Cyclic Nitrone Free Radical Traps: Isolation, Identification, and Synthesis of 3,3-Dimethyl-3,4-dihydroisoquinolin-4-ol *N*-Oxide, a Metabolite with Reduced Side Effects

Craig E. Thomas,*,† Patrick Bernardelli,‡ S. Marc Bowen, Stephen F. Chaney, Dirk Friedrich, David A. Janowick,§ Bryan K. Jones, Frederick J. Keeley, John H. Kehne,|| Bert Ketteler, David F. Ohlweiler, Leo A. Paquette,‡ David J. Robke, and Thomas L. Fevig

Hoechst Marion Roussel, Inc., 2110 East Galbraith Road, Cincinnati, Ohio 45215, and Evans Chemical Laboratories, The Ohio State University, Columbus, Ohio 43210

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A C-4 hydroxylated metabolite (**2**, 3,3-dimethyl-3,4-dihydroisoquinolin-4-ol *N*-oxide) of the previously described cyclic nitrone free radical trap **1** (3,3-dimethyl-3,4-dihydroisoquinoline *N*-oxide, a cyclic analog of phenyl-*tert*-butylnitrone (PBN)) was isolated, identified, and synthesized. The metabolite (**2**), though a less potent antioxidant than **1** in an *in vitro* lipid peroxidation assay, showed greatly reduced acute toxicity and sedative properties. Several analogs of **2** were prepared in attempts to improve on its weak antioxidant activity while retaining the desirable side effect profile. Effective structural changes included replacement of the *gem*-dimethyl moiety with spirocycloalkane groups and/or oxidation of the alcohols to the corresponding ketones. All of the analogs were more lipophilic (log k'_w) and more active in the standard lipid peroxidation assay than **2**. In addition, some of the compounds were able to protect cerebellar granule cells against oxidative damage (an *in vitro* model of oxidative brain injury) with IC_{50} values well below the value of the lead compound **1**. The ketones, as predicted, were much more potent than **2** (and **1**) in both of the above assays (up to *ca*. 200-fold). However, only compounds with a hydroxyl or an acetate group at C-4 displayed significantly reduced acute toxicity and sedative properties relative to those of **1**. Importantly, the diminishment of toxicity and sedation were not the result of a lack of brain penetration as both **2** and the corresponding ketone (3,3-dimethyl-3,4-dihydro-3*H*-isoquinolin-4-one *N*-oxide) achieved equal or greater brain levels than those of **1** when administered to rats ip.

Introduction

Free radical mediated oxidation of cellular macromolecules (lipids, proteins, DNA, etc.) has been implicated in a number of disease states, including stroke and head trauma.[1] For example, during stroke, biochemical changes arising from ischemia alter the cell such that subsequent reperfusion induces a large flux of reactive free radicals, such as superoxide anion and hydroxyl radical. These radicals, acting mainly through initiation of chain reactions, can damage the aforementioned macromolecules to such an extent that neuronal cell death ultimately occurs, with resultant neurologic impairment.

One approach to the treatment of stroke, then, is to disrupt destructive radical chain reactions by intercepting chain initiators and/or chain carriers with free radical traps (antioxidants). For example, prolonged pretreatment of animals with the lipophilic antioxidant α-tocopherol can ameliorate stroke-induced neurologic damage.[2] We are particularly interested in trapping radicals with nitrones,[3] typified by phenyl-*tert*-butylnitrone (PBN, Figure 1). Free radicals react with these

Figure 1.

compounds by adding to the nitrone double bond. The resulting products, though still free radicals (nitroxides), are much less reactive. They cannot propagate a radical chain reaction, and they have sufficient lifetimes to diffuse from the site at which they are generated, thereby presumably preventing concentrated, debilitating tissue damage. Indeed, PBN has been shown to reduce significantly neuronal cell loss and neurologic deficits in gerbil[4] and rat[5,6] models of stroke.

The low *in vitro* potency of PBN (the IC_{50} in an *in vitro* lipid peroxidation assay is 14.3 mM) and the requirement of a 100-300 mg/kg body weight dose to achieve efficacy in *in vivo* studies led us to develop a series of cyclic nitrones,[3] as outlined in the preceding paper in this issue. The simplest member of the series, compound **1** (Figure 1), was already 8-fold more potent than PBN in the *in vitro* lipid peroxidation assay. Further refinements ultimately afforded compounds up to 650-fold more potent (IC_{50} = 22 μM). During the course of this work, several structure−activity relationships were revealed. For example, a correlation was found between lipophilicity (clog *P* or log k'_w)[7] and *in vitro* potency. That is, the more lipophilic compounds were generally more potent antioxidants. Second, we found that incorporation of an additional antioxidant moiety (*o*,*o*′-dimethylphenol) into the molecule increased

* To whom correspondence should be addressed. Tel.: 317-277-8030. FAX: 317-277-4783.
† Current address: Eli Lilly and Company, Lilly Research Laboratories, Drop Code GL 45, 2001 W Main St, Greenfield, IN 46140.
‡ The Ohio State University.
§ Current address: Abbott Laboratories, 100 Abbott Park Rd., Abbott Park, IL 60064-3500.
|| Current address: Neurogen Corporation, 35 Northeast Industrial Rd., Branford, CT 06405.
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Figure 2. Metabolites of compound **1**.

the activity. Perhaps most importantly, it was observed that all of the compounds tested in animal models produced side effects.[8] These ranged from transient sedation at moderate doses to rapid death at very high doses. Although the severity of the sedative effect varied among different compound classes, the overall result was that an acceptable therapeutic index was not attained. Indeed, disappointingly, the most active compounds also seemed to be the most toxic.

The goal of the present study was to prepare a potent antioxidant which could readily penetrate the brain without causing sedation or other undesirable side effects. We describe herein our efforts to isolate, identify, and synthesize the major metabolite of **1**. We also report the synthesis of several related compounds and the evaluation of all of these compounds as free radical traps (antioxidants) for the treatment of stroke.

Results and Discussion

The search for nonsedating nitrones led us to examine the metabolism of **1** because of the unusual nature of the sedation (rapid onset and rapid decline) observed with this compound. When **1** was administered to mice and the serum was analyzed at various time points by HPLC, it was observed that the decline in sedation coincided with the appearance of a major metabolite. The fact that the antioxidant activity persisted long after sedation had subsided led to the speculation that this metabolite retained the antioxidant activity of the parent, but did not cause sedation.

Isolation and Identification of Metabolites. Sprague−Dawley rats were treated with **1**, intraperitoneally, and three components (**2**, **3**, and recovered **1**, Figure 2) were subsequently isolated from the serum, as described in the Experimental Section. Compound **1** was identified by comparison to an authentic sample, whereas the structures of **2** and **3** were assigned as follows.

