The Antimicrobial Action of Certain Glycerol Ethers and Related Compounds

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After it had been shown that the phenyl ether of ethylene glycol possessed antibacterial properties against *Pseudomonas aeruginosa* (Berry, 1944) the antimicrobial and pharmacological activities of a number of related compounds were evaluated. Two of the compounds investigated in this connection have found clinical usefulness. These are mephenesin, 3-*o*-toloxy-1,2-propanediol, widely used as a muscle relaxant (Berger and Bradley, 1946) and chlorophenesin, 3-*p*-chlorophenoxy-1,2-propanediol, used in the treatment of fungus infections of the skin (Hartley, 1947).

The object of the investigation reported here was to study the antibacterial and antifungal action, the toxicity and other biological properties of some simple methylated and chlorinated phenyl ethers of glycerol, propylene glycol and trimethylene glycol and to attempt to relate these properties with the chemical structure and physical properties of the compounds. This study has brought to light several compounds with substantially higher antibacterial and antifungal action than chlorophenesin.

Methods

The glycerol, propylene glycol and trimethylene glycol ethers used in this study were prepared by alkaline condensation of the appropriate phenolic compound with glycerol-α-monochlorohydrin, propylene chlorohydrin and trimethylene chlorohydrin respectively. The preparation and physical properties of the newer members of this series have been recently described[1] (Ludwig, *et al*, 1952).

The following microorganisms were used for the determination of bacteriostatic and fungistatic concentrations: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* (*Micrococcus pyogenes* var. *aureus*), *Streptococcus pyogenes*, and *Bacillus subtilis*, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Microsporum audouini*, *Microsporum gypseum*, and *Candida albicans*.

Compounds were tested against *E. coli*, *S. aureus*, and *P. aeruginosa* in nutrient broth, pH 7.0; and against *S. pyogenes* and *B. subtilis* in Bacto brain heart infusion broth, pH 7.0; and against fungi in broth containing 1 per cent neopeptone, 2 per cent dextrose with pH adjusted to 5.7.

A tube dilution method was used to determine inhibitory concentrations. Since most of the compounds were only slightly soluble in water, 1 per cent stock solutions were prepared by dissolving the test compound in the smallest possible amount of ethanol and diluting to volume with water. These stock solutions were further diluted in the test medium to give a final volume of 10 ml per tube. The tubes were sterilized at 121 C for 10 minutes, cooled, and inoculated with the test organism using 0.1 ml of an 18- to 24-hour culture of the bacteria in brain heart infusion broth as inoculum. *C. albicans* was grown for 18 to 20 hours in broth, then diluted to contain approximately 10×10^6 organisms per ml by direct microscopic count, and 0.1 ml used to inoculate each tube. Fungi were grown for 14 days on Sabouraud dextrose agar with 0.5 per cent yeast extract. Spore suspensions in sterile saline were diluted, after direct microscope counts had been made, to contain approximately 10×10^6 spores per ml. The standard inoculum was 0.1 ml of the diluted spore suspension.

The bacteriostatic dilution was taken as the highest dilution which completely inhibited visible growth of the test organisms in duplicate tubes for 3 days at 37 C. The fungistatic dilution was taken as the highest dilution which completely inhibited visible growth of the test fungi for 14 days at 30 C.

The irritation and sensitization action of the ethers was tested by direct application of test patches to the skin of at least ten subjects. The compounds were incorporated into petrolatum in concentrations of 2 and 5 per cent, and applied to the test patch. The patches were permitted to remain in contact with the skin for 48 hours and the area observed immediately after removal of the patch and again after an additional 24 hours. To evaluate the sensitizing action the same compounds were again applied in a similar manner to the same subjects and over the same areas of skin after an interval of two weeks.

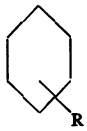
The pharmacological action and toxicity of the compounds was determined in mice using freshly prepared suspensions of the ethers in 5 per cent aqueous gum acacia solution. The use of suspensions was necessitated by the low water solubility of most of the compounds (less than 0.1 per cent).

[1] We are indebted to Dow Chemical Co. for generous samples of Dowanol 41B, 78B, and 79B.

Results

The compounds surveyed were substituted aromatic ethers of glycerol, propylene glycol and trimethylene glycol. Table 1 summarizes the activity of the compounds. The results obtained with each variation of with a single substitutent on the benzene nucleus were as a rule weaker fungistats than corresponding compounds with two or three nuclear substituents. Thus compounds 2, 5, and 7 with a single nuclear substituent were inferior to compounds 12, 14, and 16 possessing

Table 1. *The antimicrobial action of substituted phenolic ethers of glycerol, propylene glycol, and trimethylene glycol*

