Chemical and Biological Studies of 1-(2,5-Dihydroxy-4-methylphenyl)-2-aminopropane, an Analogue of 6-Hydroxydopamine

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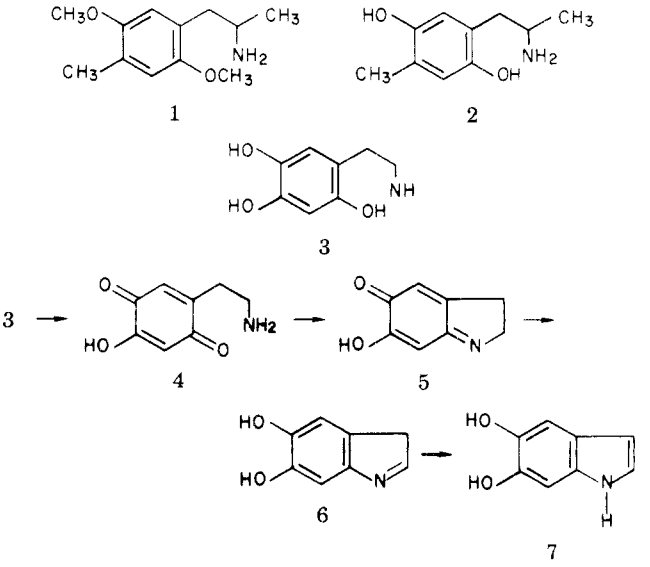
Autoxidation of the bis(*O*-demethyl)-*p*-hydroquinone metabolite of the psychotomimetic amine 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) at pH 7.4 leads exclusively to a bicyclic imino quinone. This imino quinone is a good alkylating agent, forming covalent adducts via 1,4 addition to thiols. The autoxidation appears to be dependent on trace metal catalysis and is dramatically inhibited by components of the 10000*g* supernatant fraction of rabbit liver homogenates. Incubation of tritium-labeled hydroquinone with bovine serum albumin under oxidizing conditions leads to significant amounts of nonextractable radioactivity which presumably is dependent on imino quinone alkylation of nucleophilic functionalities present on macromolecules. Incubation of tritium-labeled DOM with rabbit liver microsomes in the presence of NADPH leads to irreversible binding of the label to macromolecular components of the microsomes. Since this binding is NADPH dependent, it is likely that metabolic conversion of DOM to the hydroquinone is involved. The imino quinone oxidation product is highly lypophilic and is capable of crossing the blood–brain barrier. Intravenous administration of tritium-labeled imino quinone to rats resulted in significant nonextractable radioactivity in brain tissue. These properties of the hydroquinone metabolite parallel those reported for the structurally related sympatholytic compound 6-hydroxydopamine and have led to the hypothesis that the psychotomimetic properties of DOM may be mediated through 6-hydroxydopamine-type interactions of the hydroquinone with important macromolecules in the brain.

Studies on the metabolic fate of the potent psychotomimetic amine 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (**1**, DOM)[1] have led to the characterization of several oxidation products,[2,3] including 1-(2,5-dihydroxy-4-methylphenyl)-2-aminopropane (**2**),[3] a structural analogue of the highly selective sympatholytic agent 6-hydroxydopamine (**3**).[4] Although the precise molecular mechanisms underlying the neurodegenerative properties of 6-hydroxydopamine are not well established, it is generally accepted that the hydroquinone functionality is oxidized to the electrophilic quinone **4** (Scheme I), which then undergoes covalent bond formation with macromolecules.[5] Consistent with this mechanism, administration of radiotagged 6-hydroxydopamine to rats leads to extensive and irreversible binding of the label to spleen and heart tissues.[6] Similar binding to protein is observed in incubates of tritiated **3** with bovine serum albumin (BSA).[7] In the absence of such intermolecular reactions, **4** undergoes cyclization to the imino quinone **5**, which rearranges via indolenine **6** to 5,6-dihydroxyindole (**7**).[8] The catecholamine *O*-methyltransferase inhibitory properties of 6-hydroxydopamine are thought to be associated with cyclized products.[9]

In addition to its neurodegenerative and enzyme inhibitor properties, 6-hydroxydopamine causes significant behavioral changes in experimental animals.[10] Stein and Wise have speculated that mental disorders may be associated with the aberrant formation of 6-hydroxydopamine from dopamine.[11] Evidence supporting the "6-hydroxylation" of dopamine by both enzymatic and spontaneous chemical processes has been reported.[12] As a structural analogue of 6-hydroxydopamine, we expected that the hydroquinone metabolite **2** of DOM would display properties similar to those of 6-hydroxydopamine, in which case it would be reasonable to speculate that the psychotomimetic activity of DOM may be mediated through the covalent interactions of the oxidation product(s) of **2** with key macromolecules.

Previous work in our laboratory has established that the *p*-hydroquinone **2** undergoes a sequence of reactions which parallels the conversion of 6-hydroxydopamine to 5,6-dihydroxyindole.[13] Additionally, interstitial administration of hydroquinone **2** into the caudate-putamen nucleus of rats leads to extensive neuronal deterioration, completely analogous to that observed with 6-hydroxydopamine.[14] In order to evaluate the potential pharmacological significance of the 6-hydroxydopamine-like DOM metabolite **2**, we have undertaken further studies on its chemical and biological properties. The present paper describes the characterization of the quinone and imino quinone intermediates resulting from the oxidation of the hydroquinone metabolite **2** and the reactions of these intermediates with nucleophilic reagents. In addition, preliminary results on the interaction of these oxidation products with biological macromolecules are presented.

Scheme I



Results

Characterization of Hydroquinone Metabolite Oxidation Products. Previously, we reported that the hydroquinone metabolite **2** is rapidly oxidized in the presence of air at pH 7.4.[13] On the basis of UV spectral and cyclic voltammometric data, we speculated that the initial oxidation product observed spectrophotometrically (λ_{max} 281 nm, ϵ 27 000) was composed of an equilibrium

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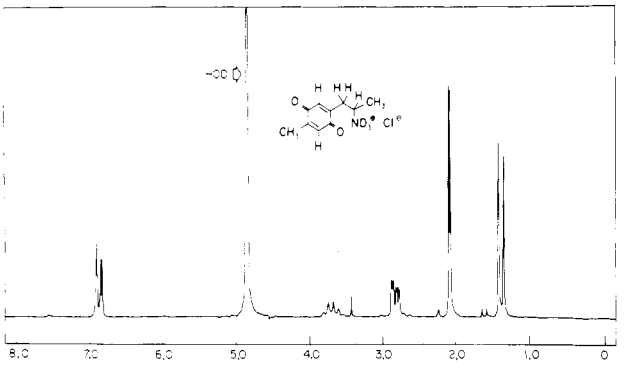


Figure 1. 100-MHz NMR spectrum of quinone 8 in D_2O containing 0.1 N DCl.

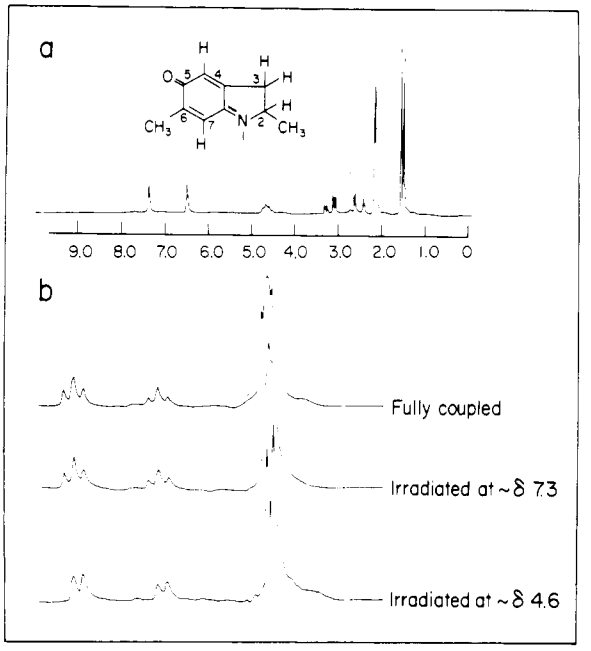


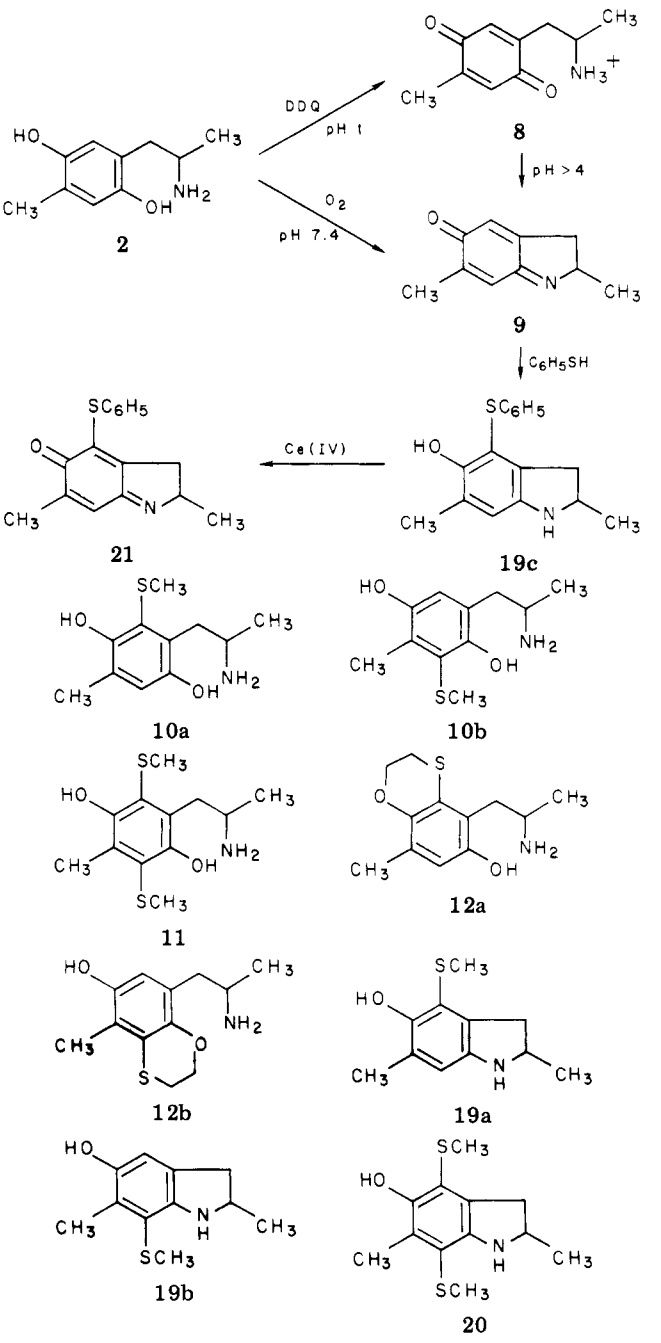
Figure 2. 100-MHz NMR spectrum of imino quinone 9.