The mass spectrum of compound **2** exhibited a molecular ion of 191, 16 mass units higher than the parent, suggesting that hydroxylation had occurred. The IR (3599, 3402, 1548, and 1172 cm^{-1}) and UV (maximum at 305 nm) spectra also indicated that a hydroxyl group was present and that the nitrone functionality was retained. Similar to the parent compound **1**, the ^1H NMR spectrum of compound **2** showed four aromatic protons (δ 7.49, br d, J = 7.5 Hz; 7.46−7.38, AB-m, 2 H; 7.24, br d, J = 7.5 Hz), consistent with an unaltered 1,2-disubstituted benzene partial structure, and a farther downfield resonance (δ 7.95, br s, $^1J_{C,H}$ = *ca.* 180 Hz), consistent with an unaltered nitrone functionality. In addition, an aliphatic methine proton (δ 4.64, br s, $^1J_{C,H}$ = *ca.* 145 Hz) and two diastereotopic methyl groups (δ 1.57, s, 3 H and 1.41, s, 3 H) were observed, indicating that the metabolite was derived from **1** by introduction of a hydroxyl group at the benzylic position. This was supported by observation of four aliphatic carbons with appropriate chemical shifts and multiplicities (δ 74.9, d, 71.5, s, 23.2, q, and 19.0, q) in the ^{13}C/APT NMR spectra. Furthermore, treatment of compound **2** with acetic anhydride and (*N*,*N*-dimethylamino)-pyridine in the NMR tube led to a substantial downfield shift of the aliphatic methine proton (from 4.64 to 5.89 ppm) and the appearance of an additional methyl group (δ 2.02, s, 3 H), consistent with transformation of a secondary alcohol into its acetate. Also consistent with structure **2** were NOED experiments in which NOE's between the methine proton (δ 4.64) and the proximal aromatic *ortho* proton (δ 7.49), as well as the *syn*-oriented methyl group (δ 1.41), were observed. The structural assignment was ultimately confirmed by synthesis of racemic **2**. We note that isolated metabolite **2** was formed as a *ca.* 90/10 mixture of enantiomers as determined by chiral HPLC (see the Supporting Information). Because of the relatively poor antioxidant activity of the compound (see below, Table 1) compared with subsequently prepared analogs, we made no attempts to separate or identify the absolute configuration of the two enantiomers.

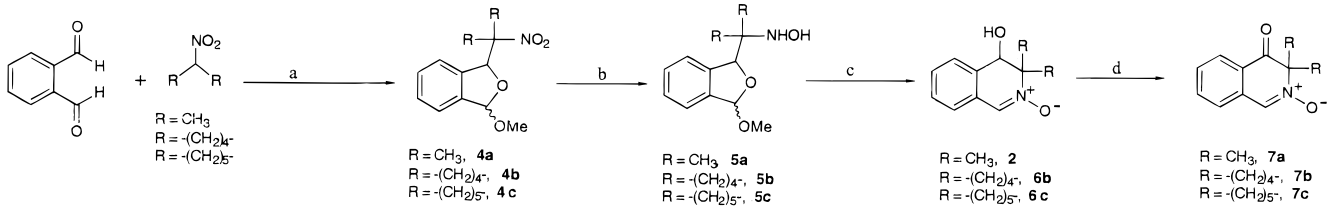
Compound **3** could be readily identified by an analysis of the following spectral data. A molecular ion of 191 and a proton NMR spectrum similar to that of **2** in the aliphatic and aromatic regions again indicated C-4 hydroxylation. In particular, an aliphatic methine proton (δ 4.58, br s, $^1J_{C,H}$ = *ca.* 145 Hz) was observed, which exhibited a substantial downfield shift (from 4.58 to 5.83 ppm) upon acetylation as before. However, the absence of the nitrone moiety could be inferred from the

Table 1. Correlation of *in Vitro* Antioxidant Activity with log k'_w and Ability To Maintain Viability of Neuronal Cells Subject to Oxidative Stress

		IC_{50} (μM)		
compd	log k'_w[a]	inhib of liposomal peroxidation[b]	inhib of neuronal peroxidation[c]	maintenance of cell viability[c]
PBN		14300	2580[d] (307)	2600[d] (292)
1	1.90	1670	307/538[e]	292/490[e]
2	0.91	5500	1900 (307)	1500 (292)
6b	1.67	1040	771 (538)	817 (490)
6c	2.10	539	520 (538)	588 (490)
7a	1.65	940	830 (307)	882 (292)
7b	2.05	157	102 (538)	105 (490)
7c	2.60	27	37 (538)	39 (490)
8	1.95	2090	839 (307)	660 (292)

[a] These values represent single determinations. The r^2 values for linear regression analyses of the raw chromatographic data were >0.999 in each case. See the Experimental Section of the preceding paper in this issue. [b] Liposomes were prepared from soybean phosphatidylcholine. Oxidation was initiated and oxidation products quantitated as described previously (see ref 17). Compound **1** was included as an internal standard in the lipid peroxidation assay of test compounds. If the value obtained for **1** varied by more than 15% from the value given above, the experiment was excluded. The value given for PBN was reported earlier (see ref 7). [c] See the Experimental Section for a description of the cerebellar granule cell experiments. [d] These PBN data were not obtained in triplicate runs (n = 1) as were those for the other compounds. [e] Compound **1** was used as an internal standard. The value obtained for **1** in a run on a test compound is given in parentheses after the value obtained for the test compound.

Scheme 1[a]



[a] Reagents: (a) NaOMe, MeOH; H_2SO_4; (b) Al/Hg, Et_2O/H_2O; (c) 2 N HCl, THF; (d) $(COCl)_2$/DMSO, Et_3N, CH_2Cl_2.

lack of the characteristic UV absorption maximum at 305 nm and the absence of the C-1 proton (singlet at δ 7.95 in **2**). Instead, an IR band (1664 cm^{-1}), the downfield shift of one of the aromatic *ortho* protons (doublet at δ 8.08), and the presence of an exchangeable proton (broad singlet at δ 5.93) were consistent with the lactam structure **3**. Although this compound was not synthesized because of its low abundance and likely lack of antioxidant activity, all spectral data obtained on the isolated metabolite are consistent with the assigned structure. We did not determine the enantiomeric composition of **3**.

Compound **2** is much more abundant *in vivo* than **3** and retains the nitrone functionality and was therefore more likely to be the putative nonsedating antioxidant. In order to obtain sufficient material for *in vivo* testing and to confirm the structural assignment, the synthesis of **2** was undertaken.

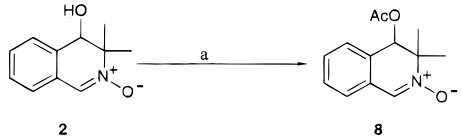
Synthesis of the Major Metabolite and Related Compounds. In addition to **2**, some more lipophilic analogs were targeted because of the lipophilicity/activity correlation mentioned earlier. Oxidation of **2** to the corresponding ketone was especially interesting since this simple modification would simultaneously increase the lipophilicity, remove the stereocenter, and introduce an electron-withdrawing group in conjugation with the nitrone, rendering it more reactive toward radicals. Acylation of the hydroxyl group was expected to increase lipophilicity but have little additional effect. Finally, replacement of the *gem*-dimethyl moiety with a spirocycloalkyl group in both the alcohol and ketone series was pursued since this approach had been shown to increase potency in other series and would require only a trivial modification of the synthetic scheme.

The target molecules were prepared in three or four steps starting from various nitroalkanes and *o*-phthalaldehyde (Scheme 1). Reaction of these two substrates in the presence of sodium methoxide,[9] followed by acidification, gave the cyclic acetals **4** (as *ca.* 1:1 mixtures of cis and trans isomers). The crude mixtures were obtained in quantitative yield and could be carried on to the next step as such with no loss in yield.

The nitro acetals **4** were reduced to hydroxylamines **5** by treatment with aluminum amalgam in ether/water according to a literature procedure.[10] Purified hydroxylamines **5** were obtained in 42−53% yield (from *o*-phthalaldehyde) after separation from variable amounts of recovered **4** and the over-reduced amino compounds. Treatment of hydroxylamino acetals **5** with aqueous HCl in THF provided the hydroxy nitrones **2** and **6b**,**c** cleanly and rapidly.[11] The crude products could be easily purified by crystallization or chromatography (52−67% yield; these yields may have been limited by the relatively high water solubility of the products).

