(prepared with acetone only), obtained from Sigma Chemical Co., St. Louis, MO. The powder (3 g) was extracted at 55–60 °C for 5 min with pH 8.0 phosphate buffer (0.05 M), and the extract was filtered through a Nalgene filter unit (0.45-μm grid membrane) to yield a clear yellow filtrate. The filtrate was fractionated with ammonium sulfate, essentially as described by Wentworth and Wolfenden,[33] and the active ammonium sulfate fraction was dissolved in 2.0 mL of phosphate buffer (0.05 M; pH 7.0). The K_m for deamination of cytidine using this preparation was found to be 5×10^{-5} M, in good agreement with the value of 7×10^{-5} M reported previously by Tomchick et al.[22] for mouse kidney cytidine deaminase. Human liver was obtained at autopsy, and cytidine deaminase was isolated and purified as previously described;[33] suitable enzyme preparations could be obtained from liver samples frozen at −20 °C. Cytidine deaminase was measured by following the decrease in absorbance at 282 nm (290 nm for crude preparations) that characterizes the conversion of cytidine to uridine;[34] all assays were performed at pH 7.0 (phosphate buffer) and 37 °C with substrate (cytidine) at 1×10^{-4} M. Spectroscopic determinations were carried out with a Beckman Model 34 kinetic spectrophotometric system with the recorder set for full-scale deflection in the range 0.0 to 0.1 absorbance unit. Candidate inhibitors were incubated routinely with the enzyme for 2 min prior to initiation of the enzymatic reaction by addition of cytidine. Studies to evaluate whether inhibition by 17 involved slow establishment of a steady state were carried out using a 10-min preincubation of enzyme with 17 prior to the additin of cytidine.

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Mammalian Metabolism of Phencyclidine

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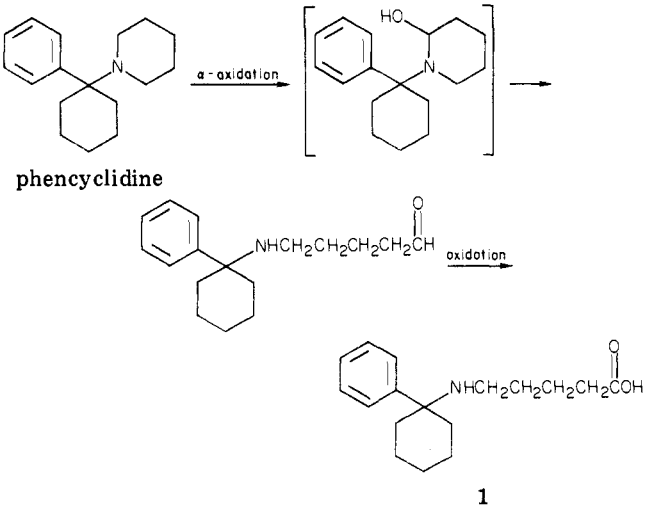
In dogs, the major metabolite of phencyclidine was found to be 5-[*N*-(1′-phenylcyclohexyl)amino]pentanoic acid (1). The γ-aminobutyric acid like metabolite was also pharmacologically evaluated to determine if the purported GABA-ergic mediated effects of phencyclidine on locomotor activity might be attributed to the metabolite. Preliminary pharmacological evaluation of 1 and its methyl ester indicates that the metabolite has little, if any, association with the effect of phencyclidine on locomotor activity.

Phencyclidine has become a widely used drug of abuse that exhibits a diverse pharmacological profile, and frequently the onset of these effects is observed long after the blood and brain levels have reached maximum values. In some cases the pharmacological effects appear to be biphasic in nature. In man, acute intoxication is characterized by hypotension, but the blood pressure may rise above normal 2 or 3 days later.[1] A schizophreniform condition appears in a small number of the subjects and the psychosis may persist for several weeks.[2-5] These observations suggest that the delayed effects of phencyclidine may be related to the production of a metabolite of phencyclidine.

