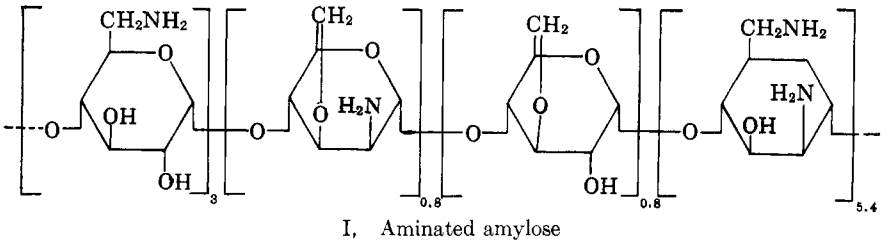
acetamido derivative was less dextrorotatory (+50.5°) than the di-*O*-*p*-tolylsulfonyl precursor (+91.5°), a fact which may be due to the expected inversion at C-2(3) during the hydrazinolysis reaction.[5]

The solubility behavior of these two modified polysaccharides is of interest. The aminated amylose was soluble only in dilute acids and no true solvent could be found for it. The *N*-acetylated aminated amylose, on the other hand, could be dispersed in water and was soluble also in dimethyl sulfoxide although not in *N*,*N*-dimethylformamide. The water solubility of the acetamido derivative would suggest that the hydrophilic amide groups are located in sterically favorable positions for solvation of the polysaccharide, in contrast to chitin where intermolecular hydrogen bonding[2] promotes insolubility.

Periodate oxidation of *N*-acetyl aminated amylose showed that approximately 30% of the pyranose residues contain contiguous hydroxyl groups at C-2 and C-3. Determination of the 5-(hydroxymethyl)-2-furaldehyde formed during acid degradation of the polymer, by the method of O'Neill and co-workers,[6] demonstrated that about 16% of the pyranose units contain the 3,6-anhydro ring. Such a ring would be formed by base-catalyzed elimination of the *p*-tolylsulfonyloxy group on C-6. Closure through a reaction involving a sulfonate ester on C-3 and a free hydroxyl on C-6 would be sterically impossible if inversion is involved and improbable since there is good evidence[3] that all the C-6 hydroxyl groups would be sulfonated. A chain structure of ten monomeric units which would accommodate the known facts is shown in I. In actuality the sequence of units in I could be expected to be random.



I, Aminated amylose

Work is in progress in this laboratory to substantiate the preceding formulation by degradative procedures.

Experimental

2(3),6-Di-O-*p*-tolylsulfonylamylose.—Amylose (Superlose, HAA-11-HV, High Viscosity, Control No. 12215, Stein-Hall and Co., Inc., New York, N. Y.), 324 g., was dissolved in 95% aqueous pyridine (2 l.) and water was removed azeotropically at 60° under reduced pressure. To the pyridine-swollen amylose was added *p*-toluenesulfonyl chloride (850 g., 2.2 equiv.) portionwise during 2 hr. The mixture was maintained at room temperature for 24 hr., then agitated with ice and methanol in a blender, and the resultant white powder was washed several times with water and dried; average yield, 720 g., $[\alpha]^{20}D$ +91.5° 2.1, dimethyl sulfoxide).

Anal. Calcd. for $[C_6H_7O_2(OH)_{1.3}(OSO_2C_6H_4CH_3)_{1.7}]_n$: C, 49.97; H, 4.75; S, 12.78. Found: C, 50.06; H, 4.51; S, 12.10.

Aminated Amylose.—2(3),6-Di-*O*-*p*-tolylsulfonylamylose (50 g.) was refluxed with anhydrous hydrazine (700 ml.) under nitrogen for 7 days. Excess hydrazine was removed under reduced pressure, the residue added to water (500 ml.), and the solution stirred with Raney nickel (20 g.) until the evolution of ammonia ceased (24 hr.). Stirring was continued and the temperature was slowly raised to 100°. The catalyst was filtered. The filtrate was concentrated to 200 ml., dialyzed against tap water for 3 days, and then against distilled water for 3 days. Lyophilization of the solution produced the aminated amylose as a white, nonhygroscopic powder; yield, 9.5 g. This product was readily soluble in dilute acids and was insoluble in water, dimethyl sulfoxide, and *N*,*N*-dimethylformamide.

