to cool to room temperature. The product was filtered, washed with a little Dowtherm, then with acetone, and dried at 100° to yield 3.9 g (92%) of **20**.

Methyl 4-Hydroxy-6,7-bis(2-methylbutoxy)-3-quinolinecarboxylate (26).—To a solution of 110 g (1 mole) of catechol in 3800 ml of dimethylformamide (DMF) in a nitrogen atmosphere was added 108 g (2 moles) of sodium methoxide powder with stirring. The reaction mixture was stirred at 40–45° for 1 additional hr and then 302 g (2 moles) of 1-bromo-2-methylbutane was added in about 45 min. A clear solution was obtained about 1.5 hr after the addition, kept under nitrogen overnight, and then poured into water. This mixture was acidified to pH 5 and extracted with benzene. The combined extracts were washed with 10% NaOH solution followed by water. After drying, the benzene was stripped *in vacuo*, yielding 98 g (39%) of *o*-bis(2-methylbutoxy)benzene.

To a solution of 200 ml of concentrated HNO_3 and 200 ml of water was added gradually 86 g (0.344 mole) of *o*-bis(2-methylbutoxy)benzene with stirring, so that the temperature never rose above 30°. Addition was completed in 1.5 hr and the reaction mixture was further stirred for 2.5 hr and then poured over cracked ice. The gummy solid was dissolved in ether and washed well with water until the washings were almost neutral. After drying, the ether was distilled to yield 87 g (86%) of dark, liquid 3,4-bis(2-methylbutoxy)nitrobenzene.

Hydrogenation of 30 g (0.1 mole) of the crude nitro compound in 200 ml of absolute ethanol over 8 g of 5% Pd-C catalyst (50% water) at 2.8 kg/cm² initial pressure was complete in 1 hr. After filtration, the filtrate was heated under reflux with 17 g (0.1 mole) of dimethyl methoxymethylenemalonate[6] for 2 hr. The solution was stripped *in vacuo* to yield 40 g of residue. A 120-g sample of the anilinomethylenemalonate prepared in this way was added to 500 ml of boiling Dowtherm A. After boiling for 10 min, the solution was allowed to cool to room temperature, the solid product was filtered, and the filtrate was again heated to boiling as rapidly as possible. After four such heating periods of 8–10 min each, 46 g (41%) of crude product was obtained. Recrystallization from 2 l. of DMF with charcoal gave 37 g of white product.

Propyl 6,7-Diethoxy-4-hydroxy-3-quinolinecarboxylate (5).—A mixture of 61 g (0.2 mole) of ethyl 6,7-diethoxy-4-hydroxy-3-quinolinecarboxylate (**3**), 1600 ml of propanol, and 2 g of *p*-toluenesulfonic acid was heated under reflux for 6 days. The mixture was filtered hot, and the solid was washed with ether and dried to give 42 g of product. A second crop of 15 g separated from the filtrate, giving a total of 57 g, (89%) of the propyl ester. Recrystallization of 55 g from DMF with charcoal gave 50 g of pure material.

Propyl 4-Hydroxy-6,7-diisobutoxy-3-quinolinecarboxylate (21). A mixture of 37 g (0.1 mole) of ethyl 4-hydroxy-6,7-diisobutoxy-3-quinolinecarboxylate (**20**) and 300 ml of 10% NaOH solution was heated under reflux for 4 hr. After filtration the hot solution was acidified with HCl and allowed to cool. The precipitate was filtered, washed well with water, and dried. A suspension of 19 g of this solid in 600 ml of benzene was heated under reflux with a Dean–Stark trap to remove any water still present. Then 5 ml of $SOCl_2$ was added and the mixture was heated under reflux for 10 hr and allowed to cool overnight. The crude acid chloride (18 g) was filtered, washed with benzene and ether, and dried at 100°.

A mixture of 6.0 g (0.017 mole) of the acid chloride in 300 ml of propanol was refluxed for 1.75 hr. The solution was filtered and cooled, and a small amount of NH_4OH was added to adjust the solution to pH 7–8. The precipitate was triturated with water, filtered, and dried at 100°. The yield was 4.8 g (76%) of the propyl ester. Recrystallization of 1 g from 100 ml of absolute ethanol gave 0.85 g of white needles.

Ethyl 4-Acetoxy-6,7-diethoxy-3-quinolinecarboxylate (4).—A mixture of 50 g (0.16 mole) of ethyl 6,7-diethoxy-4-hydroxy-3-quinolinecarboxylate (**3**) and 30 g of anhydrous sodium acetate in 1250 ml of acetic anhydride was heated under reflux for 2.25 hr and then allowed to cool. The solid was collected and washed with cold water and dried at 100°; yield, 49 g (86%) of the 4-acetoxy compound. Recrystallization from ethanol gave an analytical sample.

