Bacteriostatic Activities of α-Glyceryl and α-Glycidyl Phenyl Ethers for *Escherichia coli* B.* (32411)

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Lilley and Brewer, in 1953(1), reported the inhibitory effect of phenethyl alcohol (PEA) on gram-negative bacteria. In 1962, Berrah and Konetzka(2) reported that the effect of PEA on *Escherichia coli* was due to a specific inhibition of DNA synthesis. It was later shown by Khafagy and Lambooy(3) and by Rosenkranz *et al*(4) that the compound also inhibited RNA and protein synthesis in this microorganism.

Khafagy and Lambooy(5) found 15 ring-substituted analogs of phenethyl alcohol to be active in varying degrees in inhibiting *E. coli*. The most potent of these, p-methoxyphenethyl alcohol, was found to be 6 times as potent as PEA. They also reported phenethylamine and p-methoxyphenethylamine to be good inhibitors in the same organism; both compounds being more potent than PEA. This raises a question concerning the need for a hydroxyl group on the side chain to insure activity. Rosenkranz *et al*(3) found the organism was also inhibited by cinnamyl alcohol (the hydroxyl group removed by 3 carbon atoms from the ring) and α,α-dimethylbenzyl alcohol (the hydroxyl group removed by 1 carbon atom from the ring).

This investigation was initiated to determine further the relationship between chemical structure and biological activity. Specifically, the correlation of activity to the length of the side chain, and to the number and position of available hydroxy groups on it would be tested. For this purpose, 7 α-glyceryl phenyl ethers bearing substituents in the benzene ring, and 7 α-glycidyl phenyl ethers bearing the same substituents in the same positions were tested. The former group possesses a 3-carbon side chain, with 2 free hydroxyl groups and an ether linkage to the benzene ring. The latter group has the same structure except that the 2 hydroxyl groups are replaced by an oxygen bridge. Antimicrobial and antifungal action has previously been ascribed to some α-glyceryl phenyl ethers(6).

Materials and methods. The α-glyceryl phenyl ethers which were not commercially available were synthesized by the Wheeler-Wilson method as modified by Lambooy(7). This method employs the alkaline condensation of the appropriately substituted phenol with 3-chloro-1,2-propanediol.

The α-glycidyl phenyl ethers not commercially available were similarly prepared by condensation of the appropriate phenol with epichlorohydrin, according to the method of Chezhovskaya *et al*(8). 1-Phenoxy-2-propanol, 2-phenoxyethanol and anisole, compounds not members of the above two groups, were also purchased for testing.

Those compounds not previously described in the literature are listed in Table I.

Escherichia coli B, grown in a nutrient broth-sodium chloride media, was the bacterium used in this study. Incubation was in a Gyrotory Shaker at 37°. Agar plates were

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TABLE I. Properties of New α-Glycidyl Phenyl Ethers

R	bp	Calculated C	Calculated H	Calculated Cl	Found C	Found H	Found Cl
2-CH_3*	120-124°/10 mm	73.1	7.4		73.3	7.6	
2-C_2H_5	129-132°/ 9 mm	74.1	7.9		73.9	8.3	
2-Cl-6-CH_3	143-147°/11 mm	60.5	5.6	17.9	60.1	5.8	18.0
2-F	122-123°/12 mm	64.3	5.4		64.0	5.2	

* This compound described in Ref. 8 but reported to have a b.p. of 120-124°/2 mm.

prepared from a solution containing 15 g of agar to 1 liter of the medium.

The method for determining bacteriostatic concentrations of the compounds was as follows(5). Sterile medium (100 ml) was inoculated with 1 ml of an overnight culture of the bacteria, and incubated until a viable cell count of 1×10^8 cells/ml was reached. A series of 50 ml culture flasks were prepared by dissolving graded increments (0 for the control) of the compound being studied in 12.5 ml sterile medium. The compounds were dissolved in the medium by heating the flasks carefully over a hot plate. The α-glycidyl phenyl ethers were solubilized with Tween 80. The compound was mixed with 2.5 ml of Tween 80 and 10 ml sterile media, and a solution was produced by mixing and heating under the hot water tap. The flasks were cooled to 37°; 12.5 ml of the culture with a viable cell count of 1×10^8 cells/ml was added to each flask. (This gave each an initial cell count of about 5.0×10^7 cells/ml and, where used, a Tween 80 concentration of 10%, which has been shown to have no effect on the organism's growth.) These flasks were incubated for 4 hours. Viable cell counts were determined by taking a sample from the control flask at "zero" time, and then from all flasks at hourly intervals. These samples were plated out by the standard procedure, incubated 12-18 hours, and counted on an electronic colony counter.

