the following conditions: flask heater, 290 °C; oven, 260 °C; separator, 285 °C; source, 290 °C; ionizing voltage, 70 eV; trap current, 60 μA; accelerating voltage, 3 keV; gas flow, approximately 20 cm^3/min of helium. The retention time for triazolam and its 4-hydroxy metabolite was 10.5 and 8.0 min, respectively. In most cases a background spectrograph was taken shortly after the desired peak passed, and this was subtracted (using computerized techniques) from the mass spectrograph data taken of the desired peaks. A representative spectrum of the material of retention time, 8 min, is shown in Figure 4 with a sample of authentic 4-hydroxytriazolam.

Acknowledgment. The authors are indebted to Mrs. Cathy Solomon, Miss Sally Boukma, and Mr. B. V. Kamdar for laboratory assistance; to Dr. George Slomp for helpful discussions about the NMR studies; and to Mrs. Helen Branch for preparing this manuscript.

Supplementary Material Available: Tables V–XV (12 pages). Ordering information is given on any current masthead page.

References and Notes

(1) (a) M. Gall, J. B. Hester, Jr., A. D. Rudzik, and R. A. Lahti, *J. Med. Chem.*, **19**, 1057 (1976); (b) R. A. Lahti and M. Gall, *ibid.*, **19**, 1064 (1976).
(2) M. E. Derieg, R. I. Fryer, and S. S. Hillery, *J. Heterocycl. Chem.*, 8, 181 (1971).
(3) (a) G. R. Newkome and D. L. Fishel, *J. Org. Chem.*, 31, 677 (1966); (b) A. Walser, W. J. Zally, and R. I. Fryer, *J. Heterocycl. Chem.*, 11, 863 (1974); (c) see Tables VI and VII in the microfilm edition of this journal.
(4) For another synthesis of α-amino-α-phenyl-*o*-tolyl-4*H*-triazoles, see A. Walser, T. Flynn, and R. I. Fryer, *J. Heterocycl. Chem.*, **12**, 717 (1975). See also J. B. Hester, U.S. Patent 3 772 317; M. Gall, U.S. Patents 3 970 666 and 4 020 085.
(5) These results may be contrasted with results reported in ref 1a for two diastereomeric triazolodiphenylmethanols.
(6) H. M. McConnell and R. E. Robertson, *J. Chem. Phys.*, **29**, 1361 (1968).
(7) (a) M. R. Willcott III, R. E. Lenkinski, and R. E. Davis, *J. Am. Chem. Soc.*, **94**, 1742 (1972); (b) we thank Drs. G. Slomp and B. V. Cheney of the Upjohn Co. for use of their modified version of this program.
(8) Unpublished results of R. A. Lahti and A. D. Rudzik of these laboratories.
(9) W. R. Busing and H. A. Levy, *Acta Crystallogr., Sect. A or B*, **10**, 180 (1957).
(10) Tables V and VIII–XV of hydrogen atom coordinates, thermal parameters, and bond lengths and angles, all with standard deviations, are included in the microfilm edition of this journal.
(11) See ref 1a and references cited therein.
(12) J. B. Hester, A. D. Rudzik, and B. V Kamdar, *J. Med. Chem.*, 14, 1078 (1971).
(13) Private communication from Dr. Floyd Eberts of these laboratories.
(14) Private communication from Dr. R. J. Collins of these laboratories.

Oxidative and Cardiovascular Studies on Natural and Synthetic Catecholamines

Charles G. Chavdarian, Daiji Karashima, Neal Castagnoli, Jr.,*

Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, California 94143

and Harvey K. Hundley

U.S. Food and Drug Administration District Office, San Francisco, California 94102. *Received December 5, 1977*

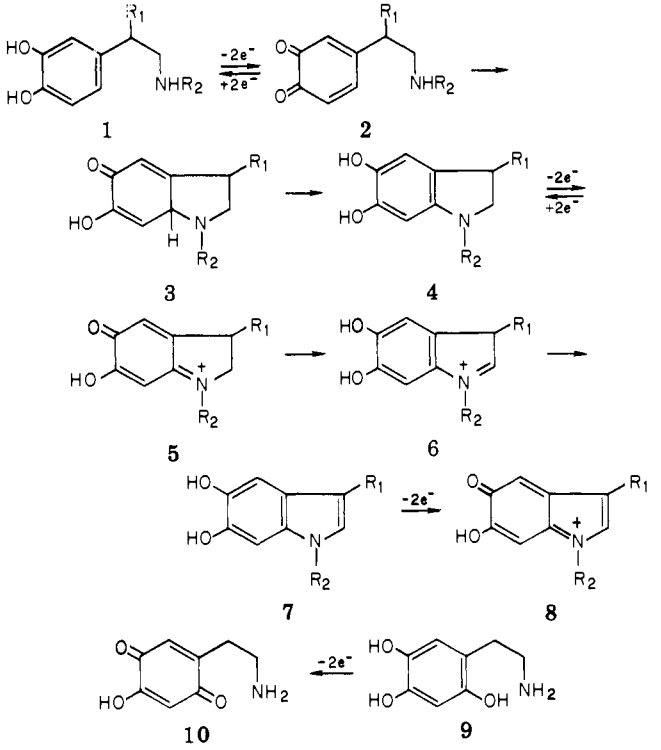
The cyclic voltammometric behavior of epinephrine, norepinephrine, dopamine, epinine, α-methyldopamine, β-methyldopamine, β-methylepinine, and β-methoxyepinine has been examined in order to evaluate substituent effects on cyclization rates of the electrochemically generated quinones. We observed that α and β substituents caused a modest enhancement of cyclization rates while an *N*-methyl group dramatically increased cyclization rates. No correlation was observed between calculated amine pK_a values, suggesting that differences in cyclization rates between the primary and secondary amine series were due to inherent nucleophilicity, a measure of which would be gas-phase proton affinities. The acute pressor effects of the newly synthesized catecholamines were compared with the native amines.

The neurotransmitter catecholamines, dopamine (**1a**), norepinephrine (**1b**), and epinephrine (**1c**), undergo the series of oxidation and cyclization reactions summarized in Scheme I.[1] Following the facile two-electron oxidation of I to the *o*-quinones **2**, cyclization via intermediates 3 to the indolines **4** occurs. The indolines are further oxidized to iminoquinones **5** which rearrange via **6** to the dihydroxyindoles **7**. The 3-hydroxyindolines **4b** and **4c** may dehydrate to the corresponding indoles. Finally, the dihydroxyindoles **7** may undergo a third two-electron oxidation to the highly unsaturated species **8**. In an analogous fashion, 6-hydroxydopamine (**9**) is readily oxidized to the *p*-quinone **10** which, following cyclization to **5a**, shares the same fate of dopamine (**1a**).