mixture of quinone 8 and the bicyclic imino quinone 9. We now have demonstrated that this oxidation product exists entirely in the cyclized form 9 at pH 7.4; the open-chain quinone 8, however, can be generated in solution by oxidation at low pH (Scheme II).

The reaction of hydroquinone 2 with DDQ in aqueous HCl (pH 1) produced a yellow solution (λ_{max} 256 nm, ϵ 16 800), consistent with oxidation to the open-chain quinone 8. The 100-MHz NMR spectrum (Figure 1) of this species generated in D_2O also is consistent with this structure, although all attempts to isolate and purify 8 have failed.

The UV spectrum of quinone 8 is stable for several hours at pH 1, but a dramatic shift in the chromophore occurs when the pH of the solution is raised to >4. The peak at 256 nm diminishes and is replaced by a new absorbance at 281 nm, ϵ 27 000, essentially the same spectrum obtained by autoxidation of 2 at pH 7.4. Extraction with methylene chloride followed by evaporation of the extract provided an orange solid which sublimed readily in vacuo to give yellow crystals, mp 68–70 °C. The 100-MHz NMR spectrum (Figure 2) of this material possesses some interesting features and conclusively proves the structure to be the cyclic imino quinone 9. In contrast to the spectrum of open-chain quinone 8, the vinylic resonances are well

Scheme II



resolved, appearing at δ 6.44 and 7.30. The most definitive evidence for the structure, however, comes from the resonances assigned to the methylene protons of the five-membered ring. These protons are chemically nonequivalent (cis and trans to the methyl group). Each proton, therefore, should be split into a maximum of eight lines (geminal, vicinal, and allylic coupling). Indeed, one of these protons (centered at δ 3.16) displayed all of the eight theoretically possible lines. The other signal (centered at δ 2.46) gave a six-line pattern, the result of two sets of overlapping pairs of doublets.

A novel feature of the spectrum of compound 9 is the presence of a long-range (seven bond) coupling between the methyl group at C-6 and the methine proton on C-2. The methyl resonance centered at δ 2.12 is an apparent triplet, whereas on the basis of simple allylic coupling one would have predicted a doublet. A homonuclear spin-decoupling experiment revealed the nature of this coupling and provided the assignment of the vinylic resonances.

Table I. Inhibition of the Autoxidation of Hydroquinone 2

incubation medium	μg of protein/mL	$t_{1/2}$ values, min
buffer	0	10
metal-free buffer	0	180
10000*g* liver homogenate	13	70
	90	*b*
liver microsomes	90	100

[a] All incubations were carried out at pH 7.4 in phosphate buffer. In all cases, the concentration of hydroquinone was 3.70×10^{-5} M. [b] No oxidation after 15 min.

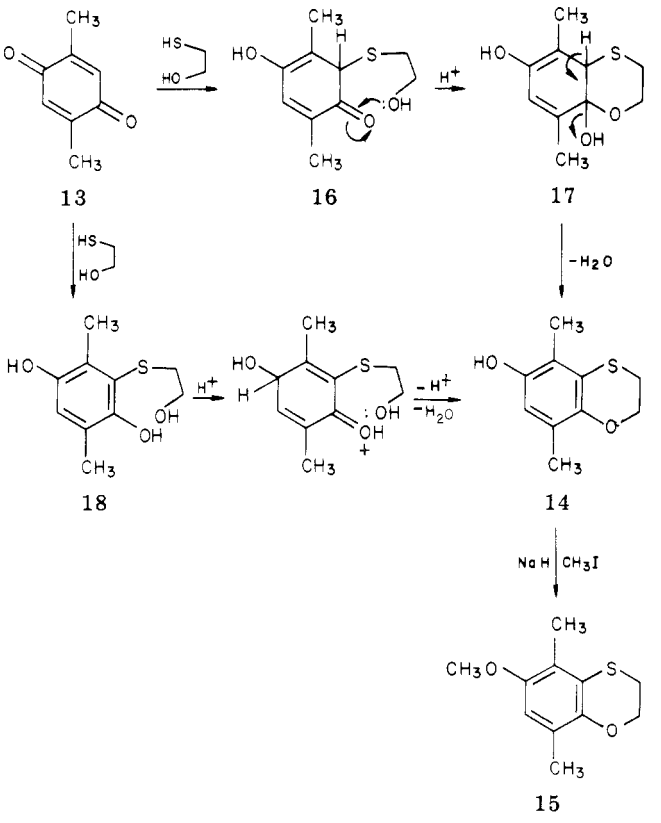
Irradiation at δ 4.6 caused the apparent triplet to collapse to a clean doublet (J = 0.7 Hz), as well as causing the six-line methylene absorbance at δ 2.46 to collapse to a four-line pattern (Figure 2). Irradiation at δ 7.3 caused the methyl "triplet" to collapse to a poorly resolved doublet. Consequently, the low-field vinylic resonance must be due to the proton adjacent to the methyl group.

Inhibition of Autoxidation of Hydroquinone 2. We have examined the rate of oxidation of the hydroquinone **2** at pH 7.4 by monitoring the increase in absorbtivity at 281 nm. A number of factors appear to influence this rate dramatically. When "tap distilled water" is used to prepare the buffer, the $t_{1/2}$ is approximately 10 min. However, when the buffer is first passed through a chelex resin to remove divalent metals, the $t_{1/2}$ for the oxidation increases to ca. 180 min. We have also found that addition of trace amounts of Cu^{2+} catalyzes the reaction, an effect that is well documented[15] for analogous oxidations. Since no particular precautions were taken with regard to trace metals in our metabolic studies, the isolation of the hydroquinone suggested that a substance or substances present in the liver homogenate incubation were protecting the hydroquinone from air oxidation. This suspicion was documented by examining the rate of oxidation of hydroquinone **2** in the presence of varying amounts of the 10000*g* supernatant fraction. The results are summarized in Table I in terms of $t_{1/2}$ values for the autoxidation of **2** in the presence of varying amounts of the 10000*g* rabbit liver supernatant fraction expressed as milligrams of protein. The precise nature of the inhibitory substance(s) is not known. The 100000*g* (microsomal) fraction also displayed an inhibitory capability, although this fraction was less active than the 10000*g* fraction (Table I). Further studies are in progress to characterize the inhibitory factor(s) responsible for the inhibition of the oxidation of **2**. It is intriguing to speculate that a substance such as superoxide dismutase[16] may provide a natural defense mechanism to protect the organism against the formation of potentially toxic species derived from xenobiotics such as the quinone **8** and imino quinone **9**. Superoxide dismutase has been shown to inhibit the autoxidation of 6-hydroxydopamine.[17]

Although components of liver preparations inhibit the air oxidation of hydroquinone **2** in vitro, we felt that under conditions of oxidative metabolism further oxidation of **2** to electrophilic species might occur. Therefore, we have examined the electrophilic properties of the oxidation products of **2** through their reactions with model nucleophiles.

Reactions of Quinone 8 with Nucleophiles. Addition of excess cysteine to a solution of quinone **8** (prepared as described above) in aqueous HCl caused a rapid discharge of the yellow color and loss of the UV chromophore at 256 nm. Unfortunately, attempts to characterize the product

Scheme III



of this reaction were unsuccessful, yielding only dark, intractable materials. Consequently, we turned our attention to simpler model nucleophiles in hopes of obtaining a characterizable adduct.

Excess methyl mercaptan was added to an aqueous solution of quinone **8** in aqueous HCl at 0 °C. The initially yellow solution turned deep orange upon addition of the thiol and then gradually faded to light yellow over a period of 1 h. Workup after 2 h provided a white solid, mp 220–222 °C. Chemical-ionization mass spectral (CIMS, MH^+ 228) and NMR analysis were consistant with the formation of a monoadduct (**10a** or **10b**). The reaction was then repeated in the same manner, except that the reaction time was increased to 18 h. In this case, a different product (mp 268–270 °C) was isolated. The CIMS of this material indicated formation of the bisadduct **11**. Apparently, the initially formed monoadduct(s) can undergo in situ oxidation to substituted quinone(s), which then add a second mole of thiol.

