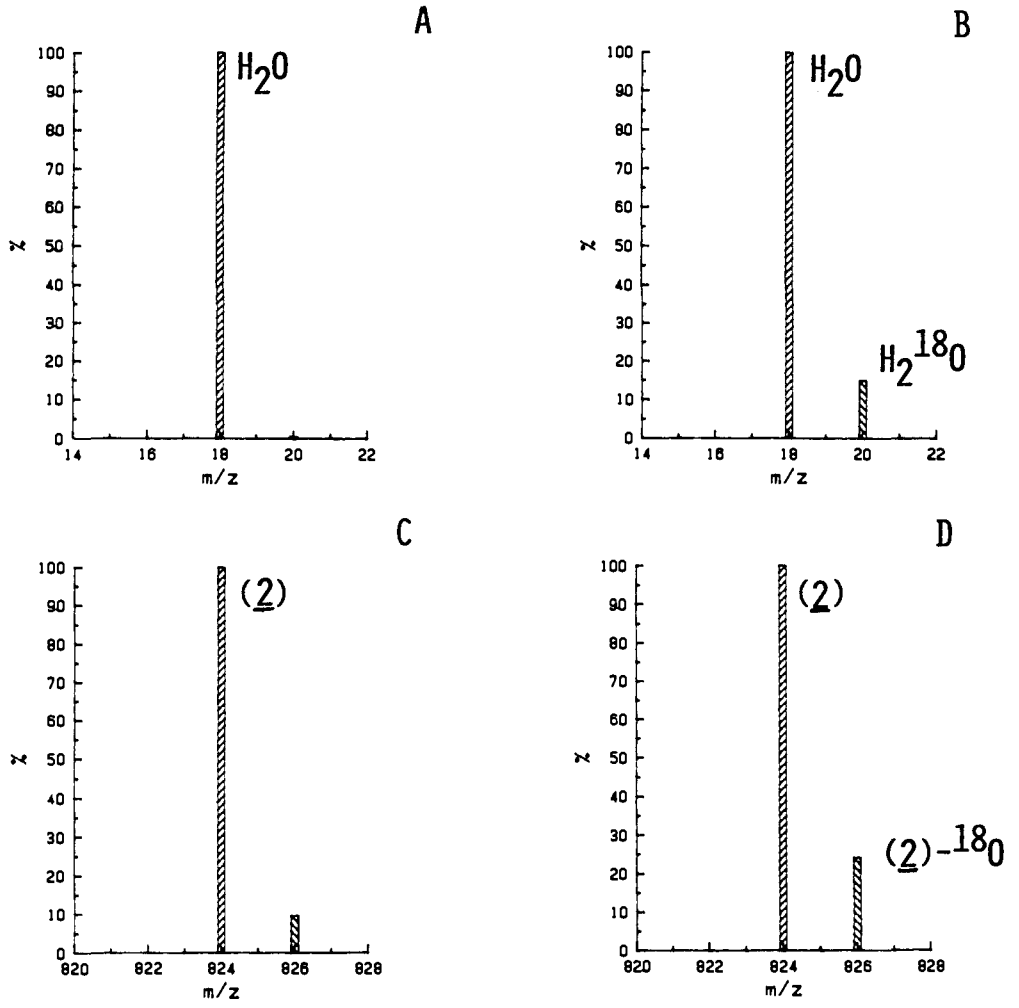

Figure 3. Relative intensities of molecular ions (M^+) and (M + 2) fragments of A, $H_2{}^{16}O$; B, $H_2{}^{16}O/H_2{}^{18}O$ mixture; C, unlabeled catharinine (**2**); and D, ^{18}O-labeled catharinine (**2**).

are expressed relative to the volume of water. The flow rate was 1 mL/min and absorbance was monitored at 220 nm. With vincristine sulfate as an internal standard, the retention volumes of VCR, VLB, and metabolite **2** were 10.4, 13.6, and 4.9 mL, respectively.