The neurodestructive[2] and enzyme-inhibiting properties[3] of 6-hydroxydopamine (**9**) appear to be dependent upon its conversion to one or more of the reactive electrophiles, **5a**, **8a**, and **10**, which are capable of alkylating nucleophilic functionalities present on macromolecules.[4] Since the aberrant in vivo formation of these reactive electrophiles from the native catecholamines could result in biochemical lesions and neurological disorders, it is important to understand how structural parameters influence the susceptibility of the parent amines to autooxidation, as well as the subsequent fate of the resulting quinones. The elegant electrochemical[5] and structural analogue studies[6] of Adams and co-workers have contributed significantly to the current state of knowledge in this area. An interesting feature observed by Adams et al.[5] is the dramatic difference in cyclization rates of the quinones **2a** vs. **2c** derived from dopamine (**1a**) and epinephrine (**1c**), respectively. In order to evaluate the influence of N, α, and β substituents on the cyclization rates of **2**, we have compared the cyclic voltammometric (CV) characteristics of a selected series of catecholamines. If the rate-determining step in the conversion of **2** to **4** is cyclization to **3**, the cyclization rate constants can be determined by measuring the appropriate peak potential currents at different scan rates (see below). We have determined these rate constants and evaluated them in terms of steric factors

0022-2623/78/1821-0548$01.00/0

Scheme I. Oxidation and Cyclization Reactions of Catecholamines 1 and 6-Hydroxydopamine (9)[a]



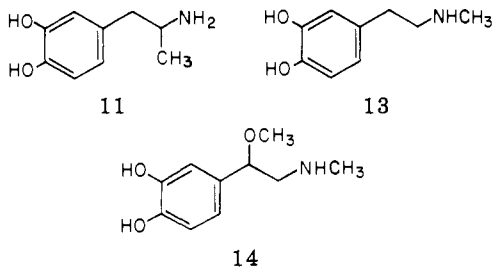
[a] a, $R_1 = R_2 = H$; b, $R_1 = OH$; $R_2 = H$; c, $R_1 = OH$; $R_2 = CH_3$; 7d, $R_1 = H$; $R_2 = CH_3$

Table I. Cyclization Rate Constants (2 → 3) and Estimated pK_a Values for Various Catecholamines

Compd	pH for CV run	$k \times 10^3$, s	Estd pK_a
Dopamine (1a)	6.0	36	10.0
α-Methyldopamine (11)	6.0	43	10.0
Norepinephrine (1b)	6.0	60	9.0
β-Methyldopamine (12)	6.0	83	10.0
Epinine (13)	3.5	19	10.5
Epinephrine (1c)	3.5	27	9.5
β-Methoxyepinine (14)	3.5	35	9.5
β-Methylepinine (15)	3.5	36	10.5

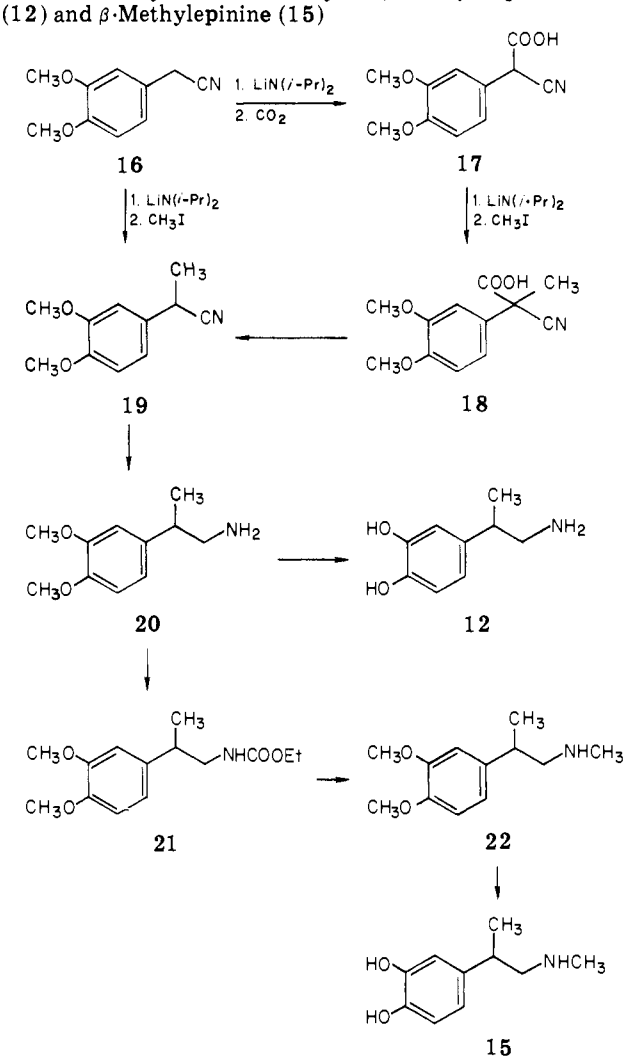
and the estimated pK_a values of the various aminoquinones 2. Finally, in an attempt to correlate the electrochemical properties of these compounds with a pharmacological parameter, we have compared their acute pressor effects in the rat.

Chemistry. The series of catecholamines examined in this study is shown in Table I. Dopamine (1a), norepinephrine (1b), epinephrine (1c), α-methyldopamine (11), and epinine (13) were commercially available. Do-



pamine-d_3 (1a-d_3), which was examined to determine if rearrangement of 3a to 4a is rate determining for the sequence 2a → 3a → 4a, was prepared by exchange of the aromatic protons in D_2O–DCl.[7] NMR established the deuterium incorporation of 1a-d_3 to be >95%.

Scheme II. Synthetic Pathways to β-Methyldopamine (12) and β-Methylepinine (15)

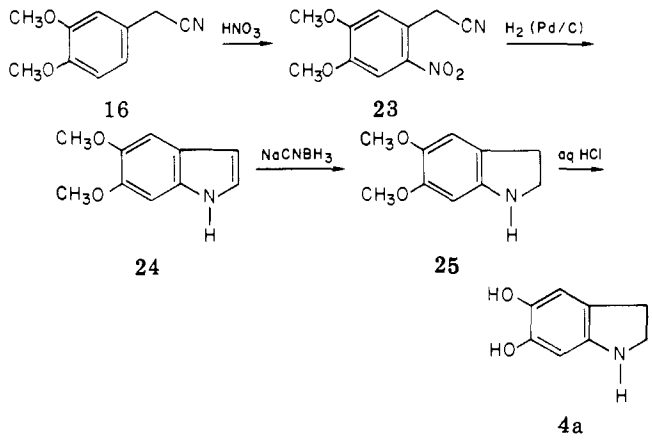


The synthesis of *N*-methyl-2-methoxy-2-(3,4-dihydroxyphenyl)ethylamine (β-methoxyepinine, 14) was readily achieved by treatment of epinephrine (1c) with methanolic HCl.[8] The hydrochloride of 14 was isolated as a light tan crystalline solid and characterized by NMR and microanalysis.