We have also investigated the reaction of quinone **8** with 2-mercaptoethanol. However, this reaction took an entirely unexpected course. Instead of forming a simple 1,4 adduct with the thiol group as observed with methyl mercaptan, the product isolated was a bicyclic compound, either **12a** or **12b**. An analogous reaction was found to occur with the model compound 2,5-dimethyl-1,4-benzoquinone (**13**) and 2-mercaptoethanol. The resulting heterocycle **14** was thoroughly characterized by its spectral properties and by conversion to the methoxy derivative **15**. We have not investigated the mechanism of this unusual cyclization reaction, but two possibilities have come to mind (Scheme III): (1) cyclization of the initial 1,4-adduct **16** to hemiketal **17**, followed by dehydration, or (2) formation of the "normal" adduct **18** by 1,4 addition and tautomerization, followed by acid-catalyzed cyclization and dehydration. Regardless of the mechanism, this reaction may find utility for the synthesis of benzo-1,4-oxathianes.

Reaction of Imino Quinone 9 with Nucleophiles.

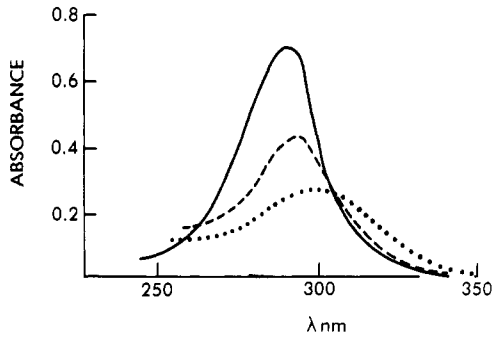


Figure 3. Changes in the ultraviolet spectrum of imino quinone **9** on reaction with glutathione: (—) spectrum of imino quinone alone; (- - -) 30 min after addition of glutathione; (···) 2 h after addition of glutathione.

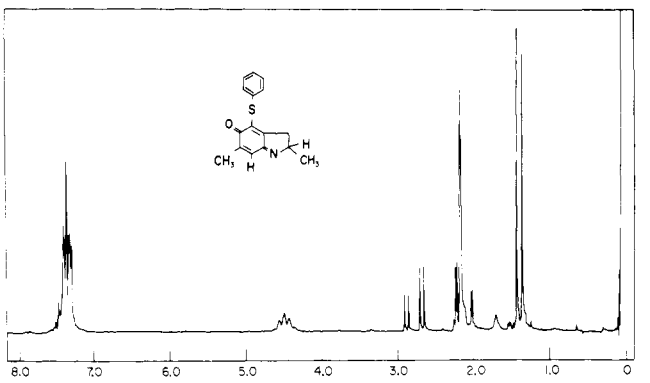


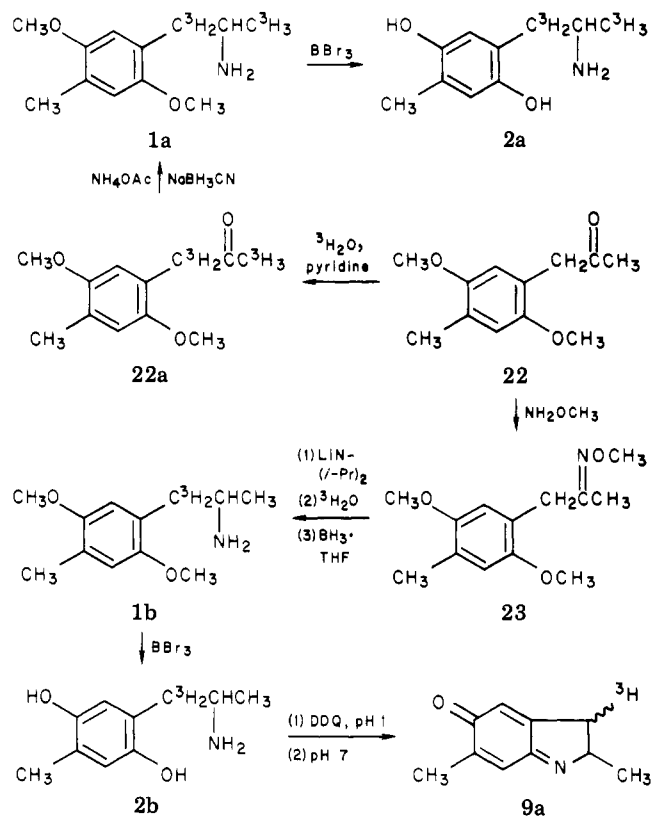
Figure 4. 100-MHz NMR spectrum of imino quinone **21**.

The susceptibility of benzoquinones to nucleophilic attack is well documented.[18] However, the corresponding reactions with imino quinones such as **9** have not been reported. According to the evidence presented above, in vivo oxidation of hydroquinone **2** would lead to the quinone **8** as a transient intermediate only; interactions with nucleophilic functionalities on macromolecules, therefore, are likely to involve the imino quinone **9**. Consequently, we examined the reactivity of **9** with several amino acids and glutathione as model nucleophiles. The reactions were carried out by adding the appropriate nucleophile to a solution of **9** in phosphate buffer (pH 7.4) at 37 °C and were monitored by measuring the decrease in the 281-nm imino quinone chromophore. No appreciable reactions were observed with lysine, histidine, glutamic acid, and tyrosine. The sulfhydryl-containing compounds cysteine and glutathione, however, reacted rapidly, with $t_{1/2}$ values of ca. 2 and 30 min, respectively (Figure 3).

Since it appeared that only sulfhydryl nucleophiles reacted with the imino quinone at an appreciable rate, we carried out reactions with simple thiols on a preparative scale in order to isolate and characterize the covalent adducts. The reaction of imino quinone **9** with methyl mercaptan in methylene chloride gave a mixture of monoadduct(s) **19a** and/or **19b** and starting material, as indicated by CIMS analysis of the crude product. The same reaction carried out in methanol, however, was much faster and gave primarily a mixture of mono- and bisadducts. Apparently, under these conditions oxidation of the initially formed monoadduct(s) to a substituted imino quinone occurs, and a second addition of thiol takes place. Formation of this bisadduct **20** suggests that imino quinone **9** may be capable of cross-linking proteins, analogous to 6-hydroxydopamine.[7b]

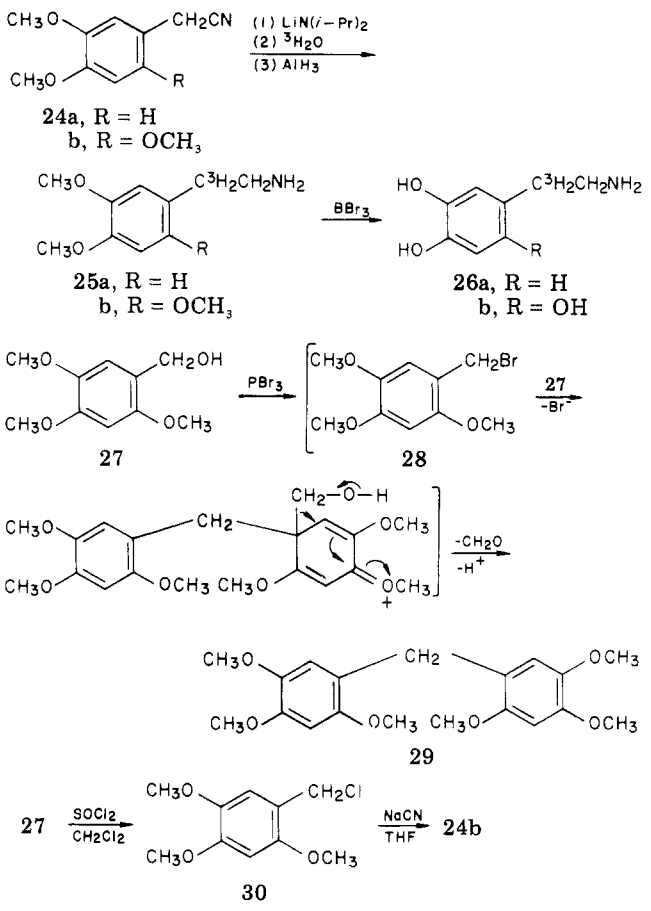
The products obtained from the reaction of imino quinone **9** with methyl mercaptan were unstable and difficult to separate. Consequently, we carried out the reaction with thiophenol in an attempt to obtain a more easily handled product. The reaction of imino quinone **9** with thiophenol in methanol was complete in ca. 5 min, as indicated by the loss of color. The product was isolated as the hydrochloride salt and has been assigned the structure **19c**. The CIMS and microanalysis clearly established that **19c** is a monoaddct. The position of attachment of the sulfur atom has been established by NMR spectroscopy. Oxidation of adduct **19c** with ceric ammonium nitrate provided an orange solution whose 100-MHz NMR spectrum is shown in Figure 4. The resonances assigned to the two methylene protons, which appeared as an eight- and six-line pattern, respectively, in the unsubstituted compound **9** (Figure 2), each appear as a four-line pattern in the substituted compound **21**. This loss of coupling upon substitution must mean that the vinylic proton adjacent to the methylene group has been replaced. As with the unsubstituted compound **9**, the C-6 methyl group gives an apparent triplet due to long-range coupling with the methine proton.

