Ultraviolet Absorption Spectra and Apparent Acidic Dissociation Constants of Some Phenolic Amines[1]

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Data are listed and discussed for the spectral properties and the apparent acidic dissociation constants of several phenolic amines. Many of the compounds are of interest because they occur naturally. Several are closely related, so the effect of structural alterations on the physical properties could be observed. The most significant observation, from the physiological viewpoint, is that the pK_a' for the first phenolic hydroxyl group of catechol compounds is about 0.8 unit lower than that of the corresponding monophenolic compounds. The increased amount of anion present at physiological pH might be partially responsible for the enhanced biological activity of the catechol amines. Representative examples are given for the preparation of phenolic amines by $LiAlH_4$ reduction of representative precursors. The use of ion-exchange resins for separation of phenolic amines from the resulting reaction mixture is described. The preparation and physical properties of two new phenolic amines, 2,4-dihydroxyphenethylamine and β-(2-hydroxyphenyl)ethanolamine (*o*-octopamine), are described.

Knowledge of the dissociation constants of phenolic amines is of interest for several reasons. Many compounds of this nature are now known to occur in tissues of animals where they exert important physiological effects. Information about their physical properties is useful for the planning of their isolation and separation with chromatographic, electrophoretic, or solvent distribution procedures. Further, some correlation between physical properties and physiological activity might exist and could provide an indication as to the mode of their physiological action. Leffler, *et al.*,[2] reported values for the dissociation constants of 27 adrenergic amines, 9 of which contained phenolic hydroxyl groups. However, only 3 of their compounds, phenethylamine, epinephrine, and *p*-sympatol, possibly occur in mammalian metabolism; the remainder were synthetic products. Lewis[3] published data on the dissociation constants for 24 amines. Values were listed for the phenolic group which was present in 17 of the compounds; 7 of his compounds possibly occur in mammals and the remainder were drugs. Shapiro[4] has examined the data of Lewis for correlations between biological activity and ionization. Finally, Tuckerman, *et al.*,[5] have reported dissociation constants for some substituted phenethylamines and have made some suggestions, based upon their data, for the internal structure of the amines.

The pK_a' values for the phenolic hydroxyl and the amine groups of phenolic amines are quite similar so it is not possible with titration methods to determine which group ionizes first, nor can accurate pK values for either be determined. An example of this uncertainty is provided by the report of Ogston[6] who assigned values incorrectly for the pK' of the phenolic and amine groups of *p*-tyramine. Lewis[3] discussed this difficulty, which will be considered in more detail later in this paper. Unfortunately, Leffler, *et al.*,[2] and Tuckerman, *et al.*,[5] failed to take into account the dissociation of the phenolic hydroxyl of the phenolic amines they studied so their values for the pK_a' of the amine dissociation of compounds which also contained a phenolic hydroxyl are erroneous. The speculations of Tuckerman, *et al.*, on the internal structure of the amines, therefore, have no experimental support and will not be discussed further.

A number of phenolic amines were obtained for studies in our laboratory. These included several which might be expected to occur in mammals and for which ionization data had not been reported. Further, they included sufficient closely related compounds that several series of amines could be arranged so that the effect of different substituents on the ionization of the phenolic and amine groups might be examined.

Results and Discussion

Spectral Properties of Phenolic Amines.—The pK_a' values for the phenolic and amine groups of most of the compounds examined here were so close that the phenol dissociation had to be determined with the spectrophotometric method. The spectral data obtained for the purpose of measuring the phenolic dissociation constants are listed, and, as representative examples, alterations in the ultraviolet absorption spectra of β-(2-hydroxyphenyl)ethanolamine, β-(4-hydroxyphenyl)ethanolamine, and β-(2,4-dihydroxyphenyl)ethylamine as a function of pH are illustrated in Figures 1–3. The maxima and isobestic points of the phenolic amines which were studied are listed in Table I. Data are not given for the three nonphenolic phenethylamines because alterations in pH had essentially no effect on their absorption spectra; this fact validates the use of the spectrophotometric method for measurement of the degree of dissociation of the phenolic group in the other compounds.

For a monophenol all the absorption spectra at different pH values pass through three isobestic points, provided certain precautions concerning the solutions are met; *i.e.*, that accurate dilutions of the amines are made from one stock solution and that buffer solutions with the same ionic strength are used. Curves made for catechol and resorcinol derivatives pass through one set of isobestic points up to a pH of 10.3–10.5. In solu-

(1) This work was supported in part by Research Grant MH-02278 from the National Institute of Mental Health, U. S. Public Health Service.

(2) E. B. Leffler, H. M. Spencer, and A. Burger, *J. Am. Chem. Soc.*, **73**, 2611 (1951).

(3) G. P. Lewis, *Brit. J. Pharmacol.*, **9**, 488 (1954).

(4) H. Shapiro, *J. Theoret. Biol.*, **1**, 289 (1961).

(5) M. M. Tuckerman, J. R. Mayer, and F. C. Nachod, *J. Am. Chem. Soc.*, **81**, 92 (1959).

(6) A. G. Ogston, *J. Chem. Soc.*, 1713 (1936).

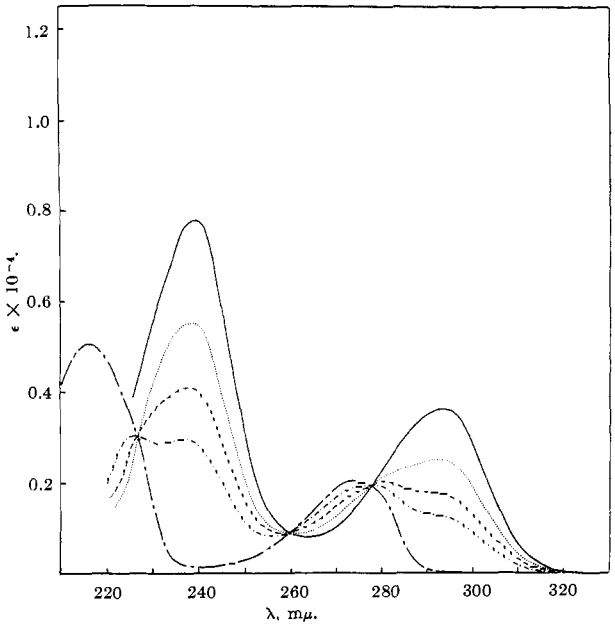

Figure 1.—Ultraviolet absorption spectra of β-(2-hydroxyphenyl)ethanolamine, 1×10^{-4} *M*: ———, 0.1 *N* NaOH; — — —, 0.1 *N* HCl; - · - · -, pH 9.05; - - - -, pH 9.33; . . . . . , pH 9.89.