Anal. Calcd. for $[C_6H_7O_2(NH_2)_{1.4}(-O-)_{0.5}(OH)(OSO_2C_6H_4CH_3)_{0.1}]_n$: N, 11.90; S, 1.94. Found: N, 11.48; S, 1.72.

N-Acetyl Aminated Amylose.—Aminated amylose (9.0 g.) was suspended in water (100 ml.) and stirred while acetic anhydride (50 ml.) was added. Stirring was continued until the solution became clear (1 hr.). After standing at room temperature overnight, the reaction mixture was dialyzed against tap water for 3 days and then against distilled water for 3 days. The solution was concentrated to 200 ml. and lyophilized to give a white, hygroscopic powder; yield, 11.5 g.; $[\alpha]^{20}D$ +50.5° (*c* 0.7, dimethyl sulfoxide). The *N*-acetyl aminated amylose was soluble in dimethyl sulfoxide and in dilute acids and swelled into water to give a clear colloidal solution. It was insoluble in *N*,*N*-dimethylformamide.

Anal. Calcd. for $[C_6H_7O_2(NHCOCH_3)_{1.4}(-O-)_{0.5}(OH)_{1.1}]_n$: N, 8.93, CH_3CO; 26.84. Found: N, 9.39; CH_3CO, 26.52; S, 0.1.

Periodate Oxidation of *N*-Acetyl Aminated Amylose.—To a sample of *N*-acetyl aminated amylose (0.1 g.) in water was added 5 ml. of an aqueous solution of sodium metaperiodate (0.3 *M*, 3.5 equiv.) and the volume was brought to 100 ml. A blank determination was prepared by omitting the sample. The solutions were maintained at room temperature in the dark and aliquots were analyzed at intervals for periodate consumption by the method of Neumuller and Vasseur.[7] Samples (5 ml.) were added to a mixture of phosphate buffer (25 ml., pH 6.98) and 20% potassium iodide (2 ml.). The resulting iodine was titrated with 0.01 *N* sodium thiosulfate, using starch as indicator. The periodate consumption in moles per saccharide unit was (time in hr., moles of oxidant consumed per hexose unit): 0.5, 0.30; 2, 0.31; 5, 0.32; 7, 0.35; 12, 0.37; 24, 0.44.

Determination of 3,6-Anhydrohexose Units in N-Acetyl Aminated Amylose.[8]—Ten samples (10 mg. each) of *N*-acetyl aminated amylose were mixed with 0.15 *N* sulfuric acid (2 ml.) and hydrolyzed in sealed tubes at 100°. Tubes were removed at intervals during a 24-hr. period, the contents were neutralized with barium carbonate, filtered into 50-ml. volumetric flasks, and diluted to 50 ml. Optical densities were read at 2850 Å. To correct for the first-order decomposition[8] of 5-(hydroxymethyl)-2-furaldehyde to formic acid and levulinic acid during the hydrolysis, the logarithm of the optical density was plotted against time and the linear portion of the curve was extrapolated to zero time. The 5-(hydroxymethyl)-2-furaldehyde detected corresponded to 11.8% of the polysaccharide or 16.4% of 2-acetamido-3,6-anhydro-2-deoxyhexopyranose units.