In studies using the rhesus monkey and ^{14}C-labeled phencyclidine, 60% of the total activity appeared in the urine in 12 h and only trace quantities of the unchanged drug was detected.[6] Though the half-life of the drug is short (2.36 h, monkey; 2.86 h, dog),[7] only trace levels of the drug and low levels of metabolites have been detected in the urine. In man and dog, the major metabolite has been tentatively identified by GC–mass spectroscopy as 4-phenyl-4-piperidinocyclohexanol, and 1-(1-phenylcyclohexyl)-4-hydroxypiperidine was observed as a minor metabolite.[8-10] Only very small quantities of the metabolite where both the cyclohexyl and piperidyl rings are hy-

Scheme I



1

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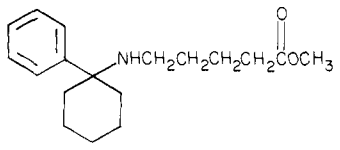
0022-2623/81/1824-0666$01.25/0

droxylated have been detected in dogs and monkeys.[8] Unfortunately, the yields of the metabolites in the earlier GC–mass spectral studies were not determined, but they would appear to be fairly low. In later studies by Martin et al. using [^{3}H]phencyclidine in mice, it was found that the quantities of the two monohydroxylated metabolites represented only a small fraction of the dose and that the vast majority of the dose had been converted to an unidentified metabolite of very high polarity.[11] It was assumed that the unidentified metabolite was the dihydroxy metabolite; however, the earlier mass spectral studies had only found trace levels of this compound.

The major objective of the present study was to determine if the metabolism of phencyclidine might lead to the formation of an amino acid metabolite (1) that would not have been detected in the GC–mass spectral studies (because of the extraction method) and that would have properties similar to the major unidentified metabolite detected using a methanol extraction procedure by Martin et al.[11]

Results and Discussion

Because of the difficulties that are frequently encountered in the extraction and analysis of amino acid metabolites, it was considered essential to synthesize a reference standard of the metabolite (1) with which to design the analytical method. Of the several methods that were attempted, the general procedure of Maddox et al.[12] for 1-arylcyclohexylamines was found to be the most useful. The first phase of the synthesis lead to the formation of the arylcyclohexylamino alcohol in 42% yield, and this was subsequently converted to **2** in a $KMnO_4$ oxidation/esterification sequence with a 35% yield. The ester was then hydrolyzed to 1 in nearly quantitative yield.



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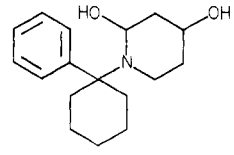

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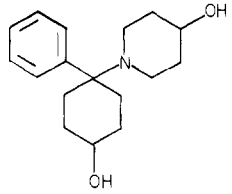

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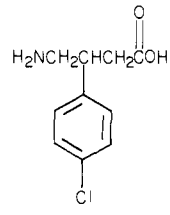

baclofen

Initial efforts on the development of an analytical method for the determination of 1 in urine samples were frustrated because of the poor extraction characteristics of the metabolite. Extractions using methylene chloride, ethanol–methylene chloride (1:10), diethyl ether, and ethyl acetate were found to give very poor recovery of the metabolite. Quantitative recovery of the metabolite was obtained when equal volumes of ethanol and urine were used. This solvent system, which is normally homogeneous, becomes biphasic when saturated with potassium carbonate, and the metabolite was found in the top layer.