Scheme IV



Synthesis of Tritium-Labeled DOM and Tritium-Labeled Hydroquinone Metabolite. In order to carry out our studies on the covalent binding of hydroquinone metabolite-derived oxidation products to macromolecules, we needed to develop syntheses for radiolabeled DOM (**1**), metabolite **2**, and imino quinone **9**. Two synthetic methods for tritiated DOM were developed (Scheme IV). The first method involved base-catalyzed exchange of the labile protons of ketone **22** with tritiated water, followed by a reductive amination with ammonium acetate and sodium cyanoborohydride to give [^{3}H]DOM (**1a**; [α,α'-^{3}H]**1**). Since this method requires relatively large amounts of tritiated water, we sought an alternative procedure. Ketone **22** was converted to its *O*-methyloxime **23** by reaction with me-

Scheme V



thoxyamine. Treatment of **23** with lithium diisopropylamide generated a carbanion, which was quenched with tritiated water. The labeled oxime was then reduced with borane in THF to give [^{3}H]DOM (**1b**). The location of the label at the benzylic carbon atom was established by carrying out the reaction with D_2O instead of tritiated water and comparing the NMR spectrum of the resulting product with the NMR spectrum of unlabeled DOM. The advantages of this method are that the conversion of oxime derivative **23** to [^{3}H]DOM can be carried out in one flask and only a stoichiometric amount of tritiated water is required. Conversion of [^{3}H]DOM to ^{3}H-labeled metabolite **2** was accomplished by a previously described procedure.[13]

Synthesis of Tritium-Labeled Dopamine and 6-Hydroxydopamine. Radiotagged dopamine and 6-hydroxydopamine were required in order to compare their alkylating properties with metabolite **2**. Our synthetic approach involved exchange of the labile protons of an appropriately substituted phenylacetonitrile with ^3H_2O, followed by reduction to the amine and ether cleavage (Scheme V). We feel that this approach offers significant advantages over previously reported methods[19a] in terms of simplicity and economy.

Preparation of the nitrile **24b**, precursor of 6-OH[^{3}H]DA, was not straightforward and deserves comment. An obvious approach would involve reaction of benzyl bromide **28** with cyanide. However, attempts to prepare bromide **28** through the reaction of PBr_3 with commercially available alcohol **27** yielded only diphenylmethane **29**, apparently formed via the coupling reaction outlined in Scheme V. Preparation of the corresponding benzyl chloride **30** was successful and nitrile **22b** was obtained in moderate yield through the reaction of **30** with sodium cyanide in THF. The preparation of nitrile **24b** by a multistep procedure has been reported previously.[19b]

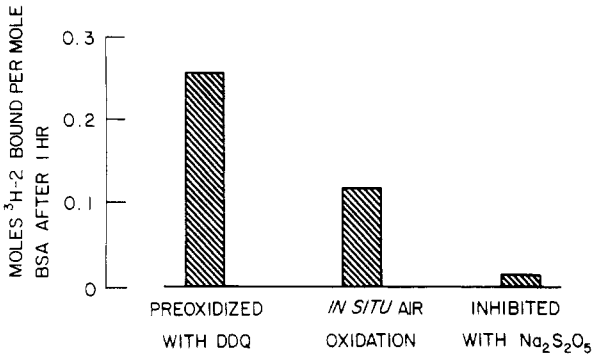


Figure 5. Covalent binding of hydroquinone-derived oxidation products to BSA.

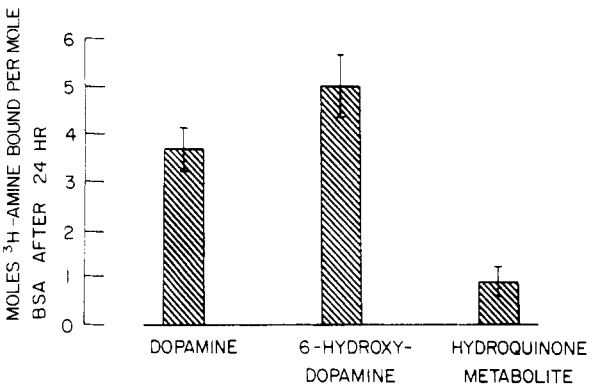


Figure 6. Covalent binding of dopamine, 6-hydroxydopamine, and hydroquinone metabolite to BSA.

"Covalent Binding"[20] of Oxidized Hydroquinone Metabolite to Bovine Serum Albumin. In their elegant studies on the binding of 6-hydroxydopamine-derived oxidation products to macromolecules, Saner and Thoenen utilized BSA as a model macromolecule.[7a] We also have used this model in our studies on the hydroquinone metabolite of DOM. Tritiated hydroquinone **2a** (4.8 mM) was incubated with BSA (0.25 mM) for 1 h at room temperature in pH 7.4 phosphate buffer. Isolation of the protein by acid precipitation followed by repeated homogenation (ten times) in alcoholic trifluoroacetic acid (TFA) led to significant amounts (0.12 equiv of **2a**/mol of BSA) of nonextractable radioactivity (Figure 5). When the incubation was carried out in the presence of the antioxidant sodium bisulfite (0.17 M), the bound radioactivity was reduced to nearly background levels. When **2a** was first converted to the imino quinone **9** by oxidative cyclization with DDQ, the amount of protein-bound radioactivity doubled (Figure 5).

These results clearly indicated that metabolite **2**, like 6-hydroxydopamine, can alkylate macromolecules under oxidizing conditions. However, the level of binding was much lower than the levels reported for 6-OHDA.[7] Consequently, we carried out parallel experiments with 6-OH[^{3}H]DA, [^{3}H]dopamine, and [^{3}H]hydroquinone **2** in order to compare the alkylating abilities of these three amines under oxidizing conditions. For these experiments, we used bovine serum albumin (BSA) at concentrations of 0.16 mM, amine concentrations of 8 mM, and 24-h incubation times, since previous studies[7] have shown that covalent binding of 6-OHDA to proteins is maximal when a large excess of amine and long incubation times are employed. The results, expressed as mole of amine bound per mole of protein, are shown in Figure 6. Clearly, 6-OHDA is a more effective alkylating agent than hydroquinone **2**, by a factor of about 5. Somewhat unexpected

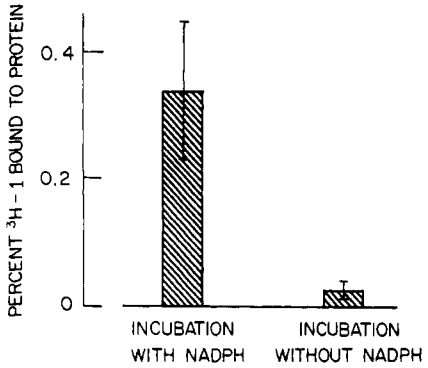


Figure 7. Covalent binding of DOM metabolites to microsomal macromolecules.

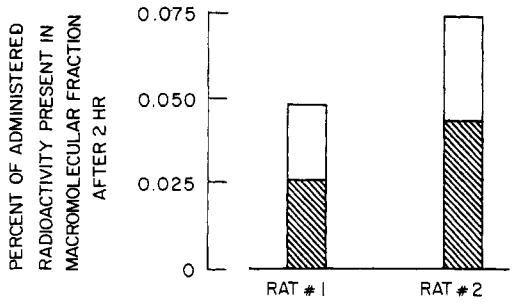


Figure 8. Covalent binding of [^{3}H]iminoquinone to rat brain tissue. Shaded area represents "covalently bound" (nonextractable) radioactivity.

was the high amount of nonextractable radioactivity obtained in the [^{3}H]dopamine incubations. This result is in contrast to the low levels of binding for dopamine reported by others.[7] It may be that the air oxidation of dopamine is sensitive to incubation conditions, such as the presence of trace metals. In any event, these data suggest that a great deal of caution is necessary when attempting to correlate in vitro experiments with events that may occur in the intact animal where oxidation processes may be more effectively controlled.

"Covalent Binding" of DOM Metabolites to Microsomal Macromolecules. Our studies on the in vitro metabolism of DOM[3] have demonstrated that the hydroquinone **2** is formed in significant amounts (up to 12%) in the 10000*g* and microsomal fractions of rabbit liver homogenates. In an effort to determine if metabolite(s) of DOM bind to microsomal macromolecules, tritium-labeled DOM (**1a**, 60 μM, 1.07 mCi/mmol) was incubated with liver microsomes (2 mg of protein/mL) in the presence and absence of NADPH for 15 min at 37 °C. Since the metabolism of DOM by rabbit liver microsomes in the absence of NADPH has been shown to be nil,[21] the level of bound radioactivity in the macromolecular fraction observed under these conditions served as a control for nonmetabolically dependent binding of radiolabeled materials. The results, which are presented in Figure 7, clearly show that a significant amount (0.2–0.4%) of the incubated **1a** becomes irreversibly bound to microsomal macromolecules under metabolizing conditions; under nonmetabolizing conditions, the bound radioactivity approaches background.

"Covalent Binding" of Tritiated Imino Quinone to Rat Brain Macromolecules. The studies described above have demonstrated that imino quinone **9** is a good alkylating agent in vitro. In order to assess the potential of these reactions occurring in the CNS, we administered tritium-labeled imino quinone intravenously to Sprague–Dawley rats and measured the radioactivity remaining in the brain after 2 h. As shown in Figure 8, a significant amount (0.05–0.08%) of the administered radioactivity was present in the brain homogenates, and of this about *50%* was nonextractable ("covalently" bound).

Discussion

At physiological pH, the hydroquinone metabolite of DOM is rapidly autoxidized to its corresponding *p*-quinone derivative **8**. This *p*-quinone apparently has only a transient existence at pH 7.4, undergoing a fast cyclization via Schiff base formation to the imino quinone **9**. As expected, imino quinone **9** is electrophilic and reacts readily with nucleophilic reagents to form covalent adducts.

The present study has demonstrated that the hydroquinone metabolite of DOM will undergo covalent binding to bovine serum albumin in vitro at physiological pH. Binding is dependent on autoxidation of the hydroquinone, since an antioxidant ($Na_2S_2O_5$) inhibits binding and preoxidation of the hydroquinone with DDQ facilitates binding. Based on our chemical model studies, binding is most likely due to alkylation of sulfhydryl groups on the albumin molecule.