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Preparation of Substituted Phenethyl Alcohols and a Study of Their Bacteriostatic Action in *Escherichia coli* B[1]

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Fifteen analogs of phenethyl alcohol have been prepared to explore the effect of ring substituents, and to a lesser degree solubility, on bacteriostatic activity. All of the monomethoxy- and dimethoxy-, all of the monohydroxy-, and three dihydroxyphenethyl alcohols have been prepared by the utilization of a variety of procedures and subjected to biological evaluation. The bacteriostatic concentration of each of the phenethyl alcohols for *Escherichia coli* B was determined and the reversibility of the bacteriostasis established. The influence of *p*-methoxy-, 2,5-, 3,4-, and 3,5-dimethoxyphenethyl alcohol in the synthesis of DNA, RNA, and protein by *E. coli* B was studied. Each of these compounds, as well as the parent compound, caused 100% inhibition of the synthesis of DNA, RNA, and protein when present at its bacteriostatic concentration. When the inhibitor was removed, these biosynthetic processes, as well as cell division, were reinitiated and resumed, rates parallel to those characteristic of the control, uninhibited culture.

In 1953, Lilley and Brewer[3] reported that phenethyl alcohol exhibited a marked inhibitory effect on gram-negative bacteria while inhibiting gram-positive bacteria only slightly if at all. They found that the incorporation of 2.05×10^{-5} moles/ml of phenethyl alcohol in a trypticase soy agar preparation produced a medium which could be used to isolate selectively the gram-positive bacteria from a mixed bacterial flora.

Phenethyl alcohol appeared to be one of the few compounds which is more inhibitory to gram-negative than to gram-positive bacteria. This unusual property of the material prompted Berrah and Konetzka[4] to

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(2) This report constitutes part of the thesis submitted by E. Z. Khafagy for the Ph.D. degree, University of Nebraska, 1966.

(3) R. D. Lilley and J. H. Brewer, *J. Am. Pharm. Assoc.*, **42**, 6 (1953).

(4) G. Berrah and W. Konetzka, *J. Bacteriol.*, **83**, 738 (1962).

undertake an investigation of the mechanism of the inhibitory action. They reported that phenethyl alcohol caused a selective and reversible inhibition of DNA synthesis, while the synthesis of RNA and protein continued at a rate which paralleled these synthetic activities in the uninhibited culture. This apparently unique status of phenethyl alcohol as a specific and reversible inhibitor of DNA synthesis has led to its widespread use as an inhibitor in a variety of studies of biological processes related to DNA synthesis.

At the time this investigation was initiated, no studies had been made of the relationship between chemical structure and biological activity of analogs of phenethyl alcohol. The absence of such studies and the apparent uniqueness of the biological activity prompted us to prepare fifteen analogs of phenethyl alcohol. These compounds, methoxy and hydroxy derivatives, are two groups of compounds which make it possible to explore the effect of some ring substituents and, to a lesser degree, the effect of solubility on their biological activity. All of the mono- and dimethoxy-, and all of the mono- and three of the dihydroxyphenethyl alcohols have been prepared and subjected to biological evaluation.

Chemistry.—*o*-, *m*-, and *p*-methoxyphenethyl alcohols were prepared by treating the appropriate bromoanisole with phenyllithium to form the methoxyphenyllithium compounds which were treated with ethylene oxide; the resulting lithium salts were hydrolyzed by acid. The products were isolated by extraction and distillation. *m*-Methoxyphenethyl alcohol was also prepared by the reduction of *m*-methoxyphenylacetic acid with lithium aluminum hydride.[5]

The preparation of the 2,3-, 2,4-, 2,5-, 3,4-, and 3,5-dimethoxyphenethyl alcohols was accomplished by condensation of the appropriate benzaldehyde with nitromethane by a procedure previously employed by Gairaud and Lappin.[6] The resultant nitrostyrenes were reduced with lithium aluminum hydride to the corresponding phenethylamines by the general procedure of Ramirez and Burger[7] with some minor modifications. The resulting phenethylamines were converted to their diazonium salts which, on hydrolysis, produced the appropriate dimethoxyphenethyl alcohols. This procedure had previously been employed by Pailer, *et al.*[8]