COMPOUND NUMBER	 R	SIDE CHAIN	FUNGISTATIC DILUTION	DILUTION INHIBITING *Candida albicans*	DILUTION INHIBITING *Escherichia coli*	DILUTION INHIBITING GRAM POSITIVE ORGANISMS
1	2-methyl	G	1:1000	—	1:500	—
2		P	1:1000–1:2500	—	1:1000	1:500–1:1000
3		T	1:2500–1:5000	1:1000	1:1000	1:1000
4	2-chloro	G	1:1000	—	1:750	1:1000
5		P	1:2500–1:5000	1:1000	1:1000	1:1000
6	4-chloro	G	1:1000	1:500	—	—
7		P	1:3000–1:4000	1:1000	1:500	1:500–1:1000
8		T	1:5000–1:7500	1:1000	1:1000	1:1000
9	2,6-dichloro	G	1:1000	—	1:500	1:500–1:1000
10		P	1:5000	1:1000	1:1000	1:1000
11	2,4-dichloro	G	1:1000–1:2500	1:1000	1:2500	1:1000–1:2500
12		P	1:10,000	1:2500	1:2500	1:2500
13	4-chloro-2-methyl	G	1:1000	—	1:1000	1:1000
14		P	1:10,000	1:1000	1:2500	1:2500–1:5000
15	4-chloro-3-methyl	G	1:2500–1:5000	1:1000	1:1000	1:1000
16		P	1:10,000	1:3000	1:2500	1:1000–1:5000
17	6-chloro-2-methyl	G	1:1000	1:500	1:500	1:500
18		P	1:2500	1:1000	1:1000	1:1000
19		T	1:1000–1:5000	1:1000	1:1000	1:1000
20	4-chloro-2,6-dimethyl	G	1:2500	1:1000	1:1000	1:1000
21		P	1:15,000	1:2500	1:2500	1:2500–1:5000
22		T	1:15,000	1:2500	1:2500	1:2500–1:10,000
23	4-chloro-3,5-dimethyl	G	1:5000–1:10,000	1:1000	1:2500	1:2500–1:7500
24		P	1:15,000–1:30,000	1:5000	1:2500	1:5000–1:10,000
25		T	1:40,000	1:10,000	1:5000	1:7500–1:10,000
26	2,6-dichloro-4-methyl	G	1:5000	1:1000	1:1000	1:1000
27		P	1:10,000–1:15,000	1:2500	1:2500	1:2500–1:5000
28		T	1:20,000	1:5000	1:2500	1:2500–1:5000

G = glycerol ether; P = propylene glycol ether; T = trimethylene glycol ether.

substituents on the benzene ring joined to the three different hydroxylated side chains are grouped together. To shorten the table only the highest and lowest bacteriostatic and fungistatic dilutions are given.

Fungistatic Action

All of the compounds examined possessed fungistatic action in dilutions of 1 in 1,000 or higher. Compounds two nuclear substituents. These latter compounds were again less active than compounds 21, 24, and 27 which had three substituents on the nucleus.

The relative effectiveness of chlorine atoms and methyl groups in the phenolic ring was of interest, in view of the effect these substituents have on the antimicrobial activity of phenol. There were only slight

differences in activity among the various mono-substituted derivatives tested.

The position of the substituent on the nucleus was also of some importance. With a single substituent there was little difference between the ortho and para isomers. When two substituents were present, however, compounds that had one substituent in the para position were superior to compounds having both substituents adjacent to the oxygen. Thus compounds 12, 14, and 16 with substituents in the 2,4, or 3,4 positions were more effective than compounds having the same substituents in the 2,6 positions (compounds 10 and 18). Trisubstituted compounds in which the substituents were as distant as possible from the ether linkage (compounds 23, 24, 25) were more effective than the corresponding compounds in which some of the same groups were adjacent to the ether linkage (compounds 20, 21, 22, and 26, 27, 28).

The effect of the number and position of added methyl groups on a chlorophenyl ether is demonstrated by the increase in activity of the several methylated derivatives of compound 7, namely compounds 14, 16, 21, and 24. The ethers of 4-chloro-3,5-dimethylphenol are the most effective of those studied.

Comparison of compounds with different hydroxylated side chains but with the same nuclear substituents indicated that the propylene glycol and trimethylene glycol ethers were always more effective than the corresponding glycerol ethers. In many instances the trimethylene glycol ethers were somewhat more effective than the corresponding propylene glycol ethers.

Action on C. albicans

Most of the compounds tested inhibited growth of *C. albicans*. All were, however, less effective in inhibiting the growth of this yeast than they were in inhibiting growth of other pathogenic fungi. The efficacy of the compounds was again related to the position of the nuclear substituent. The propylene glycol and trimethylene glycol ethers were again superior to the glycerol ethers in their toxic action on *C. albicans*.

Antibacterial Action

All of the compounds tested possessed some antibacterial action against both gram- positive and gram-negative bacteria. Most of the compounds examined had, however, weaker bacteriostatic than fungistatic properties. The less resistant organisms such as streptococci were inhibited in higher dilutions than the more resistant such as staphylococci. *Pseudomonas* was the most resistant organism tested and was not inhibited by most compounds in concentrations of 1 in 500. The relation between activity, nuclear substitution and nature of side chain was similar to that described earlier under fungistatic action.