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A Self-Condensation Reaction of 2- and 4-Hydroxymandelamines[1]

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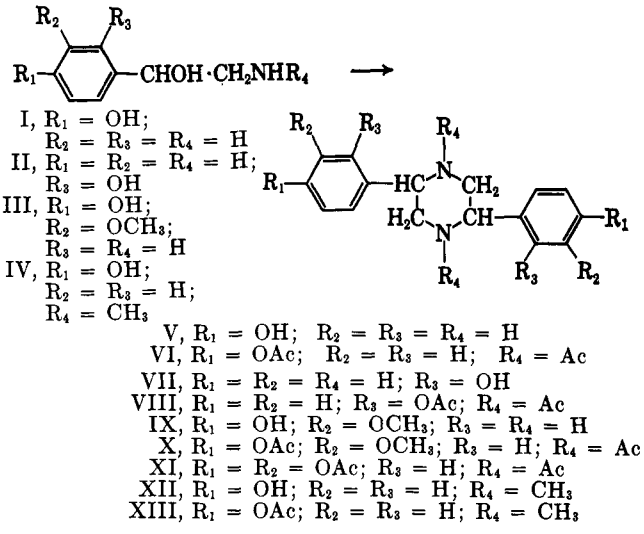
Octopamine (I) [norsympatol, norsynephrine, (α-aminomethyl)-4-hydroxybenzyl alcohol] has been

shown recently to occur in tissues of mammals[2] and is also present in extracts of the salivary gland of the octopus.[3] The free base form of octopamine has two melting points. Mannich and Thiele[4] found a melting point of 157–158° while Hinsberg[5] later recorded 250°. Racemic octopamine prepared in our laboratory melted with decomposition at 156–158°, but resolidified at this temperature and then melted at a temperature higher than 250°, while pure D- or L-octopamine each decomposed between 155–160° and formed the high-melting compound without ever melting. Despite the fact that this reaction of octopamine at its melting point probably is of little significance under physiological conditions, the behavior seemed unusual so experiments were carried out to determine the chemical reaction which apparently occurs at 155–160°.

In order to prevent overheating and to carry out the reaction smoothly on a larger scale the decomposition was effected in boiling bromobenzene (b.p. 157°). In this manner a new compound could be obtained in 84% yield. It proved to be very insoluble in most of the common organic solvents, but could be recrystallized from dimethylformamide or quinoline. Elementary analysis and a molecular weight determination showed that it was formed from two molecules of octopamine with the loss of two molecules of water. The new compound no longer reacted with ninhydrin; thus it no longer contained a primary amino group. However, it still coupled with diazotized amines and was partially cleaved by *p*-nitrobenzenediazonium chloride to form 4-hydroxy-4′-nitroazobenzene. These reactions indicated the molecule still retained a phenolic hydroxyl and a hetero atom, which in this case could only be nitrogen, in a beta position to the aromatic nucleus.[6] The only structure which is consistent with all of these data is 2,5-bis(4-hydroxyphenyl)piperazine (V).

Further experiments were then made to determine what types of mandelamines could undergo this reaction and to gain information which might make possible an explanation of the mechanism by which this self-condensation occurred. The presence of a phenolic hydroxyl seemed necessary, since mandelamine [(α-aminomethyl)benzyl alcohol] itself was recovered unchanged after it was heated at 200° for 10 min. The requirement for a phenolic hydroxyl in the *ortho* or *para* position was shown by the ready formation of a condensation product from the *ortho* isomer of octopamine (II), [(α-aminomethyl)-2-hydroxybenzyl alcohol], and from normetanephrine (III) [(α-aminomethyl)-4-hydroxy-3-methoxy-benzyl alcohol] at about 160°. The self-condensation of *p*-sympatol (IV), the N-methyl derivative of octopamine, required a higher reaction temperature, 185°, and yielded an increased amount of side products while the yield of the piperazine derivative XII was only 38%. This condensation was carried out in paraffin oil.



I, R_1 = OH; R_2 = R_3 = R_4 = H
II, R_1 = R_2 = R_4 = H; R_3 = OH
III, R_1 = OH; R_2 = OCH_3; R_3 = R_4 = H
IV, R_1 = OH; R_2 = R_3 = H; R_4 = CH_3

V, R_1 = OH; R_2 = R_3 = R_4 = H
VI, R_1 = OAc; R_2 = R_3 = H; R_4 = Ac
VII, R_1 = R_2 = R_4 = H; R_3 = OH
VIII, R_1 = R_2 = H; R_3 = OAc; R_4 = Ac
IX, R_1 = OH; R_2 = OCH_3; R_3 = R_4 = H
X, R_1 = OAc; R_2 = OCH_3; R_3 = H; R_4 = Ac
XI, R_1 = R_2 = OAc; R_3 = H; R_4 = Ac
XII, R_1 = OH; R_2 = R_3 = H; R_4 = CH_3
XIII, R_1 = OAc; R_2 = R_3 = H; R_4 = CH_3