Under metabolizing conditions, incubation of tritiated DOM with rabbit liver microsomes leads to covalently bound (nonextractable) radioactivity in the protein fraction. Although the autoxidation of hydroquinone metabolite **2** has been shown to be inhibited by components of liver homogenates, it is still quite likely that oxidation products of **2** under these conditions are responsible for the observed macromolecular binding. At the present time, we have no direct evidence for the proposed role of the hydroquinone **2**. With the possible exception of the *N*-hydroxy metabolite,[22] the other known metabolites of DOM[2,3,23] would not be expected to be capable of covalent binding to biological macromolecules.

In conclusion, it has been shown that the hydroquinone metabolite **2** exhibits 6-hydroxydopamine-like properties in vitro. Since 6-hydroxydopamine is known to produce behavioral changes[10] following central administration, it is not unreasonable to speculate that the hydroquinone metabolite **2** may contribute to the psychotomimetic effects of DOM through interactions of in vivo generated oxidation products of **2** with important macromolecules in the brain. A critical feature of this proposal concerns the transportation to the brain of any reactive metabolites formed in the liver. It is unlikely that the polar amino hydroquinone **2** would pass the blood–brain barrier. On the other hand, imino quinone **9**, which we now know possesses good electrophilic alkylating properties, is a highly lipid-soluble molecule which has been shown to partition into the CNS and alkylate brain macromolecules. Further studies on the pharmacological properties of imino quinone **9** are in progress.

Experimental Section

Melting points were taken on a Thomas-Hoover apparatus and are uncorrected. Ultraviolet spectra were recorded on a Cary 15 spectrophotometer. NMR spectra were recorded on a Perkin-Elmer R-12B (60-MHz spectra) or Varian XL-100 (100-MHz spectra). The chemical-ionization mass spectra were recorded on an AEI MS-902 spectrometer using isobutane as the reagent gas. A Büchi Kugelrohr oven was used for sublimations and bulb to bulb distillation. Incubations at 37 °C were carried out in a Dubnoff metabolic shaker. Radioactivity was determined by liquid scintillation counting (LSC) using a Packard Tri-Carb Model 3376 scintillation spectrometer with Aquasol (New England Nuclear) scintillation fluid. Combustion for radiochemical analysis was performed on a Packard Tri-Carb Model 306 sample oxidizer.

Unless otherwise noted, chemicals were obtained from commercial sources.

Oxidation of Hydroquinone 2 to Quinone 8. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ; 200 mg, 0.88 mmol) was added to a solution of hydroquinone **2**[13] (150 mg of the hydrochloride salt, 0.68 mmol) in 40 mL of 0.1 N HCl. The suspension was stirred 30 min at room temperature and then filtered to remove unreacted DDQ and its reduction product. An aliquot of the filtrate was diluted with 0.1 N HCl for UV analysis: λ_{max} 256 nm, ϵ 16 800. Attempts to isolate this compound by lyophilization of the aqueous solution led only to dark intractable gums. The 100-MHz NMR spectrum was obtained from a reaction carried out as above using 0.1 N DCl in D_2O as solvent (Figure 1).

Preparation of Imino Quinone 9. Hydroquinone **2** (150 mg of the hydrochloride, 0.68 mmol) was oxidized to quinone **8** with DDQ (200 mg, 0.88 mmol) as described above. The pH of the solution was adjusted to 7 using 1 M phosphate buffer and then extracted with CH_2Cl_2 (2 × 20 mL). The extract was concentrated on a rotary evaporator to give an orange solid, which was sublimed (0.05 mm, 100°C) to yield 40 mg of yellow powder, mp 68–70 °C. Anal. Calcd for $C_{10}H_{11}NO$: C, 74.50; H, 6.88; N, 8.69. Found: C, 74.45; H, 6.88; N, 8.62. The 100-MHz NMR spectrum is shown in Figure 2.

Reaction of Quinone 8 with Methyl Mercaptan. A solution of hydroquinone **2** (161 mg, 0.74 mmol) in 25 mL of 5% aqueous HCl was oxidized to quinone **8** as described above. Methyl mercaptan (1 mL, ~18 mmol) was condensed into a dry ice cooled test tube and added with stirring to the cold (0 °C) quinone solution. The initially yellow solution turned deep orange and then faded over a period of 1 h. The solution was stirred for an additional 2 h at room temperature. Excess methyl mercaptan was removed under aspirator vacuum, and the solution was filtered to remove a small amount of white solid. The filtrate was evaporated under reduced pressure to give a brownish solid, which was purified by dissolving in a small amount of warm isopropyl alcohol and then adding ethyl acetate until crystals began to form. After cooling the mixture in a refrigerator overnight, the product was collected by filtration and vacuum dried to give 60 mg of an off-white solid, mp 220–222 °C dec. Chemical-ionization mass spectral (CIMS) analysis (MH^+ 228) indicated formation of a monoadduct (**10a** or **10b**). Anal. Calcd for $C_{11}H_{18}NO_2SCl$: C, 50.09; H, 6.88; N, 5.31. Found: C, 49.64; H, 6.26; N, 5.16.

In a second experiment, the quinone solution was stirred with methyl mercaptan for 18 h. In this case, the bisadduct **11** was obtained: mp 268–270 °C dec; CIMS MH^+ 274.

Reaction of Quinone 8 with 2-Mercaptoethanol. Hydroquinone **2** (158 mg, 0.73 mmol) in 25 mL of 5% HCl was converted to quinone **8** as described above and then stirred with 2-mercaptoethanol (0.2 mL, 2.8 mmol) for 2 h at room temperature. The product was isolated and purified in the same manner described above for the methyl mercaptan adducts, yielding 46 mg of tan crystalline solid: mp 150–152; CIMS analysis of the pentafluoropropionyl (PFP) derivative (MH^+ 532) and of the underivitized material (MH^+ 240) indicated formation of bicyclic compound **12a** or **12b**.

Reaction of 2,5-Dimethyl-1,4-benzoquinone with 2-Mercaptoethanol. Preparation of 5,8-Dimethylbenzo-1,4-oxathian-6-ol (14). 2-Mercaptoethanol (1.6 mL, 1.8 g, 23.4 mmol) was added to a vigorously stirred suspension of 2,5-dimethyl-1,4-benzoquinone (2.8 g, 20.6 mmol) in 100 mL of 5% aqueous HCl. The mixture was stirred overnight at room temperature, during which time the yellow quinone was transformed into a flocculant white solid. The product was collected by filtration and air-dried to give 4.2 g of crude **14**, mp 105–108 °C. Recrystallization from toluene–hexane (1:2) gave 2.6 g (65% yield) of off-white crystals: mp 111.5–112.5 °C; CIMS analysis of the trifluoroacetyl (TFA) derivative of the crude product (MH^+ 293) indicated only bicyclic derivative **14** (absence of a peak at 503 due to the TFA derivative of **17**); NMR ($CDCl_3$, Me_4Si) δ 2.15 (s, 6 H, $2CH_3$), 3.1–3.3 (m, 2 H, SCH_2), 4.3–4.5 (m, 2 H, OCH_2), 4.85 (s, 1 H, ArOH), 6.40 (s, 1 H, ArH). Anal. Calcd for $C_{10}H_{12}O_2S$: C, 61.19; H, 6.16. Found: C, 61.31; H, 6.12.

5,8-Dimethyl-6-methoxybenzo-1,4-oxathian (15). Phenol **14** (1 g, 5 mmol) was added portionwise with stirring to a suspension of sodium hydride (25 mmol) in 25 mL of dry THF (H_2 evolution). Methyl iodide (3.5 g, 25 mmol) was added, and the mixture was heated under reflux for 2 h. After cooling, the mixture was poured carefully into H_2O (100 mL) and extracted with ether (2 × 30 mL). The ether extract was washed with 10% aqueous NaOH, dried over anhydrous K_2CO_3, and evaporated to give 1.1 g of yellow solid, mp 83–86 °C. Recrystallization from methanol provided 0.72 g (69% yield) of white plates: mp 85–86.5 °C; NMR ($CDCl_3$, Me_4Si) δ 2.13 (s, 3 H, CH_3), 2.20 (s, 3 H, CH_3), 3.0–3.2 (m, 2 H, SCH_2), 3.76 (s, 3 H, OCH_3), 4.25–4.45 (m, 2 H, OCH_2), 6.50 (s, 1 H, ArH). Anal. Calcd for $C_{11}H_{14}O_2S$: C, 62.82; H, 6.71. Found: 62.50; H, 6.82.

Reaction of Imino Quinone 9 with Methyl Mercaptan. Hydroquinone **2** (200 mg of the hydrochloride, 0.92 mmol) was converted to imino quinone **9** as described above. The crude product in CH_2Cl_2 was added to a solution of methyl mercaptan (1 mL, ~18 mmol) in 20 mL of ice-cold CH_2Cl_2. The solution was stirred for 1 h at 0–5 °C and then allowed to stand at room temperature overnight. Chemical-ionization mass spectral (CIMS) analysis of the solution indicated a mixture of monoadduct(s) **19a** or **19b**, their oxidized (imino quinone) forms, as well as starting material **9** and its reduced (dihydroindole) form: MH^+ (rel abundance) 162 (50), 164 (92), 208 (100), 210 (58).

The reaction was repeated using methanol as solvent. After 24 h, $NaBH_3CN$ (50 mg) followed by concentrated HCl (1 mL, dropwise) were added to reduce all products to the dihydroindole forms. CIMS indicated a mixture of mono- (**19a** or **19b**) and bis(methylthio) (**20**) adducts: MH^+ (rel abundance) 210 (75), 256 (100).

