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Received April 28, 1961. P.S.E.B.M., 1961, v107.

Activity of α-Phenoxyalkyl Penicillins Against Sensitive and Resistant Staphylococci. (26655)

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(Introduced by H. A. Feldman)
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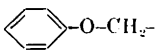
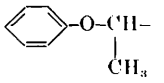
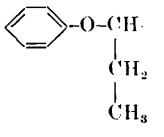
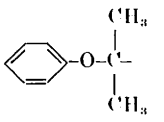
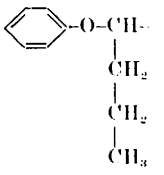
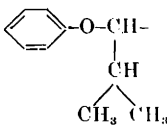
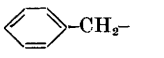
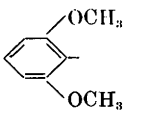
While studying the antimicrobial activity of a large number of synthetic penicillins, it became apparent that members of the α-phenoxyalkyl penicillin series were displaying significant activity against penicillinase-producing resistant staphylococci. Preliminary microbiological data and methods of synthesis of a number of such penicillins have been reported(1,2). A detailed study was done on certain members of this series of penicillins using both sensitive and resistant staphylococci in an attempt to delineate the extent of the activity and relate some of the properties of the penicillins to the structure. Comparisons of these penicillins were made with penicillin G and dimethoxyphenyl penicillin, the former being penicillinase sensitive and the latter extremely penicillinase resistant (3). Studies were also carried out to determine the *in vivo* effectiveness of the different penicillins in curing penicillin-sensitive and penicillin-resistant *Staphylococcus aureus* infections in mice.

Materials and methods. The penicillins used in this study were alkyl derivatives of phenoxymethyl penicillin substituted in the α position. The side chains of the penicillins studied are shown in Table I together with the side chains of controls, benzylpenicillin (penicillin G) and dimethoxyphenyl penicillin. The last was used as the monohydrate of the sodium salt. All the other penicillins were potassium salts. Benzylpenicillin (penicillin G), α-phenoxyethyl penicillin (phenethicillin), dimethoxyphenyl penicillin, and phenoxymethyl penicillin (penicillin V) were obtained from commercial sources, the first 3 being Bristol Laboratories production lots, the last one a Lilly product. The other penicillins were synthesized by the Organic Chemistry Dept. of Bristol Laboratories.

Cultures were maintained on porcelain beads(4), on nutrient agar slants, or frozen in milk suspension. The inoculum used for determination of the minimum inhibitory concentration was prepared by overnight incubation of a bead or a loopful from a slant culture in heart infusion broth. Standard 2-fold serial dilution technics in heart infusion broth (Difco) or in heart infusion mixed 1:1 with pooled filter-sterilized human serum were used. Usually, the overnight culture was diluted 10^4, but other dilutions were also used as noted below. Cultures for *in vivo* challenges were grown overnight in veal infusion broth. The challenge dose of *Staphylococcus aureus*, Smith strain, consisted of a dose of organism equivalent to 100 LD_{50} or approximately 8×10^5 viable cells per mouse, suspended in 5% hog gastric mucin. The challenge dose of *S. aureus*, 1633-2 strain, consisted of a dose of organism equivalent to 50 LD_{50} or approximately 1.1×10^8 viable cells per mouse prepared similarly. This latter strain was isolated from clinical material and is a penicillinase producer.

Penicillinase inactivation of the penicillins was determined by the method of Henry and Housewright(5). Acetone-ether treated staphylococcal cells prepared according to Gilson and Parker(6) from cells of the 52-75 strain of *Staphylococcus aureus* were used as the source of penicillinase. The culture was induced for 3 hours at 28°C on a rotary shaker with 100 μg/ml of benzylpenicillin added at 0 time, followed by a similar addi-

TABLE I. Side Chains of Penicillins.