The syntheses of 2-(3,4-dihydroxyphenyl)propylamine (β-methyldopamine, 12) and *N*-methyl-2-(3,4-dihydroxyphenyl)propylamine (β-methylepinine, 15) were accomplished by the pathway presented in Scheme II. Attempted monomethylation on a small scale of 3,4-dimethoxyphenylacetonitrile (16) according to the procedure reported by Watt[9] gave a 95:5 mixture of mono- to dialkylation products from which 2-(3,4-dimethoxyphenyl)propionitrile (19) could be obtained in 66% yield. Larger scale runs gave poor yields of the desired product and, therefore, an alternative approach was sought. Carbonation of the lithio derivative of 16 afforded 3,4-dimethoxyphenylcyanoacetic acid (17) which was converted to its dianion with 2 equiv of lithium diisopropylamide in THF–HMPA. Treatment of this dianion with methyl iodide gave 2-cyano-2-(3,4-dimethoxyphenyl)propionic acid (18) which upon distillation underwent decarboxylation to 19 in an overall yield of about 40%.

Reduction of nitrile 19 with $LiAlH_4$ to 2-(3,4-dimethoxyphenyl)propylamine (20) proceeded in 65% yield. Demethylation of 20 in refluxing 48% aqueous HBr followed by ion-exchange chromatography and product elution with aqueous HCl gave β-methyldopamine (12) as

Scheme III. Synthetic Pathway to Indoline 4a



its hydrochloride salt in 61% recrystallized yield. Amine **20** was converted to its ethyl carbamate **21** with ethyl chloroformate in 93% yield.[10] Reduction of **21** with $LiAlH_4$ produced *N*-methyl-2-(3,4-dimethoxyphenyl)propylamine (**22**) in 85% yield which upon treatment with BBr_3 afforded crystalline β-methylepinine (**15**) in 27% yield.

In addition to the catecholamines, we wished to examine the electrochemical properties of 5,6-dihydroxyindoline (**4a**) to confirm the structures assigned to the redox couples observed in the cyclic voltammogram of dopamine (see discussion of electrochemical studies). We achieved the synthesis of **4a** by an efficient reaction sequence outlined in Scheme III. Nitration of the readily available 3,4-dimethoxyphenylacetonitrile (**16**) provided 2-nitro-4,5-dimethoxyphenylacetonitrile (**23**) in 60% yield. Catalytic reduction of **23** yielded the indole **24** in 75% yield. Attempted reductions of indole **24** by Swan's procedure[11] with Adams catalyst worked poorly. However, application of Gribble's procedure[12] utilizing $NaCNBH_3$ gave a high yield of indoline **25** which upon treatment with concentrated aqueous HCl in a bomb at 140–150 °C provided the desired catechol product **4a** in 65% yield.

Electrochemical Studies. We have examined by cyclic voltammetry the reaction sequence outlined in Scheme I for the catecholamines listed in Table I. Our specific interests concerned the following conversions: **1** ⇄ **2** → **3** → **4** ⇄ **5**. Typical cyclic voltammograms of dopamine (**1a**) at pH 3.5 and 6.0 are shown in Figure 1a and 1b, respectively. At the lower pH, a single redox couple associated with **1a** ⇄ **2a** is observed. At pH 6.0, a second pair of peaks, tentatively assigned to the **4a** ⇄ **5a** redox couple, also appear. By changing the scan rate, the time available for the quinone **2a** to cyclize to the iminoquinone **3a** varies. At the slower scan rate the cathodic current (i_c), which is a measure of the amount of **2a** undergoing reduction to **1a**, is less than at the faster scan rate. This decrease in i_c is a consequence of the longer time period between the anodic (oxidative) and cathodic (reductive) passes which allows for more extensive cyclization of the intermediate quinone **2a**. Consistent with a relatively fast and irreversible conversion of **3a** to **4a**, one also observes increases in both the oxidative and reductive currents associated with the redox couple **4a** ⇄ **5a** at the slower scan rate. Employing the principles developed below, we established that the cyclic voltammometric characteristics of dopamine-d_0 and dopamine-d_3 were indistinguishable, thus confirming that the conversion of **3a** to **4a** is not rate determining in the overall sequence **1a** → **4a**.

The above experimental data provided an opportunity to calculate approximate cyclization rates for the various quinones **2** generated electrochemically from the corresponding catechols **1**. Nicholson and Shain[13] have formulated a dimensionless theoretical working curve for electron transfer (**1** ⇄ **2**) followed by chemical reaction (**2** → **3** → **4**) processes. The ratios of the cathodic current (i_c) to the anodic current (i_a) for the electron-transfer process (**1** ⇄ **2**) are plotted against $k\tau$, where k is the rate constant in s^{-1} for the chemical reaction (**2** → **3**) and τ is the time interval between $E_{1/2}$ (i.e., the time at half-wave potential) and E_λ (i.e., the time at the switching potential) for the process **1** → **2**. For each catecholamine we determined i_c/i_a and τ at four scan rates and plotted the corresponding values of $k\tau$ (obtained from the Nicholson and Shain curve) against τ. The plots of $k\tau$ vs. τ were computer generated and the values of k derived mathematically from the least-squares fit. In all cases straight-line relationships were observed, denoting that the cyclization reaction is first order with respect to aminoquinone.