2,6-Dimethoxyphenethyl alcohol was synthesized from *m*-dimethoxybenzene. Phenyllithium was used to introduce the lithium atom into the ring between the methoxy groups to yield 2,6-dimethoxyphenyllithium. The remainder of the synthetic sequence was the same as that used for the preparation of *o*-, *m*-, and *p*-methoxyphenethyl alcohols outlined above.

o- and *p*-hydroxyphenethyl alcohols were prepared from the corresponding hydroxyphenylacetic acids. The acids were converted to their ethyl esters as previously described,[5] and the esters were converted to the corresponding acetoxy derivatives with acetic anhydride. The acetoxyethyl esters were reduced with lithium aluminum hydride by the method described by Rohmann and Meisel.[9]

(5) J. H. Hunter and J. H. Hogg, *J. Am. Chem. Soc.*, **71**, 1922 (1949).
(6) C. B. Gairaud and G. Lappin, *J. Org. Chem.*, **18**, 1 (1953).
(7) F. Ramirez and A. Burger, *J. Am. Chem. Soc.*, **72**, 2781 (1950).
(8) M. Pailer, K. Schneglberger, and W. Reifschneider, *Monatsh. Chem.*, **83**, 513 (1952).

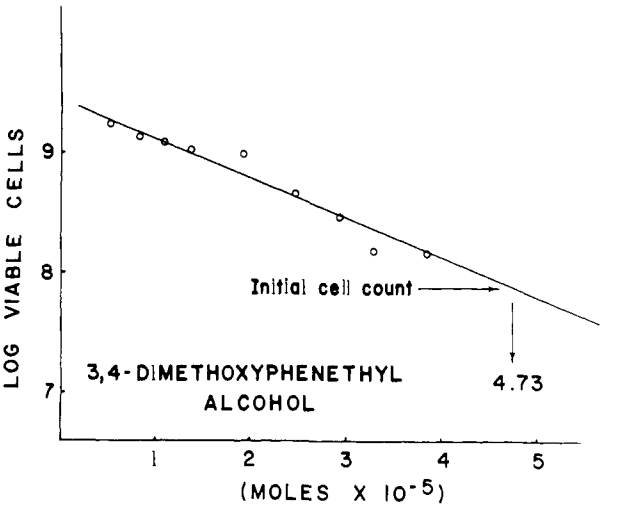

Figure 1.—Example of the method used for the estimation of the bacteriostatic concentration.

m-Hydroxyphenethyl alcohol was prepared by hydrobromic acid demethylation of *m*-methoxyphenethyl alcohol,[10] and 2,5-, 2,6-, and 3,4-dihydroxyphenethyl alcohols were prepared by demethylation of the corresponding dimethoxyphenethyl alcohols with aluminum chloride in benzene.

Biological Results.—The bacteriostatic concentration for *E. coli* B was determined for each compound by the use of a graphic analysis of the viable-cell count at the end of a 4-hr incubation period for each concentration used. The technique is described in the experimental portion and illustrated in Figure 1. Table I gives the bacteriostatic concentration of each of the compounds in moles per milliliter and rates the activity of each compound in terms of phenethyl alcohol. The most active compound was *p*-methoxyphenethyl alcohol, which was 5.5 times as potent as the parent, unsubstituted compound. The least active was *p*-

TABLE I
BACTERIOSTATIC CONCENTRATION OF PHENETHYL ALCOHOLS

Compd	Concn, moles × 10^{-5}/ml	Relative activity[a]
PEA[b]	2.64	100
o-Methoxy-PEA	1.58	168
m-Methoxy-PEA	2.18	121
p-Methoxy-PEA	0.48	550
2,3-Dimethoxy-PEA	1.38	191
2,4-Dimethoxy-PEA	1.53	172
2,5-Dimethoxy-PEA	0.97	272
2,6-Dimethoxy-PEA	4.35	61
3,4-Dimethoxy-PEA	4.73	56
3,5-Dimethoxy-PEA	1.11	237
o-Hydroxy-PEA	3.17	83
m-Hydroxy-PEA	2.72	97
p-Hydroxy-PEA	6.26	42
2,5-Dihydroxy-PEA	2.73	97
2,6-Dihydroxy-PEA	3.35	79
3,4-Dihydroxy-PEA	2.55	104
Phenethylamine	1.70	160
p-Methoxyphenethylamine	0.88	300

[a] Unsubstituted phenethyl alcohol (PEA) arbitrarily set at 100. [b] PEA = phenethyl alcohol.

(9) C. Rohmann and D. Meisel, *Arch. Pharm.*, **294**, 538 (1961).
(10) L. Schieler and R. Sprenger, *J. Am. Chem. Soc.*, **73**, 4045 (1951).