Reaction of Imino Quinone 9 with Thiophenol. Hydroquinone **2** (0.5 g of the hydrochloride) was converted to imino quinone **9** using DDQ (0.6 g) as described above. The crude product in methylene chloride was concentrated on the rotory evaporator to give an oil, which was taken up in methanol (25 mL). Thiophenol (0.5 mL) was added to the rapidly stirred solution. The initially yellow solution turned deep orange and then faded, becoming colorless in about 2 min. The solution was stirred an additional 5 min and then treated with $NaBH_3CN$ (50 mg) and concentrated HCl (2 mL, dropwise). The solution was then evaporated to dryness under reduced pressure. Methanol (50 mL) was added, and the solution was again evaporated to dryness under vacuum. (This process converts boric acid from $NaBH_3CN$ decomposition to methyl borate, which is volatile, bp 65 °C.) The residue was taken up in 25 mL of warm isopropyl alcohol, filtered to remove inorganic salts, and then evaporated to dryness. The crude product was purified by dissolving in hot isopropyl alcohol (10 mL) and then adding hexane (30 mL) portionwise, with stirring. The hydrochloride salt of adduct **19c** (130 mg) separated as a white, crystalline solid, which was collected by filtration and air-dried: mp 203.5–205 °C; NMR (D_2O) δ 1.40 (d, 3 H, $CHCH_3$), 2.20 (s, 3 H, $ArCH_3$), 2.74 and 3.30 (d of d, 1 H, $2CH_2$), 4.2–4.5 (m, 1 H, CH), 7.0–7.5 (m, ArH). Anal. Calcd for $C_{16}H_{18}NOSCl$: C, 62.43; H, 5.89; N, 4.55. Found: C, 61.99; H, 5.93; N, 4.43.

Oxidation of Phenylthio Adduct 19c to Imino Quinone 21. A solution of ceric ammonium nitrate (168 mg) in 0.5 mL of H_2O was added to a solution of adduct **10b** (29 mg) in 2 mL of H_2O. The mixture was vortexed for 5 min and then extracted with a mixture of CCl_4 (0.05 mL) and $CDCl_3$ (0.2 mL) containing a small amount of Me_4Si. The orange organic phase was washed with H_2O (1 mL) and then transferred to an NMR tube. The 100-mHz NMR spectrum is shown in Figure 4.

[^{3}H]DOM (1a) by Method 1. A solution of 1-(2,5-dimethoxy-4-methylphenyl)-2-propanone[23] (**22**; 0.7 g, 3.4 mmol) in pyridine (5 mL) containing tritiated water (1 mL, 95 mCi) was heated under reflux overnight. The pyridine and tritiated water were removed under vacuum and the crude tritiated ketone was purified by bulb to bulb distillation [150 °C (0.2 mm)]. The distillate was taken up in methanol (20 mL) and treated with ammonium acetate (6 g, 78 mmol), sodium cyanoborohydride (0.30 g, 4.8 mmol), and molecular sieves (Linde 3Å, 3 g). The mixture was stirred overnight at room temperature under a nitrogen atmosphere. The solids were filtered off, and the filtrate was poured into H_2O (100 mL). Dilute H_2SO_4 was added until the mixture was strongly acidic (hood, HCN evolved!), and the solution was extracted with CH_2Cl_2 (2 × 25 mL) to remove neutral side products. The aqueous phase was made basic with NaOH, and

the amine was extracted with CH_2Cl_2 (2 × 25 mL). The extract was concentrated under reduced pressure to give an oil, which was purified by bulb to bulb distillation [160 °C (0.2 mm)]. The distillate (which solidified in the receiver) was taken up in isopropyl alcohol (ca. 3 mL) and made strongly acidic by the addition of concentrated aqueous HCl. Addition of anhydrous ether (ca. 50 mL) led to the precipitation of the product, which was collected by filtration and air-dried. There was obtained 0.18 g (0.73 mmol, 22% based on ketone **22**) of white crystalline powder, mp 187–188 °C, 1.07 mCi/mmol.

1-(2,5-Dimethoxy-4-methylphenyl)-2-propanone *O*-Methyloxime (23). A solution of ketone **22** (2.5 g, 12 mmol) and methoxyamine hydrochloride (1.6 g, 19 mmol) in 25 mL of pyridine was stirred overnight at room temperature under N_2. The pyridine was removed under vacuum, 25 mL of H_2O was added, and the mixture was extracted with two 25-mL portions of CH_2Cl_2. After washing with H_2O (2 × 25 mL), the extract was concentrated on the rotary evaporator and distilled to give 2.14 g (9.4 mmol, 79%) of light yellow mobile liquid: bp 102–105 °C (0.3 mm); NMR ($CDCl_3$, Me_4Si) δ 1.75 (s, 3 H, CH_3), 2.22 (s, 3 H, $ArCH_3$), 3.50 (s, 2 H, CH_2), 3.80 (s, 6 H, OCH_3), 3.90 (s, 3 H, $NOCH_3$), 6.75 (br s, 2 H, ArH). Anal. Calcd. for $C_{13}H_{19}NO_3$: C, 65.80; H, 8.07; N, 5.90. Found: C, 65.74; H, 7.98; N, 5.88.

[^{3}H]DOM (1b). Method 2. A solution of *n*-butyllithium in hexane (3.1 mL of 1.6 M, 5 mmol) was added to diisopropylamine (0.7 mL, 5 mmol) in 5 mL of dry THF at −78 °C under N_2. *O*-Methyloxime **23** (1.15 g, 5 mmol) in 5 mL of THF was added, and the solution turned yellow. After stirring for 15 min at −78 °C, the anion was quenched with ^3H_2O (100 μL, 500 mCi). The solution was warmed to ~0 °C and borane–tetrahydrofuran (25 mL of 0.85 M) was added, dropwise with stirring (gas evolution). The mixture was heated under reflux for 1 h and then cooled, and excess hydride was destroyed by the careful addition of methanol (10 mL). Solvent was removed under reduced pressure, and dilute HCl (25 mL) was added, which caused a yellow, gummy material to separate. The mixture was heated under reflux for 1 h, during which time most of the gum dissolved with slow gas evolution. After cooling, the mixture was extracted with two 15-mL portions of CH_2Cl_2 and the extract was discarded. The aqueous phase was made basic with NaOH, and the product was extracted into CH_2Cl_2 (2 × 15 mL). Evaporation of the CH_2Cl_2 extract followed by a bulb to bulb distillation (0.2 mm, oven temperature 150 °C) provided 0.31 g (1.47 mmol, 30%) of colorless liquid which solidified in the receiver: mp 58–60 °C (mp[24] of unlabeled compound 60.5–61 °C); specific activity 5.3 mCi/mmol. A portion was converted to the hydrochloride salt by dissolving in isopropyl alcohol, acidifying with concentrated HCl, followed by precipitating with anhydrous ether. The product was collected by filtration and air-dried to give a white crystalline solid: mp 185–186 °C; specific activity 5.4 mCi/mmol.

1-(2,5-Dihydroxy-4-methylphenyl)-2-amino[^{3}H]propane Hydrochloride (2b). Boron tribromide (0.5 mL, 5 mmol) was added dropwise with stirring to a solution of [^{3}H]DOM free base (195 mg, 0.93 mmol, 5.4 mCi/mmol) under N_2 at ~0 °C. The solution was warmed to room temperature, stirred for 1 h, and then worked up by the dropwise addition of methanol (5 mL, ice cooling). The solvent was removed under vacuum to give crude **2b**·HBr, as a dark, gummy solid, which was purified and converted to the hydrochloride salt by cation-exchange chromatography on Dowex 50-X2 (50–100 mesh, 2 g), as described previously for unlabeled **2**.[13] The product was eluted with 30 mL of 4 N HCl. Evaporation of the eluate gave a light brown solid, which was taken up in 2 mL of warm methanol containing a few drops of concentrated HCl. Addition of ether (15 mL, portionwise) caused the product to precipitate, which was collected by filtration and air-dried to give 153 mg (0.70 mmol, 75%) of light brown powder, mp 214–217 °C. A second crystallization from methanol (2 mL) and ether (10 mL) provided 66 mg of pure product: mp 224–225 °C (lit.[13] mp 226 °C); specific activity 5.4 mCi/mmol. [^{3}H]-Hydroquinone **2b** of lower specific activity was obtained in an identical fashion by diluting [^{3}H]DOM with cold DOM prior to the demethylation reaction.

1-(3,4-Dimethoxyphenyl)-2-amino[^{3}H]ethane (25a). Butyllithium (1.5 mL of 1.6 M in hexane, 2.4 mmol) was added to a solution of diisopropylamine (0.34 mL, 2.4 mmol) in dry THF at −78 °C under N_2. A solution of 3,4-dimethoxyphenylacetonitrile (**24a**; 0.425 g, 2.4 mmol) in a small amount of THF was added, and the mixture was warmed to room temperature. The mixture was cooled again to −78 °C and tritiated water (50 μL, 2.8 mmol, 250 mCi) was added. The mixture was warmed to room temperature and stirred for 30 min. Aluminum hydride was prepared by the method of Brown and Yoon[25] in a separate flask by the addition of 100% H_2SO_4 (0.38 mL, 7.5 mmol) to a solution of $LiAlH_4$ (0.6 g, 15 mmol) in 15 mL of THF. The hydride solution was added to the nitrile solution via syringe at 0 °C. The mixture was heated at 50–60 °C for 30 min, then cooled, to 0 °C, and worked up by careful addition of H_2O (0.5 mL) in THF (10 mL). Concentrated aqueous NaOH was added until a granular precipitate had formed. The mixture was filtered, and the filter cake was washed well with THF. The filtrate was concentrated under reduced pressure, and the residue was taken up in dilute aqueous HCl (50 mL). After extracting the mixture with CH_2Cl_2 (2 × 25 mL), the acidic aqueous phase was made basic with NaOH. The liberated amine was extracted into CH_2Cl_2 (2 × 25 mL). The extract was concentrated under reduced pressure, and the product was purified by a bulb to bulb distillation (130–140 °C, 0.2 mm) to give 0.29 g (68% yield) of colorless liquid, specific activity 21 mCi/mmol.