Spectroscopic data (^1H NMR, ^{13}C NMR, IR, MS, UV) for synthetic **2** were fully consistent with the assigned

Scheme 2[a]



[a] Reagents: (a) Ac_2O, DMAP, Et_3N, CH_2Cl_2.

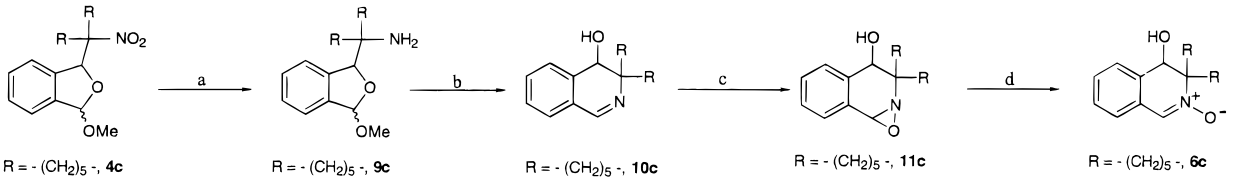
structure, but differed somewhat from those exhibited by the material isolated from rat serum (see the Experimental Section).[12] The identity of the two samples was therefore further confirmed by chiral HPLC as mentioned above and by acetylation of each sample. In the former case, the isolated sample gave two major peaks (*ca.* 10/90 ratio) with retention times of 9.3 and 11.4 min, respectively. Synthetic (racemic) **2** gave two peaks of equal intensity, also at 9.3 and 11.4 min (see the Supporting Information). Upon acetylation, the signal for the methine proton of the isolated sample shifts from δ 4.64 to 5.89 ppm as described above, whereas the corresponding signal for synthetic **2** shifts from δ 4.58 to 5.89 ppm. The two acetylated samples also have the same R_f on TLC. We consider the structure assignment of this simple molecule and the identity of the two samples to be completely unambiguous.

Hydroxy nitrones **2** and **6b**,**c** were oxidized to the corresponding ketones **7a**−**c** under Swern[13] conditions (60−74% yield), whereas only **2** was converted into the corresponding acetate, **8** (Scheme 2, 38% yield).

The syntheses described above afforded ample quantities of **2** and **6**−**8** for *in vitro* and preliminary *in vivo* testing. However, in attempting to scale-up the syntheses further, difficulties were encountered in reproducing the reduction step (**4** to **5**). For unknown reasons, over-reduction to the corresponding amine (**9c**, Scheme 3) occasionally dominated the desired process. In order to circumvent this problem, the following modified scheme was developed. The nitro acetal **4c** was deliberately converted into the amine **9c**, which, upon treatment with acid, cyclized to imine **10c**. Oxidation of the imine with Oxone afforded oxaziridine **11c** very rapidly and cleanly.[14] Attempted isomerization of the oxaziridine to the nitrone by a literature procedure (40% H_2SO_4 in MeOH, room temperature[15a]) afforded mainly the undesired amide (not shown). However, isomerization of **11c** with *p*-TsOH in MeOH in the dark[15b] gave **6c** in good yield. Although this modified route is somewhat longer than the original one, it proceeds in good overall yield and, in principle, would allow the use of mercury to be avoided.

Evaluation of 2 and Related Compounds. The target compounds **2**, **6b**,**c**, **7a**−**c**, and **8** were tested in the lipid peroxidation assay, and their relative lipophilicity was assessed by a chromatographic method (log k'_w, Table 1).[16] Their IC_{50} values for prevention of

Scheme 3[a]



[a] Reagents: (a) Al/Hg, Et_2O/H_2O; (b) 10% HCl, THF; (c) Oxone, K_2CO_3, tol/H_2O; (d) *p*-TsOH, MeOH.

Table 2. Acute Toxicity of Test Compounds at High Doses

compd	200 mg/kg (deaths/5 mice)[a]	400 mg/kg (deaths/5 mice)[a]
PBN	1[b]	4[b]
1	0	5
2	0	0
6b	0	0
6c	0	0
7a	0	1
7b	0	4
7c	0	5
8	0	0

[a] Compounds were administered intravenously. [b] All of these animals suffered convulsions.

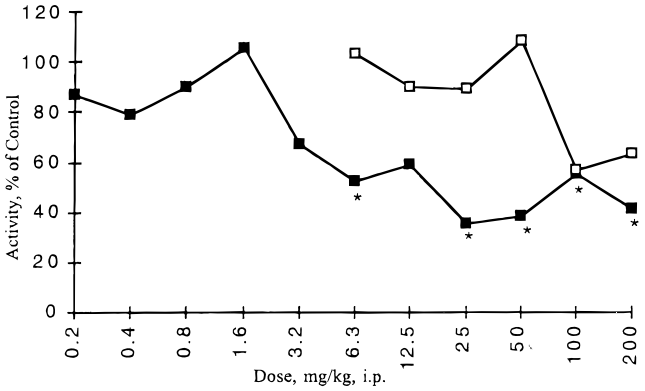


Figure 3. Effect of **1** and **2** on spontaneous locomotor activity in rats. Open squares = **2**, closed squares = **1**. For **1**, $n = 24$ for the control group and $n = 6$ for each dose group except 6.3 mg/kg ($n = 12$). For **2**, $n = 12$ for the control group and $n = 6$ for each dose group. Data points exhibiting significant differences from control ($p < 0.05$, ANOVA, followed by Dunnett's multiple comparison analysis) are noted with an asterisk. See the Experimental Section for a description of the experiment and the Supporting Information for raw data with SEM.

oxidation of neuronal lipids were determined,[17] and their ability to maintain viability of neurons subjected to oxidative stress *in vitro* was measured (Table 1). In addition, a crude assessment of the toxicity of the compounds was obtained by determining survival rates of mice dosed intravenously at either 200 or 400 mg/kg body weight (Table 2). Finally, the sedative effects of compounds **1** and **2** were measured (Figure 3), and the brain penetrability of **1**, **2**, and the ketone analog **7a** were quantitated (Table 3).[18]

The data in Table 1 (with values for PBN and **1** included for comparison) show the expected trend that increased lipophilicity generally correlates with improved *in vitro* antioxidant activity. The metabolite **2**, the most polar compound, is less active than the parent compound **1**, but is still more than twice as active as the acyclic nitrone PBN. Also, the prediction that the keto analogs would be more potent free radical traps than the corresponding methylene or hydroxy compounds is apparently correct. Compare, for example, compounds **1** and **7a** in Table 1. On the basis solely of the lipophilicity/activity correlation, the ketone **7a** ought

Table 3. Comparative *in Vitro* Activity and Brain Penetrability of PBN, **1**, the Major Metabolite **2**, and Ketone **7a**

compd	inhib of lipid peroxidation[a] IC_{50} (μM)	brain levels[b] time, min	brain levels[b] μg/g wet wt
PBN	14300	30	51 ± 3[c]
		60	38 ± 5[c]
1	1670	30	109 ± 34
		60	77 ± 21
2	5500	30	142 ± 6
		60	127 ± 5
7a	940	30	103 ± 22
		60	67 ± 7

[a] Liposomes were prepared from soybean phosphatidylcholine. Oxidation was initiated and quantitated as described previously (ref 17). [b] Rats were dosed with **1**, **2**, or **7a** at 200 mg/kg body weight (ip) and then sacrificed at the indicated time points. The brains were removed, homogenized, and extracted with $CHCl_3$/MeOH. Concentrations of **1**, **2**, and **7a**, respectively, were then determined by HPLC analysis using authentic samples of the compounds as internal standards. Values represent average ± SEM, $n = 5$. [c] These values are approximate and were taken from the literature. See ref 18.

to be less active *in vitro* than **1**, instead it is more so. Likewise, ketone **7b** has a log k'_w value comparable to that of **1**, yet is 10-fold more potent. Among the ketones, however, the lipophilicity/activity correlation holds (see compounds **7a**−**c**).