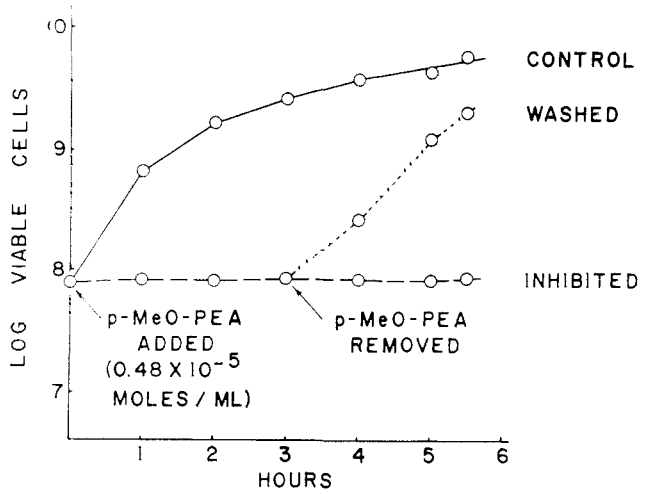

Figure 2.—Example of the bacteriostasis produced by a phenethyl alcohol and the reinitiation of cell division following the removal of the inhibitor.

hydroxyphenethyl alcohol. Two compounds of substantial activity were 2,5- and 3,5-dimethoxyphenethyl alcohols, 2.7 and 2.4 times, respectively, as active as phenethyl alcohol. It is of particular interest that 3,4-dimethoxyphenethyl alcohol was the least active of the methoxy compounds.

Since the methoxy compounds were, in general, more active than the hydroxy compounds, the suggestion that their reduced polarity enhanced their solubility in the lipoprotein cell membrane of the gram-negative *E. coli* is attractive. Solubility in the media seemed to be a less important consideration since the 2,6- and 3,4-dimethoxy compounds were of almost equal activity; the former was the least soluble and the latter the most soluble of the dimethoxy compounds. When both were converted to their dihydroxy alcohols, the 2,6-dihydroxy showed no increase in activity, although it was more soluble than the dimethoxy compound, while the very insoluble 3,4-dihydroxy compound was twice as potent as its dimethoxy derivative. Factors other than solubility and polarity are also of importance in determining the activity and potency of these materials.

The finding that the monohydroxyphenethyl alcohols were less active than the corresponding methoxy compounds limited interest in the dihydroxy analogs. The selection of the three analogs to be prepared in the dihydroxy series was based on the observation that the 2,5-dimethoxy compound was the most potent of the dimethoxy alcohols, and the 3,4- the least active dimethoxy analog. However, since the 3,4-dihydroxy groups are of interest in terms of biological activity under any circumstances, the 2,6-dihydroxyphenethyl alcohol was also made. The 2,6- and the 3,4-dimethoxy alcohols were of equally low activity.

The biological properties of phenethyl alcohol, *p*-methoxy-, and 2,5-, 3,4-, and 3,5-dimethoxyphenethyl alcohols were studied for their influence on bacteriostasis and its reversibility, and on the synthesis of DNA, RNA, and protein.

The reversibility of the bacteriostasis caused by the phenethyl alcohols was shown by the removal of the inhibitor from a culture in which it had been present for several hours. Once the inhibitor was removed from the culture, cell division was reinitiated, and it then proceeded at a rate which paralleled that of the control culture. Figure 2 illustrates the reversibility of the bacteriostasis produced by one of the compounds. The reversibility of the inhibition is believed to be characteristic of all of the compounds, but it was demonstrated for only the parent compound, phenethyl alcohol, for the most potent compounds, *p*-methoxy- and 2,5- and 3,5-dimethoxyphenethyl alcohols, and for one of the least potent compounds, 3,4-dimethoxyphenethyl alcohol.

The influence of these five compounds on the synthesis of DNA, RNA, and protein by *E. coli* B was also studied. In the case of each of the above five compounds, it was found that, when it was present in a culture at its specific bacteriostatic concentration, it caused 100% inhibition of the synthesis of the two nucleic acids and protein. When the inhibitor was removed, these biosynthetic processes were resumed and maintained rates parallel to those of control, uninhibited cultures. It is believed that this generalized action is characteristic of all of the compounds described in this report, and Table II summarizes these findings. The bacteriostatic activity of these five compounds for *E. coli* B certainly cannot be attributed to a specific inhibition of DNA synthesis.

Rosenkranz, *et al.*,[11] have reported that phenethyl alcohol has no effect on the physiochemical properties of isolated DNA. They indicated that the metabolic functions most sensitive to the inhibitory action of phenethyl alcohol appeared to be the process of enzyme induction and, possibly, the synthesis of *m*-RNA.