1-(3,4-Dihydroxyphenyl)-2-amino[^{3}H]ethane [1-^{3}H]-Hydrochloride (26a, [^{3}H]Dopamine Hydrochloride). Boron tribromide (0.8 mL, 8 mmol) was added to a solution of amine **25a** (0.25 g, 1.48 mmol) in 25 mL of CH_2Cl_2 at 0 °C. The solution was then warmed to room temperature and stirred for 3 h. The reaction mixture was worked up by the careful addition of methanol (10 mL) with ice cooling. The solvent was removed under vacuum to give a brownish, viscous residue which was purified by cation-exchange chromatography on Dowex 50-X2, 50–100 mesh. The column was prepared from 3 g of dry resin and washed successively with 4 N HCl (25 mL) and distilled H_2O (25 mL) prior to application of the crude product dissolved in ca. 2 mL of H_2O. The column was washed with H_2O (50 mL), the wash was discarded, and 30 mL of 4 N HCl was passed through the column to elute the product. The eluate was evaporated under vacuum to give a light brown solid, which was purified by dissolving in warm methanol (5 mL) and adding ether (20 mL) portionwise with stirring to precipitate the product. Filtration and vacuum drying provided 151 mg of white crystalline solid: mp 240–243 °C dec (lit.[26] mp 237 °C for unlabeled dopamine hydrochloride); specific activity 25 mCi/mmol.

(2,4,5-Trimethoxyphenyl)acetonitrile (24b). Thionyl chloride (7 mL, 11.4 g, 97 mmol) was added to a solution of 2,4,5-trimethoxybenzyl alcohol (10 g, 50 mmol) in 100 mL of CH_2Cl_2 at 0 °C. The solution was stirred for 15 min at 0 °C and then 15 min at room temperature, during which time the color changed from yellow to blue. The solvent was removed under vacuum, and NaCN (5 g, 100 mmol) was added followed by 50 mL of THF. The resulting suspension was heated under reflux overnight, then cooled, diluted with 300 mL of H_2O, and extracted with CH_2Cl_2 (3 × 50 mL). The CH_2Cl_2 extract was washed with saturated aqueous NaCl, concentrated on a rotary evaporator, and distilled (bulb to bulb, oven temperature 150–190 °C, 0.2 mm) to give 7.6 g of yellow oil that crystallized in the receiver. This material was recrystallized from ethanol–hexane to give 5.9 g of white solid, mp 65–67 °C, which was ~40% diphenylmethane derivative **29**, as indicated by NMR. A second bulb to bulb distillation (140–150 °C oven temperature, 0.2 mm) gave relatively pure (>90%) **24b**: yield 3.0 g (29%); mp 79–82 °C (lit.[20] mp 85 °C).

1-(2,4,5-Trimethoxyphenyl)-2-amino[^{3}H]ethane (25b). The preparation of **25b** was analogous to the preparation of **25a**. From 0.5 g of nitrile **24b** there was obtained 0.29 g (58%) of amine **25b**: mp[27] 63–64.5 °C; specific activity 14.6 mCi/mmol.

1-(2,4,5-Trihydroxyphenyl)-2-amino[^{3}H]ethane Hydrochloride (26b, 6-Hydroxy[^{3}H]dopamine). The preparation of **26b** was analogous to the preparation of **26a**. From 0.27 g (1.28 mmol) of **23b** there was obtained 0.20 g (1.0 mmol, 78% yield) of **24b**: mp 230–231 °C dec (lit.[28] mp 231.5–233 °C dec for unlabeled 6-hydroxydopamine hydrochloride); specific activity 14.3 mCi/mmol.

Ultraviolet Spectral Studies. A solution of hydroquinone **2** (3.3×10^{-5} M) in 0.05 M, pH 7.4, phosphate buffer contained in an open 50-mL Erlenmeyer flask was stored at 37 °C. After 1 h, oxidation to imino quinone **9** was complete, as indicated by the appearance of its characteristic UV absorption at 282 nm, ϵ 27 000. Aliquots of the oxidized solution were treated with a tenfold molar excess of the appropriate nucleophilic reagent, and the concentration of **9** was monitored by recording the UV spectra at various time intervals.

Covalent Binding of [^{3}H]Hydroquinone to BSA. Effect of Oxidation and Antioxidant. A solution of **2a** (2.07 mg, 9.5 μmol, 2.61×10^6 dpm) in 0.01 N HCl (1.0 mL) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 3 mg) was shaken periodically over 15 min. Excess DDQ and its reduction product were removed by extraction with EtOAc (5 mL). A solution of BSA (32.2 mg) in 0.2 M, pH 7.4, phosphate buffer (1.0 mL) was added, and the mixture was allowed to stand at room temperature for 1 h in an unstoppered test tube. A second incubation was carried out in an identical fashion, with the exception that no DDQ was added. A third incubation was carried out in the same manner as the second, with $Na_2S_2O_5$ (32 mg) added along with the BSA solution. The incubations were stopped by adding 1 N $HClO_4$ (2 mL) to precipitate the protein. The mixture was centrifuged, and the supernatant was separated and discarded. The precipitate was homogenized with 5 mL of 0.5 N alcoholic trifluoroacetic acid in a Teflon pestle tissue homogenizer. The mixture was centrifuged and the homogenation procedure was repeated nine times, after which radioactivity in the supernatant fluid had decreased to background levels. The final pellet was transferred to a paper combustion cup and analyzed for radioactivity by combustion–LSC. The data are summarized in Figure 5.

Covalent Binding of [^{3}H]Hydroquinone Metabolite, [^{3}H]Dopamine and 6-Hydroxy[^{3}H]dopamine to BSA under Oxidizing Conditions. Tritiated amines (**2b**, **26a**, or **26b**) were added to a solution of BSA in 0.1 M phosphate buffer (pH 7.4) to give a final concentration of 8 mM in amine and 0.16 mM in protein with a total volume of 2 mL. The solutions were incubated in open 125 × 15 mm culture tubes at 37 °C for 24 h in a Dubnoff metabolic shaker. Incubations were quenched by the addition of $HClO_4$ (1 mL of 1 M), and the precipitated protein was worked up for nonextractable radioactivity as described above, using ten 5-mL aliquots of 0.4 N $HClO_4$ in ethanol. The final pellet was dissolved in 1.0 mL of 1 N NaOH by heating at 80 °C for 30 min. Aliquots of the digested protein (100 μL) and 100 μL of glacial HOAc (to inhibit chemoluminescence) were added to 10-mL portions of Aquasol in scintillation vials for determination of radioactivity by LSC. The results are shown in Figure 6.

Covalent Binding of DOM Metabolites to Microsomal Macromolecules. Microsomes were prepared from the livers of male, brown Dutch rabbits.[21] Incubations were carried out with 5-mL aliquots (2 mg of protein/mL) in Franklin buffer (pH 7.4) containing **1a** (60 μM, 7.35×10^5 dpm) and NADPH (5 mg) at 37 °C. After 15 min, the incubations were stopped by the addition of 60% $HClO_4$ (0.2 mL). The precipitated protein was worked up and analyzed for covalently bound radioactivity as described above for the BSA binding studies. To control for electrostatic binding of DOM to microsomal protein, incubations were carried out with no NADPH added, conditions under which no metabolism should occur. The results are presented in Figure 7.

Covalent Binding of [^{3}H]Iminoquinone to Rat Brain Tissue. A suspension of DDQ (10 mg) in a solution of tritiated hydroquinone (**2b**; 6.6 mg, 5.4 mCi/mmol) in normal saline (0.66 mg) was vortexed for 15 min. The unreacted DDQ and its reduced form was removed by filtration through a plug of glass wool. Aliquots (0.020 mL) of the filtrate were injected into the tail veins of two 250-g male Sprague–Dawley rats. Two hours after the injections, the animals were sacrificed by decapitation. The brains were removed, minced, and washed with 10 mL of saline. The tissue was homogenized in 5 mL of 0.1 M, pH 7.4, phosphate buffer. A small aliquot was removed to analyze radioactivity by LSC. Aqueous $HClO_4$ (5 mL) was added, the mixture was centrifuged, and the supernatant was discarded. The precipitated macromolecular fraction was suspended in 10 mL of 0.4 N $HClO_4$ in ethanol, homogenized as described for the protein-binding studies above. A total of three homogenizations with 0.4 N $HClO_4$ in ethanol and seven homogenizations with 0.4 N aqueous $HClO_4$ were carried out. The final pellet was dissolved in 1 mL of 1 N aqueous NaOH (90 °C, 1 h), and radioactivity was determined by LSC analysis (LSC cocktail containing 0.2 mL of glacial acetic acid). The results are shown in Figure 8.