When the compounds were tested in a whole cell assay for their ability to protect cultured cerebellar granule cell neurons from radical-induced oxidation, the spirocyclic ketones (**7b** and **7c**) were substantially more effective than **1** and one of the alcohols (**6c**) was comparable (Table 1). The data for the cell-based assays were obtained in two separate experiments run approximately 6 months apart. Although we do not fully understand the rather large difference in the IC_{50} values for **1** in the two experiments, these are primary cultures, and changes in the diet of the animals, seasonal variation, etc. can lead to anomalous results in studies of this type. Within each experiment, however, the IC_{50} values were derived from several concentrations of nitrone run in triplicate, and very good reproducibility was observed. Compound **1** was included as an internal standard in each experiment so that valid comparative data could be obtained.

It is readily apparent that the majority of the IC_{50} values for antioxidant activity agree well with those for maintenance of cell viability. Thus, the inhibition of lipid oxidation by the cyclic nitrones translates to neuroprotection in this *in vitro* model of oxidative injury. The low lipophilicity of **2** would be expected to result in poor cellular uptake, so it is perhaps not surprising that this compound is the least effective in this assay.

As stated previously, a major goal of this study was to find compounds with improved side effect profiles (relative to **1**) and the ability to enter the brain. The results of an acute toxicity study, given in Table 2, show that alcohols **2** and **6b**,**c**, and acetate **8** are indeed much less toxic than **1**. No deaths occurred when these

compounds were given to mice at 400 mg/kg (iv), a dose at which **1** is always lethal. The ketone analogs **7**, however, display acute toxicity that seems to be correlated with lipophilicity. The most polar ketone, dimethyl analog **7a**, does show significantly reduced acute toxicity at the highest dose, but the more lipophilic spirocyclopentane (**7b**) is only marginally less lethal than **1** and the most lipophilic ketone (**7c**) is indistinguishable from **1** in this assay.

The compounds were also evaluated for their tendency to cause sedation. Qualitative observations from several *in vivo* experiments suggested that alcohols **2** and **6b**,**c** and acetate **8** were essentially nonsedating (though some sluggishness was observed at the very highest doses, e.g. 400 mg/kg), whereas the ketones **7a**−**c** caused significant sedation, but of a different nature than that observed with **1**. With the ketones, the onset of sedation is slower, the duration is longer, and the intensity is somewhat diminished. Only the metabolite **2** had its sedative effect measured quantitatively (inhibition of locomotor activity) in a head-to-head comparison with **1**. The results are shown in Figure 3. Linear regression analysis of these data yielded an ED_{50} value of 13.9 mg/kg for **1**. This value falls within the range of doses which elicited activity statistically different from that of the control. None of the data points obtained for **2** were statistically different from those of the control, and the ED_{50} for **2** was therefore estimated to be >200 mg/kg. Though less impressive than the qualitative observations, this single experiment nevertheless demonstrates that the metabolite **2** is, at the very least, a substantially less potent sedative than **1**, in agreement with the original hypothesis. It should be noted that the protocol (see the Experimental Section) for this experiment did not allow measurements to be taken during the peak period of sedation observed with **1**. Hence, the disparity between the sedative properties of **1** and **2** may be greater than that indicated by Figure 3.

Finally, the data shown in Table 3 suggest that the diminished toxicity and sedation exhibited by **2** is not due to poor brain penetration, since brain levels of the metabolite were equal to, or higher than, those of the parent at both time points tested. At least one of the ketone analogs, **7a**, also seems to enter the brain readily. At this time we do not have a convincing explanation for the dramatic differences in sedative properties and lethality among these series of compounds.

Conclusions

A hydroxylated metabolite of the previously described cyclic nitrone free radical trap **1** was isolated, identified, synthesized, and tested for its ability to inhibit lipid peroxidation. The metabolite (**2**), though less potent in this assay, showed greatly diminished toxic and sedative effects. Several analogs of **2** were prepared in attempts to increase the potency while retaining the desirable side effect profile. All of the compounds were more lipophilic (log k'_w) and more active *in vitro* than **2**. The ketone analogs, as predicted, were much more potent than **2** *in vitro* (up to *ca.* 200-fold), but showed relatively little improvement over **1** with respect to side effects. The two spirocyclohexyl compounds, alcohol **6c** and ketone **7c**, are undergoing further evaluation to see if they are neuroprotective in an animal model of stroke and, if so, to determine whether an acceptable therapeutic index has been obtained. The former compound has an activity profile similar to that of **1**, but with sharply reduced side effects, whereas the latter, though it causes sedation, is much more potent than **1**. The results of these studies will be reported in due course.

Experimental Section

General. General experimental methods, including determination of log k'_w values and biochemical methods, were performed as described in the preceding paper in this issue.

Protection of Cerebellar Granule Cells against Oxidative Damage. Cerebellar granule cell cultures were prepared from 8-day-old rats as previously described.[19] Briefly, 8−10 cerebella were removed and placed in a Krebs-Ringer bicarbonate medium supplemented with BSA and $MgSO_4$. Cerebella were finely chopped and digested with a trypsin/Krebs-Ringer solution. Cells were then dispersed by trituration in Krebs-Ringer containing DNase, $MgSO_4$, and trypsin inhibitor followed by plating at a density of 1×10^6 cells/well in poly-L-lysine-coated 12-well dishes in MEM with 10% fetal bovine serum/KCl/glutamine/gentamicin. Medium was replaced at 24 h and cytosine arabinoside added. Experiments were conducted with cells 8−10 days *in vitro*.

For oxidation studies the medium was removed and replaced with Na^+-free Locke's solution (154.6 mM *N*-methyl-D-glucamine, 5.6 mM KCl, 2.3 mM $CaCl_2$, 1 mM $MgCl_2$, 3.6 mM $NaHCO_3$, and 5 mM HEPES, pH 7.3) with the omission of glucose. The nitrones were added in either Locke's solution or 20% β-cyclodextrins and allowed to incorporate into the cells for 30 min. At this time, 20 μL of a 5 mM stock solution of ferrous chloride was added for a final concentration of 100 μM. After 45 min, the medium was removed and added to 1.5 mL of TBA/TCA (2:1) with 25 μL of 2% BHT, and thiobarbituric acid reactive substances (TBARS) were determined as described previously.[17] The absorbance of the control cells (no iron) was subtracted from that of the iron-treated cells, and this value was taken as the denominator to determine the concentration of nitrone required to inhibit oxidation by 50%.

After removal of the Locke's, fresh MEM media was added to the cells along with 100 μL of MTT. After 4 h, 1 mL of cold 2-propanol/0.04 N HCl was added, and the cells were scraped, mixed well, and transferred to 13 × 100 mm glass test tubes. The absorbance resulting from mitochondrial reduction of the MTT (570 nm minus 630 nm) was measured as an assessment of viability. The percent cell death was determined by comparison to the absorbance of cells to which no iron had been added. The difference between the iron-treated and control cells was taken as 100% in order to calculate the IC_{50}.

Inhibition of Spontaneous Locomotor Activity in Rats. Adult male CD rats (125−150 g) were placed in a colony room which was on a 14:10 light/dark cycle (lights on at 6:00 a.m.) and maintained at 23−26 °C. The animals were housed four per cage with free access to food and water. All animals were acclimated for at least 1 week from the date of receipt before beginning the experiments.

Compounds **1** and **2** were prepared as suspensions in distilled water containing 1% Tween 80 and given intraperitoneally (ip).