This procedure was repeated until a concentration of compound had been found which maintained the viable cell count at the initial number of cells. The bacteriostatic concentrations thus obtained are listed in Table II.

TABLE II. Bacteriostatic Concentrations of α-Glyceryl and α-Glycidyl Phenyl Ethers (All concentrations are x 10^{-5} Moles/ml).

R	α-Glyceryl R-C_6H_4-O-CH_2-CH-OH-CH_2OH	α-Glycidyl R-C_6H_4-O-CH_2-CH-CH_2 (O)
H*	3.2	3.2
2-CH_3	1.31	4.5
2-C_2H_5	.61	(10)‡
2-Cl-6-CH_3	.61	(10)‡
2-Cl	.90	2.9
2-F	3.1	1.8
4-OCH_3†	2.7	2.4

* The bacteriostatic concentration of PEA is 2.46×10^{-5} moles/ml.

† The bacteriostatic concentration of p-CH_3O-PEA is 0.48×10^{-5} moles/ml.

‡ Only approximate concentrations could be determined here due to the low solubility of the compounds.

The reversibility of the bacteriostasis caused by the bacteriostatic concentration of 2-chloro-6-methyl-1,2-propanediol was demonstrated in the following manner. Three flasks were prepared as previously described, the control containing no compound, and the other two containing the bacteriostatic concentration. The flasks were incubated for 4 hours, and viable cell count determinations made as previously described. At the end of the fourth hour, the inhibiting compound was removed from one of the experimental flasks by repeated centrifugation and washing with sterile media, and incubation was reinitiated. Viable cell counts were made for each flask at the end of every hour for the next 3 hours. The fact that bacteriostasis was reversible is demonstrated by data in Table III.

The effect of 2-chloro-6-methyl-1,2-propanediol on DNA, RNA, and protein synthesis was determined. The same procedure was employed as for the viable cell counts, but the volumes of the cultures were increased in order to permit 50 ml samples to be taken from each flask at the end of every hour for 4 hours, and after the compound had been washed out as before, 50 ml samples from the experimental flask after each succeeding hour for 3 hours. The cells from each sample were collected by centrifugation, and the nucleic acids were extracted with perchloric acid. DNA content was determined by Burton's procedure(9), with salmon sperm DNA as the standard. Mejbaum's procedure(10) was used to determine RNA, with yeast RNA being the standard. Lowry's procedure(11) was used for determining protein content; the standard was plasma bovine albumin. The fact that the compound reversibly inhibits the synthesis of DNA, RNA, and protein is shown by data in Table III. It seems a safe assumption that all of the α-glyceryl phenyl ethers act in the same way. The α-glycidyl phenyl ethers were not tested in these systems due to their uninterestingly high bacteriostatic concentrations.

TABLE III. Reversibility of Bacteriostasis and Inhibition of DNA, RNA, and Protein Synthesis by 2-Chloro-6-methyl-1, 2-propanediol.

Sample	Elapsed Time (min)	Viable Cell Count/ml Culture	DNA (ug/ml)	RNA (ug/ml)	Protein (ug/ml)
Control	0	4.0×10^7	.36	1.79	15.6
"	60	1.79×10^8	2.64	8.32	72.5
"	120	1.03×10^9	4.00	8.67	98.5
"	180	1.47×10^9	5.65	9.04	
"	240	2.2×10^9	8.60	10.30	142.5
"	300	2.9×10^9			
"	360	3.5×10^9			
"	420	4.1×10^9			
Experimental	0	4.0×10^7	.36	1.79	15.6
"	60	1.10×10^8	.88	1.87	19.5
"	120	1.70×10^8	.68	1.60	17.5
"	180	1.46×10^8	.74	1.60	27.2
"	240	1.43×10^8	.88	1.79	25.7
	*	a b			
"	300	1.34×10^8 1.2×10^8	2.44	7.39	60.2
"	360	1.19×10^8 7.8×10^8	5.56	11.01	118.0
"	420	9.2×10^7 1.78×10^9	6.68	11.20	142.5

* At this point, the compound was removed from Experimental Sample "b". The compound was not removed from Experimental Sample "a". DNA, RNA, and Protein data are for Sample "b".