Peroxidative Metabolism of VLB (1). Analytical-scale incubations of VLB with the HRP/H_2O_2 system were carried out in 50-mL Delong flasks each containing 10 mL of buffer (0.1 M sodium phosphate monobasic, pH = 7.9), 1 mL of H_2O_2 (2.2 mM), 2 mg of HRP (474 purpurogallin units), and 2 mg of VLB sulfate (2.47 μmol). The mixtures were incubated on a New Brunswick Scientific Co. G-24 gyrotory shaker at 250 rpm and 37 °C, and reactions were monitored by TLC. Samples were taken at various time intervals, adjusted to pH 10 with ammonium hydroxide solution, and extracted with equal volumes of ethyl acetate. Thirty-microliter volumes of reaction extracts were spotted onto TLC plates for analysis. Under these conditions, VLB was completely consumed in the reaction at 2 h, yielding one major product, R_f 0.62 (30%), traces of VLB *N*-oxide, R_f 0.15 (1%), and another minor product, R_f 0.65 (2%). No metabolites were formed in controls which consisted of incubation mixtures with substrate plus all reaction components except enzyme.

^{18}O-Labeling Experiment. $H_2{}^{18}O$ (95 atom % ^{18}O, 0.38 mL) was diluted to a total volume of 2 mL with pH 7.9, 0.1 M sodium phosphate buffer. Mass spectral analysis of the water contained in this buffer indicated that it contained 14.9% $H_2{}^{18}O$ (Figure 3). The incubation reaction consisted of 2 mL of the above defined buffer containing 0.5 mL of H_2O_2 (4.41 mM), 0.5 mg of HRP, and 1 mg (1.24 μmol) of VLB sulfate. The incubation and reaction workup were carried out as described before, and the isolated metabolite was subjected to EI mass spectrometric analysis to reveal ions of the following relative intensities: *m/z* (% relative abundance), 824 (100), 826 (24).

Preparative-Scale Oxidation of VLB with Peroxidase. A total of 100 mg of VLB sulfate (0.124 mmol) was added to a mixture of hydrogen peroxide (50 mL, 8.8 mM) and HRP (50 mg) in 250 mL of sodium phosphate (0.1 M, pH 7.9), and the reaction mixture was divided evenly among 25 50-mL Delong culture flasks. Incubations were carried out as described above for 2 h, when they were combined, made alkaline to pH 10 with ammonium hydroxide solution, and extracted three times with equal volumes of ethyl acetate. The ethyl acetate extract was dehydrated over anhydrous sodium sulfate and evaporated under reduced pressure to give 95.0 mg of dark brown residue. This residue was applied to a silica gel column (1.5 × 24 cm) packed with a slurry of 6.5 g of silica gel in benzene. The column was eluted with benzene/methanol (5:1, v:v) while 5-mL fractions were collected. Fractions (14–23) yielded a single component (TLC) and were combined and concentrated to dryness to give 29.3 mg of pure metabolite **2** (31.5%).

Physical and spectral properties of HRP metabolite 2: high-resolution chemical-ionization mass spectrometry, $M^+ + 1$, *m/z* 825.4090 for $C_{46}H_{57}N_4O_{10}$, calculated 825.4074; UV max 213.4 nm (log ε 4.95); IR (cm^{-1}) ($CHCl_3$) 3468 (NH, OH), 1742, 1718 (C=O), 1664 (NCHO), 1615, 1598, 1473, 1440; ^1H NMR (360 MHz, $CDCl_3$) (ppm) 0.78 (3 H, t, Me-18), 0.86 (3 H, t, Me-18′), 0.99 (1 H, m, H-14′), 1.34 (1 H, dq, H-19a), 1.80 (1 H, dq, H-19b), 3.56 (3 H, s, OMe), 3.74 (1 H, s, H-2), 3.79 (2 H, m, H-5′), 3.80 (6 H, s, OMe-16, OMe-16′), 3.82 (2 H, dd, H-17′), 5.31 (1 H, d, H-15), 5.48 (1 H, s, H-17), 5.86 (1 H, dd, H-14), 6.10 (1 H, s, H-12), 6.64 (1 H, s, H-9), 7.13 (3 H, m, H-10′, H-11′, H-12′), 7.50 (1 H, dd, H-9′), and 7.94 (1 H, s, CHO); ^{13}C NMR (Table I).

CRFF-CEM Leukemic Cell System Bioassay. Metabolite **2** was subjected to bioassay using a range of doses in the CCRF-CFM (in vitro) leukemic cell system as described by Foley[39] and Grindey.[40] The activity of **2** was compared to that of the other

dimeric Vinca alkaloids, VLB, VCR, vinrosidine, and vinleurosine, and the results are presented as IC_{50} (50% inhibitory concentrations) in Table II.