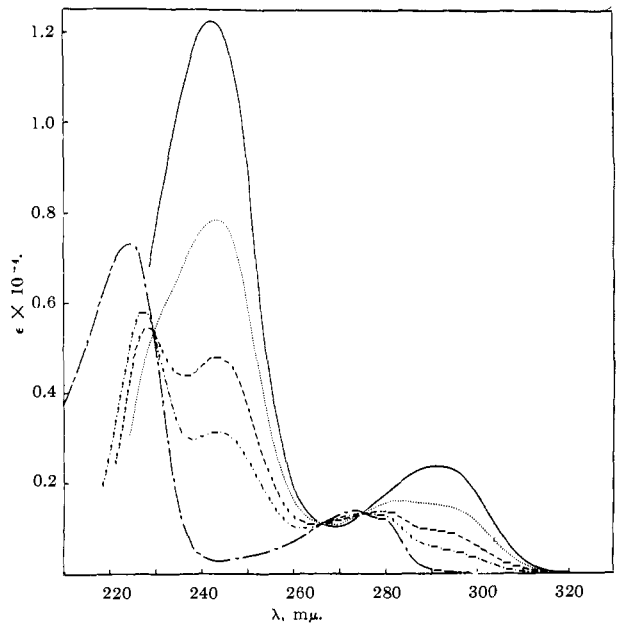

Figure 2.—Ultraviolet absorption spectra of β-(4-hydroxyphenyl)ethanolamine, 1×10^{-4} *M*: ———, 0.1 *N* NaOH; — — —, 0.1 *N* HCl; - · - · -, pH 9.05; - - - -, pH 9.33; . . . . . , pH 9.89.

tions at higher pH the maxima begin to shift and new isobestic points appear. In the case of 2,4-dihydroxyphenethylamine[7] (Figure 3) the maximum at 292 mμ (monoanion) shifts to 299 mμ and the maximum at 237 shifts to 224 mμ, and a new isobestic point develops at

(7) This is the only diphenolic compound the behavior of which was studied at higher pH values. The catechol derivatives were not examined because of their instability, as commented on in the text. The pK_a for the second phenolic hydroxyl of catechol itself has been reported to be greater than 12.0: G. Abichandani and S. K. K. Jatkar, *J. Indian Inst. Sci.*, **21A**, 417 (1938). For 2,4-dihydroxyphenethylamine, the dissociation of the first hydroxyl group ($pK_a' = 8.91$) was assumed to be essentially complete before ionization of the second hydroxyl group commenced. As commented in the text, the value for the second hydroxyl group is probably not exact. It was necessary to use the values for the phenolic groups to estimate the pK_a' for the amino group, which is, thus, even more uncertain.

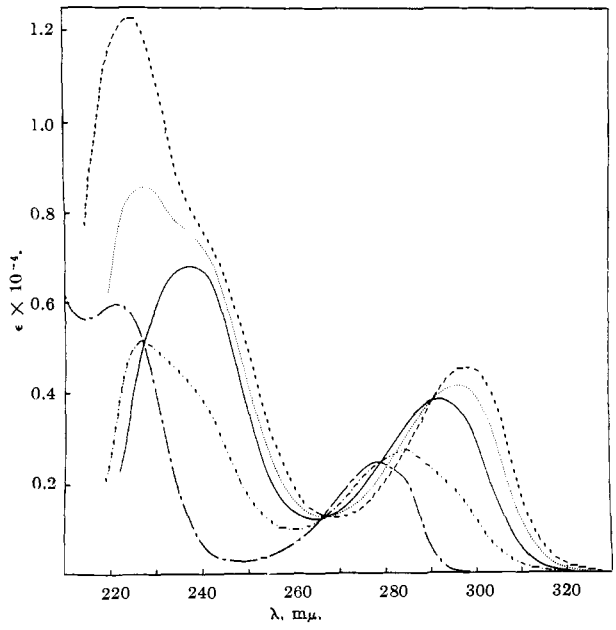

Figure 3.—Ultraviolet absorption spectra of 2,4-dihydroxyphenethylamine, 1×10^{-4} *M*: — — —, 0.1 *N* HCl; - · - · -, pH 8.95; ———, pH 10.50; . . . . . , pH 11.87; - - - -, 1 *N* NaOH (pH 14.0).

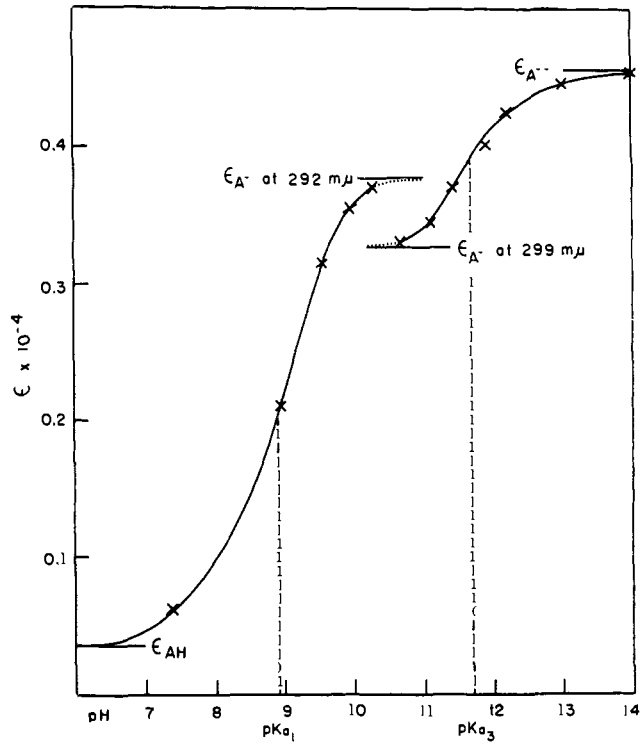

Figure 4.—Spectrophotometric estimation of dissociation of phenolic hydroxyl groups of 2,4-dihydroxylphenethylamine.