During an independent exploratory study, it was found that phenethylamine and *p*-methoxyphenethylamine were better bacteriostatic agents than phenethyl alcohol for *E. coli* B. These values are listed at the end of Table I.

Experimental Section[12]

Chemical Methods.—2,4-Dimethoxybenzaldehyde was prepared by general procedure b which is described;[13] 2,5-dimethoxybenzaldehyde was prepared by methylation[13] of 2-hydroxy-5-methoxybenzaldehyde,[14] and 3,5-dimethoxybenzaldehyde was prepared as described.[15]

The following **phenethyl alcohols** were prepared by the addition of excess ethylene oxide to the appropriate phenyllithium compounds. The procedure used was a combination of those described.[16,17] The phenyllithium compounds were prepared by the action of phenyllithium[15] on 0.25 mole of the appropriate bromoanisoles: *o*-methoxyphenethyl alcohol (12%), bp 129–133° (11 mm) [lit.[18] 133–135° (11 mm)]; *m*-methoxyphenethyl alcohol (12%), bp 135–137° (11 mm) [lit. 135–137° (12 mm)[19] and 145° (13 mm)[20]]; *p*-methoxyphenethyl alcohol (14%), bp 144–145° (11 mm) [lit.[20] 142° (12 mm)]. Lithium aluminum hydride reduction of *m*-methoxyphenylacetic acid[5] produced *m*-methoxyphenethyl alcohol (82%), bp 134–135° (10 mm).

The **dimethoxy-β-nitrostyrenes** were prepared from the appropriate dimethoxybenzaldehydes (0.15 to 0.24 mole) by a combination of procedures described.[6,7] The products were

(11) H. S. Rosenkranz, H. S. Carr, and H. M. Rose, *J. Bacteriol.*, **89**, 1354 (1965).

(12) All melting points were determined on calibrated thermometers.

(13) J. P. Lambooy, *J. Am. Chem. Soc.*, **78**, 771 (1956).

(14) H. B. Gillespie, *Biochem. Prepn.*, **3**, 79 (1953).

(15) J. P. Lambooy, *J. Am. Chem. Soc.*, **76**, 133 (1954).

(16) R. A. Barnes, *ibid.*, **75**, 3004 (1953).

(17) M. Lora-Tamayo, R. Madroñero, and G. G. Muñoz, *Chem. Ber.*, **93**, 280 (1960).

(18) S. Winstein, C. Lindegren, H. Marshall, and L. Ingraham, *J. Am. Chem. Soc.*, **75**, 147 (1953).

(19) J. B. Shoesmith and R. J. Connor, *J. Chem. Soc.*, 2230 (1927).

(20) E. R. Clark and R. D. Robson, *ibid.*, 3714 (1959).

TABLE II

INHIBITION OF SYNTHESIS OF MACROMOLECULES IN *E. coli* BY VARIOUS PHENETHYL ALCOHOLS[a]

Flask	Time, min	μg of macromolecule/ml of culture				
		PEA[a]	*p* MeO-	2,5-$(MeO)_2$-	3,4-$(MeO)_2$-	3,5-$(MeO)_2$-
		A. DNA Synthesis				
Control	0	1.92	1.90	2.08		
	60	5.72	5.92	5.44		
	120	10.50	10.20	10.24		
	180	16.00	15.50	13.90		
	240	20.30	18.96	16.96		
Inhibited	0	2.00	1.90	2.08	2.08	2.08
	60	2.04	2.00	2.24	2.32	2.24
	120	2.04	1.96	2.40	2.40	2.24
	180	2.04	2.00	2.60	2.48	2.40
	240	2.08	1.92	2.80	2.48	2.32
Inhibited (w)[b]	180[c]	7.44	8.00			
	240	10.10	12.00			
		B. RNA Synthesis				
Control	0	17.5	17.5	18.40		17.5
	60	32.2	32.2	32.6		32.2
	120	47.4	48.0	49.3		47.4
	180	56.0	55.4	49.6		56.0
	240	56.0	57.6	57.3		56.0
Inhibited	0	17.5	17.5	18.4	17.0	17.5
	60	18.0	14.1	18.0	17.0	17.6
	120	20.9	11.7	16.3	17.8	18.3
	180	21.0	11.7	18.7	17.4	18.3
	240	21.8	10.5	20.8	17.0	16.3
Inhibited (w)[b]	180[c]	34.4	32.3	*d*	*d*	*d*
	240	36.0[e]	36.8[e]			
		C. Protein Synthesis				
Control	0	27.0	27.0	25.5		
	60	65.5	65.5	65.5		
	120	108	108	133		
	180	152	152	152		
	240	170	170	167		
Inhibited	0	27.0	27.0	30.5[f]	27.2[f]	26.5[f]
	60	...	29.0	31.5	28.5	27.0
	120	21.0	28.5	30.0	30.0	28.5
	180	24.0	28.0	32.0	31.0	30.0
	240	19.5	28.5	36.0	32.0	27.0
Inhibited (w)[b]	180[c]	57.0	61.0	*d*	*d*	*d*
	240	73.0	77.0			