Irritant and sensitizing properties

The irritant and sensitizing properties of the 9 most active compounds (Nos. 20 to 28) have been examined. Each of the three trimethylene glycol ethers tested (Nos. 22, 25, and 28) produced irritation in about one-half of the subjects to whom it was applied. The propylene glycol ethers and the glycerol ethers did not cause irritation in any of the subjects. None of the compounds tested produced sensitization.

Pharmacological Properties and Toxicity

All of the compounds produced reversible paralysis when injected into mice in suitable dosage. After intraperitoneal administration of the compounds in doses of 200 to 300 mg per kg, the animals lost the righting reflex and were unable to move. Respiration and circulation were well maintained during paralysis. The animals recovered spontaneously and did not show any immediate or late ill effects. Neuromuscular conduction during paralysis was unchanged. Paralysis was due to depression of the central nervous system and was similar to that obtained after administration of mephenesin (Berger, 1949). Most of the compounds were relatively nontoxic, the LD_{50} after intraperitoneal administration being about 500 mg per kg.

Discussion

Methylated and chlorinated phenolic ethers of glycerol, propylene glycol and trimethylene glycol have a rather nonspecific activity against a number of different microorganisms. In this respect they are very similar to the phenols from which they are derived. The attachment of the hydroxylated side chain through the phenolic oxygen modifies certain important physical and biological properties of the phenols. The ethers are devoid of the characteristic odor and the corrosive properties of their phenolic parent compounds. Both groups of compounds in large doses exert an action on the central nervous system. With phenols this action manifests itself in animals by muscular weakness accompanied by tremors or mild convulsions. Death is often delayed and due to respiratory and circulatory collapse. The ethers on the other hand produce transient muscular paralysis which with most compounds is short in duration and followed by complete recovery. This train of events is probably due to the rapid inactivation of the ethers in the body in a manner similar to that occurring with mephenesin (Riley and Berger, 1949). It appears likely that the ether linkage is not broken down in the body with the liberation of the more toxic free phenols. The ethers are probably inactivated by oxidation of the alcoholic hydroxy groups of the side chain.

It has been known for a long time that the antibacterial and antifungal action of phenols can be increased by the introduction of suitable nuclear sub-

stituents (von Oettingen, 1949). The present paper shows that the antimicrobial properties of phenolic ethers behave similarly. The trimethylene glycol ethers are nearest in their antimicrobial action to free phenols but possess some of the irritant properties of the parent phenols. The propylene glycol ethers are only slightly less effective antimicrobial agents than the free phenols or their trimethylene glycol ether derivatives and appear to lack irritant and sensitizing properties. The glycerol ethers are also non-irritant but possess the weakest antimicrobial action.

Berry in 1944 drew attention to the unusual bacteriostatic properties of the phenyl ether of ethylene glycol against *P. aeruginosa*. Roushdi and Ibrahim (1950) showed that the phenyl ether of glycerol and certain other simple glycerol ethers were less active against *P. aeruginosa* than the corresponding ethylene glycol derivatives. The results reported in this paper confirm Roushdi and Ibrahim's findings and show in addition that various aryl ethers of propylene glycol and trimethylene glycol do not possess strong bacteriostatic action against *P. aeruginosa*.

One compound described in this paper, chlorophenesin, 3-*p*-chlorophenoxy-1,2-propanediol, has been recommended as an antibacterial and antifungal agent of pharmaceutical interest (Hartley, 1947). It is widely sold as a fungicidal agent under the trade name of Mycil, (British Drug Houses, Ltd.) in Great Britain and the British Commonwealth. Many compounds included in this study possess considerably greater antifungal activity than chlorophenesin. Clinical trials of some of these compounds are now in progress and preliminary results appear to bear out the *in vitro* findings.

The chemotherapeutic action of some of the more potent compounds has also been examined in mice infected with certain pathogenic bacteria, fungi and with *C. albicans*. All compounds examined were devoid of curative action in infected animals.

Most of the compounds described are stable, chemically inert and relatively nontoxic. These properties suggest that the phenolic ethers may be of value as preservatives for pharmaceuticals and other products, especially in those preparations where low water solubility of the preservative would not be disadvantageous.

Summary

The activity of a number of chloro- and methyl- substituted aryl ethers of glycerol, propylene glycol and trimethylene glycol on bacteria, fungi and yeast has been investigated.

In general all compounds examined possessed greater fungistatic than bacteriostatic activity.

The antimicrobial activity increased with the number of substituents on the nucleus and to a certain extent was a function of the position of substitution. The methyl group and chlorine atom were approximately equivalent in their effect, and maximum activity was obtained with the 4-chloro-3,5-dimethylphenoxy ethers.

Trimethylene glycol ethers were the strongest antimicrobial agents but produced irritation when applied to the skin. The propylene glycol ethers combined considerable antifungal activity with lack of irritant properties.

Etherification of substituted phenols with propylene glycol or glycerol offers a way of producing stable, nonreactive and non-toxic compounds of potential value as fungicides and preservatives for pharmaceutical and other products.

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