291 mμ. In Figure 4 is shown a plot of the extinction coefficients at 292 and 299 mμ *vs.* pH. It can be seen that the absorption for the monoanion can be determined definitively and the first phenolic pK_a' can be calculated accurately. Examination of the plot representing the absorption at 299 mμ, however, reveals that the determination of the second phenolic pK_a' is somewhat arbitrary because of the small difference in the absorption of the two species (*i.e.*, the monoanion and the dianion). The fact that the absorption curve of the monoanion corresponds much more to that of 2-hydroxyphenethyl-

TABLE I

ULTRAVIOLET ABSORPTION SPECTRA OF PHENOLIC PHENETHYLAMINES AND β-PHENYLETHANOLAMINES IN AQUEOUS SYSTEMS

No.	Compd.[a]	Anion[b] λmax, mμ (ε)		Cation λmax, mμ (ε)		Isobestic points λ, mμ (ε)	
4	2-Hydroxyphenethylamine	291 (3650)	239 (8200)	sh 277 (1850)	273 (2000)	276 (1900)	261 (1000) 227 (3100)
5	3-Hydroxyphenethylamine	291 (2850)	239 (8100)	sh 278 (1500)	273 (1700)	276 (1550)	261 (850) 227 (3100)
6	4-Hydroxyphenethylamine	294 (2650)	239 (10,500)	sh 280 (1400)	275 (1600)	278 (1500)	265 (950) 228 (5100)
7	N-Methyl-4-hydroxyphenethylamine	294 (2650)	239 (10,450)	sh 280 (1400)	275 (1600)	278 (1500)	265 (950) 228 (5100)
8	N,N-Dimethyl-4-hydroxyphenethylamine	294 (2600)	239 (10,400)	sh 280 (1400)	275 (1600)	278 (1500)	265 (950) 228 (5000)
9	2,4-Dihydroxyphenethylamine	292 (3750)[c]	237 (6700)[d]	sh 283 (2200)	278 (2450)	279 (2450)[f]	266 (1250) 228 (5000)
10	β-(2-Hydroxyphenyl)ethanolamine	293 (3700)	239 (7900)	sh 278 (1950)	274 (2100)	277 (1950)	260 (900) 227 (3100)
11	β-(3-Hydroxyphenyl)ethanolamine	292 (3000)	239 (8100)	sh 278 (1700)	274 (1800)	277 (1700)	260 (850) 227 (2900)
12	β-(4-Hydroxyphenyl)ethanolamine	292 (2400)	242 (12,200)	sh 279 (1200)	274 (1400)	275 (1350)	267 (1100) 230 (5450)
13	N-Methyl-β-(4-hydroxyphenyl)ethanolamine	292 (2400)	242 (12,100)	sh 279 (1150)	273 (1350)	275 (1300)	267 (1100) 230 (5500)
14	N,N-Dimethyl-β-(4-hydroxyphenyl)ethanolamine	292 (2400)	242 (12,200)	sh 279 (1150)	273 (1350)	275 (1300)	267 (1100) 230 (5500)
15	β-(4-Hydroxy-3-methoxyphenyl)ethanolamine	294 (4100)	247 (10,000)[e]	sh 283 (2450)	279 (2750)	280 (2700)	270 (2000) 234 (5300)
16	N-Methyl-β-(4-hydroxy-3-methoxyphenyl)ethanolamine	294 (4200)	247 (10,600)[e]	sh 283 (2500)	279 (2800)	280 (2750)	270 (2100) 234 (5350)
17	N,N-Dimethyl-β-(4-hydroxy-3-methoxyphenyl)ethanolamine	294 (4150)	247 (10,600)[e]	sh 283 (2500)	279 (2800)	280 (2750)	270 (2000) 234 (5500)
18	β-(3,4-Dihydroxyphenyl)ethanolamine	295 (4500)	243 (7100)	sh 285 (2450)	280 (2750)	281 (2750)	266 (1400) 232 (4800)
19	N-Methyl-β-(3,4-dihydroxyphenyl)ethanolamine	295 (4400)	243 (7000)	sh 285 (2500)	280 (2750)	281 (2750)	267 (1500) 232 (4900)

[a] The most commonly used synonym for these compounds are (compound indicated by numbers): **6**, *p*-tyramine; **8**, hordenine; **12**, octopamine; **13**, synephrine, *p*-sympatol; **15**, normetanephrine; **16**, metanephrine; **18**, Arterenol, norepinephrine, Noradrenaline; **19**, Adrenaline, epinephrine. [b] For the dibasic phenols (**9**, **18**, and **19**) only the data for the monoanions are given in the table. [c] The corresponding maximum for the dianion is at 299 mμ (ε 4600). [d] The corresponding maximum for the dianion is at 224 mμ (ε 12,300). [e] The cation form of these compounds shows another maximum at 208 mμ with an extinction coefficient of about 7000. [f] The isobestic points for the dianion are at 291 mμ (ε 3850) and 266 mμ (ε 1200). [g] The anion form of these compounds shows a third maximum at 218 mμ (ε 7000).

amine than to that of 4-hydroxyphenethylamine is suggestive that in this resorcinol derivative the phenolic group in the position *ortho* to the side chain is the first to dissociate.