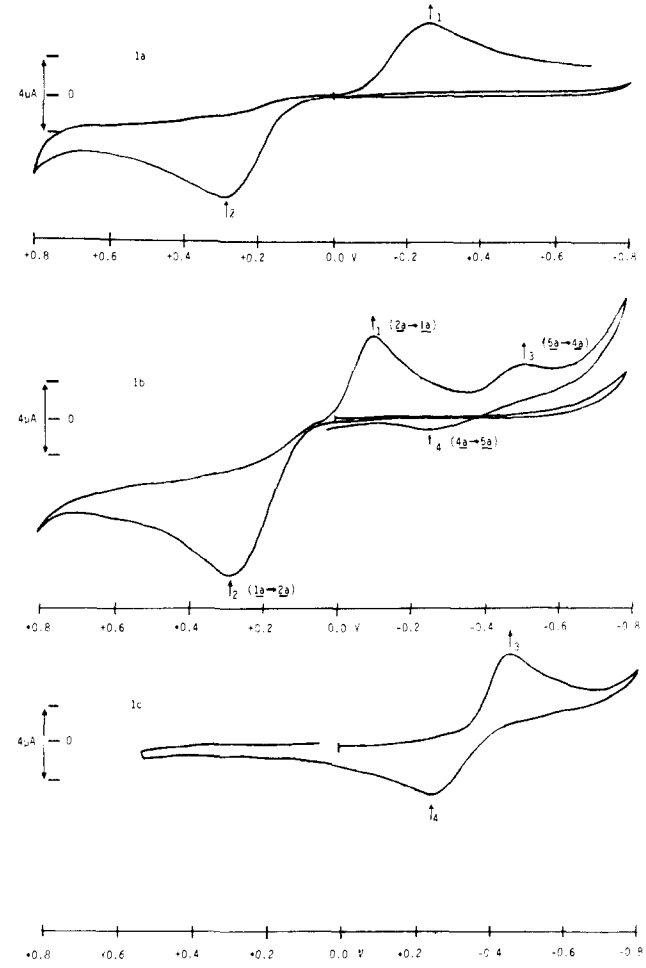


Figure 1. Cyclic voltammograms of dopamine and 5,6-dihydroxyindoline: (1a) dopamine (0.1 mM) at pH 3.5; scan rate is 2.0 V/min; (1b) dopamine (0.1 mM) at pH 6.0; scan rate is 2.0 V/min; (1c) 5,6-dihydroxyindoline **4a** (0.1 mM); nearly invariant within the pH range of 3.5–6.0; scan rate is 2.0 V/min.

Results and Discussion

The structure assignments for the two redox couples appearing in the cyclic voltammograms previously made by Adams for dopamine have been confirmed in this study.[14] The pair of peaks in Figures 1b and 1c indicated by $\uparrow_1$ and $\uparrow_2$ can be assigned to the catechol–quinone couple (**1a** ⇄ **2a**). At pH 3.5 where cyclization of **2a** to **3a** does not occur (Figure 1a), the cyclic voltammogram shows only one pair of peaks. At pH 6.0 (Figure 1b) the second pair of peaks observed (indicated by $\uparrow_3$ and $\uparrow_4$ in Figure 1b) can

Table II. Comparison of Base Strength of Various Amines in Solution and in the Gas Phase

Compd	pK_a	$-\Delta G^0_{soln}$, kcal/mol	$\Delta(\Delta G^0)_{soln}$	$-\Delta G^0_{gas}$, kcal/mol	$\Delta(\Delta G^0)_{gas}$
NH_3	9.24	12.61	1.87	198	11.8
Me_2NH_2	10.63	14.48	0.21	209.8	6.8
Me_2NH	10.78	14.69	−1.33	216.6	4.7
Me_3N	9.80	13.36		221.3	

now be unambiguously assigned to the **4a** ⇄ **5a** couple since the cyclic voltammogram of synthetic **4a** (Figure 1c) displays the same peak potentials ($\uparrow_3$ and $\uparrow_4$). By analogy, we have assigned the hydroquinone–quinone and indoline–iminoquinone couples to the corresponding peak potentials from the various catecholamines reported in this paper.

A comparison of the cyclic voltammograms of dopamine at pH 3.5 (Figure 1a) and at pH 6.0 (Figure 1b) reveals that the cyclization of quinone **2a** proceeds at a significant rate (0.036 s^{-1}) only at the higher pH. Cyclization of the norepinephrine-derived quinone **2b** is considerably faster at pH 6.0 (0.060 s^{-1}) but still undetectable at pH 3.5. On the other hand, cyclization of quinone **2c** derived from epinephrine (**1c**) at pH 6.0 is too fast to measure with the potentiostat used in these studies. At pH 3.5, however, the rate constant (0.027 s^{-1}) was conveniently measured. Based on these values it is apparent that β substituents and, much more dramatically, N substituents may lower the transition state energies for cyclization of **2** to **3**.

In order to further evaluate the influence of N, β, and α substituents on the cyclization rates of variously substituted quinone derivatives, we have examined the cyclic voltammograms of β-methoxyepinine (**14**), α-methyldopamine (**11**), β-methyldopamine (**12**), and β-methylepinine (**22**). The rate constants for the cyclization of the quinones derived from these compounds are listed in Table I. The first salient feature of these results is that the β-OCH_3 and β-CH_3 groups are, if anything, more rate enhancing than the β-OH group in both the primary and secondary amine series. It appears, therefore, that the nonbonding electrons of the β-oxygen substituents are not altering the chemical course of the cyclization reactions.

It is likely that steric effects of the side-chain substituents are contributing to the observed rate enhancement. Allinger[15] has shown that substituted chains possess a more favorable enthalpy and entropy of ring closure than unsubstituted chains. In the case of six-membered systems, the difference in enthalpies is due to the greater "gauche" interactions between the chain substituents resulting in a more favorable enthalpy of ring closure. In addition, the rotational entropy of a substituted chain is less than an unsubstituted one. As a result, the entropy decrease, which occurs on cyclization, is greater for unsubstituted chains. Allinger states that, qualitatively, this type of reasoning can be applied to five-membered ring formation. Since the substituent effects reduce the enthalpy and increase the entropy of ring closure, the change in free energy of ring closure will favor cyclization of the α- and β-substituted catecholamines.

The second important feature of these data is the dramatic effect of an *N*-methyl group on the cyclization rates. Differences in cyclization rates between the two series must be two to three orders of magnitude at the same pH values. One explanation for the different reactivities would be differences in nucleophilicity of primary vs. secondary amines, an approximate measure of which would be the pK_a values of the various compounds examined. Adams[5] has reported that the pK_a values for several primary and secondary catecholamines all range between 8.85 and 8.92. These values, however, do not reflect the ionization of the ammonium functions but rather the concomitant neutralization of all three acidic protons of the catecholamines.[16,17] Consequently, we estimated the pK_a values based on known pK_a values of related methoxy-substituted 2-phenylethylamines and the following semiempirical rules: (1) N-methylation of a primary amine increases the pK_a value by 0.5; and (2) a benzylic oxygen functionality decreases the pK_a by 1.0.[18] We have assumed that side-chain methyl groups and ring oxygen substituents do not significantly alter the basicity of the amino groups. This assumption is supported by the pK_a values for 2-phenylethylamine (9.86),[18] amphetamine (9.93),[19] and 2-(3,4-dimethoxyphenyl)ethylamine (10.0).[20]

The pK_a values calculated for the various catecholamines studied electrochemically are listed in Table I. With the likely assumption that the pK_a values of the aminoquinones **2** and the catecholamines are similar, it is clear that no correlation exists between cyclization rates of the *o*-quinones and the solution proton affinities of the amino group.