The piperazine derivatives obtained (VII and XII), from *o*-octopamine and *p*-sympatol were more soluble in organic solvents than those obtained from *p*-octopamine and normetanephrine. All the condensation products, which melt rather suddenly with complete decomposition at temperatures above 250°, were characterized as the acetyl derivatives, which crystallize nicely from ethanol and show sharp melting points. The condensation product of *p*-sympatol gives only the expected O-acetyl derivative.

2,5-Bis(4-hydroxy-3-methoxyphenyl)piperazine (IX) could be demethylated with hydrobromic acid in acetic acid and the catechol compound obtained in this way was characterized as the hexaacetyl derivative (XI).

The *meta* isomers of octopamine [(α-aminomethyl)-3-hydroxybenzyl alcohol] and of sympatol [phenylephrine, (α-methylaminomethyl)-3-hydroxybenzyl alcohol] proved to be very stable and could be recovered unchanged after they had been heated to 200 or 250°, respectively.

The results of these experiments make it possible to suggest the mechanism shown in the depicted equations. The physical properties of the free base form of octopamine are suggestive that it exists in zwitterion form. The electron-donating properties of the phenoxide group could influence the oxygen of the side chain hydroxyl so that a nucleophilic attack on a proton of the charged amino group might take place. This would result in the loss of water and formation of a carbonium ion intermediate which would contain a nucleophilic amino nitrogen. Condensation of two of these molecules would result in the observed product. The high melting point and insolubility of the products are suggestive that they might actually exist in the zwitterion form rather than as an uncharged molecule.

The stability of the *m*-hydroxy compounds and of mandelamine favor the proposed mechanism. The higher reaction temperature and the increased formation of side products in the use of *p*-sympatol indicates that initial carbonium ion formation may occur, but that the steric hindrance afforded by the methyl substituent prevents rapid dimerization of the intermediate before side reactions can occur.

(1) This research was supported in part by Research Grant MH-02278 from the National Institute of Mental Health. It was presented at the 143rd National Meeting of the American Chemical Society, Cincinnati, Ohio, January, 1963.

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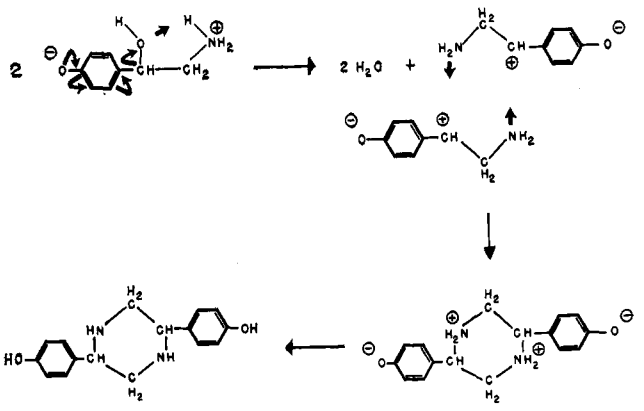

A minor by-product of the self-condensation reaction of octopamine was found to be β,β-bis(4-hydroxyphenyl)ethylamine. The origin of this substance might be explained by the elimination of the side chain of octopamine by a nucleophilic displacement by the carbonium intermediate. It is well known that the C–C linkage between nucleus and side chain in *p*-hydroxybenzyl alcohols and their derivatives is polarized to such an extent by a combination of nuclear effects of the phenolic hydroxyl in combination with a heteratom on a carbon alpha to the aromatic nucleus that it becomes vulnerable to an attack by nucleophilic reagents such as diazonium compounds.[6]

Experimental[7]

2,5-Bis(4-hydroxyphenyl)piperazine (V).—Two grams of octopamine (I) was suspended in 6 ml. of bromobenzene and heated in an oil bath at 170° for 30 min. Water and a volatile amine were liberated during the first 15–20 min. The reaction mixture was cooled, diluted with 20 ml. of petroleum ether and filtered. The brown residue was extracted three times with 20-ml. portions of hot ethanol to yield 1.48 g. (84%) of nearly pure product, m.p. 275° dec. The product was recrystallized from dimethylformamide in the form of colorless parallelograms, m.p. 275–276° dec. It also could be recrystallized from quinoline.