Apparent Dissociation Constants of Phenolic Amines. Lewis[3] used a spectrophotometric method to determine independently the dissociation of the phenolic group and a potentiometric titration to measure both phenolic and amine dissociation constants for several phenolic amines. With many of the compounds there was good agreement in the values found for the phenolic group by both methods but, with some monophenolic β-phenylethanolamines in which the pK_a' values for the two ionizing groups were within 0.5 of a unit, he observed discrepancies of the order of 0.5 for the phenolic constant as determined by the different methods. In those instances, therefore, he determined the phenolic pK_a' by the spectrophotometric method and calculated the pK_a' of the amino group from his titration data and by the use of the equation derived by Britton.[8]

In the present work the dissociation constants for the phenolic groups were determined by the spectrophotometric method. The dissociation constants of the amino groups were derived graphically from potentiometric titration curves using the spectrophotometrically determined pK_a' of the phenolic group as described later.

The apparent dissociation constants obtained for various phenethylamines and β-phenylethanolamines are listed in Table II. For comparison, the pK_a' values for some amines as found by Lewis[3] are listed. It may be noted that the values are, in general, in good agreement; this is especially true for the phenolic pK_a' values.

Although the effects of altered chemical structure on the dissociation constants of the compound are readily apparent by inspection of the data, some of the observations warrant comment.

(1) The pK_a' of the phenolic group in phenylethanolamines is lowered by 0.1–0.2 unit when the *ortho*, *meta*, and *para* isomers are compared with the corresponding isomers in the phenethylamine series. This decrease probably occurs because of a mild electron-withdrawing effect of the methylol group on the nucleus. Sprengling and Lewis[9] observed the same effect in about the same magnitude when they compared *m*- and *p*-methylolphenol with phenol itself. They found no difference between the pK_a' of *o*-methylolphenol and phenol and concluded that the phenolic hydrogen of *o*-methylolphenol is held more firmly by formation of a hydrogen bond to the α-hydroxyl so that the inductive effect of the methylol group is overcome. With *o*-hydroxyphenylethanolamine such an effect is probably repressed because of an interaction between the phenolic hydrogen and the charged amino group which results in an increased acidity of the phenolic group. In the phenethylamine series the phenolic pK_a' of the *ortho* isomer is 0.22–0.26 unit lower than those of the three *para*-hydroxy compounds, while the basicity of the amine group of the *ortho* isomers in both series is increased by 0.23–0.24 unit, as compared with the *meta* and *para* compounds. This probably occurs because of

(8) H. T. S. Britton, "Hydrogen Ions," Vol. 1, Chapman and Hall, London, 1942, p. 198.

(9) G. R. Sprengling and C. W. Lewis, *J. Am. Chem. Soc.*, **75**, 5701 (1953).

TABLE II

APPARENT DISSOCIATION CONSTANTS OF PHENETHYLAMINES AND β-PHENYLETHANOLAMINES AT 25°[a]

No.	Compd.	pK_a' Phenol	pK_a' Amine
1	Phenethylamine		9.88
2	2-Methoxyphenethylamine		10.20
3	3-Methoxyphenethylamine		9.89
4	2-Hydroxyphenethylamine	9.52	10.75
5	3-Hydroxyphenethylamine	9.58	10.50
6	4-Hydroxyphenethylamine	9.74 (9.77)	10.52 (10.73)
7	N-Methyl-4-hydroxyphenethylamine	9.76	10.71
8	N,N-Dimethyl-4-hydroxyphenethylamine	9.78	10.02
9	2,4-Dihydroxyphenethylamine	8.91, 11.7[b]	10.8[b]
10	β-(2-Hydroxyphenyl)ethanolamine	9.42	9.90
11	β-(3-Hydroxyphenyl)ethanolamine	9.56	9.63
12	β-(4-Hydroxyphenyl)ethanolamine	9.57 (9.53)	9.66 (9.70)
13	N-Methyl-β-(4-hydroxyphenyl)ethanolamine	9.55 (9.59)	9.79 (9.71)
14	N,N-Dimethyl-β-(4-hydroxyphenyl)ethanolamine	9.58 (9.56)	9.50 (9.76)
15	β-(4-Hydroxy-3-methoxyphenyl)ethanolamine	9.54	9.56
16	N-Methyl-β-(4-hydroxy-3-methoxyphenyl)ethanolamine	9.52	9.74
17	N,N-Dimethyl-β-(4-hydroxy-3-methoxyphenyl)ethanolamine	9.54	9.52
18	β-(3,4-Dihydroxyphenyl)ethanolamine	8.72 (8.90)	9.72 (9.78)
19	N-Methyl-β-(3,4-dihydroxyphenyl)ethanolamine	8.75 (8.88)	9.89 (9.90)

[a] Values in parentheses are those reported by Lewis[3]; his phenolic group values were obtained spectrophotometrically. [b] The pK_a' for the second phenolic hydroxyl was estimated from the data shown in Figure 4 in the manner described in the text. The pK_a' value for the amino group was estimated from the titration curve with the use of the values for the phenolic groups.

an attraction of the phenoxide ion for the dissociating proton of the adjacent ammonium ion so that the proton remains associated with the phenolic amine molecule rather than combining with water, while the presence of the adjacent charged amino group would promote the dissociation of the proton of the phenolic hydroxyl group.

(2) The presence of a second phenolic hydroxyl group causes a marked increase in the acidity of the first phenolic group to dissociate. The first pK_a' of the resorcinol compound is lowered about 0.6 unit and of the catechol derivatives 0.8 unit as compared with a monophenol.

(3) The presence of an alcoholic hydroxyl group in the side chain always decreases the pK_a' of the amino group. Because of this effect the pK_a' of the phenolic and amine groups of the monophenolic β-phenylethanolamines are nearly the same numerical value. In two cases (compounds **7** and **14**) the pK_a' (amino) actually appears to be lower than the pK_a' (phenol).

(4) The substitution of an amino group with one methyl group leads in all cases to about the same increase in basicity, *i.e.*, 0.13, 0.17, 0.18, and 0.19 units, respectively. The introduction of a second methyl group results in a decreased basicity of the dimethylamines as compared with the related monomethyl compounds.