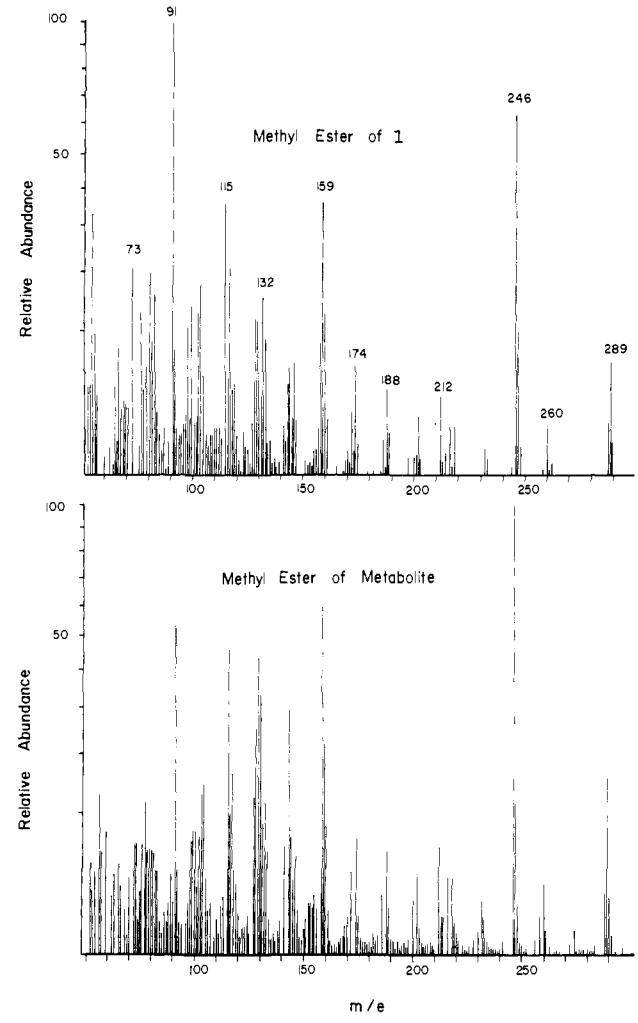

Figure 1. Mass spectra of phencyclidine metabolite. The bottom spectrum was obtained from the derivatized extract of the urine of the phencyclidine-treated dog. The top spectrum was obtained from the methyl ester derivative of 1.

For gas chromatographic analysis using a nitrogen selective detector, the methyl ester of the metabolite was formed using BF_3·CH_3OH as the reagent. It is essential that all of the excess BF_3 be removed after the esterification or the intensity of the metabolite gas chromatographic peak will be reduced and the BF_3 will destroy the rubidium bean in the nitrogen selective detector. It was also found to be essential to use a very low H_2 flow in the nitrogen selective detector to minimize the solvent peak and maximize the metabolite peak.

Using the above extraction and derivatization procedure, it was found that the urine contained a quantity of metabolite 1 representing a 27% conversion of phencyclidine. The Kovat retention index and the N_2-FID detector response index[13] of the derivative of the isolated metabolite and the synthetic standard were found to be identical. The observed detector response index (0.14) was typical of secondary amines.[13]

The GC–mass spectra (Figure 1) of the derivatized metabolite and derivatized 1 and 2 were essentially identical. A loss of 43 from the molecular ion was observed for 1, 2, and phencyclidine which could be assigned to a three carbon loss from the cyclohexyl ring.[9,10] The fragment at *m/e* 91 would be characteristic of a monosubstituted aromatic ring.

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Though the mass spectral, nitrogen detector response index, and gas chromatographic retention index measurements all clearly indicate that 1 is a major metabolite of phencyclidine, one should use caution in differentiating between 1 and the two dihydroxy metabolites (3 and 4) that have been postulated.[8,10] All three of these compounds have the same molecular weight and they would add two Me_3Si groups. In the previous mass spectral studies, authentic standards of the dihydroxy metabolites were not available and the structural assignment was deduced from fragmentation patterns. The mass spectrum of the underivatized dihydroxy metabolite (4) isolated from rhesus monkey urine was reported to show major peaks at 91, 216, and 275,[8] while underivatized metabolite (1) was characterized by a slightly different pattern: 91 (26), 216 (2.5), 232 (100), 275 (M^+, 12). The major difference between the two spectra is the presence of a peak at 232 (base peak) in the spectrum of 1 that was not observed in 4.

Wong and Biemann[10] had detected the dihydroxy metabolite previously reported and they also detected trace quantities of a second dihydroxy metabolite that was tentatively assigned the structure as shown for 3. This metabolite (and 1) exhibited a molecular ion peak at *m/e* 275 and a base peak at *m/e* 232. Unfortunately, Wong and Biemann did not report any mass spectral data other than these two peaks, and an authentic sample of the purported metabolite was not available for a direct comparison. They also reported that the product obtained from the lithium aluminum hydride reduction of the metabolite exhibited a molecular ion at *m/e* 261 and a base peak at 218. However, the lithium aluminum hydride reduction product of 1 would give the arylcyclohexylamino alcohol (also the intermediate in the synthesis of 2), which was found to produce mass spectral peaks at *m/e* 261 (M^+, 30), 218 (100), 184 (25), 159 (47), 146 (25), 117 (55), 104 (46), and 91 (77). It would appear that the metabolite reported by Wong and Biemann[10] as 3, which would be an extremely unstable carbinolamine, may have been a small portion of the major metabolite (1) that had been carried over in the methylene chloride extraction that had been used.