It is well known that proton affinities measured in the gas phase are dramatically different than the corresponding values (pK_a values) in aqueous solution. Quantitative gas-phase thermodynamic data for the proton affinities of amines have established the following order of basicities: $Me_3N > Me_2NH > MeNH_2 > NH_3$.[21] The corresponding order in solution is $Me_2NH > MeNH_2 > Me_3N > NH_3$.[22] The solution pK_a values and changes in free energy (ΔG^0) for protonation of these amines in solution and in the gas phase are summarized in Table II.[22,23] The difference in gas-phase proton affinities for primary vs. secondary amines (6.8 kcal/mol) is considerably larger than the corresponding difference in solution (0.21 kcal/mol). It is likely that similar effects are contributing to the differences observed in the cyclization rates and pK_a values for the amines examined in this study. The transition states involved in the intramolecular attack of the primary and secondary amino functionalities probably reflect their inherent nucleophilicities more closely than their pK_a values.

In conclusion, within each catecholamine series (primary and secondary) β substituents accelerate the side-chain cyclization rates following oxidation. An α substituent is less effective. The oxygen substituents closely mimic the methyl group with the differences in rates apparently being due to steric effects on the free energies of cyclization. A comparison of the basicities (assuming the validity of our calculated values) of the amines within a series reveals little, if any, correlation with rate acceleration. The dramatic difference in cyclization rates of primary vs. secondary amines is likely to be a consequence of the greater inherent nucleophilicity of secondary amines.

Biological Studies. Results and Discussion. We have examined the pressor effects of this series of catecholamines to determine to what extent amino group nucleophilic character (as revealed by the cyclization rates) may relate to this pharmacological parameter. The procedure involved intravenous drug administration via a jugular vein cannula; systemic arterial blood pressure was

Table III. Pressor Activities of Various Catecholamines

Compd	Dose × 10^6,[a] mg/kg	Potency (rel to dopamine)	Duration[b] of action
Dopamine	2.7 ± 0.4	100	32
(*S*)-α-Methyldopamine	3.0 ± 0.8	90	34
(*RS*)-α-Methyldopamine	3.3 ± 0.5	82	22
(*RS*)-β-Methyldopamine	5.5 ± 1.0	49	29
(*R*)-α-Methyldopamine	8.8 ± 1.5	31	20
(*RS*)-β-Methoxyepinine	11.0 ± 1.0	25	21
(*RS*)-β-Methylepinine	15.0 ± 5.0	18	28
Epinine	0.44 ± 0.07	614	18
(*R*)-Epinephrine	0.12 ± 0.02	2250	12
(*RS*)-Norepinephrine	0.047 ± 0.008	5745	32
(*R*)-Norepinephrine	0.026 ± 0.006	10385	32

[a] Dose required to increase arterial systolic blood pressure by 30 mmHg. [b] Seconds during which blood pressure was maintained at 30 mmHg.

monitored via a carotid artery cannula. Dose–response curves were obtained over a tenfold dose range, each of six doses being studied in four different animals.

We first studied the native catecholamines and observed that (*R*)-norepinephrine was about five times more potent than (*R*)-epinephrine and approximately 100 times more potent than dopamine (Table III). Our values are completely consistent with the data reported by Noble[24] who used the same preparation.

The dose–response curves for the catecholamine analogues are presented in Figure 2; their absolute and relative (to dopamine) potency and duration of action are listed in Table III. The pressor potencies of the catecholamine analogues were all less than dopamine, although the duration of action of (*RS*)-β-methyldopamine (**12**) was comparable to dopamine (Table III). β-Methylepinine (**15**) is considerably less hypertensive than β-methyldopamine. This result contrasts with the sixfold increase in pressor activity of epinine over dopamine. Also of interest is the nearly equipotent pressor activities of (*RS*)-α-methyldopamine (**11**) and (*S*)-α-methyldopamine. Since (*R*)-α-methyldopamine is a relatively weak agonist, simple additivity of activities would have predicted a higher dose for the racemic drug.

It is evident that the cyclization rates (Table I) do not correlate with pressor potencies. (*RS*)-β-Methylepinine (**15**) and (*RS*)-β-methoxyepinine (**14**) form quinones which cyclize at rates similar to the quinone derived from epinephrine (**16**) but demonstrate relatively weak pressor activity. (*RS*)-Norepinephrine (**1b**), which forms a quinone that cyclizes only slowly, is several orders of magnitude more hypertensive than any of the α- or β-substituted catecholamine analogues examined. It is apparent from these data that pressor agonist activity of catecholamines is not related to the redox behavior observed by cyclic voltammetry. The possible significance of substituent effects on the formation of electrophilic species with potential neurodegenerative properties is presently under investigation.

Experimental Section

A. Synthesis. All melting points and boiling points are uncorrected. ^{1}H NMR spectra were determined on either a Varian A-60A NMR spectrometer or a Perkin-Elmer R-12B 60-MHz NMR spectrometer. Chemical-ionization mass spectrometry (CIMS) was performed on an AEI MS-9 mass spectrometer. Solvent was removed with a Büchi rotary evaporator. Microanalyses were performed by the University of California Microanalytical Laboratory, Berkeley, Calif.

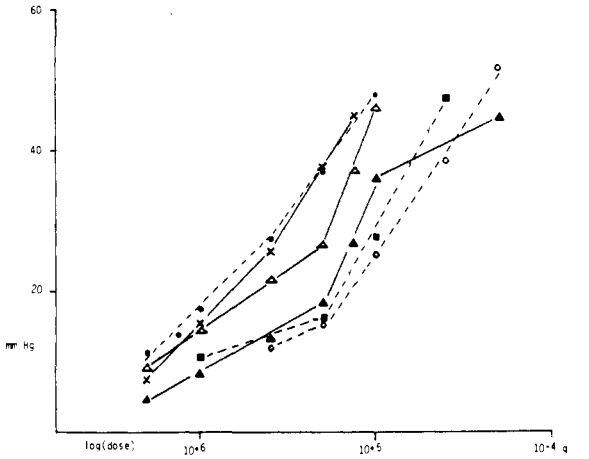


Figure 2. Dose–response curves to the catecholamine analogues: (*S*)-α-methyldopamine (●---●); (*RS*)-α-methyldopamine (×—×); (*RS*)-β-methyldopamine (△—△); (*R*)-α-methyldopamine (▲—▲); (*RS*)-β-methoxyepinine (■---■); (*RS*)-β-methylepinine (○---○).

***N*-Methyl-2-methoxy-2-(3,4-dihydroxyphenyl)ethylamine (14).** To a slurry of 135.7 mg (0.74 mmol) of epinephrine in 20 mL of anhydrous methanol was rapidly bubbled gaseous HCl for a few minutes. The resultant solution was then treated with ether to the cloud point (with scratching) to induce crystallization. Suction filtration and washing with ether afforded 129.2 mg (75%) of tan-white crystalline **14**: mp 165–166 °C; ^{1}H NMR (D_2O) δ 7.0 (s, 3), 4.59 (m, 1), 3.33 (s, 3), 3.25 (m, 2), 2.86 (s, 3). Anal. ($C_{10}H_{16}ClNO_3$) C, H, N.