In the past, efforts to determine possible correlations between ionization and physiological activity of phenolic amines have been focussed mainly on the dissociation of the amine group. The pK_a' of the amine group of all the compounds studied here is 9.5 or greater, so at physiological pH the compounds are almost completely in the cation form. It seems unlikely that even marked differences in the basicity of the amine group would have a significant effect on the physiological activity of these compounds. However, a possible significance of the markedly decreased pK_a' for the first phenolic group of catechol derivatives as compared with monophenolic compounds has not been commented upon previously in connection with the enhanced biological activity of the catechol amines. The first pK_a' (phenol) values for hydroxytyramine (dopamine, 3,4-dihydroxyphenethylamine, 8.92[3]), norepinephrine, and epinephrine are 0.82, 0.85, and 0.80 unit, respectively, more acidic than the corresponding *para*-hydroxy compounds, *p*-tyramine, octopamine, and *p*-sympatol. At the pH of blood (7.4) considerably less than 1% of the monophenols would have a phenolic group in the ionized state, but about 5% or more of the catechol compounds would have a negatively charged phenoxide ion. This might be of importance in facilitating attachment of the catechols to a receptor site where they could exert a physiological effect, or to enzymes which might inactive them.

Experimental[10]

Compounds.—Redistilled *p*-cresol (Matheson Coleman and Bell) was recrystallized from petroleum ether (b.p. 30-60°). Phenethylamine (Matheson Coleman and Bell) was converted to the hydrochloride, which was recrystallized from an ethanol-ethyl acetate mixture. *p*-Tyramine hydrochloride (Distillation Products) was recrystallized from an ethanol–ethyl acetate mixture. Hordenine sulfate hydrate and DL-metanephrine hydrochloride were obtained from Calbiochem. *p*-Sympatol tartrate (K and K Laboratories) was converted to the free base, which was recrystallized from aqueous ethanol; DL-arterenol hydrochloride, DL-normetanephrine hydrochloride, and *l*-epinephrine bitartrate were obtained from Winthrop Laboratories. 2-Methoxyphenethylamine hydrochloride,[11] 2-hydroxyphenethylamine hydrochloride[12] (*o*-tyramine), 3-hydroxyphenethylamine hydrochloride[13] (*m*-tyramine), N-methyl-4-hydroxyphenethylamine,[14] DL-3-hydroxyphenylethanolamine hydrochloride[15] (*m*-octopamine), and N,N-dimethyl-DL-4-hydroxy-3-methoxyphenyl-

(10) All melting points were measured in open capillary tubes and are corrected.

(11) K. H. Slotta and H. Heller, *Ber.*, **63B**, 3029 (1930).

(12) R. Pschorr and H. Einbeck, *ibid.*, **38**, 2072 (1905).

(13) Bayer and Co., German Patent 233,551 (July 28, 1909); *Chem. Zentr.*, I, 1334 (1911).

(14) G. S. Walpole, *J. Chem. Soc.*, **97**, 944 (1910).

(15) A. Chatterjee, S. K. Srimany, and B. Chaudhury, *ibid.*, 4576 (1961).

ethanolamine[16] were prepared by $LiAlH_4$ reduction of appropriate precursors in the manner described below; the physical properties of the compounds were in agreement with those cited in the literature and their nitrogen content corresponded closely with the calculated amounts.

2-Hydroxyphenylethanolamine (*o*-Octopamine).—A solution of 40 g. (0.266 mole) of salicylaldehyde cyanhydrin[17] in 200 ml. of absolute ether was added dropwise over a period of 6 hr. to the returning ether condensate of a well-stirred refluxing solution of 40 g. (1.05 moles) of $LiAlH_4$ in 2 l. of absolute ether; the reaction system was kept under argon at all times. After the addition of cyanhydrin was completed, the refluxing and stirring was continued overnight. The reaction mixture was cooled and hydrolyzed by the cautious addition of 2.5 l. of water, and concentrated HCl was then added slowly until all the inorganic material had dissolved. The ether layer was separated and discarded and the acidic aqueous layer was passed through a 7.5 × 60 cm. column of Amberlite CG-120 (H^+) (100–200 mesh). The resin was washed with 1 l. of water, and the amine was eluted with 4 *N* NH_4OH; 3 l. of alkaline eluate was collected. This solution was evaporated to dryness *in vacuo*, the residue was dissolved in 600 ml. of hot absolute ethanol, and a small amount of insoluble material was collected on a filter and discarded. The ethanol solution was evaporated to dryness *in vacuo*, the residue was digested with 300 ml. of water at 60°, the resulting solution was filtered to remove another 4.5 g. of insoluble material, and the aqueous solution was evaporated to dryness *in vacuo*. The residual oil crystallized when it was digested with a small volume of absolute ether. This material was contaminated with resin from the column. Pure *o*-octopamine was extracted from it with absolute ether after 8 hr. with a continuous extraction apparatus. The *o*-octopamine crystallized in the flask during the extraction and was collected after the ether solution had been concentrated to a small volume. The yield was 6.55 g. (16.3%), m.p. 97–99°. One recrystallization from isopropyl ether yielded material melting at 98.5–100°.

Anal. Calcd. for $C_8H_{11}NO_2$: N, 9.14. Found: N, 9.10.

4-Hydroxyphenylethanolamine (octopamine) was prepared from 4-hydroxymandelonitrile[17] in the same manner. The first 900 ml. of alkaline eluate from the resin column was concentrated to dryness *in vacuo*, the residue was dissolved in 450 ml. of hot absolute ethanol, the solution was filtered to remove a small amount of insoluble residue, and a solution of 15.5 g. of *d*-tartaric acid in 150 ml. of hot absolute ethanol was added. DL-Octopamine *d*-tartrate[18] was collected after the solution had remained in the refrigerator overnight; yield, 42 g. (70%); m.p. 190–197° dec.

2,4-Dimethoxyphenethylamine.—The Soxhlet extractor technique was used for the addition of 14 g. (0.067 mole) of 2,4-dimethoxy-β-nitrostyrene[19] to a solution of 11 g. (0.29 mole) of $LiAlH_4$ in 350 ml. of absolute ether. The addition required 5 hr. The reaction mixture was then cooled and was hydrolyzed by the cautious addition of 50 ml. of water. The ether layer was separated and the aqueous layer was extracted four times with 50-ml. portions of ether. The combined ether extracts were dried (Na_2SO_4), and the amine hydrochloride was precipitated by passing dry HCl into the ethereal solution; yield, 12 g. (83%); m.p. 156–158°. A sample for analysis was recrystallized from absolute ethanol; m.p. 159°.