Anal. Calcd. for $C_{16}H_{18}N_2O_2$: C, 71.10; H, 6.71; N, 10.36. Found: C, 70.77; H, 6.38; N, 10.40.

2,5-Bis(4-hydroxyphenyl)piperazine Dihydrochloride.—Two hundred milligrams of V was digested for 4 hr. at room temperature with 20 ml. of 1 *N* hydrochloric acid. Most of the dihydrochloride was separated by filtration; the filtrate yielded only a small amount more of the dihydrochloride when the solvent was evaporated. The compound melted at 345° dec.

Anal. Calcd. for $C_{16}H_{20}Cl_2N_2O_2$: N, 8.17. Found: N, 7.92.

β,β-Bis(4-hydroxyphenyl)ethylamine (XIV).—The three ethanol washings of the crude 2,5-bis(4-hydroxyphenyl)piperazine (V) were combined and passed through a 1 × 2 cm. column of Amberlite CG-120 (H^+ form). Most of the colored material passed through. The resin was washed with 500 ml. of ethanol, and the amine was eluted with a mixture of concentrated ammonia–ethanol (1:3). The first 300 ml. of alkaline eluate was collected and evaporated to dryness. The residue was dissolved in 4 ml. of ethanol and the solution was filtered after it had stood overnight. The filtrate was evaporated to dryness, and the amine crystallized after it was digested with 3 ml. of water. The yield was 81 mg. (6%) of yellow bisarylethylamine, which softened at 105–110° and melted at 199–202°, (lit.[8] 206–209°). For further purification, 80 mg. of the material and 75 mg. of *p*-toluenesulfonic acid monohydrate were dissolved in 4.5 ml. of hot water; the solution was cleaned with charcoal, cooled to 25°, and filtered. The filtrate was concentrated to 1 ml. and left in the cold overnight; 61 mg. of β,β-bis(4-hydroxyphenyl)ethylamine *p*-toluenesulfonate, m.p. 218–221°, was obtained. One recrystallization from 0.8 ml. of water yielded a product which melted at 223–225°, and did not depress the melting point of an authentic sample.

The paper chromatographic properties of the material were identical with those of the authentic compound: R_f 0.67 in butanol–acetic acid–water (4:1:1), 0.84 in isopropyl alcohol–ammonia–water (8:1:1), 0.16 to 0.25 (streak) in benzene–propionic acid–water (10:9:1); color: yellow with diazotized sulfanilic acid, red with diazotized *p*-nitroaniline, purple (turning blue) with ninhydrin.

1,4-Diacetyl-2,5-bis(4-acetoxyphenyl)piperazine (VI).—A solution of 1 g. of V and 50 mg. of anhydrous sodium acetate in 15 ml. of acetic anhydride was refluxed for 1 hr. The reaction mixture was evaporated *in vacuo* to complete dryness. The resulting oily residue crystallized when it was digested with 20 ml. of 50% ethanol. One recrystallization from 1-butanol yielded 1.25 g. of product, m.p. 223.5–225°.

Anal. Calcd. for $C_{24}H_{26}N_2O_6$: C, 65.78; H, 5.98; N, 6.39; mol. wt., 438.5. Found: C, 65.79; H, 6.12; N, 6.28; mol. wt., 393, 415 (Rast).