2-(3,4-Dimethoxyphenyl)propionitrile (19). (a) Direct Monomethylation Procedure. To a solution of 2.52 mL (18 mmol) of diisopropylamine in 25 mL of dry THF at 20 °C under nitrogen was added 8.6 mL of 2.07 M (17.8 mmol) *n*-BuLi (while maintaining the temperature below −10 °C), and this resultant solution was stirred at −20 °C for 15 min and then cooled to −50 °C. To this was added, over 10 min, a solution of 3.0 g (16.92 mmol) of 3,4-dimethoxyphenylacetonitrile (Aldrich) in 6 mL of THF, followed by an additional 5 mL of THF to improve the solubility of the generated lithio derivative. During this addition, the temperature was maintained at −50 to −40 °C. After being stirred for an additional 5 min, the dark, yellow mixture was added (via syringe over 10 min) to a solution of 1.11 mL (17.8 mmol) of methyl iodide in 18 mL of THF. The mixture was stirred at −60 °C for 1 h and at room temperature overnight. The yellow solution was poured into 50 mL of 2 N HCl and extracted with ether (3 × 25 mL). The combined ethereal layer was washed with 25 mL of water and 25 mL of brine, dried ($MgSO_4$), and evaporated to a yellow oil (2.93 g). On standing, crystallization occurred. The prisms were pressed out on porous tile to yield 2.14 g (66%) of off-white product. ^{1}H NMR revealed a 95:5 ratio of monoalkylated product (**19**) to dialkylated material. Two recrystallizations from ether–dichloromethane–petroleum ether afforded an analytical sample: mp 67–69 °C; ^{1}H NMR ($CDCl_3$) δ 6.92 (s, 3), 3.89 (s, 3), 3.86 (s, 3), 3.84 (q, J = 7 Hz, 1), 1.56 (d, J = 7 Hz, 3). Anal. ($C_{11}H_{13}NO_2$) C, H, N.

(b) Carboxylation Procedure. To a solution of 14.8 mL (0.1058 mol, 1.25 equiv) of diisopropylamine in 125 mL of dry THF under nitrogen at −20 °C was added 49.0 mL (0.1015 mol, 1.2 equiv) of *n*-BuLi such that the temperature did not rise above −5 °C. After being stirred for 15 min at −20 °C a solution of 15.0 g (0.0846 mol) of 3,4-dimethoxyphenylacetonitrile in 35 mL of THF was added over 10 min (at −10 °C), followed by an additional 5 mL of THF. After 5 min of stirring at −20 °C, CO_2 was rapidly bubbled into the solution for 30 min. The mixture was then allowed to warm to room temperature, poured into 200 mL of water, and washed with ether (twice). The aqueous layer was acidified with 70 mL of 6 N aqueous HCl and extracted with ether

(2 × 100 mL), and the combined ethereal layer was washed with brine, dried ($MgSO_4$), and evaporated to yield 14.04 g of a viscous, yellow oil. ^1H NMR was fully consistent with 3,4-dimethoxyphenylcyanoacetic acid (**17**). This material was used directly in the next step.

To a solution of 19.6 mL (0.1320 mol) of diisopropylamine in 100 mL of dry THF was added, under nitrogen at −20 °C, 66.4 mL (0.1394 mol) of *n*-BuLi as described above. After being stirred for 15 min at −20 °C, 25 mL of HMPA was added and the solution was cooled to −60 °C. To this mixture was added, over 10 min, a solution of 14.0 g (0.0633 mol) of **17** in 15 mL of THF. To the dark orange-brown mixture was added, after 20 min, a solution of 4.14 mL (0.0665 mol, 1.04 equiv) of methyl iodide in **10** mL of THF over 10 min at −60 °C. The solution then gradually lightened in color and was stirred at −60 °C for 1 h, during which time a white precipitate formed. The mixture was allowed to warm to room temperature and stirred as such for 1.5 h. The yellow mixture was poured into 200 mL of water and washed with ether (2 × 100 mL). The aqueous layer was acidified with 70 mL of 6 N aqueous HCl and extracted with ether (3 × 100 mL); the ethereal layer was washed with water (4 × 50 mL) and brine (50 mL), dried ($MgSO_4$), and evaporated to a viscous orange oil (**18**). Bulb-to-bulb distillation [oven temperature 120–130 °C (0.25 Torr)] afforded 6.11 g (38% overall based on 3,4-dimethoxyphenylacetonitrile) of **19**. The distilled oil quickly crystallized. The ^1H NMR spectrum of this product was identical with that of an analytical sample.

2-(3,4-Dimethoxyphenyl)propylamine (20). To a slurry of 1.52 g (40 mmol) of $LiAlH_4$ in 50 mL of anhydrous ether (under nitrogen) was added a solution of 2.5 g (13.1 mmol) of nitrile **19** and the resultant mixture stirred at room temperature overnight. The mixture was then heated under reflux for 4 h and stirred again at room temperature overnight. The excess hydride was destroyed by the successive addition of 1.52 mL of water, 1.52 mL of 15% aqueous NaOH, and 4.5 mL of water. The reaction mixture was filtered and the colorless solution dried ($MgSO_4$) and evaporated to 2.18 g of a clear, nearly colorless oil. Bulb-to-bulb distillation [oven temperature 100–105 °C (0.1 Torr)] afforded 1.71 g (67%) of **20**, a clear, colorless oil: ^1H NMR ($CDCl_3$) δ 6.77 (m, 3), 3.88 (s, 3), 3.87 (s, 3), 2.5–2.9 (m, 3), 1.22 (d, J = 7 Hz, 3), 1.15 (s, br, variable, 2). An analytical sample of the amine salt was prepared by crystallization from concentrated HCl–isopropyl alcohol–ether: mp 202–204 °C. Anal. ($C_{11}H_{18}ClNO_2$) C, H, N.

2-(3,4-Dihydroxyphenyl)propylamine Hydrochloride (12). To a solution of 3.75 g (19.2 mmol) of **20** in 150 mL of dichloromethane, under nitrogen at −60 °C, was added dropwise 5.46 mL (57.7 mmol) of BBr_3. The mixture was stirred at −60 °C for 5 min and at room temperature overnight. The mixture was quenched with 20 mL of anhydrous methanol and the solution evaporated to a dark oil. The oil was chromatographed through a cationic exchange column (35 g of Dowex 50W-X4, 50–100 mesh) with aqueous HCl, which resulted in the isolation of an orange semisolid (3.62 g). This residue in 50 mL of isopropyl alcohol was heated on the steam bath, cooled, and then treated with 150 mL of ether. Filtration afforded 2.36 g (61%) of **12**, an off-white, cream-colored solid with mp 171–175 °C. Recrystallization from ethanol–ether provided an analytical sample: mp 175–178 °C; ^1H NMR (D_2O) δ 6.7–7.1 (m, 3), 2.8–3.4 (m, 3), 1.33 (d, J = 7 Hz, 3); chemical-ionization mass spectrum m/e 168 (MH^+). Anal. ($C_9H_{14}ClNO_2$) C, H, N.