[a] The concentration of each phenethyl alcohol (PEA) was at or near its bacteriostatic concentration. The following concentrations expressed as moles $\times 10^{-5}$/ml were used: PEA 2.46, *p*-MeO- 0.47, 2,5-$(MeO)_2$- 1.01, 3,4-$(MeO)_2$- 4.67, and 3,5-$(MeO)_2$- 1.09. [b] A single, inhibited culture was used for the 240-min test period. At the end of 120 min of incubation, 125 ml was removed from this culture and the inhibitor was removed. The cells were then resuspended in the same volume of new medium. This culture was then designated Inhibited (w) for "Inhibited, washed." [c] This interval of 180 min is to relate the culture age to the others. The 180-min time indication is only 60 min after the completion of the removal of the inhibitor and the resumption of incubation at the end of 120 min. [d] It did not seem necessary to show the reversibility of these biosynthetic processes by the removal of the inhibitor for all cases. [e] No reason can be given for these small second-hour increases. [f] These values are higher than the true-time "zero" value of 25.5 μg/ml for this series of test cultures. The increase was due to the inadvertent failure to chill the stock culture for a short time following the removal of the zero-time sample and before the addition of the culture to the flasks containing the inhibitors.

recrystallized from 95% ethanol: 2,3-dimethoxy-β-nitrostyrene (66%), mp 83–85° (lit.[6] 84–85°); 3,4-dimethoxy-β-nitrostyrene (80%), mp 139–141° (lit.[6] 141–142°); *p*-methoxy-β-nitrostyrene (91%), mp 86–87° (lit.[6] 86–87°); 2,4-dimethoxy-β-nitrostyrene (92%), mp 104–105° (*Anal.* Calcd for $C_{10}H_{11}NO_4$: C, 57.4; H, 5.3; N, 6.7. Found: C, 57.7; H, 5.3; N, 6.7.); 2,5-dimethoxy-β-nitrostyrene (79%), mp 121–123° (*Anal.* Calcd for $C_{10}H_{11}NO_4$: C, 57.4; H, 5.3; N, 6.7. Found: C, 57.2; H, 5.2; N, 6.7.); and 3,5-dimethoxy-β-nitrostyrene (86%), mp 128–130° (*Anal.* Calcd for $C_{10}H_{11}NO_4$: C, 57.4; H, 5.3; N, 6.7. Found: C, 57.3; H, 5.0; N, 6.7.).

The **dimethoxyphenethylamines** were prepared by dissolving the dimethoxy-β-nitrostyrenes (0.15 mole) in 30 ml of a 1:1 mixture of anhydrous ether and benzene[21] per gram of compound. This solution was added to 20 g of lithium aluminum hydride in anhydrous ether. After treating the mixture with 2 *N* H_2SO_4 followed by adjusting to pH 8 with NaOH, the products were isolated from the ether solution: 2,3-dimethoxyphenethylamine (60%), bp 162–164° (20 mm); picrate mp 176–178° (lit.[22] 177–178°); 2,4-dimethoxyphenethylamine (50%), bp 161–164° (19 mm); 2,5-dimethoxyphenethylamine (42%), bp 148–150° (9 mm) [lit.[23] 148°, (8 mm)]; 3,4-dimethoxyphenethylamine (46%), bp 164° (11 mm) [lit.[24] 142° (6 mm)]; *p*-methoxyphenethylamine (35%), bp 117–118° (10 mm) [lit.[25] 113–115° (6–7 mm)]; 3,5-dimethoxyphenethylamine (58%), bp 178–180° (19 mm) (*Anal.* Calcd for $C_{10}H_{15}NO_2$: C, 66.3; H, 8.3; N, 7.7. Found: C, 65.9; H, 8.0; N, 7.5.).

The **dimethoxyphenethyl alcohols** were prepared by the hydrolysis of the diazonium salts formed from the appropriate

(21) The quantity used was 800–1200 ml. 3,4-Dimethoxy-β-nitrostyrene was dissolved in 800 ml of tetrahydrofuran.