2,5-Bis(2-hydroxyphenyl)piperazine (VII).—*o*-Octopamine (II), 500 mg., m.p. 98–100°, was heated in an open test tube in an oil bath at 170° until complete solidification had occurred; this required about 15 min. The tube was cooled and the residue was digested with 5 ml. of hot ethanol to effect complete crystallization. The suspension was allowed to stand overnight and the crystals were collected and washed with ethanol. The yield was 215 mg. (49%), m.p. 263°. Recrystallization from butanol or pyridine gave a product melting at 265° dec.

Anal. Calcd. for $C_{16}H_{18}N_2O_2$: C, 71.10; H, 6.71; N, 10.36. Found: C, 70.53; H, 6.95; N, 10.12.

1,4-Diacetyl-2,5-bis(2-acetoxyphenyl)piperazine (VIII) was prepared by acetylation as described previously. The compound melted at 174–176° after recrystallization from ethanol–water.

Anal. Calcd. for $C_{24}H_{26}N_2O_6$: C, 65.78; H, 5.98; N, 6.39. Found: C, 66.07; H, 6.22; N, 6.29.

2,5-Bis(4-hydroxy-3-methoxyphenyl)piperazine (IX).—This compound was obtained in 58% yield from normetanephrine (III) with the procedure described for the conversion of *o*-octopamine (II) to VII. Recrystallization from dimethylformamide yielded small needles, m.p. 276–277° dec.

Anal. Calcd. for $C_{18}H_{22}N_2O_4$: C, 65.45; H, 6.72; N, 8.48. Found: C, 65.20; H, 6.87; N, 8.28.

1,4-Diacetyl-2,5-bis(4-acetoxy-3-methoxyphenyl)piperazine (X).—Prisms were obtained from butanol, m.p. 222–223°.

Anal. Calcd. for $C_{26}H_{30}N_2O_8$: C, 62.45; H, 6.07; N, 5.62. Found: C, 62.46; H, 6.62; N, 5.32.

1,4-Diacetyl-2,5-bis(3,4-diacetoxyphenyl)piperazine (XI).—2,5 Bis(4-hydroxy-3-methoxyphenyl)piperazine (IX 0.80 g.) was refluxed for 2 hr. in a mixture of 30 ml. of acetic acid and 30 ml. of hydrobromic acid (48%). The reaction mixture was evaporated to complete dryness, the remaining residue was washed with absolute ethanol, dried, and refluxed for 1.5 hr. with 1 g. of anhydrous sodium acetate and 20 ml. of acetic anhydride. The solvent was removed *in vacuo*, and crystallization of the residue occurred after the addition of 20 ml. of water and overnight standing. The compound was recrystallized twice from butanol, yielding 0.95 g. (71%) of colorless prisms, m.p. 230–232°.

Anal. Calcd. for $C_{28}H_{30}N_2O_{10}$: C, 60.60; H, 5.46; N, 5.05. Found: C, 59.93; H, 5.47; N, 4.98.

2,5-Bis(4-hydroxyphenyl)-1,4-dimethylpiperazine (XII).—*p*-Sympatol (IV, 3.5 g.) was suspended in 7 ml. of paraffin oil and heated in an oil bath at 190° until the evolution of gas stopped; this required about 5 min. The paraffin oil was removed by washing the reaction mixture with ether. The remaining material was powdered, washed with 30 ml. of water, and digested twice with 15-ml. portions of ethanol. The product was then recrystallized from ethanol. The yield was 1.14 g. (38%), m.p. 265° dec.

Anal. Calcd. for $C_{18}H_{22}N_2O_2$: C, 72.45; H, 7.44; N, 9.38. Found: C, 72.29; H, 7.13; N, 9.22.

2,5-Bis(4-acetoxyphenyl)-1,4-dimethylpiperazine (XIII).—Colorless prisms were obtained from ethanol, m.p. 206–207°.

Anal. Calcd. for $C_{22}H_{26}N_2O_4$: C, 69.15; H, 6.86; N, 7.33. Found: C, 69.27; H, 6.73; N, 7.38.

(7) All melting points were made in open capillary tubes and are corrected. Mrs. Kerin N. Yates carried out the nitrogen analyses and the other analyses were made by the Weiler and Strauss Microanalytical Laboratory, Oxford, England.

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