***N*-Methyl-2-(3,4-dimethoxyphenyl)propylamine (22).** To a mixture of 1.44 g (7.39 mmol) of amine **20** in 40 mL of water at 0 °C was added 0.30 mL (3.75 mmol) of ethyl chloroformate. This was then followed by the simultaneous addition of an additional 0.30 mL of ethyl chloroformate and 0.30 g (7.5 mmol) of NaOH in 4 mL of water. The solution was stirred at 0 °C for 1.5 h and extracted with dichloromethane (2 × 30 mL). The dichloromethane solution was dried ($MgSO_4$) and evaporated to give 1.84 g (93%) of carbamate **21**, a clear, almost colorless oil: ^1H NMR ($CDCl_3$) 6.84 (m, 3), 4.11 (q, J = 7 Hz, 2), 3.89 (s, 3), 3.87 (s, 3), 3.35 (m, 2), 2.87 (m, 1), 1.27 (d, J = 7 Hz, 3p), 1.22 (t, J = 7 Hz, 3). The crude carbamate **21** was used directly in the next step.

To a slurry of 0.60 g (15.7 mmol) of $LiAlH_4$ in 30 mL of dry THF (under nitrogen) was added a solution of 1.40 g (5.24 mmol) of carbamate **21** in 15 mL of THF, and the mixture was heated under reflux overnight for 21 h. After cooling it was quenched successively with 0.6 mL of water, 0.6 mL of 15% aqueous NaOH, and 1.8 mL of water. The resultant white mixture was filtered and the solution dried ($MgSO_4$) and evaporated to give a clear, almost colorless oil (1.0 g). Bulb-to-bulb distillation [oven temperature 90 °C (0.5 Torr)] afforded 0.93 g (85%) of **22**, a clear, colorless oil: ^1H NMR ($CDCl_3$) δ 6.87 (m, 3), 3.90 (s, 3), 3.88 (s, 3), 2.5–3.0 (m, 3), 2.40 (s, 3), 1.65 (very broad, variable, 1), 1.26 (d, J = 7 Hz, 3). Anal. ($C_{12}H_{19}NO_2$) C, H, N.

***N*-Methyl-2-(3,4-dihydroxyphenyl)propylamine Hydrobromide (15).** To a solution of 390.7 g (1.87 mmol) of **22** in 20 mL of dichloromethane (under nitrogen) at −60 °C was added 0.54 mL (3 equiv) of BBr_3. The mixture was stirred at −60 °C for 5 min and then allowed to warm to room temperature where it was stirred for 2 h. The mixture was cautiously quenched with 2 mL of methanol. The resultant solution was evaporated to give a dark oil. On standing, white crystals appeared. Treatment with ether and filtration afforded 130.9 mg (27%) of **15**: mp 136–140 °C; ^1H NMR (D_2O) δ 6.75–7.2 (m, 3), 3.25 (m, 3), 2.81 (s, 3), 1.33 (d, J = 7 Hz, 3). Anal. ($C_{10}H_{16}BrNO_2$) C, H, N.

2-Nitro-4,5-dimethoxyphenylacetonitrile (23). To a solution of 30.5 g (0.172 mol) of 3,4-dimethoxyphenylacetonitrile in 125 mL of acetic acid was added 45 mL of concentrated nitric acid (in portions). An ice bath was utilized to prevent the temperature from rising above 65 °C. After the addition was complete, the dark mixture was stirred at 10 °C for 15 min and poured into 300 mL of ice water, this mixture kept in an ice bath for 30 min. Following suction filtration, the yellow product in ethanol was heated on the steam bath, cooled, and filtered. The light yellowish solid was dried overnight in vacuo, affording 22.80 g (60%) of **23**: mp 110–112 °C (lit.[25] mp 111–113 °C).

5,6-Dimethoxyindole (24). A mixture of 10.0 g (0.044 mol) of **23**, 0.7 g of 10% Pd/C, and 250 mL of ethyl acetate was shaken in a Parr hydrogenator under hydrogen (40 psi) for 2.5 h at 80 °C. The hot mixture was suction filtered through Celite and evaporated to a small volume from which the white product crystallized. The product was filtered and air-dried affording 5.85 g (75%) of **24**: mp 153–155 °C (lit.[25] mp 154–156 °C).

5,6-Dimethoxyindoline (25). To a slurry (under nitrogen) of 2.0 g (11.3 mmol) of indole **24** in 60 mL of acetic acid was slowly added 2.85 g (45.2 mmol) of sodium cyanoborohydride; the resultant solution was stirred at room temperature overnight. To the cooled mixture was added 250 mL of 4 N aqueous NaOH in portions and this mixture was extracted with dichloromethane (3 × 100 mL). The dichloromethane was washed with 50 mL of water and 50 mL of brine, dried ($MgSO_4$), and evaporated to yield 1.77 g (86%) of **25**, an off-white solid: mp 98–102 °C (lit.[11] mp 108 °C); ^1H NMR ($CDCl_3$) δ 6.81 (s, 1), 6.39 (s, 1), 3.81 (s, 6), 3.40–3.60 (m, 2), 2.79–3.15 (m, 2). Indoline **25** was used as such in the preparation of indoline 4a.

5,6-Dihydroxyindoline (4a). A mixture of 0.30 g (1.66 mmol) of indoline **25** and 4 mL of concentrated HCl (under nitrogen) was heated in a small Teflon-lined metal bomb for 5 h at 145–147 °C. After cooling, the solution was evaporated to a reddish-brown solid residue (mp 215–225 °C). The solid was dissolved in absolute ethanol and ether was gradually added until appreciable crystallization took place. After being allowed to stand for several hours to ensure complete crystallization, the crystals were suction filtered, washed with ether, and air-dried to afford 200.3 mg (65%) of **4a**: mp 230–233 °C dec (lit.[11] mp 234–236 °C); ^1H NMR (D_2O) δ 6.90 (s, 1), 6.86 (s, 1), 3.67 (t, J = Hz, 2), 3.03 (t, J = 7 Hz, 2).

B. Electrochemistry. Dopamine and epinine were obtained from Aldrich, epinephrine from Sigma, norepinephrine from Calbiochem, and α-methyldopamine from Merck, Sharp & Dohme. Britton-Robinson buffers, utilized for all runs, were prepared from double distilled water. All buffer solutions were electrolyzed prior to use. Each cell solution was purged of oxygen and maintained under an atmosphere of nitrogen during all electrochemical studies.