Penicillin	Structure
Phenoxymethyl penicillin	
α-Phenoxyethyl penicillin	
α-Phenoxypropyl penicillin	
α-Phenoxyisopropyl penicillin	
α-Phenoxybutyl penicillin	
α-Phenoxyisobutyl penicillin	
Controls:	
Benzylpenicillin	
Dimethoxyphenyl penicillin	

tion at 45 minutes and by 100 μg/ml of dimethoxyphenyl penicillin at 90 minutes. The dry preparation was stored in the refrigerator. For determination of penicillin inactivation, a suspension of cells was prepared in 0.017 M bicarbonate buffer and homogenized with teflon grinders. To the main compartment of a Warburg respirometer, 2.7 ml of this penicillinase preparation were added and 0.5 ml of penicillin solution in the same buffer was placed in the side arm. The amount of enzyme preparation in the vessel depended on the penicillinase susceptibility of the penicillin. For most penicillins, 405 μg per vessel was used. For more resistant penicillins, a larger amount was used. The vessels were equilibrated with a gas mixture containing 95% oxygen and 5% carbon dioxide. In all cases, the penicillins were present in 0.01 molar solution. Determinations were run in duplicate, and expressed in cu mm of CO_2 per mg dry material per hour. Benzylpenicillin was always used as a control and final calculations were made in per cent of the rate obtained with penicillin G.

Median curative doses (CD_{50}) were determined using acute infections produced by intraperitoneal injection of challenge doses of the organisms. The challenge was immediately followed by intramuscular treatment with graded doses of aqueous solutions of the various penicillins. The CD_{50} was determined from a probit transformation of percentage of deaths at each dose level using graphic estimation of the 50% point.

Results. In vitro *experiments.* The minimum inhibitory concentrations (MIC) of various penicillins against penicillin-sensitive (Smith) and penicillin-resistant (1633-2) strains of *S. aureus* in presence and in absence of serum are presented in Table II. Examination of the data reveals that the activity against the Smith strain decreases as the alkyl group becomes larger. This relationship holds both in absence and presence of 50% human serum. In the case of the 1633-2 strain which is penicillin G resistant, a greater activity is seen in the case of some of the higher homologs. Thus the α-phenoxypropyl and α-phenoxyisobutyl penicillins appear to be particularly active. The presence of 50% serum, however, alters these relationships somewhat, persumably due to different degrees of serum binding. Comparison of these results with that of dimethoxyphenyl penicillin shows that while this penicillin is not very active, the MIC's obtained with the resistant strain are very similar to that obtained with the sensitive strain. Also, the presence of serum had essentially no effect on

TABLE II. Minimum Inhibitory Concentrations (MIC) of α-Phenoxyalkyl Penicillins against Penicillin Sensitive (Smith) and Penicillin Resistant (1633-2) Strains of *Staphylococcus aureus* in Presence and Absence of Serum Using a 10^4 Inoculum Dilution.

	MIC in μg/ml			
	Smith strain		1633-2 strain	
Penicillin	Without serum	With serum	Without serum	With serum
Phenoxymethyl	.04	.16	6.25	25.0
α-Phenoxyethyl	.08	.16	1.6	6.25
α-Phenoxypropyl	.08	.62	.8	6.25
α-Phenoxyisopropyl	.3	1.2	1.6	25.0
α-Phenoxybutyl	.6	2.5	6.25	50.0
α-Phenoxyisobutyl	.3	1.2	.8	12.5
Controls:				
Benzylpenicillin	.04	.08	12.5	25.0
Dimethoxyphenyl penicillin	1.2	2.5	1.6	1.6

its antibacterial activity. This compound had the lowest MIC against the 1633-2 strain in the presence of 50% serum.

It has been said that determination of the antistaphylococcal action of penicillins in the presence of low numbers of organisms is not realistic(7,8). Human staphylococcal abscesses are purported to have very high bacterial count. In Table III, the influence of inoculum size on the MIC of the resistant *S. aureus*, 1633-2 strain, is shown. Of the various penicillins in this series, only α-phenoxy-isobutyl penicillin is still inhibitory at the higher inoculum levels. There is a marked increase in MIC, however, as the inoculum dilution is varied from 10^5 to 10^2. This contrasts markedly with the MIC of the dimethoxyphenyl penicillin control which increases only slightly if at all at the highest inoculum levels.

TABLE III. Effect of Inoculum Size of a Penicillinase-Producing *S. aureus* Strain on Minimum Inhibitory Concentration of α-Phenoxyalkyl Penicillins.