Anal. Calcd. for $C_{10}H_{16}ClNO_2$: N, 6.42. Found: N, 6.32.

The free base form of 2,4-dimethoxyphenethylamine had been prepared previously by Hofmann degradation of 2,4-dimethoxyhydrocinnamic acid amide.[20]

2,4-Dihydroxyphenethylamine.—A solution of 8 g. (0.037 mole) of 2,4-dimethoxyphenethylamine hydrochloride in 60 ml. of 48% HBr was refluxed in a nitrogen atmosphere for 2 hr. The mixture was then concentrated to dryness *in vacuo* and the residue was dissolved in 100 ml. of water. This solution was passed through a 4 × 8 cm. column of Amberlite CG-120 (H^+) (100–200 mesh), the column was washed with 100 ml. of water, and the product was then eluted with 4 *N* NH_4OH. The first 150 ml. of eluate was collected and evaporated to dryness in a nitrogen atmosphere under reduced pressure. The residue was recrystallized once from water (nitrogen atmosphere) and then from ethyl acetate to yield 3.7 g. (66%) of a product which had a slight pink color; m.p. 143–144°.

Anal. Calcd. for $C_8H_{11}NO_2$: N, 9.15. Found: N, 9.17.

A portion of the free amine was recrystallized from concentrated HCl acid and then from a mixture of absolute ethanol and ethyl acetate to yield **2,4-dihydroxyphenethylamine hydrochloride**, m.p. 192–193°.

Anal. Calcd. for $C_8H_{12}ClNO_2$: N, 7.38. Found: N, 7.32.

The free amine became more darkly colored after storage for 3 years, while the hydrochloride was stable. Buck[20] was unable to prepare the dihydroxy compound from the dimethoxy derivative by hydrolysis with hydriodic acid or with concentrated HCl at 180° because of side reactions.

N,N-Dimethyl-4-hydroxy-DL-mandelamide.—Ethyl 4-hydroxy-DL-mandelate[17] and an excess of anhydrous dimethylamine were sealed in a glass tube and heated at 100° for 14 hr. The excess dimethylamine was removed *in vacuo*, and the crystalline residue was washed with ether. The yield was 96%, m.p. 180–182°, of material which was sufficiently pure for the next step of the synthesis. For analysis a sample was recrystallized from 95% ethanol in the form of colorless prisms, m.p. 182–184°.

Anal. Calcd. for $C_{10}H_{13}NO_3$: N, 7.17. Found: N, 6.98.

N,N-Dimethyl-DL-4-hydroxyphenylethanolamine (N,N-Dimethyloctopamine).—A solution of 3.5 g. (0.018 mole) of N,N-dimethyl-4-hydroxymandelamide in 60 ml. of absolute dioxane was added dropwise to a well-stirred and refluxing solution of 5 g. (0.13 mole) of $LiAlH_4$ in 350 ml. of absolute ether. After the addition was completed, the stirring and heating were continued 6 hr. The reaction mixture was then cooled and processed in the manner described earlier for *o*-octopamine. After one recrystallization of the product from ether, 2.3 g. (71%) of free amine was obtained; m.p. 122–123°.

Anal. Calcd. for $C_{10}H_{15}NO_2$: N, 7.72. Found: N, 7.56.

3-Methoxyphenethylamine Hydrochloride.—The Soxhlet extractor technique was used for the addition of 10 g. (0.06 mole) of 3-methoxyphenylacetamide[21] to a well-stirred refluxing solution of 10 g. (0.214 mole) of $LiAlH_4$ in 400 ml. of absolute ether over a period of 15 hr. The reaction mixture was cooled and processed in the manner described for the preparation of 2,4-dihydroxyphenethylamine hydrochloride; yield, 8.5 g. (75%); m.p. 132–134°. After one recrystallization from a mixture of absolute ethanol and ethyl acetate the product was obtained as large glittering plates, m.p. 133–135.[22]

Anal. Calcd. for $C_9H_{14}ClNO$: N, 7.46. Found: N, 7.44.

Spectrophotometric Studies.—The ultraviolet absorption spectra were recorded with a Bausch and Lomb Spectronic 505 spectrophotometer equipped with a constant-temperature cell holder to maintain a temperature of 25°. The same matched pair of 1-cm. quartz cells was used in the same order throughout. All spectra were measured against a blank consisting of the aqueous buffer, alkali, or acid used as solvent for the phenolic compound. All the curves for one compound were recorded on the same sheet of paper. Occasionally, spectra which did not pass through the isobestic points were obtained; it was considered that dilution errors had probably occurred so a new measurement was made with a freshly prepared solution.

All spectra were recorded with an amine concentration of 1×10^{-4} *M*. In addition, the spectra of *m*-tyramine, *p*-tyramine, and octopamine were measured at a concentration of 2×10^{-4} *M*. A 1×10^{-3} *M* concentration proved to be more appropriate in most cases, since the absorbance values which were obtained allowed a calculation of the pK_a' values from the region of the maxima at about 290 mμ and also from that at 240 mμ from a single set of curves. At least two curves were recorded in buffer solutions with a pH near the pK_a' value; preferably one a little above the pK_a' and one a little below. The pK_a values were calculated according to the following equation

$$pK_a' = pH - \log \frac{\epsilon - \epsilon_{AH}}{\epsilon_{A^-} - \epsilon}$$

where ϵ_{AH} and ϵ_{A^-} are the extinction coefficients of un-ionized and

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(20) J. S. Buck, *J. Am. Chem. Soc.*, **54**, 3661 (1932).

(21) J. A. King and F. H. McMillan, *ibid.*, **68**, 2335 (1946).