Cyclic voltammetry was utilized for these studies. This is a rapid voltage-scanning technique which employs a stationary working electrode (carbon paste) in quiet solution and applies a repetitive, triangular (isosceles) wave potential sweep between it and a reference electrode (saturated calomel). The sweep intervals include a potential range of −0.8 to +0.8 V vs. SCE. The anodic and cathodic currents are recorded as a function of the

applied triangular potential sweep on an X–Y recorder.

C. Animal Study. Male Sprague–Dawley normotensive rats weighing 380 ± 20 g were used in the pressor experiments. Rats were purchased from Simonson Co. and were housed in individual cages. Water and food were given ad libitum.

The left carotid artery and right jugular vein were cannulated (under anesthesia) in order to measure blood pressure and heart rate or to administer drugs. The anesthetic used for the surgery was ip sodium pentobarbital (50 mg/kg).

(1) Cannulation of the Left Carotid Artery for Blood Pressure and Heart Rate Measurements. The left carotid artery was blunt dissected on the left neck lesion and a PE-50 tube with a 2.6-cm silastic (0.020 in. i.d. × 0.027 in. o.d.) was inserted 3 cm toward the aortic arch. The cannula was flushed with 0.1 mL of heparinized normal saline solution (50 units/mL) to deter coagulation. The cannula was then fixed on the rat's back with tape.

(2) Cannulation of the Right Jugular Vein for Continuous Drug Infusion. The right jugular vein was blunt dissected on the right neck lesion and a PE-50 tube with a 1.5-cm silastic (0.030 in. i.d. × 0.065 in. o.d.) was cannulated 3 cm toward the superior vena cava. The cannula was flushed with 0.1 mL of heparinized normal saline solution (50 units/mL) and fixed on the rat's back with tape.

(3) Drug Infusion. The rats were housed in their individual restraining cages 3 days after the recovery period. Drugs dissolved in normal saline solution were infused through the jugular vein catheter. The infusion volume was 0.25 mL (i.e., 0.1 mL of the drug was dissolved in normal saline solution and administered, and the catheter was then flushed with 0.15 mL of the normal saline solution).

(4) Blood Pressure and Heart Rate Measurements. The blood pressure was measured by the use of the carotid arterial catheter. The heart rate also was calculated from the blood pressure tracings. A Grass Model 5 polygraph equipped with a Statham P23Dc transducer was utilized for the monitoring. Calibrations were performed each time by use of both external and internal methods.

Acknowledgment. These studies were supported by NIMH Research Grant MN 21219.

References and Notes

(1) D. C. S. Tse, R. L. McCreery, and R. N. Adams, *J. Med. Chem.*, **19**, 37 (1976), and references cited therein.
(2) For a review of 6-hydroxydopamine, see R. M. Kostrzewa and D. M. Jacobowitz, *Pharmacol. Rev.*, **26**, 199 (1974).
(3) 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).
(4) (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).
(5) M. D. Hawley, S. V. Tatawawadi, S. Piekarski, and R. N. Adams, *J. Am. Chem. Soc.*, **89**, 447 (1967).
(6) C. L. Blank, R. L. McCreery, R. M. Wightman, W. Chey, R. N. Adams, J. R. Reid, and E. E. Smissman, *J. Med. Chem.*, **19**, 178 (1976).
(7) A. Kalir, C. R. Freed, K. L. Melmon, and N. Castagnoli, *J. Labelled Compd.*, **12**, 41 (1976).
(8) For a preparation of the *ethyl* benzylic ethers, see C. R. Freed, R. J. Weinkam, K. L. Melmon, and N. Castagnoli, *Anal. Biochem.*, **78**, 319 (1977).
(9) D. S. Watt, *Tetrahedron Lett.*,'707 (1974).
(10) O. M. Friedman, K. N. Parameswaran, and S. Burstein, *J. Med. Chem.*, **6**, 227 (1963).
(11) S. N. Mishra and G. A. Swan, *J. Chem. Soc. C*, 1424 (1967).
(12) G. W. Gribble, P. D. Lord, J. Skotnicki, S. E. Dietz, J. T. Eaton, and J. L. Johnson, *J. Am. Chem. Soc.*, **96**, 7812 (1974).
(13) R. S. Nicholson and I. Shain, *Anal. Chem.*, **36**, 796 (1964).
(14) Adams first reported observing these redox couples on electrochemical oxidation of dopamine at pH 7 and tentatively assigned the indoline and iminoquinone couple: R. N. Adams, E. Murrill, R. McCreery, L. Blank, and M. Karolczak, *Eur. J. Pharmacol.*, **17**, 287 (1972).
(15) N. L. Allinger and V. Zalkow, *J. Org. Chem.*, **25**, 701 (1960).
(16) We performed a potentiometric titration on dopamine hydrobromide and observed that 1.5 equiv of base was required to reach the equivalence (inflection) point (pK_a approximately 8.8).
(17) For a further discussion of the various exchangeable catecholamine species that are present at specific pH values, see C. R. Ganellin, *J. Med. Chem.*, **20**, 579 (1977).
(18) M. M. Tuckerman, J. R. Mayer, and F. C. Nachod, *J. Am. Chem. Soc.*, **81**, 92 (1959).
(19) G. P. Lewis, *Br. J. Pharmacol.*, **9**, 488 (1954).
(20) This value was determined in our laboratory.
(21) (a) M. S. B. Munson, *J. Am. Chem. Soc.*, **87**, 2332 (1965); (b) J. I. Brauman and L. K. Blair, *ibid.*, **90**, 5636 (1968); (c) J. I. Brauman, J. M. Riveros, and L. K. Blair, *ibid.*, **93**, 3914 (1971).
(22) (a) D. H. Everett and W. F. K. Wynne-Jones, *Proc. R. Soc., London, Ser. A*, **169**, 190 (1938); (b) *Trans. Faraday Soc.*, **35**, 1380 (1939).
(23) (a) E. M. Arnett, F. M. Jones, III, M. Taagepera, W. G. Henderson, J. L. Beauchamp, D. Holtz, and R. W. Taft, *J. Am. Chem. Soc.*, **94**, 4724 (1972); (b) D. H. Aue, H. M. Webb, and M. T. Bowers, *ibid.*, **94**, 4726 (1972); (c) W. G. Henderson, M. Taagepera, D. Holtz, R. T. McIver, Jr., J. L. Beauchamp, and R. W. Taft, *ibid.*, **94**, 4728 (1972).
(24) A. Noble, *Exp. Physiol. Biochem.*, **6**, 1 (1973).
(25) G. N. Walker, *J. Am. Chem. Soc.*, **77**, 3844 (1955).