The apparatus used to measure locomotor activity was a photocell-based Optivarimex "Autotrack" system (Columbus Instruments, Columbus, OH). The measure of forward locomotion used was the "distance traveled" (DT) parameter as defined by the Autotrack software. Four beam breaks were designated as the criterion for registering as one count of forward locomotion. Rats were injected ip with various doses of **1** and **2** and placed singly into one of eight clear Plexiglas boxes (16 × 16 × 8 in.) that were placed on laboratory benchtops and allowed to acclimatize for 30 min. Assessments of general depressant potential, as measured by a reduction of spontaneous locomotor activity, were made as follows: At the end of the 30 min acclimation period, each rat in a Plexiglas box was placed into the test chamber and tested for 30 min. ED_{50} values were then obtained using linear regression on the cumulative DT scores.[20] The raw data for this experiment are in the Supporting Information.

Isolation of Compounds 2 and 3 from Rat Serum. Ten male Sprague−Dawley rats (400−410 g) were dosed with **1** (75 mg/kg, intraperitoneally) at time 0 and 1 h. At time 3.5 h, the animals were anesthetized with CO_2, and the blood was withdrawn by cardiac puncture and allowed to clot on wet ice. The serum was collected and extracted with 2 volumes of $CHCl_3$/MeOH (2:1). After evaporation of the solvent, the residue was reconstituted in 300 μL of 5% MeOH/H_2O and purified by reversed phase HPLC (CH_3CN/H_2O gradient), whereupon three components were collected from multiple injections. The last compound to elute was identified as unchanged **1** (retention time 9.3 min, 4 mg) by comparison with an authentic sample. The earlier eluting compounds were assigned structures **2** (retention time 4.9 min, 3 mg) and **3** (retention time 6.5 min, 0.3 mg) on the basis of the following spectral data. ^{13}C multiplicities were determined from APT or DEPT spectra, and one-bond proton−carbon couplings ($^1J_{C,H}$) were estimated by examining ^{13}C satellites in the ^1H NMR spectra. All other spectral data are reported according to the specifications described in the preceding paper in this issue.

Compound **2**: ^1H NMR ($CDCl_3$, *ca.* 2 mg/mL)[12] 7.95 (br s, 1, $^1J_{C,H}$ = *ca.* 180), 7.49 (br d, 1, J = 7.5), 7.46−7.38 (AB m, 2), 7.24 (br d, 1, J = 7.5) 4.64 (br s, 1, $^1J_{C,H}$ = *ca.* 145), 1.57 (s, 3), 1.41 (s, 3) (O*H* not observed); ^{13}C NMR ($CDCl_3$) 132.6 (C), 130.8 (CH), 129.4 (CH), 127.5 (CH), 126.2 (CH), 74.9 (CH), 71.5 (C), 23.2 (CH_3), 19.0 (CH_3) (only five of the expected seven resonances in the olefinic region were clearly detectable, presumably due to line broadening caused by traces of DCl present in the solvent); IR ($CDCl_3$) 3599, 3402 (br), 1548, 1172, 1033; MS (EI, 70 eV) *m*/*z* 191 (M^+, base peak), 174, 145, 130.

Compound **3**: ^1H NMR ($CDCl_3$, *ca.* 0.2 mg/mL) 8.08 (d, 1, J = 7.5), 7.60 (dd, 1, J = 7.5, 7.5), 7.51 (d, 1, J = 7.5), 7.47 (dd, 1, J = 7.5, 7.5), 5.93 (exchangeable, br s, 1), 4.58 (br s, 1, $^1J_{C,H}$ = *ca.* 145), 1.33 (s, 3), 1.31 (s, 3); IR ($CDCl_3$) 3400 (br), 1664; MS (EI, 70 eV) *m*/*z* 191 (M^+), 175, 153, 105, 77 (base peak).

1-Methoxy-3-(1-methyl-1-nitroethyl)-1,3-dihydroisobenzofuran (4a). Sodium metal (12.4 g, 0.539 mol) was added to methanol (MeOH, 1 L) at 10 °C over 90 min. When a homogeneous solution was obtained, the cold water bath was removed and 2-nitropropane (256 mL, 2.85 mol) was added, followed by *o*-phthalaldehyde (120 g, 0.895 mol). The resulting solution was stirred at room temperature overnight. The solution was brought to pH 2 by adding 1 N H_2SO_4. White solids precipitated. The mixture was filtered, and the filter cake was washed with MeOH and discarded. The filtrate was stirred at room temperature for 3 h and then made basic by adding 3 N NaOH. The solution was concentrated *in vacuo* to remove the MeOH. The resulting aqueous solution was extracted twice with ether. The combined organic layers were washed once with water, dried ($MgSO_4$), and evaporated. Kugelrohr distillation of remaining solvent at 50 °C (2 mmHg) left 195 g (106% of theoretical, 86% purity by GC) of a brown liquid which was used as such in the next step. The ratio of diastereoisomers was 1:1 (^1H NMR). A portion of the crude material was purified by FC (9:1 cyclohexane/EtOAc) to give pure **4a** as a pale yellow oil: ^1H NMR ($CDCl_3$) 7.40−7.35 (m, 3), 7.15−7.10 (m, 1), 6.25 and 5.90 (isomer I, d and dd, 1 total, J = 2.4 and 2.4, 0.6, respectively), 6.01 and 5.72 (isomer II, s and d, 1 total, J = 0.6), 3.58 and 3.37 (isomers I and II, respectively, 2 s, 3 total), 1.57 and 1.56 and 1.55 and 1.48 (4 s, 6 total); ^{13}C NMR ($CDCl_3$) 138.8, 138.5, 137.6, 129.8, 129.7, 129.2, 129.1, 123.4, 122.1, 107.3, 107.0, 90.5, 86.8, 56.1, 54.0, 22.5, 22.1, 21.7, 21.0; IR (film) 1543, 1464, 1398, 1373, 1348, 1113, 1094, 1026, 974, 756; MS (CI/CH_4, 120 eV) *m*/*z* 236 (M − H)$^+$, 219, 206, 191, 175, 159, 149 (base peak), 131, 118, 91, 73. Anal. ($C_{12}H_{15}NO_4$) C, H, N.

***N*-[1-(3-Methoxy-1,3-dihydroisobenzofuran-1-yl)-1-methylethyl]hydroxylamine (5a).** Aluminum amalgam (Al−Hg) was prepared[10] from aluminum foil (Reynolds, 1.29 g, 0.048 mol) and added to ether (100 mL) and water (0.6 mL, 33 mmol) in a three-necked flask. A solution of **4a** (4.8 g, 23.0 mmol) in ether (50 mL) was then added to the stirred mixture from a dropping funnel at a rate to maintain a vigorous reflux. The bubbling which occurred initially subsided within 30 min. The mixture was filtered and the filtrate washed twice with 2 N NaOH, dried ($MgSO_4$), and evaporated to obtain a pale green oil (4.6 g, 88%). Purification by FC (1:1 cyclohexane/EtOAc) gave recovered starting material (0.48 g, 10%), and the hydroxylamine **5a** as a pale green glass (2.24 g, 43%): ^1H NMR ($CDCl_3$) 7.40−7.30 (m, 4), 6.28 and 5.55 (isomer I, 2 d, 1 total, J = 2.4), 6.03 and 5.41 (isomer II, 2 s, 1 total), 3.62 and 3.34 (isomers I and II, respectively, 2 s, 3 total), 1.32 and 0.88 (isomer II, 2 s, 3 total), 1.27 and 0.80 (isomer I, 2 s, 3 total); ^{13}C NMR ($CDCl_3$) for one isomer, 140.1, 138.2, 129.2, 128.1, 123.2, 122.3, 106.51, 85.6, 61.1, 53.2, 20.4, 19.1; for other isomer, 139.7, 138.5, 129.1, 128.0, 123.1, 122.3, 107.0, 60.3, 56.1, 20.9, 19.4; IR ($CHCl_3$) 2980, 2934, 2907, 2891, 1375, 1111, 1092, 1015, 974, 752; MS (CI/CH_4, 120 eV) *m*/*z* 224 (M + H)$^+$, 206, 192, 176, 174, 159, 149, 147, 135, 119 (base peak), 118, 102, 91, 74.