Recent studies in these laboratories on the metabolism of phencyclidine by the microorganism *Cunninghamella echinulata* (ATCC 9244) had shown that this fungus produced the two major mammalian metabolites, 1-(1-phenylcyclohexyl)-4-hydroxypiperidine and 4-phenyl-4-piperidinocyclohexanol.[14] These cultures have now been examined using the new analytical procedures, and quantities of 1 were detected that were comparable to the levels of the two monohydroxylated metabolites. The identification of 1 was based on a comparison of retention times, detector responses indexes, and mass spectra of the metabolite and an authentic sample.

It would appear that 1 is the major metabolite of phencyclidine for both dog and *C. echinulata*. The metabolic pathway leading to this product (Scheme I) could proceed through a α-oxidative hydroxylation, followed by oxidation of the aldehyde. Though the products of similar ring-opening reactions have not been frequently reported in the literature, the more recent literature[15] has recognized the importance of the reaction. In dog[8,10] and *C. echinulata*,[14] the cyclohexyl-hydroxylated metabolite was obtained with the second highest yield and the piperidyl-hydroxylated metabolite was obtained in the lowest yield.

In mice, phencyclidine has been observed to have a locomotor stimulant effect that can be blocked by baclophen (and other GABA-ergic agonists) and was not blocked by diazepam.[16] By contrast, the increase in locomotor activity produced by amphetamine was not blocked by baclophen but was blocked by diazepam. It has been suggested that some of the CNS effects of phencyclidine may result from a GABA-ergic antagonist effect. Metabolite 1 had a structure that could be associated with either GABA-ergic agonist or antagonist activity. If it were an antagonist, 1 could be responsible for the increase in locomotor activity attributed to phencyclidine. As an agonist, 1 might be associated with the biphasic effects of phencyclidine where phencyclidine was acting directly and the metabolite was countering the effect.

Table I. Effect of Phencyclidine and Metabolites on Locomotor Activity

test compd	postinjection–preinjection act. counts, mean	SE
phencyclidine hydrochloride	3062	±850
I·HCl	−1063	±198
II·HCl	−1418	±164
phencyclidine + I·HCl	2478	±688
phencyclidine + II·HCl	2388	±631
vehicle control	−1426	±112

Phencyclidine was found to greatly increase locomotor activity (Table I) as previously reported.[3] It was also found that 1 showed a slight increase in locomotor activity, but the difference would be reliable only at about the 90% level of confidence (Student's *t* test). Administration of 2 was found to have no effect on locomotor activity. Coadministration of phencyclidine with 1 and phencyclidine with 2 was found to have the same effect on locomotor activity as phencyclidine alone. These preliminary tests indicate that the metabolite (1) only has weak, if any, effects on locomotor activity. Future pharmacological evaluation will be needed to determine the role of the metabolite in the other effects of phencyclidine.

Experimental Section

Melting points were obtained on a Mel-temp apparatus and are corrected. IR data were recorded on a Perkin-Elmer Model 281B spectrometer, proton NMR data on a JEOL Model C-60-HL spectrometer, carbon-13 NMR data on a JEOL Model FX-60 Fourier transform spectrometer, and mass spectral data on a Finnigan 3221-F200 mass spectrometer. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

