

16668

Antibacterial Activity of Gentisyl Alcohol and Homogentisic Acid.

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(Introduced by J. C. Forbes)

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It has been reported recently that gentisyl alcohol¹ and numerous derivatives of gentisyl alcohol^{2,3} have practically the same antibacterial activity as patulin* against *S. aureus* in plate tests. Raistrick and coworkers⁵ had reported previously that gentisyl alcohol had only slight activity against *S. aureus*, in contrast to the strong antibacterial activity of patulin.⁶

In preparation for studies involving homologous alcohols related to gentisyl alcohol we prepared gentisyl alcohol in order to test its antibacterial activity against *S. aureus*. In contrast to the reported findings of the Swiss workers,¹⁻³ but in agreement with the original report of Raistrick and coworkers,⁵ our results with gentisyl alcohol in the cylinder plate penicillin assay technic indicated only slight antibacterial activity over a broad pH range. For this reason work with the higher homologues of gentisyl alcohol is not contemplated at the present time, but in view of the relationship of homogentisic acid to the next higher homologue of gentisyl alcohol we have also tested this compound for antibacterial activity against *S. aureus*. We found it to have definite but slight antibacterial activity of about the same order of magnitude as that of gentisyl alcohol. Curves showing the anti-

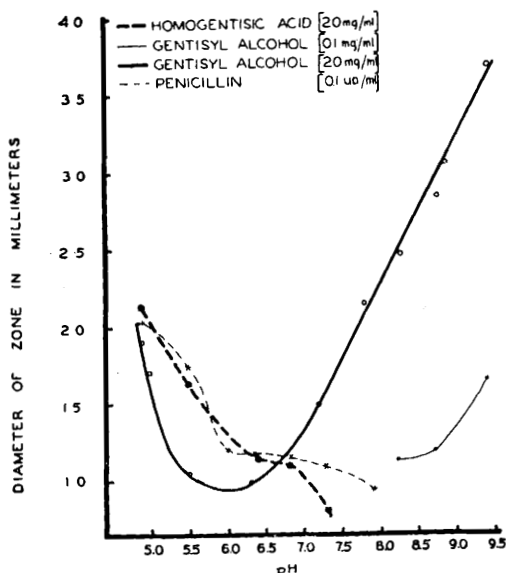


FIG. 1.

Effect of pH of the medium on the antibacterial activity of penicillin, gentisyl alcohol, and homogentisic acid.

bacterial action of gentisyl alcohol, homogentisic acid and penicillin in relation to pH of the medium are presented in Fig. 1.

Experimental. Gentisyl alcohol was prepared by catalytic hydrogenation of gentisaldehyde according to the method of Birkinshaw, Bracken and Raistrick.⁵ Synthetic homogentisic acid was prepared by a procedure which we intend to present in detail elsewhere. These compounds were purified by recrystallization several times and gave melting points agreeing with those reported in the literature.

Essentially the same cylinder plate procedure as that reported by Schmidt and Moyer⁷ for the assay of penicillin was used to de-

¹ Brack, A., *Helv. Chim. Acta*, 1947, **30**, 1.

² Renz, J., *ibid.*, 1947, **30**, 124.

³ Seebeck, E., *ibid.*, 1947, **30**, 149.

* Also known as clavacin, clavatin, claviformin, and expansin.⁴

⁴ Waksman, S. A., *Microbial Antagonisms and Antibiotic Substances*, 2nd Ed., The Commonwealth Fund, New York, 1947, p. 210.

⁵ Birkinshaw, J. H., Bracken, A., and Raistrick, H., *Biochem. J.*, 1943, **37**, 726.

⁶ Birkinshaw, J. H., Bracken, A., Michael, S. E., and Raistrick, H., *Lancet*, 1943, **2**, 625.

⁷ Schmidt, W. H., and Moyer, A. J., *J. Bact.*, 1944, **47**, 199.

termine antibacterial activity of the compounds. The only variation from this technique was in use of *Difco* "penassay" seed agar adjusted to variable hydrogen ion concentrations and the use of distilled water instead of buffer solutions to dissolve the penicillin and test compounds. Cylinders 8 mm in diameter were used, and *S. aureus* 209P was the test organism. All tests were run in quadruplicate, and an average of the 4 zone diameters was taken for the final reading.

Discussion. A concentration of 20 mg/ml of gentisyl alcohol or of homogentisic acid was necessary to produce antibacterial activity comparable to that shown by 0.1 unit/ml of the working standard of penicillin. Brack¹ reported antibacterial activity with concentrations of gentisyl alcohol of 100 γ /cc over the pH range 5.0 to 8.5. This degree of antibacterial activity was not observed in our experiments. We did note slight activity with this concentration of gentisyl alcohol in the pH range above 8.2. With 2% gentisyl alcohol antibacterial activity was more marked at low and at high pH values. Some oxidation of the compound occurs in the assay procedure, especially in alkaline media, and is evidenced by browning of the zones of inhibition. The possibility that oxidation products might have contributed to the antibacterial activity of

gentisyl alcohol noted in these cases has not been investigated. It will be noted that the pH activity curve usually observed with penicillin was obtained for control and comparative purposes in these experiments.

The various results of different laboratories might possibly be due to differences in media, test organism or to a combination of these two important factors, and they suggest that these factors may be critical in the evaluation of the antibacterial activity of gentisyl alcohol or its derivatives.

The slight antibacterial activity exhibited by homogentisic acid has not to our knowledge been described previously. This activity decreased rapidly with increase in the pH of the assay agar.

Summary. Gentisyl alcohol in our experiments was found to have only slight antibacterial activity over a broad pH range. Our results with this compound are at variance with those of Brack¹ but are in agreement with the original report of Raistrick and co-workers.⁵

Homogentisic acid was found to have a definite but slight antibacterial activity.

The effect of pH of the medium on the antibacterial activity of these compounds against *S. aureus* was studied.

16669

Purification of Psittacosis Virus by Alcohol Precipitation and Centrifugation.

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A number of procedures have been described in which chemical agents such as cal-

cium phosphate,¹ alum,² protamine,³ zinc and magnesium hydroxide⁴ are utilized in the concentration and partial purification of influenza

¹ Stanley, W. M., *Science*, 1945, **101**, 332.

² Bodily, H. L., Corey, M., and Eaton, M. D., *Proc. Soc. Exp. Biol. and Med.*, 1943, **52**, 165.

³ Chambers, L. A., and Henle, W., *Proc. Soc. Exp. Biol. and Med.*, 1941, **48**, 481.