3,3-Dimethyl-3,4-dihydroisoquinolin-4-ol *N*-Oxide (2). To a solution of **5a** (7.1 g, 31.8 mmol) in THF (20 mL) was added 2 N HCl (10 mL), and the resulting solution was stirred for 45 min at room temperature. More 2 N HCl (10 mL) was added, and the solution ws stirred for 30 min. The solution was then slowly poured into a saturated aqueous $NaHCO_3$ solution and extracted five times with EtOAc (at this point, TLC analysis of the aqueous layer showed that some desired product still remained, but it was not recovered). The combined organic layers were dried ($MgSO_4$), filtered, and evaporated to obtain 6.1 g of a yellow oil. Recrystallization from EtOAc/cyclohexane gave 3.45 g (57%) of cream-colored crystals, mp 134−136 °C. A second crop (0.62 g, 10%) was obtained by evaporating the mother liquor and recrystallizing the residue from hexane/CH_2Cl_2, bringing the total yield to 67%: ^1H NMR ($CDCl_3$, *ca.* 20 mg/mL) 7.62 (s, 1, $^1J_{C,H}$ = *ca.* 180), 7.46 (m, 1), 7.38−7.30 (AB m, 2), 7.10 (m, 1), 4.58 (d, 1, J = 6.3, $^1J_{C,H}$ = *ca.* 145, C*H*OH), 3.93 (exchangeable, d, 1, J = 6.3, CHO*H*), 1.47 (s, 3), 1.36 (s, 3); ^{13}C NMR ($CDCl_3$) 132.8 (C), 132.7 (CH), 129.8 (CH), 128.9 (CH), 127.3 (CH), 126.6 (C), 125.2 (CH), 74.7 (CH), 71.6 (C), 23.3 (CH_3), 19.0 (CH_3); IR ($CHCl_3$) 3605, 3403 (br), 1549, 1173, 1031; MS (EI, 70 eV) *m*/*z* 191 (M^+, base peak), 174, 145, 130. Anal. ($C_{11}H_{13}NO_2$) C, H, N.

3,3-Dimethyl-3,4-dihydro-3*H*-isoquinolin-4-one *N*-Oxide (7a). To a solution of **2** (3.22 g, 16.8 mmol) in CH_2Cl_2 (150 mL) was added dimethyl sulfoxide (DMSO, 23.8 mL, 336 mmol). The resulting solution was cooled to −45 °C. Oxalyl chloride ($(COCl)_2$, 11.4 mL, 131 mmol) was added over 10 min, such that the internal temperature remained below −40 °C. The mixture was stirred and maintained between −55 and −40 °C for 2 h. *i*Pr_2NEt (44 mL, 250 mmol) was added over 15 min, such that the internal temperature remained below −50 °C. The reaction mixture was then allowed to warm to room temperature, whereupon it was poured into water and extracted with CH_2Cl_2 (2×). The organic phase was washed with brine, dried ($MgSO_4$), filtered, and evaporated to give a yellow oil. The material was filtered through silica gel (EtOAc) and crystallized from cyclohexane/EtOAc to furnish **7a** as a yellow powder (2.0 g, 63%): ^1H NMR ($CDCl_3$) 8.07 (d, 1, J = 7.8), 7.86 (s, 1), 7.69 (dt, 1, J = 7.6, 1.3), 7.49 (dt, 1, J = 7.6, 1.0), 7.31 (d, 1, J = 7.8), 1.74 (s, 6); IR (KBr) 3048, 2996, 1680, 1601, 1555, 1487, 1377, 1366, 1300, 1281, 1244, 1179, 891, 872, 758, 660; MS (EI, 70 eV) *m*/*z* 191, 189 [(M^+), base peak], 172, 158, 145, 144, 130, 115, 104, 89, 77, 63, 51. Anal. ($C_{11}H_{11}NO_2$) C, H, N.

1-Methoxy-3-(1-nitrocyclopentyl)-1,3-dihydroisobenzofuran (4b). Condensation of nitrocyclopentane (5.00 g, 40.0 mmol) with *o*-phthalaldehyde (3.76 g, 28.0 mmol) in the presence of NaOMe (10 mmol) was carried out as described above for compound **4a**. The resulting pale green oil (7.21 g, 98%) was pure enough for use in the next step. The following data were obtained on a *ca.* 1:1 mixture of the cis and trans diastereoisomers: ^1H NMR ($CDCl_3$) 7.50−7.25 (m, 3), 7.10−7.00 (m, 1), 6.16 and 5.91 (isomer I, 2 d, 1 total, J = 2.3), 5.93 and 5.80 (isomer II, s and d, respectively, 1 total, J = 0.7), 3.49 and 3.30 (isomers I and II, respectively, 2 s, 3 total), 2.50−2.35 (m, 1), 2.30−1.90 (m, 3), 1.75−1.50 (m, 4); MS (CI/CH_4, 120 eV) *m*/*z* 236 (M − H)$^+$, 219, 206, 191, 175, 159, 149 (base peak), 131, 118, 91, 73; ($C_{12}H_{15}NO_2$) C, H, N.

***N*-[1-(3-Methoxy-1,3-dihydroisobenzofuran-1-yl)-1-cyclopentyl]hydroxylamine (5b).** The nitro acetal **4b** from the previous reaction (7.21 g, 27.5 mmol) was reduced with Al−Hg (from 3.31 g of aluminum foil) as described above for

compound **5a**. Purification by FC (1:1 EtOAc/hexane) provided recovered starting material (3.65 g, 35%) and the oily hydroxylamine **5b** (4.14 g, 42%): ^{1}H NMR ($CDCl_3$) 7.40−7.30 (m, 4), 6.28 and 5.55 (isomer I, 2 d, 1 total, J = 2.4), 6.03 and 5.41 (isomer II, 2 s, 1 total), 3.62 and 3.34 (isomers I and II, respectively, 2 s, 3 total), 1.32 and 0.88 (isomer II, 2 s, 3 total), 1.27 and 0.80 (isomer I, 2 s, 3 total); MS (CI/CH_4, 120 eV) *m/z* 250 ($M + H)^+$, 248, 246, 218, 200, 185, 172, 149, 135, 119, 100 (base peak), 84, 67.

3,4-Dihydroisoquinolin-4-ol-3-spirocyclopentane *N*-Oxide (6b). Hydroxylamine **5b** (4.14 g, 16.7 mmol) was converted into nitrone **6b** according to the procedure described above for **2**. The product (1.87 g, 52%) was obtained as a white solid after FC (1:1 EtOAc/hexane, then EtOAc): mp 141−143 °C; ^{1}H NMR ($CDCl_3$) 7.67 (s, 1), 7.45−7.30 (m, 3), 7.15−7.10 (m, 1), 4.52 (d, 1, J = 7.3), 3.98 (d, 1, J = 7.3), 2.70−2.55 (m, 1), 2.15−2.05 (m, 1), 2.00−1.50 (m, 6); ^{13}C NMR ($CDCl_3$) 132.7, 132.5, 129.6, 129.1, 127.8, 127.2, 125.2, 82.2, 74.3, 36.6, 30.5, 26.6, 25.9; IR (KBr) 3397, 3385, 3351, 3196, 3117, 3067, 3000, 2959, 2872, 1595, 1561, 1452, 1397, 1254, 1240, 1171, 1119, 1101, 1063, 1030, 772; MS (EI, 70 eV), *m/z* 218, 217 (M^+), 200 (base peak), 176, 170, 142, 130, 115, 104, 89, 77, 51, 41. Anal. ($C_{13}H_{15}NO_2$) C, H, N.