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Studies on Prodrugs. 10. Possible Mechanism of N-Dealkylation of N-Masked Norfloxacins Having Several Active Methylene Groups

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As a prodrug approach to norfloxacin (NFLX, **2**), we have prepared several N-masked NFLXs (**1a–f**) and studied the cleavage mechanism of the C–N bond of N-masked NFLXs utilizing the following experiments: (1) the oxidation of N-masked NFLXs (**1a–f**) with *m*-chloroperbenzoic acid (MCPBA) and their subsequent cleavage to **2** in chloroform at room temperature or at 50 °C; (2) the liberation of NFLX from N-masked NFLXs after oral administration in mice. It was found that the chemical oxidative dealkylation of N-masked NFLXs proceeded when anion-stabilizing groups (e.g., CN, COR, COOR) are present on the α carbon of the nitrogen atom. In in vivo experiments, N-masked NFLXs having acidic hydrogens on the α carbon to the nitrogen atom also liberated NFLX (**2**) after oral administration.

Norfloxacin (NFLX, **2**) has been widely used as a clinically effective antibacterial agent,[1] but it has been shown that the blood level and the urinary recoveries after oral administration of NFLX were not sufficient for use as an effective oral antibiotic.[2] We have applied the prodrug technique to NFLX.[3-5] Recently, we have reported that in in vivo experiments, *N*-(2-oxopropyl)NFLX (**1a**) was absorbed efficiently and transformed into **2** whereas *N*-(2-hydroxypropyl)NFLX (**1f**) failed to metabolize into **2**[4] (Scheme I). Definition of such a metabolic difference of compounds **1a** and **1f** may open up the possibility for developing a new prodrug approach to amines.

The metabolic N-dealkylation of alkylamines is known to be catalyzed by flavin and cytochrome P-450 monooxygenases,[6] and several conceptual pathways for enzymatic dealkylation of amines should be considered; direct hydroxylation of the methyl carbon, with or without formation of an intermediate *N*-oxide, or electron-transfer oxidation of the nitrogen is the most probable of the proposed mechanisms.[6] Recently, Burka et al. found N-dealkylation of amines via the *N*-oxide to only be a minor pathway.[7] Other workers also have studied the mechanism of N-dealkylation of amines.[8] However, no definitive work has appeared on the mechanism of oxidative cleavage of amine derivatives **1**.

We focused our interest on the structure–metabolism relationship of compounds **1a** and **1f** and have hypothesized that acidic hydrogens on the α carbon to the nitrogen atom play an important part in the N-dealkylation of **1a**. To clarify our hypothesis, we synthesized several N-masked NFLXs (**1a–f**), with or without acidic hydrogens on the α carbon atom, and measured their metabolic conversion in serum after oral administration in mice. This paper describes the possible N-dealkylation mechanism of N-masked NFLXs having acidic hydrogens on the α carbon.

Results and Discussion

Chemical Oxidation of 1a–f with MCPBA. NFLX (**2**) was synthesized in accordance with the report of Koga et al.[1a] The N-masked NFLXs **1a–f** were prepared according to a recently described method.[4]

As shown in Scheme II, the oxidation of *N*-(2-oxopropyl)NFLX (**1a**) (0.53 mM) with MCPBA (1.06 mM) in dry chloroform (20 mL) at room temperature under an argon atmosphere afforded NFLX (**2**) and *N*-formyl NFLX **3**. We also tried the oxidation of other N-masked NFLXs (**1b–f**) under similar conditions. As summarized in Table I, the oxidation of **1b–d** also afforded **2**, and in the case of **1a** and **1b**, *N*-formylNFLX (**3**) was simultaneously produced. However, no formation of **2** and **3** was observed by oxidation of **1e** and **1f**.

We focused our interest on the C–N bond cleavage by chemical oxidations of **1a–d**.

Craig and co-workers have reported that *N*-oxides are formed by the oxidation of tertiary amines with MCPBA at low temperature (<0 °C).[9] When the oxidation of **1a** with MCPBA at low temperature (<0 °C) was carried out according to the Craig method, a colorless solid (**5a**) separated. The mass spectrum of **5a** showed ions at *m/e* 392 (MH^+), 376 ($MH^+ - 16$) [O]. Compound **5a** was redissolved in organic solvent to decompose to **2** and *N*-formylNFLX (**3**) was not obtained. However, the thermolysis of **5a** was carried out in the presence of MCPBA to afford **2** and **3**.

On the other hand, **5c** obtained by a similar manner was more stable compared to **5a**. Compound **5c** was charac-

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