Results and discussion. The bacteriostatic concentrations for *E. coli* B, as determined for the α-glyceryl phenyl ethers and the α-glycidyl phenyl ethers, are listed in Table II. The parent, unsubstituted compounds have approximately the same activity. But in general, the α-glyceryl phenyl ethers are much more effective than the α-glycidyl phenyl ethers. The 2-chloro-6-methyl-, and the 2-ethyl substituted compounds are the most effective of the α-glyceryl phenyl ethers and the least effective of the α-glycidyl phenyl ethers.

In general, for the unsubstituted compounds, phenethyl alcohol (C_6H_5-CH_2-CH_2-OH) and 2-phenoxyethanol (C_6H_5-O-CH_2-CH_2-OH) are the most effective (2.6×10^{-5} moles/ml); α-glyceryl- and α-glycidyl phenyl ethers are next most effective (3.2×10^{-5} moles/ml), and 1-phenoxy-2-propanediol (C_6H_5-O-CH_2-CHOH-CH_3) and anisole (C_6H_5-O-CH_3) are the least effective (approximately 9×10^{-5} moles/ml). The effectiveness of a compound within either series appears to depend more on nuclear substitution than on the composition of the side chain. The substituted α-glyceryl phenyl ethers are generally more effective than the α-glycidyl phenyl ethers, and both groups have members more potent than PEA (2.64×10^{-5} moles/ml). p-Methoxyphenethyl alcohol is still the most potent compound yet tested in this system (0.48×10^{-5} moles/ml).

It is interesting to note in the reversible inhibition demonstrations, that the DNA content of the experimental culture doubles during the first hour after the inhibitor is added. This explains the apparent delay in inhibition, as shown by the fact that the viable cell count for the experimental culture also increased during the first hour. The same phenomenon has been observed by Treich and Konetzka during the use of PEA (12).

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A Description of Eight Feline Picornaviruses and an Attempt to Classify Them. (32412)

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The isolation of 6 distinct serotype viruses from the oropharynx(1,2) and 2 from visceral organs(3,4) of the domestic cat has previously been reported. Although some of their biologic properties are known, the viruses remain unclassified. These viruses are currently grouped as to the type of cytopathology induced in cultures of feline renal cells. The characteristic feature of their cytopathic effect is the rapidity of destruction of the monolayer without the formation of inclusion bodies or giant cells. Since other workers in the United States(5,6) and Europe(7,8,9) have isolated similar agents from the domestic cat, it appears that this group of viruses may comprise a large but undetermined number of serotypes.

The present study of some of their biochemical and biophysical properties was undertaken in an attempt to characterize these viruses. The 8 serotypes were examined with respect to: host range, plaque production, sensitivity, acid stability, nucleic acid type, ability to be stabilized against thermal inactivation by molar magnesium salts and size.

Materials and methods. Viruses: The 8 viruses are designated as California feline isolate (CFI)(2), kidney cell degenerating virus (CKD)(3), Bolin's virus (FPL)(5) and the feline respiratory isolates (FRI) 6, 12, 14, 29, and 278(1). They were used in their 6 to 13 feline cell culture passage. A feline herpesvirus, Poliovirus I and Reo I viruses were used as controls. Virus pools were prepared in cell cultures grown in 32 oz. prescription bottles. The cells and fluid were frozen and thawed once after maximum cytopathic effect (CPE) occurred. The virus suspensions were clarified at 2000 rpm for 15 minutes.

Tissue culture. Four types of cell cultures were employed. Bovine, feline and monkey† renal cell cultures, and HeLa cells. Primary bovine (BKC) and feline (FKC) renal cell cultures were prepared by the method of Madin *et al*(10). The BKC were used both as primary cells in culture tubes and subcultures prepared from bottles. The medium used for the BKC at the time of inoculation was 0.5% lactalbumin hydrolysate in modified Hank's balanced salt solution (HBSS) containing 0.1 M Tris buffer with the addition of 3% lamb serum. FKC and MKC cultures were maintained on 0.5% lactalbumin hydrolysate in Earle's balanced salt solution with 3% calf serum. HeLa cells were maintained with Ginsberg's medium. All media contained 500 units of penicillin and 0.5 mg of streptomycin per ml. All cells were incubated stationary at 36°C.

Plaque production. Plaque studies were performed by the prescription bottle technique. Culture medium was aspirated and 0.3 ml of virus was inoculated. Adsorption was carried out at 37°C for 1 hour with gentle agitation at 15-minute intervals. The cell sheet was overlaid with 10 ml of melted agar (1.5%) in Earle's salt solution containing

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† Obtained from commercial source.