(22) 3-Methoxyphenethylamine hydrochloride was reported previously to be hygroscopic and to melt at 145–146°: H. Heller, *Helv. Chim. Acta*, **7**, 948 (1924).

completely ionized species in regard to the phenolic group, and ϵ is the observed extinction coefficient of the sample in solutions at the specified pH.[3] Since the equation was derived with the assumption of a single total stoichiometric concentration for the sum of base and conjugate acid for each solution measured, accuracy was assured by making the complete series of solutions for each compound by diluting identical aliquots of the same stock solutions with equal volumes of glycine buffers so that the final concentrations of amines were 1 or 2 $\times$ 10^{-4} M and the ionic strength was 0.1. The spectra of the un-ionized form of the phenolic groups were measured in 0.1 N HCl. Spectra of the anions of the monophenols were recorded in 0.1 N NaOH; essentially the same curve was obtained in this solution as in a buffer of pH 11.35 at wave lengths longer than about 235 mμ. At shorter wave lengths, the curves measured in 0.1 N NaOH showed an increase in optical density because of end absorption and did not pass through the isobestic points if these were at wave lengths shorter than 234 mμ (see Figures 1 and 2).

The pK_a' value for the second phenolic hydroxyl group of the one diphenolic compound for which it was measured was estimated by plotting the extinction coefficients at a specified wave length most characteristic for the dianion against the pH (see Figure 4).

Because diphenols, especially the catechol derivatives, are readily oxidized in alkaline medium, all the solutions were boiled while argon was passed through them and were then cooled and stored under argon until they were used. This precaution was found to be unnecessary for the monophenols, although it was taken. For the catechol compounds this measure alone proved to be insufficient to eliminate oxidative changes in alkaline solutions. To overcome this difficulty the curves obtained with the catechol derivatives in alkaline media were measured at timed intervals, and ϵ-values at critical points were extrapolated to zero time. The curves were recorded in the region of the maximum (at 295 mμ, from which pK_a' values were calculated) exactly 2, 5, and 20 min. after mixing of the acid stock solution with the buffer or alkali. The other portions of the spectra were recorded several times between the timed recordings of the maximum.

Immediately after spectra were recorded the pH of buffer solutions were measured directly in the quartz cuvettes with a GK 2021 C concentric glass and calomel electrode assembly connected with a titrator Model TTT 1 b (Radiometer, Copenhagen). The pH measuring system was standardized before and after each measurement with Beckman No. 3501-05 standard buffers.

The most accurate pK_a' values are obtained from measurements in buffer solutions which are at a pH near the pK_a'. The values obtained in buffers other than those with the most favorable pH do, however, serve as a general check on the accuracy of the method. For instance, the pK_a' values calculated for β-(4-hydroxypenyl)ethanolamine (Figure 2) are as follows (with the pH of the buffer in parentheses): determined at 292 mμ, 9.58 (9.05), 9.55 (9.33), 9.57 (9.57), 9.59 (9.89); and 242 mμ, 9.56 (9.05), 9.56 (9.33), 9.58 (9.57), 9.63 (9.89).

Potentiometric Determination of Apparent Dissociation Constants.—Titration curves were recorded at 35° with a Radiometer Titrigraph, Type SBR 2 C. The same electrode (GK 2021 C) which was used for the determination of pH in the spectrophotometric studies was employed and was standardized with the same standard buffers. A rotating titration vessel as described by Dixon and Wade[23] was used. Solutions which contained 1 $\times$ 10^{-4} mole of amine hydrochloride (hordenine, **8**, as sulfate monohydrate) in 5 ml. of 5 $\times$ 10^{-3} N HCl were titrated under argon with approximately 1 N NaOH from a 0.5-ml. microburet. Thus, the Na^+ concentration was 4.5 $\times$ 10^{-2} M at the end of the titration and about 3.5 $\times$ 10^{-2} M in the region of the second pK_a'. Corrections for Na^+ errors at the higher pH ranges were made by means of a nomogram provided by the manufacturer of the instrument. However, the required corrections were small, *i.e.*, 0.02 unit for a pK_a' of 10.75, which was the highest encountered.

The stability of the pH meter was checked between each titration. Samples of phenethylamine hydrochloride were run as standards before and after a series of compounds was titrated, and the results were used to calculate the exact strength of the titrant. With the use of 1 N NaOH as titrant a useful pH range up to pH 11.5 was attained.

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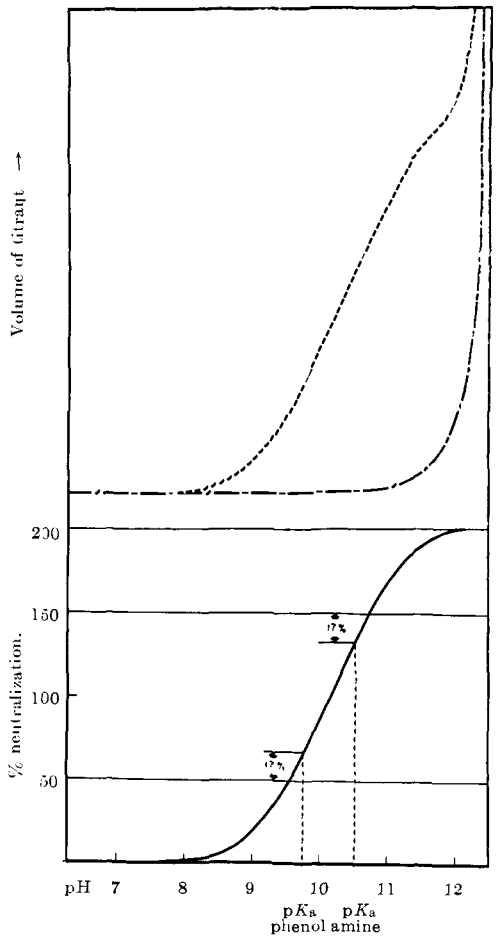

Figure 5.—Titration curve of *p*-tyramine, ———; conventional titration of *p*-tyramine, – – – –; blank titration, — — —.

The pK_a' values were estimated graphically.[24] In the top portion of Figure 5 the dotted curve is the conventional titration curve of *p*-tyramine with volume of titrant plotted *vs.* pH, and the broken curve is the blank titration of 5 ml. of 5 $\times$ 10^{-3} N HCl. The solid curve in the lower portion of Figure 5 was obtained by subtracting the blank curve from the titration curve for the amine. The total titrant added corresponds to exactly 2 equiv. of alkali, as compared with the addition of 1 equiv. for the titration of the standard phenethylamine solution or for the titration of 2- and 3-methoxyphenethylamines.