3,4-Dihydro-3*H*-isoquinolin-4-one-3-spirocyclopentane *N*-Oxide (7b). Compound **6b** (1.09 g, 5.02 mmol) was oxidized with DMSO (1.0 mL, 14.1 mmol), $(COCl)_2$ (0.5 mL, 5.73 mmol), and Et_3N (3.5 mL, 25 mmol) according to the procedure described above for **7a**. The crude product was purified by two crystallizations from hexane/EtOAc to furnish **7b** as a yellow solid (0.65 g, 60%): mp 107−108 °C; ^{1}H NMR ($CDCl_3$) 8.06 (d, 1, J = 7.8), 7.88 (s, 1), 7.68 (t, 1, J = 7.6), 7.47 (t, 1, J = 7.6), 7.30 (d, 1, J = 7.8), 2.55−2.45 (m, 2), 2.35−1.90 (m, 6); ^{13}C NMR ($CDCl_3$) 197.9, 135.6, 132.1, 132.0, 129.6, 127.2, 125.7, 125.0, 86.9, 40.4, 27.8; IR (KBr) 3441, 2976, 2945, 2870, 1682, 1595, 1555, 1485, 1360, 1323, 1281, 1252, 1181, 893, 855, 756, 662; MS (EI, 70 eV) *m/z* 215 (M^+), 198 (base peak), 174, 170, 152, 130, 127, 103, 89, 76, 63, 41. Anal. ($C_{13}H_{13}NO_2$) C, H, N.

1-Methoxy-3-(1-nitrocyclohexyl)-1,3-dihydroisobenzofuran (4c). Nitrocyclohexane (12.92 g, 100 mmol) was condensed with *o*-phthalaldehyde (8.38 g, 60.0 mmol) as described above for compound **4a**. After final drying on a vacuum pump, a yellow oil was obtained (17.29 g, 100%). The following data were obtained on a *ca*. 1:1 mixture of the cis and trans diastereoisomers: ^{1}H NMR ($CDCl_3$) 7.45−7.35 (m, 3), 7.20−7.10 (m, 1), 6.26 and 5.55 (isomer I, 2 d, 1 total, J = 2.7, and 2.3, respectively), 5.98 and 5.38 (isomer II, 2 s, 1 total), 3.59 and 3.36 (isomers I and II, respectively, 2 s, 3 total), 2.59 (m, 2), 2.22 (m, 2), 1.95−1.10 (m, 6).

***N*-[1-(3-Methoxy-1,3-dihydroisobenzofuran-1-yl)-1-cyclohexyl]hydroxylamine (5c).** Nitro acetal **4c** (14.24 g, 51.4 mmol) was reduced with Al−Hg (from 6 g of aluminum foil) as described above for compound **5a**. Purification by FC (1:1 EtOAc/hexane) gave the hydroxylamine **5c** as a pale yellow oil (7.19 g, 53%). The following data were obtained on a *ca*. 1:1 mixture of cis and trans isomers: ^{1}H NMR ($CDCl_3$) 7.45−7.30 (m, 4), 6.28 and 5.55 (isomer I, 2 d, 1 total, J = 2.4), 6.03 and 5.39 (isomer II, 2 s, 1 total), 3.63 and 3.31 (isomers II and I, respectively, 2 s, 3 total), 1.85−1.40 (m, 8), 1.20−1.05 (m, 2); MS (CI/CH_4, 120 eV) *m/z* 264 ($M + H)^+$, 262, 246, 230, 214, 199, 171, 150, 149, 135, 118, 114 (base peak), 96, 84.

3,4-Dihydroisoquinolin-4-ol-3-spirocyclohexane *N*-Oxide (6c). To a solution of **5c** (7.19 g, 27.3 mmol) in THF (100 mL) was added 10% HCl (50 mL), and the resulting solution was stirred for 20 min at room temperature. The solution was then slowly poured into saturated aqueous $NaHCO_3$ solution and extracted three times with EtOAc. The combined organic layers were dried ($MgSO_4$), filtered, and concentrated, whereupon a beige solid precipitated. This was collected and washed with hexane to furnish 3.36 g (53%) of pure product. The filtrate was evaporated, and the residue crystallized from EtOAc/hexane to give a second crop (0.72 g, 11%) of product, bringing the total yield to 64%: mp 195−197 °C; ^{1}H NMR ($CDCl_3$) 7.65 (s, 1), 7.45−7.30 (m, 3), 7.20−7.10 (m, 1), 4.93 (d, 1, J = 7.3), 3.32 (d, 1, J = 7.3), 2.47 (td, 1, J = 16.0, 4.9), 2.25−2.15 (m, 1), 2.00−1.85 (m, 1), 1.80−1.30 (m, 7); ^{13}C NMR ($CDCl_3$) 132.4, 131.6, 129.6, 129.3, 128.7, 126.9, 125.1, 74.2, 69.7, 32.0, 26.2, 25.0, 22.6, 22.1; IR (KBr) 3408, 3073, 3052, 2980, 2938, 2926, 2857, 1593, 1553, 1454, 1414, 1260, 1235, 1179, 1161, 1107, 1049, 1030, 912, 851, 764, 613; MS (CI/CH_4, 120 eV) *m/z* 232 [($M + H)^+$, base peak], 214, 198, 183. Anal. ($C_{14}H_{17}NO_2$) C, H, N.

3,4-Dihydro-3*H*-isoquinolin-4-one-3-spirocyclohexane *N*-Oxide (7c). To a solution of $(COCl)_2$ (0.50 mL, 5.73 mmol) in CH_2Cl_2 (15 mL) at −78 °C was added a solution of DMSO (1 mL, 14.1 mmol) in CH_2Cl_2 (5 mL). The resulting solution was stirred for 5 min at −78 °C. Compound **6c** (1.16 g, 5.00 mmol) was dissolved in warm DMSO, and then the solution was allowed to cool to room temperature. This solution was added to the reagent solution at a rate such that the internal temperature remained below −40 °C. The mixture was stirred and maintained in the −78 °C bath for 15 min and then treated with a solution of Et_3N (3.5 mL, 25 mmol) in CH_2Cl_2 (7 mL) at a rate such that the internal temperature remained below −50 °C. The mixture was stirred and maintained in the −78 °C bath for 15 min and then allowed to warm to room temperature. The reaction mixture was poured into water and extracted with CH_2Cl_2 (2×). The organic phase was washed with brine, dried ($MgSO_4$), filtered, and evaporated. The residue was crystallized from hexane/EtOAc to furnish **7c** as yellow needles (0.85 g, 74%): mp 92−93 °C; ^{1}H NMR ($CDCl_3$) 8.03 (d, 1, J = 7.7), 7.89 (s, 1), 7.66 (t, 1, J = 7.6), 7.48 (t, 1, J = 7.6), 7.28 (d, 1, J = 7.5), 2.55−2.35 (m, 2), 2.15−1.65 (m, 6), 1.60−1.35 (m, 2); ^{13}C NMR ($CDCl_3$) 197.0, 135.3, 132.2, 129.6, 127.2, 125.4, 125.3, 111.1, 80.2, 31.9, 24.0, 21.3; IR (KBr) 3441, 3040, 2942, 2884, 2868, 2845, 1694, 1599, 1553, 1447, 1366, 1319, 1281, 1258, 1182, 1157, 882, 752, 696, 660, 637; MS (EI, 70 eV) *m/z* 229 (M^+), 213, 212 (base peak), 188, 184, 174, 158, 132, 129, 102, 89, 76, 63, 51, 41. Anal. ($C_{14}H_{15}NO_2$) C, H, N.