(22) E. Späth, K. Riedl, and G. Kubiczek, *Monatsh. Chem.*, **79**, 72 (1948).

(23) S. Sugasawa and H. Sigehara, *Ber.*, **74**, 459 (1941).

(24) I. Kikkawa, *Yakugaku Zasshi*, **78**, 1006 (1958); *Chem. Abstr.*, **53**, 3260*i* (1959).

(25) V. G. Vinokurov, V. S. Troitskaya, and V. A. Zagurevskii, *Zh. Obshch. Khim.*, **31**, 2991 (1961); *Chem. Abstr.*, **56**, 15473*a* (1962).

dimethoxyphenethylamines[8] (0.07–0.15 mole): phenethyl alcohol (40%), bp 99–100° (10 mm) [lit.[26] 99° (10 mm)]; urethan, mp 77° (lit.[27] 79°); 3,4-dimethoxyphenethyl alcohol (63%), bp 172–174° (17 mm), mp 48° (lit.[28] 45–47°); 3,5-dimethoxyphenethyl alcohol (78%), bp 191–194° (20 mm) [lit.[29] 140° (2 mm)]; 2,3-dimethoxyphenethyl alcohol (71%), bp 172–174° (22 mm); **urethan** derivative, mp 86° (*Anal.* Calcd for $C_{21}H_{15}NO_4$: C, 67.8; H, 6.4; N, 4.7. Found: C, 67.5; H, 6.0; N, 4.8.); 2,4-dimethoxyphenethyl alcohol (46%), mp 67–70° (*Anal.* Calcd for $C_{10}H_{14}O_3$: C, 65.9; H, 7.7. Found: C, 66.1; H, 7.6.); 2,5-dimethoxyphenethyl alcohol (99%), bp 176° (8 mm), 150–153° (8 mm)[30] (*Anal.* Calcd for $C_{10}H_{14}O_3$: C, 65.9; H, 7.7. Found: C, 65.9; H, 7.7.).

2,6-Dimethoxyphenethyl alcohol was prepared by adding an excess of ethylene oxide to 2,6-dimethoxyphenyllithium.[15] The remainder of the procedure was like that given above for the monomethoxyphenethyl alcohols, giving the 2,6-isomer (23%), mp 118–119° (benzene–*n*-hexane mixture).

Anal. Calcd for $C_{10}H_{14}O_3$: C, 65.9; H, 7.7. Found: C, 65.7; H, 7.5.

***o*- and *p*-hydroxyphenethyl alcohols** were prepared by the lithium aluminum hydride reduction of the appropriate ethyl (acetoxyphenyl)acetates:[9] *o*-hydroxyphenethyl alcohol (98%), bp 125–128° (0.5 mm) [lit.[31] 125–127 (0.5 mm)]; *p*-hydroxyphenethyl alcohol (94%), mp 92° ($CHCl_3$) (lit.[9] 92°). Hydrobromic acid demethylation of *m*-methoxyphenethyl alcohol produced *m*-hydroxyphenethyl alcohol (51%), bp 124–126° (0.23 mm) [lit.[10] 168–173° (4 mm)]; 3,5-dinitrobenzoate, mp 146–148° (lit.[10] 147°).

Dimethoxyphenethyl alcohol (3 g), 9.6 g of anhydrous $AlCl_3$ and 85 ml of benzene were heated under reflux for 2 hr, cooled, and poured onto ice. The **dihydroxyphenethyl alcohols** were isolated from the benzene: 2,5-dihydroxyphenethyl alcohol (13%), mp 76–78° ($CHCl_3$) (lit.[31] 76–78°); 3,4-dihydroxyphenethyl alcohol (81%), mp 82–84° (dilute methanol) (lit.[32] 81–83°); 2,6-dihydroxyphenethyl alcohol (27%), mp 120–122° (benzene) (*Anal.* Calcd for $C_8H_{10}O_3$: C, 62.3; H, 6.5. Found: C, 62.0; H, 6.7.).

Heating 2,6-dimethoxyphenethyl alcohol to 150° for 2 hr in a sealed tube with concentrated HCl[15] did not demethylate the material but produced **2-(2-chloroethyl)-1,3-dimethoxybenzene** (78%), mp 79–81° (dilute ethanol).

Anal. Calcd for $C_{10}H_{13}O_2Cl$: C, 59.9; H, 6.5; Cl, 17.7. Found: C, 59.9; H, 6.7; Cl, 17.5.

Heating 2,6-dimethoxyphenethyl alcohol with 48% HBr at 170° for 1 hr similarly produced **2-(2-bromoethyl)-1,3-dimethoxybenzene** (67%), mp 79–82° (dilute ethanol).