The titration curve of *p*-tyramine, as seen in the figure, does not have an inflection separating the neutralization of the phenolic group and the amino group. Auerbach and Smolczyk[25] have shown how the character of titration curves is determined by the ratio of two neighboring dissociation constants. Thus when pK_1 is greater than 16pK_2 there will be an inflection in the middle of the over-all titration curve, whereas when pK_1 is less than 16pK_2 the curve will be a smooth function similar to that obtained with a monofunctional compound. All but one of the titration curves obtained in the present study proved to be continuous. The single exception was that obtained with *o*-tyramine, which had a slight inflection in the middle of the curve; the calculated pK_a' values were 9.48 and 10.75 (*i.e.*, pK_1 = 18.6pK_2). It was clear that the pK_a' values for the phenol and the amine groups for all the other compounds were too close to each other to permit their determination simply by measuring the pH at the 50 and 150% neutralization points. Therefore, for each compound the dissociation constants for the phenolic groups obtained from the spectrophotometric measurements were introduced as standard values for the estimation of the dissociation constants of the amino groups. The procedure seemed satisfactory for the following reasons: comparable results were obtained spectrophotometrically and potentiometrically for a phenolic test substance (*p*-cresol) and with *o*-tyramine,

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(25) F. Auerbach and E. Smolczyk, *Z. physik. Chem.*, **110**, 64 (1924).

where the difference between the two pK_a' values is sufficient that they do not interfere with each other in the potentiometric determination. Thus the pK_a' for *p*-cresol was found by the spectrophotometric method to be 10.14 and by potentiometric titration to be 10.08. These values correspond well with another recent determination of 10.10 (apparent) and 10.19 (thermodynamic).[3] The spectrophotometric and potentiometric phenolic pK_a' values for *o*-tyramine were in good agreement, *i.e.*, 9.52 and 9.46, respectively. The pK_a' for phenethylamine, 9.88 (average of 5 determinations), agrees well with the values reported earlier, *i.e.*, 9.86,[3] 9.83,[2] and 9.85.[26] The dissociation constants obtained from the spectrophotometric data have a relative accuracy of ±0.05 unit and the data from the titrations ±0.1 unit.

The dissociation constants for the amino groups in the phenolic amines were determined as follows; as an example the procedure used for *p*-tyramine is described. The dissociation constant (9.74) for the phenolic group was obtained from the spectrophotometric studies. At this pH it can be seen from the titration curve (Figure 5) that 67% neutralization had been effected. From this it is apparent that at half-neutralization of the phenolic group the amino group had been 17% neutralized as well. Because the titration curves for the individual groups should be symmetrical, it follows that half-neutralization of the amino group would be completed when 150 − 17% of the total 200% of alkali are added. The assumption that half-neutralization of the amino function occurs after addition of 133% of the alkali leads to $pK_a' = 10.52$. By using in this manner the spectrophotometrically determined pK_a' for the phenolic group it was possible to estimate the per cent of the amino group ionized at the pK' of the phenolic group for the different compounds. This ranged from zero or very small in the case of *o*-tyramine to 52% for **17** [N,N-dimethyl-β-(4-hydroxy-3-methoxyphenyl)-ethanolamine] and 55% for **14** [N,N-dimethyl-β-(4-hydroxyphenyl)ethanolamine].

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Antifungal Activity of a Series of Substituted [(α-Nitroalkyl)benzylthio]alkylamines[1]

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β-Nitrostyrenes, as well as compounds obtained from them by addition of thiols, are known to be antifungal agents and interest arose in preparing some water-soluble agents of this type. This was done by adding 2-aminoethanethiol hydrochloride to β-nitrostyrene (eq. 1). Strong electron-donating groups on the aryl ring prevented the addition, but a ring nitro group overcame this hindrance. Other substituents seem to have little effect. Addition is also more difficult when the amino group is replaced by dialkylamino. As antifungal agents the compounds inhibited the growth of *Trichophyton mentagrophytes*, *Candida albicans*, and *Ceratocystis ulmi in vitro*. In *in vivo* testing in mice, however, there was considerable toxicity at fungicidal levels. The compounds also showed activity *in vitro* against the bacteria *Bacillus subtilis*, *Escherichia coli*, *Diplococcus pneumoniae*, and *Erwinia carotovora;* the alga *Chlorella vulgaris;* and the protozoan *Tetrahymena geleii*. The most promising activity shown by these compounds has been against *Ceratocystis ulmi*, the organism responsible for Dutch elm disease. Several of the compounds caused inhibition of the growth of the fungus in trees, and field testing is currently in progress.

In recent years, a number of reports have been published on the antibacterial and antifungal activity of β-nitrostyrenes[2] and 2-nitro-1-phenyl-1-phenylthio(or alkylthio)alkanes[3] obtained by the addition of thiols to β-nitrostyrenes.[4] While many of these compounds have shown good antibiotic activity *in vitro*, Evans, *et al.*,[5] have shown that at least some of the nitrostyrenes are inactive in the mouse, possibly due to irreversible binding to sulfhydryl or amino groups present in blood.

We felt that the water-soluble compounds obtained by addition of 2-aminoethanethiol hydrochloride to β-nitrostyrenes might have advantages over the water-insoluble nitrostyrenes.[6] Accordingly, a series of these compounds was prepared and tested qualitatively for antibiotic activity against the following microorganisms: bacteria, *Bacillus subtilis*, *Escherichia coli*, *Diplococcus pneumoniae*, and *Erwinia carotovora;* fungi, *Candida albicans*, *Trichophyton mentagrophytes*, and *Ceratocystis ulmi;* alga, *Chlorella vulgaris;* and protozoan, *Tetrahymena geleii*.

All of the compounds displayed some degree of antibiotic action against most of the test organisms. *Candida albicans* was the most resistant organism, being affected by only 13 of the 34 compounds while *T. mentagrophytes* and *T. geleii* were universally susceptible.

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