Methyl 5-[*N*-(1′-Phenylcyclohexyl)amino]pentanoate (2). The procedure that was used was similar to "method B" reported by Maddox et al.[12] for 1-arylcyclohexylamines. A mixture of 9.8 g (0.1 mol) of cyclohexanone and 10.4 g (0.1 mol) of 5-aminopentanol was stirred with 80 g of type 4Å molecular sieves in 200 mL of anhydrous diethyl ether for 112 h. After the molecular sieve was removed by filtration, 16.8 g (0.2 mol) of freshly prepared phenyllithium[12] was added to the above mixture at reflux over a 30-min period. After a total reaction time of 1 h, the mixture was cooled, 100 mL of H_2O was slowly added, and the ether layer was extracted with two portions of 15 mL of 5 N HCl in 50 mL of H_2O. The combined aqueous extracts were made alkaline with concentrated NH_4OH and partitioned with 100 mL of diethyl ether. The ether layer was dried with anhydrous Na_2SO_4 and then evaporated to give 11.0 g (42%) of the arylcyclohexylamino alcohol product, which was shown to be pure by TLC and high-performance LC analysis: ^1H NMR (base, $CDCl_3$) δ 7.50 (phenyl, 5, m), 3.67 (OCH_2, 2, t), 3.07 (NH and OH, 2, s), 2.27 (CH_2N, 2, t), 1.4–2.0 (16, br m); ^{13}C NMR (base, $CDCl_3$) δ 146.8 (1-phenyl, s), 128.19 (phenyl, d), 126.4 (phenyl, d), 126.2 (phenyl, d), 62.2

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(OCH_2, 5), 57.6 (1-cyclohexyl, s), 41.6 ($NHCH_2$, t), 36.3 (t), 32.6 (t), 26.1 (t), 23.7 (t), 22.3 (t); IR (thin film) 3340, 1445, 1053, 760, 702 cm^{-1}.

A 15% aqueous solution of $KMnO_4$ was slowly added to a stirred solution of 11 g of the arylcyclohexylamino alcohol, 75 mL of H_2O, and 20 mL of 5 N HCl at room temperature. The solution was examined periodically to determine if the pH was acidic, if there was an excess of $KMnO_4$, and if any starting material remained (via HPLC). After the addition of 24 g (0.15 mol) of $KMnO_4$ over 2 h, the reaction was quenched with 100 mL of isopropyl alcohol. Solid $NaHSO_3$ was then added with stirring until the brown color of the MnO_2 had been dissipated. The mixture was filtered then the filtrate was concentrated to a thick slurry by evaporation at 40 °C under vacuum. To form the methyl ester and to aid in the removal of water, 3 × 75 mL portions of anhydrous methanol were added to the highly acidic residue and then evaporated at 40 °C to dryness. The crude product was chromatographed on a 3 × 30 cm silica gel column (Woelm, 0.05–0.2 mm) using 300 mL of CH_2Cl_2, 300 mL of 5% CH_3OH in CH_2Cl_2, and 300 mL of 10% CH_3OH in CH_2Cl_2 for elution to give 4.2 g of the crude methyl ester. A diethyl ether solution of the product was washed with an aqueous NH_4OH solution and dried with Na_2SO_4, and then the HCl salt of the product was precipitated with HCl–ether. The final product (**2**) was recrystallized from methanol–ethyl acetate (2:75) to give fine colorless needles: mp 189–191 °C; ^1H NMR (HCl salt, $CDCl_3$) δ 7.2–7.8 (phenyl, 5), 3.37 (OCH_3, 3, s), 1.1–2.8 (br m); ^{13}C NMR (HCl salt, $CDCl_3$) δ 173.2 (C=O, s), 135.4 (1′-phenyl, s), 129.4 (phenyl, d), 129.0 (phenyl, d), 128.4 (phenyl, s), 64.6 (1′-cyclohexyl, s), 51.5 (OCH_3, q), 41.5 (t), 33.5 (t), 33.3 (t), 26.1 (5), 25.2 (t), 22.4 (t); IR (KBr disk) 1726 (C=O), 1272, 774 (phenyl), 706 cm^{-1} (phenyl); mass spectrum (70 eV), *m/e* (relative intensity), 289 (M^+, 3.7), 246 (42), 159 (26), 115 (38), 91 (100). Anal. ($C_{18}H_{28}ClNO_2$) C, H, N.

5-[*N*-(1′-Phenylcyclohexyl)amino]pentanoic Acid (1). Though small amounts of 1 were isolated from the later fractions of the chromatographic purification of **2**, the yields were typically 10–15% of that of the methyl ester. A more useful procedure was to reflux the HCl salt of II in H_2O for 22 h to give nearly quantitative conversion to the free acid: mp 214–215 °C (from acetone); IR (KBr disk) 1733 (C=O), 1170, 771 (phenyl), 698 cm^{-1} (phenyl); mass spectrum (70 eV), *m/e* (relative intensity), 275 (M^+, 12), 232 (100), 216 (2.5). 132 (23), 91 (26). Anal. ($C_{17}H_{26}ClNO_2$) C, H. N.