Anal. Calcd for $C_{10}H_{13}O_2Br$: C, 49.0; H, 5.3; Br, 32.6. Found: C, 48.8; H, 5.6; Br, 32.7.

Biological Methods. Viable-Cell Count *vs.* Concentration of Phenethyl Alcohols.—The organism used was *Escherichia coli* B, and the medium used was nutrient broth NaCl.[33] The medium for the agar plates was prepared from the liquid medium by the addition of 15 g of agar to 1 l. of the medium. All incubations were carried out in a water-bath shaker[34a] at 37°.

An inoculum from an overnight culture was added to 200 ml of medium in a 500-ml culture flask, and the contents were incubated until the viable-cell count had reached 1×10^8 cells/ml. A series of 50-ml culture flasks had been prepared by the addition of graded increments of the phenethyl alcohol to be studied. Since most of the compounds were relatively insoluble, the following procedure was used. To each of the flasks containing the compound was added 12.5 ml of medium. All flasks were heated[35] carefully on a hot plate until the compound was in solution. The flasks were then permitted to cool to approximately 37° at which time 12.5 ml of the culture containing 1×10^8 cells/ml was added to each.[36] The flasks were then incubated for a period of 4 hr; samples for viable-cell counts were removed from the control flask at "zero" time and from all flasks at the end of each hour of incubation. The viable-cell counts were determined by the usual plate-count procedure, the counts being the average of three plates following a 12–18-hr incubation period. An electronic colony counter was used.[34b]

Bacteriostatic Concentration.—The bacteriostatic concentration of each compound was obtained by plotting the log of the number of viable cells present in the culture at the end of the fourth hour of incubation against the concentration of the compound in moles per milliliter. The point at which the plotted line transected the abscissa corresponding to the initial ("zero" time) cell count was the bacteriostatic concentration. Figure 1 is an example of this graphic determination; it is capable of excellent reproducibility. Table I summarizes the bacteriostatic concentrations for the compounds evaluated.

The reversibility of the inhibition caused by the bacteriostatic concentration was determined for phenethyl alcohol and for *p*-methoxy-, 2,5-, 3,4-, and 3,5-dimethoxyphenethyl alcohols. Three flasks were prepared for each exercise by essentially the same procedure as that used above for a determination of viable-cell count *vs.* concentration. One flask contained no inhibitor, and the other two contained the bacteriostatic concentration of the particular phenethyl alcohol. Viable-cell counts were made as before, but at the end of the second or third hour of incubation, the inhibitor was removed from one of the experimental flasks by centrifugation and washing with medium. The cells from this flask were resuspended in the original volume of medium, and incubation was resumed; viable-cell counts were determined for each flask for the next 2 hr. Figure 2 is an example of the bacteriostatic action of one of the phenethyl alcohols and the reversibility of this inhibition by removal of the inhibitor.

Synthesis of Macromolecules.—To study the influence of the phenethyl alcohols on DNA, RNA, and protein synthesis, essentially the same procedure was used as that outlined for the reversibility studies above. Larger volumes of culture were used so that 50-ml samples could be removed from the control flask and the experimental flask at the end of each hourly interval. Table II is self-explanatory. The cells were collected by centrifugation, and the DNA, RNA, and protein content were determined. It is assumed that the action of all of the phenethyl alcohols is the same, but the influence on the synthesis of these macromolecules was studied for phenethyl alcohol, *p*-methoxy-, and 2,5-, 3,4-, and 3,5-dimethoxyphenethyl alcohols. DNA was determined by Burton's procedure,[37] salmon sperm DNA being used as a standard. RNA was determined by Mejbaum's procedure,[38] yeast RNA being used as a standard. Protein was determined by Lowry's procedure,[39] crystalline bovine albumin being used as a standard. Table II summarizes the data for the effect of the five phenethyl alcohols studied on the synthesis of DNA, RNA, and protein.

These phenethyl alcohols have been sent to the Cancer Chemotherapy National Service Center for screening.

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(35) This procedure was required for all of the compounds except phenethyl alcohol, *m*-methoxyphenethyl alcohol, and 3,4-dimethoxyphenethyl alcohol. These materials were soluble without heating. For 2,6-dimethoxy- and 3,4-dihydroxyphenethyl alcohols, it was necessary to use a culture medium containing 10% Tween 80 as well as heat. This concentration of Tween 80 had been found not to influence the rate of cell division of the organism.

(36) This gave an initial viable-cell count for each flask of about 5×10^7 cells/ml.

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