Inhibition of the Autoxidation of Hydroquinone 2. All buffers (pH 7.4 and 0.1 M) were prepared using potassium phosphate, monobasic and dibasic. For the metal-free studies, the buffer (100 mL) was prepared with double glass-distilled water and was passed through a column (25 g) of Chelex 100. For studies involving liver preparations, the appropriate amount of the 10000*g* or 100000*g* fraction was added to the buffer to make up the various dilutions studied. In all experiments, the reference cell contained all components in the sample cell except the hydroquinone. Repetitive scans over the range 220 to 340 nm were recorded and the half lives ($t_{1/2}$ values) determined by inspection.

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References and Notes

(1) A. T. Shulgin, T. Sargent, and C. Naranjo, *Nature (London)*, **221**, 537 (1969).
(2) B. T. Ho, V. Estevez, L. W. Tansey, L. F. Englert, P. J. Creaven, and W. M. Mcissac, *J. Med. Chem.*, **14**, 158 (1971); B. T. Ho, V. Estevez and G. E. Fritchie, *Brain Res.*, **29**, 166 (1971).
(3) J. S. Zweig and N. Castagnoli, Jr., *Psychopharmacol. Commun.*, **1**, 359 (1975); J. S. Zweig and N. Castagnoli, Jr., *J. Med. Chem.*, **20**, 414 (1977).
(4) R. M. Kostrzewa and D. M. Jacobwitz, *Pharmacolol. Rev.*, **26**, 199 (1974).
(5) C. L. Blank, P. T. Kissinger, and R. N. Adams, *Eur. J. Pharmacol.*, **19**, 391 (1972).
(6) H. Thoenen and F. P. Tranzer, *Naunyn-Schmiedeberg's Arch. Pharmakol. Exp. Pathol.*, **261**, 271 (1968).
(7) (a) A. Saner and H. Thoenen, *Mol. Pharmacol.*, **7**, 147 (1971); (b) A. Rotman, J. W. Daly, and C. R. Creveling, *ibid.*, **12**, 887 (1976).
(8) J. Harley-Mason, *J. Chem. Soc.*, 200 (1953).
(9) R. T. Borchardt, J. R. Reid, D. R. Thakker, Y. O. Liang, R. W. Wightman, and R. N. Adams, *J. Med. Chem.*, **19**, 1201 (1976).
(10) R. E. Redmone, R. L. Hinrich, and J. W. Maas, *Science*, **181**, 1256 (1973).
(11) L. Stein and C. D. Wise, *Science*, **175**, 922 (1972).
(12) S. Senoh, B. Witkop, C. R. Creveling, and S. Udenfriend, *J. Am. Chem. Soc.*, **81**, 1768 (1959); S. Senoh, C. R. Creveling, S. Udenfriend, and B. Witkop, *J. Am. Chem. Soc.*, **81**, 6236 (1959).
(13) J. S. Zweig and N. Castagnoli, Jr., *J. Med. Chem.*, **17**, 747 (1974).
(14) L. L. Butcher, *J. Neural Transm.*, **37**, 189 (1975).
(15) P. Chaix, *Biochem. Biophys. Acta*, **4**, 471 (1950).
(16) E. K. Hodgson and I. Fridovich, *Biochemistry*, **14**, 5294 (1975).
(17) R. Heikkila and G. Cohen, *Science*, **181**, 456 (1973).
(18) K. T. Finley in "The Chemistry of the Quinoid Compounds", Part 2, S. Patai, Ed., Wiley, New York 1974, p 887.
(19) (a) A. Rotman, J. W. Daly, and C. R. Creveling, *J. Labelled Compd.*, **11**, 445 (1975); (b) A. Roberson and G. F. Bushby, *J. Chem. Soc.*, **1371** (1935).
(20) Although we cannot rigorously say that *covalent* binding has occurred, "covalent binding" to macromolecules has come to mean that radioactive compound or derived products cannot be extracted by repetitive homogenations.
(21) N. P. McGraw, P. S. Callery and N. Castagnoli, Jr., *J. Med. Chem.*, **20**, 185 (1977).

(22) J. Gal, L. Gruenke, and N. Castagnoli, Jr., *J. Med. Chem.*, 18, 683 (1975).
(23) S. B. Matin, P. S. Callery, J. S. Zweig, A. O'Brien, R. Rapoport, and N. Castagnoli, Jr., *J. Med. Chem.*, 17, 877 (1974).
(24) A. T. Shulgin, U.S. Patent 3 547 999 (1970); *Chem. Abstr.*, 71P, 12781*j*.
(25) H. C. Brown and N. M. Yoon, *J. Am. Chem. Soc.*, 88, 1464 (1966).
(26) E. Waser and H. Sommer, *Helv. Chim. Acta*, 6, 54 (1923).
(27) Unlabeled amine **25b** has been reported as a "waxy solid": J. Harley-Mason, *J. Chem. Soc.*, 200 (1953).
(28) F. G. H. Lee and D. E. Dickson, *J. Med. Chem.*, 14, 266 (1971).

Synthesis and Antihypertensive Activity of Novel 3-Hydrazino-5-phenyl-1,2,4-triazines

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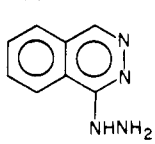
In an effort to develop antihypertensive agents with peripheral vasodilator activity, a series of 40 novel 3-hydrazino-5-phenyl-1,2,4-triazines (II) were synthesized and evaluated in the spontaneously hypertensive rat assay (SHR assay). Based on the performance of the structurally related standard, hydralazine (I), 15 triazines were active. Thirteen of these hypotensive triazines possessed LD_{50} values in the mouse greater than I (LD_{50} = 100 mg/kg); only one active triazine had an LD_{50} value greater than 300 mg/kg (**11d**). Four asymmetric triazines had moderate antihypertensive activity and LD_{50} values greater than 300 mg/kg (**6b**, **7c**, **8f**, and **9g**). Based on the relationship between toxicity and antihypertensive activity, three triazines (**8f**, **9g**, and **11d**) were chosen for dose–response studies in the SHR assay. None were as efficacious as I, but all three were less toxic, resulting in similar therapeutic indices relative to I.

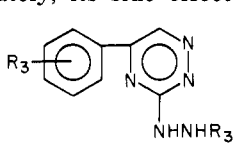
It has been estimated that approximately 15% of the adult American population is hypertensive.[1] Yet less than 10% of those considered hypertensive receive adequate hypertensive control through appropriate drug therapy.[1] Both the major Veterans Administration[2] and Framingham[3] studies demonstrated that blood reduction, through the use of drug therapy, decreases the incidence of most cardiovascular complications of hypertension. The critical need in hypertensive drug therapy is for a safe and effective antihypertensive drug, devoid of undesirable side effects, which can be used in the treatment of moderate levels of essential hypertension.

In most patients with chronic essential hypertension, abnormally high peripheral vascular resistance is the proximate cause of elevated arterial pressure.[4-6] Their cardiac output is generally within the normal range. Yet most antihypertensive drugs that elicit their hypotensive effect by depressing the sympathetic nervous system decrease cardiac output. While blood pressure decreases, the abnormally low cardiac output results in decreased tissue perfusion to the heart, brain and kidneys, a hemodynamic situation which is hardly desirable nor chronically tolerated. The hemodynamic goal in most chronic essential hypertension therapy should be to specifically reduce peripheral vascular resistance, without sympathodepression.

An antihypertensive drug presently marketed which acts directly on constricted arteriolar smooth muscle is hydralazine (I). Unfortunately, its side effect liability



I



II

(including the production of a lupus erythematosus-like syndrome) has limited its widespread use in recent years.[7] Also, the decrease in vascular resistance caused by therapy with I activates the baroreceptors, resulting in a reflex increase in sympathetic discharge which increases heart rate, stroke volume, and cardiac output.[8] Therefore, I is normally administered in combination with β-adrenergic blockers which eliminate the reflex action. A diuretic is also often added to offset the sodium and water retention caused by I. Hypertensive control is then achieved with no signs of postural hypotension, depression of cardiac output, impairment of renal or sexual function, or other adverse symptomatology.

In an effort to develop agents which act directly on arteriolar smooth muscle but do not possess the side effect liability associated with I, we synthesized a series of asymmetric triazines (II) which differ from I in the following ways: (1) One nitrogen atom has been added to the heterocyclic ring. (2) The nitrogen-containing ring is no longer fused to the phenyl ring but merely attached by a single bond. The addition of the nitrogen atom to the ring will decrease the electron density of the ring which should effect the distribution, metabolism, and intrinsic activity of the molecule. The lack of fusion between the two rings enables the aromatic rings, depending on ring substitution, to assume a nonplanar conformation, which is not possible with I. We also have investigated the effect of various substituents on the phenyl ring (R_5) and hydrazino side chain (R_3).

The antihypertensive effect of these compounds was evaluated in the spontaneously hypertensive rat assay (SHR assay), and LD_{50} values in the mouse were determined in a standard, multidimensional observational assay and calculated according to the method of Litchfield and Wilcoxon.[9]

Synthetic Aspects. The syntheses of 3-hydrazino- (**6a–g**), 3-(methylhydrazino)- (**7a**, **7c**, **7d**, and **7f**), 3-(acetylhydrazino)- (**8a**, **8d**, **8e**, **8f**, and **8g**), and 3-[(trifluoroacetyl)hydrazino]-5-(substituted phenyl)-1,2,4-triazines (**9a**, **9c**, **9d**, **9f**, and **9g**) are shown in Scheme I. Melting points and solvents of recrystallization are shown in Tables I and II.

The 3-(methylthio)-5-phenyl-1,2,4-triazines **5a–h** were synthesized according to the method of Paudler and Chen.[10] The appropriately substituted acetophenone (**3a–g**) undergoes selenium dioxide oxidation to afford the corresponding phenylglyoxals **4a–g**.[11] Unpurified glyoxal **4** undergoes facile cyclization with methylthiosemi-

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