Quantitation and Identification of Phencyclidine Metabolites. A 9-kg dog was dosed at 5 mg/kg with phencyclidine hydrochloride and this dose was repeated 60 and 180 min after the initial injection. A total of 370 mL of urine was then collected over the next 30 h.

A 10-mL portion of the urine sample was mixed with 10 mL of 95% ethanol, and then the mixture was saturated with solid anhydrous K_2CO_3, which caused the mixture to separate into two layers. After the mixture was centrifuged, the top ethanol layer was transferred to a clean conical centrifuge tube and evaporated on a steam cone under a stream of nitrogen (recovery >90%). After the sealed tubed cooled, 0.5 mL of BF_3·CH_3OH was added and the sealed tube was heated to 100 °C for 10.0 min. After the mixture cooled 0.5 mL of H_2O was added, and 10 min later the mixture was made alkaline (pH ≃10) with the dropwise addition of concentrated NH_4OH and then extracted with 3 mL of diethyl ether. After the mixture was dried with Na_2SO_4, the ether extract was evaporated under a stream of nitrogen. The residue was then taken up in 100 μL of ethyl acetate for gas chromatographic analysis (1 μL used).

A Model 900 Perkin-Elmer gas chromatograph was equipped with a 2 mm × 183 cm glass column packed with 3% OV-17 on 110–120 mesh Anachrom ABS support. The column was operated at 240 °C, the injector at 270 °C, and the manifold at 270 °C. Helium was used as the carrier (27 mL/min), and the column effluent was split equally between a flame-ionization detector (H_2 = 19 mL/min) and a rubidium bead nitrogen–phosphorus selective detector (H_2 = 2.3 mL/min). The latter detector was operated in the mode where both nitrogen- and phosphorus-containing compounds would be detected and the bead current setting of 6.8 was used. The response index of the metabolite peak on the two detectors was measured relative to caffeine as a standard as previously reported.[13]

For the purpose of quantification and identification, a known quantity of 1 in a blank sample was extracted and derivatized using the above procedure. Quantitations were based on the peak-height ratios of the standard and unknown. The retention time of the standard was found to be 5.8 min and the Kovat retention index was 2421. The detector response index of the standard was 0.14, which was typical of secondary amines (0.12–0.40).[13] The retention time, Kovat retention index, response index, and mass spectra of the metabolite peak obtained from the dog urine were also measured.

Pharmacology. The CNS stimulant effect of phencyclidine and the related compounds were measured using actometers and protocol similar to that previously reported for phencyclidine.[16] Groups of nine male Swiss mice were given the same dose of the test compound (8 mg/kg ip) in the same volume of water (0.01 mL/g of body weight). The locomotor activity of the individual mice was measured for 1 h before the injection and for 1 h after the injection. The difference in the locomotor activity (postinjection–preinjection) for the individual mice was then taken as a measure of the effect of the test compound on CNS activity. The control group receiving an injection of water was found to have an activity score of −1426 (Table I), indicating that the locomotor activity of the mice decreased in the second hour compared to the first hour when no drug was given.

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Synthesis and Preliminary Antitumor Evaluation of 5-Iminodoxorubicin

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Doxorubicin required protection as the *N*-(trifluoroacetyl) 14-*O*-*p*-anisyldiphenylmethyl ether in order to prevent extensive degradation of the α-hydroxy ketone side chain during ammonolysis of the quinone to give the stable 5-imino derivative. In the initial test against mouse leukemia P-388, 5-iminodoxorubicin showed efficacy (T/C = 173%) comparable to that of the analogue 5-iminodaunorubicin and of doxorubicin as the parent but inexplicably required at least 10 times the dose. The two imino compounds were indistinguishable by in vitro tests, which suggested weaker DNA interactions compared to doxorubicin and much weaker redox cycling with O_2 to generate free radicals.

The quinone moiety occurs in the structure of numerous anticancer agents and is often recognized as a key site of biochemical action, particularly[1-11] in the clinically important anthracyclines doxorubicin[12] (Adriamycin, 1) and

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