	MIC in μg/ml			
	Inoculum dilution			
Penicillin	10^2	10^3	10^4	10^5
Phenoxymethyl	>100	>100	6.2	.4
α-Phenoxyethyl	>100	100	1.6	.8
α-Phenoxypropyl	>100	25	.8	.8
α-Phenoxyisopropyl	>100	12.5	1.6	.8
α-Phenoxybutyl	>100	100	6.2	3.12
α-Phenoxyisobutyl	12.5	1.6	.8	.8
Controls:				
Benzylpenicillin	>100	>100	12.5	.8
Dimethoxyphenyl penicillin	3.2	3.2	1.6	1.6

Since the effect of inoculum level is thought to be due to ability of the penicillin to withstand destruction by penicillinase, the relative rates of decomposition by staphylococcal penicillinase were determined for penicillin G, α-phenoxyisobutyl penicillin, and dimethoxyphenyl penicillin using the manometric technics described above. Expressing the results in terms of benzylpenicillin (equal to 100), α-phenoxyisobutyl penicillin was 20 and dimethoxyphenyl penicillin was 0.6. Thus, the α-phenoxyisobutyl penicillin was 5 times more resistant to destruction than penicillin G and dimethoxyphenyl penicillin was approximately 30 times more resistant than α-phenoxyisobutyl penicillin.

TABLE IV. Comparison of Intramuscular CD_{50} Values of Different Penicillins Using Penicillin Sensitive (Smith) and Penicillin Resistant (1633-2) Strains of *Staphylococcus aureus*.

	CD_{50} in mg/kg	
Penicillin	Smith strain	1633-2 strain
Phenoxymethyl	.8	>500
α-Phenoxyethyl	.5	>500
α-Phenoxypropyl	1.1	>500
α-Phenoxyisopropyl	3.7	>500
α-Phenoxybutyl	4.0	>500
α-Phenoxyisobutyl	6.0	170–240
Controls:		
Benzylpenicillin	1.1	>500
Dimethoxyphenyl penicillin	9.0	28–45

In vivo *experiments*. Results of mouse protection tests are reported in Table IV. All α-phenoxyalkyl penicillins studied protect mice against infection with the sensitive *S. aureus* strain at rather low doses, the lower homologs being most active. In the case of the penicillin-resistant strain, however, only α-phenoxyisobutyl penicillin showed some protection under the conditions of this test. The CD_{50} values are, however, considerably higher than those obtained with dimethoxyphenyl penicillin. Approximately 5 times more α-phenoxyisobutyl penicillin is required

than of dimethoxyphenyl penicillin to give the same degree of protection in these tests.

Discussion. It is interesting that as the side chain of the α-phenoxyalkyl penicillins becomes larger, activity against the penicillin-sensitive strain of *S. aureus* decreases. At the same time, in the case of some of the members of the series, there is an increase in activity against a penicillinase-producing *S. aureus*. This is especially seen with the compound α-phenoxyisobutyl penicillin. Of the members in the series examined, this compound shows the greatest *in vitro* and *in vivo* activity against a penicillinase-producing resistant staphylococcus. However, the activity is considerably less than that seen with dimethoxyphenyl penicillin. In the cases examined, the activity against the resistant staphylococcus paralleled the penicillinase resistance and it would thus appear that such compounds are active against these organisms by virtue of their ability to withstand degradation by the staphylococcal penicillinase.

The penicillins in this series have an asymmetric carbon in the side chain and therefore exist as diastereoisomeric mixtures. The method of preparation was such that approximately equal amounts of each diastereoisomer would be present. The data presented therefore represent the combined activities of both diastereoisomers.

The authors wish to thank Dr. L. C. Cheney and other members of the Organic Chemistry Dept., Bristol Laboratories, for making available the various synthetic penicillins.

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Received May 1, 1961. P.S.E.B.M., 1961, v107.

Studies on Glucose Metabolism in Cartilage *in vitro*.* (26656)

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Survey of the literature reveals a paucity of *in vitro* metabolic studies on carbohydrate metabolism in cartilaginous tissue, particularly in the presence of added hormones. However, the *in vivo* effects of growth hormone and insulin on mucopolysaccharide metabolism have been intensively investigated. Dorfman and Schiller(1) reported decreased uptake of S^{35} from injected $Na_2S^{35}O_4$ and C^{14} from acetate-1-C^{14} or glucose-U-C^{14} into chondroitin sulfate isolated from skin of diabetic rats. Insulin treatment returned these values toward normal. In hypophysectomized rats, there was a rapid decline in incorporation of these labeled compounds into chondroitin sulfate and hyaluronic acid. Growth hormone given to these hypophysectomized rats restored only incorporation into chondroitin sulfate without any apparent ef-

* Supported by grants from Arthritis and Rheumatism Foundation and from Nat. Inst. Health.

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