4-Acetoxy-3,3-dimethyl-3,4-dihydroisoquinoline *N*-Oxide (8). To a solution of **2** (3.3 g, 17 mmol) in CH_2Cl_2 (100 mL) was added Et_3N (3.1 mL, 22 mmol), 4-(dimethylamino)-pyridine (210 mg, 1.7 mmol), and acetic anhydride (1.8 mL, 19 mmol). The mixture was stirred for 1 h at room temperature, then poured into water, and extracted with CH_2Cl_2 (2×). The organic phase was dried ($MgSO_4$), filtered, and evaporated to give a yellow paste. This was purified by FC (9:1 CH_2Cl_2/acetone) to afford 1.83 g of a pale yellow solid. Recrystallization from cyclohexane/EtOAc provided **8** as cream-colored crystals (1.53 g, 38%): ^{1}H NMR ($CDCl_3$) 7.76 (s, 1), 7.45−7.25 (m, 3), 7.20−7.15 (m, 1), 5.89 (s, 1), 2.02 (s, 3), 1.57 (s, 3), 1.36 (s, 3); IR (KBr) 3048, 2986, 2936, 1734, 1593, 1553, 1454, 1375, 1287, 1240, 1211, 1018, 978, 964, 770; MS (EI, 70 eV) *m/z* 233 [(M^+), base peak], 191, 190, 174, 156, 143, 130, 115, 91, 89, 77, 63, 51, 43. Anal. ($C_{13}H_{15}NO_3$) C, H, N.

Alternate Synthesis of 6c. *N*-[1-(3-Methoxy-1,3-dihydroisobenzofuran-1-yl)-1-cyclohexyl]amine (9c).[21] Nitro compound **4c** (34.4 g, 124 mmol) was reduced with Al−Hg (from 7.6 g of Al foil, 0.283 mol) as described above for compound **5a**. The crude product was purified by FC (1:1 hexane/EtOAc) to give recovered **4c** (5.9 g, 17%) and amine **9c** (21.5 g, 70%) as a mixture of diastereoisomers: ^{1}H NMR ($CDCl_3$) 7.40−7.30 (m, 4), 6.24 and 5.10 (isomer I, 2 d, 1 total, J = 2.2), 5.94 and 5.02 (isomer II, 2 s, 1 total), 3.58 and 3.31 (isomers II and I, respectively, 2 s, 3 total), 1.62−1.18 (m, 10); IR ($CHCl_3$) 3691, 3597, 3080−2860, 1601, 1508, 1451, 1376, 1087, 1011; ^{13}C NMR ($CDCl_3$) 139.7, 139.3, 138.9, 138.7, 128.8, 128.7, 127.9, 127.9, 123.0, 122.9, 106.6, 106.3, 91.1, 90.7, 54.8, 53.8, 53.1, 35.0, 33.7, 33.5, 33.4, 25.9, 25.8, 21.4, 21.3, 21.2, 21.1.

3,4-Dihydroisoquinolin-4-ol-3-spirocyclohexane (10c). Amine **9c** (5.10 g, 20.6 mmol) was dissolved in THF (70 mL) and treated with 10% HCl. The mixture was stirred overnight at room temperature, then poured into saturated $NaHCO_3$ solution, and extracted with EtOAc (3×). The organic phase was dried ($MgSO_4$), filtered, and concentrated to give 4.31 g (97%) of imine **10c** as a white solid: mp 120−123 °C (EtOAc/hexanes); ^{1}H NMR ($CDCl_3$) 8.12 (s, 1), 7.40−7.20 (m, 4), 4.41 (s, 3), 4.11 (br s, 1), 1.85−1.25 (m, 10); ^{13}C NMR ($CDCl_3$) 157.2, 137.0, 131.4, 128.3, 127.7, 127.0, 126.3, 70.5, 60.5, 33.4, 31.1, 25.6, 21.7, 21.3; IR ($CHCl_3$) 3300, 3076−2859, 2209, 1628, 1577, 1454, 1376, 1218, 1013.

Conversion of Imine 10c into Nitrone 6c. The imine **10c** (10.13 g, 47.0 mmol) from the previous reaction was dissolved in warm toluene (300 mL), and an aqueous solution of K_2CO_3 (52.1 g, 377 mmol, in 250 mL of H_2O) was added. To the resulting vigorously stirred mixture was added dropwise a solution of Oxone (34.7 g, 56.5 mmol) in H_2O (250 mL). After the addition was complete, the mixture was stirred for 15 min and then the layers were separated. The aqueous phase was extracted with EtOAc, and the combined organic phase was washed with 10% Na_2SO_3 solution, dried ($MgSO_4$), filtered, and evaporated to furnish the intermediate oxaziridine **11c** (11.1 g, quant) as a semisolid containing a trace of toluene. This material was dissolved in MeOH (500 mL) and treated with a catalytic amount of *p*-toluenesulfonic acid (2.25 g, 11.82 mmol). The mixture was stirred at room temperature in the dark for 1.5 days. The reaction mixture was then poured into saturated $NaHCO_3$ solution and extracted first with CH_2Cl_2 (4×) and then with 50% MeOH/CH_2Cl_2 (3×). The extracts were combined, dried ($MgSO_4$), filtered, and evaporated. The resulting solid was dissolved in MeOH and diluted with EtOAc to induce crystallization. Two crops of yellowish crystals (**6c**) were collected to give a total yield of 7.42 g (68%) from **10c**. The spectral data for this material matched those given above for **6c**.

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Supporting Information Available: Chiral HPLC chromatograms for isolated and synthetic **2**, additional ^{1}H NMR data for synthetic **2**, the ^{1}H NMR spectrum of isolated **2**, and raw data for Figure 3 (10 pages). Ordering information is given on any current masthead page.

References

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(11) Treatment of **4a** with Zn/HOAc, known conditions for *intermolecular* reactions of aldehydes with nitro compounds to give nitrones (Hinton, R. D.; Janzen, E. G. Synthesis and Characterization of Phenyl-Substituted C-Phenyl-*N*-*tert*-butylnitrones and some of Their Radical Adducts. *J. Org. Chem*. **1992**, *57*, 2646−2651), failed to afford **2**.

(12) A reviewer expressed concern about the discrepancies between the NMR chemical shifts reported for the isolated metabolite **2** and synthetic **2** (see the Experimental Section). We note that ^{1}H NMR spectra of synthetic **2** obtained in $CDCl_3$ at different concentrations and/or using different batches of solvent show significant variations in chemical shifts and line widths, particularly in the aromatic/olefinic region. These variations are presumably caused by small amounts of acidic impurities, specifically DCl, which is typically present in this solvent unless removed immediately prior to use. This is illustrated by a series of spectra of synthetic **2** in $CDCl_3$ under different conditions. These spectra, and a spectrum of the isolated metabolite **2** for comparison, are included in the Supporting Information. Similar variations have also been observed for the parent compound **1**. This is in fact a very common phenomenon that can be observed for many compounds containing basic functionality which can be protonated and can engage in intra- or intermolecular tautomeric equilibria.

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